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FREE FLUID PARTICLE ELECTROPHORESIS ON APOLLO 16

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INTRODUCTION

One of the more promising possibilities identified to date for processing of materials in the space environment is the separation and/or purification of biological materials by free fluid 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 surface charge, the particles migrate either to the cathode or anode. Differences in migration velocities provide a sensitive means of separating from their mixtures substances that are otherwise difficult or impossible to separate.

Electrophoresis was first performed under conditions of zero gravity when the separation of red and blue dye was attempted on the return trip from the moon on Apollo 14[1]. The resulting photographs showed that the boundary dividing the dyes was sharper and better defined than was possible to achieve with comparable equipment on earth. It was also 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 on the design and operation of the apparatus for the Apollo 16 mission began as soon as the Apollo 14 results were analyzed.

Electrophoresis done in space can 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 heat 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. At zero gravity, electrophoresis can be applied to molecules or particles suspended in fluid media without the occurrence of gravity-induced mixing or of sedimentation. These conditions may make electrophoretic separation in space practicable for the separation of medical and biological products of high social and economic value, such as living cells.

Cell electrophoresis is one of the few physico-chemical processes that can be applied to living cells without producing permanent damage. Since a living cell is large and dense, the separation of living cells according to charge is limited by gravity-induced sedimentation and convection on earth. The successful performance of electrophoresis of small particles with the Apollo

16 apparatus is a crucial step in the development of the methodology for electrophoreis at zero gravity of biological particles.

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

ELECTROPHORESIS THEORY

Practically all particles acquire either a positive or negative surface charge when suspended in an aqueous medium. This holds true for visible bubbles or drops, to microscopic colloidal particles as well as 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 between ionized groups on the particle surface and the free ions that are dissolved in the medium. The measurable charge at the plane of shear of the particles (the ζ potential) may not be fixed and varies according to the pH and ionic strength of the medium.

When an electric field is applied to an aqueous suspension of particles, the charged particles migrate in the potential gradient to the oppositely charged electrode at a velocity principally dependent upon their surface charge. Since two species may acquire different net charge densities in the solvent, particles that otherwise have similar physical and chemical properties may move at different rates.

Basically, the electrophoretic migration is described by

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

where:

U = the particle velocity

E = the electric field

η = the viscosity of the medium

D = the dielectric constant of the medium

ζ = the potential of the diffuse double layer at the plane of shear

For particles that are large in comparison to the thickness of their double layers, the field distortion due to the particles plays an appreciable role and the constant changes from 6π to 4π, (see Shaw;[2] Henry,[3] Overbeek,[4] Wiersema, Loeb and Overbeek;[5] Overbeek and Wiersema[6]). Thus, while the ζ potential of particles is independent of their size, the electrophoretic mobility, U/E, of large particles can under given conditions of ionic strength be up to 50% faster than that of small particles. In addition, the drag (or relaxation) exerted by medium-sized ionic double layers, may even further slow down the electrophoretic mobility of particles with ζ potentials higher than 25 mV.[4] Dependent upon the ionic strength (which governs the thickness of the electrical double layer), latex particles of identical ζ potentials but of different sizes may therefore manifest different electrophoretic mobilities.

ELECTROOSMOTIC COUNTERFLOW

The electrophoresis of particles in a closed cylindrical tube or cell, such as used on Apollo 16 or for micro-capillary electrophoresis, is in addition affected by the electrokinetic phenomenon of electroosmosis in the closed liquid system, which 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.[2] 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.

