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TITLE: Development of Trace Enrichment Techniques for Trace Metals in Natural Waters Using High Pressure Liquid Chromatography

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An on-line trace enrichment (TE) system, based on high pressure liquid chromatogroaphy (HPLC) and flame atomic absorption (AA), has been developed for trace metal analysis. The sample is pumped through a column filled with a cation exchange resin which traps metal ions. The ions are subsequently stripped from the column with citrate solution and pass directly into the AA for quantitation. Calibration is achieved with an 87 µ L sample loop which introduces standard solutions directly onto the column without going through the pump. A microcomputer system interface was constructed which performs instrumental control and data handling functions.

The system was tested with three representative elements - Cu, Cd, and Mn. Detection limits with the sample loop were found to be about 5 ng for all three test metals. The limitation on detection of dilute analyte solutions was tested by determination of 0.3 µg/L solutions for each metal, using a 50 mL TE. Lower concentrations could presumably be determined by use of a larger TE volume. Precision for replicate runs is typically better than 5-10% relative standard deviation (RSD), and linearity of the system was found to extend from the detection limit to approximately 5 mg/L for all three metals. Thus a sample can be

determined if its concentration is in the range of at least 0.3 μg/L to 5000 μg/L, or over 4 orders of magnitude. Sample throughput time depends on sample concentration, and it takes about 20 minutes to analyze samples in triplicate at the μg/L level. About 20 minutes are necessary to generate a calibration curve.

An interference study was made of species most commonly found in natural water samples. The system was found to be free from interference effects from them at levels higher than that expected in most real samples.

The 3 metals were also determined in river and tap water samples. The concentration of Cu was found to be 1.2 mg/L in tap water and 2.6 μg/L in river water. Cd was found to be 0.25 μg/L in tap water and 0.22 μg/L in river water, while Mn was 2.3 μg/L in tap water and 9.5 μg/L in river water. These results were verified by a standard addition procedure to assure that the system was not subject to significant interference effects.

# Development of Trace Enrichment Techniques for Trace Metals in Natural Waters Using High Pressure Liquid Chromatography

by

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## LIST OF ABBREVIATIONS USED

| | |
|---|---|
| AA | atomic absorption |
| ADC | analog-to-digital converter |
| DAC | digital-to-analog converter |
| dw | deionized water |
| HPLC | high pressure liquid chromatography |
| I/O | input/output |
| LED | light emitting diode |
| MP | mobile phase |
| OA | operational amplifier |
| PB | port B |
| PC | printed ciruit |
| RAM | random access memory |
| ROM | read only memory |
| RSD | relative standard deviation |
| SD | standard deviation |
| SR | stripping reagent |
| SS | stainless steel |
| TE | trace enrichment |
| UV | ultraviolet |

# Development of Trace Enrichment Techniques for Trace Metals in Natural Waters Using High Pressure Liquid Chromatography

## INTRODUCTION

Transition metals are important constituents of the biosphere. Several metals are known to be toxic at low levels to humans and other species. They often interfere with cellular biochemical systems by tying up important sites on enzymes or hindering cellular nutrient uptake, and the continuing expansion of industrialization is adding increasing amounts of them into natural waters. On the other hand, some metals are important bio-nutrients at low levels and their availability is being threatened by chemicals which remove them from the environment. Thus there has been a good deal of attention given to trace metals and a large commitment, both in capital costs and man-hours, toward monitoring trace metal concentrations in natural waters.

There are many methods for trace metal analysis, but several of the more convenient ones have detection limits which are above environmental levels of some metals. In addition, they are often subject to considerable interference effects from concomitant species in the sample. As a result of these problems, a separate clean-up and/or a preconcentration step (e.g. solvent extraction, column chromatography) is often necessary prior to analysis. This adds to the analysis time and often requires considerable operator skill. Also, these extra analysis steps increase the possibility of compromising the reliability of the results due to contamination or loss of the analyte.

In response to this twofold need - detection limit improvement and separation of the analyte from concomitant interferents - this research has involved the development of an integrated preconcentration and detection system. The purpose of the system is to enhance the utility of flame atomic absorption (AA) detection and broaden the scope of suitable sample compositions and analyte concentrations. Preconcentration is based on a chromatographic stationary phase which is specifically denoted trace enrichment (TE).

Flame AA offers several inherent advantages as a technique for total metal analysis. It is one of the most selective of all techniques because it is based on absorption over narrow atomic line profiles and therefore is less susceptible to spectral overlap than molecular spectral methods. It has wide applicability and can be used to determine about 70 elements (1). Convenience of the technique is twofold. Not only is the measurement process rapid, but also the technique is fairly tolerant to sample composition and matrix effects. Finally, it requires little operator skill or expertise. It is a relatively sensitive technique and many samples may be analyzed directly, but detection limits for other metals are still higher than their environmental levels. Thus a flame AA with an expanded TE capability makes it an even more powerful tool for trace metal determination.

The developed system uses a high pressure liquid chromatography (HPLC) pump to pass the sample through a cation exchange column. The column concentrates the analyte metal and simultaneously separates it from some concomitant species. A change in the mobile phase then strips the metal from the column and the AA is used to detect and quantitate it. Calibration of the instrumental response is

accomplished with a sample loop to introduce analyte standard solutions directly to the column. In order to enhance convenience of operation, a microcomputer system was constructed which automates analysis by controlling the timing and switching of valves. The computer also collects and analyzes data, then reduces it to the form of statistical summaries.

The performance of the system was tested with three metals - Cu, Cd, and Mn - in order to prove it capable of providing reliable analytical information for a sample. The range of linearity was tested since it determines the restraints on the sample in terms of analyte concentration. A wide range of linearity will allow greater flexibility of analyte concentration. A study was also conducted to determine how low an analyte concentration can be reliably detected by the system as was its degree of tolerance to co-existing species in a sample during a determination of analyte concentration. Finally, several real samples were collected and analyzed for the test metals. A standard addition procedure was used to verify the validity of the determinations and to ascertain whether the system was subject to error due to interferences.

# BACKGROUND

## Introduction

The need for preconcentration arises when an instrumental analysis method fails to provide the necessary sensitivity for a particular sample or application. In recent years advances in instrumental design have improved the capabilities for convenience and speed of analysis, but many instrumental techniques are still limited by the sample itself. In addition, as environmental and health scientists increase their attention on trace metals, more stringent requirements for lower detection limits with good selectivity are being made on analytical instrumentation and methodology.

The following discussion of the background to this research will deal primarily with inorganic analytes, although many of the considerations and techniques apply to the analysis of organic species as well. The historical summary is not intended to be a comprehensive discussion of work done in the particular topic area. Rather, it is intended to cite some typical examples and applications of common methods currently employed for preconcentration of trace metals in aqueous samples in order to give the reader a feeling for the subject.

Examples of the various methods of preconcentration will be presented along with the major advantages and limitations of them. The discussion will focus primarily on the preconcentration techniques themselves and little attention will be paid to the subsequent measurement step. However, the physical and chemical form of the analyte and

its matrix environment are important factors for some instruments, and where appropriate, this will be commented on.

In most cases, the basic idea of preconcentration is to transfer the analyte in the original sample into a smaller volume before presentation to the measurement instrument. Ideally, this transfer is quantitative and does not increase matrix interference. In many cases, the preconcentration step can simultaneously isolate the analyte from many matrix species and thus improve selectivity.

## Evaporation

Evaporation is conceptually the simplest concentration technique, and the intent is to evaporate a large fraction of the solvent without affecting the number of analyte ions, thus increasing the analyte concentration. The primary advantage to the technique is that usually no complicated glassware or reagents are required and contamination is minimized. The technique has been used to prepare samples for x-ray analysis by evaporating them to dryness (2, 3), but for most analyses of aqueous samples, it has several distinct disadvantages. Due to the large heat of vaporization for water, it is quite slow for aqueous samples and, in addition, the concentration of total dissolved solids will of course be increased along with the analyte. Thus matrix effects and problems such as precipitation and coprecipitation can result. Increased viscosity and clogging (4) can adversely affect techniques such as flame atomic spectrometry.

## Coprecipitation

Simple precipitation of the analyte may be accomplished by manipulation of the solution conditions such that the solubility product is exceeded for the analyte by adding another ion. Thus it is useful for techniques that require solid samples, such as x-ray spectroscopy and neutron activation. However, in many dilute solutions particle size of the precipitate may be too small and the particles may pass through the filtering device, or supersaturation may occur and particles do not form at all. These effects result in a less than quantitative recovery and are often not predictable or reproducible.

A solution to these problems is often found by the addition of a carrier ion to the sample in order to cause coprecipitation of the analyte by causing it to bind to or in a predetermined precipitate or colloid. General schemes have been developed (3) to achieve general and selective precipitation by using a variety of reagents. Iron has been used to coprecipitate both negatively and positively charged species (4). Ferric ion is initially precipitated with ammonium. At high pH the precipitate forms a negatively charged colloid which attracts and precipitates positive ions.

$$Fe(OH)_3 + OH^- \rightarrow Fe(OH)_4^-$$

$$Fe(OH)_4^- + M^+ \rightarrow Fe(OH)_4M$$

At low pH the ferric hydroxide loses an $OH^-$ to form a correspondingly positive colloid which selectively precipitates anionic species. Others (7) have used ferric ion and organic anions to collect Cd, Co, Cr, Cu, Ni, and Pb with a precision of 2-7% RSD.

Once the analyte is precipitated it may be collected by filtration or as an emulsion. Hiraide et al. (8) co-precipitated Cr(III), Mn(II), Co, Ni, Cu(II), Ce and Pb in indium hydroxide and collected the precipitate in an oleate and dodecyl sulfate emulsion with nitrogen bubbling to enrich by a factor of 240 for analysis by an inductively coupled plasma emission spectrometer.

One of the main advantages of coprecipitation is the ability to extend the capacity of the system in order to accommodate the analyte in the presence of chemically similar concomitant species which may be present in large excess. For instance, it may be desired to analyze for Sr in the presence of a large excess of Mg. The main disadvantage to the technique is that the procedures are often tedious and labor intensive, making them inconvenient for routine analysis. Also the collected precipitate must be redissolved for most measurement techniques, which often results in a small enrichment factor. Contamination due to analyte in the precipitation reagent or reagents used to redissolve the precipitate can be a problem.

## Electrodeposition

Electrodeposition is a technique which takes advantage of the electro-activity of the transition metals and was used extensively in electrogravimetry (9), an early application of the principle. The object is to deposit the analyte metal directly onto a cathode by application of a constant potential which is more negative than the metal's reduction potential. The reduced metal is often redissolved for measurement and the technique finds the most extensive use for

electrochemically well behaved metals such as Cd, Pb, Cu, Co and Ni. Typical deposition time is 2-30 minutes and analyte metals have been deposited onto such surfaces as Pt(10) and W(11) filaments and into hanging Hg drops (12, 13). In the latter case, the Hg may be subsequently distilled off, or the reduced metal may be re-oxidized and its resultant current measured for anodic stripping voltametry (14-18).

A rather novel application of the principle is to deposit the metal onto a graphite tubular electrode which subsequently serves as a carbon rod for electrothermal AA. The graphite tube is usually fixed to an apparatus whereby the sample circulates through the tube during deposition. Various combinations of flow rate and electrochemical configurations have been tried in order to achieve a uniform deposition (19-21).

The primary disadvantages to the technique are that it is applicable to only a few metals, it is very slow, and precision is poor for repetitive analysis with precision typically worse than 10% RSD (20). Also for fresh water samples, an electrolyte must be added, which introduces a potential source of contamination. The main advantages are that it requires few reagents and the instrumentation is simple.

## Donnan Dialysis

The basis of Donnan dialysis is a membrane which exhibits permselectivity - the difference in diffusibility between ions of opposite charge. With such a membrane, analyte ions are selectively passed from the sample into a smaller volume. On the membrane are either positive or negative (not both) fixed ionic groups. Ions with the same charge

as the groups (co-ions) are excluded from the membrane phase and exist in relatively low concentration compared to exchangeable counterions that have the opposite charge of the fixed groups. Thus the co-ions are slow to diffuse through the membrane (flux rates typically $10^{-9}$ mol $cm^{-2}$ $s^{-1}$) whereas the counter ions are fast ($10^{-6}$ mol $cm^{-2}$ $s^{-1}$).

The analyte receiving solution is an electrolyte which is selected such that the concentration of analyte in it is initially zero. For each counterion in the electrolyte that traverses the membrane, an analyte ion passes into the receiving solution (assuming the same magnitude of charge for both ions). The concentration of the electrolyte is much higher than the ionic strength of the sample and eventually the slower co-ions and the counter-ions will establish a concentration equilibrium across the membrane, but the process is stopped before this equilibrium is established. Thus in a matter of hours, the analyte ions are essentially quantitatively transferred into the electrolyte solution and an equal number of electrolyte ions (though a much smaller fraction of their total number) have traversed into the original sample solution.

The benefits to the procedure are twofold: not only is there an enrichment of the analyte ion concentration but a matrix normalization of the sample is also achieved (22). This lends itself quite well for measurement techniques which require a high ionic strength sample such as ion selective electrode analysis. A primary limitation to the utility of the technique is that it is limited to samples with low ionic strength, usually less than 0.01 M.

One application of the Donnan principle is an "amplifier" for ion selective electrodes (23). For this a Donnan membrane is wrapped

around the exterior of an electrode which is then dipped directly into the sample solution. Enrichment factors of 5-100 and a half-life to steady state of less than one minute are claimed. Another application of the principle is to pre-concentrate the analyte for AA analysis, as has been done for Cu, Cd, and Zn (24, 25). Anions have also been enriched (26).

## Solvent Extraction

By differences in solubility, an analyte can be selectively transferred from one phase to another, preferably into a smaller volume. The receiving phase is usually an organic solvent, and a carrier molecule is often bound to the analyte to enhance its solubility in it. If the carrier molecule is selective for the analyte, the bulk of sample matrix species are left behind. Thus the analyte is both concentrated and separated from the bulk of the interferents.

A multitude of solvent and carrier molecule systems have been developed (27-36). Typical carrier molecules are chelating agents, which are very general and will simultaneously extract a variety of transition metals. The primary advantage of these agents is removal of the analyte from alkali and alkaline earths. Other agents, such as dimethylglyoxime and oxine (32) are more specific.

Typically after extraction, the analyte is quantitated by some absorption technique in the organic liquid phase. In one experiment (27) Cd, Fe, Zn, Cu, Mn and Pb were extracted from 500 mL of river water into 50 mL of isoamyl alcohol and the metals were subsequently

determined by flame AA. Only Cu transfer was quantitative, though, and precision varied from 5-15% RSD, with an enrichment factor of only 10.

Common problems with the technique include a less than quantitative separation of the small volume of organic phase from the larger volume of aqueous phase, the adherence of the organic phase to vessel walls, and the formation of emulsions (37). A rather novel approach to these difficulties (30, 31) is to use an organic solvent which melts a few degrees above room temperature. Conventional solvent extraction is carried out at a temperature above its melting point. After extraction, the system is allowed to cool and the organic phase solidifies. Napthol and diphenyl compounds will form a compact bead which is easily collected and subsequently dissolved for metal determination.

## Ion Exchange and Chromatographic Techniques

A family of techniques which offer the potential for much larger enrichment factors than those previously mentioned are based on collecting the analyte ion on a stationary phase. By virtue of their greater affinity for the stationary phase, analyte ions are exchanged with a co-ion and retained at active sites on the stationary phase. The exchange sites usually retain the metal ion by an ion exchange or chelating mechanism. Both batch extraction of the ions (mixing of the sample with the stationary phase in a beaker) and column chromatography (where the stationary phase is placed in a column and the sample percolated through the column as a mobile phase) have been used.

In some cases, the metal ions are measured directly on the solid stationary phase by such means as molecular absorption (38), x-ray

spectrometry (39, 40) or neutron activation analysis (41). More often, the ions are removed from the resin with a small volume of eluent and this fraction is then analyzed (42-52, 123).

Although application of the metal ion to the stationary phase is usually the same, elution schemes have involved a greater variety of methods. Backflushing (42) has been used in order to minimize the elution volume for greater enrichment factors. In situ reduction was used to selectively remove a single oxidation state of Cr from anion exchange (42) and chelating (43, 52) resins.

An ion exchange method with a novel configuration for the retention material is found in resin filter papers (40, 53, 54). For this application, paper disks are impregnated with ion exchange resins, and offer the convenience of simply filtering the sample through the paper, often repeatedly (54).

Metals may also be adsorbed on stationary phases by converting them with a reagent to a chemical form that has an affinity for a stationary phase. Neutral metal complexes of 8-hydroxyquinoline have been adsorbed onto activated carbon which is then filtered and directly analyzed with x-ray spectroscopy (55). Also oxine complexes from seawater samples have been adsorbed onto a $C_{18}$-bonded silica gel and subsequently eluted with methanol for determination by atomic spectroscopy (56).

## High Pressure Liquid Chromotography (HPLC)

With the advantages offered by many resins for sample pre-concentration and the instrumental advantages offered by HPLC technology, it seems a logical extension to combine the two. The tremendous advances in the role of HPLC as a separation technique cannot be overstated (57-59), but most use of HPLC as a pre-concentration technique has thus far been confined to the determination of organic compounds.

Several materials such as activated carbon (60-62), porous organic polymers (63) and ion exchange resins (64-66) which were initially used for off-line analysis have recently been applied to on-line HPLC applications (67). Several applications (68-71) have also been made of bonded phase resins, and Frei et al. (72, 73, 76) have discussed several of them. Samples have included both river water (68, 71, 74) and seawater (75).

HPLC techniques have been used to separate organo-metallic species (77-84, 91) and some work has been done on HPLC separation of metal ions (85-87). TE with HPLC is somewhat embryonic, but some work has been done with both bonded phase (88, 89) and conventional (88-90) resins.

There are many possible detection systems for quantitation of HPLC effluents (92-95). Electrochemical detectors have been employed (96, 97), which offer sensitivity and specificity. In post-column derivatization (88, 89, 98, 99) a reagent which forms a chromaphore with the analyte is added to the effluent as it elutes from the column. The resultant species then enters a molecular absorbance or fluorescence detector. Sensitivity can be good, but specificity may be lacking due

to possible spectral overlap since the derivatizing agent may not be selective for only the analyte. Atomic spectroscopic detection is a logical choice for determination of metals since there is generally less potential for spectral overlap than molecular methods. Detection systems have been devised based on atomic emission (100, 101), fluorescence (102), and absorption (86, 103-105). Some work has been done to interface the effluent of HPLC to electrothermal AA (106-108), but since non-flame atomization generally requires discrete sampling, the inherent technical difficulties suggest flame AA unless low analyte levels require the increased sensitivity offered by electrothermal methods. TE can usually preclude this necessity.

# INSTRUMENTAL

## Overview

The basic component to this TE system is a pump which passes a sample through a column containing a stationary phase. The stationary phase removes the analyte from the sample as it passes by. When the desired volume of sample has passed through the column, a valve directs deionized water (dw) to flush the sample from the pump and tubing (see Figure 1). A second valve then changes the mobile phase (MP) to a stripping reagent (SR), supplied via another pump, which removes the analyte from the stationary phase and sends it to a couple of detectors (see Figure 2). Since the SR elutes the metal in a small plug, the resultant detector response is a peak. In order to calibrate the response of the detector to the analyte, a third valve is inserted which contains a sample loop that is filled with a standard analyte solution by a smaller pump (see Figure 3). Finally, a microcomputer is added which collects and processes the data from the detector and also controls the timing and function of the various components (see Figure 4).

The chromatographic aspects and then the computerization of the system will be discussed.

## Chromatograph

The entire chromatographic system is situated on a cart, allowing it to be wheeled up to the atomic absorption (AA) spectrometer for an

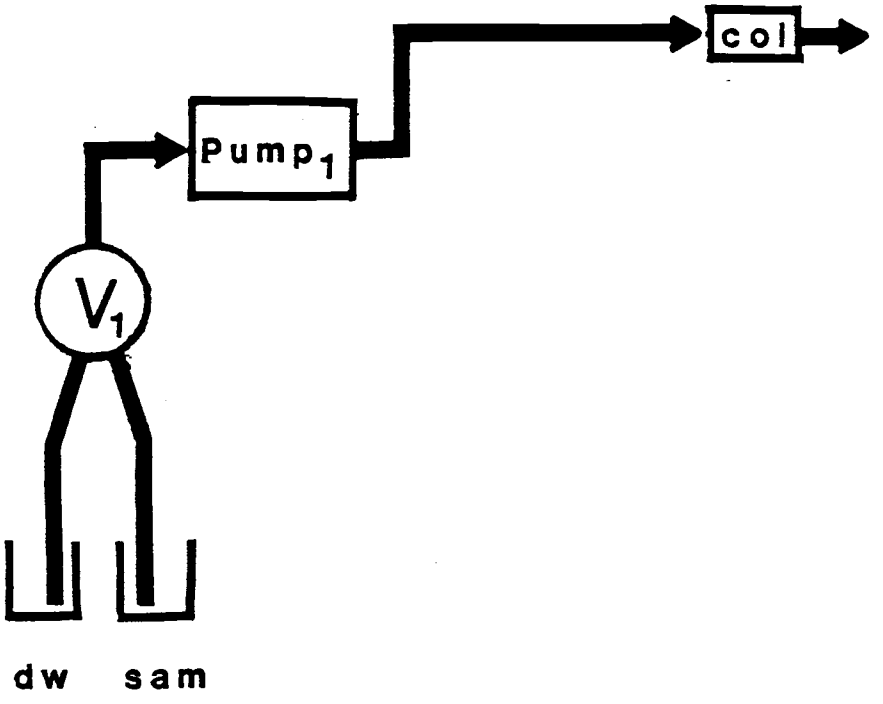



Figure 1. Conceptual overview of TE system

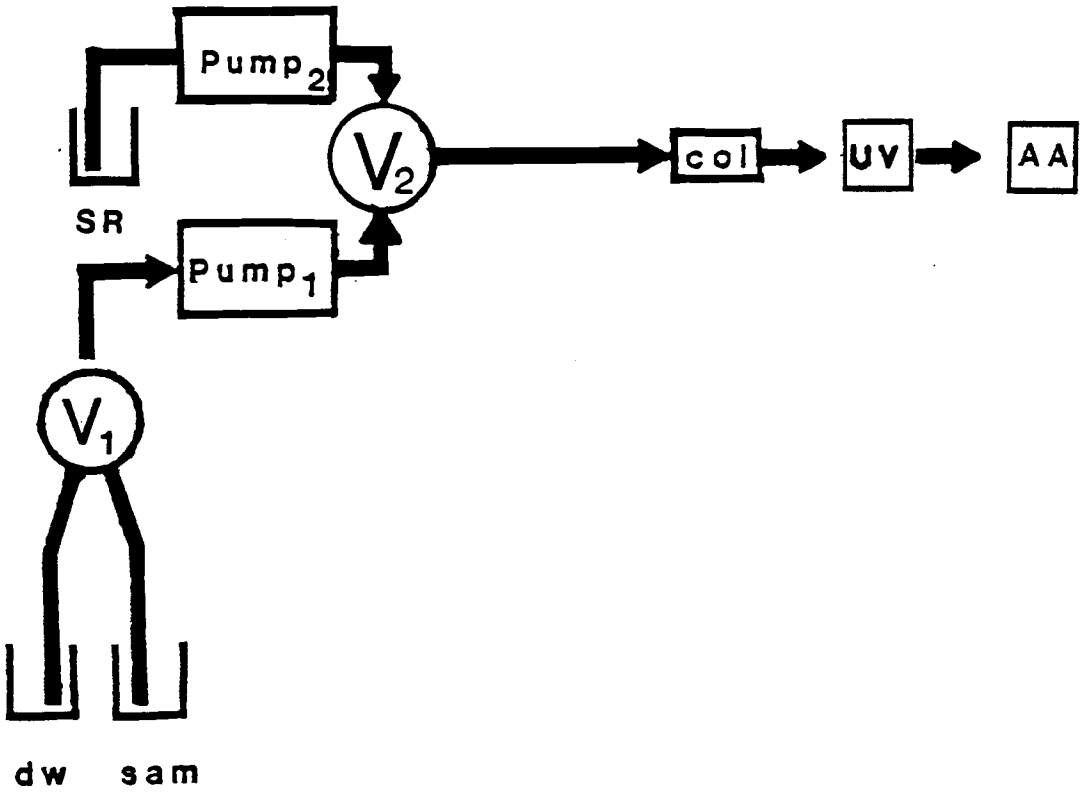



Figure 2. Conceptual overview of TE system (cont'd.)

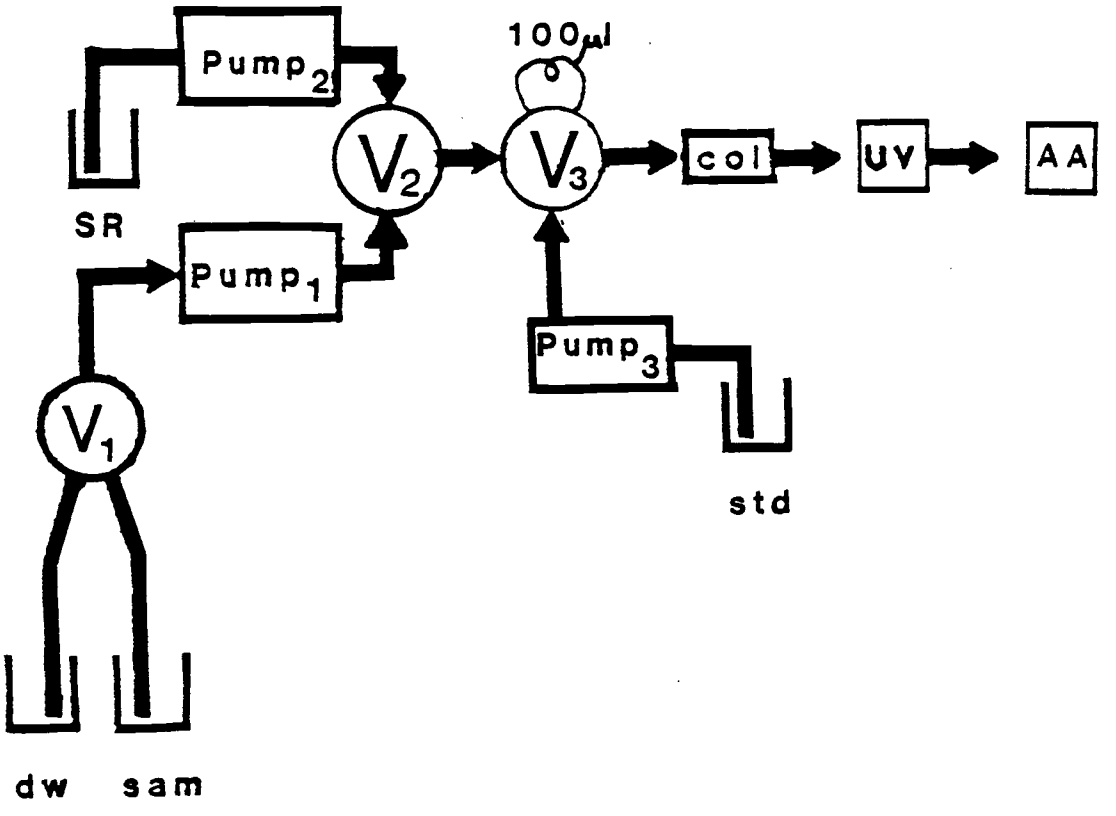



Figure 3. Conceptual overview of TE system (cont'd.)

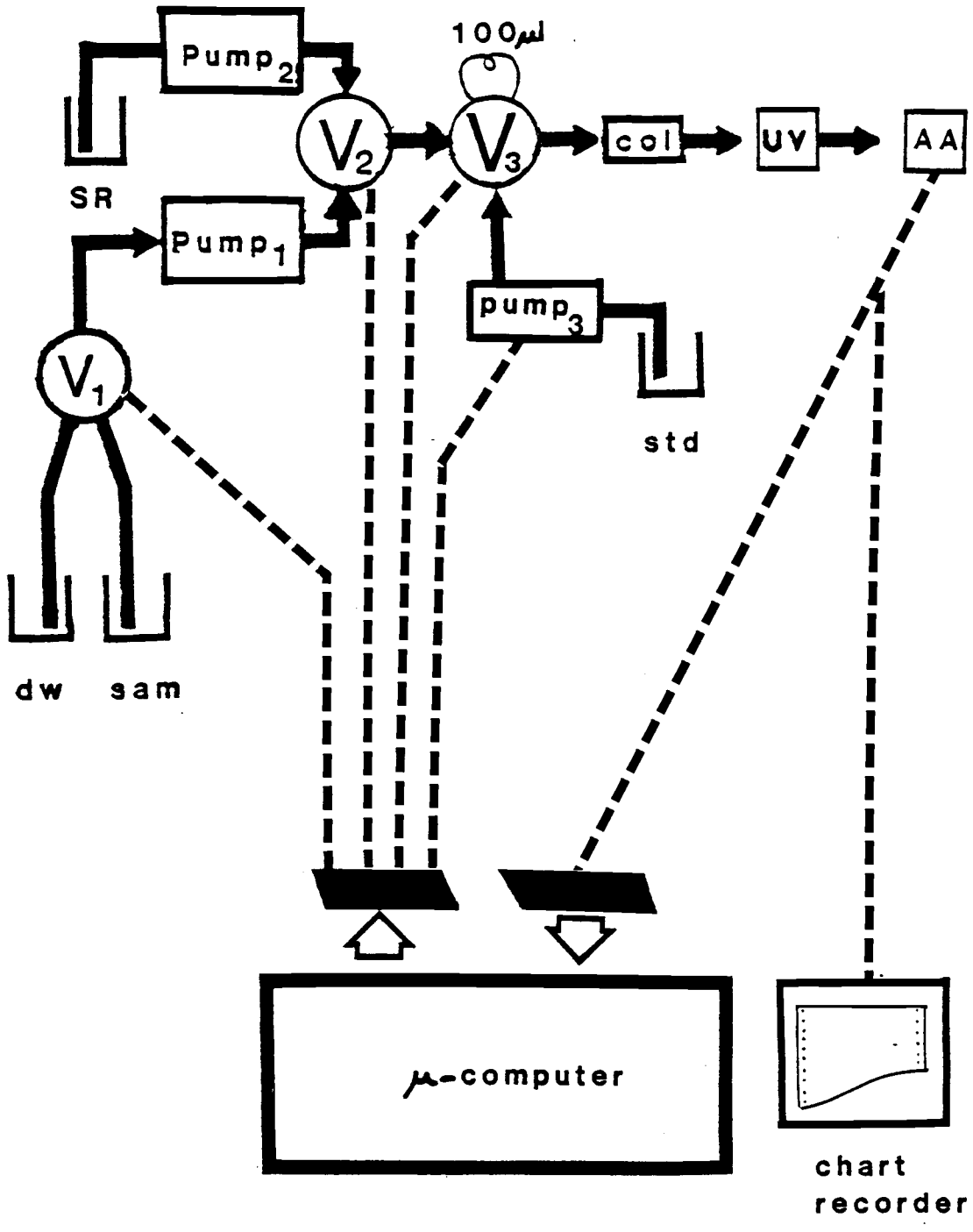



Figure 4. Conceptual overview of TE system (cont'd.)

analysis and removed from it when not in use. The overall orientation of the system is depicted in Figure 5, and the components are identified in Table I.

The TE system is based on two HPLC pumps (referred to as $P_A$ and $P_B$). The pump inlet tubing is Teflon and was fitted with Omnifit Teflon shut-off valves ($V_4$ and $V_5$) to prevent siphoning of the solvent when the pumps are turned off.

The majority of plumbing connections are a standard flanged tube type with a ¼-28 threaded bushing holding the flared tubing against the body of the connecting port. All valve and column fittings are made to accept this type of tubing connection.

In order to minimize solution contact with metal surfaces, stainless steel (SS) wire mesh filters located inside the pump inlet manifolds were removed, and the outlet tubing, which is SS on exit from the pump, was adapted to Teflon close to the discharge manifold ($A_1$ and $A_2$). Thus the only metal contact is within the pump body, the short piece of SS exit tubing, and the SS to Teflon adaptor at the pump outlet.

The two HPLC pumps are controlled by a solvent programmer (SP). In initial experiments the programmer was used to change the composition of the MP for the analysis, but now it is used with the 10 mL/minute flow rate and 50% start B settings. This provides a 5 mL/minute flow rate to each pump and is used in this way because it was found that the nominal flow rates more closely resemble the measured ones than if each pump were controlled by its own flow rate dial. The external control provided by the programmer is more accurate than the individual flow adjustment potentiometers.

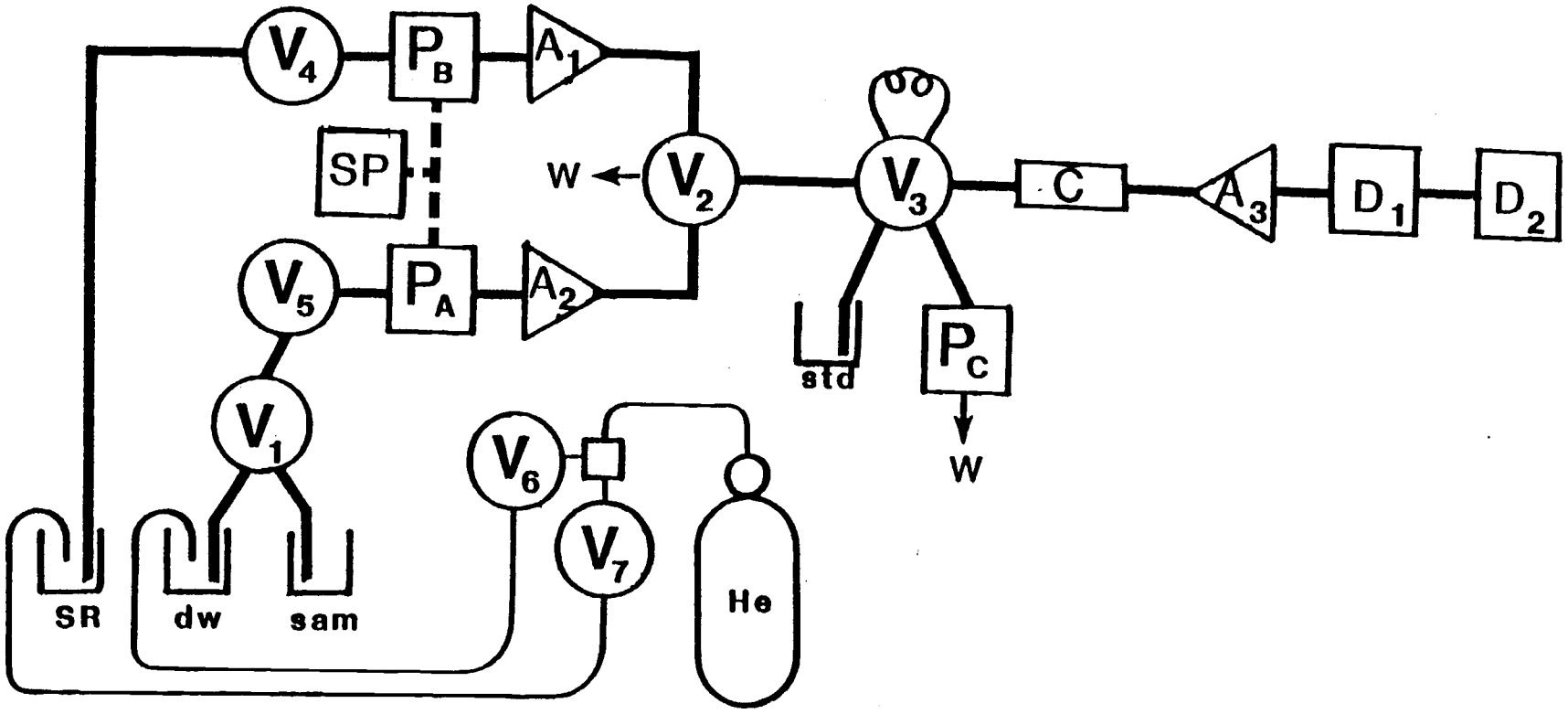



Figure 5. Chromatograph block diagram. Components identified in Table I.

Table I: Chromatograph Inventory

| Figure 5 Symbol | Item | Source | Comment |
|---|---|---|---|
| | Pumping system | | |
| $P_B$ | Constametric I | Laboratory Data Control<br>Riviera Beach, FL | 0-10 mL/min<br>5000 psi max |
| $P_A$ | Constametric II | Laboratory Data Control<br>Riviera Beach, FL | 0-10 mL/min<br>5000 psi max |
| SP | Constametric II G<br>Solvent Programmer | Laboratory Data Control<br>Riviera Beach, FL | Controls flow rates of<br>$P_A$ and $P_B$ |
| | Valves | | |
| $V_4$, $V_5$ | Omnifit 2-way | Rainin Instr. Co.<br>Emeryville, CA | #45-1101<br>Shut-off |
| $V_1$, $V_2$ | 4-way | Dionex<br>Sunnyvale, CA | #30524, Slider type<br>pneumatic activation |
| $V_3$ | Sample injection | Dionex<br>Sunnyvale, CA | #30520, Slider type<br>pneumatic activation |
| | Skinner 3-way solenoid | Buchanan Fluid Systems<br>Seattle, WA | 110 VAC activation |
| $V_6$, $V_7$ | Swagelok needle | Portland Valve<br>Portland, OR | |

Table I: Chromatograph Inventory (cont'd)

| Figure 5 Symbol | Item | Source | Comment |
|---|---|---|---|
| | Tubing | | |
| | Teflon - 1/8 in. od 1.5 mm id | Lab Data Control | $V_2$ to waste, $P_C$ to waste ($^2$(3 in. ea)<br>$V_2$ to $V_3$ (20 in.), $V_3$ to $P_C$ ($^3$5 in.)<br>Sample and Solvent res. to $P_A$ and $P_B$ |
| | 316 SS - 1/16 in. od 0.02 in. id | Lab Data Control | $A_3$ to $D_1$ (5 in.)<br>$P_A$ to $A_1$ (2 in.ea)<br>$P_B$ to $A_2$ |
| | Teflon - 1/16 in. od 0.8 mm id | Rainin<br>Emeryville, CA | $A_1$ and $A_2$ to $V_2$ (15 in. ea)<br>std to $V_3$ (10 in.)<br>$V_3$ to C (10 in.)<br>C to $A_3$ (10 in.)<br>$D_1$ to $D_2$ (6 in.) |
| | Tygon - 7/16 in. od 5/16 in. id | VWR Scientific<br>San Francisco, CA | $V_6$ and $V_7$ to res. (70 in. ea) |
| $A_1$,$A_2$,$A_3$ | tubing adaptor (1/16 in. SS to 1.5 mm Teflon) | Rainin<br>Emeryville, CA | #200-41 |

Table I: Chromatograph Inventory (cont'd)

| Figure 5 Symbol | Stem | Source | Comment |
|---|---|---|---|
| | Column | | |
| C | glass column | Rainin<br>Emeryville, CA | Altex #251-84<br>3 mm x 50 mm borosilicate |
| | Detectors | | |
| $D_1$ | UV | Lab Data Control<br>Riviera Beach, FL | Model 1203<br>fixed wavelength - 254 nm |
| $D_2$ | AA | Varian Instrument Co.<br>Palo Alto, CA | Model AA6 |

A solvent and valve module was designed and constructed (see Appendix A), providing covered space for the solvent reservoirs (1000 mL Erlenmeyer flasks), the samples, and the standards. Clear plexiglass construction of the front and top allow visual inspection of solvent levels at all times, and a multiple hinged top gives the operator access to the standards without opening the solvent section. Inside the module are mounted the valves ($V_1$, $V_2$ and $V_3$) and their corresponding pneumatic solenoids to change the MP during the analysis. On the side of the box is mounted a "sidecar" containing the computer control circuitry for the valves, and on the front is a bracket to hold the column (C).

