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REPORT MDC E2000

INVESTIGATION OF THE FREE FLOW ELECTROPHORETIC PROCESS

FINAL REPORT

VOLUME I
EXECUTIVE SUMMARY

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MCDONNELL DOUGLAS ASTRONAUTICS COMPANY - ST. LOUIS DIVISION

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INVESTIGATION OF THE FREE FLOW ELECTROPHORETIC PROCESS

FINAL REPORT

MAY 1979 **REPORT MDC E2000**

VOLUME I
EXECUTIVE SUMMARY

SUBMITTED TO: NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MARSHALL SPACE FLIGHT CENTER
HUNTSVILLE, ALABAMA

CONTRACT NO: NAS 8-32200

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PREFACE

This MDC report entitled "Investigation of the Free Flow Electrophoretic Process" is submitted under NASA Contract Number NAS 8-32200. It consists of two volumes as specified below:

Volume I - Executive Summary
Volume II - Technical Analysis

Prepared as the final report of a seven-month study, with the same title, performed by McDonnell Douglas Astronautics Company - St. Louis Division, this document summarizes the results of a study that focused on demonstrating the effects of gravity on the process and comparing the demonstrated effects with predictions made by mathematical models. This contract was administered by the NASA Marshall Space Flight Center, Huntsville, Alabama.

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1.0 INTRODUCTION

The microgravity environment of space may provide advantages to the production and purification of biological materials in terms of greater availability and higher purity of therapeutic, research, and diagnostic materials. Experiments conducted in space have already demonstrated the advantages of using static (1) and free-flow (2) electrophoresis to separate biological materials in a microgravity environment. Cells separated using static electrophoresis showed increased production of urokinase and erythropoietin when subsequently subcultured in earth based laboratories (3). The previously noted experiments demonstrated the positive results that the space environment has on materials processing, but they were not intended to focus on process parameters. A necessary step toward NASA's goal of space industrialization is an in-depth study of the effects of gravity on the process. Understanding these effects will facilitate quantification of the advantages of space processing, allowing ground-space economic trade-off analyses to be made. The purpose of this study is to demonstrate the effects of gravity on the free-flow electrophoretic process and to compare the demonstrated effects with predictions made by mathematical models.

The free flow electrophoresis chamber used to demonstrate the effects of gravity on the process is of a proprietary design developed by McDonnell Douglas Astronautics Company - St. Louis Division. This chamber is 120 cm long, 8.25 cm wide, and 0.3 cm thick. The chamber and its supporting hardware are shown in Figure 1, AN Electrophoresis Test Setup. Flow in this chamber is in the upward direction and exits through 105 outlets at the top of the chamber. During electrophoresis a stream of sample is injected into the flow near the bottom of the chamber and an electrical field is applied across the width of the chamber. The field causes a lateral force on particles in the sample proportional to the inherent charge of the particle and the electrical field strength. Particle lateral velocity is then dependent on the force due to viscous drag which is proportional to particle size. The characteristic that describes particle motion is electrophoretic mobility, which is the lateral velocity divided by electrical field strength.

The free flow electrophoretic process depends on maintenance of a steady laminar flow of the carrier fluid. Time variant velocity fluctuations will cause corresponding fluctuations in the particle paths spoiling the intended separation. On

AN ELECTROPHORESIS TEST SET-UP

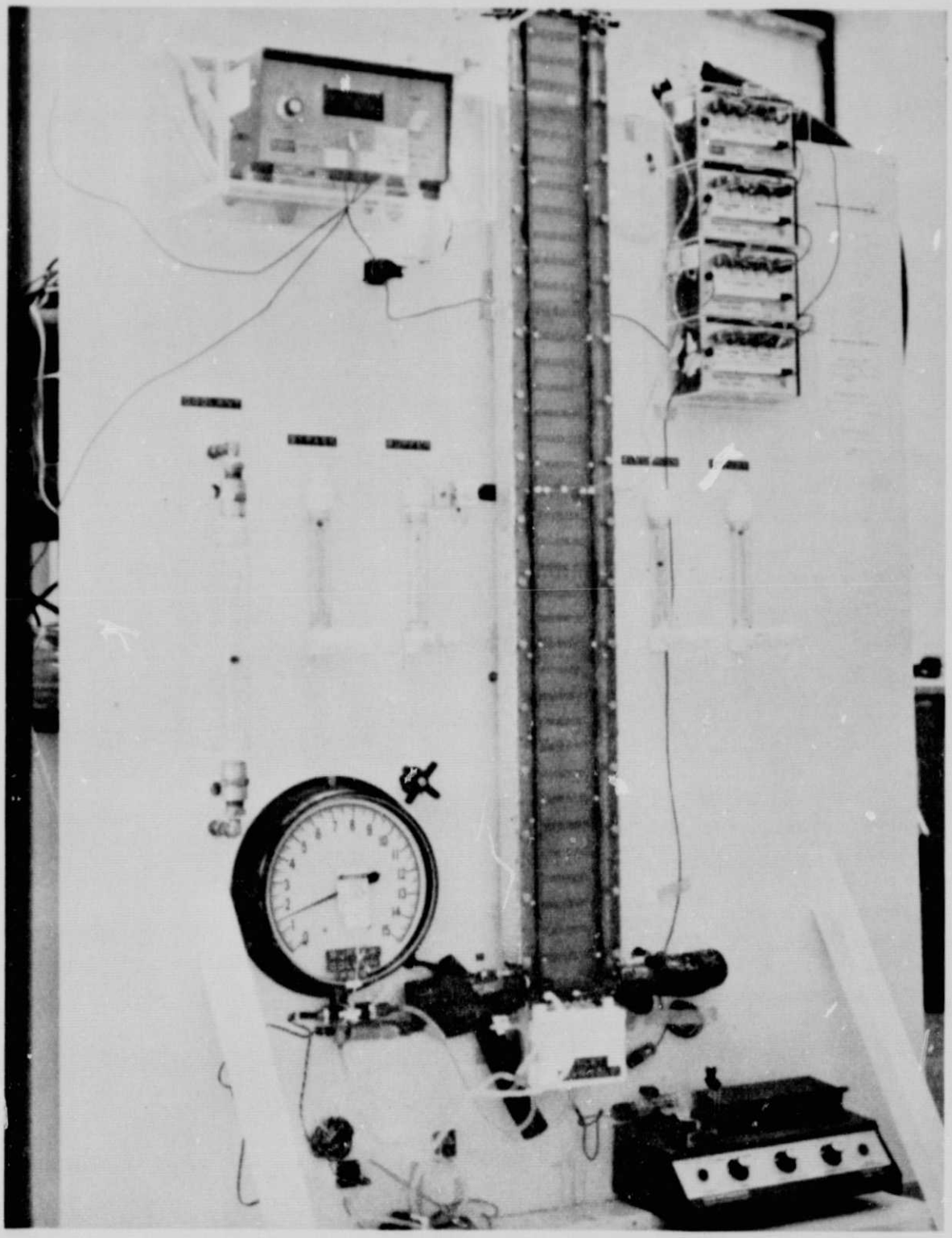

Figure 1

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earth the primary source of velocity variations in the carrier fluid is convection currents. Free convection in turn is caused by density variations due to temperature differences in the fluid. These temperature differences are caused by Joule heating of the fluid by the electrical field. This problem is aggravated by the requirement that the carrier fluid must have sufficient ionic strength to insure stability of the biological materials being separated. This carrier fluid, or buffer, is therefore an electrically conductive medium.

