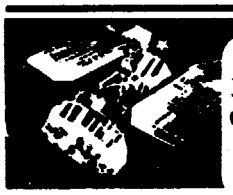
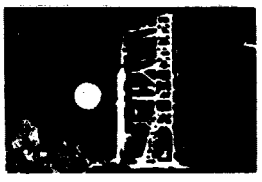
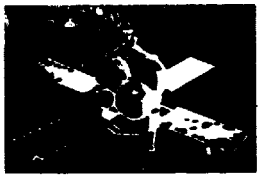
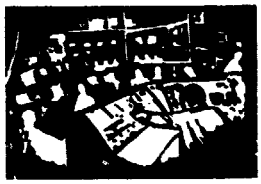
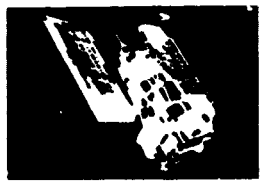
SPACE DIVISION

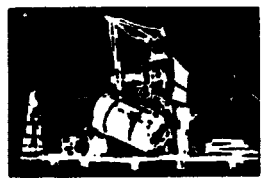


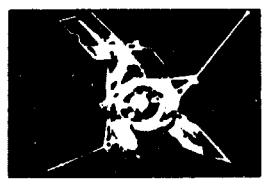












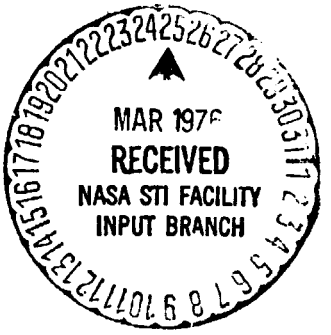
FLUID FLOW ELECTROPHORESIS IN SPACE

FINAL REPORT

CONTRACT NAS 8-29878

Prepared for the

National Aeronautics and Space Administration
Marshall Space Flight Center
Huntsville, Alabama 35812



by

General Electric Company
Space Sciences Laboratory
P. O. Box 8555
Philadelphia, Pennsylvania 19101

GENERAL ELECTRIC

This report was prepared by the Space Sciences Laboratory of the General Electric Company under Contract NAS 8-20878, "Fluid Flow Electrophoresis in Space" for the George C. Marshall Space Flight Center of the National Aeronautics and Space Administration. The author was Dr. Richard N. Griffin.

For NASA the work was administered under the technical direction of the Materials Laboratory by Mr. Percy H. Rhodes.

ABSTRACT

Four areas relating to free-flow electrophoresis in space were investigated. The first was the degree of improvement over earthbound operations that might be expected. It was shown that accurate estimates of the improvement in resolution to be expected in space are best made by analyzing a set of input data by means of a computer program developed under another contract. Limitations on sample throughput were described.

The second area of investigation covered the problems in developing a flowing buffer electrophoresis apparatus. It was shown that no adverse effects should be expected when using a free-flow apparatus in an intermittent or static mode. Application of an external electric field produced changes in the streaming current in capillary tubes (hence, presumably, the zeta potential of the capillary wall), giving some hope that wall zeta potentials might be changeable at will. Problems were identified in changing samples or buffers in sequential experiments. Other studies included cooling requirements in space, fluid sealing techniques, and measurement of voltage drop across membranes.

The third area of investigation was the problem of testing on the ground equipment designed for use in space. Functional tests of most components are possible, but proper operation of the entire system including electrophoretic separation of test specimens is unlikely.

The fourth area of investigation was the improvement to be expected in space for purification of biologicals. The results of some ground-based experiments are described.

TABLE OF CONTENTS

LIST OF FIGURES

I. INTRODUCTION

The objective of this work is the definition and investigation of the advantages as well as the scientific and engineering problems of developing a flowing buffer electrophoresis apparatus and doing electrophoresis in the device in space. The topics considered include:

1) the improvement in resolution and/or throughput expected in space,
2) the problems expected to be encountered in design and construction of a zero-gravity free-flow electrophoresis device,
3) the problems inherent in testing flight-type equipment on the ground,
and 4) the improvement that can be expected from purifying some biologicals in space.

The work therefore constitutes a survey of what must be done to develop zero-gravity free-flow electrophoresis devices, how much better such devices may operate, and what this may mean in terms of purer biologicals.

While there is little doubt that free electrophoresis is hampered by sedimentation and thermal convection, and there is little doubt that free

electrophoresis in zero-g may be a uniquely powerful tool for separating some biological materials, nevertheless there is little agreement about the best method for conducting electrophoresis in space. In some systems the medium remains motionless, in some it moves intermittently, while in some others it moves continuously. Each system has its proponents, and each system has its advantages and problems.

