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REPORT MDC E2275

INVESTIGATION OF THE FREE FLOW ELECTROPHORETIC PROCESS

FINAL REPORT

MCDONNELL DOUGLAS ASTRONAUTICS COMPANY - ST. LOUIS DIVISION

MCDONNELL DOUGLAS CORPORATION

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FINAL REPORT

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PREFACE

This MDC report entitled "Investigation of the Free Flow Electrophoretic Process" is submitted under NASA Contract Number NAS 8-33713.

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 continuation of the work described in MDC Report E2000, dated May 1979, that extends the scope of the work to include the separation of mixtures of standard particles. This contract was administered by the NASA Marshall Space Flight Center, Huntsville, Alabama.

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SECTION 1.0
BACKGROUND AND APPROACH

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 (Ref. 1) and free flow (Ref. 2) electrophoresis to separate biological materials in a microgravity environment. Cells separated during static electrophoresis showed increased production of urokinase and erythropoietin when subsequently subcultured in earth-based laboratories (Ref. 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 research 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 trade-off analyses to be made. The purpose of the proposed work 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.

MDAC-St. Louis recently completed a seven month investigation of the free flow electrophoretic process (Ref. 4). This work included experiments designed to demonstrate the effects of gravity on the carrier buffer flow and on sample separations. Samples of mixed proteins were separated and live cells were exposed to electrophoresis. As a result of this work with biological materials the desirability of gathering comparative data for standard particles was recognized.

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, 16 cm wide, and 0.15 cm thick. The chamber and its supporting hardware are shown in Figure 1-1, Electrophoresis Test Setup. Flow in this chamber is in the upward direction and exits through 197 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