2.0 EFFECTS OF GRAVITY ON CARRIER BUFFER

The purpose to Task 1.0 was to determine the effects of gravity induced thermal convection on the carrier buffer flow. Tests were performed to measure vertical centerline velocity as gauged by the motion of dye fronts in the carrier buffer flow. A dye front near the entrance of the chamber with no field applied is shown in Figure 2 and one at a field strength of 10 volts/cm near the outlet is shown in Figure 3. The results for the zero voltage case are what would be expected for flow between closely spaced parallel plates i.e. a nearly flat profile that falls off only near the sides of the chamber. With voltage however, peaks develop in the profile near the sides of the chamber. These peaks were found to be caused by heating of the fluid at the membranes, this conclusion was based on correlation with velocity predictions from a three dimensional mathematical model of the chamber flow velocities, pressures, and temperatures developed by McDonnell Douglas Astronautics Company - St. Louis Division.

Good correlation of test results with the mathematical model with no field applied may be demonstrated by comparing the observed data of Figure 4 with the model predictions of Figure 5. The mean of observed data (0.1890 cm/sec) is approximately one standard deviation (0.0135 cm/sec) less than the predicted mean velocity (0.2066 cm/sec).

When power was applied to the chamber the centerline velocities were significantly reduced by the return flow of the gravity induced convective cells evidenced by the velocity peaks seen in Figure 3. In this case the mathematical model centerline velocities were predicted to be significantly higher than the observed test data. This indicates that the mathematical model may have underestimated the return flow for the upward convection currents at the membranes. Therefore, the model predictions for sample residence times during test separations were less than the actual case.

VELOCITY PROFILE (0 VOLTS/CM)

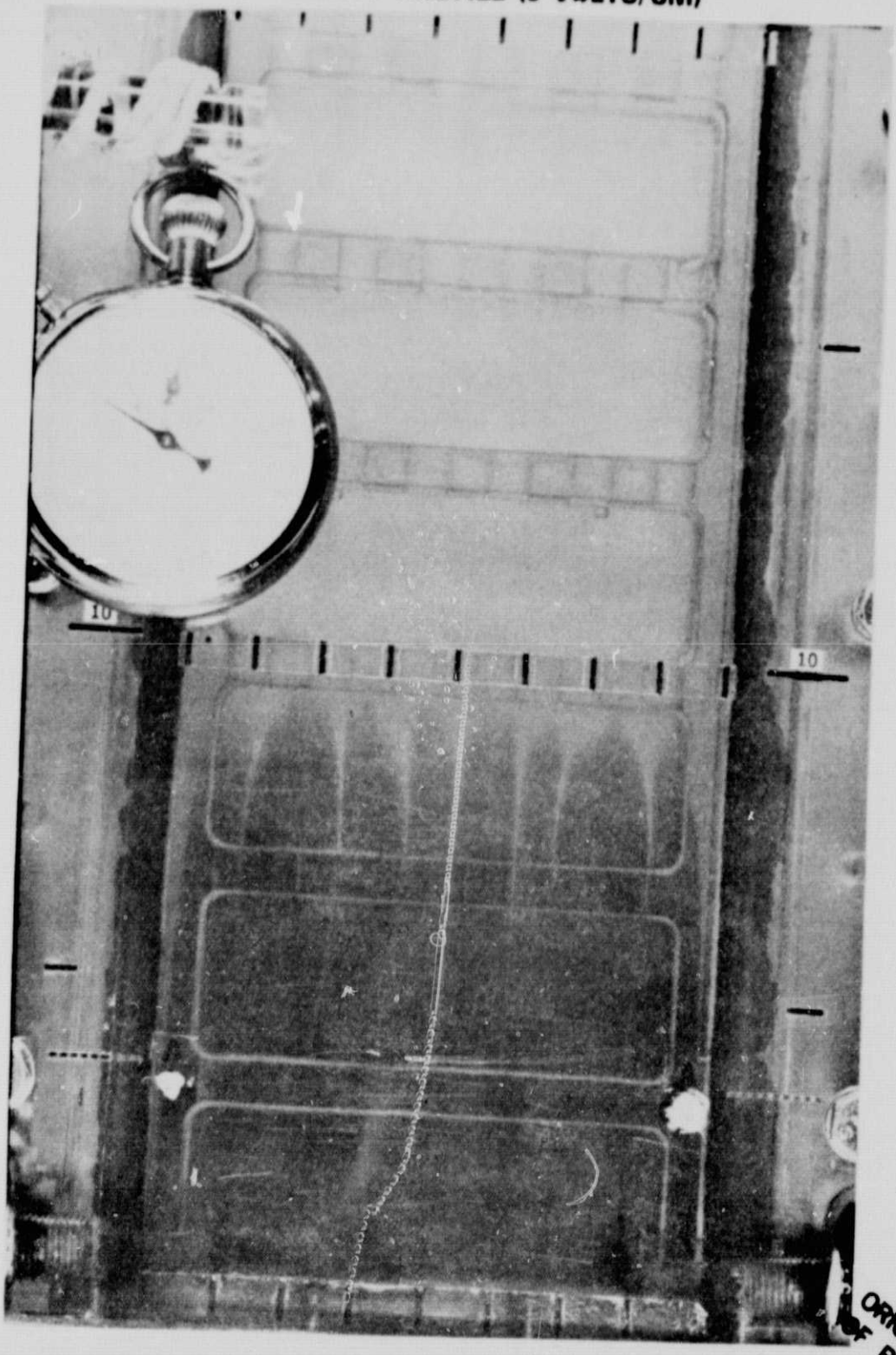

Figure 2

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VELOCITY PROFILE (10 VOLTS/CM)

