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ELECTROPHORESIS SEPARATION IN SPACE-APOLLO 14

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16. ABSTRACT

Fluid electrophoresis separation, based on the motion of particles in an electric field, was suggested as a promising process to make practical use of the near-zero-gravity condition in space. Hence, an apparatus was developed to demonstrate the principle and possible problems of electrophoresis on Apollo 14. It applied 30 volts per centimeter to samples of mixed red and blue dye, hemoglobin, and deoxyribonucleic acid, in aqueous solutions of boric acid. A filter system was provided to remove gas bubbles formed in the electrolyte. Photographs of the action in the tube indicated that the shape and sharpness of the separation boundary between the dyes was better in space than on earth. Difficulties with bacterial action precluded observation of the other samples, based on post-flight ground tests. Much was learned about the potential and the requirements of doing electrophoresis in space.

* General Electric Company

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INTRODUCTION

Fluid electrophoresis is a separation process based on the motion of particles in a fluid due to the force of an electric field. This process was suggested as one of the more promising ideas in a survey of potential materials processes which could provide practical uses of the space environment. It uses the near-zero-gravity condition of space to improve a well known chemical separation process by reducing sedimentation and thermal convective mixing. It was first suggested in a study contract, NAS8-24683, in March 1970, by the General Electric Company (Ref. 1). This study investigated several physical and chemical processes for the preparation of materials in space. Electrophoresis was chosen as one of the simplest processes to perform in space with the greatest potential benefit to mankind. It was postulated that, after demonstration of the basic advantages of electrophoresis separations in zero gravity, small but significant quantities of biological materials such as vaccines, viral insecticides, and other valuable materials could be purified and separated in space, economically.

THEORY OF ELECTROPHORESIS

Electrophoresis means "borne of electricity" (Ref. 2). Most materials which can be divided into fine particles take on a charge when dispersed in an aqueous solvent. The charge may be due to partial ionization, adsorption of ions on the dispersed solid particles, or ion-pair formation. Particles move through a fluid to the oppositely charged electrode at a velocity dependent upon their accumulated charge, size and shape. After a period of time, particles separate according to their velocity into distinct zones, just as runners in a race spread out over the course. The solvent must be an electrolyte, usually a buffer, whose pH is adjusted to a value that provides the optimum surface charge density and the least chemically aggressive environment to the solute.

The particles move with a velocity, U, which is proportional to ζ, the particle zeta potential; to E, the electric field strength; to D, the dielectric contant of the medium; and is inversely proportional to η, the viscosity of the medium. The exact relationship between electrophoretic velocity and these factors is also a complex function of the size and shape of the particles. Discussion of these relationships is beyond the scope of this paper, but an excellent analysis can be found in reference 3.

When the ratio of particle radius, a, to thickness, δ, of the ionic atmosphere is greater than about 100,

$$U = \frac{\zeta ED}{4\pi\eta}$$

which is the generally accepted Holmholtz-Smoluchowski relation. When the ratio a/δ is less than about 0.1 (the Debye-Huckel range) electrophoretic mobility is characterized by a similar relationship:

$$U = \frac{\zeta ED}{6\pi\eta}$$

But between these extremes the variation of electrophoretic velocity with zeta potential is a complex function of a/δ and therefore of particle size. In the case of two dyes in the Apollo 14 demonstration, however, the above Debye-Huckel relation applied. Separation of the two dyes in the Apollo 14 experiment can be theoretically characterized as a linear function of electrophoresis time and difference in the zeta potentials (other conditions being held constant). At the same time, separation is adversely affected by diffusion, sedimentation, and convective mixing. The separation may be speeded by increasing voltage, but Joule heating increases as the square of the voltage. Hence, means were sought to improve the sharpness of the resolution without thermal convection.

DEVELOPMENT OF APOLLO 14 DEMONSTRATION

Based on the great potential uses for this process, a simple demonstration of the principle and possible problems was needed at the earliest possible date. Since the sustained near-zero-gravity conditions could be achieved only on a space flight, a demonstration was suggested by Dr. James Bredt to take place during the return from the moon of Apollo 14. This required a fast response in designing, developing, and building the demonstration apparatus on a short schedule. The necessary agreements to consider flight on Apollo 14 were made in September 1970. A laboratory prototype was designed, built and operated in October and described in detail to the Apollo Change Configuration Boards. By keeping the management requirements and the physical interface with the Apollo 14 spacecraft as simple as possible, the interface control documentation and the contract end item specifications were completed (Ref. 4) by the end of October 1970. In November, a qualification test model was delivered and tested to assure that it would safely meet all the requirements for the Apollo spacecraft. In early December, 1970, the flight hardware was delivered to Kennedy Spacecraft Center along with

the complete acceptance data package. Except for the replacement of the specimens in mid January to provide for a shorter storage time before flight, no further changes were made. The demonstration was made as scheduled on February 7, 1971, on the return trip from the moon.