ELECTROPHORESIS TEST SETUP

17-677

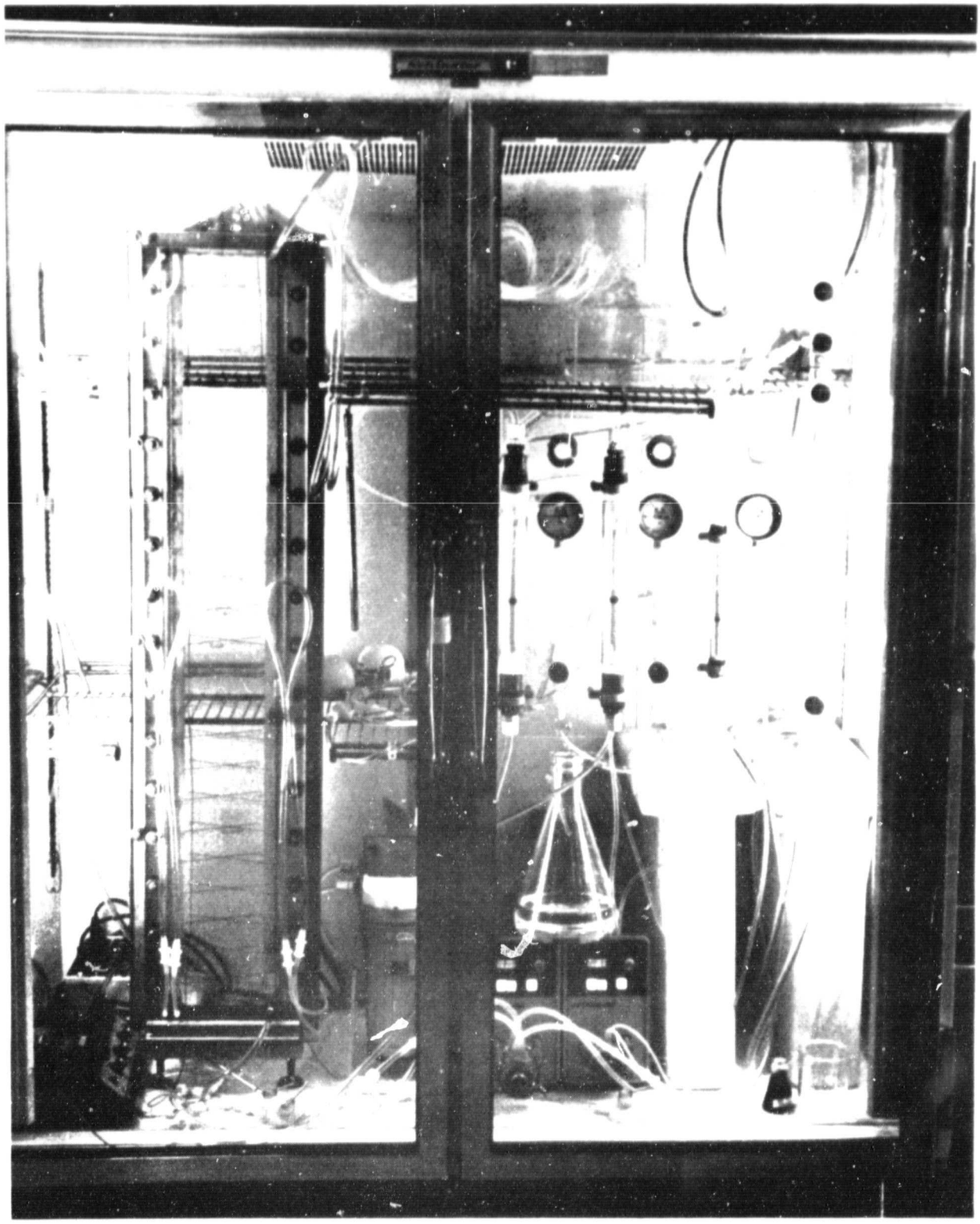

Figure 1-1

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 and particle shape dependent. The characteristic that describes particle motion is electrophoretic mobility, which is the lateral velocity divided by electrical field strength.

This effort is a continuation of the work described in MDC Report E2000, dated May 1979 (Ref. 4). It extends the scope of this work to include the separation of mixtures of standard particles. The results of these separations of particles, which are not soluble in the carrier buffer, will be compared with those for protein mixtures, which are normally soluble in the buffer.

SECTION 2.0
TEST PROGRAM

Several tests were conducted to effect the separation of mixtures of proteins and mixtures of standard particles. The purpose of these tests was to provide comparative date for proteins that are normally soluble in the carrier buffer fluid and for particles which are not. The proposed work was divided into two tasks: Task 1.0, Electrophoretic Separations; and Task 2.0, Mathematical Analysis.

2.1 Electrophoretic Separations - The electrophoretic separation tests consisted of the twelve runs described in Figures 2-1 for protein mixtures and 2-2 for mixtures of standard particles. The protein separation tests used a mixture of human fibrinogen and human albumin in the R-1 buffer suggested by the MSFC Space Sciences Laboratory. The test parameters, 40 and 80 ml/min buffer flow rate and 0.10% and 0.03% sample concentration are also selected for continuity with previous work. The distribution of the proteins in the outlet fractions were determined by a modified Lowry (Ref. 5) protein assay technique.

Figure 2-2 describes the four test runs to determine the separability of standard particles. These tests were performed using R-1 buffer so that the data could be compared with data obtained by MSFC Space Sciences Laboratory. The distribution of the particles in the outlet fractions were determined using standard particle counting techniques. The standard particles were furnished by MSFC Space Sciences Laboratory for continuity with their previous work in neutrally buoyant particle development.

2.2 Mathematical Analysis - The results of the electrophoretic separation in Task 1.0 were compared with predictions made using an MDAC-St. Louis mathematical model. This three-dimensional simulation models the flow and concentration in the vicinity of the sample stream. The results of the comparisons of test data and analytical predictions are presented in Section 3.0 for evaluation with comparable programs at MSFC in order to facilitate prediction of performance in zero g operation.

FIGURE 2-1
TASK 1.0 TEST MATRIX, RUNS 1-8

BUFFER FLOW ML/MIN / PROTEIN CONCENTRATION	40	80
0.12%	2 / 1	4 / 3
0.034%	6 / 5	8 / 7

FIELD STRENGTH

20 VOLTS /CM / 0 VOLT/CM

FIGURE 2-2
TASK 1.0 TEST MATRIX, RUNS 9-12

BUFFER FLOW ML/MIN	40	80
STANDARD PARTICLES	10 / 9	12 / 11

SECTION 3.0
RESULTS AND CONCLUSIONS

The results of the electrophoretic separations and the mathematical data correlations are presented in Figures 3-1 through 3-6. Although listed second in the Statement of Work, the standard particle separations were accomplished first and are discussed before the protein separations.

3.1 Mixed Standard Particle Separations - Figure 3-1 shows the results of a separation of standard particles at a large product of field strength and residence time. These separations were performed using R-1 phosphate buffers, because data are available at the MSFC Space Sciences Laboratory on the mobility of standard particles in this buffer and the wall electro-osmotic mobility for this buffer in contact with polycarbonate (Lexan). The mobilities of fixed cow and turkey red blood cells as measured by the MSFC Space Sciences Laboratory are 2.0×10^{-4} and 2.9×10^{-4} cm^2/volt-sec, respectively. The wall electro-osmotic mobility, as measured by J. W. Vanderhoff, was -3.1×10^{-4} cm^2/volt-sec (Ref. 5). It was apparent from the initial correlation attempts that the apparent mobilities from the Run 10 and Run 12 test data were considerably larger or that the residence times or field strengths were greater. Field strength variation was ruled out, because it is measured directly using voltage probes in contact with the carrier buffer inside the chamber. It was suspected, however, that chamber thickness could be greater than nominal, increasing residence time. Theoretical residence time for a 0.15 cm thick chamber at 20 ml/min is 14.4 minutes. Actual residence times measured using dye tracers at three different flow rates averaged about 80% larger, so for the comparison runs a chamber thickness of 0.275 cm was used. Even with this increase the apparent mobilities were higher than indicated by the MSFC data. To investigate this problem, the mobilities of the fixed cow and turkey red blood cells were measured using a Laser Zee 500. The mobilities, which measured about 40% greater than the MSFC data, were 2.7×10^{-4} and 4.3×10^{-4} cm^2/volt-sec for the fixed cow and turkey red blood cells, respectively. Therefore, it was decided to increase both the electrophoretic and electro-osmotic mobilities to achieve better correlation. The mobilities used for the correlation runs were 3.0×10^{-4} and 4.7×10^{-4} cm^2/volt-sec for cow and turkey red blood cells, respectively, and -5.2×10^{-4} cm^2/volt-sec for wall electro-osmotic mobility.

