CONTINUOUS FLOW ELECTROPHORESIS SYSTEM EXPERIMENTS
ON SHUTTLE FLIGHTS STS-6 AND STS-7

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ABSTRACT

In 1978, McDonnell Douglas Astronautics Company (MDAC) began discussions with NASA on the opportunities to develop a space continuous flow electrophoresis system (CFES) that would incorporate specific modifications to their laboratory instruments to take advantage of weightlessness. A Joint Endeavor Agreement (JEA) that allocated certain flights on the Space Shuttle to MDAC in return for opportunities for NASA and interested scientists to do research in the MDAC laboratory and on their space instruments was made.

Under terms of the JEA, NASA was provided an opportunity to process two samples on STS-6. All experiment objectives and operational parameters, such as applied field, sample residence time in the field, and buffer composition had to accommodate the MDAC capabilities and NASA flight constraints. The NASA objectives were formulated so as to include investigation of the sample concentration effects reported by MDAC on STS-4. The specific objectives were (1) to use a model sample material at a high concentration to evaluate the continuous flow electrophoresis process in the MDSC CFES instrument and compare its separation resolution and sample throughput with related devices on Earth and (2) to expand our basic knowledge of the limitations imposed by fluid flows and particle concentration effects on the electrophoresis process by careful design and evaluation of the space experiment. Because the MDAC instrumentation did not include sample mixing facilities, cell separation procedures were precluded and after a variety of soluble materials were considered, hemoglobin and polysaccharide were selected as primary samples. The results from space show a large band spread of the high concentration of the single species

of hemoglobin that was principally due to the mismatch of electrical conductivity between the sample and buffer.

The seventh mission of the Space Shuttle carried two additional NASA experiments in the CFES instrument. The major objective was to evaluate the influence of the electrical properties of the sample constituents on the resolution of the continuous flow electrophoretic device. As expected, the polystyrene latex microspheres dispersed in a solution with three times the electrical conductivity of the curtain buffer separated with a significantly larger band spread than in to the second experiment under matched conductivity conditions. The structure of the bands is also different between the samples and laboratory experiments have been conducted to further evaluate the phenomena affecting the electrophoresis. The analysis of both flight results is nearing completion and a qualitative explanation based upon the non-gravity dependent electrical conductivity mismatch is being developed.

1.0 Introduction

A series of electrophoresis experiments on Apollo [1], Apollo Soyuz Test Project (ASTP) [2], and Space Shuttle [3] have been carried out in space to show that disturbances due to buoyancy-induced thermal convection and sample sedimentation during the separation process are negligible in reduced gravity. The experiments to date have been small-scale demonstrations of specific principles that have increased our knowledge of electrokinetic and fluid dynamic phenomena and have supported our long range electrophoresis goals [4]. Simultaneously, the limitations of ground-based electrophoretic separators have been documented [5], and new concepts have been proposed for future experiments [6].

Several laboratory instruments have been constructed utilizing past developments in the design and operation of continuous flow electrophoretic separators by Strickler [7] and Hannig [8] combined with innovative developments in the field of fluids analysis by Saville [9] and Ostrach [10]. Building on these developments the McDonnell Douglas Astronautics Co. (MDSC) designed a continuous flow electrophoresis system that evolved from a detailed survey of the requirements for fractionation of biological materials.

In 1978, MDAC began discussions with NASA on the opportunities to develop a space continuous flow electrophoresis system (CFES) that would incorporate specific modifications to their laboratory instruments to take advantage of weightlessness. The first MDAC flight experiments with the CFES

on STS-4 in June 1982 fractionated a proprietary tissue culture medium and evaluated the effect of sample concentration using mixtures of rat and egg albumin. MDAC concluded that there was no loss of resolution at the higher concentrations processed in space. In addition, MDAC reported that the quantity of albumin that could be fractionated in the CFES in space was significantly higher (over 400 times) than the quantity that could be processed in their ground laboratory instrument during the same time interval. They proposed that these improvements originated from both instrument modifications and increased sample concentrations permitted by weightlessness.