A truly static system for use in zero-gravity, in which the sample and suspending medium were frozen in place, thawed, electrophoresed, and refrozen, was demonstrated by the NASA-Marshall Space Flight Center experiment on the ASTP flight. Almost any other system must involve flow of some sort in order to permit introduction of sample and removal of separated sample fractions. It matters little whether the removal of sample fractions is accomplished by pumping the fluid out of the electrophoresis cell, or whether a change in the size or shape of the electrophoresis cell is used to permit collection of separated fractions.

All such devices can be characterized as "intermittent flow" devices. A flowing curtain electrophoresis device can be used in either an intermittent or continuous flow mode. Obviously, its normal application is in continuous flow free electrophoresis. However, if desirable, the flow of electrolyte through the cell can be interrupted during electrophoresis. This equipment then becomes, in effect, a static electrophoresis device

with introduction and removal of sample by means of a pump. In addition to ease of operation, one of the advantages of such a system is that the sample can be introduced in a very narrow band. It is self-evident that in electrophoresis the ultimate resolution cannot be better than the sample band width. With a flowing curtain device a sample band width of about 75 microns is easily achieved.

II. IMPROVEMENT IN RESOLUTION AND/OR THROUGHPUT EXPECTED IN SPACE

The resolution obtainable in free-flow electrophoresis is generally limited by band distortion due to electroosmotic and hydrodynamic effects (Reference 1). With recent improvements in equipment it is now possible to obtain one fraction band free of distortion. All other bands must, however, be more or less distorted. Detailed analysis of the amount of distortion requires consideration of a number of interrelated factors, and the calculations involve simultaneous second-order differential equations. A computer solution to these equations was developed under another contract (Reference 5). However, a crude approximation is that the distortion of an unfocused band is inversely proportional to the square of the cell thickness at any given sample stream thickness. The implicit assumption is that the sample stream diameter is less than the cell thickness, the sample stream is centered in the cell, and both walls have identical zeta potentials.

The thicker electrophoresis cells which should be possible in zero-g should lead to improved resolution. They could also be used to increase the throughput of the device. The ratio between sample stream thickness and cell thickness can be varied to produce the ultimate resolution or the highest throughput without increase in resolution, or some compromise between the extremes.

In free-flow electrophoresis on the ground, sample concentrations are limited by at least two factors. The first is density. If the density of the sample stream is sufficiently larger than the density of the surrounding medium, a "string of pearls" results. In our experience, this is the most common evidence of excessive sample flow rate or concentration. This effect should disappear in the absence of gravity.

There is, however, another limit to sample concentration which is the conductivity of the sample relative to that of the surrounding medium. The following is quoted from Reference 2.

> "Electrical conductivity of the sample, especially in relation to curtain conductivity, plays an important role. This is partly due to local distortion of the electric field at the sample stream position. The conductivity of the sample has two components. One of these is conductivity due to the ionic content; the other is due to "surface conduction" of the particles. Surface conduction arises from the mobility of ions in the double electric layer which surrounds the charged particles. It increases with the particle zeta potential and the total particle surface area in a unit of sample volume. It is therefore greater for small particles and increases in proportion to particle

concentration. In suspensions with about 0.1% "solids" in typical buffers, surface conduction may account for 10% or more of the observed conductivity.

In general, for moderate sample flow rates, the conductivity of the sample suspension medium may be twice or more that of the curtain, or as low as one-third or less, before one sees greater spreading than observed when curtain and suspension medium are equal in molarity."

Thus, even in the absence of gravity, sample concentration cannot be increased without limit. However, it is likely that concentrations can be increased by about an order of magnitude over those used on earth.

III. PROBLEMS IN DEVELOPING A FLOWING BUFFER ELECTROPHORESIS APPARATUS

A. Fluid Dynamics of Intermittent Flow

In order that a flowing curtain device be considered as practical for use as a static electrophoresis device, it was necessary to investigate the fluid dynamics of starting and stopping the buffer and sample flows. We elected to take an empirical approach to the problem since we felt that a theoretical approach to the hydrodynamics of intermittent flow could be very time consuming and would probably leave many questions unanswered. Therefore, we set up a mock-up of a flow electrophoresis cell with the buffer flow controlled by a tubing pump. This was chosen deliberately as a sort of worst case since this type of pump produces very large pulsations in the flow. A dye simulating the sample was injected by hand from a hypodermic syringe. Close-up photographs taken of the dye stream during flow, upon stopping, and on re-starting are shown in Figures 1, 2 and 3. Figure 1 shows the sample stream as it was injected. Immediately after the sample was photographed, the movement of the buffer was abruptly stopped. It remained motionless for 30 seconds before the second photograph (Figure 2) was taken. No disturbance of the sample was evident. After another 30 second interval the buffer was abruptly set in motion again -- as if to

remove separated fractions from the apparatus. Figure 3 shows the sample stream being pumped out of the apparatus with no disturbance beyond the expected diffusion. This appears to support the thesis that in a non-compressible (i.e., closed, liquid-filled) fluid system transient disturbances, as from stopping and starting the flow, can be made negligible. It would therefore seem that the same basic design might be applied to both static and flowing electrophoresis devices.