With these mobilities the predicted outlet peaks are close to the actuals, however, the actuals do indicate more spreading than predicted. This probably results from lack of homogeneity among the cells of each type; in fact, the small peaks between the major peaks were identified under a microscope as cells that differed from the others in their characteristics.

Spreading of the sample with no field applied indicates that at this low flow rate the sample is affected by gravity, which could also account for some of the spreading in the separated samples.

Separations at higher flow rates such as that shown in Figure 3-2 for 40 ml/min buffer flow exhibit tighter distributions and peaks closer to the predicted values. In this separation, the field strength was increased by 33% while the residence time was halved, resulting in a separation potential two-thirds as great as for Run 10. Here again, the small peaks between the major peaks are real as evidenced by counting under a microscope.

3.2 Mixed Protein Separations - The results of the mixed protein separations and mathematical analyses are shown in Figures 3-3 through 3-6. These same proteins, human albumin and human fibrinogen, were separated in the previous work reported in MDC Report E2000. Those separations used barbital buffer at pH 8.6, but the latest runs were in R-1 buffer at pH 7.2. Because of the lower pH decreased mobilities could be expected, probably less than one-half of those for barbital. However, since the mobilities in R-1 were unknown, the values which best correlated the data in the previous work in barbital were used for comparison. As evidenced in the figures, the predicted deflection under electrophoresis is greater than the actual. However, because of the pH change the predicted deflection would be less than one-half of the indicated deflections and the predicted peaks consistently to the left of the actual peaks. The differences between the theoretical and actual residence times quantified for the standard particle separations is also applicable to the mixed protein separations, since the chamber geometry was unchanged. Therefore, the deflections should at the same time be increased by 80%, which would result in predicted deflections closer to the actual than are shown in the figures.

3.3 Conclusions - The principal difficulties in the correlation of data continues to be lack of both electrophoretic and electro-osmotic mobility data. Most of the available data for standard particles were taken at 25°C and temperature corrections were made assuming that mobility varied inversely with the viscosity as determined by Vanderhoff for A-1 buffer (Ref. 6), a variant of the R-1 buffer used. Future work should involve the determination of mobilities at working temperatures to confirm this assumption.

