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ELECTROPHORESIS DEMONSTRATION ON APOLLO 16

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16. ABSTRACT

Free fluid electrophoresis, a process used to separate particulate species according to surface charge, size, or shape was suggested as a promising technique to utilize the near-zero-gravity condition of space. Fluid electrophoresis on earth is disturbed by gravity-induced thermal convection and sedimentation. An apparatus was developed to demonstrate the principle and possible problems of electrophoresis on Apollo 14 and the separation boundary between red and blue dye was photographed in space. The basic operating elements of the Apollo 14 unit were used for a second flight demonstration on Apollo 16. Polystyrene latex particles of two different sizes were used to simulate the electrophoresis of large biological particles. The particle bands in space were extremely stable compared to ground operation because convection in the fluid was negligible. Electrophoresis of the polystyrene latex particle groups according to size was accomplished although electro-osmosis in the flight apparatus prevented the clear separation of two particle bands.

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SUMMARY

The electrophoresis of polystyrene latex particles was demonstrated during the flight of Apollo 16. Two sizes of the spherical, submicron particles were used as single species and combined in a three-cell geometry in order to model the electrophoresis of particulate materials. Photographs were taken periodically during the demonstration for comparison with similar experiments done on earth. The demonstration in space showed that eliminating gravity-induced thermal convection and sedimentation allowed cohesive bands with sharply defined particle fronts to be formed during the electrophoresis. Comparable experiments done on earth show the lack of particle band stability in a gravity field. Electrophoresis of the polystyrene latex particles according to size occurred although a clear separation of the particle bands was not observed because of extensive electro-osmosis in the flight apparatus.

INTRODUCTION

One of the most promising ideas identified to date for processing of materials in the space environment is the separation and/or purification of biological materials by electrophoresis. Electrophoresis is the movement of charged colloidal particles and macromolecular ions in a solution under the influence of an electric field. Depending upon the sign of their net charge, the particles migrate either to the cathode or the anode. Differences in migration velocities provide a sensitive means of separating substances from their mixtures which are otherwise difficult or impossible to separate.

Electrophoresis done in space will alleviate at least two major problems that occur on earth:

1. The electric field produces an electric current in the liquid medium which results in joule heating. This heating generates convection currents in the solution which mix the components already separated.

2. Large biological particles of high density, such as living cells, settle to the bottom of liquid electrophoresis beds and cannot be effectively separated. Under conditions of weightlessness, electrophoresis can be applied to molecules or particles of arbitrary size suspended in fluid media without gravity-induced mixing and sedimentation. The advantages are expected to make electrophoretic separation in space practical for preparing medical and biological products of high social and economic value.

Electrophoresis was demonstrated on Apollo 14 when red and blue dyes were separated on the return trip from the moon [1]. Photographs showed that the boundary dividing the dyes was better defined than in comparable equipment on earth. The apparatus also contained samples of hemoglobin and DNA which were not observed to separate. Subsequent examination of the apparatus indicated that these specimens were destroyed by bacteria, probably during the long storage time before the demonstration in space actually took place.

The Apollo 14 experiment demonstrated that the component parts of the apparatus worked as designed. The electrical and electrolyte circulation systems of the apparatus operated successfully and gas bubbles generated at the electrodes were filtered and absorbed as planned. Much was learned on Apollo 14 about the problems and requirements for doing electrophoresis in space, and laboratory investigations began soon after the Apollo 14 results were analyzed to improve the design and operation of the apparatus for the Apollo 16 mission.

The Apollo 16 demonstration was designed to use the basic operating elements of the Apollo 14 unit for the electrophoresis of large particles that could be a model experiment for the separation of fragile biological particles during some future mission. Hardware modifications were made to increase the amount of data that could be obtained from the demonstration but the changes did not decrease the reliability of the unit. Photography of the demonstration in space with commentary by the astronaut was the sole source of data for comparison with ground results. The electrophoresis demonstration was done the day after launch of Apollo 16 and the hardware was jettisoned in the Lunar Module so that additional storage could be provided in the Command Module for lunar material.

A long range objective for electrophoresis in space is the separation, classification and analysis of living cells. Electrophoresis is one of the few physico-chemical measurements which can be made on living cells without producing permanent damage. Since a living cell is large and dense, the spearation of living cells according to size, induced charge, or surface characteristic is limited by gravity-induced sedimentation and convection on earth. The Apollo 16 apparatus demonstrated the electrophoresis of large, dense particles of a nonbiological model system in order to evaluate the future electrophoresis of biological particles such as living cells.

ELECTROPHORESIS THEORY

Practically all particles acquire either a positive or negative surface charge when suspended in an aqueous solvent. This applies to visible bubbles or drops, to microscopic collodial particles, or to individual molecules provided they are charged or ionized. The electrical charge at the surface of ionic or ionizable solids is due to the interaction of ionic species on the particle surfaces and in the water. Ions are either absorbed on the dispersed solid particles, removed preferentially by the solvent, or exchanged with other ions in the solution, depending upon the nature of the particle and surroundings. The net charge on the particles is not fixed and can be varied by changing the pH and ionic strength of the solution.

When an electric field is applied to the aqueous suspension of particles, the charged particles migrate in the potential gradient to the oppositely charged electrode at a velocity dependent upon their size, shape, and accumulated charge. Since two species may acquire different net charge densities in the solvent, the particles that otherwise have similar physical and chemical properties will move at different rates. The mobility of a particle is the distance a particle will travel in a unit of time per unit electric field strength. Although a particle's mobility is approximately proportional to its surface charge, size, and shape, individual particle characteristics and interactions between the particle and supporting medium make quantitative calculations of mobility difficult.

Basically, Stokes law describes the electrophoretic migration:

$$U = \frac{QE}{6 \pi \eta}$$

where U is the particle velocity, Q is the charge on the particle, E is the electric field, and η is the viscosity of the fluid. An ionic double layer that forms at the particle/solvent interface causes an additional drag such that if the field distortion due to the particle is negligible, the velocity decreases to

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

where D is the dielectric constant of the solvent and ζ is the characteristic potential across the double layer. If the thickness of the double layer and the radius of the particle have comparable dimensions, the constant 6π becomes a complex function of both the size and the shape of the particle. For spherical particles in this dimensional range, separation according to size can be accomplished. This was an objective of the Apollo 16 demonstration. The functional relationship becomes a new constant, 4π, for larger particles.

The electrophoresis of particles in a closed cylindrical cell, such as used on Apollo 16 or for microcapillary electrophoresis, is affected by the fluid motion which is not uniform across the cell and the migration of particles thus varies across the cell. The electrokinetic phenomenon of electro-osmosis in the closed liquid system causes the solvent in which the particles are suspended to flow along the surface of the cell in one direction and then return through the center of the cell in the opposite direction. This flow of liquid causes the group of electrophoretically migrating particles to assume a paraboloid shape. The extent of deviation from uniform cross section to paraboloid depends primarily upon the characteristics of the cell wall, and materials are available as cell wall coatings that diminish the electro-osmosis under most conditions.

Detailed descriptions and analyses of electrophoresis principles and electrokinetic phenomena beyond the scope of this report are contained in the two volumes on electrophoresis edited by Bier [2] and the other texts on the subject.

EXPERIMENT DESIGN AND SAMPLE SELECTION

At the conclusion of the Apollo 14 demonstration analysis, it was recommended that a second demonstration on Apollo 16 with comparable apparatus could increase out knowledge of electrophoresis in space. Stable, nondegradable sample particles and a tripod arrangement for holding the camera were prerequisite.

A group of scientists with extensive electrophoresis experience was assembled at the Marshall Space Flight Center in August 1971 to examine the Apollo 14 demonstration apparatus and design, to discuss electrophoresis on earth and the opportunities in space, and to assess the value of an Apollo 16 demonstration. The participants were Dr. Henry L. Leidheiser, Lehigh University; Dr. Milan Bier, University of Arizona; and Dr. Carel van Oss, State University of New York at Buffalo. Dr. Sydney Ross, Rensselaer Polytechnic Institute, and Dr. Alan Johnson, New York University, joined subsequent meetings.

The concept of electrophoresis in a weightless state for large, dense particles, such as living cells, was endorsed, and it was agreed that a model material would effectively demonstrate particle electrophoresis in space. Monodisperse polystyrene latexes of two different sizes were suggested as the sample material. It was recommended that three simultaneous experiments be done: one with a mixture of the two latexes; one with the large size latex; and one with the smaller size latex. The approximate sizes to be considered were 0.2 micron diameter and 0.8 micron diameter. The design of the electrophoresis cell allowed the polarity to be reversed to determine the reversibility of the separation of the two sizes. The same experiments would be done on the ground with the cells in a vertical position and stabilized with a sucrose gradient in order to establish the ground control for later comparison with the flight.

Monodisperse polystyrene latexes have been prepared and distributed for many years by the Dow Chemical Company. The narrow distribution of particle sizes (diameter variations measure about 1 percent) has led to diverse applications such as calibration of electron microscopes, ultracentrifuges and microscopic particle counters. Since the characteristics of the particle surfaces, in addition to the size, would determine electrophoretic mobilities, Dr. John Vanderhoff (of Lehigh University, formerly associated with polystyrene latex research at Dow [3]) was also consulted on experiment design. The particles selected for Apollo 16 were the 0.8 micron polystyrene latex from Lot #LS-1200-B and 0.2 micron polystyrene latex from Lot #LS-1047-E.

DESCRIPTION OF THE DEMONSTRATION APPARATUS

The engineering design, manufacture of a qualification unit, flight and flight backup unit, and ground testing of flight prototype apparatus were done by the Space Sciences Laboratory of the General Electric Co. under the direction of Dr. Richard N. Griffin. The supporting experiments, done by

Dr. Griffin and his associates, included verification that the two sizes of polystyrene latex were separable in flight prototype apparatus with the inclusion of a sucrose density gradient, assessment of the long time stability of reversible electrodes and salt bridges which could have eliminated the electrolyte circulation system, and testing on the operation and reliability of significant items such as the Kapton film sample retainer.

The demonstration apparatus developed for Apollo 16 had the same dimensions [10 × 12.7 × 18 cm (4 × 5 × 7 in.)] and comparable weight [3.4 kg (7.5 lb)] as the Apollo 14 unit but several modifications were made to obtain more data. (Figures 1 and 2 show sketches of the unit.) A detachable tripod with two M-21 Hasselblad lens extension tubes was included (Fig. 3) so that the electric Hasselblad camera would take closeup pictures with the correct range and focus settings. Pictures were taken automatically every 20 seconds by means of an attachable Mauer Intervalometer. The window was enlarged to 6.4 × 11.4 cm (2.5 × 4.5 in.) so that the electrodes as well as the three separation columns could be seen. An Accutron watch to confirm the interval between photographs, a thermometer to measure the ambient temperature in the unit, and three milliammeters to indicate current in each of the electrophoresis columns were also added. Markings one centimeter apart were scribed on the tubes to aid subsequent measurements and the marking closest to the anode was painted yellow to indicate the cell location for reversal of the voltage.

The apparatus used 32 watts of power (115 volts, 400 Hertz) to run the pump which circulated buffer through the electrode regions, two fluorescent lights for side lighting the electrophoresis tubes, and a voltage double plus rectifier which provided the 300 volts dc to the electrodes. The platinum 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 and 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 hydrophobic filter was recirculated, while the gas passing through the hydrophilic filter was removed from the system by absorption of hydrogen in palladium black and release of oxygen to the environment.

The electrophoresis cells were three Lexan (polycarbonate) tubes 0.636 cm (0.25 in.) inside diameter, 0.159 cm (0.06 in.) wall thickness, and 10 cm (4 in.) long. Dialysis membranes of cellulose acetate enclosed the ends of each tube to separate the buffer in the cells from the circulating

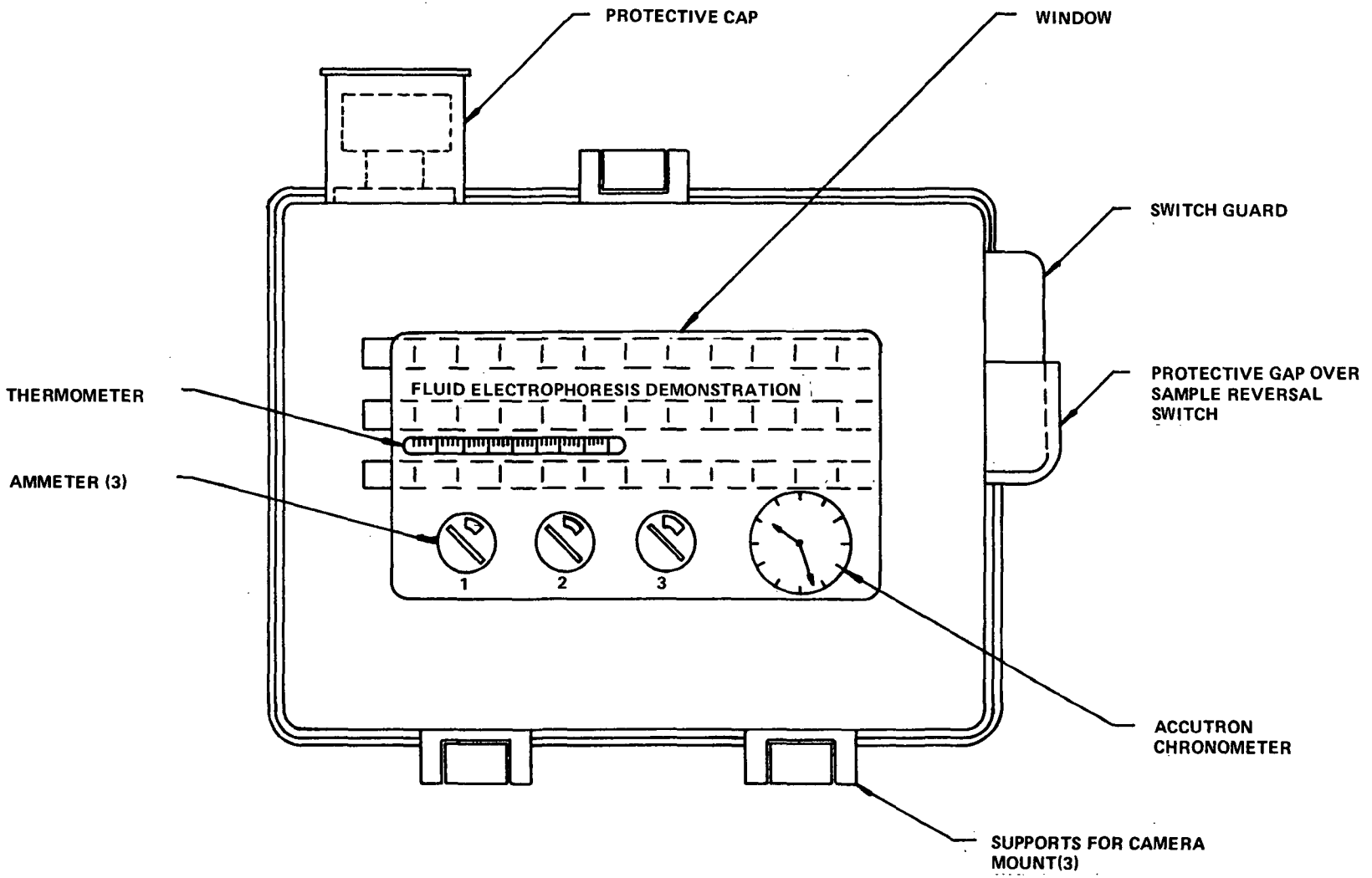


Figure 1. Electrophoresis demonstration — front view.