A solvent degassing system was constructed using fritted gas bubblers and He. Dust covers were made from Teflon which solidly hold the bubbler as well as the solvent inlet tubing (see Appendix A) over the solvent reservoir. Brass fittings and needle valves were then assembled and mounted to the chromatograph cart to connect and control helium flow to each of the solvent reservoirs. A short cylinder of He supplies the gas and is regulated to about 3 psi.

The column bracket is mounted on the exterior of the solvent/valve module and has positions for up to three clamps in order to hold a variety of column lengths and diameters.

The valves ($V_1$, $V_2$ and $V_3$) are all pneumatically actuated Dionex slide valves and are made of Teflon. The valve for selection of dw or the sample for $P_A$ (referred to as $V_1$) is a 4 way valve with one port plugged (see Figure 6). Another 4 way valve ($V_2$) is used to change the MP and is stacked together with the sample injection valve so that the two are switched simultaneously with a single set of actuators. In

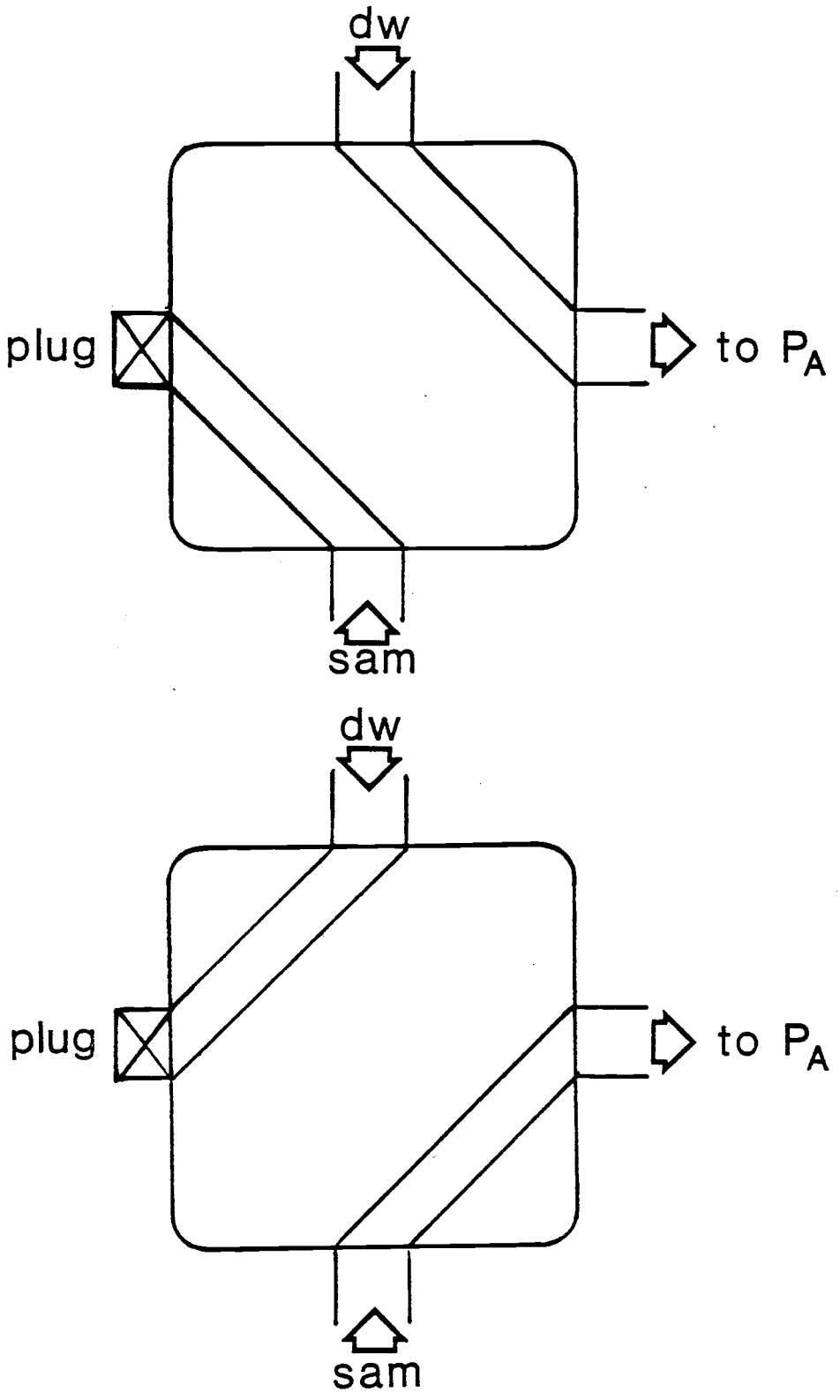



Figure 6. Connections to $V_1$ in TE and dw positions. Top: dw position; Bottom: TE position

order for the holes in the sliders to line up with the ports in the valve bodies, a plastic spacer (0.4 in. x 0.4 in. x 0.125 in.) was placed between the sliders of the two valves.

Pneumatic activation is achieved with the use of Skinner electric solenoid 3 way valves, activated by computer. The valve slider has holes in it which line up with and connect different ports in the body, depending on which of two positions it is in. Compressed air is always pushing on one (and only one) end of the slider (see Figure 7). Thus two solenoid valves are required for each valve. The solenoids are switched by application of 110 VAC and will be discussed in the computer hardware section. It was found that supply pressures greater than 80 psi are needed to push the sliders. A common compressed air supply line feeds each of the four solenoids by means of a home-made manifold (see Appendix A).

In the "load" position, $V_2$ directs dw to the column while the SR is routed to waste (see Figure 8). Meanwhile the sample loop is off line and filled with standard by a mini peristaltic pump ($P_c$), which pulls the standard from its flask through the loop. By stacking $V_2$ and $V_3$, the injection and stripping steps are combined. Putting the valve in the "inject" position places the sample loop contents "on line" to begin to be carried to the column at the same time that the MP is switched to the SR. A 1 mL delay loop between $V_2$ and $V_3$ allows the column to be rinsed with that volume of dw before the SR reaches the column to remove the metal.

The volume of the sample loop for $V_3$ was calibrated by finding the difference between the empty weight and the weight of the loop filled with dw. A subsequent experiment checked the loop volume <u>in situ</u> on

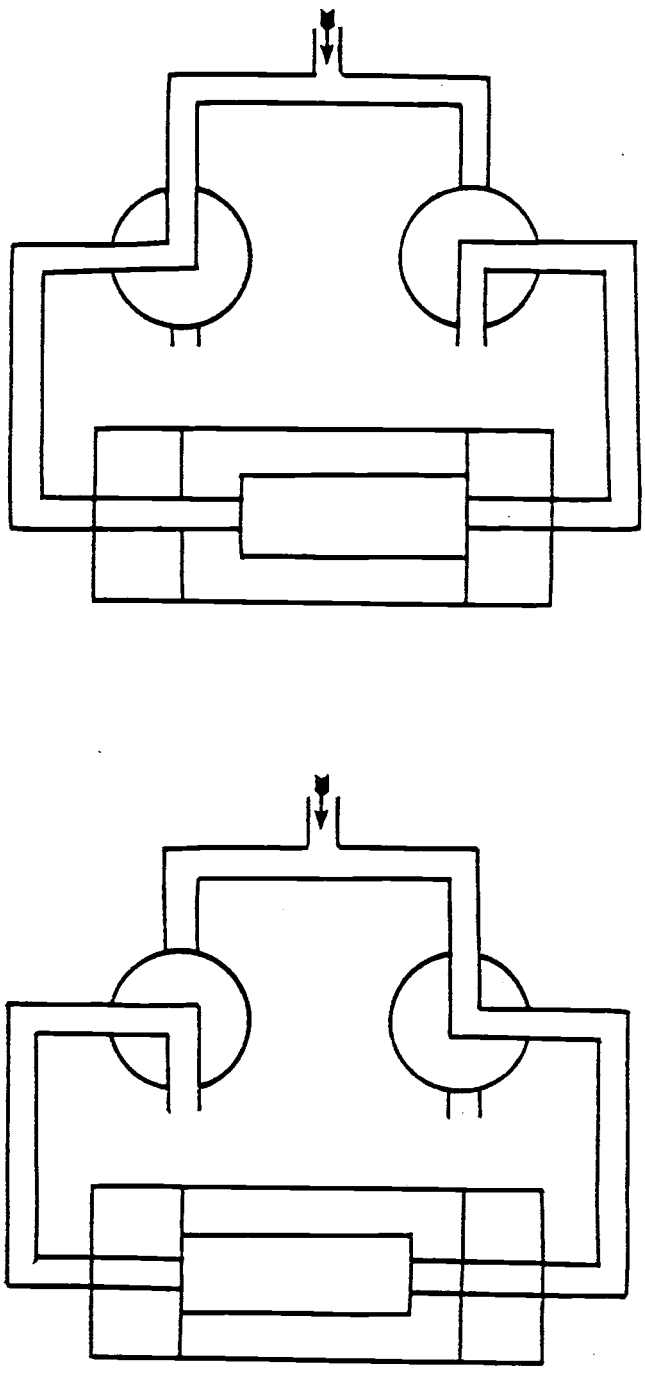


Figure 7. Pneumatic actuation of slider valves

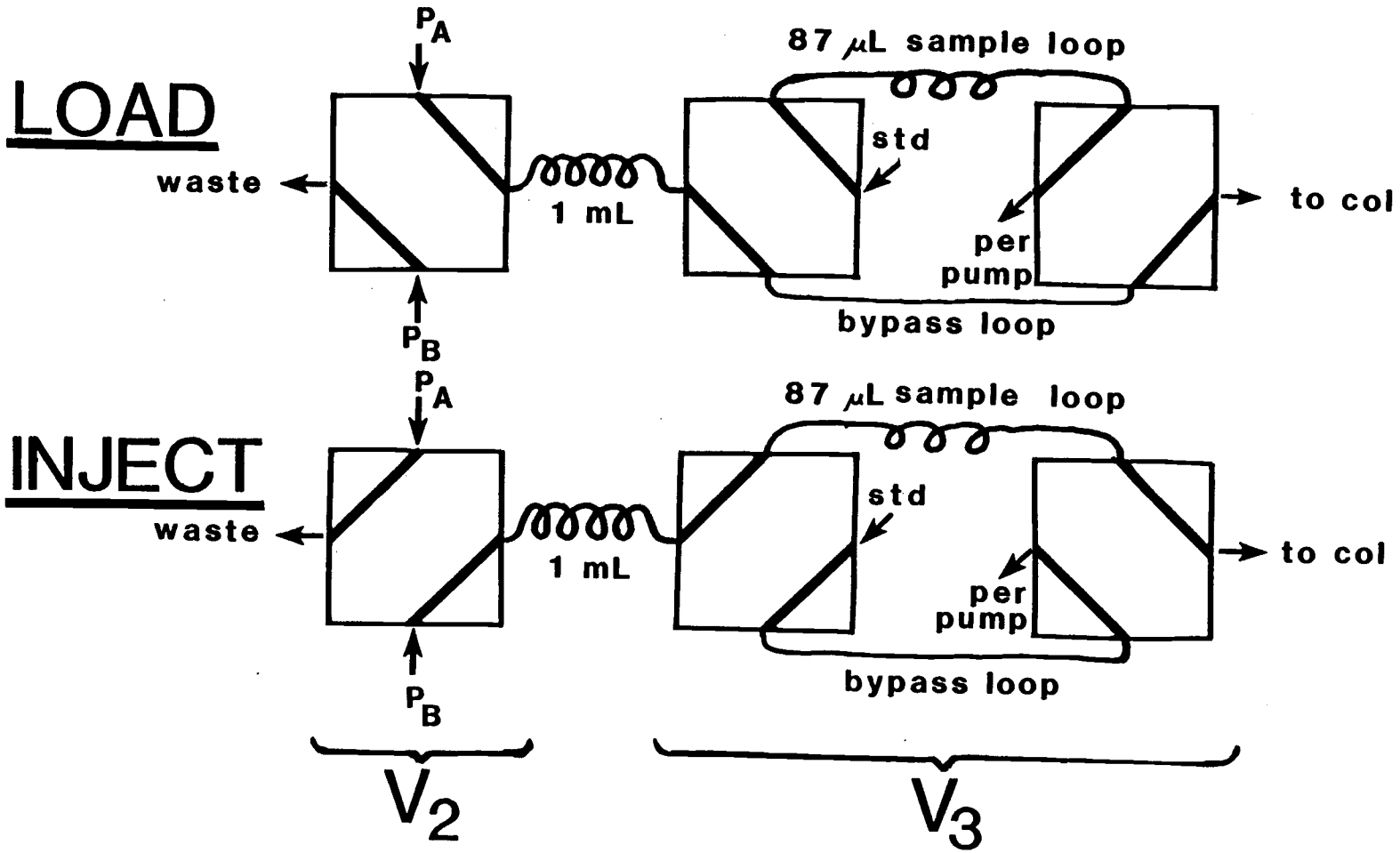



Figure 8. Connections of $V_2/V_3$ in load and inject positions.

the system. With the column removed, the loop was filled with a solution of $10^{-2}$ M $KMnO_4$ as it normally would be with a standard. The contents of the loop were then "injected" with dw as the MP and the plug of $KMnO_4$ was collected in 25 mL volumetric flasks. Several known volumes of the $10^{-2}$ M $KMnO_4$ were also diluted to 25 mL to serve as a reference. The $KMnO_4$ content was then determined on a Cary 118C spectrometer using 526 nm. The volume by weighing is 87.5 µL and 87.6 µL by $KMnO_4$ injection. The exact bypass loop volume was not determined.

Upon being stripped from the column, the metal travels to an ultraviolet (UV) detector, which has SS inlet tubing and a Teflon to SS tubing adaptor ($A_3$) is thus necessary. The detector monitors a fixed wavelength of 254 nm, and since dw has no absorbance at this wavelength but most dissolved species do, the detector indicates the composition of the MP leaving the column and is thus used to monitor the column condition.

Upon leaving the UV detector, the metal passes through a short length of Teflon tubing and goes directly into the AA spectrometer. The original fixed flow rate aspirator was replaced with a variable rate aspirator, which is set to null flow. Thus all the aspiration force is supplied by the HPLC pumps.

The AA spectrometer is used in the absorbance mode with the damp setting on "A". For all metals studied, the flame was acetylene-air, using flow rates of 3 L/minute and 11 L/minute, respectively. This is an oxidizing ratio. A hollow cathode lamp of the metal to be measured is used at the recommended current and the absorbance is zeroed with

the system running dw into the flame. The wavelength setting is 324.7 nm for Cu, 228.8 nm for Cd, and 279.5 nm for Mn.

## Computer Hardware

### Overview

The computer system is primarily situated on an instrument cart, providing the same mobility as for the chromatograph, and is depicted in overall perspective in the block diagram shown in Figure 9. The components are listed in Table II. From a general perspective, the computer system can be seen as consisting of five modules.

An expanded microcomputer is the brain of the entire system and does all calculations and controls data acquisition and timing through numerous input-output (I/O) lines. An analog interfacing module contains an analog-to-digital converter (ADC) and digital-to-analog converter (DAC) to allow the microcomputer system to communicate with external analog devices and signals. A signal conditioning module manicures the AA detector signal before it enters the analog interfacing module. An oscilloscope is used for visual inspection of the data and selection of processing parameters by the operator. A digital interfacing module provides circuitry to control valves, a pump, and a beeper. Each of these modules will now be discussed in greater detail.

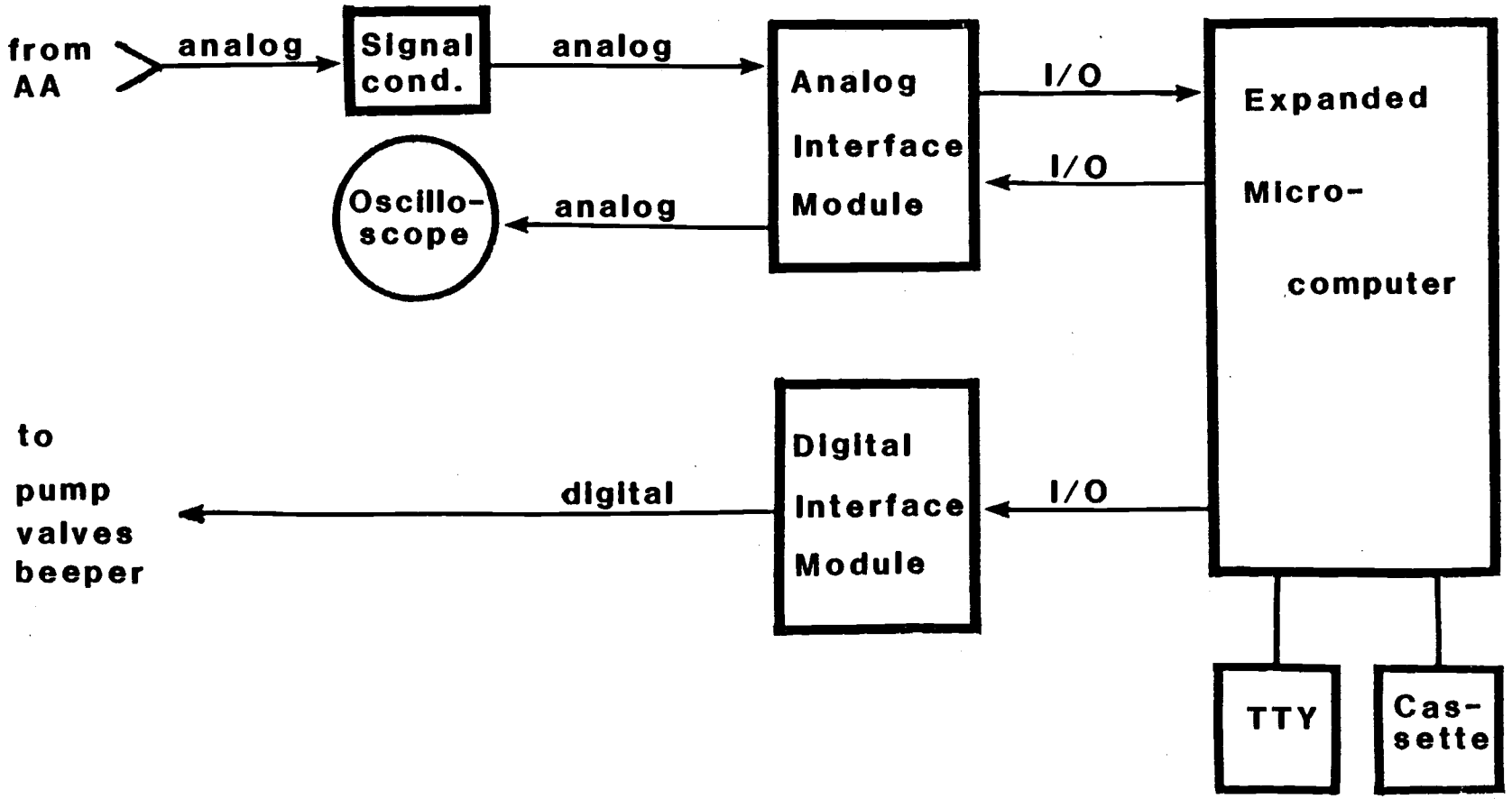



Figure 9. Computer system module block diagram

Table II: Computer System Inventory

| Module | Source | Comments |
|---|---|---|
| **Microcomputer System** | | |
| AIM 65 | Rockwell Intl.<br>Anaheim, CA | 8 bit with LED display and thermal printer |
| Cassette recorder | J.C. Penney<br>New York | Model 681-6568 |
| Teletype | Teletype Corp<br>Portland, OR | Model 43 |
| Mother Board | Seawell Marketing<br>Seattle, WA | LBM with<br>4K RAM<br>5 expan. slots |
| Memory board | Seawell Marketing<br>Seattle, WA | SEA - 16<br>16K RAM |
| I/O board | In House | 2 6522 chips<br>variable address |
| Boxer fan | Newark Electronics<br>Chicago, IL | Model PWS 2107FL |
| **Signal Conditioning Module** | | |
| Dynamic filter | Spectrum<br>Newark, DE | 1021 filter/amp |
| 60 Hz filter | In house | TL 081 OA<br>x10 follower<br>with gain |
| **Analog Interfacing Module** | | |
| DAC board | In house | 12 bit; based on Burr Brown DAC 80CB1 |
| ADC board | In house | 12 bit; based on Analog Devices AD 574 |

Table II: Computer System Inventory (cont'd.)

| Module | Source | Comments |
|---|---|---|
| **Digital Interfacing Module** | | |
| Solid state relay | Grayhill<br>LaGrange, Il | #7052-03-B02-H<br>switches 120 VAC<br>from 3-15 VDC |
| Beeper | Radio Shack<br>Corvallis, OR | Archer #273-060<br>solid state 4.7 kHz<br>piezo type 2-20 VDC |
| **Oscilloscope** | | |
| Body | Tektronix<br>Beaverton, OR | Type 561A |
| Time base plug-in | Tektronix<br>Beaverton, OR | Type 3B3 |
| Amplifier plug-in | Tektronix<br>Beaverton, OR | Type 3A1 |
| **Chart Recorder** | | |
| Dual pen | Tegal Scientific, Inc<br>Concord, CA | Linear Model 585 |

Expanded Microcomputer

The heart of the computer system is a Rockwell AIM 65 single board microcomputer, which is based on a 6502 central processing unit. The 6502 is an 8 bit microprocessor and operates at 1 MHz, providing a minimum instruction execution time of 2 μs. With a 16 bit address bus there are 64K bytes of possible memory space. On board the microcomputer are 4K bytes of user random access memory (RAM), a 6522 I/O chip, providing 16 bidirectional I/O data lines and 4 I/O control lines, a 20 character LED display through which most operator feedback is accomplished, 16K of read only memory (ROM) containing a BASIC interpreter and monitor, and a 20 column thermal printer which records all data calculations and statistical summaries.

On the rear of the microcomputer board is a 44 pin application edge connector which allows computer interfacing. It provides connections to the I/O data and control lines, the cassette tape interface lines (for loading programs), 20 mA loop teletype interface lines (for hard copy program listing), and power connections. A 44 pin expansion edge connector, also at the rear of the microcomputer board, brings out the address, data, and control lines for expansion of memory and I/O capability. Further descriptions of the AIM 65 and its operation are in the User's Guide (109).

The AIM 65 single board microcomputer is expanded by connection to a mother board into which is plugged a 16K RAM board and an I/O board to provide additional memory and further I/O capability. The mother board plus memory and I/O boards are housed in an aluminum and plexiglass box which is cooled by a boxer fan and will be denoted as

Pandora's box. The AIM-65 expansion and application connectors are linked to the mother board with ribbon cables and three 25 pin female amphenol connectors on Pandora's box. The cassette recorder and teletype are connected directly to the application connector of the AIM-65. The analog interfacing module is mounted inside of Pandora's box while the 60 Hz filter and beeper circuits are mounted in a box attached to the outside of Pandora's box.

The commercial mother board has 4K of static RAM on board, buffered expansion slots for five printed circuit (PC) boards, and regulates the 5 V for the computer from the unregulated 8 V provided by the power supply. The commercial memory board has 16K of static RAM, consisting of 2 blocks of 8K, and the address of each block is independently selectable. In the present design, the system uses only 4.6K of the available memory on this board, which leaves significant space for system expansion. Further specifications and instructions for the mother board and memory board are in their respective manuals (110, 111).

The I/O board was designed and constructed in house and contains two 6522 I/O chips, each of which provide 16 additional I/O lines. The board occupies 1K of memory and its address is user selectable. The address lines are buffered on board and all I/O data and control lines of the 6522's are brought out to a 44 pin edge connector. A more complete description of the board is in Appendix B. For this system, the board is used for timing between each event of the analysis and the I/O lines are used for valve switching and activation of a beeper to gain operator attention at the appropriate time.

The expanded microcomputer provides a total of 24K of RAM and 48 I/O lines, although only 20 I/O lines are used. Further discussion on use of memory is in the following software section.

Appropriate BNC and banana plug connectors on the left side of Pandora's box are used to connect the I/O lines to external devices. Additional connectors are provided for connection to power supplies and to the DAC and ADC. These connections are summarized in Table III.

## Analog Interfacing Module

The analog interfacing module consists of a DAC PC board, an ADC PC board, and an auxiliary power supply, all of which are mounted inside of Pandora's box. The ADC is used to digitize the AA output data for storage by the computer, and is based on a 12 bit converter with a 25 μs conversion time. Data are read in a high 8/low 4 bit format. The board is modeled after the original Marino design (112) except that it is used in a bipolar, -5 to +5 V mode (resolution of ±2.4 mV). Since only one signal source is digitized, the 1 of 8 channel multiplexer was removed and a wire jumper installed.

The DAC is used to convert stored data from a digital pattern of bits to the corresponding analog value in order to be displayed on the oscilloscope. The chip is latched, 12 bit, and used in the same ±5 V range as the ADC (with the same ±2.4 mV resolution).

Digital communication between the analog interface module and the microcomputer is made through the I/O lines of the 6522 on the microcomputer board. Since only one of the devices in the analog interface module is active at any one time, the same lines go to both

Table III: Connectors for Pandora's Box

| Connector Type | Internal Connection | External Connection |
|---|---|---|
| **Left Side** | | |
| 4 female banana | Auxiliary power supply (used to power DAC, ADC, TL 081 OA circuit) | Ground used for digital interface others available for breadboarding |
| 4 female banana | CA1, PA0, PA1, PA2 of main board 6522 | PA0 - trigger scope<br>PA1 - Ch 2 scope |
| 5 female banana | -PB0, PB1, PB2, PB3 of I/O board 6522<br>-beeper input | digital I/O:<br>PB0 - $V_1$<br>PB1 - $V_2$<br>PB2 - peristaltic pump<br>PB3 - beeper |
| 5 female BNC | -DAC output<br>-OA circuit | DAC - Ch 1 scope<br>OA - Spectrum 1021 |
| **Front** | | |
| 25 pin female amphenol (A) | -LBM edge connector & ADC/DAC edge connectors | I/O & control lines of AIM application connector |
| 25 pin female amphenol (E1) | -LBM edge connector | AIM expansion connector (mostly top side) |
| 25 pin female amphenol (E2) | -LBM edge connector | AIM expansion connector (mostly bottom side) |
| **Back** | | |
| 2 holes | -+8 V and gnd LBM power connectors | +8 V and gnd of main power supply |
| 110 VAC power line | -boxer fan and auxiliary power supply | line voltage |

devices. When a device is inactive, it neither senses nor affects what is on the I/O lines. Software commands the direction of data on these time-shared I/O lines and is further discussed in the software section.

Power for the analog interface module is provided by the auxiliary power supply mounted on a PC board inside Pandora's box. The Marino design (112) supplies ±15 V at 200 mA and 5 V at 500 mA. It is fused with a 3/4 A slow blow fuse and access to it along with an on/off switch and indicator light are located on the front of Pandora's box.

## Digital Interface Module

There are two basic functions served by several of the I/O lines from one of the 6522 chips on the I/O board. An I/O line may be attached directly to a device, or attached to a relay switch which in turn controls a device. One of the 6522 I/O lines is attached to a solid state beeper circuit, as shown in Figure 10. A logic 1 signal on the I/O line turns the beeper on, and a logic 0 turns it off. (A logic 0 means low or 0 V on the line and 1 means high or 5 V on the line.) A switch allows isolation of the beeper from the control line.

Three other I/O lines are used to turn AC power on and off via connection to solid state relays. A logic 1 signal on the I/O line turns the solid state relay on which causes 110 VAC to be applied to the peristaltic pump or 3-way solenoid valves that control valves $V_1$, $V_2$, and $V_3$. (Refer to chromatography section for control of valves.) The solid state relays are fused to 1 A, have an on/off switch, and are mounted in a box attached to the exterior of the solvent box. The

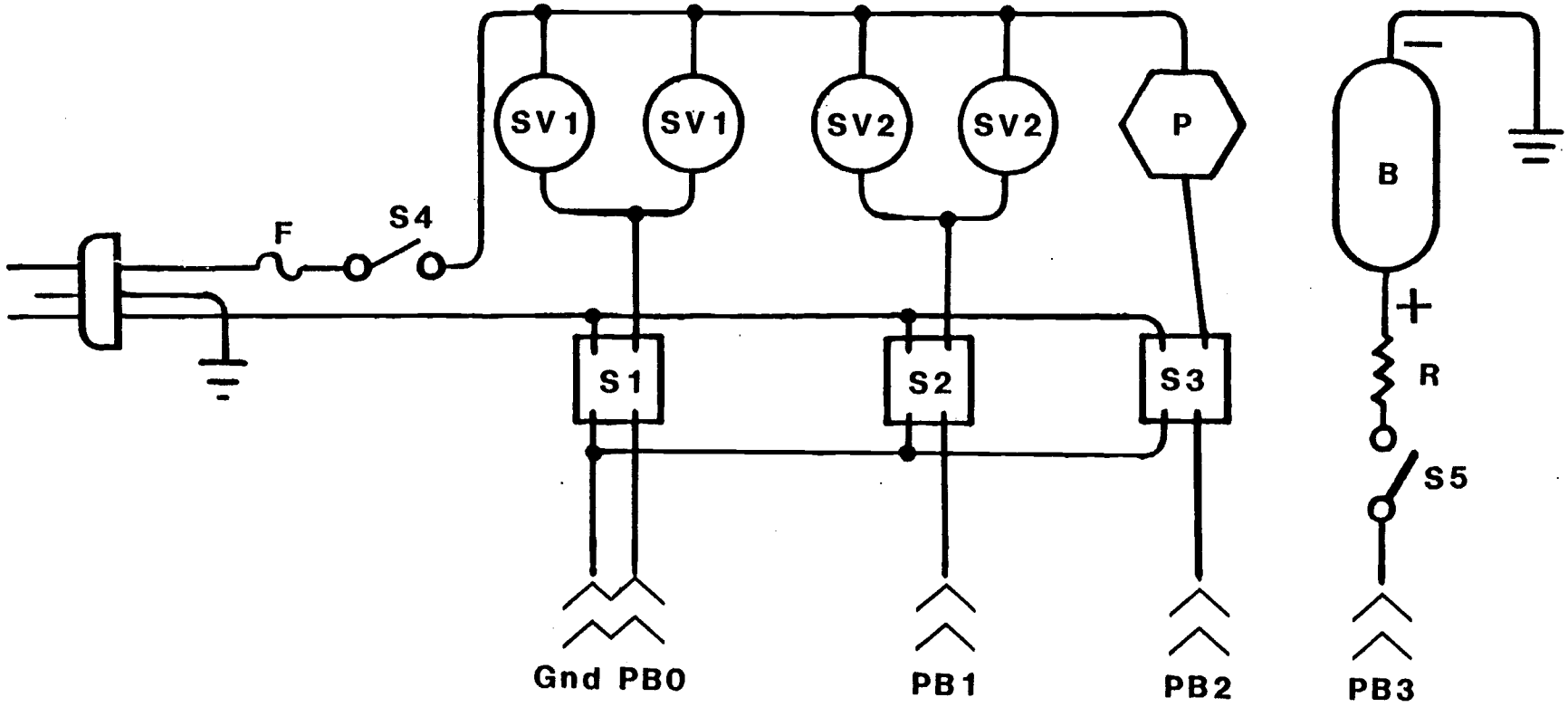



Figure 10. Digital I/O module schematic diagram. PB0, PB1, PB2, PB3 = digital output lines from 6522 on the I/O board in Pandora's box; S1, S2, S3 = solid state relays; S4, S5 = SPST; B = solid state buzzer; R = 4.7 kΩ; F = 1 A slow blow fuse; SV1 = solenoids to control $V_1$; SV2 = solenoids to control $V_2$ and $V_3$; P = mini peristaltic pump

solenoid valves and peristaltic pump which they control are located inside the solvent box.

## Signal Conditioning Module

The output from the Varian AA is 100 mV for 1 AU, and this signal is first brought to the Spectrum active noise filter (see Figure 11). The purpose of the Spectrum is twofold - to filter the signal and amplify it by 10 (so that 1 V = 1 AU).

Since the filter is a high frequency cut-off type, it is desired to filter all frequencies down to the lowest possible without attenuating the detected peak. This smooths out the random fluctuations in the baseline caused by source and flame transmission noise, and thus facilitates quantitation. By repeated injection and stripping of the same solution while varying the cut-off frequency, it was found that the peak is attenuated at frequency cut-off settings lower than 0.1 s, and at this setting the peak to peak baseline noise was a factor of 2 smaller than it was without the filter. Thus 0.1 s is the selected cut-off frequency.

The output of the noise filter goes to the chart recorder for a hard copy of the eluted peaks and also to the input of a 60 Hz filter circuit (see Figure 12). The operational amplifier (OA) is configured as a follower with gain, and the signal is amplified by 10 again, making 10 V = 1 AU. A zener diode with breakdown voltage of 5.2 V protects the ADC from possible overvoltage damage by limiting the signals to the range of -0.6 V to 5.2 V (-0.12 to +1.04 AU on ADC ±5 V

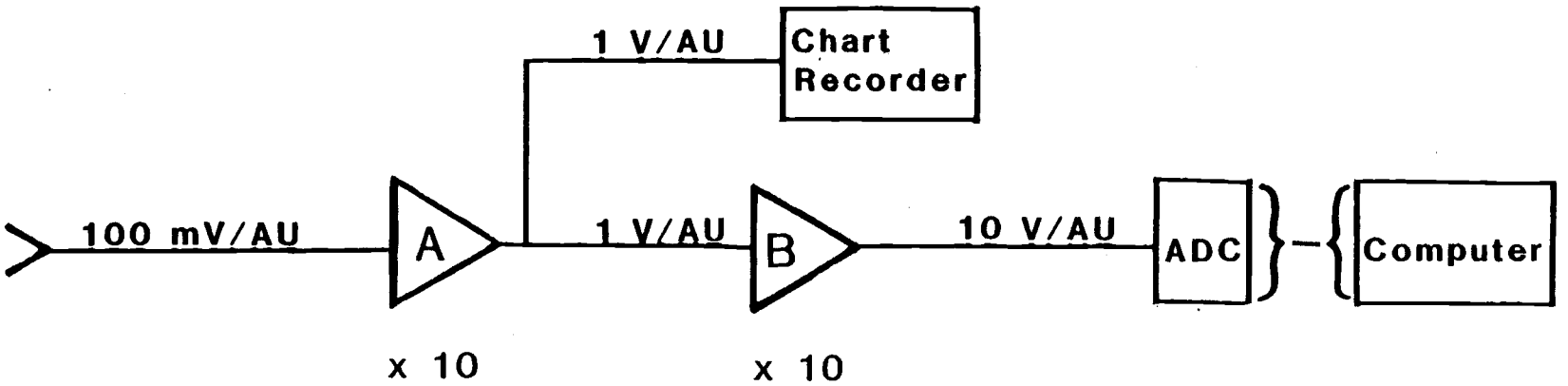



Figure 11. AA signal conditioning module. A = Spectrum 1021 active noise filter/amplifier; B = OA circuit (see Figure 12 for expanded circuit diagram); ADC = 12-bit analog-to-digital converter; Computer = AIM-65 microcomputer

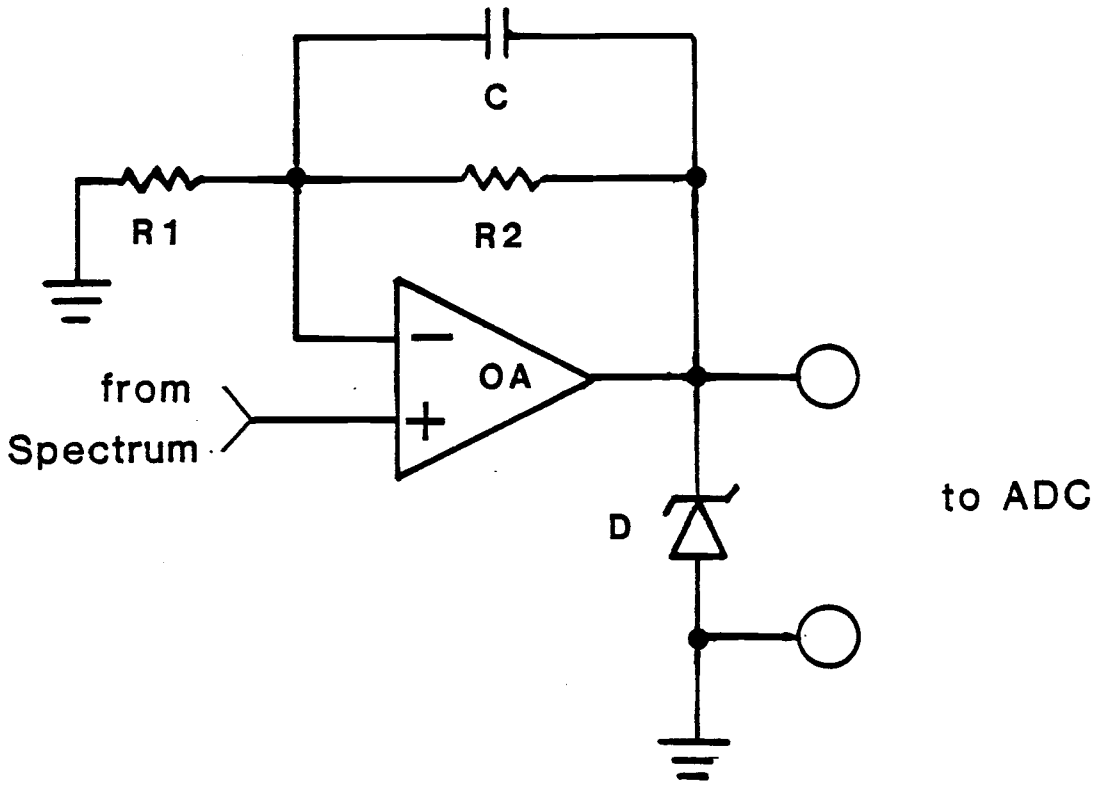



Figure 12. Circuit diagram of OA noise filter. R1 = 1 kΩ; R2 = 9 kΩ; C = 1 μF; OA = 081 operational amplifier; D = zener diode, 5.2 V breakdown

range). This range of absorbances will encompass a negatively drifting baseline and any reasonably large signal.