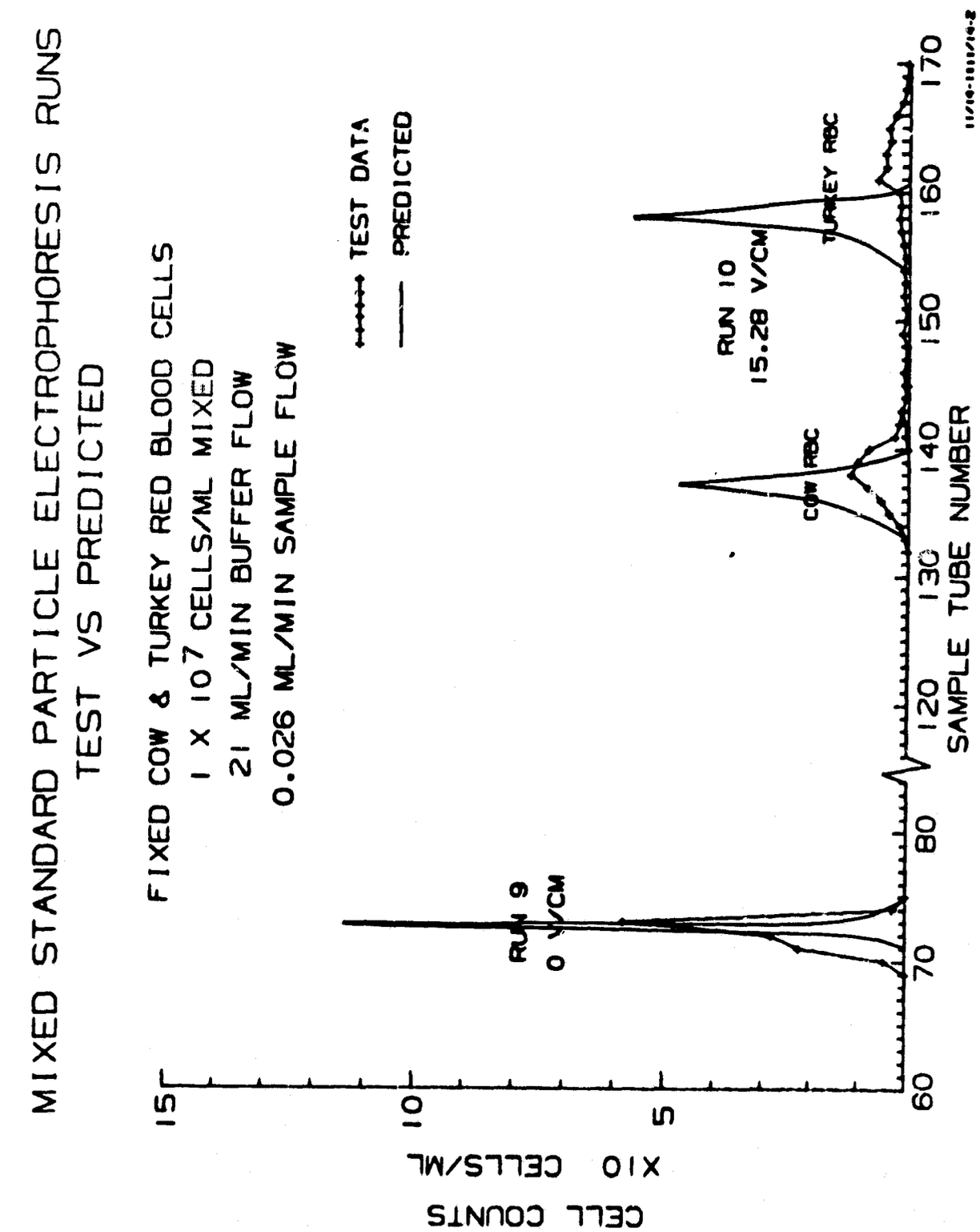
MIXED STANDARD PARTICLE ELECTROPHORESIS RUNS
TEST VS PREDICTED
FIXED COW & TURKEY RED BLOOD CELLS
1 X 10⁷ CELLS/ML MIXED
21 ML/MIN BUFFER FLOW
0.026 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 9
0 V/CM
RUN 10
15.28 V/CM
COW RBC
TURKEY RBC
CELL COUNTS
X10 CELLS/ML
SAMPLE TUBE NUMBER
15
10
5
60
70
80
120
130
140
150
160
170

Figure 3-1

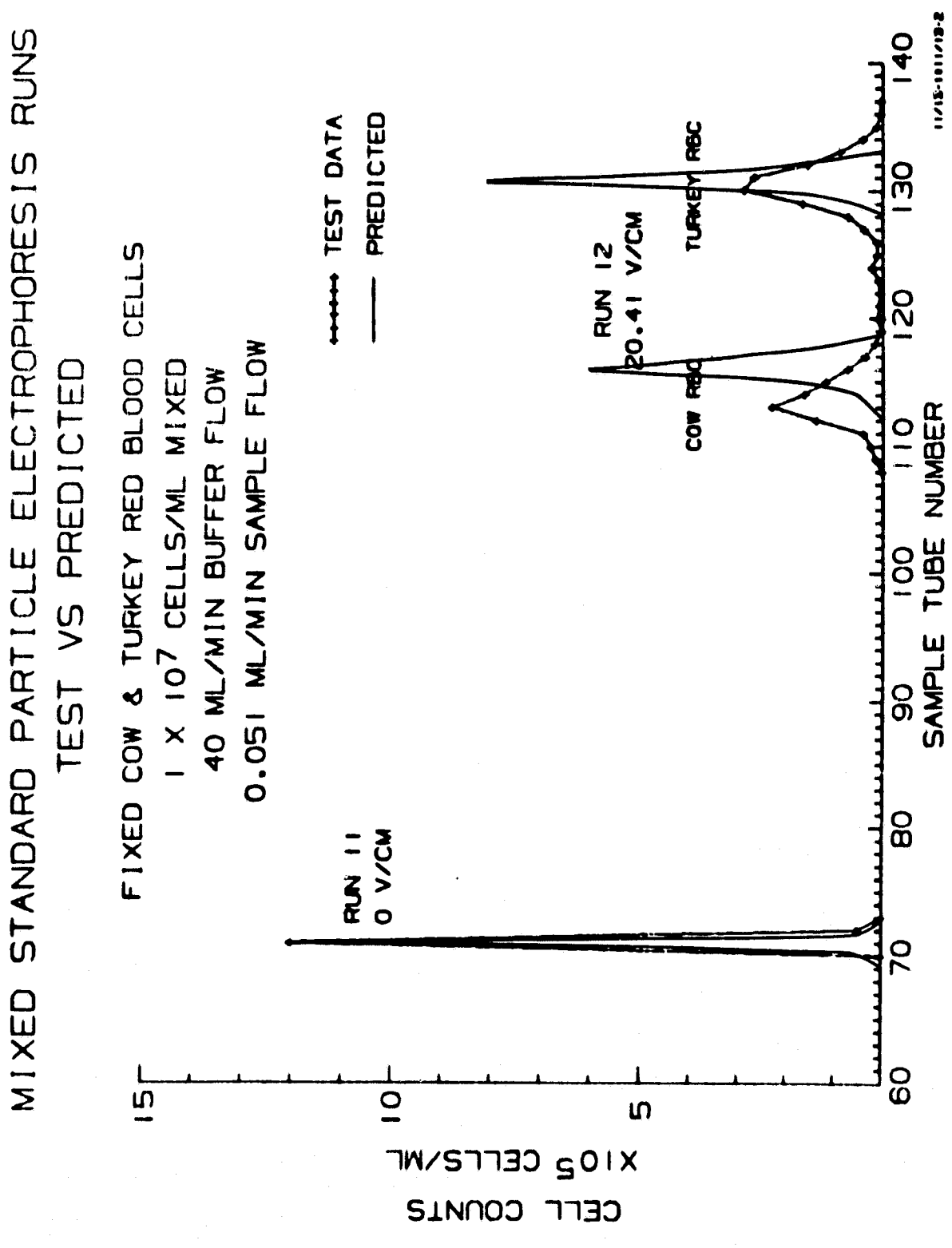
MIXED STANDARD PARTICLE ELECTROPHORESIS RUNS
TEST VS PREDICTED
FIXED COW & TURKEY RED BLOOD CELLS
1 X 10⁷ CELLS/ML MIXED
40 ML/MIN BUFFER FLOW
0.051 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 11
0 V/CM
RUN 12
20.41 V/CM
COW RBC
TURKEY RBC
CELL COUNTS
X10⁵ CELLS/ML
SAMPLE TUBE NUMBER
15
10
5
60
70
80
90
100
110
120
130
140

