FINAL REPORT

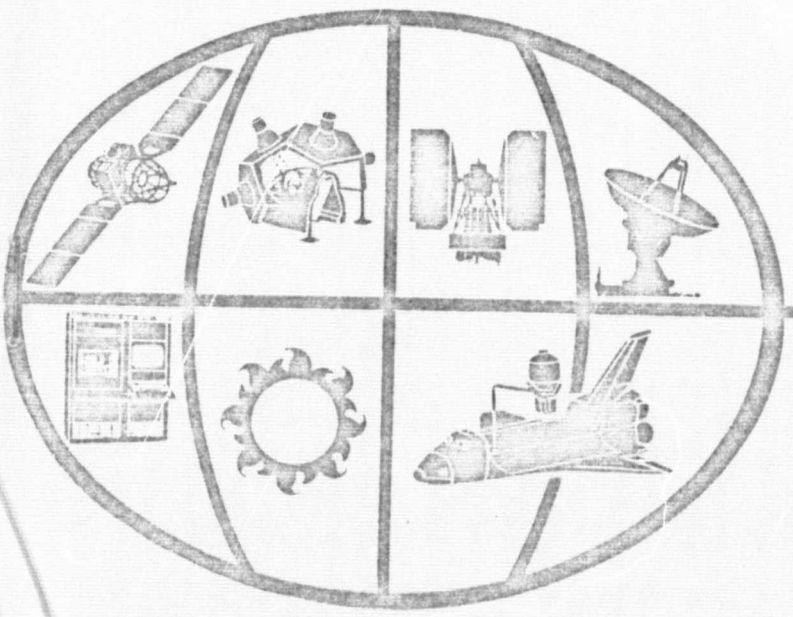
FOR

SPAR ELECTROPHORETIC SEPARATION EXPERIMENTS

CONTRACT NAS8-31036

PART II

17 JULY 1978



space division

GENERAL ELECTRIC

FINAL REPORT

FOR

SPAR ELECTROPHORETIC SEPARATION EXPERIMENTS

CONTRACT NAS8-31036

17 JULY 1978

Prepared For

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I. INTRODUCTION

The opportunity to use a sounding rocket for separation experiments is a logical continuation of earlier electrophoresis demonstrations and experiments. A free-flow electrophoresis system, developed under the Advanced Applications Flight Experiment (AAFE) program, was designed so that it would fit into a rocket payload. The SPAR program provides a unique opportunity to complete the initial stages of microgravity testing prior to any Shuttle applications.

The objective of the work described in this report was to ensure proper operating parameters for the defined experimental samples to be used in the SPAR Electrophoretic Separation Experiment. This was to be accomplished by using the best available equipment, namely, the Beckman CPE Mark II as modified by General Electric. Ground based experiments were undertaken not only to define flight parameters but also to serve as a point of comparison for flight results. Possible flight experiment problem areas were also studied such as sample interaction due to sedimentation, concentration effects and storage effects. Late in the program anomalies of field strengths and buffer conductivities were also investigated.

Since the exact samples to be used for the SPAR Electrophoresis Separation Experiment remains undefined, this report is limited to the sample materials made available for study.

II. ELECTROPHORESIS OF FIXED RED BLOOD CELLS

A. Separations

The fixed human red blood cells to be possibly used on the SPAR Electrophoretic Separator were supplied by Dr. Geoffery Seaman, Department of Neurology, University of Oregon Health Sciences Center. A description of the RBC's are listed in Table I.

All separations of fixed RBC's were done in R-1 buffer as described below:

Substance	Concentrate
Na_2HPO_4	2.5 g/ℓ
KH_2PO_4	0.5 g/ℓ
$Na_2EDTA \cdot 2H_2O$	1.25 g/ℓ

Solutions were made up with H_2O from a continental 3-stage deionizing system. Solutions of the concentrated buffer (used as electrode rinse) have an average conductivity of 39.0×10^{-4} MHO cm^{-1} and a pH of ≈ 7.2, a 10:1 dilution of the buffer concentrate is used for the curtain buffer (buffer in which separation is run). The average conductivity of this solution is $4.80 \pm 0.20 \times 10^{-4}$ MHO cm^{-1}. The pH is ≈ 7.2.

The first samples received from Dr. Geoffery Seaman were fixed RBC's, Lots RH82277FV and RH72777FL. In order to discriminate between the two cell populations in mixtures, the RH72777FL cells had been lysed prior to fixation making them less dense and optically translucent. It was necessary to add methylene blue to sample solutions in order to accurately observe the FL cells when doing a hemacytometer count.

In the first separation (CPE # 092077-A) tried in the CPE Mark II, using the 30 tube collector, the mobility of the FL cells was underestimated and half the

Table I. Electrophoresis Particle Standard Specification Sheet

Specifications	Code		
	RH82277FV	RH72777F	RH72777FL
Cell Type	Human Red Blood Cells	Human Red Blood Cells	Human Red Blood Cells
Stabilizing Agent	Formaldehyde	Formaldehyde	Formaldehyde
Additional Treatments	Neuraminidase Digestion		Lysis prior to fixation
Form Supplied	10 - 15% v/v Suspension in formaldehyde fixative		
Properties:			
*Elect. Mobil. in R-1 Buffer	2.15 $\pm$ 0.15 (30)	4.16 $\pm$ 0.25 (30)	4.55 $\pm$ 0.23 (30)
Elect. Mobil. in 0.15 M NaCl	0.38 $\pm$ 0.05 (30)	2.13 $\pm$ 0.16 (30)	1.18 $\pm$ 0.05 (30)
Density	$\sim$ 1.10 g/cc $\sim$ 1.10 g/cc	$\sim$ 1.1 g/cc $\sim$ 1.1 g/cc	$\sim$ 1.02 g/cc $\sim$ 1.02 g/cc
Optical Features	Opaque	Opaque	Translucent

*Mean $\pm$ stand. dev. for number of measurements in parentheses. Conditions: T = 25°C, 0.15 M NaCl buffered to pH 7.2 $\pm$ 0.2 with 0.15 M $NaHCO_3$.

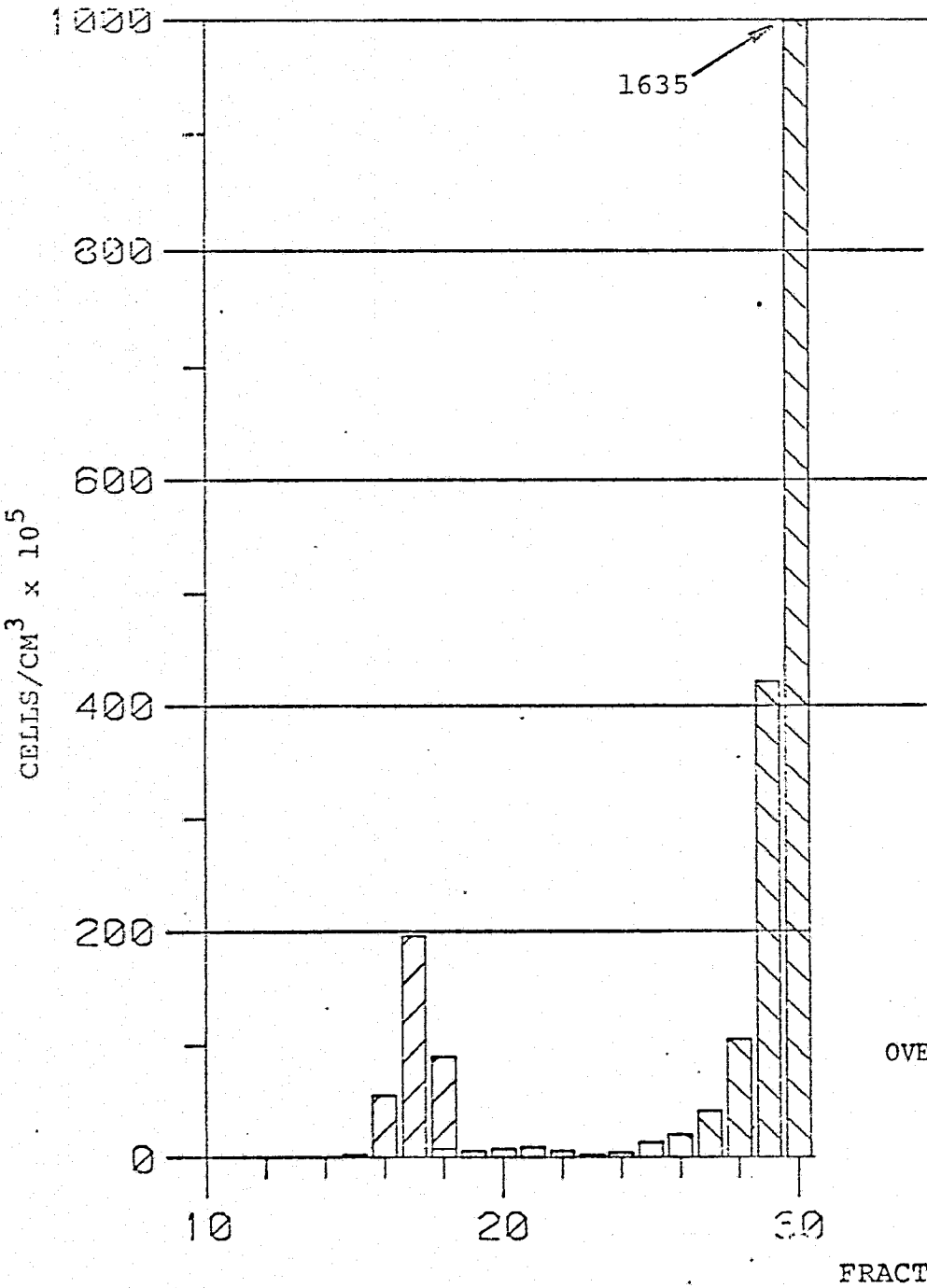
peak was driven into the overflow. Although the separation was good, there was definite tailing of the more mobile sample (RH72777FL cells), see Figure 1.

A second separation (CPE # 092177-A) was run using the same starting materials as CPE 092077-A. Applied voltages were as before but the curtain buffer flow rate was increased from 9.5 cc/min to 15.0 cc/min to reduce the displacement of the faster moving fraction. As can be seen in Figure 2, a good separation was achieved but still some tailing of the leading peak was present.

In an attempt to narrow the mobility distributions of the RBC sample a separation (101877-A) was run in the CPE using RH82277FV and RH72777FL cells at concentrations of 117.5 x 10^6 cells/ml and 325 x 10^6 cells/ml, respectively. The "center-cut" fractions (FV = 11, 12; FL = 19, 20) from each peak were combined and rerun (102177-A) in the CPE using the same parameters as the initial separation (101877-A). No improvement in resolution was observed. See Figure 3.

A mixture of fixed human red blood cells from lots RH82277FV and RH72777FL was electrophoresed (CPE # 101377-A) in the CPE, using only the "B" electrode region, in a field of 64 V/cm and with a residence time in the field of 35 seconds. The temperature was between 3 and 4°C. Except for the one-third longer electrode region (CPE electrode "B" is 15 cm long and SPAR ES electrode is 10 cm long) these conditions are similar to those that would be suggested should this sample mixture be used in a SPAR experiment. Separation was nearly complete as is shown in Figure 4.

Fixed human RBC's designated lot RH72777F were also supplied by Dr. Geoffery Seaman. As described in Table I, these cells are quite similar in density and physical appearance to RH82277FV cells but have a mobility near that of the FL cells.



PARAMETERS

SAMPLE: FIXED H RBC's
RH82277 FV
RH72777 FL

RATE APPLIED: 0.8 cc/hr

AMOUNT APPLIED: --

CURTAIN BUFFER: R-1 4.0×10^{-4} MHO cm^{-1} pH 7.2

ELECTRODE BUFFER: R-1 39.0×10^{-4} MHO cm^{-1} pH 7.2

ELECTRODES USED: A, B, C

FIELD STRENGTH: $E_a = 39$, $E_b = 39$, $E_c = 20$ V/cm

RESIDENCE TIME: A = 180 sec, B = 90 sec, C = 90 sec

COLLECTOR: 30 tube

CELLS RECOVERED: --

Figure 1. CPE # 022077-A

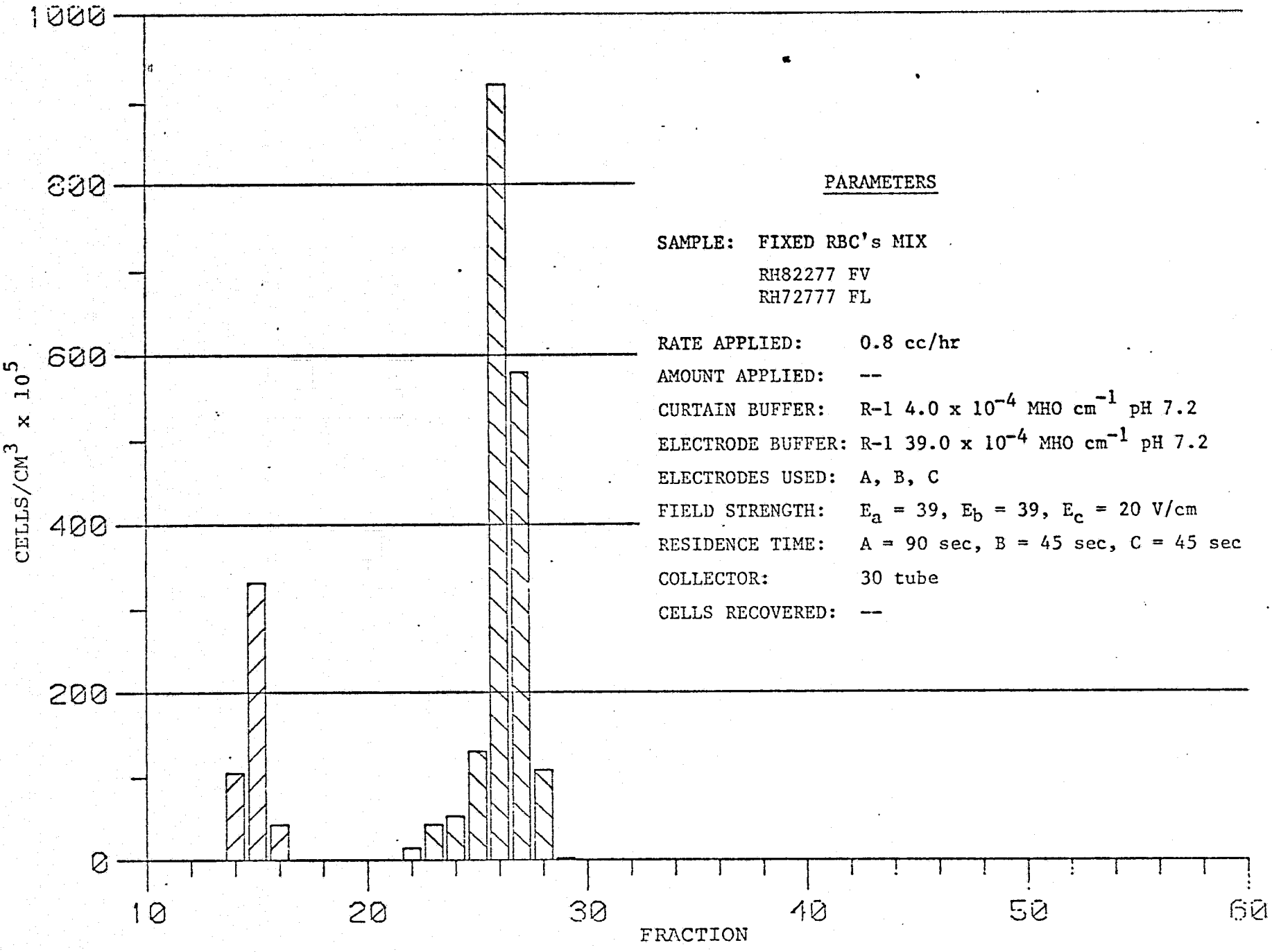


Figure 2. CPE # 092177-A

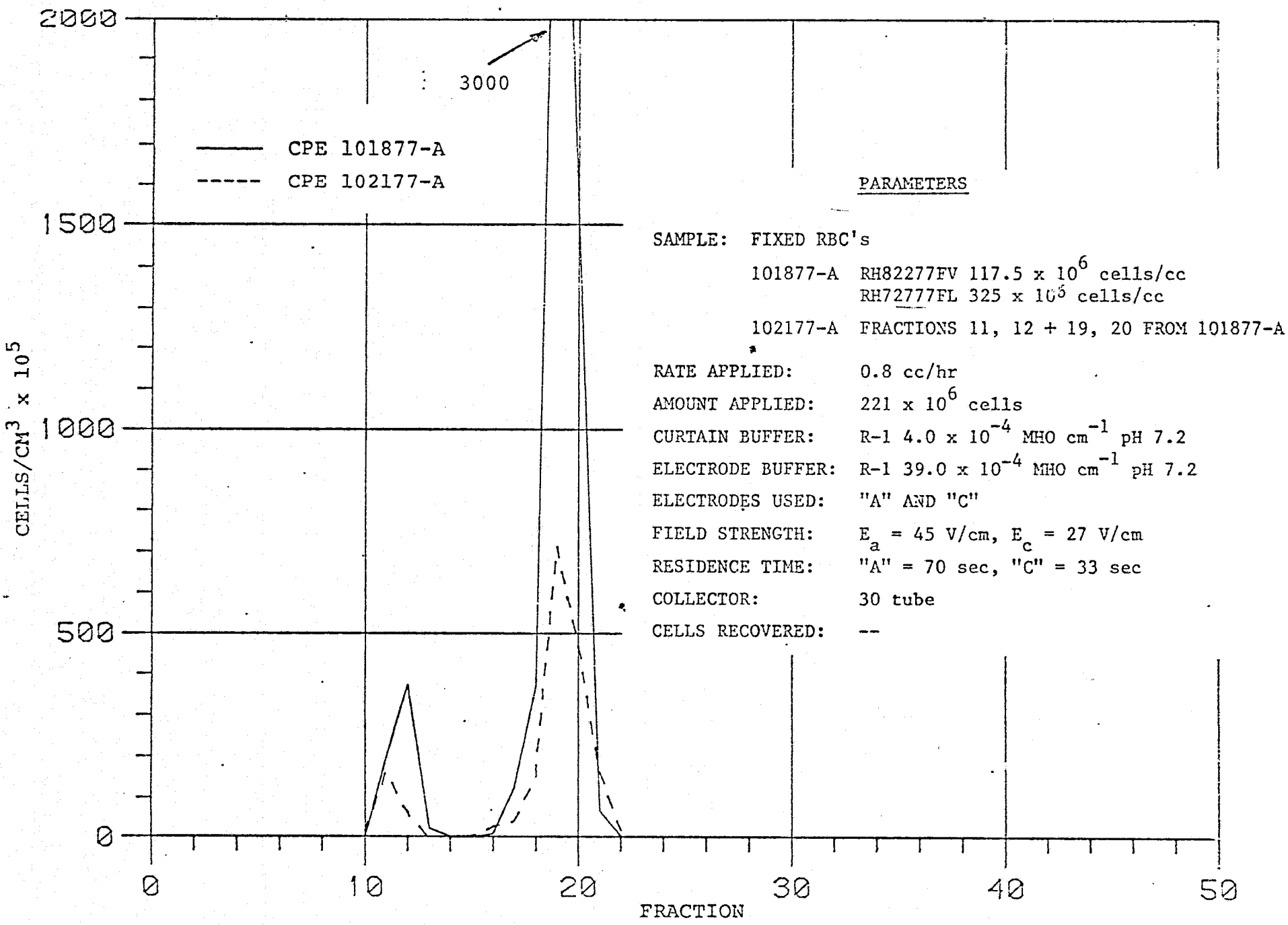


FIGURE 3. CPE #101877-A AND 102177-A

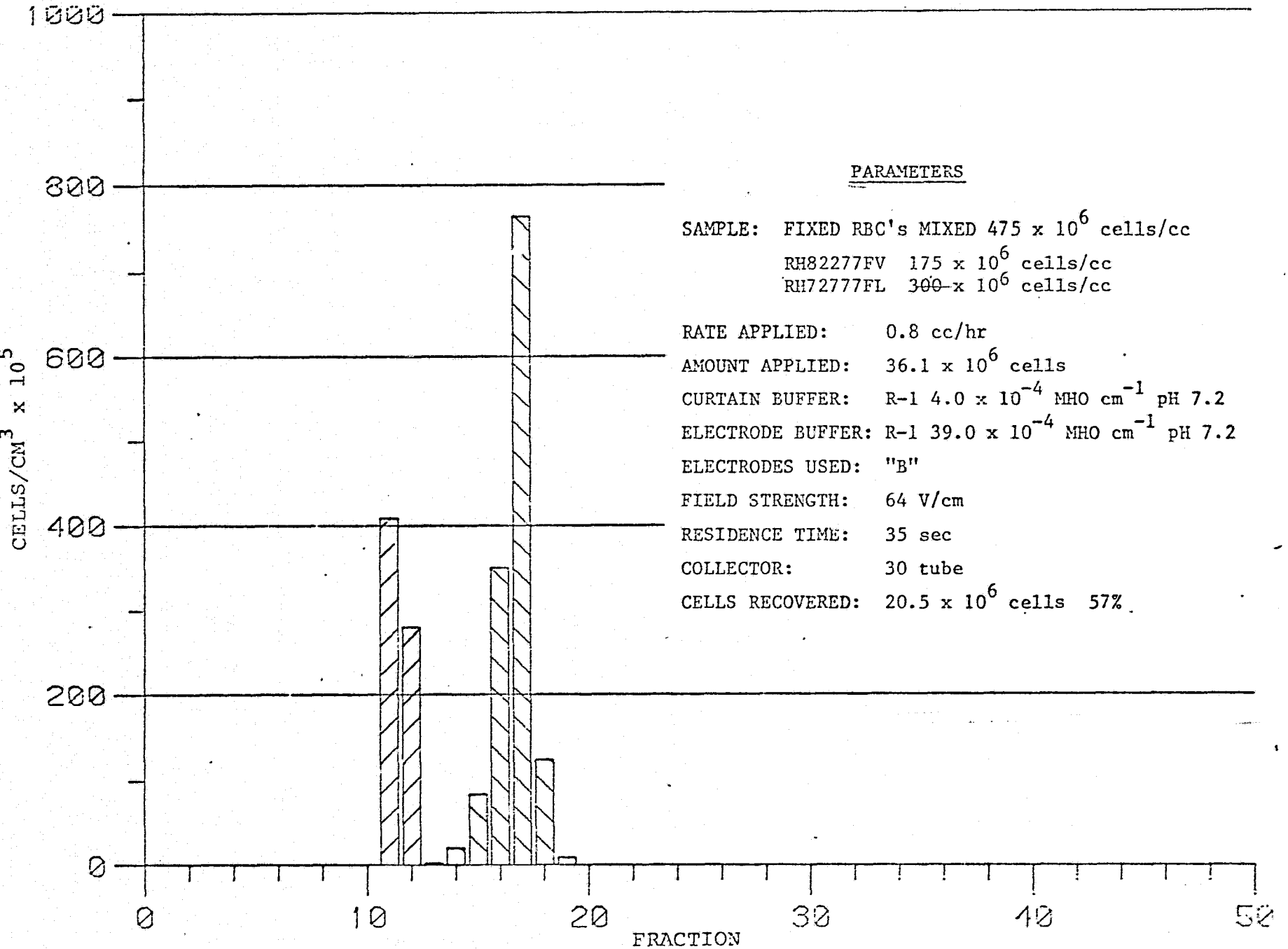


Figure 4. CPE # 101377-A

Several separations were conducted in the CPE to assess the separability of the FV and F fixed red blood cells under approximate conditions that would exist in the SPAR electrophoresis experiment.

First, each cell type (FV and F) was run separately (CPE # 020878-A and B) at a field strength of 50 V/cm (electrode "B") and with a residence time in the electrode region of 35 seconds. The RH82277FV cells were collected in fractions 9 and 10; the RH72777F cells in fraction 12. Under a no applied voltage condition, cells were collected in fraction 5. These results were by visual inspection. No precise cell counts were made.

A mixture of RH82277FV and RH72777F RBC's was separated (020978-A) using parameters closely approximating those to be used in the SPAR flight electrophoretic separator. The separation was not as good as those obtained previously with RH82277FV and RH72777FL fixed cells and not as good as the mobilities described by Dr. Geoffery Seaman (Table I). See Figure 5 for parameters and results.

Using the same sample mixture and parameters as in CPE separation 020978-A, a separation (CPE 020978-B) was run using the 94 tube collector instead of the 30 tube collector with overflow, described in Figure 6.

The 94 fractions (and a curtain buffer sample) from this separation were sent to Dr. Seaman for analysis. Tests of the conductivity (results discussed under Anomalies) and pH were conducted. Hemacytometer cell counts and counts carried out in a celloscope/electrozone particle size analyzer were made. The pH of the curtain buffer control was 7.25 while the pH of the fractions collected was 7.38 ± 0.02. Results of the cell counts are shown in Figure 7.