When data were collected at certain frequencies (e.g. 5 points/second), a pattern of intermittent noise (0.02 V in magnitude which corresponds to $2 \times 10^{-3}$ AU) was introduced into the collected data. This extraneous noise interferred with measurements of small signals, and was found to be 60 Hz pickup from the AC power cord for the cooling fan and ADC/DAC power supply board. Several remedial steps were taken but were unsuccessful at solving the problem, including covering the 120 V line with grounded Cu screen, adding 0.1 μF decoupling capacitors to the OA power supply lines, and locating the circuit on the exterior of the interface box. What finally reduced the pickup was the addition of a 1 μF capacitor to the feedback loop, a rather unorthodox adaptation of a follower with gain. The choice of value for this capacitor was based on the desire to attenuate or eliminate the noise pickup but leave the signal unaffected. Since the response time of the circuit is not a simple RC product in this configuration, the value was chosen empirically.

By using a 0.5 V peak-to-peak square wave with 40 ms peak width as the input to the OA circuit, the output was observed with an oscilliscope. It was found that with the 1 μF capacitor, a 60% rise in the output signal took 8 ms. Thus a signal with rise time less than 8 ms will be attenuated. Since the RC time constant of the AA is 0.2 s, the signal should not be attenuated at all, because the OA responds to a signal change much faster than the signal changes.

To see how well the circuit suppresses noise, a 0.5 V peak-to-peak sine wave was input and the frequency varied while measuring

attenuation. As the frequency was increased from 0.1 to 5 Hz, the attenuation was zero, at 30 Hz it was 56% attenuated, and signals with line frequency of 60 Hz were about 75% attenuated. This reduces the 0.02 V noise to 5 mV, which is small enough to be unseen by the ADC, and thus the problem was eliminated.

## Oscilloscope

In order to display the collected data, the DAC converts it to an analog voltage which is sent to the oscilloscope. The output of the DAC goes to channel 1 of the scope and since the original design of the board was made to be KIM compatible, the data are inverted and channel 1 must be in the inverted setting. Were an oscilloscope used that does not have this feature, a software modification would be necessary to invert the data before being sent to the DAC. A digital cursor signal controlled by software is connected to channel 2 of the scope from PA1 of the 6522 on the microcomputer board. The scope is triggered by a software controlled digital signal from PA0 of the same chip. Since it takes about 14 ms for the DAC to display the data file, the time base is set to 2 ms/division. The trigger settings are "ext" and "DC".

## Computer Software

The computer software will be discussed at three levels: an overview, an operational description, and a more technical discussion of how the program works.

Overview

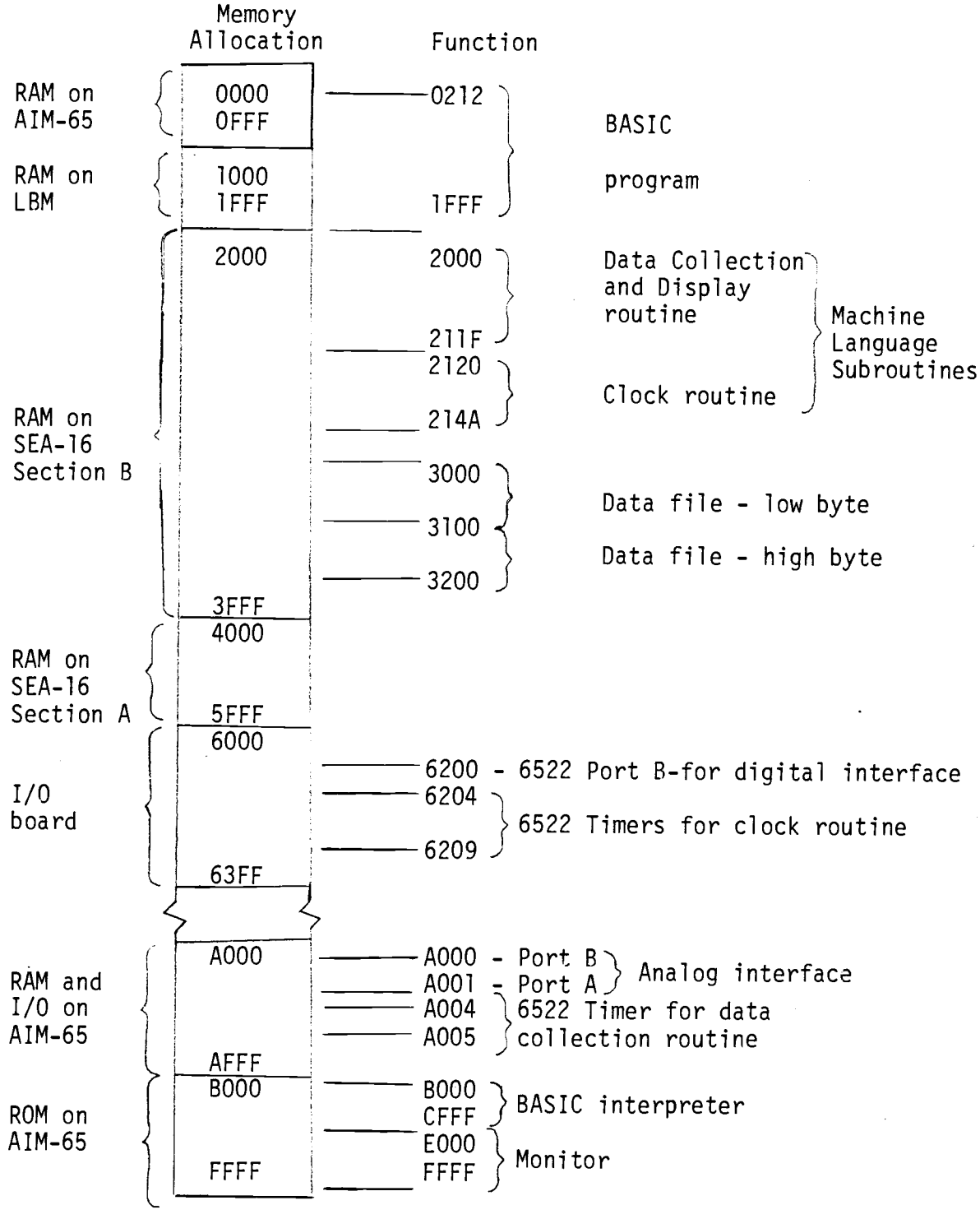
The TE software package used for this system is an operator interactive program that performs data collection and reduction as well as instrument control. Communication with the operator is performed through the keyboard and LED display (both of which are on the main computer board) and with the oscilloscope. In addition, the beeper mounted on Pandora provides for uni-directional verbal communication.

As is typical in many similar uses of microcomputers, two language levels are used. BASIC is the master language and is responsible for operator interaction such as to input data collection parameters. It also performs all calculations, including individual peak analysis and statistical summaries, as well as printing the results of those calculations. Finally, instrument control such as valve switching and display of the time remaining before the next event is also performed at the BASIC level.

Machine language subroutines are frequently called by BASIC, and some of the more rudimentary tasks are performed at this level. Data collection (i.e. control of the ADC) is a machine level chore as is subsequent data display.

The memory map for the computer system is given in Table IV. The AIM uses the highest addresses for its BASIC interpreter and monitor firmware. The BASIC program is at the lowest addresses, and the machine language subroutines are wedged between it and the data files. There is obviously plenty of room for expansion, since less than half of the 16K of memory space on the memory board is currently used. The BASIC program uses virtually all the memory space allocated to it

Table IV: Computer System Memory Map



(7.7K), for there are only 63 bytes free. Thus any expansion of the BASIC program will require shifting the blocks of machine language routines and data files above it to higher memory addresses in order to give it room to grow.

## Operational Description

Figures 13-15 are a flow chart for the operational use of the program package. The program first prompts the operator to type in heading information such as the date, the experiment number, the flow rate, and the column pressure as well as any other desired information.

Two experimental parameters - collection frequency (how often to take a point) and collection delay (how long after initiating the strip step to wait before commencing data collection) - are then entered. Default values of 10 points/s for frequency and 15 s for delay may be used if desired. A short machine language routine then zeroes the data file and back in BASIC the user declares which of the two procedures (TE of a sample or injection of standards) will be done, and is prompted to type in a label to describe the solution being determined. If a TE is chosen, the computer prompts the operator to enter the TE volume ($V_{TE}$). The program calculates the TE time needed to yield $V_{TE}$ based on the flow rate of $P_A$ and then switches $V_1$ to the proper position to allow the sample to pass through the column for this period. A 60 s column rinse with distilled water follows and after this the operator's attention is gained for the imminent strip step by flashing a "start AA" message on the LED display with concurrent beeping. During this message, the peristaltic pump flushes the sample loop with distilled

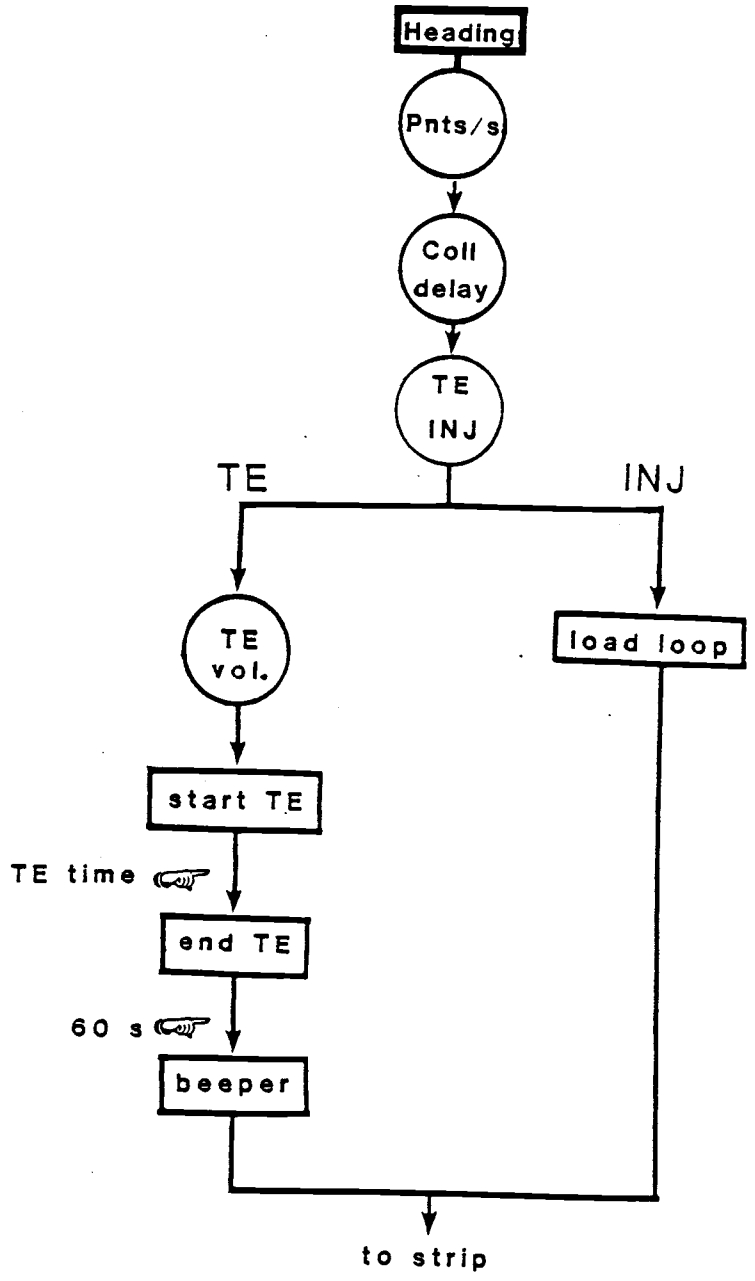



Figure 13. Computer program flowchart

water. (The LED displays letters in all capitals, but will be indicated here with lower case letters in quotes.)

In the case that the injection mode is chosen rather than TE, the computer simply turns on the peristaltic pump for 10 s in order to fill the sample loop, and follows this with a 10 s wait period, which allows relief of the partial vacuum in the tubing. (This vacuum effect will be further discussed in the results and discussion section.) The beeper is not used to warn of imminent sample strip in the injection mode because the time between each sample is not long enough to warrant turning the AA off between them.

As stated in the chromatograph part of this section, the sample injection and strip initiation are combined into a single valve switch since $V_2$ and $V_3$ are stacked together. In the injection mode, the sample loop is filled with standard solution when $V_2/V_3$ are switched and in the TE mode, the loop has been filled with distilled water during the "start AA" message. But in either case, the contents of the sample loop are placed "on line" and the strip step is initiated by switching $V_2/V_3$, which occurs 10 s after the "start AA" message is over in the TE mode or 10 s after the peristaltic pump is turned off in the injection mode. Switching $V_2$ allows the SR to be directed to the column.

As shown in Figure 14, the computer waits the previously input delay time after initiating the strip step before commencing data collection. After the delay period, the program jumps to a machine language routine to collect data at the previously chosen collection frequency. When not actually digitizing and storing a data point, the program directs the DAC to repetitively display the data file. Since

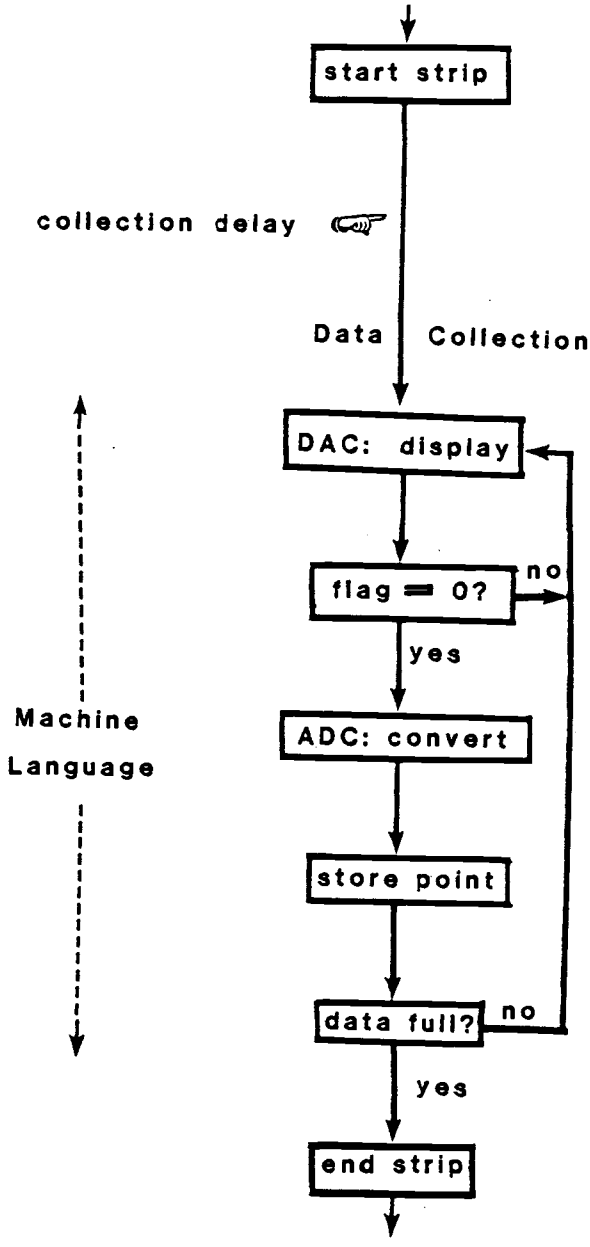



Figure 14. Computer program flowchart (cont'd.)

the data were initialized to zero, the peak will grow from the zero line as it comes off the column. Since conversion and storage take only 70 μs and data are typically collected once every 100 ms, most of the computer time is spent continuously displaying the data file. Data collection is terminated when the data file is full (255 points or typically 25 s of data) or when any key on the keyboard is pushed. The program jumps back to BASIC and immediately switches $V_2$ to distilled water in order to end the strip step and wash the column with distilled water in preparation for the next sample.

Figure 15 shows that following the strip step and while the column is being rinsed with distilled water the LED display lists the master menu to offer the operator 4 choices: "View Repeat New Manual". Choosing the view mode causes the program to jump to a machine language subroutine that extracts the data from memory and again displays it on the scope. In the meantime the LED display is left with a sub-menu on it: "Cursor: R L A B I X". While the machine language routine is displaying the data via channel 1 on the scope, it is also constantly scanning the keyboard. If an X (or any key not on the menu) is pushed, the program exits the view mode and returns to the master menu. If an R or L are pushed, the program (via machine language) will do two things; it will move a single cursor dot on the scope screen (input through channel 2) right or left, for R and L respectively, and indicate on the LED display the cursor location (between 0 and 255) and the magnitude of the data (between 0 and 4095) for that point in the file. Thus the cursor may be moved to any point in the data file. When the cursor is located right in front of the peak rise, the operator pushes A on the keyboard, which sets one of the integration limits. Pressing

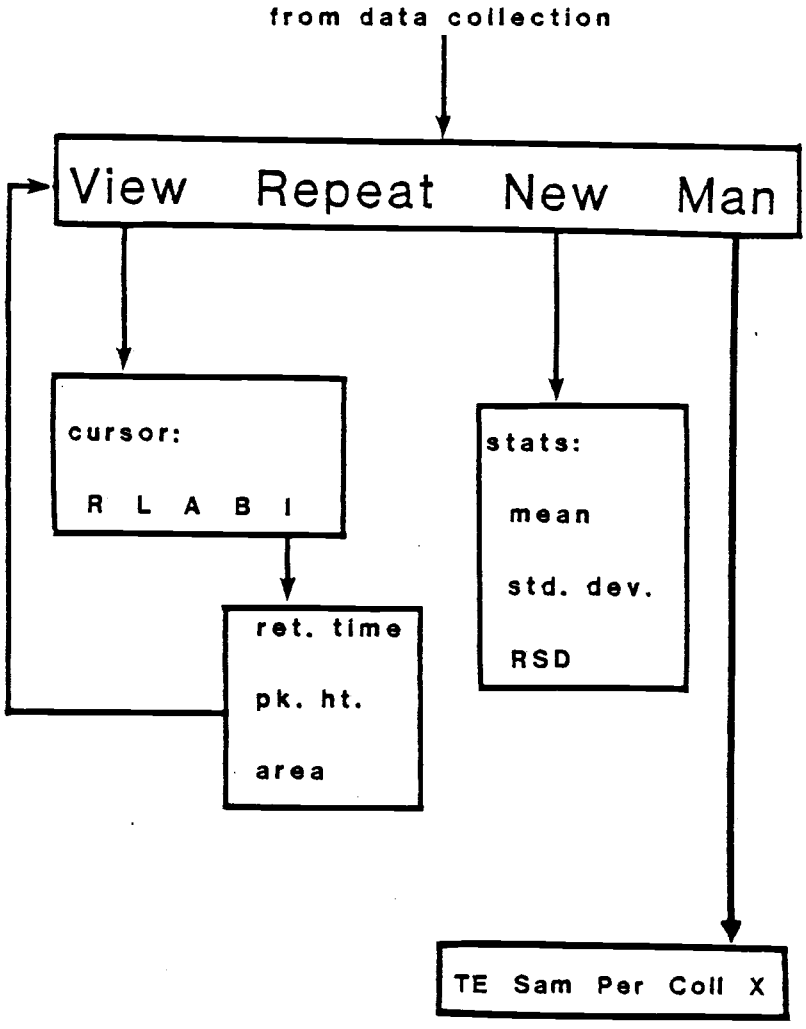



Figure 15. Computer program flowchart (cont'd.)

B when the cursor is on the other side of the peak similarly sets the other integration limit. Figure 16 is a reproduction of a typical peak and shows the relative positions of the A and B integration limits.

If the A limit is set and B has not been set yet, the program (in BASIC) offers a couple of choices. It will display the value of the peak width W (= B - A) from the previous run (W = 0 initially) and ask if it is to be changed. If not, a residual dot is left at A and a new one appears at A + W, where the cursor is then located. This saves the operator from having to move the cursor the entire width of the peak each time and allows each use of the same integration limits for all peaks. If the operator choses to change W, a pre-selected width may be entered, again saving time.

When both integration limits are set, the I key is pushed, and several things happen. The peak is removed from the screen while the computer calculates a baseline from the 10 points before A. Then it determines the location of the peak maximum (relative to the time of strip initiation), and calculates a baseline corrected peak height and the area between A and B. To do this, the program (in BASIC) sequentially scans the data from A to B and subtracts the calculated baseline from each point. The differences are summed for the area, and the largest difference is called the peak height, the location of which is called the retention time (converted to seconds and added to the collection delay). Upon printing these values and storing them in an array, the program returns to the master menu.

If "Repeat" is chosen from the master menu, the program returns to the point subsequent to the selection of the TE or injection mode. There is an opportunity to change the collection frequency and

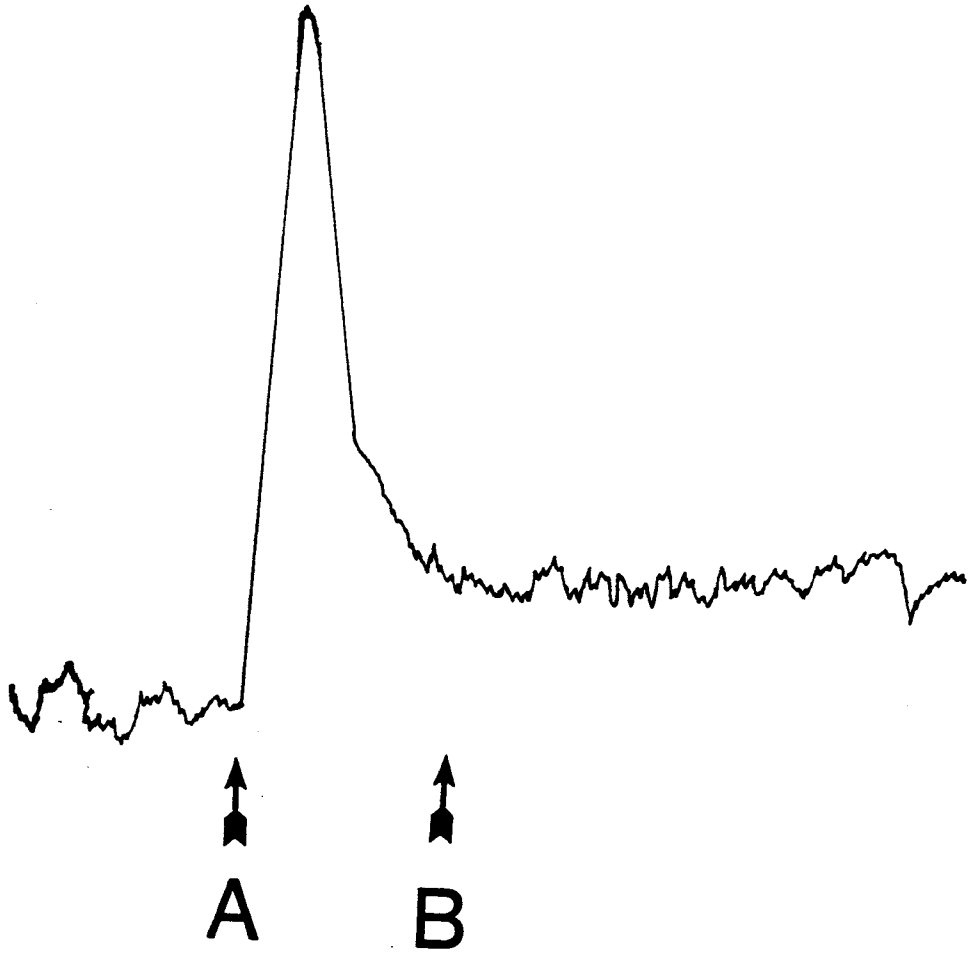



Figure 16. Typical stripping peak showing integration limits

collection delay before the appropriate routine is repeated. Calculations of each peak are stored in an array after each quantitation.

When enough repetitive measurements of a solution have been made for statistical validity (usually 3 or 4, although there is array space for 10), selection of "New" from the master menu offers an optional statistical summary. The operator may delete any "wild" values and repeat the summary as many times as desired. A print-out of the mean, standard deviation (SD) and relative standard deviation (RSD) for peak height and area is given as well as the mean and standard deviation of the retention time. Figure 17 is a sample of the printout for each of the repetitive measurements and their summary. After the printout, the program returns to the TE/injection choice for mode selection and label printing. An optional sample flush is then used when a new TE sample is to be analyzed. Five mL of new solution are passed through the tubing in order to flush out the old sample and a strip is done to clean and prepare the column.

The final choice on the master menu is the manual mode, which allows the operator to independently operate the valves and peristaltic pump, or collect data without being in the injection or TE mode. Upon pressing "M" from the master menu to enter the subroutine, the manual mode sub-menu is displayed: "TE Sam Per Coll X". While pressing "X" will cause the program to return to the main menu, pressing "T" gives the prompt "DW or TE Soln?," and subsequent pressing of "D" or "T" will cause $V_1$ to pass dw or the sample, respectively, to $P_A$. The display then returns to the manual mode sub-menu. Similarly, pressing "S" from the manual mode menu displays the choice "Load (DW) or Inj (SR)?" Pressing "L" or "I" will cause the $V_2/V_3$ value combination to

```
* * * * * * * * * *

TE: 10ML 2.5 PPB

NO. 1
PK HT= 19.54
PK AREA= 1164
AT 33.8 SEC
BASELINE= 3.54
W= 140 ( 14 SEC)
 10 PNTS/SEC

NO. 2
PK HT= 19.05
PK AREA= 1077
AT 33.6 SEC
BASELINE= 2.08
W= 140 ( 14 SEC)
 10 PNTS/SEC

NO. 3
PK HT= 22.11
PK AREA= 1087
AT 34.2 SEC
BASELINE= -.99
W= 140 ( 14 SEC)
 10 PNTS/SEC

SUMMARY: ALL POINTS

     PK HT  AREA
MEAN  20.2    1109
  SD  1.6     48
 RSD  8       4

RET TIME= 33.9 SEC
      SD= .3

* * * * * * * * * *
```

Figure 17. Typical data printout and statistical summary

respectively pass dw to the column and be in the load position for the sample loop or pass SR and inject the loop contents on the column. The choice for the peristaltic pump (by pressing "P" on the sub-menu) is to press 1 to turn it on or 2 to turn it off. The "Coll" choice returns to the optional collection frequency input and then immediately starts collecting data (avoiding a collection delay).

## Technical Description

Most of the technical details to be discussed are related to machine language subroutines, but a few of the unique characteristics of the BASIC program will be discussed first. In order to decrease the number of keystrokes necessary to respond to computer prompts, most decision inputs such as menus and yes/no answers use the GET statement. This command scans the keyboard for a single key entry and can be used to prevent crashing the program due to pushing the wrong key. The program repeatedly scans until one of the possible choices is entered, and will ignore incorrect entries.

There are two 6522 chips used by the computer. The one on the main microcomputer board is used for data collection and transfer between the microcomputer and the analog interface module. The other one is on the I/O board in Pandora's box and serves two functions: digital instrument control and clock display for time keeping of events in the analysis.

In order for the 6522 in Pandora's box to control the valves, peristaltic pump and beeper, the I/O ports are initialized as outputs at the beginning of the program and BASIC must be able to individually

change the logic state of a desired I/O line without affecting the others. This individual bit control is achieved in BASIC language subroutines using POKE commands with logical AND and OR statements. The top of Table V shows the states of each device resulting from the two logic states on the corresponding I/O line.

In order to illustrate the software steps needed to alter an individual bit, an example follows for turning the peristaltic pump on and off when the beeper is on (Logic 1) and the other I/O lines are logic 0. Inspection of Table V reveals that the bit pattern on port B (PB) is initially 1000 for this set of conditions. At the address corresponding to PB, logic 1 at bit 2 corresponds to the decimal number 4 ($0100_2$). In order to set bit 2 high while masking out the others, bit 2 is OR'd with 1 and the other bits are OR'd with 0. The resultant pattern of bits is the same as the original except bit 2 is now high. Similarly, to turn the pump off without affecting the other devices, PB2 is AND'd with 0 and the other bits are AND'd with 1.

| | Turn On | | Turn Off |
|---|---|---|---|
| Initial | 1000 | Initial | 1100 |
| OR 4 | 0100 | AND 11 | 1011 |
| Result | 1100 | Result | 1000 |

The bottom of Table V shows the BASIC statements used to control each of the 4 I/O lines used.

The other use of the 6522 in Pandora's box is to act as a countdown clock for timing the events of the analysis, and this function is entirely independent of the digital interface function just described. The countdown clock is a BASIC language subroutine, entered with the

Table V: Digital I/O Control

| PB bit [a] | Device | Position for Logic State: 0 | 1 |
|---|---|---|---|
| 0 | $V_1$ | dw | TE |
| 1 | $V_2/V_3$ | load | inj |
| 2 | per pump | off | on |
| 3 | beeper | off | on |

| Device | BASIC Statement | Effect[b] PB 3 | 2 | 1 | 0 |
|---|---|---|---|---|---|
| $V_1$ | AND 14 | X | X | X | 0 |
| | OR 1 | X | X | X | 1 |
| $V_2/V_3$ | AND 13 | X | X | 0 | X |
| | OR 2 | X | X | 1 | X |
| Per Pump | AND 11 | X | 0 | X | X |
| | OR 4 | X | 1 | X | X |
| Beeper | AND 7 | 0 | X | X | X |
| | OR 8 | 1 | X | X | X |

a. Address $6200_x$

b. 0 = low (0 V); 1 = high (5 V); X = unaltered by statement

desired delay time, which is displayed on the LED display as the number of minutes and seconds remaining until the next event. The 6522 has two timers. In a machine language subroutine, timer 2 (T2) is loaded with 10 and timer 1 (T1) is loaded with a value such that it decrements T2 every 0.1 s. To do this, PB7 and PB6 are hardwired together with a wire jumper on the edge connector of the I/O board, and T2 decrements on pulses input from PB6 which T1 outputs onto PB7 whenever it times out.

Thus in one second, T2 has been decremented to 0 at which time the program jumps back to BASIC to decrease the displayed time by 1 s. The program continues the cycle of jumping to machine language for 1 s and back to BASIC to decrease the displayed time until the displayed time reaches zero. Then BASIC jumps back from the clock subroutine and does whatever is next on the schedule to do.

The 6522 on the AIM board is used to tell the computer when it is time to collect a point. As previously mentioned, the computer spends most of the data collection time displaying the data file. After each sweep of the file (see Figure 14) a flag is checked. If the flag is zero, the computer collects a point, reloads the flag, and continues displaying the data. Independent of this, the timer on the 6522 counts to zero and an interrupt service routine decrements the flag. The proper values for the flag and the timer depend on the collection frequency and are calculated and loaded by BASIC.

The 6522 on the AIM board is also used for data conversions. As can be seen in Figure 18, many I/O lines of the 6522 are shared by the ADC and DAC. The ports, which are bi-directional, are made inputs when the ADC collects a point and data are transferred through them from the

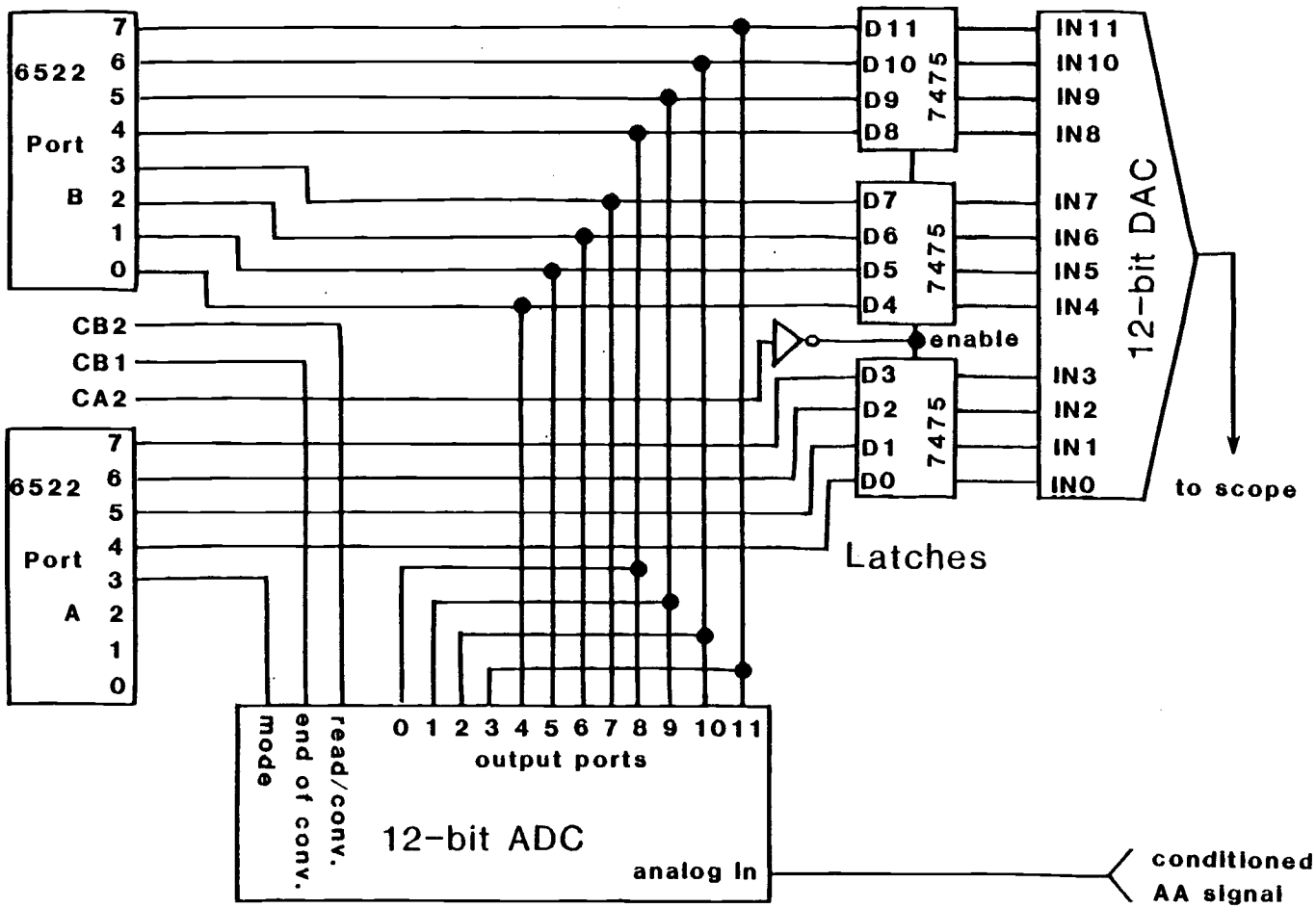



Figure 18. Wiring schematic of ADC and DAC

ADC to memory. When the DAC displays data, the I/O lines are made as outputs and the data are transferred from memory to the DAC latches. Thus the I/O lines are timed shared to serve both functions.

The 12 bits of a data point cannot all fit in one address location so the data are stored according to the structure given in Figure 19. The 8 most significant bits for each point are stored in the lower page (255 bytes) of the file and the 4 least significant bits are stored in the higher page. This leaves 4 bits for each location in the higher page available for use. Bit 0 of each location is set high and is used to trigger the oscilloscope. Bit 1 is used for the cursor, and only one of the 255 points has this bit set high, corresponding to the data point to which the cursor is pointing. When the cursor is moved right or left, the point that has the cursor bit set is a higher or lower address point, respectively. Bits 2 and 3 are not used and are always set low.

In order to facilitate loading the programs into the computer and starting it running, both the BASIC and machine language programs were saved on tape as one file. All memory locations from $00_x$ to $FF_x$ (pg 0) and $200_x$ to $2200_x$ (pgs 2 thru 21) were recorded as a machine code dump. (A subscript x denotes hexadecimal number. All other numbers are in decimal.) Page 1 is used by the AIM for the stack, so reading this region of memory back into the computer causes problems and it is not needed anyhow. Once the programs are loaded in this way, the necessary vectors, etc. are automatically set. This allows a "warm start" entry into BASIC by pushing 6 and RUN (CR) after the program is loaded rather than the normal "5" entry and subsequent answering of BASIC prompts.

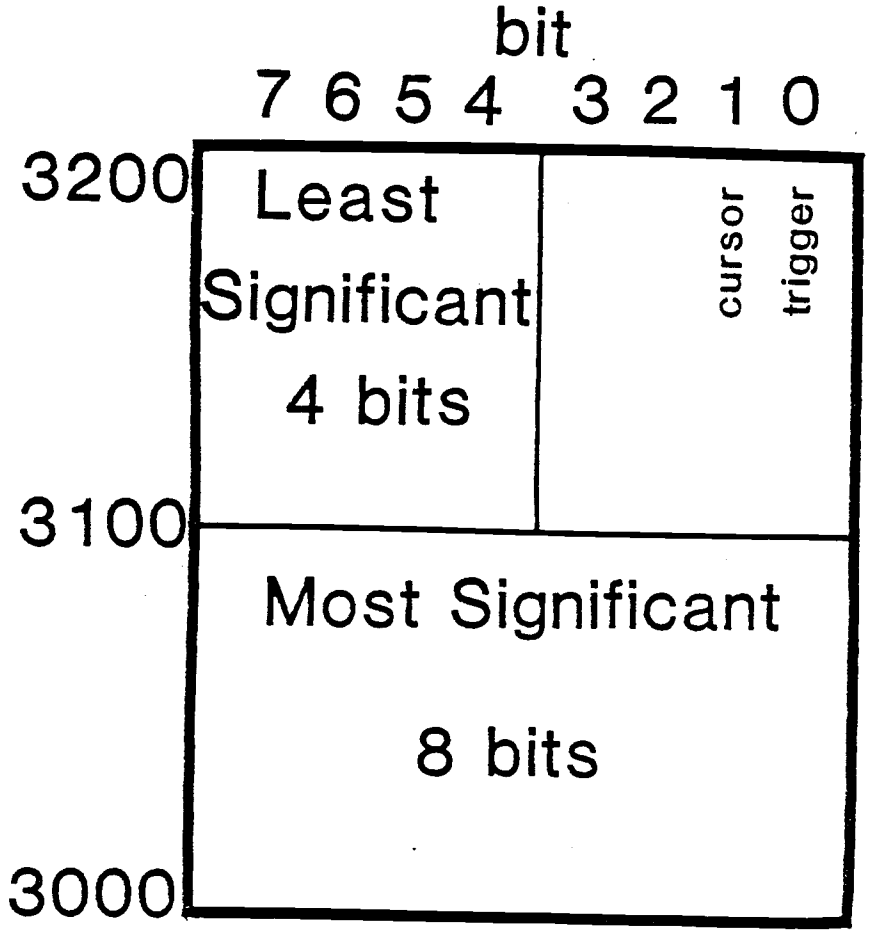



Figure 19. Data file structure

A listing of the programs is found in Appendix C. Since the assembler/editor is better and easier to use on the KIM system than the AIM, the majority of the machine language subroutines were developed and assembled on the KIM. Due to memory allocation discrepancies in the two computers, an assembler offset of $30_x$ was put in memory location $0A_x$ of the KIM prior to assembly. This causes the assembler to shift the object code up $3000_x$ from the starting address indicated in the editor. After assembly, the object code, starting at location $5000_x$, was dumped onto a tape and subsequently loaded into the AIM (in KIM format). A byte shift program was then used by the AIM to move the program from the starting address of $5000_x$ to $2000_x$. The BASIC program was loaded next and the two jointly saved as one machine code file.