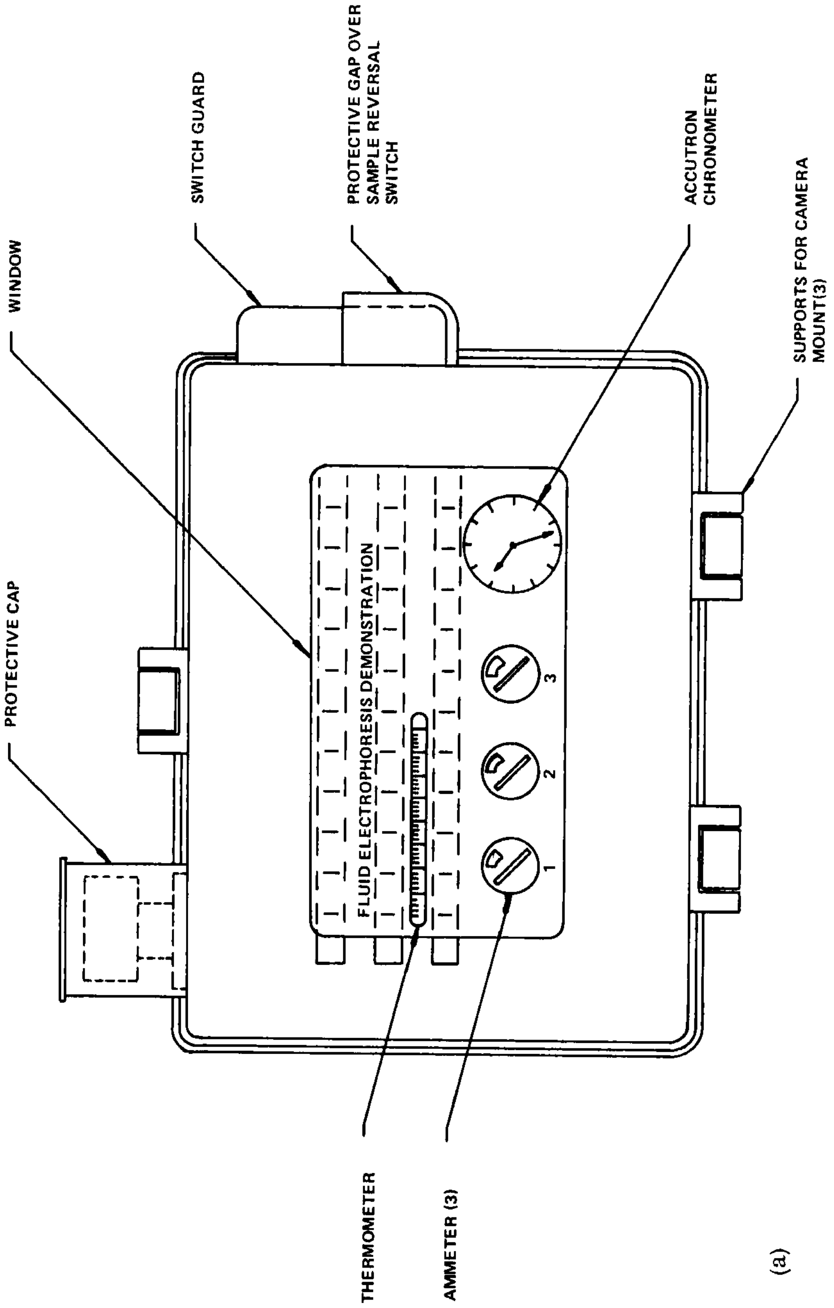
EXPERIMENT SAMPLE SELECTION

Stable, non-degradable monodisperse polystyrene latices of two different sizes were chosen as the sample materials. Three simultaneous experiments were done: one with the large size latex (0.8μ); one with the smaller size latex (0.2μ), and one with a mixture of the two latices. Control experiments were done on the ground with the tubes in a horizontal or a vertical position and stabilized with a sucrose density gradient in order to establish the ground control for later comparison with the flight. Experiments on the ground with the tube in a horizontal position and without a stabilizing gradient were also done. Microscopic electrophoretic mobility measurements were also done on the ground with both particles.

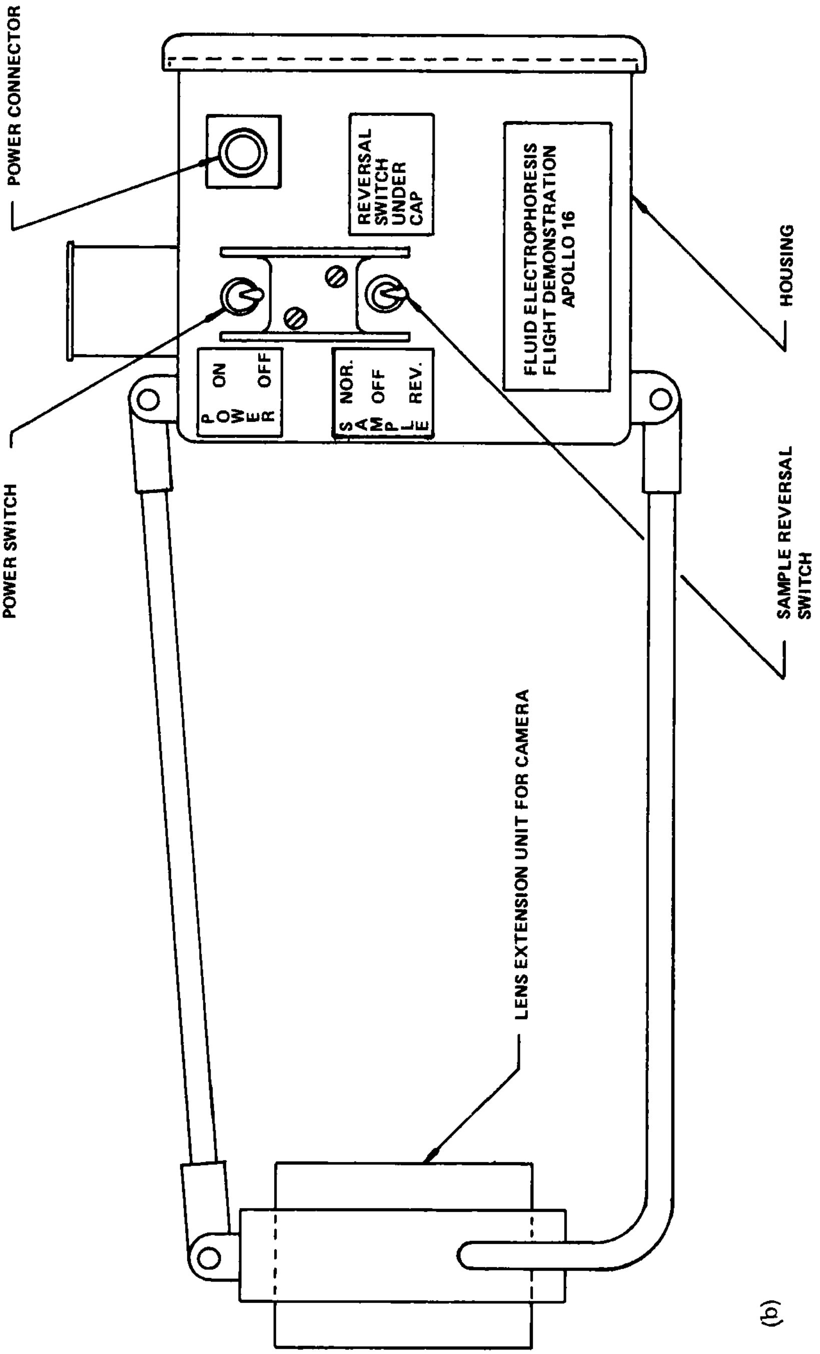
Monodisperse polystyrene latices have been prepared and used for many years.[7] The narrow distribution of particle sizes (diameter variations amount to no more than about ±1%) has led to various applications such as calibration of electron microscopes, ultracentrifuges and microscopic particle counters.[7] The particles selected for Apollo 16 were the 0.8μ diameter polystyrene latex from Lot # LS-1200-B and 0.2μ diameter polystyrene latex from Lot # LS-1047-E from Dow Chemical Company, Midland, Michigan.

DESCRIPTION OF THE APPARATUS

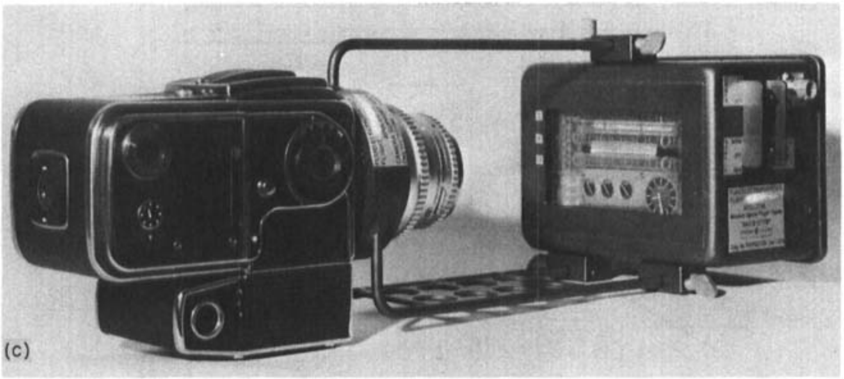
The engineering design, manufacture of a qualification unit, flight and flight back-up unit and ground testing of flight prototype apparatus were done by the Space Sciences Laboratory of the General Electric Company.[8] The supporting ground experiments included verification that the two sizes of polystyrene latex were separable in flight prototype apparatus by the use of a sucrose density gradient. Also assessed on the ground was the stability of the reversible electrodes and the salt bridges as well as the