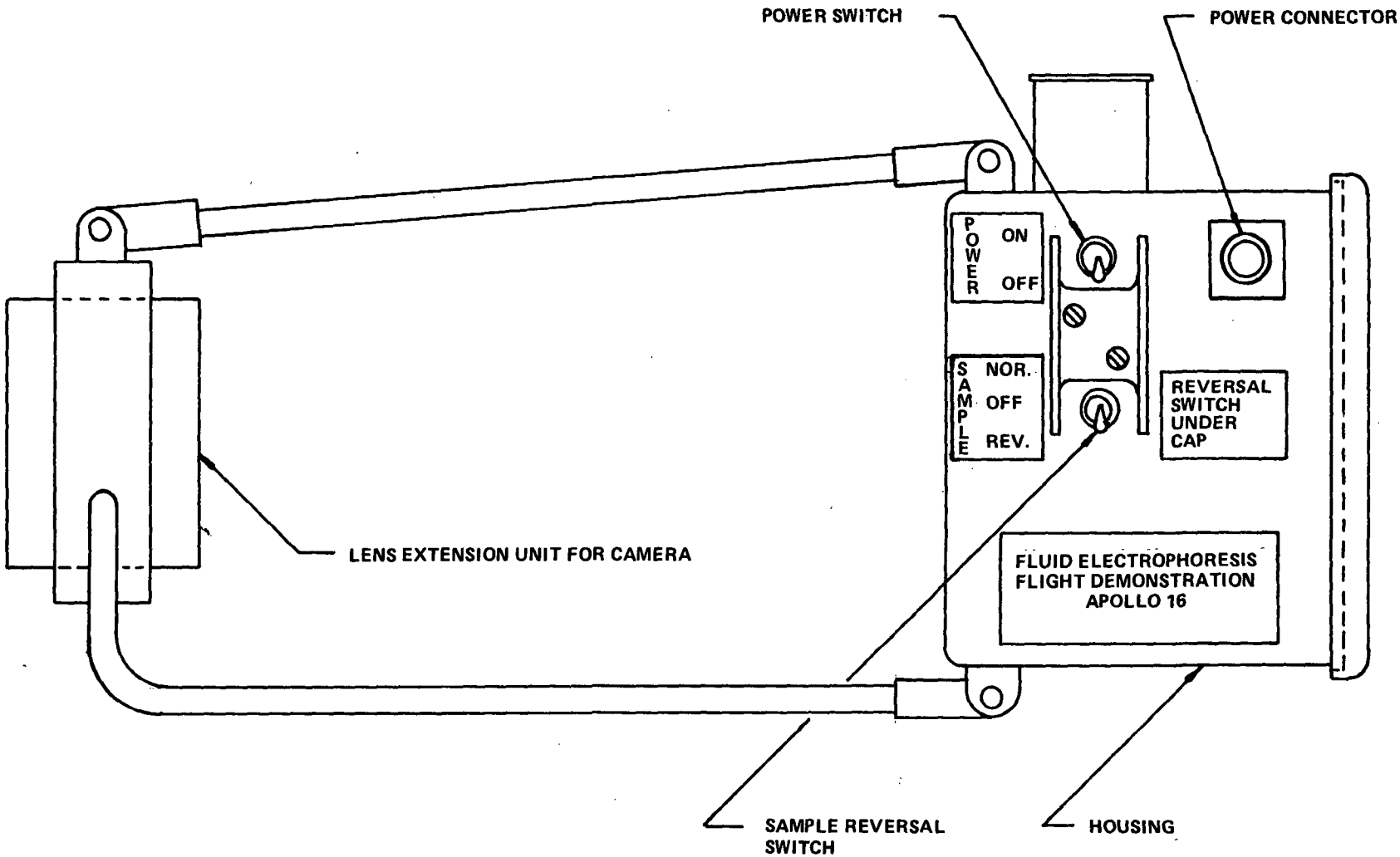


Figure 2. Electrophoresis demonstration — left side view.

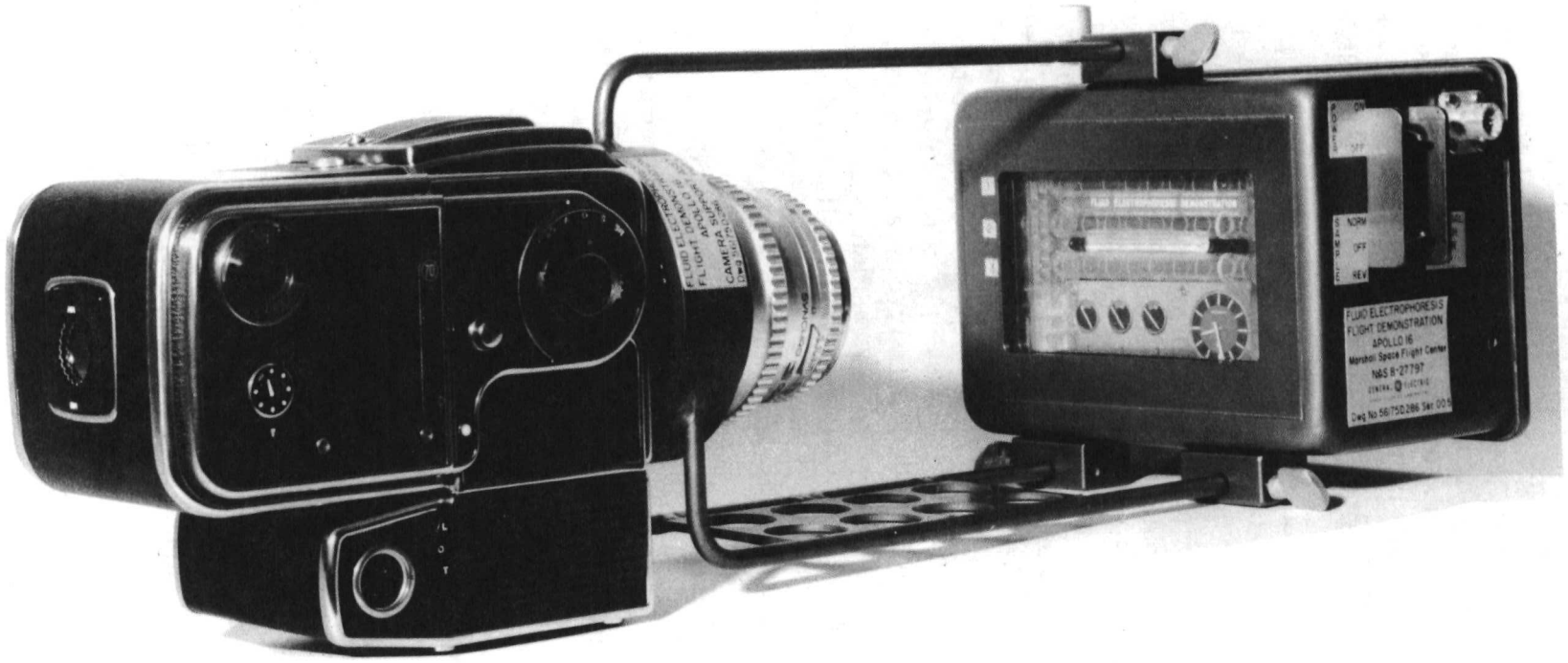


Figure 3. Assembled Apollo 16 electrophoresis demonstration.

buffer in the electrode regions. The same dilute borate buffer (H_3BO_3/NaOH) was used throughout the system. The ionic strength of the buffer was 0.0024, the specific conductivity was 0.13×10^{-6} $ohm^{-1}cm^{-1}$ and the pH was 8.4. Although polystyrene latex is substantially immune to bacterial degradation, 0.1 percent formalin was added to the solutions as a bactericide. The addition of 0.02 percent sodium lauryl sulfate to the buffer and periodic turning of the unit were required to prevent agglomeration of the polystyrene latex particles.

The polystyrene latex particles were retained at the membrane closest to the cathode by a Kapton film. The three disk-shaped sample containers had a smaller diameter [0.477 cm (0.18 in.)] than the inside diameter of the cell so that the initial insertion of the particles and subsequent electrophoresis would take place down the center of the cells and away from the walls where the electro-osmotic flow would retard the particle migration. The cathode membrane and Kapton film were separated by 0.15 cm (0.06 in.) which defined the initial band length. Before electrophoresis was to begin in space, the Kapton film retained the cylindrical disk of particles and prevented current flow in the cells. When the experiment was to take place, the Kapton film was slowly pulled across the sample/buffer interface of each chamber simultaneously. Each group of polystyrene latex would then migrate electrophoretically to the anode. A polystyrene latex sample concentration of 3 percent was selected for the single species cells and 6 percent (3 percent concentration of each size) was selected for the combination cell to give the same number of particles in each band for comparison to the other two cells.

The mixture of the 0.2 micron and 0.8 micron polystyrene latex was in the upper cell, identified as number 1. The center and lower electrophoresis cells contained each size of polystyrene latex separately so that interactions between the particle groups in the upper cell could be detected and any experiment anomalies attributable to each polystyrene latex sample could be identified. The center cell contained the 0.8 micron diameter sample and the lower contained the 0.2 micron diameter sample. The larger size particles have a higher electrophoretic mobility and, hence, were expected to migrate faster than the smaller particles.

The Apollo 16 hardware developed serious problems about a month before launch. The elecrrophoresis cells, electrode compartments, and phase separators were constructed of Lexan. These parts cracked at machining and assembly points releasing electrolyte inside the box. The loss of fluid caused the formation of bubbles in the cells and electrode circulation system which impaired the experiment operation. The electrolyte lowered insulation resistances and condensed on the window during testing, causing additional viewing problems. Lexan specialists subsequently attributed the cracking to machining and assembly stresses that caused stress corrosion

type cracks due to environmental fluids such as sterilization chemicals and skin oils. Careful annealing, elimination of sterilization thus relying on the formalin in the buffer, handling with cloth gloves, and coating all Lexan parts with RTV 168 to prevent leakage of buffer were done to the flight and flight backup units.

The primary flight unit was delivered to the Kennedy Space Center on April 5, 1972, but the discovery of large bubbles in each tube, lowered insulation resistances, and cloudiness indicative of polystyrene latex in the center cell caused withdrawal of this unit on April 13 in favor of the flight backup unit. Subsequent failure analysis resolved that the Lexan parts had not cracked but that the electrolyte escaped through the gap between the Kapton sample release film and the O-ring seals. The Lexan parts at this location had not been screwed together tightly enough because of the inherent possibility of Lexan cracking. The bubbles observed in each cell were probably due to the loss of electrolyte since the buffer had been degassed prior to filling. The hydrophobic membrane in the phase separator had also failed, providing another source of electrolyte leakage of the system.

Although small bubbles had also developed in each cell of the backup unit during testing, the insulation resistances were still high. It was determined that the presence of these bubbles in flight would not interrupt the electrophoresis of the particles and this backup unit was stowed in the Apollo 16 spacecraft the day before launch. The General Electric Final Report [4] describes the hardware development in more complete detail.

RESULTS OF THE APOLLO 16 DEMONSTRATION

Approximately 25 hours into the flight of Apollo 16, Astronaut T. K. Mattingly set up the electrophoresis apparatus on one of the stowage lockers and began the photographic sequence. Fifty-two photographs were taken during the four traversals of the latex particle groups down the cells and back (Fig. 4). The transmitted commentary of Command Module Pilot Mattingly helped to explain several phenomena observed later in the photographs and gave continuity to the photographic data which were obtained every 20 seconds. The Appenxix contains the complete transmission between Apollo 16 and Houston during the demonstration. For reference, the first picture, Number 17001 was taken at 01:01:29:19 corresponding to 11:12:14 on the Accutron watch. The first reversal was done at Frame 17017 (time 11:17:37) and successive reversals at 11:20:26 and 11:23:12. Pictures 17001 and 17002 were taken before the samples were released into the cells (tubes) and before cell voltage was applied.

