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Final Report

Wet Chemistry Instrument Prototype

Report No. 23197-6001-RU-00
31 January 1974

Prepared under Contract No. NAS 2-7198
for
Ames Research Center
National Aeronautics and Space Administration

TRW
SYSTEMS GROUP

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PREFACE

The Breadboard Study Phase of the Wet Chemistry Instrument Prototype described in this document was undertaken for the National Aeronautics and Space Administration, Ames Research Center, under Contract No. NAS2-7198. The study was under the direction of Mr. G. Thorley, Technical Monitor, NASA/Ames Research Center. The experimental concepts, chemistry of the experiment, procedures and processes were provided by the experimenters, Mr. G. E. Pollock, Dr. R. D. Johnson and Dr. K. Kvenvolden, NASA/Ames Research Center and Dr. S. L. Miller, University of California, San Diego. We wish to acknowledge the many valuable discussions and contributions of the experimenters, the Technical Monitor, and Mr. T. H. Harmount of NASA/Ames Research Center.

The program at TRW Systems Group was performed by the Instrument Systems Department under the direction of Dr. H. S. Suer. Mr. S. L. Korn acted as Study Manager; Dr. R. J. Day was responsible for the experimental investigations.

1. INTRODUCTION

This document is the final report for a study of a Wet Chemistry Instrument Prototype capable of detecting amino acids in planetary soil samples. The program was carried out by TRW Systems for the National Aeronautics and Space Administration, Ames Research Center (NASA/ARC) under Contract No. NAS2-7198.

Experiments capable of analyzing amino acids in planetary soils and of determining whether the amino acids are optically active are important to establish whether life as we know it does currently exist or has existed in the past on other planets.

Amino acids and their condensation products, the proteins and peptides, are indispensable to the forms of life found on Earth, and the identifying characteristic is their optical activity. The determination of the optical purity of amino acids becomes, therefore, a suitable candidate for a purely "chemical" life detection experiment, as compared to the currently planned biology experiments in the Viking Lander Biology Instrument (VLBI) which attempt to detect metabolic, growth or reproductive activities of organisms. The presence or absence of optically active or racemic amino acids in planetary soils should have not only far-reaching consequences on present theories in biology, but also on chemical evolution and geochemistry.

The purpose of the breadboard program which was carried out between August 1972 and January 1974 was to conduct tests and design updates to provide quantitative information on the performance of a flight-type wet chemistry instrument system. To accomplish this, a breadboard based on an earlier flight instrument concept, presented in the Final Report for NASA Contract NAS2-6218 (TRW Report No. 16660-6001-R0-00), was designed, fabricated, and tested. Various laboratory studies and materials compatibility tests were carried out in support of the breadboard design. Based on breadboard program results, the earlier flight design concept was updated and revised.

In the 14th month of the study NASA/ARC directed a change from Processing Sequence No. 1 (described in the Appendix) to a new baseline Processing Sequence No. 2 (described in the Appendix). All breadboard testing was conducted using Processing Sequence No. 1 which requires an acid hydrolysis directly on the soil sample while Processing Sequence No. 2 which hydrolyzes a water extract of the soil was adapted for the updated flight design concept presented herein.

This report is divided into five major subsections. Section 2, Summary summarizes the basic breadboard system and flight system concepts, the breadboard program objectives, and the major program accomplishments. Also given are a test results and flight design summary, and the conclusions and recommendations resulting from the program.

Section 3, Breadboard Test Program, provides a description of the major breadboard component design and of the total breadboard system. This is followed by a detailed discussion of the breadboard test results in the order of component tests, system tests, and supporting laboratory studies.

At the end of each appropriate section, the impact of the change from Processing Sequence No. 1 to the new baseline Processing Sequence No. 2 is discussed. Remaining problems are pointed out and recommendations for further investigations are given.

Section 4, Flight Design Update, provides the updated system requirements and instrument system definition. In addition, the instrument system design and the design of all major subsystems and components are described, and layout drawings are provided. A discussion of modification to the baseline instrument concept, to provide improvement in instrument performance and to broaden the instrument analysis capabilities, is also included in this section. A summary of major conclusions of this study is presented in Section 5, Conclusions and Recommendations, which also includes recommendations for further development of the instrument.

A brief Appendix, Processing Sequences, concludes this Final Report.

2. SUMMARY

The chemical approach of life detection carried out with the wet chemistry instrument consists of searching for optically active amino acids in planetary soils.

The amino acids present in terrestrial soils, and it is expected also in planetary soils, are at least partially in the form of biopolymers (peptides and proteins). Thus, the first step in the separation and detection is to break down the polymers into the individual amino acids by acid hydrolysis. In Processing Sequence No. 1 the hydrolysis is carried out directly on the soil. (In the new baseline Processing Sequence No. 2 the hydrolysis is carried out on a water extract of the soil.)

Acidic hydrolysis carried out directly on the soil sample precludes any loss of material and is gentle enough to minimize the destruction or racemization of the amino acids. The soil can be removed by filtration and the excess acid (HCl) removed by evaporation. The product of the soil hydrolysis contains materials such as organic acids, neutral organic compounds, and salts which may interfere in the subsequent steps of the analysis. The interfering materials are separated from the amino acids through precipitation with HF/NH_4OH and subsequent desalting in an ion exchange column. (Processing Sequence No. 2 produces significantly less interfering material, and the precipitation step can be eliminated and desalting is accomplished with an ion exchange column only.)

The amino acids in a solution at a suitably low pH are injected onto a strong cation exchange resin column in the acid form. They are absorbed as ammonium ions RNH_3^+ while any anions and neutral organic species are passed through. Following this, the amino acids are washed off the column with NH_4OH. The NH_4OH can be removed by evaporating the solution to dryness, leaving a residue of relatively pure amino acids.

Once the amino acid is extracted and purified, its enantiomers are resolved chemically by derivatization with an optically active esterification reagent, and after acylation the enantiomers separated by means of a gas chromatograph based on the differences in the properties of the resultant diastereoisomers. Gas chromatography has proven to be a sensitive and simple method for separating the individual amino acid derivatives,

identifying them by a characteristic retention time, and resolving the diastereoisomers.

The objective of the wet chemistry instrument breadboard program has been to obtain data on the performance of a prototype instrument system comparable to that which would be obtained from an actual flight instrument. The following tasks were carried out to accomplish this objective:

- A breadboard based on the flight instrument concept presented in the Final Report for NASA Contract NAS 2-6218 was designed and fabricated.
- Laboratory materials compatibility tests to support breadboard design were carried out.
- Step-by-step evaluation of the performance of each breadboard component for its function in the process sequence was conducted.
- Laboratory tests in glassware for control analyses were carried out.
- Breadboard performance demonstration tests with ratiotracers, NASA/ARC supplied soils, and amino acid-free soils were accomplished.
- The flight design concept based upon the breadboard program results and the new baseline Processing Sequence No. 2 was revised and updated.

A major accomplishment of this study was the demonstration that the updated flight instrument concept is basically able to meet the experimental objectives specified in NASA/ARC Specification A-16231, Rev. 3 from October 15, 1973, under the conditions specified therein. Several remaining problems were identified, and solutions to those problems are proposed.

The design of the wet chemistry instrument system was revised to incorporate the change from three sets of processing cells in the previous instrument concept to just one set of reusable cells, and also to meet the new interface requirements which, with minor exceptions, are identical to the current VLBI interface. Major instrument components such as the processing cells and the ion exchange column are flight-weight versions of the equivalent components used successfully in the breadboard. Design concepts and detailed designs proven on VLBI have been incorporated when deemed appropriate. The proposed use of gas actuated tantalum diaphragm valves and the presentation of a conceptual design is an important step

towards solving the valve problem on the wet chemistry instrument, which was identified during this study.

3. BREADBOARD TEST PROGRAM

Our basic approach to the breadboard test program has been to conduct a series of tests on the breadboard instrument to provide test data comparable to that which would be obtained from a flight instrument. The objective of these tests has been to determine the performance of the instrument - initially at the component level, with modifications made in each unit as required. As the functionality of each cell was demonstrated, the units were combined ultimately up to complete system level. In both component level and system level testing, experiments were run to determine the percent recovery of amino acids, the sensitivity for trace amounts of amino acids, the presence or absence of material interfering with gas chromatographic analysis and the absence of racemization. It was intended that the test results would demonstrate that the materials and methods used to carry out the processing, derivatization and analysis steps in an automated spacecraft instrument would not significantly degrade the experimental results demonstrated with laboratory glassware and that the various requirements pertinent to a flight instrument could be satisfied.

For these tests, Processing Sequence No. 1 listed in the Appendix was used, which consisted of 6N HCl hydrolysis on the soil in the hydrolyzer unit, filtration into the evaporator unit, evaporation, redissolution in water, HF/NH_4OH (initially NaOH) desalting, filtration into the ion exchange column, washing of the IEC, elution of the amino acids with an ammonia solution into the derivitizer unit, evaporation to dryness and derivatization with 2-butanol/HCl and then by trifluoracetic anhydride in methylene chloride, evaporation of the derivatives into a gas chromatograph column and GC analysis. This processing scheme results in the exposure of the processing cells to much more severe conditions than those of the new baseline process which is Processing Sequence No. 2, listed in the Appendix.

In addition to breadboard testing, a variety of supporting tests were conducted. Standards and methods were established for radiotracer work to follow breadboard and procedural losses. A series of material

compatibility tests were run on candidate container, seal and valve materials. The need and procedures for rigid reagent clean-up were established for low level amino acid work. A standard protein, ribonuclease, was run. A long-term (8-month) storage test was conducted on racemic 2-butanol/HCl in a sealed glass ampoule. Soil tests were run in laboratory glassware on different soils provided by NASA/ARC.

Racemic 2-butanol was used for derivatization of samples prior to gas chromatographic analysis for most of the testing for convenience, ease in interpretation of the chromatograms, and reduction of costs. Tests using derivatization with optically active 2-butanol were carried out which demonstrated that there were no observable racemization effects in the breadboard or lab processing.

3.1 BREADBOARD DESIGN

The breadboard design was based on the original flight design concept reported in TRW Final Report No. 16660-6001-R0-00 and developed for Processing Sequence No. 1 in which an acid hydrolysis was carried out directly on the soil. The design duplicated, as closely as practicable, all aspects of the flight design which might affect instrument performance, such as internal cell geometry, interconnecting valves and plumbing, materials exposed to the analysis process, sequencing, and vent pressures. In the breadboard, one set of cells was used which had to be capable of undergoing in excess of 50 analyses. The extensive use of the breadboard required certain modifications to the hardware to accommodate extended operational lifetime, cleaning, servicing, and parts replacement. Simulated flight type components, such as hand operated valve simulators were used in some places, and commercial components such as some valves, lines, and detectors were incorporated where they did not affect the process.

A schematic of the breadboard is shown in Figure 3-1. Figure 3-2 shows a photograph of the breadboard, which was mounted on an 18-by-30 inch panel which was self-supporting and which could be used on a laboratory bench counter top. The set of processing cells (hydrolyzer,

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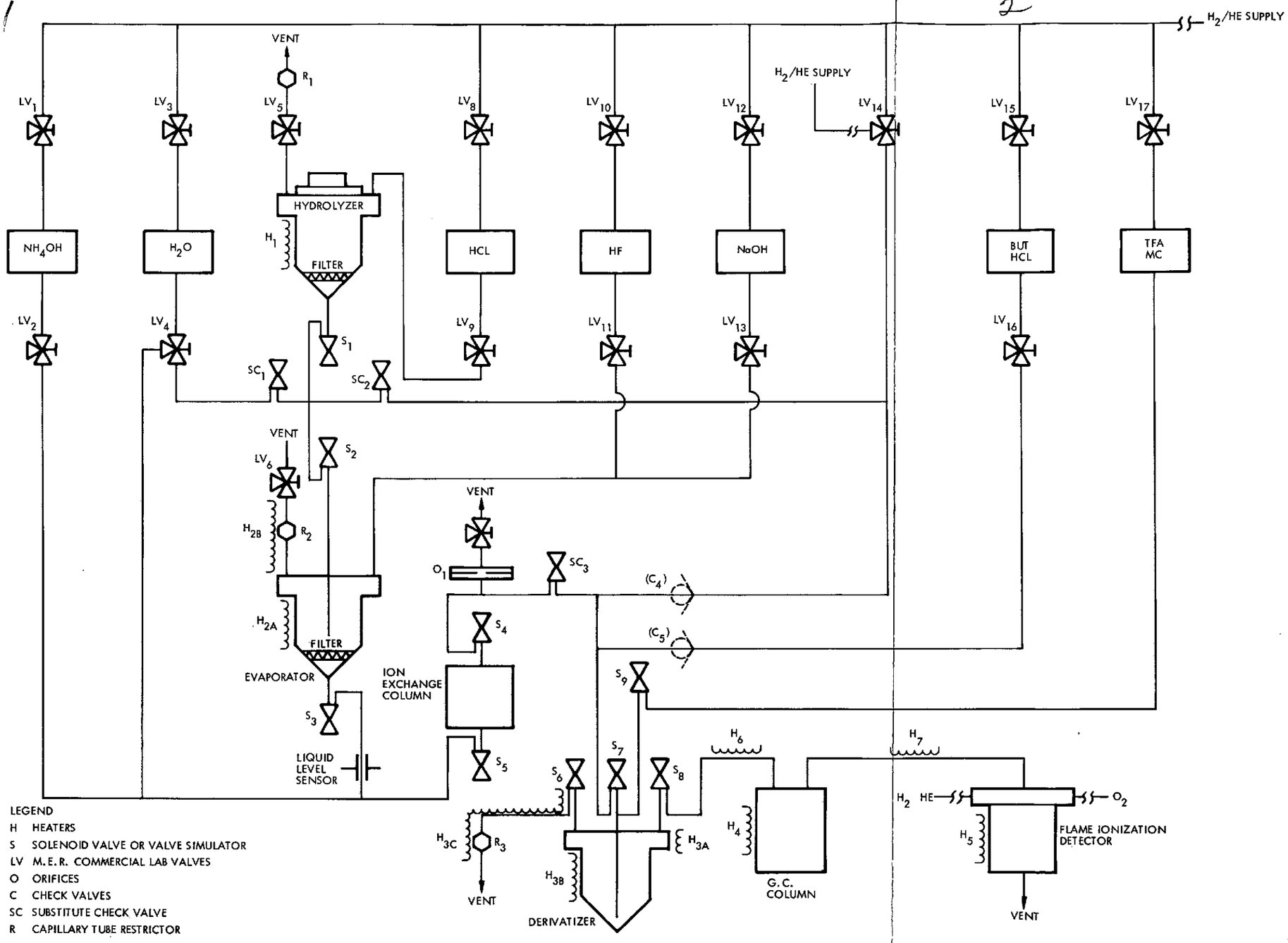

Figure 3-1. Wet Chemistry Prototype Instrument Schematic (Initial System Test Version)

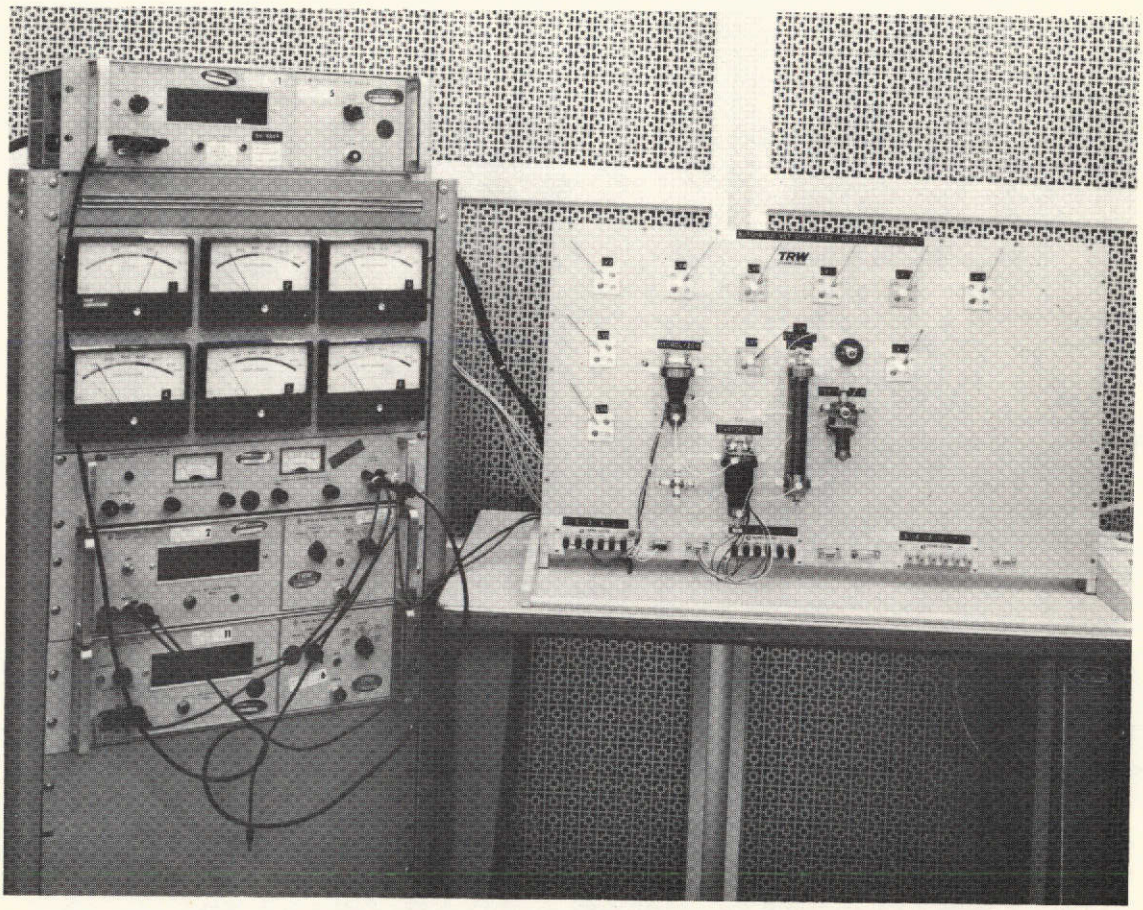

Figure 3-2. Wet Chemistry Instrument Breadboard

evaporator, ion exchange column and derivatizer) were mounted on the front side of the panel. All of the hand valves required for running an analysis were operated from the front panel. Service valves, lines, reagent injectors and electrical connectors and take-apart fluid joints were provided for each test cell to permit cell removal for cleaning and servicing even while an analysis was in progress elsewhere on the breadboard. Soil loading was accomplished manually through the top plate of the hydrolyzer.

The breadboard could be used with either a flight prototype self-heated gas chromatographic column and flame ionization detector or with a commercial gas chromatograph.

3.1.1 Breadboard Component Design

The breadboard component design and test results reported herein are directly applicable to the new flight design concept even though the breadboard was designed for the Processing Sequence No. 1. The flight design described in Section 4 of this report is based on the Processing Sequence No. 2, which has many advantages including easier filtration requirements, fewer and less corrosive reagents, and significantly less interfering material, especially from the smaller ion exchange column. Differences between the two processing sequences, however, do not signficantly impact the engineering design of the flight instrument components, even though the function of the first two cells is changed somewhat.

3.1.2 Hydrolyzer

The design of the breadboard hydrolyzer is shown in Figure 3-3. The processing cell in the new flight design that uses the same internal geometry and material is the extractor.

Some features peculiar to the breadboard hydrolyzer are the increased wall thickness to increase life and to facilitate serviceability. The soil inlet port accepts a closure plate in place of a gas actuated cover plate since the soil is loaded manually, and the valve at the outlet is a hand-operated solenoid valve simulator. The fluid joint of the inlet port for the HCl calibration amino acid injection is an M.E.R. commercial

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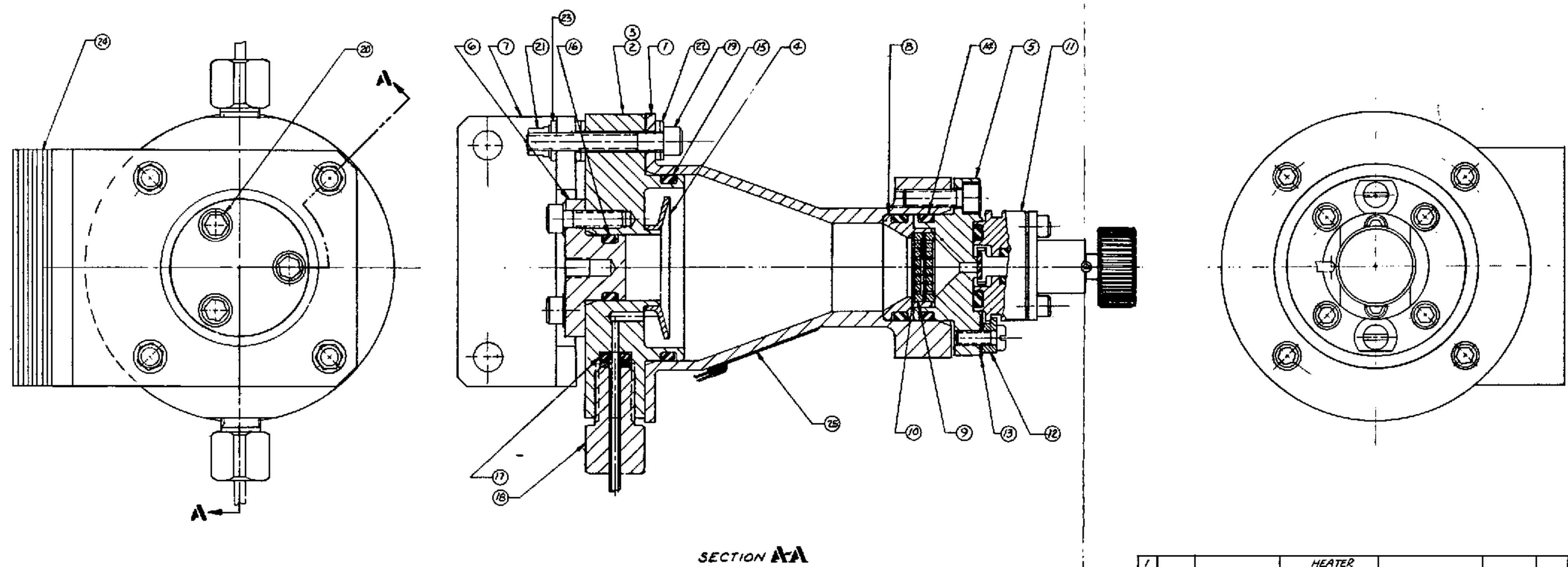

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16			FIBER WASHER	#4	HW6302			23
11		NAS620C4L	WASHER	#4	HW1240			22
4		MS21043-04	NUT	4-40 LOCK CRES	HN31287			21
4			SOC.HD. CAP SCR	4-40x.38 LG CRES	HS58025			20
7			SOC.HD. CAP SCR.	4-40x.875 LG CRES	HS58026			19
2			MER FITTING					18
2		PARKER #2-004	O RING	VITON V747-75				17
1		PARKER #2-011	O RING					16
1		PARKER #2-023	O RING					15
2		PARKER #2-015	O RING	VITON V747-75				14
2		AN960ZL	WASHER	#2 LIGHT-CRES	HW1012			13
2	06775	Z0405Z-325	MINI-SYNCLAMP					12
1		X409972-1	HAND VALVE ASSY					11
2		-20	FILTER DISC					10
2		-19	FILTER PLATE					9
1		-18	FILTER HOLDER					8
1		-17	MOUNTING PLATE					7
1		-16	HEAD CAP					6
1		-15	VALVE PLATE					5
1		-14	DEFLECTOR					4
1		-13	HEAD PLATE					3
1		-12	HEAD ASSY					2
1		-11	BODY					1
-1								

PARTS LIST

DRAWN L.B. GODDARD

TRW

HYDROLYZER ASSY
AWCI BREADBOARD

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Figure 3-3. Hydrolyzer Assembly

fitting to allow easy removal of the cell from the breadboard. Hydrolyzer parts and the assembled breadboard cell are shown in Figure 3-4.

3.1.3 Evaporator

The breadboard evaporator is shown in Figures 3-5 and 3-6. The processing cell in the flight design that uses the same internal geometry and material is the hydrolyzer/evaporator.

Features peculiar to the breadboard evaporator are again the increased wall thickness to increase life and to facilitate serviceability, the hand operated solenoid valve simulators at the inlet and outlet, and the commercial M. E. R. fittings at the fluid joints to allow easy removal of the test cell from the breadboard. The evaporator layout in Figure 3-5 does not show modifications to the cell described in Section 3.2.1.2. After initial testing a cylindrical tantalum section was added between the cell body and the head end plate to increase the volume of the cell by 24 milliliters. The purge tube was lengthened accordingly. In addition, a tantalum spacer was installed downstream of the filter stack to reduce the volume between the filter and the outlet valve.

3.1.4 Ion Exchange Column

The ion exchange column (Figures 3-7 and 3-8) consisted of a tantalum cylindrical section with removable, flanged end pieces and Creavey Seals to facilitate repacking and experimentation with different spring loads on the packed resin bed. The only non-tantalum materials were the Teflon filters used as bed retainers and the Teflon-coated retainer spring at the IEC inlet. The size of the resin bed was 30 milliliters. Thirty milliliters of resin was required to achieve adequate desalting in the No. 1 Processing Sequence in which HCl hydrolysis was performed on the soil. Hand-operated valve simulators were used at the inlet and outlet to facilitate column reuse. The fluid joints were commercial M. E. R. fittings.

3.1.5 Derivatizer

The breadboard derivatizer layout is shown in Figure 3-9. It consisted of an all tantalum cell with flanged head end accommodating four hand-operated valve simulators. Originally the derivatizer was outfitted with the prototype, hand-operated valve simulators which were used

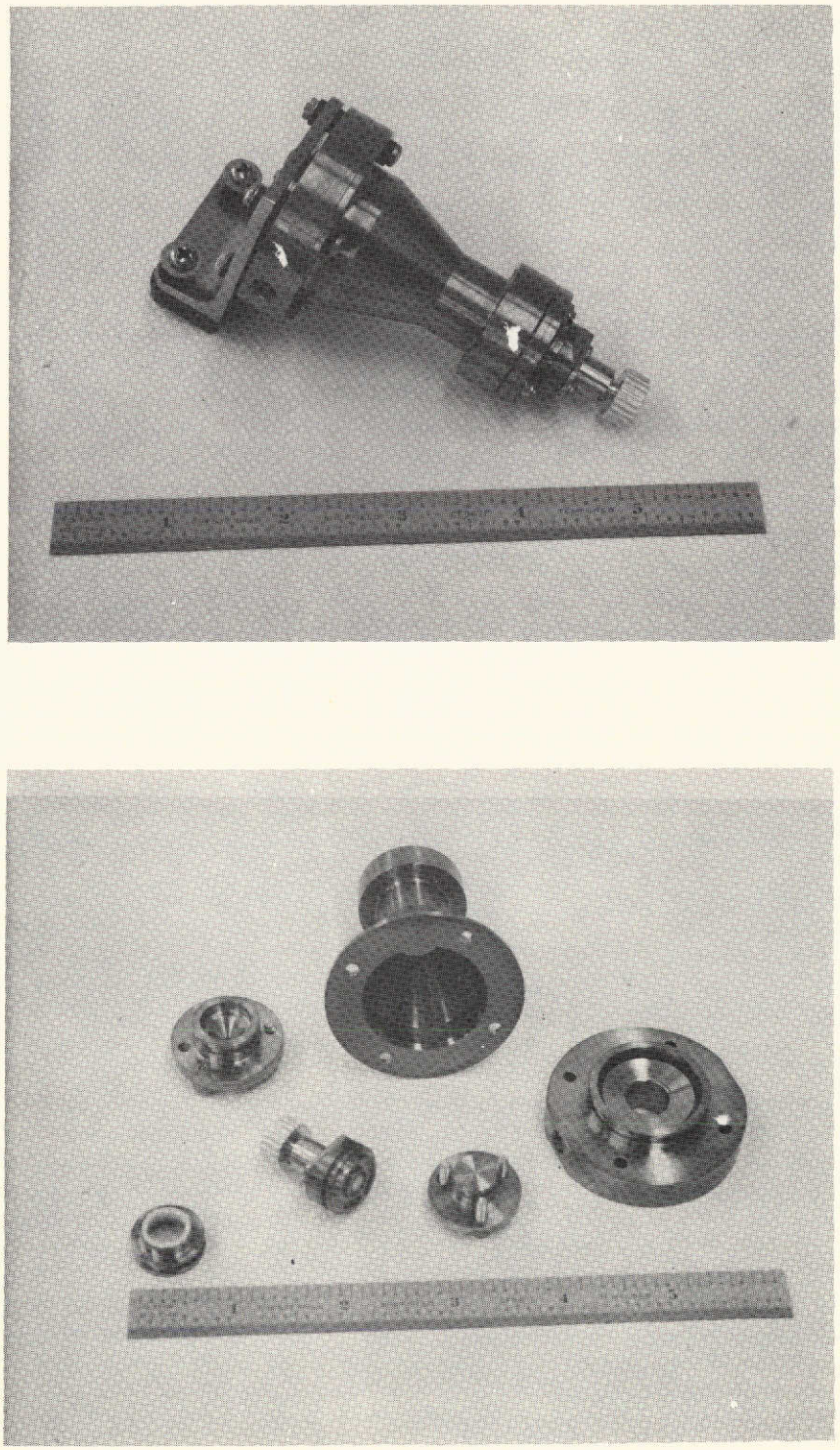

Figure 3-4. Photographs of Breadboard Hydrolyzer

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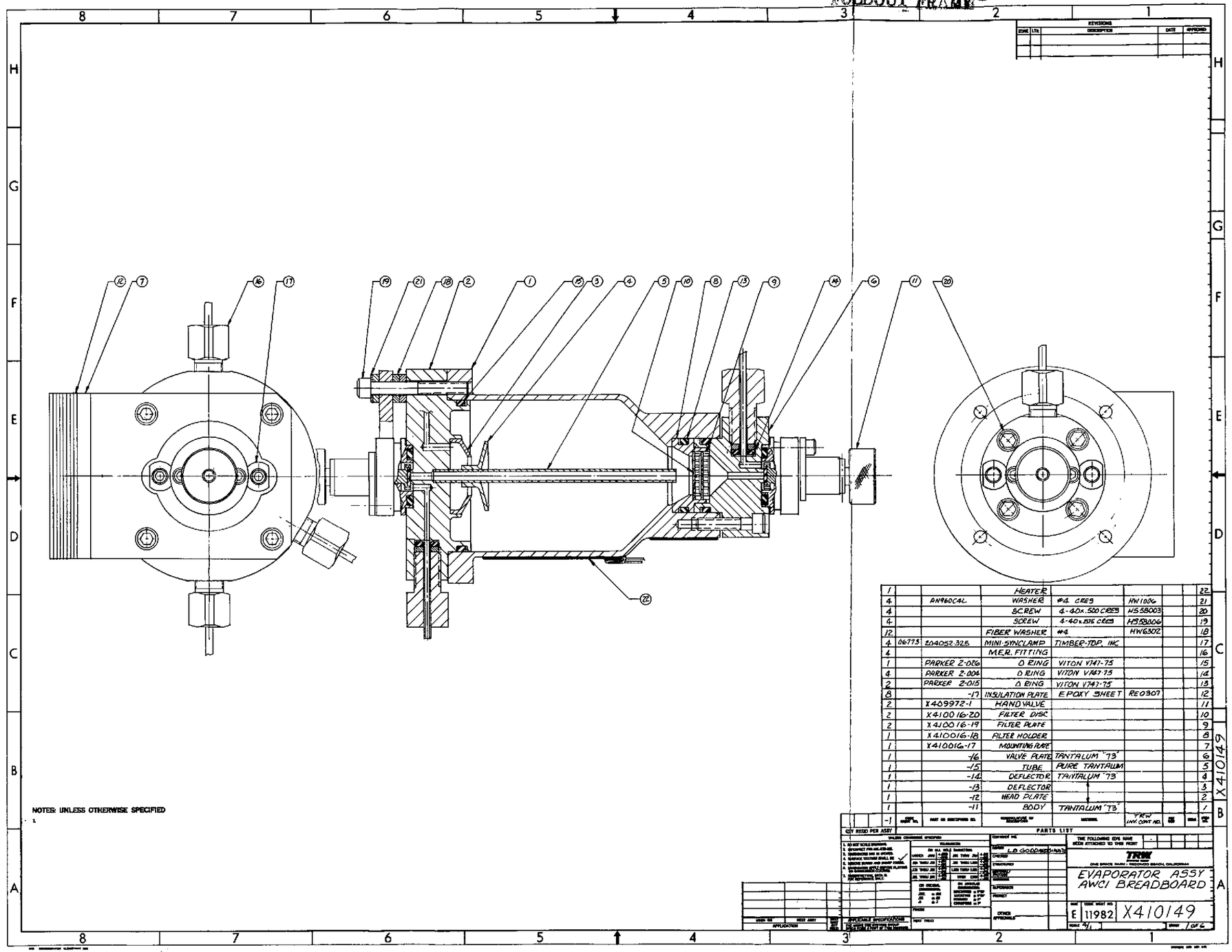

Figure 3-5. Evaporator Assembly

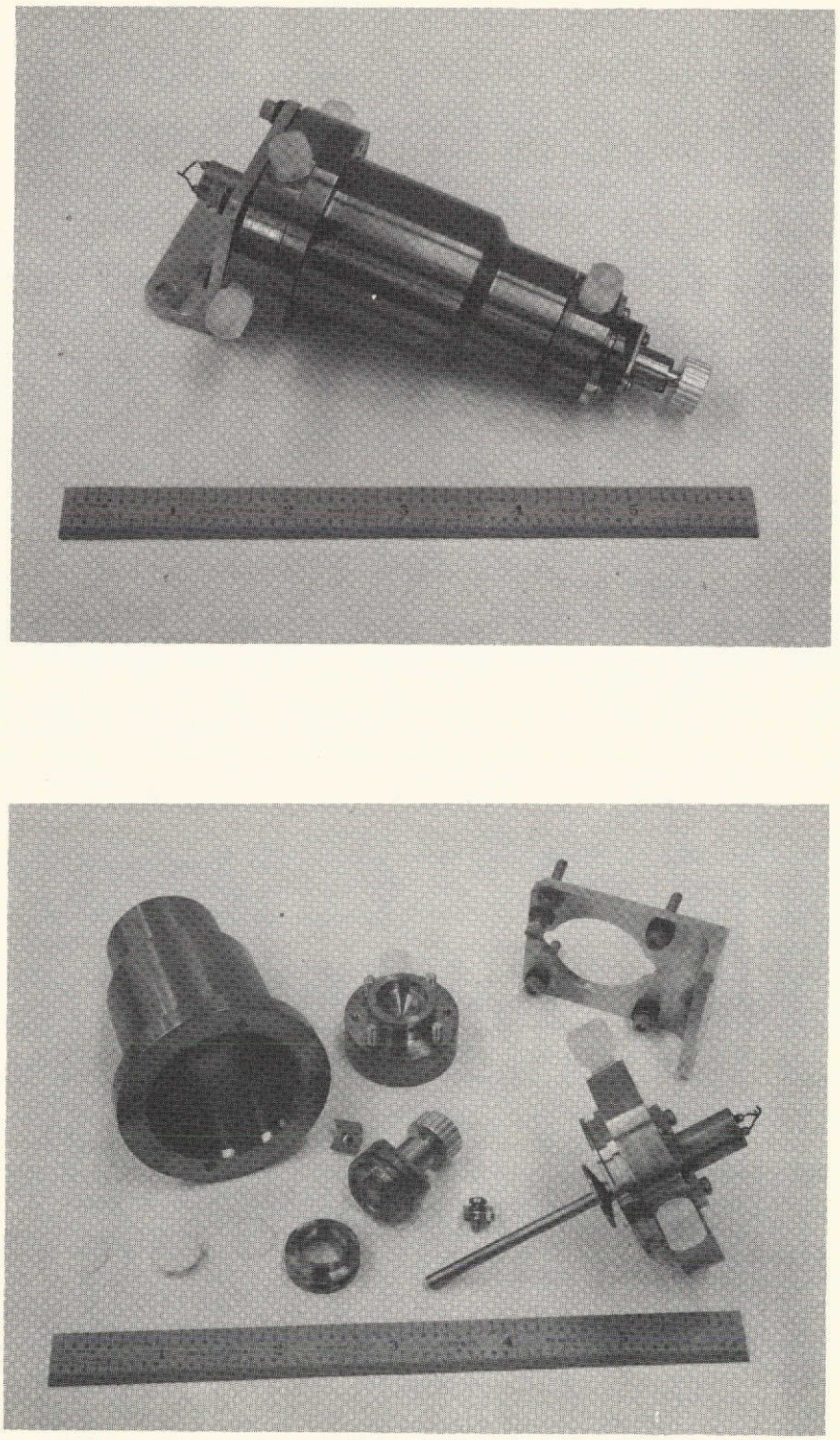

Figure 3-6. Photographs of Breadboard Evaporator

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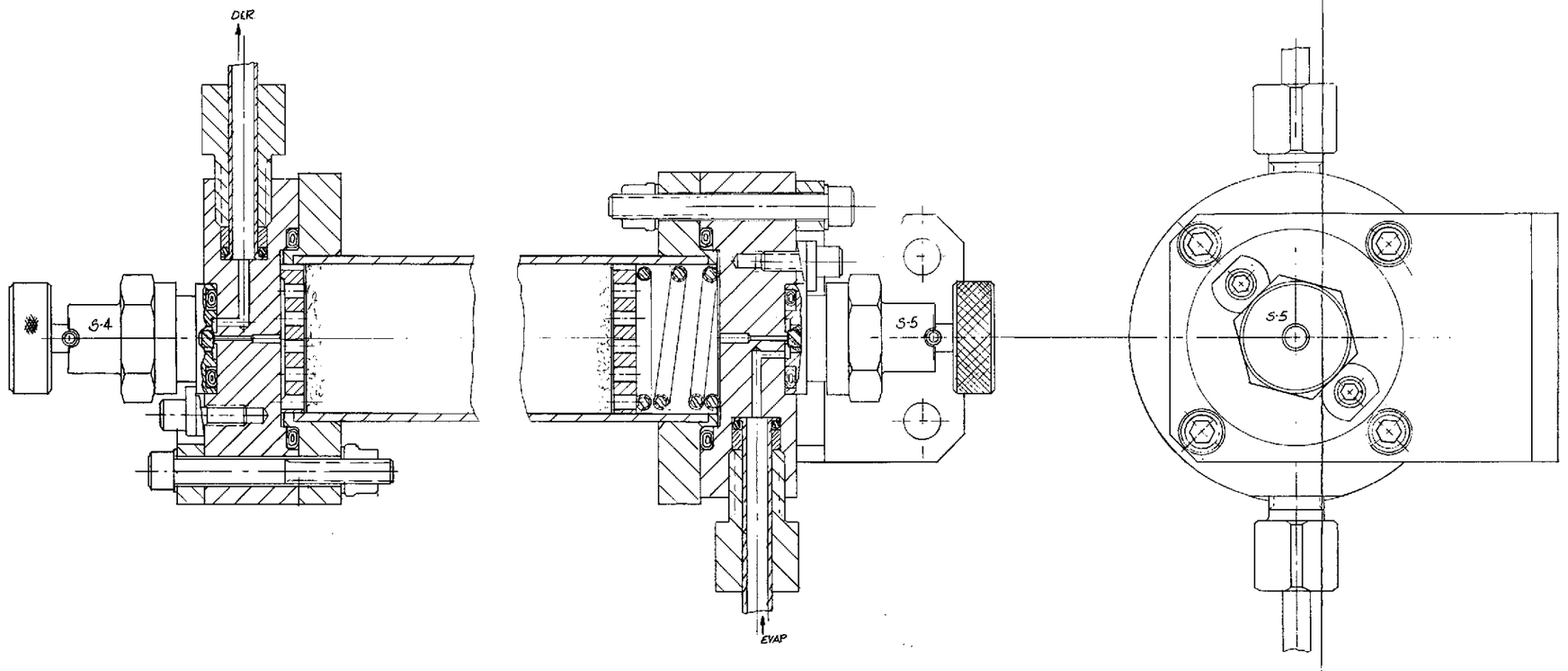

NOTES: UNLESS OTHERWISE SPECIFIED

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PARTS LIST

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4. SURFACE TEXTURE SHALL BE ✓
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.014 THRU .129	+.004 −.001	.751 THRU 1.600	+.010 −.001
.126 THRU .250	+.005 −.001	1.600 THRU 2.600	+.012 −.001
.253 THRU .500	+.006 −.001	OVER 2.600	+.015 −.005

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ENGINEER

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BREADBOARD

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Figure 3-7. Ion Exchange Column Layout

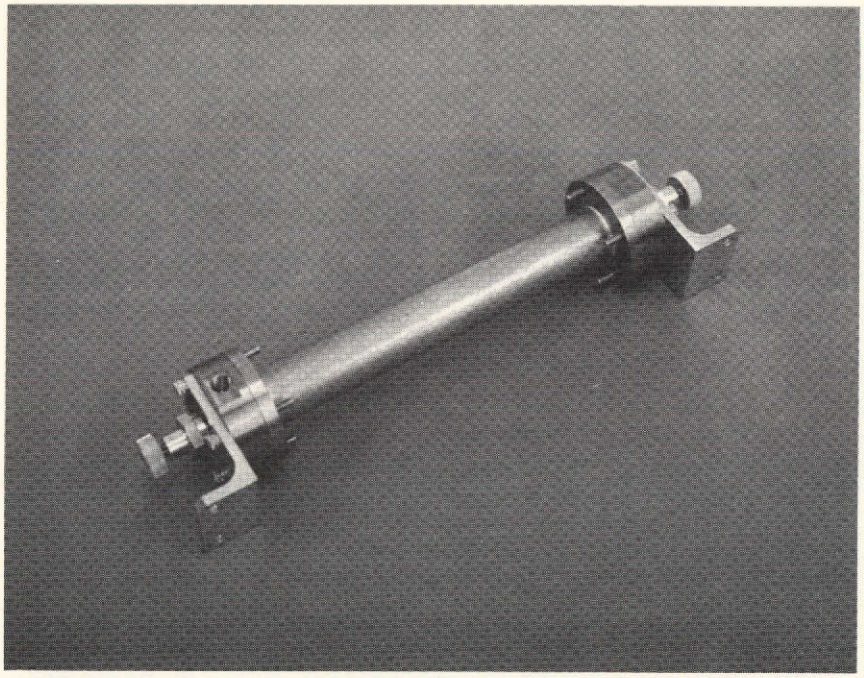

Figure 3-8. Prototype Ion Exchange Column

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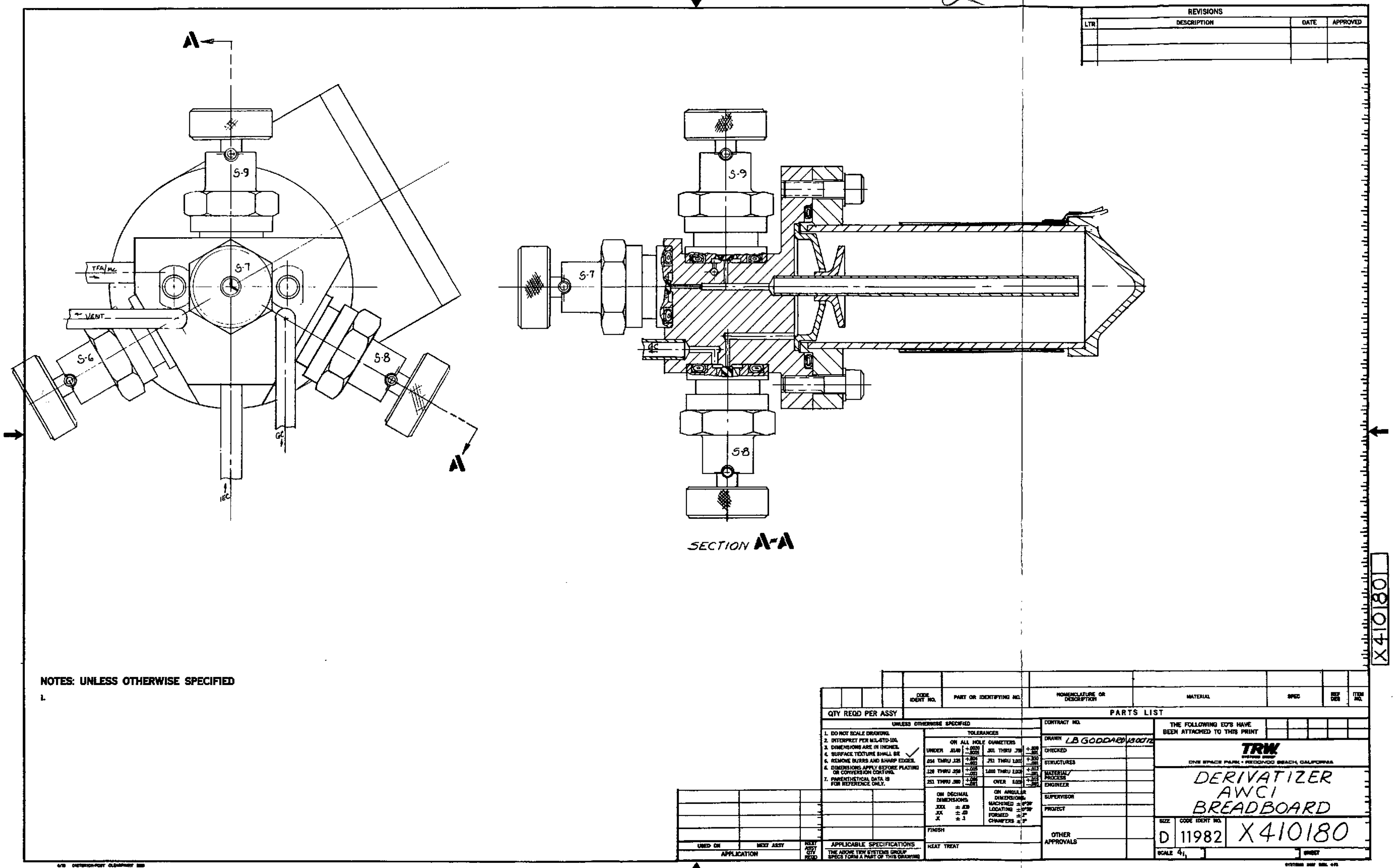


Figure 3-9. Derivatizer Layout

successfully on the other test cells and the IEC. It was determined, however, that the valve seals on the derivatizer were responsible for high background peaks in the gas chromatogram. The seal problem was rectified by the use of hand-operated all tantalum diaphragm valves with Teflon seats. Figure 3-10 shows the breadboard derivatizer with the all tantalum valves.

3.1.6 Valves

Valves proposed for the original flight design (TRW Final Report No. 16660-6001-R0-00) were modified VLBI solenoid valves with tantalum front end for reagent compatibility. The valves used successfully on the breadboard hydrolyzer, evaporator and ion exchange column were hand operated solenoid valve simulators. The valve design concept was based on available compatibility data which indicated that Viton elastomer was satisfactory for all reagents except ammonia. Viton was also a preferred selection because of its good high temperature characteristics. Figure 3-11 is a cross sectional schematic of the valve assembly. The valve housing was fabricated of 316 Stainless Steel. The center shaft (pintle) and valve body were machined from Tantalum. The poppet seal was Teflon and the elastomeric seals were Viton E-60C initially. The shaft seal was later replaced by an EPR O-ring and the static seal at the base of the valve was changed to a Creavey seal. Figure 3-12 shows several assembled hand-operated solenoid valve simulators.

Because of contamination problems on the derivatizer and after extensive valve and system studies, a gas-actuated tantalum diaphragm valve was selected for the new flight design, and hand-operated, all tantalum prototype diaphragm valves with Teflon seats were constructed and used on the breadboard derivatizer. The hand-operated prototype tantalum valve is pictured in Figure 3-13 installed on a test fixture. Figure 3-14 is an exploded view of the component parts. A cross sectional schematic is presented in Figure 3-15. The design is the result of several iterative attempts to machine an integral diaphragm and housing that had a sufficiently low force/stroke ratio to be functional. Because of the relatively high spring rate of the machined diaphragm a screw jack is utilized to actuate the valve to the open positions. The trapped knurled nut is

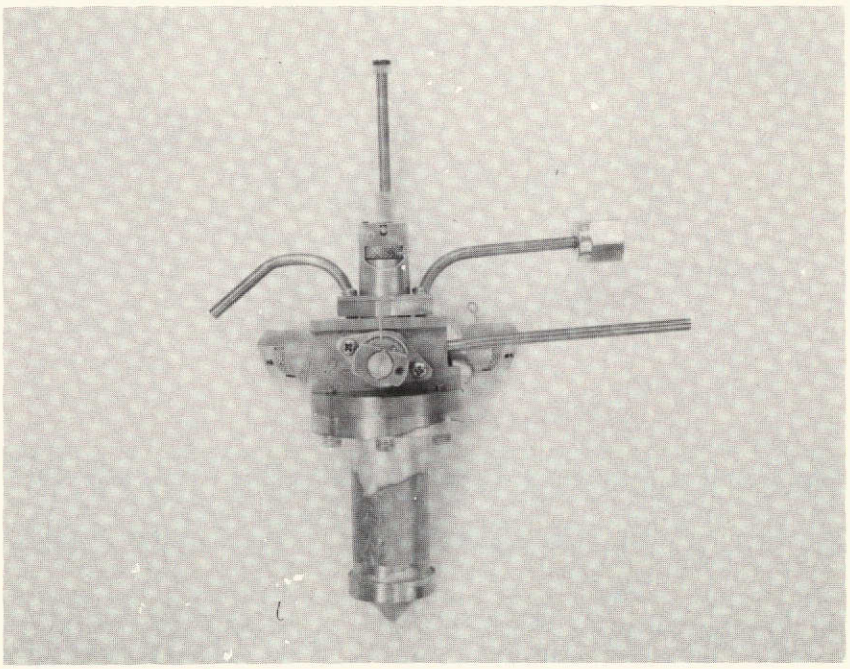


Figure 3-10. Prototype Derivatizer, Shown With Hand-Operated Tantalum Diaphragm Valves

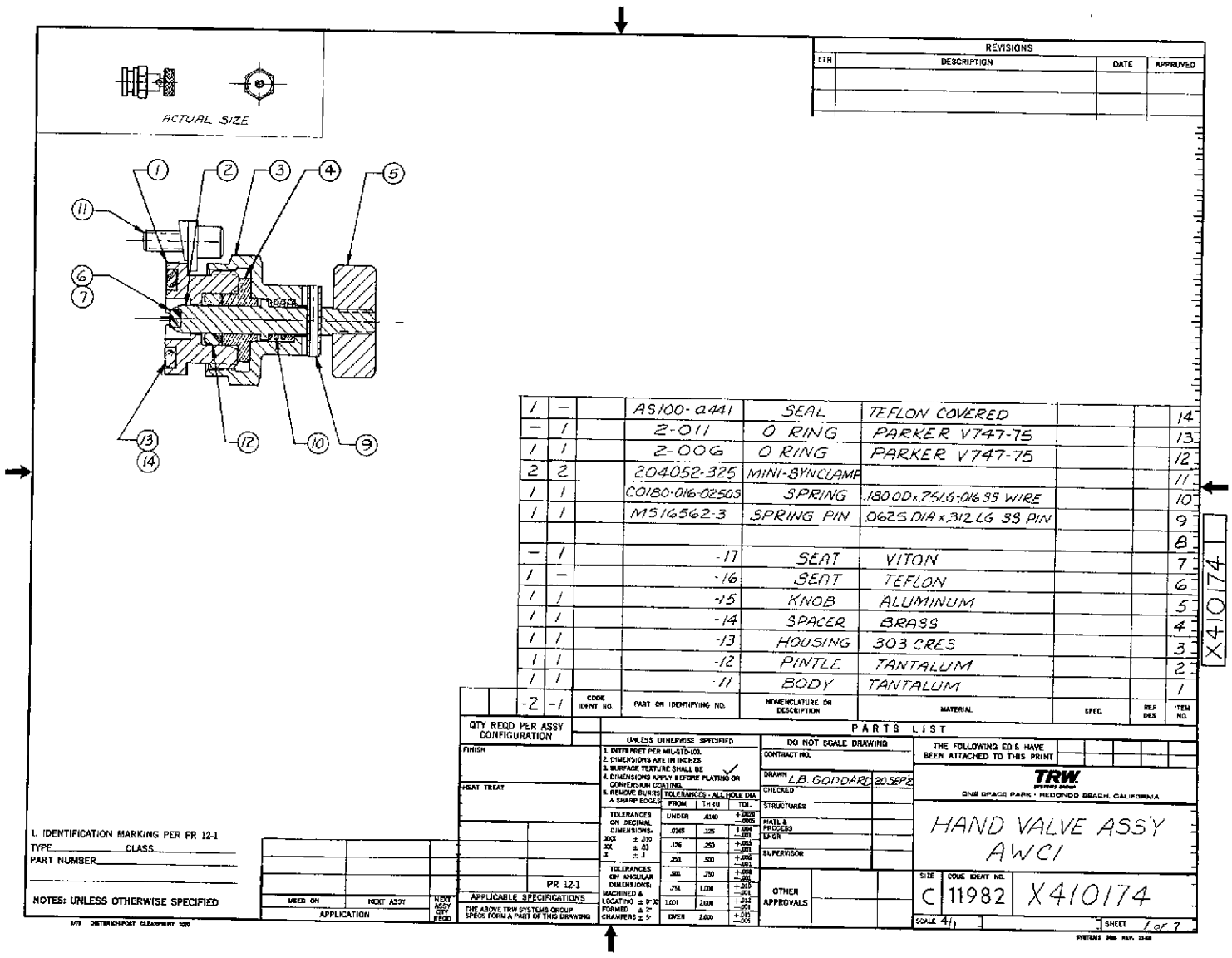


Figure 3-11. Solenoid Valve Simulator Assembly

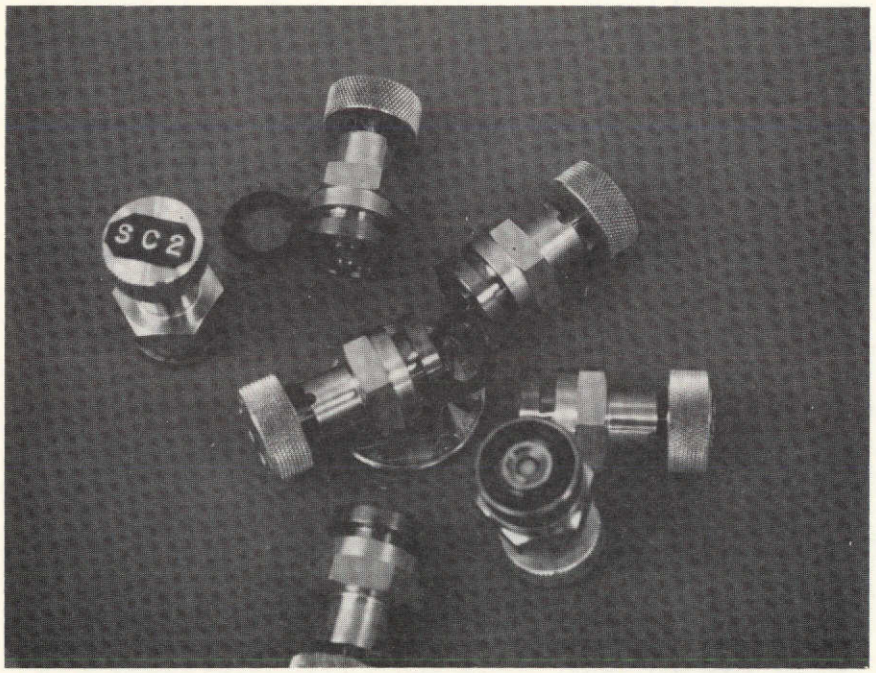
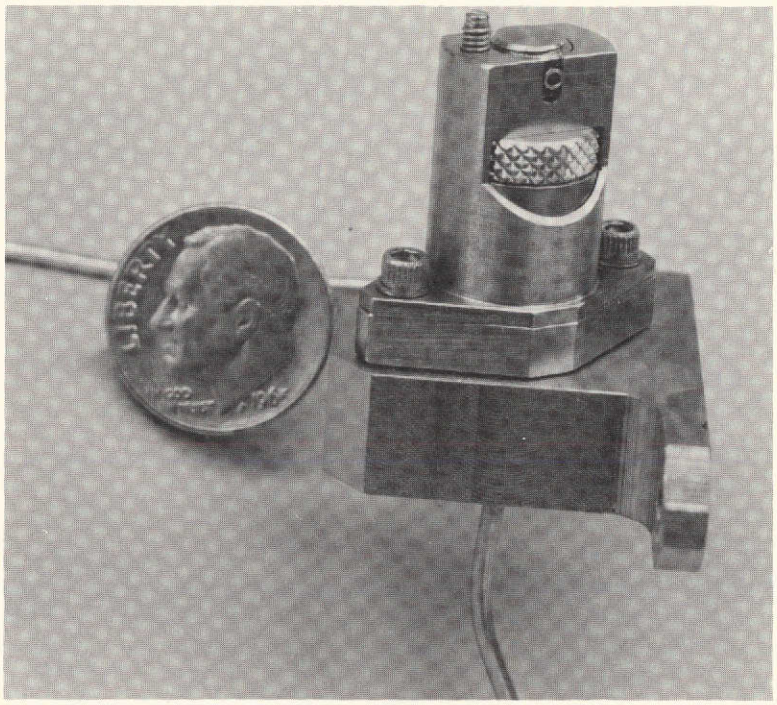
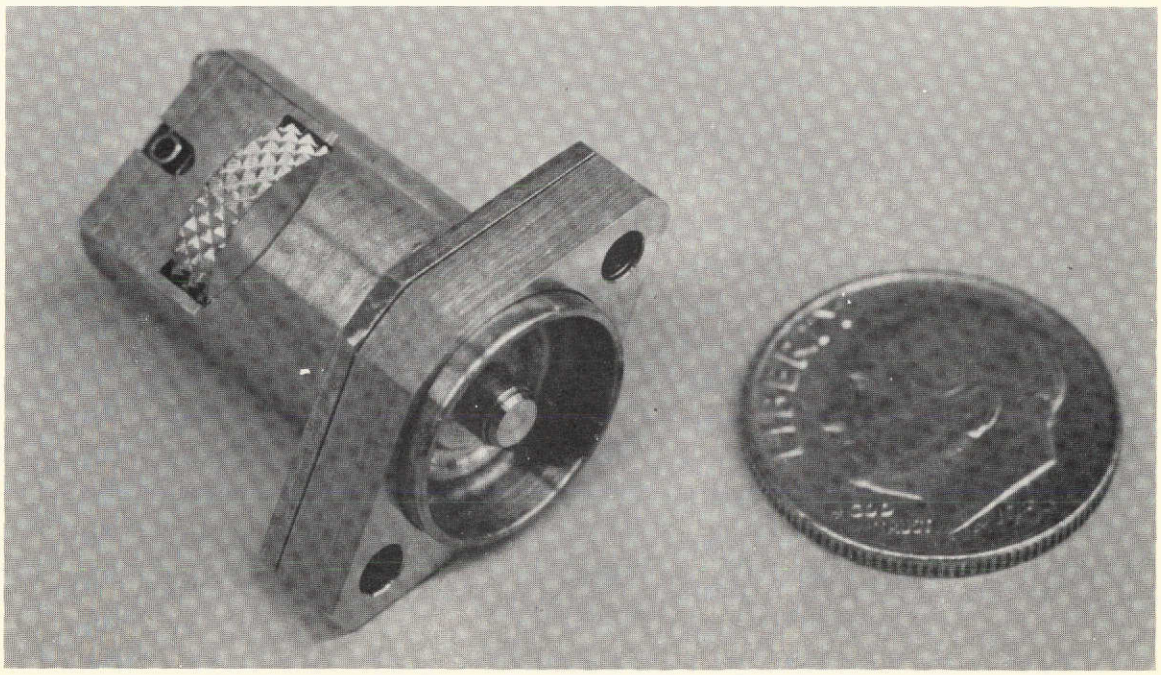