Under the terms of the JEA, NASA was provided an opportunity to process two samples on STS-6 in April 1983 [11]. All experiment objectives and operational parameters, such as applied field, sample residence time in the field, and buffer composition had to accommodate the MDAC capabilities and NASA flight constraints. The NASA objectives were formulated so as to include validation of the sample concentration effects reported by MDAC on STS-4. The specific objectives were (1) to use a model sample material at a high concentration to evaluate the continuous flow electrophoresis process in the MDAC CFES instrument and compare its separation and sample throughput resolution with related devices on Earth and (2) to expand our basic knowledge of the limitations imposed by fluid flows and particle concentration effects on the electrophoresis process by careful design and evaluation of the space experiment. Hemoglobin and a polysaccharide were selected as primary samples for STS-6 by the Marshall Space Flight Center (MSFC).

The NASA experiments on STS-7 in June 1983 were intended to build upon the results obtained on STS-6 [12]. Because MDAC changed the curtain buffer on STS-7 from a barbital buffer (pH 8.3) to a propionate buffer (pH 5.2), it was not possible to perform a follow-up experiment using hemoglobin and polysaccharide as processed on STS-6. The change in pH would have resulted in both the hemoglobin and polysaccharide becoming positively charged and migrating toward the cathode. Polystyrene latex particles (PSL) were, therefore, chosen for separation on STS-7 since they are known to be negatively charged at pH 5.2 and are produced in a range of sizes with different surface charge groups and surface charge densities.

2.0 Materials and Methods

2.1 Electrophoresis Instruments

The internal dimensions of the space CFES (Figure 1) plexiglas separation (flow) chamber is 16 cm wide, 120 cm long, and 0.3 cm thick. A cooling jacket covers each broad chamber face with one electrode in each cooling chamber positioned diagonally across from each other to provide the electric field across the length of the chamber. The platinum electrodes are in contact with the separation chamber via slots cut into the chamber plates and are covered with a proprietary, porous membrane. The circulation of cooled electrolyte, from the bottom (flow entrance) of the chamber to the top through a serpentine passageway, establishes a uniform lateral temperature gradient and removes the bubbles formed at each electrode. Rectangular
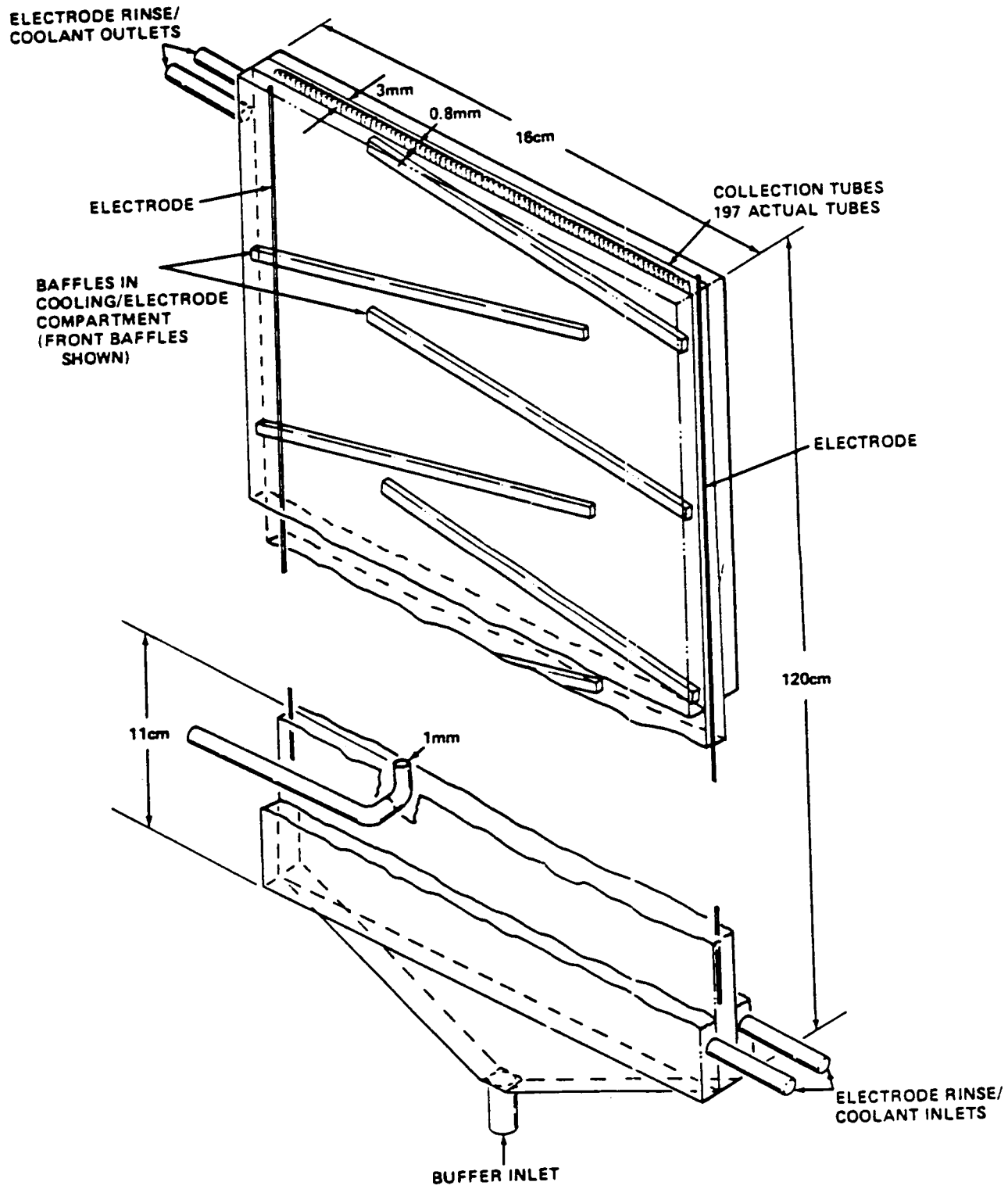
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Figure 1   Continuous Flow Electrophoresis System (CFES)