B. Control of Electroosmosis

Several years ago it was suggested (Reference 3) that it might be possible to control electroosmosis by application of an external electric field to the electrophoresis cell. Electroosmosis has always been a problem in electrophoresis, both in microscope electrophoresis where electroosmosis causes different rates of migration to occur at different depths in the electrophoresis cell, and in paper and cellulose acetate electrophoresis where electroosmosis can make low mobility fractions move in the direction opposite to that which is expected. In zone or microelectrophoresis the elimination of electroosmosis by a zero-zeta potential on the electrophoresis cell wall is desirable. It has, however, been shown that in free-flow electrophoresis a certain amount of electroosmosis is required to give the optimum results. (Reference 1). While the ideal situation in free-flow electrophoresis occurs when the zeta potential of the cell wall and of the sample are the same,

practical considerations make such a zeta potential match unlikely. A means of producing an effective zeta potential match has been devised. This involves the use of two separate sets of electrodes with the cell area between each pair coated with a material whose zeta potential is either higher or lower than that of the desired sample fraction. The details of this procedure are included in Reference 1. A wider latitude of adjustment, however, hight be obtainable if the cell wall zeta potential could be controlled by application of an external electric field.

Thin walled plastic and glass capillary tubes were used in an Alternating Current, Streaming Current Analysis (ACSCA) apparatus in experiments intended to determine the effect of external applied voltage. The ACSCA technique is based on the same electrokinetic phenomena as the conventional streaming potential measurement but uses forced sinusoidally oscillated electrolyte flow and measures a current proportional to surface charge density on the capillary wall. Significant advantages of 1) increased sensitivity, 2) freedom from electrolyte conductive effects permitting measurement at physiological electrolyte concentrations, 3) insensitivity to fluid viscosity variations, 4) decreased temperature dependence and 5) better control of hydraulic and electrical parameters for improved reproducibility are thus achieved.

In a normal streaming potential system care is exercised to isolate electrically the input and output ends, and thus to measure the potential difference by electrodes connected to a high impedance potentiometric circuit. However, there is always a back leakage path through the capillary and at equilibrium the number of ions being separated by the streaming flow is equal to the number leaking back through the internal capillary resistance. Obviously if the electrolyte resistance is decreased, as by increasing the salt concentration, the ionic back-leakage increases and the measured streaming potential decreases. Because of this effect streaming potential systems become quite insensitive at electrolyte ionic strengths approaching physiological levels.

In ACSCA, this problem has been greatly reduced by collecting the charge, as fast as it is separated, on large area reversible electrodes connected externally through a very low impedance current measuring circuit. The separated ionic charge is quickly neutralized by electronic charge flowing through the external measuring circuit and the capillary virtually operates at zero potential difference at all times. Thus, back leakage is eliminated and good sensitivity is obtained even in the physiological range of ionic strength.

An oscillating electrolyte flow is forced through a capillary by a reciprocating positive displacement pump driving the electrolyte in a push-pull mode. The pump displacement rate is made sinusoidal by means of a Scotch yoke and electric motor drive, thus producing a sinusoidal capillary electrolyte velocity and a resultant charge displacement, the magnitude of which also varies sinusoidally with time. The displaced ionic charge is converted essentially instantaneously into a sine wave alternating current in the low impedance external circuit by the large area reversible electrodes.

The oscillating concept makes use of a positive displacement pump practical so that electrolyte is forced to flow at a predetermined rate regardless of fluid viscosity. The coefficient of viscosity is thus eliminated from the governing relationships with the practical result that the system becomes very much less temperature dependent, and the hydraulic flow control problems of continuous flow streaming potential systems are essentially eliminated.

No effect on wall zeta potential was observed with acrylic or glass capillaries and half-strength Beckman B-2 buffer at external field strengths up to $\pm$ 3000 V/cm. At 3333 V/cm negative applied potential, a slight increase in streaming current was noted; and at around 10^4 V/cm the streaming current was twice that observed in the absence of the field.

With deionized water in the streaming current apparatus the application of an external field of -6.7 KV/cm produced a ten-fold increase in the streaming current. Surprisingly, the same result was obtained when the polarity of the external field was reversed.

Finally, with Beckman B-2 buffer diluted 10:1 we observed a steady increase of streaming current with increasing negative applied potential and a minimum value of streaming current with positive applied potential. The data are summarized below where the voltage gradient is the gradient across the cell wall due to an external applied field, the polarity is that of the outside of the cell relative to the inside and the ACSCA reading is an arbitrary scale proportional to the effective cell wall zeta potential.