Figure 3-12. Hand-Operated Solenoid Valve Simulators



(A)



(B)

Figure 3-13. Tantalum Diaphragm Hand Valve

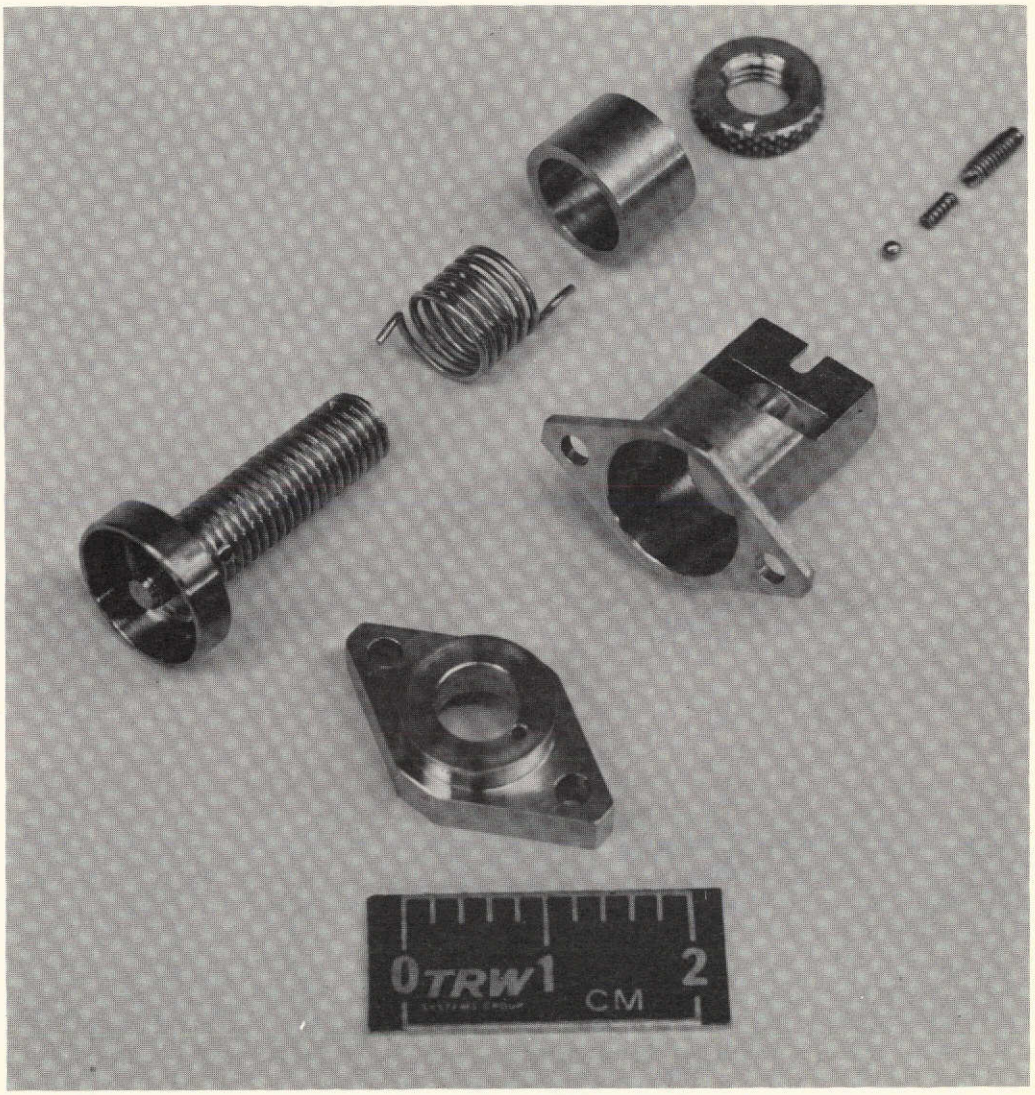


Figure 3-14. Tantalum Diaphragm Hand Valve Disassembly

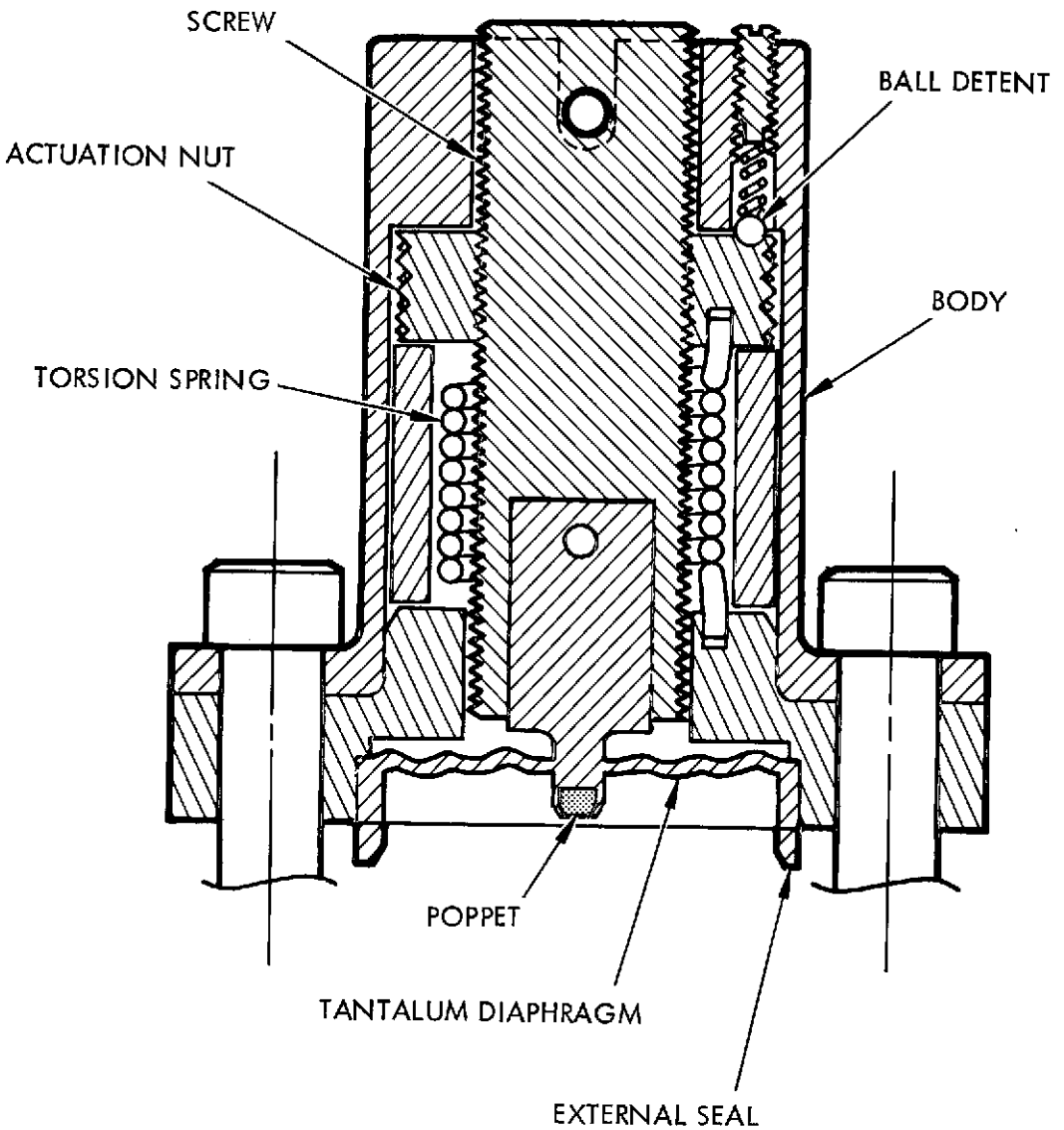


Figure 3-15. Tantalum Diaphragm Hand Valve Layout

rotated approximately 90 degrees which lifts the screw and attached poppet seal. A preloaded torsion spring serves to return the poppet and maintain seal load on the valve seat when in the closed position. A ball detent located at the top is provided to hold the nut in the open position. Figure 3-16 is a picture of the head end of the derivatizer with the four valves in place. As can be noted, a small lever has been added to the actuator nut to facilitate operation.

3.1.7 Self-Heated GC Column

The breadboard self heated gas chromatographic column is shown in Figures 3-17 amd 3-18. The column assembly contains 200 feet of 0.062-inch OD by 0.030-inch ID 316 stainless steel tubing covered with a 0.012-inch-thick Teflon tube to provide an electrically resistant coating between adjacent coils. The column is wound on an aluminum mandrel and installed in a thin-walled aluminum can. The can is packed with aluminum oxide spheres (hollow) for thermal insulation. Commercial feedthroughs are used for the thermocouples and power return electrical lines. The thermocouples will provide measurement of longitudinal and radial thermal gradients in the wound column. Power taps are located at the center and ends of the column. Special insulated fittings which are attached to the column with commercial swageloks are used to connect to the derivatizer and detector.

This design differs from the flight version in the final report to NASA/ARC Contract No. NAS2-6218 in several respects. For convenience, Teflon tube is used for the column insulator instead of an insulating paint such as conventionally used for solenoid coils. The column is wound on an aluminum spool instead of directly on the packed insulating material (Fiberfrax) in the flight design. This is to facilitate column replacement. The column tubing used is thick-walled, commercially available chromatographic grade tubing in place of the special order thin-walled tube in the flight design.

3.1.8 Reagent Injectors

In the breadboard, cylinders of Kel-F were used to contain the reagents. For those applications where the volume of the injector was important (such as for HCl, NaOH or NH_4OH, HF, or TFA/MC), the breadboard injector internal dimensions were like those for the previous

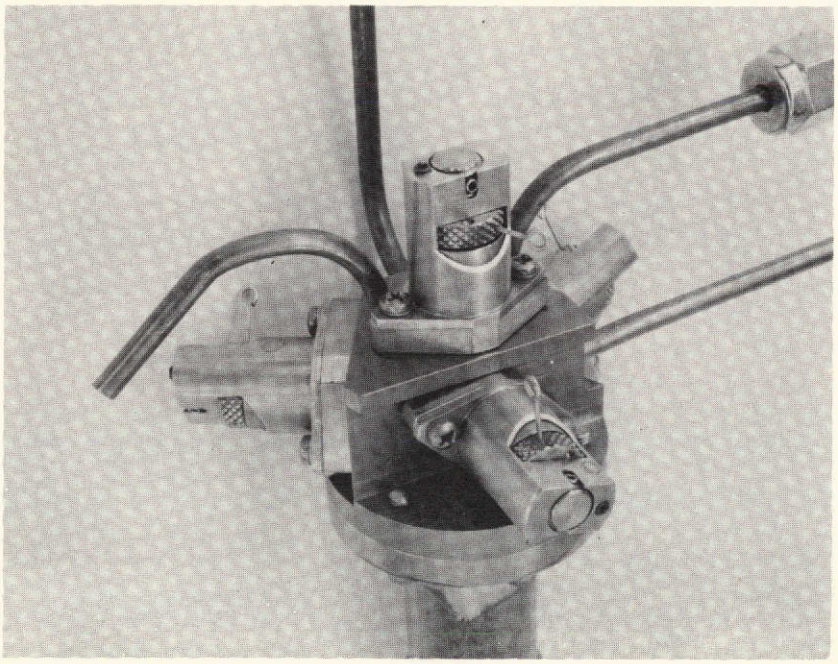


Figure 3-16. Derivatizer Head End With Tantalum Hand Valves

FOLDOUT FRAME

1

FOLDOUT FRAME

2

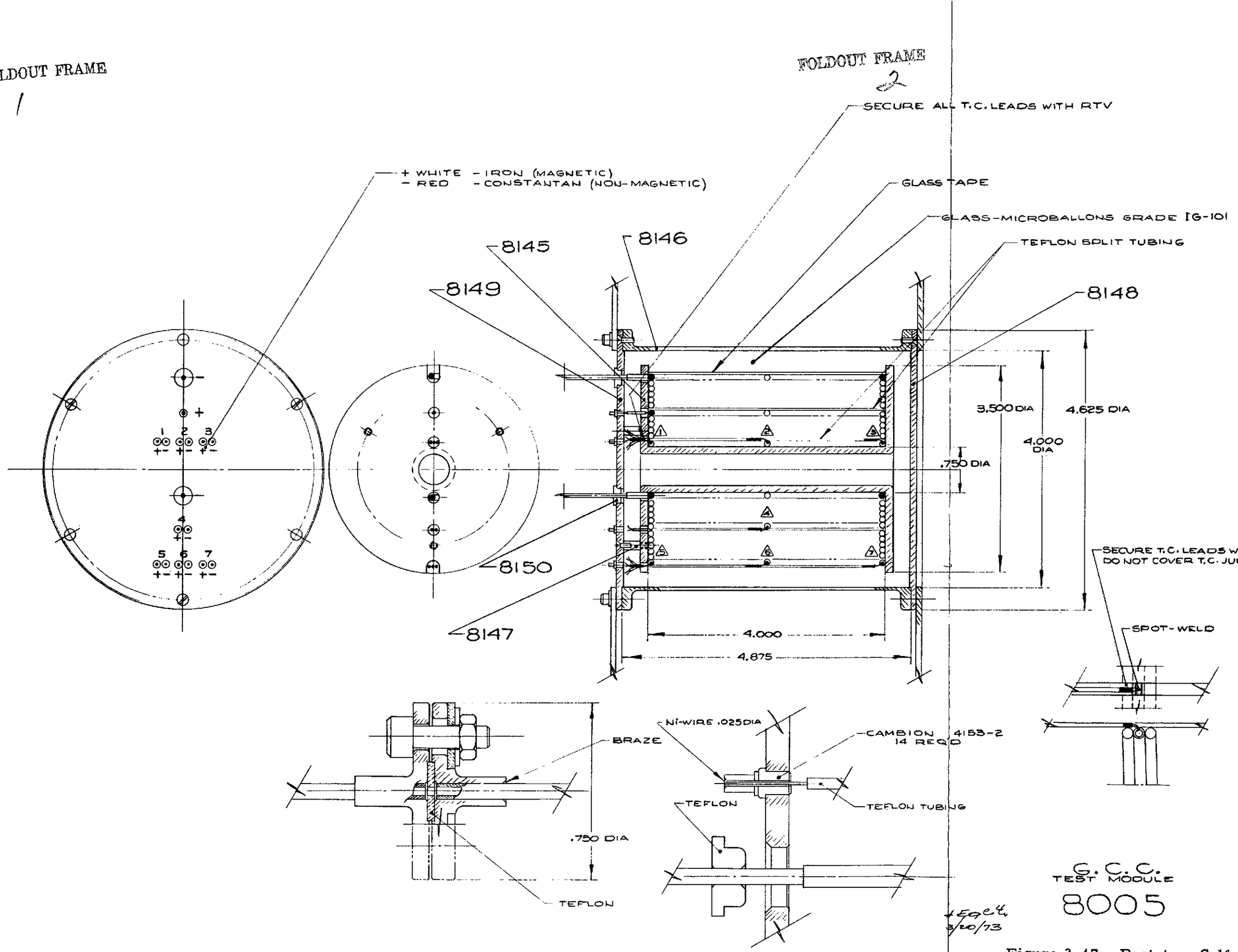


Figure 3-17. Prototype Self-Heated Gas Chromatographic Column Design

Figure 3-18. Self-Heated Gas Chromatographic Column

flight design. M. E. R. commercial laboratory valves were used instead of the one-shot gas-actuated isolation valves for reasons of both cost and serviceability. M. E. R. valves were also used at the upper end of the injectors to provide fill ports for reagents and pressurant gas.

3.1.9 Flame Ionization Detector

A laboratory type flame ionization detector in a commercial gas chromatograph was used for the breadboard testing.

3.2 BREADBOARD TEST RESULTS

Major results of the breadboard test program can be summarized as follows:

1) No major problems were encountered with the hydrolyzer and evaporator cells. Test results with both cells were comparable with laboratory glassware results, and a high degree of confidence in the adequacy of the design and compatibility of the basic cell material (tantalum) has been established. Furthermore, there are indications that the design and materials will function even better with Process No. 2.

2) NH_4OH was found to be superior to NaOH in neutralizing HF in the precipitation step because of the greater ease of attaining the desired pH with NH_4OH.

3) Tests with a 30-milliliters Biorad AG 50W-x8 (Dowex 50W) 200-400 mesh ion exchange column (IEC) showed better performance than equivalent glassware columns. However, the ion exchange resin was identified as a major soure of interfering materials, leading to interference in the gas chromatographic analysis. The need for cleanup immediately prior to use via NaOH elution followed by HCl regeneration was established. The use of a 5-milliliter ion exchange column with with Process No. 2 should greatly decrease the amount of interfering material produced during ion exchange desalting.

4) The complete absence of observable racemization effects anywhere in the breadboard was demonstrated.

5) Some material incompatibilities were identified during testing of the derivatizer; e.g., elastomeric valve seals and the Creavey Seal used for the cell seal contributed to high background. These problems were rectified by using all tantalum valves with Teflon seats and a tantalum/Teflon cell seal.

   Some interference problems remain however, which are associated with heating the derivatizer to evaporate the

derivatives, obtained from samples derived from soils processed in the breadboard system, onto the GC column. At least one solution does exist for this problem.

6) Successful derivatizations of amino acid mixtures (25 nanomole levels) were performed after extensive cleaning and vacuum bakeout of the derivatizer, although some interference was still present in most tests.

7) Additional components that were identified as contributors of interferring materials were the Teflon filters and possibly the Teflon/Kel-F reagent containers.

8) Changing from Process No. 1 to Process No. 2 should reduce or eliminate most of the remaining problems and while further work is needed, we have a high degree of confidence that the remaining problems can be solved and that a flight instrument can be produced which will successfully carry out the experiment within the mission constraints.

3.2.1 Component Tests

3.2.1.1 Hydrolyzer

Summary: The hydrolyzer was tested with soil-free blanks, with radiotracer doped Waukena soil and with Ribonuclease on blank soil. All functions of the hydrolyzer were successfully carried out and the test results were comparable to conventional glassware results. Good amino acid recovery (90 percent) and freedom from racemization or corrosion effects were demonstrated. The only problems observed with the hydrolyzer were concerned with the Teflon filters which gave high background blanks even after extensive clean up. The hydrolyzer was still in good working order after more than 20 hydrolyses.

Detailed Test Results: Initial testing of the hydrolyzer consisted of engineering proof, leak, and heater tests. The associated M.E.R. fittings were checked as well. The M.E.R. fittings were hand-tightened only and leak checked at 165 psig. All were bubble tight and all combinations were checked; i.e., M.E.R. fittings in M.E.R. hand valve, M.E.R. fitting in Kel-F injector head, M.E.R. fitting in TRW-machined tantalum components and modified M.E.R. fitting, tantalum tube combination in a tantalum component. The "O" rings in both of these applications can be removed and replaced. Two assemblies consisting of 1/16 Teflon tube and M.E.R. fittings at each end were hydrostatically tested to destruction.

Both failed at approximately 1500 psig with rupture occurring in the Teflon tube. Next, engineering oriented Waukena soil tests were carried out which demonstrated overall functionality. The cell seals performed adequately, with no visible loss of solution occurring during the hydrolysis. Fluid handling techniques were satisfactory including injection of the wash H_2O up through the filter stack. Inspection of the unit indicated no evidence of chemical attack after 45 hours of exposure to 6N HCl at 105° to 110°C. The hydrolysate from one of the above soil tests was carried through the rest of the processing scheme in laboratory glassware. The sample was analyzed by gas chromatography and compared to a sample processed completely in laboratory glassware. The two chromatograms were generally similar with the amino acid concentrations being somewhat larger in the sample hydrolyzed in the hydrolyzer than in the sample hydrolyzed in glassware. Radiotracers (^{14}C labeled amino acids) had also been added to the soil prior to hydrolysis. The net recovery of the radiotracers in the hydrolysate and the wash was 90 percent.

After thorough cleaning, contamination blank was carried out by injecting 7.5 milliliters of 6N HCl into the hydrolyzer and heating for several hours at 100°C. The solution was collected and a 2.5 milliliter portion was evaporated to dryness and derivatized. The derivatization blank for the batch of 2-butanol/HCl in use had become excessively large and precluded observation of trace contaminants below the one nanomole range. The chromatogram for the hydrolyzer contamination blank did not show any observable amino acid peaks and only three non-interfering unidentified contaminant peaks.

The hydrolyzer was then rinsed with redistilled water and loaded with 1 cubic centimeter of the ARC blank soil. One milliliter of aqueous bovine pancreatic ribonuclease (2×10^{-5} M) was added to the soil to provide 20 nanomoles of the standard enzyme. The cell was then closed and 6.5 milliliters of 6.9N HCl was injected to provide a net HCl concentration of 6N for the 7.5 milliliters of solution. Air was removed by pressurizing with He/H_2 and then venting several times. The hydrolyzer was heated to 110°C and held at this temperature for 16 hours, after which the hydrolysate was forced out and collected along with the 5 milliliter H_2O rinse for further processing in laboratory glassware.

Radiotracers consisting of ^{14}C labeled alanine (2 nanomoles), valine (12 nanomoles) and leucine (4.5 nanomoles) were added to the hydrolysate before continuing the processing in laboratory glassware in order to monitor losses in the subsequent steps.

While the hydrolysis was being carried out, a set of controls was hydrolyzed in laboratory glassware. These controls were: (1) 1 cubic centimeter of blank soil, 20 nanomoles of ribonuclease, (2) blank soil without ribonuclease, and (3) 20 nanomoles of ribonuclease without soil. After hydrolysis, the ribonuclease sample without soil was evaporated to dryness and derivatized without further processing. The other two laboratory samples were filtered as usual and then the same radiotracers as above were added to the combined filtrates and rinsings for the two laboratory hydrolysates. The three solutions were then evaporated and carried through the subsequent HF-NaOH and ion exchange processing steps. Radiotracer results are given in Table 3-1. The recoveries are good up to the derivatization step, but significant losses occurred during the derivatization process. Gas chromatograms are shown in Figure 3-19.

Comparison of the chromatograms indicates that the hydrolysis in the hydrolyzer was equivalent to the hydrolyses in laboratory glassware.

After the hydrolysis was completed, the hydrolyzer was disassembled for cleaning and inspection. The Teflon filters had inadvertently been left out. However, very little soil escaped the hydrolyzer because of the small orifice size in the valve and because of the tendency of the soil to act as a filter. It is doubtful that the absence of the filters had any deleterious effects on the experiment. Inspection of the hydrolyzer showed no changes in appearance.

Further testing with the hydrolyzer indicated that a small leak had developed in the outlet valve (S-1). This leak may have been a result of a small amount of soil passing through the valve. Valve rework cured the problem. The S-1 valve is different from the other valves in that it has a larger poppet and hence has less sealing pressure. It is recommended that if this unit is to be used in the future that the unit be reworked to accept valve simulators with the smaller poppet.

Table 3-1. Radiotracer Recoveries for Hydrolyzer Test with Ribonuclease ARC Blank Soil Labeled After Hydrolysis with Alanine, Valine, Leucine, Total cpm 1,036,000

	Breadboard Processed Soil + RNase (20 nmole)		Lab Processed Soil + RNase		Lab Processed Soil, No RNase	
	cpm*	% original cts.	cpm*	% original cts.	cpm*	% original cts.
Combined filtrates after HF/NaOH step	1,001,000	96.6	988,000	95.4	967,000	93.3
Ion Exchange						
Precut	5,790	0.6	5,360	0.5	21,160	2.0
Prime filtrate	1,052,000	101.5	1,021,000	98.6	951,000	91.8
Post cut	1,670	0.2	1,250	0.1	1,240	0.1
Derivative**	601,000	58.0	515,000	49.7	514,000	49.6

*cpm corrected for background and for volume sampled

**After evaporation of TFA/CH_2Cl_2 and dissolution in CH_2Cl_2

HYDROLYZER TEST

1 CC "BLANK" SOIL PLUS 20 NANAMOLES RIBONUCLEASE HYDROLYZED IN HYDROLYZER WITH REMAINING PROCESSING CARRIED OUT IN LAB GLASSWARE. CHROMATOGRAM RUN ON CARBOWAX 20M COLUMN PROGRAMMED 100° TO 200°C AT 2°C/MIN. 1.5% INJECTED.

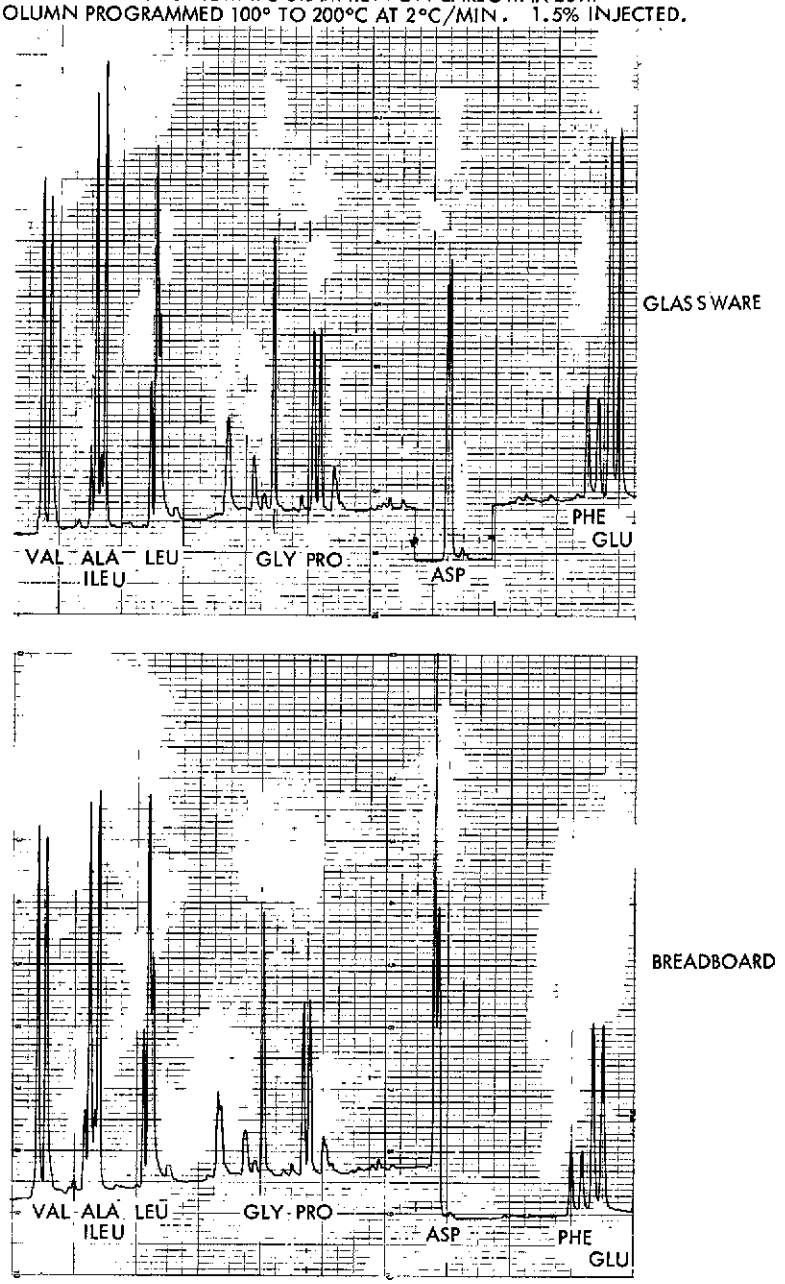


Figure 3-19. Hydrolyzer Test Chromatogram

Subsequent work with the hydrolyzer including tests for possible racemization effects was either in conjunction with other units as a part of various levels of system tests or as a part of filter material tests. The results of subsequent testing are discussed in the appropriate sections.

In going from Process No. 1 to Process No. 2 the temperature and pressure are higher, but the exposure time is much shorter. Also, the environment is less corrosive. Otherwise, the same basic functions are required of the processing cell. Thus, the hydrolyzer used for Process No. 1 is expected to work very well as the extractor for Process No. 2.

3.2.1.2 Evaporator

Summary: The evaporator was tested with soil-free blanks, with radiotracer doped standard soil hydrolysate, and with hydrolysates from soils hydrolysed in the hydrolyzer. After some small modifications in the unit after initial engineering tests, all functions of the evaporator were successfully carried out, and the test results were comparable to results obtained in parallel tests with conventional glassware. Much greater ease in attaining the desired pH was obtained by substituting NH_4OH for NaOH in the desalting step. Good amino acid recovery (94 to 100 percent) using either NaOH or NH_4OH in the processing and freedom from racemization effects were demonstrated. The only continuing problems concerned with the evaporator were with the filter stack which required a higher pressure drop than desired to achieve adequate flow, and which gave high background blanks from the Teflon filter material even after extensive clean-up. Despite the large number of tests with HCl, HF, and NH_4OH, no significant corrosion effects were found at the end of the test program after more than 20 HF-NH_4OH (or NaOH) processes and more than 25 HCl evaporations.

Detailed Test Results: Initial testing of the evaporator consisted of engineering proof, leak and heater tests. Two engineering oriented processing tests were carried out on the unit using Waukena soil hydrolysate (processed in the hydrolyzer) doped with ^{14}C labeled alanine, valine, and leucine. Several problems were encountered in these tests both from procedural errors and from deficiencies in the unit itself. Because of the

unavailability of tantalum capillary tubing, a heated stainless steel capillary tube was used for the vent restrictor in these tests. This restrictor plugged during HCl evaporation, and therefore was removed. In the absence of the restrictor, difficulties occurred during evaporation and reagent mixing which resulted in losses into the vent system. This confirmed that a restrictor was required. During the injection of the HF and the NaOH solutions, portions of the reagents went into the vent instead of the cell, apparently because of a flow surge as the last of the reagent entered the cell head end. The problem was more pronounced with the NaOH solution probably because of inadequate volume in the cell. Another deficiency noted was that a portion of the solution escaped the HF/NaOH processing by being trapped in a small volume between the bottom of the filter stack and the outlet valve.

Only one of the two tests was carried to completion. In this test, the pH of the solution pushed out of the bottom of the evaporator after HF/NaOH processing was 5. An additional 0.5 milliliter of NaOH solution was required to reach the desired pH of 9. After pH adjustment, the solution was reintroduced into the cell and pushed out through the filter again. The remainder of the processing and derivatization was completed in glassware. Radiotracer measurements after ion exchange desalting showed 73 percent recovery for the breadboard hardware processing as compared to 92 percent recovery for a sample processed entirely in glassware. This was not surprising in view of the various difficulties encountered.

In contrast to the radiotracer results, gas chromatographic analysis of the resulting derivatives indicated larger yields for most of the amino acids in the breadboard hardware sample. This indicated that the cleaning procedures used prior to this test were inadequate.

Based on the results of these tests, several modifications were made. a tantalum disk with 0.010-inch sharp-edged orifice was fabricated for use as the vent restrictor. This disk was inserted in the bottom of the M. E. R. fitting receptacle in the evaporator head end. The restrictor was retained by the standard M. E. R. tube fitting which was inserted into the receptacle. Since the restrictor was located within the cell head end, it did not require

a separate heater. An additional 24 milliliters of volume was provided by adding a cylindrical tantalum section between the cell body and the head end plate and extending the length of the standpipe. Finally, a tantalum spacer was fabricated and installed downstream of the filter stack to reduce the volume between the filter and the outlet valve.

After the modifications were completed, the evaporator and associated reagent injectors, valve blocks and interconnecting tubing were cleaned by repeated soaking and flushing with 6N HCl. Heat was applied during some of the soak periods. This was followed by repeated rinses with redistilled water. Then a contamination blank was carried out by injecting 7.5 milliliters of 6N HCl into the evaporator. The HCl solution was heated several hours at 100°C and then pushed out of the cell through the filter stack. The solution was evaporated to dryness and the residue derivatized. Gas chromatographic analysis showed a number of the common amino acids, typically at about the 20 nanomole level. In comparison, a similar contamination blank run on the hydrolyzer but without Teflon filters present did not show similar quantities of contaminants. Therefore, it was concluded that the Teflon filters were the source of the contamination. Later tests confirmed this conclusion and demonstrated that these filters continually released contaminants when heated in the presence of HCl and were extremely difficult to clean to an adequate level.

After the above test, the evaporator was washed with water and a processing blank was carried out to test procedures and to obtain further blank information. 7.5 milliliters of 6N HCl and 5 milliliters of H_2O were injected into the evaporator and a nominal evaporation was carried out with vacuum applied to the evaporator vent. Several different temperatures were used during the course of the evaporation; the final temperature was 100°C. No evidence for bumping was observed during the evaporation process which required approximately 2 hours. After standing overnight, 10 milliliters H_2O was injected and the evaporator heated to 60°C. Mixing by gas bubbling was carried out by alternately evacuating the cell and then injecting He/H_2 gas through the center tube. No loss of liquid into the vent was observed as a part of the gas bubbling. After cooling, 9 milliliters of 5N HF was injected and the 5-minute mixing period was carried out by performing bubbling cycles twice each minute. Next, an equivalent

amount of 5N NaOH was injected. Some liquid went into the vent line at the end of this injection. The solution was mixed as before by gas bubbling cycles for 15 minutes. The solution was then pushed out with gas pressure, and the pH checked and adjusted. An additional 0.25 milliliters of 5N NaOH was required to reach pH 8.5, indicating that this quantity of solution was lost or consumed. A yellowish precipitate formed during pH adjustment. The 10 cubic centimeters wash was injected into the evaporator and collected. Its pH was neutral, and it was slightly cloudy. Upon standing, a small amount of white precipitate settled out. The filtrate and the 10 milliliter wash were placed on an ion exchange column by decanting the solutions away from the precipitates.

Derivatization and gas chromatography were completed in laboratory glassware. Amino acids were present, mostly in the 2-nanomole range, although approximately 12 nanomoles each of glycine, aspartic acid and glutamic acid were present. This was significantly better than the previous contamination blank, but still greater than ion exchange blanks, indicating continued release of contaminants by the Teflon filters.

The evaporator was disassembled and inspected. It did not show any visible changes. The unit was reassembled with an additional seal in the filter holder to insure that some of the solution was not bypassing the filter (a possible cause for the precipitate found in the wash solution). The evaporator was washed with a series of 6N HCl flushes. During some of these, "dummy" injections were carried out with the HF and NaOH injectors. It was observed that in some, but not all instances, some liquid blew into the vent line as the last portion of the solution rushed into the cell. Better results were obtained by injecting with the residual headspace pressure rather than by applying constant pressure to the injector, but it was not possible to entirely prevent liquid from going into the vent.

After rinsing the evaporator and the HF and NaOH injectors repeatedly until the washings were neutral, another mixing test was carried out with 9 milliliters of 5N HCl and an equivalent amount of 5N NaOH. The standard procedure was carried out including injection of 10 milliliters H_2O except that some of the times were shortened. Some liquid went into the vent in both the HCl and the NaOH injections. After mixing for a while

by gas bubbling the solution was pushed out through the filter and titrated. Again, additional NaOH was required, approximately 0.5 milliliter this time.

Possible causes of the NaOH discrepancy include loss into the vent during injection and losses from the more viscous NaOH solution clinging to the injector walls. Loss into the vent was probably not the cause. Visual estimates of the quantity of liquid which went into the vent indicated that less than 0.1 milliliter was lost in this manner. Since the NaOH follows the HF into the cell, there is some degree of extra washing of HF into the cell that does not occur for the NaOH, but it is doubtful that this could account for the 0.25 to 0.5 milliliter difference observed. It was decided to add an extra 0.4 milliliter of NaOH to make up for the amount lost.

Next, a soil hydrolysate processing test was carried out using the standard Waukena hydrolysate to which ^{14}C labeled alanine, valine and leucine had been added. The evaporation of the hydrolysate proceeded smoothly without apparent bumping. After the evaporation was complete, the traps in the ancillary vacuum system were washed out and the solution counted. The total radioactivity found in the traps was 0.2 percent of that added to the hydrolysate, indicating negligible loss during evaporation. Processing was continued using the nominal procedure. Because of previous tests which indicated a NaOH discrepancy of approximately 0.4 milliter, the amount of NaOH solution loaded into the injector was increased by 0.4 milliliter over the amount nominally required. The HF-NaOH processing was carried out without difficulty (small amounts of HF and NaOH went into the vent during injection as had occurred in previous tests).

The filtration step after processing was umusually slow with the flow rate being only 0.5 ml/min at 25 psi differential. However, after injecting the wash water and mixing, the wash solution flowed through the filters very rapidly which indicated that a partial filtration blockage occurred during filtration of the processed solution. The uppermost filter in the filter stack was a 50-micron Teflon filter bonded to a perforated Teflon plate. The filter and plate were intended as a pre-filter and support for the finer filters. For this experiment, the filter had been loaded with the

perforated plate upwards. This greatly reduced the effective surface area of the 50-micron filter and also made the system prone to blockage by the precipitate filling up the holes in the Teflon plate. It was initially concluded that the reversal of the filter so that the 50-micron filter was up and the perforated plate was down would still provide adequate support for the finer filters and would be much less likely to be blocked by the precipitate. (Later results indicated filter clogging would occur regardless of the filter type.)

Following collection of the primary filtrate and the 10-cubic centimeter wash, the pH of the filtrate was found to be 9. This confirmed the need for the additional 0.4 milliliter of NaOH to achieve the desired pH. The solutions were then placed on a laboratory ion exchange column and the processing was completed in laboratory glassware. Simultaneously two control processing experiments were carried out entirely in lab glassware. One control was a similar sample of radiotracer labeled Waukena soil hydrolysate. The other was a laboratory blank without soil hydrolysate but with HF-NaOH processing. Radiotracer results comparing recoveries of the breadboard processed sample with the lab processed sample are given in Table 3-2. No significant losses occurred during the breadboard portion of the processing. Recovery through the ion exchange step was good for both samples.

Gas chromatography of the derivatives from the lab and the evaporator processed soil samples showed most of the relative areas to be equivalent. An interfering peak (trifluoroacetamide) at the leucine position prevented reproducibility for this amino acid.

At the end of this experiment, the evaporator was inspected and cleaned. There were no visible signs of attack or degradation.

After discussion of these results, NASA/ARC suggested the use of NH_4OH for HF neutralization and pH adjustment. Following laboratory verification (Section 3.3.1) a breadboard processing test was carried out with the HF-NH_4OH process. The sample was again standard Waukena soil hydrolysate with added radiotracers. The processing proceeded nominally except that filtration problems occurred again. This time, the precipitate was not stopped by the filter stack in the breadboard evaporator.

Table 3-2. Comparison of Sodium Hydroxide and Ammonia as Desalting Reagents. (Recent desalting results measured "combined filtrate" radiotracer recoveries.)

Sample	HF Neutralized with	Soil	Radiotracer	Percent Recovery Filtrate after HF/OH^- Step
BB Soil + RNase	Sodium hydroxide	ARC blank	Ala, Val, Leu	96.6
Lab Soil + RNase	Sodium hydroxide	ARC blank	Ala, Val, Leu	95.4
Lab Soil	Sodium hydroxide	ARC blank	Ala, Val, Leu	93.3
BB processed	Sodium hydroxide	Stock Waukena hydrolysate	Ala, Val, Leu	100.4
Lab processed	Sodium hydroxide	Stock Waukena hydrolysate	Ala, Val, Leu	99.4
Sample B	Ammonia	Stock Waukena hydrolysate	Ala, Val, Leu	102.9
Sample A	Ammonia	Stock Waukena hydrolysate	Pro, Glu, Phe, Lys	92.8
BB Processed	Ammonia	Stock Waukena hydrolysate	Pro, Glu, Phe, Lys	94.1
Lab processed	Ammonia	Stock Waukena hydrolysate	Pro, Glu, Phe, Lys	94.9

The sample and the 10-milliliter wash were filtered through our standard lab filtration set-up to get rid of the precipitate and the processing was completed normally in laboratory glassware along with laboratory glassware controls. Radiotracer results are given in Table 3-2.

Equivalent 94 percent total recoveries were obtained in the lab and on the breadboard. At least half of the missing counts were accounted for in handling errors. The gas chromatographic results for the evaporator processed sample versus the lab processed sample were equivalent.

A comparison of sodium hydroxide versus ammonia processing in both the lab and in the breadboard is given by the summary Table 3-2. The alanine, valine, leucine radiotracer mixture seems to give slightly higher recoveries than the other labeled amino acid mixture. However, most losses seem to be accountable as handling errors. The overall conclusion was that the use of NH_4OH did not show any deficiencies and had shown a number of advantages. All further processing used NH_4OH in place of NaOH.

The evaporator was disassembled to see if the cause of the filtration failure could be determined. It was found that the filter stack in use (coarse Teflon bonded to backup, 10-micron Teflon, 5-micron Teflon, 5-micron Teflon, coarse Teflon bonded to backup) was not adequately compressed to get good sealing against the flanges. Therefore, the filtration failure probably occurred because the solution was able to go around rather than through the filter stack.

Because of the failure of the filters to stop the precipitate in the above test, additional tests were carried out to cure this problem. The initial attempt was to add additional filters to the filter stack with the idea being that the increased compression would ensure that the stack was sealed into the filter retainer. This was initially successful, but it was found that after heating the assembled evaporator for several hours there was sufficient thermal relaxation in the filters so that the filter stack was no longer compressed after the heating. Therefore, an O-ring (initially Viton, later changed to Teflon) was placed at the bottom of the filter stack, and the unit reassembled. Flow tests indicated that the filters were properly sealed.

Another processing test was then carried out in the evaporator for the purpose of testing the filtration, and also for the purpose of providing a sample solution for a test of the breadboard ion exchange column. The evaporator processing was carried out by the nominal procedure using a portion of the standard Waukena soil hydrolysate. NH_4OH was used to neutralize the HF. The filtration after the HF-NH_4OH was carried out successfully although the flow rate through the filter stack was slow due to the large number of filters in the filter stack. The evaporator wash and filtration step was also carried out successfully. Therefore, it was concluded that an O-ring would provide adequate filter stack sealing.

Further testing of the evaporator was carried out as a part of the systems level tests.

The basic conclusion from the component level evaporator testing was that except for a higher pressure drop during filtration and release of contaminants by the Teflon filters, the unit functioned well and gave excellent results.

Most of these problems are effectively eliminated with the new baseline process because there is no precipitation step and hence no precipitate to clog. A filter may not be needed at all, and if one is needed, it should be possible to change filter materials or to change the design so that interference material from Teflon filters will not be a problem. Further comparison of Process No. 1 and Process No. 2 indicates that the conditions in Process No. 2 are milder than in Process No. 1, and that all of the functions required for Process No. 2 have already been demonstrated for the evaporator (which becomes the hydrolyzer/evaporator in Process No. 2).

3.2.1.3 Ion Exchange Column

Summary: The breadboard ion exchange column (IEC) was tested with NH_4OH blank elutions, with HF-NH_4OH processing blanks, and with soil samples processed through the HF-NH_4OH step in either laboratory glassware or in the breadboard. In general, the prototype IEC worked well. Good recovery (90 to 96 percent) of amino acids was demonstrated with samples containing ^{14}C labeled amino acids. Flow

rate variations did occur because of resin volume changes with different reagents, but proper flow control could be achieved via an external flow restrictor. The teflon coated stainless steel spring performed satisfactorily in maintaining bed packing with upward flow through the column. The only significant problem with the IEC was that the resin itself was a source of interfering material. The interference from the resin was reduced by elution and regeneration of the IEC immediately before use. The breadboard IEC contained 30 milliliters of resin which is the amount required for Process No. 1. The new baseline process requires only 5 milliliters of resin. Thus, the flight IEC would be expected to produce significantly less interference.

Detailed Test Results: After proof and leak checks, the breadboard ion exchange column was loaded with ion exchange resin from one of the laboratory columns then in use. At the time of loading, the resin was in the H^+ form and was loaded in a 4N HCl slurry since the resin occupies minimum volume in the HCl solution. Sufficient resin was added to just slightly compress the Teflon-covered spring after the 30- to 60-micron Teflon sliding filter (bed support) was installed. Before installing the inlet valve block, the volume of the bed was observed as water was washed through it via suction on the outlet. As the resin went from the acid to the neutral environment, the filter was pushed down the tube as the resin expanded. After the column effluent was neutral and had expanded to its greatest extent, the spring compression was checked. (It was found that the spring was not fully compressed and, therefore, there was adequate spring travel.) The rest of the assembly was completed, and the column was mounted on the breadboard. The first test carried out was to monitor the column flow rate as the column was eluted with NH_4OH, washed with water, and regenerated with HCl. Initially, 40 psi was required to obtain significant flow, but after a short period of time, the flow increased. Some irregularities in flow also occurred when flow was stopped and then restarted. These effects were apparently a result of air trapped in the 0.1-inch-thick Teflon filters used as bed supports. After several cycles of HCl, H_2O, NH_4OH, H_2O, all of the trapped air escaped and the flow behavior was reproducible. The flow did vary depending on the reagent in the column because of bed expansion and contraction.

To better define the flow variations, an HF/NH_4OH blank was run through the IEC while monitoring pressure and flow rate as the various reagents passed through the column. The pressures required to maintain a flow rate of 0.5 ml/min varied from 6.5 to 3.5 psi from the initial NH_4F solution through the ammonia elution. This is not an excessive pressure variation, and the flow could be controlled reasonably well with the addition of an external flow restrictor. For a time, a 1.5-micron Teflon filter held in a M.E.R. union was used as a restrictor; but for most testing, a restrictor was not used and the pressure applied was adjusted to maintain the proper flow rate.

The collected filtrate from an evaporator test was labeled with ^{14}C proline, glutamic acid, phenylalanine and lysine. After adding phenolphthalein, the filtrate was placed in the breadboard ammonia reservoir and was pushed onto the ion exchange column. This was followed by the evaporator wash, 30 milliliters of H_2O and 4N NH_4OH. During the NH_4OH elution the pressure was adjusted to maintain the flow at 0.5 ml/min. The pressure required varied from 3.8 to 6.6 psi.

Results of the radiotracer measurements are shown in Table 3-3. The overall results were quite good, with all the radioactivity being accounted for within experimental error. The volume in the column exit tube was small (<100 µl) so that prime eluent (7 ml) included virtually no solution prior to the appearance of the phenolphthalein color. This accounts for the fact that the precut contained 3.5 percent of the radioactivity, since a small quantity of the amino acids usually immediately precedes the appearance of the indicator color. Therefore, a short length of 1/8-inch OD teflon tubing was added to the outlet tube to increase the volume in the line and hence to retain more solution preceding the appearance of the phenolphthalein color.

Some losses occurred during subsequent laboratory processing of the prime eluent (evaporation and derivatization). The evaporation loss represents bumping problems on the rotary evaporator. Radiotracer measurements made during laboratory derivatization are also shown in Table 3-3.

Table 3-3. Breadboard Ion Exchange Column Radiotracer Recovery, Initial Activity - 928,500 cpm

Ion Exchange	cpm*	Percent Recovery
Precut	32,640	3.5
Prime Eluent	895,240	96.4
Post Cut	2,270	0.2
Column Wash ($NH_4OH + H_2O$)	14,910	1.6
Ammonia Reservoir	0	0
Total Accounted for	945,060	101.8
Derivatization		
Losses in Evaporation	72,560	7.9
Recovery after HCl/BuOH Step	784,900	87.7
Final Derivative	613,900	78.2

*cpm corrected for background and volume sampled

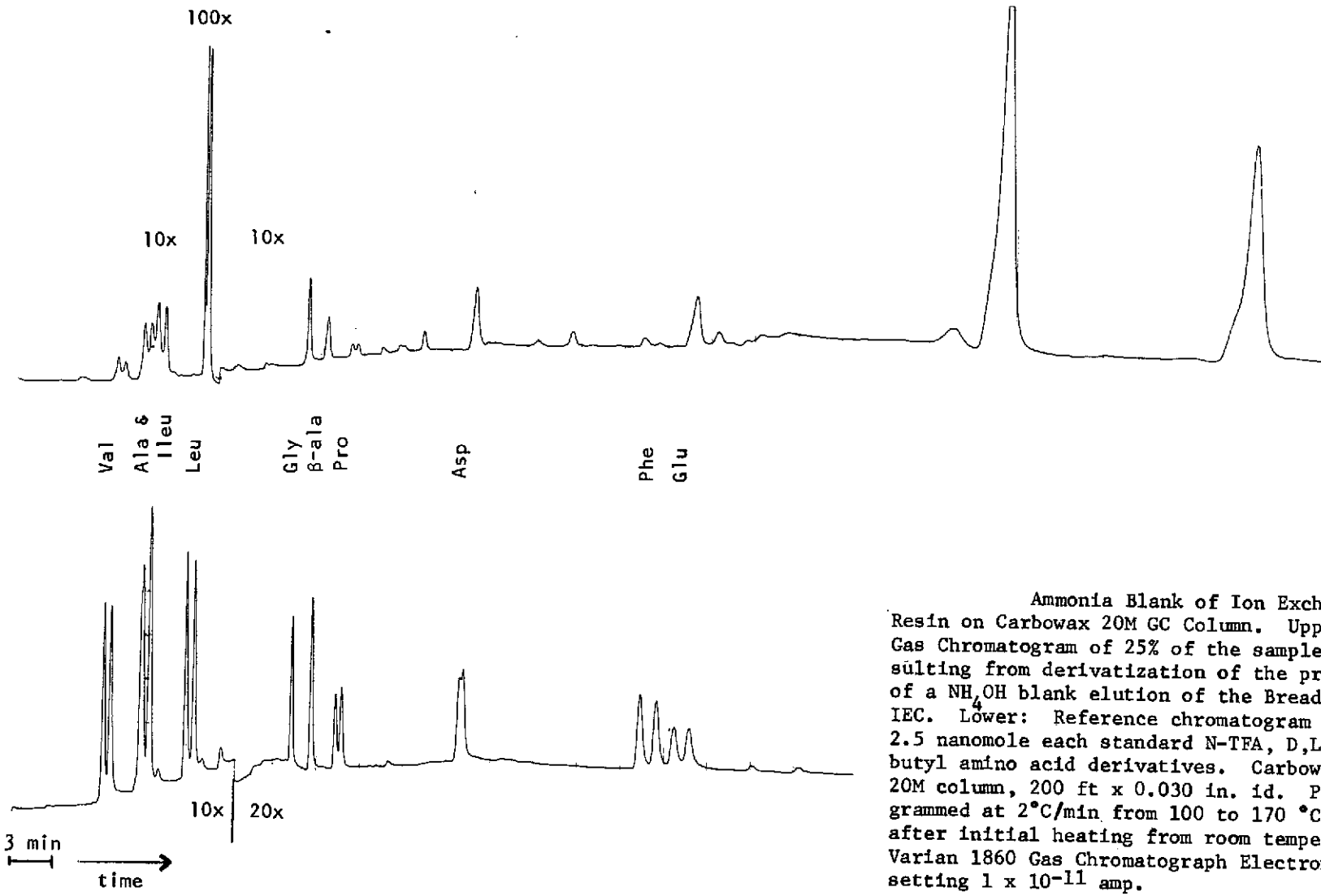
The gas chromatogram resulting from the derivatized product was very similar to those of derivatives resulting from other tests such as those from the previous evaporator test, except that the trifluoroacetamide interference peak was much larger for the breadboard ion exchange column sample than for samples processed in the lab columns. The larger interference peak with the breadboard column was felt to be caused by the fact that the breadboard column had less use than the lab columns, (the trifluoroacetamide peak decreases with frequent column use).

Next, the column was cycled (NH_4OH-H_2O-HCl-H_2O) several times for cleaning purposes. During one of these cycles, gas was inadvertently passed into the column. The column was back-flushed for a while and then forward flow was resumed. Approximately 4 hours of flow were required before the flow rate returned to its initial value.