struts used to form the coolant passage also provide some support to the thin, separation chamber plates. The sample enters the chamber through a thin-wall glass tube (0.1 cm inside diameter) located 11 cm from the curtain buffer entrance of the chamber. The buffer and separated sample fractions exit at the top of the chamber through a collection array of 197 Tygon tubes (0.068 cm inside diameter, 0.078 cm outside diameter) which span the width of the chamber.

2.2 Samples and Buffer for STS-6

Hemoglobin was selected as the primary sample candidate based upon its: availability in large quantities as a single, molecular species; visibility for easy analysis; utility as an electrophoresis standard in laboratories; availability as variants with different electrophoretic mobilities; and stability in the cyanmethemoglobin form. Hemoglobin A (HbA) provided for ground and space use was prepared at the Centers for Disease Control (CDC). Selected from a single donor, the HbA was purified on an ion exchange column, converted to cyanmethemoglobin and concentrated to nearly 11%. The hemoglobin was sent to MSFC where it was then dialyzed against the flight buffer and stored for use in the planned experiments.

The second sample was one type of pneumococcal capsular polysaccharide (PCP) obtained from Lederle Laboratories, Pearl River, New York. PCP type 6 had a distinctly higher mobility than HbA with minimal variation and, although not colored, could be detected in low concentrations immunologically. The purity of

the PCP was determined by specific antibody tests. Since it is known that the PCP's have multiple repeating units of the basic saccharide chain, an attempt was made to obtain PCP with constant molecular weight using chromatography and laboratory continuous flow electrophoresis.

The buffer selected by MDAC for laboratory and flight experiments was 2 mM barbital consisting of 0.386 mg/ml sodium barbiturate, 0.070 mg/ml barbituric acid, and 50 µg/ml gentamycin sulfate, pH 8.3 and electrical conductivity 160 µmho/cm at 25 °C.

2.3 Samples and Buffer for STS-7

Monodisperse polystyrene latex (PSL) particles less than 1.0 µm in diameter were chosen to minimize sedimentation and eliminate any requirement for resuspending the sample during flight. Three particle sizes were ultimately chosen, and two of the particle populations were dyed to enhance photographic detail and aid in experimental analysis.

The latex particles with the highest mobility (nominal diameter of 0.56 µm) were dyed red; the particles with the lowest mobility (nominal diameter 0.80 µm) were dyed blue. The particles with intermediate mobility (nominal diameter 0.30 µm) were not dyed. The latexes were then suspended in the curtain buffer containing Brij using a procedure of buffer exhange with filtration. Appropriate volumes of each latex were combined to yield equal concentrations for the final flight sample with the following properties: total latex concentration, 5.0%; pH, 5.6%; and conductivity, 155 ± 5µmhos/cm. The

conductivity of a second sample of PSL was increased approximately three times that of the initial sample to 455 ± 5 mhos/cm using 0.10 M NaCl, while maintaining a total latex concentration of 5.0% by weight.