DESCRIPTION OF THE DEMONSTRATION APPARATUS

The Apollo demonstration apparatus as shown in Figure 1 and drawing No. 56175D26 in the end item specification consisted of four sub-systems. The first was a metal case for safety and containment about 10 X 12.7 X 18 cm (4 X 5 X 7 inches) with a window approximately 5 X 7.6 cm (2 X 3 inches). The weight of the unit was about 2.26 kilograms (5 pounds). This size and weight fit within the limitations imposed for storage on Apollo 14. The appratus required 32 watts of power from the 400 Hertz Hycon camera circuit. Second, the electrical system powered a pump - motor to circulate the electrolytic fluid, a fluorescent light for viewing the action in the electrophoresis tubes, and a voltage doubler/ rectifier to supply 270 volts d.c. to the electrodes. Third, the electrophoresis cells were in a polycarbonate block, 12.7 X 7.6 X 1.27 cm (5 X 3 X 1/2 inches) with 3 holes drilled through the long dimension to provide the 0.63 cm (1/4 inch) diameter test tubes. The fluid in the cells or test tubes did not flow and was enclosed at the ends by membranes of regenerated cellulose with a pore size of 4 to 5 microns. Hence, the electrodes at each end of the tubes were separated from the specimens in their solution.

The fourth subsystem provided circulation of electrolyte through the six electrode compartments. In operation, the electrodes were continuously flushed by the flowing electrolyte which had the same composition as the solvent. The flowing electrolyte maintained a relatively constant pH in the electrode compartments by being interchanged between the anode and cathode ends. It also served to remove gaseous electrolysis products from the vicinity of the electrodes. Gas bubbles were removed by passage of the electrolyte through a phase separator consisting of concentric hydrophilic and hydrophobic filters. The electrolyte passed through the hydrophilic filter and was recirculated, while the gas passing through the hydrophobic filter was removed from the system to be absorbed in palladium black and charcoal.

Data on the progress of electrophoresis were collected by taking a sequence of photographs at intervals of 2.5 to 5 minutes of the action in the tubes through the window of the case with the 70 mm Hasselblad camera normally used on Apollo. The total time required to demonstrate the separations was 57 minutes.

SELECTION OF SPECIMENS

The specimens or samples were selected to satisfy several criteria. First of all, the decision was made to run three experiments in parallel to permit experiments of varying degrees of difficulty. Two dyes mixed together, Brilliant Blue and Amaranth Red, were chosen for their intense color (to facilitate detection), their stability, and because it was shown by experiment that both dyes are highly mobile at the desired pH (9). Furthermore, they differ in mobility enough so that they should separate in the relatively short distance available in the Apollo 14 demonstration unit. Of the three, this experiment was considered to have the highest inherent probability of success. Although the samples were not representative of the types of materials for which near zero-g electrophoresis may eventually be used, the experiment was chosen to provide a highly visible demonstration of the principles involved.

A second experiment contained a high molecular weight biological material whose natural color would permit visual and photographic detection. Dr. Ruth Rappaport of Wyeth Laboratories suggested the use of whole formalinized red blood cells. However, storage tests showed that the cells settled and agglomerated in a few days, and therefore lost most of their mobility. Dr. Rappaport suggested as an alternate the use of the colored constituent of the cells, hemoglobin. While small compared to the whole red blood cells, hemoglobin is, nevertheless, a large molecule by most standards, having an effective molecular weight of about 68,000. It is a highly colored, naturally-occurring material of biological origin and closely akin to materials of practical interest for electrophoresis.

The third and most difficult experiment involved a very high molecular weight material which is difficult to electrophorese by conventional zone methods. Dr. Ben Rubin of Wyeth Laboratories suggested salmon sperm deoxyribonucleic acid, DNA, with a molecular weight of about 20×10^6. DNA has been electrophoresed by microscopic methods, with only moderate success. Also, Dr. Rubin felt that the DNA might survive the unusually long storage period prior to operation of the demonstration. The photographic limitations on sample detection equipment made it necessary to render the DNA visible without grossly denaturing it. Many stains are merely adsorbed on the DNA and are quickly removed by electrophoresis. Other stains require conditions which would precipitate and denature the DNA, so visible staining did not appear practical. On the other hand, fluorescent moieties can sometimes be chemically bonded to materials such as DNA. A reaction was obtained between the DNA and dimethylaminonaphthalene sulfonyl chloride (DANSYL chloride) and this fluorescent tag was not readily removed from the DNA. No attempt was

made to determine the point of attachment of the DANSYL moiety, though we presumed that it reacted with some unhindered amine group. Detection of this third sample therefore depended on observation of fluoresence activated by the "black light" in the demonstration unit.