PROTECTIVE CAP
WINDOW
SWITCH GUARD
PROTECTIVE GAP OVER SAMPLE REVERSAL SWITCH
ACCUTRON CHRONOMETER
SUPPORTS FOR CAMERA MOUNT(3)
FLUID ELECTROPHORESIS DEMONSTRATION
1
2
3
THERMOMETER
AMMETER (3)

(a)


POWER CONNECTOR
POWER SWITCH
REVERSAL SWITCH UNDER CAP
FLUID ELECTROPHORESIS FLIGHT DEMONSTRATION APOLLO 16
HOUSING
POWER ON OFF
SAMPLE NOR. OFF REV.
SAMPLE REVERSAL SWITCH
LENS EXTENSION UNIT FOR CAMERA

(b)



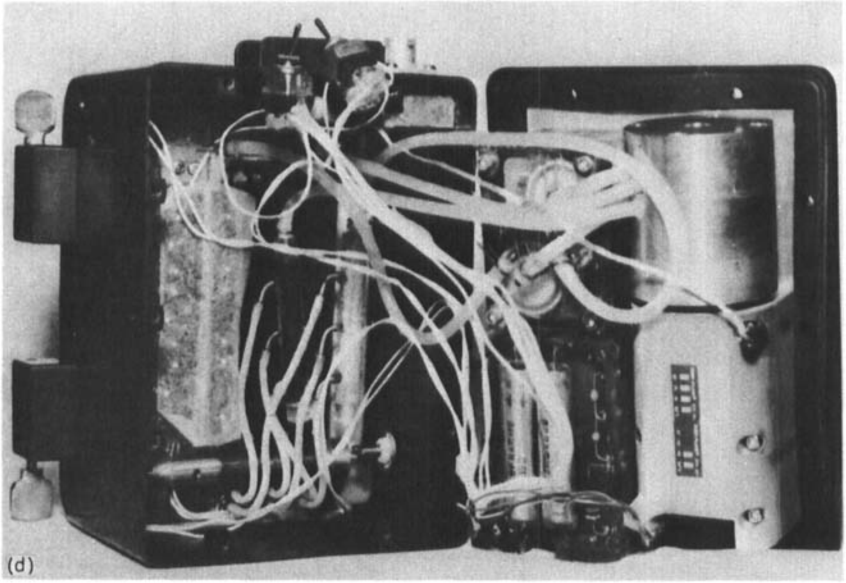


FIGURE 1

(a) Diagram of electrophoresis apparatus - front view. (b) Diagram of electrophoresis apparatus - left side view. (c) Assembled Apollo 16 electrophoresis apparatus. (d) Interior of Apollo 16 electrophoresis apparatus.

the reliability of significant items such as the polyimide film (Kapton, General Electric Co.) sample retainer.

The apparatus developed for Apollo 16 has the same dimensions (10 x 13 x 18 cm) and weight (3.4 kg) as the Apollo 14 unit.[1] Figure 1 shows photographs of the unit. A detachable tripod with two M-21 Hasselblad lens extension tubes were used to take close-up pictures with the correct range and focus settings. Pictures were taken automatically every 20 seconds by means of a Mauer Intervalometer. The window in the electrophoresis apparatus was 6.4 x 11.4 cm, so that the electrodes as well as the three separation columns could be seen. Also added were an Accutron watch (Bulova Watch Company, New York, New York) 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. Markings one centimeter apart were scribed on the tubes to aid subsequent measurements.

The platinum electrodes were continuously flushed by electrolyte which had the same composition as the solvent to maintain a constant pH in the electrode compartments to remove gaseous electrolysis products from the vicinity of the electrodes. Gas bubbles were removed from the electrolyte by passage through a phase separator with concentric hydrophilic (microporous polyethylene, Porex Corporation) and hydrophobic (Gore-Tex TFE Teflon, W. L. Gore, Incorporated) filters. The electrolyte passing through the hydrophilic filter was recirculated, while the gas passing through the hydrophobic filter was removed from the system by hydrogen absorption on palladium black and release of oxygen to the environment.

Three polycarbonate (Lexan, General Electric Co.) tubes, 0.64 cm inside diameter, 0.16 cm wall thickness and 10 cm long were used for electrophoresis. (Shortly before the Apollo 16 flight, the tubes and other parts made of Lexan developed cracks at machining and assembly points releasing electrolyte inside the apparatus. This loss of fluid caused bubbles to form in the tubes as shown in the flight photographs). Dialysis membranes of cellulose acetatenitrate (Union Carbide Corp.) enclosed the ends of each tube to separate the buffer in the cells from the circulating buffer in the electrode regions. Dilute borate buffer ($H_3BO_3/NaOH$) was used throughout the system. The molarity of the buffer was 0.001, the specific conductivity was 0.13 $\frac{\text{mmho}}{\text{cm}}$ and the pH 8.4. Although polystyrene latex is substantially immune to bacterial degradation, 0.1% formaldehyde was added to the solutions as a bactericide. Sodium dodecyl sulfate (0.02%) was added to the buffer to forestall flocculation and periodic turning of the unit every two days helped to prevent sedimentation of the polystyrene particles prior to the flight.

Before electrophoresis in space, the polystyrene latex particles were retained at the membrane closest to the cathode by a polyimide (Kapton) film. The three disk-shaped sample containers had a smaller diameter (0.47 cm) than the inside diameter of the cell so that electrophoresis of the particles would take place down the center of the cells and away from the walls, where the electroosmotic fluid flow tends to reverse the direction of the particles' migration. The cathode membrane and polyimide film were separated 0.15 cm,which defined the initial band length. Before electrophoresis was to begin in space, the polyimide film retained the cylindrical disk of particles and prevented current flowing in the cells. When the experiment took place, the polyimide film was slowly pulled across the sample/buffer interface of each chamber simultaneously. Each group of polystyrene latex particles then migrated electrophoretically to the anode. The concentration of polystyrene latex was 3% for the single species tubes and 6% (3% concentration of each size) for the combination tube.