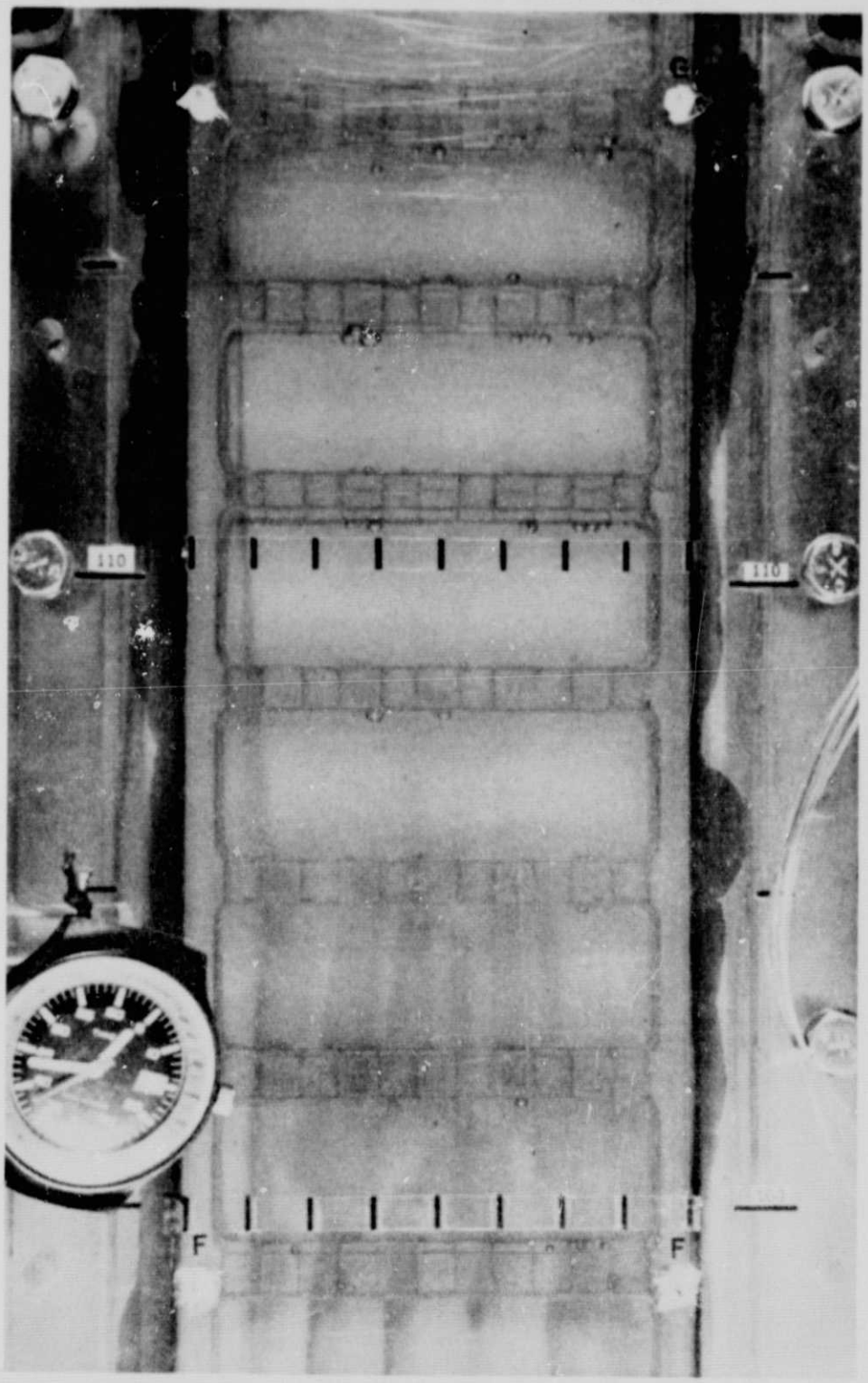


Figure 3

VERTICAL CENTERLINE VELOCITY DATA - CM/SEC

(0 VOLTS/ CM)

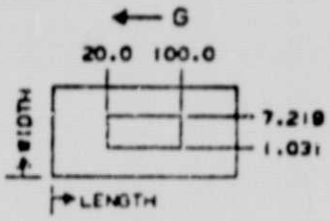
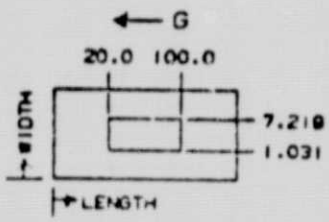


Figure 4

PREDICTED VERTICAL CENTERLINE VELOCITY - CM/SEC

(0 VOLTS/CM)



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8.225	.1367E+00	.6907E-01	.6996E-01	.7006E-01	.7044E-01	.7081E-01	.7114E-01	.7155E-01	.7182E-01	.7211E-01	.1367E+00
8.200	.1367E+00	.1114E+00	.1134E+00	.1201E+00	.1206E+00	.1211E+00	.1216E+00	.1221E+00	.1225E+00	.1229E+00	.1367E+00
8.150	.1367E+00	.1773E+00	.1771E+00	.1773E+00	.1777E+00	.1781E+00	.1785E+00	.1789E+00	.1793E+00	.1796E+00	.1367E+00
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4.125	.1367E+00	.2059E+00	.2067E+00	.2063E+00	.2066E+00	.2065E+00	.2067E+00	.2069E+00	.2071E+00	.2077E+00	.1367E+00
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.430	.1367E+00	.2059E+00	.2067E+00	.2066E+00	.2068E+00	.2068E+00	.2069E+00	.2071E+00	.2076E+00	.2080E+00	.1367E+00
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.100	.1367E+00	.1771E+00	.1776E+00	.1778E+00	.1784E+00	.1790E+00	.1795E+00	.1801E+00	.1807E+00	.1812E+00	.1367E+00
.050	.1367E+00	.1134E+00	.1193E+00	.1209E+00	.1217E+00	.1225E+00	.1233E+00	.1239E+00	.1245E+00	.1250E+00	.1367E+00
.025	.1367E+00	.6964E-01	.7016E-01	.7066E-01	.7133E-01	.7191E-01	.7247E-01	.7295E-01	.7329E-01	.7366E-01	.1367E+00
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LENGTH	3.00	6.00	10.00	20.00	40.00	60.00	80.00	100.00	110.00	115.00	120.00

Figure 5

Correlation of test results with the mathematical model requires reduction of the velocity profile data at the same locations within the chamber. This was accomplished by first measuring the profile coordinates for input to a data reduction program. These profile coordinates as a function of time were curve fitted and interpolated by computer at the analytical coordinate values. Velocities were interpolated at approximately one centimeter increments across the width of the chamber and 20 centimeter increments along the length of the chamber. The velocities are close to being constant, as would be expected from the flatness of the velocity profile. Analytical predictions were made for 1463 locations in a half thickness chamber model.

Tests were also conducted to determine horizontal centerline velocity. In these tests seven dye streams were injected into the carrier buffer flow at equal increments across the width of the chamber as shown in Figure 6 . The tangent of the angle of the stream away from the vertical and the vertical velocity were used to calculate the horizontal velocity. It should be noted that in order to avoid the introduction of error due to the scatter in the vertical velocity test data, a constant analyzed vertical velocity was used for the calculation. Figure 6 shows that the dye streams are vertical with no applied electrical field indicating negligible horizontal velocity. The corresponding data reduction is shown in Figure 8 . The horizontal velocities from the reduced test data are generally about 10^{-4} cm/sec in magnitude and either positive or negative in sign, indicating the limiting accuracy of the test method. The corresponding analytical predictions of velocity showed even lower values indicating residuals in the iterated solution. Figure 7 shows the dye streams near mid-chamber with an applied electrical field of 10 volts/cm. The corresponding data reduction is shown in Figure 9 where the test velocities approximate the predicted value of 0.002 cm/sec. Discrepancies are due to the values being almost of the same order of magnitude as the accuracy of the test method, as illustrated by the zero voltage case.

DYE STREAMS (0 VOLTS/CM)