A mixture of RH82277FV and RH72777F RBC's was separated (031378-A) again using the 94 tube collector. A field strength of 60 V/cm was applied to the "B" electrode with a residence time in the electrode region of 35 seconds. A

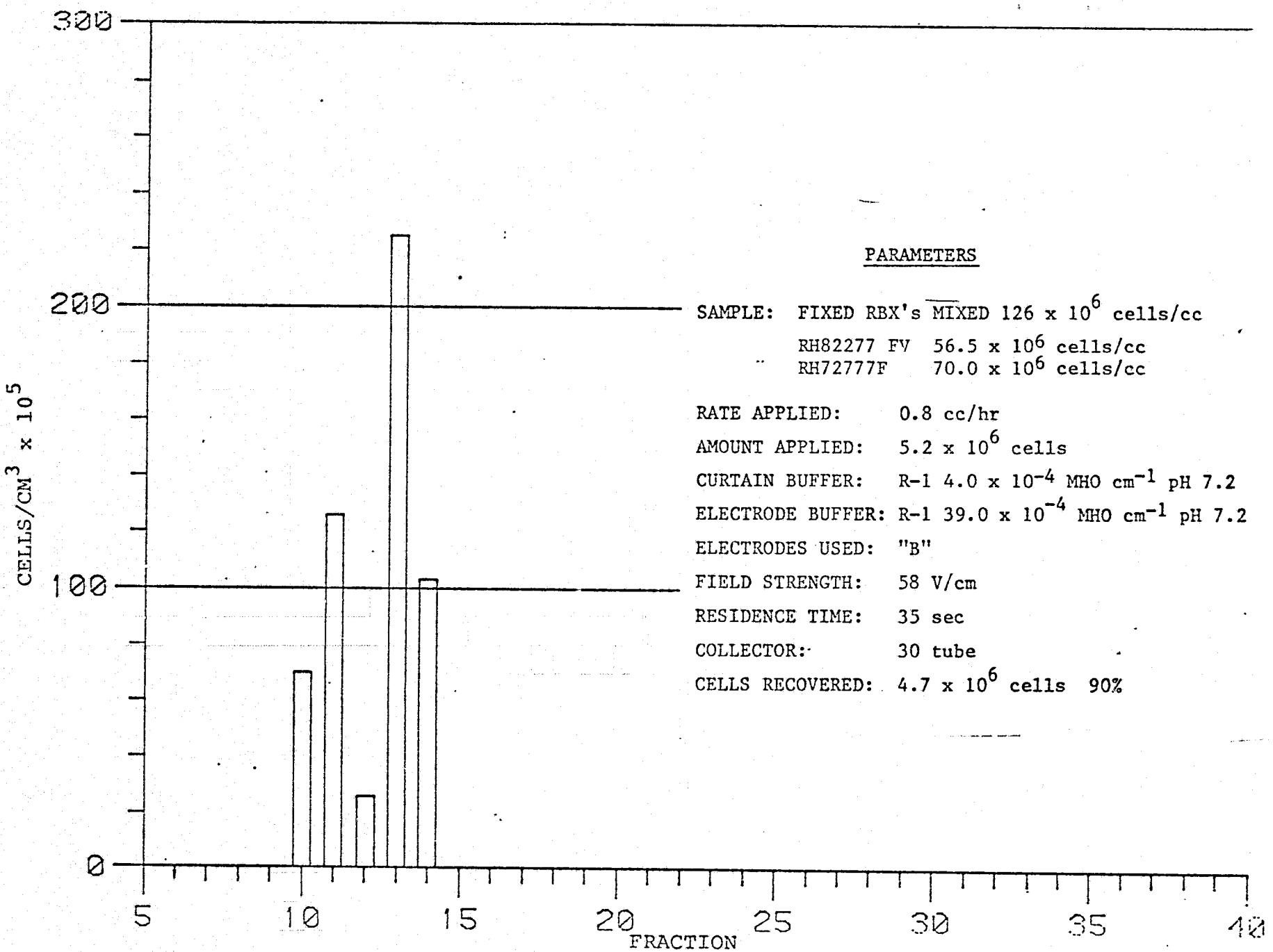
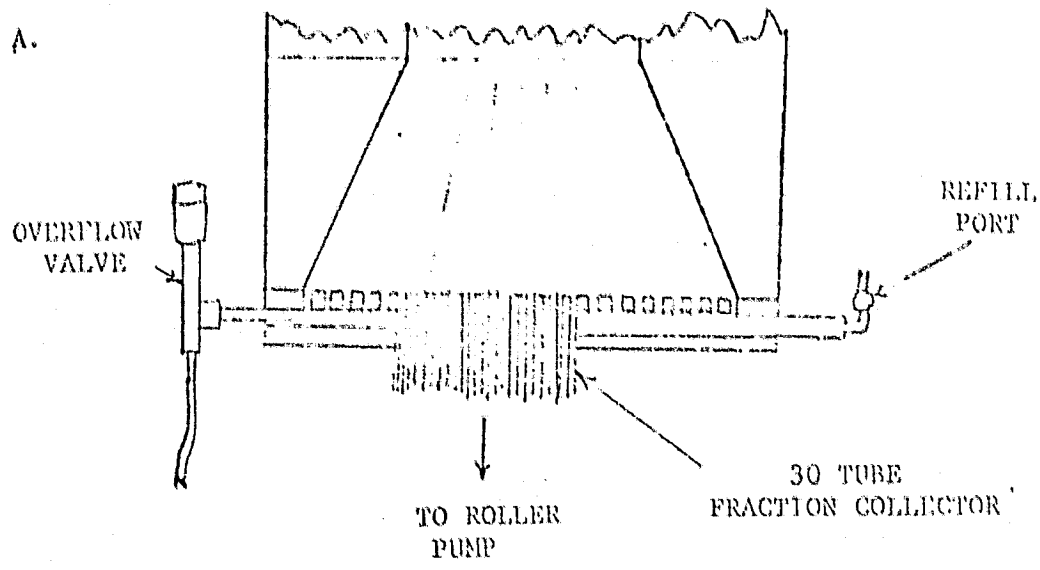
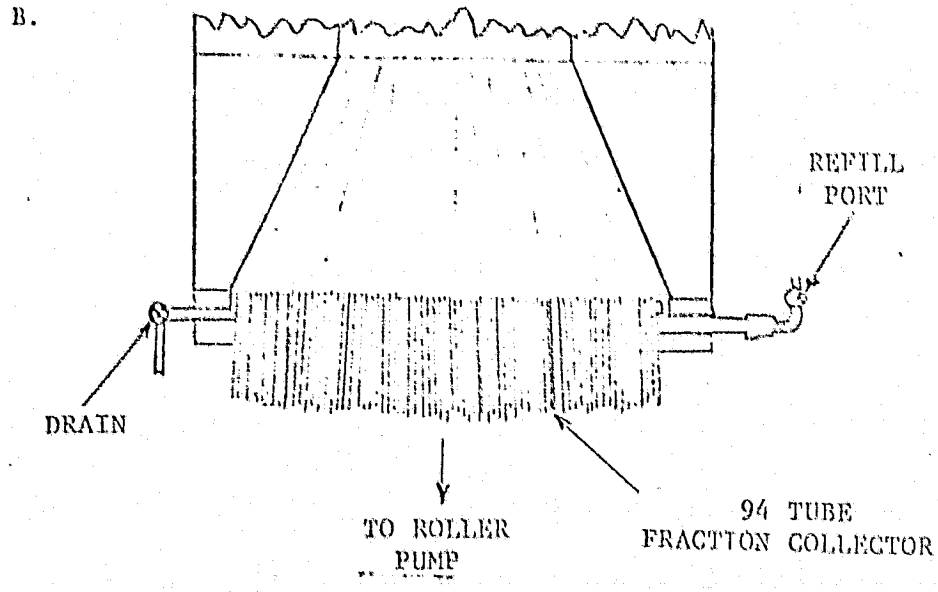


Figure 5. CPE # 020978-A



CPE 30 TUBE FRACTION COLLECTOR



CPE 94 TUBE FRACTION COLLECTOR

Figure 6. CPE Fraction Collectors

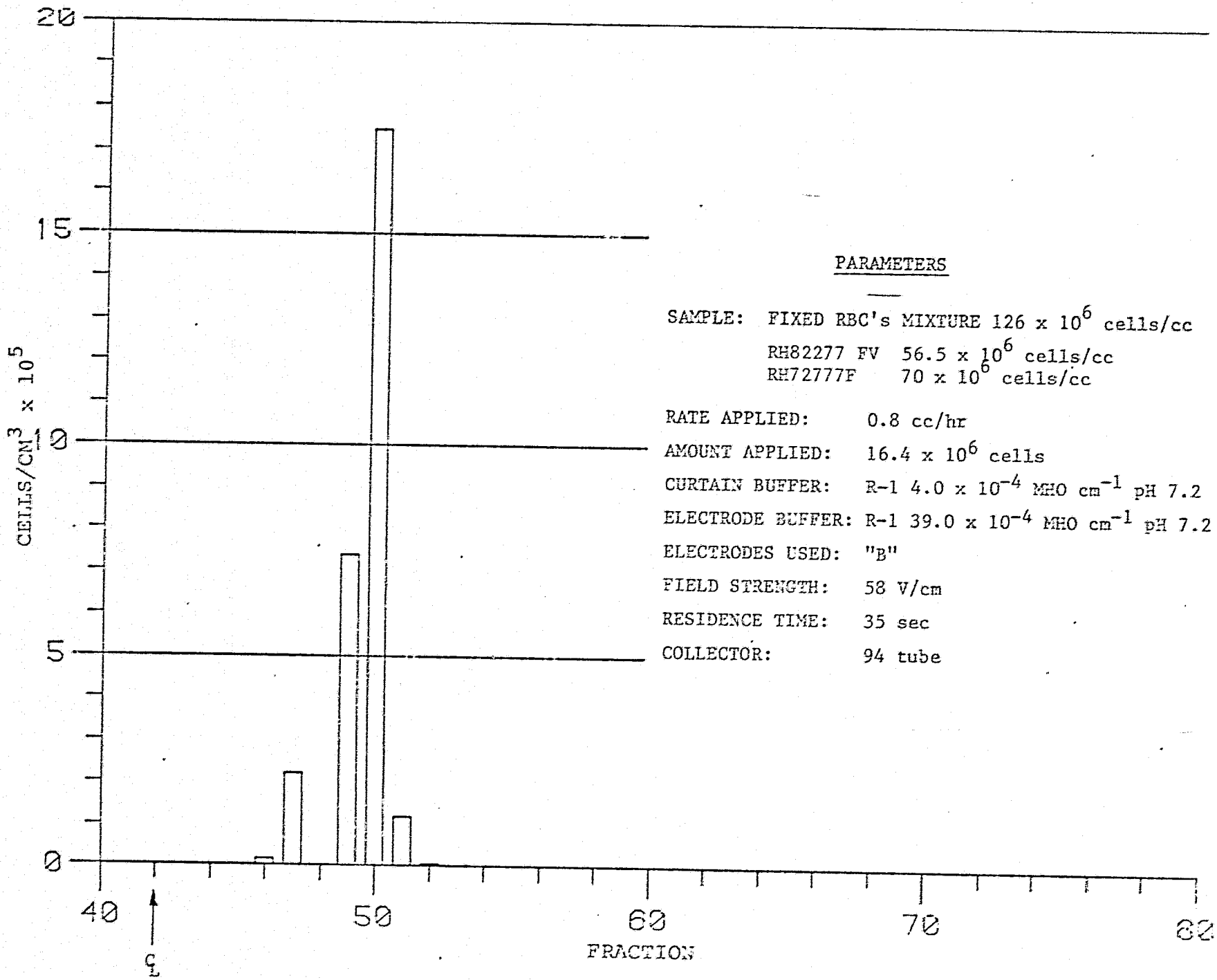


Figure 7. CPE # 020978-B

separation was accomplished but the peaks were only 3 fractions apart. See Figure 8 for results.

One constant problem in the separation of the fixed RBC's was the low percentage of cells recovered as compared to the calculated number applied. One suspect area was the inlet tubing leading from the sample syringe to the sample inlet. Due to the position of the sample syringe, the tubing (micro-bore silicone) leading to the sample inlet was approximately four inches long and positioned horizontally. At the end of a separation, it often appeared that some sedimentation of the cells was taking place in the tube. To eliminate this possibility, the sample syringe was relocated above and facing down toward the inlet tube. See Figure 9. The syringe and sample inlet tube were then connected by a two inch length of tubing.

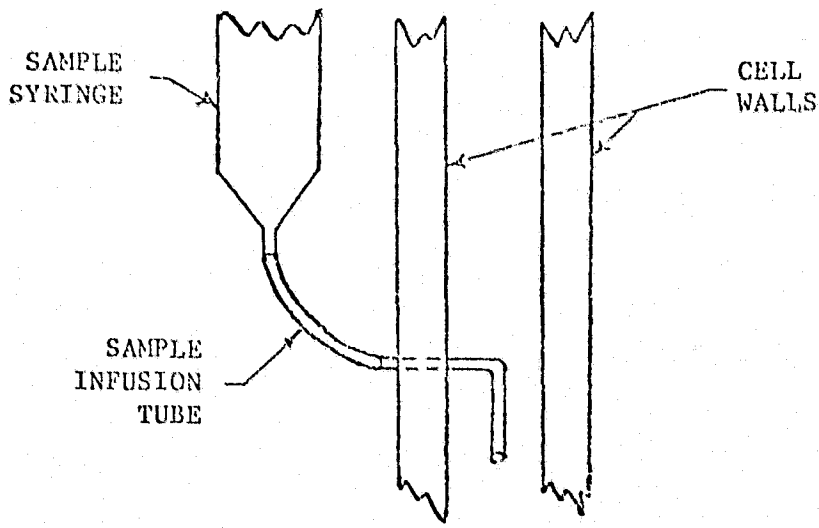


FIGURE 9. SAMPLE SYRINGE ARRANGEMENT

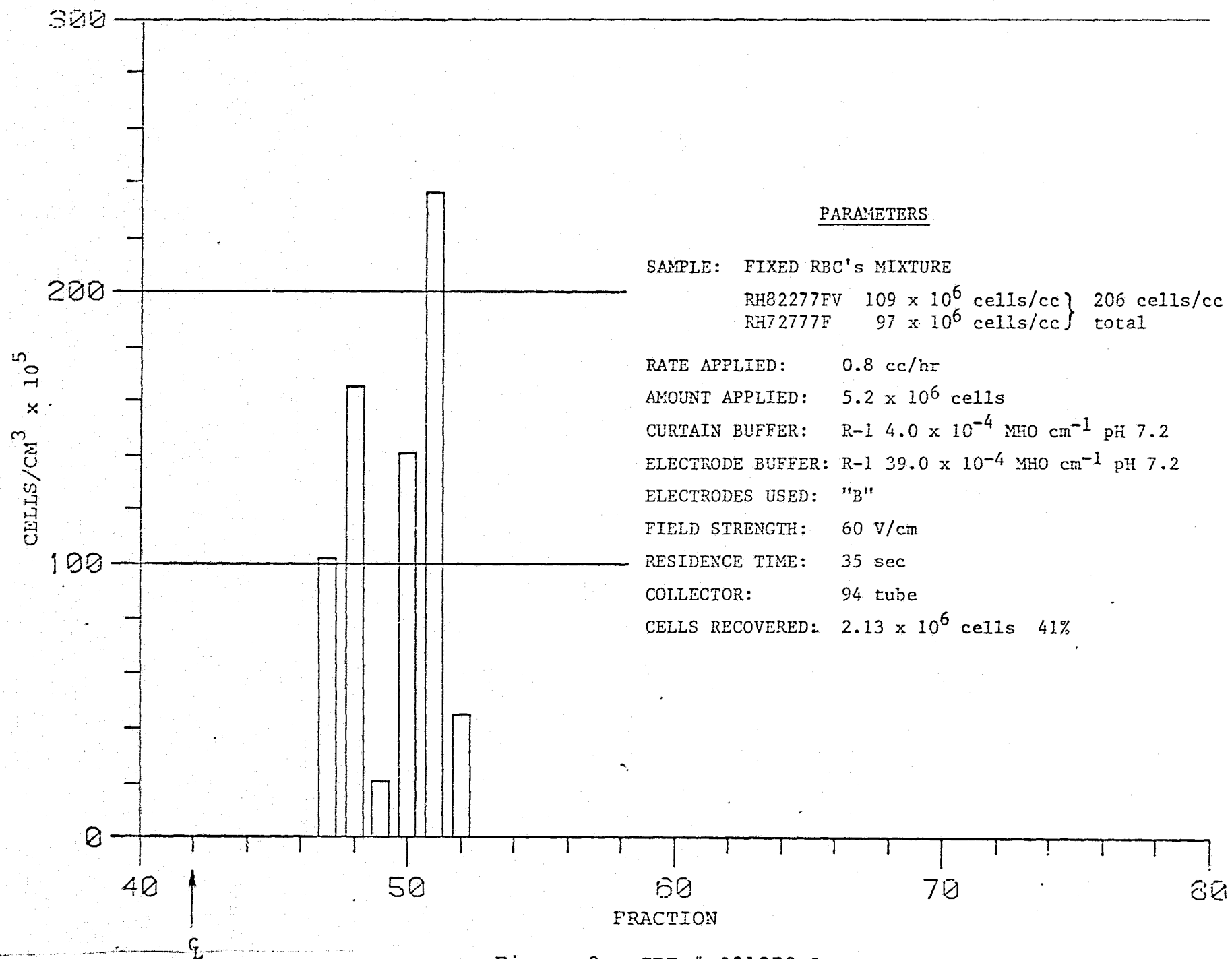


Figure 8. CPE # 031378-A

Three more separations (032178-B and C, 040478-A) of fixed RBC's (RH82277FV and RH72777F) were run.

Separations 032178-B and C were run using the same samples and conditions. Samples from separation 032178-B were sent to Dr. Geoffery Seaman for analysis while separation 032178-C was retained and cell counts obtained using a hemocytometer. Although a total separation was not achieved in CPE run 032178-C the percentage of recovered cells was 87% of the calculated number of cells applied. This was a marked improvement over previous separations. For complete parameters and results see Figure 10.

In separation 040478-A a 40 second residence time in the electrode region was tested in hopes of a better separation. Also sample infusion rate was 1.5 cc/hr an increase of 0.7 cc/hr over previous tests.

The separation of FV and F cells in run 040478-A was definitely better than that in run 032178-C. Again the percentage of recovered cells was good, being ≈ 84% of the calculated number of cells applied. See Figure 11 for details and results.

B. Storage and Handling

Sedimentation rates of the RH82277FV, RH72777FL and the RH72777F fixed RBC's were carried out using 1.0 ml aloquots taken directly from the sample bottles supplied to us from Dr. Geoffery Seaman.

The Westergren method for determining sedimentation rates of erythrocytes (Gradwokls Chemical Laboratory Methods and Diagnosis, Volume 2) was used. The results are as follows: FV cells 6.7 mm/hr, F cells 6.5 mm/hr and FL cells 0.0 mm/hr.

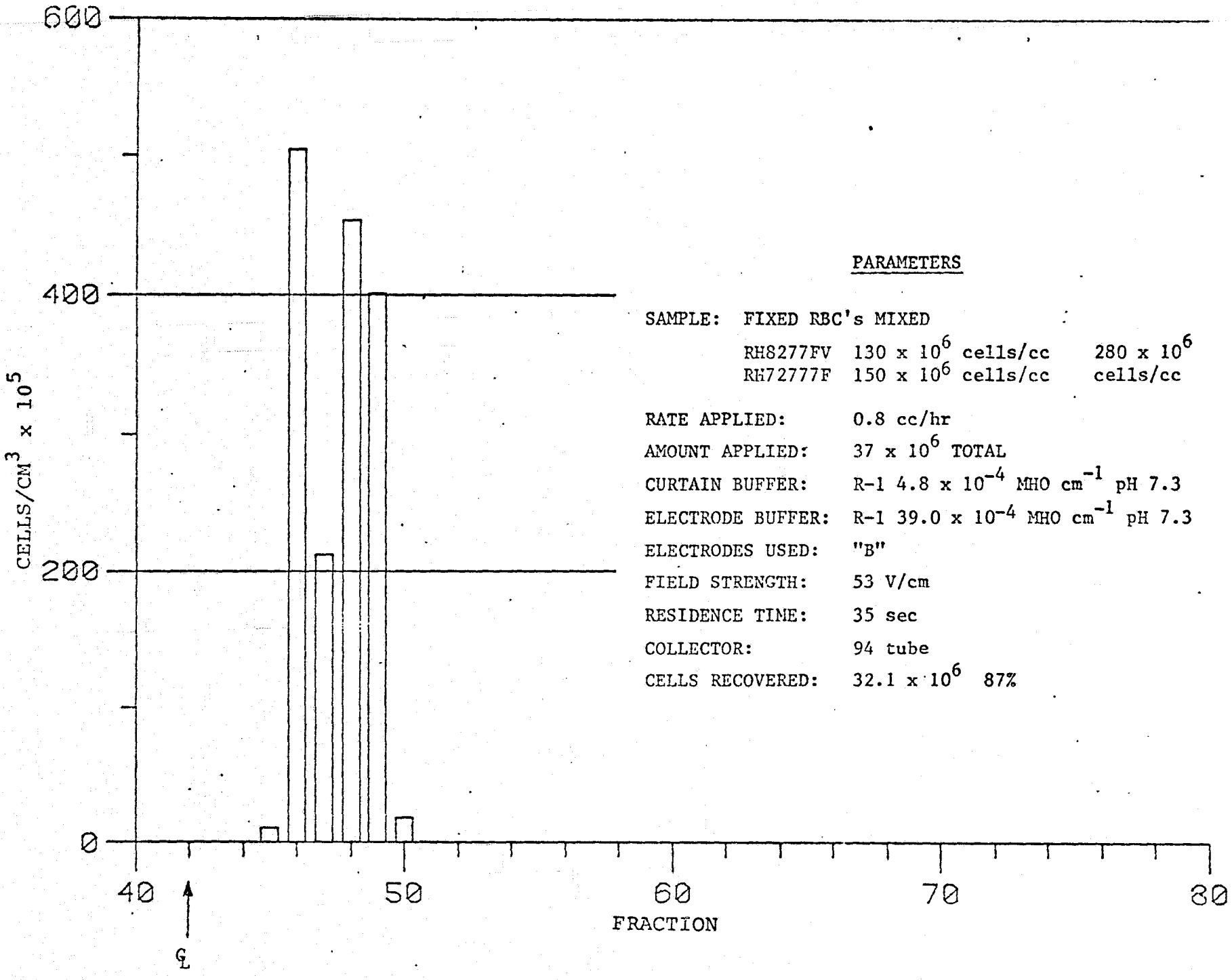
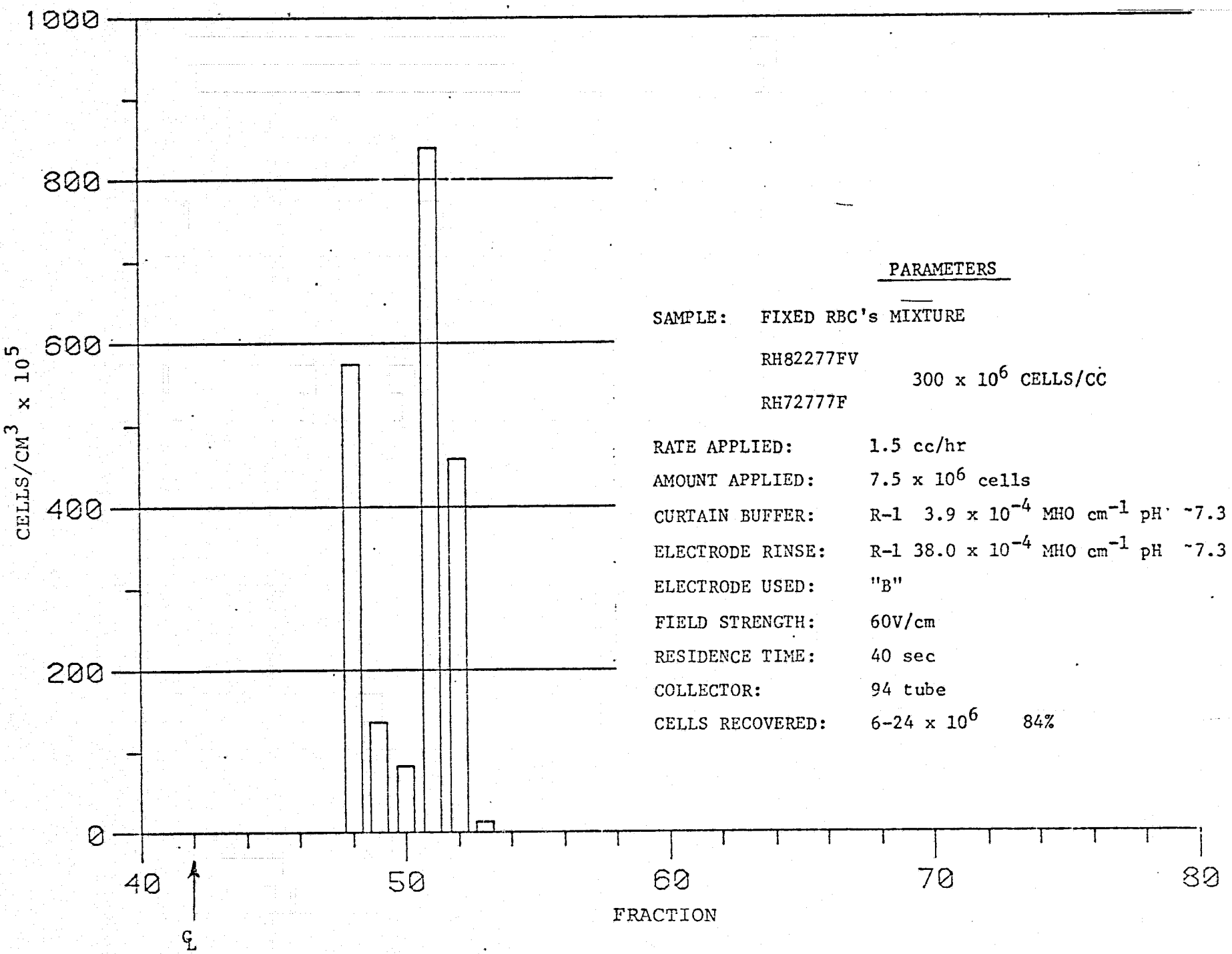


FIGURE 10. CPE #032178-C



PARAMETERS

SAMPLE: FIXED RBC's MIXTURE
RH82277FV
RH72777F
300 x 10^6 CELLS/CC

RATE APPLIED:	1.5 cc/hr
AMOUNT APPLIED:	7.5 x 10^6 cells
CURTAIN BUFFER:	R-1 3.9 x 10^{-4} MHO cm^{-1} pH ~7.3
ELECTRODE RINSE:	R-1 38.0 x 10^{-4} MHO cm^{-1} pH ~7.3
ELECTRODE USED:	"B"
FIELD STRENGTH:	60V/cm
RESIDENCE TIME:	40 sec
COLLECTOR:	94 tube
CELLS RECOVERED:	6-24 x 10^6 84%

FIGURE 11. CPE #040478-A

Two tests were performed to determine the type of pre-flight handling that might be required for samples which sediment at the rate of RH82277FV cells.