# EXPERIMENTAL

The experimental section will be covered in four parts - column packing, solution preparation, real sample analysis, and the actual running of an analysis.

## Column Packing

The column is slurry packed in order to get a uniform packing of the resin bed. Column packing is considered by some to be a "black magic". However, a couple unique factors favor success. The slurry packing technique is relatively simple and rapid, requiring no sophisticated column packing equipment. Also, the TE application for the column is less demanding of chromatographic performance than would be if the column were used for a regular separation.

A 10 mL polystyrene beaker cup is filled about half full with $10^{-2}$ M $HNO_3$ and one scupula of resin is placed in the acid. Meanwhile, the downstream end of the column is assembled with a homemade filter disc placed over the Teflon bed support. About 0.5 mL of an agitated resin slurry from the acid solution is then drawn into a Pasteur pipet.

With the column held at about a 45° angle, the pipet tip is carefully inserted along the side of the column until it reaches the bottom. (Care must be taken not to jam the pipet in hard enough to puncture the filter disc.) While trying to prevent any air bubbles from forming in the column, the slurry is slowly discharged from the pipet until the column is filled with liquid and the pipet is withdrawn.

A rubber hose (1/4 in. i.d. 3/8 in. o.d.) fitted to house air which is regulated to 5-10 psi is then placed over the flanged column end. The air pressure forces the liquid out through the column and settles the resin bed. When the liquid level is about 2 mm above the top of the bed, the hose is removed from the column and the pipet refilled with slurry in order to again fill the column. The process of filling the column with slurry and extracting the liquid with compressed air is repeated until the resin is about 2 mm from the top of the column. The small dead volume is left to allow for any slight resin swelling. If, during the repeated slurry packing process, the liquid level drops below the top of the bed, more $10^{-2}$ M $HNO_3$ is added and the top of the bed is mixed to insure that no air remains entrapped.

When resin addition is complete, the remaining column volume is filled with acid and the upstream end of the column is carefully assembled to insure that no air is left at the top of the resin bed. After assembling the column, all fittings are hand tightened to prevent leakage. If the column is properly packed, initial back pressure should be no higher than 100-120 psi when fitted to a pump at a 5 mL/min flow rate.

## Solution Preparation

All solutions were made with deionized water (dw) from a Millipore Milli-Q system connected to the house deionized water. The Millipore system delivers 18 MΩ water. All weighings of less than 5 g were made with a Mettler type H 15 balance to 0.1 mg. Weighings of more than 5 g were made with a Satorious type 1106 top loader balance to 0.01 g. All

pH measurements were made with either a Chemtrix type 60A or a Beckman model 96 Zeromatic pH meter and a Broadley-James combination pH electrode (#9124).

Dilution of stock solutions for sample preparation were made in a filtered input, positive air pressure clean room, and sample solutions in the sub-ng/mL range were made in a class 100 laminar flow hood located in the clean room. With rare exception blank values due to metal contamination were barely detectable.

The stripping reagent (SR) is a citrate buffer. A 0.2 M solution was made by dissolving 38.42 g of anhydrous citric acid (Baker reagent grade 1-0122) per L of dw. (It was found that the hydrated form is much slower to dissolve and more hygroscopic than the anhydrous form.) The powder was weighed into a beaker and dissolved in dw, then transferred to a volumetric flask which was filled about 80% full with dw. A magnetic stir bar was put in the volumetric flask and concentrated $NH_4OH$ (Baker reagent grade 3-9721) added while stirring until the desired pH was attained. If too much $NH_4OH$ was added and the desired pH overshot, then concentrated $HNO_3$ (Baker reagent grade 3-9601) was used to correct it. The electrode was calibrated with commercially supplied pH 4 and 7 buffers.

At this point, one drop of 20 g/L $NaN_3$ solution was added per liter of citrate. The azide solution is somewhat unstable and so was stored in a refrigerator. The citrate SR solution was then filtered through a Millipore 0.45 μm HA filter (mixed esters of cellulose acetate and nitrate) in a 300 mL suction apparatus and the first 40 mL of filtrate discarded in case of metal contamination in the paper. The

volumetric flask was then rinsed out with dw and SR was stored in it until used for analyses.

For sample solutions, glassware was dedicated to a set sample concentration as much as possible. When a new sample concentration was to be made the glassware for it was acid washed and rinsed with dw several times. The test solution was then made and allowed to sit for awhile, then discarded and remade. This process was repeated several times before the solution was used.

Stock solutions of 1000 mg/L of each test metal were individually made. For Cu the metal was dissolved in about 5 mL of concentrated $HNO_3$ and diluted with dw. For Cd and Mn, $Cd(NO_3)_2 \cdot 4H_2O$ (Malk 4008) and $MnSO_4 \cdot H_2O$(Malk 6192) were dissolved in dw. The stock solutions were diluted to an intermediate 10 mg/L concentration from which the injection standards were diluted into 25 mL volumetric flasks for analysis. TE samples were made in volumetric flasks from dilutions of the standards and acidified by addition of one mL concentrated $HNO_3$ per L sample.

Interference studies were often made of several species at once, especially with chemically similar groups (e.g. the alkali metals). Conditions and concentrations varied with the experiment, but usually the interferent solution was adjusted to pH 2, and Table VI summarizes the stock solutions used to make them.

None of the interferent solutions were filtered with the exception of the humic acid solution. After the stock solution was diluted and acidified, it was filtered through a 0.45 μm Millipore HA filter and the first 50 mL discarded. The analyte was then added to the filtrate,

Table VI: Interferent Stock Solutions

| Solution Number | Species | Conc. | Source | Comment |
|---|---|---|---|---|
| 1 | $Fe^{+3}$ | 88 g/L | $FeCl_3$(Malk 5029) | 1.9 g to 100 mL |
| 2 | $Fe^{+3}$ | 10 g/L | $Fe_2(SO_4)_3 \cdot H_2O$ (Malk 5036) | 1.79 g to 25 mL |
| 3 | $PO_4^{-3}$ | 1 g/L | $H_3PO_4$ (Malk 2704) | 0.75 mL to 1 L |
| 4 | $SO_4^{-2}$ | 1 g/L | $NaHSO_4 \cdot H_2O$ (Malk 7432) | 1.7 g to 1 L |
| 5 | $Mg^{+2}$ | 10 g/L | $MgCl_2 \cdot 6H_2O$ (Malk 5958) | 8.4 g to 100 mL |
| 6 | $Ca^{+2}$ | 10 g/L | $CaCl_2 \cdot 2H_2O$ (AC 1511) | 3.7 g to 100 mL |
| 7 | $Cr^{+3}$ | 10 g/L | $Cr(NO_3)_3 \cdot 9H_2O$ (Malk 2570) | 7.7 g to 100 mL |
| 8 | $K^+$ | 1 g/L | KCl (Malk 6858) | 1.9 g to 1 L |
| | $Na^+$ | 1 g/L | $NaNO_3$ (Malk 7808) | 3.7 g to 1 L |
| 9 | Humic Acid | 1 g/L | Soil Extracted (CPL H5820)[b] | 0.1 g to 100 mL + 5 mL con $NH_4OH$ |
| 10 | $Ni^{+2}$ | 1 g/L | c. | |
| 11 | $Zn^{+2}$ | 1 g/L | c. | |
| 12 | $Mn^{+2}$ | 1 g/L | c. | |
| 13 | $Cd^{+2}$ | 1 g/L | c. | |
| 14 | $Pb^{+2}$ | 1 g/L | c. | |
| 15 | $Mg^{+2}$ | 10 g/L | $MgCl_2 \cdot 6H_2O$(Malk 5958) | 8.4 g to 100 mL |
| | $Ca^{+2}$ | 10 g/L | $CaCl_2 \cdot 2H_2O$(AC 1511) | 3.7 g to 100 mL |
| 16 | $Na^+$ | 1 g/L | $NaNO_3$ (Malk 7808) | 3.7 g to 1 L |
| | $K^+$ | 1 g/L | KCl (Malk 6858) | 1.9 g to 1 L |
| 17 | $Cr^{+3}$ | 1 g/L | $Cr(NO_3)_3 \cdot 9H_2O$ (Malk 2570) | 1.54 g to 200 mL |
| 18 | NaCl | 1 M | NaCl (Ama SS270) | 14.5 g to 250 mL |

a. All Chemical reagent grade except as noted
Malk - Mallinckrodt CPL - Chemical Procurement Labs
Ama - Amachem AC - Allied Chemical
b. Technical grade
c. Borrowed stock solutions; unsure of chemical source

which had a measured pH of 2.1 for the 10 mg/L solution. Interferent solutions containing the analyte metal were usually made several times at the concentration to be tested before being analyzed.

Real Sample Analysis

In order to test the performance of the TE system, river and tap water samples were analyzed for content of each of the three metals tested. The river samples were taken from the Willamette River at the north boat ramp in Corvallis, Oregon. One L polyethylene bottles were soaked with 2.5 M $HNO_3$ for 3 days and rinsed with dw prior to use. One mL of concentrated $HNO_3$ was added to each empty bottle before sample collection to ideally prevent metal loss on the sides of the container. For sampling, the mouth of the bottle was pointed downstream and partially submerged until full. It was then quickly withdrawn so that the water flow would be solely into the bottle and none of the acid preservative could escape.

A summary of collection times is given in Table VII. Sample 5 was filtered through a 0.4 μm Nucleopore polycarbonate filter and the other river samples were filtered through 0.45 μm Millipore HA filters. A new filter paper was used for each sample and the first 50 mL of the filtrate was discarded in case the papers were contaminated with metal. The rest of the filtrate was then analyzed with the TE system as will be described in the next section.

The tap water samples were taken from a faucet in the teaching laboratory of Gilbert Addition hall, and 100 μL concentrated $HNO_3$ was

Table VII: Real Sample Collection

| Sample Number | Source | Collection Date | Collection Time | Analyzed Metal |
|---|---|---|---|---|
| 1 | Tap | 3/16/82 | 1:30 pm | Cu |
| 2 | River | 4/ 1/82 | 8:30 am | Cu |
| 3 | River #1 | 8/ 5/82 | 12:15 pm | Cd |
| 4 | River #2 | 8/ 5/82 | 12:15 pm | Cd |
| 5 | River #3 | 8/ 5/82 | 12:15 pm | Cd |
| 6 | Tap | 8/ 6/82 | 1:30 pm | Cd |
| 7 | River #1 | 9/ 8/82 | 2:30 pm | Mn |
| 8 | River #2 | 9/ 8/82 | 2:30 pm | Mn |
| 9 | River #3 | 9/ 8/82 | 2:30 pm | Mn |
| 10 | Tap | 9/ 8/82 | 2:30 pm | Mn |

added to 100 mL water in a beaker. The sample was not filtered prior to analysis.

In order to verify detector response linearity of the real samples, a standard addition procedure was employed. After the sample was analyzed and the metal content determined, another portion of the sample was spiked with a small volume of a standard solution such that the mass of metal in the spike was similar to the calculated mass of metal in the original sample. The spiked sample was then analyzed by the same procedure as the original sample to see if the instrumental response increased in a linear fashion. For Cu, the river sample (number 2 in Table VII) was used for standard addition, for Cd the tap water sample (number 6) was used, and for Mn the tap (number 10) and a river sample (number 8) were both used.

## Analysis Procedure

Standards were made directly prior to analysis, as were the artificial TE samples. The injection blank was dw and TE blank was $10^{-2}$ M $HNO_3$. When experiments are carried out soon after each other, the TE system apparatus is left in place adjacent to the AA. (The AA is the only system component which is not mobile.) If the system has been moved elsewhere, the chromatograph cart is placed on the left facing the AA, and the computer cart on the right. Power cords for each cart must be plugged in to the power strip behind the AA, and the UV detector placed on the AA table and also plugged in. Gas cylinders of He, acetylene, and compressed air must be connected to the bubbler manifold, AA, and solvent box, respectively. The He regulator is set

to about 5 psi, the compressed air to 85-90 psi, and the acetylene to 12 psi. Compressed air for the AA is house and left on at 40 psi.

A shielded 4 conductor cable connects the PB0-PB3 ports on Pandora's box to the corresponding valve control inlets on the side of the solvent box. Three harnessed wires connect the trigger, Ch 1 and Ch 2 of the scope to the labeled ports on Pandora's box, and a BNC cable connects the Spectrum 1021 to the ADC inlet. Finally, three amphenol connectors link Pandora's box with the AIM.

The two solvent reservoirs are filled with the SR and dw, and are degassed with the He bubblers while the hollow cathode lamp warms up and the program is loaded (starting at 10 on the tape counter, F=COLLN & T=1). When the program is done loading, power is turned on to the pumps, valve control box, oscilloscope, Spectrum 1021, chart recorder, UV detector, auxillary power supply, oscilloscope, and beeper. Turning power on to these while the program is loading may put a spike on the line which crashes the tape reading. RUN (CR) is then entered and a heading printed. An initial strip in the injection mode with the injection inlet tube in dw cleans the column and prepares it for analysis.

Many operator responses to computer input prompts may be entered by pressing a single key, such as Y or N. When the program wants an answer that cannot easily be contained in one key, such as description of a solution or the collection delay, then the response must be typed in and entered with the return key. If in doubt, a single key entry remaining on the LED display indicates that the return key must be pressed to enter the response. If a return key is not needed, then a single key entry will elicit immediate computer response, such as

asking another question or switching a valve. The operator should be forewarned that due to a peculiarity with AIM Basic, pressing return without a preceding entry for a return-requiring response will crash the program.

When the standards are analyzed, the flame is left on the entire time and the injection tubing put directly into the respective volumetric flasks. Since the blank peak is so small that only the computer can find it, a standard in the middle of the concentration range is analyzed first and the same limits used for the blank. Then the rest of the standards are sequentially analyzed. Collection delay is left as 15 s.

When doing TE, the AA flame is turned off after the completion of each stripping step in order to save gas. The computer tells the operator when to turn it on with the "Start AA" message. During TE analysis, the injection inlet tubing is left in a beaker of dw since the sample loop is flushed with it prior to each strip. Choice of collection delay depends on the retention time, which for pH 2 samples is strictly TE volume dependent and covered in the results and discussion section.

If the peak height is less than $5 \times 10^{-3}$ AU for Cu and Mn or $8 \times 10^{-3}$ AU for Cd, a greater TE volume is necessary. The "New" selection must be chosen since only through it is a new TE volume entered which is necessary to calculate a new TE time. However, if the same sample is still to be analyzed, the "sam flush" sequence which is offered with "New" is not necessary. As is done in the injection mode, the blank is usually analyzed after a sample so the same integration limits can be used to integrate the humanly indiscernible blank peaks.

# RESULTS AND DISCUSSION

## Introduction

The results and discussion will be treated in several parts. First the calibration procedures will be discussed and justified, followed by a description of the optimization of various chromatographic and computer parameters. Third, the performance of the system will be reviewed (e.g. calibration curves and system limitations) and then interference studies and real sample analysis will be presented.

## Calibration Procedure

Calibration is based on comparison of the AA response for the sample (determined by TE) to that of the standards. The following steps are followed:

1. Standards of several different concentrations, including a blank, are "injected" with the same fixed volume sample loop and measured.
2. The mass of metal delivered by each standard is calculated as follows:

   mass (ng) = concentration (ng/μL) x volume (μL)

   and used as the abscissa for the calibration curve. Blank corrected response is the ordinate.
3. The calibration curve is then used to convert the blank corrected TE signals to their corresponding mass.
4. The original sample concentration is found by dividing the calculated mass by the TE volume:

   concentration (ng/mL) = mass (ng) ÷ TE volume (mL)

Standards are not measured by TE for several reasons. Analysis time is considerably shorter for a given mass by injection than TE. It takes about five minutes to make three repetitive measurements by injection and 15-20 minutes by TE. Thus an entire calibration curve takes 15-20 minutes by injection and an hour or more by TE. Solution preparation is more convenient for injection solutions, and only 10-25 mL are required, whereas about 100 mL are needed for TE. Finally, solution concentration integrity is easier to insure in the mg/L range needed for injection than it is for the μg/L range for TE.

Since all TE recoveries are referenced to the injected standards, it is assumed that the standards are 100% retained and recovered. This assumption was verified by comparing the AA response by a normal injection procedure to that from a modified system where the injected sample passes straight through the system. The latter situation was created by injecting the standard into a stream of SR rather than the normal dw. Inspection of Table VIII indicates that although the peaks are wider and shorter using the normal configuration, the areas, and thus roughly the total integrated signals are very similar to the unretained peaks.

## Optimization of Variables

In most cases, results for the various optimization parameters are the same for all three metals tested. Copper was the "prototype" metal (i.e. it was used for most of the system development) and by reason of analogy initial choices for parameter values during tests with

Table VIII: Verification of Quantitative Recovery for Injection

| Metal[a] | Configuration[b] | pk ht[c] average | pk ht[c] SD | Area[d] Average | Area[d] SD | W[e] |
|---|---|---|---|---|---|---|
| Cu | Normal | 11.4 | 0.8 | 449 | 31 | 78 |
| | Modified | 13.4 | 0.6 | 399 | 26 | 70 |
| Cd | Normal | 25.4 | 0.5 | 1412 | 24 | 99 |
| | Modified | 35.7 | 1.7 | 1373 | 62 | 75 |

a. Instrumental conditions:
Sample loop volume = 87.5 µL
SR: 0.2 M citrate at pH 4 for Cu, 5 for Cd, and 6 for Mn
Flow rate = 5 mL/min
Concentration: 0.3 mg/L each

b. Normal: sample injected onto column with subsequent stripping step
Modified: Sample injected into SR stream so no retention by column

c. Absorbance x 1000

d. Arbitrary units

e. W = peak width (number of points at 10 pnts/s)

subsequent metals were often those chosen for Cu. Some parameters must be element specifically optimized and will be discussed later.

## Stationary Phase

The heart of the chromatographic system and thus the most important parameter to optimize is the choice of stationary phase. It is important that the selected resin have a strong affinity for the metal ion in order to quantitatively retain it, but also the resin must be convinced to release the metal in a small, concentrated plug during the stripping step.

It is also important that the resin have a high capacity for the analyte and be selective for it, since in many real samples, the concentration of concomitant species may be several orders of magnitude greater than the analyte concentration. In addition, the resin should have long term physical and chemical stability - the former to prevent formation of voids and fissures or bed collapse and the latter to obtain reproducible chromatographic behavior, both during an analysis and also from day to day. It is not desirable that frequent repacking of the column be required.

Particle size is important also. The analyte will be retained in a narrower band and hence stripped in a narrower zone if the particle size is small. However, larger particles make column packing easier and create a smaller back pressure, allowing faster flow rates and therefore shorter analysis times. Thus, the choice of particle size involves a trade-off. Finally, an obvious desirable trait for the resin is a low cost per column.

The final choice of resin for this system is Bio-Rad AG 50W-X8 cation exchange resin. The mesh size is "minus 400" which corresponds to a particle size range of 37-44 μm. It is polystyrene based with 8% cross linking (see Figure 20). The selectivity coefficients for some metal ions on the resin, as listed by Dean (113), are given in Table IX. The values are based on the data of Bonner, et al. (114, 115) and express the selectivity of the resin for the given ion in preference to hydrogen ions from a solution containing equal concentrations of each. Basically a gel type structure, the rigidity and thus resistance of the resin to swelling and shrinking increases with a larger degree of cross linking. The exchange sites are a sulfonic acid type, classified as a "strong cation exchanger." A one pound bottle cost $80.00 in December 1981.

Since the resin comes with 50% moisture content and has a consistency not unlike a paste, it is not possible to dry pack it. Thus, the slurry packing technique described in the experimental section is necessary. The first time the resin was used, the slurry was made simply with dw. However, when a TE sample (which is $10^{-2}$ M in $HNO_3$) was subsequently passed through the packed column, the resin swelled and extruded through bed support at the end of the column. The resin is most swollen in the H form and the TE sample is the most acidic solution of the analysis. Henceforth the resin slurry has been made in $10^{-2}$ M $HNO_3$ so the column is filled with the resin already swelled to its maximum value. No "blow-outs" have occurred since.

The column is repacked with new resin when it shows chromatographic or physical signs of deterioration, such as high back pressure, peak doublets, or tailing. How long a column lasts depends on what

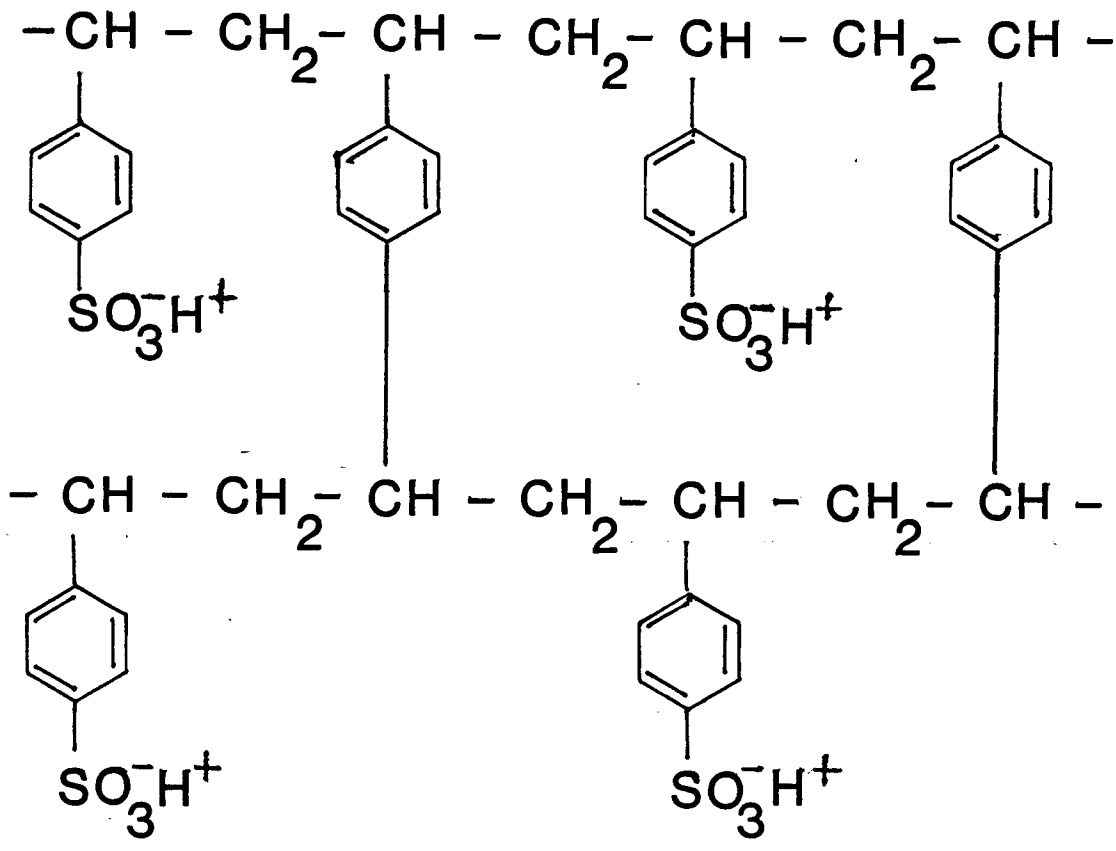


Figure 20. Structure of Bio-Rad AG50W-X8 cation exchange resin.

Table IX: Selectivity Coefficients for Dowex 50[a]

| Ions | K [b,c] |
|---|---|
| **Univalent** | |
| Li | .79 |
| H | 1.00 |
| Na | 1.56 |
| $NH_4$ | 2.01 |
| K | 2.28 |
| Rb | 2.49 |
| Cs | 2.56 |
| Ag | 6.70 |
| **Bivalent** | |
| Mg | 1.15 |
| Zn | 1.21 |
| Co | 1.31 |
| Cu | 1.35 |
| Cd | 1.36 |
| Ni | 1.37 |
| Mn | 1.43 |
| Ca | 1.80 |
| Sr | 2.27 |
| Pb | 3.46 |
| Ba | 4.02 |
| **Trivalent** | |
| Cr | 2.0 |

a. Divenyl benzene type with 8% cross-linking

b. $K = \frac{MR \times H}{M \times HR}$ where MR, H, M and HR refer to molar concentrations of bonded metal, hydrogen ion, free metal, and resin in the H form, respectively

c. Values from reference 113.

sort of abuse it is subjected to (such as high flow rates and sample clarity), but rarely does a column require repacking with less than 10 days use.

The column chosen for the system is a borosilicate glass column made by Altex. An i.d. of 3 mm and length of 50 mm yield a bed volume of 0.3 mL. The column comes with a Teflon disc bed support assembly that contains a SS filter insert with a 2 μm pore size. Between this assembly and the resin bed goes a 5 μm pore size Teflon filter disc (see Figure 21). Since these filter discs are overpriced (about $1.00 each), Mitex filter paper media (10 μm pore size) was purchased and a 9 mm punch made in order to cut out "home-made" filter discs. In order to approach a more metal free system, the SS filter insert was removed from the bed support, relying on the Teflon filter disc for physical support of the resin. However, the home-made discs are thinner than the commercial ones (0.004 in. thickness compared to 0.007 in.) and so the SS insert is necessary on the downstream end of the column to provide the necessary physical support. No detrimental effects on the chromatography have been noted by the inclusion of the insert.

Other stationary phases that were tried include a silica based resin manufactured by E. Merck, Darmstadt, Germany. The bonded phase sulfonic acid type cation exchanger has a 10 μm particle size and is packed by Brownlee Labs in a 3 cm x 4.5 mm i.d. SS cartridge. Use of the column was discontinued because peak shape problems (the injection stripping peaks were shaped differently than the TE ones) and anomalous results (memory effects noted with repetitive measurements and contamination evident in TE peaks). It was suspected that the column was

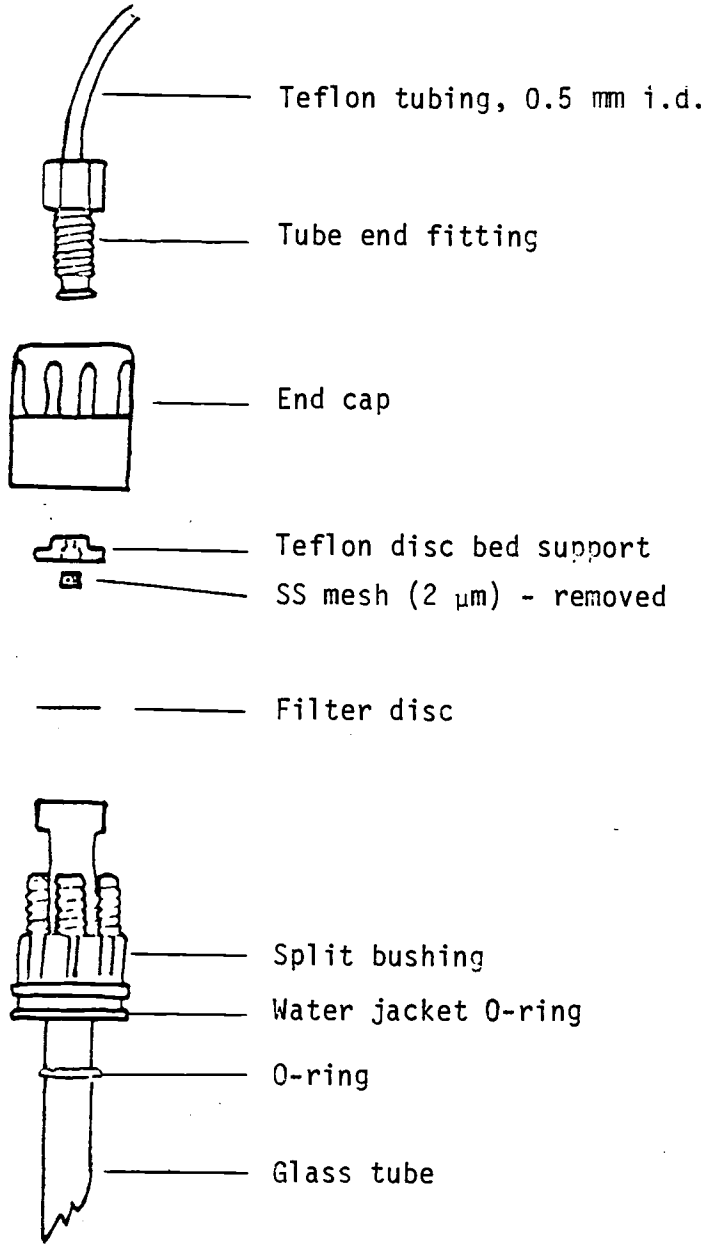



Figure 21. Diagram of Altex microbore glass column

leaching small but significant amounts of metal from the SS walls and end frits.

A similar type resin is made by Vydac. It is also a silica based bonded phase sulfonic acid cation exchanger but has an average particle diameter of 40 μm, allowing it to be easily dry packed. Most experiments with it were conducted in the same 3 mm x 50 mm glass column as was used with the Bio-Rad resin. The resin showed promising results with Cu in reproducibility and sensitivity but failed to quantitatively retain the analyte in the presence of concomitant cations. Since the problem seemed to do with an inadequate number of available exchange sites, a larger bed volume of the resin was tried. A column with the same 3 mm i.d. but 250 mm in length, giving a fourfold increase in bed volume, was tested but could not be operated at flow rates greater than 2 mL/minute without exceeding the system pressure limitation of 500 psi. A wider column (6 mm i.d. with adjustable end set to a bed volume of 1.8 mL) produced a small pressure drop but produced poorly shaped stripping peaks for the TE sample.

Stripping Reagent

If choice of stationary phase is the most important chromatographic parameter to optimize, then choice of stripping reagent (SR) is easily the second. The most important characteristic of the SR is that it be able to compete effectively with the resin for the metal. If it does, then the metal will be removed from the resin during the strip step in a small concentrated plug and the resultant peak will be sharp. A sharp peak is desirable because it is easier than a broad flat one to distinguish from baseline noise and measure.

It is also important that the SR be non-destructive to the system components and the resin. An example of the latter might be an irreversible bonding of the SR to the resin sites and thus deactivation of them. An example of the former is our experience with the use of 1 M $HNO_3$ as a SR. The acid at that strength dissolves a seal in the pump causing extensive down time and making the cost of the analysis prohibitively high.

The metal stripping process is of course a competition of equilibria. For the TE process it is desired that the stationary phase win the competition so that essentially all the metal is retained by the resin. During the strip step though when the mobile phase (MP) is switched to the SR, it is desired that the equilibrium favor the MP so that complete recovery of the metal in a small SR volume is possible. If the SR removes the metal by a complexation mechanism, then ease of stripping will be enhanced by a large stability constant of the metal complex. In addition to thermodynamic favorability, kinetics is also important, for if a complex with a large formation constant is slow to form, it will not be useful as a SR.

Table X is a tabulation of log $\alpha$ constants for several ligands and test metals, where $\alpha$ is the ratio of total metal in solution to free metal (unbound by ligand). Thus a larger $\alpha$ corresponds to a larger bound fraction. Stability constants cannot be used directly to determine ligand favorability to metal complexation, because acid dissociation constants of the ligand also play a key factor and thus the fraction of bound metal in a system is pH dependent.

It would appear from Table X that oxalate would be the best ligand for metal stripping. However, in a personal communication (116) with

Table X: Values of Log $\alpha$ for Selected Metals and Ligands [a, b]

| Metal | Ligand | $\mu$ [c] | pH 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Cu | Citrate | 0.5 | 0.8 | 2.9 | 5.1 | 6.7 | 8.0 |
| | Oxalate | 0.5 | 4.9 | 6.3 | 6.9 | 6.9 | 6.9 |
| | Tartrate | 1.0 | 1.0 | 2.5 | 3.1 | 3.2 | 3.2 |
| | Acetate | 1.0 | | 0.3 | 0.9 | 1.1 | 1.1 |
| Cd | Citrate | 0.5 | 0.1 | 0.8 | 2.0 | 2.7 | 3.0 |
| | Oxalate | 0.5 | 1.8 | 2.2 | 2.6 | 2.7 | 2.7 |
| | Tartrate | 0.5 | 0.6 | 1.4 | 1.8 | 1.8 | 1.8 |
| | Acetate | 1.0 | | 0.1 | 0.3 | 0.4 | 0.5 |
| Mn | Citrate | 0.5 | 0.1 | 0.7 | 1.5 | 2.1 | 2.4 |
| | Oxalate | 0.5 | 0.6 | 1.8 | 2.2 | 2.2 | 2.2 |

a. $\alpha$ is defined as M'/M where M' = concentration of total metal
M = concentration of free metal
Concentration of ligand is 0.1 M in all cases

b. Values are from reference 120.

c. Ionic strength, M

the manufacturers of the resin, citrate was suggested and so it was chosen as the SR in this system. Since it is a triprotic organic acid, it has a considerable buffering range with $pK_a$'s of 3.12, 4.76, and 6.40. The formula to determine the $[H^+]$ at the equivalence points (where the acid and base forms are in equal concentration) is:

$$[H^+] = \frac{K_1 K_2 F}{K_1 + F}$$

where $K_n$ are the acid dissociation constants and F is the formal concentration of the acid (117). At 0.2 M, the pH at the first and second equivalence points is 3.9 and 5.6, respectively.

Bio-Rad (116) suggested that after stripping the metal, the column be reconditioned with NaOH and then $H_2SO_4$. This would leave the resin in the H form for subsequent TE and H is the most easily displaced cation. When the column was rinsed with pH 8 water followed by a pH 2 $H_2SO_4$ wash after stripping, no significant effects on column performance were noted. The pH range is limited to these bounds for prevention of SS and glass dissolution.

Once the choice of SR has been made, there remains the concentration and pH of the SR solution to optimize. These parameters are more metal specific in nature and are optimized more empirically. For instance, citrate was initially pH adjusted with NaOH. Peak tailing was a problem, though, and this was resolved by using $NH_4OH$ for pH adjustment. The ammonium provides an apparent enhancement in stripping by formation of ammonia complexes as well as citrate ones (e.g. the first stepwise formation constant for Cu with $NH_3$ is about $10^5$). Also, the resin is more selective for ammonium ions than Na (see Table IX) and the ammonium supplies an extra impetus to evict the metal.

The SR concentration was chosen as 0.2 M from the results of an experiment where the SR concentration was varied from 0.05 M to 0.2 M at pH 4. The peak height of injected Cu was increased by a factor of 2.5 at 0.2 M compared to 0.05 M citrate. At 0.2 M there is no evidence of incomplete recovery, such as a memory effect - a high initial response for a low concentration standard when proceeded by a high concentration standard. If there were incomplete recovery during the repetitive analysis of a high concentration standard, then the "left-over" metal would appear when a lower concentration was analyzed, adding to it and causing an initially high response. Since 0.2 M citrate produced narrow peaks for analysis and showed no memory effect, a higher SR concentration would only increase the cost of analysis with no further benefit and was therefore not tried.

The SR pH was optimized in a similar way as the concentration. Figure 22 shows the dependence of peak height on SR pH by injection of 0.3 mg/L of each metal. The chosen optimum values of SR pH are 4 for Cu, 5 for Cd and 6 for Mn. Although Cd has a slightly higher peak height at pH 6 SR, pH 5 was chosen as optimum because the TE recoveries were better there. This behavior might be due in part to a lower SR pH (i.e. higher $[H^+]$) leaving more resin sites in the $H^+$ form which might facilitate a more quantitative retention during TE. The fact that Mn follows a pH optimization curve very similar to Cd but works fine at pH 6 might be due to the higher selectivity coefficient for Mn by the resin (see Table IX). Note that at the chosen SR pH values $\log \alpha$ is between 2 and 3 for all 3 test metals (Table X), implying that for parameter optimization of a new test metal, initial selection of SR pH might be chosen such that $\log \alpha$ values are in this range.

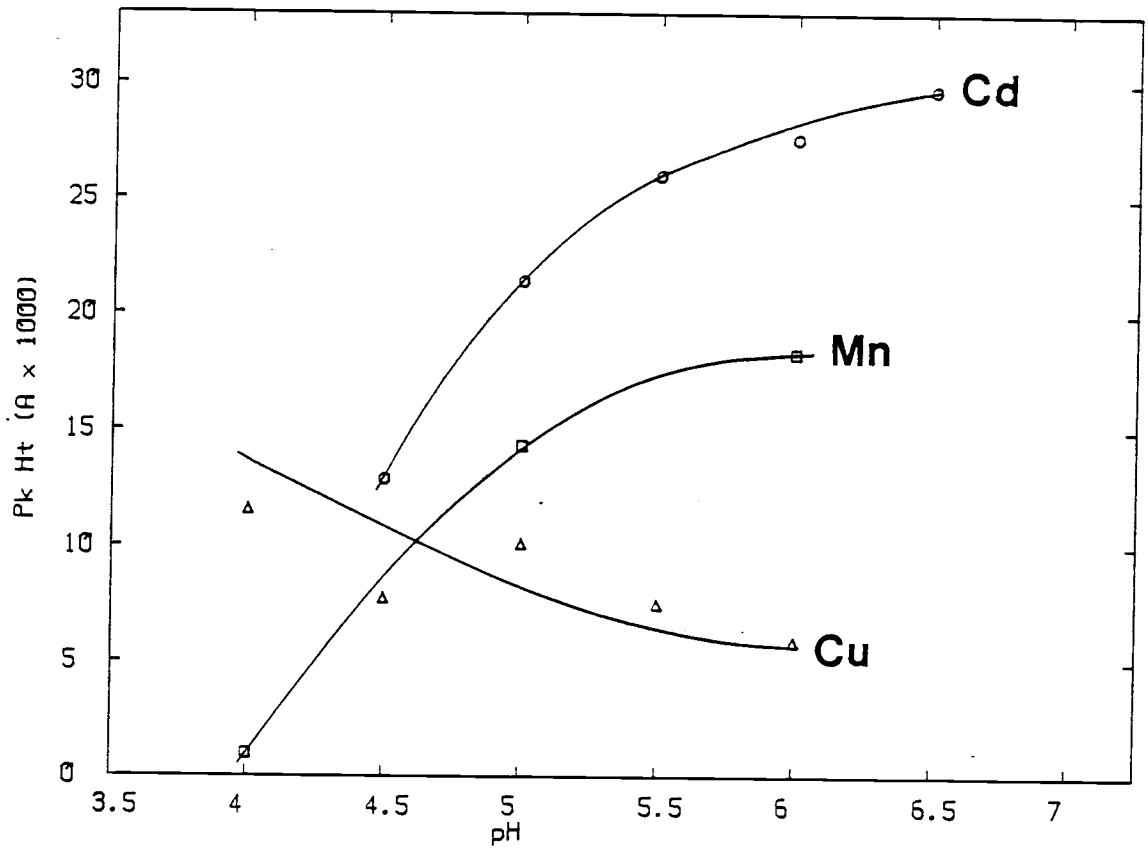



Figure 22. Dependence of peak height on stripping reagent pH. Points are the averages of repetitive injections of 0.3 mg/L of each metal. Other instrumental conditions as in Table VIII

Several of these observed experimental results were compared to those predicted by complex equilibrium equations. Since there are many equilibrium reactions occurring simultaneously, a multiple equilibrium chemical modeling computer program (118) was utilized. The user defines all species in solution as a matrix representation of the appropriate combination of components. The computer then applies a Newton-Raphson iterative technique to improve initial estimates of each species concentration until all equilibrium expressions are satisfied within a specified degree.