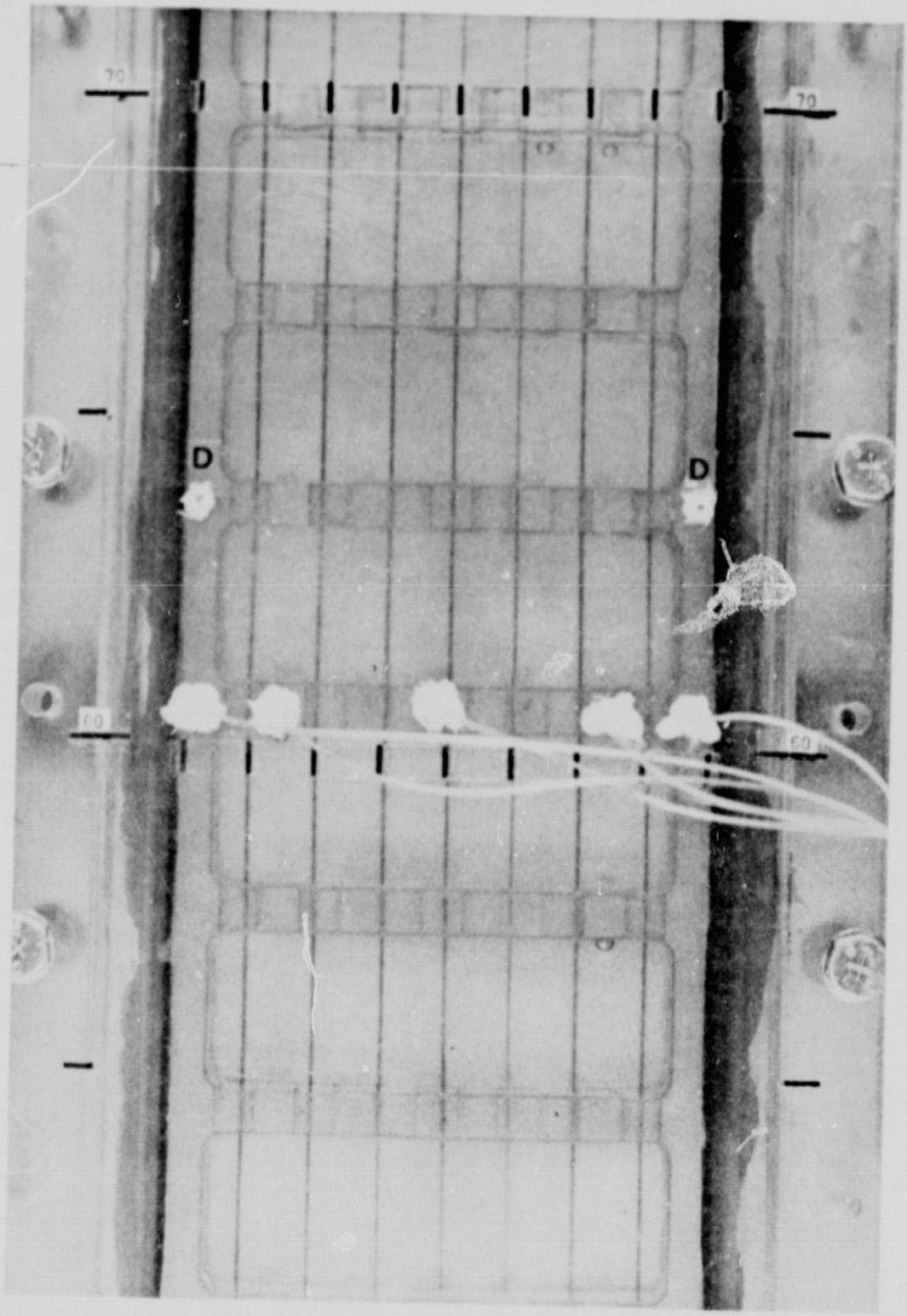

Figure 6

DYE STREAMS (10 VOLTS/CM)

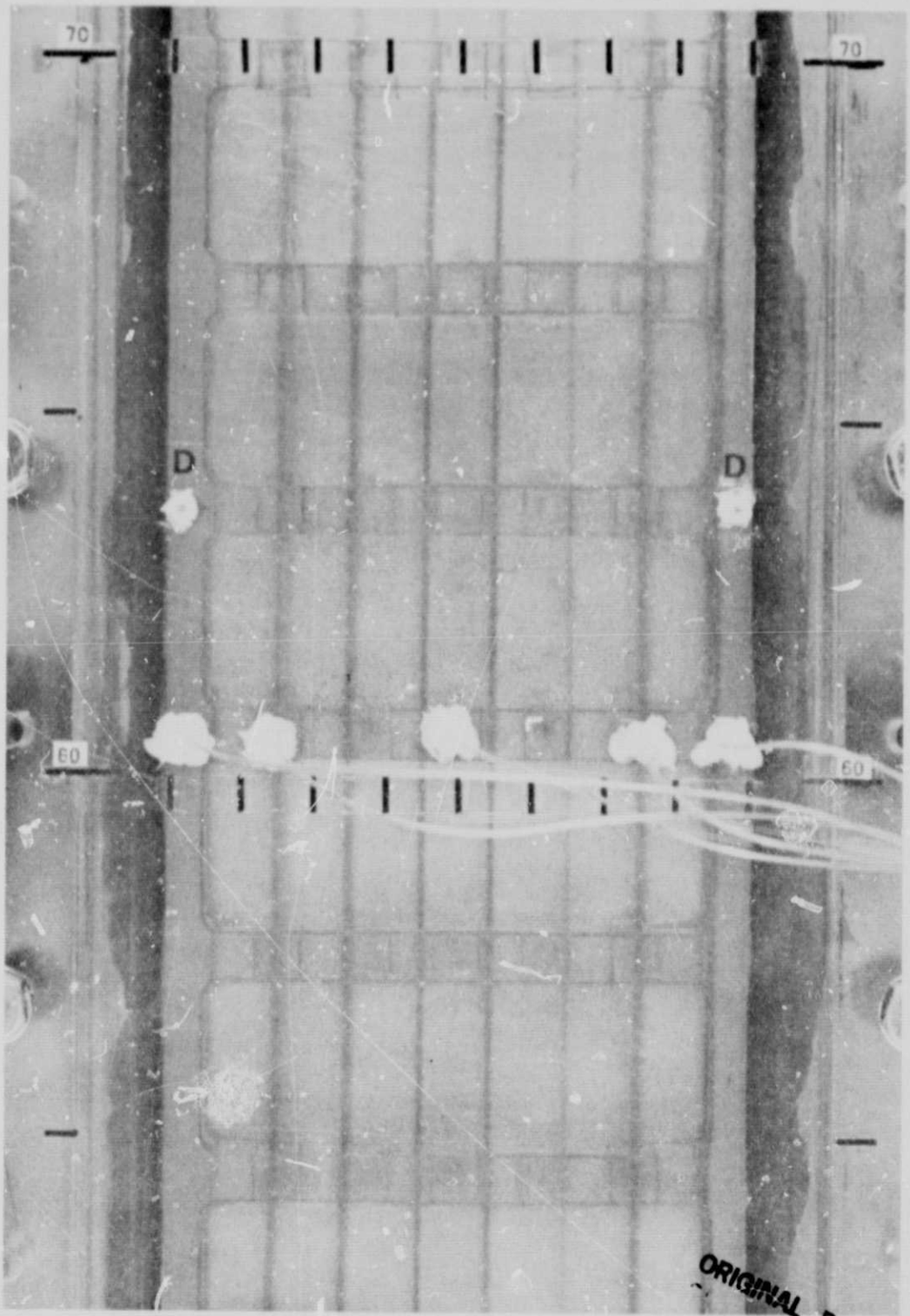

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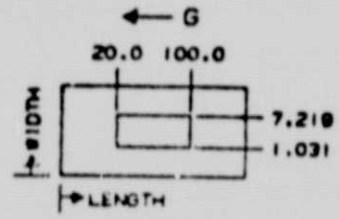
Figure 7

HORIZONTAL CENTERLINE VELOCITY DATA - CM/SEC

(0 VOLTS/CM)



MDAC-STL ELECTROPHORESIS DATA REDUCTION
ORIGINATOR: D.W. RICHMAN 12/79

RUN NO. 16, TEST ENGINEER: C.D. WALKER
BUFFER FLOW 40.0 ML/MIN, INLET TEMP 7.4 C
FIELD STRENGTH 0.0 V/CM
BUFFER HORIZONTAL CENTERLINE VELOCITY

WIDTH											
8.250											
8.225											
8.200											
8.150											
8.050											
7.850											
7.219	0.	0.	.2929E-03	0.	0.						
6.188	.2155E-02	.1327E-02	-.2967E-03	-.1097E-02	-.3514E-03						
5.156	.1850E-02	.1112E-02	-.2021E-03	-.1197E-02	-.1896E-02						
4.125	.1848E-02	.1312E-02	-.3247E-03	-.1704E-02	-.1927E-02						
3.094	.4641E-02	.1365E-02	-.1047E-02	-.1904E-02	-.2124E-02						
2.063	.4447E-02	.7821E-03	-.8932E-03	-.1097E-02	-.1737E-02						
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.200											
.100											
.050											
.025											
0.000											
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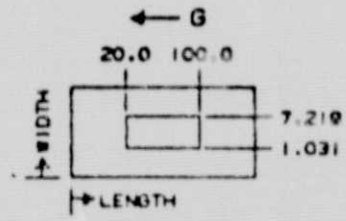
WIDTHS OUTSIDE DATA = 0

Figure 8

HORIZONTAL CENTERLINE VELOCITY DATA - CM/SEC

(10 VOLTS/CM)



MDAC-STL ELECTROPHORESIS DATA REDUCTION
ORIGINATOR: D.W. RICHMAN 12/79

RUN NO. 17, TEST ENGINEER: C.D. WALKER
BUFFER FLOW 40.0 ML/MIN, INLET TEMP 6.9 C
FIELD STRENGTH 10.0 V/CM
BUFFER HORIZONTAL CENTERLINE VELOCITY