The ion exchange column (IEC) was then repacked by removing the spring-loaded end and stirring the resin bed until all of the resin was in suspension. The bed was then allowed to settle, and the IEC was reassembled, washed, and regenerated. A NH_4OH blank elution was carried out to monitor the condition of the IEC with respect to interfering material in the 7 milliliters of NH_4OH eluent. This test was also partially in response to a question from NASA/ARC as to how much reduction in resin volume would be required to reduce to an acceptable level the amount of interfering material coming out of the resin. The 7 milliliters NH_4OH sample (containing phenolphthalein) from the IEC elution was evaporated and derivatized by the usual laboratory method and the derivative was chromatographed on both Carbowax 20M and Dexsil 400 columns. Figures 3-20 and 3-21 show the chromatograms obtained from each column. Underneath the IEC sample chromatograms are shown reference chromatograms of a standard derivative mixture. The standard represents 2.5 nanomoles of each amino acid (based on the amount of amino acid originally derivatized and the fraction of the derivative injected), while the IEC sample chromatograms are from injections of 25 percent of the sample.

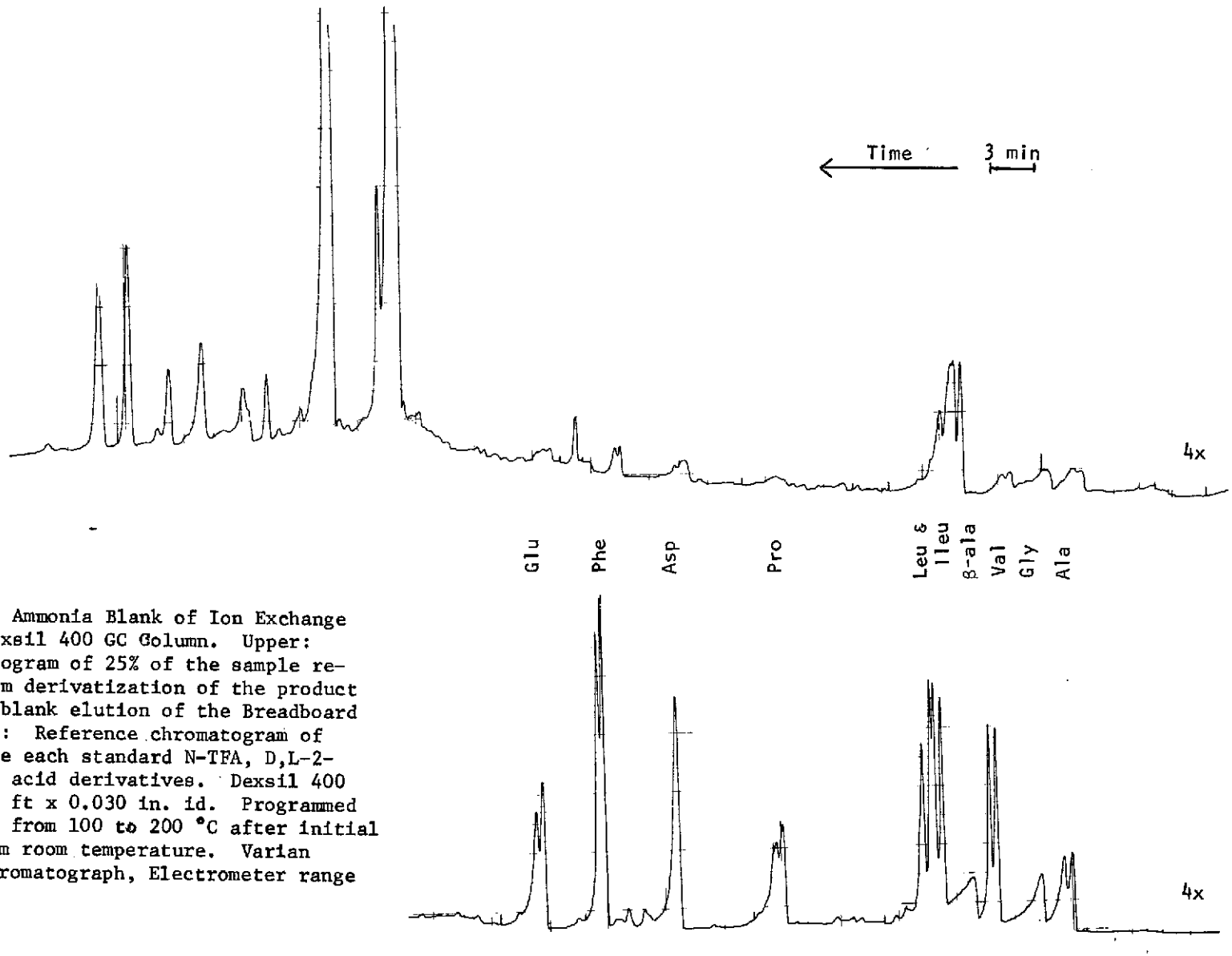
On Carbowax 20M, the large interfering peak which occurs at the leucine position (and is ascribed to trifluoroacetamide) is very prominent. This peak is equivalent in size to an amino acid derivative peak of approximately 100 nanomoles. Most of the other peaks in the region of interest (valine through glutamic acid) are identifiable as being from trace amino acid contamination and represent (except for alanine) less than 5 nanomoles each in the total sample. There are only a few non-identified peaks in the region of interest and they represent less than 5 nanomoles in each case. Other major unknown peaks do occur in the chromatogram, but their retention times are longer than those of the amino acids of interest and hence are non-interfering.

In the case of the Dexsil 400 chromatogram, virtually all of the peaks seen in the region of interest are identifiable as resulting from trace amino acid contamination, and no major interfering peaks occur until shortly after the retention time of the lysine derivative.



Ammonia Blank of Ion Exchange Resin on Carbowax 20M GC Column. Upper: Gas Chromatogram of 25% of the sample resulting from derivatization of the product of a NH_4OH blank elution of the Breadboard IEC. Lower: Reference chromatogram of 2.5 nanomole each standard N-TFA, D,L-2-butyl amino acid derivatives. Carbowax 20M column, 200 ft x 0.030 in. id. Programmed at 2°C/min from 100 to 170 °C after initial heating from room temperature. Varian 1860 Gas Chromatograph Electrometer setting 1 x 10^{-11} amp.

Figure 3-20. Ammonia Blank of Ion Exchange Resin on Carbowax 20M GC Column



Ammonia Blank of Ion Exchange Resin on Dexsil 400 GC Column. Upper: Gas Chromatogram of 25% of the sample resulting from derivatization of the product of a NH_4OH blank elution of the Breadboard IEC. Lower: Reference chromatogram of 2.5 nanomole each standard N-TFA, D,L-2-butyl amino acid derivatives. Dexsil 400 column, 200 ft x 0.030 in. id. Programmed at 2 °C/min from 100 to 200 °C after initial heating from room temperature. Varian 1527 Gas Chromatograph, Electrometer range 1.

Figure 3-21. Ammonia Blank of Ion Exchange Resin on Dexsil 400 GC Column

On the basis of gas chromatographic results, it appears that the primary interference from the ion exchange resin is trifluoroactamide. Stringent cleaning should reduce the amount of amino acid contamination, and a reduction of the resin volume to the 5 milliliters in the new baseline process should reduce the amount of other interfering material to the acceptable level in the region of interest. Elution and regeneration of the column immediately before use appears to be necessary to minimize the amount of interfering material produced. The results of the IEC testing were encouraging, and in fact, even with the full 30 milliliters of resin, large portions of the chromatograms approach the level of derivatization blanks except for the amino acids and the trifluoroacetamide. Also, no corrosion of any of the IEC components was observed including the Teflon-coated stainless steel spring.

The remainder of the IEC testing was carried out as a part of systems level testing.

There are two significant differences between Process No. 1 and No. 2 for the ion exchange column. The reduction in resin volume to 5 milliliters in the baseline process should not only reduce the amount of interfering material produced by the resin but also should reduce the amount of trace contamination of amino acids because the volumes of the various reagents, especially water, used with the ion exchange column are decreased. The other significant difference is that the salts passing into the column in Process No. 2 should not have a significant quantity of fluoride present. Thus, no HF would be produced in the column via the ion exchange process. Thus, the conditions to which the column materials and the resin are exposed to will be significantly milder with Process No. 2.

While a new ion exchange column will have to be fabricated to test Process No. 2 with the breadboard, the overall operation will be the same as before, so good results can be anticipated with a new 5-milliliter ion exchange column.

Liquid Level Sensor

The recovery of amino acids and the efficiency of the desalting is degraded somewhat if gas is inadvertently passed through the ion

exchange column. Since it was difficult to predict the flow rate of solutions out of the evaporator into the IEC in Process No. 1, the design included a liquid level sensor to determine when all of the filtrate from the HF/NH_4OH processing and the evaporator wash were pushed into the IEC. The breadboard sensor operated by measuring the conductivity of the solution passing through it. It was constructed from a Teflon union threaded to accept M. E. R. type fittings. The electrodes were two gold wires press-fitted into the Teflon. When gas is present in the line, the conductivity decreases to a very small value.

The operation of the sensor was tested with both DC and AC resistance measurements. In the DC mode using a standard ohmmeter, reliable indications were obtained as to the presence or absence of liquid in the sensor. Because of electrode polarization effects, the actual resistance values obtained with various solutions had little significance. Typical DC resistance values were from 0.1 to 50 megohms depending of the type of solution and the length of time the DC voltage was applied. While the device worked reliably at first in this mode and was simple to operate, it was felt that the use of DC is undesirable because of the formation of electrolysis products from the solution or the electrodes and because of the possibility of electrode fouling.

AC measurements overcome most of these problems since there is no steady-state current. However, typical laboratory instrumentation for the measurement of AC impedance or resistance does not allow for convenient operation of the sensor. The sensor had a cell constant of approximately 10 so the resistances occurring with most solutions exceeded the range of devices which have simple meter readouts. AC bridges worked satisfactorily (except with pure water because of the high resistance) but were inconvenient because of the need to balance both resistance and capacitance. Use of total impedance rather than AC resistance should be adequate and should permit simpler instrumentation. The sensing of capacitance would be another alternative.

After the sensor had been in use for a while it developed an internal conductive path which made its indication unreliable, so it was not used further. Visual observation of the Teflon line between the evaporator

and the IEC was used in its place. For further work, it is recommended that the sensor be redesigned with a smaller cell constant and larger electrode area.

Flow rates into the ion exchange column should be much more predictable with Process No. 2. Hence, if a reduction in recovery is acceptable (because of allowing for some margin of error by not planning to collect all of the sample), then the liquid level sensor may be dispensed with entirely. Further testing is required, however, before such a decision can be made, and it is recommended that this be evaluated in the next breadboard phase.

3.2.1.4 Derivatizer

Summary. The derivatizer was tested for basic function, for its ability to transfer standard derivatives onto GC columns, and for derivatization of pure amino acids and samples derived from soil processing. Interference peaks caused by elastomeric materials occurred and, as a result of further testing, the derivatizer seals were replaced with various types until all materials present were tantalum and Teflon. This was accomplished by fabricating new hand-operated, all tantalum diaphragm valve simulators. At this point, most of the derivatizations with pure amino acids and optically inactive 2-butanol showed one remaining interference peak. However, the final derivatization which was with optically active 2-Butanol, in addition to showing that no racemization effects occurred, also did not produce any significant interference peaks. Derivatization of samples derived from soil processing continued to produce large interference and further work is needed in this area. However, it is felt that a solution to this problem is available. The change from Processing Sequence No. 1 to the new baseline process should also result in reduced interference.

Detailed Test Results. Preliminary proof and leak tests indicated that some modifications were needed in the vent line and vent restrictor. The initial restrictor was a Teflon orifice in a Teflon Swagelok fitting, and it tended to leak with thermal cycling. The Teflon orifice was replaced with a tantalum orifice press fitted into the end of the tantalum

vent tube coming out of the derivatizer head end. In order to have more reliable temperature control, the meter relay type temperature controllers were replaced with a better type of thermocouple controller which incorporated thermocouple break protection. Heater tests were carried out which demonstrated that the proper temperatures could be obtained. Cooling tests were carried out to develop a convenient method of cooling the derivatizer to 0°C or below. An insulated box was constructed to surround most of the derivatizer: by placing crushed dry ice into the box, but not in direct contact with the unit, it was possible to reduce the derivatizer temperature below -5°C. The unit could be controlled at this temperature with the temperature controllers. The magnitude of the thermal gradients was not determined.

Evaporation tests carried out with a 4N NH_4OH solution containing phenolphthalein proceeded smoothly with no apparent bumping. There was no evidence of phenolphthalein color in the condensate found in the vacuum system trap. Thus, it appeared that the NH_4OH and butanol/HCl reagents could be successfully evaporated. Later tests also confirmed this although bumping did occur in a few instances when the vent valve was opened when the solution in the cell was at too high a temperature (>70°C for H_20).

Tests were then set up to monitor TFA/CH_2CL_2 evaporation. The evaporation of NH_4OH and butanol/HCl can be monitored by the presence of condensate in the unheated portions of the vacuum system. This condensation does not occur with CH_2Cl2 and hence some tests were carried out with a pressure transducer added to the derivatizer so that evaporation times could be determined as a function of reagent quantity and of cell temperature. These evaporation tests were carried out separately since it was undesirable for the transducer to be present during an actual derivatization because of materials problems. These tests were based on the fact that, as long as there is reagent in the cell, the pressure was significantly above zero because of the flow restrictor in the vent line. The times required for evaporation of TFA and methylene chloride, individually, and as a mixture from the derivatizer at -5°C, were determined with the transducer connected to the S-8 outlet line. The lower portion of the cell was surrounded by dry ice (but not in direct contact

with it), and the heater ($H3_B$) controller was set at -5°C. The cell was evacuated and the vent closed. Next, 0.4 milliliter total quantities of CH_2Cl_2, TFA, or a mixture of the two, were introduced through valve S-9 via a syringe attached to the inlet line. After a short waiting period to allow the reagent to cool, the cell was opened simultaneously to vacuum and to the pressure transducer. The output of the transducer was recorded at 15- to 60-second intervals. Figure 3-22 illustrates the transducer output as a function of time in four such tests, two of them being replicate experiments with a mixture of TFA and methylene chloride. It was concluded from these tests that 10 to 12 minutes was a sufficient length of time for the reagents to evaporate at -5°C. (In later tests, the evaporation time for TFA/CH_2Cl_2 was increased to 30 minutes to insure complete evaporation of any last traces of TFA.)

Tests were carried out to demonstrate that methylene chloride could be evaporated off without significant losses of amino acid derivatives, and that the derivatives could be evaporated out of the derivatizer and into a GC column. Four microliters of a standard amino acid derivative solution (= 2 nanomoles each) were injected with a syringe through a chilled inlet line into the evacuated, chilled cell followed by a total of 0.4 microliters CH_2Cl_2. The cell was opened to vacuum for 10 minutes at -5°C in order to evaporate the CH_2Cl_2. A lab Carbowax 20M column was attached to the S-8 outlet, and the gas was allowed to flow through the cell and the column at a rate of about 15 cc/min. The cell and S-8 outlet were slowly heated to and then held at 150°C for about 5 minutes. The column was then removed from the breadboard and reconnected to the gas chromatograph for data collection.

Two such tests were performed initially. The first chromatogram showed a broad interference peak during the elution of valine through proline which was not present in the second run. This interference may have been from residual matter in the cell since only limited heating of the cell had been carried out prior to this test. The chromatogram from the second test was, in general, good although the glutamic acid peaks were either missing or smeared out. (Later tests with standard derivatives carried out to monitor the derivatization condition generally were free of interferences and all expected peaks were present and well shaped.)

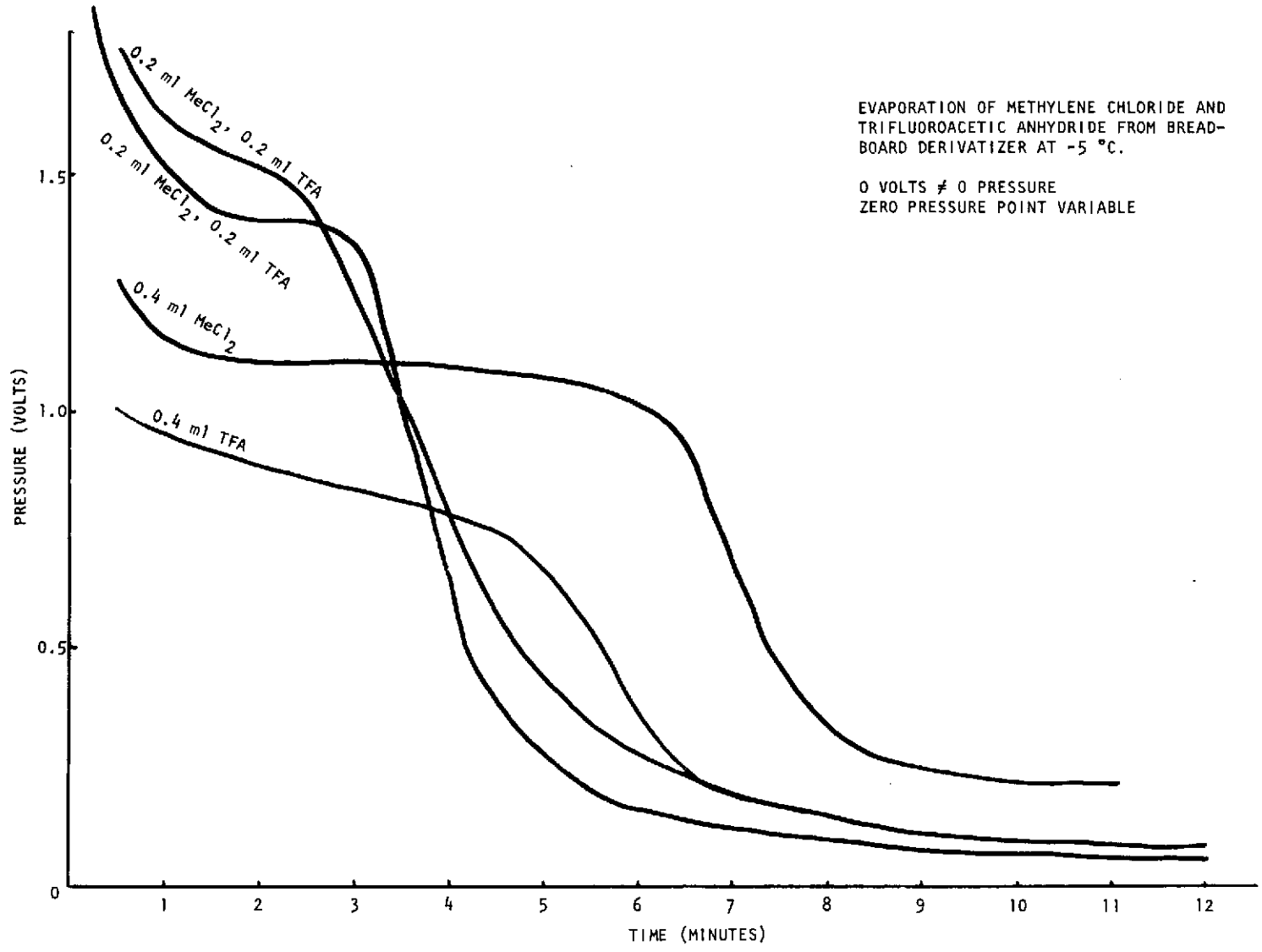


Figure 3-22. Evaporation of Methylene Chloride and Trifluoroacetic Anhydride from Breadboard Derivatizer at -5°C.

Since the derivatizer appeared to be capable of performing the basic processes required, two complete derivatizations were carried out on three amino acids in the derivatizer followed by evaporation of the products into a laboratory GC column and subsequent gas chromatographic analysis. The complete process included injection of 1 milliliter of an aqueous solution of 2 nanomoles each of valine and phenylalanine and 10 nanomoles of proline followed by injection of 6 milliliters of 4 N NH_4OH to provide the proper solution conditions. The 7 milliliters of aqueous NH_4OH solution were evaporated in the nominal mode by heating the vent (H_{3C}) to 120°C, opening S-6 and then slowly heating the cell (H_{3A}, H_{3B}) to 100°C. (H_{3A}, H_{3B}, H_{3C} are heater designations in Figure 3-1.) The evaporation required 40 to 60 minutes for completion as evidenced by no visible vapor in the vacuum vent lines. The heaters were turned off, and when the cell had cooled to about 50°C, 2 milliliters of 2-butanol/HCl were injected. Next, the lower cell was gradually heated to 100°C with the cell closed. The upper cell temperature was set (H_{3A}) about 5°C less than that of the lower cell to encourage refluxing. Three hours later, the heaters were turned down and the cell allowed to cool to below 70°C before being opened to the vacuum for the evaporation step. The evaporation of the 2-butanol/HCl required from 15 to 35 minutes before appearing complete.

During this process, the cell was heated at 70°C and the vent line was heated to 110°C. After the evaporation was completed and the derivatizer had cooled to room temperature, 0.2 milliliter CH_2Cl_2 and 0.2 milliliter TFA were injected into the cell, and the acylation step was allowed to proceed for 1 hour. The cell was then cooled (lower cell temperature, H3B = -5°C) and opened to the vacuum for 10 to 12 minutes in order to allow the TFA/CH_2Cl_2 to evaporate. The derivatives were transferred to a GC column by slowly heating the derivatizer to 150°C with 15 cc/min He/H_2 flow through the derivatizer into the column. Finally, the column was transferred to the Varian 1800 GC and a standard GC program carried out. The resulting chromatogram showed an extremely large amount of interference (approximately one thousand times the size of a 2-nanomole amino acid derivative peak) throughout the chromatogram. At the conclusion of this test, the derivatizer was disassembled for inspection and cleaning. The inside of the cell was covered with a grey dusty

residue which was easily wiped out. In addition, the cell walls were somewhat streaked and spotted. These spots remained even after extensive soakings and washings with various reagents, and may have been present before the derivatization was carried out.

After the derivatizer was cleaned, dried, and reassembled, it was baked out under vacuum for 30 minutes at 150°C to insure that any volatile impurities were removed. The derivatization was then repeated, but the chromatographic results were similar to the first derivatization. Although the interfering material was an order of magnitude lower than the first test, it was still 4 orders of magnitude larger than can be accepted.

After running the chromatogram at the conclusion of the first derivatization test, it was found that the column (Carbowax 20 M) no longer functioned properly. At the time no special note was taken of this fact since this particular column was near the end of its life, having been used for a considerable period of time. However, after the second derivatization test, the column used (Dexsil 400) was again damaged. Therefore, it was concluded that the material coming out of the derivatizer either by partially remaining in the column or by reacting with the liquid phase had altered the column. Since previous results have demonstrated that TFA can be passed through the column, it was concluded that the interfering material had been formed during the derivatization from something in the derivatizer and the peaks seen were not a result of unevaporated TFA.

The primary suspects as a source of interfering material formed during derivatization were the elastomeric seals used in the valve simulators. These valves had two external seals, which in the case of the two derivatization tests were of Viton E-60, which is the material used in the VLBI solenoid valves. It was hoped that even if there were some materials problems with the Viton, the metal-to-metal contact surfaces between the cell contents and the seals would prevent any serious reactions. Apparently this was not the case. Therefore the static body seal was replaced with a Creavey type seal (Teflon encased metal spring).

Initially, it was thought a Creavey type could not be used here because sufficient force to effect the seal might distort the tantalum body. However, further study indicated that the valve could withstand the force required.

Because replacement of the dynamic shaft seal with a non-elastomeric type was very difficult, a brief materials test was carried out to see if ethylene propylene rubber (EPR) would be a more suitable material. Portions of Viton and EPR O-rings were put in derivatization vials and derivatizations carried out. The O-ring materials were removed, the derivatization products were dissolved in CH_2CL_2, and small portions were chromatographed. A 0.5 percent injection of the sample from the derivatization in the presence of Viton resulted in a number of relatively large peaks, while in the case of EPR, a 0.5 percent injection was relatively clean. However, significant peaks were observed from the EPR sample when 25 percent of the sample was injected. Thus EPR was a superior material, but it was not certain that it could be successfully used for the shaft seal. All four valve simulators were, therefore, removed from the derivatizer and disassembled. The Viton O-ring shaft seals were replaced with ethylene-propylene rubber O-rings. The Viton O-ring external seals were replaced with Creavey seals.

The derivatizer itself was cleaned, reassembed, and baked out. A standard mixture of amino acid derivatives (2.5 nanomole each of Ala, Val, Leu, Gly, β-Ala, Pro, Phe, Glu) dissolved in 0.4 milliliters of CH Cl was introduced through S-9, and the methylene chloride was evaporated off at -5°C through the vent (S-6). (See Figure 3-1 for the derivatizer flow schematic and valve notation.) A Carbowax 20M GC column was connected to the GC port (S-8), and a gas line was connected to S-9 port. Gas was then swept into S-9 through the derivatizer and out S-8 and then into the column. Atfer flow was started (15 cc/min), the derivatizer was slowly heated to 150°C to evaporate the derivatives onto the column. Subsequent gas chromatographic analysis gave good results with all the amino acid derivative peaks being present at the expected level including glutamic acid (which had not been observed in earlier tests with standard derivatives). There were several other unidentified peaks

present in the chromatogram at levels similar to the amino acid derivatives which may have been the last traces of the interfering material produced in previous tests, or it may have been some material outgassing from theS-8 and S-9 valve shaft seals.

After baking the derivatizer under vacuum, another full derivatization test was carried out. Twenty-five nanomoles each of Val, Pro, Phe and Glu were introduced through S-9 in 1 milliliter of H_2O. The H_2O was evaporated off, and the S-9 inlet line dried. Two millileters of 2-butanol/HCl was then injected through S-9 using the breadboard 2-butanol/HCl reservoir. After heating at 100°C for 3 hours, the reagent was evaporated with the heater controllers set at 70°C. The S-9 inlet line was dried, and atfer the derivatizer had cooled to 30°C, 0.4 milliliter of TFA/CH_2Cl_2 was injected through S-9 using the TFA/CH_2Cl_2 reservoir. After 1 hour, the unit was cooled to -5°C, and the TFA/CH_2Cl_2 was evaporated for 30 minutes. The S-9 line was briefly heated, and then the product of the derivatization process was evaporated onto a Carbowax 20M column attached to GC column port (S-8) with He being swept through the unit entering via S-9. The subsequent chromatogram was much better than those from the previous two derivatization tests, although there was interference through much of the chromatogram. No amino acid derivative peaks were identified. The Val and Pro derivatives could not have been detected because of the interferences. However, the baseline was sufficiently stable in the time period where the Phe and Glu derivative peaks would be expected for the peaks to be detected if present. It was hypothesized that the large amount of material in the column resulted in a change in the retention characteristics in the column so that the Phe and Glu peaks were not eluted at the expected time.

The GC column was purged for several hours at 200°C and then tested with standard derivatives. Unlike the first two derivatization tests, the column was found to be functioning normally which again indicated significant reduction in the amount of interfering material produced.

While the changes made thus far had improved the result, it was obvious that major problems remained. Further consideration of the derivatizer operation led to the conclusion that use of the same inlet line and hence the same valve for both reagents and for the helium might be

contributing to the problem since the valve shaft seal was exposed to a degree to the reagents and then was exposed while the cell was heated to evaporate the derivatives into the GC column. By bringing the helium into the derivatizer through S-7 as was designed for full breadboard operation, S-9 would be closed during the evaporation onto the column step and hence any reaction products between the O-ring and the reagents which were adsorbed onto the O-ring would not be volatilized and swept into the column. The helium line going to S-7 had not been used in the past because it was connected with the line going to SC-2 (to provide He for the hydrolyzer and evaporator), and occasionally aqueous solutions got into the line. Therefore, the gas lines were rerouted to prevent this. Prior to the changes, the He lines going to SC-2 and to S-7 were connected after LV-14. In the new routing, LV-14 was used as a three-way valve with separate lines to SC-2 and S-7 to prevent any water from getting into the derivatizer via the He lines.

The overall post-test appearance of the derivatizer was good. However, the Creavey seal between the cell body and the head-end was found to have failed. The Teflon had taken a set and had cracked. Upon close examination, corrosion of the inner stainless steelspring was observed. Previous tests with unstressed Creavey seals had indicated that they were reasonably compatible with the derivatization process. A test was carried out with a stressed Creavey seal. This seal also developed a set and cracked during the derivatization, leading to the conclusion that a replacement seal for the Creavey seals in the derivatizer was required.

After a new Creavey seal was installed for the cell seal, the unit was baked out and another standard derivative evaporation test was carried out to check out the new flow routing and to check for any interfering material. 2.5 nanomoles each of the eight standard amino acid derivatives used in the previous evaporation test were introduced and the test carried out in a similar manner except for the use of S-7 as the He inlet. The chromatogram after evaporation of the derivatives on the Carbowax 20M column was similar to that from the previous standard amino acid derivative evaporation test. All the amino acid derivative peaks were observed and there were several interfering peaks present

at levels similar to the amino acid derivative peaks. Thus it appears that a small amount of interfering material was produced even in a simple evaporation test. This material is thought to come from the seals either by simple outgassing or because of action of methylene chloride on the seals.

After a vacuum bakeout, a full derivatization procedure was again carried out. The procedure was identical to the previous full derivatization test except for the use of S-7 rather than S-9 for the He inlet. The gas chromatographic results were much better than the previous test. The amount of interfering material while still present in large quantity was greatly reduced and, for the first time, the peaks corresponding to the derivatives of the four original amono acids (25 nanomoles each of Val, Pro, Phe and Glu) were identified in the chromatogram. This was a significant result because it meant that the breadboard derivatizer would be functional once the large amount of interfering material produced was eliminated. The remaining interferences were similar in size to the 25 nanomole amino acid derivative peaks so a significant reduction in the interfering material was still required.

It was felt that most of the problem still resulted from the breadboard seals and hence would not occur or could be avoided in a flight type unit. The need for frequent disassembly and cleaning on the breadboard caused the selection of the type of seals then in use, but further work on improved seals was needed.

Effort was then directed at finding replacement seals for the valve simulator shaft seals and for the main cell body seal. It was desirable, however, to determine what part of the derivatization process was causing the remaining interference in order to attack the problem in an effective manner. This was accomplished by breaking down the various steps and carrying them out partially in the derivatizer and partially in lab glassware. To determine if some of the interference was being washed in with the reagents through the injection lines, the reagents were injected into the derivatizer head end with the bottom removed so that they could be collected in a standard lab derivatization vial and a normal lab derivatization carried out.

The test was carried out by injecting 2 milliliters of 2-butanol/HCl, collecting in the derivatization vial and heating the reagents for 3 hours at 100° followed by evaporating to dryness in the vial using the normal lab procedure. In the meantime the derivatizer head end and associated lines were dried by heating and gas flow. The vial was taken back to the derivatizer and 0.4 milliliter of TFA/CH_2Cl_2 was injected through the derivatizer head end. The derivatization was continued in the normal lab manner and the resulting derivative was analyzed by gas chromatography. The chromatogram did show some interfering peaks, but it, in general, was not significantly different from a regular lab derivatization blank which was carried out at the same time. Therefore, it appeared that heating of the reagents in the derivatizer was required to form the large amount of interfering material found in derivatizer derivatizations. The shaft seal on the vent valve (S-6) was especially suspect since it is exposed to hot 2-butanol/HCl during the 2-butanol/HCl evaporation step; and since this seal is upstream of the flow restrictor, it would be easy for material formed from attack by 2-butanol/HCl to get back into the cell.

Following the head end test, the entire derivatizer was cleaned and returned to the engineering laboratory for work to develop new non-elastomeric seals. Since the Creavey seal used as the main cell body seal had not held up well, a special tantalum metal seal was constructed. This seal in conjunction with Teflon gaskets (to prevent galling) appeared to seal satisfactorily. As an expedient, split Teflon o-rings were installed in place of the elastomeric O-rings on the valve shafts. Initial leak tests indicated that they might seal adequately so the derivatizer was reassembled using split Teflon O-rings as the shaft seals; the tantalum seal was used at the cell body seal; and Creavey seals were used for the valve external seals. After further leak tests in the engineering laboratory, the unit was brought back for further derivatizer testing. The unit was baked out for a short period and cooled. A standard derivative mixture in CH_2Cl_2 was introduced into the cell and the CH_2Cl_2 was evaporated off at -5°C. The sample was then evaporated onto a Carbowax 20M GC column. Seal performance during this test was marginal. The S-6 shaft seal leaked badly when the unit was chilled. Either or both the S-7 and S-8 shaft seals leaked moderately at room temperature. The seals did appear to seal adequately at elevated temperatures. The

resulting chromatogram showed a large, very broad interference peak, but no identifiable amino acid peaks. The derivatizer was cleaned as a total unit and the cell was baked out and the derivative mixture test was repeated. The results were similar to the first test. The broad interference peak was somewhat smaller, but there were still no amino acid derivative peaks. Since earlier derivatizer testing with derivative mixtures was successful, it appeared that the changes made on the seals had caused some sort of problem.

A third trial at derivative evaporation led to a repeat of prior results – a major broad peak about half the size of the previous run but with small (about 1 percent full scale) humps occurring at locations appropriate for amino acids.

Because the last run gave some indication that amino acids were coming out of the derivatizer but that most were being destroyed, another run was made to test the hypothesis that water was getting into the cell and destroying the amino acids. Valve leaks were observed in the above tests, especially in cooling. For this test 10 nanomoles each of the standard a. a. derivatives was injected into the derivatizer followed by a 200 microliter methylene chloride wash. Without cooling or evaporating the solvent, the sample was evaporated into the GC column. This test too resulted in no amino acid peaks. The cell was dismantled and badly corroded valves were discovered. Thus the split Teflon O-rings were not effective in preventing the reagents from attacking the back stainless steel portion of the valve simulators. Apparently the corrosion products caused the decomposition of the derivatives when evaporation of the derivatives onto the column was attempted. Therefore, the old valve simulators were abandoned and new all tantalum valves described in Section 3.1.6 were built as replacements.

For the first use with all tantalum and Teflon the standard derivative evaporation test was run with 25 nanomoles of each amino acid. Upon heating to evaporate the amino acids onto the column, thermal expansion caused the valve to close above 75°C, preventing He flow. Therefore, evaporation onto the column was carried out at 70°C. The resulting chromatogram was quite good however, with only glutamic acid smaller

than expected. This indicates that only 75°C is necessary to evaporate derivatives through phenylanine.

After some additional valve modifications, full derivatization was then attempted in the unit on an aqueous solution of amino acids (25 nanomoles each). The solution was injected into the breadboard derivatizer, evaporated to dryness at 50°C under vacuum and derivatized with butanol/HCl and trifluoracetic anhydride/methylene chloride. Valves were barely functional for this test, sticking closed on several occasions. The GC results, however, were positive, as shown in Figure 3-23. One large unknown peak is present between Leucine and Glycine and the glutamic peak is somewhat smaller than expected.

On the basis of this good result, an end to end system test was run in order to use the full breadboard (several hydrolyzer through IEC tests had already been completed successfully) as a system and to run a soil derivative in the derivatizer cell. Valves were reworked prior to this test so that they functioned reliably. The soil for this end to end test was Waukena diluted with blank soil (25 percent Waukena) to reduce the amino acid content in the sample below 50 nanomoles since the GC column starts to show overloading effects with more than 50 nanomoles. Small quantities of ^{14}C labeled amino acids were also added to the soil to aid in monitoring the intermediate steps. The chromatogram ultimately resulting from the end to end test showed a very large broad peak which masked any possible amino acid peaks. Upon opening the derivatizer cell, approximately one-third the radioactivity was recovered, indicating a large percentage of the sample was not transferred to the GC column. Furthermore, it was observed in the laboratory glassware control that the sample contained a waxy solid, apparently peculiar to the blank soil as it has not been observed in Waukena soil samples.

After thorough cell cleanup, two standard derivative mixtures were injected into the derivatizer cell to confirm the unit was still functional. First 25 nanomoles were chromatographed successfully followed by 2.5 nanomoles as seen in Figure 3-24 (new chromatogram 4). These tests confirmed that the cell was sealed, no obvious material problems existed, and the nominal injection procedure was functional.

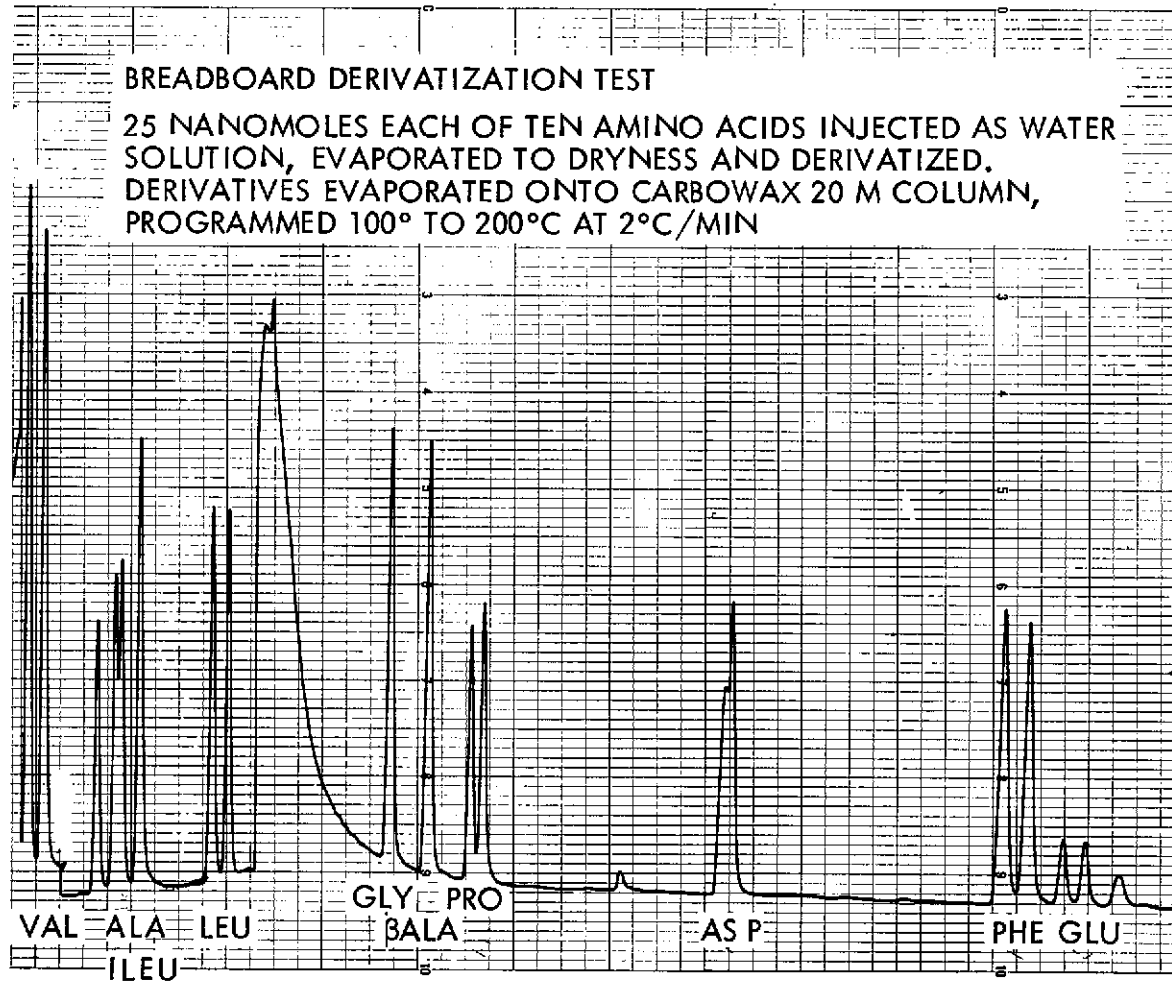


Figure 3-23. Chromatogram, Breadboard Derivatization Test

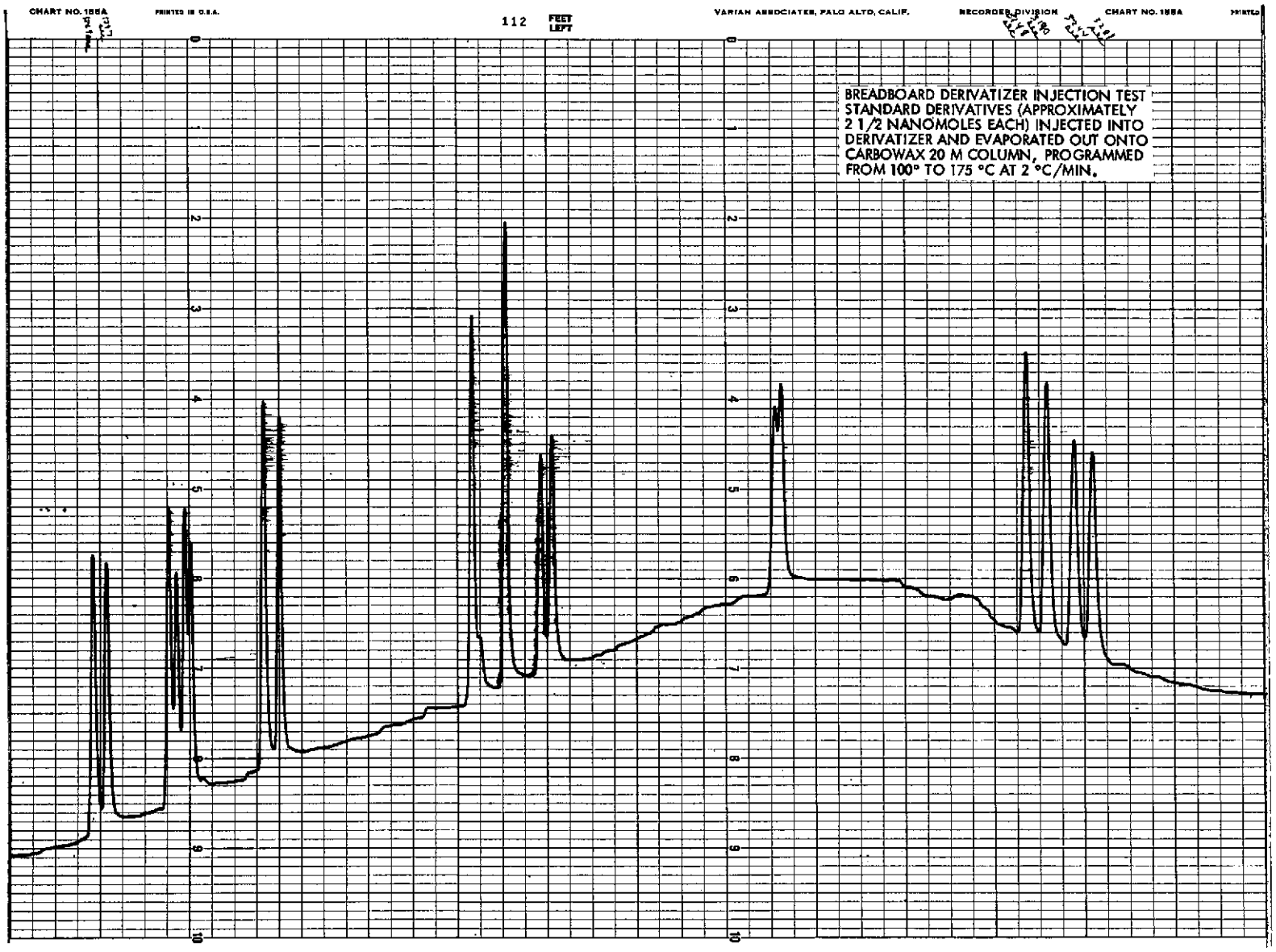


Figure 3-24. Chromatogram, Breadboard Derivatizer Injection Test

A derivatization starting with a pure aqueous solution of amino acids (25 nanomoles each) was then performed. The chromatogram (on Carbowax 20M) showed all the amino acids and in addition had an unknown peak following Leucine as was observed previously.

As another derivatization test on a sample derived from soil (and also as a modified end-to-end system test), 25 percent of a ^{14}C labeled Waukena soil sample processed from hydrolyzer through IEC was injected into the derivatizer. The ammonia solution was evaporated to dryness and derivatized, using normal reagent volumes. The derivatization proceeded smoothly. The chromatogram, however, again had a very large impurity peak. No amino acids were observed. Radiotracer measurements showed a significant portion of the radioactivity again remained in the cell.

Based on a comparison of the results from pure amino acids with those from samples derived from soil processing and with results from lab processing, it was concluded that some sort of material was present in the samples derived from soil processing that was interfering with transfer of the amino acid derivatives to the column and with the subsequent gas chromatographic analysis. Furthermore, it was concluded that this material was not soluable in CH_2Cl_2 and hence did not interfere with lab samples since the derivatives in those samples were dissolved in CH_2Cl_2 in order to inject them into the gas chromatograph. Thus the problem with soil derived samples arose as a part of the heating of the derivatizer to volatize the derivatives. The interfering material may have come from the soil itself, or it may have come from the ion exchange column. It did appear that the amount of material present in lab derivatization vials was soil dependent. However, the material still could be coming from the ion exchange resin since the amount and nature of the salts from the soil could affect the amount of material released by the resin.

The hypothesis that the problem with soil derived samples was from volatization of an interfering organic material was tested as follows: a 25 percent aliquot of a sample derived from ^{14}C labeled Waukena soil processed as usual through the IEC step was injected into the derivatizer

cell and derivatized. The cell was then opened and the final product dissolved in methylene chloride. The chromatogram of 20 percent of total sample (Figure 3-25) showed all amino acids with no extraneous peaks prior to glutamic acid.

This demonstrated that the derivatizer was functional up to the point of derivative injection onto the CG column, and tends to confirm that the extraneous peaks seen in previous tests were caused by volatilization of material not soluble in methylene chloride.

Several days later, the derivatizer was reassembled and heated with the nominal CG injection procedure to volatize any material remaining in the cell onto the CG column for analysis. Only a small baseline increase was observed in the chromatogram indicating that a large amount of material was not driven out of the derivatizer into the CG column. It is not known why so little material was seen in this test. Possibly the non-CH_2Cl_2 soluble material was lost or decomposed while the derivatizer was open and exposed to air.

It is possible that the interfering material seen in the end to end tests with Process No. 1 may not occur with the new baseline process. Further testing with the current cell may elucidate methods of circumventing the interfering material. Removal of all non-metallic components may be of value since the interfering material may be absorbed in the Teflon. Use of a different CG liquid phase may also reduce the effect of the interfering material.

If other solutions are unsatisfactory, it should be possible to carry out sample removal from the derivatizer and sample introduction onto the CG column in a manner similar to laboratory methods by extracting the derivatives out of the derivatizer with CH_2Cl_2 and transferring the solution to another small cell which would then be used to evaporate the derivatives into the column. (Prior low temperature evaporation of the methylene chloride into the vent might be necessary.)

After cleaning the derivatizer and installing new seals, the final test which was derivatization of optically active amino acids with optically active 2-butanol/HCl was carried out. The only difference between this test and previous tests with pure amino acids was the use of optically

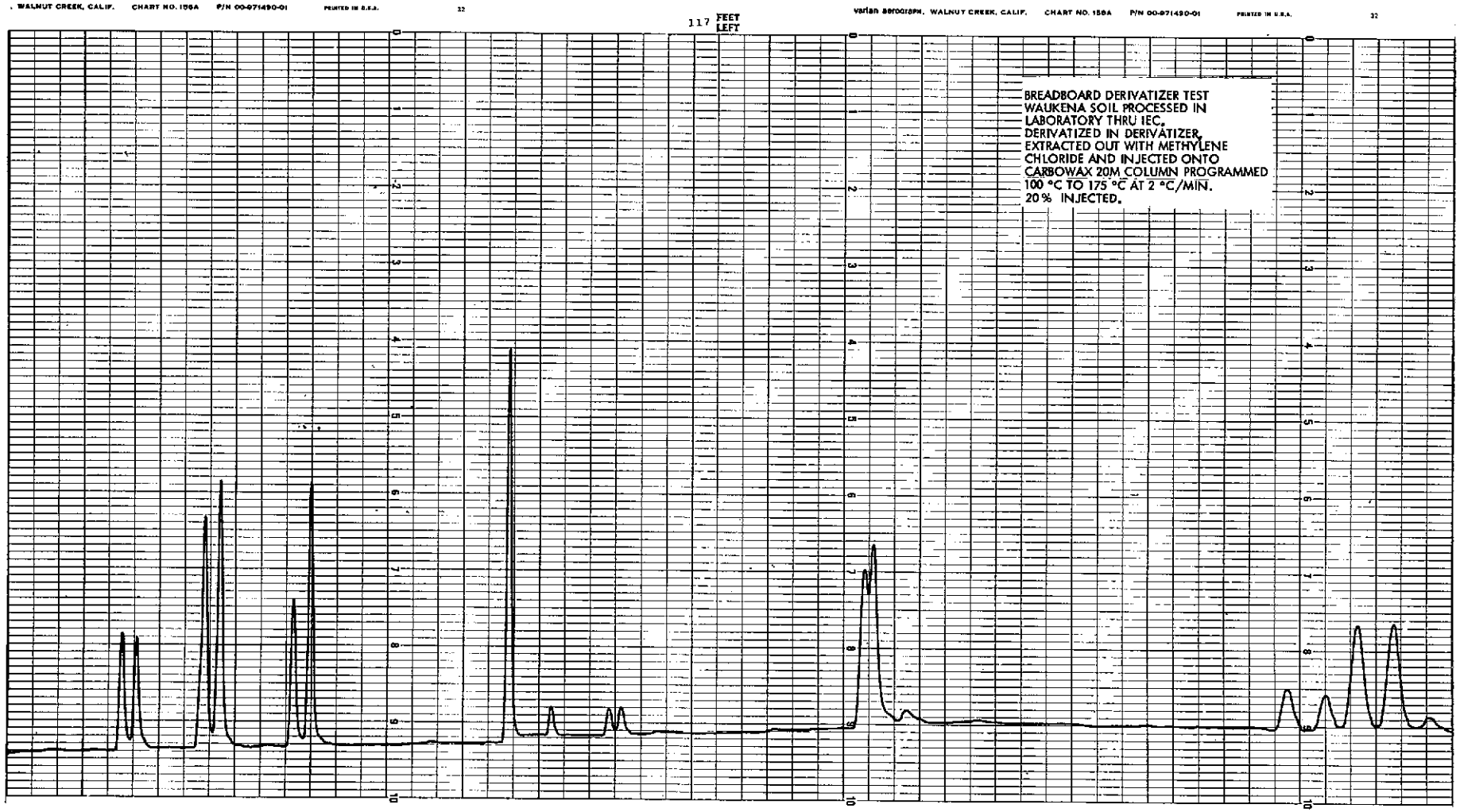


Figure 3-25. Chromatogram, Breadboard Derivatizer Test

active 2-butanol instead of inactive 2-butanol, and the replacement of the tantalum seals with new ones of the same type.

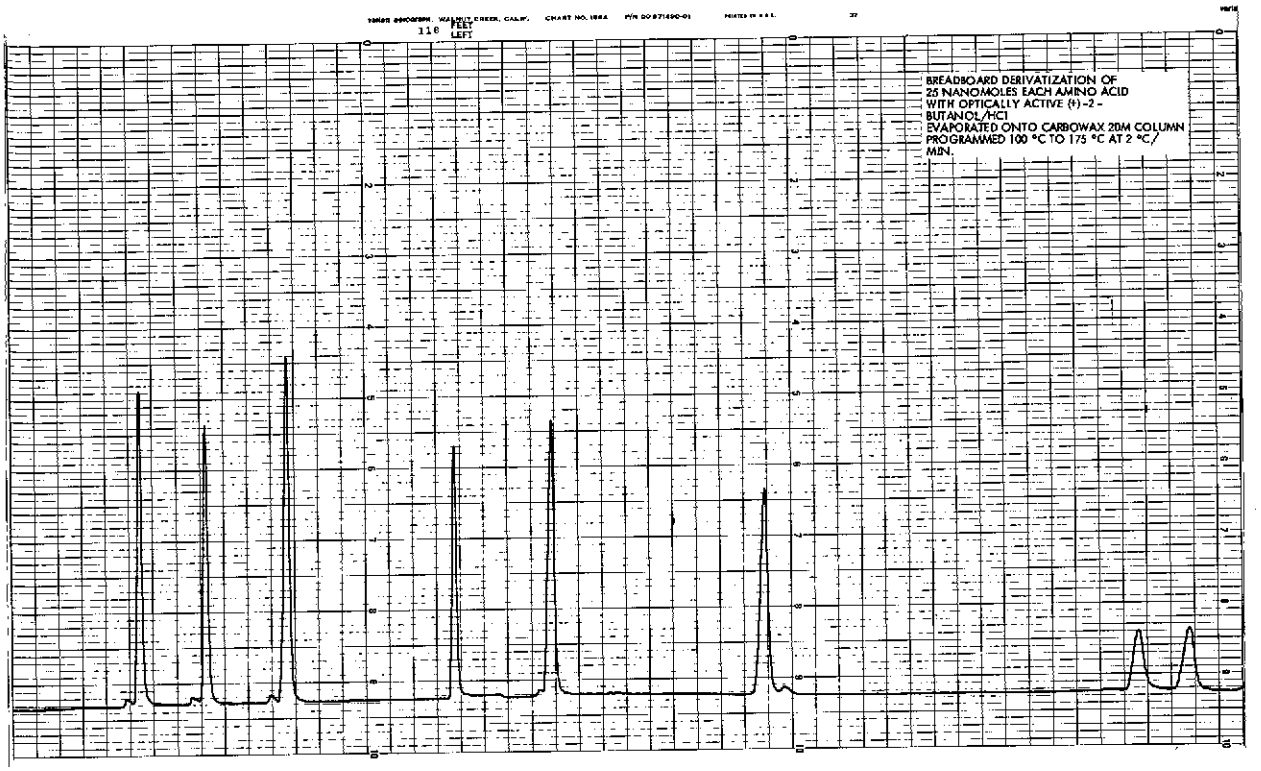
The chromatogram resulting from the test is shown in Figure 3-26. No observable racemization had occurred. The small peaks in the (D+), (L-) position are caused by the residual (-) butanol isomer in the alcohol rather than from amino acids racemized to the D configuration. The other encouraging result is that there were no significant interfering peaks to the chromatogram at the 25 nanomole level. Thus it appears that the only outstanding problem with the derivatizer is the interfering material observed with soil derived samples, and it is felt that at least one solution exists for this problem.

3.2.1.5 Self-Heated Gas Chromatographic Column

Prior to fabrication of the actual breadboard self-heated column, a short engineering model was fabricated for preliminary evaluation. This model was constructed from a 100 foot of 0.062 inch OD by 0.0023 inch ID section of stainless steel tube wound on a fiberglass mandrel and was instrumented with five thermocouples. The tests were conducted in a vacuum to provide a thermal environment similar to the heavily insulated flight design concept. The column was programmed over a range of 25° to 250°C in the self-heated mode. No temperature control problems were observed.

Based on the satisfactory performance of the model, the prototype self-heated column described in Section 3.1.7 was designed and fabricated. After the prototype column was completed, except for installation of the spool in the can and packing with the liquid phase, engineering thermal tests were conducted with the column packed in insulation to simulate the final configuration. The temperatures recorded (at approximately steady state) using the center thermocouple for the control (TC No. 4, Figure 3-17) are shown in Table 3-4.