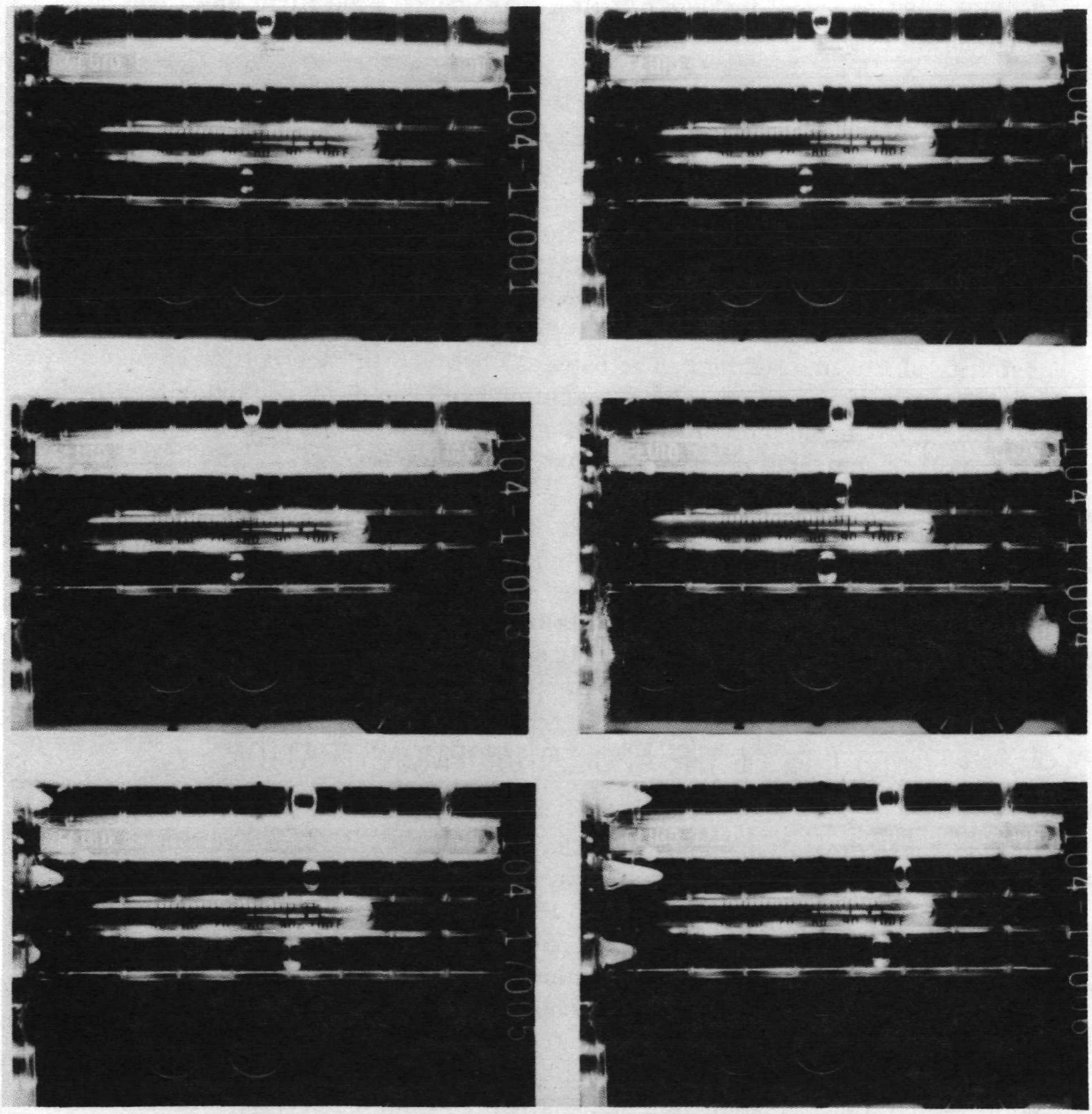
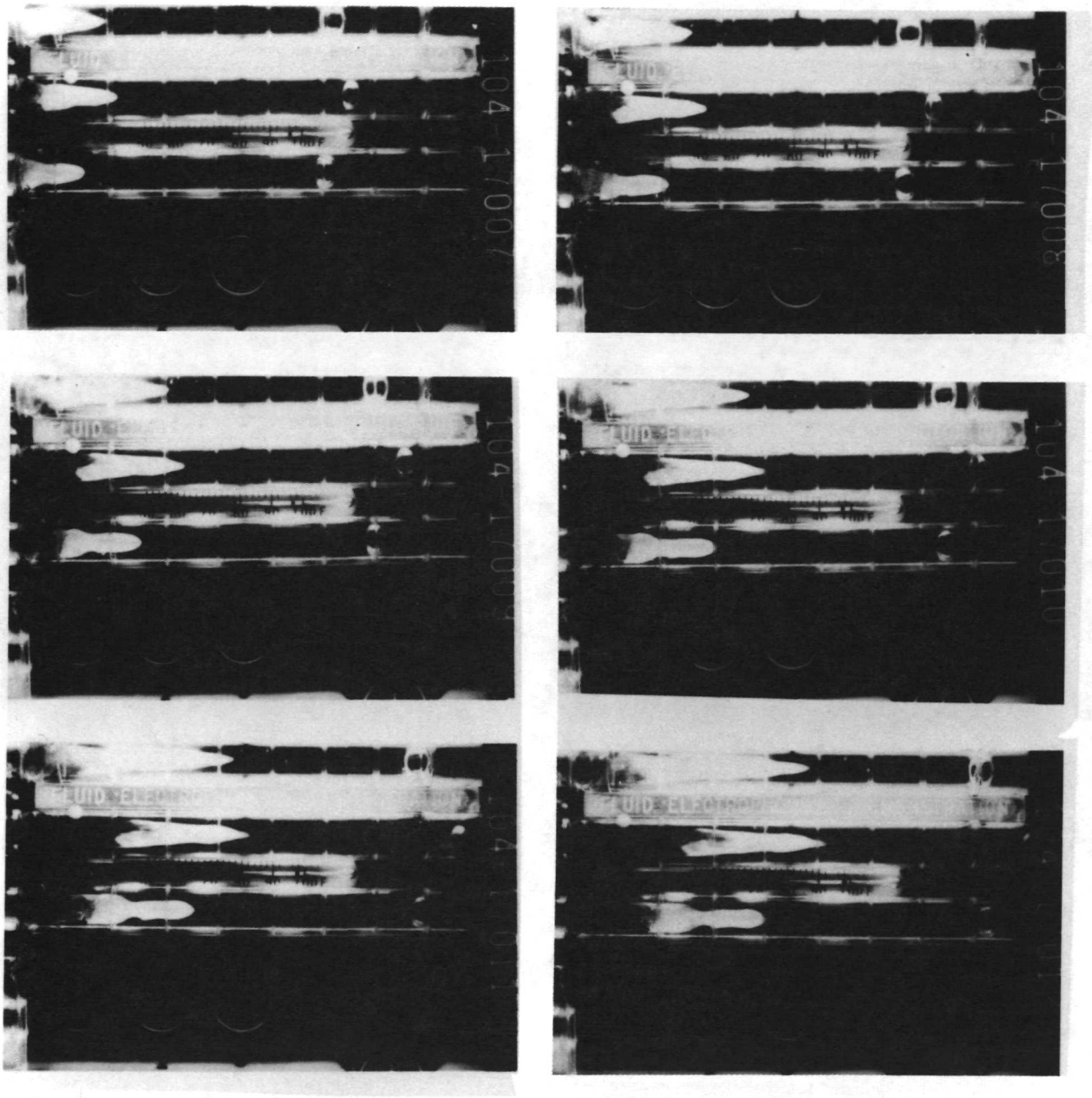


Figure 4. Apollo 16 electrophoresis pictures.



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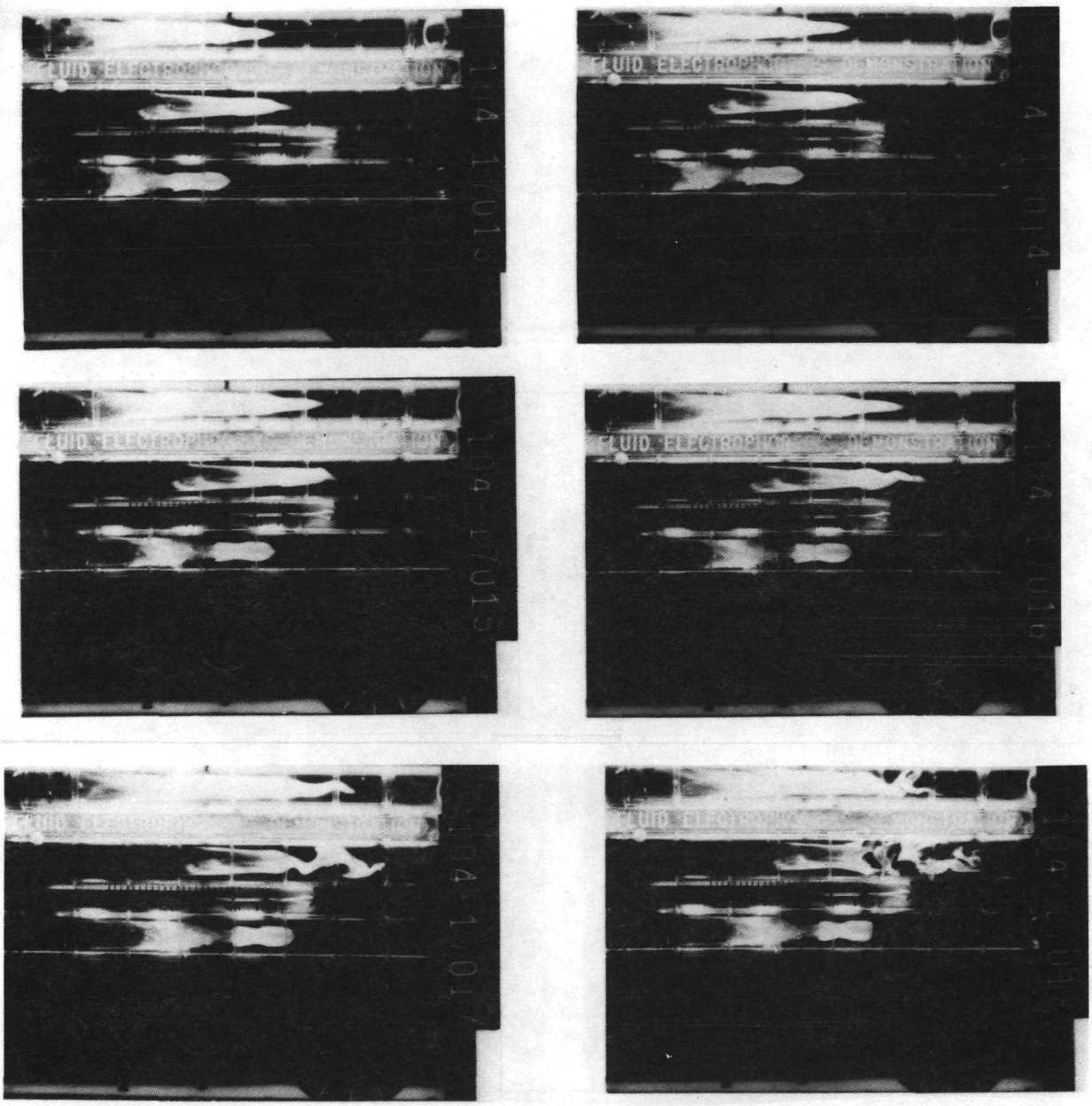

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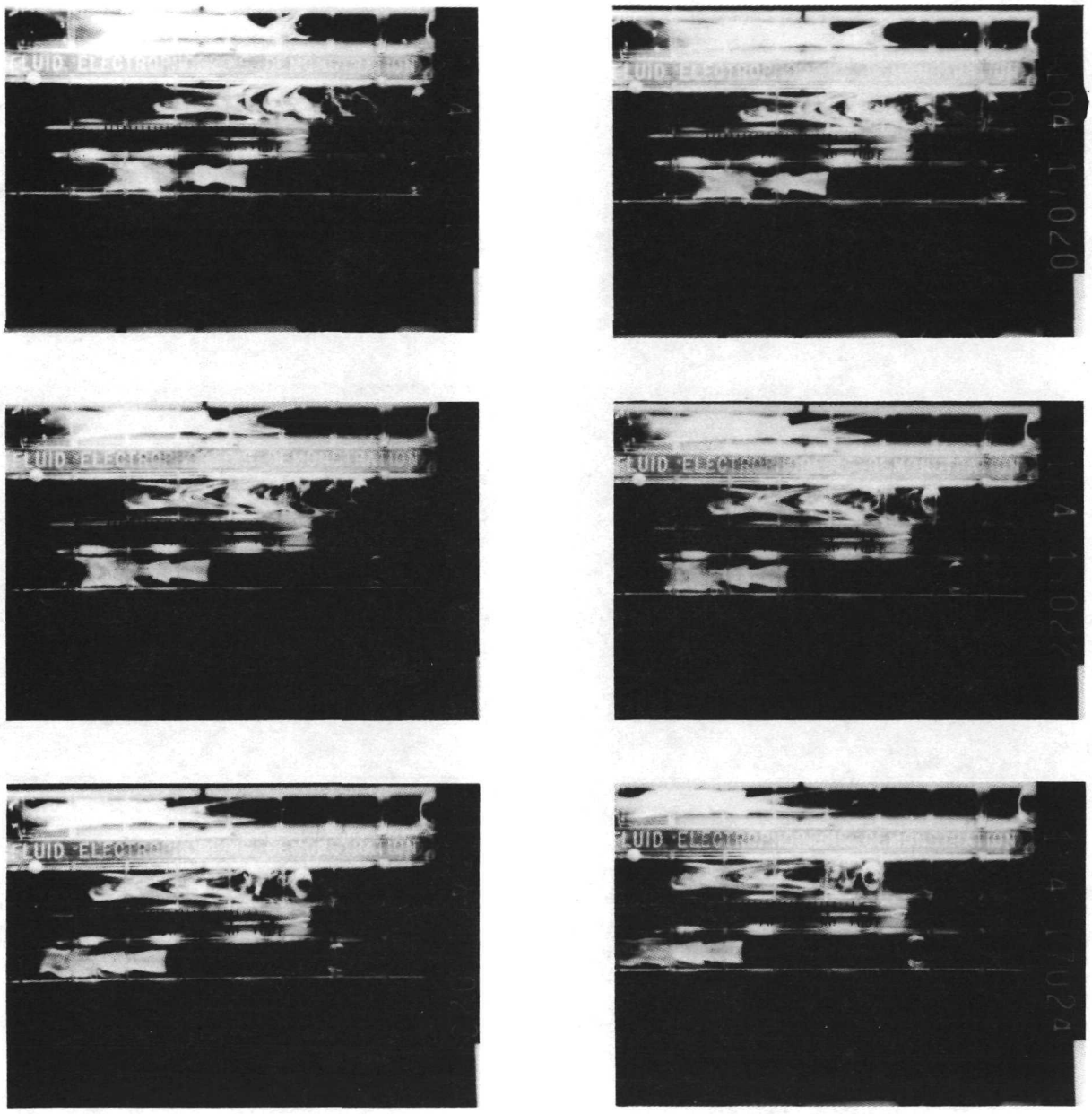