Gradient KV/cm	ACSCA
0	0.80
-1.7	0.85
-3.3	1.00
-5.0	4.00
-6.7	3.80
+1.7	0.50
+3.3	0.48
+5.0	0.42
+6.7	1.45

C. Precautions in Changing Buffers and Biological Samples

The usefulness of an electrophoretic separator in space will be greatly enhanced if it can be used to separate a variety of different materials under a variety of different conditions, much like an ordinary piece of laboratory apparatus. The one possible problem is cross contamination between one sample and the next. Human serum albumin was used as a model contaminant which might be found on the inside surface of an electrophoretic device. The human serum albumin was applied by soaking capillary tubes in a 0.1% solution for one hour. The degree of residual contamination after various cleaning procedures was assessed by comparing the zeta potential of the capillary wall with the zeta potential of the same wall after thorough cleaning.

The following sequence demonstrated (as expected) that simply rinsing with buffer or deionized water would not suffice. The buffer used was Beckman B-2 diluted 10:1.

Treatment	$-\zeta$, mv
1) Clean capillary	109.87
2) Soak 1 hour, .1% albumin. Rinse	47.1
3) Soak 10 minutes, rinse with 20 ml buffer	47.1
4) Repeat (3)	47.1
5) Repeat (3)	50.2
6) Repeat (3)	50.2
7) Repeat (3)	50.2
8) Soak 18 hours in buffer. Rinse 20 ml buffer	53.4
9) Rinse with 500 ml deionized water	54.9
10) Rinse with 2000 ml deionized water	59.6

Because a truly clean glass surface is probably an unrealistic simulation of a piece of apparatus, we decided to start with a capillary which had been cleaned with a commercial laboratory glass cleaner, Micro, (TM International Products Corp.) in a manner that would be possible with a piece of apparatus. The following sequence of tests was then performed:

Treatment	$-\zeta$, mv
1) Clean with Micro	97.3
2) Soak in .1% albumin, rinse	50.2
3) Soak 10 minutes in buffer, rinse with 30 ml buffer	58.1
4) Repeat (3)	62.8
5) Repeat (3) except soak 80 minutes	62.8

On the chance that deionized water might be more (or less) effective than buffer in removing the albumin we ran a similar series of tests with water soaks and rinses.

Treatment	$-\zeta$, mv
1) Clean with Micro	100.4
2) Soak in .1% albumin, rinse	50.2
3) Soak 20 minutes in deionized water, rinse with 10 ml deionized water	59.6
4) Soak 10 minutes in deionized water, rinse with 10 ml deionized water	64.4
5) Soak 10 minutes in deionized water, rinse with 40 ml deionized water	65.9
6) Rinse with 500 ml deionized water	65.9

Since it was obvious that none of these procedures was capable of removing the albumin we tried soaking in a 0.1% solution of Micro for 10 minutes followed by 10 minutes soaking in deionized water followed by rinsing in 20 ml of deionized water. The results were quite striking as indicated below:

Treatment	$-\zeta$, mv
1) Cleaned with Micro	100.4
2) Soaked in 0.1% albumin	53.4
3) Soaked 10 minutes in Micro, 10 minutes in deionized water, rinsed with 20 ml deionized water	100.4

However, since it is not unusual for parts of electrophoresis apparatus to be made of plastic, we ran some similar experiments in acrylic capillaries. Although the results clearly showed that human albumin is removed from acrylic much more easily than from glass, the measurements made with acrylic capillaries lacked the degree of repeatability found in the experiments with glass.

Treatment	$-\zeta$, mv
1) Clean with Micro	81.6
2) Soak in 0.1% albumin	62.8
3) Soak 20 minutes in deionized water, rinse with 10 ml deionized water	65.9
4) Repeat (3)	78.5
5) Repeat (3). Rinse with 40 ml	81.6

Treatment	- ζ, mv
1) Clean with Micro	91.0
2) Soak in 0.1% albumin	59.6
3) Soak 10 minutes in .1% Micro, 10 minutes in deionized water, rinse with 20 ml deionized water	100.4

Treatment	- ζ, mv
1) Clean with Micro	141.3
2) Soak in 0.1% albumin	100.4
3) Soak 10 minutes in buffer, rinse with 30 ml buffer	103.6
4) Repeat (3)	119.3
5) Repeat (3)	133.4

All the zeta-potential values reported herein are based on a value of -109.87 mv for clean glass (Reference 4). For our purposes there seemed to be no reason to obtain an absolute calibration of streaming current output vs. zeta potential.

With the exception of the sample inlet and fraction collection tubes, no contact should occur between the sample and the inside of a continuous flow electrophoresis device designed for use in space. Contact does occur in some, but not all, continuous flow electrophoresis devices designed for use on earth.