The temperature difference between the middle of the column (TC No. 4 and TC No. 6) and the portions of the column at the ends of the spool appeared to be caused by the higher conductive losses resulting from contact with the aluminum spool. The flight design, which uses a thermal insulator for the spool, should have much lower thermal losses



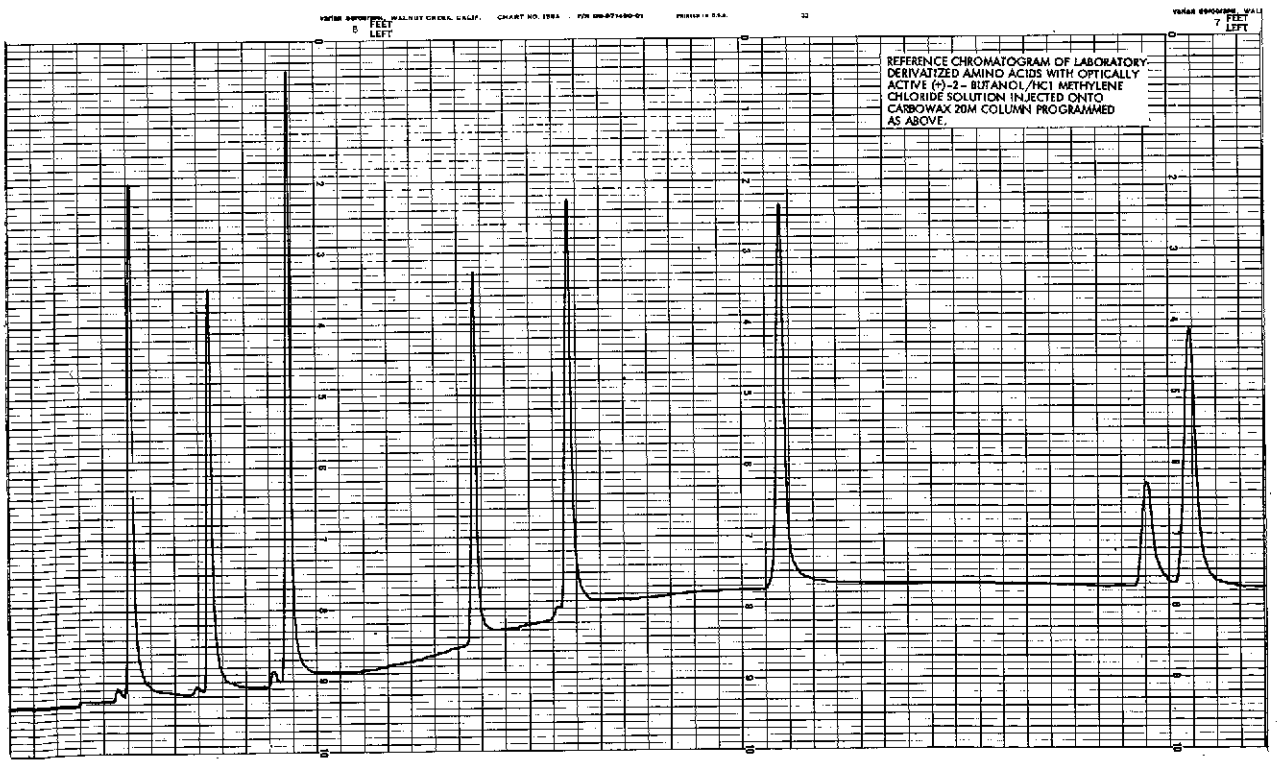


Figure 3-26. Chromatogram, Breadboard Derivitization

Table 3-4. Steady-State Temperatures

Control (TC No. 4)	TC No. 1	TC No. 2	TC No. 3	TC No. 5	TC No. 6	TC No. 7
92°C	80	84	80	77	92	81
140°C	122	128	124	122	137	122
204°C	179	189	184	182	190	179

at the spool ends. Also, some improvement was expected with the prototype when it was installed in the can and packed with the aluminum oxide insulation. After installation in the can and addition of the aluminum oxide sphere insulation, the column was coated with Carbowax 20M via the standard procedure resulting in a 46 milligram loading. It had been planned to heat the column using an SCR type linear temperature programmer. However, AC feedback through the control thermocouple occurred and interfered with operation of the temperature programmer. Therefore, the column was heated with a DC power supply and a thermocouple on-off controller. Temperature programming was accomplished by applying sufficient power to the column to obtain the approximate rate of rise desired. The thermocouple controller was used to set the upper temperature limit.

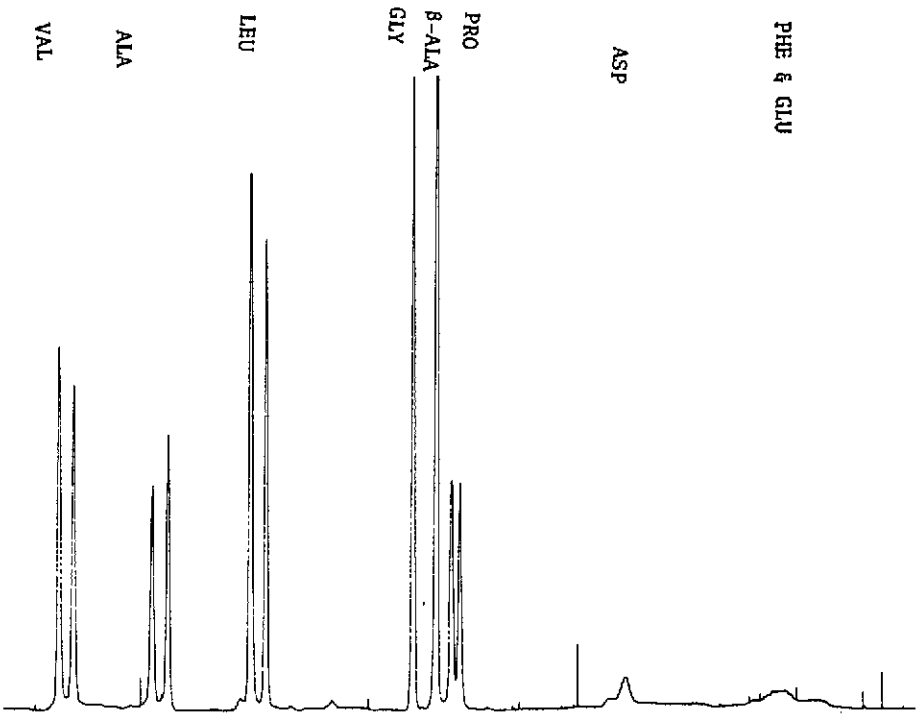
To evaluate if the column was properly coated, it was installed in a GC oven and held isothermally and operated as a normal column. The resulting chromatograms with standard derivatives showed good resolution and separation. Next, several chromatograms were run in a self-heated mode, but they were not interpretable, apparently because of insufficient conditioning of the column. There did not appear to be any interaction between the column heating power supply and the GC detector electrometer even though insulating unions were not used (power was applied to the middle of the column with the two ends being at ground potential).

After additional conditioning, the column gave much better results. Injection of standard derivatives into the cold column followed by programming the column in a self-heated mode at approximately 2°C/min (ballistically) gave good results through proline, but the later peaks were

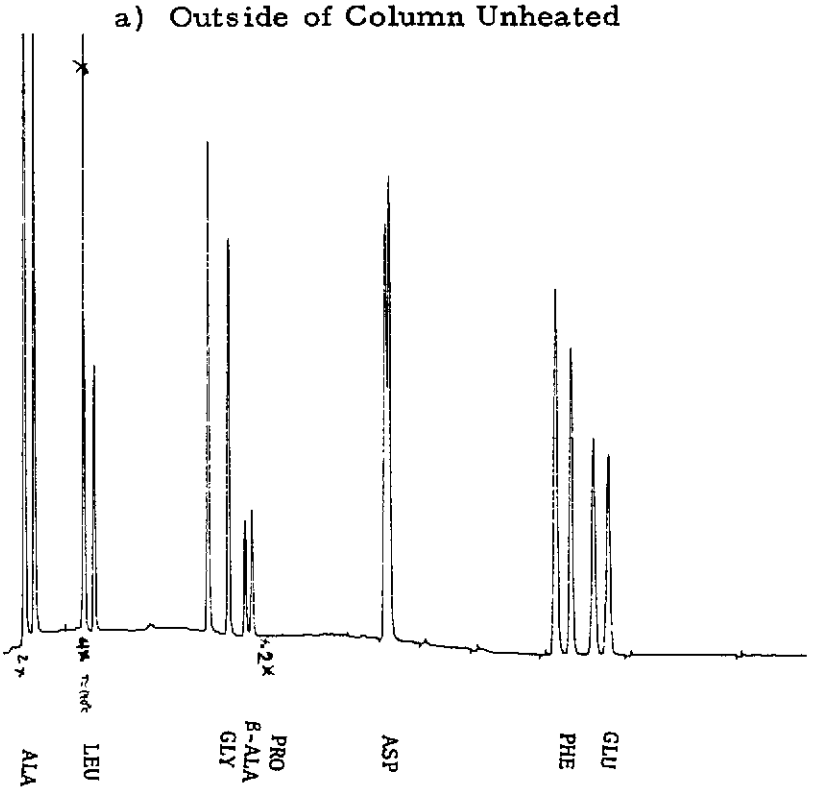
poor as shown in the upper curve in Figure 3-27. It was felt that this was a result of cold spots somewhere in the system either in the column itself or in the line between the column and the detector. Modifications were made to the connecting line heater to ensure that it was adequately heated which did not affect the chromatogram. Thus, it was concluded that there must be cold spots within the self-heated column itself. The most likely area for these cold spots was the interface plate where the column and the electrical leads were fed through. To test the cold spot hypothesis, the column was put in a GC oven such that after injection onto the cold column, the outside of the column was heated by heating the GC oven to 170°C. This external heating had only a small effect on the column temperature as a whole because of the high degree of insulation. The column itself was operated self-heated in the same manner as before. The chromatogram in this case was excellent (Figure 3-27), lower curve) for all the sample constituents (Ala, Leu, Gly, β-Ala, Pro, Asp, Phe, Glu) recorded. The aspartic acid resolution of 37 percent was typical for our results with 0.030-inch ID Carbowax 20M columns. This confirmed that the only problem with the self-heated column was the presence of some cold spots.

Additional heaters on the ends of the spool were added to remove the cold spots. The prototype column was then reassembled and tested again with standard derivatives. The sample was injected in CH_2Cl_2 via a heated injection port into the column which was at 30°C. The column was then heated in the self-heated mode with power also applied to the auxiliary heaters. The chromatogram shown in Figure 3-28 demonstrates satisfactory performance. Thus, the basic concept and design has been confirmed. Some redesign of the prototype is desirable, however, to reduce the amount of auxiliary heat needed.

The prototype column was not tested for life, but laboratory Carbowax 20M columns have storage times of at least several years. The columns last 2 to 5 months in heavy use so that both the storage life and the number of analyses available for calibration and test as well as mission use are expected to greatly exceed that which is required for a successful mission.



a) Outside of Column Unheated



b) Outside of Column Heated

Figure 3-27. Chromatograms Obtained with Breadboard Self-Heated Column

200 FT LONG X 0.030 INCH I.D. COATED WITH CARBOWAX
20 M TEMPERATURE PROGRAM APPROXIMATELY 3°C/MIN

INJECTION OF STANDARD AMINO ACID DERIVATIVE MIXTURE
(20 NM LEVEL)

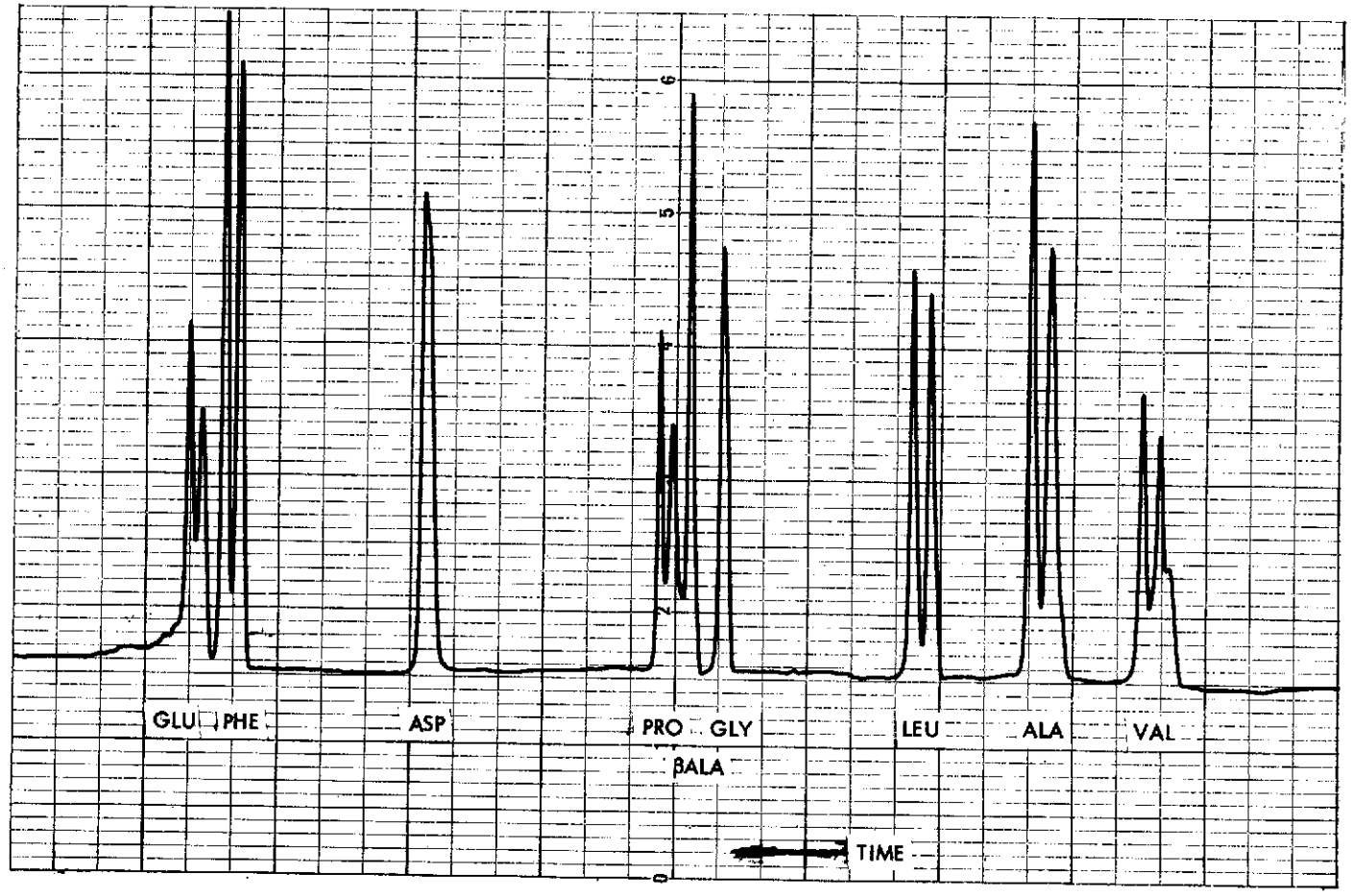


Figure 3-28. Prototype Self-Heated Column Chromatogram

3.2.1.6 Valve Simulators

No special testing of the valve simulators was carried out except for functional and leak checks. Instead, evaluation of the valve simulators is based on their performance while in use on the breadboard. In general, the original valve simulators worked reasonably well in that part of the breadboard where they were only exposed to aqueous reagents. The valve simulator used on the hydrolyzer did have some leakage problems but this was probably caused by soil particles getting in the valve. The breadboard hydrolyzer valve was a slightly larger design than those used in the rest of the system and hence had a larger sealing surface and was more prone to leakage. It is recommended that the outlet valve block on that unit be replaced with one which will accommodate the smaller valves. No significant problems were encountered with the valve simulators on the evaporator, ion exchange column, and the SC-1, SC-2 valve block. Some external corrosion of the outer stainless steel parts occurred because of the generally corrosive environment of the breadboard. This external corrosion did not cause any problem except in one case where the spring in the simulator degraded and did not apply adequate force. This was corrected by replacing the spring.

As discussed in Section 3.2.1.4, the original valve simulators were not satisfactory when used on the derivatizer. The original valve would not function without an elastomeric shaft seal and the elastomers produced interfering material in the derivatization. These results lead to the design and fabrication of a valve simulator which had only tantalum and Teflon exposed to the derivatization reagents. Early versions of the all tantalum valves had some mechanical problems which caused the valve to close when heated, but the final version worked quite well and much better results were obtained with the derivatizer when these valves were used. It appears that flight valves with characteristics similar to the all tantalum/Teflon valve simulators will work satisfactorily throughout the system with either Process No. 1 or the new baseline process.

3.2.2 Breadboard System Tests

Following breadboard component tests, various system level tests were carried out, primarily with the hydrolyzer, evaporator, and ion exchange column combined so that the entire soil processing and desalting portion of the experiment was tested as a system. Also, two complete breadboard "end-to-end" tests were conducted with the entire breadboard tested as a unit with only the gas chromatographic analysis being performed outside of the breadboard. The number of system tests and the number of soils used were limited because the interfering material problems from the ion exchange resin and the Teflon filters, and the interference problems with the derivatizer prevented meaningful tests from being conducted earlier in the program.

The basic conclusions reached concerning the system level breadboard testing was that most of the results were very favorable. The radiotracer recoveries for the hydrolyzer through IEC portion of the processing ranged from 54 to 82 percent. However, the recoveries were reduced by the various operational problems, primarly, channelization of the IEC caused by gas being pushed through it. Based on component tests and the known losses, 85 to 95 percent recovery could be expected in the absence of problems associated with manual operation. Freedom from racemization was demonstrated which is a most important result. Problems observed were the continuing low level contamination produced from the Teflon filter material, and the derivatization problems which occurred in the end to end tests. In changing from Process No. 1 to Process No. 2 most of the difficulties encountered in the system level testing should not occur or should be minimized. Because of the deletion of a precipitation step in Process No. 2, there will be no difficulty in transferring processed solution into the 5 milliliter IEC, and hence channelization can easily be avoided and losses of amino acids from this cause will be eliminated. Thus, amino acid recovery (excluding extraction efficiency considerations) should be high (~90 percent) for the processing part of the breadboard with Process No. 2. As discussed in component testing, contamination from the Teflon filters can probably be reduced or eliminated. The remaining significant problem is that associated with evaporating derivatives out of the derivatizer and onto the GC column, and, as discussed in Section

3.2.1.4 (derivatizer component testing), at least one solution does exist for this problem.

In summary, with either Process No. 1 or Process No. 2, we have great confidence that a successful experiment can be carried out and in changing to Process No. 2, most of the problems we have experienced will not occur or can be solved readily.

3.2.2.1 Hydrolyzer Through Ion Exchange Column System Tests

System Blank: The hydrolyzer, the SC-1, SC-2 valve block, the evaporator, and the ion exchange column were connected in the nominal system configuration as shown in Figure 3-1. (At this time NH_4OH had been substituted for NaOH). The only deviation in the system flow path was the absence of the liquid level sensor. The filters used in the hydrolyzer and evaporator were the usual combination of backup, 10 micron, 5 micron, backup Teflon filters. These filters were soxhlet-extracted with 6N HCl prior to use. After assembly, the hydrolyzer and evaporator were heated with 6N HCl and rinsed with distilled water. These rinsings were not passed into the ion exchange column (IEC). The processing was carried out for the standard times with the reagents specified for Process No. 1. The only difference between this test and a soil test was the absence of soil.

Phenolphthalein was added to the 10 milliliter evaporator wash to monitor the IEC effluent. The collection of the 7 milliliters of IEC effluent following NH_4OH breakthrough included the 0.3 milliliter of solution which was in the 1/8-inch Teflon line connected to the IEC outlet prior to the appearance of the phenolphthalein color.

Evaporation of the NH_4OH and the subsequent derivatization steps were carried out in the lab glassware. There were several significant interfering peaks in the gas chromatogram (Carbowax 20M column) similar to those observed in ion exchange column blanks (Section 3.2.1.3). The two largest interferences were one of approximately 30 nanomoles near valine, and one of 130 nanomoles near leucine (later demonstrated to be trifluoroacetamide).

A number of amino acids were detected, including 16 nanomole Ala, 25 nanomole Gly, 4 nanomole β-Ala, 6 nanomole Pro, 6 nanomole Phe,

11 nanomole Glu. Interfering peaks prevented the measurement of Val, Leu, and Asp, but the maximum quantity of Asp plus interference is 18 nanomole. These amino acid quantities were lower than previous breadboard evaporator blanks but still were excessive. The Teflon filters are still felt to be a likely source of amino acid contamination.

Waukena Soil Test: Except for the IEC, only minor cleaning was required to ready the breadboard for the soil test after the system blank. The hydrolyzer was rinsed with redistilled water, the evaporator was flushed with 6N HCl and then redistilled water. The liquid level sensor was washed with HCl and water and installed in the liquid path out of the evaporator. The IEC was regenerated with HCl and then washed to neutrality. During the wash, the column was backflushed for a while to attempt to reduce any channelization which might have occurred in earlier testing.

The Teflon filters in the hydrolyzer and evaporator were not replaced for this test since after rinsing they should have been even cleaner than before the system blank.

The sample used for the test was 1 cubic centimeter of Waukena soil to which 2 microliter quantities of ^{14}C labeled amino acid solutions were added. The radiotracers provided approximately 200,000 DPM each of proline, phenylalanine and glutamic acid. The hydrolysis, evaporation and HF/NH_4OH processing steps were carried out satisfactorily. The filtration step was very slow, approximately 0.2 cc/min at 20 psi pressure differential. The restriction was apparently caused by the precipitate clogging the Teflon filters. The time required for filtration at this rate was excessive, and it was felt desirable to change the filter configuration or to increase the pressure across the evaporator. Gas was pushed through the IEC during transfer of the evaporator rinse. Channelization of the IEC evidently occurred because of this gas event since early breakthrough of some radioactivity (~5 percent of the initial quantity) was detected in the IEC effluent during the 30 milliliter column wash. The amount of radioactivity detected in the 7 milliliters of solution after ammonia breakthrough was 81 percent of the initial quantity. Another 7 percent was accounted for in the accumulated column wash and pre and post 7 milliliter samples.

A similar sample of Waukena soil was processed in laboratory glassware as a control. Some of the filtrate from the HF/NH_4OH processing was lost so the overall radiotracer recovery was reduced. The 7 milliliter fraction of the ion exchange effluent after NH_4OH breakthrough contained 76 percent of the initial radioactivity. Derivatization in laboratory glassware and gas chromatographic analyses were completed on the breadboard and lab Waukena soil processing samples. Table 3-4 summarizes the radiotracer results. The derivatized samples were analyzed by gas chromatography on a Carbowax 20M column. The chromatograms from the breadboard and lab samples were very similar except that the breadboard sample gave larger amino acid derivative peaks as would be expected from the fact that the overall radiotracer recovery was greater for the breadboard sample. The average ratio of the breadboard sample peak areas to the lab sample peak areas was 1.41 (Table 3-5), close to the ratio of overall radiotracer recovery which was 1.37. The interfering peak thought to be caused by trifluoroacetamide (which occurs at the leucine position on Carbowax 20M) was larger in the lab sample than in the breadboard sample. The overall results of the test are considered good.

Table 3-4. Breadboard Hydrolyzer Through IEC Waukena Soil Test-Radiotracer Results

	Breadboard Sample		Lab Sample	
	DPM	Percent Recovery	DPM	Percent Recovery
Initial quantity (Pro, Phe, Glu)	614,000		614,000	
Ion Exchange				
Column Wash	27,200[a]		27,200[b]	
Precuts	11,600		5,058	
Prime Fraction	495,000	80.6	470,000	76.5
Postcut	800		1,350	
Recovered in Clean up	31,300		5,410	
Total counts accounted for thru ion exchange step	566,500	92.3	481,800	78.5
Final Derivative	355,500	57.9	259,500	42.3

[a]Collected during 30 milliliter column wash prior to NH_4OH elution.
[b]Approximately 25 percent of sample lost by spillage.

Table 3-5. Breadboard Hydrolyzer Through IEC Waukena Soil Test-Chromatographic Results

Amino Acid	Peak Area		Ratio Breadboard/Lab
	Breadboard Processed Sample	Lab Processed Sample	
Val[a]	161,400	120,600	1.33
Ala & 1 Leu	746,100	493,100	1.51
Leu + NH_3	667,700	1,268,000	0.53[b]
Leu[a]	213,500	173,200	1.23
Gly	546,000	346,100	1.31
β-Ala	74,400	52,500	1.42
Pro + unknown	323,700	170,300	1.90[b]
Asp	693,900	400,200	1.73
Phe	203,000	182,500	1.11
Glu	604,400	368,700	1.64
			average ratio 1.41

Conditions: Carbowax 20M column 0.030 id x 200 ft., Varian 1860 Gas Chromatograph Electrometer setting 1 x 10^{-11} amps. N-TFA, D, L-2-butyl derivatives. Because of D, L-2-butanol, the two peaks obtained for optically active amino acids are summed except:

[a]When an interference occurs on one peak only the non-interferred peak was used.

[b]Not used to obtain average ratio.

An extensive cleanup was carried out at the conclusion of this test which included replacement of most of the 1/16th-inch Teflon interconnecting tubing. Post-test inspection of the breadboard components during cleaning indicated that the components were still in good condition. The Creavey seal in the hydrolyzer showed some corrosion of the inner stainless steel spring, so it was replaced with a new one. The Teflon-coated stainless steel spring used in the ion exchange column was inspected during repacking and was found to be in excellent condition with no evidence of attack.

Cleaning Tests. The hydrolyzer filters were replaced with new soxhlet-extracted ones of the same type. In the case of the evaporator, a new type of Teflon filter cloth was used as the upper filter in an attempt to prevent clogging that occurred with the type of filter cloth previously used.

Because the previous system blank had shown significant quantities of amino acids and other material, some cleaning tests were conducted to see if additional cleaning in the assembled units would reduce the amount of contamination. After the usual cleaning procedure which consists of several short soaks with hot 6N HCl and rinses with distilled water, prolonged heatings of the hydrolyzer and evaporator were carried out with 6N HCl in the units. After these heatings, the HCl was collected, evaporated to dryness and derivatized. The resulting derivatives were analyzed by gas chromatography to determine how much material was present and if the samples from the second heatings of the two units were any better than the first. In the case of the hydrolyzer, 7.5 milliliters of 6N HCl was used with the cell being heated for 18 hours at 100°C. The resulting derivatives showed a number of peaks, both amino acid and non-amino acid. The amount of amino acids was smaller in the second sample than in the first, but the results were complex for the non-amino acid material, and the overall quantity of non-amino acid material appeared to be larger in the second sample than in the first.

In the case of the evaporator, 30 milliliter of 6N HCl was used, again for 18 hours at 100°C. After this period, most of the HCl solution was evaporated from the evaporator. The remainder (5 milliliter in one case, 10 milliliter in the other) was collected, evaporated and derivatized in the derivatization vials. The gas chromatographic results were similar to those from the hydrolyzer in that the amount of amino acids seen in the first sample were greater than in the second, while the results for the non-amino acid material were complex. The overall level of material found was greater for the evaporator than for the hydrolyzer. Most of these problems should not occur with the new baseline process because the filtration requirements are much different from Process No. 1 and the filter design can be altered or in the case of the hydrolyzer/evaporator, the filter may be unnecessary.

System Blank. After the above cleaning tests, the hydrolyzer and evaporator were rinsed with redistilled water, the IEC was regenerated and a no-soil system blank was run. The hydrolysis and evaporation part of the processing proceeded normally. The filtration of the solution after the HF-NH_4OH processing was slow, and 50 psig of He pressure was required to obtain 0.4 mil/min flow rate. Apparently the new Teflon filter cloth was also blocked by the NH_4F precipitate. The liquid level sensor failed to operate during this test, apparently because of the formation of a conductive path which was present even when gas was passing through the sensor. In this test, only a small amount of gas entered the IEC. The reaminder of the IEC processing was carried out without difficulty and the 7ml of NH_4OH effluent was evaporated and derivatized in the lab. It was obvious after evaporation of the NH_4OH that a large amount of material was present in the sample so that only 2.5 percent (rather than 25 percent) was injected onto the Carbowax 20M column for gas chromatographic analysis. The chromatogram showed a number of interfering peaks, some of which were very large. The peak ascribed to trifluoroacetamide indicated a quantity of approximately 2 micromoles, and in general this system blank was considerably worse than the one run earlier. The major difference between the two system blanks was the use of a new type of Teflon filter cloth in the evaporator. The source of this filter and some of the other filters is Chemplast. We have since learned that Chemplast prepares Teflon filters which are replicas of cellulose type filters; that Teflon is sintered over the cellulose material to be replicated and that the cellulose is "burned off" during the sintering process. We did not discuss this with Chemplast, but if such a process has been used to prepare the filters, there may have been sufficient organic matter left after the sintering process to result in the contamination that was observed in these tests. Such material may not be easily removed by the HCl cooking procedure used for cleaning and may come out in large amounts during the HF-NH_4OH processing step. This could account for the fact that the system blank was much worse than the individual hydrolyzer and evaporator HCl blanks.

Because of the filter problems, a series of filter tests was carried out in the evaporator which is discussed in Section 3.3.25. Based on these tests, 10-micron Millipore Teflon filters were selected for use in

the remainder of the testing as being the best reagent compatible material available at that time even though they were not completely satisfactory.

The breadboard was cleaned, the IEC was repacked and regenerated, and then a hydrolyzer through IEC system blank was run. Two nanomoles each of ^{14}C labeled alanine, proline phenylalanine, and glutamic acid were added to monitor amino acid recoveries in a very low level situation.

After the HF/NH_4OH step was complete and transfer of the solution to the IEC had started, a large volume of gas was pushed through the evaporator and into the IEC even though approximately half of the solution was still in the evaporator. Apparently the evaporator standpipe was so close to the filter that the gas was able to push through the liquid and precipitate and flow directly out of the cell. The cell was not being pressurized via the standpipe, but a leak in the external gas lines allowed the standpipe to be pressurized. This problem was solved by disconnecting and capping off the line leading to the standpipe through valve S-2. The flow rate of liquid out of the evaporator was very slow when it was flowing properly, being 0.1 ml/min. Increasing the pressure on the cell did not appear to cause much change in flow rate. Apparently the additional pressure compresses the precipitate so that it becomes more of a restriction at high pressures. As usual, the IEC effluent was collected throughout column operation. The precuts (solution prior to NH_4OH breakthrough) contained 11 percent of the original radioactivity indicating that channeling in the IEC was very severe. The 7 milliliter prime cut contained 65 percent of the radioactivity. After the sample was removed for radiotracer measurements, the bulk of the prime cut was transferred to the derivatizer and the nominal derivatization process was carried out. The product was evaporated onto a lab GC column. As discussed in Section 3.2.1.4, only a very large interference was seen in the gas chromatogram. In light of the severe channeling of the IEC and other problems, the recovery of 65 percent in the prime cut is not surprising. The total recovery from the breadboard including pre and post cuts, and known losses was 84 percent. No soil was used in the test, instead 7 to 5 milliliters of 6NHCl containing the radiotracers was heated in the hydrolyzer for the nominal 16 hours at 110°C. The remainder of the preprocessing was carried out nominally. The flow rate out of the evaporator through

the IEC after HF/NH_4OH processing was 0.7 ml/min at 46 psig. When the filtration of the liquid was finally complete at that pressure, and the gas penetrated the filter, the remaining liquid in the lines moved very rapidly, and it was not possible to prevent some gas from entering the IEC, again causing channeling and somewhat reduced recovery in the 7 milliliter prime cut from the IEC elution (81.5 percent of the original radioactivity recovered). The evaporation and derivatization was carried out in the lab. The chromatogram (run on Carbowax 20M) was relatively clean with both the amino acid contamination and the interfering material peaks being at a low level (except for the trifluoro-acetamide peak) prior to the glutamic acid peaks.

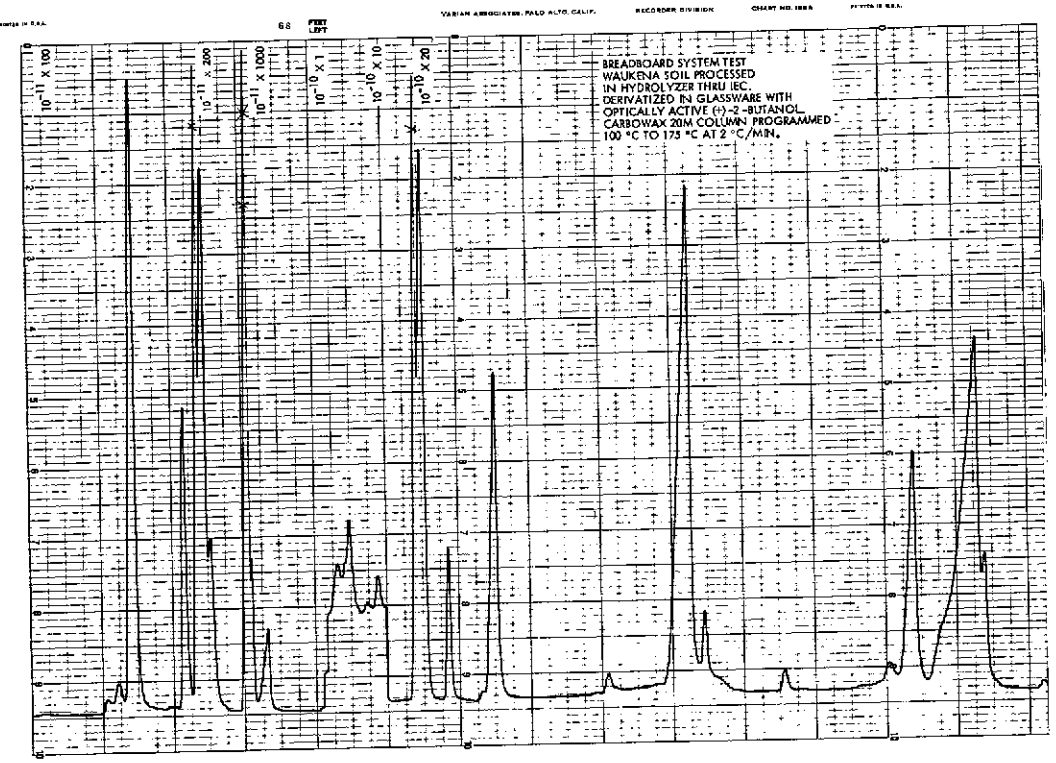
3.2.2.2 End to End System Tests

The next test was designed as a complete end to end (hydrolyzer through derivatizer) test. Because the quantity of amino acids in Waukena soil (>200 nanomoles/cc for some amino acids) is sufficient to overload the column, the Waukena soil was diluted with blank soil in a 25:75 ratio in order that the sample used would contain no more than approximately 50 nanomoles of any given amino acid. The one cubic centimeter of 25 percent Waukena/75 percent blank was innoculated with 2 nanomoles each of ^{14}C labeled alanine, proline, phenylalanine and glutamic acid to monitor the intermediate processing steps.

The quantity of NH_4OH used in the HF/NH_4OH processing was changed from 14 milliliters to 16 milliliters based on laboratory processing of blank soil samples which showed that an extra 2 milliliter of 4N NH_4OH was required to reach pH9 for the blank soil as compared to Waukena soil. There were several hardware and procedural problems which occurred during this test.

After removal of 0.25 inch of tubing from the end of the evaporator standpipe, cleaning of the breadboard, repacking and regeneration of the IEC, the final system test was carried out. This test had two goals, one of which was to carry out another end to end system test, and the other was to provide a sample processed from the hydrolyzer through the IEC to be derivatized with optically active 2-butanol for information on possible racemization effects in the breadboard processing. The soil sample was 1 cubic centimeter of Waukena soil innoculated with 2 nanomoles each

of the same ^{14}C labeled amino acids as those used in the previous two tests. Procedural problems occurred which resulted in extra losses. The hydrolysate evaporation process was erroneously started with the evaporator at too high a temperature which resulted in bumping when the vent valve was opened. Post-test sampling of the vacuum system traps revealed a 13 percent loss of radioactivity because of this problem. The flow rate out of the evaporator through the IEC after the HF-NH_4OH processing was 0.25 ml/min at 47 psig. The spring on the S-6 valve simulator (IEC inlet) caused difficulties during IEC operation. The spring had become corroded because of the general environment surrounding it. Because of the spring, the valve did not operate reliably, and gas was pushed through the IEC again. Eighteen percent of the radioactivity was collected in the IEC effluent prior to NH_4OH breakthrough. The 7 milliliter prime cut contained 54 percent of the radioactivity. After collection and radiotracer sampling, the 7 milliliters were divided into two aliquots. Twenty-five percent of the sample was introduced into the derivatizer and a nominal derivatization carried out. As discussed in Section 3.2.1.4, the chromatogram resulting from this derivatization again showed only a large interference. The remaining 75 percent of the 7 milliliter IEC prime cut was derivatized in laboratory glassware using optically active (+)-2-butanaol/HCl. The resulting chromatogram (Figure 3-29, upper) indicates that no significant racemization had occurred as a part of the breadboard processing. Where measurable, the diastereomer ratios for the test sample and for standard amino acids (Figure 3-29, lower) are equivalent.



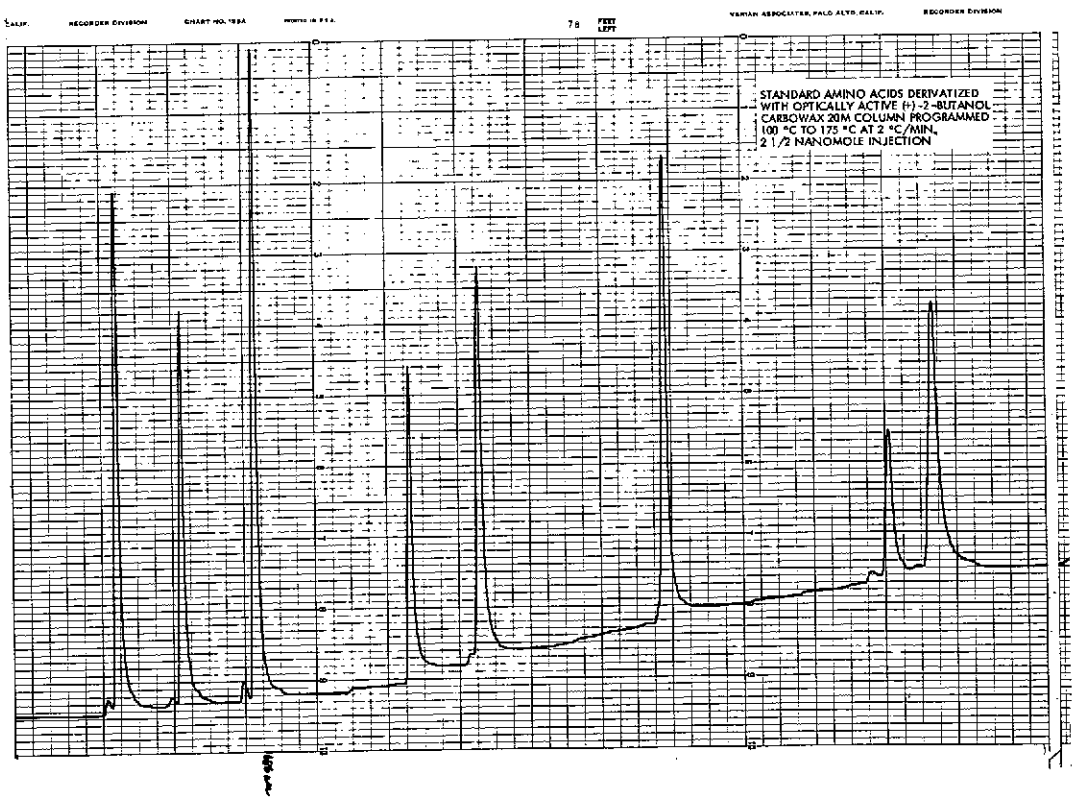


Figure 3-29. Chromatogram, Breadboard System Test

3.3 SUPPORTING LABORATORY STUDIES

In addition to the laboratory processing tests run as controls in parallel with breadboard tests, a variety of supporting laboratory work was performed. Standard procedures and methods were established, reagents purified, and material compatibility tests run.

3.3.1 Soil Tests

Of the soils provided by NASA/ARC/Waukena, Aiken, Hazen, Death Valley, Volcanic Ash and blank), Waukena and the blank were used for most of the lab testing and all of the breadboard testing. The other soils except volcanic ash were all run at least once in the lab. No unusual problems occurred with any of the soils although considerable variation (±2 milliliters) was found in the amount of 4N NH_4OH needed to achieve the desired pH of 9 in the HF/NH_4OH processing. The effect of soil variability is expected to be much less with the baseline process, but this needs to be evaluated.

Earlier in the program before the breadboard components were ready for testing, lab processing tests were carried out to gain familiarity with the Process No. 1. A test of soil processing efficiency was run in duplicate (Samples A, B), with labeled amino acids to determine losses at various steps in laboratory glassware. 0.05 μC each of glycine-^{14}C, lysine-^{14}C, and glutamin-^{14}C, equivalent to about 164,500 cpm were added to 1 cubic centimeter Waukena soil samples which were then processed. Results are in Table 3-6. Samples were taken and numbered according to the experimental sequence. Hydrolysis rinses A and B consisted of 15 to 20 milliliters wash water rinses of the hydrolysis vessel after the prescribed 5 milliliters rinse of Step 5. This water was not added to the hydrolysate.

Sample A-9 was 0.1 milliliter taken prior to filtration of the NaOH/HF processed solution (contained salts). Sample B-9 was 0.1 milliliter taken after filtration of the NaOH/HF solution. Some of the radiotracers were stuck onto the precipitate, but the next step, Samples A-9W, B-9W proved these amino acids were washed off with the 10 milliliters water wash.

Table 3-6. Soil Processing Recoveries

Step		Measured cpm	Calculated cpm From Volume Sampled	Calculated Recovery Percent	Notes
Hydrolysis					
Step 5	Extra rinse A	548	2,200	Some loss	Extra rinse not added to hydrolysate
	Extra rinse B	1,764	2,200		
Step 7	A-7	1,478	143,000	87	Sampled after 10 milliliters H_2O added to evaporated hydrolysate
	B-7	1,398	135,000	82	
Desalting					
Step 9	A-9	578	147,000	89	A-9 before filtration
	B-9	521	130,000	79	B-9 after filtration
Wash	A-9W	158	11,000	6.6	
	B-9W	302	15,000	9.1	
Sum	A-9 + A-9W		158,000	96	
Sum	B-9 + B-9W		145,000	88	
After Ion Exchange					
	A10 α	648	600	Negligible loss	α = 2 milliliters prior to ammonia collection
	A10	1,854	126,000	77 percent	Ω = 2 milliliters after the first 7 milliliters of NH_3 solution
	A10 Ω	438	390	Negligible loss	
	B10 α	10,629	10,580	Early breakthrough	
	B10	518	32,800	20 percent	
	B10 Ω	607	560	Negligible loss	

It is seen that little was lost during processing until the ion exchange resin where anomalous results were obtained for replicate samples. Resin column B was allowed to go dry briefly and flow stopped whereas with column A, ammonia was added to keep head pressure constant. The early breakthrough and the low recovery are both ascribed to allowing column B to go dry during elution, and hence this was not a result of a problem that would have any significance to a flight instrument.

Much better recoveries were obtained in subsequent testing. The early lab and evaporator processing results (described in Section 3.2.1.2) were discussed in a meeting at NASA/ARC. Based on these results and other considerations (simplicity of pH control, fewer reagents, and ease of purification), it was decided that 4N NH_4OH should be substituted for the NaOH solution. Preliminary laboratory experiments were carried out to confirm the desirability of this step.

An HF/NH_4OH titration with and without Waukena soil hydrolysate, Figure 3-30, showed the system to be well buffered and less subject to wide pH variations compared to HF/LiOH titrations observed previously (Design Study, TRW Final Report No. 16660-6001-R0-00, Figures 4.1.2-1 through -4). Based on these curves it was decided to use 2 milliliters 4N NH_4OH more than required to reach the equivalence point. This leads to a pH of 8.8 with samples derived from Waukena soil which is in the desirable range and allows for large variations in the residual acidity from the evaporated soil hydrolysate. Use of a large excess of NH_4OH is undesirable because it would lead to higher pH's, and would reduce the effective capacity of the ion exchange resin.

Next, laboratory radiotracer processing experiments were carried out with two samples of Waukena soil hydrolysate: sample A with ^{14}C labeled proline, glutamic acid, lysine, and phenylalanine; and sample B with ^{14}C labeled alanine, leucine and valine. Approximately one million cpm total were added to each sample.

The labeled soil hydrolysates were evaporated to dryness, desalted, ion-exchanged and derivatized in laboratory glassware along with appropriate controls: a system blank, an ion exchange blank, and a derivatization blank. Samples were taken for radiotracer counting at various points to follow amino acid losses.

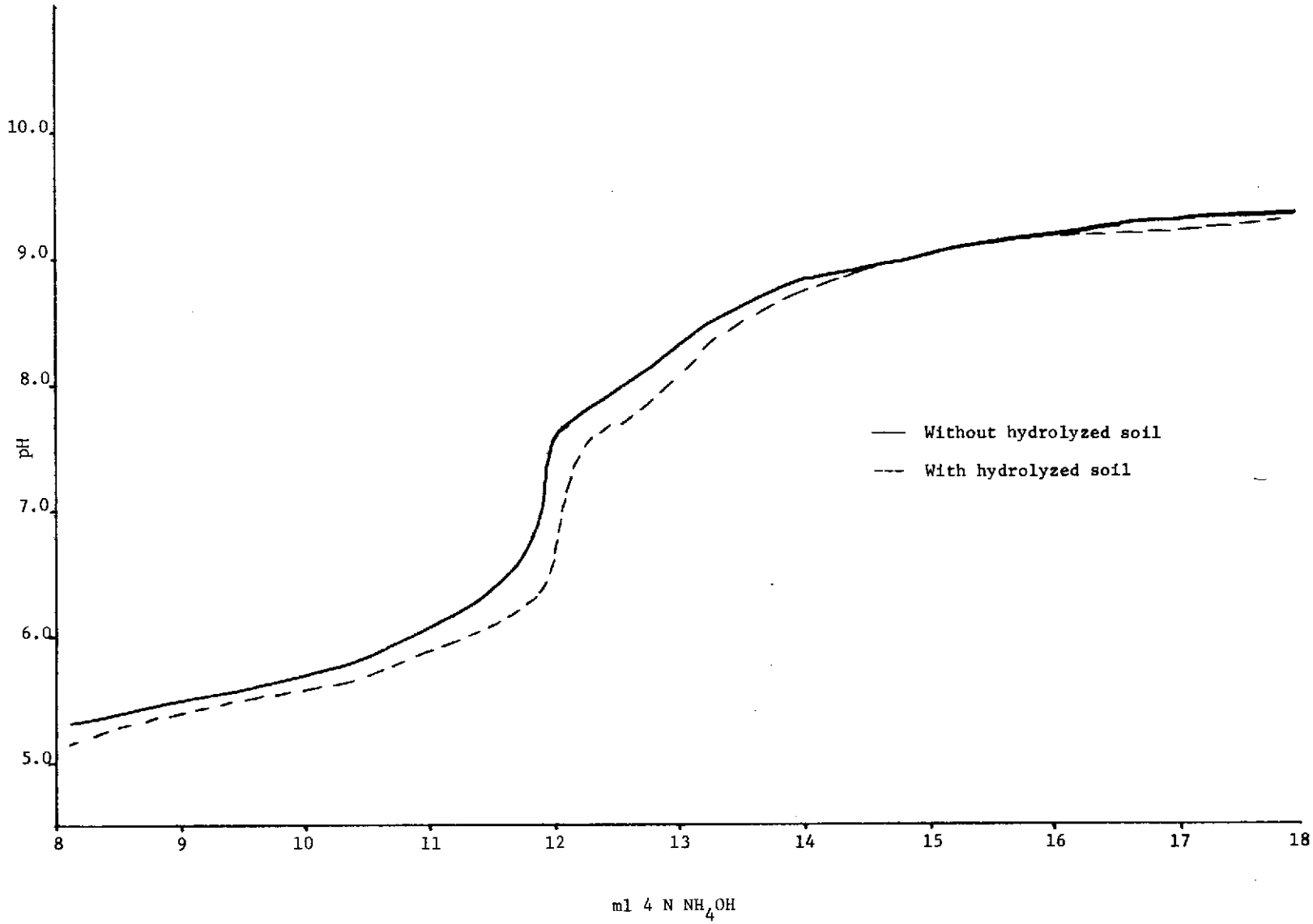


Figure 3-30. Titration of 4 N NH_4OH with 9 milliliters 5 N HF

The results of the radiotracer work are given in Table 3-7. Some variation in recovery is seen for the two samples. This is felt to result from handling losses rather than losses due to the ammonia reagent as the data compare with that obtained earlier using NaOH as a desalting reagent.

The chromatograms for this series of derivatives were run simultaneously on Carbowax and Dexsil columns to test a new Dexsil column and to collect background data on it while running under the same conditions as on the Carbowax column.

The two soil samples A and B gave nearly identical chromatograms, and no particular abnormalities were observed. On Dexsil, the major interference peaks were beyond the amino acid peaks. The interference peaks were not large, indicating that the system blank was improving.

Comparison with chromatograms from samples processed using NaOH to neutralize the HF showed no significant changes in either amino acid appearance or impurity peak size or location. Thus, the change from NaOH to NH_4OH in the precipitation step eliminated all reagent injection problems in the evaporator by greatly minimizing the difficulty in achieving the desired pH without causing any loss in performance either in amino acid recovery or in interfering material.

3.3.2 Material Testing

Material tests were carried out as required throughout the program to determine which materials were most suitable for cell construction, seals, valve components and filters.

3.3.2.1 Tantalum and Fansteel 63

Based on preliminary compatibility testing which showed that tantalum was unaffected by the derivatization reagents, tantalum was chosen as a candidate material for the derivatizer. Mixtures containing selected amino acids (at 10 micromole quantities) were derivatized using existing reagents with or without tantalum foil present. Semi-quantitative GC analysis gave peak heights within ±10 percent for the two samples for valine, glycine, proline, methionine and phyenylalanine, which was within experimental error.

Table 3-7. Radiotracer Recoveries for Soil Desalted with Ammonia

	Sample A		Sample B	
	cpm*	Percent Original Cts.	cpm*	Percent Original Cts.
Hyd flask rinse	9,070		3,400	
Prime filtrate	1,222,480	92.8	1,010,420	102.9
Filtrate rinse	58,410		44,330	
Ion Exchange				
Pre-cut	3,290		1,400	
Prime eluent	1,213,730	88.0	929,960	90.8
Post cut	1,400		730	
Transferring losses		-13.2		- 6.0
After HCl/2-Butanol	1,064,200	77.2	962,000	93.9
After TFA	973,200	70.6	1,016,600	99.2
Derivative**	982,400	71.3	871,400	85.1

*Corrected for background and for volume sampled.
Sample A labeled with proline ^{14}C, glutamic acid ^{14}C, phenylalanine ^{14}C, lysine ^{14}C.
Total cpm 1,378,490.
Sample B labeled with alanine ^{14}C, valine ^{14}C, leucine ^{14}C. Total cpm 1,024,480.

**After evaporation of TFA/CH_2Cl_2 and dissolution in CH_2Cl_2.

Next, solutions containing 1μM each of the following amino acids (alanine, valine, leucine, glycine, β-alanine, proline, aspartic, phenylalanine, glutamic acid, lysine) were prepared for testing with ~0.5 gram samples of tantalum and fansteel 63. Derivatives were prepared along with appropriate controls.

Neither of these metals significantly altered the chromatograms of the resulting derivatives within the reproducibility of the results.

Later in the program a possible alternate method involving acid hydrolysis in the presence of HF was discussed. At the request of NASA/ARC, the effect of this process on tantalum and Fansteel 63 was tested.

Two samples each of Fansteel 63 and pure tantalum were heated separately for 16 hours in sealed Teflon containers at 100° to 110°C in HF/HCl reagent prepared according to the procedure of Experiment Sequence A-1, Fluoride Method, supplied by G. E. Pollock of ARC.

- The Fansteel dissolved completely.
- The tantalum corroded to a small fraction of initial size and lost all structural integrity.
- The Teflon containers for these experiments survived the treatment but seemed to absorb some quantity of the solution.

These results indicate that it would be impossible to hydrolyze with HF present using either of these materials. Furthermore, the degree of attack was so great that it is considered unlikely that HCl/HF could be evaporated from Fansteel or tantalum processing cells. These results do not contradict our earlier tests of tantalum with HF in the HF-NaOH (or HF-NH_4OH) processing step since the exposure time is very short and the solution is cool.

3.3.2.2 Ion Exchange Column Spring

A Teflon-coated spring for use in the ion exchange column was tested with solutions it would be exposed to in this configuration. The solutions showed no discoloration and the coated spring suffered no obvious damage. The spring was incorporated into the breadboard ion exchange column.

At the completion of the program, after more than 15 tests and regenerations, many of which involved multiple acid/alkaline cycling, and more than 10 disassemblies and reassembly operations, the spring remained in excellent condition.

3.3.2.3 Seals and Valve Seat Materials

Viton E-60 and Creavey Seals:

At the first meeting with NASA/ARC, a question was raised about the compatibility of Viton E-60 which was proposed for use in the breadboard derivatizer and evaporator. Therefore, some brief tests were carried out with several Viton E-60 O-rings and several O-ring seals of an alternate type known as Creavey seals. The seals were refluxed with 6N HCl for 18 to 24 hours and then inspected. The Viton seals, while not seriously affected, did become swollen and showed evidence of some surface flaking. However, the Creavey seals, which consist of a stainless steel spring sealed inside a Teflon (FEP) tube, (Sealol Co., Culver City, California) were essentially unaffected. Thus the Creavey seals were adopted in preference to Viton E-60 for exposure to hot HCl solutions.

Viton E-60 was also the material selected initially for derivatizer valve simulator body seals. It was hoped that even if there were some materials problems with the Viton, the metal-to-metal contact surfaces between the cell contents and the seals would prevent any serious problems. Apparently this was not the case and early derivatizer tests (described in Section 3.2.1.4) showed the Viton produced a large amount of interfering material.

Therefore, the valve body seal was replaced with a Creavey seal. Further testing of the derivatizer with Creavey seals present showed some degradation of the seal with repeated use (especially the Creavey seal used between the derivatizer body and head end). After several derivatizations, the Creavey seal between the cell body and the head end was found to have failed. The Teflon had taken a set and had cracked. Upon close examination, corrosion of the inner stainless steel spring was observed. Previous tests with unstressed Creavey Seals had indicated that they were reasonably compatible with the derivatization process.

However, a test carried out with a stressed Creavey seal showed that this seal also developed a set and cracked during the derivatization, leading to the conclusion that a replacement seals for the Creavey seals in the derivatizer were necessary. This led to the development of an all tantalum/Teflon seal.

For hydrolyzer and evaporator use it had been observed that Creavey seals must be replaced after several tests. Thus, the tantalum/Teflon seals are probably desirable in these locations as well.

Ethylene Propylene Rubber:

When problems with Viton in the derivatizer were first encountered, attempts were made to see if ethylene propylene rubber (EPR) would be a more suitable material for the shaft seals on the initial valve simulators since replacement of the valve shaft seals with a non-elastomeric type would have been very difficult. Portions of Viton and EPR O-rings were put in derivatization vials and derivatizations carried out. The O-ring materials were removed, the derivatization products were dissolved in CH_2Cl_2, and small portions were chromatographed. A 0.5 percent injection of the sample from the derivatization in the presence of Viton resulted in a number of relatively large peaks; while in the case of EPR, a 0.5 percent injection was relatively clean. However, significant peaks were observed from the EPR sample when 25 percent of the sample was injected. Thus EPR was a superior material, but it was not completely certain that it could be successfully used for the shaft seal. Tests were then carried out with the derivatizer using EPR shaft seals in the valve simulators to see if acceptable results could be obtained. However, as discussed in Section 3.2.1.4, the interference was still excessive. These results lead to the fabrication of the all tantalum/Teflon valve simulators described in Section 3.2.1.6.

Earlier in the program, samples of the ethylene propylene terpolymer diaphragm material used in the VLBI "-4" configuration solenoid valves were tested with some of the Wet Chemistry reagents.

Separate samples were subjected to one of the following:

1) 6N HCl overnight at 100°C
2) 2-butanol/HCl overnight at 100°C
3) TFA/CH_2Cl_2 overnight at room temperature.

In each case, the initially colorless solutions turned various shades of yellow indicating that something was being extracted out of the diaphragms. The diaphragms themselves changed color and swelled with the largest swelling having occurred in 6N HCl. This swelling is undesirable because it could prevent the valve from opening. These tests indicated that the VLBI "-4" configuration solenoid valves would be unsatisfactory for use with the Wet Chemistry reagents.

Omniseal:

One of the candidate seals to replace the derivatizer main cell body seal was a type called an Omniseal (Aeroquipt Corp., Jackson, Michigan) which is a Teflon seal backed up with a stainless steel spring. The Teflon is thicker in an Omniseal than in a Creavey seal so it was hoped that the Omniseal might have better performance. A derivatization test was carried out in a small tantalum fixture sealed with an Omniseal. The primary test evaluation was the appearance of the seal. At the end of the test the stainless steel spring was somewhat discolored which indicated attack by the 2-butanol/HCl. Therefore, it was concluded that Omniseals probably were unsatisfactory for use in the derivatizer.