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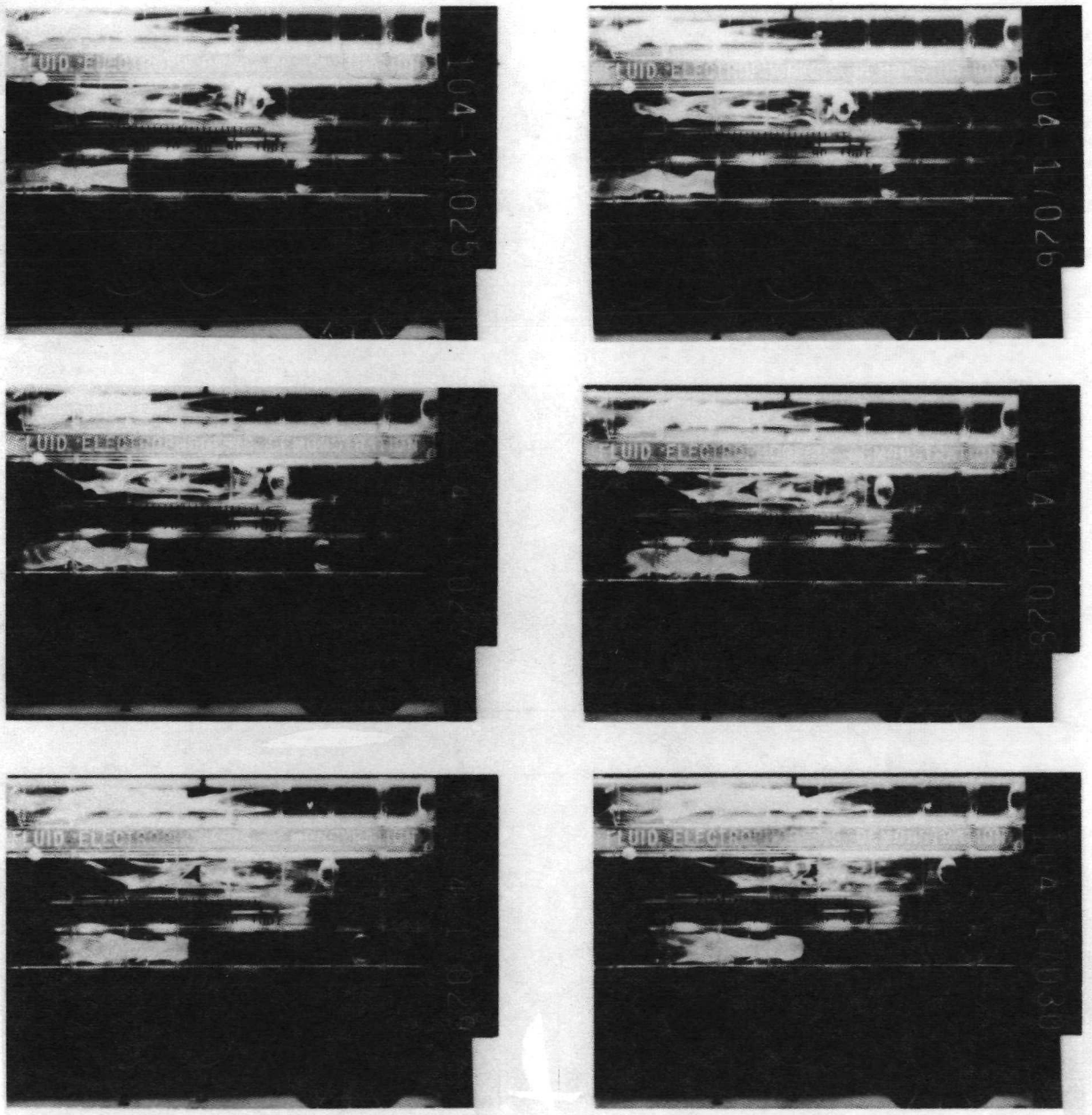

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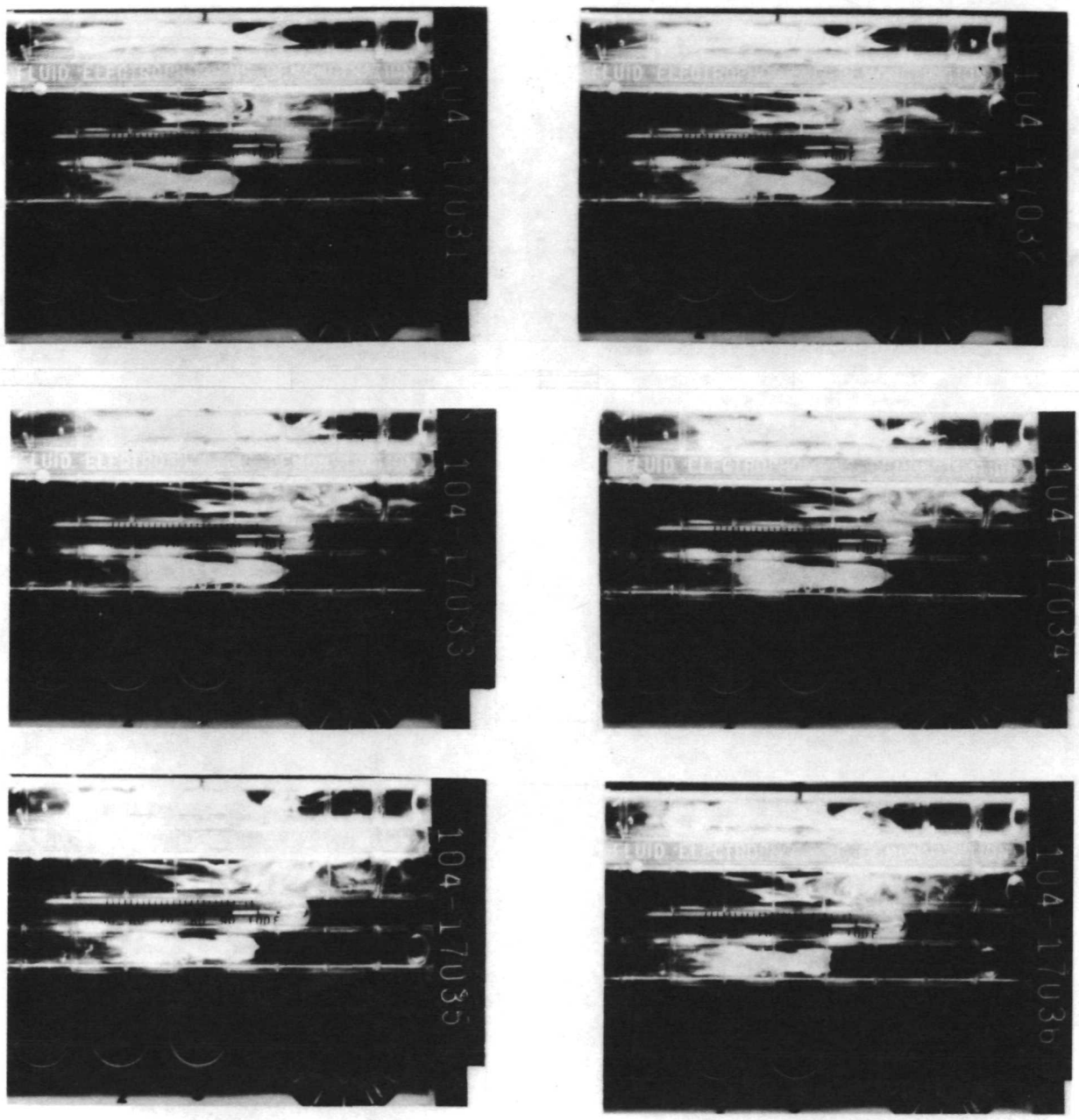

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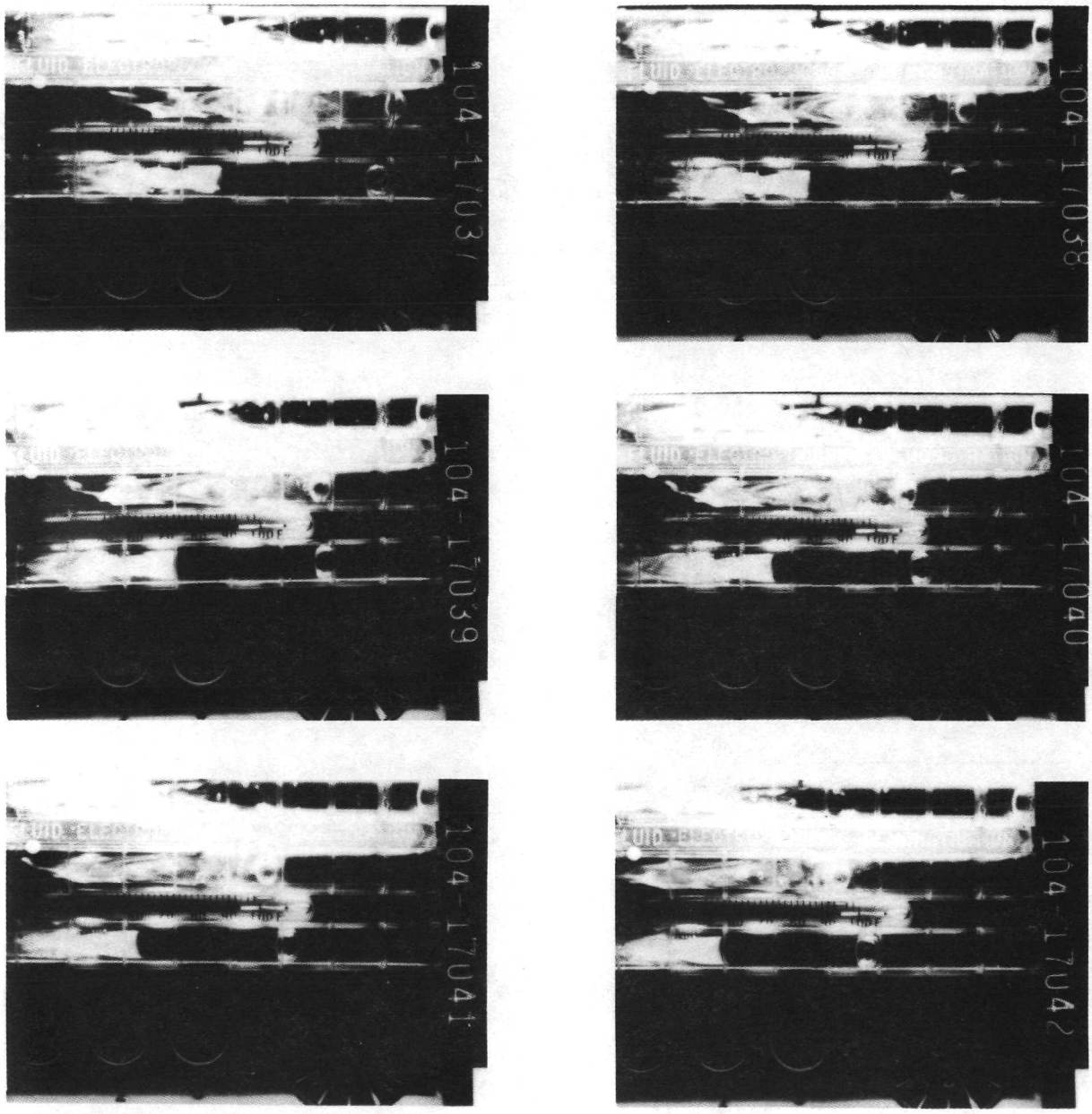

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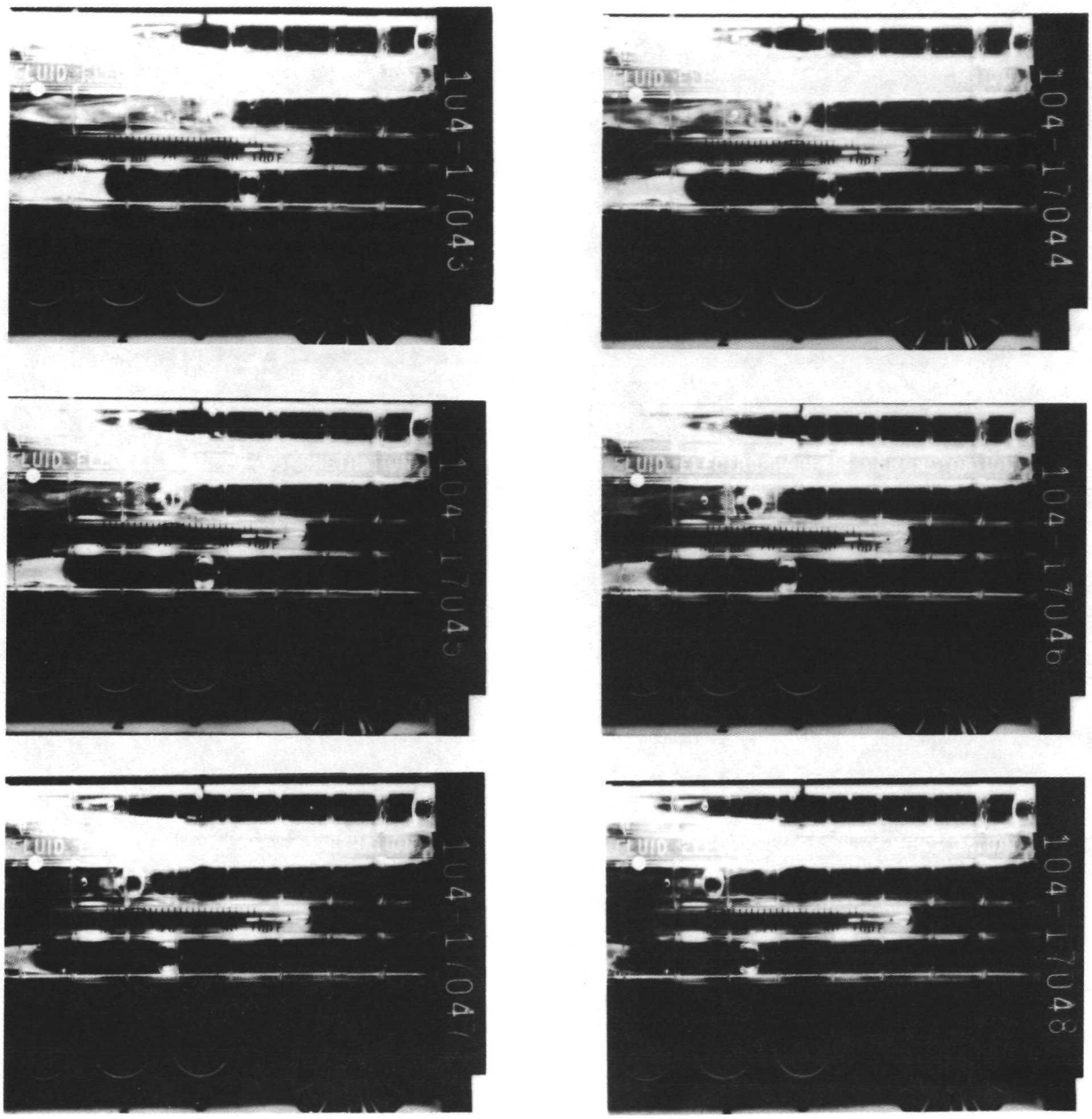