Figure 3-2

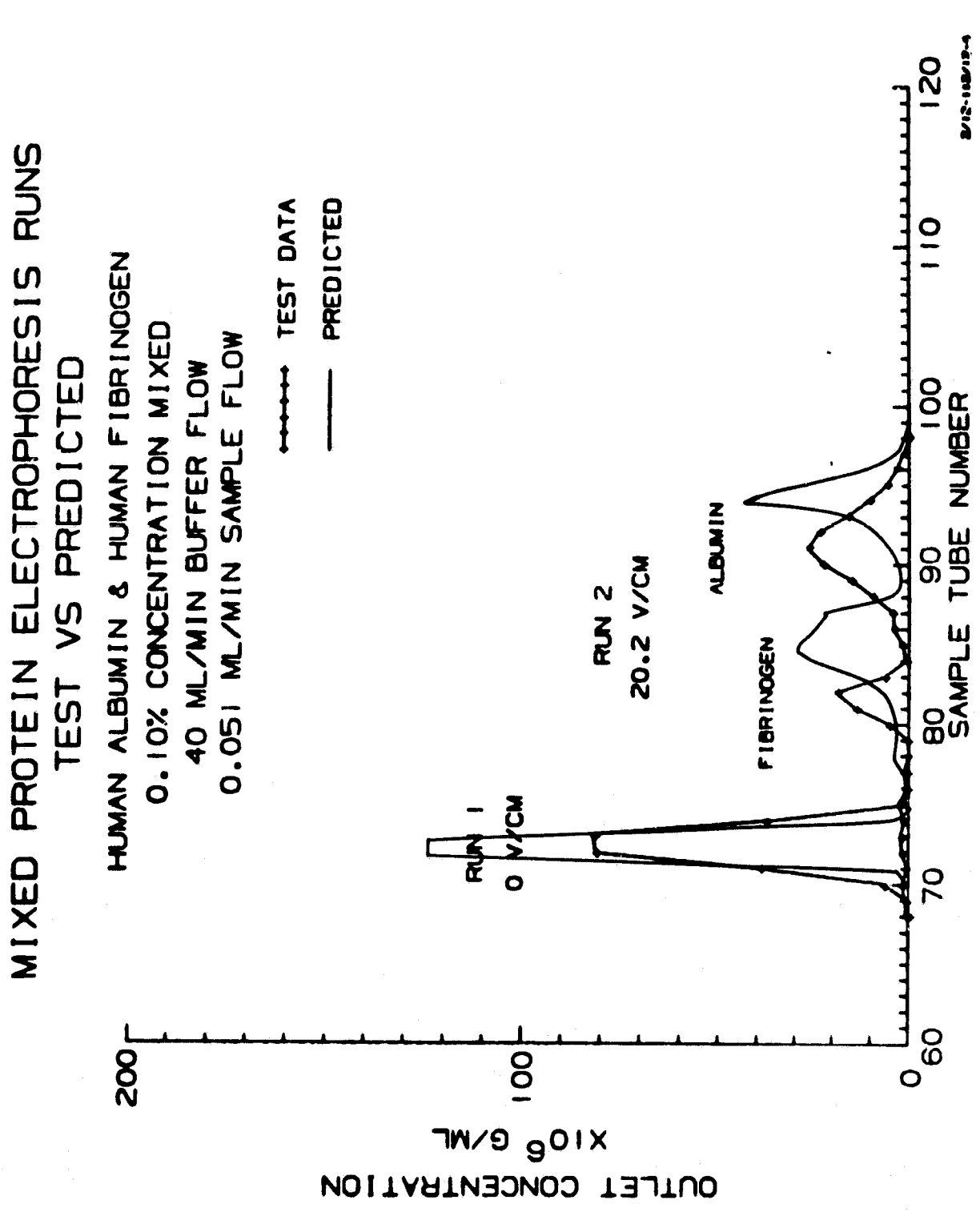
MIXED PROTEIN ELECTROPHORESIS RUNS
TEST VS PREDICTED
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.10% CONCENTRATION MIXED
40 ML/MIN BUFFER FLOW
0.051 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 1
0 V/CM
RUN 2
20.2 V/CM
ALBUMIN
FIBRINOGEN
OUTLET CONCENTRATION
X10⁶ G/ML
SAMPLE TUBE NUMBER
200
100
0
60
70
80
90
100
110
120