The mixture of the 0.2 micron and 0.8 micron polystyrene latex was in the upper tube, so that interactions between the particle groups in the upper tube could be detected. The center tube contained the 0.8μ diameter sample and the lower contained the 0.2μ diameter sample. The larger size particles have a higher electrophoretic mobility under the prevailing conditions and hence were expected to migrate faster than the smaller particles.[2-6]

GROUND EXPERIMENTS AT 1 X GRAVITY

For comparison, the electrophoretic mobility of the individual 0.8μ and 0.2μ polystyrene latex particles were measured in a micro-capillary electrophoresis apparatus (Rank Brothers, Ltd., Cambridge, England) in the standard buffer solution used in the Apollo 16 experiment, and modified forms of it, at a potential of 1.7 volts/cm. The capillary tube had a diameter of 2 mm and extremely thin walls, $<$ 0.1 mm, to provide distortionless viewing at all levels. The zero solvent flow level in this tube was accurately defined by measuring the parabolic profile for particles of different electrophoretic mobility. Illumination was provided by a dark field with a quartz-iodine light source, but a 5 mV He:Ne laser (Model ML411, Metrological Instruments, Inc.) was necessary as light source for the 0.2μ particles. Thus, the electrophoretic velocity of a large number of individual particles could be measured directly.

An electrophoresis cell was constructed from polycarbonate (Lexan) tubing so as to eliminate bubbles more easily from the channel and electrode compartments. The main features of this cell were identical to the apparatus used on Apollo 16. The experimental results obtained from this apparatus indicated that the semi-permeable membranes used on Apollo 16 did not affect the potential gradient. However, when bubbles were intentionally introduced into the polycarbonate tubing, the value for E decreased from 26 volts/cm down to values as low as 18 volts/cm. Furthermore, air bubbles that were in physical contact with the semi-permeable membrane had a greater effect in reducing the value for E. It should be noted that when a gravitational field is present, the air bubbles tend to rise and flatten out, thus offering less resistance than might be expected in space.

The electroosmosis at a polycarbonate channel wall/buffer solution interface was measured by placing a cylindrical electrophoresis cell on a microscope stage and observing the particle velocity with a 250 x magnification. To facilitate the observation of the particles near the channel wall, 2.0μ diameter polystyrene particles, with an electrophoretic mobility of 10μ cm $volt^{-1}$ sec^{-1}, were used. The results, which showed that the particles changed direction near the channel wall, indicated that the solvent electroosmotic value at the polycarbonate/buffer solution interface was greater than -10μ cm/volt sec, (i.e., towards the cathode, as opposed to the negatively charged polystyrene particles which move toward the anode).

In order to obtain the best comparison with the flight experiment, an electrophoresis cell similar to the cell used on Apollo 16 was immersed in a constant temperature bath in a number of different orientations and with different applied potentials. When the cell was fixed with the channels either in a horizontal position or a vertical position with the sample chamber located on the top, electrophoresis could not be carried out because release of the polyimide film resulted in a turbulent exodus of the latex particles from the sample chamber, even in the absence of a gradient. The latex particles under these conditions did not behave as individual particles, i.e., they did not settle according to Stokes' law, but the latex dispersion rather acted as a continuous fluid of higher density than the buffer solution. The orientation finally adopted was the vertical one, with the sample chamber located at the bottom. Upon removal of the polyimide film, the sample remained in the sample chamber until the current was turned on.

An attempt was made to duplicate experimental conditions of the Apollo 16 flight by applying 300 volts across the electrodes while circulating buffer solution through the electrode compartments. Although the cell was completely immersed in a constant termperature bath, the latex particles were strongly agitated by what appeared to be thermal convection forces. When the applied potential was subsequently decreased to 10 volts, thermal convection was virtually eliminated. The latex particles migrating from

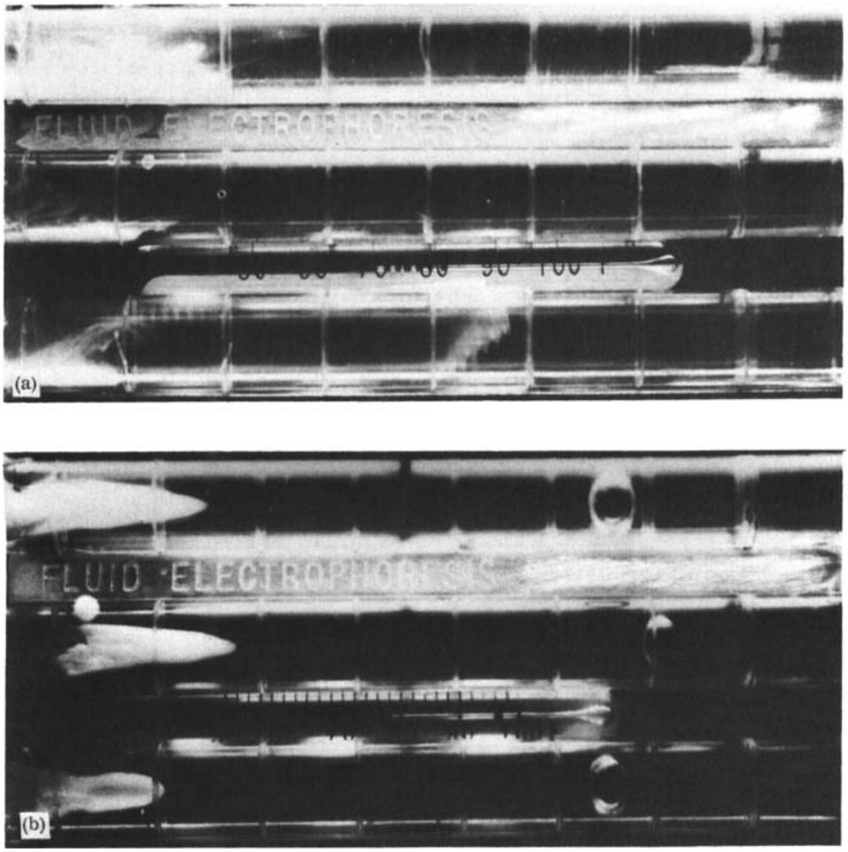


FIGURE 2

(a) Ground test. (b) Apollo 16 electrophoresis after 100 seconds.

the sample chamber in the first half hour of the experiment had a well-defined parabolic shape similar to what was observed within the first minutes of the Apollo 16 flight films. The well-defined shape of the parabola, however, became more and more diffuse with time so that it was difficult accurately to measure the velocity of the apex, and to compare velocities in the different tubes which contained 0.2μ and 0.8μ particles. The photograph in Figure 2 shows the results of this experiment after four hours and clearly demonstrates that the tail of the parabola in all three tubes extends to the sample chamber. The observed velocity of the particles in the center of the channel was estimated to be 0.04 cm/min at 10 volts. This velocity is in reasonably good agreement with the velocities calculated from the Apollo 16 flight films.