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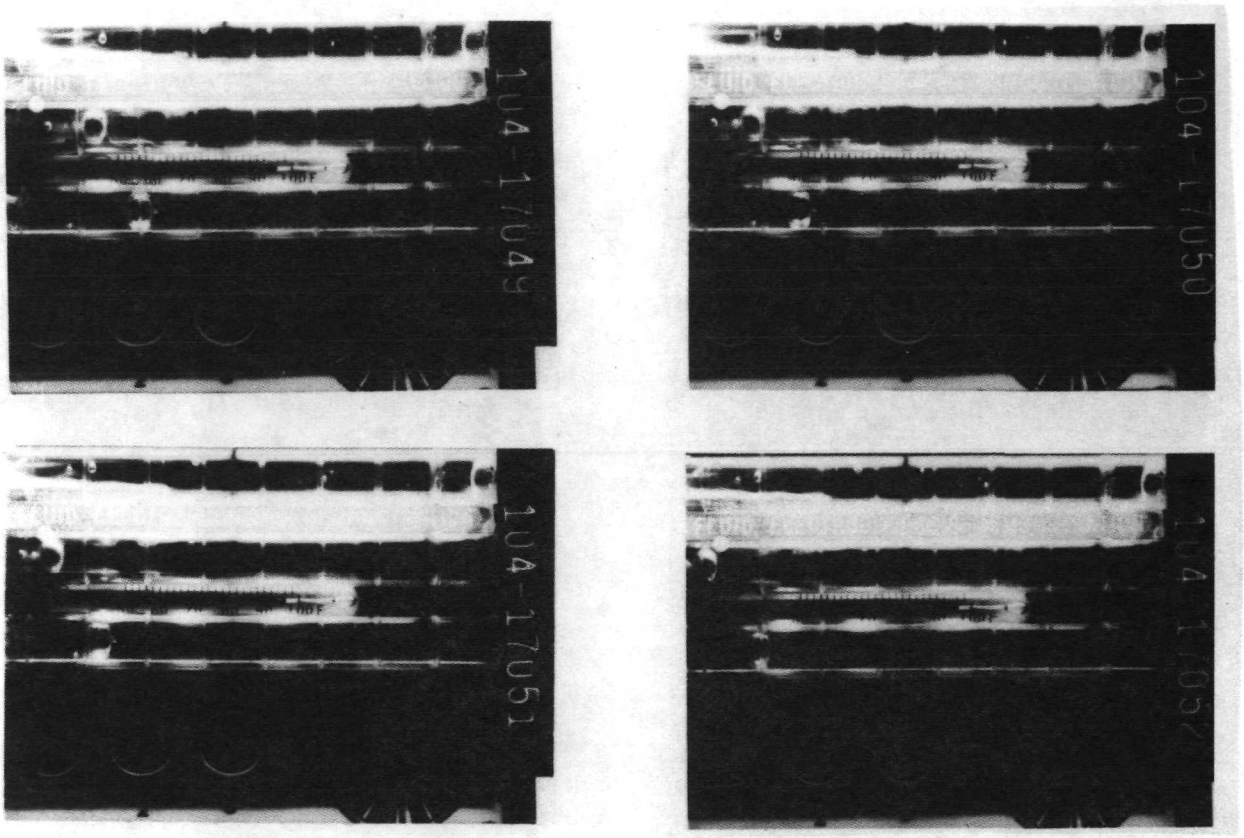


Figure 4. (Concluded).

The polystyrene latex was released into the cells precisely when Frame 17003 was taken, by pulling a strip of Kapton film out of the cell and exposing the sample to the cell buffer and electric field. The bubbles observed in each cell were about midway down each cell and motionless before electrophoresis began. During sample release in space, some polystyrene latex apparently escaped from the cell through the loose seal and hit the inside of the window. This is the "blob of stuff" referred to by Mattingly. Concurrently, the bubbles inside the tubes got larger since the ambient pressure in the Command Module in space is one-third earth pressure. The meter for Cell 3 did not indicate any current when the insulating Kapton film was removed nor did the meter needle move during the voltage reversals. However, this particle band migrated in accordance with the other bands and meter needle binding or failure of the meters to indicate was designed to have no impact on the experiment operation.

By the time the samples were visible in the photographs (less than half a centimeter from the sample input) the front of each sample group had become pointed. The parabolic shape acquired by the nose of each sample ("bullet shape" referred to by Mattingly) was due to electro-osmosis of the buffer. Although the sample was injected as a cylindrical disk, the electro-osmotic flow quickly modified the shape of the particle bands into the paraboloids.

The initial displacements of the sample bands were not as expected nor are they consistent with their subsequent steady velocity because the release of sample varied slightly from cell to cell. Figure 5 plots the velocity of the nose of each sample during the initial electrophoresis, and shows that the combined particles in Cell 1 and the 0.2 micron sample in Cell 3 were initially retarded while the 0.8 micron sample in Cell 2 migrated with its uniform velocity. It cannot be determined by study of the photographs or behavior of the sample which cell(s) lost the polystyrene latex during release.

One of the objectives of the experiment was to measure any interactions between identical and different particles during electrophoresis in space. The theoretical foundations of particle electrophoresis do not explicitly deal with multiple particle interactions, and experiments on earth must include the interaction of gravity. The nose of the combined particle band in Cell 1 is composed primarily of 0.8 micron particles and does migrate slower than the 0.8 micron particle band in Cell 2. The particle bands also acquired a well-defined nose that was different for the two sizes. The 0.8 micron particles in Cell 2 and the leading part of the band in Cell 1 became significantly more pointed than the 0.2 micron particles in Cell 3. Nearly identical electrophoresis and fluid flow conditions can be assumed in each cell based upon experiment design and general results of the electrophoresis demonstration. These phenomena, attributable to a mutual interaction among particles, were expected to occur but the extent of interaction has not been confirmed by ground evaluation.

The bubbles also migrated with constant velocity toward the anode when the electric field was imposed. The bubbles acquired a surface charge in the buffer due to the orientation of the lauryl sulfate at the bubble/liquid interface and the electric field moved them similar to the particle bands. The large bubbles in Cells 1 and 3 moved slower than the smaller bubble in Cell 2 because of electro-osmotic fluid circulation and viscous drag.

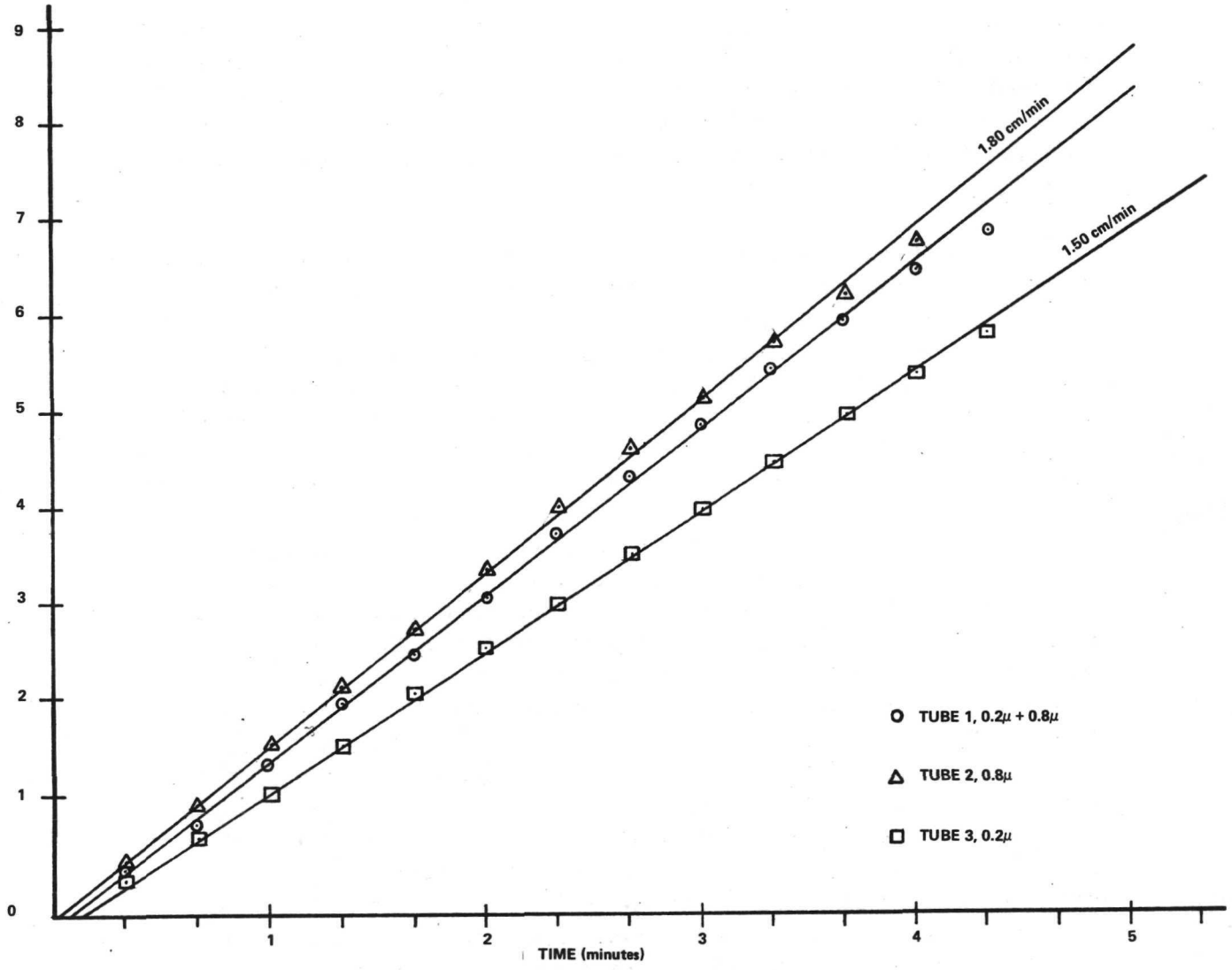


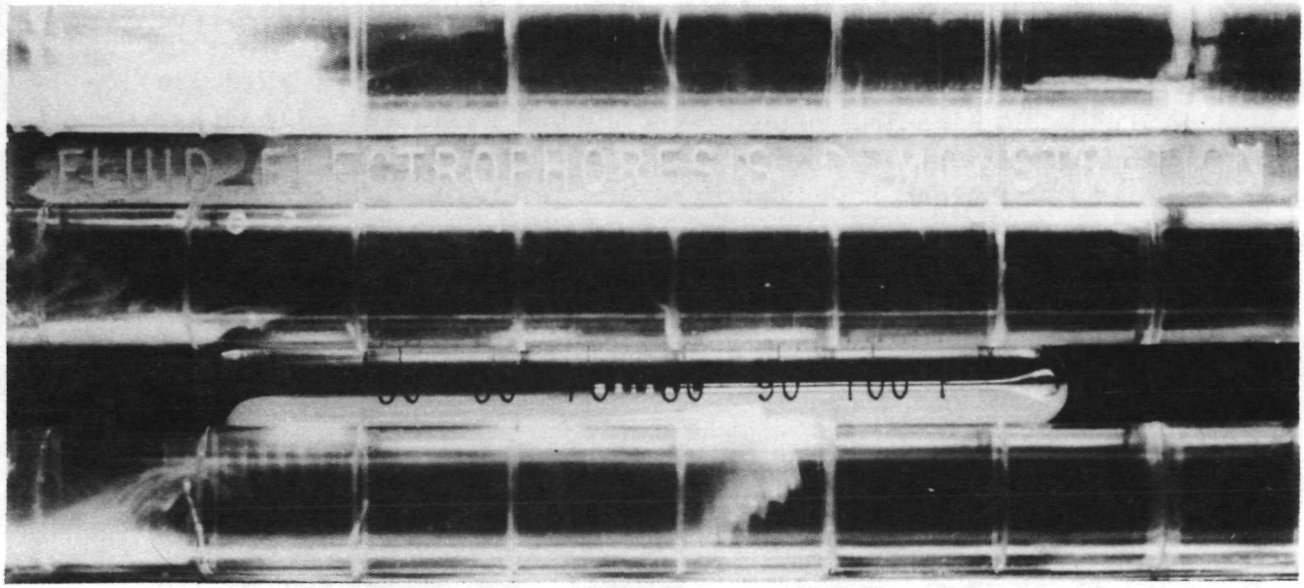
Figure 5. Measured displacements of the particle bands during Apollo 16 electrophoresis.

The large bubbles distorted the electric field in their vicinity, but this field asymmetry did not disturb the particle bands until the bands got closer to the bubbles which were stopped at the anode membrane. It is believed that this modified electric field slowed the bands and produced the corkscrew motion of the 0.8 micron particles in Cells 1 and 2. The extent and assymmetry of any field distortion in Cell 3 was not sufficient to modify the structure of the 0.2 micron particle band. In fact, a very fine stream of particles can be seen in the original pictures preceding the bullet-shaped band by almost half a centimeter that remains unchanged in the center of the cell through the first reversal.

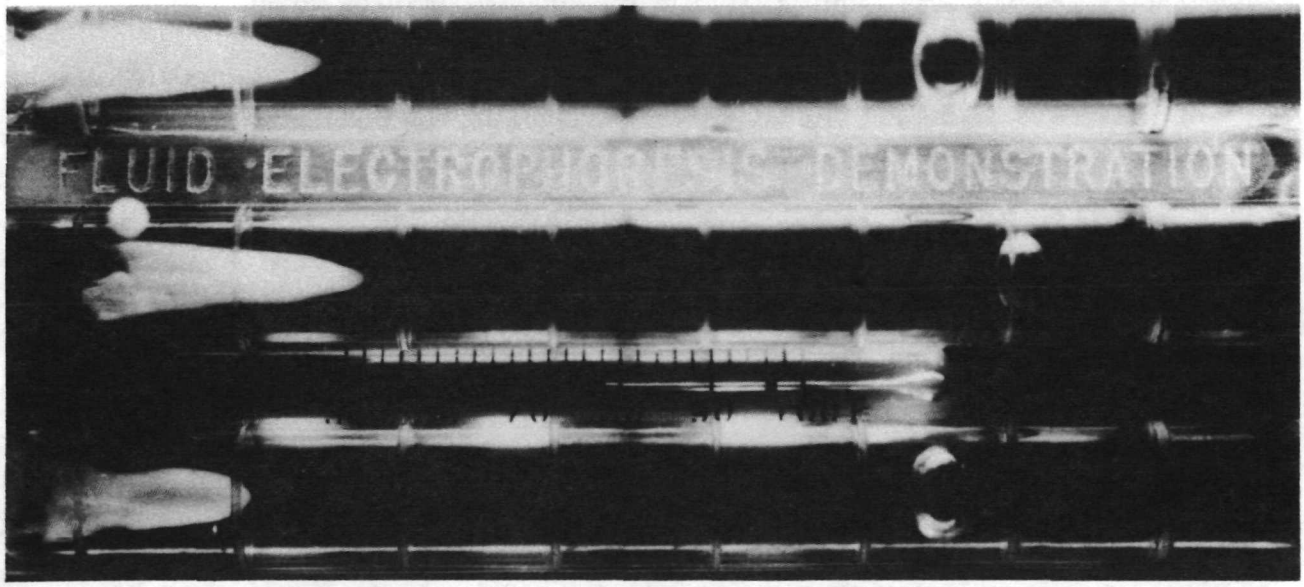
The corkscrew motion of the particle bands in Cells 1 and 2 became turbulent when the voltage was reversed and the particle profiles are difficult to interpret. Although there are several fluid phenomena of curiosity in these cells after the first voltage reversal, they are discussed in this report only as they pertain to electrophoresis. The 0.2 micron band in Cell 3 retained its coherent shape through several reversals. The cone shape of the band is apparent during the inversions of the nose caused by combined electrophoresis and electro-osmosis. Although the largest bubble in Cell 1 adhered to the anode membrane, the other major bubbles reversed direction as the field was changed. The bubbles also increased in size as the temperature in the cells increased.