WIDTH											
8.250											
8.225											
8.200											
8.150											
8.050											
7.850											
7.219	0.	.3196E-02	.1877E-02	.2792E-03	.4034E-03						
6.188	.6806E-02	.3633E-02	.1473E-02	.1607E-02	.2260E-02						
5.156	.7117E-02	.2964E-02	.8553E-03	.1240E-02	.2141E-02						
4.125	.6526E-02	.2884E-02	.1729E-03	.9015E-03	.5345E-03						
3.094	.5587E-02	.2967E-02	.1351E-02	.9459E-03	.2644E-03						
2.063	.6000E-02	.3071E-02	.1654E-03	.1050E-02	.2764E-03						
1.031	0.	0.	0.	0.	0.						
.400											
.200											
.100											
.050											
.025											
0.000											
LENGTH	0.00	5.00	10.00	20.00	40.00	60.00	80.00	100.00	110.00	115.00	120.00

WIDTHS OUTSIDE DATA = 0

Figure 9

3.0 EFFECTS OF GRAVITY ON SAMPLE

The purpose of Task 2.0 was to determine the effects of gravity on the particle streams during electrophoresis. The limiting effects of gravity on sample streams in upward flow are illustrated by Figure 10. For a sample that is heavier than the carrier buffer, the sample falls back around the sample input tube. A sample stream that is lighter than the carrier buffer, however, is buoyed

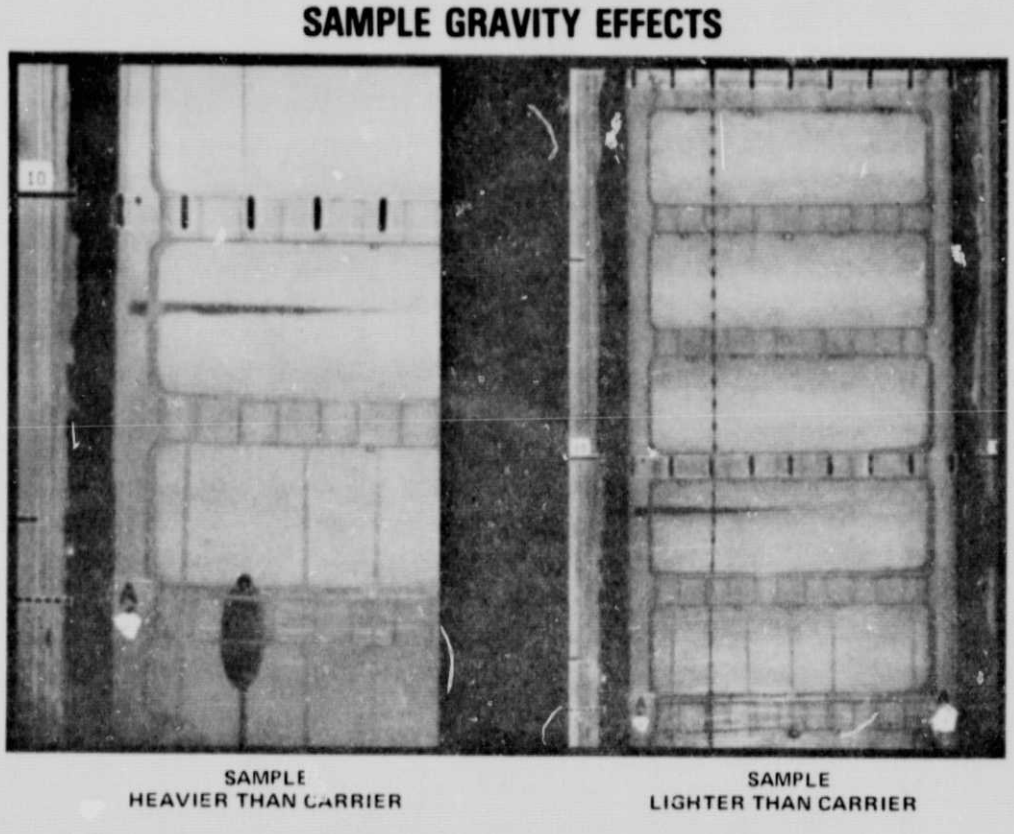


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Figure 10

up in the flow and breaks up into beads. To assure realism and applicability of the results, biological materials, both proteins and cells, were used for these experiments. The selected materials had specific gravities greater than unity, as do most biological materials. This meant that fall back limited, for example, sample protein concentrations, to about 0.15% by weight per unit volume. To obtain good laminar sample streams, the protein samples were diluted to 0.12% maximum protein by weight.

The effects of gravity persist, however, even after the sample is diluted. The effect of gravity on sample streams that are heavier than the surrounding buffer in upward flow is to increase residence time and to widen the particle streams. Widening of the particle streams can cause overlapping so that the desired separation can not be obtained.

The mechanism involved in these gravity effects is that a negative buoyant force results on the particle when the buffer is displaced by a particle with a higher specific gravity. For equilibrium, this force must be balanced by the viscous shear due to a particle velocity that is less than that of the surrounding buffer. For particles the size of proteins and to some extent for cells, the velocity difference is negligible. However, for equilibrium of the particle stream with the surrounding buffer the force of viscous shear on the outside of the stream must be equal and opposite to the sum of the buoyant forces on the particles within the stream. For particle specific gravities higher than buffer specific gravity in upward flow, the particle stream velocity will be less than that of the surrounding buffer. Particle stream widening occurs under these conditions, because the particles with lower velocities will have longer residence times and greater lateral movement than particles with higher velocities near the edges of the particle stream. In upward flow therefore, the middle of the sample continuously overtakes the leading edge, while the trailing edge falls farther and farther behind in the lateral direction. The expected result is that the apparent mobility of the particle stream will increase with both increasing concentration and decreasing buffer flow rate.

Two proteins were separated at various concentrations and flow rates to demonstrate the gravity effects. The two proteins used were human albumin and human fibrinogen. In preparation for the separation of a mixture of fibrinogen and albumin, electrophoresis was performed on each of the proteins using a range of field strength and buffer flow rates. The test data was correlated with the mathematical model by using the apparent electrophoretic mobility at the maximum flow and minimum concentration as a constant input. The three dimensional mathematical model used for this correlation is similar to the buffer flow model except that it calculates conditions at 1001 points in the vicinity of the particle stream and it includes both particle diffusion and gravity effects. Test versus predicted outlet concentration distribution for human albumin and human fibrinogen are shown in Figure 11.

In general, the predicted spreading of the samples is less than for the test data, both with and without applied electrical field, indicating that this spreading is a characteristic of the MDAC-St. Louis chamber. In addition, the smaller predicted movement of the proteins with the field applied is caused by actual residence times being greater than predicted, as evidenced by the buffer gravity effects data correlation.

Predicted gravity effects on electrophoresis of mixed proteins are shown in Figure 12. The greater movement under electrophoresis in one-g is caused by the increased residence times due to the particle streams slipping with respect to the buffer. Widening of the particle streams is not evident, however, because each of the separating streams was only at a fraction of the limiting concentration.

The effects of gravity on cell samples at varying concentrations and flow rates were demonstrated using lymphocytes. Test versus predicted outlet concentration distributions for 33H human lymphocytes are shown in Figure 13. Again, the predicted spreading of the sample is less than the test data, both with and without electrical field, indicating that the spreading is characteristic of the chamber. And as in the case of proteins, the predicted movement is less than the measured movement due to the actual residence time being greater than predicted.

Predicted gravity effects on electrophoresis of cells are shown in Figure 14. The greater movement under electrophoresis in one-g is caused by the increased residence time due to the particle streams slipping with respect to the buffer. As in the case of proteins, widening of the particle streams would probably become evident at higher concentrations or at greater electrophoretic movement.