The problem of changing from one buffer to another may be more severe than that of changing from one sample to another. In the course of shake-down experiments on our Beckman Mark II CPE free flow

electrophoresis machine, we ran some polystyrene latex in barbital buffer. For a subsequent experiment we switched to a tris buffer, and then back to the original barbital buffer. The equipment was thoroughly flushed with deionized water between each of the buffer changes. However, the results obtained with the barbital buffer subsequent to the use of the tris buffer were quite different than those which had been obtained before use of the tris buffer. We were forced to conclude that the tris buffer adsorbed in the cell wall coatings and/or the electrode membranes. The original results were duplicated only after we dismantled the entire cell and replaced both the cell wall coatings and the electrode membranes. It is probable that such results would not be obtained with other buffers or other buffer combinations, but this incident emphasizes that due care must be taken when proposing a series of experiments involving different buffers.

D. Cooling Requirements in Space

The requirements for cooling electrophoresis devices in space are no different than the requirements on the ground. In essence, the cooling system must be capable of removing whatever energy is put into the system. In space a free electrophoresis device can tolerate a much larger temperature variation through the thickness of the cell than can equipment operated on the ground. This, of course, is the basis for being able to use a thick cell in zero-g. If the cooling system is capable of maintaining the inside cell wall at or just above the freezing point of the buffer, then the temperature gradient through the cell will be a unique function of the cell thickness and the power input. The cooling requirements therefore are a function of individual equipment design and experiment parameters such as voltage and buffer composition. At first glance it appears that the temperature gradient through the cell is relatively unimportant in zero gravity so long as the temperature at the center of the cell does not exceed a value compatible with the sample. This, however, is not quite true. Due to changes in viscosity of the medium with temperature and changes in conductivity of the medium with temperature, there are deleterious effects due to temperature variations through the thickness of the cell. The degree of effect depends on a large number of interrelated variables,

and is not susceptible to simple analysis. A mathematical model programmed for computer solution was developed under another contract. The program solves simultaneous second order differential equations to calculate the effect of temperature variation through the cell among other things. The program, however, requires specific data inputs. There is no single cooling requirement in space which is susceptible of a generalized solution.

E. Fluid Sealing

The same techniques that are used on earth to seal fluid-filled systems should be adequate for most applications in space. With proper care sealing arrangements such as O-rings are adequate to prevent the formation of bubbles due to leakage. Bubbles are, however, a common problem in free electrophoresis. In terrestrial applications the bubbles normally occur as a result of uneven wetting and entrapment of air, or as a result of degassing of the fluid. In space, however, we believe that bubbles in free electrophoresis normally will occur as a result of evaporation of fluid through the wall of the container. This is a reflection not of some difference due to zero gravity or vacuum, but rather due to the materials of construction which must be used to satisfy safety requirements as well as the normally long times that the fluid must be contained prior to operation of the experiment. Bubbles can best be eliminated by choosing materials of

construction which have low water absorption and a low rate of water vapor transmission, as well as by minimizing the length of time between filling of the apparatus and operation of the experiment. Free-flow electrophoresis has a potential advantage over static electrophoresis in elimination of problem bubbles because it is at least possible that bubbles can be driven from the apparatus by the flowing buffer. In practice on earth this is relatively rare since bubbles normally adhere sufficiently strongly to the cell walls that they are not readily driven out by the flow of buffer.

F. Voltage Drop Across Membranes

At the request of the NASA Program Manager we undertook an investigation of the effective electrical conductivity of semipermeable membranes in aqueous solutions. This has been a subject of some curiosity since the time of Apollo 14 and 16 when very non-reproducible results were sometimes obtained. Accordingly, a glass cell with platinum electrodes was set up. The inside diameter of the cell was 12 mm, distance between electrodes 140 mm. The cell was filled with phosphate buffer, pH 7.7, $\gamma/_2$ = .15, containing 3.99% glucose, .062% sodium chloride, and 4% glycerol. The conductivity of the solution was determined at a series of ten voltages between 20 and 240 volts. A slight deviation from linearity of the current/voltage curve was observed. This was undoubtedly due to temperature

changes in the unthermostatted cell. A single thickness of Fisher dialysis membrane #8-667, average pore size 4-8 millimicrons, thickness .025 mm, was interposed at the midpoint between the electrodes. The same sequence of measurements was repeated. No deviation greater than 2.5% was observed between values obtained with and without the membrane.

Similar experiments were carried out in an apparatus containing two membranes (a better simulation of the Apollo electrophoresis demonstrations). Again, no significant resistance was found with a .05 mm thick dialysis membrane, a .25 mm Nafion membrane, or even with two double thickness #8-667 Fisher dialysis membranes.