3.3.2.4 Teflon

Teflon retains structural integrity upon repeated exposure to reagents used in the processing scheme, but it is known that materials (in particular, amino acids at trace levels) can be lost into the pores under some conditions. Under other conditions contaminant materials are released from the Teflon. For these reasons, Teflon has not been a candidate material for cell construction. It has been used, however, for filter material, gaskets and seals.

Some problems have been experienced in its use as filter material. In particular, the background impurity level has been high, even after soxhlet extraction with 6N HCl. (Discussed in Section 3.2.2, system blank).

Tests were run on Teflon rod and sheet to determine whether they were responsible for unidentified peaks observed upon derivatizer heating to evaporate the sample onto the CG column. The samples were first derivatized in reaction vials, then removed. Methylene chloride was added to the vials to dissolve any residue and 25 percent of the solution was injected into the GC column. These showed no significant interfering peaks.

The Teflon samples were then heated while attached to a GC column, in a manner equivalent to derivatizer cell heating. Some baseline shift was observed, but the magnitude of the broad baseline hump is considerably less than observed on soil processing runs, leading to conclusion that Teflon may not be the sole cause of the interfering material seen in most of the derivatizations of pure amino acids in the derivatizer, or that the interfering material may decompose on standing while exposed to air.

3.3.2.5 Filter and Filtration Tests

Filtration out of the hydrolyzer and evaporator presented two problem areas; first that of the impurities released by the Teflon filters; and second, that of clogging or leaking created by inadequate arrangement of the filter stack. For these reasons, a variety of filtration tests were run, both to find appropriate filter stack arrangement, and to find possible substitute material.

Tests were then run on the following filter arrangements by filtering a mixed solution of water, HF and NH_4 through filters placed in the evaporator:

1) 2 perforated Teflon plates bonded to porous backup material. 2 millipore Teflon filters (5 μ, 10 μ) previously extracted in 6N HCl. Filtration was successful.

2) Whatman glass fiber filter GF/A - 1 filter support (Teflon, perforated plate). Filtration fast but unsuccessful; the precipitate came through.

3) Support - Whatman glass fiber filter GF/C support. Filtration fast but unsuccessful; the precipitate came through.

4) Support - Whatman glass fiber filter GF/B support. Filtration fast but unsuccessful; the precipitate came through.

5) GF/B - 10μ Teflon - 4 (new) supports. Successful filtration but slow (about 1 ml/min).

6) GF/B - supports. Filtrate appeared clear but precipitate not clearly visible on the top filter and the GF/B filter appeared degraded.

7) 10μ Teflon - 4 supports - Teflon O-ring. Clear filtrate at about 0.5 ml/min.

The following tests consisted of processed soil (after water, HF/NH_4OH treatment) filtration:

8) Same filter arrangement as (6) above. Filtration incomplete - very fine white precipitate came through. Unit dismanteled following day - discovered GF/B filter perforated and degraded. Teflon O-ring squeezed out of shape, filtrate chalky white.

9) 1 GF/B - 10μ Teflon - 3 supports - fresh Teflon O-ring. Filtration rate about 0.25 ml/min for 13 milliliters, then slowed to less than 0.1 ml/min.

10) 1 GF/B - 3 supports. Flow fast for ~2 ml then slowed significantly.

The following test was run on a GF/B filter which had been soaked in hot HCl, and had hot HCl filtered through it followed by redistilled water. The test consisted of 30 ml 6N HCl pushed through with pressure.

11) GF/B - 3 supports. Procedure punctured large hole in filter.

Thus, Teflon filters performed successfully although impurities have been a problem. Whatman glass fiber filters were unsuccessful either because of inadequate strength or because of inadequate filtration.

For breadboard use the following arrangements were selected as functional, but not ideal:

Hydrolyzer:

1 perforated Teflon support - 10μ Teflon filter
- 3 perforated Teflon supports.

Evaporator:

10μ Teflon filter - 4 supports - Teflon O-ring.

3.3.3 Radiotracer Methods

Because of the problems inherent in working with extremely low concentrations, it was felt that radiotracer tests using selected labeled amino acids were a necessary part of the program. By combining radiotracer tests with GC analysis, independently analyzable data was obtained. Furthermore, the use of radiotracers allowed the separation of amino acid recovery problems from derivatization and GC analysis problems. It was felt to be unnecessary to carry out radiotracer studies with all of the required amino acid types. Two standard amino acid mixtures were developed to accomplish radiotracer testing in an efficient manner.

3.3.3.1 Handling Techniques

Initial radiotracer work was done to establish both efficiency and reproducibility of our laboratory procedures. A variety of transferring and measuring devices were tested to determine the most reproducible method of handling small quantities of radioactive solutions in conjunction with thin layer chromatography. The microliter-syringe was the measuring device selected as most accurate and convenient.

The scintillation cocktail selected was 12 milliliters New England Nuclear Aquasol plus 4 milliliters 0.1 N HCl. The amino acid, plain or absorbed onto cellulose (in the case of thin layer chromatography), was first dissolved in the aqueous HLc, then Aquasol was added, and the cocktail shaken to gel, which provided a homogeneous reproducible counting medium.

Two standard mixtures of labeled amino acids were established:

Mixture 1 -	Glutamic acid	Mixture 2 -	Alanine
	Lysine		Leucine
	Phenylalanine		Valine
	Proline		

The first mixture provided a variety of amino acid types: acidic, basic, aromatic, and imino. The second mixture provided three well-behaved but volatile components. In addition, thin layer chromatographic techniques were developed to permit the isolation of each individual amino acid in the mixture. Thus, if unexplained losses occurred, it was possible to identify which amino acids were lost.

These radiotracers could be added at any point in a processing test, and could be sampled at any point.

3.3.3.2 Thin Layer Chromatography

Thin Layer Chromatography was selected as a method to separate amino acids in the radiotracer mixture when necessary to pinpoint specific losses and to establish the purity of our radiotracer compounds.

Analtech 100-micron Avicel microcrystalline cellulose plates were used for this work.

Identification was accomplished with autoradiography using Kodak X-ray film (no screen), as this is non-destructive identification allowing later scraping off of the cellulose and counting the radioactivity for quantitative recovery information.

Initial work with glutamic acid on TRW plates showed a recovery of about 80 percent glutamic acid from the cellulose. Cellulose added to the three references resulted in a 1 percent decrease in counts, indicating that it was not the cellulose itself that was significantly interfering with the counts. The plates from which the cellulose squares had been scraped were washed with H_2O and 0.1 N HCl and the wash water was counted to demonstrate that no significant losses occurred in the scraping process.

Further tests with glutamic acid spotted on cellulose were made in order to discover a means for recovering a greater percentage of the amino acid with the following results:

1) Adding unlabeled amino acid or 0.1 N NaOH to the cellulose before spotting with labeled amino acid increased the recovery from the cellulose.

2) Solvent (i.e., H_2O) was required in order to both better dissolve the amino acid and to form a gel when Aquasol was added, so that the cellulose and amino acid would not all settle to the bottom of the vial. When one milliliter of Aquasol was added to the sample of cellulose in the vial before water had been added, a 20 to 30 percent count reduction resulted.

In later tests with Avicel plates, reproducible measurements were achieved, and in each sample vial a gel was formed of 4 milliliters of aqueous solution and 12 milliliters of Aquasol. The results with all three labeled amino acids showed good recovery from the cellulose after it was simply spotted with the amino acid, scraped into the counting vial, dissolved in a solvent and made into a gel. Loss of counts was seldom greater than 2 percent.

Included in these recovery tests was an investigation into which solvent of three: H_2O, 0.1 N HCl, or 0.1 N NaOH, best extracted the amino acids from the cellulose. NaOH was not satisfactory because it had a significant quenching effect. Both H_2O and HCl gave good results, and it was decided to use 0.1 N HCl in subsequent tests.

Two TLC solvent systems were tried:

1) Butanol/acetic acid/water: 60/20/20
2) Chloroform/methanol/17% ammonia: 40/40/20

Better separations were obtained with system 1, and it was used for subsequent TLC work. Using pure, unlabeled amino acids, adequate one dimensional separations were obtained for lysine, glutamic acid, proline and phenylalanine.

3.3.3.3 Trapping Tests

Some tests of trapping techniques were carried out to evaluate methods of trapping radiotracer labeled derivatives so as to have a method of monitoring the derivatizer in addition to gas chromatography.

These tests were conducted by connecting traps of various types and configurations to a heated injection port of a gas chromatograph. With carrier gas flow in the usual range, the radiotracer labeled amino acids were injected, and after a period of carrier gas flow, the traps were disconnected, washed with solvent (usually toluene) and the solutions counted. Various trapping temperatures used included room temperature, ice bath, and liquid nitrogen temperatures. The recoveries were not satisfactory. It appears that traps using a GC liquid phase may be necessary to get adequate recovery although these may be difficult to wash out.

An alternate method would be to combust the derivatives to CO_2 in a reactor and quantitatively trap the $^{14}CO_2$ in hyamine hydroxide for counting. One test of this concept was carried out. An old laboratory version of the copper/firebrick organic vapor trap (OVT) used in the Viking pyrolytic release experiment was used to oxidize the derivatives. The OVT was used in both trapping/oxidation and straight oxidation modes. Both He and O_2 were used as carrier gases. The amount of radioactivity collected in the hyamine hydroxide trap ranged from 20 to 80 percent, and the results were not consistent in replicate experiments. Therefore, it was concluded that a more efficient oxidizing unit would be required if these experiments were to be pursued further. No further oxidation type trapping tests were carried out.

Thus, the nominal method of evaporating the derivatives onto a GC column and running a gas chromatogram was the only method used to monitor the derivatizer with the exception of post mortem cell washing to measure the amount of sample remaining in the derivatizer.

3.3.4 Reagents

In order to evaluate reagent purity status, various reagent blanks were run with existing reagents. The intent was to place the initial purification emphasis on those reagents which had the worst blanks. The

ion exchange resin was the most significant contributor to GC impurity peaks initially, but improvements were made throughout the program.

3.3.4.1 Ion Exchange Resin

Ion exchange resin was cleaned according to the following procedure: a large chromatography column with a coarse porosity glass frit was set up with heating tape attached to a Variac. About one-half pound Biorad AG50W X8 (200 - 400 mesh) in a slurry of 0.1 N HCl was poured into the column and allowed to soak overnight. The following reagents were then washed through at 50°C as received (except Arrowhead distilled water redistilled in glass before use): water, 2N NaOH, water to neutrality, 4N HCl, water to neutrality, 50% glacial acetic acid, water to neutrality. The resin was then soxhlet extracted with methanol for 1 hour and dried under vacuum overnight.

Following initial purification the same resin was reused throughout the program with normal processing reagent recycling between uses. It appears that impurities occur on standing, and that recycling prior to uses provides a cleaner sample.

At the end of the program the only major interference peak occurring was that ascribed to trifluoroacetamide.

3.3.4.2 Other Reagents

The following was the final status of reagent purification:

HCl, aqueous - Mallinckrodt Reagent diluted to constant boiling mixture and distilled in all glass system

HCl, anhydrous - Air Products, electronic grade

Hydrofluoric acid - Baker Reagent

Ammonium hydroxide - Baker Reagent

2-butanol (racemic) - Baker grade

(+)-2-butanol (optically active) - Norse Laboratories

The 2-butanol was purified before use by decanting from molecular sieve and distilling from 2, 4-dinitrophenylhydrazine and sodium sulfate.

Methylene chloride - Mallinckrodt Nanograde

Trifluoroacetic anhydride - PCR, Inc. Distilled before use in glass system.

H_2O - Redistilled arrowhead distilled water in all glass system.

2-butanol/HCl - prepared in all glass system by bubbling anhydrous HCl through concentrated H_2SO_4 then through butanol protected from air by butanol plus NaOH pellets and an Aquasorb outlet tube.

A long-term storage test was carried out on several samples of racemic 2-butanol/HCl to determine if interfering materials were produced on standing (the low rate of racemization of 2-butanol/HCl on long term storage at room temperature was demonstrated in the previous study). The butanol/HCl was stored in sealed glass ampoules to prevent exposure to air and water and thus more closely modeled storage in the flight instrument. After 8 months, the ampoules were opened and derivatization blanks were prepared and chromatographed. The chromatograms were not significantly different from the ones obtained with samples for the same butanol/HCl batch before it was sealed into the ampoules. Some peaks were present in both sets of chromatograms since our early 2-butanol/HCl reagent was not as well purified and produced as later reagent was. The key point is the fact that neither the number nor the size of the peaks significantly increased. Thus it appears that 2-butanol/HCl is stable in terms of interference production as well as in terms of racemization, and it should last through a nominal 90-day mission.

3.3.5 Gas Chromatography

Two commercial gas chromatographs with flame ionization detectors were used on this program: a Varian 1800 and a Varian 1527. A mixture of 60/40 He/H_2 was used as carrier gas, plus an additional quantity added as volume make-up at the detector.

Early in the program it had been decided on consultation with NASA/ARC that Carbowax 20M would be used as the primary column, so our old Carbowax 20M columns were briefly reconditioned and tested. Qualitatively, there did not appear to have been any degradation of the columns during 18 months of storage without any special precautions. The resolution of diastereomeric pairs remained excellent for most of the common amino acids, and the sensitivity was good.

While the storage life of these columns is expected to be good, it is important to confirm this fact prior to spacecraft usage.

Several Carbowax 20 M and Dexsil 400 columns were prepared during the program. For the 200 foot, 0.030-inch ID columns, 65 to 75 milligrams Carbowax was appropriate. Since the columns were generally used for analytical purposes only, conditions (temperature programming rate, carrier gas flow) were not optimized to establish maximum resolution per coating weight.

The Dexsil Columns were coated with 78 to 85 milligrams material. The Dexsil columns did not have a long life under the conditions that they were used. This is apparently caused by damage of the coating in the column inlet by injection of large volumes of methylene chloride (50 microliters sample injections were frequently used). These Dexsil columns also appeared to tail excessively with small quantities of amino acids. Good peaks were obtained with quantities greater than 10 nanomoles and fair to good peaks with 2.5 nanomoles. However peaks of 1 nanomole or less are not well shaped. This effect did not occur with Carbowax 20M.

Because different results were obtained for the ion exchange column blank with Carbowax 20M and Dexsil 400, an attempt was made to reactivate an old Poly A-101A column to see what results would be obtained for a third liquid phase. However, the old column had degraded badly and was not worth using. A fresh Poly A-101A column was prepared using a 66-milligrams coating which may have been slightly light. While this column does not resolve amino acids as well as Carbowax, it is useful as an alternative type column because impurity peaks are shifted to other locations. The peak ascribed to trifluoroacetamide occurs beyond the amino acid range.

4. FLIGHT DESIGN UPDATE

4.1 SYSTEM REQUIREMENTS AND INSTRUMENT SYSTEM DEFINITION

The Automated Wet Chemistry Instrument is required to analyze at least three soil samples for optically active amino acids under the conditions specified in NASA/ARC Specification A-16231, Revision 3, Automated Wet Chemistry Instrument for Landed Planetary Missions. A list of mandatory and desirable amino acids the instrument shall be capable of analyzing as well as the basic sequence for accomplishing the analyses, including types and amounts of reagents, order of addition, and processing times and temperatures are also defined in Specification A-16231. Performance requirements such as resolution and separation of and sensitivity to amino acids, and internal calibration requirements are also specified in the document. Portions of the specification are included in Table 4-1 for reference.

The processing sequence contains the following basic operations: internal calibration, soil handling, amino acid extraction, hydrolysis, purification, derivitization and gas chromatographic analysis. The components necessary to perform these operations are:

- Soil distributor for soil handling
- Extractor cell for amino acid extraction
- Hydrolyzer/Evaporator cell for hydrolysis and then HCl evaporation
- Ion exchange column for desalting
- Derivatizer cell for derivitization of the amino acids
- Gas Chromatographic Column and detector for gas chromatographic analysis.

Table 4-1. Analysis Requirements (From Specification A-16231, Rev. 3)

Analysis Sequence:

Step 1. Place a 1 cubic centimeter soil sample in a chamber

Step 2. Add 10 milliliters of water.

Step 3. Heat to 165 5°C for 1 hour.

Step 4. Allow to cool and filter off the insoluble soil residue.

Step 5. Add 10 milliliter of 6N HCl to the filtrate from Step 4.

Step 6. Heat solution to 110°C for 5 hours.

Step 7. Evaporate to dryness.

NOTE: The following step is performed on the ion exchange column prior to proceeding to Step 9. The reagent volumes for Step 8 are given for a 5 milliliter Dowex 50H+ column.

Step 8. Place 10 ml of 4N NaCH on ion exchange column. Follow this with 20 ml of water directly onto the ion exchange column. Follow this with 15 ml of 6N HCl directly onto the ion exchange column. Follow this with 20 ml of water directly onto the ion exchange column.

Step 9. After evaporating to dryness (Step 7), dissolve the amino acids and residual salts in 5 milliliters of water. (It may be necessary to heat a short time to assure solution).

Step 10. Place solution (Step 9) on strong cation exchange column for amino acid exchange, cation and neutral organic removal. Follow the amino acid solution immediately with 15 milliliters of water directly onto the ion exchange column. Follow this with 10 milliliters of 4N NH_4OH and start collecting the amino acids when the ammonia begins to break through the ion exchange column. Collect only the first 1 to 2 milliliters.

Step 11. Evaporate the amino acid solution to dryness at 100°C

Step 12. To the dried sample add 0.5 milliliter of (+) 2-butanol containing sufficient anhydrous HCl to make it 4N.

Step 13. Heat solution to 100°C in a closed chamber for 2 hours.

Step 14. Evaporate to dryness. Cool to below 35°C.

Step 15. To the dried sample add 0.1 milliliter of trifluoracetic anhydride and 0.4 milliliter of methylene chloride. Heat in a closed chamber for 1 hour at 35 to 40°C.

Step 16. Evaporate the solvents at a temperature below 10°C.

Table 4-1. Analysis Requirements (From Specification A-16231, Rev. 3) (Continued)

Step 17. The resultant sample is analyzed for composition by gas chromatography.

Sample Size - The size of the soil sample shall be between 1 and 10 cubic centimeters.

Gas Chromatography - The analysis shall be performed meeting the following conditions:

Carrier Gas - TBD

Columns - The column(s) shall be capable of separating the amino acids listed below. All of the amino acids on both the mandatory and goal lists must be identifiable by retention time. The instrument is required to work only for the mandatory list. The goal list is both a goal and to identify the most probable compounds that might also be present and require some identification.

Mandatory Detectable Amino Acids	Design Goal Detectable Amino Acids
1. Alanine	1. Ornithine
2. Valine	2. ϵ - Amino Caproic Acid*
3. Isoleucine	3. δ - Amino Valeric Acid*
4. Leucine	4. γ - Amino Butyric Acid*
5. Glycine*	5. α - Amino Adipic Acid
6. Proline	6. Alloisoleucine
7. Aspartic Acid	7. β - Amino- -Butyric
8. Methionine	8. β - Amino - Isobutyric
9. Phenylalanine	9. N - Methyl-alanine
10. Glutamic Acid	10. Isovaline
11. Beta alanine*	
12. Norvaline	
13. Norleucine	
14. α - Amino-η-Butyric	
15. Lysine	
16. Pipecolic acid	
17. α - Amino isobutyric*	

*Denotes that the amino acid is not optically active.

Table 4-1. Analysis Requirements (From Specification A-16231, Rev. 3) (Continued)

GC Peak Resolution and Separation. Definitions: Resolution is used to denote the separation of the diastereomeric peaks of a single racemic amino acid and is to be determined according to R. Kaiser, Gas Chromatography Vol. 1, p. 39 (1963) Butterworth, Washington. Separation is used to denote the separation of a diastereomeric pair of one amino acid from the diastereomeric pair of another amino acid.

The resolution of the peaks of a single racemic amino acid shall be 90 percent or better for all optically active amino acids on the mandatory and goal lists except for aspartic acid (40 percent) lysine and ornithine (65 percent), β-amino-isobutyric (TBD), Isovaline, N-methyl-alanine, and β-amino-η-butyric (0 percent). This resolution is to be obtained for amino acid concentrations up to 50 nanomoles per amino acid. For concentrations above 50 nanomoles, the resolution may be degraded.

The separation of a mixture of the racemic mandatory amino acids shall be such that of these acids all will be separated with resolution being no less than 50 percent in any conflict. Such conflicts shall not involve more than three of the amino acids on the mandatory list. The goal shall be 100 percent separation of all acids on the mandatory list.

Column Temperature. The temperature programming and readout accuracy shall be ±0.5°C.

Column Retention Time. The retention time precision for each amino acid shall be within 0.5°C of its retention time.

Detector. The overall sensitivity (total scheme, sensitivity at detector output after data processing) shall be such that 0.1 nanomoles and less than 50 nanomoles of each of the amino acids on the mandatory list in Table 4-1 can be detected in the soil sample. It is a design goal that 0.01 nanomole or less of each of the amino acids in a soil sample be detected.

Detector Dynamic Range. The detector dynamic range shall be six decades. The peak area accuracy for each decade of peak height (concentration) shall be 1 percent.

Internal Calibration. A mixture of two (TBS) racemic amino acids or peptides shall be carried in the instrument for calibration of the experiment system for each of the processed soil samples. The (TBS) racemic amino acids or peptides shall be placed in each hydrolysis chamber with the soil sample prior to the addition of HCl.

Additional components necessary to implement the functions in an automated system are:

- Reagent storage containers and injection systems
- Gas supply subsystems
- Interconnecting plumbing and valves
- Electronic subsystem.

A system block diagram of the basic system components, including summaries of the process operations performed with each component, is presented in Figure 4-1. A complete instrument system schematic is shown in Figure 4-2. A short description of the system is presented in the following paragraphs, which also point out differences to the previous flight design (TRW Final Report No. 16660-6001-R0-00).

A major difference is the use of only one set of reusable processing cells as compared to three sets of separate, non-reusable cells in the previous design. The soil metering and distribution subsystem which is based on the VLBI soil distributor receives soil from the Lander soil processor via the soil feed tube, meters out a fixed volume of the soil sample and deposits the sample in the extractor after removal of the top cover of the extractor. Leftover soil is deposited in a dump cell. The soil distributor can deliver at least three soil samples of identical size to the extractor. The extractor cover is held in place by a gas actuated piston and return spring. The spring holds the cover closed and gas pressure on the actuator raises it. Actuation is provided by a solenoid valve.

A set of processing cells which can be used repeatedly is provided for preparing the sample for gas chromatographic analysis. The set consists of the extractor, hydrolyzer/evaporator, ion exchange column and derivatizer, and the associated plumbing and valves. In the previous process hydrolysis was carried out directly on the soil, so the first cell was the hydrolyzer. The hydrolysate was transferred into the second cell, the evaporator, for HCl evaporation and a subsequent desalting process. The following steps in the previous process are the same as in the current baseline process.

The extractor, hydrolyzer/evaporator and ion exchange column each contain filters at their outlets. It might be possible to eliminate the filter in the hydrolyzer/evaporator which would remove one possible source of

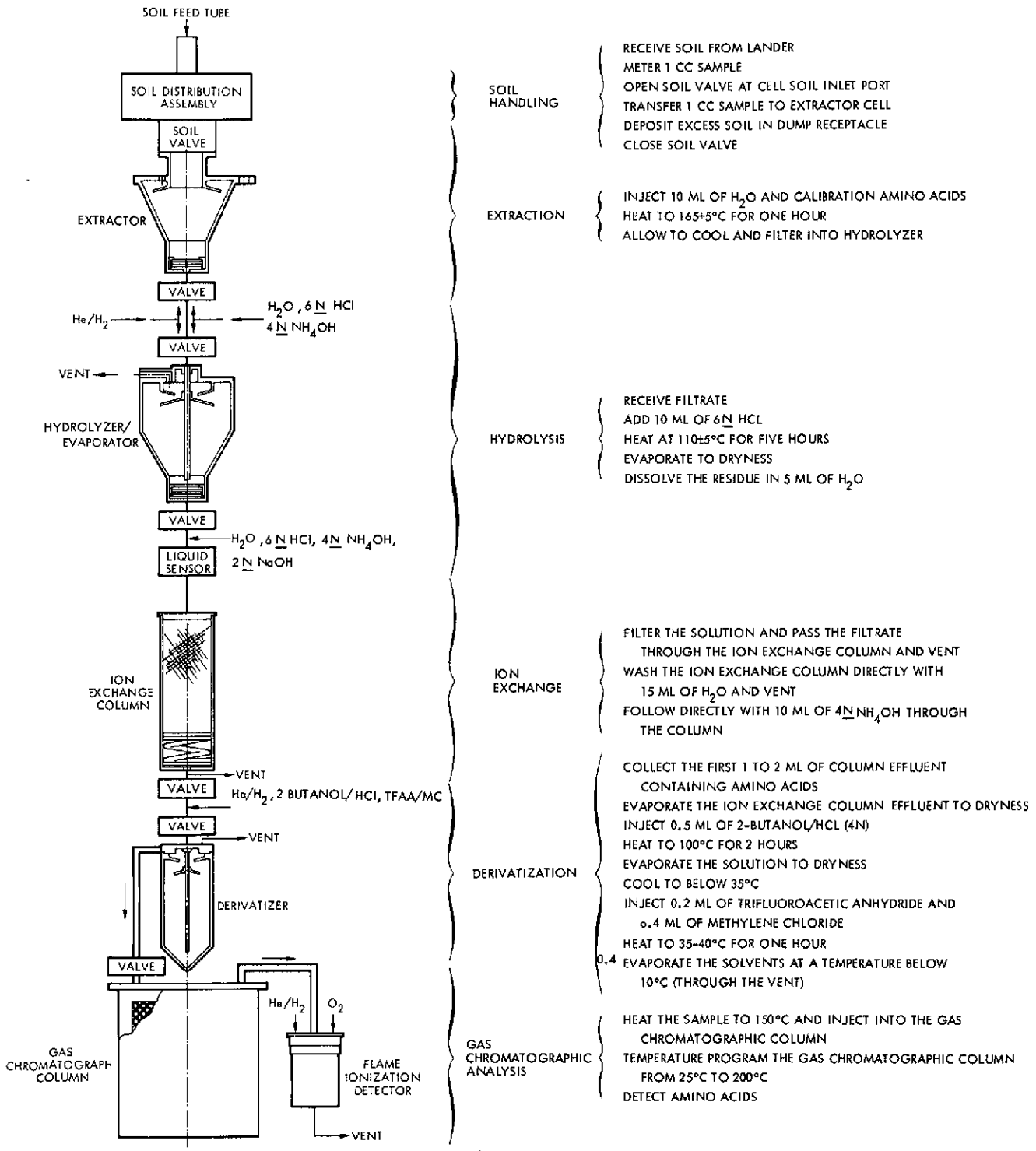


Figure 4-1. Wet Chemistry System Block Diagram

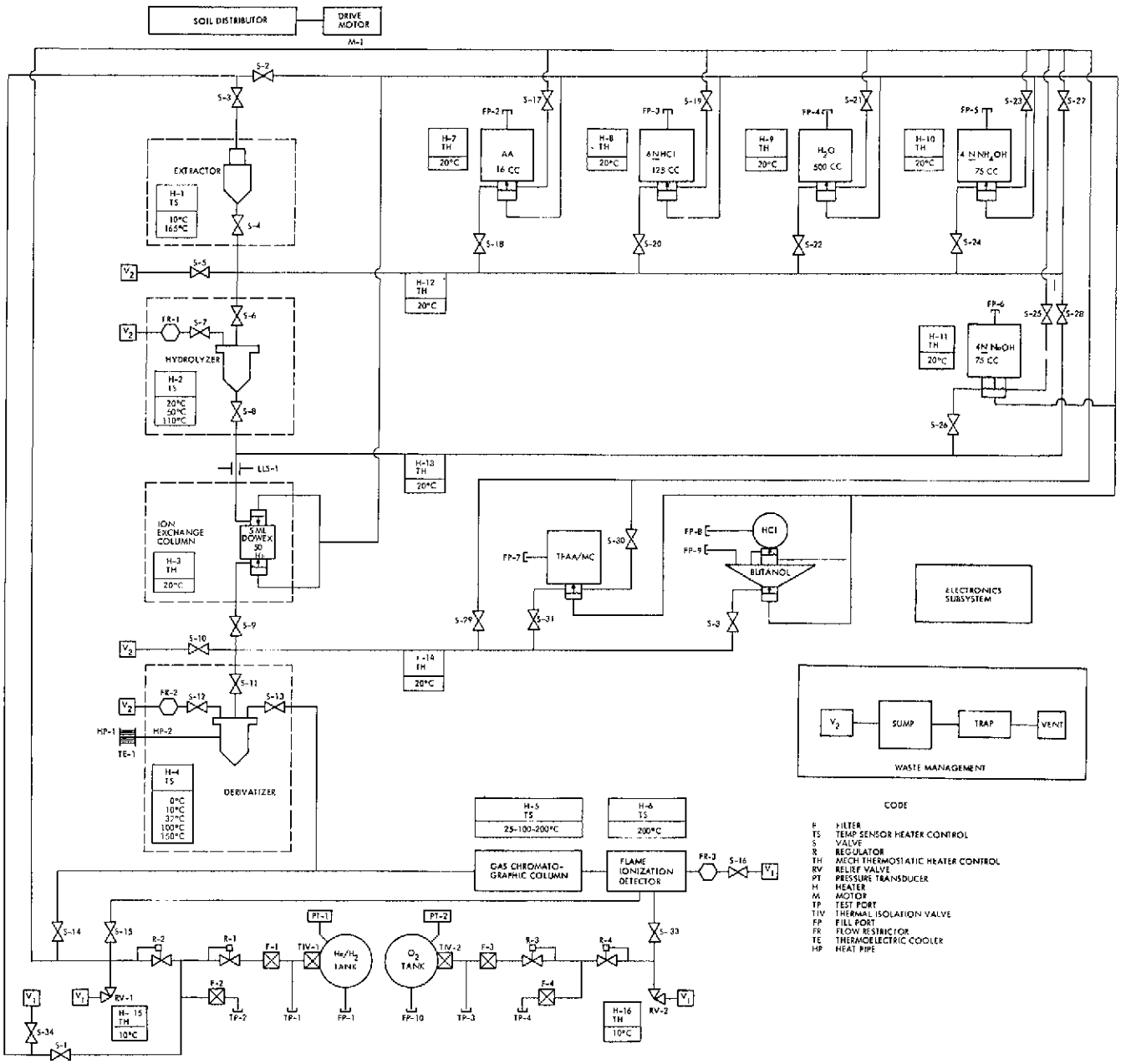


Figure 4-2. Instrument System Schematic

contamination. The extractor, hydrolyzer/evaporator, ion exchange column, and derivatizer are equipped with heaters to maintain the required temperature environment. The derivatizer, in addition, is connected via a heatpipe to a thermoelectric cooler to maintain the cell temperature below 10°C during evaporation of solvents after amino acid derivitization. The ion exchange column has gas actuated isolation valves at inlet and outlet to preclude drying of the resin during interplanetary cruise.

The instrument contains a single capillary tube self-heated gas chromatographic column which is used, in the baseline configuration, in conjunction with a flame ionization detector. The use of a state-of-the-art electron capture detector or the addition of a mass spectrometer is also under consideration. The stainless steel column is used as a resistance element heater and is designed to provide uniform column temperatures during steady state and programmed temperature control. Active temperature control is maintained by sensing the change in column electrical resistance as the column is heated. The column inlet and outlet are close coupled to the derivatizer outlet and detector, respectively. The amino acids are injected directly into the column from the derivatizer. Cold trapping of amino acid derivatives is provided by the column liquid phase.

The baseline instrument contains a single flame ionization detector. The detector has inlets for the He/H_2 carrier gas from the gas chromatographic column, makeup He/H_2 gas, and O_2 gas. A single outlet is provided with a gas actuated valve and flow restrictor. The detector has a separate heater and temperature sensor to maintain the detector temperature at a constant value, slightly above the maximum programmed gas chromatographic column temperature.

Two gas supplies are used in the instrument. One provides oxygen for the flame ionization detector. For long term storage, the oxygen supply is isolated by a thermally actuated isolation valve which is actuated at the start of the experiment. A control valve is used to turn the gaseous oxygen on and off as required.

A second gas supply contains a mixture of helium and hydrogen (approximately 44 percent H_2 by volume). This system has three branches. The first branch is connected downstream of the first pressure regulator (approximately 165 psia) and is used to pressurize the gas actuated isolation valves on all reagent containers and on the ion exchange column. It

will also be used for all gas actuated tantalum diaphragm valves (control valves) in the instrument, and for the gas actuated extractor cover. The other two branches are connected downstream of the second stage regulator (18 psia); one branch supplies carrier gas through the gas chromatographic column and then through the flame ionization detector. Gas from each of these branches is controlled by a separate valve. Low pressure gas is also used for pressurization of the various cells, for purging and gas drying, and for fluid routing and reagent injection. The gas supply is sealed with a thermally actuated isolation valve for long term storage.

Identical reagent storage containers and injectors are used in the current design. The reagents are stored in compatible metallic containers and a gas actuated isolation valve is used to seal the container for long term storage. After actuation the flow of the reagent is maintained by the low pressure He/H_2 gas, and the amount of reagent delivered to the cell is determined by the open time of the control valve of the outlet.

Because of shelf life limitations, the 2-butanol and anhydrous HCl are stored separately. The anhydrous HCl is stored in a small pressure vessel at approximately 900 psi. Ten percent of this volume contains helium gas. The 2-butanol is stored in a separate container under its own vapor pressure. The two vessels are isolated from each other and from the rest of the system by gas actuated isolation valves. Upon actuation of the valve between the two vessels, the HCl gas and 2-butanol in the adjacent reservoir are allowed to react. The inert helium is used as the driving pressure (blowdown mode) for reagent injection. Injection is accomplished by first opening the gas actuated isolation valve downstream of the 2-butanol reservoir and then opening the control valve for a predetermined time to meter the mixture to the derivatizer.

Since this injector operates in a blowdown mode, the time required to inject equal volumes into the derivatizer in subsequent analysis sequences will vary with successive injections. This characteristic is repeatable and can be accommodated in the electronics by changing the valve open time for each application.

The instrument contains two vent systems internal to the package (as compared to three previously). These are connected to a single outlet vent for interfacing with the Lander. A common vent manifold is used

for the extractor, hydrolyzer/evaporator, derivatizer, and ion exchange column. Valves at the outlets of these cells prevent backflow and cross contamination between cells. The second vent system is used on the outlet of the gas chromatographic column and flame ionization detector and for the two gas supply systems.

Control and routing of reagents, gases and samples are accomplished with a fluid system consisting of small diameter tubing and gas actuated control and isolation valves. The plumbing system is designed to contain a minimum number of valves and to prevent cross contamination between various cells. Two and three way solenoid valves, most thermally actuated isolation valves and (passive) check valves incorporated in the previous design have been eliminated and/or replaced.

The electronic subsystem provides the regulated power, instrument control functions and data processing. This subsystem receives, decodes and distributes commands from the Lander.

4.1.1 Instrument Analysis Capabilities

The following paragraphs contain discussions on the analysis capabilities of the instrument as compared to the specification requirements for Process No. 2. The items covered are soil sample size, carrier gas composition, number of amino acids detectable, gas chromatographic column resolution and separation, column temperature control, column retention time, overall system sensitivity and detector dynamic range, peak area integration accuracy, optically active resolving agent, and internal calibration. A summary of the specification requirements is included in Table 4-1.

We believe the instrument is capable of satisfying the specification requirements.

The separation and resolution of all of the amino acids on the mandatory list have not yet been demonstrated but columns are available which should provide adequate separation and resolution. While the detector sensitivity is better than required, the overall sensitivity is determined by interference, and further reduction of the amount of interference is

needed to obtain sensitivities below 1 nanomole. It is felt that the interference can be reduced sufficiently to reach the 0.1 nanomole requirement.

4.1.1.1 Soil Sample Size

The soil distribution subsystem meters soil volumetrically. A metering cavity size of 1.0 cubic centimeter was selected for the instrument design. Based upon the Viking Program Mars Engineering Model, the density of Martian soil varies from 1.0 g/cm^3 (Loess) to 3.2 g/cm^3 (rock). For design purposes, a soil density of 1.5 g/cm^3 was selected and the reagent injector volumes were sized accordingly.

For a given system detection sensitivity, increasing the soil sample size will provide some increase in the capability to analyze lower amino acid concentrations. This is not necessarily a linear relationship for all of the amino acids because the amount of interfering species may also increase. From this standpoint, the optimum soil sample size has not been determined.

Using the analytical sequence of Process No. 2, increasing the soil sample size has the following impact on the system. The H_2O volume for extraction probably would increase linearly with soil sample size as would the amount of HCl solution required for hydrolysis.

Some increase in ion exchange resin volume would be required. The volume of the reagents associated with the ion exchange column would increase linearly with resin volume.

4.1.1.2 Carrier Gas

The specification requirement for the carrier gas is to be determined. The instrument, as currently designed, uses a mixture of helium and hydrogen (56 percent He, 44 percent H_2). The helium and hydrogen gases are mixed together and stored in a single tank and are used for pressurization, mixing, purging, gas chromatographic column carrier gas and flame ionization detector operation. The reason for combining the gases was to minimize the number of gas supply subsystems. Laboratory tests continue to verify the validity of this approach.

Other carrier gases could be used if a different detector type were desired, or if the experiment was operated in conjunction with a mass spectrometer.

4.1.1.3 Column Performance

Identification. The requirements for amino acid identification by retention time and for retention time precision can be satisfied with the columns evaluated.

The retention time precision for each amino acid is required to be within 0.5 percent. Normally, variations in the column temperature profile are the most significant variable affecting retention time. However, in this instrument, the column temperature profile is closely controlled (within $\pm 0.5^{\circ}C$) and should not be a large source of retention time variation.

The other significant variable with respect to the retention time in this instrument is the carrier gas flow rate. The current design uses a carrier gas supply system which is basically the same as that used for Viking Lander Biology Instrument. With this system the flow can vary as much as 2.2 percent because of variations in regulator pressure. The effect of flow rate on retention time depends on column operating parameters. At constant temperature, the retention time varies approximately as the square root of the flow rate (i.e., 2.2 percent flow variation equivalent to a 1.1 percent variation in retention time). For a column which is temperature programmed, the effect of flow variations on retention time is reduced. Thus, 2.2 percent flow variation will result in a significantly smaller variation in retention time. Furthermore, some of the pressure variation is caused by temperature changes at the regulator. These effects can be corrected for in the returned data by use of temperature measurements and the calibration of the regulator pressure/temperature dependence. If further development testing shows that the fluctuation of retention time is greater than desired, additional flow control is feasible.

The nature of the column can also affect the retention time precision because of aging effects. The retention times obtained for a given column varies over the life of the column. However, the effect over several operating cycles is small as long as the column is not near the end of its life. Retention time precision of 0.5 percent is achieved in the laboratory if care is taken to maintain reproducible operating conditions.

Resolution and Separation. General column testing was, not carried out as a part of this program and all of the amino acids in the new mandatory list were not run. Based on column tests in the previous program and on results from NASA/ARC, columns are available which can meet most if not all resolution and separation requirements of the specification. However, it is recognized that the possibility that the column most suited to the overall mission goals might not meet the separation and resolution requirements in their entirety. Selection of the GC column to be used in the instrument should await further instrument development.

Column Temperature. The temperature programming will be controlled so that at any time, the actual temperature will be within ±0.5°C. of the nominal temperature for that point on the time-temperature profile. The temperature readout will provide the requirement ±0.5°C accuracy.

4.1.1.4 Detector

Sensitivity. The overall sensitivity requirement for the total instrument after data processing is that between 0.1 and 50 nanomoles of each of the amino acids on the mandatory list can be detected. The design goal is to be able to detect 0.01 nanomole or less of each amino acid in the soil sample. Laboratory studies indicate that the gas chromatographic column detector system is capable of providing the required sensitivity. Based on the data obtained with a conventional laboratory gas chromatograph from samples in the 2 nanomole range and extrapolating a 2:1 signal-to-noise ratio for an amino acid 100 percent in one enantiomer, 0.005 to 0.05 nanomole of the mandatory amino acids should be detectable (in the absence of conflicts) depending on the column, column operating conditions and the particular amino acid.

The major uncertainties in estimating the total overall sensitivity lies in the recovery of the amino acids in the soil, and in the amount of interferences present in the gas chromatogram. The recovery is influenced by the extraction efficiency, the amount of amino acid breakdown during hydrolysis, losses during ion exchange desalting, and the derivatization efficiency. The extraction efficiency depends upon the nature of the soil sample and the degree of polymerization of the amino acids. Extraction efficiency is high for abiologic samples, but for life derived samples, the efficiency may be lower. Based on breadboard test results extrapolated

to Process No. 2, the recovery in the rest of the processing is expected to be high (85 to 90 percent) and the derivatization efficiency is expected to be good (85 to 90 percent average). Based on NASA/ARC information, the extraction efficiency should be adequate so that less than 0.1 nanomole can be detected.

The sensitivity with the current breadboard with Process No. 1 is limited by the presence of interferences in the gas chromatogram. The change to process should lead to the reduction or elimination of most of the interference problems. Known solutions are available for the remaining interference problems. Thus, it is felt that the 0.1 nanomole requirement can be satisfied.

Dynamic Range. The dynamic range requirement for the detector is six decades. One percent area integration accuracy must be maintained in each decade over this entire range. This requirement means, first, that the detector must handle signals over this range with reasonable linearity, and its operating parameters must be adequately controlled. The suitability of the ATC HYFID design for this requirement has been demonstrated by its incorporation in an organic analysis system covering seven decades of linear range (NASA/ARC Contract NAS 2-5469).

Secondly, the detector electrometer must be range-switched to prevent overload and guarantee correct utilization of the analog/digital (A/D) converter to maintain the 1 percent area integration accuracy in each decade of range. (For example, a 10-bit A/D converter has a resolution of only one part in 1024 and cannot be used to cover the entire six decades of range.) Low level signal conditioning, A/D conversion and data formatting are discussed in Section 4.4.7.

Accuracy. The entire chromatogram will be sent back in a manner basically identical to that used to send back the entire chromatogram from the gas exchange experiment of the Viking Lander Biology Instrument. The concepts involved in reconstruction of the chromatogram were discussed in the final report on the previous program (NASA/ARC Contract NAS 2-6218). These concepts have been proven by reconstruction of actual digitalized chromatograms from VLBI development instruments.

The basic difference between the wet chemistry and the VLBI chromatograms is the greater dynamic range with the wet chemistry instrument which will require additional range switching before the A/D converter, and the increased length of the chromatogram. There is also a greater variability in peak shapes in the wet chemistry instrument if column overloading occurs, and some column bleed is present at the upper end of the temperature program. However, the analysis of these factors performed in the previous program indicates that the reconstruction process is not significantly affected. Thus the entire chromatogram will be available with better than 1 percent accuracy, and hence the best ground based integration methods can be applied to the reconstructed chromatogram which should allow peak area accuracy to be better than the specified 1 percent.

4.1.1.5 Optically Active Resolving Agent

The (+) 2-butanol shall consist of 90/10 ratio of the enantiomorphic pairs as specified. In order to preserve the optical purity of the 2-butanol during terminal sterilization and storage, the 2-butanol/HCl reagent will be made after landing from 2-butanol and anhydrous HCl.

4.1.1.6 Internal Calibration

A mixture of two racemic amino acids or peptides are to be included in the instrument for calibration. They will be added as an aqueous solution to the extractor after loading of soil and before the start of the extraction.

4.1.2 Operating Sequence

A step-by-step operating sequence has been developed for the instrument system. This sequence contains the basic functions for analysis of a single soil sample. Additional functions are required to open the isolation valves for the gas supplies and H_2O, NH_4OH and 2-butanol/HCl injectors the first time the instrument is operated.

The operating sequence is described in the following paragraphs. This sequence is for a Lander thermal plate temperature of $-15^{\circ}F$. The sequence will vary slightly as a function of Lander plate temperature because of differences in heat-up and cool-down times. The $-15^{\circ}F$ condition is a worst case from a power standpoint (longer heat-up times, higher

thermal losses). Table 4-2 contains a listing of the sequence steps, the operating time requirements for each step, and valve and heater actuation requirements.

The following paragraphs describe the processing steps listed in Table 4-2.

1) Activate Instrument

An initiation sequence is performed prior to the first analysis to actuate the thermal isolation valves for the gas supply systems: He/H_2 (TIV-1) and O_2 (TIV-2). Line heaters H-12 and H13 and the He/H_2 supply heater, H-15 are also turned on at this time.

2) Receive Soil from Lander

Operation of the instrument begins on receipt of the initiate command and electrical power from the Lander. This command is to be generated after the Lander has deposited the soil sample into the instrument soil feed tube. (Any mechanical processing, such as crushing of soil, is carried out by the Lander.) The command starts the automatic sequence for processing the first sample through processing cells.

3) Transfer Soil Sample into Extractor

Soil from the Lander soil processor is transferred through the soil feed tube into the soil distributor assembly where it is metered and dumped into the extractor. This operation is described in more detail in Section 4.3.1.

4) Heat Extractor

To prepare the sample for processing, the extractor is heated to 10°C. Since the minimum specification temperature of -32°C (-25°F) could cause freezing of any of the reagent solution to be injected into the processing cells, all reagent containers are heated in preparation for the next step at this time by turning on heaters H-7 through H-11.

5) Open Gas Actuated Isolation Valves

To prevent loss or contamination prior to launch and in flight, all reagents are stored in sealed containers, which are opened prior to use with high pressure He/H_2 by opening S-1 and S-2 to operate the gas actuated isolation valves on the reagent containers. After the isolation valves have been opened, excess gas pressure is vented through S-34. The injectors are heated during this process so the reagents will be liquid when the disks are punctured.

FOLDOUT FRAME 1

Event No.	Event and Steps	Time To Next Step	Elapsed Time	Valves	Heaters
1.	Activate Instrument				
	a. Apply operate power	1	1		
	b. Turn on gas supply heaters	15	2		H-15 (on)
	c. Activate thermal isolation valves	5	17	TIV-1,TIV-2	
2.	Receive soil from Lander				
	a. Deposit soil sample into feed tube	1	22		
	b. Start automatic sequence	1	23		
3.	Transfer soil sample to extractor				
	a. Open high pressure He/H_2 supply valve	1	24	Op S-1	
	b. Open soil valve	1	25	Op S-3	
	c. Drive distribution motor	1	26		
	d. Deposit sample into extractor	1	27		
	e. Reverse drive motor	1	28		
	f. Close high pressure He/H_2 supply valve	1	29	Cl S-1	
	g. Open high pressure He/H_2 line vent valve	1	30	Op S-34	
	h. Close soil valve	1	31	Cl S-3	
	i. Close high pressure He/H_2 line vent valve	1	32	Cl S-34	
4.	Warm extractor				
	a. Turn on extractor, line and reagent heaters	-	33		H-12, 7 thru 11 (ON)
	b. Heat extractor to 10 °C and maintain	15	33		H-1 (10)
5.	Open gas actuated isolation valves				
	a. Open high pressure He/H_2 supply valve	1	48	Op S-1	
	b. Open actuation valve	1	49	Op S-2	
	c. Close high pressure He/H_2 supply valve	1	50	Cl S-1	
	d. Open He/H_2 vent valve	1	51	Op S-34	
	e. Close Actuation Valve	1	52	Cl S-2	
	f. Close He/H_2 vent valve, turn off reagent injector heaters	1	53	Cl S-34	H-8,10,11 (OFF)
6.	Inject standard amino acids into extractor				
	a. Open amino acid injector pressurization valve	2	54	OP S-17	
	b. Close amino acid injector pressurization valve	1	56	Cl S-17	
	c. Open amino acid injection valve	1	57	Op S-18	
	d. Open extractor valve	1	58	Op S-4	
	e. Close amino acid injection valve, turn off injector heater	1	59	Cl S-18	H-7 (OFF)
	f. Close extractor valve	1	60	Cl S-4	
	g. Open line vent valve	1	61	OP S-5	
	h. Close line vent valve	1	62	CL S-5	
7.	Inject H_2O into extractor				
	a. Open H_2O injector pressurization valve	2	63	OP S-21	
	b. Close H_2O injector pressurization valve	1	65	Cl S-21	
	c. Open H_2O injection valve	1	66	Op S-22	
	d. Open extractor valve	9	67	Op S-4	
	e. Close H_2O injection valve	1	76	Cl S-22	
	f. Close extractor valve	1	78	Cl S-4	
8.	Clean Line				
	a. Open line vent valve	1	79	Op S-5	
	b. Close line vent valve	1	80	Cl S-5	
	c. Open H_2O injection valve	1	81	Op S-22	
	d. Open line vent valve	1	82	Op S-5	
	e. Close H_2O injection valve	1	83	Cl S-22	
	f. Open low pressure He/H_2 supply valve	1	84	Op S-27	
	g. Close low pressure He/H_2 supply valve	1	85	Cl S-27	
	h. Repeat steps b through g four times	24	86		
	i. Close line vent valve, turn off line, H_2O injector and gas supply	1	110	Cl S-5	H-9,12,15 (OFF)
9.	Carry Out Extraction				
	a. Change extractor heater set point	-	111		H-1 (165)
	b. Heat extractor to 165 $\pm 5^{\circ}C$ and maintain	90	111		
	c. Change extractor heater set point	-	201		H-1 (10)
	d. Allow extractor to cool below 50°C	30	201		
10.	Transfer Extract to Hydrolyzer/Evaporator				
	a. Turn on hydrolyzer/evaporator, line, and gas supply heaters	-	231		H-2 (20), H-15, 12 (ON)
	b. Heat hydrolyzer/evaporator to 10°C and maintain	15	231		
	c. Open hydrolyzer/evaporator inlet valve	1	246	OP S-6	
	d. Open line vent valve	1	247	OP S-5	
	e. Close line vent valve	1	248	CL S-5	
	f. Open extractor valve	5	249	OP S-4	
	g. Open hydrolyzer/evaporator vent valve, turn on HCl injector heater	1	254	OP S-7	H-8 (ON)
	h. Close hydrolyzer/evaporator vent valve	1	255	CL S-7	
	i. Close hydrolyzer/evaporator inlet valve	1	256	CL S-6	
	j. Open low pressure He/H_2 supply valve	2	257	OP S-27	
	k. Close low pressure He/H_2 supply valve	1	259	CL S-27	
	l. Open hydrolyzer/evaporator inlet valve	5	260	OP S-6	
	m. Open hydrolyzer/evaporator vent valve	1	265	OP S-7	
	n. Close extractor valve	1	266	CL S-4	
	o. Close hydrolyzer/evaporator vent valve	1	267	CL S-7	
	p. Turn off extractor heater	1	268		H-1 (OFF)

FOLDOUT FRAME 2

Table 4-2. Typical Instrument Sequence (-15°F Lander Thermal Plate Case)

FOLDOUT FRAME 1

11.	Inject HCl Solution Into Hydrolyzer/Extractor				
	a. Open HCl injector pressurization valve	2	269	OP S-19	
	b. Close HCl injector pressurization valve	1	271	CL S-19	
	c. Open HCl injection valve	10	272	OP S-20	
	d. Close HCl injection valve, turn off injector heater	1	282	CL S-20	H-8 (OFF)
	e. Close hydrolyzer/evaporator inlet valve	1	283	CL S-6	
12.	Start Hydrolysis				
	a. Change hydrolyzer/extractor set point	-	284		H-2 (110°C)
	b. Heat hydrolyzer/extractor to 110°C and maintain for .5 hours	5	284		
	c. Turn on H_2O injector heater	15	289		H-9 (ON)
13.	Clean Line				
	a. Open line vent valve	1	304		
	b. Close line vent valve	1	305		
	c. Open H_2O injection valve	1	306		
	d. Open line vent valve	1	307		
	e. Close H_2O injection valve	1	308		
	f. Open low pressure He/H_2 supply valve	1	309		
	g. Close low pressure He/H_2 supply valve, turn on line and NaOH injector heaters	1	310		H-13, 14, 11 (ON)
	h. Repeat steps b through g four times	24	311		
	i. Close line vent valve	1	335		
14.	Pre-elute Ion Exchange Column				
	a. Open NaOH injector pressurization valve	2	336	OP S-25	
	b. Close NaOH injector pressurization valve	1	338	CL S-25	
	c. Open NaOH injection valve	1	339	OP S-26	
	d. Open line vent valve	1	340	OP S-10	
	e. Open IEC outlet valve	20	341	OP S-9	
	f. Close IEC outlet valve	1	361	CL S-9	
	g. Close NaOH injection valve, turn off injector heater	1	362	CL S-26	H-11 (OFF)
15.	Wash Ion Exchange Column				
	a. Open H_2O injector pressurization valve	2	363	OP S-21	
	b. Close H_2O injector pressurization valve	1	365	CL S-21	
	c. Open H_2O injection valve	1	366	OP S-22	
	d. Open line interconnect valve	1	367	OP S-28	
	e. Open IEC outlet valve	17	368	OP S-9	
	f. Turn on HCl injector heater	13	385		H-8 (ON)
	g. Close IEC outlet valve	1	398	CL S-9	
	h. Close H_2O injection valve	1	399	CL S-22	
16.	Regenerate Ion Exchange Column				
	a. Open HCl injector pressurization valve	2	400	OP S-19	
	b. Close HCl injector pressurization valve	1	402	CL S-19	
	c. Open HCl injection valve	1	403	OP S-20	
	d. Open IEC outlet valve	30	404	OP S-9	
	e. Close IEC outlet valve	1	434	CL S-9	
	f. Close HCl injection valve, turn off HCl injector heater	1	435	CL S-20	H-8 (OFF)
	g. Close line interconnect valve	1	436	CL S-28	
	h. Open line vent valve	1	437	OP S-5	
	i. Open low pressure He/H_2 supply valve	1	438	OP S-27	
	j. Close low pressure He/H_2 supply valve	1	439	CL S-27	
	k. Close line vent valve	1	440	CL S-5	
17.	Wash Ion Exchange Column				
	a. Open H_2O injector pressurization valve	2	441	OP S-21	
	b. Close H_2O injector pressurization valve	1	443	CL S-21	
	c. Open H_2O injection valve	1	444	OP S-22	
	d. Open line interconnect valve	1	445	OP S-28	
	e. Open IEC outlet valve	40	446	OP S-9	
	f. Close IEC outlet valve	1	486	CL S-9	
	g. Close line interconnect valve	1	487	CL S-28	
	h. Close H_2O injection valve, turn off H_2O injector heater	1	488	CL S-22	H-9 (OFF)
	i. Close line vent valve	1	489	CL S-10	
	j. Open line vent valve	10	490	OP S-5	
	k. Open low pressure He/H_2 supply valve	10	500	OP S-27	
	l. Close low pressure He/H_2 supply valve	1	510	CL S-27	
	m. Close line vent valve, turn off line and gas supply heaters	93	511	CL S-5	H-12,13,14,15 (OFF)
18.	End Hydrolysis, Evaporate HCl Solution				
	a. Change hydrolyzer/evaporator heater set point	-	604		H-2 (20)
	b. Allow hydrolyzer/evaporator to cool below 50°C	30	604		
	c. Open hydrolyzer/evaporator vent valve	1	634	OP S-7	
	d. Change hydrolyzer/evaporator heater set point	-	635		H-2 (110)
	e. Heat hydrolyzer/evaporator to 110°C and maintain	200	635		
	f. Change hydrolyzer/evaporator heater set point	1	835		H-2 (50)
	g. Close hydrolyzer/evaporator vent valve	15	836	CL S-7	
	h. Allow hydrolyzer/evaporator to cool to 50°C	-	836		
	i. Turn on gas supply, line and H_2O injector heaters	14	851		H-9,12,15 (ON)

FOLDOUT FRAME 2

Table 4-2. Typical Instrument Sequence (-15°F Lander Thermal Plate Case) (Continued)