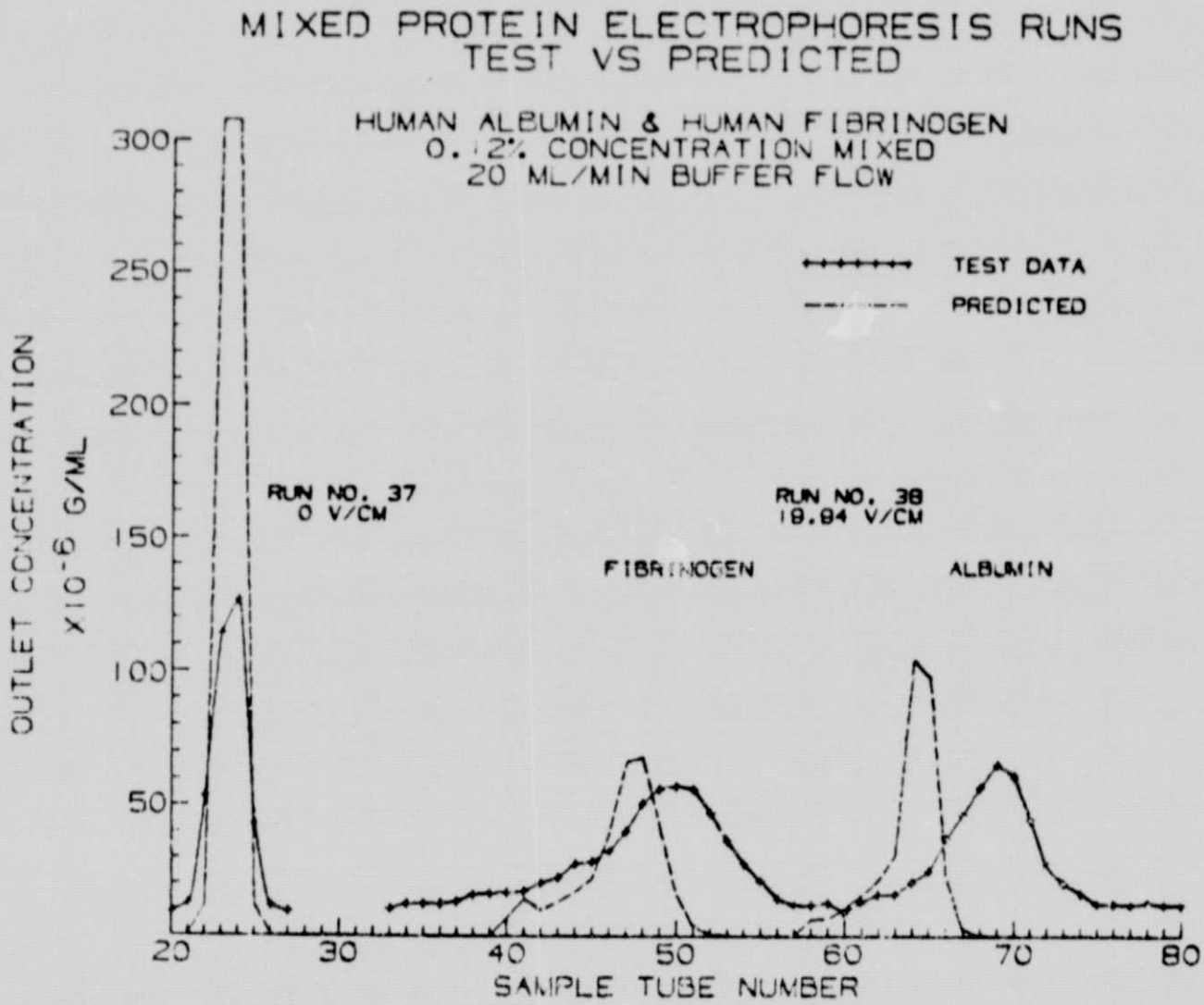
MIXED PROTEIN ELECTROPHORESIS RUNS
TEST VS PREDICTED
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.12% CONCENTRATION MIXED
20 ML/MIN BUFFER FLOW
TEST DATA
PREDICTED
RUN NO. 37
0 V/CM
RUN NO. 38
19.84 V/CM
FIBRINOGEN
ALBUMIN
OUTLET CONCENTRATION
X10-6 G/ML
300
250
200
150
100
50
20
30
40
50
60
70
80
SAMPLE TUBE NUMBER

FIGURE 11

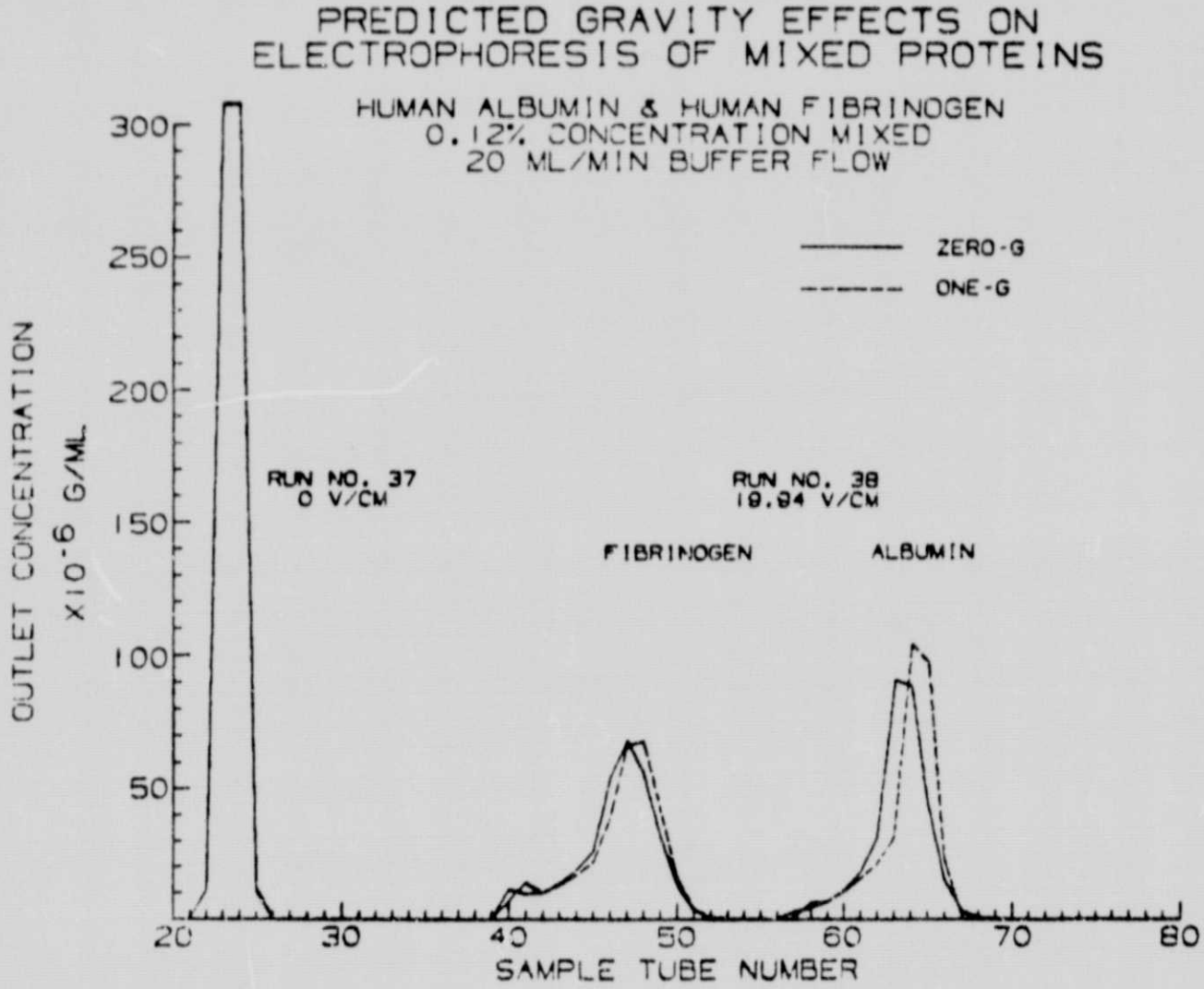
PREDICTED GRAVITY EFFECTS ON
ELECTROPHORESIS OF MIXED PROTEINS
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.12% CONCENTRATION MIXED
20 ML/MIN BUFFER FLOW
ZERO-G
ONE-G
OUTLET CONCENTRATION
X10-6 G/ML
300
250
200
150
100
50
RUN NO. 37
0 V/CM
RUN NO. 38
19.84 V/CM
FIBRINOGEN
ALBUMIN
20
30
40
50
60
70
80
SAMPLE TUBE NUMBER