The curtain buffer was prepared from a 100X stock solution of 225 mM sodium propionate, pH 5.2 by 100X dilution with distilled water to give a 225 mM solution of pH 5.0 and conductivity of 140 ± 5 μmhos. Laboratory tests were initially performed with a noionic surfactant, 0.05% w/v Brij 35 (polyoxyethylene lauryl ether 35) added to the sample and curtain buffer. Shortly before flight, however, it became necessary for MDAC to remove the Brij from the curtain buffer. Since the sample latex had been selected according to their separation in buffer with Brij, the flight samples included Brij in the suspension medium although Brij was excluded from the curtain buffer.

3.0 Space Electrophoresis-Experiments

The two samples processed in the first NASA space experiment on CFES were (1) a high concentration of hemoglobin alone and (2) a mixture of polysaccharide and hemoglobin at a lower concentration. The band behavior obtained from processing the single species was used to define the performance of the space instrument. The low concentration mixture, 1.9% Hb and 0.5% PCP-6, although still higher than can be processed at high resolution on Earth would permit a comparison of the separations achieved before flight in the various laboratory units.
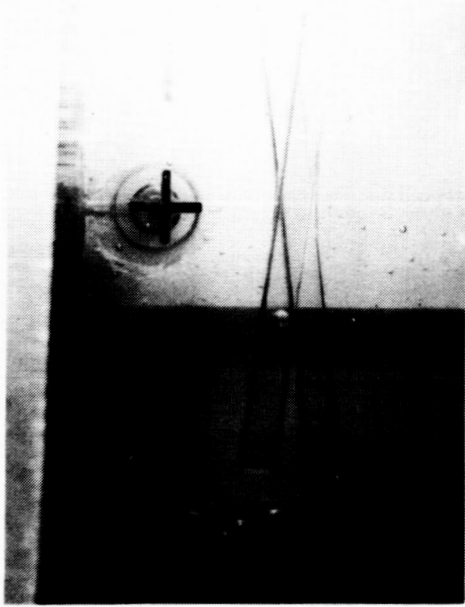
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In orbit, the NASA hemoglobin sample of 8.7% concentration was processed first to establish the initial CFES performance. The hemoglobin experiments each started with an initial zero voltage segment. The sample band spread only slightly during its 8 minute passage through the chamber which can be attributed to diffusion. After the zero run, an electric field of 25 V cm^{-1} was applied for an interval of about 32 minutes in order to establish steady state conditions. Photographs of the electrophoresis runs were taken at sample insertion, midway up the chamber and at the collection end. A portion of the separated samples were also collected for later analysis.

Photographs of the high concentration HbA, taken at the same chamber region are shown in Figure 2 with the time in hours and minutes shown in the lower right hand corner of each picture. Figure 2a shows the zero voltage position of the sample stream. With the cathode on the left, the sample should proceed only toward the right to the anode when the electric field is applied. However, Figures 2b-2d were taken over a period of ten minutes and show that the band spreading is so extensive that some of the sample is in a retrograde migration. This condition could only exist if the sample spread in the chamber thickness past the electroosmotic stationary layer. Based on comparison of the photographs there appears to be a build up of sample near the walls as the more intense color of the later photograph seems to suggest.
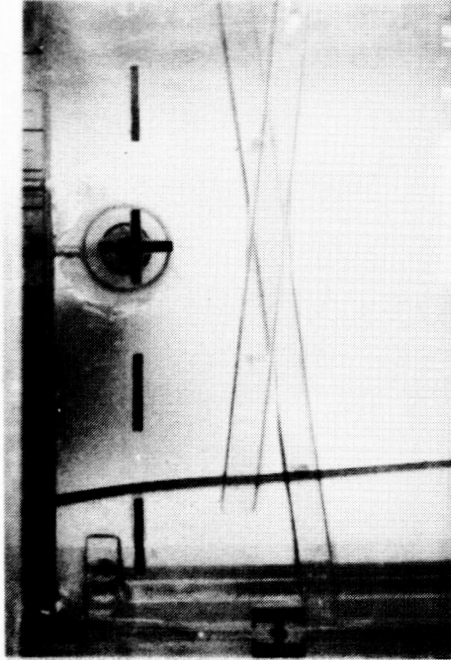
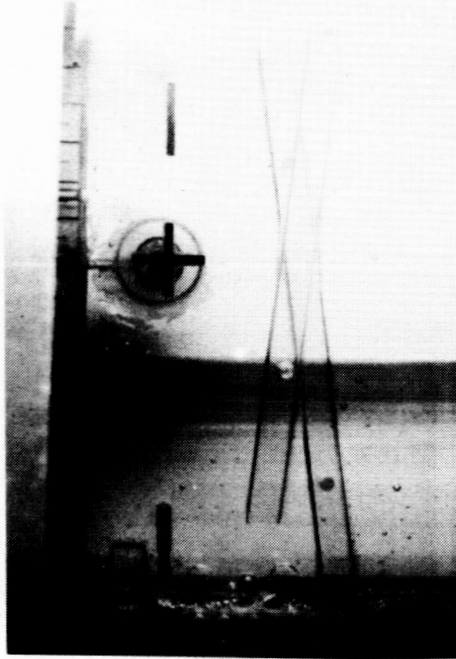After completing half the MDAC proprietary samples, the second NASA preparation, the HbA and PCP mixture, was