RESULTS OF THE APOLLO 14 DEMONSTRATION

The electrophoresis photographs were returned shortly after the Apollo 14 splashdown and the apparatus was returned after 60 days of quarantine at the Lunar Receiving Laboratory. The red and blue dyes separated as expected, but no action was seen in the hemoglobin or DNA tubes. While the successful completion of all these experiments was, of course, the goal, the success of the one experiment involving the dyes was sufficient to meet the objectives of the demonstration. Although some of the pictures were out of focus, information was provided which could be compared to pictures taken of the apparatus on earth.

The most obvious differences in the pictures shown as Figures 2a, 2b, 3a and 3b are the shapes of the boundary. On earth the boundaries are highly irregular due to a combination of electroosmosis, thermal convection, and sample density. The tendency of the dyes to stratify due to density differences, thermal convection, or both, can be observed in Figure 2b. The boundaries photographed in space are much blunter, in spite of difficulties with injection of the samples. The important fact is that no lateral motion of the fluid is evident which can be attributed to thermal convection or sedimentation, in Figure 2a taken in space.

One measure of success of an electrophoresis (or almost any separation) method is the sharpness of the sample boundaries. Densitometer measurements were made at the leading edges of the blue dye in the photographs of the space and earthbound experiments since it was expected that the lack of convection would provide a sharper boundary in the near-zero-gravity condition. Indeed, the typical data in Figure 4 verify that the sample boundary in space was sharper and better defined than on earth. These data were obtained by making photodensity measurements along the length of the tube in the vicinity of the boundary.

The data shown in Table I are taken from the returned photographs. The position of the forward boundary of the red and blue dyes was determined from each photograph for each specific time. The difference in the position of the boundary from one photo to the next provided the distance traveled during that period of time, and that distance divided by the time interval provided the incremental velocity data shown. The

velocity data were not reduced to mobilities by dividing by the field strength because field strength was a constant. The applied voltage of 270 volts d.c. over a length of 9 cm provided an average field strength of 30 volts per centimeter. However, there is a slight reduction of field strength due to the membranes.

The velocity of the dyes in the space experiment was less than half as great as the velocity measured on earth. It is extremely unlikely that this difference represents a fundamental change in the process in the absence of gravity. Between the time the electrophoresis unit was loaded and the time it was returned for analysis 11 weeks later, a major change occurred in the pH of the electrolyte. If this change occurred prior to operation of the unit it could account for the lower mobility of the dyes in the space experiment. It could also account for the fact that no hemoglobin was observed, but could not account directly for the apparent failure of the DNA to migrate.

Upon return of the apparatus, the fluid system was examined to determine if the solution containing these specimens had leaked out. The check revealed that the system was completely filled with fluid, there were no leaks, there were no bubbles, but there was no evidence of hemoglobin or DNA, either. It should also be noted that the post-flight analysis showed no evidence of the two dyes, though they were obviously present when the experiment was run. The pH of the fluid in each compartment was measured. Before flight, the pH was 8.95. The post-flight pH was 7.55 in tube No. 1 and 7.75 in tubes No. 2 and 3. The pH of the fluid in the cathode compartments was 7.80, 7.88 and 7.85 respectively. In the anode compartment of tube 1 the pH was 7.80; in the anode of tube 2, 7.98. The cause of the pH change was sought. Calculations show that if all the DNA and hemoglobin were converted bacterially to an acid, at least half enough acid would be produced to account for the pH change. Enough other organic materials were present in the fluid system to provide the additional food for conversion to the acid. Subsequent culture tests indicated that the bacteria, E. Coli, were present along with butyric acid, a product of the cultured bacteria. Additionally, the reduced pH could have retarded the observed velocity of the dye samples in space.

In ground tests there was no evidence of pH change or sample deterioration after laboratory storage of the electrophoresis unit for up to three weeks (the time between loading of the flight unit and the Apollo 14 flight). At one time benzalkonium chloride was added to the electrolyte to inhibit growth of the organisms, but this addition upset the ionic strength of the electrolyte. Since no evidence of a contamination problem was found at that time the benzalkonium chloride was subsequently omitted. Additional post-flight tests, involving a variety of storage conditions are in progress.

The ultraviolet absorption spectrum of the fluid in each of the three tubes was measured during post-flight analysis. All spectra were substantially identical and were characterized by an absorption maximum at about 275 $m\mu$, the region normally associated with an aliphatic carbonyl. This absorption was not present in the original electrolyte. Although attempts to reproduce the reaction are still in progress, it is believed that the absorption may have been caused by butyraldehyde, although it did not form a 2, 4 -dinitrophenyl hydrozone.

Additionally, ultraviolet fluorescence of the fluids and the various parts of the system, including the gas phase separators, was employed. This test also indicated that no specimen was left in the fluid or in the system. Dr. William Carroll of the National Institutes of Health suggested that bacteria in these tubes may have consumed the hemoglobin and DNA during the long storage period. He also indicated that this problem could be corrected in the future by adding a bactericide to the solution. However, it will be necessary to find one that does not affect the pH or the ionic strength.