In the first test a cell suspension (FV) containing 97 x 10^6 cells/cm^3 was placed in the ES sample pump. The pump was oriented with its outlet in a downward position. The sample was continuously stirred for six hours, after which a 0.5 cc sample was collected from the pump outlet. The cell count of this sample was 92 x 10^6 cells/cc. This indicates that the ES pump is capable of keeping RBC cells in suspension with little or no settling effects.

In the second test the ES pump was oriented as before and filled with a cell suspension (FV) containing 87 x 10^6 cells/cm^3. This was allowed to stand for 4 days. At the end of that time, the stirrer was operated for 45 minutes. A 0.5 cc sample was collected as before and counted in a hemocytometer. The count was 132 x 10^6 cells/cm^3 indicating that once the cells have sedimented, something over 45 minutes of stirring will be necessary to resuspend them.

It was noted in the separation experiments in the CPE that the mobilities of the RBC's seemed somewhat lower than their predicted mobilities. On re-testing RBC lots RH82277FV and RH72777F, Dr. Geoffery Seaman found a significant decrease in mean mobilities.

The mean mobility for the RH82277FV cells in R-1 buffer on 8/23/77 was -2.15 ± 0.15 μm sec^{-1} V^{-1} cm which by January 1978 was reduced to -1.92 ± 0.16 μm sec^{-1} V^{-1} cm. The RH72777F cells changed even more drastically, from -4.16 ± 0.25 μm sec^{-1} V^{-1} cm to -3.04 ± 0.22 μm sec^{-1} V^{-1} cm. The data suggest that these cells are not ideally suited for a SPAR flight. The degradation of mobility must be factored in to the planning of the flight test if possible. Perhaps more stable particles should be sought for the crucial first tests of the flight hardware.

III. ANOMALIES

A. Field Strength

On the first attempt to use R-1 buffer as described by G. V. F. Seaman in the Bekman Mark II CPE an unusually low field strength was observed in the separation chamber compared to the applied field, about 60% of the expected value. Although low field strengths had been noted before, replacing the electrode membranes had always solved the problem. The cause probably being either contaminated membranes or the almost total removal of surfactant from the membrane material causing a reduction in its hydrophilic properties. It was also noted that in the past this problem of reduced field strength was most prevalent when phosphate buffers were used.

Increasing the concentration of the buffers (curtain and/or electrode rinse) did little to improve the low field strength.

The replacement of the phosphate (R-1) buffer with borate/glycine buffer immediately eliminated the problem.

The membranes (Millipore type VS 0.025 μ-pores) on the CPE cell were replaced with fresh ones of the same type, but the field strengths using R-1 buffer remained low.

The Millipore VS membranes were removed from the CPE and replaced with Gelman "Acropor" type. Due to its superior strength, the "Acropor" membranes had been a prime candidate for use on the ESE chamber. Its one shortcoming is its greater porosity (0.2 μ pores) which allows excessive fluid exchange between the electrophoresis chamber and the electrode compartments. This is generally handled by coating the membrane with a 0.1% agar gel, but because of the possible long storage time for the flight cell, an acrylamide gel was tested instead.

Accordingly, a piece of "Acropor" membrane impregnated with 7.5% polyacrylamide gel was used to divide a "U" tube cell. The resistence of R-1 buffer measured across the cell with a 1000 hertz bridge (type 1650A General Radio Co.) was only 5% higher with the membrane in place than it was without it. "Acropor" membranes impregnated with 7.5% polyacrylamide gel were then installed in the CPE. R-1 buffer was again used in the electrophoretic chamber and a voltage applied. The field was 70% of the applied voltage. Even the addition of NaCℓ to the R-1 buffer, increasing the conductance to 9.0×10^{-4} MHO cm^{-1}, did not substantially increase the field strength. With the same membranes and borate/glycine buffer (3.0×10^{-4} MHO cm^{-1}) in the electrophoresis chamber and the electrode rinse compartments, the field strength was 94% of the applied voltage.

The acrylamide impregnated "Acropor" membranes were removed from the CPE and replaced with "Acropor" membranes impregnated with 0.1% agar gel. The results with R-1 buffer were not significantly different than before.

Finally, a prototype of the ESE electrophoresis chamber was fitted with two platinum electrodes for field strength measurements. "Acropor" membranes filled with agar were used to isolate the electrophoresis chamber from the electrode compartments. Two things became immediately obvious. First, there was no evidence of a prolonged (5-15 min.) equilibration period as there is at times with the CPE. The field strength observed when power was applied to the electrodes was the same at the beginning as it was fifteen minutes later. Second, no difference was observed between the behavior of phosphate (R-1) and of borate/glycine buffers. The field strength observed with agar-filled "Acropor" membranes and R-1 buffer (4.0×10^{-4} MHO cm^{-1}) both in the curtain and in the electrode rinse was 72% of that observed when no membranes were present. But if the electrode rinse was changed to a concentrated R-1 buffer (39.0×10^{-4} MHO cm^{-1}), the observed field strength rose to 82% of the no-membrane value.

Having learned how to apply Millipore type VC (0.1 micron pore size) membranes to the ESE chamber without fear of leakage or cracking, they were applied to the prototype chamber. With these membranes in place, the observed field strength in R-1 buffer (4×10^{-4} MHO cm^{-1}) was 74% of the no-membrane value if R-1 buffer (4.0×10^{-4} MHO cm^{-1}) was used in the electrode compartments, but rose to 97% of the no-membrane value if an R-1 concentrate (39.0×10^{-4} MHO cm^{-1}) was used in the electrode compartments. The same results were obtained when the VC membrane was filled with agar gel to reduce permeability. It does not appear that the VC membrane is permeable enough to need the agar filler, but this option can be taken at any time without affecting other system parameters.

It is recommended that Millipore VC membranes be used in the ES, and that the electrode rinse be a 5-10 fold concentrate of R-1 buffer.

B. Conductivity Profiles

Another anomaly surfaced when samples from CPE separations (020978-B and 032178-B) of fixed RBC's (RH82277FV and RH72777F) were sent to Dr. Geoffery Seaman for analysis. In testing the conductivity of the ninety-four samples from each run, anomalies in the conductivity profiles were observed.

These anomalous conductivity profiles were later confirmed by the Separations Processes Branch of the Space Sciences Laboratory at the Marshall Space Flight Center and here at the General Electric Space Sciences Laboratory.

In an attempt to determine any further effects coinciding with the conductivity profiles such as possible cross-flow in the curtain buffer two electrophoresis separations were run using RBY dye (Gelman Instrument Co. #51250) (the yellow dye has a zero-mobility), as a sample.

In separation "A" the curtain buffer and electrode rinse buffers were both R-1 buffer with a conductivity of 4.12×10^{-4} MHO cm^{-1}.

In Separation "B" the curtain buffer (R-1) conductivity was 4.12×10^{-4} MHO cm^{-1} and the electrode rinse buffer (R-1 10X) was 43.2 MHO cm^{-1}. All conductivities were measured using a General Radio Impedance Bridge Model 1650-A and a YSI model 3400 series conductivity cell.

The conductivity of each of the 94 fractions from both experiments was measured. Also the location of the dyes in the various fractions was noted.

The conductivity profiles of the fractions from both experiments were similar with the conductivity at the anode side being low and the conductivity at the cathode side being high.

The yellow dye showed zero migration in separation "B" and a slight reverse migration in separation "A". See Figure 12 for details.

The noted differences in solution conductivity across the CPE may be explained by the following illustration. Conductivity measurements provide only the sum of the ionic mobilities in a solution. Certainly, individual ions will have characteristic mobilities and faster moving ions will contribute more to conductance than slower ions. Changes in concentration (near the electrodes) during electrolysis was first observed by Hittorf in 1853. These changes take place without violating the electrical neutrality of the solution. Consider the electrolysis of a dilute HCℓ solution between platinum electrodes in a cell divided by porous partitions into three compartments (Figure 13).

Let the mobility of H^+ be $\sim$ 4X the mobility of $C\ell^-$ and let five Faradays pass through the solution. Four-fifths will be carried by H^+ and one-fifth by $C\ell^-$. At the same time, 5 moles of each ion are deposited at the electrodes. As a result the following changes occur (see Figure 14).

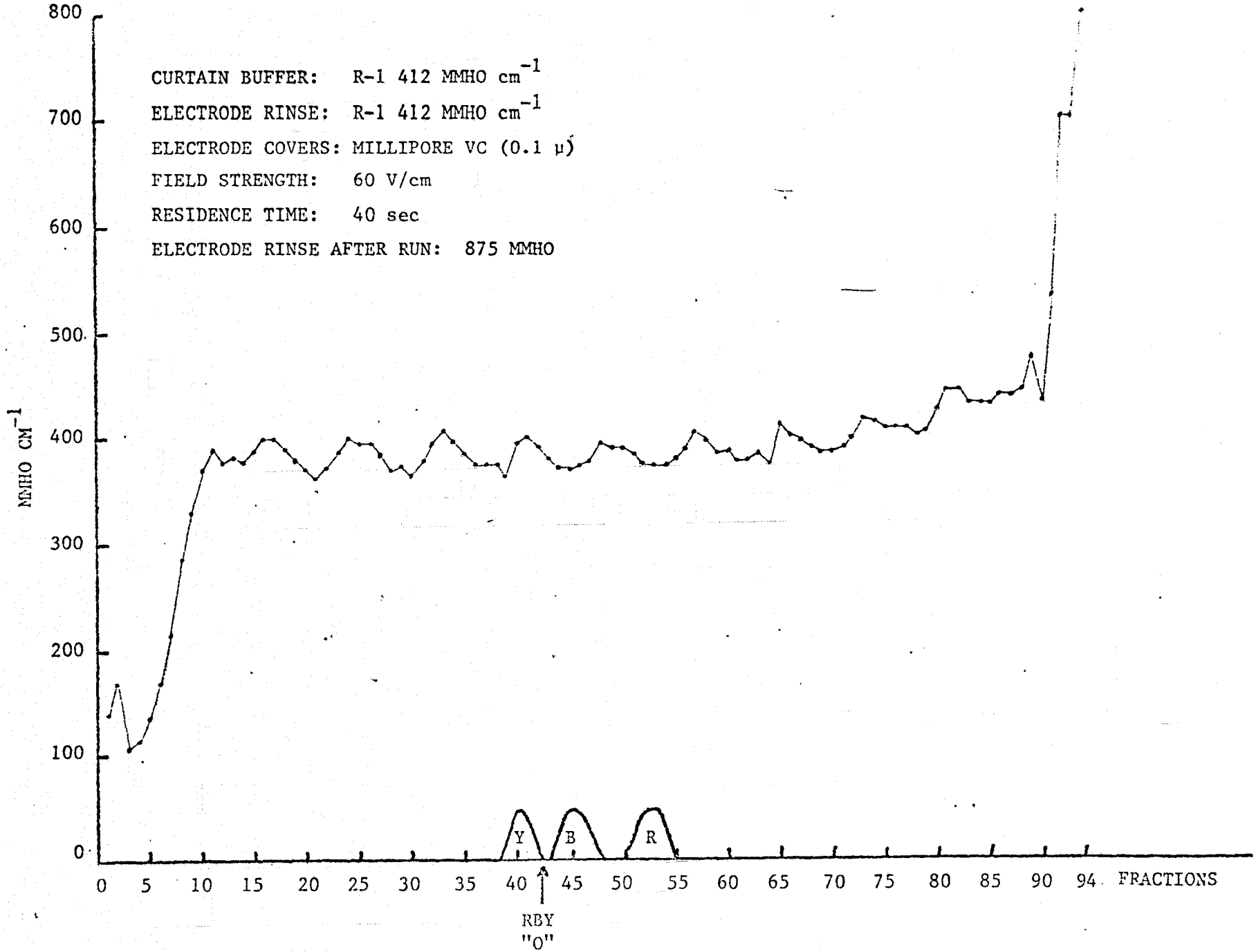


Figure 12A. Conductivity Profile of Fractions from CPE

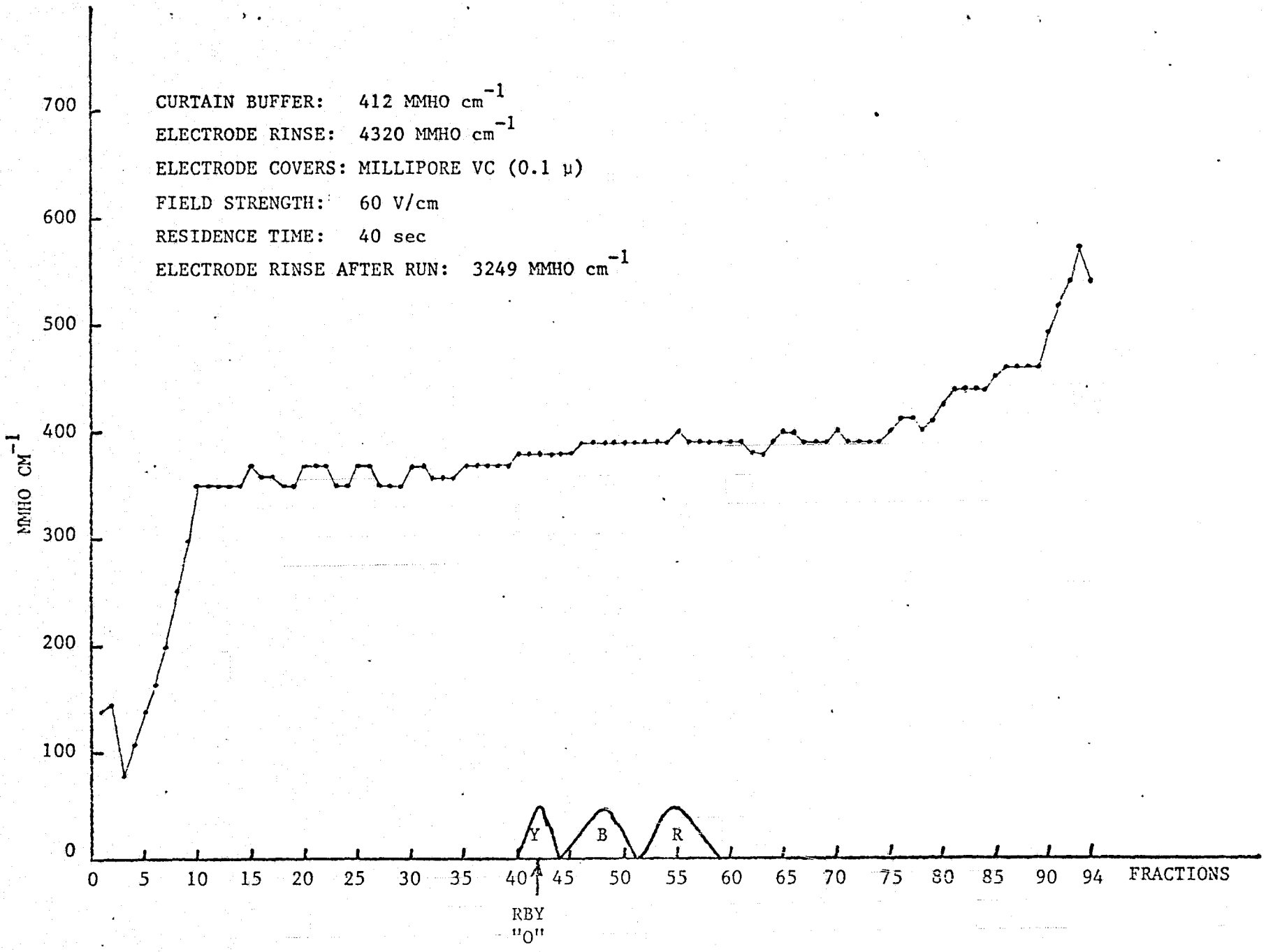


Figure 12B. Conductivity Profile of Fractions from CPE

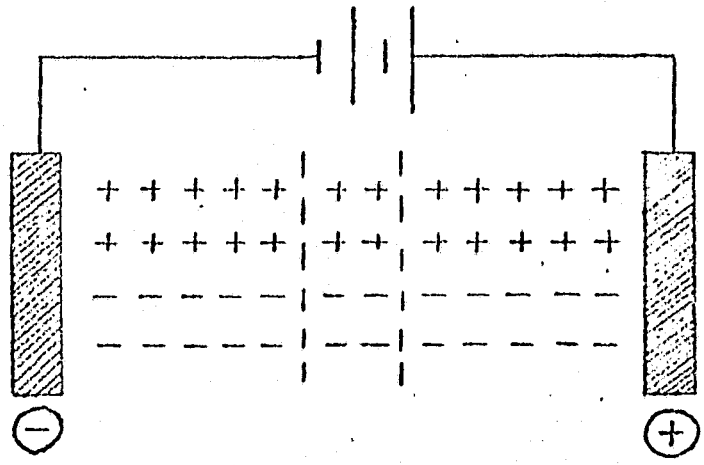


FIGURE 13. NO CURRENT FLOW

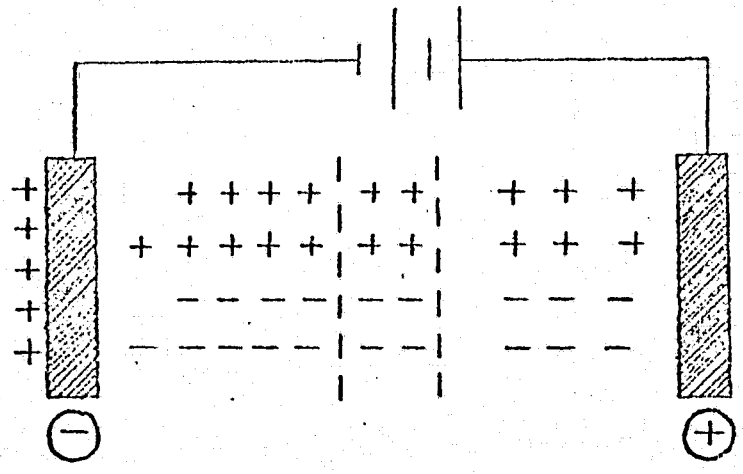


FIGURE 14. AFTER CURRENT FLOW

	Anode	Middle	Cathode
Discharge	$-5\ Cl^-$	0	$-5\ H^+$
Enters	$+1\ Cl^-$	$+4\ H^+ + 1\ Cl^-$	$+4\ H^+$
Leaves	$-4\ H^+$	$-4\ H^+ - 1\ Cl^-$	$-1\ Cl^-$
Total	-4 HCl	0	-1 HCl

In this example the anode concentration decreases more than the cathode and these differences would be observed in conductivities. Along with concentration changes, alterations in volume would also occur, therefore these changes are usually expressed as grams per weight of solvent rather than moles per liter.

Obviously, the R-1 buffer used in the CPE is a much more complex system than a simple dilute HCl solution. R-1 contains: phosphate, having three equilibria, sodium chloride and disodium EDTA which can exist as five different species depending on pH. Complicate the system further with a slow laminar flow and one should not be surprised to find differences in conductivities outside the electrode areas. Evidence of the associated volume change is seen in the increase of volume of electrode buffer during prolonged CPE operation.

It is reasonably certain that these mobility differences account for the conductivity differences observed in the CPE. The slight polarization caused by this effect is the reason for using KCl salt bridges in some electrochemical techniques. K^+ and Cl^- have almost identical mobilities and thus the use of a KCl salt bridge virtually eliminates errors due to junction potentials arising from ionic mobility differences.

Some further studies of the effects on electrophoretic separations caused by the anomalous conductivity profiles in the CPE might be in order.

IV. ESE ELECTRODE COVERS: HANDLING AND INSTALLATION

As in the Beckman CPE Mark II, the electrode areas in the SPAR Electrophoretic Separator must be kept separated from the separation chamber by a membrane which will maintain electrical field integrity across the chamber but will keep electrode rinse buffer and electrode generated gases out of the separations area. To this end several membrane materials have been tested and at this time either the "Acropor" membrane by Gelman or the Millipore VC type membrane seem to produce the best results.

Depending on the membrane materials to be used in the flight separations chamber, the techniques for application vary slightly.

The easiest material to handle is the "Acropor" membrane by Gelman (Part # 61820, 0.2 μ pore size). This material is very resistent to tearing or cracking and may be applied directly to the electrophoresis chamber spacer by means of Scotch Brand Silicone Adhesive (#4653DRF733R41) strips. See Figure 15. Due to the porosity of this material it is necessary that it be impregnated with either acrylamide gel (7.5% solution) or with a thin agar gel.

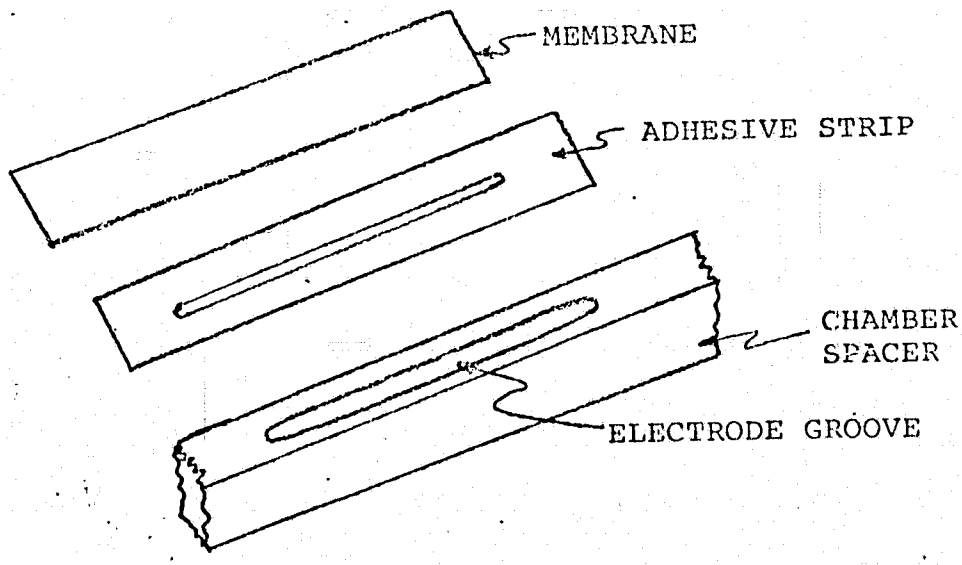


Figure 15. Electrode Cover

Formula for acrylamide gel is as follows:

Part #1

7.5 g Cyanogum 41

0.2 cc TEMED

0.6 g NaCl

in 80 cc H_2O (deionized or distilled)

Part #2

0.15 g Ammonium Persulfate in 20 cc H_2O

Mix both solutions and use within 10 minutes. Apply to membrane in place on chamber spacer with a soft brush, taking care to thoroughly wet membrane. One application is sufficient. Allow to cure approximately 20 minutes before using.

Formula for agar gel coating is as follows:

0.10 g Agar

0.05 cc glycerine

100 cc H_2O (deionized or distilled)

Heat to 90°C while stirring. While solution is at 90°C apply to membrane with a soft brush taking care to completely wet membrane. Allow to stand one hour before using.

The Millipore VC type (0.1 μ pore size) membrane has a lower porosity than the Gelman "Acropor" membrane, therefore, eliminating the problem of treating with acrylamide or agar gels but this type of membrane must be "softened" with glycerine to eliminate cracking during installation on the ES chamber spacer.

After applying a membrane strip (≈ 16 x 180 mm) to a precut piece of Scotch Brand Silicone Adhesive (≈ 16 x 180 mm with a 1 mm slot 120 mm long, 30 mm from each end), the membrane is wet evenly with a 5% aqueous solution of glycerine. This process is repeated usually between 3 - 5 times or until the membrane strip

feels fairly flexible when just still damp. The protective backing is then removed from the adhesive strip and the membrane is carefully affixed to the cell, taking care to line up the slot in the silicone adhesive with that of the electrode.

Millipore membranes should be kept moist at all times once applied.