The two sizes (0.2 micron and 0.8 micron) of polystyrene latex in the upper cell did not clearly separate into two distinct bands as anticipated. Early testing in flight prototype cells in a one-gravity environment showed that polystyrene latex formed irregular streams of particles swirled by thermal convection during electrophoresis in a moderately high electric field (26 volts per centimeter). Operation of the tubes horizontally caused much of the polystyrene latex to sediment to the bottom of the tubes due to the higher density of the polystyrene (1.05 grams per cubic centimeter). It was not possible to separate the two sizes on the ground in buffer alone. The photographs shown in Figures 6 through 9 compare pictures taken in flight with those taken of flight prototype apparatus operated horizontally in the laboratory and the effects described above are readily visible. Each pair of pictures was taken at the same time after beginning electrophoresis.

A separation of the two sizes occurred in Cell 1 but more sensitive photographic techniques were required to resolve the separation. Since the electrophoretic force was uniform across the cell and only electro-osmosis created the parabolic band shape, it was reasonable that tucked within the core of the 0.8 micron particles should be a parabolic band of the 0.2 micron particles. Careful exposure of flight original negatives revealed that the nose of the combined band was indeed less dense and, at a location behind the nose corresponding to the beginning of the 0.2 micron band in Cell 3, was a higher particle density that could be the nose of the separating band of 0.2 micron particles. Figure 10 shows an enlargement of Frame 17013 in which these features are visible. Color contour densitometry was used to display these features more distinctly. Figure 11 is a color contour photograph of 17013 for comparison with Figure 10. This technique allocates a color to each of four ranges in the exposure density distribution of the film. The yellow band in the upper cell signifies the highest particle density and corresponds to the supermicron particle bands. The yellow parabola thus defines the 0.2 micron particle distribution in Cell 1.

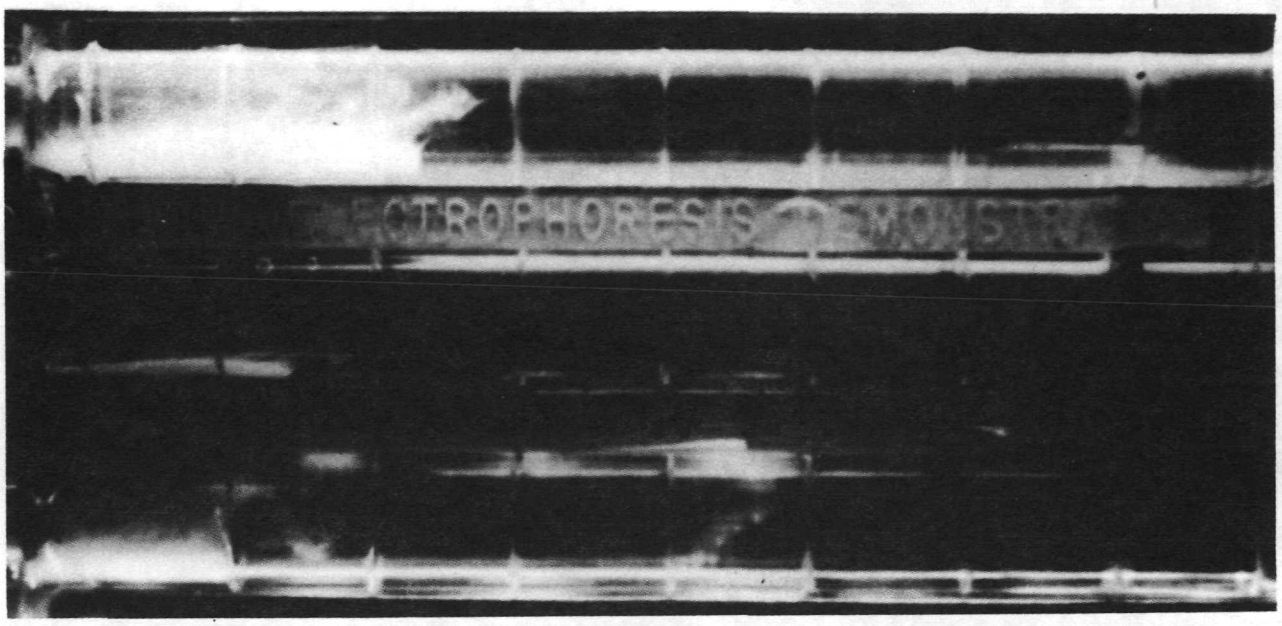


a. Ground test.

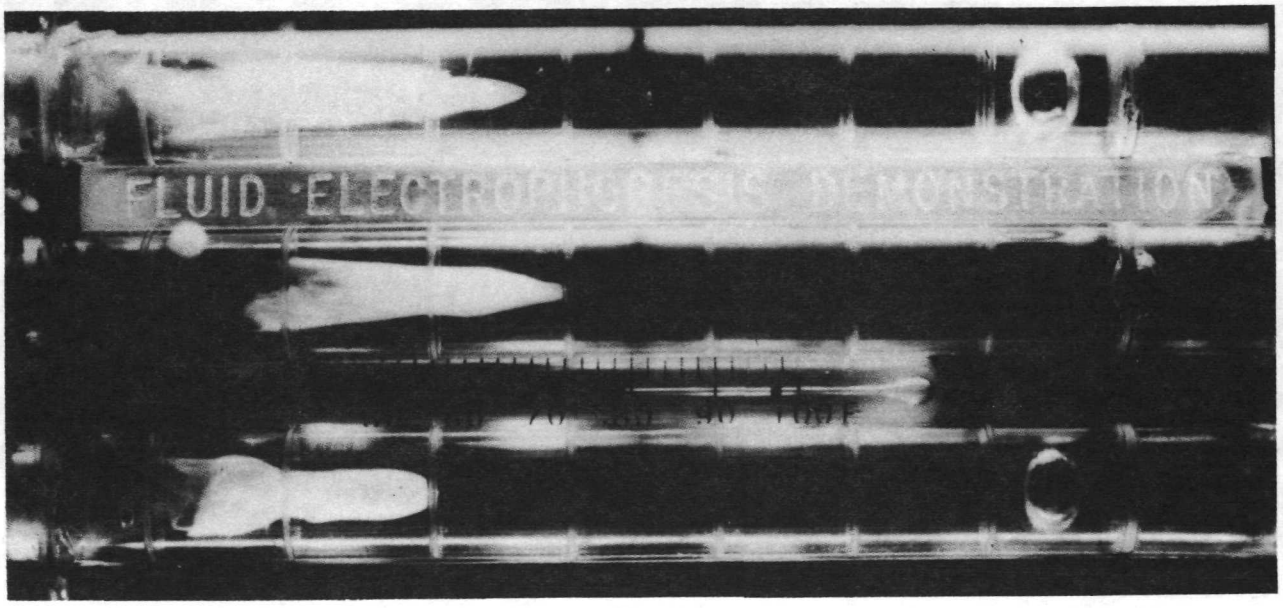


b. Apollo 16 photograph.

Figure 6. Apollo 16 electrophoresis after 100 seconds.



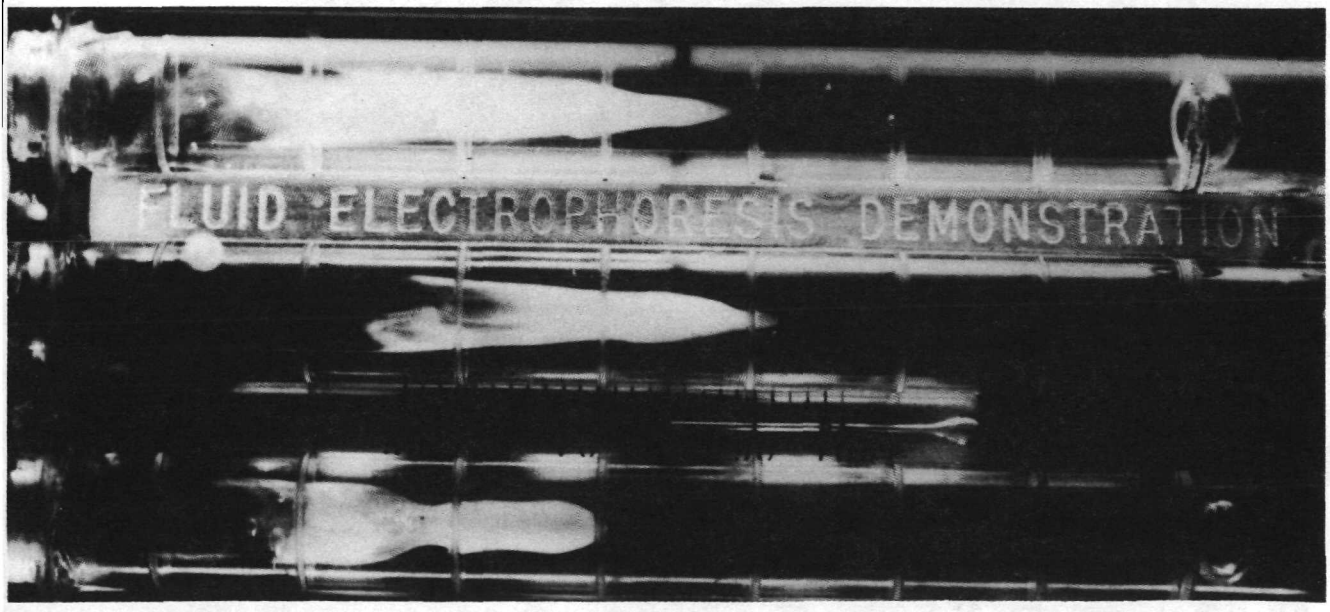
a. Ground test.



b. Apollo 16 photograph.

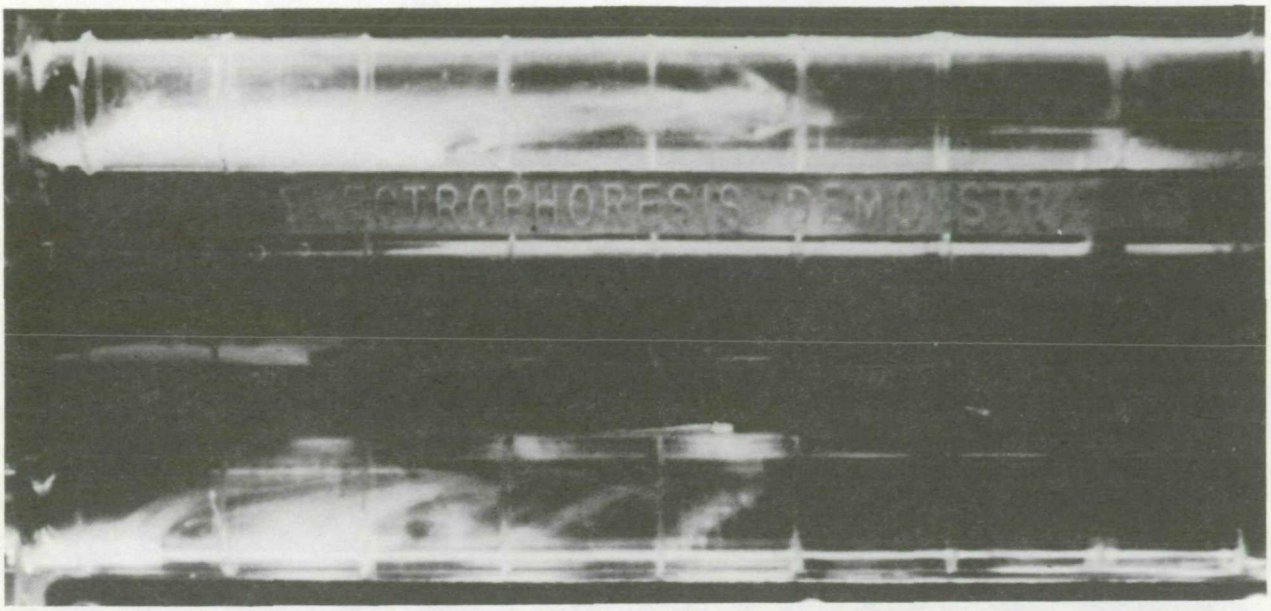
Figure 7. Apollo 16 electrophoresis after 140 seconds.

a. Ground test.

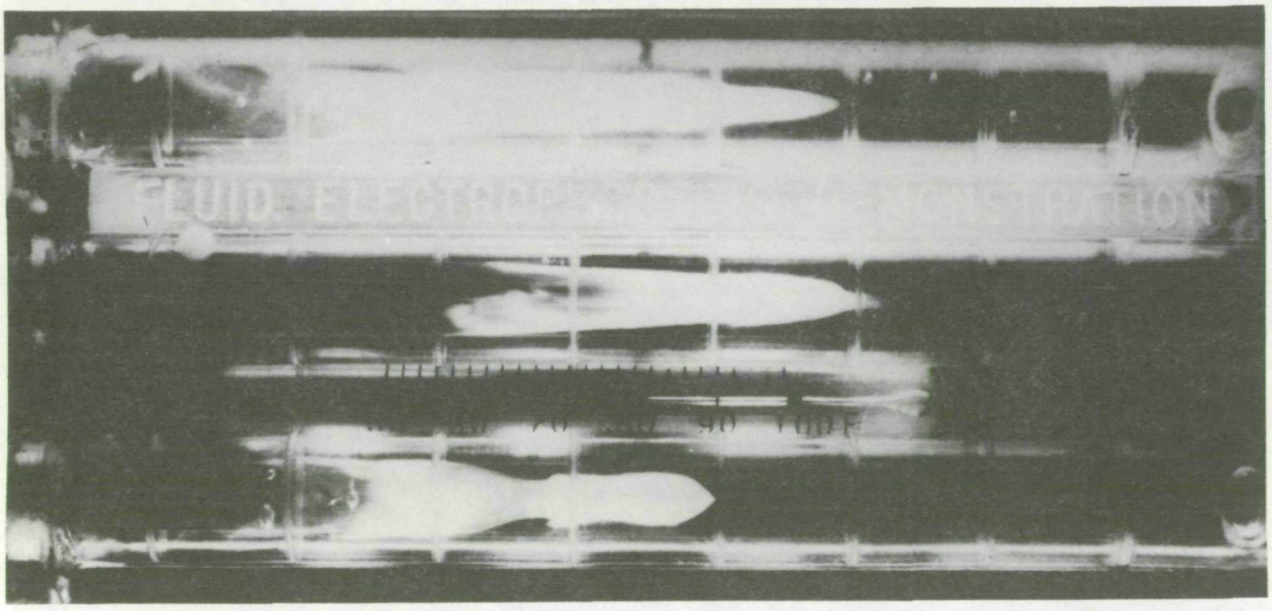


b. Apollo 16 photograph.