As shown in Figure 5, it was obvious that the slide valve did not fully open due to a misalignment between the cell block and the case. This put the dye sample in the region of maximum electroosmotic shear along the wall of the tube. Had the valve operated properly, the sample would have been injected into the center of the tube in the volume characterized by a radius of 0.25 cm from the center. By being injected from approximately the tube center to one wall, the sample was subjected to osmotic flow with three times the shear expected at the center of the tube. This means that band broadening due to electroosmosis was three times as great as it should have been. In light of this difficulty the results obtained are quite encouraging. The valve problem will be corrected in the future by the use of indexing pins or a larger bearing surface for the foot of the screw actuator which drives the gage valve.

CONCLUSIONS

It is concluded that, (1) the electrical and fluid flow systems of the apparatus worked as designed, and that gas bubbles were filtered and absorbed even in near-zero-gravity. (2) In the red-blue dye separation, the resolution in space was better than on earth. (3) The shape and sharpness of the advancing boundary of separated material was improved in space by the lack of sedimentation and convection currents which were suppressed by the near-zero-gravity condition. In future precision separations, it is this improvement in shape and sharpness of the boundary which may prove to make fluid electrophoresis in space a valuable process. (4) The hemoglobin and DNA may have been consumed during storage by bacterial action which changed the pH of the electrolyte. This suggests that storage conditions for biological samples

must be improved. Much was learned about the problems and requirements for doing electrophoresis in space. Subsequent laboratory investigations and pre-flight preparations will be specifically aimed at solving these problems most of which can be specifically and readily corrected.

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1. McCreight, L. R. and Griffin, R. N.: "Survey of the Preparation of Materials in Space," Contract NAS8-24683, March, 1970.

2. Dean, John A, "Chemical Separation Methods," Chapter 14, pages 323-328, Van Nostrand-Reinhold, New York, 1969.

3. Wiersema, P. H., Loeb, A. L. and Overback, J. Th. G., "Calculation of the Electrophoretic Mobility of a Spherical Colloid Particle," J. Colloid and Interface Sci., 22, 78 (1966).

4. McKannan,E. C., "Apollo 14 Electrophoresis Demonstration, End Item," Specification 002001.

APPENDIX

Astronauts Debriefing - Apollo 14

McKannan - Did you see the blue dye get all the way across the tube?

Roosa - Yes, red and blue.

When using the 70 mm Hasselblad at short distances you worry about focus; is it (the film) in focus?

McKannan - Some shots were slightly out of focus.

Do you remember about where you held the camera? or did you have a check point?

Shepherd - We measured it.

Roosa - I took a gage on the strut.

I varied a couple of shots to get it bracketed. I was real sure.

Shepherd - We did not have a yardstick or measuring device on board.

McKannan - Did you look through the view finder?

Roosa - No, you just have to hold the camera out there and snap it.

McKannan - I have a question on the valve. Do you remember if it took a long time to open the valve, or how many turns it took to open the valve?

Roosa - It took a lot of turns and a descrete amount of time. It got harder to turn toward the end, so I really torqued down near the end. It turned the same (torque) until 2 or 3 turns to the end. It got stiff. I got a couple of extra turns. It never did completely snub down. After a couple of minutes, I went back and tried again.

McKannan - Do you recall when setting up, did the apparatus get bumped, shaken or turned?

Roosa - Didn't we do something with that before we started?

Mitchell - It had far more severe handling getting stored aboard than during zero "g".

Roosa - We waited 10 minutes and went right by the times (in the procedures). Things happen pretty slow in the spacecraft. Any action at all in cell #1? (UV light on DNA)

McKannan - We haven't seen it, yet.

Roosa - Okay, I guess you got the data sheet that showed things happened a lot slower than you were expecting.

TABLE I

ELECTROPHORESIS DATA SHEET

Apollo Frame A-512-76-103	Time Min.	Position cm	Red Dye Distance cm	Velocity cm/min.	Blue Dye Position cm	Distance cm	Velocity cm/min.
I. Apollo 14 in Space							
-37	0.5	1.4	-	-	0.6	-	-
	-	-	1.0	0.40	-	0.9	-
-38	3.0	2.4	-	-	1.5	-	-
	-	-	0.8	0.32	-	0.8	0.32
-39	5.5	3.2	-	-	2.3	-	-
	-	-	0.8	0.32	-	0.6	0.24
-40	8.0	4.0	-	-	2.9	-	-
	-	-	1.7	0.34	-	1.5	0.30
-41	13.0	5.7	-	-	4.4	-	-
	-	-	1.4	0.28	-	1.5	0.30
-42	18.0	7.1	-	-	5.9	-	-
II. Earthbound							
-3	2.0	1.1	-	-	-	-	-
	-	-	0.6	0.6	-	-	-
-4	3.0	1.7	-	-	0.6	-	-
	-	-	0.9	0.9	-	0.8	0.8
-5	4.0	2.6	-	-	1.4	-	-
	-	-	1.3	0.7	-	1.4	0.7
-6	6.0	3.9	-	-	2.8	-	-
	-	-	1.4	0.7	-	1.4	0.7
-7	8.0	5.3	-	-	4.2	-	-
	-	-	-	-	-	1.4	0.7
-8	10.0	-	-	-	5.6	1.4	0.7