INSTRUCTION MANUAL

FOR

AAFE ELECTROPHORETIC SEPARATOR EXPERIMENT

Including

FUNCTIONAL TEST

ACCEPTANCE/CHECK-OUT TEST

AND

BIOLOGICAL HANDLING PROCEDURES

CONTRACT NAS8-31036

Prepared for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Marshall Space Flight Center

Huntsville, Alabama

by

General Electric Company
Space Sciences Laboratory
P. O. Box 8555
Philadelphia, Pennsylvania 19101

INSTRUCTION MANUAL

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II. OPERATIONAL PROCEDURES (continued)

LIST OF FIGURES

I. GENERAL DESCRIPTION

A. System Operation

1. General

The objective of this free-flow electrophoretic separator is to take advantage of the lack of convection and sedimentation in space and to use this advantage to fractionate and collect samples, which may be live biologicals, with greater resolution and/or throughput than currently available from state-of-the-art terrestrial equipment.

The apparatus described here is an automated unit which can perform the separation of biological material during a sounding rocket flight.

In this process, the sample is injected into the electrophoresis chamber and is carried into the electric field by the buffer. Under the influence of the field the various sub-fractions of the sample will be deflected according to their mobilities. After leaving the field, the separated fractions enter a 50 channel collector at the end of the cell, ideally each fraction in a different channel. They are then pumped through tubing by a peristaltic pump, to collection bags in a thermally controlled container, which can be removed after the mission, for further examination.

All the systems described in 1.B. will have been activated prior to launch with the exception of the sample pump, which may have been run briefly in order to fill the sample feed tube and then turned off. At the time of launch, a time delay relay is activated. This relay turns the sample pump on at a predetermined time so that sample enters the chamber during the "micro g" portion of the flight. At the end of that time, another time delay relay removes all power from the ESE unit.

2. A "typical" Sequency of Events:

T-24 hours
- o Start ground coolant
- o Start N_2 purge
- o Exchange buffer for antiseptic

T-3 hrs.	o	Install collection container
	o	Install sample pump
	o	Run check of detector and other systems
T-2.5 hrs.	o	Start internal coolant
T-1 hr.	o	End ground coolant
	o	End N_2 purge
TBD by P.I.	o	Run sample pump to fill sample feed tube then stop pump
T-2 min.	o	Begin operation: Buffer flow, Electrolyte rinse and High Voltage
T-0	o	Launch (time delays activated)
T + 1 min.	o	Automatic Start : Sample Pump
T + 5 min.	o	End of "micro g" portion of flight - all functions OFF
T + X_o hrs.	o	Recovery
T + X hrs.	o	Remove collection container

B. System Elements

This section will describe the individual elements which comprise the total ESE units.

1. ESE Unit (Figure 1)

All of the system elements are contained in a single structural assembly consisting of a pan and shroud which measures 14.44" in diameter by 19" high. The interior structure consists of aluminum hexrods, anchored to the pan, which support two aluminum mounting plates. The pan (lower section of the assembly) is also an interface with the flight vehicle. The shroud (upper section) is a load bearing structure which is also a pressure tight container. The shroud further serves through a snubber to support the top of the hexrod/plate structure. The top plate of the internal assembly is actually an optical bench, to which the electrophoresis chamber, optics, lamp and detector are mounted. The other components are distributed between the mid-plate and the pan. On the side of the shroud is a

6.4" x 13.2" access door, held in place by captive screws. Immediately below the door (on a bulkhead on the pan) are the electrical and fluid connectors. The shroud is normally bolted to the pan and the joint is sealed by an "O" ring.

2. Electrophoresis Chamber (Figure 2)

The electrophoresis chamber consists of 3 sub-assemblies; the frames, the faceplates and the spacer (see Figure 2). The spacer is a machined plexiglas piece which houses the electrodes and sets the cell thickness. The electrodes are thin platinum strips which are physically, but not electrically, isolated from the electrophoresis chamber by porous membranes.

The faceplates are machined from fiberglas reinforced epoxy, with alumina inserts in the area of the electric field to increase the thermal conductivity there. Both faceplates have fused silica windows and one of the plates has the 50 channel collector attached to it. The sample enters through one face. The curtain buffer enters through the opposite face. The faceplates are assembled one above and one below the spacer and "O" rings maintain the fluid seal. The faceplate/spacer assembly is held together by two frames. These are machined aluminum and also incorporate cavities used for cooling the assembly during operation. Also attached to the assembly are the inlet and outlet ports for the electrolyte circulation.

3. Peristaltic Pump (Figure 3)

The curtain buffer is circulated through the system by a 50 channel peristaltic pump. Those channels collect the curtain buffer (and separated fractions) from the chamber and pump them into the sample recovery bags. The speed of the pump can be set to give residence times in the cell of 25 sec. to 400 sec. The use of a peristaltic pump on the outlet of the 50 channel collector serves to equalize the flow through the individual channels to insure

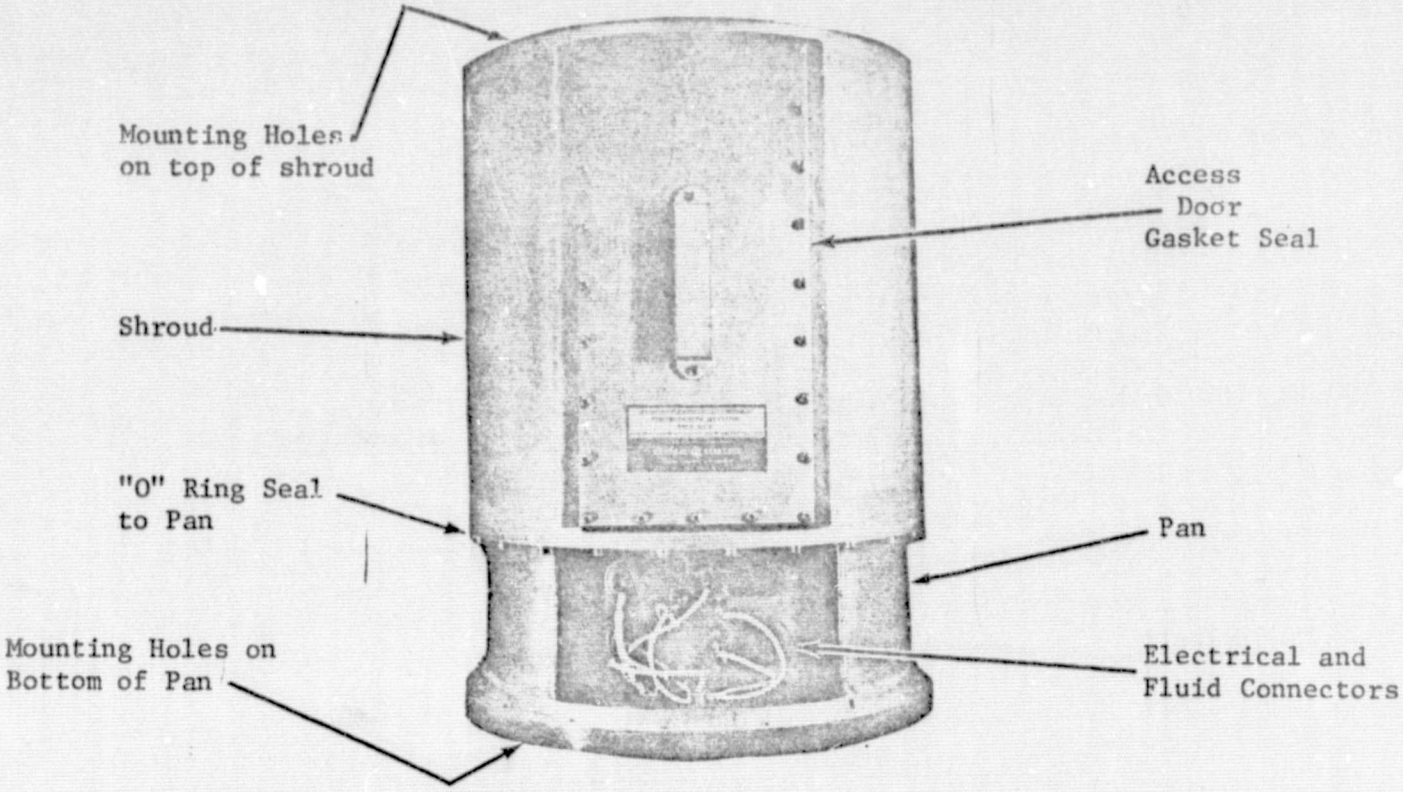


FIGURE 1. ESE UNIT - EXTERIOR VIEW

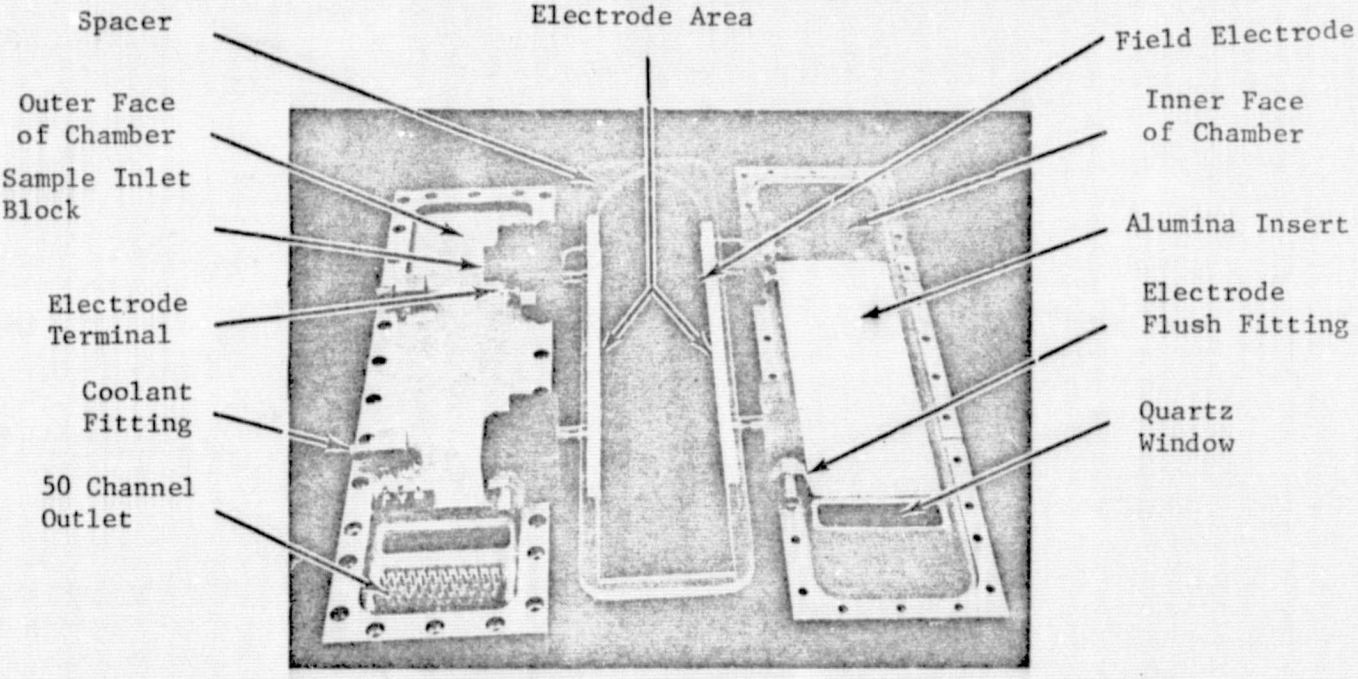


FIGURE 2. ELECTROPHORESIS CHAMBER

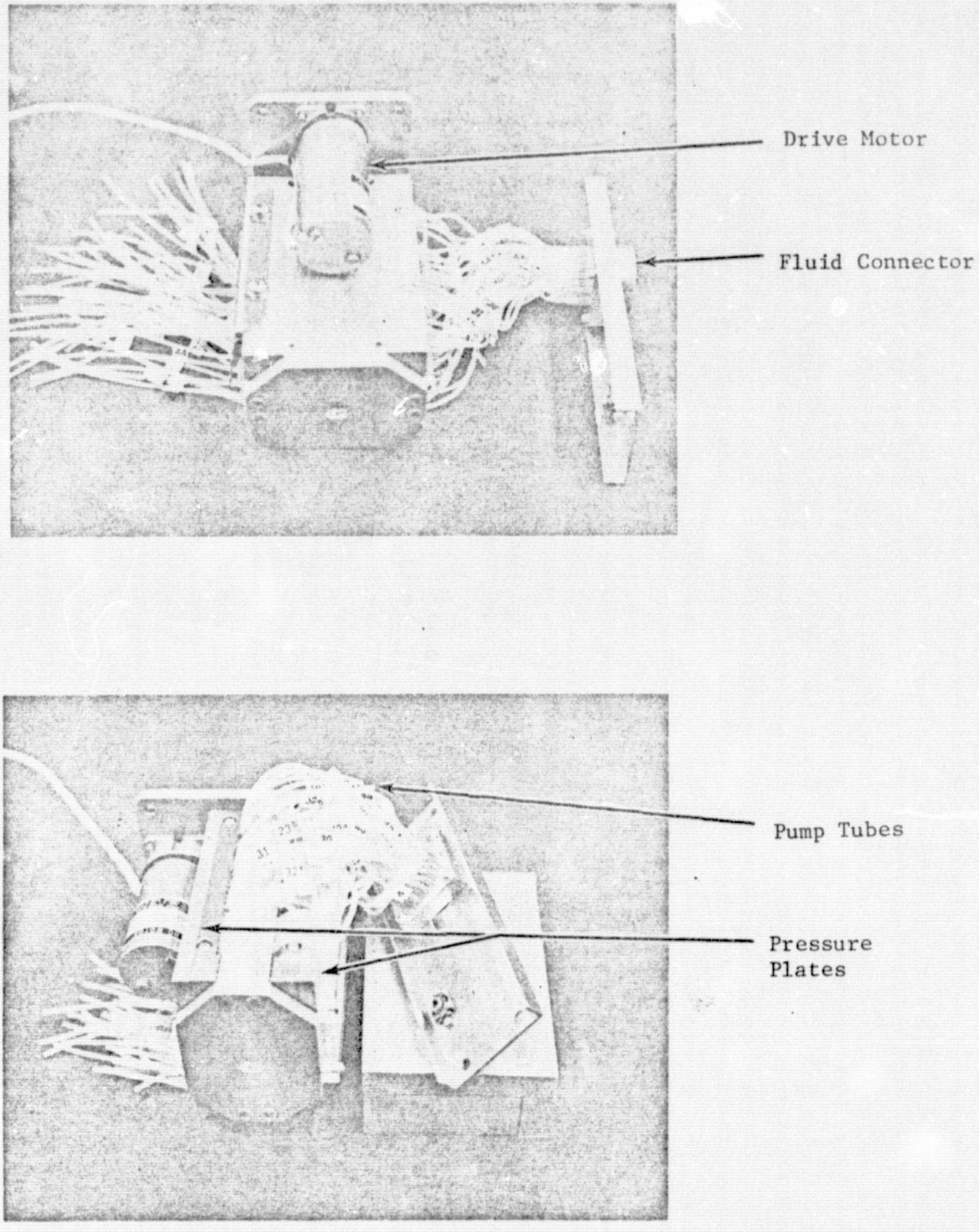


FIGURE 3. 50 CHANNEL PERISTALTIC PUMP

maximum uniformity of the flow in the cell. It also serves to isolate the cell from the collection container since the pump actually pinches off the tubing that passes through it.

4. Buffers, Electrolyte and Storage

The medium in which the sample is electrophoresed is a buffer. The buffer used will be selected by the Principal Investigator. However, some general guidelines for buffer properties can be given. The buffer used should have a conductance no less than 2×10^{-4} $ohm^{-1}cm^{-1}$. This is dictated by the nature of the electrophoresis process. Severe sample distortion is to be expected, at any lower conductance. The ESE system is designed for a maximum power density of 3.5 $watts/cm^3$ in the chamber. This number was derived from computer calculations that assumed the temperatures at the center of the chamber could not exceed 37°C (body temperature), that the walls were at a uniform temperature of 4°C and that the buffer was stationary. Obviously, other assumptions could have been made to yield a different result. It is believed, however, that the 3.5 $watts/cm^3$ is a useful number that will come very close to describing the chamber capabilities under operating conditions which are yet to be defined. On this basis we may use the following equation:

$$(E)^2 \, ke = 3.5 \ W/cm^3 \qquad (1)$$

E is the voltage gradient in volts/cm
ke is the conductance in $ohm^{-1}cm^{-1}$

So for a minimum conductance of 2×10^{-4} ohm^{-1} cm^{-1}, the maximum field would be 132 V/cm or an applied voltage of 660 volts. Of course, any lower gradient may be chosen and the conductance of the buffer adjusted to match.

In the case where a living, mammaliam cell is to be the sample, the osmolarity (related to osmotic pressure) of the buffer must be in the range of 0.270 Om to 0.310 Om. Higher or lower osmolarities will cause irreversible

damage to the cells. Sorbitol is recommended to bring up the osmolarity of a buffer, although other sugars may work as well. The amount needed will depend upon the other solutes in the buffer.

There is another fluid in the system which is used to flush electrolysis products from the electrodes and is called electrolyte. This fluid may be simply a salt solution or another buffer. Use of a buffer is recommended to keep pH changes to a minimum. For simplification it may be the same buffer used in the chamber but it should be about 10X as concentrated.

Both the buffer and electrolyte are stored in plastic bags inside a thermally controlled storage compartment.

The buffer and electrolyte bags can be filled externally through the use of switching valves located inside the unit.

5. Sample Injection (Figure 4)

The sample injection is achieved through the use of a specially designed, refrigerated, motor driven syringe. The refrigerant is passive and is contained in a jacket surrounding the syringe. The speed of the motor may be adjusted to provide a sample linear velocity appropriate to the buffer linear velocity. Another significant feature is that a mechanism for stirring the sample is provided for samples which may be subject to sedimention in normal gravity (e.g. mammalian cells).

6. Sample Collection Box (Figure 5)

After having passed through the electrophoresis chamber, the 50 channel collector and the peristaltic pump, each fluid channel is directed to an individual collection bag located within a collection container. This container is also passively refrigerated and is connected to the peristaltic pump through a specially designed fluid connector.

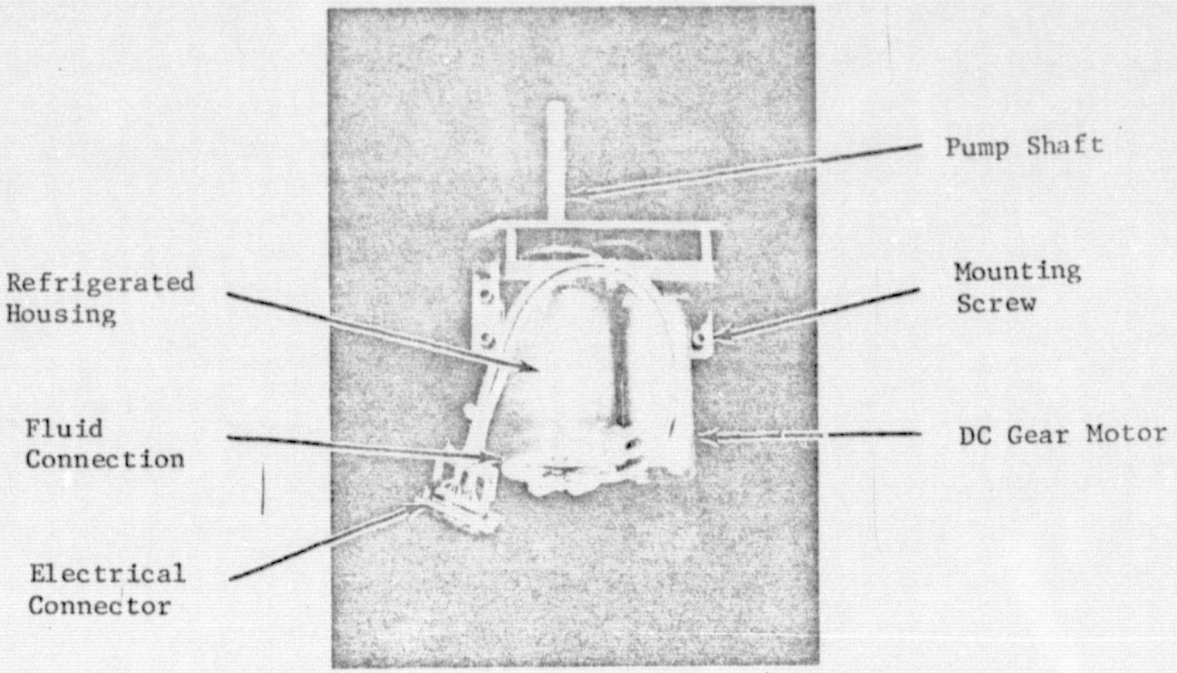


FIGURE 4. SAMPLE PUMP

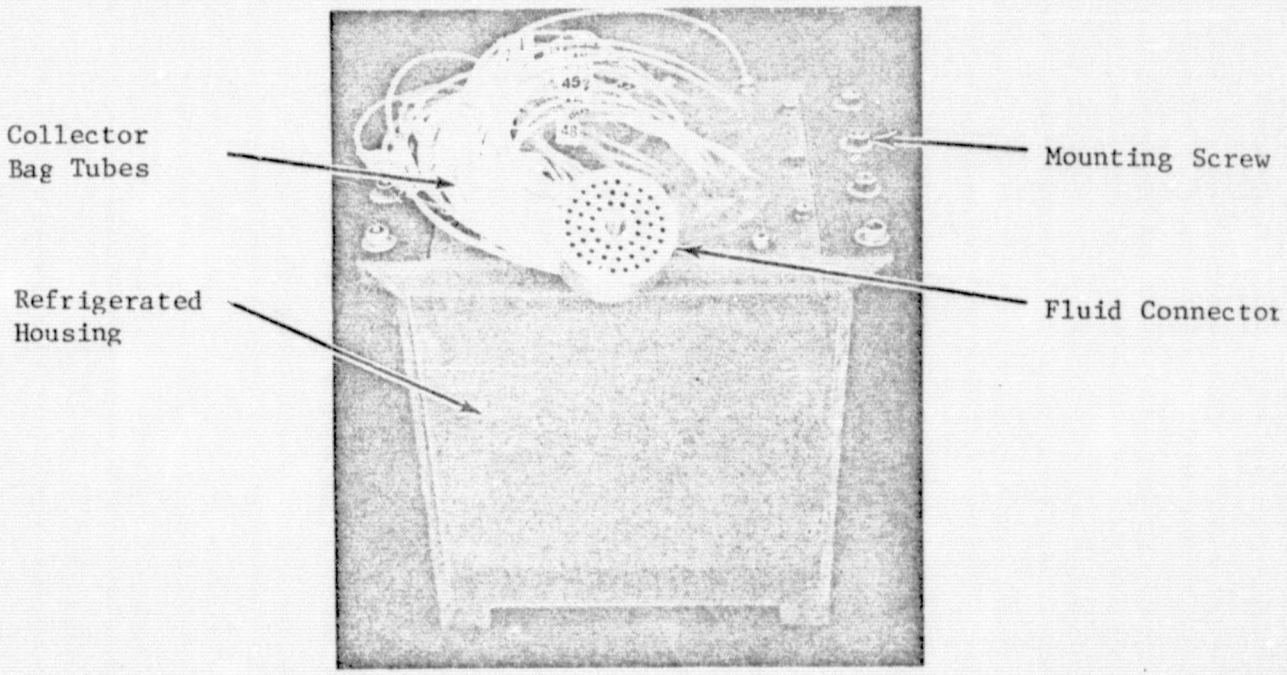


FIGURE 5. COLLECTION CONTAINER

7. Fluid Storage and Refrigeration System

The fluid storage assembly is constructed as a tube within a tube. The buffer and electrolyte fluids are stored in plastic bags in the inner tube. The space between the inner and outer tubes is filled with a passive refrigerant (eutectic solution) which serves to keep the fluids cool and as a heat sink for cooling the electrophoresis chamber during operation. Two sets of fluid coils are provided in the refrigerant chamber. One set of fluid coils is connected through quick disconnect fittings to an external source of cooled fluid prior to launch. This is used to freeze the refrigerant initially. The second set of coils is connected through a circulating pump to the cooling jackets on the electrophoresis chamber. This set of coils serves to cool the electrophoresis chamber during operation.

The material used at present will maintain a constant temperature of $4^{\circ}C$ for about 6 hours, depending on ambient conditions. Other solutions may be substituted with different eutectic or freezing points.

8. Detector

The detector system is composed of a linear photodiode array, optics and an ultraviolet source. The ultraviolet source (a mercury vapor lamp) is imaged into the middle of the chamber where sample fractions may block the radiation. On the other side of the cell, the photodiode array "looks" into the middle of the chamber and will detect any blockage by the sample. The light to the detector is optically filtered to pass only 253.7 nm wavelength. The output from the array is processed electronically and a video signal is available at the telemetry connector.

The U.V. source is powered by a DC to AC converter which employs a single photodiode in a feedback circuit to maintain the source intensity constant.