Figure 8. Apollo 16 electrophoresis after 180 seconds.



a. Ground test.



b. Apollo 16 photograph.

Figure 9. Apollo 16 electrophoresis after 220 seconds.

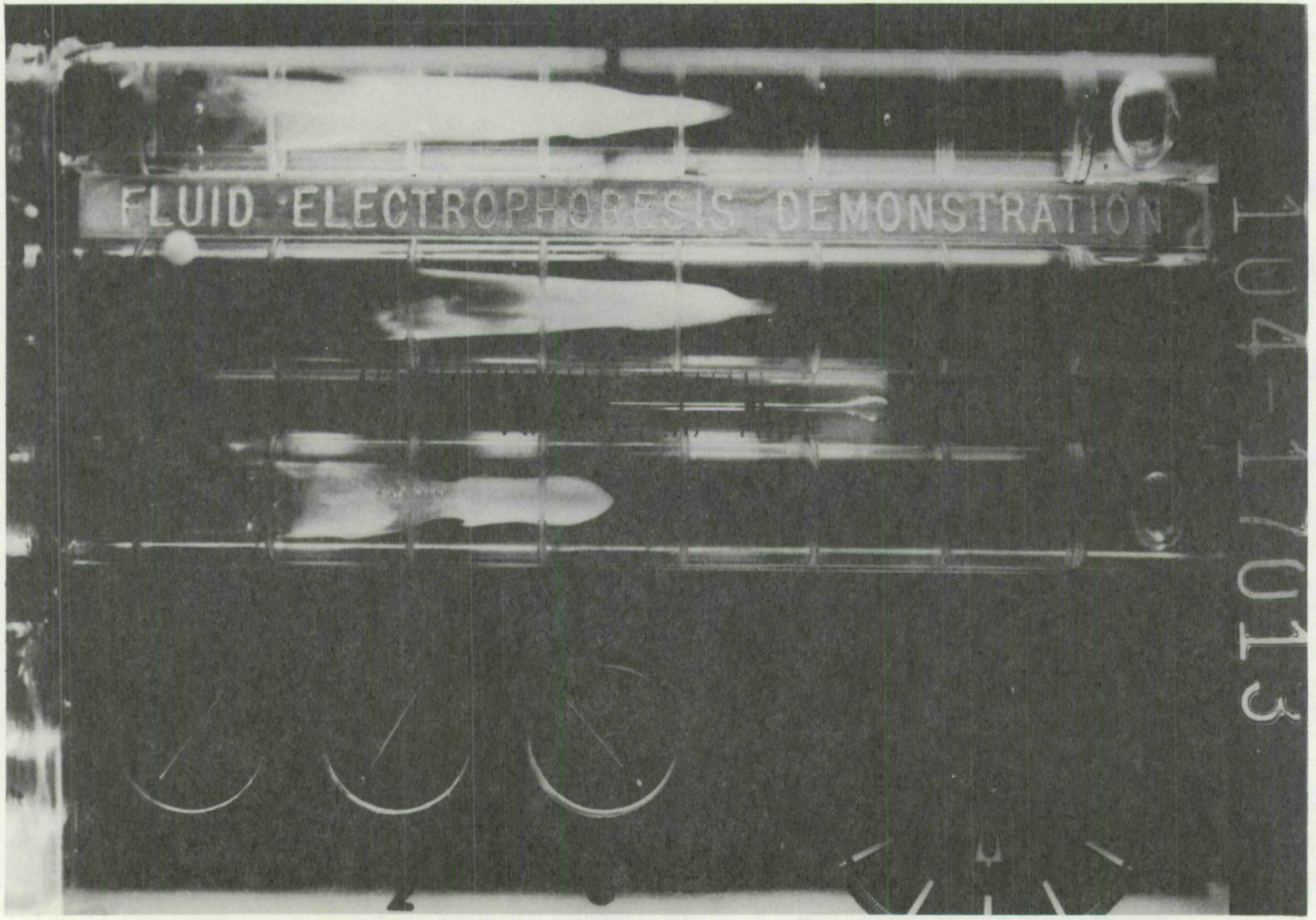


Figure 10. Apollo 16 electrophoresis picture number 17013.

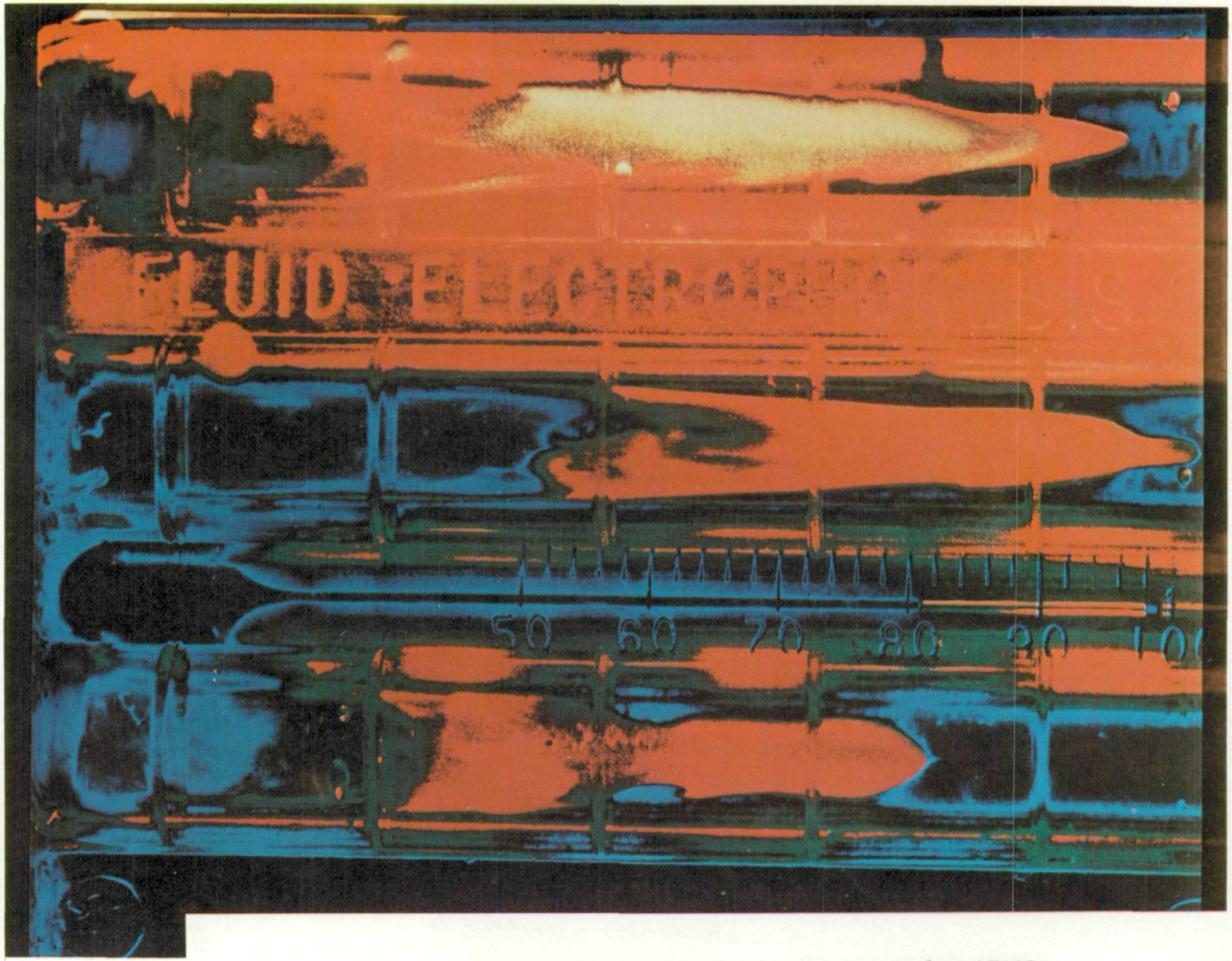


Figure 11. Color contour photograph of Apollo 16 picture number 17013.

Although it appears that the bullet-shaped nose of the 0.2 micron particles in Cell 3 is trying to separate from its more diffuse tail, this polystyrene latex sample was found to be homogeneous when received from Dow and after storage tests with simulated flight apparatus. It has been postulated that some of the particles agglomerated in groups of two or three but storage tests are not conclusive. The phenomenon is real because several of the later pictures show structure in the particle bands of Cells 1 and 3 that could be attributed to different distinct species; measurements are continuing.

A clear separation of the two sizes of polystyrene latex particles was expected based upon ground based testing in a density gradient [4]. The same electrophoresis experiments were done vertically in flight protytype apparatus and buffer with sucrose layered into the cells to establish a density gradient. Experiments done in a sucrose density gradient were intended to minimize the effect of gravity-induced thermal convection and sedimentation on earth and, therefore, simulate the flight demonstration. Repeatable separations of the two sizes were obtained with this technique although the sucrose gradient altered the experimental conditions. The increase in buffer viscosity slowed the particle migration and effectively eliminated the electro-osmosis. Although the density gradient tests produced clearly-separated cylindrical disks of polystyrene latex, there was still considerable swirling of the latex in the vicinity of the bands.

It was not possible to design or conduct an experiment in the laboratory which eliminated the irregular and unpredictable swirling of particles during electrophoresis. Density gradient electrophoresis in a water jacket controlled to 4° C reduced the thermal convection currents which stirred the particle groups. Reducing the electric field lowered the joule heating but slowed the electrophoretic migration sufficiently that diffusion became a problem. Only during the electrophoresis demonstration in space can distinct particle profiles be predicted based upon previous particle patterns obtained at precisely timed intervals. Selected features of the flight pictures, such as a rooster-tail structure at the end of the 0.8 micron band in Cell 2 and the line of particles preceding the 0.2 micron band in Cell 3, did not change during the initial electrophoresis run. As soon as the electrophoresis of the particles and electro-osmosis of the buffer were initiated, the migration of the particle bands was predictable. The stability of the particle groups during electrophoresis in space was expected, and the flight film clearly demonstrates the phenomenon.

It was recognized during Apollo 16 planning that electro-osmosis would distort the polystyrene latex bands and careful selection of the cell wall material could minimize the effect. Gelatin and methyl cellulose were proposed as coatings with a low zeta potential and, consequently, small electro-osmotic flow. Unfortunately, neither of these materials are stable for more than a few days and the Apollo requirements of loading several weeks before launch precluded their use. Collodion (cellulose nitrate) applied to the inside of the Lexan cells during testing was shown to be stable over many weeks, easily applied as a uniform coating, and compatible with the borate buffer. Collodion has a moderate zeta potential and, therefore, some electro-omosis was expected. Electrophoresis investigators, such as A. Stickler of Beckman Instruments, have considerable experience with electrophoresis of polystyrene latex in collodion-coated cells and were available to assist in the experiment evaluation. However, the collodion could have cracked the Lexan cells [4]. Tests indicate that the zeta potential for uncoated Lexan ranges from values comparable to collodion to values about twice as high.

A significant amount of electro-osmosis was suspected as soon as the bullet shaped bands were described by the astronaut in space. An electro-osmotic flow pattern in the cell would produce two of the effects observed: (1) The particle bands would be parabolic due to the reverse fluid flow along the walls; and (2) the nose of the bands would move faster because of return flow down the center of the cells.

The amount of electro-osmosis that occurred during the Apollo 16 demonstration can be calculated [5] and compared with expected results. The observed velocity profile of the particles, V_{obs}, was composed of the velocity of the buffer due to electro-osmosis, V_w, and the electrophoretic velocity of the particles relative to the buffer, V_e, i.e.,

$$V_{obs} = V_w + V_e \quad .$$

Within a closed cylindrical cell of inner radius a, the buffer velocity will vary with radius r such that

$$V_w = U \left(\frac{2r^2}{a^2} - 1 \right) \quad .$$

When r = 0, at the axis of the cell, $V_w = -U$. The particle profiles to be expected based upon these equations, particles velocities measured by microcapillary electrophoresis, and the observed velocity of the particle bands during the flight demonstration measured along the axis of the cells have been calculated and are shown in Figure 12. The profiles in each cell are calculated one minute apart. Profiles obtained from the flight pictures are shown for comparison. The calculated profiles duplicate significant features of the flight film, such as the parabolic shape of the nose of the 0.8 micron particles in Cells 1 and 2 and the outline of the particle tails in all cells. The calculations do not agree with the blunt bullet shape of the 0.2 micron particle band nor the different migration velocities in Cells 1 and 2.

Although the slight difference in velocities could be attributed to variations in the cell properties or bubble accumulation, the blunt nose of the 0.2 micron band and the leading thin stream of particles can be explained only in terms of some sample insertion anomaly. More study is being devoted to this unusual occurrence.

The electrophoretic mobilities used to calculate the particle profiles were measured by microcapillary electrophoresis [6]. Using a laser to illuminate the particles, the average mobility of the polystyrene latex species are 5.8 micron centimeters per volt second for the 0.2 micron batch and 9.2 micron centimeters per volt second for the 0.8 micron batch. These mobility measurements were constant within experimental error estimated to be ±5 percent.

Questions remain on several details observed in the flight pictures. For example, the behavior of the particle bands after the electric field was reversed reveal reproducible features common to more than one cell. Understanding these phenomena will provide insight into doing fluid electrophoresis in reduced gravity.

CONCLUSIONS

It is concluded that the electrophoresis of model particles in a free liquid in a weightless environment was demonstrated on Apollo 16. The flight pictures clearly show the stability of the bands and sharpness of the particle fronts during electrophoresis in space. The effects of gravity-induced sedimentation and thermal convection on particle electrophoresis can be seen in the comparisons of the flight demonstration and laboratory experiments.