Figure 2 Photographs of the Collection of High Concentration
Sample of Hemoglobin
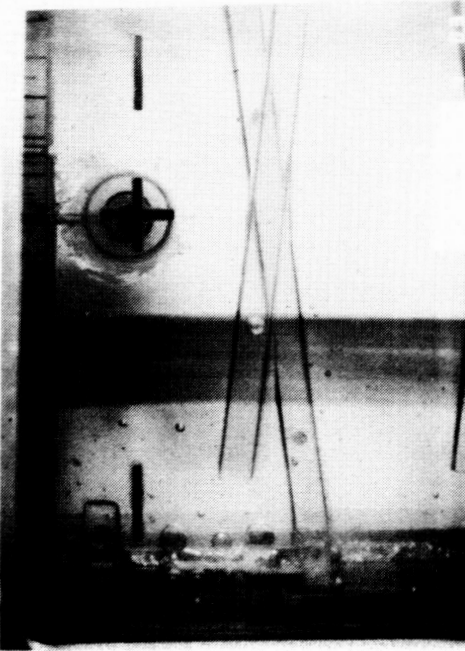
A. Zero Voltage

B. Deflection at 4 hours, 3 minutes

C. Deflection at 4 hours, 10 minutes

D. Deflection at 4 hours, 13 minutes

processed. Although faint, the stream was more compact with
little indication of spreading. The migration of the leading
edge of the sample was roughly the same as in the previous
experiment. However, there does not appear to be the large
retrograde migration observed in the high concentration
experiment.

In addition to the photographs just described, the sample
was collected for the two experiments. The entire fluid output
of the space chamber was collected, as noted previously, during
the middle of each separation run in 197 small (1.5 ml)
polypropylene pockets arranged in a steel tray. The trays
containing the NASA samples were analyzed for hemoglobin and
polysaccharide with the results shown in Figures 3 and 4.

Figure 3 shows a very broad single peak with some sample
deflected toward the cathode side of the zero field location.
However, this does not show the total amount of retrograde sample
migration, since some of the sample near the walls clearly
remained in the chamber. This is apparent by observing the color
extending to the left (cathode) wall in Figure 2d.