At a voltage gradient of 7 volts/cm the conductivity of such a cell with two membranes in place increased slightly with time up to at least 45 minutes. This we attribute to temperature rise. In order to increase the voltage gradient without excessive temperature rise we immersed the cell in an ice bath. Under these conditions we were able to operate the cell for an hour at 15 volts/cm with no evidence of increased membrane resistance. One significant difference between these experiments and the experiments at NASA-Marshall Space Flight Center

where high resistance was measured is the current density through the membrane. NASA reported that at 15 volts/cm current of 20 ma was measured in a .635 cm dia. tube, or a current density of 66 ma/cm^2 if the membrane was the same size. The highest current density achieved in our experiments was 61.2 ma/cm^2; and 24.7 ma/cm^2 was the highest achieved in the one hour tests.

We believe that at a certain current density the rate of ionic migration exceeds the diffusion rate of the ions through the membrane. The resulting polarization of the membrane, akin to Donnan equilibrium, will have a potential

$$E = \frac{RT}{F} \ln \frac{a_1}{a_2}$$

where a_1 and a_2 are the activities of the principal current-carrying ions on the two sides of the membrane. If this is correct, there is some current density at which every membrane will become significantly resistive. The value of this current density will depend both on the nature of the membrane and on the ions in the conductive solution. Under any given set of conditions, the problem can be mitigated by increasing the membrane area, effectively decreasing the current density in the membrane without changing it anywhere else in the system.

IV. INVESTIGATION OF THE PROBLEMS OF DEVELOPING AND TESTING FLIGHT PROTOTYPE EQUIPMENT ON THE GROUND

Many components of a zero-g fluid electrophoresis system can be adequately tested on the ground. The operation of most electrical circuits should be unaffected by zero gravity so long as they do not depend on convective cooling. Pumped fluid systems which operate properly with, against, or normal to the gravity vector can be expected to operate properly in the absence of gravity. For example, a forced flow closed cooling loop powered by a vane pump which operates in any orientation on the ground should operate properly in space, as long as the coolant flow is turbulent and not laminar. Peristaltic or tubing pumps operate in any orientation relative to gravity on the ground, and can be expected to operate properly in zero-gravity. Some of the difficulties of conducting ground tests on equipment for zero gravity use are exemplified in our experience in testing a pneumatic system for controlling buffer flow. This system was investigated because it is potentially completely pulseless and free of vibration.

While peristaltic pumps have the disadvantage of inducing pulsations in a flow, they have the advantage that the flow rate is extremely reproducible and predictable. In an attempt to determine whether fluid flow induced by

pressure could be made equally reproducible, we conducted experiments with a system consisting of a 1 liter bladder suspended inside a 4 liter flask. Pressure in the flask was regulated by a 2 stage regulator and was monitored with a Bourdon-type gauge. The flow of fluid was controlled by a micrometer valve and was measured both by collection of fluid in a graduated cylinder and by means of a flow meter. A number of experiments were run at various pressures and throttle valve settings. Results were generally similar with a tendency for the fluid flow-rate to diminish during the course of the experiment. Subsequent experiments showed that the tendency for the flow-rate to decrease could be eliminated if the bladder and pressure flask were operated in a horizontal position.

Having determined that a fairly constant flow could be achieved from a pressurized bladder system, we then attempted to determine the reproducibility of the flow from one run to another. The bladder was half filled with water, the system operated for 20 minutes, then shut down by closing the gas supply valve. The system was started again by opening the gas valve. No additional fluid was added to the bladder between runs. The fluid control needle valve setting was undisturbed. However, the flow-rate varied from one run to another as much as about 6%. Since this discrepancy might be explained by a small bubble or particle of dirt lodging in the small orifice of the fluid control valve, another set of

experiments was performed in which the flow-rate was controlled by replacing the fluid control valve with a short section of small bore tubing. Little advantage over the micrometer valve was gained by the use of the capillary tube for control.

The problems in maintaining constant flow in a pressurized pneumatic system were shown to depend on two factors. Most ordinary pressure regulators are not sufficiently precise or reproducible for this purpose. Furthermore, our attempts to eliminate changes in the fluid head were apparently inadequate. A precision, low-pressure regulator was used to maintain the bladder pressure constant at 0.55 psi. Instead of trying to maintain a constant head, we allowed the fluid height to change and recorded the head each time the flow rate was measured. Correction of the observed flow rates for the change in head resulted in a calculated flow rate which remained constant during the 45 minute experiment.

Having determined that the pneumatically-controlled flow system is practicable, we decided to investigate its use in controlling an intermittent flow cell 6" x 12" x .185" having five buffer inlets 0.112" ID evenly spaced on one-inch centers and twenty outlets made from 21 gauge hypodermic needles. This is the type of system that might prove useful for free isotachophoresis or isoelectric focusing. Flows were controlled by the pneumatic system without problems.