19.	Dissolve Amino Acids and Residual Salts				
	a. Open H_2O injector pressurization valve	2	865	OP S-21	
	b. Close H_2O injector pressurization valve	1	867	CL S-21	
	c. Open hydrolyzer/evaporator vent valve	1	868	OP S-7	
	d. Close hydrolyzer/evaporator vent valve	1	869	CL S-7	
	e. Open hydrolyzer/evaporator inlet valve	1	870	OP S-6	
	f. Open H_2O injection valve	5	871	OP S-22	
	g. Close H_2O injection valve, turn off H_2O injector heater	1	876	CL S-22	H-9 (OFF)
20.	Mix by Gas Bubbling				
	a. Open low pressure He/H_2 supply valve	1	877	OP S-27	
	b. Close hydrolyzer/evaporator inlet valve	0.1		CL S-6	
	c. Open hydrolyzer/evaporator vent valve	0.1		OP S-7	
	d. Close hydrolyzer/evaporator vent valve	0.1		CL S-7	
	e. Open hydrolyzer/evaporator inlet valve	0.1		OP S-6	
	f. Repeat steps b through e, nine times	4			
	g. Close hydrolyzer/evaporator inlet valve, turn on line heaters	1	881	CL S-6	H-13,14 (ON)
	h. Change hydrolyzer/evaporator heater set point	-	882		H-2 (20)
	i. Allow hydrolzyer/evaporator to cool to 20-25°C	30	882		
21.	Place Solution on Ion Exchange Column				
	a. Open hydrolyzer/evaporator inlet valve	1	912	OP S-6	
	b. Open hydrolyzer/evaporator outlet valve	1	913	OP S-8	
	c. Open line vent valve	1	914	OP S-10	
	d. Open IEC outlet valve, turn on H_2O injector heater	-	915	OP S-9	H-9 (ON)
	e. Activate liquid level sensor	10	915		
	f. Close hydrolyzer/evaporator and IEC outlet valves (by LLS or by time)	1	925	CL S-8, S-9	
	g. Close hydrolyzer/evaporator inlet valve	1	926	CL-6	
	h. Turn hydrolyzer/evaporator heater off	1	927		H-2 (OFF)
	i. Open line vent valve	2	928	OP S-5	
	j. Close low pressure He/H_2 supply valve	1	930	CL S-7	
	k. Close line vent valve	1	931	CL S-5	
22.	Wash IEC				
	a. Open H_2O injector pressurization valve	2	932	OP S-21	
	b. Close H_2O injector pressurization valve	1	934	CL S-21	
	c. Open H_2O injection valve	1	935	OP S-22	
	d. Open line interconnect valve	1	936	OP S-28	
	e. Open IEC outlet valve	18	937	OP S-9	
	f. Turn on NH_4OH injector heater	12	955		H-10 (ON)
	g. Close IEC outlet valve	1	967	CL S-9	
	h. Close H_2O injection valve, turn off H_2O injector heater	1	968	CL S-22	H-9 (OFF)
23.	Elute Ion Exchange Column				
	a. Turn on derivatizer heater	-	969		H-4 (10)
	b. Heat derivatizer to 10°C and maintain (in parallel with subsequent steps)	1	969		
	c. Open NH_4OH injector pressurization valve	2	970	OP S-23	
	d. Close NH_4OH injector pressurization valve	1	972	CL S-23	
	e. Close line interconnect valve	1	973	CL S-28	
	f. Open line vent valve	5	974	OP S-5	
	g. Open low pressure He/H_2 supply valve	5	979	OP S-27	
	h. Close low pressure He/H_2 supply valve	2	984	CL S-27	
	i. Open low pressure He/H_2 supply valve	5	986	OP S-27	
	j. Close low pressure He/H_2 supply valve Turn off He/H_2 supply heater	2	991	CL S-27	H-15 (OFF)
	k. Close line vent valve	1	993	CL S-5	
	l. Open derivatizer vent valve	2	994	OP S-12	
	m. Close derivatizer vent valve	1	996	CL S-12	
	n. Open NH_4OH injection valve	1	997	OP S-24	
	o. Open line interconnect valve	1	998	OP S-28	
	p. Open IEC outlet valve	14	999	OP S-9	
24.	Collect Amino Acids in Derivatizer				
	a. Close line vent valve	1	1013	CL S-10	
	b. Open derivatizer inlet valve	4	1014		
	c. Close derivatizer inlet and IEC outlet valves	1	1018	CL S-9,11	
	d. Close line interconnect valve	1	1019	CL S-28	
	e. Close NH_4OH injection valve Turn off NH_4OH injector heater	1	1010 1020	CL S-24	H-10 (OFF)
	f. Open line vent valves	60	1021	OP S-5,10	
	g. Close line vent valve	1	1071	CL S-10	
	h. Close line vent valve Turn off line heaters	1	1072	CL S-5	H-12,13,14 (OFF)
25.	Evaporate Ammonia Solution				
	a. Open derivatizer vent valve	1	1073	OP S-12	
	b. Change derivatizer heater set point	-	1074		H-4 (100)
	c. Heat derivatizer to 100°C and maintain	120	1074		
	d. Close derivatizer,vent valve	1	1194	CL S-12	
	e. Change derivatizer heater set point	1	1195		H-4 (10)
	f. Allow derivatizer to cool to 20°C or below	48	1196		
	g. Turn on line and He/H_2 supply heaters	12	1244		H-14,15 (ON)

Table 4-2. Typical Instrument Sequence (-15°F Lander Thermal Plate Case) (Continued)

FOLDOUT FRAME

FOLDOUT FRAME

FOLDOUT FRAME 1

26.	Inject Butanol/HCl				
	a. Open line vent valve	1	1256	OP S-10	
	b. Open butanol/HCl injection valve	1	1257	OP S-32	
	c. Close butanol/HCl injection valve	1	1258	CL S-32	
	d. Open He/H_2 supply valve	1	1259	OP S-29	
	e. Close He/H_2 supply valve	1	1260	CL S-29	
	f. Repeat steps b through e two times	8	1261		
	g. Close line vent valve, Turn off He/H_2 supply heater	1	1269	CL S-10	H-15 (OFF)
	h. Open derivatizer vent valve	2	1270	OP S-12	
	i. Close derivatizer vent valve	1	1272	CL S-12	
	j. Open derivatizer inlet valve	1	1273	OP S-11	
	k. Open butanol/HCl injection valve	1	1274	OP S-32	
	l. Close butanol/HCl injection valve	1	1275	CL S-32	
	m. Close derivatizer inlet valve	1	1276	CL S-11	
	n. Open line vent valve	1	1277	OP S-10	
27.	Esterify Amino Acids				
	a. Change derivatizer heater set point	-	1278		H-4 (100)
	b. Heat derivatizer to 100°C and maintain	30	1278		
	c. Close line vent valve Turn off line heater	120	1308	CL S-10	H-14 (OFF)
	d. Change derivatizer heater set point	-	1428		H-4 (37)
	e. Allow derivatizer to cool below 50°C	30	1428		
28.	Evaporate Butanol/HCl				
	a. Open derivatizer vent valve	90	1458	OP S-12	
	b. Change derivatizer heater set point	1	1548		H-4 (10)
	c. Close derivatizer vent valve	-	1549	CL S-12	
	d. Allow derivatizer to cool below 20°C	15	1549		
	e. Turn on line and He/H_2 gas supply heaters	15	1564		H-14,15 (ON)
29.	Inject Trifluoroacetic Anhydride/Methylene Chloride				
	a. Open TFAA/MC injector pressurization valve	2	1579	OP S-30	
	b. Close TFAA/MC injector pressurization valve	1	1581	CL S-30	
	c. Open line vent valve	1	1582	OP S-10	
	d. Open He/H_2 supply valve	5	1583	OP S-29	
	e. Close He/H_2 supply valve Turn off He/H_2 supply heater	1	1588	CL S-29	H-15 (OFF)
	f. Close line vent valve	1	1589	CL S-10	
	g. Open TFAA/MC injection valve	1	1590	OP S-31	
	h. Close TFAA/MC injection valve	1	1591	CL S-31	
	i. Open line vent valve	1	1592	OP S-10	
	j. Repeat steps f through i	4	1593		
	k. Close line vent valve	1	1597	CL S-10	
	l. Open derivatizer inlet valve	1	1598	OP S-11	
	m. Open TFAA/MC injection valve	1	1599	OP S-31	
	n. Close TFAA/MC injection valve	1	1600	CL S-31	
	o. Close derivatizer inlet valve	1	1601	CL S-11	
	p. Open line vent valve	10	1602	OP S-10	
	q. Close line vent valve Turn off line heater	1	1612	CL S-10	H-14 (OFF)
30.	Acylate Amino Acid Esters				
	a. Change derivatizer heater set point	-	1613		H-14 (37)
	b. Heat derivatizer to 37°C and maintain	45	1613		
	c. Change derivatizer heater set point	30	1658		H-4 (0)
31.	Evaporate TFAA/MC				
	a. Activate thermoelectric cooler	-	1688		TE-1 (ON)
	b. Cool derivatizer to 0°C and maintain	15	1688		
	c. Open derivatizer vent valve	30	1703	OP S-12	
	d. Close derivatizer vent valve	1	1733	CL S-12	
	e. Turn off thermoelectric cooler	1	1734		TE-1 (OFF)
32.	Evaporate Derivatives Onto GC Column				
	a. Turn on flame ionization detector heater	-	1735		H-6 (ON)
	b. Heat FID to 200°C and maintain	45	1735		
	c. Turn on GC column, He/H_2 gas supply heaters	15	1780		H-5 (25), H-15 (ON)
	d. Open FID He/H_2 supply valve	1	1795	OP S-15	
	e. Open FID outlet valve	1	1796	OP S-29	
	f. Open He/H_2 supply valve	1	1797	OP S-16	
	g. Open derivatizer inlet valve	1	1798	OP S-11	
	h. Opend derivatizer outlet valve	1	1799	OP S-13	H-16 (ON)
	i. Turn on derivatizer heater Turn on O_2 supply heater	-	1800		H-4 (100)
	j. Heat derivatizer to 100°C and maintain	40	1800		
	k. Turn off derivatizer heater	1	1840		H-4 (OFF)
	l. Close derivatizer inlet and outlet valve	1	1841	CL S-11,13	
	m. Open GC He/H_2 supply valve	1	1842	OP S-14	
	n. Close He/H_2 and FID supply valves	1	1843	CL S-15,29	
33.	Start Flame Ionization Detector				
	a. Open O_2 supply valve	1	1844	OP S-33	
	b. Activate hot wire ignitor	-	1845		
	c. Open FID He/H_2 supply valve	1	1845	OP S-15	
34.	Carry Out Gas Chromatographic Analysis				
	a. Heat GC column at 7.5°C/min to 100°C	10	1846		H-5 (Ramp 1)
	b. Change temperature-time profile to 1°C/min	25	1857		H-5 (Ramp 2)
	c. Start data collection	45	1881		
	d. Hold GC column temperature at 170°C	15	1925		H-5 (Hold)
	e. Stop data collection	1	1941		
	f. Close O_2 supply valve Turn off O_2 supply heater	60	1942	CL S-33	H-16 (OFF)
	g. Turn off column heater	30	2002		H-5 (OFF)
	h. Close FID He/H_2 supply valve	1	2032	CL S-15	
	i. Turn off FID heater	10	2033		H-6 (OFF)
	j. Close FID outlet valve	1	2043	CL S-16	
	k. Close GC column He/H_2 supply valve Turn off He/H_2 supply heater	TBD	2044	CL S-14	H-15 (OFF)

NOTE: As discussed in Section 4.3.6, the valves in contact with the processing or reagent solutions are gas actuated by a manifolding system. Thus, for example, OP S-4 represents the opening of the pilot and master supply solenoid valves, followed shortly thereafter by their closure. CL S-4 represents the opening of the pilot and master vent solenoid valves, followed shortly thereafter by their closure.

FOLDOUT FRAME 2

Table 4-2. Typical Instrument Sequence (-15°F Lander Thermal Plate Case) (Continued)

6) Inject Standard Amino Acids Into the Extractor

Prior to the extraction step, standard amino acids (TBD) are injected onto the soil to provide an internal reference.

The reference amino acid solution is injected by first pressurizing the reagent container via S-17, closing S-17 and opening S-18 and S-4 to permit flow. This reagent is not used again until the next analysis. Excess reagent in the line is vented through S-5.

7) Inject H_2O Into Extractor

The first step of the analysis consists of water extraction. This process has the advantage over the previously used first step of acid hydrolysis in that less interfering material from the soil is present during the actual hydrolysis.

This allows for milder desalting conditions: elimination of the HF/OH^- desalting step and a volume reduction in the ion exchange resin.

The water is injected into the extractor by opening S-21 to pressurize the reagent container, closing S-21, then opening S-22 and S-4.

8) Clean Line

After reagent injection, housekeeping details are carried out to wash and vent injection lines. The line is vented by opening and closing S-5. It is then flushed with water by opening S-22 and dried by gas purge through S-27.

9) Heat Extractor

The extractor is then heated to 165 $\pm 5^{\circ}C$ for an hour, then cooled to below $50^{\circ}C$, in order to conduct the extraction step.

10) Transfer Extract to Hydrolyzer/Evaporator

The extract is then filtered away from the soil. The hydrolyzer cell is heated to $10^{\circ}C$ with H-2.

Filtration is accomplished by opening valves S-4 and S-6 between the two cells and allowing the solution to flow after venting the hydrolyzer/evaporator. Completion is assured by subsequent pressurization of the extractor, then allowing flow through S-4 and S-6 again into the hydrolyzer/evaporator.

11) Inject HCl Into the Hydrolyzer

The amino acids present in life containing soils are at least partially in the form of biopolymers (peptides and proteins).

Thus, a major step in the separation and detection is to break down any polymers in the extract into the individual amino acids by acid hydrolysis.

Reagent injection is accomplished by pressurizing the HCl reservoir and then injecting the HCl with the volume controlled by the time the HCl injection valve is open.

12) Start Hydrolysis

The hydrolysis is conducted by heating the hydrolyzer/evaporator at 110°C for 5 hours. While the hydrolysis is being carried out a pre-elution and regeneration of the ion exchange column is conducted to ready the IEC for use.

13) Clean Lines

Once again any HCl or extract residue remaining in the lines is removed by washing and drying the lines.

14) Pre-elute Ion Exchange Column

During storage, ion exchange resin by-products develop which interfere with gas chromatograph data. Much of these by-products can be eliminated by cycling the ion exchange column through base and then acid (with water washes between reagents) immediately prior to experimental use.

Ion exchange column cycling is initiated by first pressurizing the NaOH container via S-25, closing S-25 and injecting 10ml through S-26. The IEC effluent is vented through S-10, S9.

15) Wash Ion Exchange Column

The NaOH elution is followed by a 20 milliliter water wash accomplished by pressurizing the reservoir, then flowing through S-22 and S-28. Valve S-9, which was closed at completion of NaOH injection to prevent gas from entering the IEC, is reopened to allow liquid flow.

16) Regenerate Ion Exchange Column

The resin is then returned to the acid form necessary for actual use by washing with 15 milliliters 6N HCl.

This is done by pressurizing the HCl injector, then flowing through S-20. Valve S-9, closed as a protective measure, is again opened to allow flow.

After the HCl injection, the line is vented through S-5 to eliminate HCl contamination of following injections.

17) Wash Ion Exchange Column

The ion exchange column is then washed to neutrality with 20 milliliters H_2O injected in the same manner as above. At the completion of the washing step, the water injector heater is turned off, and the line heaters are turned off to conserve power.

18) End Hydrolysis, Evaporate HCl Solution

At the completion of the hydrolysis step the cell heater power is reduced by changing the set point to 20°C and allowing the cell to cool below 50°C so that the vent valve can be opened without bumping.

Evaporation is then accomplished by opening S-7 and heating to 100°C. The evaporation rate is controlled by a flow restrictor located downstream of the valve (FR-1).

The heater is designed to provide enough heat input for the evaporation process and any heat transfer to the attaching hardware. A 50 percent evaporation time margin is provided to assure complete evaporation.

At the completion of evaporation the cell is then allowed to cool below 50°C. Heaters are again powered to gas supply line, fluid line and water injector (H-9, H-12, H-15).

19) Dissolve Amino Acids and Residual Salts

The residue from the preceding step is dissolved in 5 milliliters water which is injected in the nominal manner as above.

20) Mix by Gas Bubbling

The solution is stirred alternately by pressurizations with He/H_2, then venting at 0.1 minute intervals. This process is repeated for a total of ten cycles to insure complete dissolution of sample. The hydrolyzer is allowed to cool to 20° to 25°C. Line heaters are turned on (H-13, H-14) for subsequent use.

21) Place Solution on Ion Exchange Column

The amino acid solution is then placed on a strong acid-type cation exchange resin column which is in the acid form. As long as the solution is not too acidic or too alkaline, the amino acids and residual cations are retained and can be washed. The amino acids can be eluted with an ammonia solution while the inorganic cations are still retained (except aluminum). In order for the ion exchange purification procedure to be effective, the flow rate through the column must be slow enough (less than 0.5 ml/min) to allow the exchange reactions to equilibrate.

Flow is highly restricted at the IEC outlet to assure that liquid in the column does not flash into a two-phase mixture. Flow direction is from the bottom up to provide a slight increase in column efficiency and to insure maintenance of a liquid column even if the evaporator flow resistance dominates (i.e., no flow from the column caused by gravity).

Because of the possibility of flow variations out of the hydrolyzer/evaporator into the IEC, and because simple timed sequencing is not an adequate method for recovering all of the liquid contents of the cell, a liquid sensor is provided between the hydrolyzer/evaporator and IEC. This sensor consists simply of two electrodes. While fluid is in contact with both electrodes, it will provide a low resistance circuit. A gas interface will interrupt this circuit, indicating completion of the flow process. This open circuit will trigger the closing of the hydrolyzer/evaporator outlet valve and IEC vent valve.

22) Wash Ion Exchange Column

Water is injected through the IEC to wash out non-retained material. This is accomplished in the nominal manner, first by pressurizing the water injector, then injecting 15 milliliter water by opening S-22, S-28 and S-9. The ammonia injector heater (H-10) is also turned on in this step.

23) Elute Ion Exchange Column

Elution of the amino acids is accomplished with ammonia. Enough reagent must be supplied to displace all of the liquid within the column (if flow were from the top down, a smaller quantity of NH_4OH solution, followed by gas, might be used), and also account for the reaction of the ammonia with the ion exchange resin which is still in the acid form. Injection after injector pressurization through S-23 is accomplished by opening the IEC vent valve S-9. After a sufficient quantity has been injected, the water wash will have been displaced, and the amino acids will be in the first portion of the NH_4OH effluent.

Prior to amino acid collection, the derivatizer is heated to $10^{o}C$ and is vented via S-12.

24) Collect Amino Acids in Derivatizer

At the point of ammonia breakthrough, and accompanying amino acids, the vent valve S-10 is closed and the inlet valve to the derivatizer S-11 is opened.

The baseline design uses a timed sequence to perform this operation. The first 2 millimeters of ammonia contain virtually all the amino acids. Four minutes after ammonia

breakthrough the appropriate 2 millimeters will have eluted. Collecting additional solution would increase the amount of salts collected with the amino acids. At this time the derivatizer inlet valve, the IEC outlet valve and the ammonia injection valve are closed. Line vent valve S-5 is opened for a time to clean the line.

25) Evaporate Ammonia Solution

After the effluent from the purification procedure is transferred to the derivatization cell, it is dried by a timed heating period with vapor removal through an orifice as in the previous evaporation step. The vent valve is opened, and the derivatizer is heated to 100°C for 120 minutes. The derivatizer is then cooled to below 20°C.

The amino acids in their free state are very polar and hence cannot be volatilized without decomposition nor separated by gas chromatography directly. However, by carrying out reactions such as esterification of the carboxylic acid group and acylation of the amino group, volatile derivatives are formed which can then be separated by gas chromatography. In addition to separating the amino acids from each other, it is also desirable to separate the two optical isomers of those amino acids which contain an asymmetric site since strong interferences as to the possibility of biogenic origin of the amino acids can be made based on the isomer ratios. However, the two optical isomers of a given amino acid are equivalent in a symmetric environment. If an alcohol such as 2-butanol which is also optically active is used to esterify the carboxylic acid function, the diastereomers are formed with those amino acids which also contain asymmetric sites.

Now the derivatives with the same optical configuration at both asymmetric sites (DD and LL) are chemically and physically different from those derivatives with different optical configuration at the two asymmetric sites (DL and LD) and can be separated by a variety of gas chromatographic columns. Ninety percent pure 2-butanol is used so that two peaks are obtained for each optically active amino acid regardless of the optical purity of the amino acids in order to confirm the separation and identification by the gas chromatograph.

26) Inject 2-Butanol/HCl Into Derivatizer

Since the 2-butanol/HCl mixture cannot withstand the sterilization conditions without excessive reaction, the anhydrous HCl gas is not mixed with the 2-butanol until the instrument is functioning at its destination. The temperatures experienced by the 2-butanol/HCl solution at the destination are moderate enough so that the solution will be stable through the 90-day life of the instrument. Hence, this reagent need only be prepared once. This is done the first time the experiment is performed.

Prior to reagent injection, the lines are cleaned of any residue water vapor or other contaminants that would interfere with the derivatization process. This is done by alternately venting butanol/HCl through S-32 and S-10 and purging with He/H_2 through S-29. This is done a total of three times.

The injection of 0.5 milliliter 2-butanol/HCl is accomplished by opening the derivatizer inlet valve S-11, followed by opening the injection valve S-32 for 1 minute and then closing it.

27) Esterify Amino Acids

The actual esterification of the amino acids is accomplished by heating to 100°C for 2 hours. At the end of the 2-hour period the derivatizer is allowed to cool to below 50°C to prevent bumping when the vent valve is opened to start the subsequent evaporation.

28) Evaporate Butanol/HCl

The butanol/HCl is then evaporated through S-12 while heating to 100°C again. When evaporation is complete, the cell is cooled to below 20°C.

29) Inject Trifluoroacetic Anhydride/Methylene Chloride Solution into Derivatizer

After the carboxylic acid function has been esterified and excess reagent has been removed, the amino function is acylated with trifluoroacetic anhydride with methylene chloride present as a solvent. The resulting N-TFA-2-butyl derivatives of the amino acids are quite volatile and are readily separated and resolved by gas chromatography. Other acylating agents could be used in place of the trifluoroacetic anhydride, but because of its short reaction times and lack of interference in the gas chromatographic separation, it is the reagent of choice.

The fluid lines are cleaned prior to use in the same manner as with the preceding reagent, cycling between TFA/methylene chloride and He/H_2 purge gas twice before injection.

The injection process is accomplished through S-31 and S-11.

30) Acylate Amino Acid Esters

The final derivatization step is accomplished by heating the cell to 37°C for 1 hour. At the end of this step the cell is allowed to cool.

31) Evaporate Trifluoracetic Anhydride/Methylene Chloride

Prior to injection of the derivatized amino acids into the gas chromatographic column, the reaction reagents must be eliminated. To prevent loss of the volatile derivates, evaporation is carried out below 10°C. The thermo-electric cooler is used to control the temperature at 0°C. Evaporation occurs through S-12.

32) Evaporate Derivatives Onto GC Column

The current sequence calls for direct evaporation of the derivatives out of the derivatizer cell onto the GC column for ultimate separation and an analysis. Prior to this evaporation the GC is turned on: the detector heater (H-6) and gas supply, the GC column gas supply and heaters, and the O_2 supply heater.

The derivatizer is then opened (S-11, S-13) to the GC column and heated to 100°C to evaporate the amino acid derivaties onto the column, while the GC column is kept cool enough to trap the derivatives. The pressurant gas supply is used to sweep the derivatizer and act as a carrier gas during this event. At the conclusion of this event, the derivatizer heater and solenoid valves are turned off. This procedure results in trapping the entire sample quantity in the first few feet of the GC column.

In order to achieve a good separation in a gas chromatograph, the sample vapor in the column at the start of the separation must be concentrated in a small volume of carrier gas. If the volume of carrier gas is large (broad), poorly resolved peaks are obtained. This factor becomes increasingly important as the size of the column is decreased, so that with capillary columns sample introduction can be a serious problem.

Effective dead volume, i.e., that part of the volume in the active portion of the introduction, column and detector flow paths which is not effective in the separation, is also an important consideration. The effect of dead volume is to spread the sample components into a greater volume of carrier gas, thus degrading resolution

By trapping the derivatives in the cool column and then heating the column to start the separation, any problem from slow introduction of the sample into the carrier gas and any dead volume effects prior to the column are eliminated. This introduction technique is that the column be cool enough to immobilize the derivatives during the sample introduction and that contaminant volatiles are not introduced from the derivatizer.

The overall system sensitivity requirement is to detect 0.1 nanomole of each amino acid in the original soil sample (with a goal of 0.01 nanomole). To meet this requirement it is necessary to introduce the entire quantity of the resulting amino acid derivatives into the gas chromatograph. On the other hand, the possibility of a relatively rich soil sample cannot be discounted. In this case, the amount of derivatives present might exceed the capacity of the column so that it would no longer give good resolution and separation. (The specification allows for this possibility by relaxing the resolution requirements for samples containing greater than 50 nanomoles).

When evaporation is completed, the gas flow path is switched from the derivatized cell to a valve leading directly to the GC column.

33) Start FID

The FID is started by opening the oxygen gas supply valve S-33, and then activating the hot wire ignitor. If combustion is not initiated, the ignitor shuts off the fuel and after a suitable delay again opens the fuel valve and again attempts to ignite the mixture. After ignition, the FID heater and temperature sensor are used to maintain the detector temperature at 200°C.

34) Carry Out Gas Chromatographic Analysis

To carry out the gas chromatographic separation, the column which now contains the trapped derivatives is heated in a controlled manner. First, the column is heated rapidly to the temperature at which the separation of the more volatile amino acid derivatives begins, then the temperature is raised at a rate that will give good separations and short retention times.

The derivatives have a wide range of volatility so that the ideal temperature for resolution of each amino acid derivative is different. If a column is operated isothermally to resolve the low boiling derivatives, the high boiling derivatives emerge as broad peaks of low sensitivity and long retention time. By the use of a controlled increase in the temperature of the column during the analysis (temperature programming), each component is separated close to its optimum temperature. The result is that high boiling compounds are eluted earlier and as sharp peaks, thus decreasing analysis time and increasing the overall sensitivity of the system. However, as the temperature of the column is increased, volatility of the liquid phase increases. If appreciable, noise and an upward drift in the base line result.

The amount of bleeding which can be tolerated acts as an upper bound to the temperature profile. The exact temperature profile will be determined by the particular column ultimately selected to carry out the separation. Data collection will start at a predetermined time after column temperature programming is initiated. As an example, a profile used in the laboratory with a Carbowax 20M column is: heat the column oven from 25°C (trapping temperature) to 100°C in 10 minutes, then heat from 100°C to 170°C at 1°/min. Finally, hold the column at 170°C until the analysis is completed. In contrast to laboratory practice of using a forced convection oven, the instrument GC column will be heated directly, using the column tube as the resistance heater element.

This method of heating is more efficient and compact than laboratory practice and should also give better control and reproducibility. The FID is maintained above the maximum column temperature for the entire analysis.

Upon the conclusion of data collection, the oxygen is turned off. The column and detector heaters are left on for a time period which will depend on the column ultimately chosen (for example, 1/2 to 1 hour). The purpose of this step is to purge any material of low volatility in the column so that it will not interfere with subsequent analyses. Upon completion of this purging period, the column, and then the FID heater are shut off.

35) System Cleanup

The system requires cleanup of the processing cells and connecting lines and posibly a heating and purge of the GC column between analyses. The detailed cleaning steps will require further laboratory development so a detailed sequence for these steps has not been defined. However, the general operations and requirements are known. The cells and lines will be cleaned with a series of reagent soaks, heatings and flushes. The IEC would be regenerated and possibly cycled one or more times. Thus the overall time and power requirements for the cleaning sequence will be similar to that of the actual operating sequence. The power and time requirements for the cleaning sequence would not be expected to exceed that required for the operating sequence.

4.2 INSTRUMENT SYSTEM DESIGN

The original flight design presented in TRW's Final Report No. 16660-6001-R0-00 was based on Processing Sequence No. 1, which was also used for the breadboard test program. Ongoing research at NASA/ARC resulted in process modifications and Processing Sequence No. 2 was selected as the baseline flight sequence.

There are significant differences between the two sequences: In the previous sequence, acidic hydrolysis was carried out directly on the soil. This was followed by filtration, evaporation of HCl and then by a $HF-NH_4OH$ desalting process. This was followed in turn with final desalting on a rather large (30 milliliters) ion exchange resin column. Elution of the column, derivatization, and a gas chromatographic analysis completed the procedures. In the new process, the soil is extracted with H_2O at 165°C for 1 hour, the extract is filtered off and HCl is added to the extract. After 5 hours of hydrolysis at 110°C, the HCl solution is evaporated to dryness, the product redissolved and passed directly onto a small (5 milliliters) ion exchange column. Subsequent steps are unchanged except for volume adjustments. While the new procedure may have somewhat reduced recoveries with some types of samples and may cause a small amount of racemization, it has many advantages including easier filtration requirements, fewer and less corrosive reagents, and significantly less interfering material, especially from the smaller ion exchange column.

Because of these differences and because of problems uncovered in the breadboard test program, the flight design has been significantly modified. A major modification is the elimination of the three separate sets of non-reusable processing cells, and the introduction of only one set of processing cells which can be used repeatedly. As pointed out before, the basic design of all instrument components was not changed due to the modifications in the flight processing sequence and the current test cells, ion exchange column, and GC-column are flight weight designs of the prototype breadboard components.

While the previous design of the instrument is modified, the basic design philosophy remains unchanged. The adopted modular design approach facilitates testibility, and repair and replacement capability at the component, subsystem and system level. Viking '75 hardware and technology are chosen whenever this leads to the elimination of duplicate engineering development efforts and to potential cost savings. Margins are provided for each component design and contingencies are built in for weight, volume, electronic parts and board space, valves, heaters, etc.

The instrument is designed to simplified VLBI interface specifications and the VLBI structural design concept is used, including the provision for accommodating the lander soil processor loads. Thermal control is achieved by isolators, emissivity coatings, heaters and one thermoelectric cooler for the derivatizer. The average instrument power for one analysis is approximately 18 watts (cold case) averaged over 1 day, but can easily be brought down to below the maximum specified 16.3 watts. The instrument weight with reagents is 29.4 pounds.

The instrument system package consists of two major subsystems. The mechanical subsystem (MSS) which contains the soil distribution assembly and the processing assembly, and the electronic subsystem (ESS). A view of the assembled instrument depicting the two subsystems and the location of major instrument components is shown in Figure 4-3. Also shown is how the Lander soil processor interfaces with the Wet Chemistry Instrument.

The primary structure in the mechanical subsystem is an aluminum experiment mounting plate to which all major instrument components are attached. The experiment mounting plate attaches with titanium struts to the upper mounting plate which interfaces with the Lander mounting plate, and which provides mounting points and load support for the Lander soil processor.

The electronic subsystem is contained in a single module which fastens directly to the Lander mounting plate. The electrical connection between the ESS and MSS is established with a cable harness ending in electrical connectors inside the MSS.

4.2.1 Interfaces

An interface control drawing of the instrument is presented in Figure 4-4. The interface to the Lander is identical to the VLBI interface with the following exceptions: There is only one thermoelectric cooler instead of four on VLBI, and the thermal interface plate which is required for the VLBI soil illumination lamp has been eliminated. The external dimensions of the instrument are the same as those specified for the VLBI instrument: 13.50 x 10.75 x 11.60 inches.

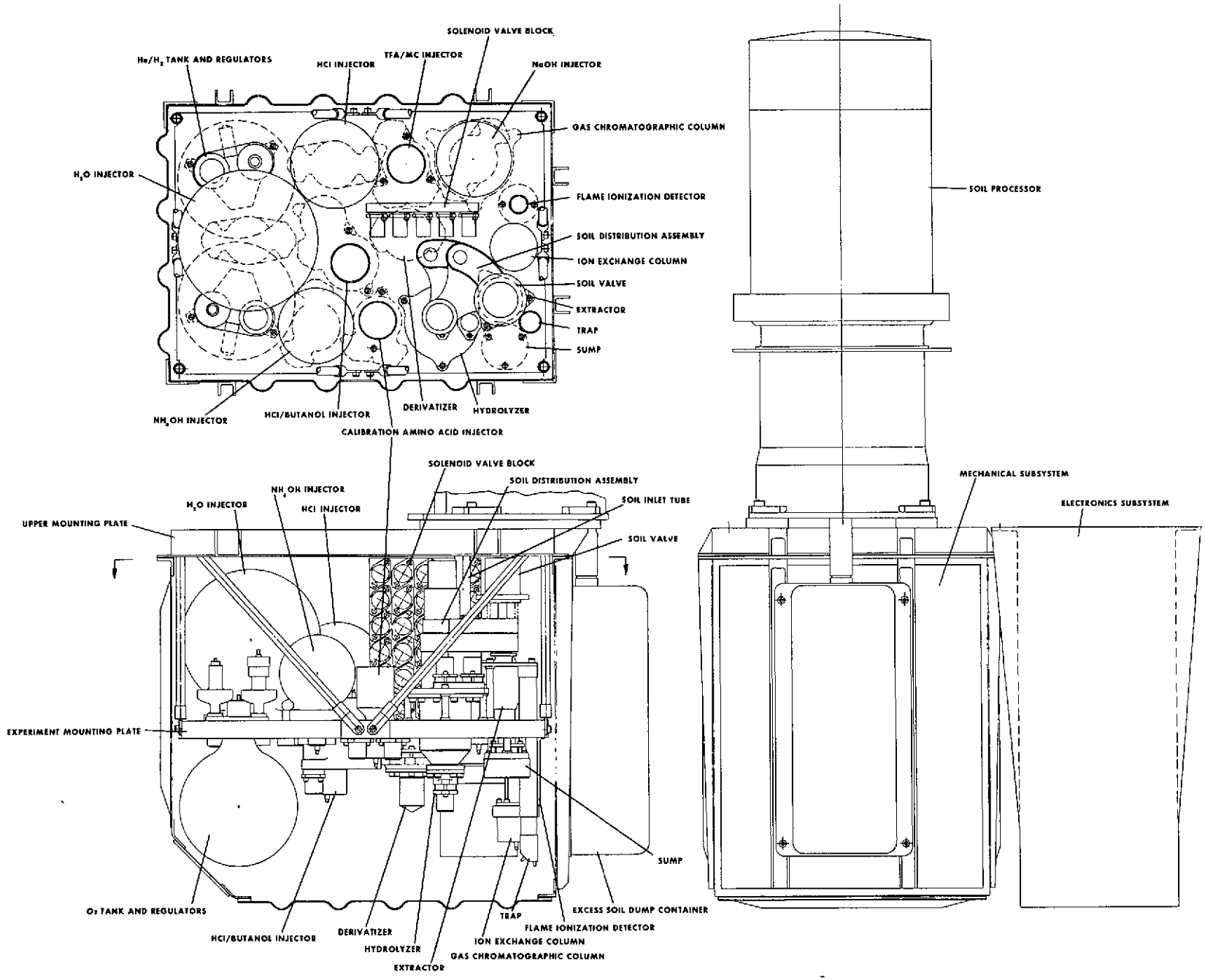


Figure 4-3. Wet Chemistry Instrument

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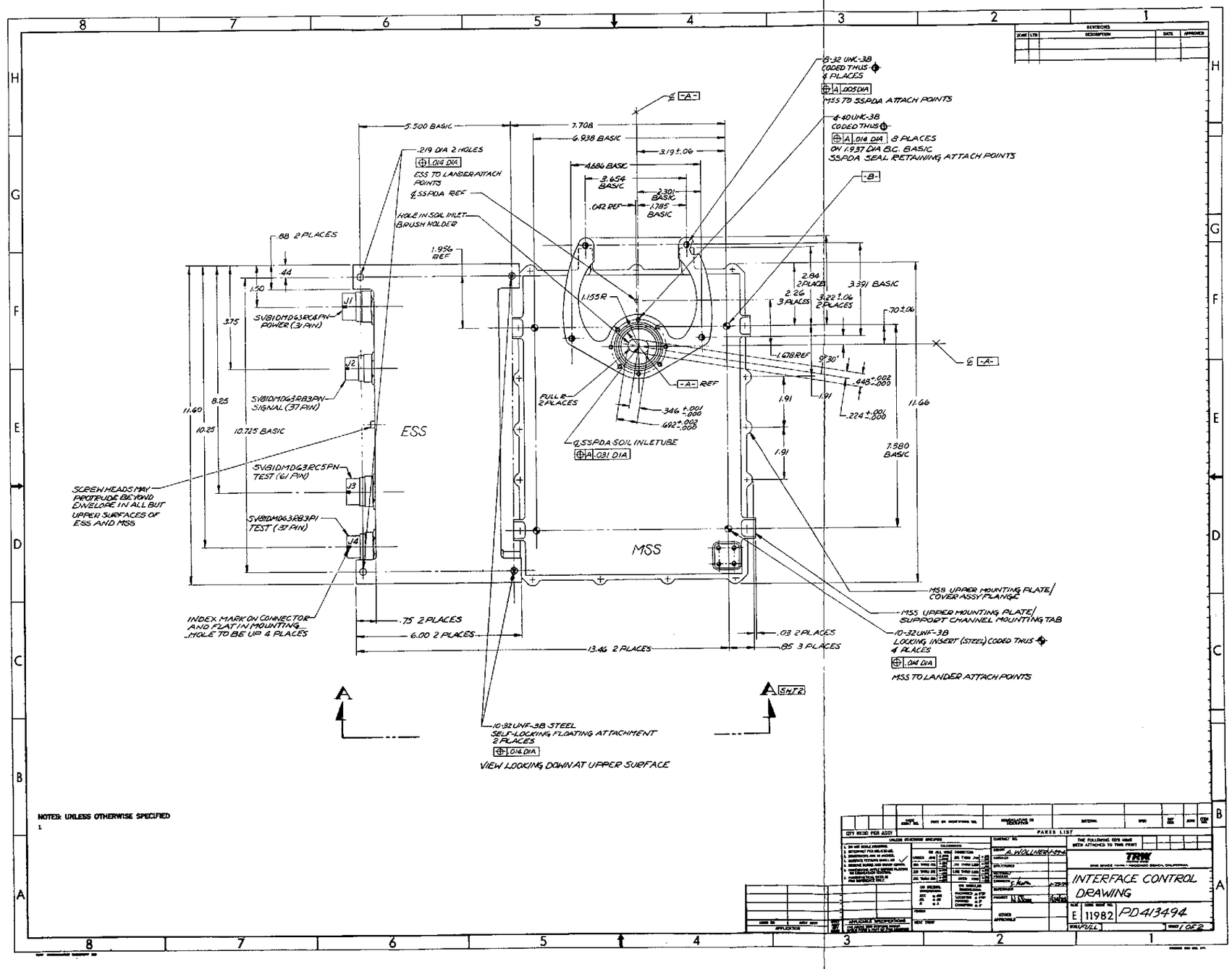

Figure 4-4. Interface Control Drawing

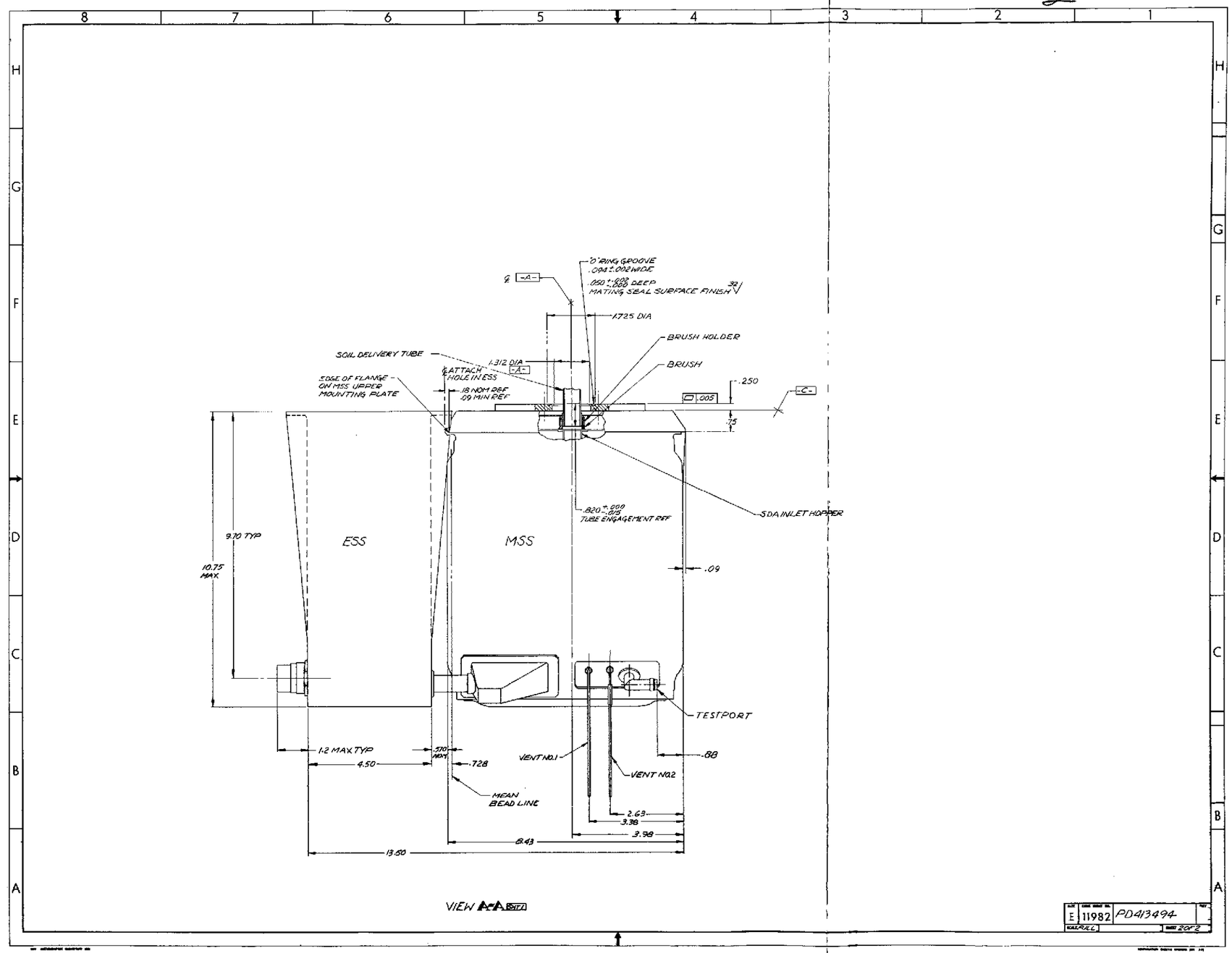

Figure 4-4. Interface Control Drawing (Continued)

4.2.2 Mass Properties

The computed weight of the instrument, including consumables, is 29.4 pounds. The weight computations were derived from the detailed drawings for the instrument components and from actual weight numbers of existing VLBI hardware. A detailed weight breakdown is given in Table 4-3. An assessment of the 27.3 pounds dry weight estimate is given in Table 4-4. It shows that more than 50 percent of the dry weight is based on actual VLBI hardware and that only 3.3 percent is based on conceptual design. The maximum dry weight specified is 30 pounds so that on almost 10 percent weight margin is left.

4.2.3 Thermal Design and Power Profile

TRW's overall approach to the thermal control of the Wet Chemistry instrument is essentially unchanged from that formulated in Section 3.2.5 of the Contract NAS 2-6218 Final Report, i.e.:

- Thermally isolate the instrument from the Lander mounting plate to minimize the effect of variations in mounting plate temperatures.
- Physically mount and isolate components (or series of components) within the instrument according to their specific requirements.

The thermal model constructed during this previous contract assumed metallic conduction to be the dominant mode of heat transfer within the instrument. Experience gained in the thermal design of the VLBI instrument from 1971 to 1974 indicates that if a temperature controlled component is mounted to a colder platform, conduction across the mount amounts to around half of the total heat loss, with most of the remainder attributable to gas conduction. Losses due to IR radiation and natural convection are usually small but not negligible. Accordingly an approximate analysis accounting for all heat transfer modes was conducted for each component of the revised instrument to determine worst cold case power requirements. Each component was considered separately connected to a mounting plate at -15°F (-26°C). This procedure should produce conservative cold case power since the heat lost by each component will tend to warm the surroundings of all components, thus reducing subsequent losses.

Table 4-3. Weight Summary

	Weight (Pounds)
Soil Distributor	0.3
Test Cells	
Extractor	0.4
Hydrolyzer	0.6
Derivatizer	0.4
Ion Exchange Column	0.2
Gas Chromatographic Column	0.5
Flame Ionization Detector	0.2
Reagent Injectors	
Amino Acids	0.3
HCl	0.6
H_2O	0.5
NH_4OH	0.5
NaOH	0.5
HCl/Butanol	0.3
TFAA/MC	0.4
Isolation Valves	0.8
He/H_2 Subsystem	1.3
O_2 Subsystem	1.3
Solenoid Valves and Block	0.8
Gas Operated Valves	1.5
Plumbing	0.5
Waste Management	0.2
Thermoelectric Installation	0.3
Primary Structure	5.9
Electronics Subsystem	8.4
Electrical Installation	0.6
Total Dry Weight	27.3
Consummables	
Gases	0.3
Liquids	1.8
Total Weight	29.4

Table 4-4. Weight Assessment

	Pounds	Percentage
Weight Based on VLBI Components	14.4	53
Weight Based on Modified Components	7.3	27
Weight Based on Prototype Components	2.3	8
Weight Based on Conceptual Design	3.3	12
Total Dry Weight	27.3	100%

Figure 4-5 shows the cold case power profile for a single analysis. The profile assumes a -15°F instrument interface temperature and is largely based on the thermal hand calculations described above. These calculations were based on worse case assumptions. Somewhat lower power requirements would be indicated by a thermal analyzer program model in which heat dissipated in each component could soak to adjacent components.

The average power for a single analysis is 18.1 watts averaged over the 2073 minute duration of a compact sequence. This exceeds the total 16.3 watts available in the cold case according to specification NASA/ARC A-16231 Rev. 3 (October 15, 1973). Programming a 400 minute hold between extraction and hydrolysis will cause the average power to fall below the specified 16.3 watt level. Thus 2473 minutes are required for a single analysis.

Paragraph 2.1.3.7.4 of the specification requires that operating power consumption excluding heaters not exceed 10 watt averaged over 15 days or 7.6 watts averaged over one day. If operating power is defined as power to operate electronics and actuate valves only 4.6 to 5.0 average watts of this category will be used. On the other hand, the formula given for heater power allows 8.72 watts in the cold case (-15°F interface). Clearly the heater power situation for the Wet Chemistry instrument is somewhat different than for VLBI. In VLBI heaters typically are used to

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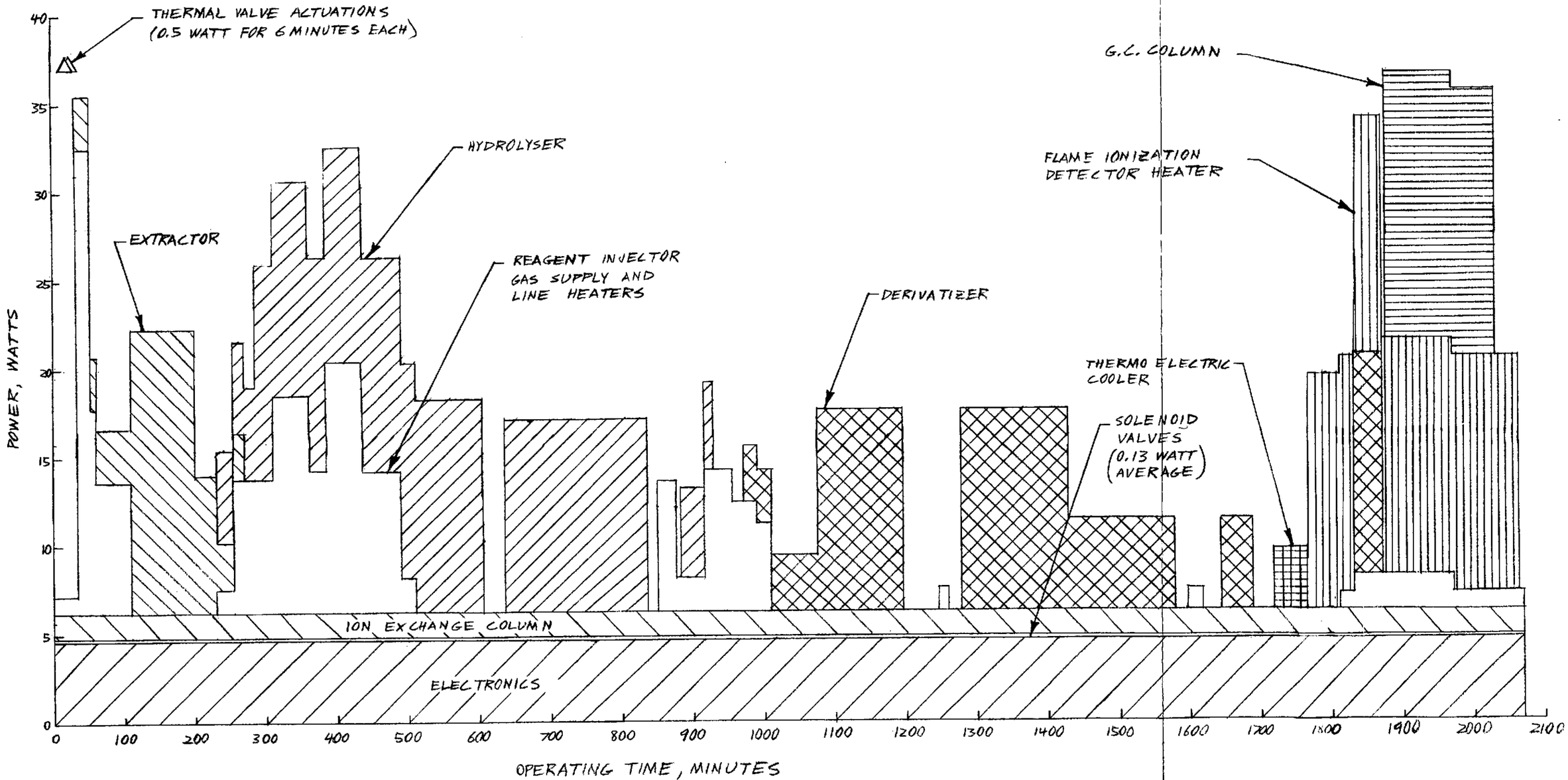


Figure 4-5. Power Profile, -15°F Lander Plate

hold low incubation temperatures for hours at a time and much more heat is required in a cold case environment than in the hot case. In the wet chemistry instrument the reaction vessel heaters are used to actually operate the instrument, i.e. to heat solutions quickly to over 100°C and to evaporate samples and solvents. Nearly as much heater power is required to evaporate a solution at 100°C in -26°C surroundings as in +4°C surroundings. Indeed hot case heater power is expected to be about 85 percent of the cold case requirement. Thus the specification can not be satisfied in the hot case with present power allowances.

4.3 MECHANICAL SUBASSEMBLY AND COMPONENTS DESIGN

An exploded view of the mechanical subsystem is shown in Figure 4-6. It illustrates the assembly of the mechanical components including the soil distributor onto the experiment mounting plate, which attaches to the upper mounting plate with titanium struts. The assembled MSS is enclosed by a metal enclosure as shown in Figure 4-3.

4.3.1 Soil Distribution Assembly

A layout drawing of the proposed soil distribution assembly (SDA) is shown in Figure 4-7. It shows a greatly simplified version of the VLBI soil distribution assembly. The latter (Figure 4-8) is required to deliver three soil samples of different sizes to three different locations, while this experiment requires the delivery of three soil samples of only one size to only one location. Otherwise the same design parameters and requirements as for VLBI have been specified.

The SDA consists of a soil inlet tube for receipt of the bulk soil from the Lander soil processer, a metering tube for measuring the bulk soil, a soil carrier (shuttle) for transporting the sample to the extractor, a dump cell for the excess soil, and a drive motor. These components are mounted on an aluminum bracket. Also on the bracket is the gas actuated extractor cover.

The prototype of the VLBI SDA flight units has now completed more than 2300 cycles at from -200°F to 97°F and at 8 millibars to ambient air pressure without a single failure of any sort. It has never failed to deliver the correct soil samples using four different soil models at various humidities, made up to VLBI specifications.