Figure 3-3

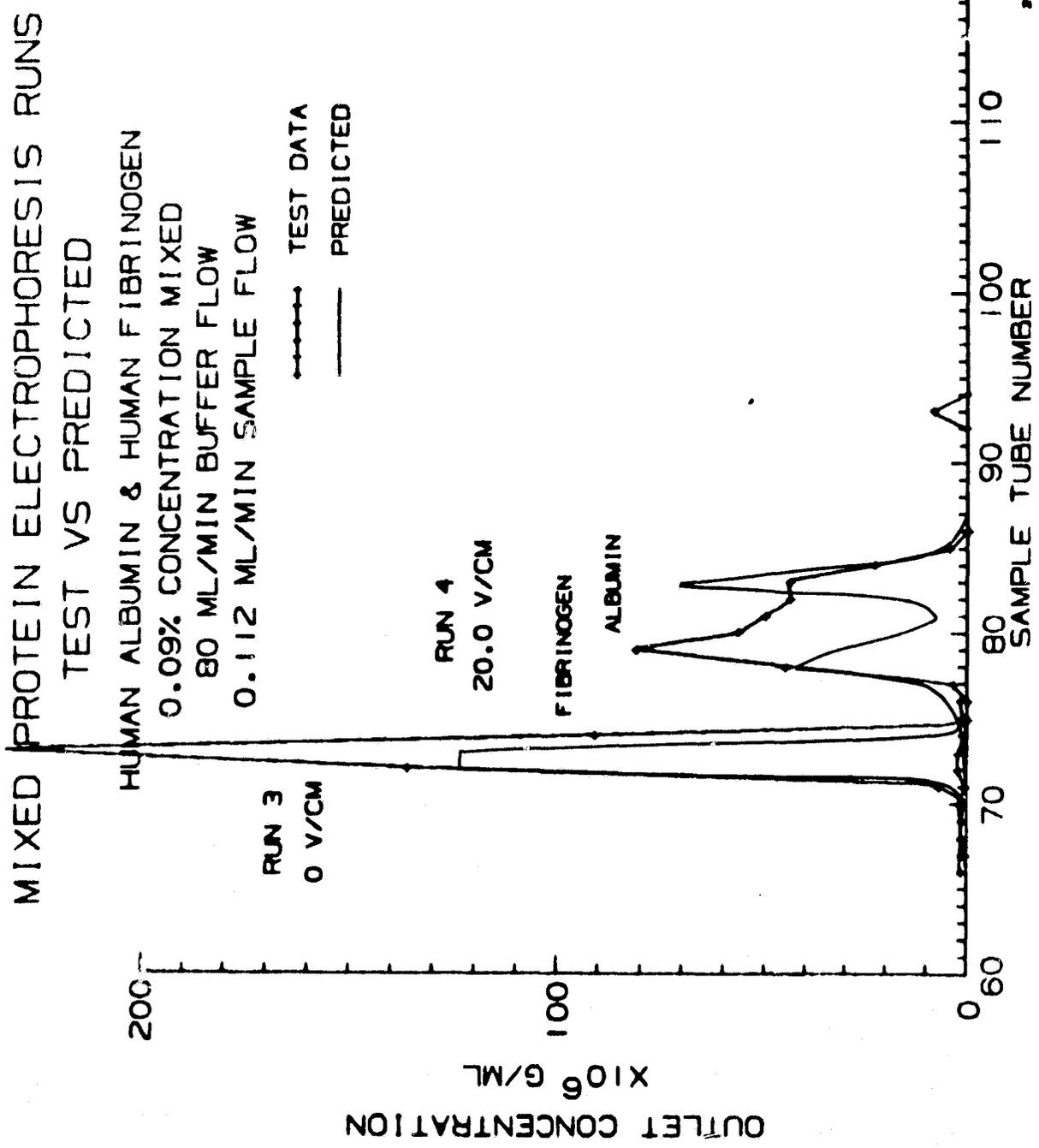
MIXED PROTEIN ELECTROPHORESIS RUNS
TEST VS PREDICTED
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.09% CONCENTRATION MIXED
80 ML/MIN BUFFER FLOW
0.112 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 3
0 V/CM
RUN 4
20.0 V/CM
FIBRINOGEN
ALBUMIN
OUTLET CONCENTRATION
X10⁶ G/ML
200
100
0
60
70
80
90
100
110
120
SAMPLE TUBE NUMBER