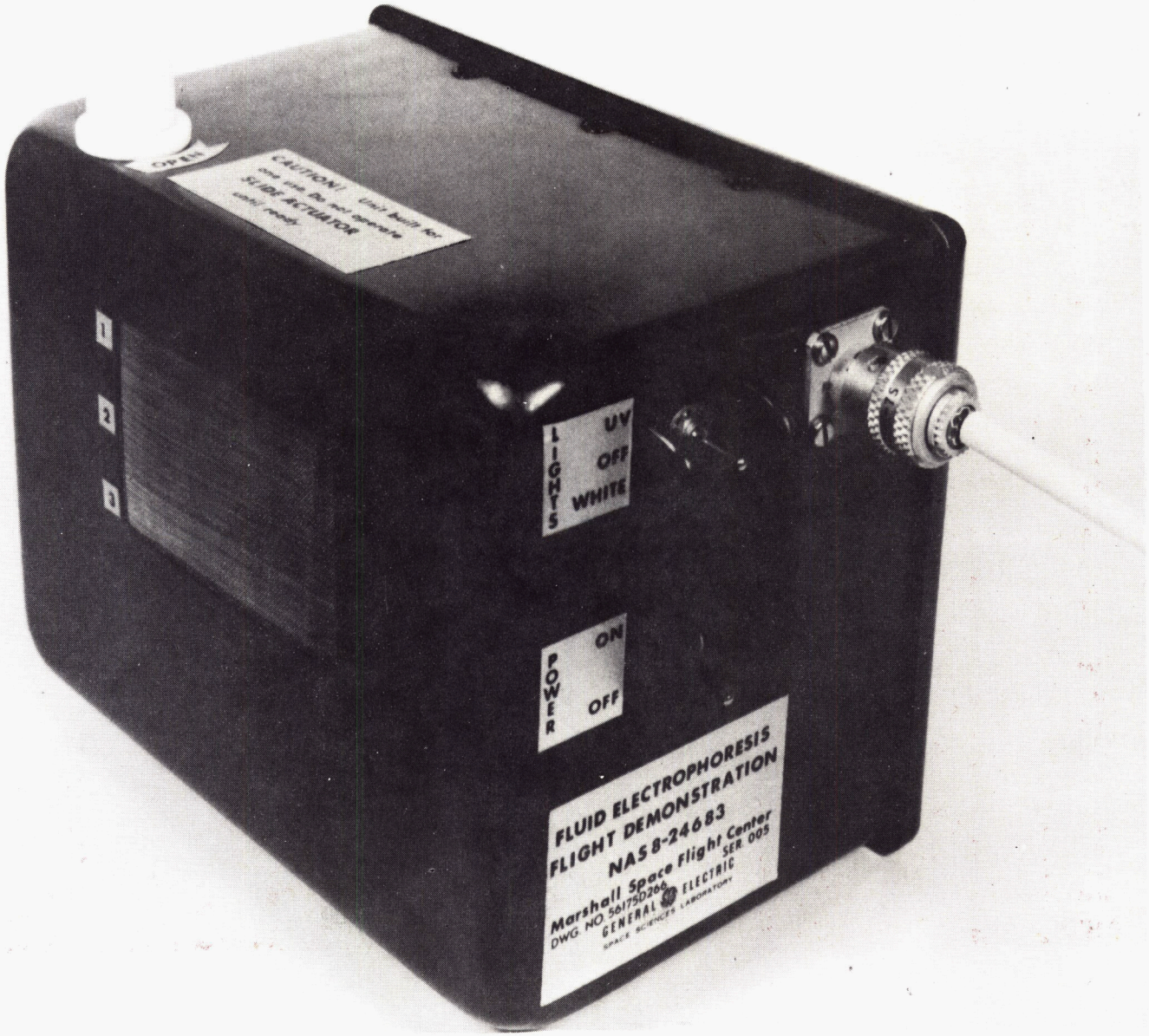

Figure 1 - Apollo 14 Demonstration Unit

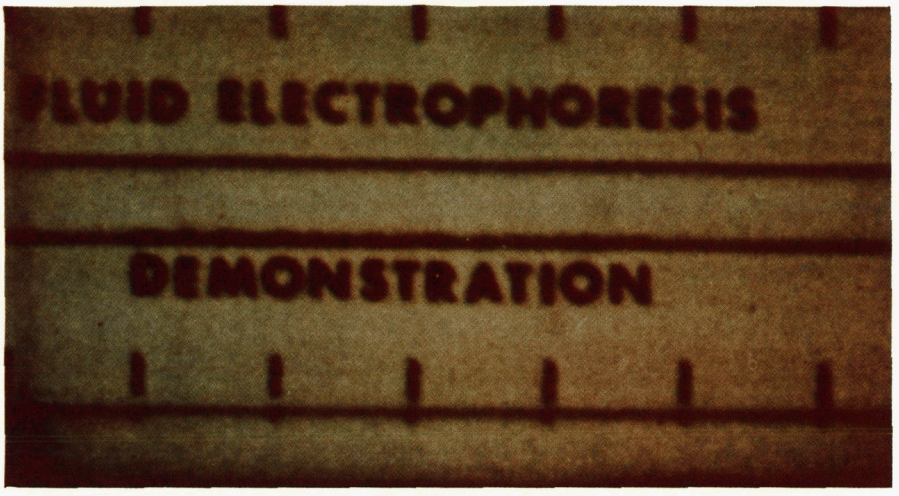

Figure 2a - Apollo 14 Flight, Electrophoresis of Red and Blue Dyes at 3 Minutes, Apollo Frame A-512-76-103-38