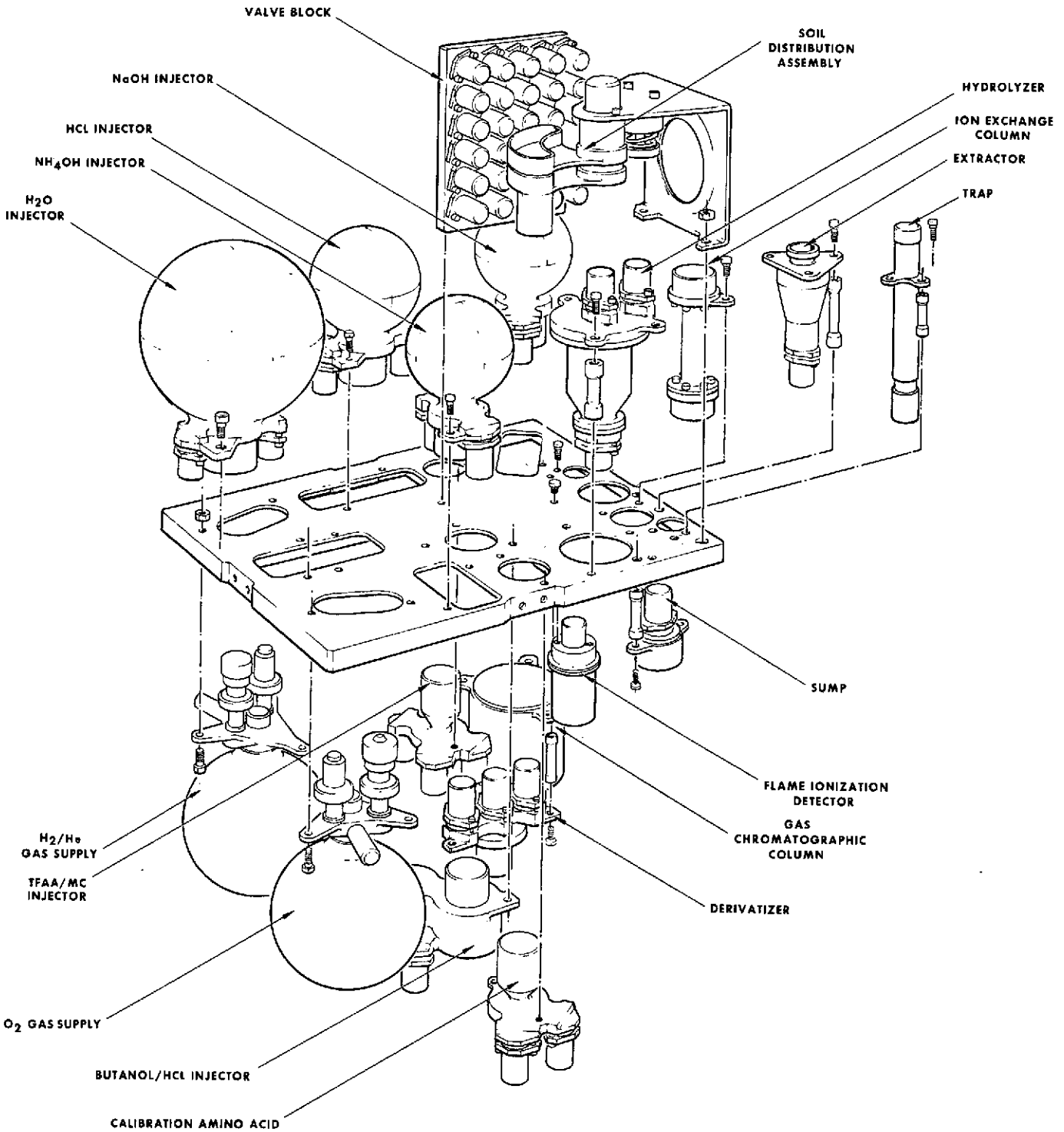


Figure 4-6. Wet Chemistry Instrument (Mechanical Subassembly)

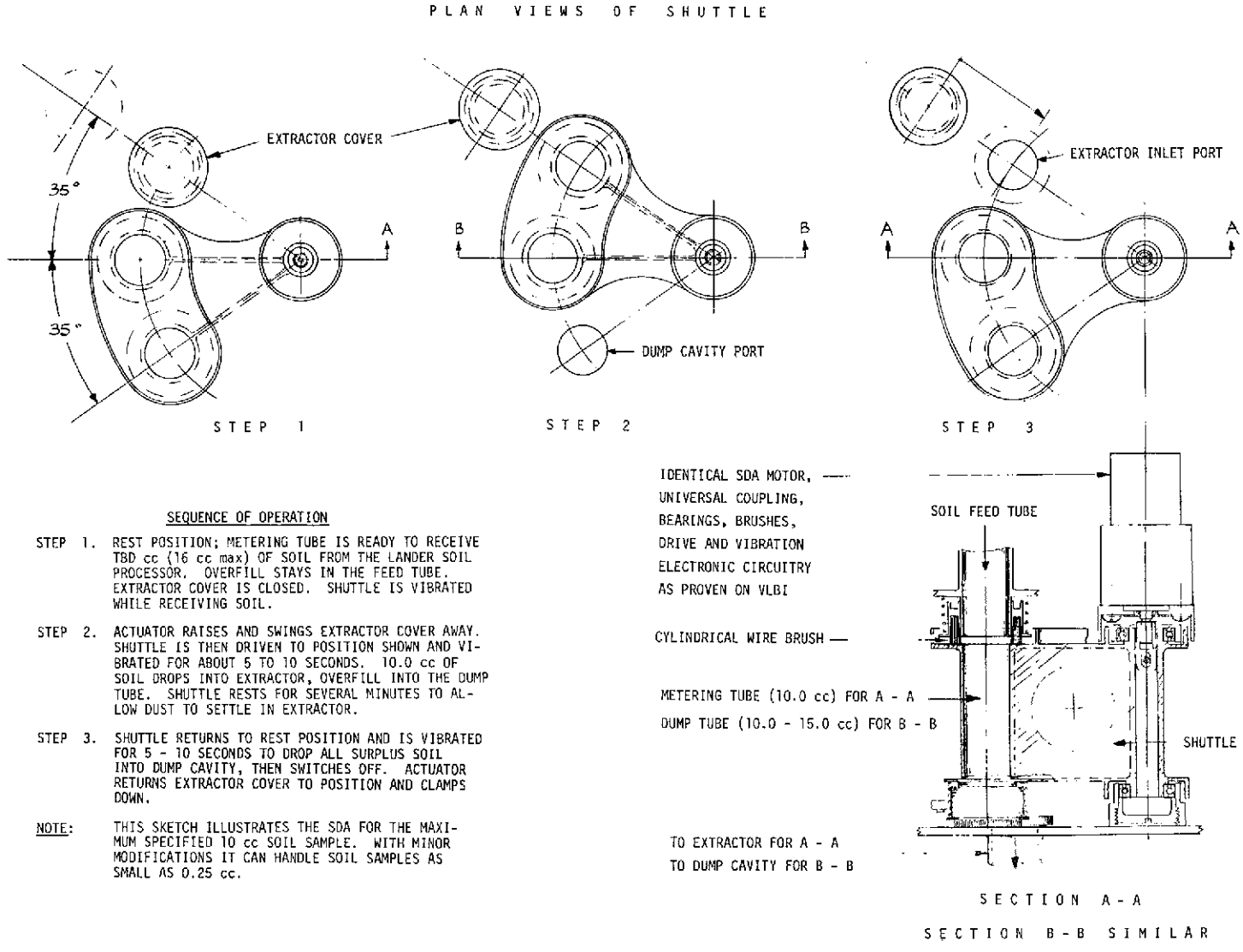


Figure 4-7. Proposed Soil Distribution Assembly (SDA)

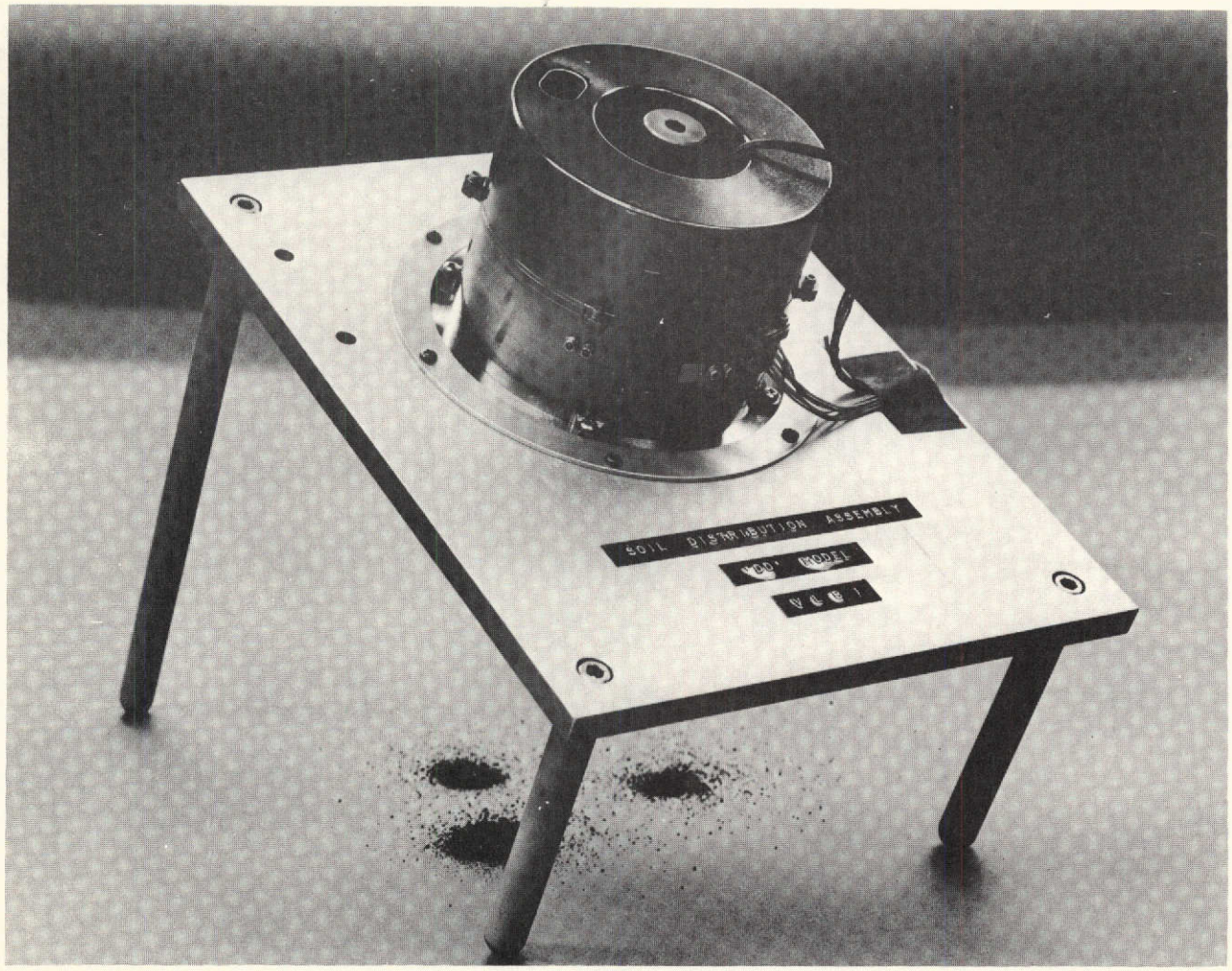


Figure 4-8. VLBI Soil Distributor

Based upon experience gained during the past 3 years of development for VLBI the following key points can be substantiated:

- The mechanism cannot be jammed by large soil particles since cylindrical CRES wire bristle brushes permit moving metal interfaces in the vicinity of the soil to be never closer than twice the sieve size through which the soil is processed by the Lander.
- Metered soil samples are accurate and repeatable within 5 percent.
- Soil losses during transportation are negligible.
- Oscillating the shuttle electronically at 10 Hz, while receiving soil, prevents cavitation and ensures that the correct volume has been received.
- Oscillating the shuttle while delivering soil ensures complete evacuation of the metering tube into the extractor. It also ensures complete emptying of all surplus soil into the dump cavity in preparation for the next bulk sample.
- Carry-over from one bulk sample to the next is thus far less than 5%.

4.3.2 Processing Cells

The general design requirements for the three types of processing cells (extractor, hydrolyzer/evaporator, and derivatizer) are similar. The three cells share the same basic material problems of resistance to attack by corrosive reagents, and prevention of sample contamination or sample loss. All three components need reagent and He/H_2 gas injection ports, vent ports, and outlet ports. Each of the three cells is required to maintain thermal control over the various steps in the individual processes. The internal configurations must be designed to insure proper cell operation over the ±35-degree range of Lander tilt angles. The extractor has the unique requirement for a mechanism at the top to accept the sample from the soil distribution device. The extractor and hydrolyzer/evaporator require filters at their outlets. The hydrolyzer/evaporator and derivatizer require evaporation of fluids as a part of their processing cycle.

4.3.2.1 Extractor

A layout drawing of the extractor is presented in Figure 4-9. The extractor is a flight weight version of the breadboard hydrolyzer (described in Section 3.1.2), and consists of an all tantalum structure

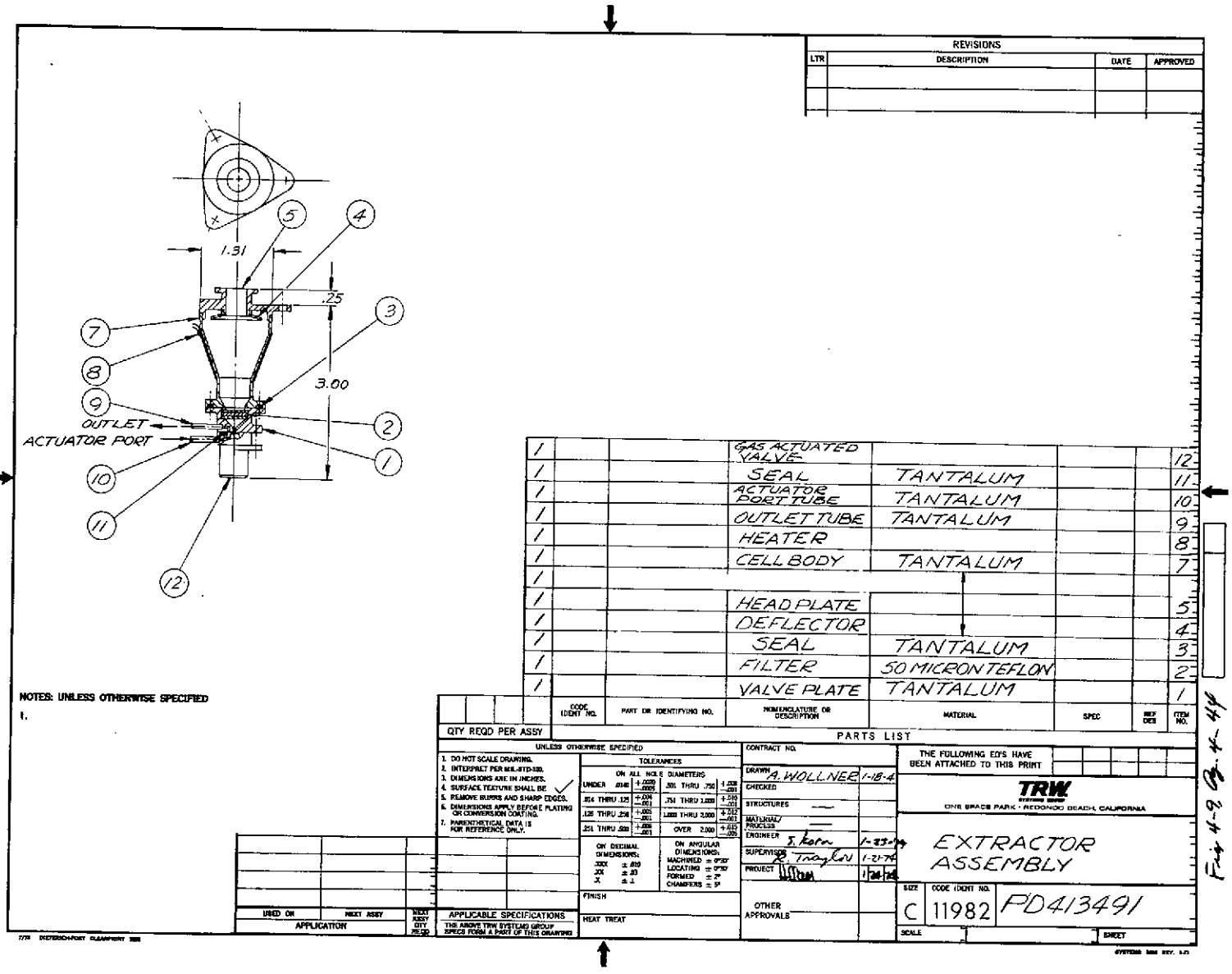


Figure 4-9. Extractor Assembly

with multiple Teflon filters. Major modifications to the breadboard design are:

1) The extractor head end is welded to the cell body and the inlet port accepts the gas actuated extractor cover which is part of the soil distribution assembly.

2) The hand operated solenoid valve simulator is replaced by a gas actuated tantalum diaphragm valve.

The extractor is currently sized for a 1 cubic centimeter soil sample to be extracted with 10 milliliters of H_2O, which is injected through the outlet port. A TBD amount of calibration amino acids is also injected and included in the 10 milliliters total H_2O volume. The outlet port is also used to inject pressurization gas for fluid transfer. An external heater and temperature sensor are mounted to the cell for temperature control. Tantalum tubing connected to the test cell will be joined by EB welding. The filter in the bottom of the extractor will be Teflon since no interference is expected to result from the extraction process. If planned breadboard investigation of the new baseline process should show interference from the Teflon filter, different filter materials and designs will be investigated.

4.3.2.2 Hydrolyzer/Evaporator

A layout drawing of the hydrolyzer/evaporator is shown in Figure 4-10. This processing cell is a flight weight version of the breadboard evaporator (described in Section 3.1.3), and consists of an all tantalum structure with a filter stack in the bottom of the cell. Major modifications are in the detailed design of the flanged head end and bottom plate. The head end accepts two tantalum diaphragm valves for the inlet and vent lines. The purge tube in the center of the cell serves as the entrance for the extract, as support for the deflector plate, and as purge tube through which the carrier gas is injected to provide agitation during dissolution of the residue with H_2O. Tantalum tubing connected to the cell will be joined by EB welding.

The filter in the bottom of the hydrolyzer/evaporator is made out of Teflon in the current design. If future breadboard testing using the new baseline processing sequence shows that the Teflon filter leads to unacceptable interference levels, different filter materials and designs

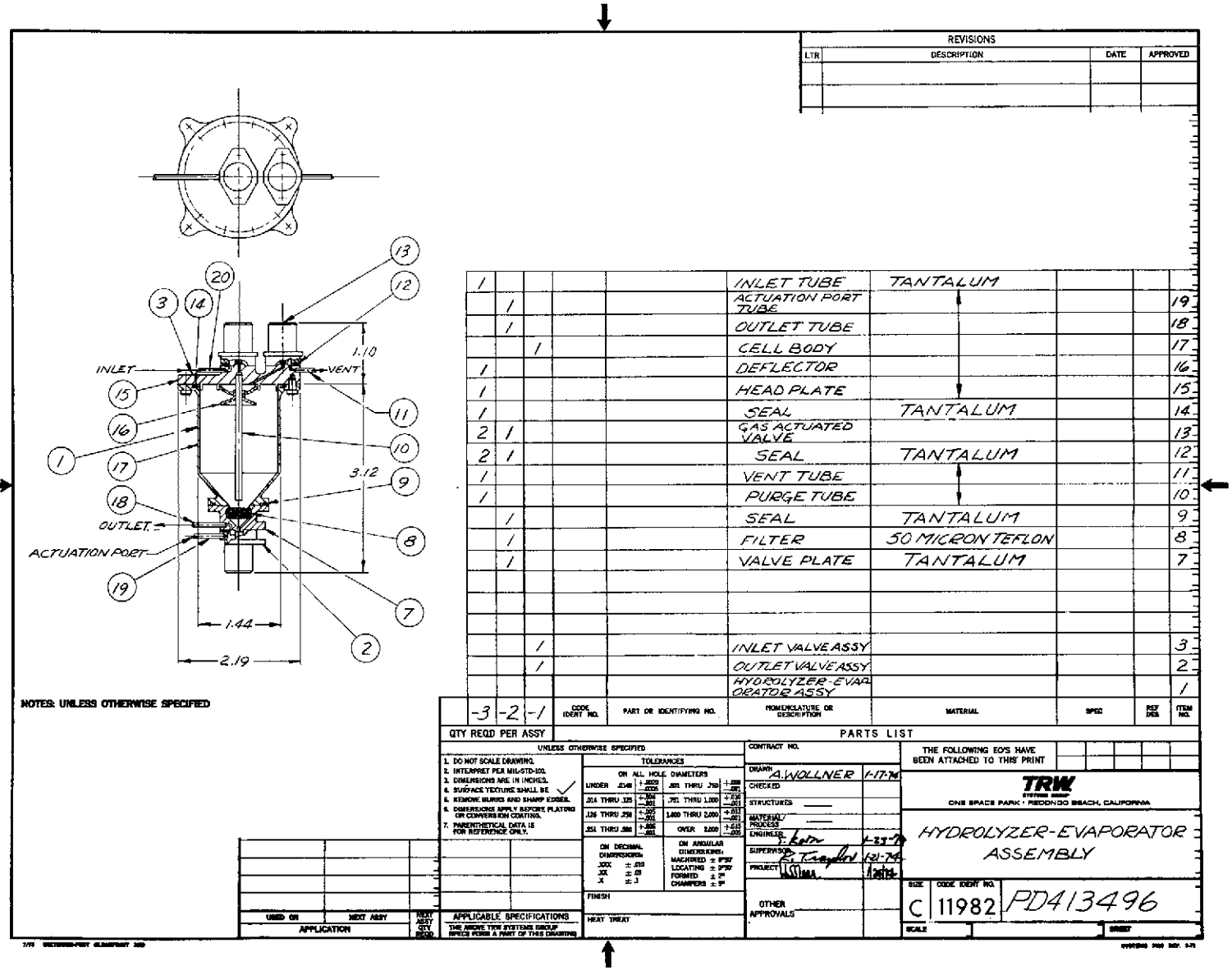


Figure 4-10. Hydrolyzer/Evaporator Assembly

will be investigated. There is a possibility to eliminate a filter altogether with the new baseline process since no precipitate might be formed in the hydrolyzer/evaporator. Further tests are proposed to investigate this.

An external heater and temperature sensor (not shown in Figure 4-10) are provided for temperature control.

4.3.2.3 Derivatizer

Figure 4-11 presents the layout of the derivatizer, which is a flight weight version of the breadboard design (described in Section 3.1.5). The derivatizer is an all tantalum cell with flanged tantalum head end. Major modifications to the breadboard version are in the detailed design of the head end which has three gas actuated tantalum diaphragm valves in the vent line, inlet, and outlet to the GC column. Because the reagent volumes in the new baseline process are smaller than in the process used in the breadboard, the volume of the flight design derivatizer can be reduced, pending the outcome of further breadboard tests. As in the hydrolyzer/evaporator, the center tube is used for reagent and gas injection, and also to receive the effluent from the ion exchange column.

Contamination problems experienced with the breadboard derivatizer due to the use of elastomeric seals in the hand-operated valve simulators are minimized with the all tantalum diaphragm valves (with Teflon seats), which are sealed to the derivatizer head end with tantalum/Teflon seals.

4.3.3. Ion Exchange Column

A layout drawing of the ion exchange column (IEC) design is shown in Figure 4-12. The design is again a flight weight version of the ion exchange column used in the breadboard (described in Section 3.1.6). The IEC consists of a thin walled tantalum tube with gas actuated tantalum diaphragm valves at the inlet and outlet to hermetically seal the IEC during interplanetary cruise. The major difference to the breadboard IEC is the size of the resin bed which is 5 milliliters as compared to 30 milliliters in the breadboard. Reduction in resin volume is possible in the new baseline processing sequence because hydrolysis is carried out not on the soil but on the water extract of the soil, and because the HF/NH_4OH precipitation step is eliminated. This results in the formation

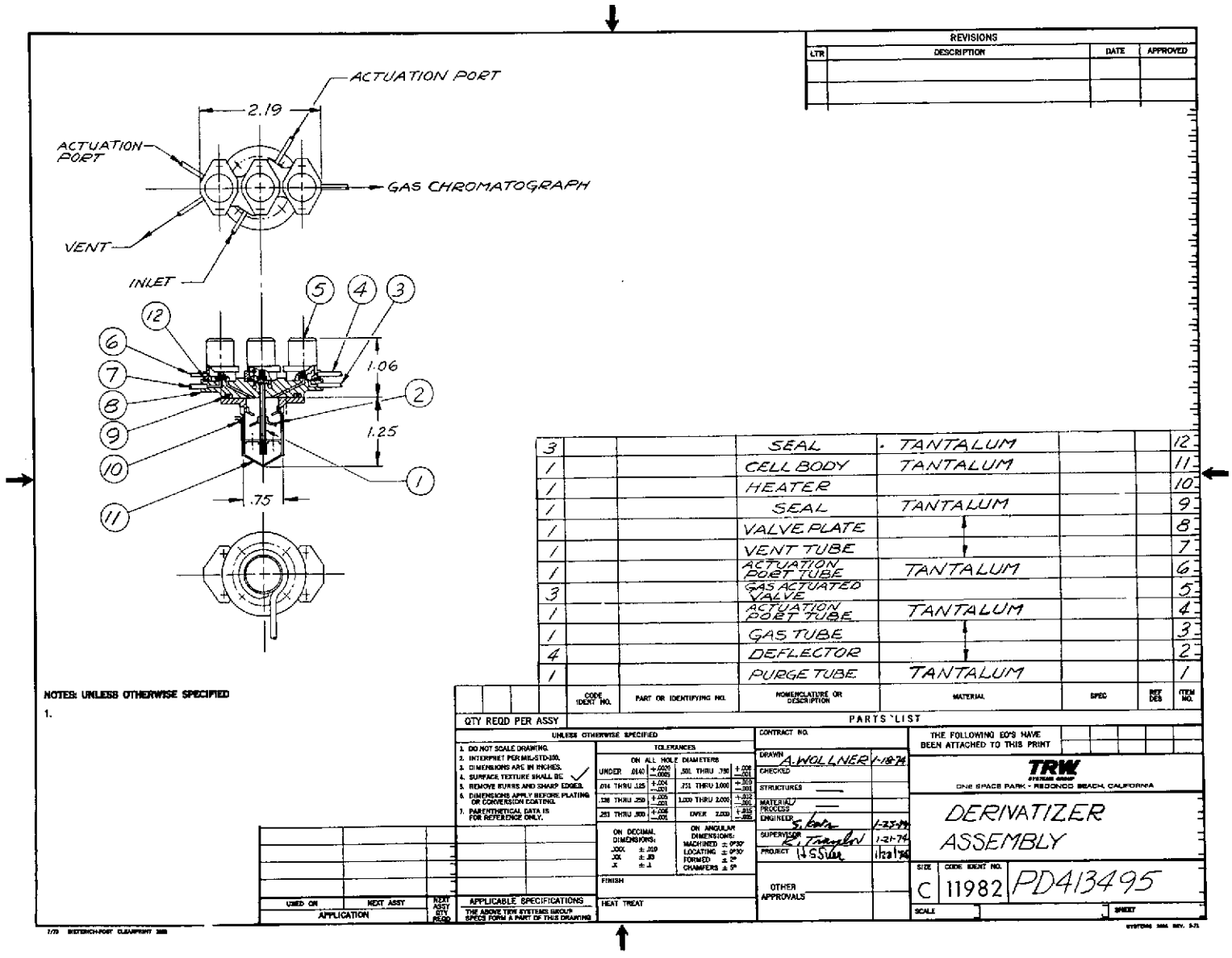


Figure 4-11. Derivatizer Assembly

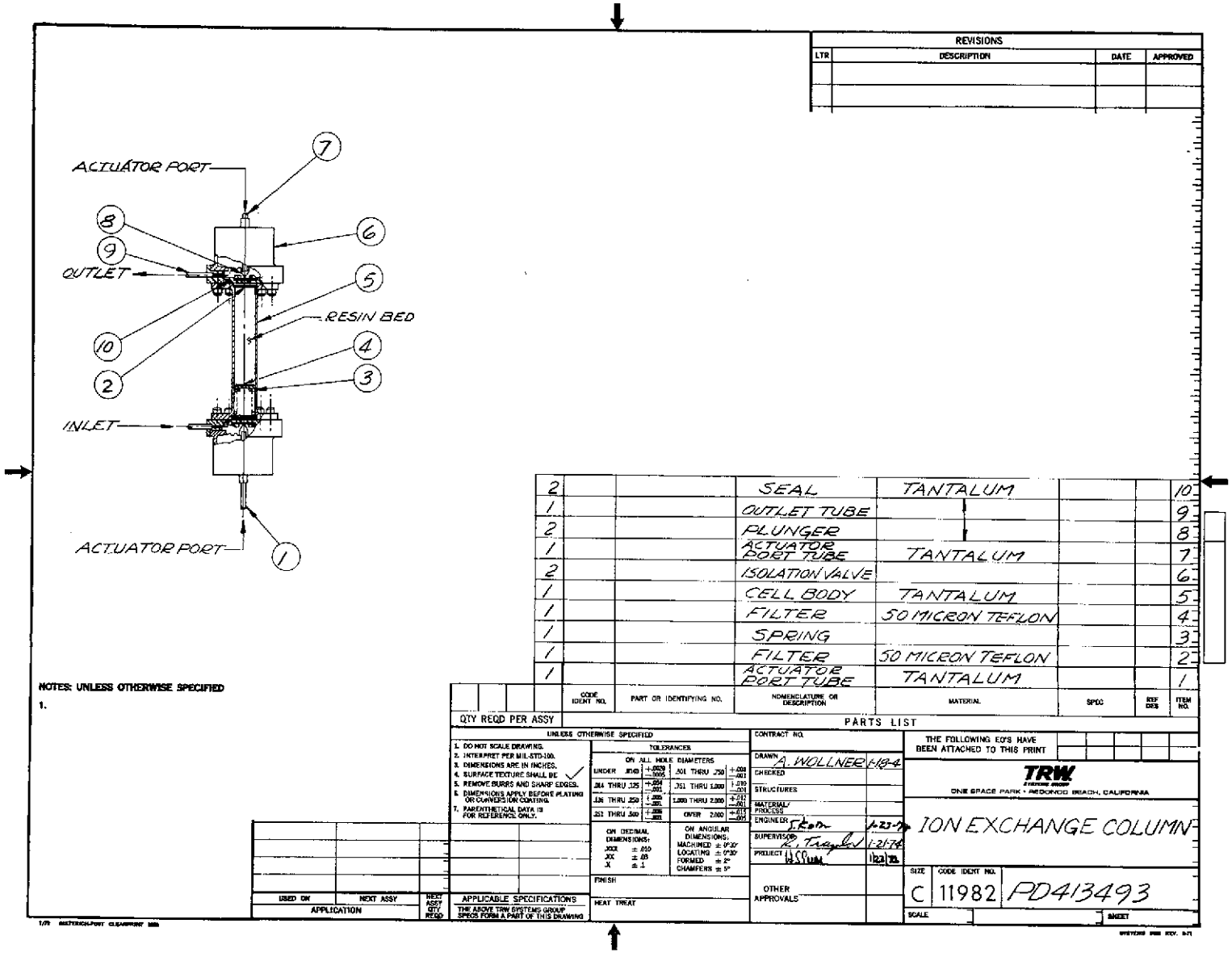


Figure 4-12. Ion Exchange Column

of a mcuh smaller amount of salt which has to be removed in the ion exchange column.

The column is packaged with Biorad AG30W-x8 (Dowex 50W), 200 to 400 mesh ion exchange resin. The resin is maintained between two Teflon filters and held under compression by a Teflon coated retainer spring in the inlet port of the IEC to minimize the dead volume in the outlet. The retainer spring also allows for expansion of the resin during column operation.

An external heater and temperature sensor are provided for temperature control during cruise (to prevent freezing of the H_2O) and operation of the column.

4.3.4 Thermoelectric Cooler/Heat Pipe Assembly

In order to provide cooling of the derivatizer test cell to below $10^{o}C$ a thermoelectric cooler (TEC) will be utilized in conjunction with a heat pipe as schematically illustrated in Figure 4-13. The proposed design is similar in concept to that employed in the VLBI instrument. Experience gained during development testing of the VLBI unit will permit the incorporation of minor design improvements to increase the efficiency of the cooler.

The cold side of the thermoelectric cooler is attached directly to the side of the test cell with a special conductive grease film coating at the interface. Heat extracted from the test cell is transmitted from the hot junction side of the TEC to the upper instrument mounting plate by means of a heat pipe. The inherent insulative properties of the TEC will prevent any significant heat loss to the heat pipe when the test cell is heated during the evaporation portion of the derivitization sequence.

Both the thermoelectric cooler and the heat pipe are static devices utilizing no moving parts. The TEC makes use of semi-conductor material properties that initially were developed to convert thermal energy to electricity. Thus by reversing the operation and, applying an electrical potential across the semiconductor couple, the action is reversed and heat is forced from one side of the couple to the other. The heat pipe which in turn conducts the heat to the spacecraft structure, by means of the Lander mounting plate, consists of a tubular structure containing a capillary-wick and a small amount of vaporizable fluid. The heat pipe employs an essentially isothermal boiling condensing cycle with the capillary wick pumping the condensate from the cool end of the "pipe" to the hot end where it is vaporized. The heat transported is thus by means of the latent heat of vaporization which is many times greater than the heat that can be transferred in a conventional conduction system resulting in a large saving in weight and volume.

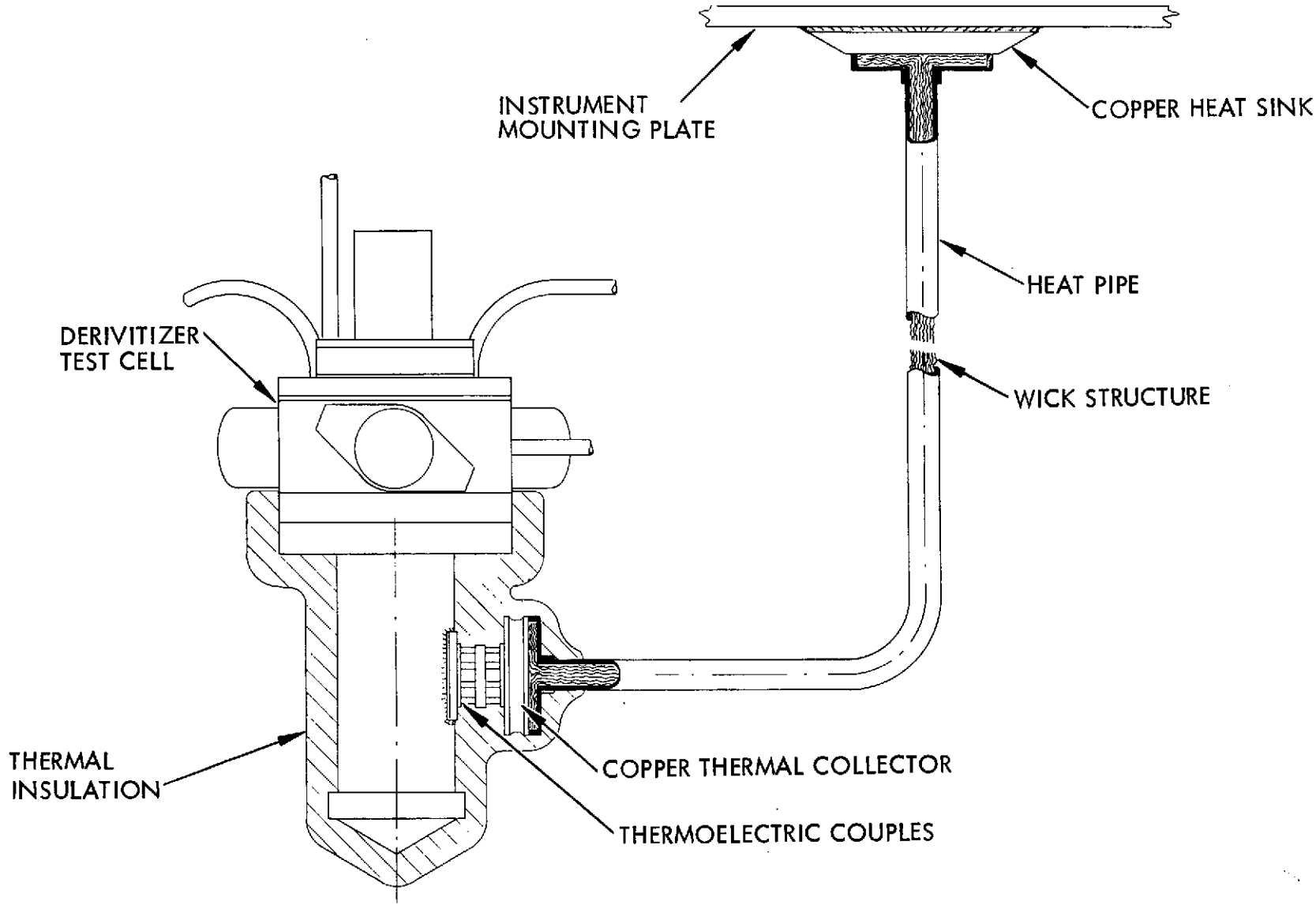


Figure 4-13. Thermoelectric Cooler/Heat Pipe Assembly

4.3.5 Gas Chromatographic Column

The gas chromatographic column consists of a stainless steel capillary tube coated internally with a liquid phase. It must be maintained below a critical temperature (approximately 25° C) for amino acid condensation during sample injection and then temperature programmed at selected slew rates and within the specified temperature tolerance of ±0.5° C.

The flight design GC column consists of 200-feet by 0.030-inch ID capillary stainless steel tube temperature programmed from 25° to 170° C. Thermal control is achieved by resistance heating of the stainless steel tube.

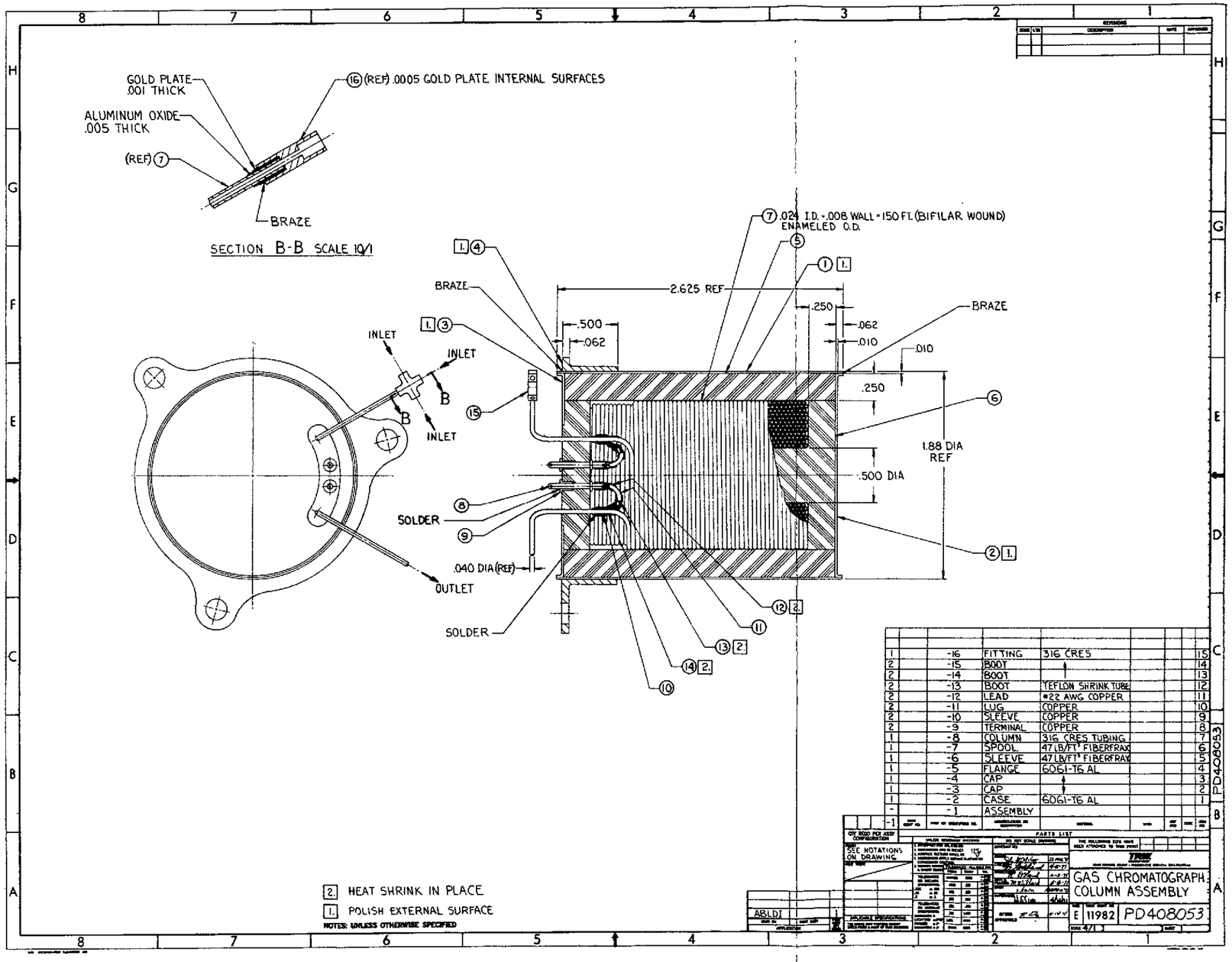
The design of the GC column shown in Figure 4-14 is the same presented in TRW's Final Report No. 16660-6001-RU-00. The dimensions shown in the GC column assembly drawings are actually based on 150 feet of 0.024-inch ID tube. The design can, of course, easily be modified to accommodate the 200 feet of 0.030-inch-diameter tube which was used in the breadboard GC column which has been described in previous sections. A detailed discussion of the design given in Section 3.3.7, page 3-199, of the previous Final Report.

4.3.6 Valves

The currently proposed valve concept is the result of our experience from the breadboard tests and of extensive valve and system studies. The initial valve concept was to use VLBI solenoid valves modified with tantalum front end for reagent compatibility. Manually operated prototype solenoid valve simulators were fabricated and tested in the breadboard. The results indicate that the final version of this valve is satisfactory for all applications except the derivatizer. There the elastomeric, dynamic poppet shaft seal caused a high contamination background in the gas chromatogram. A split ring Teflon shaft seal was evaluated but excessive leakage into the valve cavity was experienced.

It was concluded the two options exists to solve the problem:

1) Protect the magnetic components in the valve cavity with a combination of gold plating and an improved Teflon dynamic seal.



1	-16	FITTING	316 CRES		15
2	-15	BOOT			14
2	-14	BOOT			13
2	-13	BOOT	TEFLON SHRINK TUBE		12
2	-12	LEAD	#22 AWG COPPER		11
2	-11	LUG	COPPER		10
2	-10	SLEEVE	COPPER		9
2	-9	TERMINAL	COPPER		8
1	-8	COLUMN	316 CRES TUBING		7
1	-7	SPOOL	47 LB/FT³ FIBERFRAX		6
1	-6	SLEEVE	47 LB/FT³ FIBERFRAX		5
1	-5	FLANGE	6061-T6 AL		4
1	-4	CAP			3
1	-3	CAP			2
1	-2	CASE	6061-T6 AL		1
-	-1	ASSEMBLY			-

PARTS LIST

Figure 4-14. Gas Chromatographic Column Assembly Drawing

2) Isolate the magnetic components in the valve cavity with a Teflon or metallic diaphragm or bellows.

The first option, in the case of the wet chemistry instrument, does not present a completely satisfactory solution because contamination of the reagents or sample due to even minor reactions cannot be tolerated. In addition, the reliability of valve operation is adversely affected to a significant degree by the probability of plating imperfections, resulting in exposure of the magnetic material and the subsequent generation of corrosion products which can readily impair actuation of the valve. The only reliable solution to this problem is to isolate the reagents from the magnetic components of the solenoid actuator, and the tantalum diaphragm valve concept was adopted for further design studies. The fabrication techniques for forming diaphragms are well founded and only minimal development is anticipated.

Actuation of the diaphragm will require significantly higher forces than are available with standard solenoids due to the relatively large effective areas of this device when pressurized. Consequently it is proposed to actuate the valve remotely by pneumatic pressure using small solenoid pilot valves. A schematic layout of the proposed valve is shown in Figure 4-15. Various methods will be studied to implement the alternate pressurization and depressurization required to effect on-off operation. In lieu of utilizing two valves on each diaphragm assembly, one to pressurize and one to vent, a scheme similar to that used on the VLBI vertical actuators is planned. As shown in Figure 4-16 each individual diaphragm assembly is controlled by its own valve in conjunction with either the master gas control valve to open or the master vent valve to effect closure. Thus only two extra valves are required per system.

The proposed approach is to use the current VLBI solenoid valve for gas control functions and as a pilot valve for suppling gas to actuate the tantalum diaphragm valve, which offers total isolation of the solenoid valve components from the sample and corrosive reagents.

To prove that the proposed design approach is reliable and fulfills all requirements TRW has proposed to NASA/ARC to build prototypes of gas actuated tantalum diaphragm valves and test them during the next phase of breadboard testing with the new baseline process. In addition,

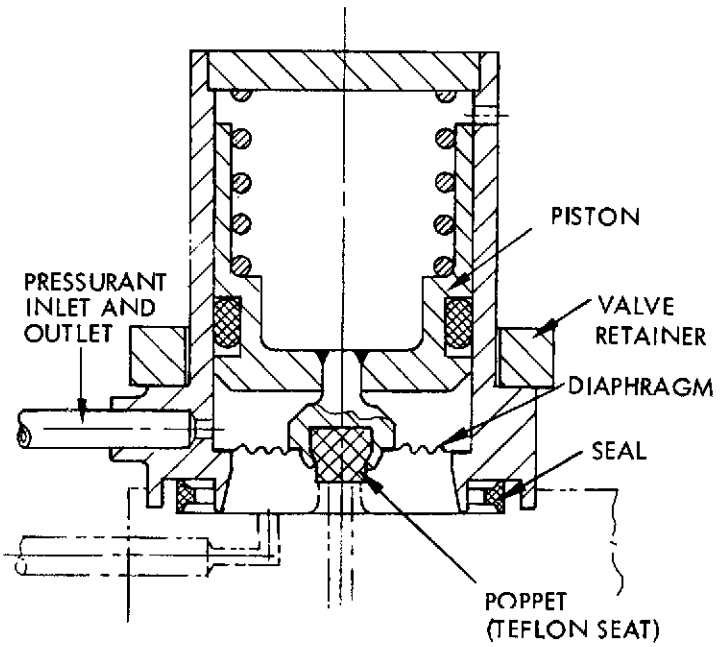


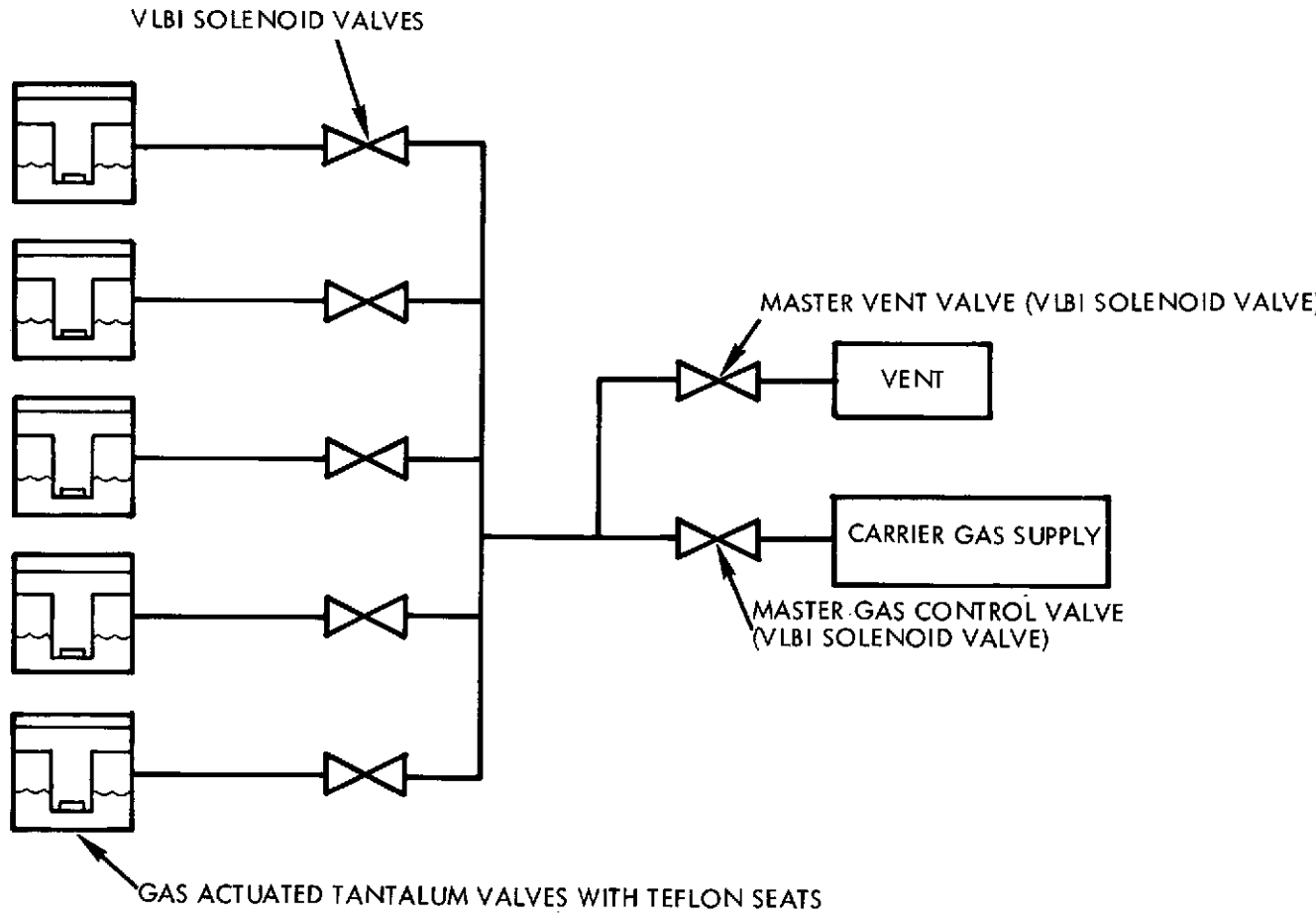
Figure 4-15. Proposed Tantalum Diaphragm Valve Schematic

various other methods should be studied, including bellows isolation members with both direct solenoid actuation and remote pneumatic actuation.

4.3.7 Reagent Storage and Injection

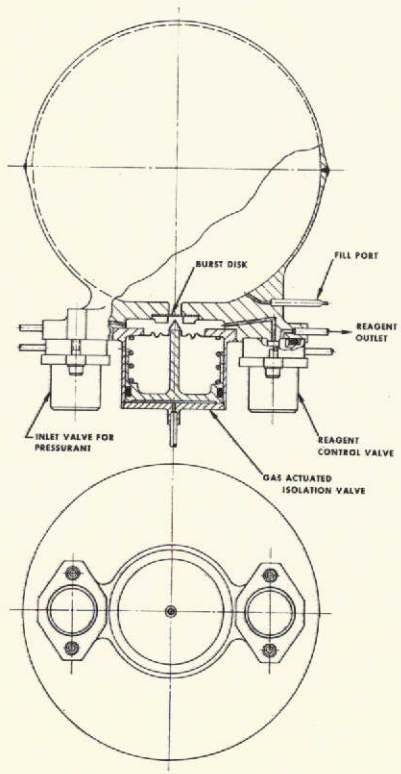
It is currently planned to store the reagents in compatible metallic containers. The layout of the proposed reagent injector is shown in Figure 4-17. A gas actuated isolation valve is used to provide hermetic sealing of the container. After the frangible disk at the outlet has been punctured, the outlet is used for both pressurization and flow of the reagent to the processing cell. The amount of reagent delivered to the cell is determined by the control valve open time.

This design is a simplification of the concept used on VLBI, where insertable glass containers are used for nutrient storage. The disassembled VLBI nutrient injector is also shown in Figure 17. If long term compatibility of the reagents in metallic containers proves to be a problem, use of the VLBI type reagent injectors with glass ampoules



- INDIVIDUAL TANTALUM VALVE CONTROL ACHIEVED BY PROPER SEQUENCING OF VLBI SOLENOID VALVES TO SUPPLY AND VENT PRESSURIZING GAS
- THIS CONTROL SCHEME IS IDENTICAL TO THE ONE USED FOR THE VLBI TEST CELL POSITIONING MECHANISM VERTICAL ACTUATORS

Figure 4-16. Valve Actuation Diagram



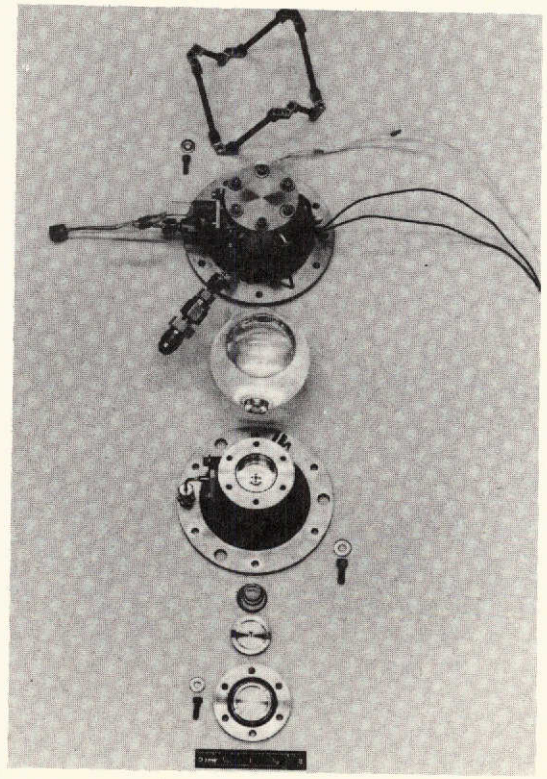


Figure 4-17. Reagent Storage and Injection System and VLBI Nutrient Injector

may be required. Long term compatibility test are proposed to evaluate whether reagent contamination or gas evolution results from reagent reaction with metallic containers.

4.3.8 Gas Supply

The gas supply consists of two subsystems supplying oxygen to the flame ionization detector; high pressure He/H_2 (165 psia) to pressurize all gas activated tantalum diaphragm valves, gas actuated isolation valves on the reagent containers and ion exchange column, and the gas actuated extractor cover plate; and finely regulated low pressure He/H_2 (18 psia) for the gas chromatographic column and flame ionization detector, and for the pressurization of the processing cells, for purging and gas drying, and for fluid routing and reagent injection.

The O_2 supply system and the He/H_2 carrier gas supply system each contain the following major components:

- Pressure vessel
- Isolation valve
- Filter
- Pressure regulator
- Fill valve
- Relief valve

The He/H_2 carrier gas and O_2 supply system design is identical to that for the VLBI instrument, with the exception of tank capacities and materials, and the use of two regulators instead of three. The VLBI carrier gas supply is shown in Figure 4-18. The modular approach conserves space, minimizes weight, and reduces the number of joints, thereby reducing potential leak paths. The gas supply assembly incorporates welded joints at component interfaces to minimize leakage. Metallic diaphragms are used in lieu of moving piston seals and the use of isolation valves prevents pressurization of the system over long periods.

The first stage regulator was selected for simplicity to provide the 165 psia actuation pressure for the H_2/He system. The design incorporates a Ni-Span-C corrugated diaphragm as a sensing unit. Ni-Span-C

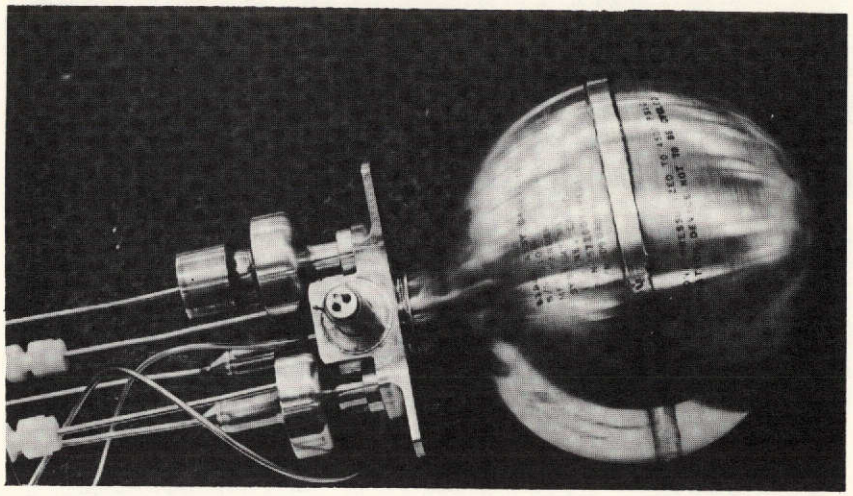


Figure 4-18. Gas Supply System

was selected to minimize thermal effects on the spring rate of the sensing unit. A spherical poppet and plastic seat make up the valve portion of the regulator. The poppet is a 1-millimeter tungsten carbide ball. The seat is polyimide, Vespel SP-1, selected to obtain the sealing properties of an elastomer combined with the stability of a hard seat.

The second stage regulator is designed similarly, but with a much higher sensitivity. Outlet pressure is closely regulated to meet the resolution requirements of the gas chromatographic column. Because of the lower pressure, the diaphragm spring rate has been reduced in order to obtain as precise a regulated flow as possible. The regulator has no sliding surfaces and requires no lubrication. The only metal-to-metal contacts are the diaphragm guide and the poppet seat. The guide maintains poppet-to-seat concentricity and supports the diaphragm assembly against lateral shock and vibration forces. Regulation is anticipated to be less than 2 percent.

The O_2 supply system uses the same first and second stage regulators to control the flow of oxygen into the flame ionization detector.

The tanks are pressurized through the fill valve in gradual stages to prevent overheating. External cooling is usually required to produce pressurization in a reasonable time and to minimize the number of fatigue cycles. Generally, convection cooling with cool air or CO_2 is effective. The fill valve consists of an AM 355 housing, tungsten carbide poppet, and

a polyimide (Vespel SP-1) seat. A piece of ground support equipment is installed on the threaded fitting using an O-ring seal. A probe is used to unseat the poppet and allow the tank to be filled. Removal of the probe results in the poppet returning to the seat by means of a follower spring. The poppet is then pressure-loaded closed as the supply pressure is decreased. Subsequently, the inlet tube is welded shut, effecting a redundant, positive seal for the long term storage. This item is in production at this time.

A test port is located between the isolation valve and the filter at the regulator inlet. This port is used to pressurize the system for functional tests. The tube is sealed by the same process as the fill tube.

The utilization of the flame ionization detector as the baseline detector has significantly affected the selection of materials from which the pressure supply tanks will be fabricated. Inconel 718, the selected material for the VLBI, although satisfactory for helium, is not considered compatible with hydrogen because of embrittlement. Hydrogen embrittlement occurs through a process identified in conjunction with high pressure vessels and is most severe at room temperature. It can be distinguished from the more common effects of stress corrosion by: (1) ionic hydrogen in interstitial solution in the metal lattice, and (2) gaseous hydrogen surrounding a nucleating and advancing crack front. These characteristics have subsequently been described as "hydrogen-environment embrittlement. Its general characteristics are:

a) The effect is one of embrittlement and only when in a hydrogen environment.

b) It is a surface effect.

c) It is an immediate effect.

d) It is a function of hydrogen pressure.

e) The effect is not increased by temperature.