Figure 3-4

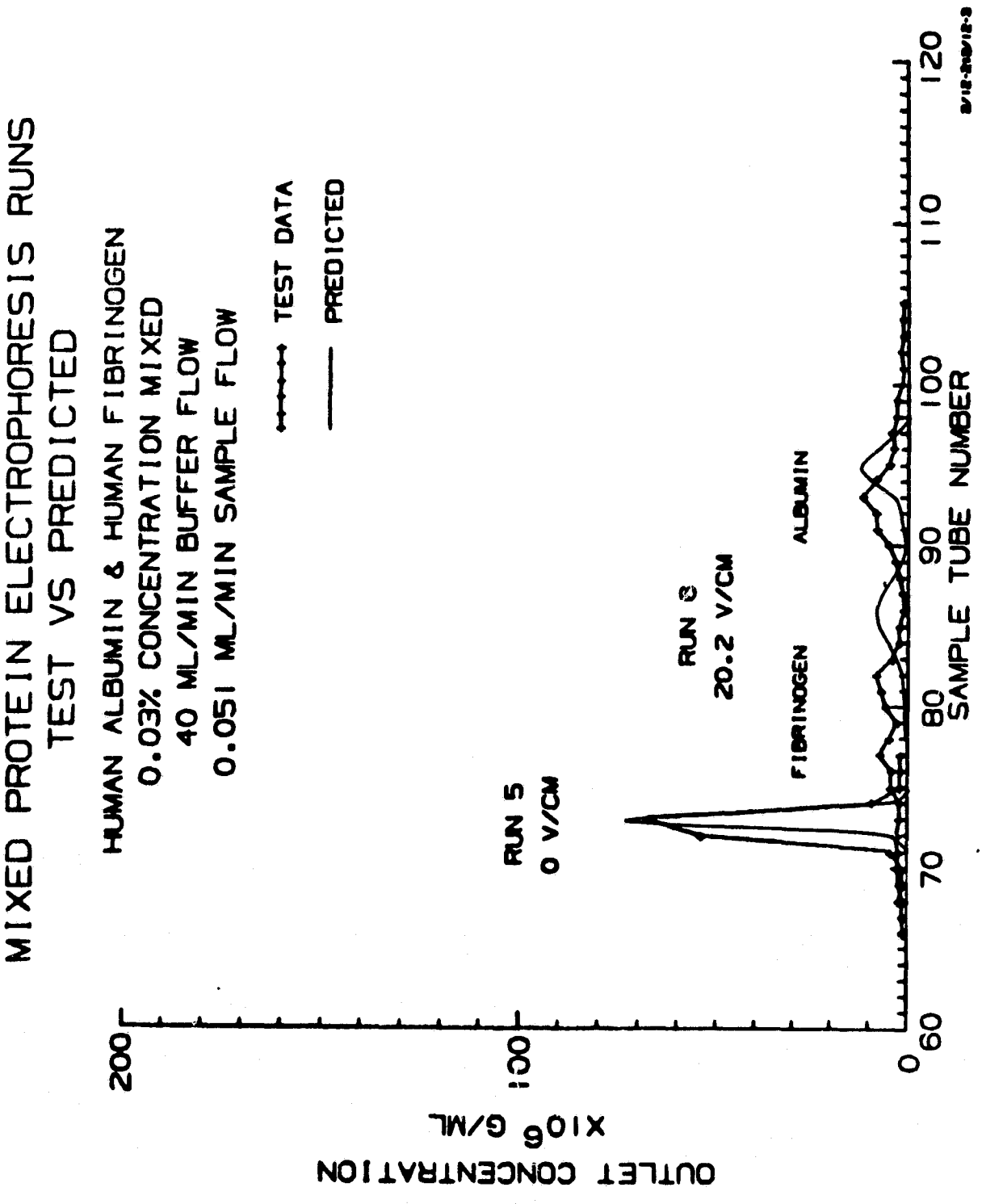
MIXED PROTEIN ELECTROPHORESIS RUNS
TEST VS PREDICTED
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.03% CONCENTRATION MIXED
40 ML/MIN BUFFER FLOW
0.051 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 5
0 V/CM
RUN 8
20.2 V/CM
FIBRINOGEN
ALBUMIN
OUTLET CONCENTRATION
X10⁶ G/ML
200
100
0
60
70
80
90
100
110
120
SAMPLE TUBE NUMBER