FIGURE 12

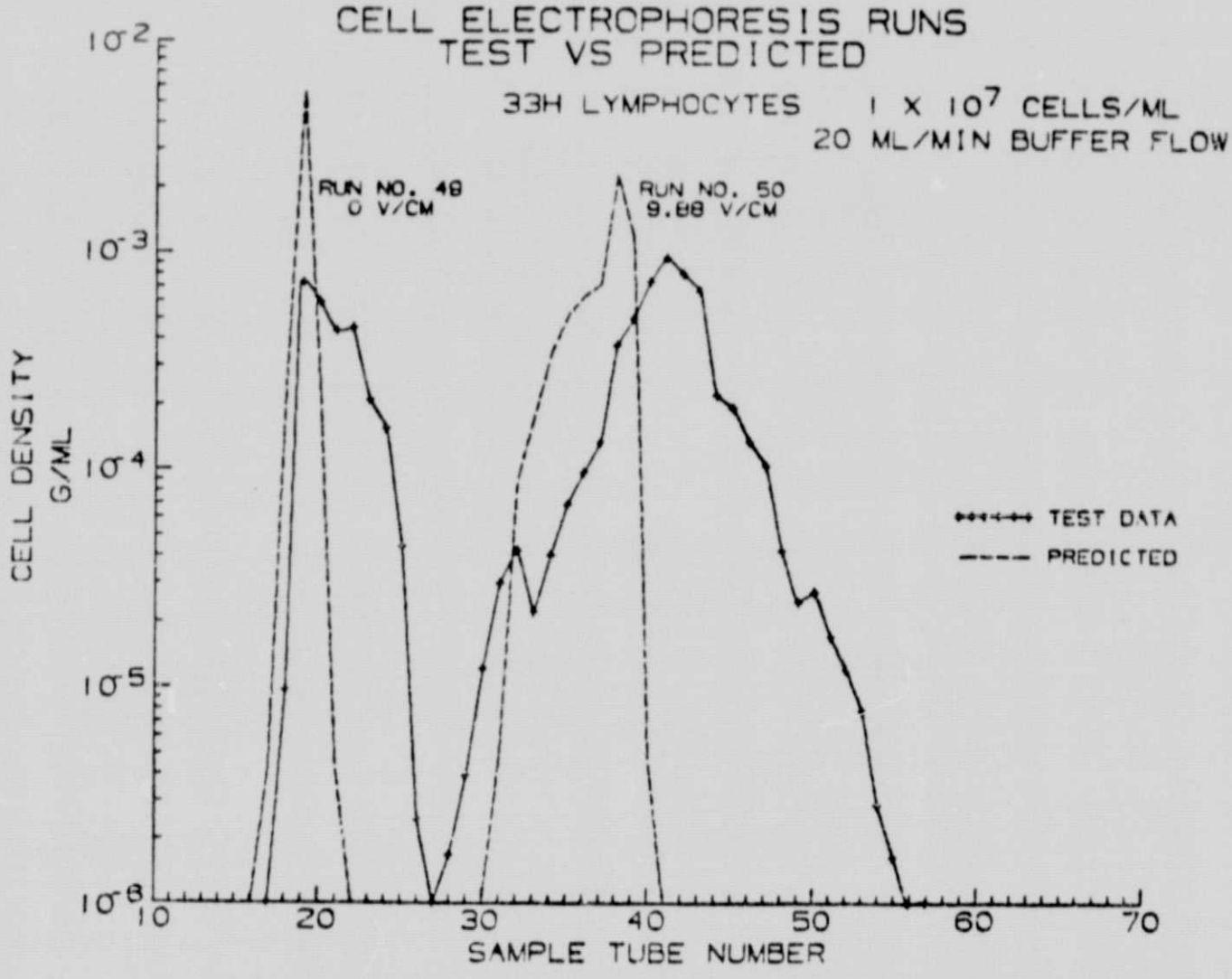
CELL ELECTROPHORESIS RUNS
TEST VS PREDICTED
33H LYMPHOCYTES 1 X 10^7 CELLS/ML
20 ML/MIN BUFFER FLOW
RUN NO. 48
0 V/CM
RUN NO. 50
9.88 V/CM
TEST DATA
PREDICTED
CELL DENSITY
G/ML
10^-2
10^-3
10^-4
10^-5
10^-6
10
20
30
40
50
60
70
SAMPLE TUBE NUMBER

FIGURE 13

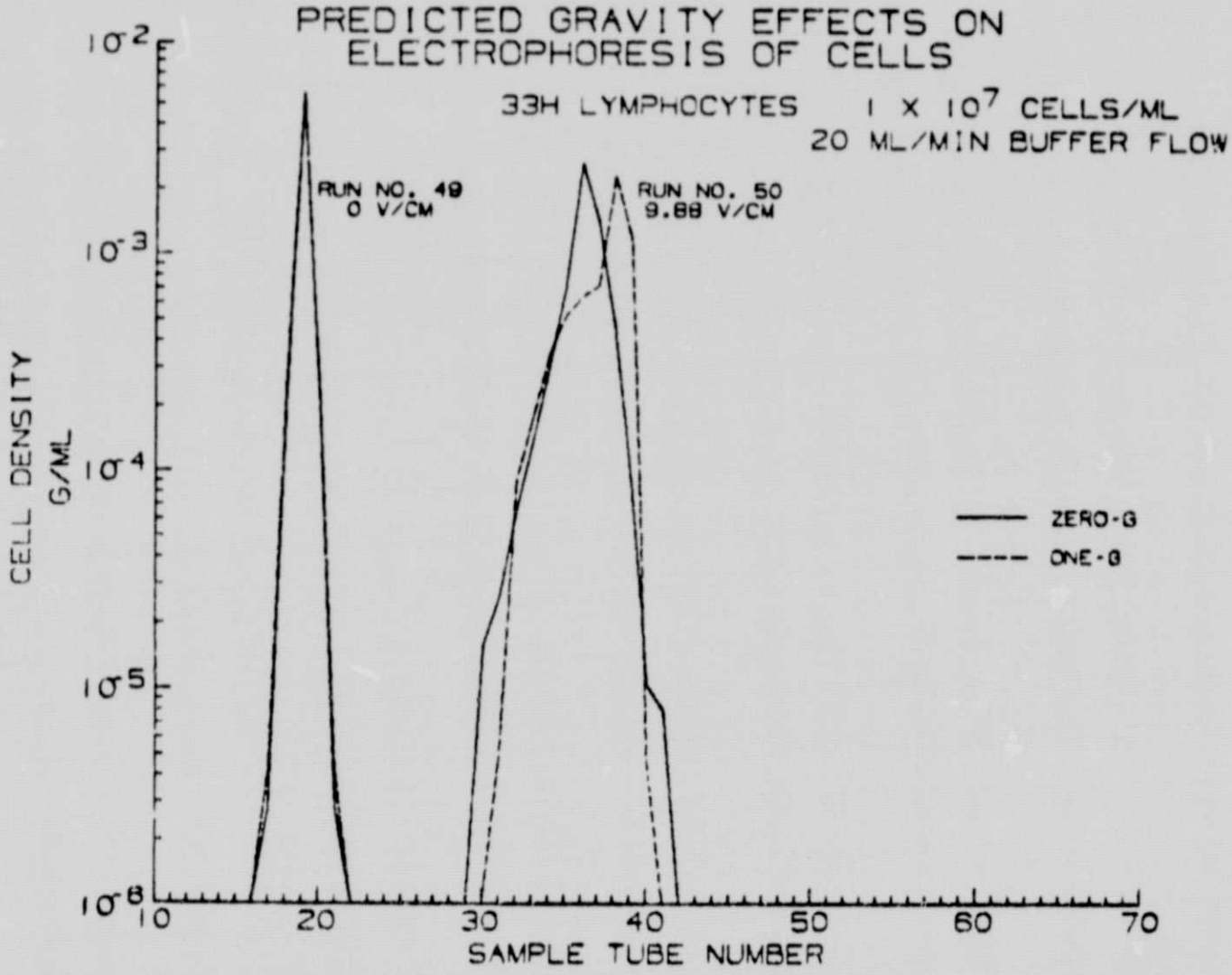
PREDICTED GRAVITY EFFECTS ON
ELECTROPHORESIS OF CELLS
33H LYMPHOCYTES 1 X 10^7 CELLS/ML
20 ML/MIN BUFFER FLOW
RUN NO. 49
0 V/CM
RUN NO. 50
9.88 V/CM
ZERO-G
ONE-G
CELL DENSITY
G/ML
10^-2
10^-3
10^-4
10^-5
10^-6
10
20
30
40
50
60
70
SAMPLE TUBE NUMBER