The same electrophoresis experiments were done vertically in flight prototype apparatus and buffer with sucrose layered into the cells to establish a density gradient[9], in order to minimize the effect of gravity-induced thermal convection and sedimentation on earth. Repeatable separations of the two particle sizes were obtained with this technique although the sucrose gradient increased the buffer viscosity and slowed the particles' migration. Swirling of the latex occurred in the vicinity of the bands.

THE APOLLO 16 EXPERIMENT

Approximately 25 hours into the flight of Apollo 16, the electrophoresis apparatus was secured to one of the storage lockers and electrophoresis in space begun. Photographs of the latex particle groups were taken every 20 seconds. The transmitted commentary of Command Module Pilot Mattingly[10] helped to explain several phenomena observed later in the photographs and gave continuity to the photographs which were obtained every 20 seconds.

The polystyrene latex was released into the tubes by pulling the strips of polyimide film out of the tubes, thus exposing the sample to the cell buffer and the electric field. Bubbles were evident about midway down each tube; they were motionless before the electrophoresis began. When the electric field was imposed the bubbles migrated with constant velocity toward the anode. Presumably, the bubbles had acquired a charge in the buffer from the sulfate groups at the bubble/liquid interface and moved in the electric field with the same mobility and in the same direction as the particles.

Although the sample was injected as a cylindrical disk, by the time the latex particles were visible in the photographs

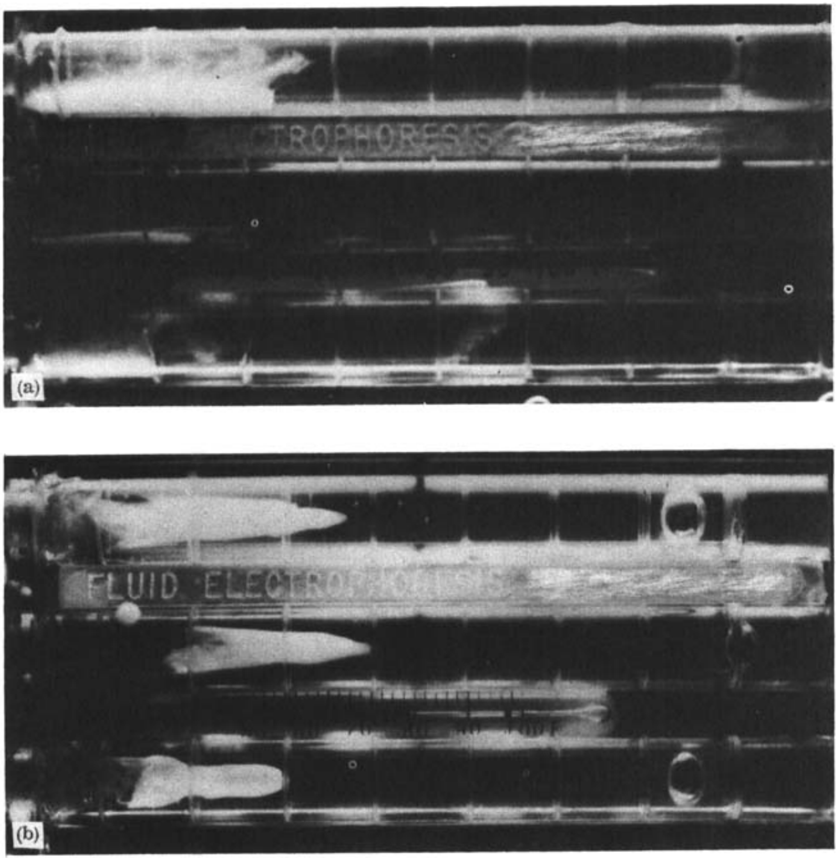


FIGURE 3

(a) Ground test. (b) Apollo 16 electrophoresis after 140 seconds.

(less than 0.5 cm from the sample input) the front of each group of particles was already parabolic. Presumably, the electroosmotic flow pattern of the buffer in the electrophoresis tubes quickly modified the shape of the particle bands into parabolas. The photographs shown in Figures 2-5 compare pictures taken in flight with those taken in a flight prototype apparatus operated horizontally on the ground. Each pair of pictures was taken at the same time after beginning electrophoresis. These photographs clearly show