Claudia Seyfert of the OSU Chemistry Department used the program to determine the modeling for 0.2 M citrate as a SR with the Bio-Rad resin. Cu was chosen as the test metal. For this model, the resin is treated as though it were a ligand in solution rather than in the solid phase. Also, the system is treated as static and in thermodynamic equilibrium and the volume occupied by the resin was not considered in a rigorous manner. Since citric acid has a hydroxy H which dissociates in addition to the three acid groups, the parent molecule is denoted $H_4L$.

The speciation of citrate as a function of pH was examined and the speciation of citrate and Cu-citrate complexes at pH 4 was also determined. Figure 23 shows the speciation of citrate as a function of pH and from Table XI it can be seen that at pH 4 (the optimum value for stripping Cu), citrate exists primarily in the $H_2L$ form. In the primary form of the metal complex at that pH, however, Cu is bound to HL, representing a loss of a proton.

Next, the equilibrium distribution of Cu between the mobile and stationary phase during a strip was examined. The concentration of Cu

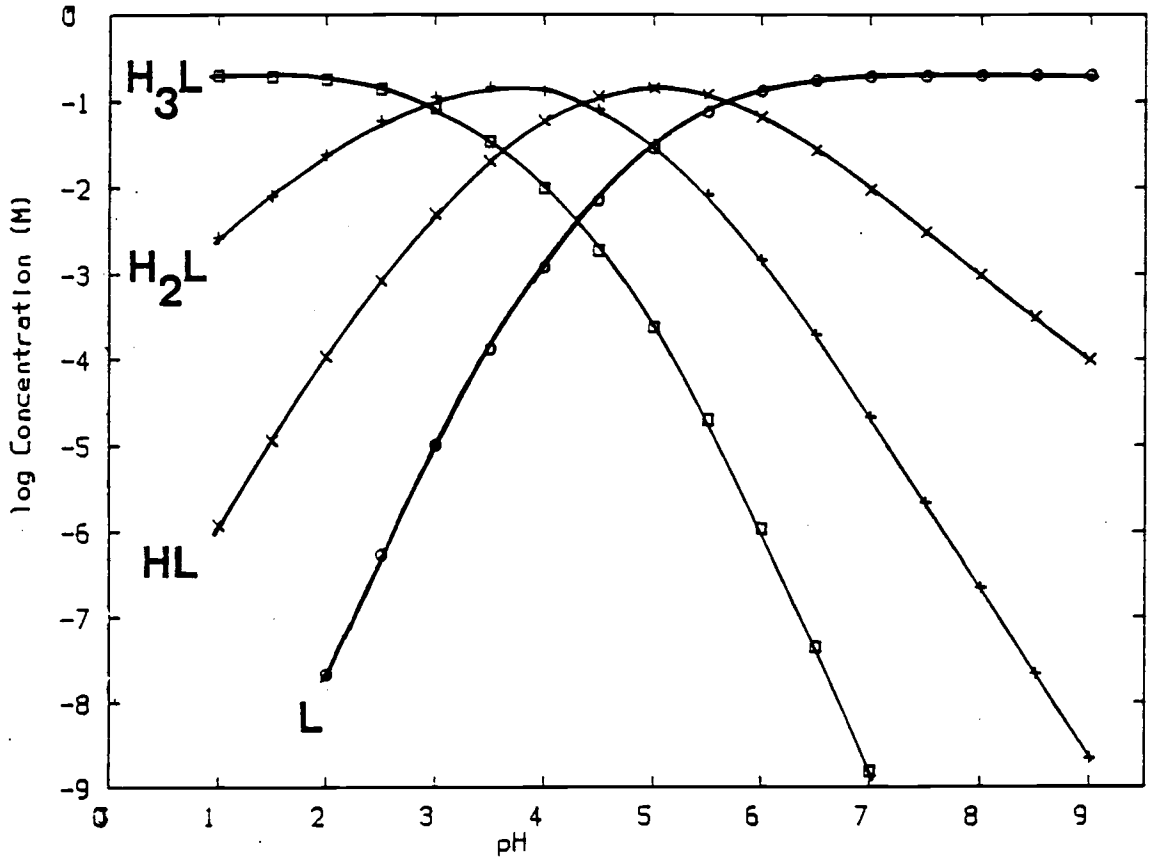



Figure 23. Dependence of speciation of citrate as a function of pH. Values determined by computer modeling (reference 118) for 0.2 M citrate solution

Table XI: Distribution of Citrate and Cu Citrate Complexes at pH 4 [a]

| Form [b] | Concentration (M) | % |
|---|---|---|
| $H_4L$ | 0.00001 | 0.01 |
| $H_3L$ | 0.00969 | 4.85 |
| $H_2L$ | 0.13072 | 65.36 |
| HL | 0.05839 | 29.19 |
| L | 0.00119 | 0.60 |
| Total Citrate | 0.20000 | 100.02 |
| Cu | $2.11 \times 10^{-12}$ | $2.11 \times 10^{-3}$ |
| CuL | $2.00 \times 10^{-9}$ | 2.00 |
| CuHL | $9.79 \times 10^{-8}$ | 97.95 |
| $CuH_2L$ | $5.02 \times 10^{-11}$ | 0.05 |
| $Cu_2L$ | $6.69 \times 10^{-21}$ | - |
| $Cu_2L_2$ | $1.00 \times 10^{-18}$ | - |
| Total Cu | $1 \times 10^{-7}$ | 100.00 |

a. System consists of Bio-Rad AG 50W-X8 resin (exchange capacity = 1.7 eq/L), 0.2 M citrate at pH 4, $10^{-7}$ M (6.35 µg/L) Cu; resin selectivity coefficient = 1.35 for Cu; Cu complexes with $OH^-$ and $CO_3^{-2}$ were not considered in model but are negligible at pH 4.

b. $H_4L$ = citric acid

in the MP is the sum of free Cu and all forms of the Cu citrate complex, while the stationary phase concentration is that Cu bound to the resin. Table XII lists the concentration of resin-bound Cu and its percent of the total Cu at stripping pHs between 1 and 8. It can be seen that the resin bound fraction is minimal (and hence stripping most efficient) near the empirically chosen value of SR pH. This modeling illustrates how the behavior of a species involved in a complex equilibrium cannot be predicted with a single constant as would be convenient. If one were to predict a SR pH optimum from Table X alone, the erroneous conclusion would be that stripping is continously improved as pH increases, since $\alpha$ increases with pH. What Table X fails to consider is the competition of the resin for the metal, and experimental evidence indicates that log $\alpha$ values between 2 and 3 correspond to the optimum pH of this competition.

The 68% distribution of metal in the MP at pH 4 SR is not to be taken as the stripping efficiency, for the modeling is applied to a static system whereas in actuality the SR is flowing through the column at 5 mL/min. Since 26% of a volume of closest packed spheres is the interstitial volume (119), then one column dead volume for the 0.3 mL bed volume is 0.078 mL. If one assumes that equilibrium between mobile and stationary phases is fast, then each dead volume plug of SR will remove 68% of the remaining bound metal and hence 99% of the metal will be removed in 4 column dead volumes. This corresponds to a 0.31 mL stripping volume which compares reasonably well with the actual typical peak width of 0.63 mL (7.5 s) after considering the influence of normal post-column zone spreading.

Table XII: Percent Cu in Stationary Phase with 0.2 M Citrate

| pH | CuR [a] ($\times 10^{8}$ M) | Percent Retained [b] |
|---|---|---|
| 1.0 | 9.04 | 90.4 |
| 1.5 | 8.61 | 86.1 |
| 2.0 | 7.19 | 71.9 |
| 2.5 | 5.16 | 51.6 |
| 3.0 | 3.64 | 36.4 |
| 3.5 | 3.06 | 30.6 |
| 4.0 | 3.26 | 32.6 |
| 4.5 | 4.34 | 43.4 |
| 5.0 | 6.31 | 63.1 |
| 5.5 | 8.26 | 82.6 |
| 6.0 | 9.36 | 93.6 |
| 6.5 | 9.79 | 97.9 |
| 7.0 | 9.93 | 99.3 |
| 7.5 | 9.98 | 99.8 |
| 8.0 | 9.99 | 99.9 |

a. Total Cu = $10^{-7}$ M (6.35 µg/L)
CuR = molar concentration of resin-bound Cu

b. % retained = CuR x 100% / $10^{-7}$

Once the SR has removed the metal ions from the resin, the metal should be delivered to the detector in a form that is readily detectable. In the case of using AA for detection, this is not a problem. Although atomization and hence sensitivity can vary somewhat with chemical form entering the atomizer, the metal is always in the same form leaving the column, regardless of what form it is in when it arrives. Thus choice of SR is not critically dependent for its effect on detection. However, if molecular spectroscopic or electrochemical detection is used, this becomes an important factor in choice of SR.

In the present design of the system, the SR is continuously flowing. During TE and column washing, the SR goes through $V_2$ to waste and for a strip $V_2$ directs the flow on line to the column. In an earlier system design, the SR flow was stopped except during the strip step. When large and anomalous blanks were obtained, it was suspected that when static, the SR was leaching metal ions from the SS lines and pump, causing unpredictable contamination. Keeping the SR continuously flowing minimizes the contamination and keeps it at a constant background level, rather than variable and dependent on such things as the time it is statically in contact with the pump. A further embellishment of the automation aspect of the system would be computer on/off control of the pump, where the pump could be left off except for just prior to the strip step. This would considerably reduce the consumption of citric acid, which although is comparatively inexpensive, nonetheless is required in considerable volume.

Other Chemical Variables

When a water sample is collected, it is often "preserved" by adjusting it to pH 2. This prevents the loss of analyte metal ions due to adsorption on the walls of the collection vessel. Since the system analyzes samples at pH 2, it offers the convenience of not requiring any further sample pre-treatment other than filtration. Also, if the sample pH had to be adjusted to a higher value for analysis, there is a chance that adsorption would compromise the integrity of the sample concentration before it could be measured. In this research, synthetic TE samples were typically adjusted to pH 2 when made in order to prevent adsorption losses on the glassware. When a 3 μg/L Cu sample made in this way was neutralized just prior to analysis by addition of an amount of NaOH equivalent to that of the acid, it was found that about 50% of the metal was lost by what is apparently a kinetically fast process.

For calibration, it is desired to have conveniently prepared standards, and a pH adjustment would be an inconvenience, especially for the small volume of standards required (10-25 mL). In this system, no pH adjustment is required for the standards, and no further pH adjustment is necessary for the sample.

To verify that the system responds the same to a given mass of metal delivered by either TE or injection, equal masses of a Cd solution were analyzed by both methods. Normal concentrations are at the μg/L level for TE and mg/L level for injection standards. The test solution had to be such that its volume and concentration for TE were close to the range normally used and at the same time deliver an

adequate mass with injection to be detected. The solution chosen was 0.04 mg/L Cd and the 87 µL sample loop was replaced with one that is 0.94 mL. The resultant injected mass of 40 ng was delivered by TE by using a TE volume of 0.94 mL. Since the solution was a standard which had been made numerous times, the glassware was "dedicated" and well equilibrated with the metal. Thus adsorption loss in the non-acidified sample was not a problem. Table XIII indicates that the response for each method was essentially the same.

In order to test the dependence of the retention efficiency on TE sample pH, 3 µg/L Mn solutions were made at pH 1, 2 and 3. A 10 mL TE volume was used, and Table XIV indicates that retention decreases slightly with sample pH of 1. Thus samples should not be over acidified.

The dependence of sample pH on retention efficiency may also be predicted from theory by using the selectivity coefficient of the resin. The expression for selectivity coefficient is:

$$K = \frac{MR \times H}{M \times HR}$$

where K is the coefficient and MR, H, M and HR refer to molar concentrations of bonded metal, hydrogen ion, free metal, and resin in the H form, respectively. HR remains constant for a resin with a low amount of loading and can be estimated as the exchange capacity. Since K and H are also known, one can find the ratio MR/M from the expression and then for a total metal concentration of 3 µg/L for each one:

$$MR + M = 4.72 \times 10^{-8} \text{ M for Cu}$$

$$= 2.67 \times 10^{-8} \text{ M for Cd}$$

$$= 5.46 \times 10^{-8} \text{ M for Mn}$$

Table XIII: TE vs Injection of Equivalent Mass [a]

| Mode [b] | n [c] | Peak Height [d] ave | SD | RSD | Area [d] ave | SD | RSD |
|---|---|---|---|---|---|---|---|
| Injection | 3 | 22.3 | 0.5 | 2 | 1325 | 98 | 7 |
| TE | 2 | 21.4 | 0.2 | 1 | 1382 | 141 | 10 |

Table XIV: Experimental Retention Efficiency Dependence on Sample pH [e]

| Soln | Measured pH | n [c] | Peak Height ave | SD | RSD | Area ave | SD | RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.04 | 3 | 12.3 | 1.3 | 10 | 540 | 67 | 12 |
| 2 | 1.87 | 3 | 13.9 | 0.7 | 5 | 463 | 74 | 16 |
| 3 | 2.69 | 4 | 13.4 | 0.9 | 7 | 579 | 116 | 20 |

a. Unacidified 0.04 mg/L Cd used for both methods. Instrumental conditions as in Table VIII

b. 0.94 mL sample loop for injection
0.94 mL TE volume for TE

c. Number of runs

d. In this and all subsequent tables, units for average and SD are absorbance x 1000 for peak height and arbitrary units for area.

e. All solutions 3 µg/L Mn: Solutions 1, 2 & 3 made by addition of 1, 0.1 and 0.01 mL concentrated $HNO_3$ to 100 mL sample; 10 mL TE volume each; instrumental conditions as in Table VIII.

which yields 2 equations and 2 unknowns for each metal. When these equation systems are solved for all 3 metals at the 3 pH's tested, the results are the values listed in Table XV. It can be seen that at sample pH values near one, hydrogen ion begins to effectively compete with the metal for resin sites.

## Physical Parameters

In addition to chemical parameters final selection of several physical variables warrants mention. An important one is the flow rate for the TE and stripping reagent pumps. Ideally, one would desire the maximum possible flow rate during TE, especially for dilute samples, so as to minimize the analysis time. In reality, the flow rate can be limited by maximum pump output, pressure limitations of system components, and decreased retention efficiency during TE with high flow rate. The selection of 5 mL/min represents the maximum flow rate that keeps the back pressure (caused primarily by the column packing) around 200-300 psi, comfortably below the 500 psi pressure limitations of the system components (e.g. valve and column end fittings). Variation of the TE flow rate from 2 to 5 mL/min had no effect on TE efficiency.

Maximizing the flow rate during the strip step is less important for minimizing analysis time since the metal ion comes off the column in a relatively short time (less than a minute after initiating the strip step compared to typical HPLC and gas chromatography retention times of 15 minutes to one-half hour). If the peak height were critically dependent on flow rate, then small variations in the SR pump flow rate could result in poor precision. Although it was expected

Table XV: Theoretical Retention Efficiency [a] Dependence on Sample pH

| | Metal | | |
|---|---|---|---|
| pH | Cu | Cd | Mn |
| 1 | 95.8 | 95.9 | 96.1 |
| 2 | 99.6 | 99.6 | 99.6 |
| 3 | 100.0 | 100.0 | 100.0 |

a. Retention efficiency = bound metal (MR) x 100/total metal (M + MR).

Selectivity coefficients are listed in Table IX.

HR = 1.7 eq/L for AG 50W-X8 resin

that the peak height would decrease with decreasing flow rate due to a slower mass transfer, the height remained the same from 5 down to 2 mL/min for repetitive stripping of 0.3 mg/L Cd. Perhaps a decrease in the rate of metal removal from the resin at slower flow rates is offset by an increasing efficiency of atomization with slower mass transfer.

The retention time (measured as the time from switching of $V_2$ to the time of peak maximum) is solely dependent on the volume of TE and not on sample concentration. One would likely expect that with a greater TE volume, the metal ions would make their way further down the column and hence have a shorter journey to the detector during the strip, resulting in a shorter retention time. In actuality the opposite effect was found - retention time increases with TE volume. One possible mechanism that would be consistent with this observation is that a greater TE volume (longer TE time) would allow deeper penetration of metal ions to the interior of the resin beads. During the strip, a longer time would then be required for the metal ions to diffuse to the surface and be carried away by the SR.

Nonetheless, the retention time varied most with Cu, as shown in Figure 24 and general formulas for prediction of retention time are:

$$R_t = 26.6 + 1.10\,(V_{TE}) - 0.012\,(V_{TE})^2 \qquad \text{Cu}$$

$$= 27.7 + 0.50\,(V_{TE}) - 0.0043\,(V_{TE})^2 \qquad \text{Cd}$$

$$= 25.1 + 0.58\,(V_{TE}) - 0.0061\,(V_{TE})^2 \qquad \text{Mn}$$

where $V_{TE}$ is TE volume in mL and $R_t$ is the retention time in seconds. The linear terms dominate at normal TE volume (10 mL or less).

The major importance for retention time prediction is in selection of a value for "collection delay", since the computer collects data in

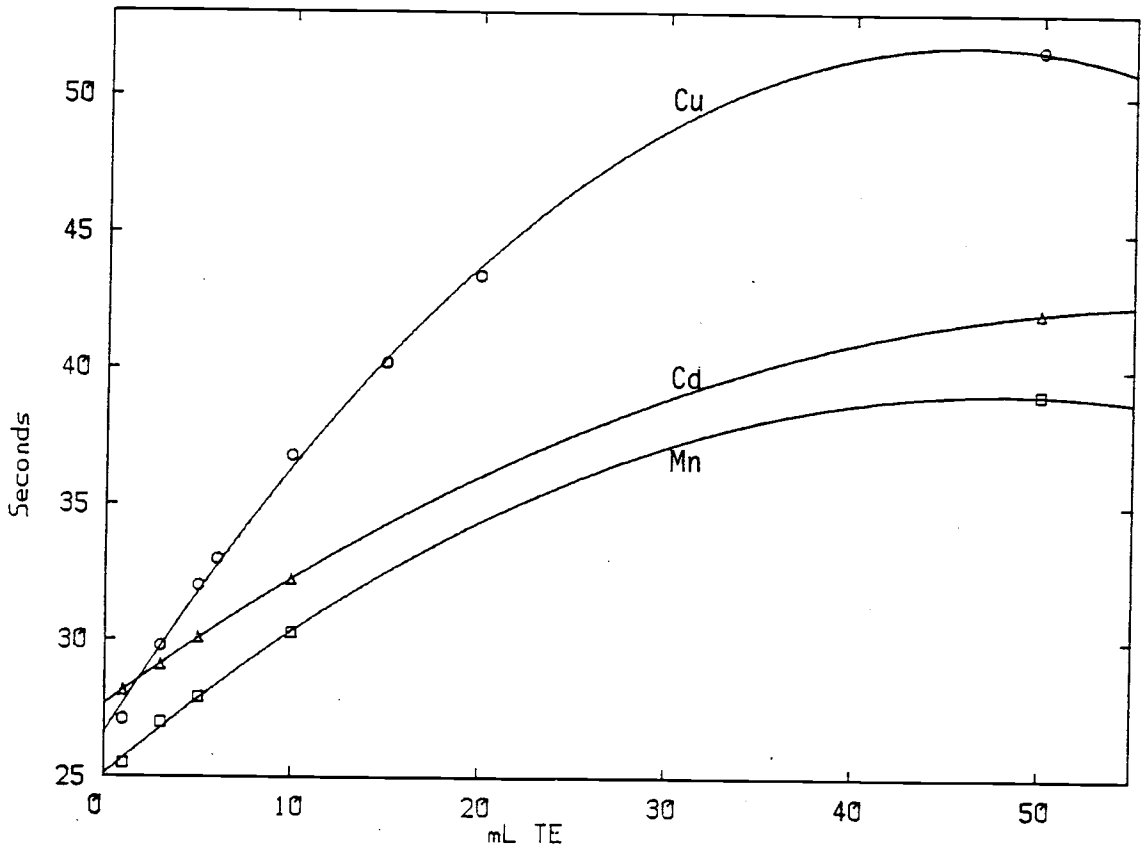



Figure 24. Dependence of retention time of metals during strip step on TE volume. Instrumental conditions as in Table VIII

a 25 s window and it is necessary for the metal to elute within this time. A rule of thumb for getting the peak to fall in the middle of the collection window is to chose the collection delay, D, such that

$$D = R_t - 15 \text{ s}$$

The peristaltic pump fills the sample loop by suction, that is by creating a partial vacuum in the line and drawing the standard through the loop. This partial vacuum is not immediately removed when the pump is turned off, and if the vacuum is not completely relieved by the time the sample injection valve is switched, then this residual vacuum will partially fill the bypass loop with standard. When the strip step is over and the valve is subsequently returned to the "load" position, the contents of the bypass loop are then passed through the column. This may cause erroneously high values for the standards.

Since the pump turns at a fixed 60 revolutions per minute, the flow rate is determined by the diameter of the peristaltic tubing. A tube with an i.d. of 5 mm produces a flow rate of 25 mL/min. With this large of a flow rate, it was found that 25 µl of standard were inadvertantly delivered to the bypass loop and hence to the column by this residual vacuum effect. Increasing the delay time between peristaltic pump off and switching of standard injection valve did not solve the problem but when the 5 mm tubing was replaced with 2.5 mm i.d. tubing, (12 mL/min), the problem was resolved. The smaller tubing passes 2 mL of standard through the loop in the 10 s load period and with a sample loop volume of 87 µL, this is more than adequate.

The selection of sample loop volume of 87 µL was based on several criteria. In order to get the masses of standards in a desirable range for a calibration curve, one can adjust the sample loop volume or the

concentration of standards. It is desired to have a small enough loop volume to allow using standards with convenient concentrations. If the standards have too low a concentration, then contamination and loss become potential problems. A small loop volume also consumes less standard. On the other hand too small a loop volume many reduce the precision of delivered volume. By using an 87 µL loop, standards normally are in the range of 0.1 to 1 mg/L for a calibration mass of 10 to 100 ng.

The volume for TE is determined by the duration that $V_1$ is in the TE position. The more dilute the sample, the longer the TE time that is necessary in order to collect a mass of metal that is within the calibration curve range. Although the discharged flow rate of the HPLC pumps are very precisely controlled and pulsationless, the intake is not. When the inlet tubing was placed in a 10 mL graduate cylinder filled with dw, it was found that the pump withdrew short segments of 0.12 mL, pausing for about 0.5 s between each one. For this reason it is necessary to have a total TE volume large enough to average these pulsations out. TE volumes down to 1 mL have been used without any loss of precision. The upper limit for TE volume would seem to be open and dependent on sample concentration, and volumes of 50 mL have been used with quantitative recoveries. Thus the tested range of TE volumes is between 1 and 50 mL.

Since the interface between HPLC and AA is simply a Teflon tube, there is little to optimize there. However, it was found that replacement of the standard aspirator on the AA with a variable flow aspirator set to null causes a peak height enhancement of about a factor of 2 compared to the fixed flow. There is an apparent increase

in nebulization efficiency when the HPLC pump supplies all the driving force.

## Computer Variables

One of the most important computer variables to optimize is the frequency of data collection. Since the data file is limited to 255 points (for reasons of programming convenience), the amount of time the computer spends collecting data is determined by the collection frequency - a higher frequency results in a shorter time spent collecting data to fill the file. A collection frequency that is too high will shorten the collection window such that it becomes easier to miss the peak. A lower collection frequency makes it easier to insure catching the peak in the window, but if too low, the peak will be defined by fewer points and hence accuracy of the data is compromised. A general rule of thumb is that 30 points are the minimum required to accurately define a peak. The chosen value of 10 points/s defines a fairly generous collection window (25 s) while maintaining a normal peak width of greater than 70 points, more than enough to insure accurate determination of peak parameters.

Most of the other computer variables involve timing steps. In the TE mode, the computer waits 60 s after switching $V_1$ to dw at the end of the TE time (see Figure 13) before initiating the beeper attention cycle. This time allows dw to flush the sample from the tubing between $V_1$ and the column to insure that the entire TE volume makes it to the column (see Figure 5). As stated in the instrumental section, dw has no absorbance at 254 nm but most dissolved species do. During a TE,

when the sample is flowing through the column, the UV will read about 0.060 AU. By the end of this 60 s wash and the 15 s beeper cycle, the UV detector response goes below 0.010 AU, indicating that the sample has been flushed through the system and the column is ready for the strip step.

In the injection mode, the peristaltic pump is on for 10 s to fill the sample loop, followed by a 10 s delay period before the strip step is initiated. The former time period allows an ample volume of standard to flush out and fill the sample loop, as discussed under physical parameters. The latter delay time allows relief of the partial vacuum created in the standard tubing line when the pump is on.

Once the stripping step is initiated, the computer waits the collection delay time before starting to collect data. The default value of 15 s puts the earliest eluting peaks in the middle of the collection window. Samples with TE volume less than 5 mL and all the standards will fit in the window using this delay value.

The final delay time is not determined by the computer but by the operator. This is the post-strip time - the time between termination of the stripping step and initiation of the next column loading, either via TE or injection. It is necessary to wash the SR from the column before attempting to load it with another dose of metal. The column is considered washed and the delay period deemed over with when the UV response drops from greater than 0.030 AU during the stripping step to below 0.010 AU. The time to set the integration limits for a peak normally fills most of this post-strip delay, and therefore no tests

## System Performance

One of the most desirable criteria of an analytical technique is linearity. A wide range of linearity allows versatility in choice of concentrations for standards and a wide range of sample concentrations without the need for sample pre-treatment such as dilution or pre-concentration. In this system, it is desired to analyze a sample such that its TE volume is convenient and delivers a mass to the column that is within the range of the standards.

For all three metals tested, the normal concentration range of standards for calibration is from 0.04 mg/L to 1 mg/L (3.62 ng to 90 ng with an 87 μL sample loop). The linearity for injected standards extends to the detection limit on the low end. To find the upper limit of linearity, calibration curves were generated from 0.04 mg/L (0.1 mg/L for Cu) to 10 mg/L of each metal. The absorbances measured for 4 and 10 mg/L were compared to the values expected from linear extrapolation of the absorbances for 1 mg/L and below. The data and "expectation values" are listed in Table XVI. With the exception of the lowest concentration standard, precision is generally better than 10% RSD.

The data from Table XVI are graphically depicted in Figures 25-27 where the plotted calibration curves are from the blank corrected peak heights of the standards up to 1 mg/L. Plots for area looked essentially identical. The calibration curves were calculated by linear regression programs on either a hand held calculator or an IMSAI 8080 microcomputer system. The plots were made with a Houston digital plotter and the IMSAI.

Table XVI: Data for Standards [a]

| Metal | Conc (mg/L) | n[b] | Peak Height ave | Peak Height SD | Peak Height RSD | Area ave | Area SD | Area RSD | Peak Height net[c] | Peak Height $\bar{Y}$[d] | Area net[c] | Area $\bar{Y}$[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cu | 10 | 2 | 190.3 | 0.1 | 1 | 7913 | 40 | 1 | 189 | 207 | 7892 | 8624 |
| | 4 | 3 | 78.4 | 1.6 | 2 | 3330 | 89 | 3 | 77.3 | 83 | 3309 | 3510 |
| | 1 | 2 | 22.8 | 0.2 | 1 | 967 | 15 | 2 | 21.7 | - | 946 | - |
| | 0.3 | 3 | 8.6 | 0.3 | 3 | 411 | 7 | 2 | 7.5 | - | 390 | - |
| | 0.1 | 3 | 4.1 | 0.5 | 12 | 181 | 26 | 14 | 3.0 | - | 160 | - |
| | blk | 2 | 1.1 | 0.1 | 10 | 21 | 29 | 142 | - | - | - | - |
| Mn | 10 | 2 | 335.9 | 6.6 | 2 | 15108 | 146 | 1 | 333 | 361 | 15034 | 15472 |
| | 4 | 3 | 158.5 | 1.9 | 1 | 6900 | 153 | 2 | 156 | 145 | 6826 | 6232 |
| | 1 | 3 | 39.6 | 1.2 | 3 | 1687 | 64 | 4 | 37.2 | - | 1613 | - |
| | 0.3 | 4 | 13.8 | 0.9 | 6 | 594 | 42 | 7 | 11.4 | - | 520 | - |
| | 0.1 | 4 | 8.3 | 0.5 | 6 | 362 | 20 | 5 | 5.9 | - | 288 | - |
| | 0.04 | 3 | 4.0 | 0.6 | 16 | 162 | 69 | 43 | 1.6 | - | 88 | - |
| | blk | 3 | 2.4 | 0.7 | 27 | 74 | 79 | 107 | - | - | - | - |
| Cd | 10 | 3 | 298.2 | 3.1 | 1 | 20091 | 190 | 1 | 296.4 | 335 | 20017 | 21725 |
| | 4 | 3 | 128.5 | 1.8 | 1 | 8595 | 108 | 1 | 126.7 | 135 | 8521 | 8749 |
| | 1 | 3 | 36.1 | 1.2 | 3 | 2320 | 179 | 8 | 34.3 | - | 2246 | - |
| | 0.3 | 3 | 14.7 | 1.3 | 9 | 883 | 102 | 12 | 12.9 | - | 809 | - |
| | 0.1 | 4 | 5.7 | 0.4 | 6 | 360 | 31 | 9 | 3.9 | - | 286 | - |
| | 0.04 | 3 | 3.9 | 0.3 | 7 | 241 | 68 | 28 | 2.1 | - | 167 | - |
| | blk | 4 | 1.8 | 1.0 | 53 | 74 | 67 | 89 | - | - | - | - |

a. Instrumental conditions as in Table VIII.
Units defined in Table XIII
b. Number of Runs
c. Net = Blank corrected average for this and all subsequent tables
d. $\bar{Y}$ = expectation value of Y extrapolated from calibration curve

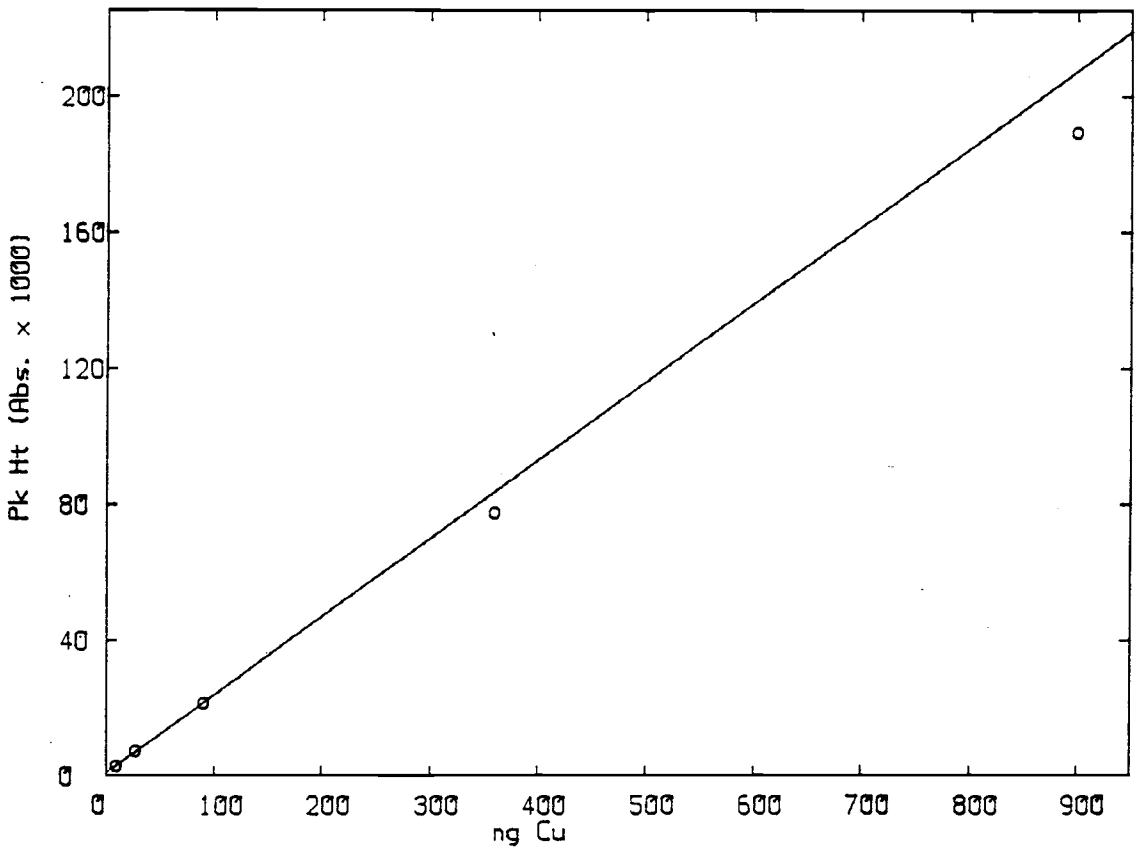



Figure 25. Dependence of peak height on mass of injected Cu. Instrumental conditions as in Table VIII

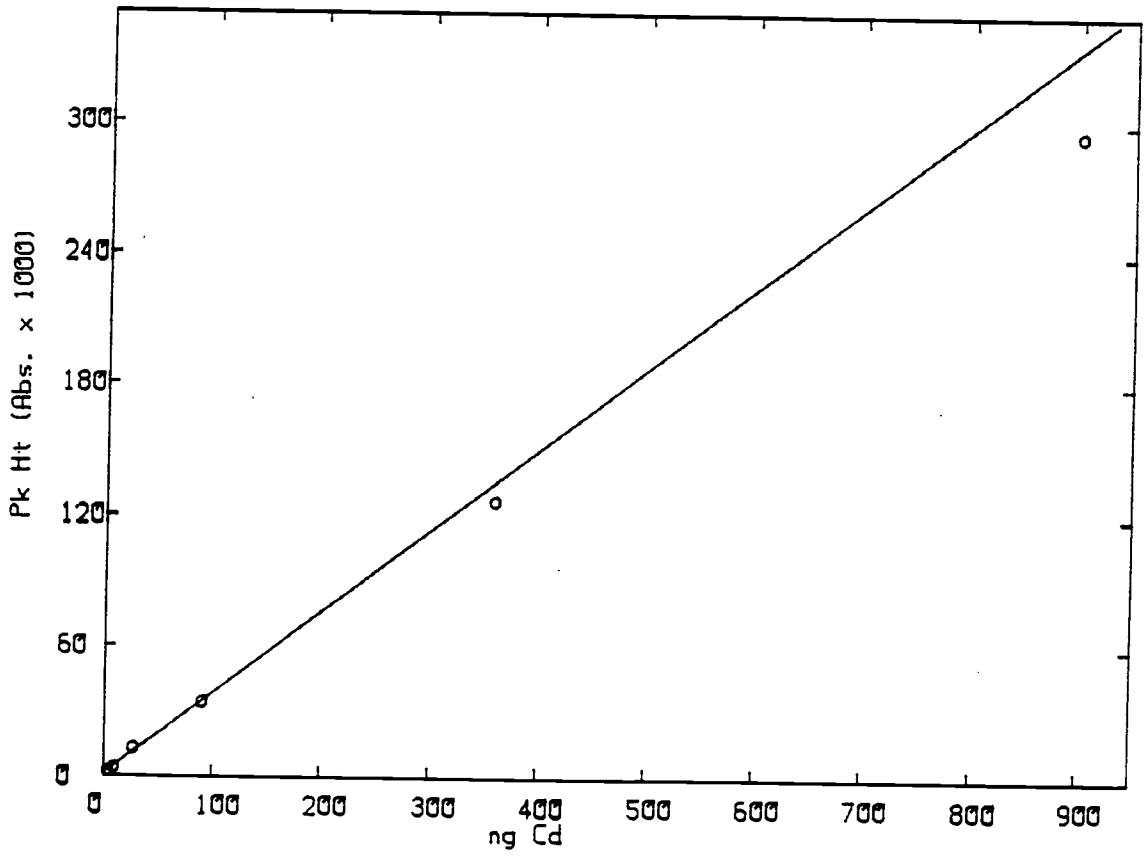



Figure 26. Dependence of peak height on mass of injected Cd. Instrumental conditions as in Table VIII

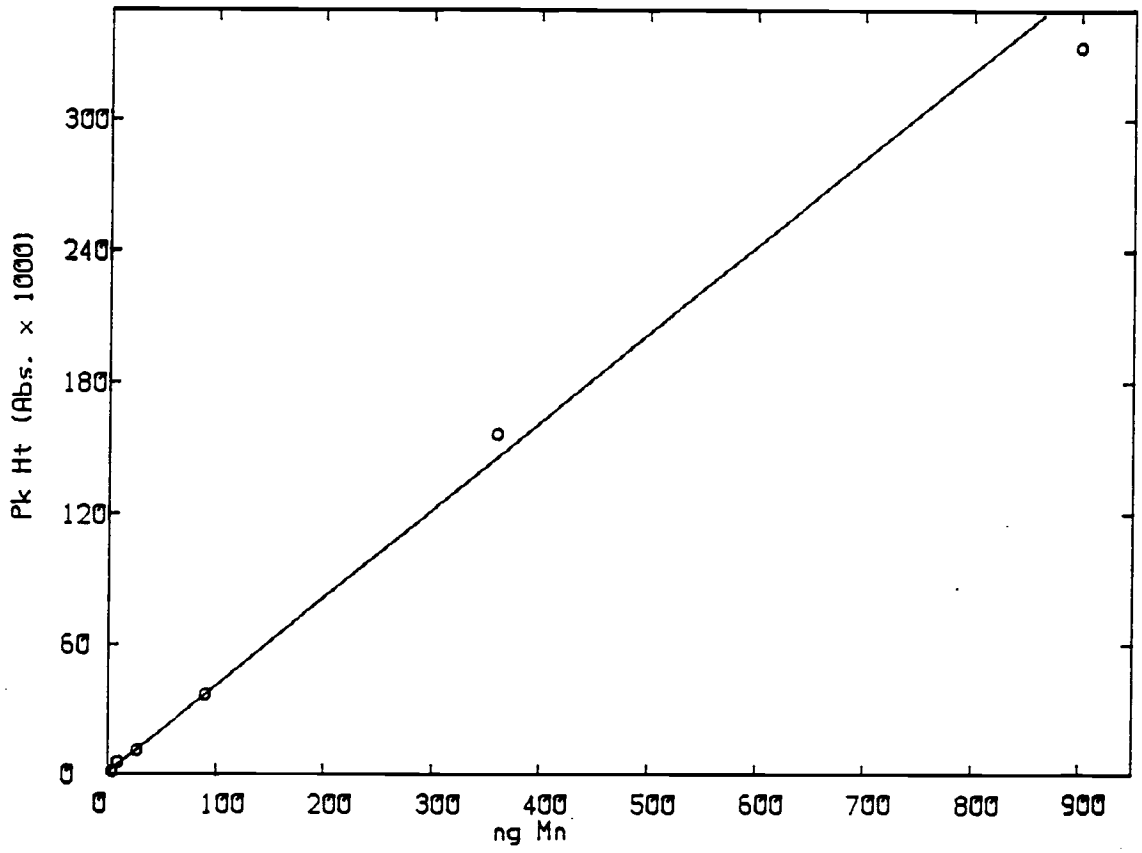



Figure 27. Dependence of peak height on mass of injected Mn. Instrumental conditions as in Table VIII

Inspection of the curves indicates that Mn is linear beyond 4 mg/L, but that Cu and Cd begin to deviate from linearity near that concentration. For Cd, the measured peak height is 94% of the expectation value at 4 mg/L and 88% at 10 mg/L. For Cu, it is 93% at 4 mg/L and 91% at 10 mg/L. The peak height for 10 mg/L Mn is 92% of the expectation value. The decrease in linearity at high concentrations is minimal and possibly due to inherent non-linearity in the AA response versus concentration or due to a kinetic limitation in reaching the exchange sites in the interior of the resin beads. Any sample that is concentrated enough to appear on the upper end of the calibration curve could probably be analyzed directly with flame AA.