While many components of a free flow electrophoresis device may work equally well on the ground and in zero-g, and some components (such as described above) may actually work better in zero-g than on the ground, adaptations of some fluid handling systems must be made to compensate for the lack of gravity in space. For example, the buffer flow and electrode rinse systems are normally operated open on the ground. In space, of course, such systems must be closed and provision must be made for changes of volume during operation of the equipment. Adaptation of such systems to zero gravity is not a difficult problem.

One of the principal reasons for wanting to do free flow electrophoresis in zero gravity is that the absence of gravity permits us to use a cell with a much thicker dimension (e.g. 5 mm) than can be used on earth. The advantages of the thick cell have been described numerous times, as for example in Reference 5. It is axiomatic that if such a cell could be used successfully on earth there would be little or no reason to perform experiments in space. Therefore, ground-based tests involving the electrophoresis cell are limited to those which can be conducted without an electric field or which require only a very minimum power input. The difficult separations which are of practical interest cannot be performed on the ground in an electrophoresis cell designed for use in space. Predictions of separation in space for such materials must be based upon experiments performed on the ground in the best available equipment designed for use on the ground.

V. IMPROVEMENT TO BE EXPECTED IN SPACE FOR PURIFICATION OF BIOLOGICAL MATERIALS

A. Rationale

There is, of course, a bewildering array of biological materials which might benefit from an improved purification method. This list is in no way intended to be comprehensive, but rather represents a few materials of real or potential value - monetary or medical - which present unsolved problems in their purification. Furthermore, as far as possible we have tried to select materials for which there is evidence of possible electrophoretic purification.

B. Suggested Materials

Many of the following materials have been discussed at great length during the past few years, and a lengthy discussion herein is considered unnecessary.

1. Viable cells - the isolation of pure cell fractions is the endeavor which comes closest to meeting universal acceptance.

2. Absolute separation of nucleic acid from protein - This is an old problem associated with the production of certain virus vaccines. New solutions are being actively sought (e.g. by Merrill National Laboratories) but there does not appear to be any conclusive evidence that the problem has been solved or that free electrophoresis will provide the solution.

3. Erythropoietin - This potentially valuable hormone has resisted purification attempts for a decade. Although universal agreement by experts on the value of free electrophoresis has not been obtained, the Erythropoietin Advisory Committee of the National Institutes of Health has indicated in the past their willingness to commit some of this rare hormone for attempted purification by free electrophoresis.

B. Suggested Materials

Many of the following materials have been discussed at great length during the past few years, and a lengthy discussion herein is considered unnecessary.

1. Viable cells - the isolation of pure cell fractions is the endeavor which comes closest to meeting universal acceptance.

2. Absolute separation of nucleic acid from protein - This is an old problem associated with the production of certain virus vaccines. New solutions are being actively sought (e.g. by Merrill National Laboratories) but there does not appear to be any conclusive evidence that the problem has been solved or that free electrophoresis will provide the solution.

3. Erythropoietin - This potentially valuable hormone has resisted purification attempts for a decade. Although universal agreement by experts on the value of free electrophoresis has not been obtained, the Erythropoietin Advisory Committee of the National Institutes of Health has indicated in the past their willingness to commit some of this rare hormone for attempted purification by free electrophoresis.

4. Interferon - This glycoprotein protects cells against attack by a variety of viruses. Although it was first identified in 1957 it has not yet been obtained in a pure form. Dr. Anfinsen at NIH indicates that he is making progress in the purification. It may be, then, that purification by non-electrophoretic means will be achieved in the next few years; or it may be that electrophoresis would be a useful adjunct to such purification, particularly for research purposes. (Sialated glycoproteins seem to be often electrophoretically inhomogeneous and therefore susceptible to further purification or fractionation.)

5. Antihemophilic factor - Two schools of thought exist in regard to AHF. Many experts who are concerned with the production and clinical use of AHF generally see no need of further purification. Others, however, believe that purification should be attempted in order to permit determination of the structure of AHF as a necessary prelude to synthesis of material to supplement the chronically inadequate and expensive supply.

6. Somatomedin or sulfation factor - This is a current separation problem which Dr. Wickerhauser of the ANRC Blood Fractionation Center suggested as a possible candidate for free electrophoresis.

7. Immunoglobulin fractions - The desirability and feasibility of separting immunoglobulin subfractions electrophoretically appear to be fairly universally accepted. The question of whether this need be done in space, however, has occasioned lengthy debate.

C. Ground Based Experiments

In our laboratory we have the finest free flow electrophoretic separator in existence. It is a Beckman Mark II CPE, which incorporates all of the state-of-the-art improvements known to improve separation resolution. The truly remarkable capability of this machine is demonstrated in Figure 4 by the separation of two polystyrene latices, and the further separation of one of these latices into two subfractions. A qualitative measure of resolution can be taken as the width of the fraction bands and the separation distance between them.