Figure 3-5

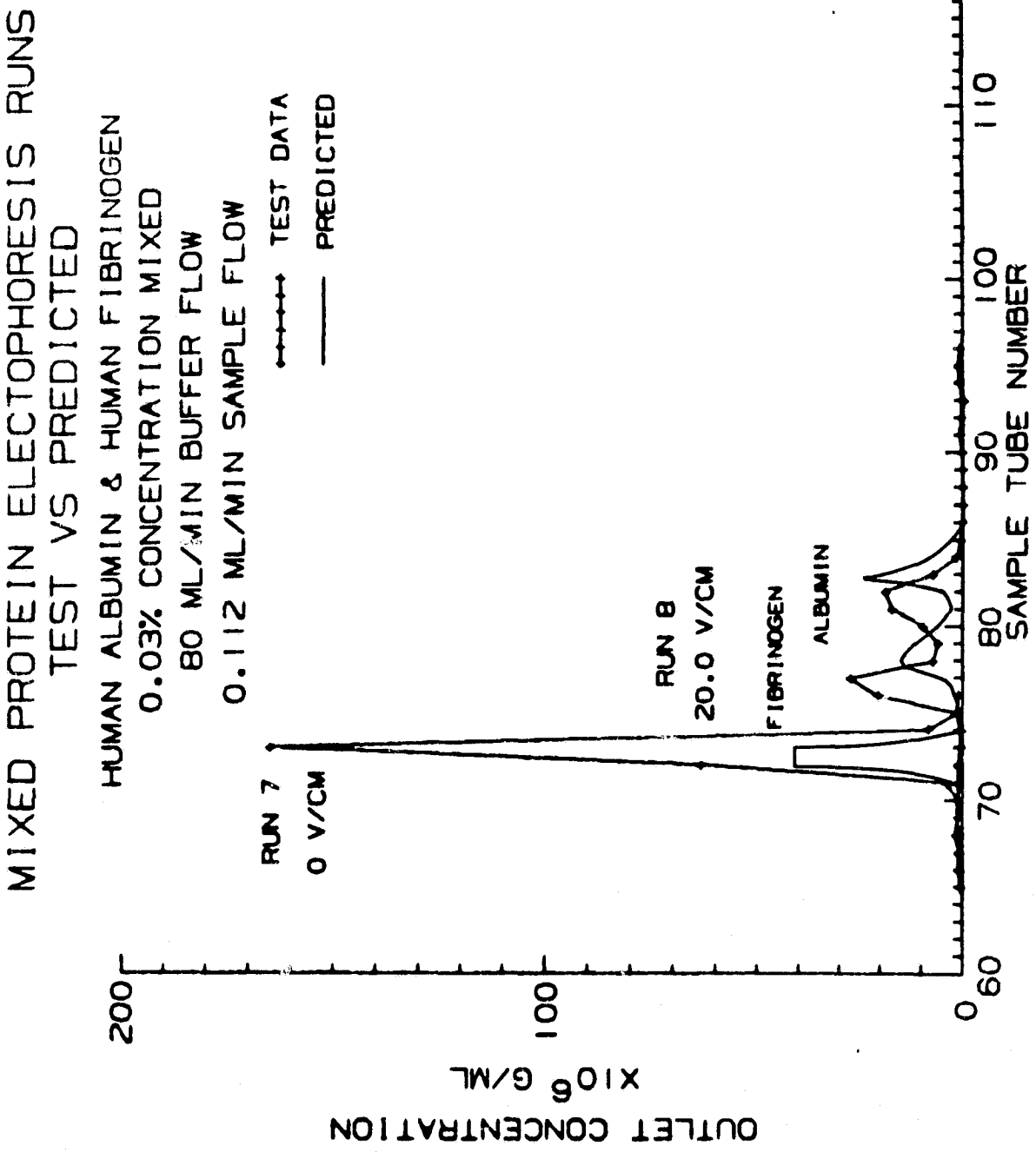
MIXED PROTEIN ELECTOPHORESIS RUNS
TEST VS PREDICTED
HUMAN ALBUMIN & HUMAN FIBRINOGEN
0.03% CONCENTRATION MIXED
80 ML/MIN BUFFER FLOW
0.112 ML/MIN SAMPLE FLOW
TEST DATA
PREDICTED
RUN 7
0 V/CM
RUN 8
20.0 V/CM
FIBRINOGEN
ALBUMIN
OUTLET CONCENTRATION
X10⁶ G/ML
200
100
0
60
70
80
90
100
110
120
SAMPLE TUBE NUMBER

Figure 3-6

4.0 REFERENCES

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