The limiting factor in measurement of dilute injected solutions is in distinguishing the eluted peaks from baseline noise. As mentioned in the experimental section, blank peaks are usually so small that only the computer can find them. The standard deviation of such baseline noise is estimated as 20% of the peak-to-peak fluctuations. The peak-to-peak baseline fluctuations for the 3 metals tested and the standard deviations estimated by them are listed in Table XVII along with the values of the blank standard deviations extracted from Table XVI. The standard deviations estimated from the peak to peak fluctuations and those calculated for peak height by the computer agree within a factor of 2 or so which indicates that baseline noise rather than reproducibility in blank peaks is the limiting factor at low concentrations.

Since area is just an integration of these baseline fluctuations, it has the same limitation. However, as solution concentration is reduced, area standard deviations remain essentially constant and

Table XVII: Limiting Noise and Detection Limits by Injection

| | Cu | Cd | Mn |
|---|---|---|---|
| Baseline | | | |
| Peak-to-peak[a] | 1.0 | 2.5 | 1.5 |
| 1/5 peak-to-peak[a] | 0.2 | 0.5 | 0.3 |
| Peak Height | | | |
| $s_{blk}$ [a] | 0.1 | 1.0 | 0.7 |
| Slope [b] | 0.23 | 0.37 | 0.40 |
| Detection Limit [c] | 0.9 | 5 | 4 |
| Area | | | |
| $s_{blk}$ [d] | 79 | 67 | 29 |
| Slope [e] | 9.5 | 24.0 | 17.1 |
| Detection Limit [c] | 6 | 6 | 9 |

a. Absorbance x 1000
b. Absorbance x 1000/ng metal
c. $c_1$ (ng) = 2s/m
d. Arbitrary units
e. Arbitrary units/ng metal

become significant fractions of net area signals sooner than peak height standard deviations do, as reflected in Table XVI by higher RSD's in area than peak height for low concentration solutions. Thus area appears less reliable at low concentrations (below 0.1 mg/L or so) than peak height.

The detection limit is usually defined (120) as:

$$c_l = 2s/m$$

where s is the standard deviation in the blank and m is the slope of the calibration curve. Based on this formula, standard deviations from the data in Table XVI, and slopes generated from them, the detection limits for the 3 metals calculated from peak height and area are tabulated in Table XVIII. Since areas are less reliable than peak heights at low concentrations, the detection limits calculated from peak height are probably more valid.

In addition to the injected standards, TE should also behave in a linear fashion. Here the delivered mass is a function of volume as well as concentration, and combinations of them which deliver equivalent masses should yield the same response. Table XVIII lists the data and Figures 28-30 are plots of peak heights for various concentrations of each metal at several volumes which give masses in the range of the calibration curve. For each metal, the corresponding calibration curve is superimposed over the TE points.

The absorbance of the TE blank is dependent on the TE volume, but for volumes of 10 mL or less, the values are similar to the injected blank. Cd has the noisiest baseline, and so its blank standard deviation is the highest, whereas Cu has the smoothest baseline and a

Table XVIII: TE Correlation to Calibration Curves

| Metal | | Ref. Sym.[a] | Conc[b] | Vol[c] | n[d] | Peak Height ave | Peak Height SD | Peak Height RSD | Area ave | Area SD | Area RSD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cu | Stds | 1 | 0.04 | | 2 | 2.4 | 0.3 | 12 | 90 | 30 | 33 |
| | | 2 | 0.1 | | 3 | 5.2 | 0.7 | 13 | 170 | 25 | 15 |
| | | 3 | 0.3 | | 2 | 12.1 | 0.5 | 4 | 469 | 33 | 7 |
| | | 4 | 1.0 | | 3 | 33.2 | 0.2 | 1 | 1207 | 20 | 2 |
| | | - | blk | | 4 | 1.3 | 0.3 | 24 | 34 | 15 | 43 |
| | TE | F | 3.0 | 10 | 3 | 10.4 | 0.5 | 5 | 364 | 9 | 2 |
| | | D | 3.0 | 5 | 2 | 5.8 | 0.2 | 4 | 190 | 7 | 4 |
| | | B | 3.0 | 3 | 3 | 3.7 | 0.5 | 13 | 121 | 40 | 33 |
| | | A | 3.0 | 1 | 2 | 1.4 | 0.1 | 7 | 17 | 11 | 64 |
| | | G | 10.0 | 5 | 2 | 16.9 | 0.3 | 2 | 563 | 19 | 3 |
| | | E | 10.0 | 3 | 2 | 10.8 | 0.4 | 3 | 350 | 7 | 2 |
| | | C | 10.0 | 1 | 2 | 3.9 | 0.5 | 12 | 142 | 33 | 24 |
| | | - | blk | 10 | 4 | 1.0 | 0.2 | 22 | 25 | 27 | 108 |
| Cd | Stds | 1 | 0.04 | | 3 | 4.5 | 0.7 | 15 | 212 | 13 | 6 |
| | | 2 | 0.1 | | 3 | 7.6 | 0.2 | 2 | 345 | 47 | 14 |
| | | 3 | 0.2 | | 3 | 10.4 | 0.4 | 4 | 551 | 38 | 7 |
| | | 4 | 0.3 | | 3 | 17.6 | 0.9 | 5 | 949 | 99 | 10 |
| | | - | blk | | 5 | 1.0 | 0.5 | 51 | -61 | 66 | 108 |
| | TE | E | 1.5 | 10 | 3 | 12.0 | 1.4 | 12 | 510 | 25 | 5 |
| | | C | 1.5 | 5 | 2 | 5.5 | 0.5 | 10 | 297 | 72 | 24 |
| | | B | 1.5 | 3 | 2 | 2.7 | 0.4 | 15 | 87 | 30 | 35 |
| | | F | 3.0 | 5 | 2 | 9.6 | 1.0 | 10 | 446 | 15 | 3 |
| | | A | 3.0 | 1 | 2 | 2.3 | 0.3 | 12 | 76 | 11 | 14 |
| | | H | 10.0 | 5 | 3 | 31.9 | 2.2 | 7 | 1589 | 175 | 11 |
| | | G | 10.0 | 3 | 2 | 17.8 | 0.8 | 4 | 894 | 122 | 14 |
| | | D | 10.0 | 1 | 2 | 7.0 | 0.2 | 2 | 361 | 32 | 9 |
| | | - | blk | 10 | 2 | 0.7 | 0.5 | 63 | -58 | 29 | 49 |

Table XVIII: TE Correlation to Calibration Curves (cont'd.)

| Metal | | Ref. Sym.[a] | Conc[b] | Vol[c] | n[d] | Peak Height ave | Peak Height SD | Peak Height RSD | Area ave | Area SD | Area RSD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mn | Stds | 1 | 0.04 | | 3 | 2.8 | 1.0 | 37 | 108 | 66 | 61 |
| | | 2 | 0.1 | | 5 | 3.8 | 0.6 | 15 | 137 | 22 | 16 |
| | | 3 | 0.3 | | 3 | 12.1 | 0.5 | 4 | 502 | 23 | 5 |
| | | 4 | 1.0 | | 3 | 30.7 | 0.8 | 3 | 1220 | 22 | 2 |
| | | - | blk | | 2 | 1.0 | 0.3 | 30 | 2 | 1 | 56 |
| | TE | G | 1.5 | 10 | 4 | 7.0 | 0.5 | 7 | 245 | 55 | 23 |
| | | C | 1.5 | 5 | 3 | 3.5 | 0.3 | 9 | 106 | 40 | 38 |
| | | B | 1.5 | 3 | 2 | 2.6 | 0.1 | 4 | 95 | 33 | 34 |
| | | H | 3.0 | 10 | 3 | 13.3 | 0.5 | 4 | 459 | 19 | 4 |
| | | F | 3.0 | 5 | 3 | 7.9 | 1.0 | 12 | 276 | 42 | 15 |
| | | D | 3.0 | 3 | 3 | 4.3 | 0.3 | 8 | 177 | 35 | 20 |
| | | A | 3.0 | 1 | 2 | 2.3 | 0.6 | 25 | 74 | 17 | 24 |
| | | J | 10.0 | 5 | 2 | 20.4 | 0.3 | 2 | 738 | 32 | 4 |
| | | I | 10.0 | 3 | 3 | 12.2 | 0.8 | 7 | 465 | 23 | 5 |
| | | E | 10.0 | 1 | 2 | 3.6 | 0.5 | 13 | 128 | 55 | 43 |

a. See Figure 28 for Cu, Figure 29 for Cd and Figure 30 for Mn.

b. Concentration in mg/L for standards, µg/L for TE solutions; 0.01 M $HNO_3$ blank for TE solutions, dw blanks for standards.

c. Volume in mL for TE; 87.5 µL sample loop for all standards.

d. Number of runs

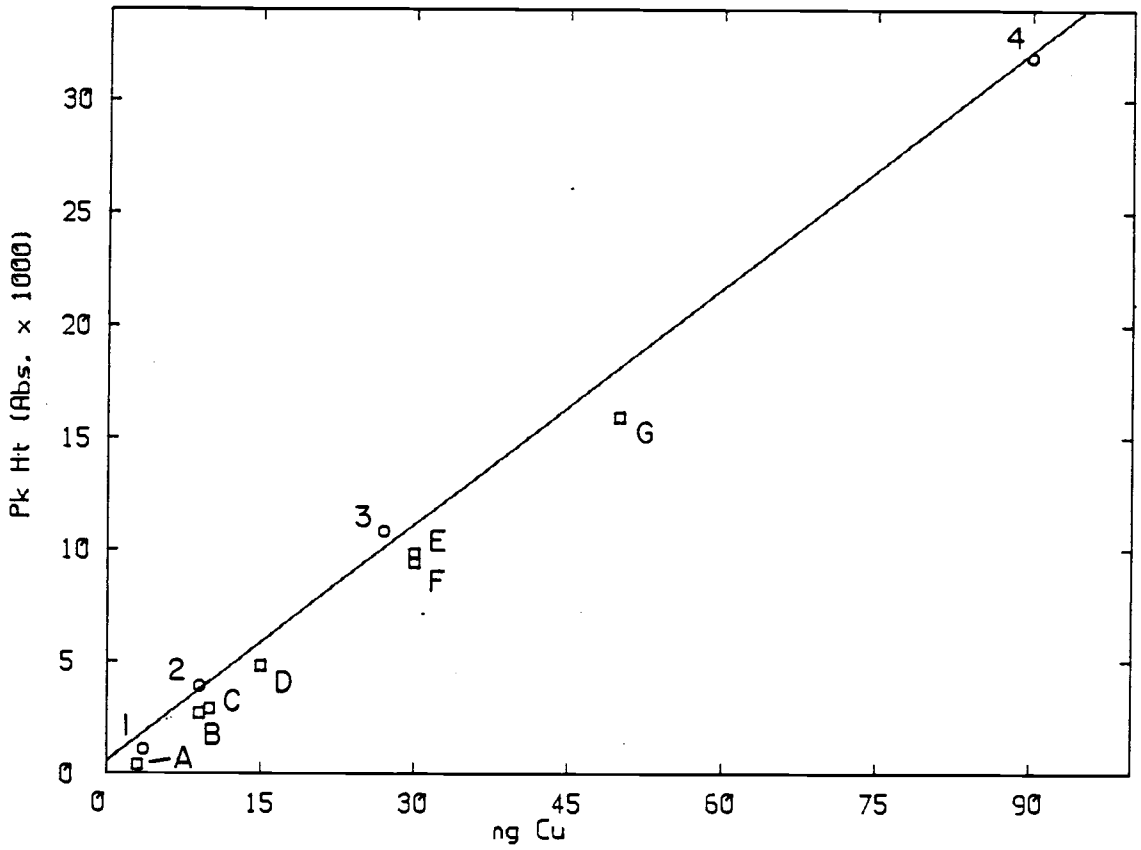



Figure 28. Comparison of response of injected and trace enriched Cu. Instrumental conditions as in Table VIII

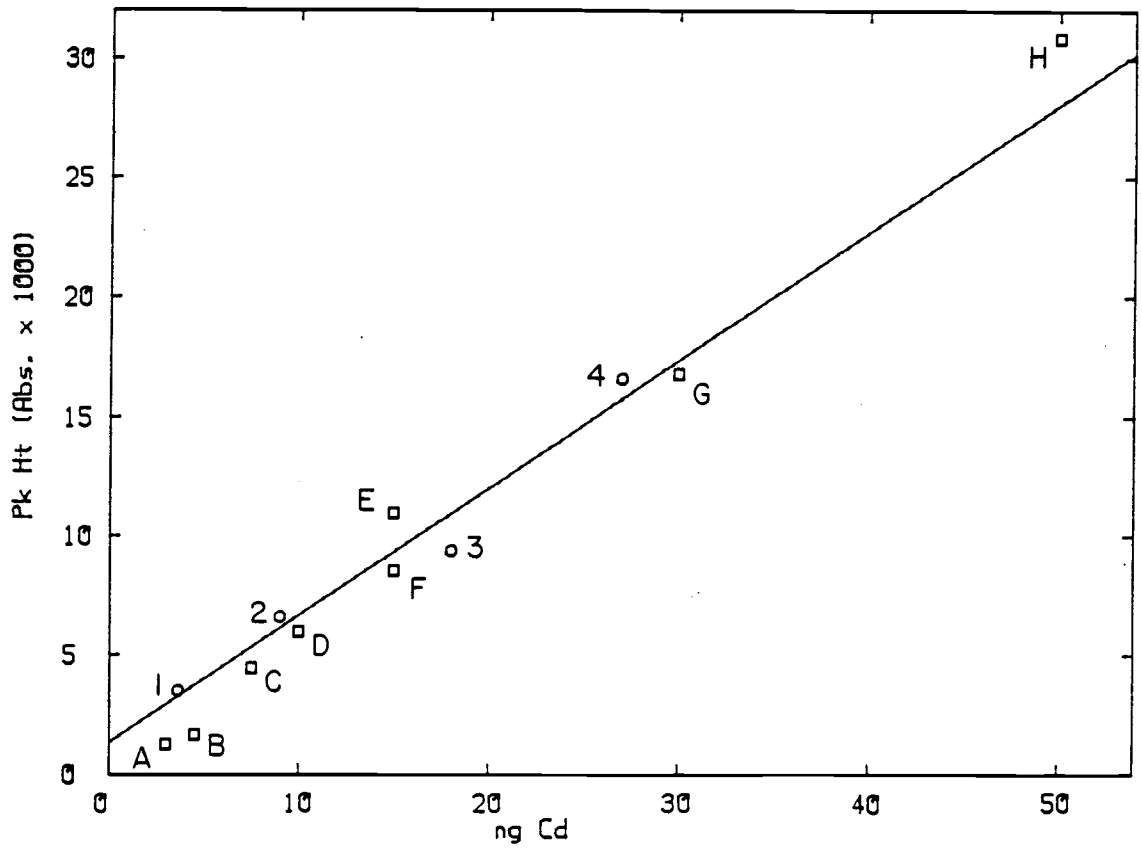



Figure 29. Comparison of response of injected and trace enriched Cd. Instrumental conditions as in Table VIII

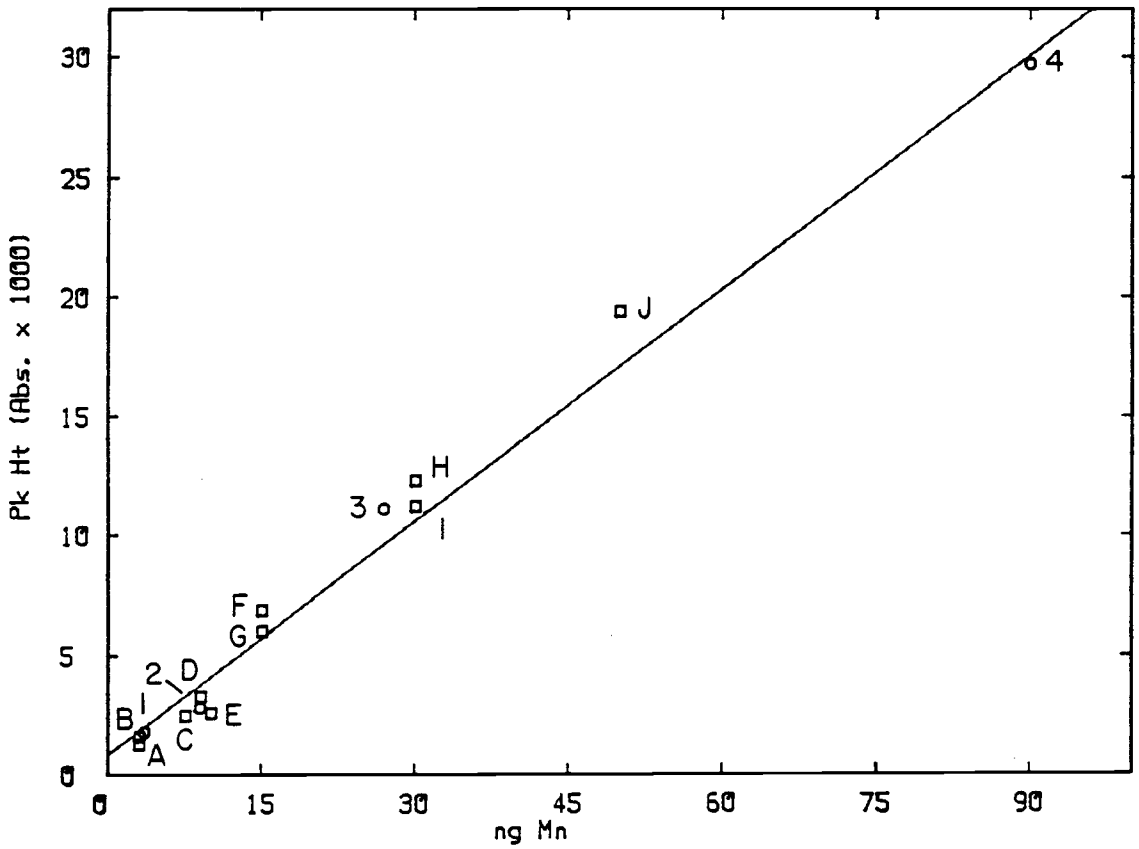



Figure 30. Comparison of response of injected and trace enriched Mn. Instrumental conditions as in Table VIII

lower calibration slope relative to the other test metals and so its blank and blank standard deviation are the lowest.

As shown in Table XVIII and Figure 30, the TE sample concentration of Mn varied from 1.5 to 10 µg/L and volume was varied from 1 to 10 mL. TE is linear and correlates well with the calibration curve from 3 to 50 ng. However, the small deviations of TE signals from the calibration curve become a more significant fraction of the total at masses below 10 ng, and the ratio of measured signal to expectation value of the signal drops to about 70% at masses below 5 ng. For this reason, the mass delivered by TE should be greater than 10 ng in order to get accurate calibration from the standards.

In the case of Cu, Figure 28 indicates that TE is linear but the signals are consistently below the calibration curve. If the calibration curve is shifted so that it goes through the origin, then the TE points fall closer to it and the same limitations on calibration accuracy below 10 ng will apply as for Mn. The blank that was used for the standards was distilled water, and the non-zero intercept of the calibration curve could be due to inadequate blank correction.

The TE of Cd shows the most deviation from linearity as Figure 29 indicates, and the deviation becomes significant again below 10 ng. For instance, the ratio of measured to expectation response values is 91% at 10 ng (point D), 85% at 7.5 ng (point C), 46% at 4.5 ng (point B) and 45% at 3 ng (point A).

Detection limit in the injection mode is definitive and has a lower concentration limit which is dependent on the ability to differentiate a peak from baseline noise. However, a TE detection limit is a more open ended topic since presumably TE volume could be

increased to whatever is necessary for a sample in order to trace enrich a mass that is on the calibration curve. In practice, there is probably a threshold concentration below which the resin will not retain the metal. Since the search for this lower concentration of acceptability would involve extensive difficulty in ultra-trace concentration solution handling to prevent contamination, the system performance of low concentration level TE was instead tested with metal concentrations below any anticipated in real samples. Table XIX shows that good recoveries were made, especially for peak height, with precision of better than 10% RSD for most metal solutions.

As seen in Table XVIII, precision was good for repetitive measurements of a given solution and in general all TE samples had precision better than 10% RSD. In addition to this precision for repetitive measurements, responses for the standards were generally reproducible from day to day, as exemplified by the following sequence of responses for 0.3 mg/L Mn:

| Date | Net Pk Ht | Net Area |
|---|---|---|
| 8/10/82 | 10.9 | 481 |
| 8/11/82 | 11.1 | 500 |
| 8/13/82 | 8.6 | 358 |
| 9/8/82 | 11.4 | 520 |

In short, then, the major limitations for an analysis by this system are to keep the mass for TE greater than 10 ng, and to keep the volume of TE greater than 1 mL. As mentioned in the Results and Discussion section under the calibration procedure discussion, a calibration curve takes about 20 minutes. The sample throughput time

Table XIX: TE Detection Limit Studies

| Metal | Conc | TE Vol | Peak Height ave | Peak Height SD | Peak Height RSD | Area ave | Area SD | Area RSD | Pk ht Yield[a] | Area Yield[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cu | 0.3 µg/L | 50 mL | 7.5 | 0.3 | 4 | 317 | 37 | 12 | 97 | 110 |
| | blk | 50 mL | 4.1 | 0.6 | 15 | 167 | 37 | 22 | | |
| Cd | 0.3 µg/L | 50 mL | 12.4 | 1.3 | 10 | 536 | 104 | 20 | 102 | 74 |
| | blk | 50 mL | 3.0 | 0.4 | 13 | 96 | 64 | 67 | | |
| Mn | 0.3 µg/L | 50 mL | 6.3 | 0.5 | 8 | 235 | 22 | 9 | 102 | 110 |
| | blk | 50 mL | 1.4 | 0.2 | 13 | 20 | 38 | 188 | | |

a. $\frac{\text{measured response x 100\%}}{\text{calibration curve response for same mass}}$

depends on the TE volume necessary (i.e. how dilute it is), but a sample with concentration greater than 1 μg/L requires a 10 mL or less TE volume and takes about 15 minutes to analyze with three measurements.

## Interference Study

Probably the greatest test of a proposed analytical technique or instrument is its ability to reliably determine the analyte concentration in the presence of co-existing species. The test is often made even more challenging when the analyte must be determined in a sample where the co-existing species are present in much greater abundance than the analyte.

There are at least two opportunities for a species to interfere with the determination of an analyte in this system. The species may prevent the analyte from being fully retained by the column or it may interfere with the measurement of the analyte during the stripping step.

Lack of retention can occur if a species binds to or alters the analyte metal so as to render it unable to adhere to the resin exchange sites (e.g. by complexing it), or if it competes with the metal for the sites themselves and causes the metal to pass unretained through the column during the enrichment step. To prevent the former, the column has to have a strong enough affinity for the metal to sequester it from any form it exists in upon entering the column. To prevent the latter, either the column affinity has to be preferentially stronger for the metal than the interferent, or the column must have enough available

exchange sites to accommodate both the analyte and the interferent. If it can do neither of these for samples with interferent concentrations found in real samples, then a pre-analysis cleanup must be performed. Consideration of Table IX indicates that ion selectivity by the column is not highly favorable for the three test metals compared to other species in the Table and so column capacity must also play a part in preventing significant interferences from moderate concentrations of common interferent species which co-compete for column exchange sites.

The other opportunity for an interferent to impede accurate determination is by co-eluting with the analyte and interfering with its detection during the strip step. Element selective detection such as the AA used here helps minimize this potential problem so that co-eluting species with identical retention times as the analyte are not detected. This is a primary advantage of this system over non-specific detection used in other methods (88, 89, 98, 99).

The choice of species for the interference study was based on their occurrence in real samples. Interferent species chosen for this study are the main constituents of most natural water samples, the application to which this research is primarily directed. Also species were chosen which are most likely to pose potential interference problems, either due to their chemical similarity to the analyte (such as other transition metals) or their excessive abundance relative to the analyte in typical samples.

The approach to this study was to make direct comparisons of analyte solutions containing the interferents to unadulterated analyte solutions. This was accomplished with four solutions for each experiment.

1. The interferent solution was made with interferent concentrations higher than those expected in most real water samples. Usually several interferent species were tested simultaneously, especially in chemically similar groups such as the transition metals. The solution matrix was made approximately pH 2 with $HNO_3$ as would typically be the case for a water sample from its acid preservation treatment. Then an amount of analyte metal was added to the solution such that the added concentration of analyte was on the low end of the expected real sample concentration range.

2. An interference blank solution was identical to the interferent solution matrix, including the pH adjustment, except that no analyte was added.

3. A reference analyte solution was composed of the analyte at the same concentration as that added to the interferent solution and it was also adjusted to pH 2.

4. A reference blank solution was simply a pH 2 $HNO_3$ solution. The pH adjustment was made for each solution by addition of 1 mL concentrated $HNO_3$ per L solution. The theoretical pH of this dilution is 1.80 and the measured pH was about 1.9.

It is important to distinguish between the added analyte concentration and the total analyte concentration in the interferent solution. Some of the chemicals used to make the interferent stock solutions have traces of analyte in them present as low level contamination. However, due to the high concentration of interferents relative to the analyte, the amount of analyte present as contamination may be similar to that added to the solution. The interferent blank should account for the difference, but since it is desired to test the

interference effects at low analyte concentrations, the added analyte concentration should be 2-3 times that present as contamination.

When significant interference effects were noted for a tested interferent, its concentration was lowered while maintaining the same added analyte concentration and the experiment was repeated. This process was continued until the interferent concentration was such that it no longer posed an interference problem. If the initially chosen interferent level produced no interference and its level was already higher than that expected in any normal sample, then the study of that interferent was considered complete.

The data for the various interferent concentrations are given in Tables XX, XXI, and XXII for Cu, Cd, and Mn, respectively. For the one or more interferent solutions and the reference and corresponding blank solutions of each experiment, the average, standard deviation and RSD for peak height and area are given. The net values are the averages minus their corresponding blank values and the yield is the ratio of the net interferent value over the net reference value. The highest levels for non-interference (which is when peak height yields are within 10% or so of 100%) are summarized in Table XXIII, along with typical freshwater levels of each species.

When humic acid was initially tested with Cu, it was not filtered before use. Quantitative yields were obtained for a 1 mg/L humic acid solution, but at 5 or 10 mg/L, the yield was reduced to 50% and system back pressure built up enough to cause the valves to leak. (The pressure transducer on the HPLC pump was malfunctioning at the time so it was not possible to see on the gauge what the pressure was.) Subsequent disassembly of the column revealed that the top of the resin

Table XX: Interference Study for Cu

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield[d] pk ht | Yield[d] area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1000 mg/L Na K<br>100 mg/L<br>Mg Ca | Inter | 4 | 16.8 | 0.1 | 1 | 814 | 13 | 2 | 6.0 | 287 | 113 | 115 |
| | | Ref | 4 | 7.2 | 0.4 | 5 | 312 | 23 | 7 | 5.3 | 249 | | |
| | Fe Cr | Inter blk[b] | 3 | 10.8 | 0.5 | 5 | 527 | 13 | 3 | | | | |
| | | Ref blk[c] | 4 | 1.9 | 0.4 | 20 | 63 | 14 | 23 | | | | |
| 2 | 1000 mg/L<br>$SO_4^{-2}$<br>$PO_4^{-3}$ | Inter | 4 | 8.9 | 0.4 | 5 | 381 | 28 | 7 | 5.5 | 228 | 90 | 91 |
| | | Ref | 4 | 8.6 | 0.4 | 5 | 345 | 25 | 7 | 6.1 | 251 | | |
| | | Inter blk | 4 | 3.4 | 0.2 | 7 | 153 | 14 | 9 | | | | |
| | | Ref blk | 3 | 2.5 | .3 | 12 | 94 | 10 | 11 | | | | |
| 3 | 10 mg/L<br>Humic Acid | Inter | 3 | 10.5 | 0.2 | 2 | 392 | 26 | 7 | 8.5 | 318 | 104 | 106 |
| | | Ref | 4 | 10.0 | 0.5 | 5 | 370 | 30 | 8 | 8.2 | 299 | | |
| | | Inter blk | 4 | 2.1 | 0.5 | 23 | 74 | 21 | 28 | | | | |
| | | Ref blk | 2 | 1.8 | 0 | 2 | 71 | 22 | 31 | | | | |
| 4 | 1 mg/L<br>Ni<br>Mn Zn<br>Cd Pb | Inter | 3 | 18.8 | 0.8 | 4 | 707 | 9 | 1 | 14.7 | 544 | 113 | 107 |
| | | Ref | 3 | 16.2 | 0.2 | 1 | 643 | 8 | 1 | 13.0 | 506 | | |
| | | Inter blk | 3 | 4.1 | 0.5 | 11 | 163 | 17 | 10 | | | | |
| | | Ref blk | 3 | 3.2 | 0.3 | 10 | 137 | 5 | 4 | | | | |

Table XX: Interference Study for Cu (cont'd)

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield[d] pk ht | Yield[d] area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | (1)-1 M NaCl | Inter (1) | 2 | not detected | | | not detected | | | - | - | 0 | 0 |
| | (2) 0.5 M NaCl | Inter (2) | 3 | 5.5 | 0.6 | 10 | 257 | 37 | 14 | 2.5 | 128 | 63 | 80 |
| | | Ref | 5 | 5.9 | 0.9 | 13 | 212 | 41 | 19 | 4.0 | 161 | | |
| | | Inter blk | 4 | 3.0 | 0.4 | 13 | 129 | 29 | 23 | | | | |
| | | Ref blk | 3 | 1.9 | 0.3 | 13 | 51 | 7 | 13 | | | | |

a. All interferent and reference solutions are 3 µg/L Cu: TE volume = 10 mL for all solutions
b. Interferent blank is same as interferent solutions except without added Cu
c. Reference blank is $10^{-2}$M $HNO_3$
d. Yield = $\frac{\text{net interferent signal}}{\text{net reference signal}}$ x 100%

Table XXI: Interference Study for Cd

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield pk ht | Yield area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1000 mg/L Na K<br>100 mg/L<br>Mg Ca<br>Fe Cr | Inter | 3 | 29.2 | 1.3 | 4 | 2485 | 77 | 3 | 15.3 | 1368 | 46 | 118 |
| | | Ref | 3 | 39.1 | 0.2 | 1 | 1356 | 32 | 2 | 33.4 | 1159 | | |
| | | Inter blk | 3 | 13.9 | 0.2 | 2 | 1118 | 109 | 10 | | | | |
| | | Ref blk | 3 | 5.7 | 1.5 | 26 | 197 | 62 | 32 | | | | |
| 2 | 100 mg/L Na K<br>10 mg/L<br>Mg Ca<br>Fe Cr | Inter | 3 | 37.0 | 0.6 | 2 | 1593 | 23 | 1 | 33.5 | 1438 | 84 | 106 |
| | | Ref | 3 | 45.3 | 0.3 | 1 | 1484 | 51 | 3 | 40.1 | 1355 | | |
| | | Inter blk | 3 | 3.5 | 0.2 | 7 | 155 | 18 | 12 | | | | |
| | | Ref blk | 3 | 5.2 | 0.7 | 14 | 129 | 39 | 30 | | | | |
| 3 | 1000 mg/L Na K<br>100 mg/L Ca Mg | Inter | 4 | 32.7 | 1 | 3 | 2879 | 137 | 5 | 14.4 | 913 | 41 | 81 |
| | | Ref | 3 | 38.5 | 0.8 | 2 | 1214 | 103 | 8 | 34.7 | 1128 | | |
| | | Inter blk | 3 | 18.3 | 1.5 | 8 | 1966 | 76 | 4 | | | | |
| | | Ref blk | 4 | 3.8 | 1.9 | 48 | 86 | 94 | 109 | | | | |
| 4 | 100 mg/L Na K<br>10 mg/L Ca Mg | Inter | 4 | 24.8 | 1.7 | 7 | 841 | 68 | 8 | 22.0 | 737 | 108 | 118 |
| | | Ref | 3 | 22.5 | 0.2 | 1 | 672 | 38 | 6 | 20.2 | 627 | | |
| | | Inter blk | 3 | 2.8 | 1.0 | 35 | 104 | 59 | 57 | | | | |
| | | Ref blk | 6 | 2.3 | 1.0 | 45 | 45 | 66 | 148 | | | | |
| 5 | 1 mg/L Cr | Inter | 4 | 25.4 | 1.1 | 4 | 758 | 51 | 7 | 23.0 | 712 | 102 | 99 |
| | | Ref | 4 | 25.6 | 1.4 | 6 | 777 | 35 | 5 | 22.6 | 720 | | |
| | | Inter blk | 4 | 2.4 | 0.6 | 24 | 46 | 27 | 59 | | | | |
| | | Ref blk | 3 | 3.0 | 0.8 | 28 | 57 | 26 | 46 | | | | |

Table XXI: Interference Study for Cd (cont'd)

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield pk ht | Yield area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 10 mg/L Fe | Inter | 4 | 25.9 | 1.5 | 6 | 806 | 91 | 11 | 23.4 | 767 | 102 | 111 |
| | | Ref | 3 | 26.5 | 0.8 | 3 | 819 | 38 | 5 | 23.0 | 687 | | |
| | | Inter blk | 3 | 2.5 | 0.7 | 27 | 39 | 41 | 105 | | | | |
| | | Ref blk | 4 | 3.5 | 0.8 | 22 | 132 | 57 | 43 | | | | |
| 7 | 1 mg/L Ni Zn Mn Pb Cu | Inter | 3 | 33.8 | 0.9 | 3 | 977 | 7 | 1 | 29.0 | 827 | 110 | 101 |
| | | Ref | 4 | 29.2 | 1.6 | 5 | 884 | 55 | 6 | 26.3 | 822 | | |
| | | Inter blk | 3 | 4.8 | 0.3 | 6 | 150 | 22 | 14 | | | | |
| | | Ref blk | 4 | 2.9 | 1.0 | 34 | 62 | 35 | 57 | | | | |
| 8 | 1000 mg/L $SO_4^{-2}$ $PO_4^{-3}$ | Inter | 3 | 26.0 | 0.2 | 1 | 1001 | 73 | 7 | 19.8 | 656 | 106 | 107 |
| | | Ref | 4 | 21.1 | 1.5 | 7 | 657 | 71 | 11 | 18.7 | 615 | | |
| | | Inter blk | 4 | 6.2 | 1.1 | 17 | 345 | 56 | 16 | | | | |
| | | Ref blk: | 6 | 2.4 | 0.8 | 35 | 42 | 75 | 180 | | | | |
| 9 | 10 mg/L Humic Acid | Inter | 4 | 16.5 | 0.9 | 5 | 582 | 42 | 7 | 13.9 | 539 | 101 | 104 |
| | | Ref | 4 | 15.5 | 1.2 | 7 | 528 | 42 | 8 | 13.7 | 518 | | |
| | | Inter blk | 4 | 2.6 | 0.8 | 32 | 43 | 49 | 114 | | | | |
| | | Ref blk | 4 | 1.8 | 0.9 | 49 | 10 | 68 | 687 | | | | |

a. All interferent and reference solutions are 3 µg/L Cd; TE vol = 10 mL for all solutions. All other definitions are the same as for Table XX

Table XXII: Interference Study for Mn

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield pk ht | Yield area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 (1) | 1000 mg/L Na K 100 mg/L Ca Mg | Inter (1) | 2 | 10.3 | 0.5 | 4 | 1087 | 8 | 1 | 2.5 | 478 | 24 | 117 |
| (2) | 100 mg/L Na K 10 mg/L Ca Mg | Inter (2) | 4 | 10.4 | 0.2 | 2 | 541 | 53 | 10 | 9.1 | 512 | 86 | 125 |
| | | Ref | 3 | 11.9 | 1.1 | 9 | 418 | 19 | 5 | 10.6 | 409 | | |
| | | Inter blk(1) | 2 | 7.8 | 0.3 | 4 | 609 | 53 | 9 | | | | |
| | | Inter blk(2) | 4 | 1.3 | 0.4 | 34 | 29 | 33 | 113 | | | | |
| | | Ref blk | 3 | 1.3 | 0.8 | 62 | 9 | 33 | 381 | | | | |
| 2 | 1 mg/L Cr | Inter | 3 | 17.3 | 0.4 | 2 | 627 | 26 | 4 | 15.3 | 542 | 103 | 100 |
| | | Ref | 3 | 16.5 | 0.8 | 5 | 565 | 43 | 8 | 14.8 | 539 | | |
| | | Inter blk | 3 | 2.0 | 0.5 | 26 | 85 | 56 | 66 | | | | |
| | | Ref blk | 3 | 1.7 | 0.3 | 17 | 26 | 26 | 100 | | | | |
| 3 | 10 mg/L Fe | Inter | 4 | 76.6 | 1.7 | 2 | 3118 | 116 | 4 | 13.9 | 524 | 118 | 97 |
| | | Ref | 4 | 13.0 | 0.9 | 7 | 557 | 87 | 16 | 11.8 | 540 | | |
| | | Inter blk | 2 | 62.7 | 0.3 | 0 | 2594 | 84 | 3 | | | | |
| | | Ref blk | 3 | 1.2 | 0.3 | 23 | 17 | 19 | 112 | | | | |
| 4 | 2 mg/L Fe | Inter | 3 | 38.8 | 0.8 | 2 | 1345 | 42 | 3 | 18.3 | 591 | 108 | 96 |
| | | Ref | 3 | 18.7 | 0.7 | 4 | 688 | 25 | 4 | 16.9 | 616 | | |
| | | Inter blk | 3 | 20.5 | 1.1 | 6 | 754 | 46 | 6 | | | | |
| | | Ref blk | 2 | 1.8 | 0.3 | 14 | 72 | 36 | 50 | | | | |

Table XXII: Interference Study for Mn (cont'd)

| Exp. No. | Composition of Interferent Solution | Solution[a] | # of runs | Pk ht ave | Pk ht SD | Pk ht RSD | Area ave | Area SD | Area RSD | Net pk ht | Net area | Yield pk ht | Yield area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 mg/L CU Ni Zn Pb Cd | Inter | 3 | 20.2 | 0.9 | 5 | 711 | 31 | 4 | 18.9 | 684 | 101 | 100 |
| | | Ref | 2 | 19.8 | 1.5 | 8 | 656 | 121 | 18 | 18.7 | 683 | | |
| | | Inter blk | 3 | 1.3 | 0.3 | 25 | 27 | 23 | 84 | | | | |
| | | Ref blk | 2 | 1.1 | 0.3 | 25 | -27 | 2 | 9 | | | | |
| 6 | 1000 mg/L $SO_4^{-2}$ $PO_4^{-3}$ | Inter | 4 | 21.0 | 1.4 | 7 | 848 | 141 | 17 | 17.1 | 660 | 92 | 89 |
| | | Ref | 4 | 19.6 | 1.6 | 8 | 704 | 51 | 7 | 18.6 | 738 | | |
| | | Inter blk | 4 | 3.9 | 0.8 | 20 | 188 | 25 | 13 | | | | |
| | | Ref blk | 3 | 1.0 | 0.3 | 32 | -34 | 73 | 24 | | | | |
| 7 | 10 mg/L Humic Acid | Inter | 3 | 17.0 | 0.4 | 2 | 646 | 57 | 9 | 16 | 666 | 107 | 115 |
| | | Ref | 4 | 17.0 | 1.1 | 6 | 651 | 46 | 7 | 14.9 | 581 | | |
| | | Inter blk | 3 | 1.0 | 1.0 | 93 | -20 | 91 | 461 | | | | |
| | | Ref blk | 5 | 2.1 | 0.6 | 29 | 70 | 30 | 42 | | | | |

a. All interferent and reference solutions are 3 µg/L Mn.
All other definitions are the same as for Table XX.