The combination of these effects is to embrittle the exposed surface to a limited-finite thickness. This surface layer cannot, as a consequence, under plastic deformation to the same degree as it can in air. For a

susceptible metal, an existing crack or one formed through pressurization in an hydrogen environment will propogate at a lower stress level and at a more rapid rate than in an inert environment, even at low pressures below 14.7 psia. Test results of studies conducted to investigate these effects on typical pressure vessel materials has resulted in the selection of A-286, a high strength corrosion resistant steel as the material from which the tanks will be fabricated. Low cycle fatigue and notch impact test results indicate that the strength ratio of A-286 when exposed to helium versus hydrogen is 1.0. For comparison, Inconel 718 has a He/H_2 ratio of 0.46 for the notched strength test and 0.04 for the fatigue cycle test.

Inconel 718 has been selected for use in fabricating the oxygen tank based on compatibility studies and results from programs such as Apollo. Thus, this tank will be identical to that used on the VLBI.

The VLBI tanks have been sized to hold in excess of 80,000 scc's at 4500 psi. The tanks also serve as structural supports for the other components in the gas supply subsystem. The use of welded joints and the isolation valve in the supply line will insure a long-term leak-tight assembly.

4.3.9 Gas Chromatographic Column Detector

The baseline detector proposed for the flight instrument is still the flight version of a hydrogen flame ionization detector (HYFID) developed under NASA Contract NAS2-5469. TRW has conducted tests with a protoptype unit under simulated flight instrument conditions to establish and verify operating parameters for satisfactory performance. The results of these tests and a detailed description of the design are incorporated in TRW's Final Report No. 16660-6001-R0-00. No new information on the HYFID detector has been accumulated since then. A drawing of the flight version of the detector is shown in Figure 4-19.

Use of a state-of-the-art electron capture is also considered and it is suggested that an electron capture detector will be evaluated with respect to sensitivity, linearity, and response to interfering substances. There are several reasons for evaluating an electron capture detector. The most important reason is that some of the interfering material

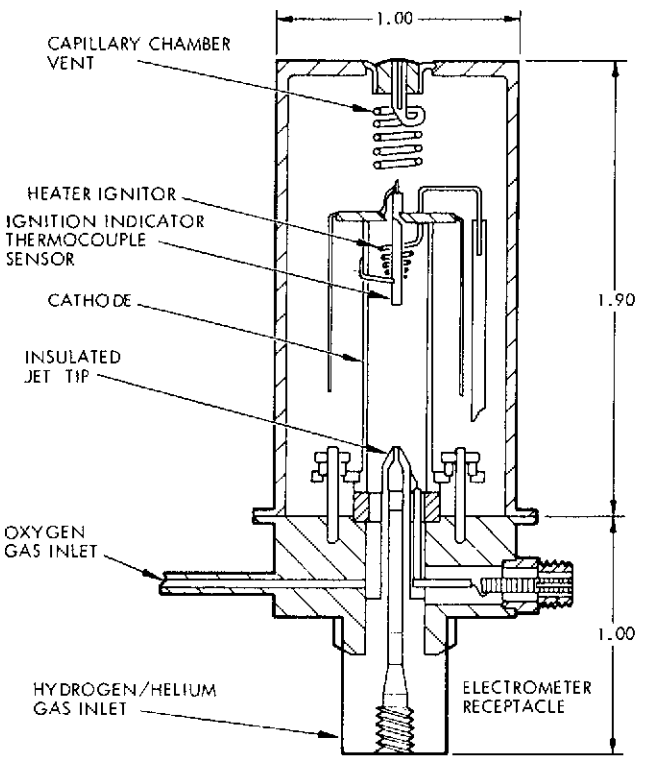


Fig. 4-19. Flame Ionization Detector

observed by flame ionization detection may be less sensitive to electron capture detection and hence an improved signal to background ratio might be obtained. Electron capture detection might also offer some design simplifications, especially if the instrument were used in conjunction with a mass spectrometer.

The use of an electron capture detector would require certain modifications to the instrument system in the following areas: The electronic subsystem needs to be modified in the front end of the analog data system to adapt to the particular detector finally chosen. The hydrogen admixture in the carrier gas would not be required and pure He could be used. The oxygen for the flame ionization detector would be replaced by the electron capture gas which would be nitrogen or an argon/methane mixture. Finally the methylene chloride would have to be replaced by ether as a solvent for the trifluoroacetic anhydride used in the derivatizer.

Another alternative which is currently under study by TRW under NASA/ARC Contract No. NAS2-7695 is incorporating the Viking 75 GCMS Mass Spectrometer into the wet chemistry instrument. Mass spectrometry of the gas chromatograph effluent would provide more definitive identification of the actual chemical composition of the amino acid derivatives identified in the gas chromatogram. A preliminary assessment of the addition of the mass spectrometer shows an approximate weight addition of 22 pounds and an additional volume requirement of 600 cubic inches. The integration of the mass spectrometer in the wet chemistry instrument is shown schematically in Figure 4-20.

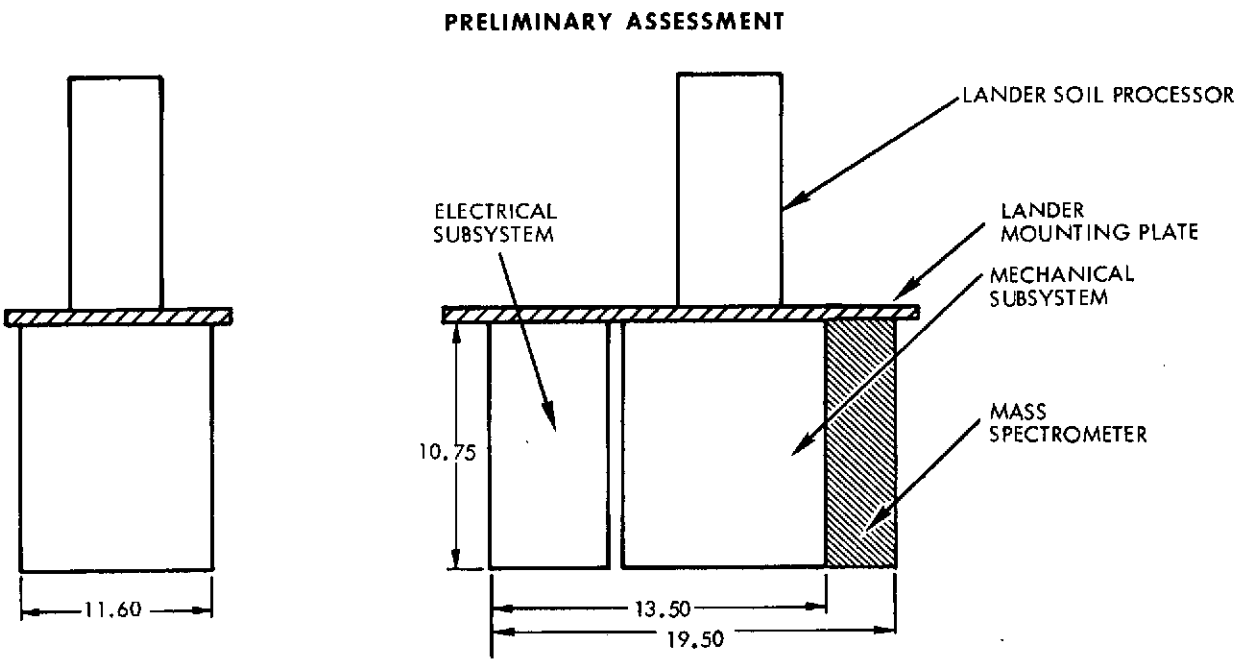


Figure 4-20. Wet Chemistry/Mass Spectrometer Integration

4.4 ELECTRONIC SUBSYSTEM

The Electronic Subsystem for the Wet Chemistry experiment has been updated to incorporate the latest Viking 75 program requirements and to conform to the updated instrument system design. The major changes to the Electronic Subsystem from our previously reported design (TRW Final Report No. 16660-6001-R0-00), include:

- Micro-programmed sequencing
- Addition of a memory
- New A/D converter design
- Improved definition of subsystem weight and power requirements.

The instrument electronic subsystem will provide the regulated power, data processing and all control functions for the instrument operation. The subsystem design is based on flight-proven designs and design techniques.

A block diagram of the main elements which compose the electronic subsystem is shown in Figure 4-21. Digital techniques are used extensively to eliminate the parameter drift problems associated with analog circuitry. These techniques allow the subsystem to be packaged in the allotted volume using conventional packaging methods. To make effective multiple use of functions, some functions are time-shared to minimize the parts count.

The command processor receives, decodes, and distributes commands from the lander. The commands are stored in the instrument memory and relayed to Earth for command verification.

The sequencer directs the conduct of the instrument and the collection of data in accordance with a pre-established routine as modified by commands and on-board instrument redirection.

All scientific and engineering data are analog by nature and are processed by a 10-bit analog-to-digital converter, formatted and stored in the instrument memory for subsequent transfer to Earth via the lander data handling and telemetry system.

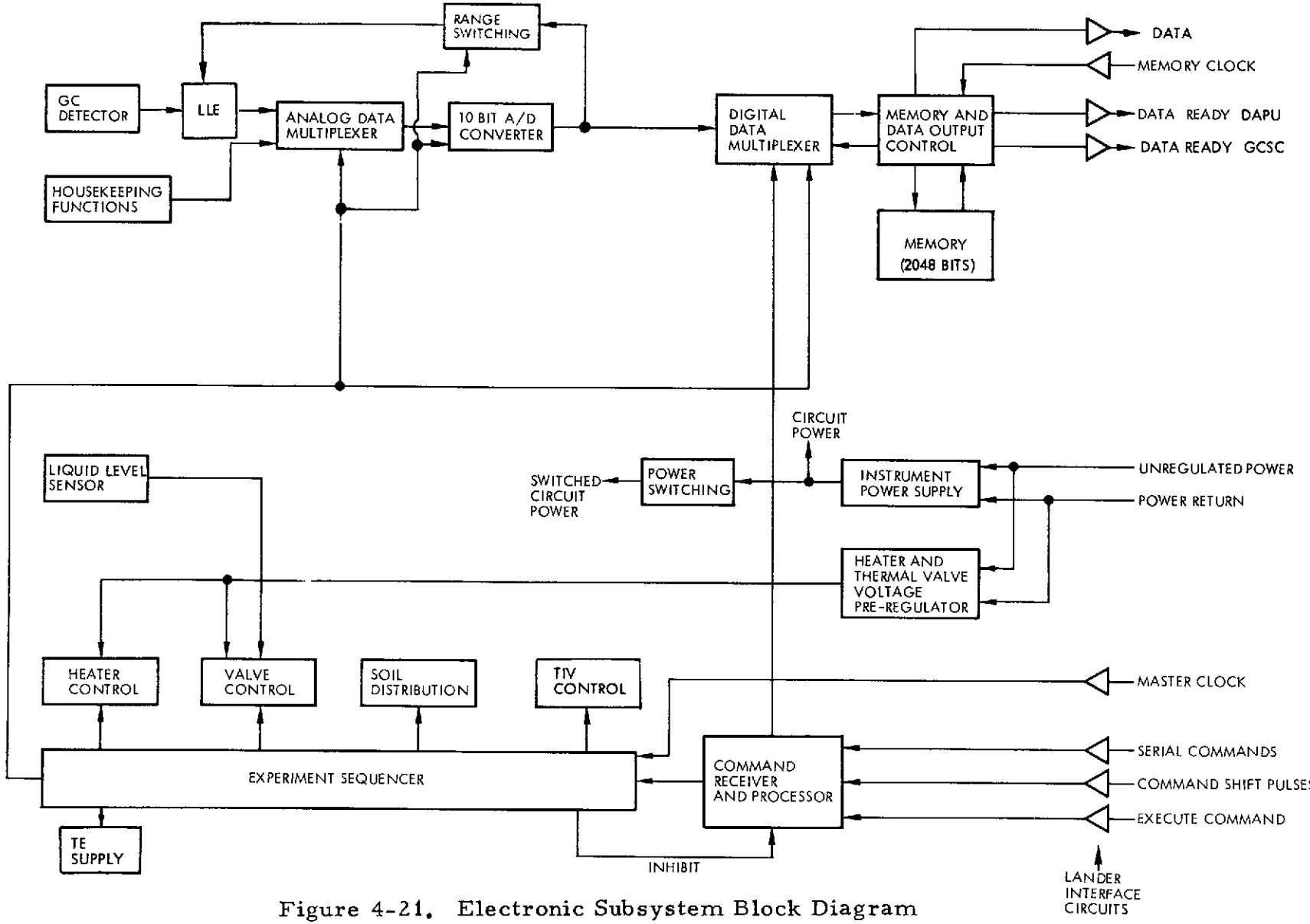


Figure 4-21. Electronic Subsystem Block Diagram

Digital data generated by the instrument along with commands from the Guidance Control and Sequencing Computer (GCSC) will be stored in a solid state buffer memory. The storage capacity will be minimized to reduce power consumption but without necessitating real time transfer to the lander Data Acquisition and Processor Unit (DAPU).

Extensive use of power switching on low duty cycle circuits is used to reduce average power consumed by the instrument. Also, system heaters and valves are operated directly off of the raw bus to eliminate conversion efficiency losses. The electronic subsystem power is presently estimated at 4.6 watts continuous.

4.4.1 Command Processing

Commands issued to the instrument from the GCSC have a serial 24-bit word structure. The commands, which are shifted into the instrument with a GCSC clock, are bracketed by a Command Enable signal. The rise of the Command Enable is used to power up the Command Processor and the falling edge triggers the command decoding circuitry.

Upon activation of the decoding circuitry, the processor checks the last 12 bits of the command for odd parity (the first 12 bits are discarded). If the command passes parity check, the command is executed and is stored in the memory. If the parity test is not passed, the instrument loads the command directly into the memory without executing it. A simplified block diagram of the Command Processor is shown in Figure 4-22.

The required instrument commands that have been identified are listed below. The Command Processor is not limited to the following list and can be easily expanded to meet any requirement.

Command Number	Command Function
1	Dump Memory
2	Activate Thermal IV's
3	Distribute Soil
4	Access Sequencer
5	Analysis No. 2
6	Analysis No. 3
7	Flight Status Activation

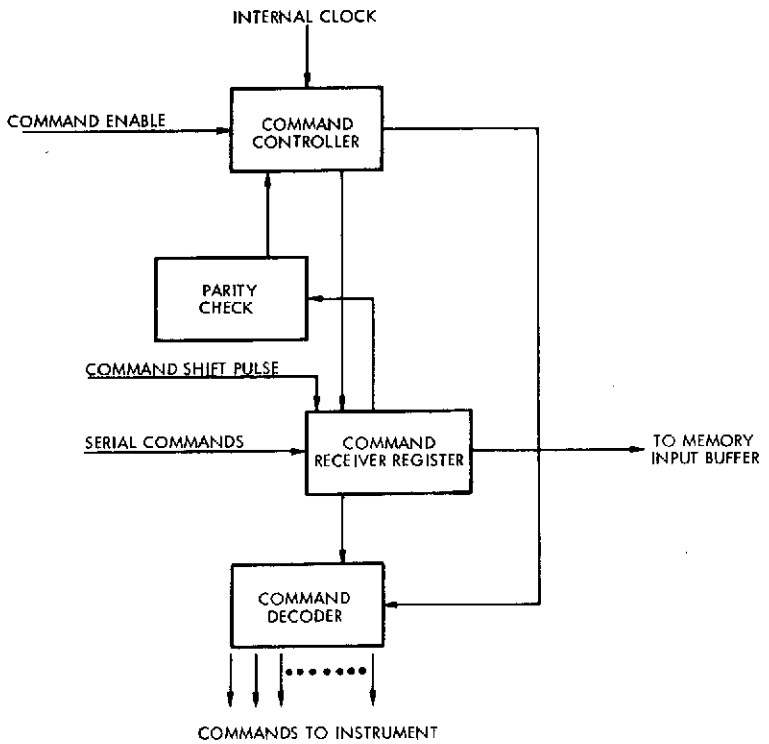


Figure 4-22. Serial Command Processor Block Diagram

Commands 1, 2 and 3 are self-explanatory. Command 4 allows access to any step within the sequence thus providing commandable sequence changes. The fifth and sixth commands identify which analysis is to be run with the first analysis as the default mode. Command 7 is used to insure all latching relays and valves are in the correct flight configuration.

4.4.2 Memory

The instrument will contain a solid-state MOS buffer memory capable of storing 2048 bits of data. The memory size (identical to VLBI) represents a reasonable compromise between instrument power and lander data rate compatibility. With the instrument running at its maximum data taking mode, a data transfer to the DAPU will only be required approximately once every 1.5 minutes. Although the transfer rate is well within the capability of the present lander, the instrument memory size can easily be expanded or contracted if required by 79' mission constraints.

The memory is organized into 128 16-bit words. The 16-bit word structure contains 12 data bits, 3 identification bits and a parity bit. The data output formatting contains the required sync and identification words as required by the lander and is completely compatible with the DAPU hardware and software.

A block diagram of the memory is shown in Figure 4-23. Data are written into the memory when either the Command Processor or A/D Converter sends the memory an Access Request signal. When available, the memory powers up, clocks in the data at 96 Kbps, assigns the ID and parity bits and returns to a low power hold mode.

A memory read cycle will occur either by a commanded dump or automatically when the memory is 3/4 full. Data transfer is accomplished through the standardized DAPU interface system as described in the Wet Chemistry Instrument specification A-16231.

4.4.3 Instrument Sequencer

The Wet Chemistry sequencer is conceptually identical to the sequencer presently used in the Biology Instrument. The sequencer was

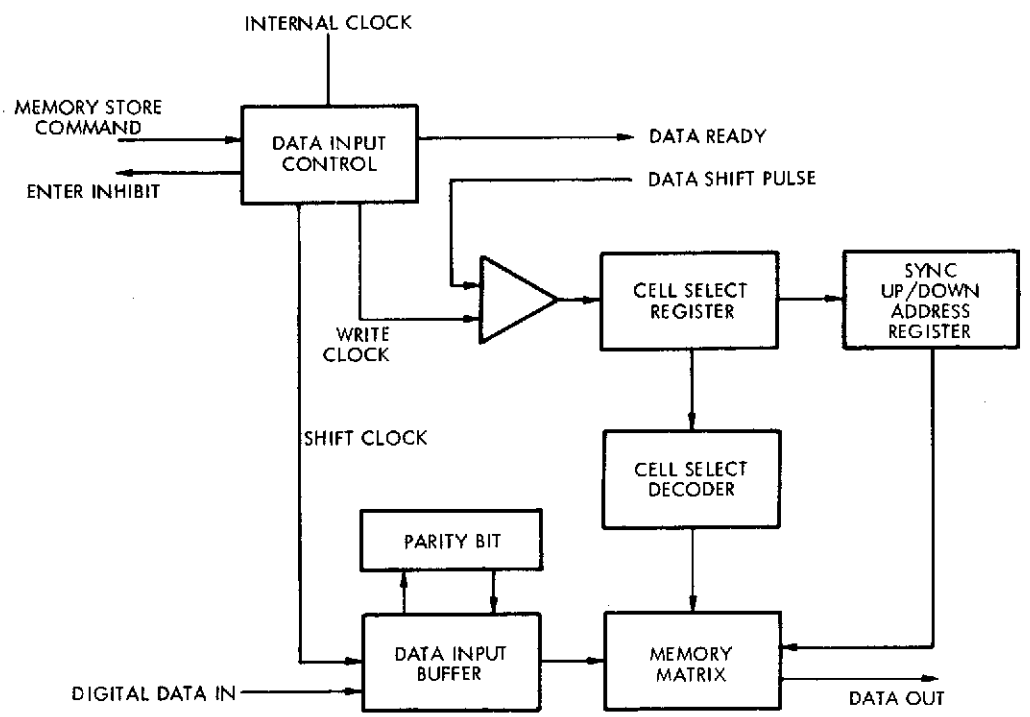


Figure 4-23. Random Access Memory Block Diagram

micro-programmed for operational control. The sequencer operates synchronously with the lander master clock and thus provides time related control of all instrument functions.

The sequencer is organized in a sequential manner such that if not commanded to change, it will proceed to run the experiment on a predetermined, internally stored, time basis. It is commandable through the lander and can be made to start, stop, mark time (hold its position), change the way an experiment is to be run.

The micro-programming is performed with the use of 256 X 10 programmable read-only memories (ROM's). The sequencer contains 5 ROM's which are parallel addressed and thus provide 256 discrete program steps (words). Each program step provides 50 bits of information, which when decoded, are used to control the internal operation of the sequencer and provide event signals to the instrument. (Events are command signals produced by the sequencer which cause valve operations, heater switching, etc.) Each program step is subdivided into two micro-instructions. The first is 10-bits long (one ROM output) and is used directly to provide time for the sequencer. This instruction determines the time (in 30-second increments) between successive events and has a range of approximately 30 to 30,000 seconds. If required, provisions will be made to provide time intervals of less than 30 seconds. The second micro-instruction of each program step contains both control and event information. This instruction contains 40 bits of data and is decoded to produce the required events.

A functional block diagram of the sequencer is shown in Figure 4-24. The operation of the sequencer is as follows:

Every 30 seconds after it is activated, the sequencer turns on and compares the output of the Time ROM (first 10-bit micro-instruction) with its internal time counter. If they do not match, the sequencer updates the time counter by one bit (equal to 30 sec) and powers down. It then waits 30 seconds and makes another comparison repeating this process until the outputs do match. When the outputs do match, the second micro-instruction in that program step is decoded and the event commands are sent to the rest of the system. The sequencer then clears the time counter and updates

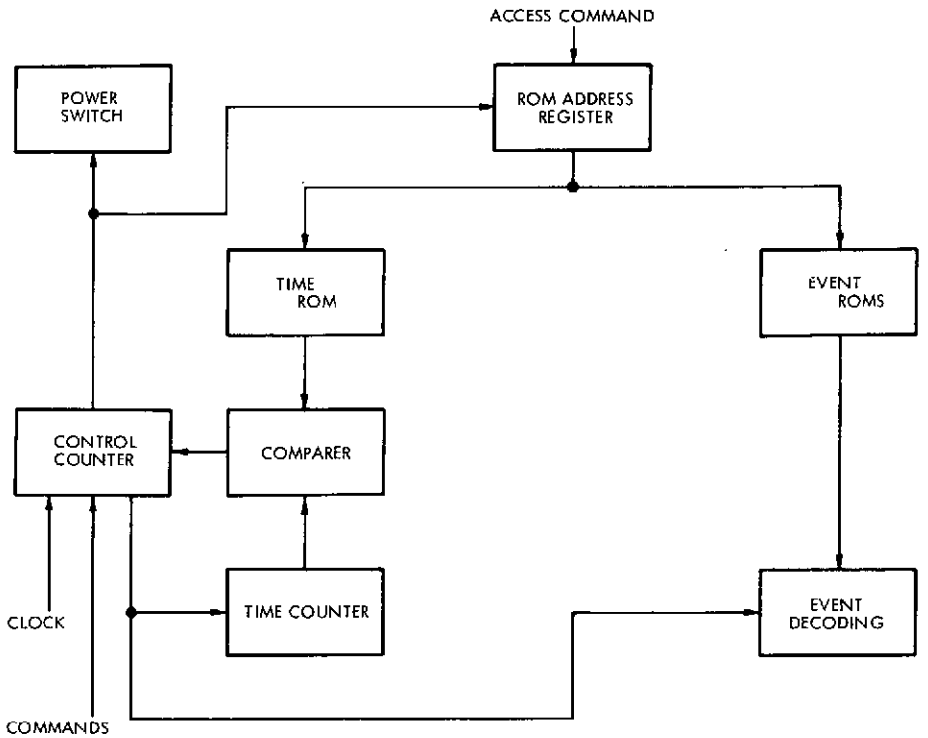


Figure 4-24. Experiment Sequencer Block Diagram

the ROM address register thus providing access to the next sequential program step. It then powers down and waits for the next 30-second period before it repeats the process. At the end of the experiment, a self-contained disable command is decoded and the sequencer stops.

4.4.4 Heater Control

The system contains 16 heaters which will be controlled by two different methods. Eleven of the heaters control to $10^{\circ}C$ or $20^{\circ}C$, respectively, and exact temperature control is not required. These heaters will be controlled by mechanical thermostats which will be mounted to the controlled surface. If the control range is determined to be more critical or if the physical size of the thermostat makes mounting prohibitive (as may be the case with the H-13 line heater), an electronic thermostat will be used. The electronic thermostat (presently used on VLBI) is extremely efficient (≈ 90 percent) and provides a control capability of $\pm 1^{\circ}C$.

The remaining five heaters (H-1, H-2, H-4, H-5 and H-6) will be powered by pulse width modulated (PWM) heater controllers. This method of control is very efficient for high power heaters and provides close control. A functional block diagram of the PWM controller is shown in Figure 4-25. A platinum sensor is used to provide temperature feedback information to the controller error amplifier. The error amp output is then amplified by a gain control buffer and fed into a comparator. The amplified error signal is then compared with a ramp voltage generated by the heater preregulator. The heater preregulator is required because of the wide variation in lander bus voltage (24 to 37 volts). The circuit produces a PWM signal which is proportional to the bus voltage. The comparator output controls the heater driver stage through an optically coupled isolator. The isolator is required because of lander grounding isolation constraints. The gas chromatographic column heater (H-5) also will require profile control. This will be accomplished by a 10-bit digital ramp control counter which will be fed into the gain control buffer as an error signal. The ramp generator will provide a step resolution of approximately $0.2^{\circ}C$.

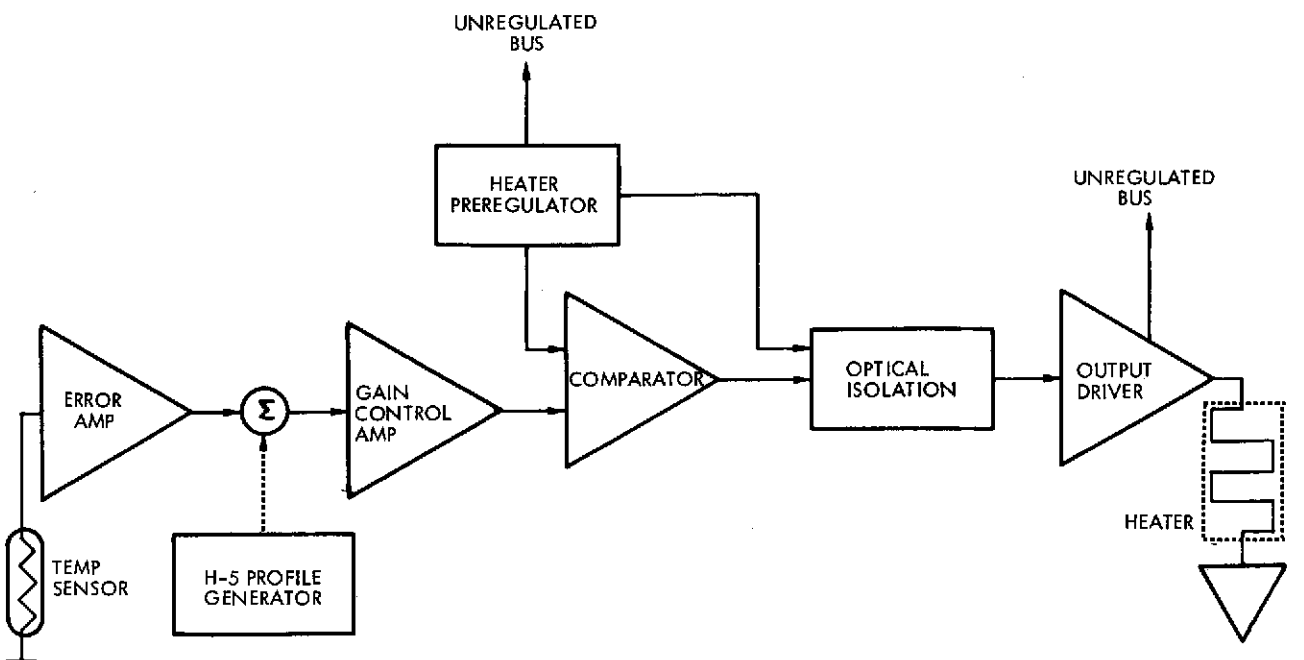


Figure 4-25. PWM Heater Controller

4.4.5 Solenoid Valve and Soil Distribution Control

The instrument's solenoid valves for control of the gas actuated control valves are controlled by a 6 by 7 relay matrix. The matrix can control 42 valves. The matrix is controlled by the instrument sequencer which provides the column and row signals which actuate the selected relays and thus the selected valves. Relays are used in the matrix instead of transistors because their inherent isolation capability allows us to drive the valves directly from the lander bus resulting in a significant power savings.

The soil distribution controller is a simple four-phase stepper motor driver. Upon command from the sequencer, the controller drives the stepper motor at a 40 Hz stepping rate until it receives a reverse signal from the soil distribution assembly. It then reverses the phasing of the drive pulses to the motor and returns it to its starting position. The feedback position signals both from the reversal and stopping position are provided by photodiode-phototransistor pairs.

4.4.6 Liquid Level Sensors

The time required for the evaporator effluent cannot be forecast with any reasonable accuracy. For this reason a liquid level sensor is installed in the line between each evaporator and ion exchange column.

The sensor is a conductivity detector consisting of a pair of closely spaced electrodes in the liquid path. A layout design of the liquid sensor is presented in Figure 4-26. The conductance ranges from approximately 10^{-4} mhos for wash water to 10^{-2} mhos for the HF-LiOH effluent. Even with wet walls in the detector, the conductivity will be several orders of magnitude less than that of water in the absence of a liquid stream. This allows us to use simple conductance comparator circuits which control the vent valves which in turn allow passage of the effluent into the ion exchange column.

4.4.7 Analog Data Processing

Two kinds of data are produced by the instrument in the form of analog voltages. These data are derived from the gas chromatography low level electronics and the housekeeping functions. The housekeeping

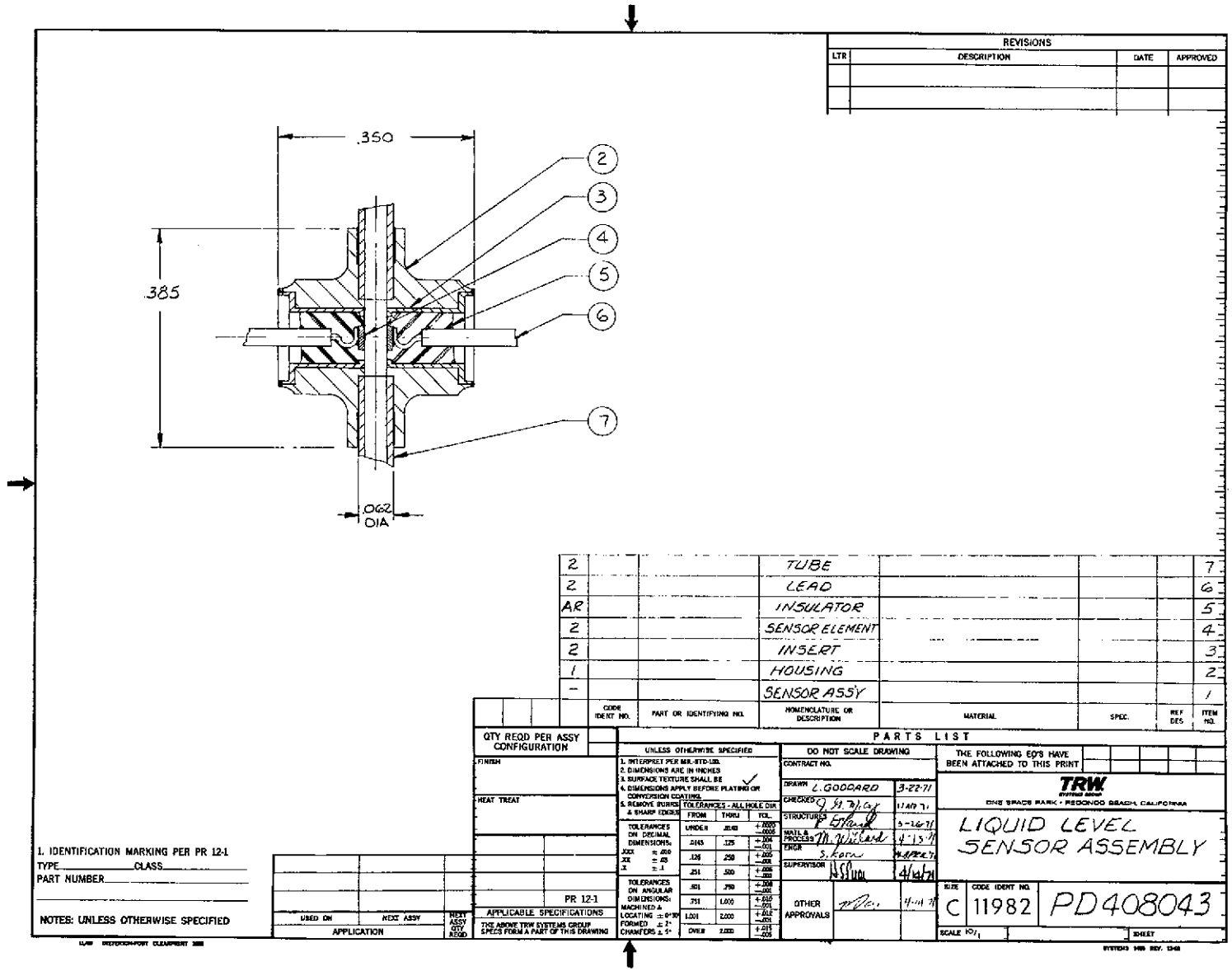


Figure 4-26. Liquid Level Sensor Assembly

functions such as the data from the pressure transducers and temperature sensors will be read out directly by the lander through the DAPU 8-bit A/D converter.

The gas chromatography low level electronics output will be read by the instruments internal 10-bit A/D converter. This conversion is done internally because the DAPU converter can not provide the accuracy and resolution required, nor can it sample the chromatogram at a fast enough rate to resolve all of the peaks unambiguously.

The Analog to Digital Converter used in the instrument will be identical to the converter used in VLBI. It is a 10-bit (i.e. 1 part in 1023) dual slope converter with an automatic zeroing correction loop. The converter has an accuracy of 0.1 percent ± 1/2 LSB and when commanded by the sequencer will sample the chromatography sensor output a at a rate of 1 per second. The output of the converter is a 12-bit digital word. Ten of the bits are used for the analog measurement and the remaining two bits are used for range switching identification. A functional block diagram of the Wet Chemistry converter is shown in Figure 4-27.

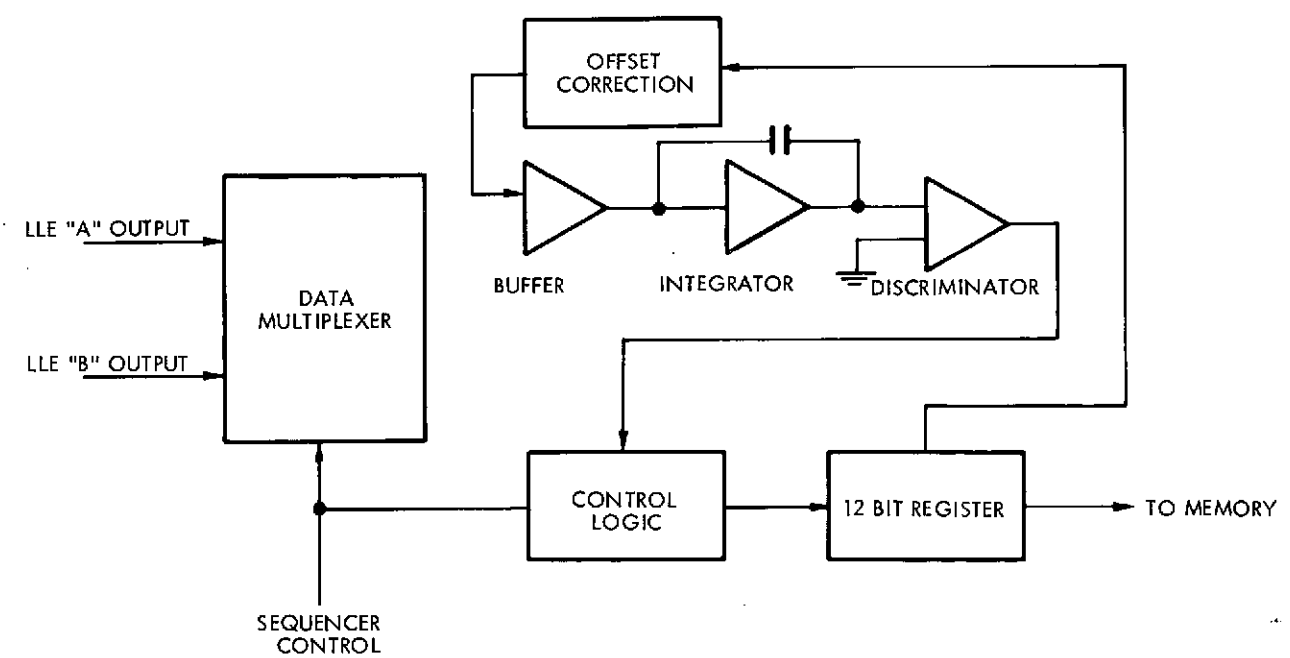


Figure 4-27. A/D Converter

The low level electronics for the gas chromatography flame ionization detector will have to be specially designed for this instrument. The electrometer will have to be sensitive down to the 10^{-13} ampere range just to reach a signal to noise ratio of 1. The electrometer must also have a range of 6 decades which will be handled by automatic gain switching.

Figures 4-28 and 4-29 show a basic design configuration which would be used in the instrument. It uses an LM108A op-amp whose input characteristics are improved by a FET source-follower pair. The electrometer connection, with current feedback to the inverting input, eliminates the effect of stray input and ionization chamber capacities on performance; it also makes cable and input connector leakages non-critical. Since the FET input resistance is 10^{11} ohms, the loop gain almost equals the op-amp gain and linearity is better than 0.01 percent. Common-mode rejection of supply variations and input voltage drift are both guaranteed by the use of current source biasing by another FET at the zero-temperature-coefficient point. The crucial parameter in an electrometer circuit is the input bias current drift; the input circuit has been designed to minimize it.

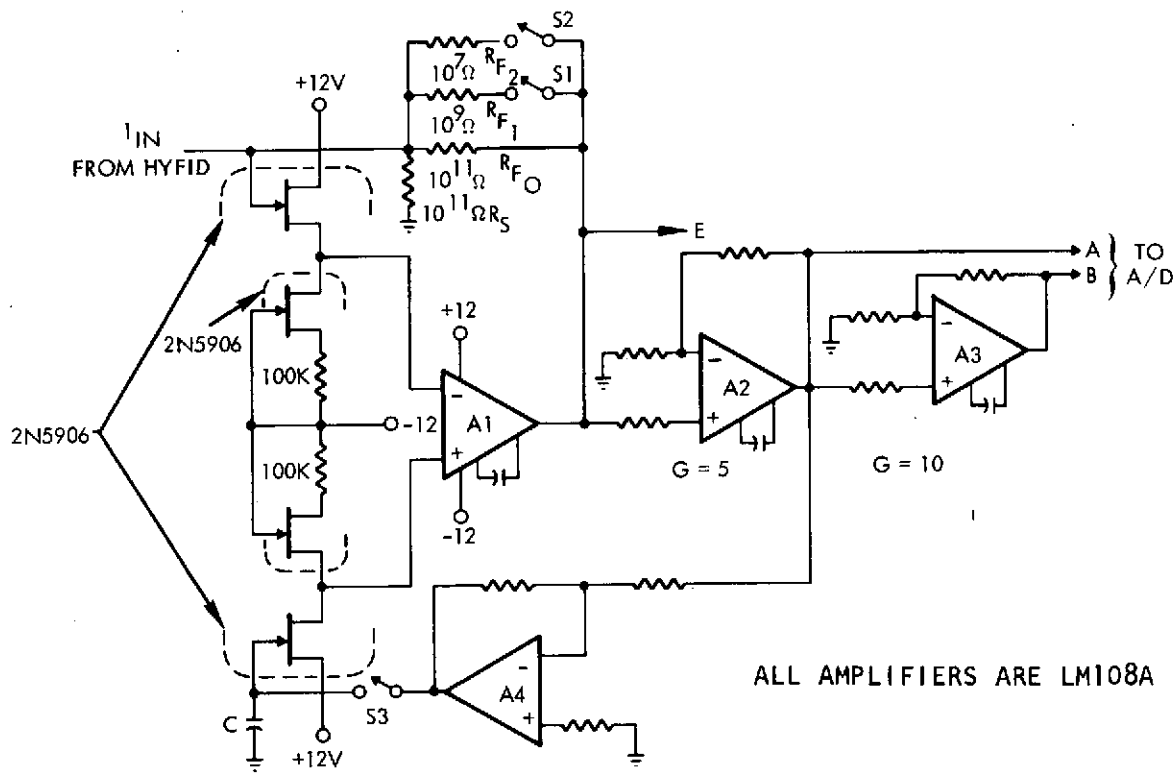


Figure 4-28. Wide Dynamic Range Electrometer

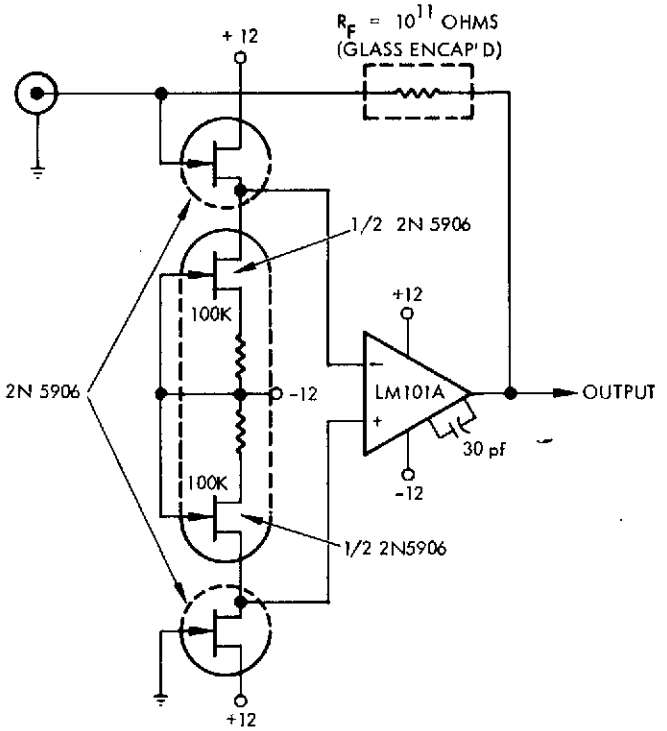


Figure 4-29. Basic Electrometer Circuit

4.4.8 Power Conditioning and Distribution

The Wet Chemistry Instrument contains a primary power supply to provide the regulated bias voltages required by the instrument and a separate thermal electric power supply. The thermal electric cooler requires a separate supply because the power it requires (≈ 1.3 v at 1 amp) could not be efficiently provided by the primary supply.

The primary supply for the instrument is identical to the supply used in VLBI. A block diagram of the primary supply is shown in Figure 4-30. This configuration combines a switching regulator and a dc-dc converter by pulse width modulating the drive waveform to the inverter transistors. The average voltage at each of the converter transformer secondaries is held constant to the desired precision. This configuration requires an inductance input filter for each of the output voltages. The size and weight of the magnetic components is minimized by using ferrite core material and operating at a switching frequency of approximately 30 KHz. The overall power conversion efficiency will be about 75 percent.

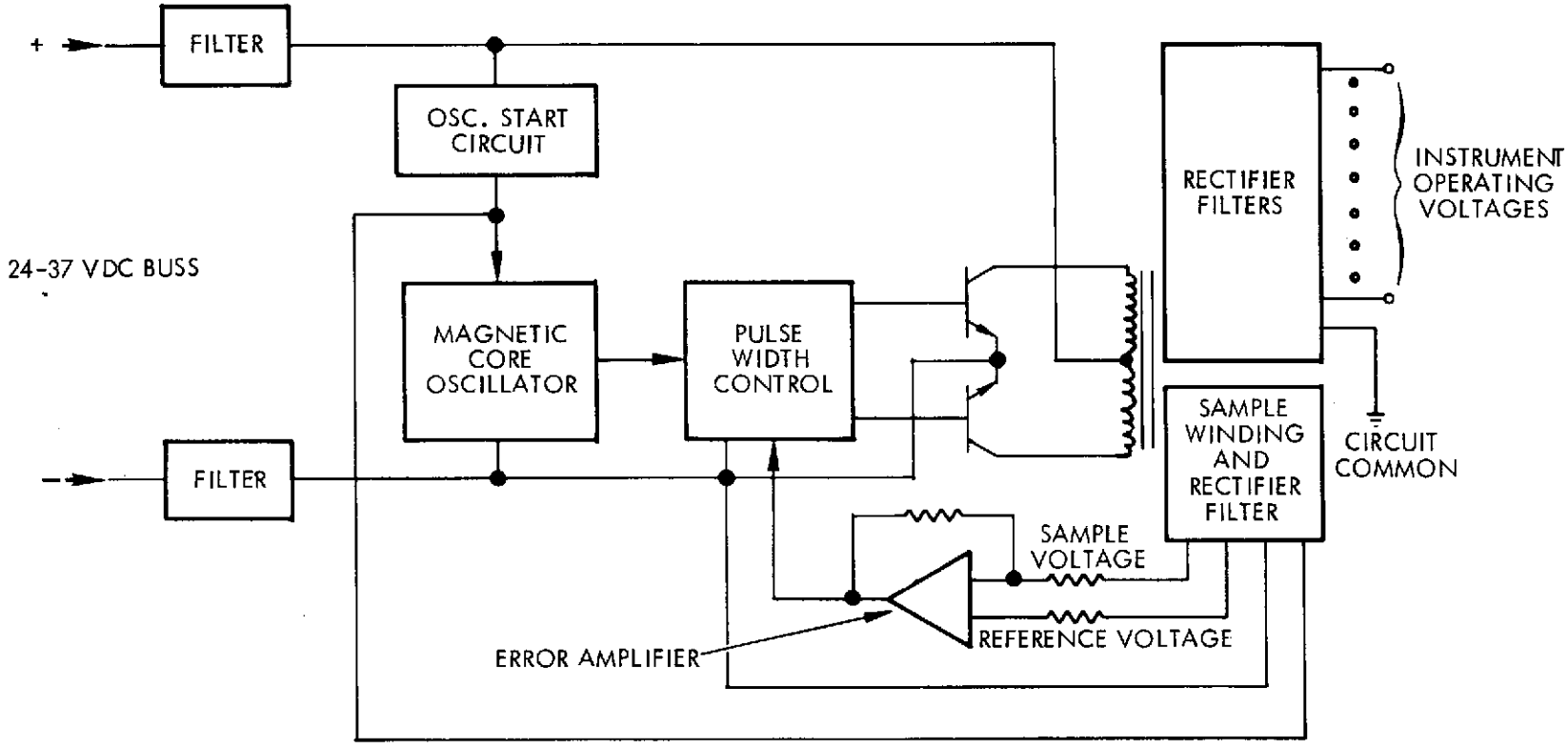


Figure 4-30. Instrument Power Supply Block Diagram

The thermal electric (TE) cooler power supply will be a modified version of the TE supply presently in VLBI. The supply combines a switching ripple regulator with a thermal control circuit. The thermal control circuit is used to sense the temperature of the derivatizer and to turn the TE supply on or off to maintain a predetermined temperature. By controlling the supply directly, we are able to reduce the efficiency losses due to external cooler power switching.

4.4.9 Mechanical Packaging Design

The mechanical design of the electronic subsystem affords low design cost, low manufacturing cost, ease of component replacement and excellent flexibility to incroporate development circuit design changes. The component to volume ratio is such that circuits may be added, changed or deleted as required during the development phase without causing a major design cost impact. There is a growth factor allowance of 10 percent for the package. The preliminary design is to have the power supply and regulating circuits on one circuit board, 60 percent of the flat packs on one board, and the signal conditioning circuits on three circuit boards. The board placement is such that one additional flat pack or signal conditioning board may be added. All circuit boards are multi-layered to reduce size and thus the weight of the instrument. The initial component count is 1160. The volume allocated to the electrical subsystem is 11.6 by 10.75 by 4.5 inches.

The packaging design will be similar to that in VLBI. The boards will be mounted in machined housings and interconnected by an ESS/MSS harness. Internal wiring between boards located in the same housing will be done by flex cable. EMI shields will be used between housing for minimizing circuit cross-talk and interference problems.

High density Cannon "Golden D" connectors will be used for internal instrument (ESS to MSS) wiring and the Viking standard connectors for lander interface.

The present estimated weight of the electronic subsystem and interconnect harness is 9 pounds.

5. CONCLUSIONS AND RECOMMENDATIONS

TRW believes that the breadboard study program reported herein has clearly demonstrated the feasibility of an automated wet chemistry instrument for the isolation and measurement of amino acids and their optical isomer ratios at the nanomole level within the constraints of the weight, power, volume and other lander interface specifications. The performance of the critical components of the design has been demonstrated with a prototype breadboard whose design duplicated as closely as practicable all aspects of the flight design which might affect the instrument capabilities, including internal cell geometry, interconnecting valves and plumbing, materials exposed to the reagents, sequencing, and vent pressures. Conceptual designs and design adaptions of Viking Lander Biology Instrument hardware have been prepared for the remaining components required to support the analysis. These include gas supplies, reagent injectors and the thermoelectric cooler/heat pipe assembly.

The breadboard testing was carried out with Process No. 1 which uses acid hydrolysis directly on the soil, rather than the new baseline Process No. 2 in which hydrolysis is performed on a water extract of the soil, but the overall processes are similar, and Process No. 2 does not have any operations that are significantly different from the operations in Process No. 1. Furthermore, Process No. 2 is significantly simpler, and certain problems did arise from interferences in Process No. 1 which will be eliminated or greatly reduced with the baseline Process No. 2. Some processing problems still remain, but we feel that although further study is required, solutions for these problems can be obtained within the context of a breadboard study program.

We recommend that to continue the orderly development of this instrument that the wet chemistry instrument breadboard fabricated and tested in this study be redesigned, refurbished and modified for optimum use with the new baseline Process No. 2, and that additional testing be carried out to solve the remaining interference problems and to demonstrate the performance of the instrument with the new baseline process.

The test program should include the following tasks: 1) laboratory tests to evaluate process modifications developed by NASA/ARC and to

establish prototype instrument operating conditions for the revised process; 2) evaluation of filter requirements and filter materials for the baseline Process No. 2; 3) identification of the source of and solution of the interference problem with the derivatizer; 4) investigation of the use of a state-of-the-art electron capture detector in place of/or in addition to the flame ionization detector, especially in regard to their relative sensitivities for interferences; 5) step-by-step evaluation of the performance of each breadboard component for its function in the process sequence, and laboratory tests in glassware for control analyses; 6) breadboard performance demonstration tests with radiotracers, NASA/ARC supplied soils and amino acid-free soils; and finally, 7) revision of the flight design concept based upon the breadboard program results.

APPENDIX

Processing Sequence No. 1 - used for all breadboard tests (from NASA/ARC Specification A-16231, Rev 1, Jan. 4, 1972).

Experiment Sequence - The following is a typical experiment sequence. The reagent volumes are given for a 1 cubic centimeter soil sample. Derivatization assumes one (1) micromole of each of the protein amino acids for the reagent volumes.

Step 1. Place a 1 cubic centimeter soil sample in a chamber.

Step 2. Add 7.5 milliliters of 6N HCl (aqueous).

Step 3. Heat to 110°C in closed chamger for 16 hours.

Step 4. Filter off the insoluble soil residue.

Step 5. Wash the soil residue with 5 milliliters of water and filter off the soil residue.

Step 6. The amino acids and dissolved salts in HCl (Combined filtrates from steps 4 and 5) are then evaporated to dryness.

Step 7. After evaporating to dryness (final heat at 100°C), dissolve the amino acids in 10 milliliters of water. (It may be necessary to heat a short time to assure solution.)

Step 8. Allow to cool to below 35°C. Add 9 milliliters of 5N HF. Mix for 5 minutes. (Gas bubbling is acceptable.)

Step 9. Add 9.2 milliliters of 5N NaOH to adjust pH to 7-11. Continue to mix for 15 minutes.

Step 10. Filter solution and place filtrate on strong cation exchange column for amino acid exchange, cation and neutral organic removal. Follow the filtrate immediately with 10 milliliters of water added to the previous chamber (Steps 6 to 9) and forced through the precipitate and onto the ion exchange column. Follow this with 30 milliliters of water directly onto the ion exchange column. Follow this with 10 milliliters of 4N NH_4OH and start collecting the amino acids when the ammonia begins to break through the ion exchange column. Collect only the first 7 milliliters. (The reagent volumes for Step 10 are given for a 30 milliliter Dowex 50 x 8 (H+) column.

Step 11. Evaporate the amino acid solution to dryness at 100°C.

Step 12. To the dried sample and 2 milliliters of (+) 2-butanol containing sufficient anhydrous HCl to make it 4N.

Step 13. Heat solution to 100°C in a closed chamber for 3 hours.

Step 14. Evaporate to dryness. Cool to below 35°C.

Step 15. To the dried sample add 0.2 milliliter trifluoroacetic anhydride and 0.2 milliliter of methylene chloride. Heat in a closed chamber for one hour at 35-40°C.

Step 16. Evaporate the solvent at a temperature below 10°C.

Step 17. The resultant sample is analyzed for composition by gas chromatography.

Processing Sequence No. 2. New baseline processing sequence adopted for the updated flight concept (from NASA/ARC Specification A-16231, Rev 3, October 15, 1973).

Experiment Sequence. The following is a typical experiment sequence. The reagent volumes are given for a 1 cubic centimeter soil sample. Derivatization assumes one (1) micromole of each of the protein amino acids for the reagent volumes.

Step 1. Place a 1 cubic centimeter soil sample in a chamber.

Step 2. Add 10 milliliters of water.

Step 3. Heat to 165 $\pm 5^{\circ}$C for 1 hour.

Step 4. Allow to cool and filter off the insoluble soil residue.

Step 5. Add 10 milliliters of 6N CHl to the filtrate from Step 4.

Step 6. Heat solution to 110°C for 5 hours.

Step 7. Evaporate to dryness.

NOTE: The following step is performed on the ion exchange column prior to proceeding to Step 9. The reagent volumes for Step 8 are given for a 5 milliliter Dowex 50M+ column.

Step 8. Place 10 milliliters of 4N NaOH on ion exchange column. Follow this with 20 milliliters of water directly onto the ion exchange column. Follow this with 15 milliliters of 6N HCl directly onto the ion exchange column. Follow this with 20 milliliters of water directly onto the ion exchange column.

Step 9. After evaporating to dryness (Step 7), dissolve the amino acids and residual salts in 5 milliliters of water. (It may be necessary to heat a short time to assure solution.)

Step 10. Place solution (Step 9) on strong cation exchange column for amino acid exchange, cation and neutral organic removal. Follow the amino acid solution immediately with 15 milliliters of water directly onto the ion exchange column. Follow this with 10 milliliters of 4N NH_4OH and start collecting the amino acids when the ammonia begins to break through the ion exchange column. Collect only the first 1 to 2 milliliters.

Step 11. Evaporate the amino acid solution to dryness at 100°C.

Step 12. To the dried sample add 0.5 milliliters of (+) 2-butanol containing sufficient anhydrous HCl to make it 4N.

Step 13. Heat solution to 100°C in a closed chamber for 2 hours.

Step 14. Evaporate to dryness. Cool to below 35°C.

Step 15. To the dried sample add 0.1 milliliters of trifluoracetic anhydride and 0.4 milliliters of methylene chloride. Heat in a closed chamber for one hour at 35-40°C.

Step 16. Evaporate the solvents at a temperature below 10°C.

Step 17. The resultant sample is analyzed for composition by gas chromatography.