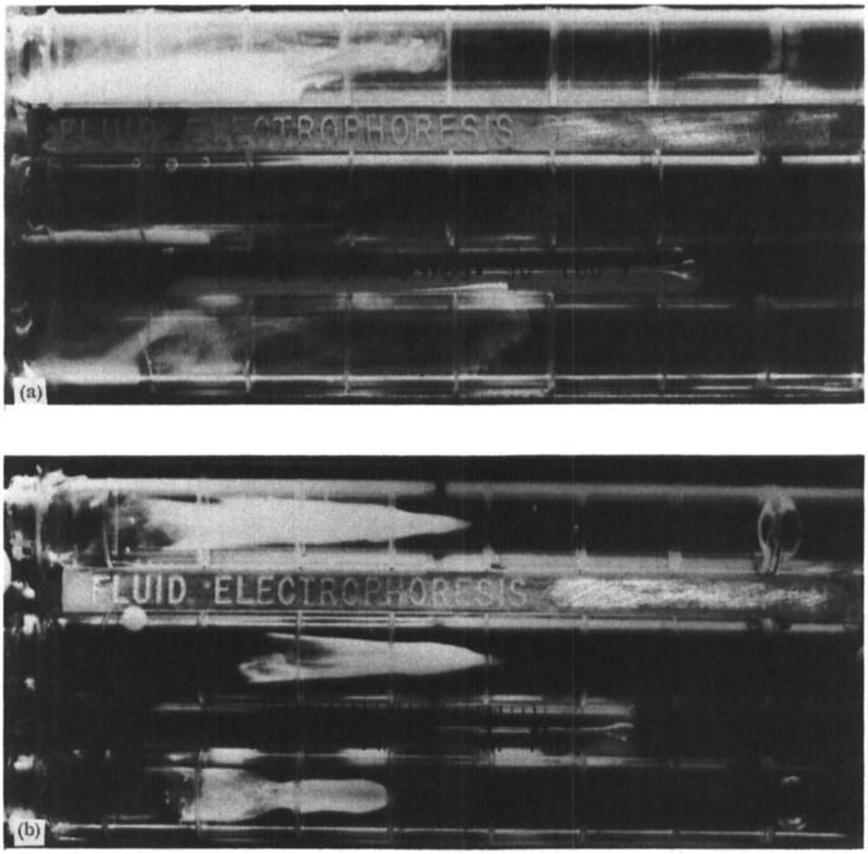


FIGURE 4

(a) Ground test. (b) Apollo 16 electrophoresis after 180 seconds.

the influences of sedimentation and thermal convection on the polystyrene latex.

A separation of the two sizes occurred in the upper tube but more sensitive photographic techniques are required to resolve the separation. Careful exposure of flight original negatives revealed that the nose of the combined band was much less dense and, at a location behind the nose corresponding to the beginning of the 0.2μ band in tube 3, there indeed was a higher particle density that

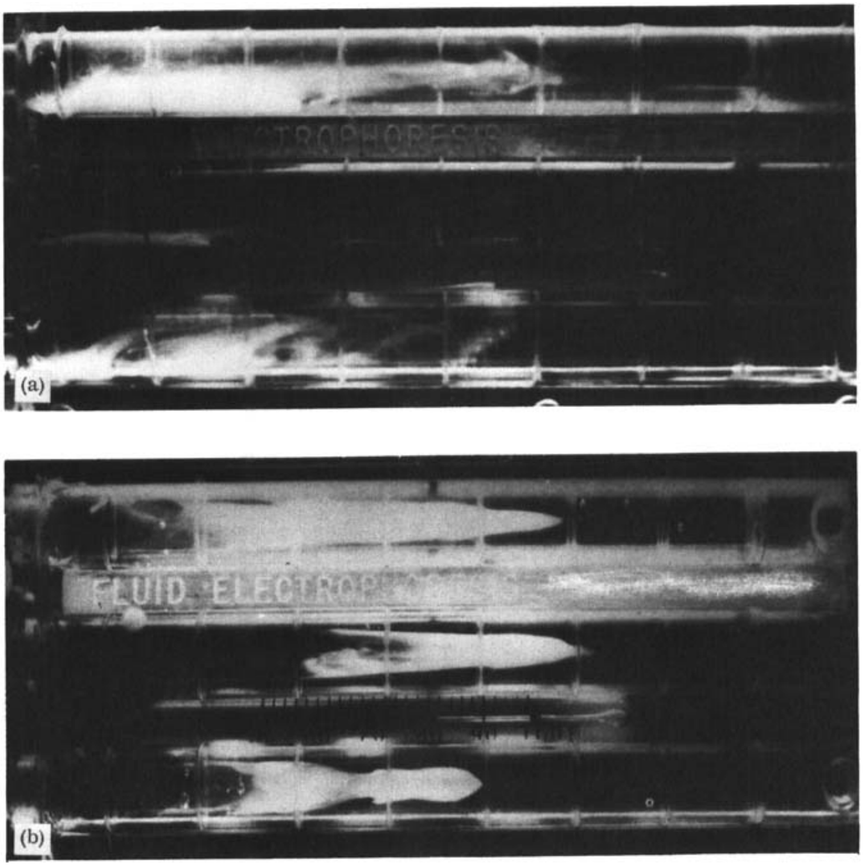


FIGURE 5

(a) Ground test. (b) Apollo 16 electrophoresis after 220 seconds.

could only correspond to the nose of the slower column of 0.2μ particles. Figure 6 shows an enlargement of the thirteenth frame in which these features are visible. Color contour densitometry was used to display these features more distinctly. Figure 7 is a black and white print of a color contour photograph of Figure 6 for comparison. This technique allocates a color to each of four ranges in the exposure density distribution of the film. The lightest band in the upper tube signifies the highest particle

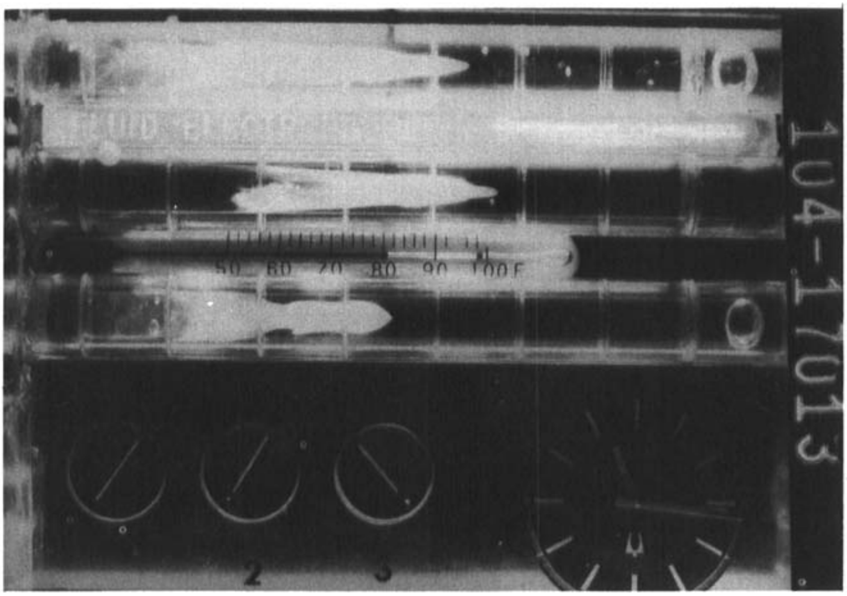


FIGURE 6

Apollo 16 electrophoresis after 200 seconds.

density and corresponds to the superimposed particle bands. The white parabola defines the 0.2μ particle distribution in the upper tube.