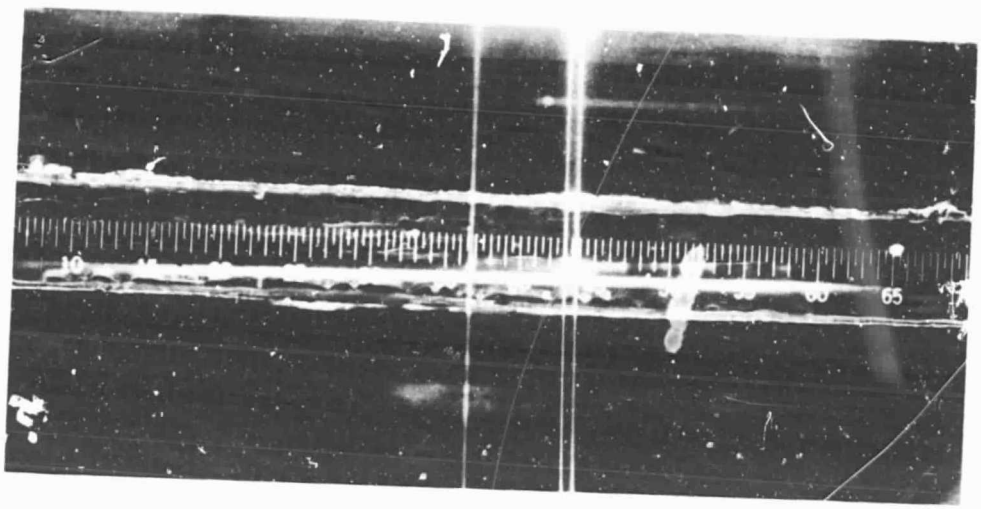


Figure 4. Separation of Two Polystyrene Latices in the Mark II CPE.

This equipment was used in some laboratory experiments to determine the feasibility of electrophoretic separation of some of the above biological materials. While we were unable to obtain a sample of virus vaccine contaminated with nucleic acid, we were able to obtain samples of an influenza B vaccine contaminated with bacterial endotoxin. Complete electrophoretic separation of the virus antigens from the bacterial endotoxin contaminant was successfully demonstrated. The antigen band as determined by chick RBC agglutination was remarkably sharp. Successive fractions had antigen concentrations of 0, 32, 512, 64, 8 and 0 (no units), and even sharper resolution is undoubtedly possible. Since the antigen consists of fragments of the virus, the possibility existed that the antigen would be very inhomogeneous electrophoretically and difficult or impossible to separate from contaminants. This is not a problem, and separation of antigen and nucleic acids seems a very real possibility. We also found that neuraminidase activity corresponds identically to the hemagglutination activity, leading to the tentative conclusion that in this vaccine all the neuraminidase is in spikes which are still attached to the virus protein. The presence of only one neuraminidase fraction was puzzling to the manufacturer, and several further experiments were suggested. However, we feel that sufficient work has been done to establish the ability of electrophoresis to remove the impurity from the vaccine, and we have in

effect provided the ground-based reference data for comparison with a possible future flight experiment. Although in this case improved resolution does not seem to be important, the potential of a thick cell to process up to 80 times as much material per unit time may very well be of interest in this case. The amounts of material actually processed in these experiments was too small to detect by any means except biological assay.

A partial purification of sheep plasma erythropoietin was accomplished in the Beckman Mark II CPE. The principal contaminant in the raw material is the sheep albumin. By electrophoresis we were able to move the peak of the sialic acid activity one fraction to the anodic side of the peak of the albumin concentration. Thus it appears that it may be possible to effect an electrophoretic separation, but more work needs to be done before it is accomplished. It may well be in this case that the earthbound electrophoresis device is not capable of effecting clean separation of the sheep albumin and the sheep plasma erythropoietin. On the basis of the results obtained to date it does not appear that even a factor of five improvement in resolution would be sufficient to produce a clean fractionation. There is, however, no reason to believe that in this brief period we have managed to optimize the earth-bound separation.

VI. REFERENCES

1. A. Strickler and T. Sacks, "Focussing in Continuous Flow Electrophoresis System by Electrical Control of Effective Cell Wall Zeta Potentials", Annals of the N.Y. Acad. of Sciences, Vol. 209, p. 497, June 15, 1973.

2. T. Johns, Beckman CPE Exchange #4, September 1, 1969.

3. G. E. Veen, Final Report Contract NAS 8-28365, 1972.

4. A. C. Krupnick, "Development of Coatings to Control Electroosmosis in Zero Gravity Electrophoresis", NASA TM X-64807, February 14, 1974.

5. Annual Report, Contract NAS 8-31036, September 1975.

END

DATE

FILME

Y 5 197