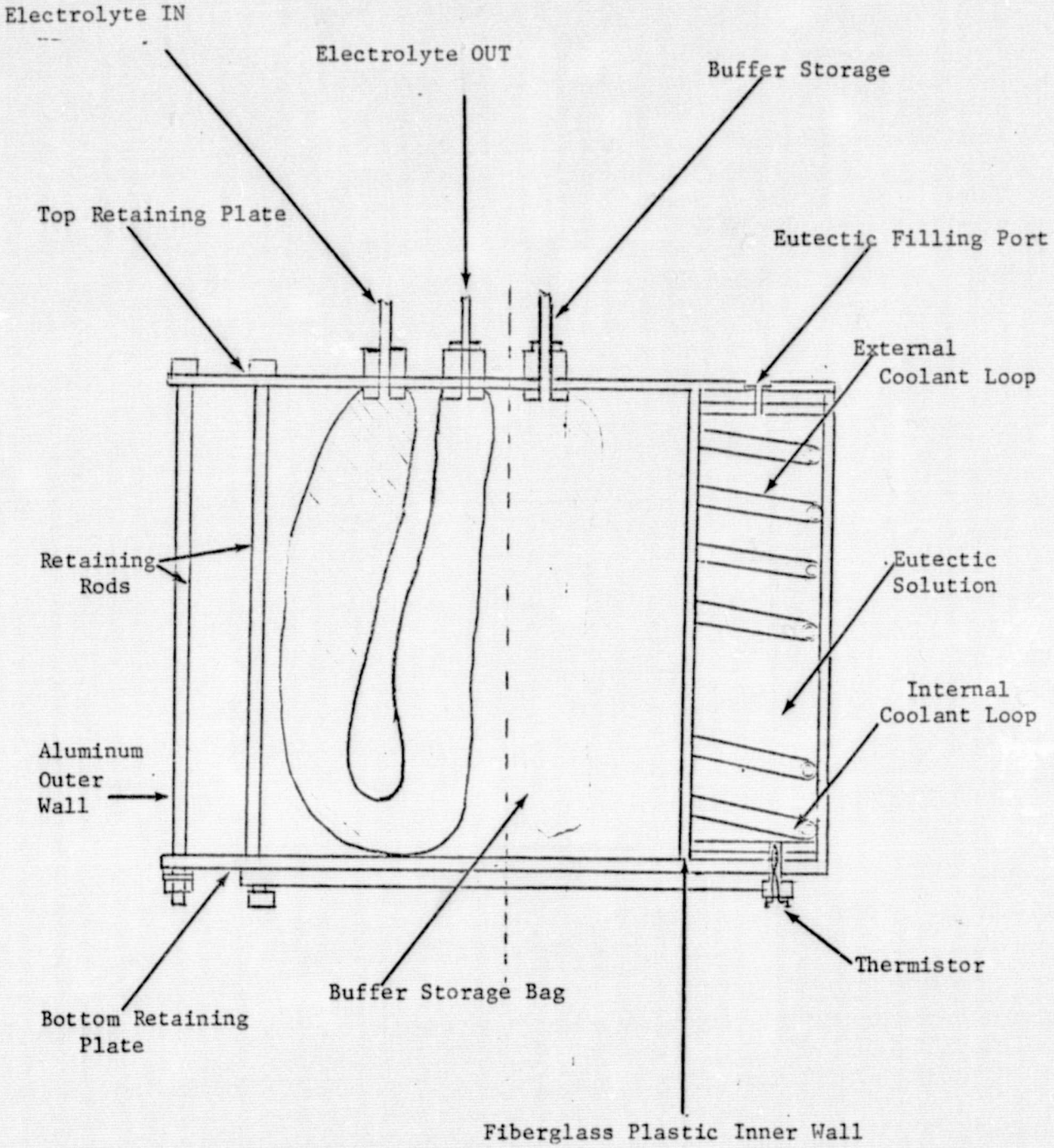


FIGURE 6. CUT AWAY VIEW: STORAGE CONTAINER

The photodiode array is about .512" long and is optically focused on a 0.75 inch width in the cell. Resolution is about 0.4% or 1 mm. Figure 7 depicts a typical output.

9. Electrical

The power distribution system in the ESE unit is shown in block diagram form in Figure 8. There are six separate supplies to provide the necessary voltages, currents and frequencies to operate the unit. The main supply is a 50 watt DC to DC type manufactured by Tecnetics that provides $\pm$ 15 volts DC and +5 volts DC. This unit supplies power for most of the low level electronics. A second supply, also manufactured by Tecnetics, is a 3 watt unit supplying $\pm$ 15 volts DC. It is used to power the operational amplifier on the photodiode that senses the output of the UV lamp. The third supply is a Tecnetics 28V DC, 3 watt unit used to power the current sensor that measures the electrophoresis current. Separate power supplies are necessary to provide isolation between high level and low level return lines to minimize noise in the telemetry signals. The fourth supply is a DC to DC unit manufactured by Abbott that supplies the polarizing voltage for the electrophoresis chamber. This may be a 50 or a 90 watt unit depending on the chamber voltage and resistance parameters established by the Principal Investigator. The fifth supply is a custom built low power unit intended to supply two phase, low frequency power required to run the stirrer in the sample pump. The sixth supply is the already mentioned high voltage AC supply for powering the UV lamp. This supply was also built in-house.

10. Telemetry

Several monitoring points are brought out on the pan at a connector and are available for the vehicle telemetry system. The points monitored are:

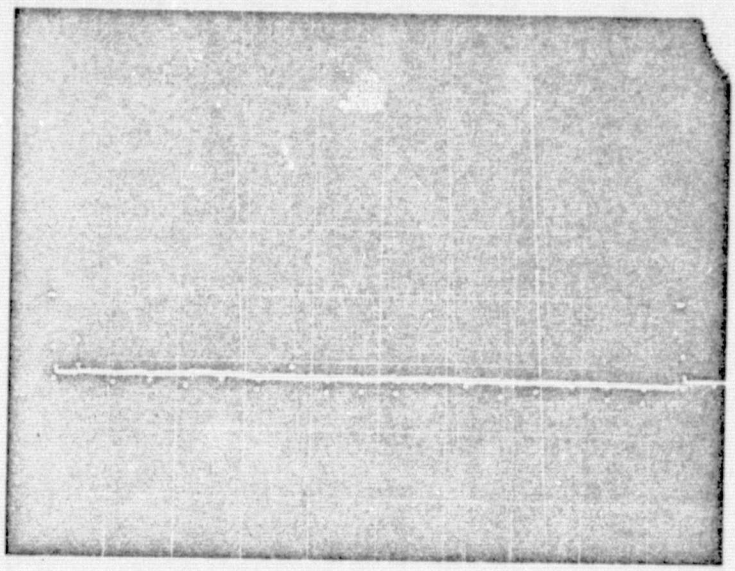


Figure 7. Typical Reticon Output
No sample present. Full Scale deflection = 1.2 volts at 1 V/cm

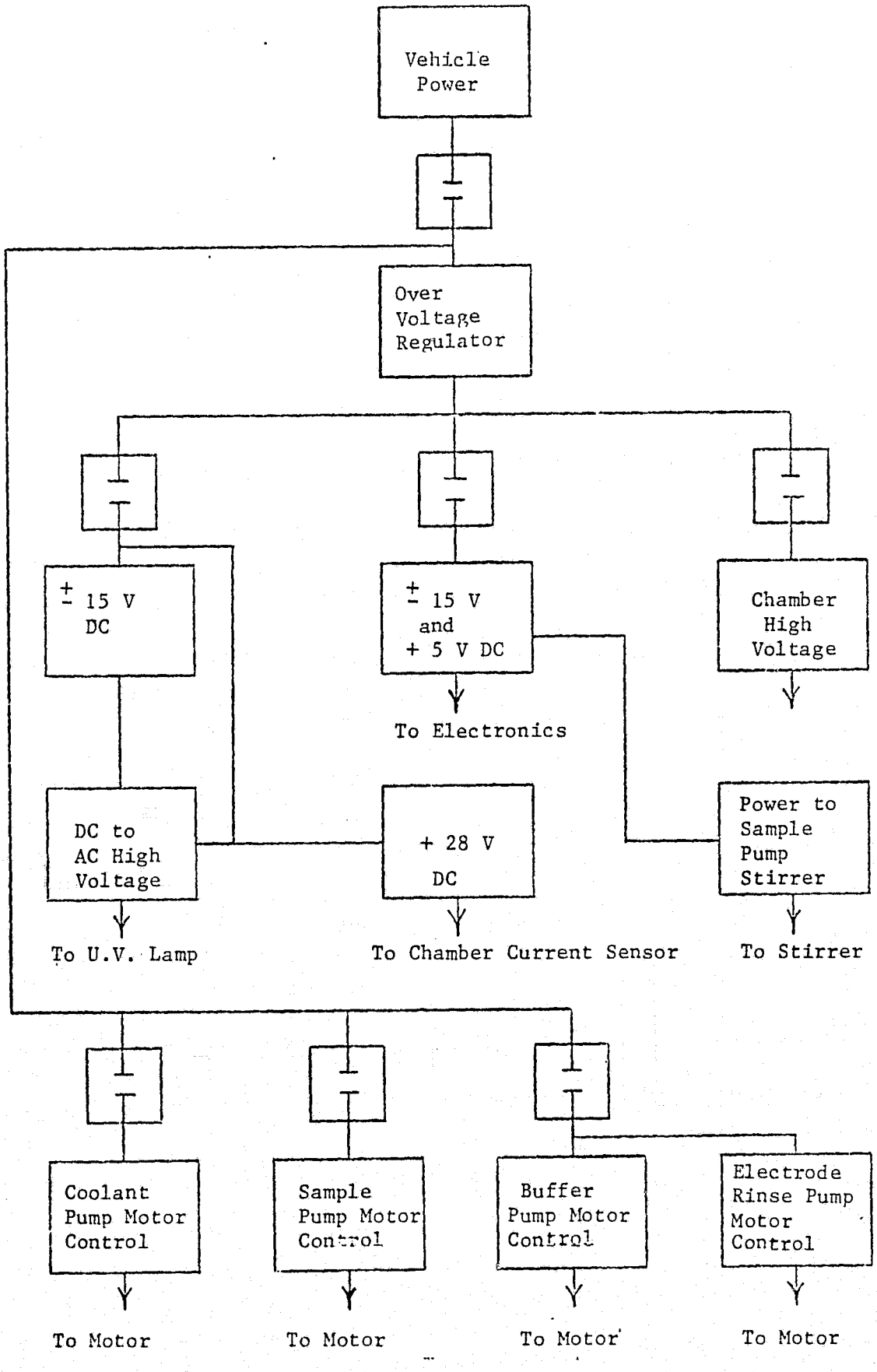


FIGURE 8. POWER DISTRIBUTION DIAGRAM

buffer pump motor, sample pump motor, coolant pump motor, electrode rinse circulator, sample stirrer, UV source, video, buffer storage temperature, sample temperature, chamber inlet temperature, chamber outlet temperature, chamber field voltage and internal pressure.

11. Control Console

The control console for the ESE is shown in Figures 9 and 10. It consists of two sub-assemblies; a control panel and a test box. The control console, with both sub-assemblies in place, will be used for bench tests and pre-integration checkout. The test box, which is removable from the console, is designed to be used for test and control functions which need to be performed through the access door on the launch vehicle.

The test box incorporates a 21 position rotary selector switch, a digital voltmeter, seven control switches with red and green illumimated switch position indicators and the necessary electrical plugs for making connections to the ESE package. Control switches are wired in such a way as to actuate the various ESE functions without going through the relays that normally control those functions.

This feature provides a method of control through the access door of the SPAR vehicle when in the vertical position on the launch tower just hours prior to launch. The ESE assembly may be totally isolated from the SPAR internal power system so that power can be applied through the access door to operate the functions within the apparatus.

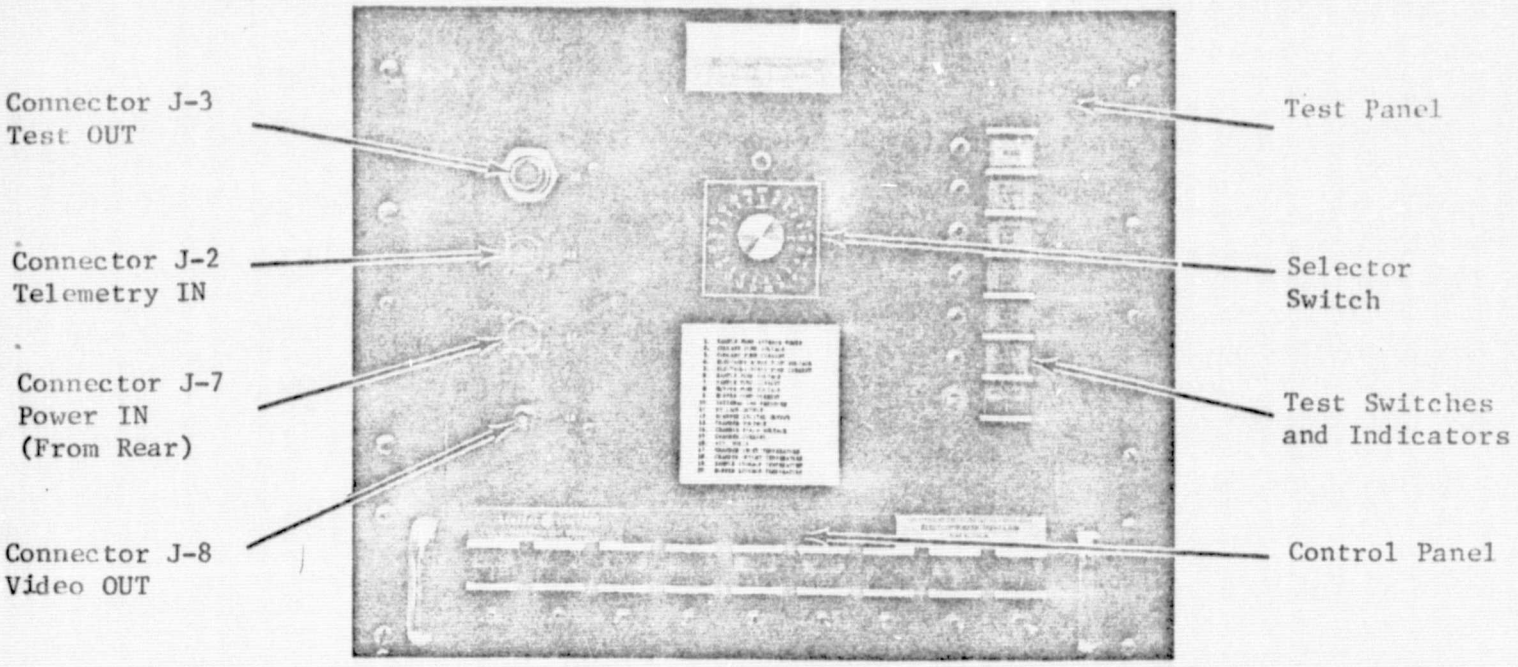


FIGURE 9. CONTROL CONSOLE - FRONT VIEW

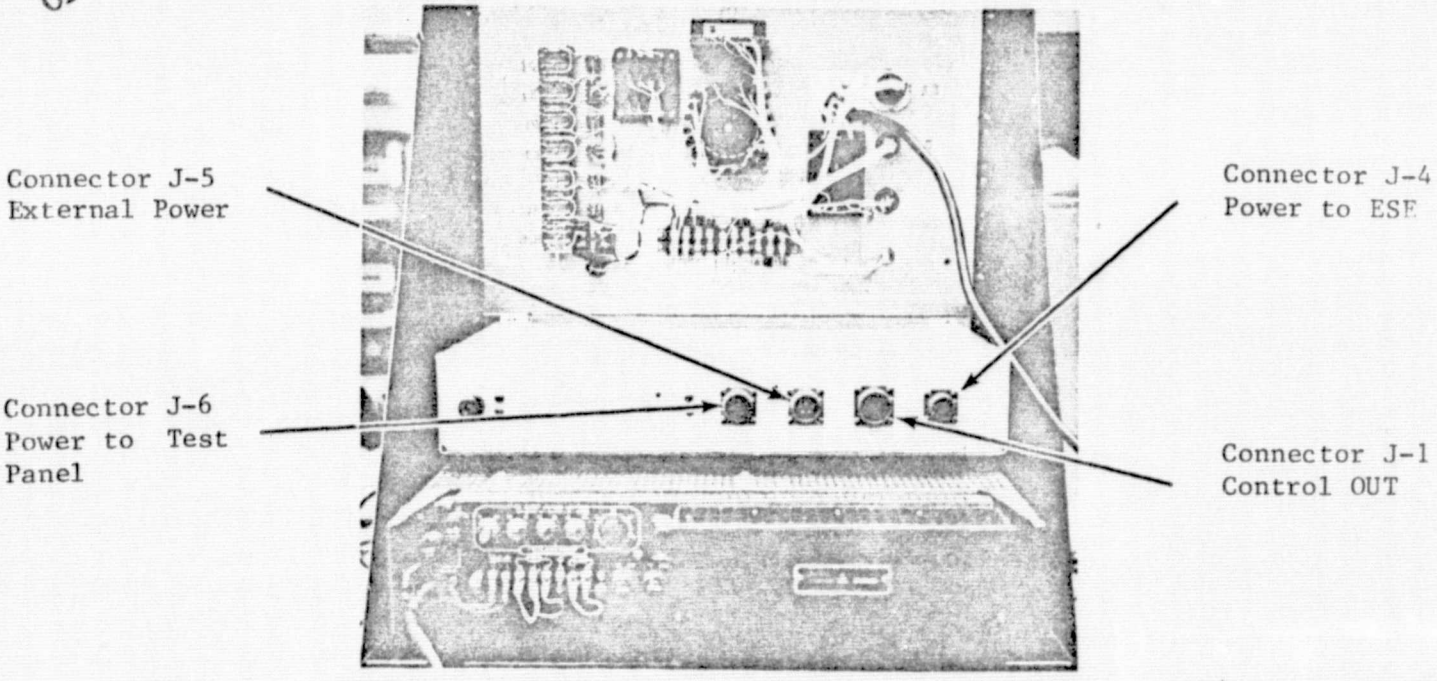


FIGURE 10. CONTROL CONSOLE - REAR VIEW

The control panel on the console has 9 switches with red and green illuminated switch position indicators. These switches also actuate the various ESE functions but work through the relays internal to the ESE. Thus, tests using the entire console are slightly more rigorous than tests using only the test box.

It should be noted that the console does not incorporate its own power supply. A suitable supply is described in the Appendix

12. Ground Support Systems

These elements are needed to properly conduct an experiment with the ESE unit and are not supplied. Suggested equipment is listed in Appendix A.

a) Sterilization Equipment

A gas sterilizing set up is needed for sterilizing the ESE unit itself, the sample pump and the collection container. The buffers and electrolytes preferably should be sterilized by autoclaving, in the bags provided.

b) Fluid Transfer Equipment

A twin head peristaltic pump is needed to transfer the buffer and electrolyte solutions. During transfer, the effluent from the electrophoresis cell is to be monitored for pH and electrical conductivity and, therefore, a pH meter and a conductance bridge are needed.

c) Power Supplies

Power supplies are needed to conduct tests outside of a flight vehicle.

d) Refrigeration and Storage Equipment

The passive refrigerant in the storage compartment is frozen from a circulating refrigeration unit external to the ESE. Using the equipment suggested in Appendix A, a reservoir temperature of $-10^{o}C$ and an ambient temperature of $27^{o}C$,

it takes about seven hours to freeze the eutectic. The temperature of the reservoir and ambient conditions will cause this time to vary.

A freezer is needed to initially freeze the eutectic solutions in the sample pump and the collection container. Afterward, an insulated chamber (such as a picnic cooler) may be needed to store the pump and collection container, if the freezer is not convenient to the assembly point.

e) Tools

Two hex drivers and a torque wrench are needed to remove and install the access door, sample pump and collection container.

C. Programmable Functions

Several functions of the ESE unit are programmable to meet the needs of the Principal Investigator and the mission profile. These are: peristaltic pump speed to control residence time, sample pump speed to match the curtain velocity, electrode rinse pump speed, coolant pump speed, the chamber high voltage power supply, the time delay relays, and the intensity of the UV lamp. The pump speeds are set by adjustable resistors located on circuit board TB2A. The high voltage load resistor is located on TB3. The time delay relays are programmed using two connectors, one located near the sample pump and one located inside the pan.

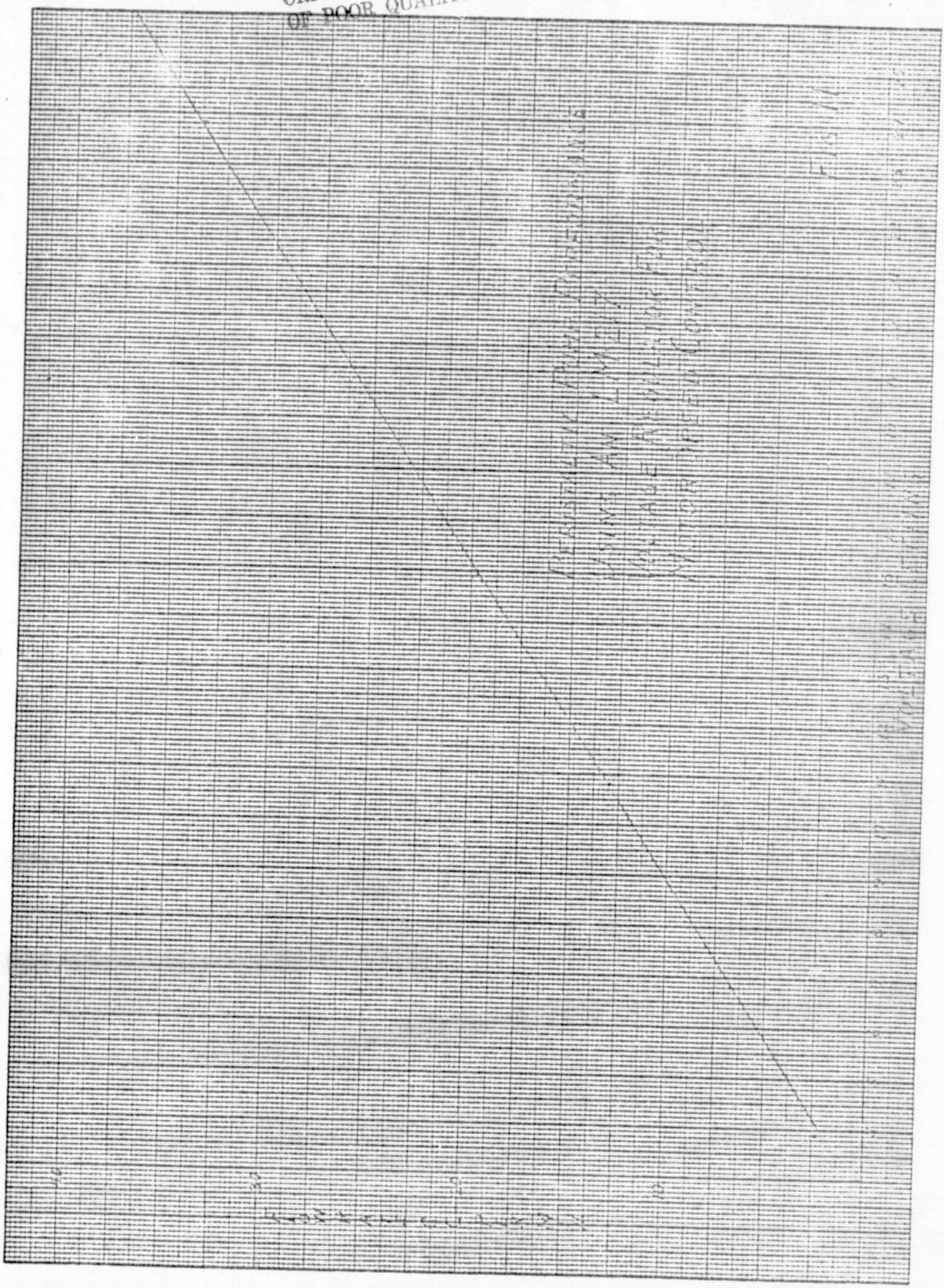
1. Pump Control System

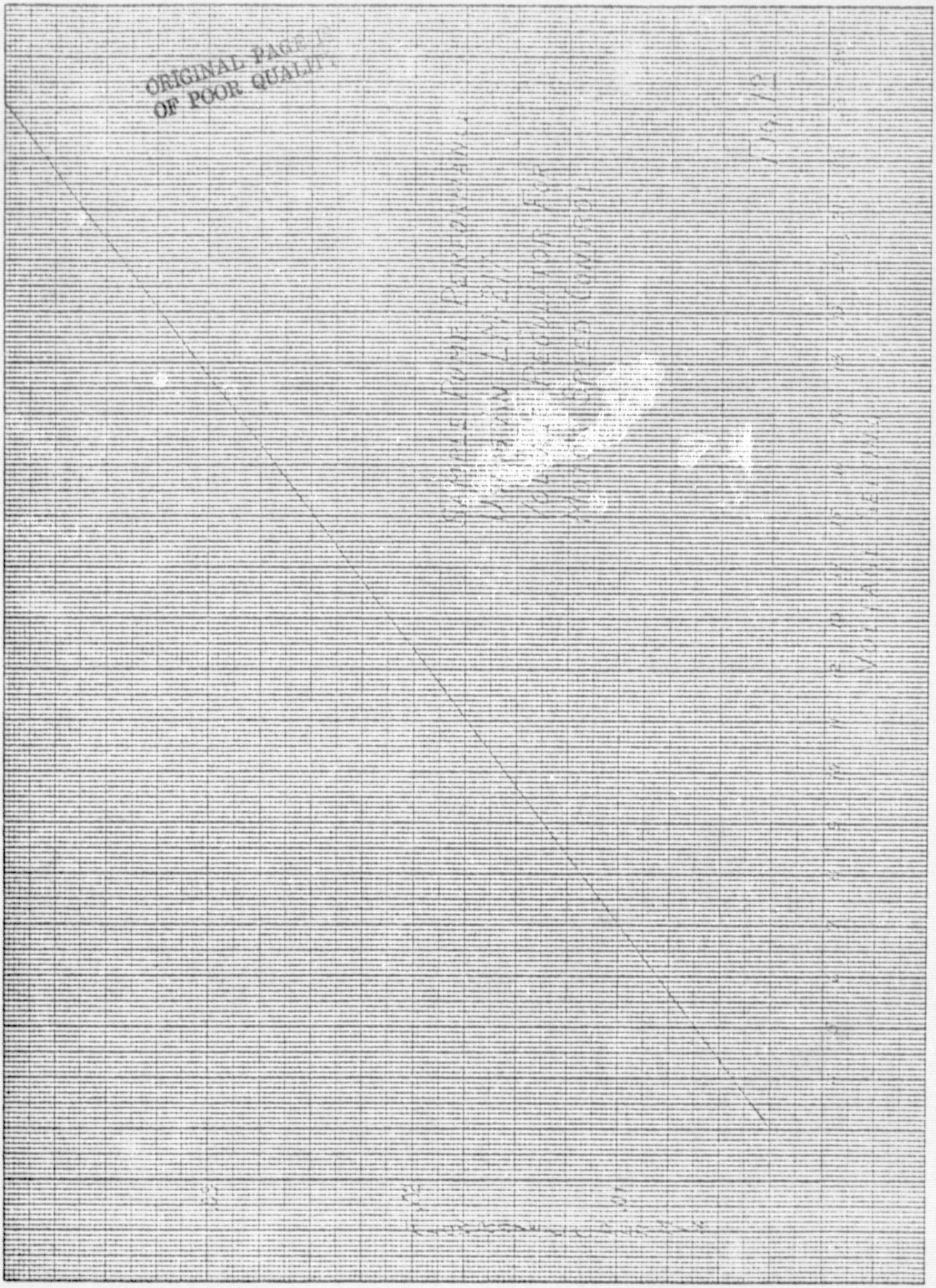
The pumping rate of the peristaltic pump must be adjusted to achieve the desired sample residence time. Because of boundary layer effects, rigorous calculation of residence time is complicated. However, for practical purposes we can use:

$$Tr = \frac{16.6}{F_b} \text{ where } F_b \text{ is volumetric flow rate is } \frac{cm^3}{sec} \qquad (2)$$

The speed is adjusted by varying Trimpot No. 26 on Tb-2A. The resistor is adjusted until the desired flow rate is achieved. Determine the voltage necessary to give the desired flow rate using Figure 11 and adjust to that voltage using the voltmeter in the test console. Note that this voltage is actually ten times the voltage indicated on the meter.