The strong influence of electroosmosis was confirmed as soon as the experiment was described in space. An electroosmotic flow pattern in the cell must produce two of the effects observed (1) the particle boundary must be parabolic due to the reverse fluid flow along the walls in a closed tube; and (2) the nose of the boundary must move faster than it would under the single influence of electrophoresis, because of return flow down the center of the tubes. The amount of electroosmosis that occurred during the Apollo 16 experiment can be calculated[9] and compared with expected results. The observed velocity profile of the particles, V_{obs}, was composed of the velocity of the buffer due to electroosmosis, V_w, and the electrophoretic velocity of the particles relative to the

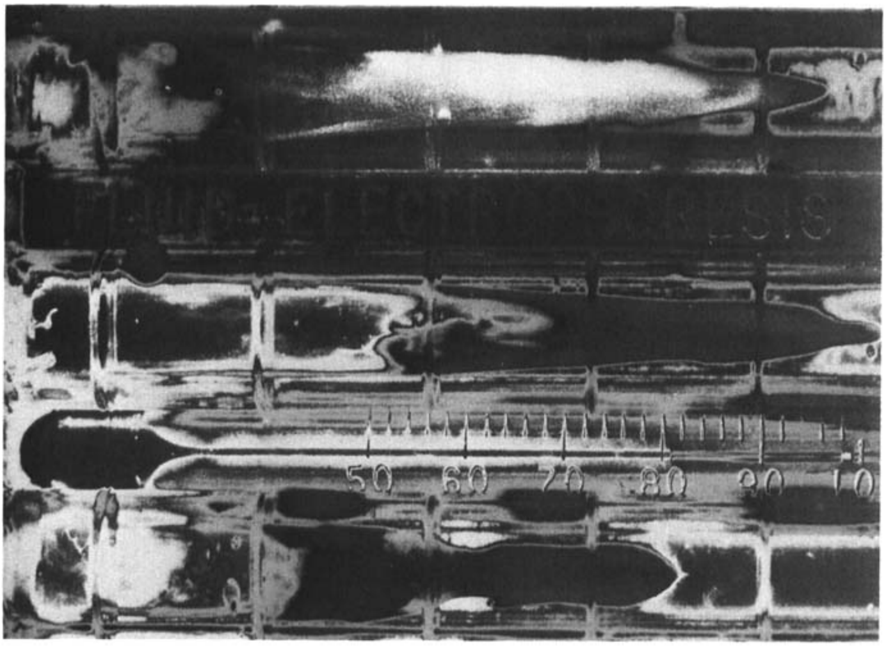


FIGURE 7

Black and white reproduction of color contour photograph of Apollo 16 electrophoresis after 200 seconds (see Fig. 6).

buffer, V_e:

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

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

$$V_w = U \left[\frac{2r^2}{a^2} - 1 \right].$$

Where:

$$V_w = = U, \text{ when } r = 0, \text{ at the axis of the tube.}$$

The particle profiles to be expected based upon these equations have been calculated, using the electrophoretic velocities measured by micro-capillary electrophoresis, and the observed velocity of the particle bands during the Apollo 16 flight experiment measured along the axis of the tubes and are compared with the profiles obtained from the flight pictures in Figure 8. The profiles in each cell are calculated at one minute intervals. The calculated profiles strikingly duplicate the significant features of the Apollo 16 film such as the parabolic shape of the nose of the 0.8μ particles and the outline of the particle tails in all tubes. The results of the calculations are:

1. The actual electrophoretic mobility of the 0.2μ polystyrene latex in the borate buffer was 6.5μ cm/volt sec, in the Apollo 16 experiment.
2. The actual electrophoretic mobility of the 0.8μ polystyrene latex in the borate buffer was 9.2μ cm/volt sec, in the Apollo 16 experiment.
3. The potential gradient in each channel must have been reduced by approximately 40%, probably because of the presence of bubbles.
4. Electroosmosis at the polycarbonate/buffer solution interface was the primary factor that determined the parabolic shape of the latex particles.
5. The separation of the 0.2μ and 0.8μ latex particles in Tube 1 did in fact occur according to theory (see Figure 8 for the comparison between the calculated and the actual profile of the particles for the three tubes).

DISCUSSION AND CONCLUSIONS

It was not possible to design or conduct an experiment on earth that would eliminate 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 drastically slowed the electrophoretic migration. It was only at zero gravity