Figure 4 shows a separation of the low concentration
hemoglobin and polysaccharide. The separation, however, was not
as distinct as obtained in the laboratory CFES or in the Desaga
FF48 [11]. Also, none of the Earth-based laboratory experiments
had sample appear on the cathode side of the zero field
location. It is interesting to note that Figure 4 shows about
the same amount of retarded and retrograde sample between tubes
#28 and #55 as observed for the high concentration experiment in
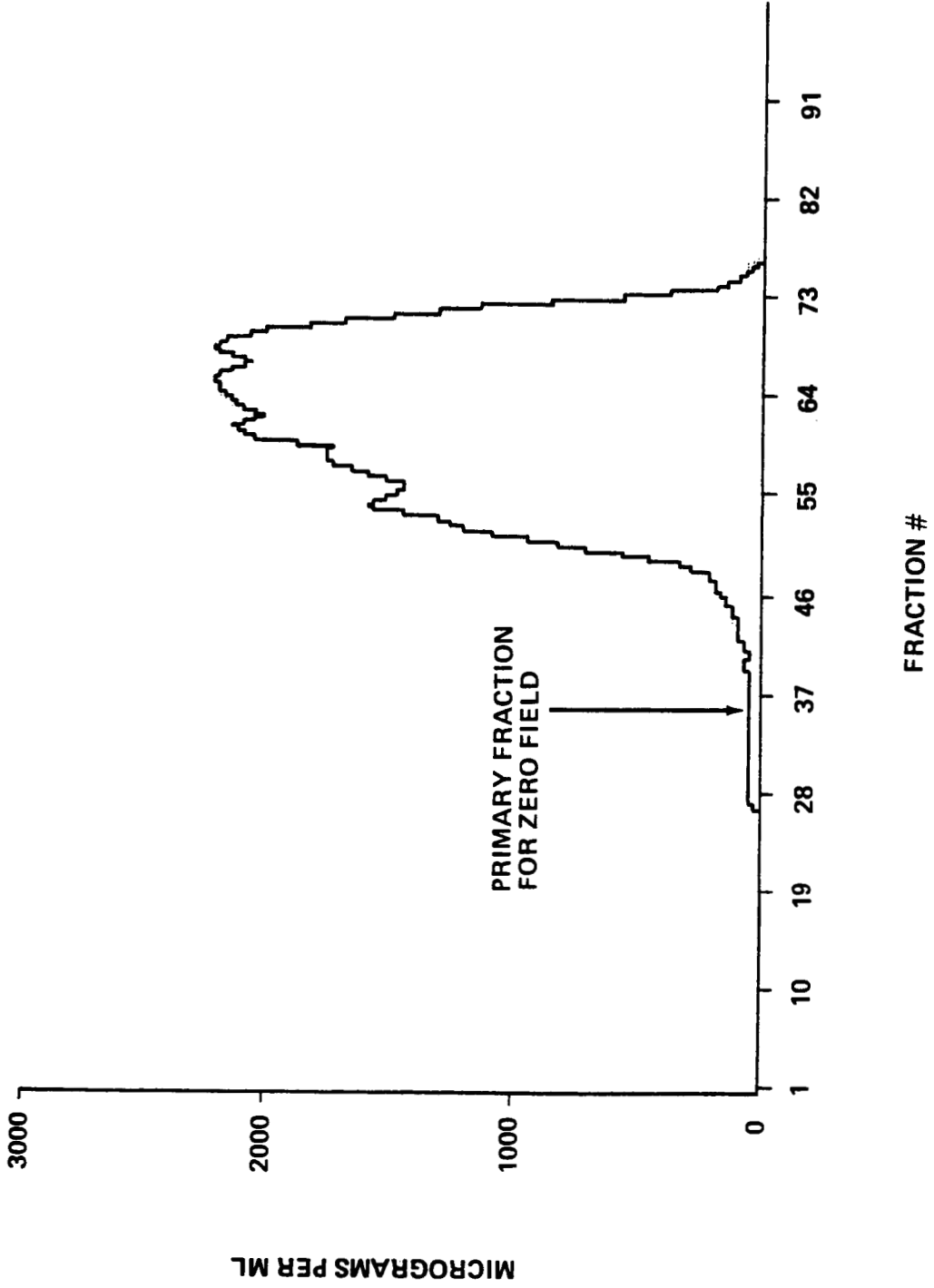Figure 3.

MDAC CFES STS-6 EXP-1
HEMOGLOBIN A (8.7%)