Once the flow rate of the buffer is established it is necessary to set the sample pump speed to give a sample injection velocity appropriate to that buffer flow rate. The present understanding of the behavior of a small stream when it is injected into a larger stream appears not to be sufficiently detailed to determine the ideal injection velocity from theory alone. It seems rather certain that the sample stream velocity should be somewhat less than the local buffer velocity and that any velocity below that will be all right. Obviously, the lower the sample stream velocity, the lower the throughput of the cell so it is desirable to keep it as high as possible without causing unsteady conditions. Experience to date seems to indicate that an average sample stream velocity of approximately 0.6 times the average buffer stream velocity works best. In the ESE chamber, the geometry is such that a sample pump flow rate that is 0.05% of the buffer flow rate appears to be satisfactory. It is not presently known whether the selection of buffer and/or sample has a significant effect on the optimum ratio of buffer to sample flow rate. It is believed, therefore, that the optimum ratio should be determined experimentally using real buffer and real sample and with other parameters as close as possible to those to be expected in flight. Actual adjustment of the sample pump flow rate is similar to that for the buffer pump by setting Trimpot No. 39 on Tb-2A. Flow rates can be set by referring to the sample pump calibration curve (Figure 12) and setting the voltage to that appropriate to the desired flow rate.





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The electrode rinse pump and the coolant pump are equipped with voltage regulator type speed control systems similar to those used with the buffer and sample pumps. These will normally be preset and will not require changing. Should change be necessary, rheostat R27 controls the electrode rinse motor and R-38 controls the coolant pump. Adjustment procedure is similar to that for the buffer and sample pumps.

2. Cell High Voltage Power Supply

The ESE unit is designed to use an Abbott 28 VDC to DC high voltage power supply for the chamber. Either the B5AS---A (50 watt) or the B10AEH---A) (22-32V) (90 watt) series of supplies may be used. The particular power supply chosen will be determined by the requirements of the Principal Investigator and the design equation given in Section I.B.4. The supply to be chosen is designated as B5AEVDCA or B10AEVDCA, where VDC is given below:

$$VDC = 5\,\bar{E} \qquad (3)$$

where $\bar{E}$ is the desired voltage gradient and is $\pm$ 132 V/cm

It is necessary to always have a load present at the output of this type of supply. This load should be 4% of the supply output. Therefore a resistor is to be installed across points 1 and 2 on TB3. See Figure 13. It must be rated for at least 5 watts and its value is calculated using Equation 4.

$$R_i = \frac{(VDC)^2}{.04W} \qquad (4)$$

where R_i is in ohms and W = Power Supply Output rating

The high voltage supply is only adjustable to $\pm$ 6% about the center voltage. The proper supply must be purchased for the required center voltage. This power supply is to be installed prior to final assembly.

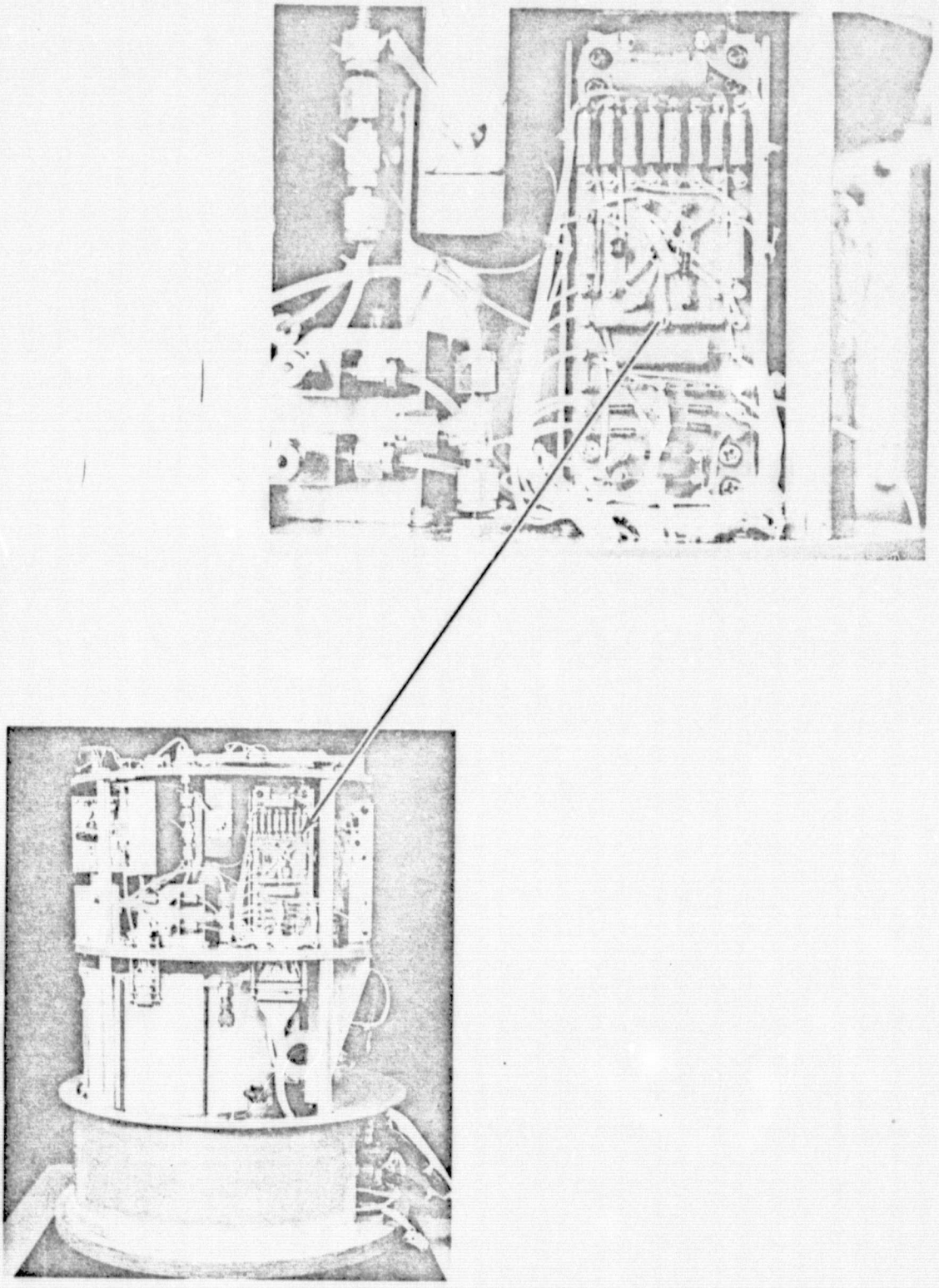


FIGURE 13. LOCATION AND VIEW OF TB-3

3. Time Delay Relays

The ESE unit uses time delay relays to 1) start the sample pump after launch and 2) shut the system down after the "micro-g" portion of flight. The ESE apparatus was designed with the expectation that it would be used initially on SPAR flights using the Black Brant vehicle. The system was also designed to be as flexible as possible by permitting "last minute" changes to the resistors which control the settings of the time delay relays which are mounted on a plug P18 on the sample pump mounting plate. Furthermore, to accommodate longer flight times, such as might be provided by an ARIES launcher, two additional time delay relays were incorporated into the system. Thus three relays may be connected to operate in sequence to provide for operation times up to 1500 seconds. A plug, P-13, inside the pan section of the ESE package, (see Figure 14) is provided for selecting the number of time relay relays to be used.

Time delays for each of the four time delay relays may be set by using the formula:

$$R = 2T - 100$$

where R = resistance in kilohms
T = the desired time delay in seconds

Note that time delays cannot be set below 50 seconds (zero resistance). The selected resistors, which should be 1/8 or 1/6 watt 1% precision metal film types, are mounted on a standard connector plug, see Figure 15. This plug is installed below the sample pump connection, see Figure 16. System wiring is such that the resistor (R-11) for the turn-on time delay relay (K-4) is connected to pins 1 and 6 on the plug. R-12 for the first operating time relay (K5) is connected to pins 2 and 7. R-13 for the second operating time relay (K-6) is connected between pins 3 and 8. R-14, for the third operating

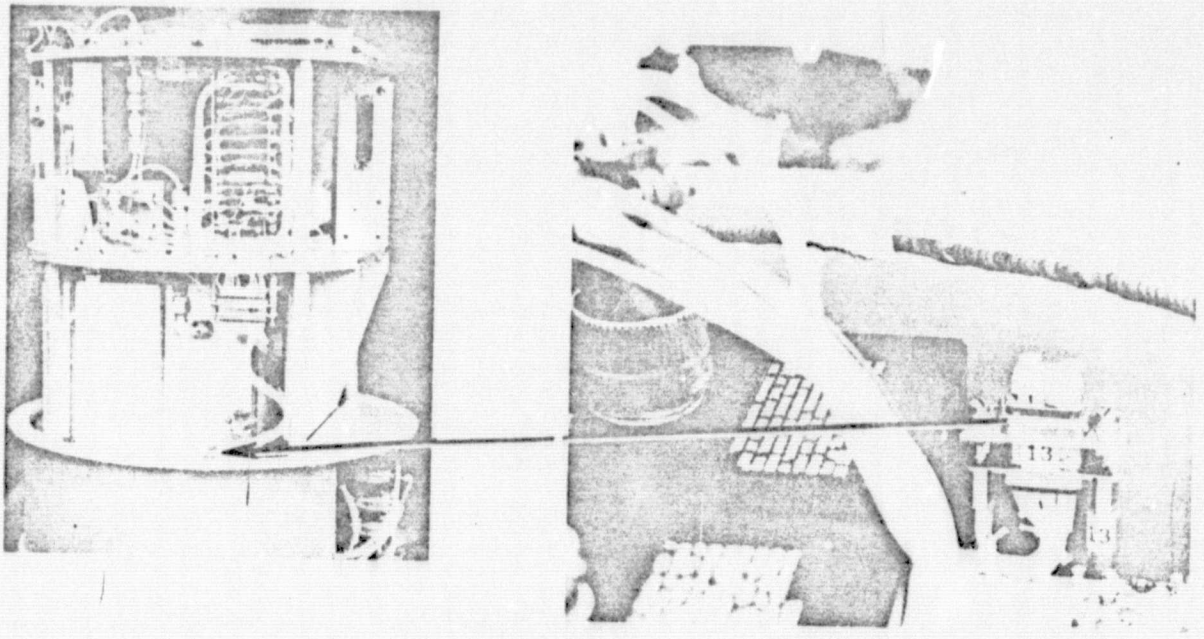


FIGURE 14. LOCATION AND VIEW OF SHORTING PLUG

FIGURE 15. TIME DELAY PLUG

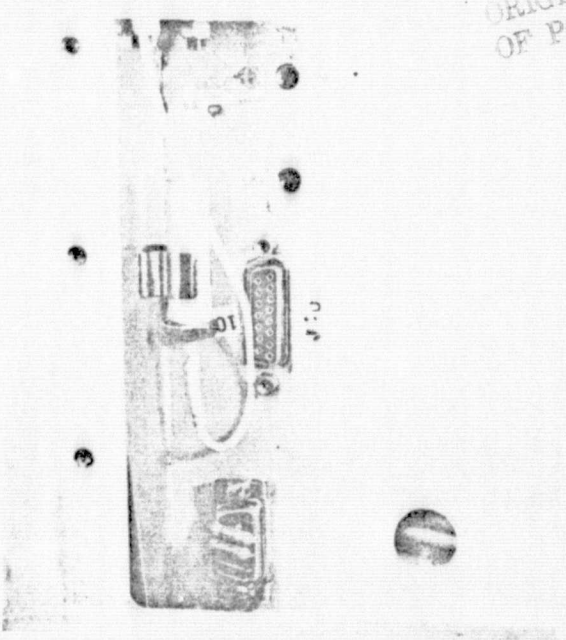
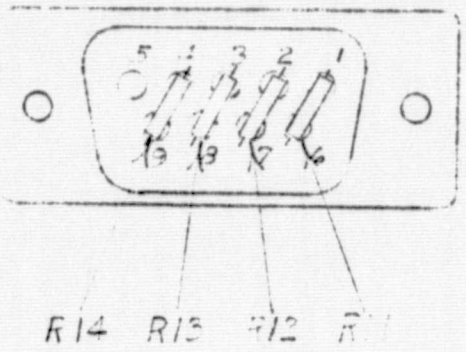


FIGURE 16. TIME DELAY PLUG INSTALLED

time relay (K-7) is connected between pins 4 and 9 (see Figure 17). Because the ESE apparatus is expected to be flown first with a Black Brant vehicle, the timing plug will normally be equipped with a 20K resistor for R-11, a 500 K resistor for R-12, an 800K resistor for R-13 and a 900K resistor for R-14. This will give a delay of sixty seconds after lift-off before the sample pump starts and a running time of 200 seconds if K-5 is used alone, 750 seconds if K-5 and K-6 are used and 1150 seconds if K-5, K-6 and K-7 are used. The selector plug, inside the pan, will normally be delivered with Pins 1 and 7 connected together so that K-5 will usually be used alone. This arrangement should be satisfactory for flights on Black Brant, For longer operation times up to the capacity of two delay relays (1000 seconds) the jumper in the selector plug should be unsoldered from pin 1 and soldered to pin 4. Then pins 1 and 2 should be connected together. For still longer delays, up to 1500 seconds, the jumper from pin 7 should be unsoldered and rewired so as to connect pins 6 and 7 together. Then jumpers should be installed between pins 1 and 2 and between pins 4 and 5 as necessary. It will be noted that to achieve the maximum delays each relay must be connected to a 900K control resistor. Relay K-7 will normally be supplied with a 900K control resistor (500 seconds delay) and relay K-6 will normally be supplied with an 800K control resistor. It is recommended, therefore, that time delay selections be made by changing R-11 and R-12 as necessary, leaving R-14 always as 900K and changing R-13 only if an operating time between 950 and 1000 or between 1450 and 1500 seconds is necessary.



R_{11} - Variable pump start-up delay

R_{12} - Operation - up to 8 min.

R_{12} & R_{13} -

Operation - up to 16 min.

R_{12} - R_{13} - R_{14} -

Operation - up to 25 min.

Figure 17. Connector P_4, Time Delay Programming Plug.

4. UV Lamp Intensity

The output intensity of the UV lamp is adjustable from the potentiometer mounted on the lamp supply board (see Figure 18). The intensity should be adjusted with the cell filled with the buffer chosen by the Principal Investigator. With the Reticon/lamp system operating, observe the video output from the Reticon (see Section II.B.1) on an oscilloscope and adjust the potentiometer for a 4.8 volt full scale signal from the Reticon array. Once this is set, the feedback circuit will act to keep the intensity constant.

II. OPERATIONAL PROCEDURES

A. Biological Handling

1. Electrophoresis Chamber and Ancillary Equipment

a) Initial Filling

The ESE normally operates with the chamber and all associated plumbing completely filled with fluid. Trapped air pockets can have a serious effect on proper operation. Therefore it is strongly recommended that the initial filling of the fluid systems (and refilling if the ESE is emptied or partially emptied) be done under reduced pressure. This is most conveniently done in a vacuum chamber or bell jar as shown in Figure 19. A mechanical vacuum pump is adequate.

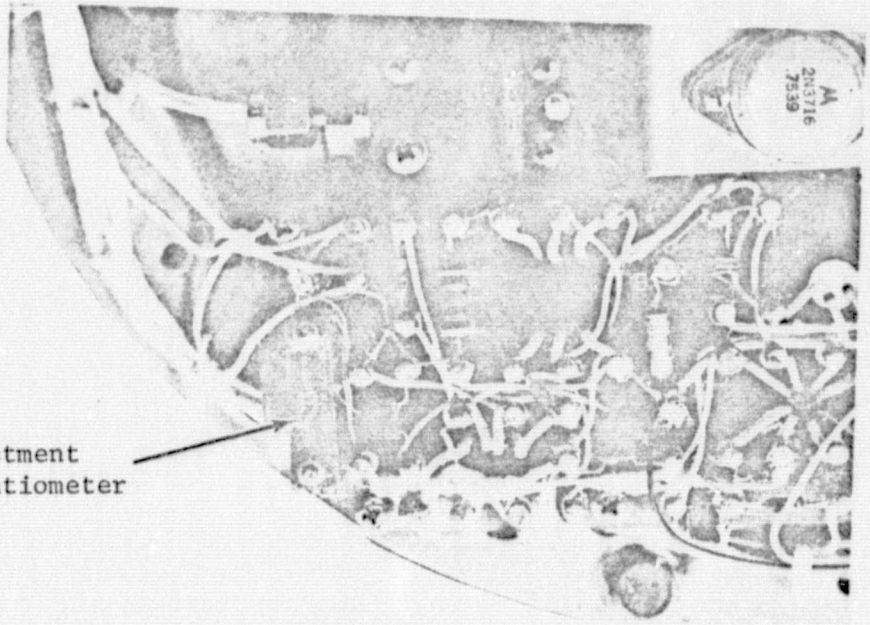


FIGURE 18. VIEW OF TOP DECK SHOWING INTENSITY ADJUSTMENT FOR U.V. SOURCE

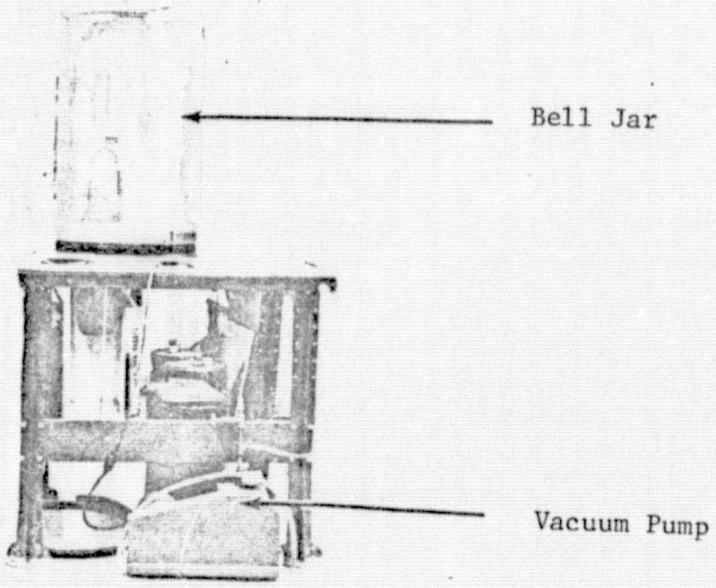


FIGURE 19. VACUUM CHAMBER FOR INITIAL FILLING OPERATIONS

Standard practices should be followed to protect oil pumps from water contamination. The vacuum chamber and all fittings and hardware should be as clean as possible in order to minimize bacterial and fungal load in the ESE. Where possible, surfaces should be wiped with Betadine Solution (Povidone Iodine, Purdue Frederick Co., Norwalk, Conn.), using clean cotton gauze. Inaccessible places (e.g. valves and fittings) should at least be rinsed with 95% ethanol.

If the vacuum system is located in a Class 1000 or better clean area, further precautions are probably unnecessary. If in an unfiltered environment, the use of a submicron filter on the chamber vent is necessary. This can be of any type with a pore size $\leq$ 0.45 μ. Gelman type 12106 filters are satisfactory.

The sample pump (Figure 4) and the fraction collection assembly (Figure 5) are removed from the ESE if installed. Valve A with the red handle, should be in the down position (see Figure 20). In order to prevent any pressure differentials which might damage the unit, all three fluid systems should be filled simultaneously. This requires breaking into each system at some point. For the buffer system, the most convenient place is at the chamber inlet; for the electrolyte, any one of the four electrode connectors and the coolant loop at the circulating pump.

Fluids for each system are placed inside the vacuum chamber with the unit. Buffer and electrolyte solutions should normally be sterile but coolant need not be. Autoclaved silicone tubing is connected at each break and placed into the appropriate fluid. The sample injection tube should also be immersed in the buffer.

Pressure in the chamber is slowly reduced to avoid "bumping" of the fluid. Pressure of a few torr or less should be held for a few minutes. (The pressure reading will depend on the sensitivity of the detector to water vapor.

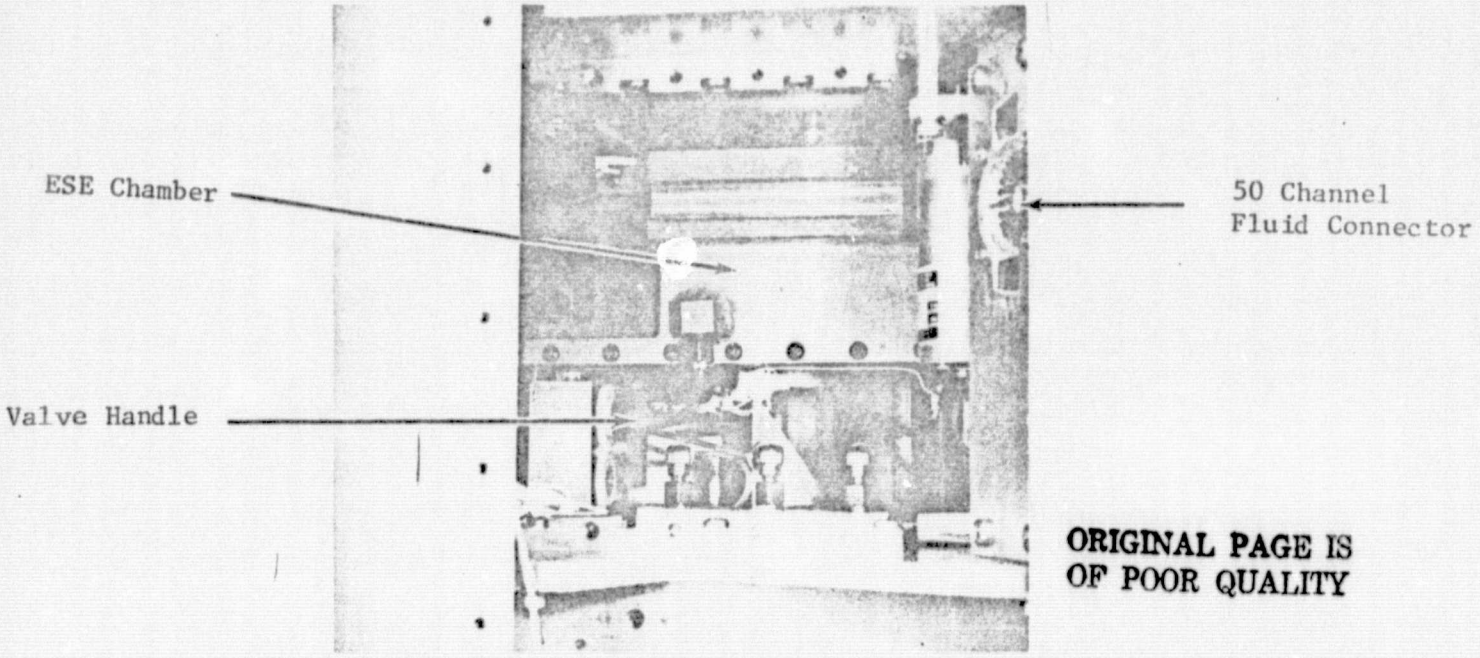


FIGURE 20. VIEW THROUGH ACCESS DOOR
VALVES IN OPEN (HANDLE DOWN) POSITION

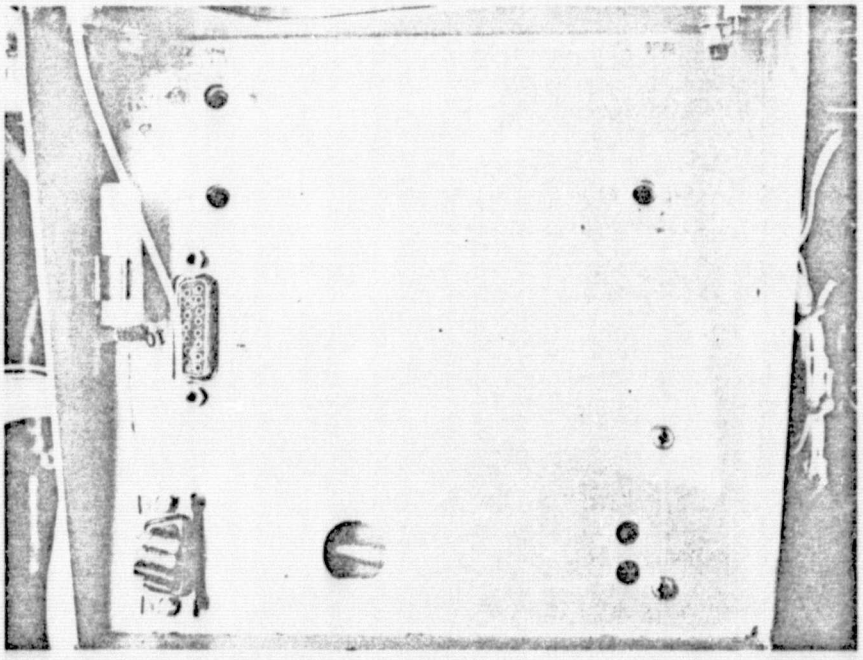


FIGURE 21. VIEW THROUGH DOOR SHOWING TEFLON CAP FOR INJECTION NEEDLE

A simple mercury manometer should be adequate.) The vacuum pump is valved off and the chamber slowly vented. Pressure rise should be limited to about 100 torr/minute, to avoid excessive pressure on the electrophoresis chamber walls and windows.