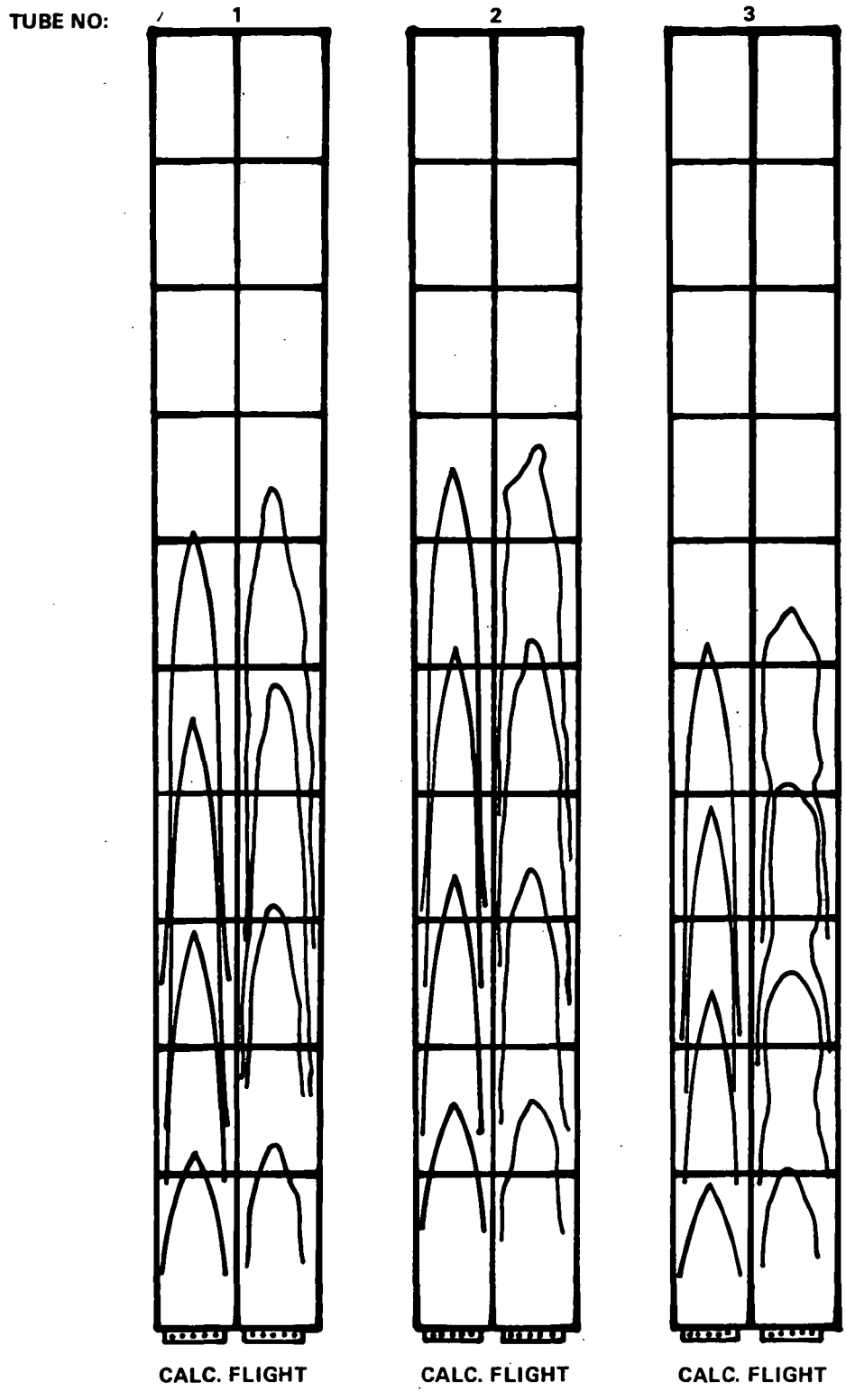


Figure 12. Polystyrene latex parabolic profiles at 1, 2, 3, and 4 minute intervals.

The difficulties that limited the results obtained from the Apollo 14 mission did not recur during Apollo 16 although new hardware and experimental problems arose that reduced the effectiveness of the demonstration. For example, if a suitable low zeta potential cell wall coating could have been used, electroosmosis would have been significantly reduced and a distinct separation of the two sizes of polystyrene latex would have been obtained.

The ramifications of this demonstration for any future endeavor in free fluid electrophoresis in space are:

1. This engineering design developed for Apollo 16 differs very little from Apollo 14. Many variations were tried during development but most were found unworkable. The advantages and limitations of this design for future electrophoresis are now well understood.

2. The principal limitations identified during the ground testing and flight demonstration include a negligible zeta potential cell wall coating, more precise sample insertion, and means of collecting adjacent separated samples.

3. Characterization of the samples before electrophoresis, preservation in their native state, and analysis after electrophoresis were not accomplished as routinely as expected with the model polystyrene latex particles and will be significantly more difficult with viable biological materials.

The need for large quantities of precisely separated biological materials will be a research objective for several years. Although the invention of specialized techniques to overcome gravity-induced thermal convection and sedimentation will continue, zero-gravity electrophoresis is not similarly restricted. This flight electrophoresis and the analytical work that support it have shown that free fluid electrophoresis can be conducted in space in a controlled manner, thus demonstrating the utility and potential of this process for the future.

APPENDIX

TRANSMISSION BETWEEN APOLLO 16 AND MANNED SPACECRAFT CENTER DURING ELECTROPHORESIS DEMONSTRATION

Tape 17/3

01 01 18 14 CMP	Okay, Tony. We're on the electrophoresis now, and we're now just about to hook up the power cable and turn the power on, and it says at that point "Hold for instructions from Houston."
01 01 18 29 CC	Okay, instructions I have there are to press on through that hold and go on down to just before starting the camera and then hold again and give us a call.
01 01 18 41 CMP	Okay.

Tape 17/4

01 01 23 57 LMP	Houston, we're down to the step before Ken turns on the electrophoresis. Where do you want us to hold that? Over.
01 01 24 12 CC	Okay, we'd like you to hold just prior to starting the camera.
01 01 24 20 LMP	Okay, just prior to starting the camera.
01 01 24 22 CC	Roger. On the next page.
01 01 24 25 CMP	Okay; how about telling us where we're going here, because I've got to turn this thing on, and I'd like to have it in my mind what it is we're going to do.
01 01 24 34 CC	Roger. The note here was, at that point, you're supposed to observe the current meters, and if there's no indication of a current flow in any tube, you tap the box gently along the axis, or parallel with the face,

and then you allow the whole unit to lie motionless for additional 3 to 5 minutes before proceeding. They're afraid there may be a bubble in one of the tubes, and you don't get a current.

01 01 25 06 CMP — Okay. Well, actually, there's a bubble in each tube.

01 01 25 10 CC — Say that again.

01 01 25 12 CMP — Actually, there's a bubble in each tube. They are in exactly the same place. They're lined up in a row, and they are directly over meter number 3. And the bubbles are about - oh, one-eighth of an inch in diameter.

Tape 17/5

01 01 25 41 CC — Okay, the PI says that's okay, and we should go ahead and proceed.

01 01 25 47 CMP — Okay. Now, the question that you had for me was that if any of the meters do not go into the green, we turn the power on. Did you want me to tap the box, and the do what?

01 01 26 02 CC — Okay. The instructions were to tap the box gently, allow the unit to remain motionless for an additional 3 to 5 minutes, and then proceed.

01 01 26 18 CC — According to the instructions, that's correct.

01 01 26 22 CMP — All righty.

01 01 27 44 CMP — Okay, Tony. It turns out that meter number 1 is just barely into the red; meter number 2 didn't come up quite into the red; meter number 3 is about a needle width below the red.

01 01 28 06 CC — Okay. We'd like you to go on with the experiment.

01 01 28 14 CMP — Okay, I've jiggled it a little bit and I'm gonna let it settle here for a second, and then we'll start. We'll give you a mark when we start.

01 01 28 23 CC — Roger, we concur.

01 01 30 16 CMP — Okay, Houston; we have started the experiment. And as soon as we got it rotating - got it running, and I turned according to the decal on the box, which is counterclockwise, half rotation; and, soon as I did, the orange film disappeared and - I see white particles coming through as a stream. It looks much like a latex.

01 01 31 01 CC — Okay. We copy that. Any difference in rates between the different tubes?

01 01 31 06 CMP — Yeah. The first thing that happened, as soon as I opened it, I got a big blob of this stuff inside of the window

Tape 17/6

here between where it shows - the decal on the outside says "sample 1 and 2." It's got a couple of big blobs in there....the number 1 sample is approaching it. The number 3 sample is about halfway between ring 2 and 3. Also have current meter number 1 is in the green, current meter number 2 is in the green, and number 3 is still about a needle width below the red line and didn't move at all. The bubbles are moving at about the same rate as the white material, and the first bubble in tube number 2 has just reached the yellow band, and as I understand this, I'm going to have to wait until the white material reaches that yellow band.

01 01 32 28 CC — That's affirmative. The white material in the fastest tube.

01 01 32 33 CMP — Okay.

01 01 32 36 CC — And we had some bad comm right there in the middle when you were describing the rates and the difference in the three tubes of the white material. If you could say a little bit of that again, it might help.

01 01 32 47 CMP

Okay. It's moving much more rapidly than I had anticipated it would, Tony. Right now, the number 2 sample is leading by about a nose. It's just crossed the one - two - three - four - fifth ring inscribed on that center tube. The number 3 sample has just crossed the fourth one; the number 1 sample has just crossed the fifth one now; and number 2 is about halfway between five and six. Number 3 sample is maintaining a very cohesive shape and looks like a little cylinder with a pointed nose on it, and it's maintaining its white consistency. I guess that the length of the group of particles in there that's maintaining a solid appearance is about the width of these lines. Then, it tails out to a very diffuse gaseous - just a swirl material behind it that goes all the way back to the Lexan. The faster samples are diffusing much more rapidly, and they have a little nose on them, which is very thin and leads ahead of the larger mass of material. And they form sort of a cone shape. And they are about

Tape 17/7

two and a half to three ring lengths in length, and - I'm talking about the distance between sets of rings. And they both appear to be diffusing about the same amount. The number 2 sample is really starting to break up now and starting to twist the - looks like it's taking on a corkscrew appearance as it approaches the yellow line. And it's approaching the yellow line, and now number 1 is approaching one...., so I'm gonna hit the REVERSAL SWITCH.

01 01 34 38 CC

Okay. You say there is no difference in diffusion between 1 and 2?

01 01 34 48 CMP

Well, there wasn't when we started, now that we've hit the REVERSAL SWITCH, I guess all bets are off. They've just really broken up in number 2 and in number 1 is holding together a little better. They really looked very, very similar; except that just as it crossed the last ring before the yellow ring, number 2 started to get an elongate nose on the point, and it

was starting to twist - I say it was looking like a corkscrew. And then about the same time, when - just about the time I hit the REVERSAL SWITCH, the sample in number 1 did the same thing. The sample in number 3 is doing entirely a different operation. It retained sort of a bullet shape all the way down as far as it went, and now - that we've reversed it, the point end, which was on the right side, the direction of motion, has now become a flat blunt end, and it's picking up - kind of an arrow-shaped head on the left side as it goes back towards the container. But it's still retaining its cohesiveness. The sample number 2 just really got all diffused and spread around. And number 1 is holding together a little bit better. It's starting to take shape that looks like number 3; in fact, the trailing edge - that's the one on the right side now, or sample number 1 - has just about caught up and looks very much like sample number 3, except that you can tell that some of the material in sample 1 has been diffused.

01 01 36 31 CC — Outstanding.

Tape 17/8

01 01 36 32 CMP — And we're about to approach the original end. Do you want me to reverse it again, or what do you suggest at this point?

01 01 36 48 CC — Yeah, Ken. We'd like you to reverse it again.

01 01 36 51 CMP — Okay, and I'll do that when the first large portion of the sample reaches the Lexan manifold; is that okay? That's - some of the diffused material will already coincide.

01 01 37 05 CC — Okay, that sounds good.

01 01 37 33 CMP — Okay, I've reversed it, and I reversed it when the pointed end of sample number 3 reached the first marked ring before reaching the Lexan manifold.

01 01 37 42 CC — Okay.

01 01 37 45 CMP — And it's starting to snake now. These little blobs don't seem to take this reversal so well. Another thing that was a little different after I reversed it, I mentioned that all three had bubbles who were right together when we started. The bubble in them all passed over to the extreme right end, except that number 1, when we reversed the samples, it remained over the right end, and numbers 2 and 3 traveled with the material.

01 01 38 21 CC — Okay. Copy that.

01 01 40 08 CMP — Okay, Tony, number 2 has reached the end again. I'm going to reverse it for the last time.

01 01 40 16 CC — Okay.

01 01 40 17 CMP — It's reversed at this time. Mark it.

01 01 40 19 CC — Okay.

01 01 40 23 CMP — Number 2 looks like an emulsion. Number 1 still has a central core that's holding together, and number 3 is doing a good job of staying together. It's diffused very little.

01 01 40 41 CC — Okay, we copy that.

Tape 17/9

01 01 40 45 CMP — Okay, and it looks to me like it's so diffused that at the end of this run when we get it back, I'll just go ahead and secure it.

01 01 42 37 CC — Yeah, Ken, I think they're gonna have fun analyzing that one.

01 01 42 42 CMP — I think they've got their work cut out for them. Are there any questions that you might want to get resolved that maybe were obvious to me but weren't obvious to you before we put it all away? We're going to be closing down here in a couple of minutes.

01 01 43 00 CC — Okay. The PI is back there, and hopefully he's working on some questions.

01 01 44 25 CC — Ken, Houston.

01 01 44 29 CMP — Go ahead.

01 01 44 31 CC — Okay. One, you said you tapped the box there at the beginning to try to get rid of the bubbles. How long did you wait before you started? I know you gave a mark, but we'd like to verify that.

01 01 44 45 CMP — Between the time we tapped the bubbles and the time we started the experiment?

01 01 44 49 CC — That's affirmative.

01 01 44 51 CMP — Is that the time frame you - ? Okay. That time frame was - I would guess it was about a minute, Tony. Because when I tapped it, I just couldn't get them to move. I had already - I had already tapped that thing once before, for the bubbles, and - because when - as soon as we unpacked it, we saw the bubbles out there, and I banged it a little bit to try and see if I could get them to move and didn't have any luck at all. So we didn't wait any 3 or 5 minutes, it was about 2 minutes, I guess.

01 01 45 19 CC — Okay, we copy that; 2 minutes. And on the tube 1, did you notice any separation of the two sizes?

Tape 17/10

01 01 45 29 CMP — Not unless that's what this diffuse and central feature turns out to be. But the dark - oh, let me rephrase that, the higher concentration of material that makes it look more solid - if that's a large particle and the diffuse material is the finer particles, then I would say that perhaps there was a separation of small particles from larger ones in tube number 2 just about the time I reversed it, just starting to show up; and number 1 perhaps the same. And number 3, I would

say, if that's the proper interpretation, that there was no appreciable separation of any kind. And I'm not sure that number 1 ever exhibited some of the symptoms that number 2 did. I can't tell you right now which of these tubes splurted these blobs of particles under the window unit.

01 01 46 30 CC

All right, we copy that. I sort of expected from the information we got here that 1 would be the one that split up in the two sizes, but I guess we'll have to look at that later.

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APPROVAL

ELECTROPHORESIS DEMONSTRATION ON APOLLO 16

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.

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