Figure 3   Band Spread of High Concentration Hemoglobin Sample

MDAC CFES STS-6 EXP-4
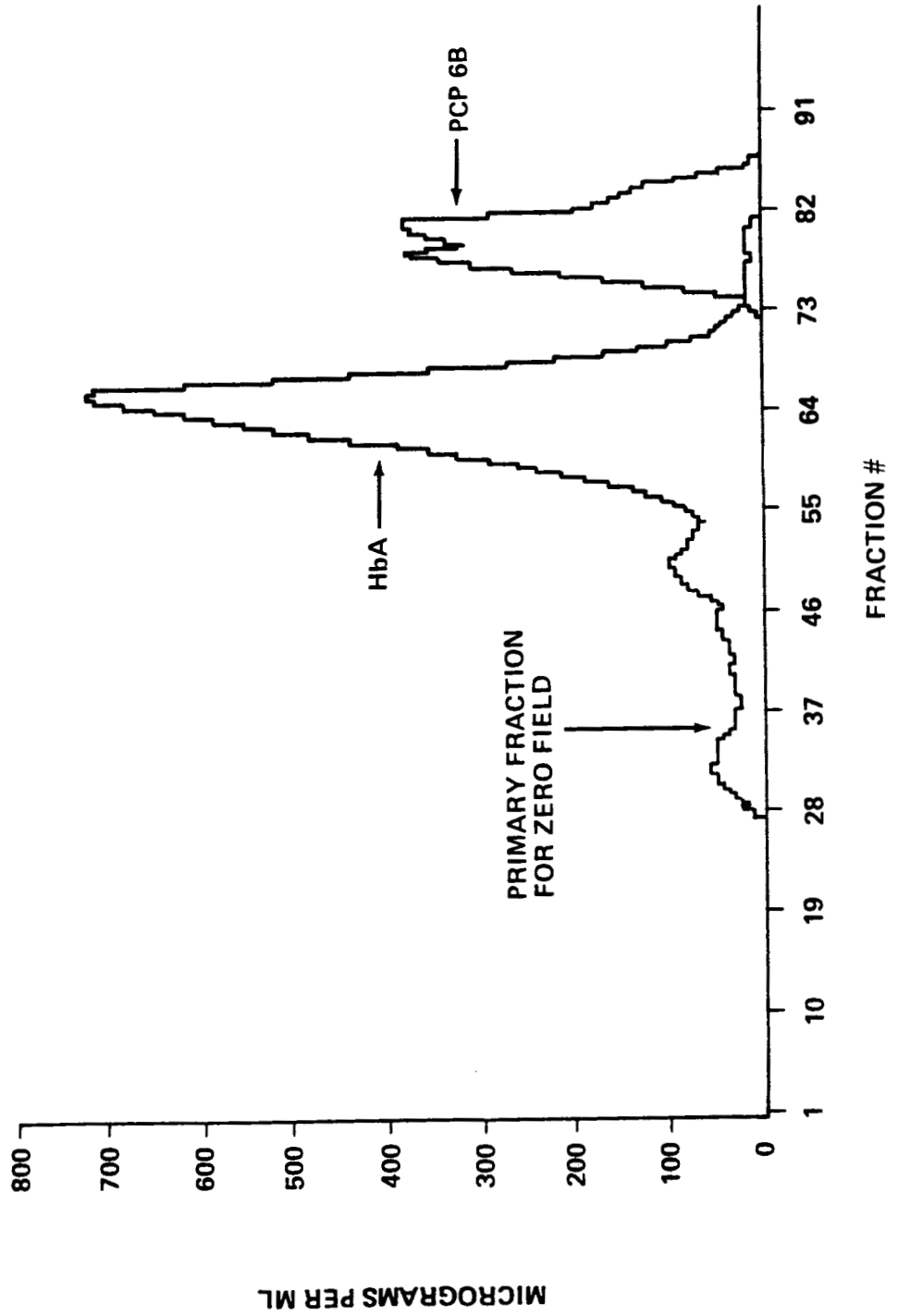HEMOGLOBIN A (1.8%)
POLYSACCARIDE 6B (0.5%)

Figure 4   Band Spread of Low Concentration Hemoglobin and
Polysaccharide Sample

The same procedures were followed with the polystyrene latex samples on the next flight, STS-7. The results of the earlier flight demonstrated considerable band broadening when the sample conductivity was approximately three times that of the curtain buffer. These results, as well as prior ground-based data, provided the justification for the design of the STS-7 experiment which evaluated the relationships between the sample and curtain buffer properties and the fractionation resolution.

Figure 5 shows a series of photographs of the unmatched sample conductivity (left side) and matched sample conductivity (right side). These photographs are of the separated latexes just before entering the collection tray at the top of the CFES. The band distortion and spreading of the PSL samples when the conductivities of the samples and curtain were not matched is evident from the photographs and was confirmed by analysis of the collected fractions (Figure 6).

4.0 Discussion and Conclusion

These two experiments in the MDAC CFES were intended to confirm two previous space observations: only electrophoresis and electroosmosis determined particle migration in an electric field during zone electrophoresis on Apollo 16 and electrophoresis was independent of sample concentration with albumin up to 20% on STS-4. Instead, these experiments showed that the previous conclusions were too simplistic.