When the vacuum chamber has been vented to atmosphere and opened, check for bubbles in the cell and tubing to the extent possible. If bubbles are seen, check for leaks and repeat the vacuum filling process. If no bubbles are seen, reconnect the fittings for the three fluid systems. Fill the cap for the injection tube (Figure 21) with fluid, make sure the silicone tubing sleeve is in place over the needle, and fasten the cap to the sample injection tube.

b. Fluid Exchange

There may be a number of occasions for changing the fluid contents of the ESE. If the ESE is free of air bubbles, the vacuum procedure outlined in a) above is not necessary. In this case a simple procedure for exchanging fluids should suffice. In the following discussion equipment and fluids may or may not be sterile, depending on the nature and reason for fluid exchange. The operator should take whatever steps are necessary to produce and maintain the required degree of sterility. In any event, care must be taken not to introduce bubbles or random macroscopic contaminants into the fluid system.

Silicone tubing in a reversible double peristaltic pump (Section I,B,12) is connected to fluid connectors J25 and J26. See Figure 22. The pump is operated to withdraw fluid from the ESE until the buffer and electrolyte storage bags are substantially empty. The tubes are moved to containers of the fluid(s) to be installed in the ESE, care being taken that no air bubble exists in the tubing. The pump is then reversed and fluid is pumped into the

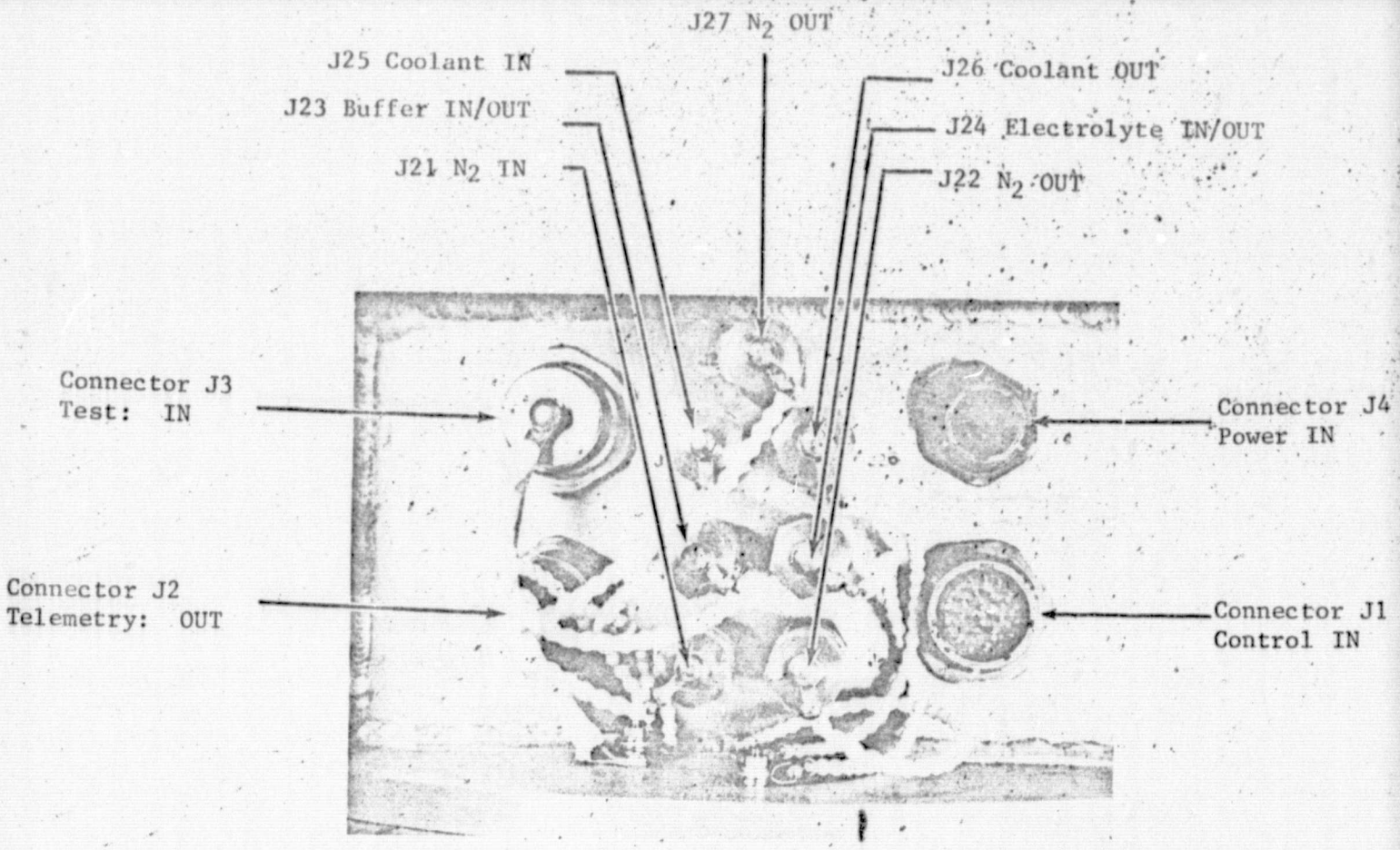


FIGURE 22. ELECTRICAL AND FLUID CONNECTIONS

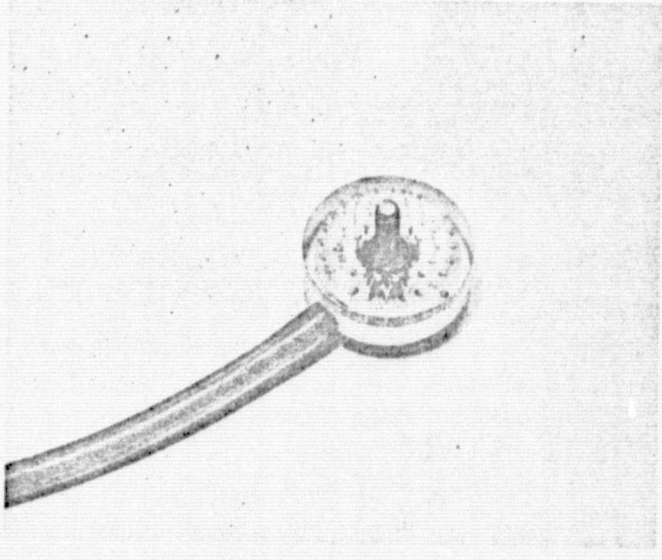


FIGURE 23. ACCUMULATOR

bags. It is suggested that about 200 ml be pumped into each bag. The pump is again reversed and the fluid withdrawn. This procedure is repeated until the nature of the fluid being withdrawn is substantially the same as that being installed as determined by conductivity, pH, or other appropriate measure.

The storage bags are then filled with 200 ml of the desired fluid(s) by reversing the pump as above. A tube is connected to fluid connection J27, and the accumulator (Figure 23), is connected where the sample recovery connector would normally go. Valve A (Figure 24) is rotated to the UP position and power is supplied to the ESE peristaltic pump through the Control Panel. Fluid emerging from the accumulator is measured. When the outflow from accumulator tube indicates the buffer storage bag is nearly empty, the ESE peristaltic pump is turned off. This is necessary to purge the chamber and the lines in the pump. The electrode rinse pump will run when the peristaltic pump is running and will pump fluid in the pump and associated plumbing back into the electrode rinse bag and will be removed when the bag is drained. Repeat the process as necessary until the drained fluids meet in the criteria established for flushing the storage bag.

c. Sterilization and Storage

The ESE chamber, storage bags, and associated tubing are conveniently sterilized with formaldehyde or similar fluid solutions. The following procedure is one which we have used successfully, however, it is to be expected that the Principal Investigator will alter this procedure or make substitutions as may be required.

The buffer storage and electrode flush (EF) storage bags are filled by whichever method, above, is appropriate with the primary sterilizing solution, formaldehyde-borate (See Appendix A). If method II.A.1.b is used it

FIGURE 24. VIEW THROUGH ACCESS DOOR
VALVES IN THE CLOSED (HANDLE UP) POSITION

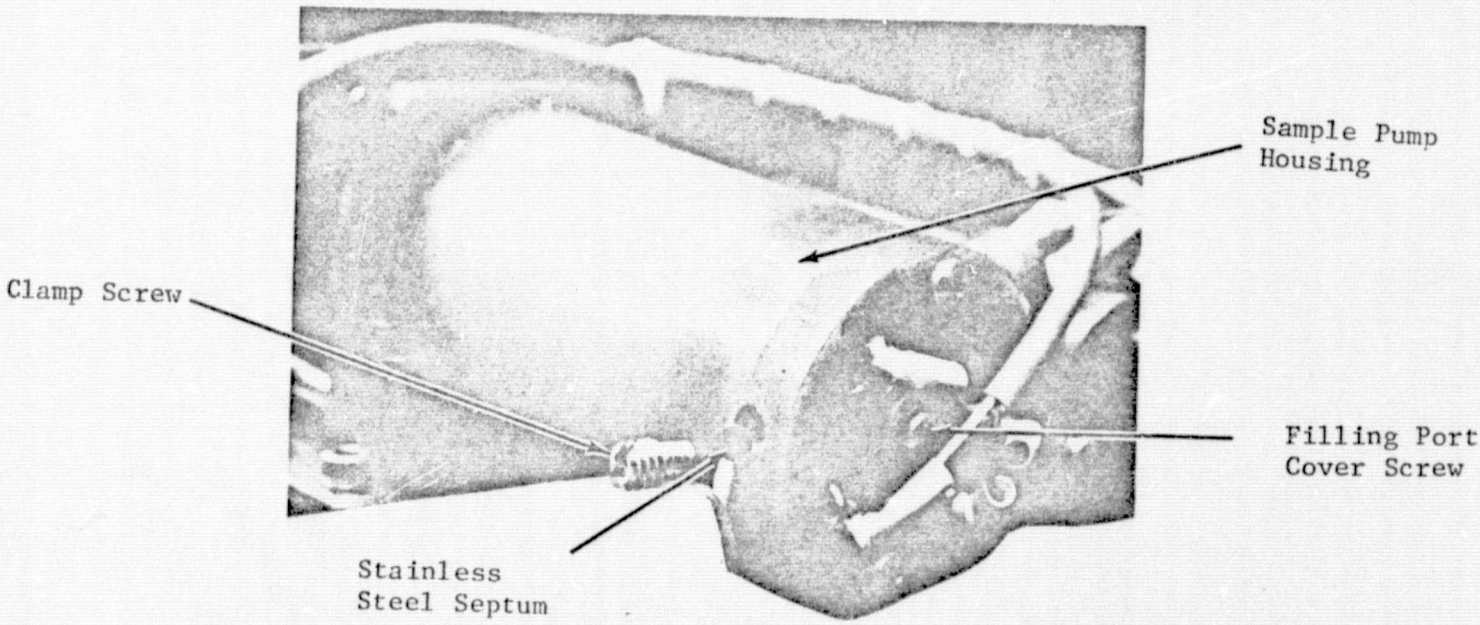


FIGURE 25. SAMPLE PUMP SEPTUM

will be necessary to operate the ESE peristaltic pump until about 200 ml of fluid have been withdrawn in order to ensure full potency of the sterilizing solution in the ESE chamber and electrode passages.

The formaldehyde solution must remain in the system at least sixteen hours. The primary sterilizing solution is removed from the storage bags by the method of II.A.1.b and is replaced by sterile distilled or demineralized water. This is pumped by the ESE pumps through the electrophoresis chamber and electrode chambers. The storage bags are refilled with sterile water and the process is repeated. When the storage bags have been filled and emptied twice with sterile water the effluent from the electrophoresis chamber is tested with Schiff's reagent for the presence of formaldehyde. The rinsing process is continued until a negative Schiff test is obtained.

At this time the secondary sterilizing solution is introduced by the procedure of II.A.1.b. It is a dilute solution of Zephiran (TM Winthrop Laboratories) in sterile water (1:7000). This solution is left in the apparatus for at least sixteen hours. The secondary sterilizing solution is then replaced by sterile water according to II.A.1.b and rinsing is repeated until the specific conductance of the chamber effluent is less than 3×10^{-6} ohm^{-1} cm^{-1}.

If it is desired to check sterility at this point, it can be done by catching about ten drops of effluent in a Trypticase Soy Broth tube (BBL #21093) or on Trypticase Soy Agar (BBL #11045). Other culture media, of course, may be equally satisfactory.

If the ESE is to be stored, the sterile water should be exchanged for a solution 0.1% w/v of sodium azide in sterile water. Steps should also be taken to minimize the bacterial load inside the ESE but outside the fluid

system. At a minimum the shroud and access door should be sealed and the apparatus flushed for four hours or more with sterile-filtered nitrogen. It is necessary to torque the screws when replacing the shroud. The screws are randomly torqued to 10 inch-oz to set the gasket and then in an alternating pattern they are torqued to 22 inch-oz to achieve metal to metal seal. If prolonged storage (a week or more) is planned, gas sterilization with ethylene oxide followed by flushing with sterile-filtered nitrogen is recommended. Standard gas sterilization procedures and precautions should be observed.

ii. Buffer Exchange

For long term storage the ESE unit should be kept filled with a solution of 0.1% w/v sodium azide and this must be removed prior to testing.

The exchange procedure is covered in great detail in Section II.A.1.b.

After exchange, it is recommended that the sterility of the unit be checked before proceeding. Samples of buffer effluent are taken and cultured in Trypticase Soy Broth tubes. If the unit needs to be resterilized, refer to Section II.A.1.c.

iii. Ground Coolant and Atmosphere

The passive refrigerant in the storage compartment must be frozen by an external source prior to operation. Connect an external refrigeration unit to #J25 (coolant in) and #J26 (coolant out), see Figure 22. It takes about 7 hours to freeze the unit with the external reservoir set at -10°C. The actual time will vary according to reservoir temperatures and ambient conditions.

The temperature of the passive refrigerant can be monitored from the control panel by setting the meter selector to position 20 and observing the thermistor output. The temperature will slowly decrease until it reaches the freezing point of the refrigerant where it remains constant. The external cooling is continued until the reading begins to drop again. Stop the external coolant

at this point. It is undesirable to lower the temperature so that the buffer might freeze. If the temperature should increase, external cooling may be resumed.

Concurrent with this cooling operation, replace the access door and flush the unit with dry N_2. This is to keep condensation off the optics while cooling the unit. Connections are made through J22 (atmos. in) and J21 (atmos. out), see Figure 22. Always connect the atmosphere exhaust line first because pressure build-up inside the apparatus could be disastrous.

d. Preparation for Mission or Simulation

Depending on the nature of the experiment to be performed, it may be desirable to remove substantially all the sodium azide from the system before the ESE is integrated into the launch vehicle. Substitute an antibiotic solution for the azide solution using the procedure of II.A.1.b. For most purposes, the exchange can be considered complete when a negative test for azide is obtained on the effluent. (H. W. Arnold, Ind. Eng. Chem., Anal. Ed. 17, 215 (1945)).

2. Sample Pump

a. Sterilization and Storage

The plunger of the sample pump should be retracted as far as possible. The stainless steel septum (Figure 25) should be sealed. The filling screw should be removed. The entire sample pump assembly, along with the filling screw, a screw driver and a heat-sealable plastic bag, should be placed in a collapsible chamber such as a Glove Bag and gas sterilized using standard procedures and precautions. When sterilization is complete and the chamber wall flushed with sterile-filtered nitrogen (including particularly, the inside of the sample pump), the filling screw is secured, the sample pump assembly placed in the sterile bag, and the bag heat sealed immediately on removal from the sterilization chamber.

b. Preparation for Mission or Simulation

Under most circumstances the sample pump assembly, still in the sealed bag, should be placed in a freezer at -10°C for six hours or longer to freeze the refrigerant. It is then removed from the freezer and allowed to warm to ~ 0°, the filling screw withdrawn, and the pump completely filled with sample. No air bubble must remain. The filling screw is then replaced and the assembly kept between 0° and 4°C until ready for use.

3. Collection Container

a. Sterilization and Storage

The collection bag assembly (optionally in the collection box) is placed in a manipulatable sterilization chamber as in 2a, above. The fluid connector keeper should be placed in the chamber but should not be fastened in place. The collection bag assembly is gas sterilized using standard procedures and precautions. Care should be taken that both the sterilizing and purging gases are directed into the inside of the bags. The fluid connector keeper is placed in a sterile plastic bag before the sterilization chamber is opened, and the bag sealed immediately after it is removed from the chamber. The collection bag assembly should also be sealed in a sterile plastic bag.

b. Preparation for Mission or Simulation

The collection container is removed from storage and placed in a freezer at -10°C for twelve hours or more. Remove it from the sealed bag and mount it in the ESE through the open access door. (See Figure 26) Fasten in place with the eight captive socket-head screws. NOTE: If the collection container does not enter the assembly properly, check to be sure valve A is in the fully upright position (Figure 24). Remove the covers from both halves of the connector and swab or mist the connector halves with disinfectant. (The

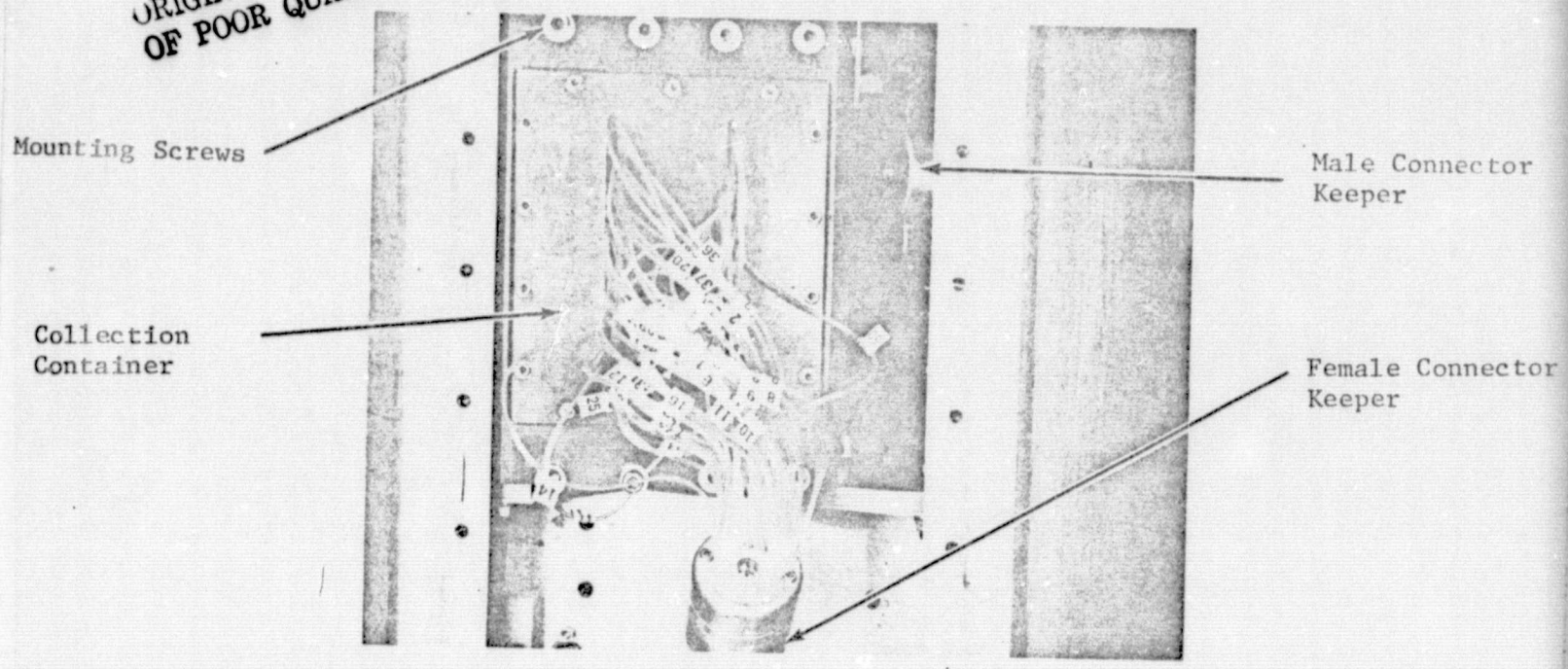


FIGURE 26. COLLECTION CONTAINER BEING INSTALLED THROUGH DOOR

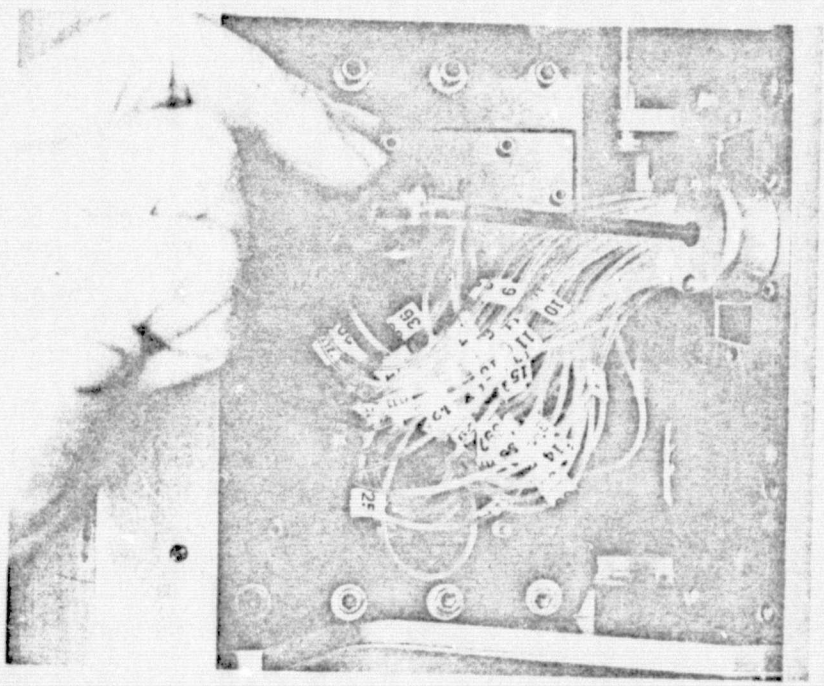


FIGURE 27. CONNECTOR HALVES BEING MATED

procedure and the choice of disinfectant, if any, is the province of the Principal Investigator.) Join the connector halves carefully and fasten together with the captive screw. (Figure 27)

c. Post-Flight Recovery

As soon as possible after a rocket flight, access should be gained to the ESE. After the access door is removed, the fluid connector (Figure 27) should be unfastened and covered with a sterile keeper firmly screwed in place and the collection container removed by unfastening the eight captive screws (Figure 26). Note that simply backing out the assembly screw in the connector will force the connector halves apart. The collection container should be placed in a cold or refrigerated location and returned to the Principal Investigator as soon as possible. More specific details on sample preservation may be supplied by the Principal Investigator.

4. Buffers and Electrolytes

Many buffer solutions can be autoclaved and stored in sealed glass containers. If the buffer can tolerate autoclaving without significant change in pH or conductivity this will generally be the method of choice. In order to minimize the amount of dissolved gases, autoclaved buffer should be sealed in airtight containers while still hot. If the buffer cannot be autoclaved, sterile filtration may be an acceptable alternative. Sterile filtration should be accomplished by vacuum rather than by pressure.