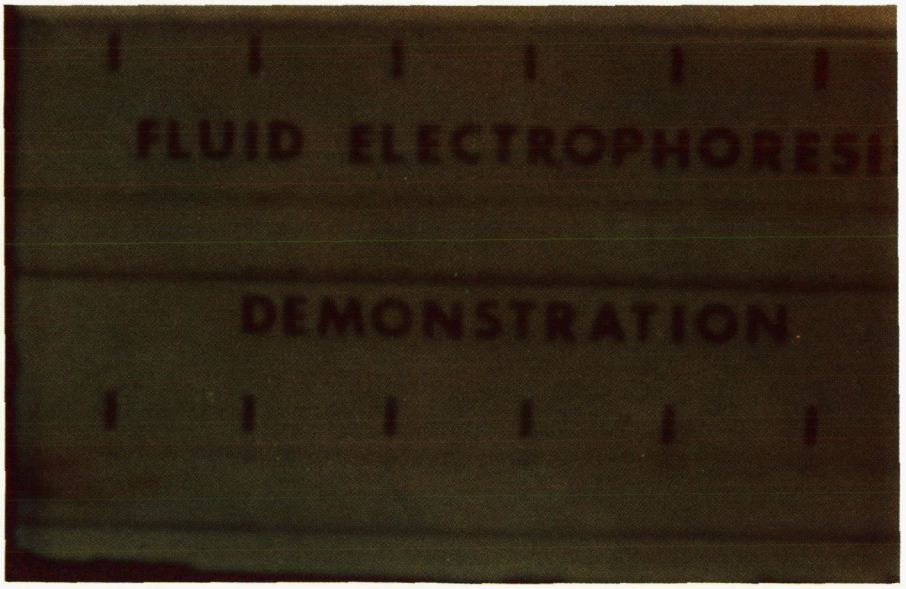

Figure 2b - Earthbound Electrophoresis of Red and Blue Dyes at 3 Minutes

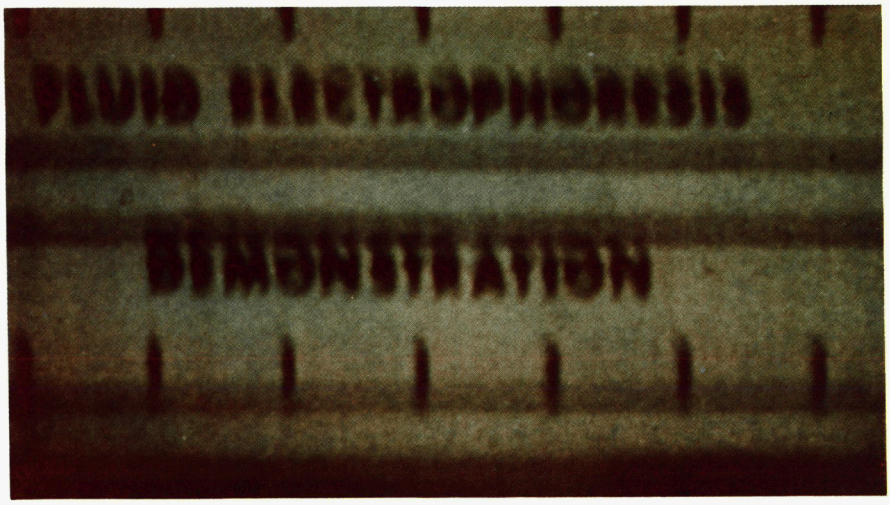

Figure 3a - Apollo 14 Flight, Electrophoresis of Red and Blue Dyes at 5.5 Minutes, Apollo Frame A-512-76-103-39