FIGURE 14

4.0 EFFECTS OF SAMPLE CONCENTRATION ON ELECTROPHORETIC MOBILITY

The purpose of Task 3.0 of this study was to determine if sample concentration has a significant effect on the electrophoretic mobility of the individual protein components of the sample. This is of interest because previous MDAC-St. Louis studies showed that only very dilute samples can be processed in a one-g environment by free flow electrophoresis, and that throughput in the microgravity environment could be increased substantially by processing more highly concentrated samples. Instead of diluting human plasma 70 times with water, for example, it may be possible to process concentrated plasma samples thus increasing the sample concentration from about 0.1% on Earth to 28% or even higher in the micro-gravity space environment.

Questions have arisen, however, concerning protein-protein interaction at concentrations above 0.1% which may change the electrophoretic mobility of individual proteins or in some other way interfere with their electrophoretic separation. If interfering interactions do occur, then the benefits of purity, attainable in space, would be offset by poor resolution as a result of these protein interactions.

In order to detect the possible effects of sample concentration on electrophoretic mobility three common ground based electrophoretic procedures were employed and the mobilities of various proteins in human plasma at several concentrations ranging from 0.109% to 28% total protein were studied.

Two of these methods, agar gel plate and polyacrylimide disc gel electrophoresis, gave consistent reliable test results and were used to evaluate mobilities of the various proteins. A third method, using cellulose acetate strip electrophoresis provided erratic data from day to day and was not used for evaluation.

Test results obtained using agar gel plate electrophoresis are summarized in Figure 15 and 16 . No significant differences in protein mobilities were noted at any of the concentrations tested over a range of 0.875% to 28%.

MIGRATION OF PLASMA PROTEINS ON CORNING AGAR GEL PLATES
RUN 1

% PLASMA CONCENTRATION	PROTEIN MEASURED					
	ALBUMIN	α-1 GLOBULIN	α-2 GLOBULIN	β-1 GLOBULIN	FIBRINOGEN	γ GLOBULIN
	DISTANCE MOVED (cm) AT 85 VOLTS; FIELD STRENGTH 15 VOLTS/cm					
7.0	1.8	1.5	1.0	0.60	0.25	−0.25
3.5	1.8	1.5	1.0	0.60	0.25	−0.25
1.75	1.8	N.V.	1.0	0.60	0.25	N.V.
0.875	1.8	N.V.	N.V.	0.55	0.25	N.V.

N.V. — BANDS WERE NOT VISIBLE DUE TO DILUTION

Figure 15

MIGRATION OF PLASMA PROTEINS ON CORNING AGAR GEL PLATES
RUN 2

% PLASMA CONCENTRATION	PROTEIN MEASURED					
	ALBUMIN	α-1 GLOBULIN	α-2 GLOBULIN	β-1 GLOBULIN	FIBRINOGEN	γ GLOBULIN
	DISTANCE MOVED (cm) AT 85 VOLTS; FIELD STRENGTH 15 VOLTS/cm					
7.0	1.9	1.55	1.0	0.65	0.25	−0.25
28.0	1.9	1.55	1.0	0.65	0.25	−0.25
7.0 (REDILUTED)	1.9	1.55	1.0	0.65	0.25	−0.25

Figure 16

Results obtained using polyacrylamide gel electrophoresis are shown in Figure 17. In this procedure one anomaly occurred. Albumin appeared to have increased mobility in the higher concentrated samples. However, this apparent increased mobility is probably the result of an overloading of the gel capacity and resultant exclusion of a portion of the albumin molecules from the molecular sieve action of the gel.

MIGRATION OF PLASMA PROTEINS IN POLYACRYLAMIDE DISC GEL ELECTROPHORESIS

% PLASMA CONCENTRATION	PROTEIN MEASURED					
	PRE-ALBUMIN	ALBUMIN	α-1 GLOBULIN	β-1 GLOBULINS (HEMOGLOBIN AND TRANSFERRIN)	α-2 GLOBULIN	γ GLOBULIN
	DISTANCE MOVED (cm) AT 150 VOLTS, FIELD STRENGTH, 12 VOLTS/cm					
7	5.8	4.4	3.3	2.1	1.4	0
3.5	5.8	4.4	3.3	2.2	1.4	0
1.75	N.V.	4.3	3.4	2.3	1.4	0
0.875	N.V.	4.2	3.3	2.3	1.4	0
0.437	N.V.	4.2	N.V.	2.2	N.V.	0
0.218	N.V.	4.2	N.V.	2.2	N.V.	0
0.109	N.V.	4.1	N.V.	2.1	N.V.	0

N.V. – NOT VISIBLE DUE TO DILUTION

Figure 17

5.0 CONCLUSIONS AND RECOMMENDATIONS

Principal conclusions of this investigation are that the carrier buffer flow is affected by gravity induced thermal convection and that the movement of the separating particle streams is affected by gravity induced buoyant forces. Although much has been written about the fluid convection effects, the gravity effect on the particle streams is probably more important. It is this effect that limits the allowable density difference between the particle stream and the surrounding buffer to a fraction of a percent. And even within this allowable range of density difference, velocity variations within the stream cause widening of the particle streams during electrophoresis. Widening of the particle streams can cause the streams to overlap, limiting separation capability.

One finding of this investigation is that mathematical models, if they include the gravity induced buoyancy forces, should be able to effectively predict electrophoresis chamber separation performance. Additional work is recommended in the areas of correlation with the upward flow velocities with field applied and in testing to reliably determine wall electroosmotic flow velocities using microelectrophoresis for the tested combinations of wall material and buffers.

Another finding of this investigation is that sample concentration, using ground based electrophoresis procedures does not affect protein electrophoretic mobility over the range of 0.1% to 28%.

This investigation should provide a starting point for meaningful comparisons of free-flow electrophoresis chamber performance, i.e. output and separation capability, on the earth and under microgravity conditions and additional work in this area is planned.

6.0 REFERENCES

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