Containers of buffer and electrolyte should contain at least 1 liter. Larger volumes (e.g. 5 liters) may be advantageous in minimizing the risk of contamination during fluid exchanges in the ESE.

B. Ground Testing

1. Functional and Operational Testing

a. General

This set up and test procedure is designed to demonstrate that the ESE package is properly assembled and ready for installation in a launch vehicle. The general procedure is as follows:

- o Connect unit to control console
- o Remove storage fluids and install operational fluids in unit
- o Cool refrigerants in buffer storage assembly, sample pump and sample collection container down to operating temperature
- o Perform pretest conditioning to temperature stabilize the unit
- o Run through a test sequence that simulates an actual mission.

b. Ground Support Equipment

The following equipment is needed to perform the functional test. This is the same equipment that will be used in an actual launch sequence and has already been described in Section I.A.12.

- o Presterilized buffer and electrolyte
- o Peristaltic pump for fluid transfer
- o Refrigeration unit
- o pH meter
- o Conductance bridge
- o Power supplies

C. Set-up Procedure

1. Control Console Hook-up

The control console does not incorporate its own power supply system so test power must be provided from external supplies. For functional testing two supplies PS-1 and PS-2 are needed. PS-1, which should supply 32 $\pm$ 3 VDC at 10 amperes, simulates the rocket battery. PS-2, which should supply 28 $\pm$ 0.3 VDC at 3 amperes provides power for lamps and relays in the test console. The power supplies should be connected to J-4 on the ESE unit and to J-5 on the test console respectively as shown in Figure 28.

The data to be collected during these tests should be recorded on a data sheet similar to the one supplied in Appendix D.

Start the test by turning on SW1 (labeled VEH PWR T). It should light up green. All the other switches in the horizontal group along the base of the console will be red except SW6 (SAM PMP T) and SW 9 (LO Test), which will be green. This arangement was selected because the Test Console is also expected to be used for launch control and a green board is desirable at launch. Therefore the sample pump switch and the lift-off switch, which should be open at launch are wired to show green when off. They should show red only during ground testing.

ii. Buffer Exchange

For long term storage the ESE unit will normally be kept filled with a 0.1% solution of sodium azide in either distilled or deionized water. This must be removed prior to testing. The fluid exchange procedure is covered in detail in Section II.A.1.b.

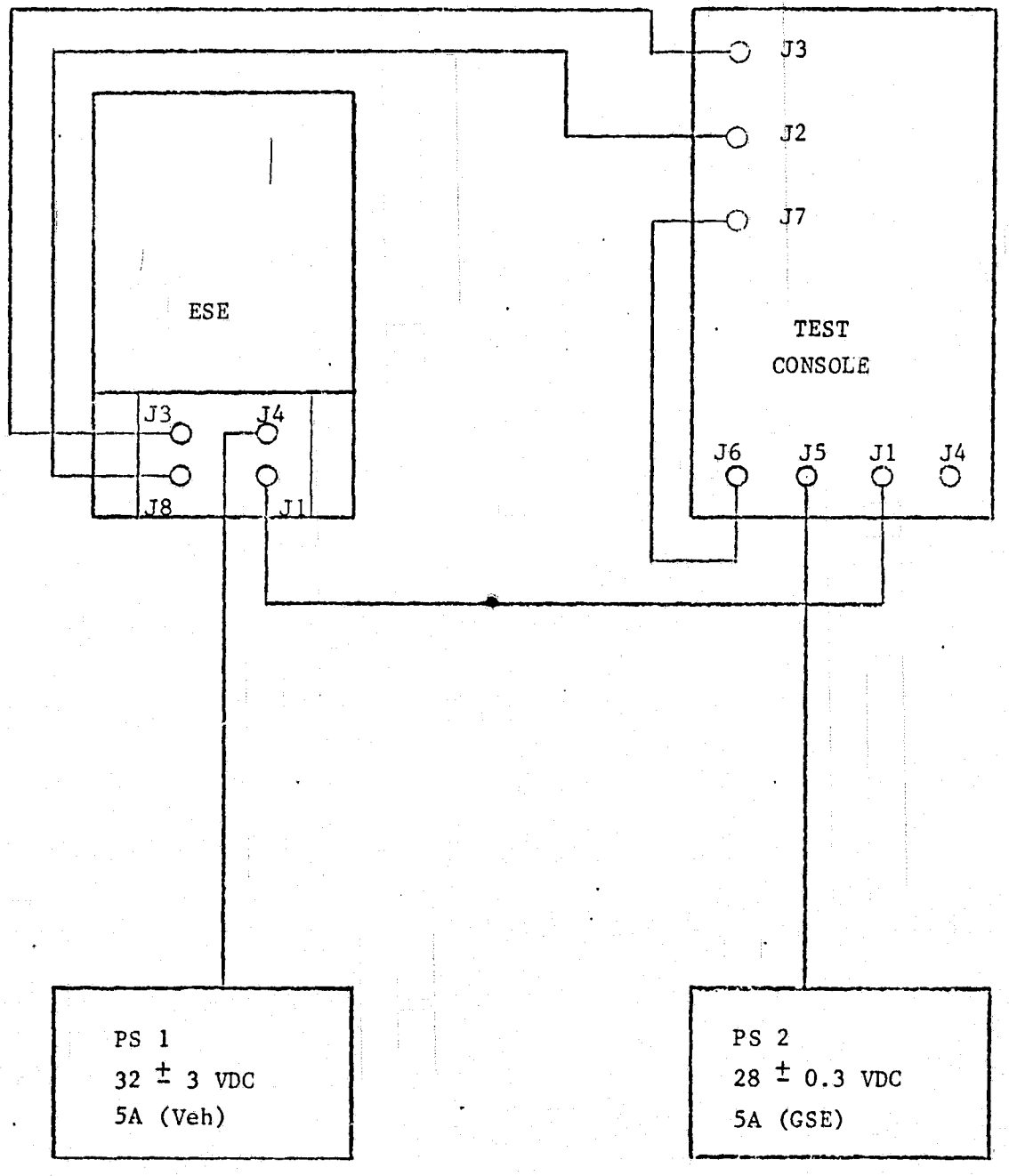


FIGURE 28. ESE EQUIPMENT INTERCONNECTIONS FOR ENVIRONMENTAL TESTS

After exchange, it is recommended that the sterility of the unit be checked before proceding. Samples of buffer effluent should be taken in Trypticase Soy Broth tubes. If necessary, the unit should be resterilized in accordance with the proceudre given in Section II.A.1.c.

iii. Ground Coolant and Atmosphere

The passive refrigerant in the buffer storage compartment must be frozen by an external source prior to operation. Connect tubes to J-25 (coolant in) and J-26 (coolant out) to circulate the cooling fluid through the coolant coil in the buffer storage assembly. A 50/50 mixture of ethylene glycol anti-freeze and water is a suitable coolant fluid. With the coolant fluid coming in at -10°C it takes about 7 hours to freeze the unit.

The temperature of the passive refrigerant can be monitored from the control console by setting the meter selector to position 20 and observing the thermistor output. This reading will be in volts. See Figure 30 to convert this to temperature. The reading will slowly increase (temperature decreases) until it reaches the freezing point of the refrigerant where it will remain constant until all the refrigerant is frozen. Stop the cooling when the temperature begins to start going down again. Do not allow the temperature to go as low as 0° where the buffer may freeze. If the temperature starts to rise again after cooling has stopped, resume cooling.

Concurrent with the cooling operation, replace the access door on the ESE and flush the unit with dry nitrogen. This is necessary to keep condensation off the optics while cooling the unit. Bring the nitrogen in through J-22 (atmosphere in) let it escape through J-22 (atmosphere out). See Figure 22. Note that J-21 and J-22 are sealing types of quick disconnect fitting. Be sure there is an opening to let the gas out of the ESE package (J-22, J-27 or open door) to avoid building up pressure in the ESE package. Even a relatively low excess pressure could be disastrous.

2. Acceptance/Check-Out Procedure

This procedure is to assure that all of the systems within the ESE unit are operating within normal limits. It is, in essence, the functional test without environmental or launch simulations. The control panel is connected to the unit via the control and telemetry cables. Power must be applied to the panel through power connector J5 on the rear (see Figure 10) from PS2. Operate SW1 on the lower panel to activate the system. The detailed procedure for the test can be found in the previous section (II.B.1).

D. Pre-Launch

This section covers pre-launch operations where the ESE has been integrated into a launch vehicle. Operations and installations are performed at the launch site through the vehicle and ESE access doors. System operations and check-out are performed using the test panel.

1. Test Panel Hook-Up

The test panel is removed from the Control Console and taken to the access area of the launch vehicle by the Operator. Power is supplied to the test panel at the access area. Open the access door to the ESE. Connect the test cable between the panel and the test connector on the ESE pan (J3). All systems may now be operated from the panel. The panel is wired to the ESE so that a system function cannot be inadvertently left ON. The panel can also monitor selected telemetry points. These are: storage compartment temperature, peristaltic pump voltage and current, sample pump voltage, coolant pump voltage, electrode rinse pump voltage, sample temperature and internal pressure.

2. Refrigeration and Dry N_2 Purge

The passive refrigerant in the storage compartment must be frozen from an external source. Details covering this operation are found in

Section II.B.1.C. Care must be taken to avoid freezing the buffer and electrolyte in the storage bags. The temperature of the eutectic can be periodically monitored from the test panel using selector position #20.

Simultaneously, the unit should be purged with dry N_2. Hook-up is described in Section II.B.1.C. The internal pressure may be monitored through selector position #10 on the test panel.

3. Buffer Exchange

Prior to launch (see Section I.A.2), remove the antibiotic solution from the unit and substitute the Principal Investigator's buffer and electrolyte solutions. A detailed procedure for fluid exchange is given in Section II.A.1.b. The current monitor for the peristaltic pump may be observed during this operation.

Once the buffer and electrolyte have been exchanged there will be a period of little activity. During this time, the ground coolant and the N_2 purge should continue. The coolant is monitored to prevent buffer freezing. The access door is replaced loosely to allow a high N_2 purge rate.

4. Installation of Collection Container

This is covered in Section II.A.3.b.

5. Filling of Sample Pump

This is covered in Section II.A.2.b.

6. Installation of Sample Pump

Remove the filled and frozen pump from its storage bag and make the fluid connection to the ESE first. Before installing the sample pump, plug in the time delay connector as shown in Figure 17. Remove the cover from the needle (Figure 21). Disinfect the needle and septum before assembly. Put the needle

into the pump and thread the captive screw into the hole. As the screw is tightened, it forces the needle to pierce the stainless steel septum in the pump. With the needle secured, make the electrical connection next (see Figure 29). Finally, mount the pump to the unit. The temperature of the sample pump may now be checked from the test panel. Now seal the access door to the ESE for mission operation. The procedure is as follows: torque randomly to 5 inch-oz to set gasket. Then, in an alternating pattern, torque screws to 7-8 inch-oz. Remove the test cable from the pan. The unit is now ready for a mission sequence.

E. Post-Flight

1. Removal of Collection Container

This is covered in Section II.A.3.c.

2. Preparation for Re-use and Storage

Remove the sample pump. Any residual buffer and electrolyte should be removed from the unit and the system rinsed several times with sterile, deionized water (see Section II.A.1.b. and c). After rinsing, the unit is then filled with a 0.1% sodium azide solution (Appendix B) for storage. The unit should be purged with dry N_2 before sealing it up.

The sample pump should be thoroughly cleaned to remove any residual sample. A new stainless steel septum inserted and the pump should be resterilized (see Section II.A.2.a.) and stored.

New collection bags are installed in the collection container. It is then resterilized (See Section II.A.3.a.) and stored.

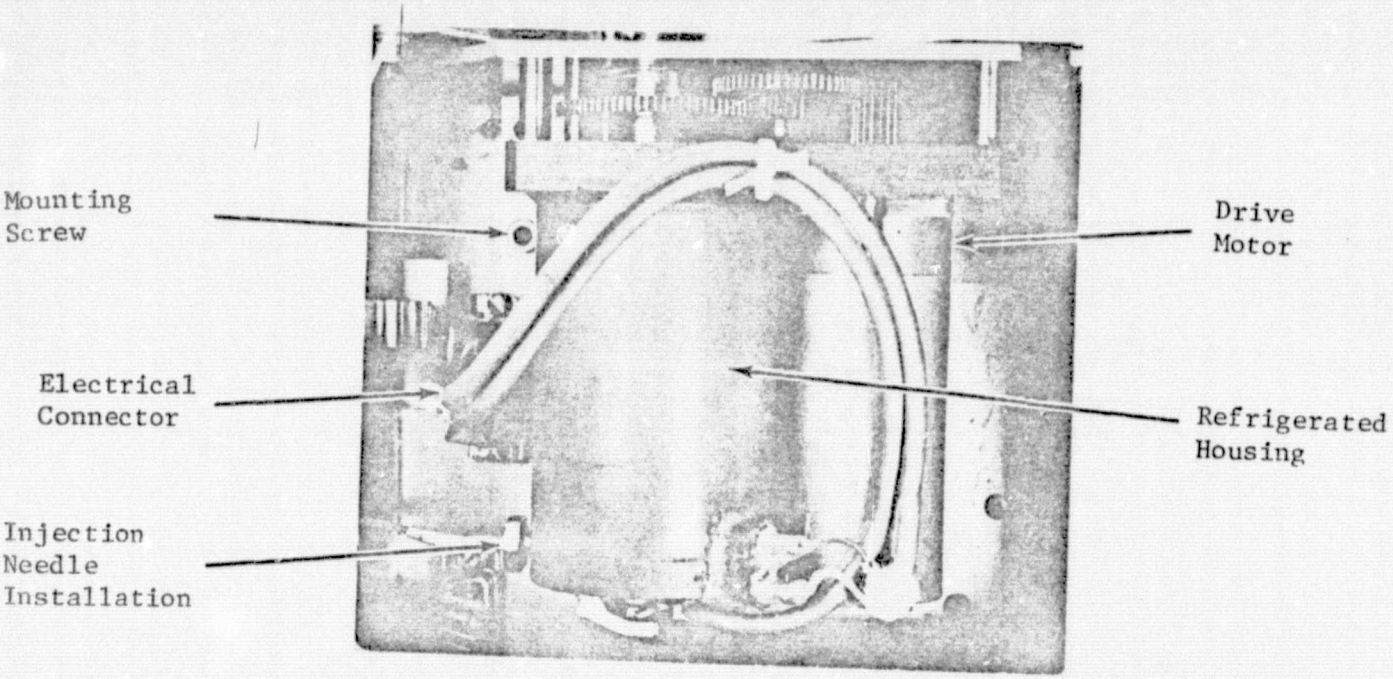
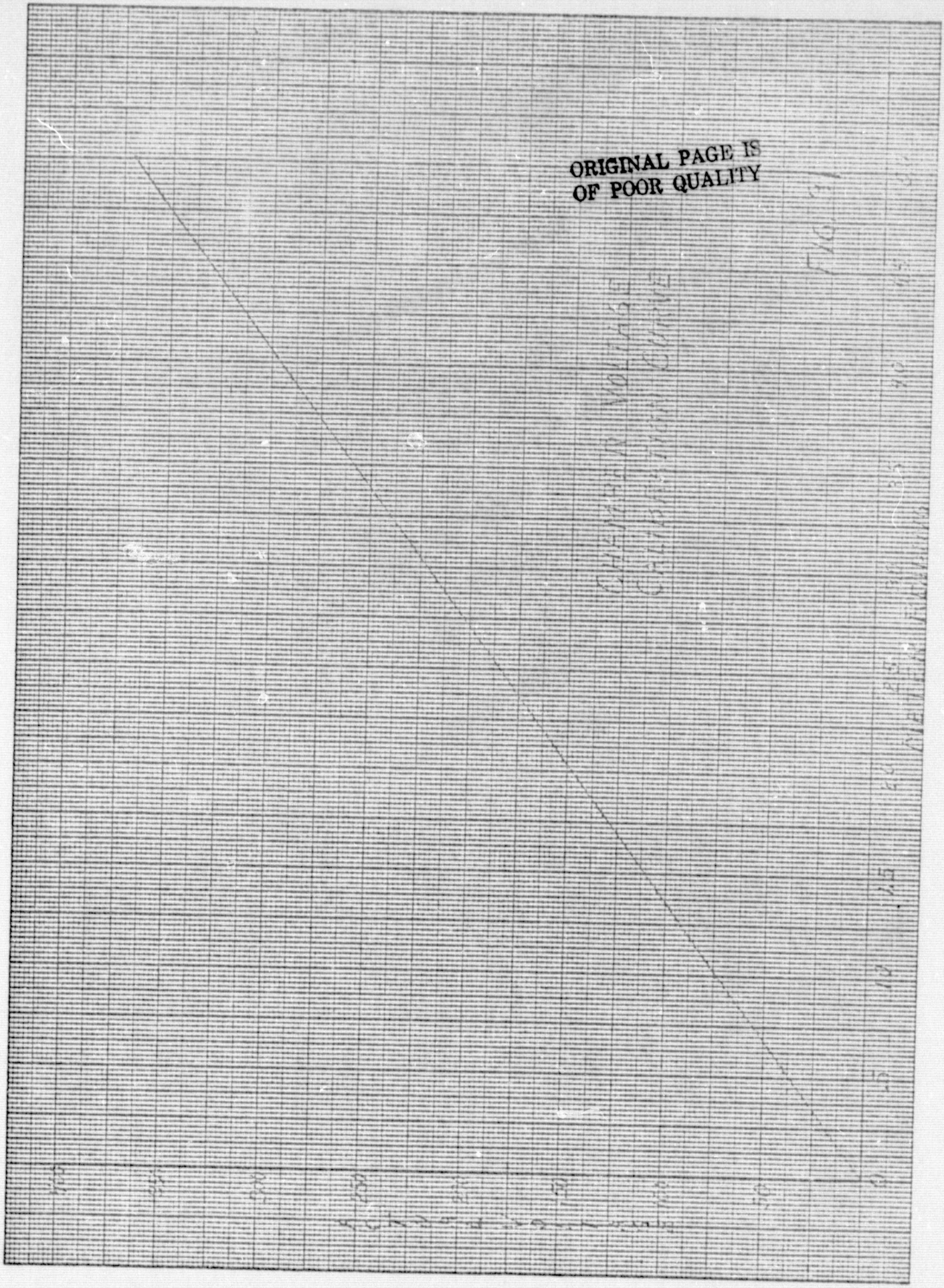


FIGURE 29. SAMPLE PUMP INSTALLATION
VIEW THROUGH DOOR

Fig 20



CHAMBER FIELD VOLTAGE CALIBRATION CURVE

FIG. 32.

III. APPENDIX

A. Ground Support Equipment: Suggested Equipment

1. Masterflex Pump (Cole Parmer) #7545 with #7014 pump head and #7014-20 add on head

2. Portable Cooling Unit, Blue M Model #1241, 1800 BTU/hr with a 6 gallon reservoir. Coolant is 50% V/V Ethylene Glycol/ H_2O. Insulated storage box for sample pump and collection container ~ 12" x 10" x 9" (Cole Parmer #3719)

3. pH Meter

   Micro-combination electrode: Fisher #13-639-92

   Line operated meter - Fisher Accumet 140

   Battery operated - Fisher Accumet 150

4. Conductivity Meter

Micro-cell	YSI Model #3403
Portable Meter	YSI Model #33 S-C-T meter
Line Operated	YSI Model #31

5. Glove bag (I^2R) for gas sterilization

   Use 12% Ethylene Oxide as sterilizing agent

6. Power Supplies PS1 32VDC$\pm$3VDC
   Hewlitt Packard 6433D Variable Output

   PS2 28VDC$\pm$0.5 @ 3 amps Acopian
   B28GT300
   Fixed Output

7. Tools

   5/32" Allen wrench for sample pump installation

   9/64" Allen wrench for collection container installation

   Torque wrench (range 0 to 15 inch-oz) with #2 Phillips driver for access door.

III. APPENDIX (continued)

B. Sterilizing Solutions

1. Primary Sterilizing Solution

Formaldehyde-Borate

Formalin (33% aq. formaldehyde)	100 cm^3	to make
Sodium Tetraborate	50 gm	1 liter of solution

2. Secondary Sterilizing Solution

Zephiran - Chloride (17%) 1:7000

0.5 cm^3 Zephiran Chloride (17%) solution to 700 cm^3 water

3. Long Storage Solution (Antiseptic Solution)

Sodium Azide 0.1% W/V

C. Trouble Shooting

The following section will describe areas in which failures may occur, their probable cause and recommended repairs.

If any of the systems fails to operate from the control panel, it is a simple matter to test the relays which supply power to these systems. Disconnect the control cable from the unit and connect the test cable. The test cable is wired around the relays and will activate a system if the relay is faulty. It is recommended that this procedure be used first to eliminate the possibility of relay failure. If a relay has failed, it is not recommended that a replacement be made in the field. In fact, due to the highly sophisticated nature of this apparatus, there are many repairs which should not be attempted in the field and these will be denoted by an asterisk (*).

System	Symptom	Probable Cause	Recommend
Pan and Shroud	Low Pressure	1. Access door not sealed	Reseal and/or replace gasket
		2. Shroud not sealed to pan	Replace gasket and reseal
		3. Fitting on pan leaks	*
Power Distribution	No ± 15 and/or +5 VDC	Open circuit at input or output or converter failed	*
	No Chamber High Voltage	Open circuit at input or output or converter failed	*
Coolant	Pump does not run	No power, open circuit, short circuit, regulator set too low	Check power supply *
	Pump runs but no circulation	Air bound	Refill loop
		Magnetic drive slipped	Stop and Re-start
		No coolant, leak in loop	Refill and tighten
	Thermistor not within limits	Open Circuit	*
		Short Circuit	*
		Eutectic too hot	Re-freeze
		Eutectic too cold	Allow to warm
Sample Injection	Pump doesn't run	Not connected	Collect to unit
		End of travel	Remove and reset
		Open circuit	Cleck leads & repair
		Short circuit	*
		Bound	Check gears
		Voltage set too low	Reset
	Motor runs but doesn't pump	Leaks out of fill port	Replace gasket
		Leaks out of fill port	Tighten needle
	Thermistor reading not within limits	Open circuit	*
		Short circuit	*
		Eutectic too hot or too cold	Re-freeze or warm up

* Not recommended for field repairs

System	Symptom	Probable Cause	Recommend
Sample Injection (cont'd.)	Stirrer doesn't run	No power to 2ϕ driver	Check ± 15 & +5 Volt P.S.
		One or more timers failed	*
		Open lead to coil	*
Peristaltic Pump	Does not run	No DC to motor	Check power supply
		Open circuit	Repair
		Short circuit	*
		Pump bound	*
		Voltage set too low	Reset
	Runs but doesn't pump	Faceplates not tightened	Tighten
		Tubing leaks	*
		Tubing pinched off	*
	Runs, pumps, but not constant	Faceplates too tight	*
	Draws air or leaks fluid	Fitting not tight	Check all fittings
		Fitting stripped	*
		Hole in tubing	*
Mercury Vapor	Does not light	No power to converter	Check +28 and +15 volt supply
		Converter not oscillating	*
		Output voltage not high enough	*
		Open lead to lamp	Repair
		Lamp failed	Replace
	Lights but not constant intensity	Feedback sensor failed	*
		Feedback amp. failed	*
		Open circuit in feedback loop	*
Detector	No signal	No power to board	Check -15 and +5 supplies
		Binary divider not set correctly	Reset per Reticon data
	Signal present	Low level:binary divider set incorrectly	Reset per Reticon data
		High level:array partially blocked	Remove obstruction
		Array partially failed	*

*Not recommended for field repairs.

System	Symptom	Probable Cause	Recommend
Detector (cont'd.)	Full frame signal but low level	Lamp not lit	Check
		Array totally blocked	Remove obstruction
		Array unplugged	Check cable
		Array destroyed	*

*Not recommended for field repairs.

DATE _____

MSFC - 95M16900
Par _____

ESE DATA SHEET

Performance Test: ☐ Pre ☐ During ☐ Post _____ Environment

A. Item

Item	Make	Model	IC No.	Out of Cal Date
PS #1				
PS #2				
DVM				
Timer				
Fixture/Chamber				

B. Condition:

PS #1	35 ± 0.4 V	29 ± 0.4 V
PS #2	28 ± 0.3 V	28 ± 0.3 V

C. Data

Start Time Delay	_____	sec	_____	sec
End Time Delay	_____	sec	_____	sec

SW Position

1. Sample Pump Stirrer Power	_____	_____
2. Coolant Pump Voltage	_____	_____
3. Coolant Pump Current	_____	_____
4. E.R. Pump Voltage	_____	_____
5. E.R. Pump Current	_____	_____
6. Sample Pump Voltage	_____	_____
7. Sample Pump Current	_____	_____
8. Buffer Pump Voltage	_____	_____
9. Buffer Pump Current	_____	_____
10. Internal Gas Pressure	_____	_____
11. U.V. Lamp Output	_____	_____
12. Scanner Digital Output	_____	_____
13. Chamber coltage	_____	_____
14. Chamber Heid Voltage	_____	_____
15. Chamber Current	_____	_____
16. +15 Volts	_____	_____
17. Chamber Inlet Temp.	_____	_____
18. Chamber Outlet Temp.	_____	_____
19. Sample Storage Temp.	_____	_____
20. Buffer Storage Temp	_____	_____

Data Recorded by: _____ Approved by: _____