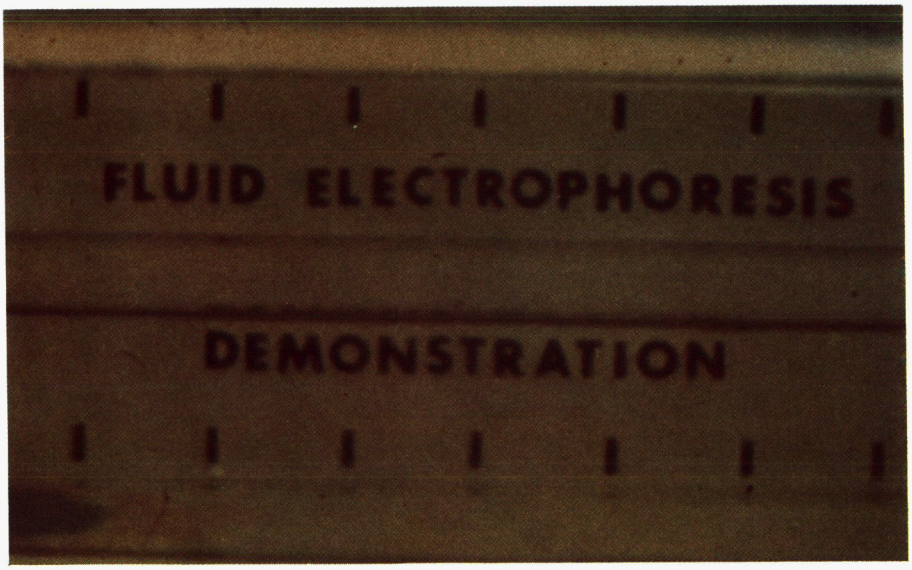

Figure 3b - Earthbound Electrophoresis of Red and Blue Dyes at 4 Minutes

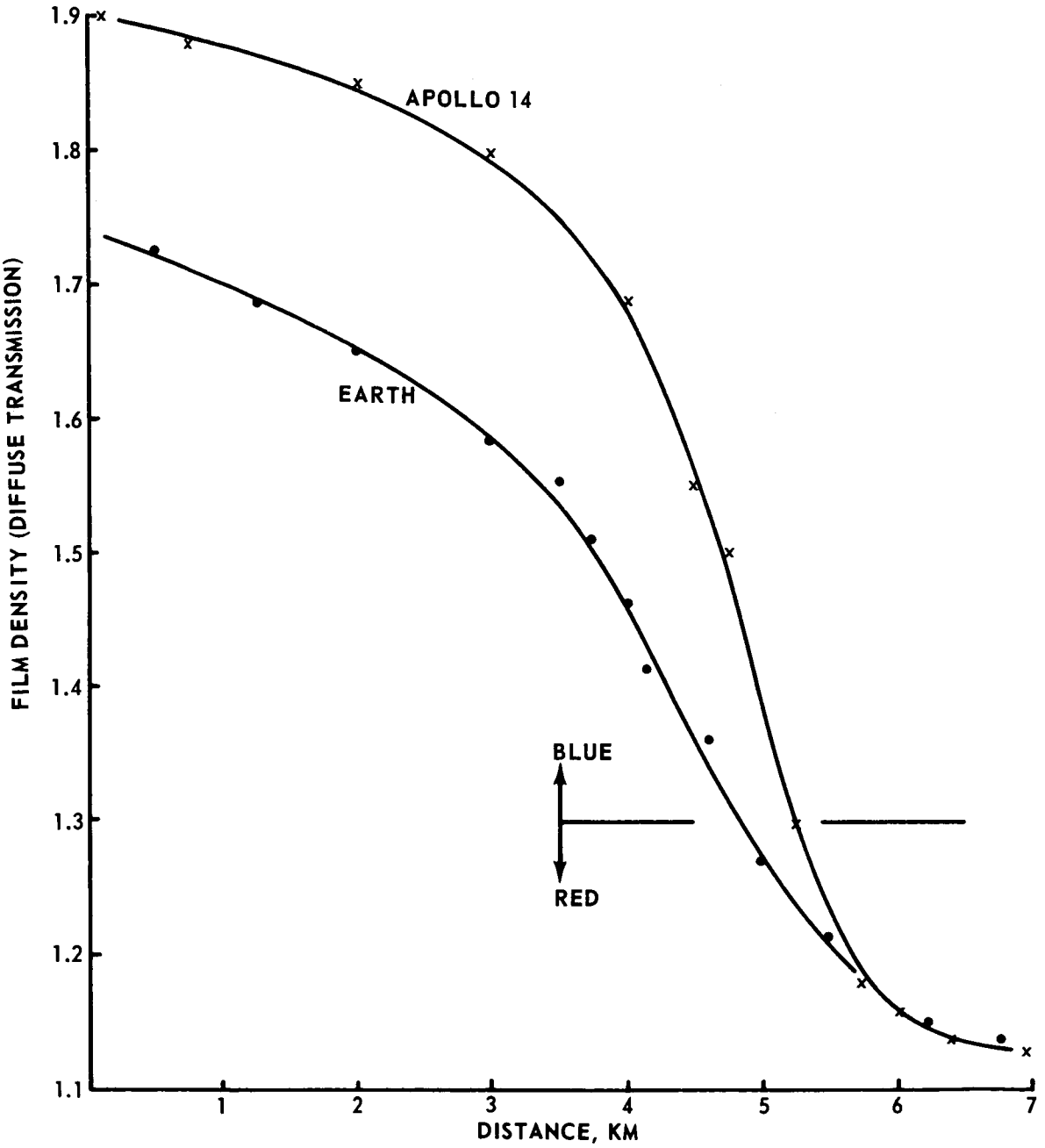

FIGURE 4 SHARPNESS OF ELECTROPHORESIS BOUNDARIES BY DENSITOMETRY

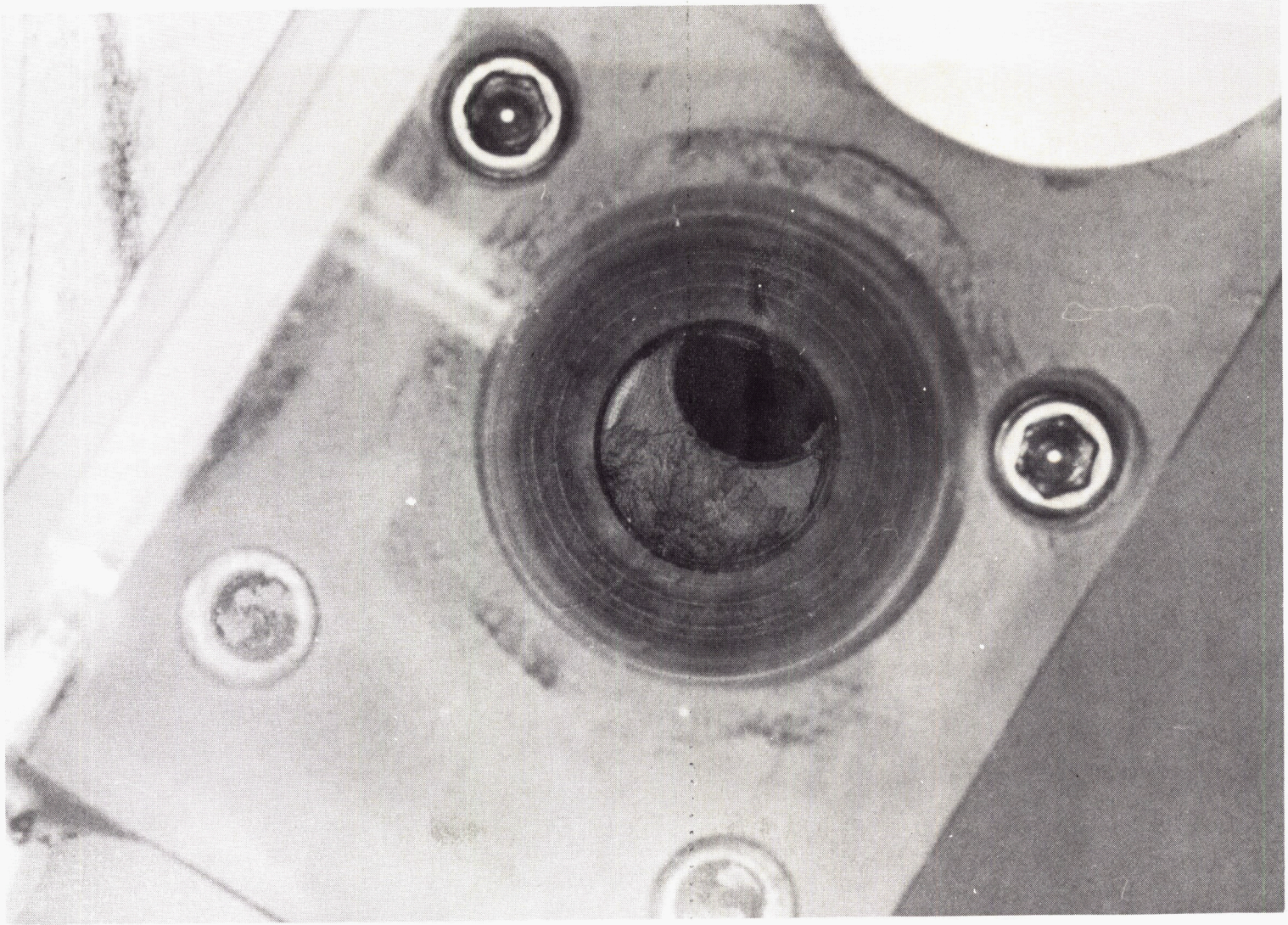

Figure 5 - Condition of Partially Opened Gate Valve
on Return of Apollo 14 Electrophoresis Demonstration Unit

APPROVAL

ELECTROPHORESIS SEPARATION IN SPACE - APOLLO 14

By

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The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.

R. J. Schwinghamer
Chief, Materials Division

Karl L. Heimburg
Director, Astronautics Laboratory

MSFC—RSA, Ala