Table XXIII: Minimum Non-interference Levels [a]

| Species | Cu | Cd | Mn | Freshwater[b] |
|---|---|---|---|---|
| $PO_4^{-3}$ | 1000 | 1000 | 1000 | 0.02 |
| $SO_4^{-2}$ | 1000 | 1000 | 1000 | 10 |
| $NO_3^-$ | 2700 | 270 | 270 | 0.2 |
| $Cl^-$ | 1550 *[c] | 140 | 140 | 8 |
| Na | 1000 * | 240 * | 240 * | 6 |
| K | 1000 | 100 * | 100 * | 2 |
| Mg | 100 | 10 * | 10 * | 4 |
| Ca | 100 | 10 * | 10 * | 2 |
| Fe | 100 | 10 | 2 | 0.7 |
| Cr | 100 | 1 | 1 | 0.0002 |
| Ni | 1 | 1 | 1 | 0.01 |
| Zn | 1 | 1 | 1 | 0.01 |
| Pb | 1 | 1 | 1 | 0.005 |
| Cu | - | 1 | 1 | 0.01 |
| Cd | 1 | - | 1 | 0.01 |
| Mn | 1 | 1 | - | 0.01 |
| Humic Acid | 10 | 10 | 10 | 2 |

a. Interferent species concentrations are in mg/L. Minimum level for non-interference is concentration with peak height yield equal to or greater than 90%. Yields greater than 100% are likely due to random error or incomplete blank compensation

b. Values from reference 125.

c. * indicates that interference was found at higher levels. Other interferents were not tested at higher levels and so the level where interference occurs may be higher than the listed value.

bed was discolored brown and the filter disks, especially the upstream one, had particulates trapped on them. It appears that there are particles in the humic acid solution which are greater in size than the 5 μm pore size of the filter disks, and these particles irreversibly bind to Cu.

Since the same 10 mg/L humic acid solution with the particles removed delivered a 100% yield of the Cu from this soluble fraction, it might be concluded that approximately 50% of Cu in an unfiltered humic acid sample is bound by particles greater than 5 μm in size. At the sample pH of 2, there would not be expected to be much metal complexation, so the other 50% is at most loosely associated with the humic acid.

The alkali metals and alkaline earths have a potential to interfere not so much by virtue of a more favorable exchange coefficient than the analyte metal (see Table IX) as by simple mass action of their sheer excess in concentration compared to the analyte in many samples. The most environmentally prevalent members of the two groups were chosen, and the tested metals have tolerances to them at much higher than environmental levels, especially Cu. Cd was initially tested for the two groups simultaneously with Fe and Cr, but when low yields were obtained, even with concentrations one tenth those in the Cu experiment, the groups were investigated separately from Fe and Cr. Inspection of blanks in experiments 3 and 4 of Table XXI and experiment 1 of Table XXII indicates that the stock solution of the active metals is somewhat contaminated with Cd and Mn.

In order to see if the tolerance of Cu could be pushed as far as seawater levels, a seawater matrix was simulated with 0.5 M NaCl and

tested with 3 µg/L Cu. A solution preparation calculation error was responsible for the inadvertant testing of 1 M NaCl which resulted in a Cu yield of zero. The 0.5 M NaCl produced some interference, which indicates that the interference tolerance is on the edge for seawater levels of salt. Were seawater analysis of Cu desireable, it might be possible to dilute the sample slightly, by a factor of 2 or so, and get freedom from interference, though seawater analysis was not the major intent of this project and it was not investigated further.

Since Mn is physically and chemically similar to Fe (121), the two often coexist in nature and as a result, chemical sources of Fe are often contaminated with Mn. This posed a problem when it was desired to test Mn interference from Fe and the latter was to be 4 orders of magnitude greater in concentration. It was found that reagent grade $FeCl_3$ at the 10 mg/L level was also 0.03 mg/L in Mn and the added concentration of Mn to the interferent solution is only 0.003 mg/L. $Fe_2(SO_4)_3$ was only half as contaminated, but it had to be diluted to 2 mg/L Fe in order to make a good measurement. Thus Mn is probably more tolerant to Fe than the 2 mg/L level listed in Table XXIII.

Anions have the potential to interfere by binding with the metal and preventing its retention by the column exchange sites. When the anion interference study was initially undertaken, it was intended to include carbonate, but when $NaHCO_3$ was added to the stock solution with $NaHSO_4$ and $H_3PO_4$ already in it (pH 2.0), the solution fizzed extensively, indicating release of $CO_2$ by the process:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow H_2O + CO_2\uparrow$$

This rather dramatically illustrated that at pH 2, a sample has negligibly small amounts of carbonate in a form capable of binding a metal, and it was henceforth excluded from the anion study.

By nature of the reagents used to make the interferent solutions, counterions of the interferent species were automatically included in the interference experiment. A couple of these concomitant interferent species were tested at higher levels than when intentionally tested. The values listed in Table XXIII reflect the highest tested concentration that produced no interference, whether it was during a test for that specific ion or as a concomitant test result. Table XXIV lists these concomitant sources of interferents.

The highest intentional test level of Na for Cd and Mn was 100 mg/L, but during the anion tests, Na was present at 240 mg/L due to the $NaHSO_4$ used to provide $SO_4^{-2}$. Nitrate came with the Na, and the primary sources of $Cl^-$ were the Mg, Ca, and K used in the groups I and II experiment. Thus the sum of $Cl^-$ provided by these three ions is the value of $Cl^-$ in Table XXIII.

In several of the tested solutions, such as the Group I and II metals (see experiments 1 and 3 in Table XXI and experiment 1 in Table XXII), yields for peak height are low while those of area are acceptable. This indicates that interference is not due to failure of the column to retain the metal from the interferent matrix but rather that the shape of the stripping peak is changed relative to the reference solution stripping peak. Thus for samples with very high concentrations of interferents, more accurate values for analyte concentrations might be obtained by using areas rather than peak height.

Table XXIV: Concomitant Sources of Ions

| Ion | Source | Conversion |
|---|---|---|
| $NO_3^-$ | $Cr(NO_3)_3 \cdot 9\ H_2O$ | 1 mg/L Cr = 3.6 mg/L $NO_3^-$ |
| | $NaNO_3$ | 1000 mg/L Na = 2700 mg/L $NO_3^-$ |
| $Cl^-$ | $FeCl_3$ | 10 mg/L Fe = 18.8 mg/L $Cl^-$ |
| | $MgCl_2 \cdot 6\ H_2O$ | 100 mg/L Mg = 300 mg/L $Cl^-$ |
| | $CaCl_2 \cdot 2\ H_2O$ | 100 mg/L Ca = 170 mg/L $Cl^-$ |
| | KCl | 1000 mg/L K = 900 mg/L $Cl^-$ |
| $Na^+$ | $NaHSO_4$ | 1000 mg/L $SO_4$ = 240 mg/L $Na^+$ |

## Real Sample Analysis

The results for analysis of river and tap water for Cu, Cd, and Mn by TE are summarized in Table XXV. Metal concentrations were determined from the injection standards calibration curve and standard addition in some cases. Sample numbers correspond to Table VII in the experimental section.

The pH of several initial acidified river water samples was measured with a pH meter and found to be near pH 2. For the sake of protecting the electrode from being fouled by adsorbed organics (which are abundant in the Willamette River), the pH of the remaining river sample was not measured. It was assumed that the same ratio of 1 mL concentrated $HNO_3$ per L sample for all subsequent sample adjusted the pH to correct values for accurate analysis.

Many of the dissolved organics and particulates in the river sample were greater than 0.45 μm in size, as evidenced by rapid deterioration of filtration rate and considerable discoloration of the filter paper. By the time 500 mL were filtered, the filtration rate was reduced to a couple drops per second. No analysis of the residue was performed. Some metals may be contained in the retained material although at pH 2, the majority of metals should be released from natural organic complexing agents.

The concentration of an analyte in a sample is usually reported as within a range, the magnitude of which depends on the number of measurements, the deviation in them and the desired statistical confidence. The true analyte concentration lies in the range defined (120) by:

Table XXV: Summary of Results of Real Sample Analysis

| Sample number[a] | Metal | Source | TE vol (mL) | n[d] | Conc (µg/L) by cal. curve and pk ht[b] | Conc (µg/L) by cal. curve and area[b] | Conc(µg/L) by std add & pk ht[b] | Conc (µg/L) by std add & area[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | Cu | Tap | Injection | 2 | 1200 | 1000 | - | - |
| 2[c] | Cu | River | 10 | 3 | 2.6 ± 0.8 | 2.0 ± 0.1 | 2.3 | 3.8 |
| 3 | Cd | River #1 | 20 | 2 | 0.49 ± 0.12 | 0.09 ± 0.03 | - | - |
| 4 | Cd | River #2 | 20 | 2 | 0.20 ± 0.35 | 0.02 ± 0.71 | - | - |
| 5 | Cd | River #3 | 20 | 3 | 0.24 ± 0.44 | 0.03 ± 0.11 | - | - |
| 6[c] | Cd | Tap | 30 | 2 | 0.25 ± 0.50 | 0.44 ± 0.37 | 0.23 | 0.23 |
| 7 | Mn | River #1 | 10 | 3 | 10.9 ± 0.2 | 9.3 ± 1.1 | - | - |
| 8[c] | Mn | River #2 | 10 | 3 | 8.9 ± 0.2 | 7.8 ± 0.6 | 7.5 | 7.7 |
| 9 | Mn | River #3 | 10 | 3 | 9.8 ± 0.8 | 8.4 ± 1.0 | - | - |
| 10[c] | Mn | Tap | 10 | 3 | 2.3 ± 0.4 | 1.9 ± 0.3 | 2.3 | 2.1 |

a. Same numbering sequence as in Table VII
b. Using 95% confidence level based on $ts/\sqrt{n}$; see explanation in text
c. Standard addition
   For 2, 100 mL sample spiked with 0.25 mL of 1.0 mg/L Cu to provide 2.5 µg/L increase in concentration
   For 6, 100 mL sample spiked with 0.1 mL of 0.3 mg/L Cd to provide 0.3 µg/L increase in concentration
   For 8, 100 mL sample spiked with 1.0 mL of 1.0 mg/L Mn to provide 10 µg/L increase in concentration
   For 10, 100 mL sample spiked with 0.2 mL of 1.0 mg/L Mn to provide 2 µg/L increase in concentration
d. n = number of runs

$$c \pm \frac{ts}{\sqrt{n}}$$

where c is the mean concentration, n is the number of measurements and s is the standard deviation in c. The value of t is found in a table of student t statistics and a 95% confidence level was used here.

The standard addition technique is a way to compensate for non-spectral interferences which affect the calibration sensitivity. Comparison of the results obtained from a standard calibration curve to those from standard addition indicates the degree to which a technique is subject to these types of interferences.

A formula for standard addition that assumes negligible dilution (120) is:

$$c_x = \frac{S_x c_s}{(S_{x+s} - S_x)}$$

where $c_x$ is the analyte concentration in the original sample, $S_x$ is its instrumental response, $c_s$ is the spiked analyte concentration and $S_{x+s}$ is the response of the spiked sample. The mass of spiked metal should be similar to that originally present in the sample. Since spiked volumes were at most 1 mL in 100 mL sample, the assumption of negligible dilution is valid for this study.

A slope is the ratio of instrumental response to mass of analyte, and there are several slopes of interest here. The TE slope ($S_x/c_x$) should be the same as calibration slope of the standards and this was proven with the TE linearity study in the system performance section. There should also be the same TE response before ($S_x/c_x$) and after ($S_{x+s}/c_{x+s}$) standard addition. Finally the net system response to the

added metal $((S_{x+s}-S_x)/c_s)$ should be the same as to the total metal after standard addition $(S_{x+s}/c_{x+s})$. The results of the calculations of these various ratios for standard additions of the three test metals are listed in Table XXVI.

Cu

In the analysis of tap water, the Cu content was much higher than the calibration curve range. Since the peaks for a 10 and then a 5 mL TE were way off scale, the sample was analyzed by injection. The response was still out of the range of the standards, but the concentration was estimated by extrapolation to be 1.1 mg/L. Conventional flame AA analysis indicated the Cu content to be 1.7 mg/L. This is considered to be above the safe limits for drinking water.

The Cu content in river water was about 3 orders of magnitude lower than the tap water, which might indicate plumbing as the source of Cu for the latter. As can be seen in Table XXV, reasonable agreement, especially for peak height, was obtained between the calibration curve and standard addition data for the concentration. From Table XXVI, it can be seen that the calibration standards slope for peak height is slightly less than the other slopes. The larger scatter in slopes based on area may be due to the greater imprecision of peak area measurements at low analyte concentrations.

Table XXVI Standard Addition Slopes[a]

| | Cu river | | Cd tap | | Mn river | | Mn tap | |
|---|---|---|---|---|---|---|---|---|
| Slope | pk ht[b] | area[c] | pk ht[b] | area[c] | pk ht[b] | area[c] | pk ht[b] | area[c] |
| Stds ($S_x/c_x$) | 0.22 | 12.4 | 0.48 | 26.3 | 0.40 | 17.1 | 0.40 | 17.1 |
| TE before std addn ($S_x/c_x$) | 0.25 | 15.6 | 0.62 | 57.1 | 0.41 | 18.0 | 0.44 | 20.1 |
| TE after std addn ($S_{x+s} / c_{x+s}$) | 0.27 | 11.6 | 0.65 | 36.4 | 0.45 | 17.6 | 0.44 | 19.9 |
| Net std addn ($(S_{x+s} - S_x) / c_s$) | 0.29 | 8.3 | 0.67 | 28.6 | 0.49 | 17.3 | 0.45 | 19.1 |

a. $S_x$ = signal from analyte solution
$c_x$ = analyte concentration in original sample
$S_{x+s}$ = signal from analyte solution spiked with standard
$c_{x+s}$ = analyte concentration in spiked sample

b. Absorbance x 1000/ng metal

c. Arbitrary units/ng metal

Cd

For Cd, river samples were collected and analyzed in triplicate in order to check the reproducibility in sample collection and handling. The Cd concentration in river sample number 1 is about twice as great as in samples numbers 2 and 3. This difference could be due to contamination or just due to random error since all concentrations are at levels where precision is poor. Areas gave anomalous results and were not useful for analyte determination, perhaps again because of the greater imprecision of area measurement at low analyte concentrations.

The Cd content in tap water was much lower than Cu, which is fortunate considering their differences in toxicity. The calibration slope for the standards by peak height was somewhat less than the other slopes, but differences could be ascribed to random error. As can be seen from Table XXVI, the slopes for area exhibit more imprecision than those of peak height. Most sample TE masses were about 5 ng, well below the normal 10 ng mass limit, and all sample concentrations were less than the 0.3 μg/L solutions used to test for the detection limit of TE.

Mn

For Mn, river samples were again collected and analyzed in triplicate and standard addition tests were run on both river and tap water samples. The concentrations were higher than Cd and so 10 mL TE was adequate for all samples. From Table XXV there is better agreement

between the 3 river samples, which may be due to either better sample handling technique or because the metal concentration is more above the detection limit than for Cd.

Good agreement between the calibration curve and standard addition data was achieved for both peak height and area, especially in the tap water sample, and as indicated in Table XXVI, the slopes remained consistent with standard additions in both samples.

These experiments indicate that the TE system has the ability to make precise and accurate determinations of Cu and Mn in natural water samples and for Cd larger TE volumes would be required to obtain accurate results. Even in this case though, an upper limit on Cd concentration is established. All sample concentrations were well below the detection limit for conventional flame AA, and rapid determinations were made without need for sample cleanup or separate pre-concentration steps. The standard addition experiments indicate that the system is not subject to serious analyte interference in natural water samples.

# CONCLUSIONS

Many samples have analyte concentrations which are below the practical detection limit of conventional methods of analysis. The TE system just described has been shown to be an effective technique to lower the detection limit of conventional flame AA such that concentrations of the test metals conveniently measured with the system are the concentrations normally found in most real water samples. Solutions were successfully analyzed at analyte concentrations of 0.3 µg/L for each test metal which is below the practical detection limit for AA. It should be noted that practical detection limits, which a person with only a moderate expertise would achieve, are usually 2-5 times higher than those stated in the literature. This is because the stated detection limits are usually obtained under optimum instrumental conditions and with ideal samples.

The described system also shows good freedom from interference. Samples of the analyte test metals were accurately analyzed in a matrix of commonly occurring concomitant species at concentrations which are higher than expected in most real samples. The system achieves a certain degree of matrix normalization since elution of the analyte metal is always in the same 0.2 M citrate solution, regardless of what environment the analyte is in when it arrives at the column. Thus a variety of samples matrices can be analyzed with the same instrumental conditions.

Sample analysis is convenient, since the concentration and measurement steps are combined and no pre-measurement separation or cleanup, other than normal filtration, is required of most samples.

Water samples are typically preserved by adjustment to pH 2, and since the system can directly analyze samples at this pH, no further pH adjustment is necessary. This prevents potential contamination from added reagents and sample loss which might occur with additional pH adjustment. Another benefit of the combination of the concentration and measurement steps is the time savings. Conventional sample cleanup steps are generally time consuming and labor intensive. With the described system, a sample can typically be measured in triplicate in 15 to 30 minutes, depending on analyte concentration.

There are several additional advantages of the described system over a conventional alternative such as manual ion exchange preconcentration followed by a separate flame AA analysis of the eluent for the analyte metal. In the automated TE system, all experimental variables related to enrichment and elution (such as flow rate and timing) are rigidly controlled by a precision delivery pump and a microcomputer. This results in faster enrichment times and greater precision in volumes than what is obtainable with gravity flow and manually measured solution volumes. Also, the AA senses a peak concentration of the analyte metal during elution with the TE system, but with a separate manual preconcentration the elution volume must be large enough to contain all the metal. Hence the concentration of analyte in the eluted plug must be greater for the manual case in order to obtain the same instrumental response and therefore a greater volume must be preconcentrated for the same detection limit.

Linearity of injection was demonstrated in the system over 2½ orders of magnitude for all test metals using a fixed 87.5 µL sample loop. Quantitative TE was achieved over 2 orders of magnitude and

response is linear with differing volumes of a fixed concentration as well as with varying concentrations of a fixed volume. Since the normal concentration of injected standards extends down to 40 µg/L, any sample with a concentration greater than that is better analyzed by the faster injection procedure. Thus a sample can be measured directly without concentration or dilution if its concentration is within either the normal TE or injection range. This composite range extends from at least 0.3 to 5000 µg/L, or 4 orders of magnitude.

Accuracy of the system is demonstrated by generally good agreement between TE and injection. Responses from TE are referenced to those from injection and so if recovery for injection was not quantitative, calibration results would still be good as long as TE recovery was the same as injection. However, recovery for injection was shown to be essentially quantitative for the metals tested.

What was somewhat surprising in this study was that there were more similarities than differences for the metals in experimental parameter values. Basically the only parameter which varies with the metals tested is the SR pH, and based on the experience of this study, even the value of this parameter is somewhat predictable from the log $\alpha$ values of the citrate complexes. Thus the system appears to be readily expandable to many metals as long as the chosen element has a resin selectivity coefficient (Table IX) similar to those of the three test metals and forms stable citrate complexes (Table X). There could be potential problems if the citrate SR causes a significant background absorption on the AA at the wavelength corresponding to the chosen element, although no significant spectral interferences were found from it at the wavelengths for the metals tested.

The primary components of the system (HPLC, AA and microcomputer) are found in most reasonably equipped laboratories and the extra hardware items required to construct the system (valves, electronic components, etc.) are moderately priced. Thus the cost of detection limit enhancement of existing equipment for environmental analysis is much lower than that of original purchase of more sensitive instrumentation.

The degree of automation employed and the self-directed software lower the operator expertise required to use the system. Further automation embellishments might include a computer generated flag that warns the operator of a need for a greater TE volume when the TE response is too low. Also, the addition of an auto-sampler system would eliminate the need to manually change solutions when measuring the standards. Software additions could then perform blank corrections and automatically calculate calibration curves and hence sample concentrations. Presently these manually performed calculations take about 15 minutes.

Given the 50% recovery for Cu in 0.5 M NaCl, a procedure for seawater analysis using this system could probably be developed with little difficulty. The system might be modified slightly, such as by using a larger column (more exchange sites) so as to be able to analyze a sample directly, or the sample might require minor pre-measurement preparation such as a twofold dilution. If the latter course is followed, the degree of dilution should be kept minimal, since typical concentrations of the tested metals in seawater are already sub-µg/L (122). The more the sample is diluted, the greater the TE volume (and

hence analysis time) would be necessary for an adequately measurable response.

Other possible embellishments to the system include shortening the analysis time by the use of higher flow rates, at least for the enrichment step (assuming that the kinetics of ion exchange are fast enough). For example, even increasing the flow rate to 20 mL/min, a four-fold increase over the present case, would decrease the time for the enrichment step from 10 minutes to 2½ minutes for a 50 mL TE volume (which is used for the 0.3 µg/L solutions). If it were desired to analyze a sample for more than one element, a more specific SR could be used to selectively strip the metals from the column one at a time after a single enrichment. The AA lamp would need to be switched between each strip and the various metals would have to be present in similar concentrations since only one TE volume would be used. Alternatively, the more general citrate could be used as the SR with multi-element detection such as ICP so that all elements of interest are determined simultaneously. The latter option might require a compromise in SR parameters in order to accommodate all the metals at once.

If a totally inert pumping system were used, stronger reagents could be utilized, which would broaden the choice of analysis parameters. For instance, metals are typically stripped from Chelex-100 (a common resin in column enrichment techniques) with 2.5 M $HNO_3$ (42-52, 123). The present TE system is limited to an acid strength of $10^{-2}$ M. An inert pumping system would also reduce potential blank problems, although blanks are not a significant problem for the three metals tested with the present degree of system inertness.

The SR could possibly be loaded in a large sample loop and "injected" for a strip, eliminating the need for a SR pump altogether. Since the primary SR limitation is with the pump, this would also allow use of a stronger SR. Although the sample for TE could even be delivered to the column in this manner, versatility in selection of TE volume would be reduced to the pre-determined sample loop volume.

Probably the main drawback to the system in its current design is its inability to provide information on the speciation of a metal in a sample. However, the system does show promise of being adapted in order to provide this ability. In order to determine the metal speciation in a sample, it cannot be preserved at pH 2 in the normal manner since this process in itself radically alters the speciation. At pH 2 metal complexation is likely to be insignificant, and in fact the assumption of total retention during TE is based on this premise in conjunction with the resin's ability to sequester the metal from any residual weak association. This premise was proven to be valid with the humic acid study.

The system could distinguish free metal from complexed metal by coupling two columns together. An unacidified sample would first pass through a column which retains only free metal, such as Chelex 100, and then would pass through a strong cation exchange resin which sequesters the metal from any form it is in. Each column could then be stripped individually to yield the two fractions. A fair amount of additional valves and plumbing would be required to achieve this.

The coupled column idea might also be applied to samples that contain positively and negatively charged forms of the metal. The simplest speciation of this type is by oxidation state. For example,

Mn was studied in this work in the cationic +2 state, but the +7 state is an anion, $MnO_4^-$. If a sample containing both forms was first passed through a cation exchange resin, the $MnO_4^-$ would presumably pass through where it could be subsequently trapped on an anion exchange column. Each of these two columns could then be individually stripped and measured. The +7 state is unstable relative to the +2 state though (121) and so is likely to be absent in most natural samples.

The ultimate speciation information would be the separation and measurement of every analyte species in a sample. Presumably the separation could be accomplished with a general stationary phase such as a reversed phase column. However, since this would require the direct measurement of a discrete sample without the advantage of TE, a fundamental problem would be insufficient sensitivity of detection. For many samples, the total metal concentration, let alone individual fractions, is often below the detection limit. Thus a much more sensitive detector would have to be found, or else fractions would have to be collected and subsequently trace enriched for quantitation.

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# Appendix A

## Solvent/Valve Module

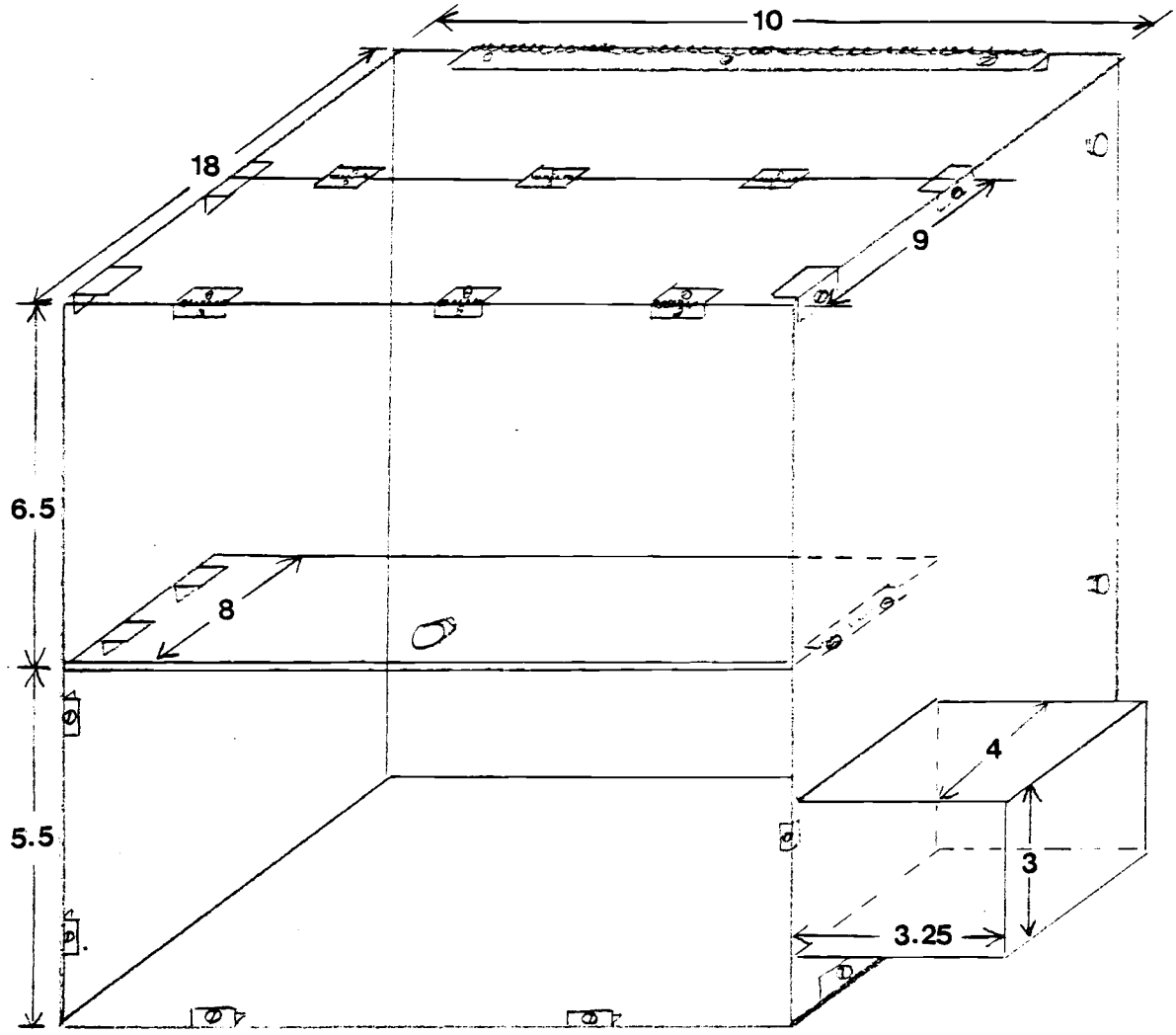



Figure 31. Solvent/valve module. All dimensions in inches. Materials of construction: sides, bottom, back = 3/16 in. aluminum; top, front = 1/8 in. clear plexiglass; sidecar box = 1/16 in. aluminum and Vector cage

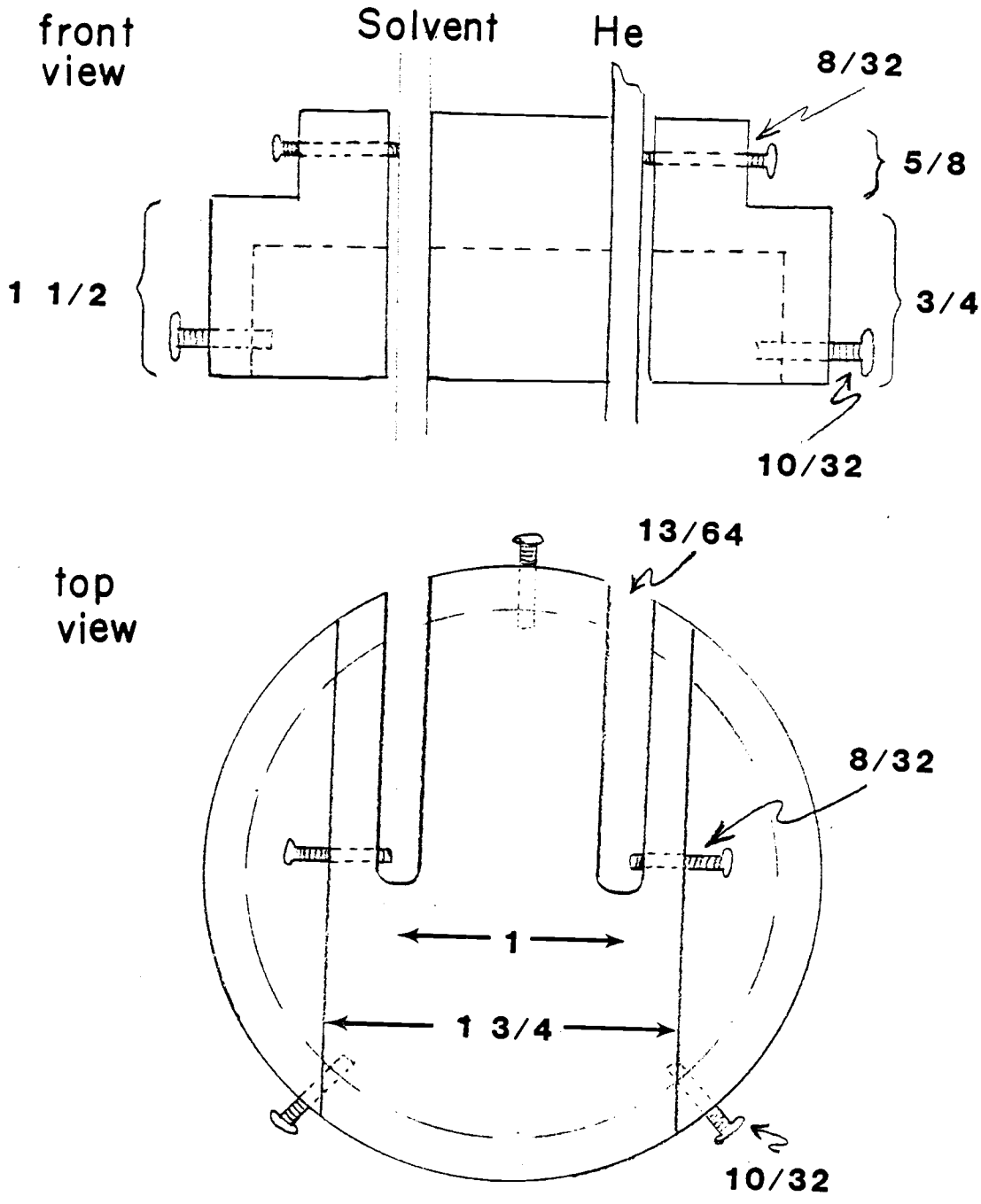



Figure 32. Teflon solvent covers. All dimensions in inches

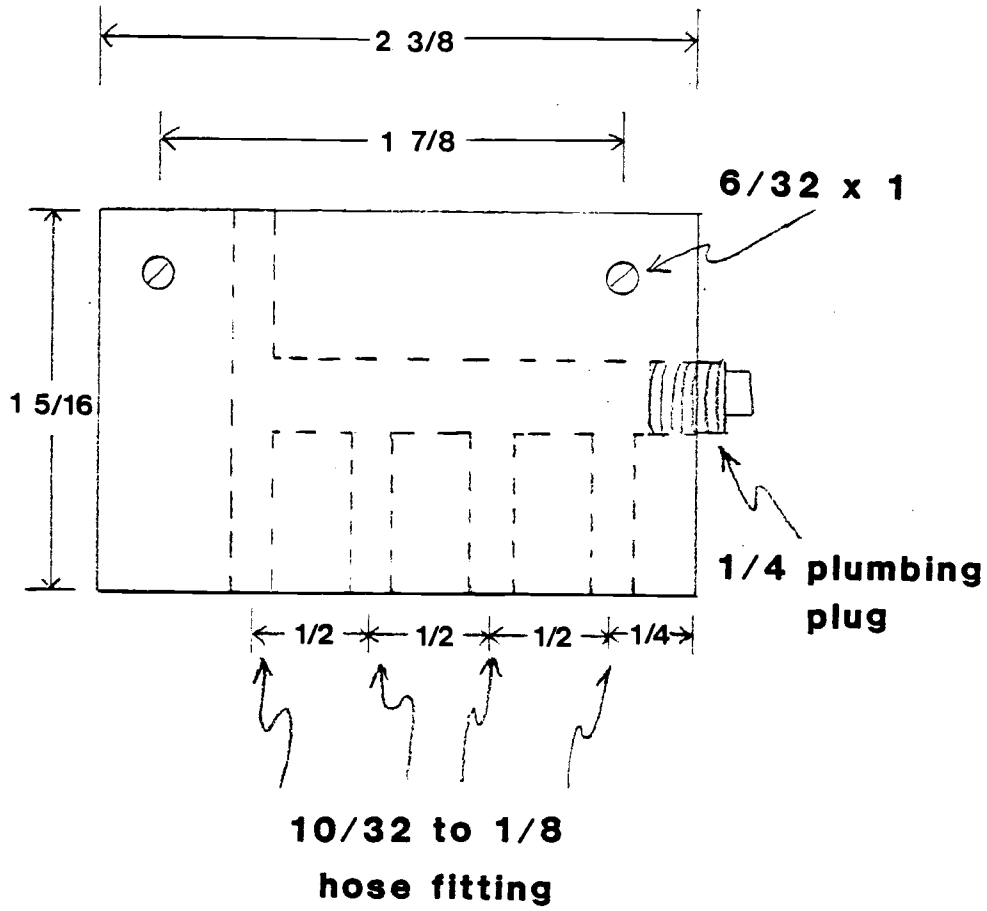



Figure 33. Compressed air manifold. All dimensions in inches

# Appendix B

## Variable Address I/O Board

Description

This board allows computer access to two 6522 versatile interface chips and hence 32 I/O lines. By means of two 8 pin switches, any address can be assigned to the chips and the board occupies 1K ($400_X$) of memory, ½K ($200_X$) per chip.

The board is 7 in. x 10 in. and arranged with 2 44-pin edge connectors on opposite sides of the board. The bottom connector is related to the bus system of the expansion connectors on the AIM 65 and is meant to plug into a mother board such as Seawell's LBM. The other edge connector brings out the 16 data and 4 control I/O lines of each of the 2 6522 chips. Table XXVII gives the pin descriptions for both edge connectors, and Figure 34 shows the board layout.

The address lines are buffered by tri-state buffers (74367) and the decoding is by means of 1 of 8 decoders (74138). The data lines are not buffered. A simple NOR gate (7402) accomplishes additional logic and an inverter function. Power of +5 V is supplied by an on-board regulator (LM 340-5) which requires unregulated +8 V.

Address lines 10-15 (A10 - A15) are used to select the address of the board and A9 selects the appropriate chip. Thus one chip is located in the "upper" ½K of the block (A9 = 1) and the other is in the "lower" ½K of the block (A9 = 0). Address lines 0 through 3 select the 16 registers of the chip, whereas A4 - A8 are not utilized (decoded) and are thus ignored. Hence if the board was given an address of $8000_X$, then $80F0_X$, $8130_X$ . . . $80X0_X$ would still address the board. In this example, the lower chip would be $80X0_X$ (or $81X0_X$) and the upper would be $82X0_X$ (or $83X0_X$).