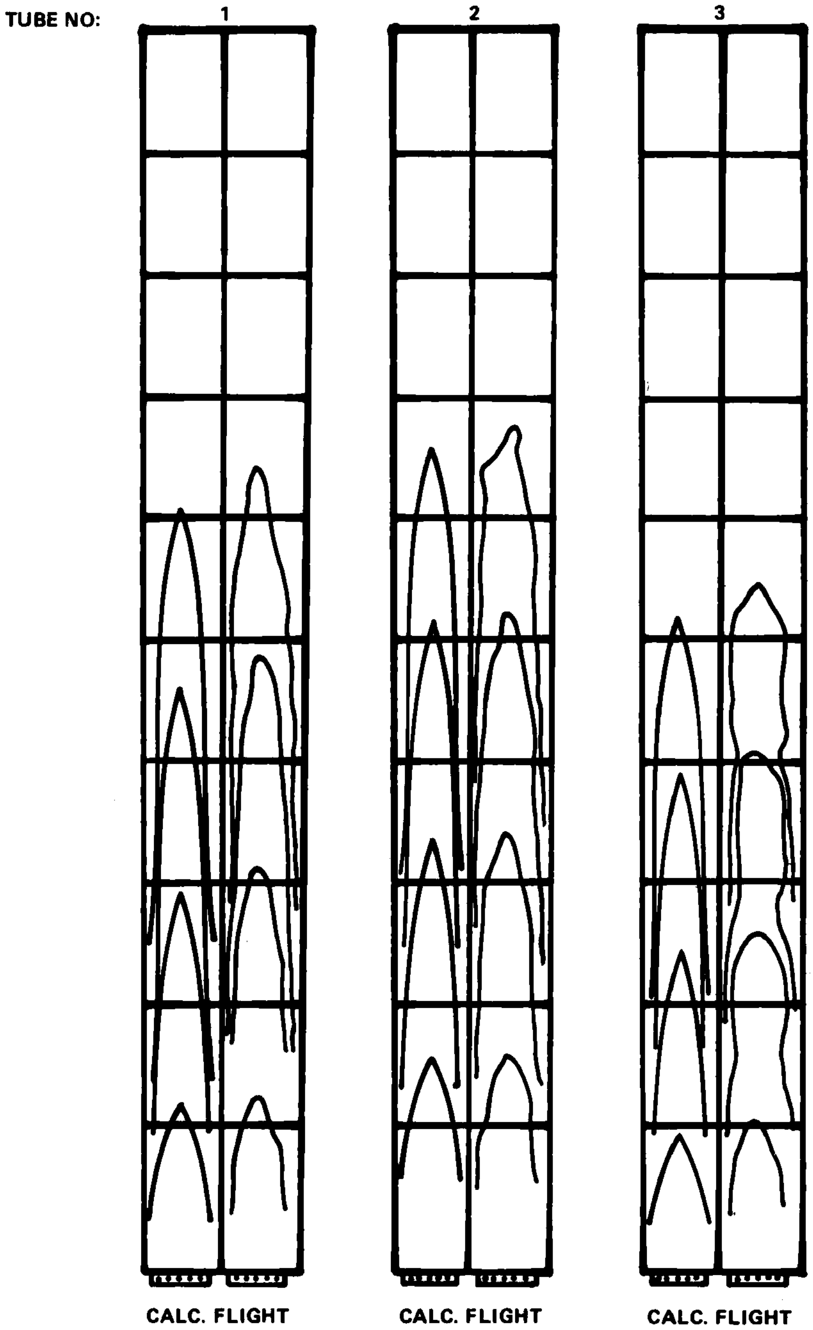


FIGURE 8

Polystyrene latex parabolic profiles at 1, 2, 3 and 4 minute intervals. Left, the calculated profiles and right, the observed flight profiles, for each tube.

that the boundaries of the electrophoresed particles proved to conform closely to the profiles predicted on the basis of the simple combination of the electrophoretic and electroosmotic movements, (Figure 8). The separation and the conserved identity of the two groups during electrophoresis in space also followed the theoretically predicted patterns, as the flight photographs clearly show, (Figures 2 to 7) .

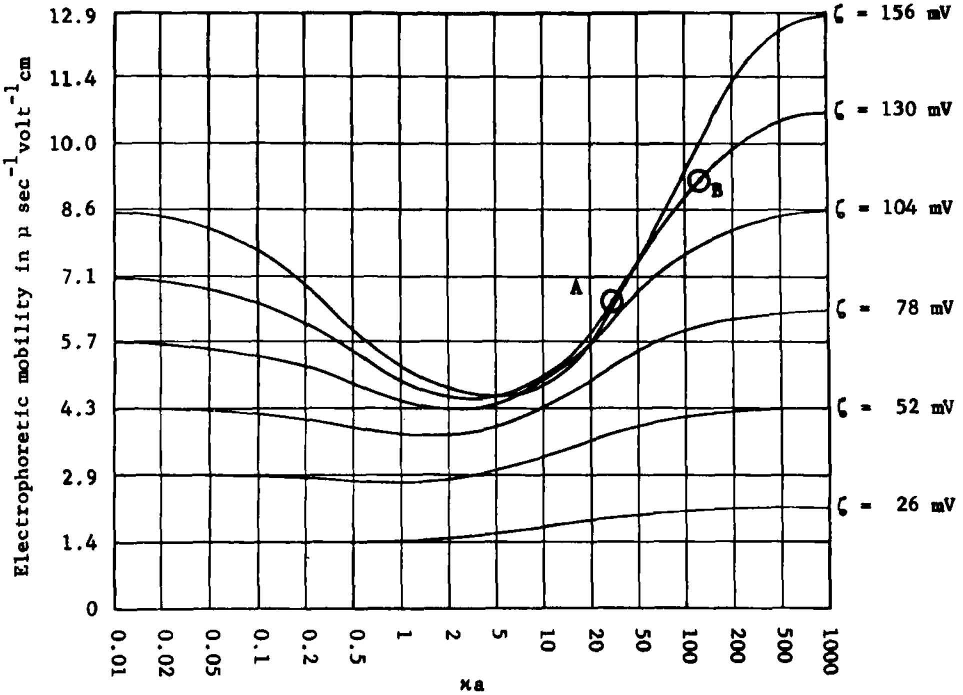


FIGURE 9

Graph of ζ potential (in mV) versus electrophoresis mobility (in μ sec^{-1} volt^{-1} cm), after Wiersema[11], with the electrophoretic mobilities of the 0.2μ and the 0.8μ particles (resp. 6.5 and 9.2 μ sec^{-1} volt^{-1} cm) from the Apollo 16 electrophoresis experiment indicated on it. With an estimated thickness $1/\kappa$ for the ionic double layer of 3mμ, the ratios of the diameter of the particles to the double layer are $\kappa a \approx 33$ and $\kappa a \approx 133$. When these values are superimposed on the graph (see points A for the 0.2μ particle, with $\kappa a \approx 33$, and B for the 0.8μ particle with $\kappa a \approx 133$), a ζ potential of ≈ 130 mV is found for both particles. (A slight correction necessitated by the presence of some trivalent co-ions[5,6,11] has not been made).

As soon as the electrophoresis of the particles and the electroosmosis of the buffer was initiated, the migration of the particle boundaries proved indeed predictable. The separation and the conserved identity of the two groups of particles during electrophoresis in space was expected and the flight photographs clearly confirm it.

The electrophoretic mobilities attained by the 0.2μ and the 0.8μ diameter particles were respectively 6.2 and 9.2 $\mu\ \mathrm{sec}^{-1}$ volt^{-1} cm. If one may estimate the diffuse ionic double layer $1/\kappa$ to be approximately 3mμ thick, a ζ potential of about 130 mV is found for both particles, when both the Henry retardation[3] and the Overbeek relaxation[4] are taken into account[5,6,11] (see Figure 9). This high surface potential may be for an important part attributable to adsorbed dodecyl sulfate ions.

Electrophoresis of model particles in a free liquid in a weightless environment was done on Apollo 16. The flight pictures clearly show the separation and conserved identity of the boundaries and sharpness of the particle fronts during electrophoresis in space. The deleterious effects of gravity-induced sedimentation and thermal convection on particle electrophoresis can be seen by comparing the results of the Apollo 16 with those of the ground experiments.

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