Table XXVII: Pin Descriptions

| Mother Board Connector | | | | Output Connector | | |
|---|---|---|---|---|---|---|
| A | | 1 | | A | 1 | |
| B | A-0 | 2 | | B | 2 | |
| C | A-1 | 3 | | C | 3 | CB 2 |
| D | A-2 | 4 | IRQ | D | 4 | CB 1 |
| E | A-3 | 5 | | E | 5 | PB 7 |
| F | | 6 | | F | 6 | PB 6 |
| H | | 7 | RES | H | 7 | PB 5 |
| J | | 8 | D-7 | J | 8 | PB 4 |
| K | | 9 | D-6 | K | 9 | PB 3 |
| L | | 10 | D-5 | L | 10 | PB 2 |
| M | A-9 | 11 | D-4 | M | 11 | PB 1 |
| N | A-10 | 12 | D-3 | N | 12 | PB 0 |
| P | A-11 | 13 | D-2 | P | 13 | PA 7 |
| R | A-12 | 14 | D-1 | R | 14 | PA 6 |
| S | A-13 | 15 | D-0 | S | 15 | PA 5 |
| T | A-14 | 16 | | T | 16 | PA 4 |
| U | A-15 | 17 | | U | 17 | PA 3 |
| V | $\phi$-2 | 18 | | V | 18 | PA 2 |
| W | R/W | 19 | | W | 19 | PA 1 |
| X | | 20 | +8V | X | 20 | PA 0 |
| Y | | 21 | +8V | Y | 21 | CA 2 |
| Z | | 22 | Gnd | Z | 22 | CA 1 |
| | | | | upper $\frac{1}{2}$K | lower $\frac{1}{2}$K | |

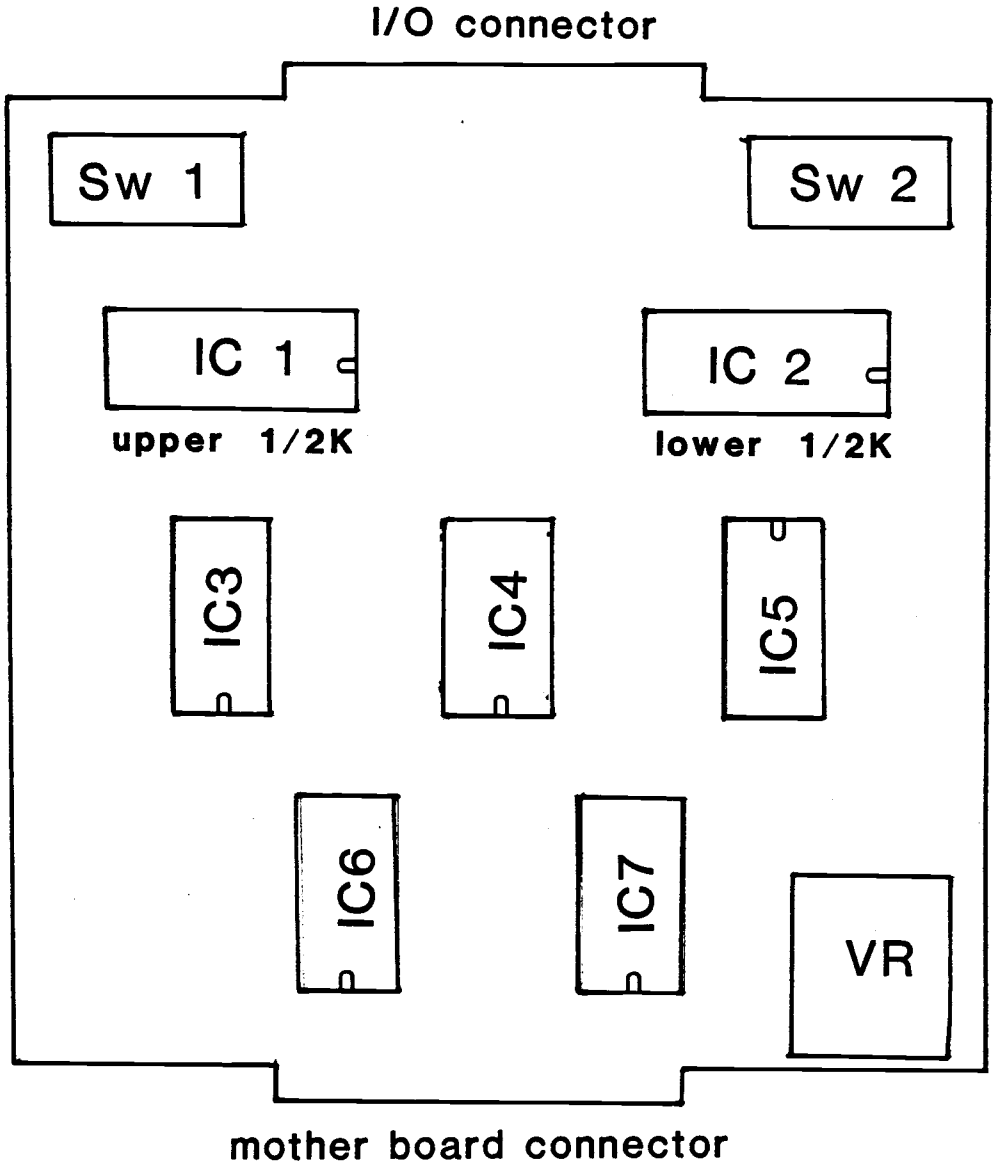
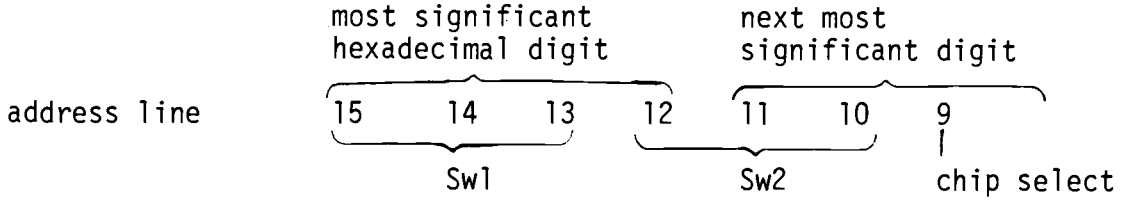



Figure 34. Layout and orientation of I/O board components. Sw 1, Sw 2 = 8-pin DIP switch; IC1, IC2 = 6522; IC3, IC5 = 74138; IC4 = 7402; IC6, IC7 = 74367; VR = +5 V voltage regulator

Figure 35 is a circuit diagram for the address decoding logic. When the pattern of A10 - A15 corresponds to the selected address, a high is sent to the normally low chip select line (CS1) on both chips. A9 is used for the normally high CS lines. CS1 must be high and CS2 low for the chip to become active.

Operation

It takes 4 hexadecimal digits (16 address lines) to define an address. The top 1½ hexadecimal digits (composed of the 6 highest address lines) define the address of the board. Since there are 6 address lines to decode and each 74138 decodes 3, each switch does not define a hexadecimal digit of the address. Rather, there is overlap of the 1½ address bytes as follows:




Thus the first step in operating the board is to convert the desired board address into the corresponding pattern of the top 6 address bits. For example, if the board is desired to reside at $D500_x$, the bit pattern for A15 - A10 is 110101. From Table XXVIII it is found that the 110 corresponds to 7 for switch 1 and the 101 corresponds to 6 for switch 2. Also in Table XXVIII are four common functions for the 6522 registers. Further information on operation of the 6522 can be found in the manual (124).

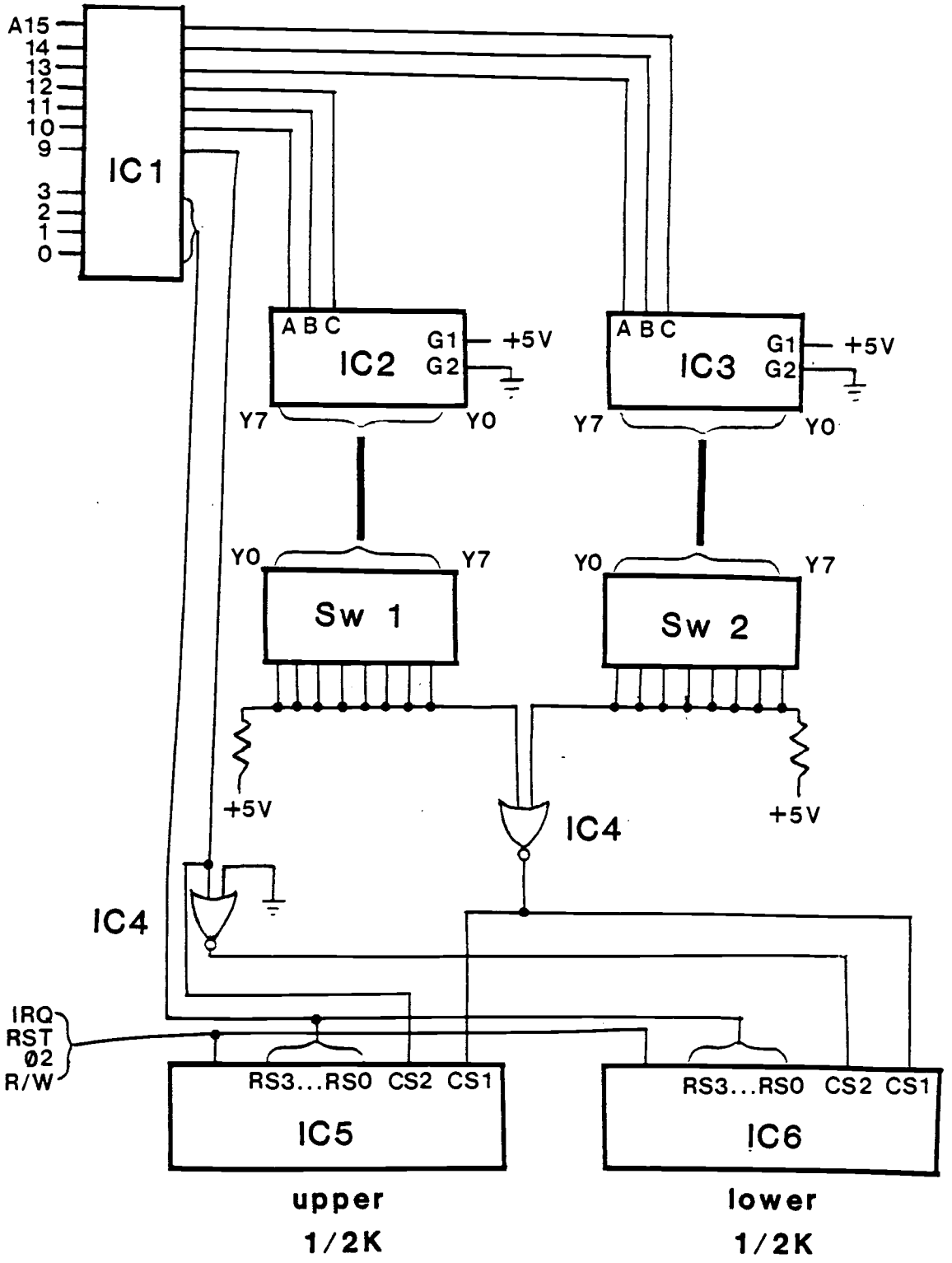



Figure 35. Circuit diagram of I/O board. IC1 = 74367; IC2, IC3 = 74138; Sw 1, Sw 2 = 8-pin DIP switch; IC4 = 7402; IC5, IC6 = 6522

TABLE XXVIII: Address Selection and Register Function

Address Switches

| | Address Lines | | | |
|---|---|---|---|---|
| $S_1$: | 15 | 14 | 13 | |
| $S_2$: | 12 | 11 | 10 | Switch |
| | 0 | 0 | 0 | 1 |
| | 0 | 0 | 1 | 2 |
| | 0 | 1 | 0 | 3 |
| | 0 | 1 | 1 | 4 |
| | 1 | 0 | 0 | 5 |
| | 1 | 0 | 1 | 6 |
| | 1 | 1 | 0 | 7 |
| | 1 | 1 | 1 | 8 |

Registers

| A0-A3 | Function | |
|---|---|---|
| 0 | Data port B | |
| 1 | Data port A | |
| 2 | Data direction register B | 0 = in |
| 3 | Data direction register A | 1 = out |

# Appendix C

## Computer Program Listings

BASIC Language Program

```
1 REM                    Trace Enrichment
2 REM                Control and Data Collection
3 REM                        Version 7
4 REM
5 REM                      by Art Ambrose
6 REM                          8/31/82
7 REM
8 REM
10 ZERO=8395:SETUP=8416:VU=8227:CURS=8442
11 REM   zero=20CB   setup=20E0   vu=2023   cursor=20FA
12 BF=12288:DM=12287:KY=42027
13 REM   bf=3000   dm=2FFF   ky=A42B
15 C=25088:REM =6200
16 T1=194:T2=176:REM     T1=B0C2
19 DIMA(10),P(10),R(10)
20 POKEC+2,15:REM  PB 0,1,2,3 - outouts
50 DEFFNH(T)=INT(T/256)
52 DEFFNL(T)=T-FNH(T)*256
60 DEFFNV(X)=(10*X/4095-5)/1E-2
70 DEFFND(I)=16*PEEK(BF+I)+INT(PEEK(BF+256+I)/16)
75 DEFFNP(T)=INT(10*T+.5)/10
98 GOTO910
110 PRINT"NEW COLL FREQUENCY?"
112 GETR$:IFR$="N"THEN131
114 IFR$<>"Y"GOTO112
116 INPUT"PNTS/SEC";PS
118 TS=256/PS:PRINT"SCAN TIME=";INT(TS);"SEC"
120 GOSUB900
130 REM          calculate and load values for clock
131 IFPS=0THENPS=10
132 X=1E6/PS:I=INT(X/5E4+.5):IFI<1THENI=1
134 P=X/I-2:Q=FNH(P):R=P-256*Q+.5
136 POKE240,I:POKE241,R:POKE242,Q
140 REM          setup and zero
142 POKE4,FNL(SE):POKE5,FNH(SE):Q=USR(0)
144 POKE4,FNL(ZE):POKE5,FNH(ZE):Q=USR(0)
145 IFS$="C"GOTO415
146 IFATHENPOKE255,A
147 PRINT"D=";D;"NEW COLL DELAY?"
148 GETR$:IFR$="N"THEN152
149 IFR$<>"Y"THEN148
150 INPUT"COLL DELAY(SEC)";D
152 IFD=0THEND=15
200 IFM$="T"GOTO250
205 IFM$<>"I"GOTO442
208 REM          Injection
209 REM     Load Loop
210 PRINT"INJ ROUTINE":GOSUB900
```

```
215 X=1:GOSUB990:REM      per on
220 T=10:GOSUB700
225 X=0:GOSUB990:REM      per off
227 T=10:GOSUB700
230 GOTO350:REM            to strip
249 REM           TE
250 IFS$="R"GOTO290
265 INPUT"TE VOL (ML)";V
270 IFF=0THENINPUT"ML/MIN";F
275 TE=V/F*60
290 IFTE=0THENPRINT"ZERO TE TIME":GOSUB900:GOTO265
292 X=1:GOSUB970:REM      begin TE
295 T=INT(TE+.5):ER%=0:GOSUB700
297 IFER%=1GOTO430
300 X=0:GOSUB970:REM      end TE
305 T=60:GOSUB700:REM       60 sec
307 IFER=1GOTO430
309 X=1:GOSUB990:REM      per on
310 FORI=0TO10
315 PRINT"START AA"
317 X=1:GOSUB960
320 FORJ=0TO400:NEXTJ
322 X=0:GOSUB960
325 FORK=0TO100:PRINT" ":NEXTK
330 NEXTI
334 X=0:GOSUB990:REM      per off
335 T=10:GOSUB700
349 REM           Strip
350 X=1:GOSUB980
399 REM           Data Collection
410 T=0:GOSUB700
415 PRINT"HIT KBRD TO ABORT"
420 POKE4,8:POKE5,32:Q=USR(0):REM      start coln
421 IFS$="C"GOTO425
422 X=0:GOSUB980
425 A1=0:A2=0
429 REM           # # # # # # # # # #
430 PRINT"VIEW REPEAT NEW MAN"
432 GETS$:IFS$="R"GOTO110
434 IFS$="M"GOTO800
436 IFS$="V"GOTO475
440 IFS$<>"N"GOTO432
441 N%=N%-1:GOSUB2000
442 PRINT"TE OR INJ"
444 GETM$:IFM$="T"GOTO450
446 IFM$<>"I"GOTO444
448 L$="INJ:":GOTO455
450 L$="TE:"
455 INPUT"SOLUTION":A$
460 PRINT!" * * * * * * * * * *"
465 PRINT!TAB(63)L$SPC(1)A$:X=3:GOSUB905
470 GOSUB3000:GOTO110
```

```
475 PRINT"CURSOR:R L  A  B  I  X"
480 POKE4,FNL(VU):POKE5,FNH(VU):Q=USR(Ø)
485 I=PEEK(DM)*PEEK(KY)
490 IFI=759THENM=1:GOTO512
492 IFI=1506THENM=Ø:GOTO512
494 IFI=1771THENA=PEEK(255):A1=1:PRINT"A SET":GOSUB9ØØ:GOTO525
496 IFI=1270THENB=PEEK(255):A2=1:PRINT"B SET":GOSUB9ØØ:GOTO521
5ØØ IFI<>1ØØ4GOTO43Ø
5Ø2 IFA1*A2=ØTHENPRINT"SET LIMITS FIRST":GOSUB9ØØ:GOTO475
5Ø5 GOTO55Ø
512 POKE4.FNL(CU):POKE5.FNH(CU):POKE246,M:Q=USR(Ø)
515 I=PEEK(255):PRINT"CURS=";I;"HT=";INT(FND(I)+.5)
52Ø GOTO48Ø
521 IFA1THEN475
522 GOSUB1ØØØ:GOTO512
525 IFA2THEN475
526 PRINT"W=";W;"NEW LIMITS?"
527 GET$:IF$="Y"THENGOSUB1ØØØ:GOTO512
528 IF$<>"N"THEN527
53Ø B=A+W-(-1)↑M:IFB>255GOTO54Ø
535 POKE255,B:GOTO512
54Ø PRINT"B OFFSCALE BY";B-255:GOSUB9ØØ:B=255
545 A2=1:GOTO562
55Ø IFA<BGOTO562
555 PRINT"LIMITS REVERSED":GOSUB9ØØ:GOTO475
558 REM          # # # # # # # # # #
56Ø REM          baseline calculation
562 Z=Ø:BA=Ø:PA=Ø
565 PRINT"CALCULATING"
57Ø FORI=A-1ØTOA
58Ø IFI<ØTHENZ=Z+1:NEXT
585 BA=BA+FND(I):NEXT
59Ø BA=BA/(11-Z)
595 BA=FNV(BA)
6ØØ REM          ok ht and area calculation
6Ø2 W=B-A:PH=BA
6Ø5 FORI=ATOB
61Ø PA=PA+(FNV(FND(I))-BA)
615 IFFND(I)<=PHTHEN625
62Ø PH=FND(I):PK=I
625 NEXT
63Ø PH=FNV(PH)-BA
632 R=(D*1Ø+INT(1Ø*PK/PS+.5))/1Ø
635 REM          calculations printout
638 PRINT!"NO.";N%
64Ø S=SGN(PH):PRINT!"PK HT=";INT(1ØØ*ABS(PH)+.5)/1ØØ*S
645 PRINT!"PK AREA=";INT(PA+.5)
647 PRINT!"AT"R"SEC"
65Ø S=SGN(BA):PRINT!"BASELINE=";INT(1ØØ*ABS(BA)+.5)/1ØØ*S
655 PRINT!"W=";W;"(";INT(W/PS+.5);"SEC)"
66Ø PRINT!PS;"PNTS/SEC"
665 X=3:GOSUB9Ø5
```

```
670 IFN%>10GOTO430
672 A(N%)=PA:P(N%)=PH:R(N%)=R
675 N%=N%+1:IFN%=10THENPRINT"START NEW FILE":GOSUB900
680 GOTO430
696 REM
697 REM          # # # # # # # # # #
698 REM          Timer Subroutine
699 REM     arrive with T sec
700 PRINTINT(T/60);"MIN"SPC(2)T-INT(T/60)*60;"SEC LEFT"
705 POKE4,32:POKE5,33:Q=USR(0):REM     2120
740 T=T-1
750 IFT=0THENRETURN
752 GETS$:IFS$<>""THENER%=1:X=0:GOSUB970:GOSUB980:GOSUB990:RETURN
760 PRINTINT(T/60);"MIN"SPC(2)T-INT(T/60)*60;"SEC LEFT"
761 POKE4,57:Q=USR(0):REM  2139
762 GOTO740
798 REM
799 REM          Manual Valve Control
800 PRINT"TE SAM PER COLL X"
802 IFS$="C"THEN110
805 GETS$:IFS$="T"THENGOSUB850
810 IFS$="S"THENGOSUB860
815 IFS$="P"THENGOSUB870
820 IFS$<>"X"GOTO800
825 GOTO430
848 REM
849 REM          TE valve routine
850 PRINT"DW OR TE SOLN?"
852 GETT$:IFT$="D"THENX=0:GOSUB970:RETURN
854 IFT$<>"T"GOTO852
856 X=1:GOSUB970:RETURN
859 REM          sam inj valve routine
860 PRINT"LOAD(DW) OR INJ(SR)?"
862 GETU$:IFU$="L"THENX=0:GOSUB980:RETURN
864 IFU$<>"I"GOTO862
866 X=1:GOSUB980:RETURN
869 REM          per pump routine
870 PRINT"OFF(1) OR ON(2)"
872 GETV$:IFV$="1"THENX=0:GOSUB990:RETURN
874 IFV$<>"2"GOTO872
876 X=1:GOSUB990:RETURN
```

```
899 REM           Subroutines
900 FORI=0TO900:NEXT:RETURN:REM      1 sec pause
905 FORJ=1TOX:PRINT!" ";NEXT:RETURN:REM      blank lines
909 REM           heading sub
910 X=8:GOSUB905
912 N%=1
915 INPUT"DATE";D$
920 PRINT!TAB(8)D$
925 INPUT"REFERENCE NUMBER";D$
930 PRINT!TAB(8)D$
932 X=1:GOSUB905:INPUT"FLOW RATE (ML/MIN)";F
933 INPUT"PRESSURE";D$
934 PRINT!TAB(8)F"ML/MIN",TAB(8)D$SPC(1)"PSI"
935 PRINT"MORE?"
940 GET$$:IF$$="N"THENX=3:GOSUB905:GOTO430
945 IF$$<>"Y"GOTO940
950 INPUT"WHAT";D$
952 PRINT!TAB(8)D$
955 GOTO935
959 REM           Beeper
960 ONX+1GOTO962,964
962 POKEC,PEEK(C)AND7:RETURN
964 POKEC,PEEK(C)OR8:RETURN
969 REM           TE(V1)
970 ONX+1GOTO972,974
972 POKEC,PEEK(C)AND14:RETURN:REM      DW
974 POKEC,PEEK(C)OR1:RETURN:REM      TE
979 REM           sam(V2)
980 ONX+1GOTO982,984
982 POKEC,PEEK(C)AND13:RETURN:REM load
984 POKEC,PEEK(C)OR2:RETURN:REM inj
989 REM           per pump
990 ONX+1GOTO992,994
992 POKEC,PEEK(C)AND11:RETURN:REM off
994 POKEC,PEEK(C)OR4:RETURN:REM on
```

```
999 REM          Manual W Set
1000 PRINT"SET W?"
1005 GETS$:IFS$="N"THEN1060
1010 IFS$<>"Y"THEN1005
1015 INPUT"W (R OR L)";W$
1020 IFRIGHT$(W$,1)="L"THENW$=LEFT$(W$,LEN(W$)-1):W=VAL(W$):GOTO1045
1025 IFRIGHT$(W$,1)<>"R"GOTO1015
1030 W$=LEFT$(W$,LEN(W$)-1):W=VAL(W$)
1035 B=A+W-(-1)*M:IFB<256THENPOKE255,B:RETURN
1040 PRINT"B OFFSCALE-W=";255-A:GOSUB900:B=255:POKEB,B:RETURN
1045 A=B-W-1:IFA>5THENPOKE255,A:RETURN
1050 PRINT"A OFFSCALE-W=";B-5:GOSUB900:A=5:POKE255,A:RETURN
1060 IFA1THENPOKE255,A+1:RETURN
1065 IFA2THENPOKE255,B-1:RETURN
1994 REM                    Statistics
2000 Z%=0
2002 IFH%=1THENH%=2:N%=1:RETURN
2004 PRINT"STATS?"
2005 GETR$:IFR$="N"THENN%=1:RETURN
2006 IFR$<>"Y"GOTO2005
2008 PRINT"ERASE ANY?"
2010 GETR$:IFR$="N"GOTO2050
2015 IFR$<>"Y"GOTO2010
2020 INPUT"WHICH ONE";J%
2025 IFJ%>N%THEN2035
2030 IFJ%THEN2040
2035 PRINT"ERROR! N=";N%:GOSUB900:GOTO2008
2040 A(J%)=0:P(J%)=0:R(J%)=0:Z%=Z%+1:GOTO2008
2050 S1=0:S2=0:S3=0:S4=0:R1=0:R2=0
2055 FORI=1TON%
2060 S1=S1+A(I):S2=S2+P(I):R1=R1+R(I):NEXT
2062 IFN%-Z%=0THENN%=1:RETURN
2065 S1=S1/(N%-Z%):S2=S2/(N%-Z%):R1=R1/(N%-Z%)
2066 REM     S1=ave area   S2=ave pk ht
2070 FORI=1TON%
2075 IFA(I)*P(I)=0THEN2090
2080 S3=S3+(A(I)-S1)*(A(I)-S1)
2085 S4=S4+(P(I)-S2)*(P(I)-S2)
2087 R2=R2+(R(I)-R1)*(R(I)-R1)
2090 NEXT
```

```
2100 IFN%-Z%=1THENSA=0:SP=0:SR=0:GOTO2112
2104 SR=SQR(R2/(N%-Z%-1))
2105 SA=SQR(S3/(N%-Z%-1))
2110 SP=SQR(S4/(N%-Z%-1))
2111 REM     SA=std dev(area)   SP=std dev(pk ht)
2112 IFS1=0THENRA=0:GOTO2116
2114 RA=SA/S1*100
2116 IFS2=0THENRP=0:GOTO2120
2118 RP=SP/S2*100
2119 REM     RA=RSD(area)   RP=RSD(pk ht)
2120 IFZ%=0THENPRINT!"SUMMARY: ALL POINTS":GOTO2130
2125 PRINT!"SUMMARY: ALL BUT";J%
2130 X=1:GOSUB905
2132 PRINT!SPC(5)"PK HT"SPC(2)"AREA"
2135 PRINT!"MEAN"SPC(1)FNP(S2)SPC(2)INT(S1+.5)
2140 PRINT!SPC(2)"SD"SPC(1)FNP(SP)SPC(2)INT(SA+.5)
2141 PRINT!SPC(1)"RSD"SPC(1)INT(RP+.5)SPC(2)INT(RA+.5)
2142 X=1:GOSUB905
2143 PRINT!"RET TIME="INT(10*R1+.5)/10"SEC"
2144 PRINT!SPC(6)"SD="INT(10*SR+.5)/10
2145 X=3:GOSUB905:PRINT"AGAIN?"
2146 GETR$:IFR$="Y"THEN2008
2147 IFR$<>"N"GOTO2146
2160 N%=1:RETURN
2999 REM     Sample Flush
3000 PRINT"SAM FLUSH?"
3002 GETS$:IFS$="N"THENRETURN
3005 IFS$<>"Y"THEN3002
3010 X=1:GOSUB970:T=INT(5/F*60):GOSUB700:X=0:GOSUB970:T=85:GOSUB700
3015 X=1:GOSUB980:T=INT(D+25):GOSUB700:X=0:GOSUB980:RETURN
```

# Machine Language Programs

```
DATA COLLECTION......PAGE 0001

LINE # LOC     CODE       LINE

0002  0000                                    ;VERSION SIX
0003  0000                                    ;
0004  0000                                    ;8/28/81

0006  0000                    *=$2000
0007  2000                E0=$E0
0008  2000                F0=$F0
0009  2000                KBRD=$ED00
0010  2000                TIM=$A000
0011  2000                INT=$A400
0012  2000                DMMY=$2FFF
0013  2000                STL=$30
0014  2000                STH=$31
0015  2000                HI=$3100

0017  2000  4C          COUNT  PHA             ;SAVE ACCUM
0018  2001  22 21 4C           LDA TIM+4       ;CLEAR INTER FLAG
0019  2004  3B 21              DEC F0+$A       ;COUNT DOWN
0020  2006  84                 PLA             ;RECOVER ACCUM
0021  2007  F4                 RTI

0023  2008  A0 00        START  LDA #$C0
0024  200A  81 ER              STA F0+3        ;IER
0025  200C  48 20              LDA #STL        ;LOAD LO AND
0026  200E  75 2A              LDX #STH        ;HI ADC PNTRS-HI BYTE
0027  2010  68 A4              STA F0+5
0028  2012  F4 60              STX F0+9
0029  2014  A5 ED              LDA #00
0030  2016  A6 EE              STA F0+4        ;LO BYTE-ADC PNTRS
0031  2018  85 EB              STA F0+8
0032  201A  86 EC              STA F0+$A       ;INMEDIATE ADC CONVERSION
0033  201C  60 84 F4           JSR CLOCK       ;SET UP AND START CLOCK
0034  201F  20                 CLI             ;SET INTER. ENABLE ON
0035  2020  25 20 A4           JMP DCON

0037  2023  F4 60              LDA #$11        ;VIEW ENTRY PNT
0038  2025  6C 86              STA F0+$A

0040  2027  17 84 F4     DCON  JSR PAUSE
0041  202A  48 20 32     B     JSR DPREP
0042  202D  20 68        AGAIN LDY #00         ;PNT TO 1ST OF DATA TABLE
0043  202F  A4 F4              LDA #STL        ;RESET DAC PNTRS
0044  2031  60 6C              STA F0+$E
0045  2033  84 17              LDA #STH
0046  2035  A9 0D              STA F0+$C
0047  2037  20 28 20           JSR MORE        ;SEND OUT ENTIRE TABLE
0048  203A  A9 0A 4C           JSR PAUSE       ;SEE IF READY FOR NEXT PNT
0049  203D  28 20 60           JSR KBRD        ;SCAN KEYBOARD
0050  2040  EA EA 84           STY DMMY
0051  2043  F4                 TYA
0052  2044  20 4A              BEQ AGAIN
0053  2046  20                 SEI
0054  2047  A4                 RTS             ;RETURN TO BASIC
```

DATA COLLECTION.....PAGE 0002

```
LINE # LOC     CODE        LINE

0056  2048  F4 60 6C    PAUSE  JSR TCHECK
0057  204B  82 17 84           DEC DMMY        ;WASTE TIME
0058  204E  F4 48               BNE PAUSE
0059  2050  20                 RTS

0061  2051  57 20        CLOCK  LDA F0+3        ;GET  IER
0062  2053  68 A4 F4           STA TIM+$E      ;AND LOAD IT
0063  2056  60 6C               LDA #$40        ;FREE RUN CODE
0064  2058  80 17 A9           STA TIM+$B
0065  205B  0D 20               LDA F0+1
0066  205D  4D 20 A9           STA TIM+4       ;LOAD AND START TIMER
0067  2060  0A 4C               LDA F0+2
0068  2062  4D 20 60           STA TIM+5
0069  2065  EA                 RTS             ;BACK TO JSR CLOCK

0071  2066  EA 60        TCHECK LDA F0+$A       ;IF TIMER DONE, GO TO
0072  2068  EA EA               BEQ READY       ;ADC
0073  206A  60                 RTS             ;IF NOT, GO BACK TO JSR CHECK

0075  206B  EA EA        READY  LDA F0          ;RELOAD TIMER
0076  206D  60 EA               STA F0+$A
0077  206F  EA 60               LDX #01
0078  2071  EA EA 4C           STX TIM+1       ;SET MODE TO 1
0079  2074  31                 DEX             ;X=0
0080  2075  2A 20 2A           STX TIM+2       ;B=INPUTS
0081  2078  53 45               LDA #$E0        ;READ CODE
0082  207A  41 2D 36           STA TIM+$C      ;READ MODE NOW
0083  207D  35 2A 3B           LDA TIM         ;GET 1ST BYTE, HI
0084  2080  45 4E               STA (F0+4,X)    ;STORE HI BYTE (TOP 8 BITS)
0085  2082  44 3B               LDA #09         ;GET INTO LO MODE
0086  2084  20 4F 4B           STA TIM+1
0087  2087  3F 20 3B           ORA TIM         ;GET LO BYTE AND SET BITS 0 AND 3
0088  208A  20 20               STA (F0+8,X)    ;STORE LO BYTE (LO 4 BITS)
0089  208C  46                 INX
0090  208D  52 4F 4D           STX TIM+1       ;MODE 1 AGAIN
0091  2090  3D 3B               INC F0+4        ;INCR LO PNTRS
0092  2092  20 20               INC F0+8
0093  2094  54 4F               BNE DPREP

0095  2096  3D           END    PLA             ;CLEAR STACK SCROLL
0096  2097  3B                 PLA             ;FROM DCON
0097  2098  4D                 PLA
0098  2099  45                 PLA
0099  209A  4D                 SEI             ;DISABLE INTERUPTS
0100  209B  4F                 RTS             ;BACK TO BASIC

0102  209C  52 59 20     MORE   JSR MDAC        ;SEND OUT DATA PNT
0103  209F  46                 INY
0104  20A0  41 49               BNE MORE
0105  20A2  4C 20 40           STY TIM+1       ;TURN OFF TRIGGER FOR OSCILL.
0106  20A5  20                 RTS             ;BACK TO AGAIN

0108  20A6  3B 43        DPREP  LDA #$FF
0109  20A8  4F 50 59           STA TIM+2       ;B=OUTPUTS
0110  20AB  52 49               LDA #$CA
```

```
DATA COLLECTION.....PAGE 0003

LINE # LOC     CODE       LINE

0111  20AD  47 48 54          STA TIM+$C
0112  20B0  20               RTS              ;BACK TO DCON

0114  20B1  37 38       MDAC  LDA (F0+$D),Y    ;GET A BYTE
0115  20B3  20 53 45          STA TIM          ;SEND IT TO DAC PORT
0116  20B6  41 57              LDA (F0+$B),Y    ;ANOTHER
0117  20B8  45 4C 4C          STA TIM+1        ;LATCH
0118  20BB  20 4D              LDA #$CC
0119  20BD  4B 54 2E          STA TIM+$C       ;MAKE CA2=0
0120  20C0  2C 49              LDA #$CE
0121  20C2  4E 43 2E          STA TIM+$C       ;MAKE CA2=1
0122  20C5  3B 45              LDA #$CC
0123  20C7  44 49 54          STA TIM+$C       ;MAKE CA2=0
0124  20CA  3B                RTS

0126  20CB  42 20       ZERO  LDA #STL
0127  20CD  42 55              STA F0+$E        ;LOAD PNTR
0128  20CF  46 20              LDX #02          ;2 PAGES TO EMPTY
0129  20D1  20 43              LDA #01
0130  20D3  20 4C              LDY #00          ;INDEX=0
0131  20D5  4E 40       PASS  STA (F0+$D),Y    ;FILL A SPOT
0132  20D7  20                 INY
0133  20D8  20 45              BNE PASS         ;DONE?
0134  20DA  20 46              INC F0+$E
0135  20DC  49                 DEX              ;ONE PAGE LESS
0136  20DD  4C 20              BNE PASS
0137  20DF  20                 RTS

0139  20E0  45 20       SETUP LDA #$FF         ;MAKE PORT A OUTPUTS
0140  20E2  62 55 46          STA TIM+3
0141  20E5  20 20              LDA #00          ;INTER ADDR FOR COUNT (LO BYTE)
0142  20E7  43 20 4C          STA INT
0143  20EA  6E 23              LDA #$20         ;HI BYTE COUNT ADDR
0144  20EC  20 20 4C          STA INT+1
0145  20EF  20 43              LDA #00
0146  20F1  4E 54              STA F0+$B
0147  20F3  3B 4E              STA F0+$D
0148  20F5  45 57              LDA #$7D
0149  20F7  20 46              STA F0+$F        ;CURSOR TO CH 125
0150  20F9  49                 RTS
```

```
CURSOR......PAGE 0004

LINE # LOC     CODE        LINE

0152  20FA  4C 45       CURSOR LDY F0+$F
0153  20FC  3F 20              LDA #$FD
0154  20FE  20 3B 59           AND HI,Y        ;TURN OFF OLD CURSOR
0155  2101  45 53 3B           STA HI,Y
0156  2104  4E 4F              LDA F0+6        ;GET MOVEMENT INSTR
0157  2106  3B 53              BEQ UP
0158  2108  59 4D              CPY #00         ;IS IT AT BOTTOM NOW?
0159  210A  42 4F              BEQ J
0160  210C  4C                 DEY             ;NO-MOVE IT DOWN
0161  210D  20 54              BCS J           ;WILL ALWAYS BRANCH

0163  210F  41 42       UP     CPY #$FF        ;IS IT AT THE TOP?
0164  2111  4C 45              BEQ J
0165  2113  3B                 INY             ;NO-MOVE IT UP

0167  2114  20 52       J      LDA #02         ;GET CURSOR BIT
0168  2116  45 41 44           ORA HI,Y        ;TURN CURSOR ON
0169  2119  59 3F 20           STA HI,Y
0170  211C  3B 20              STY F0+$F       ;SAVE IT
0171  211E  49                 RTS             ;BACK TO BASIC
0172  211F                     .END


SYMBOL TABLE

SYMBOL   VALUE

AGAIN    202D   B        202A   CLOCK    2051   COUNT    2000
CURSOR   20FA   DCON     2027   DMMY     2FFF   DPREP    20A6
E0       00E0   END      2096   F0       00F0   HI       3100
INT      A400   J        2114   KBRD     ED00   MDAC     20B1
MORE     209C   PASS     20D5   PAUSE    2048   READY    206B
SETUP    20E0   START    2008   STH      0031   STL      0030
TCHECK   2066   TIM      A000   UP       210F   ZERO     20CB
```

Countdown timer

```
2120 A9 LDA #E0
2122 8D STA 620B
2125 A9 LDA #0A
2127 8D STA 6208
212A A9 LDA #00
212C 8D STA 6209
212F A9 LDA #C2
2131 8D STA 6204
2134 A9 LDA #B0
2136 8D STA 6205
2139 A9 LDA #20
213B 2C BIT 620D
213E F0 BEQ 213B
2140 A9 LDA #0A
2142 8D STA 6208
2145 A9 LDA #00
2147 8D STA 6209
214A 60 RTS
```