First, the sample insertion disk used for the Apollo zone electrophoresis experiments is not a valid model for the sample
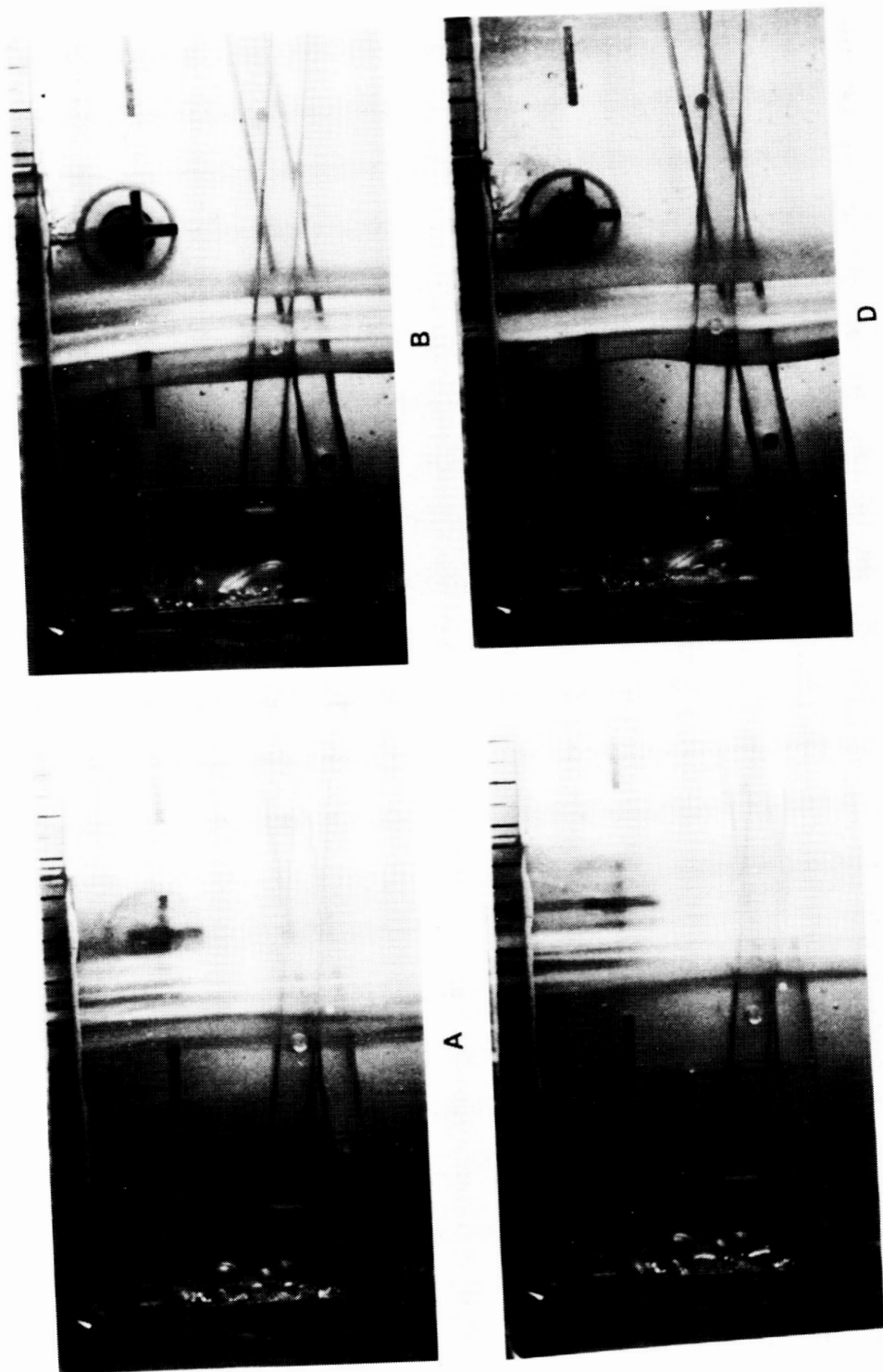
Figure 5   Photographs of Polystyrene Latex Samples With Unmatched Conductivity (A and C) and Matched Conductivity (B and D)
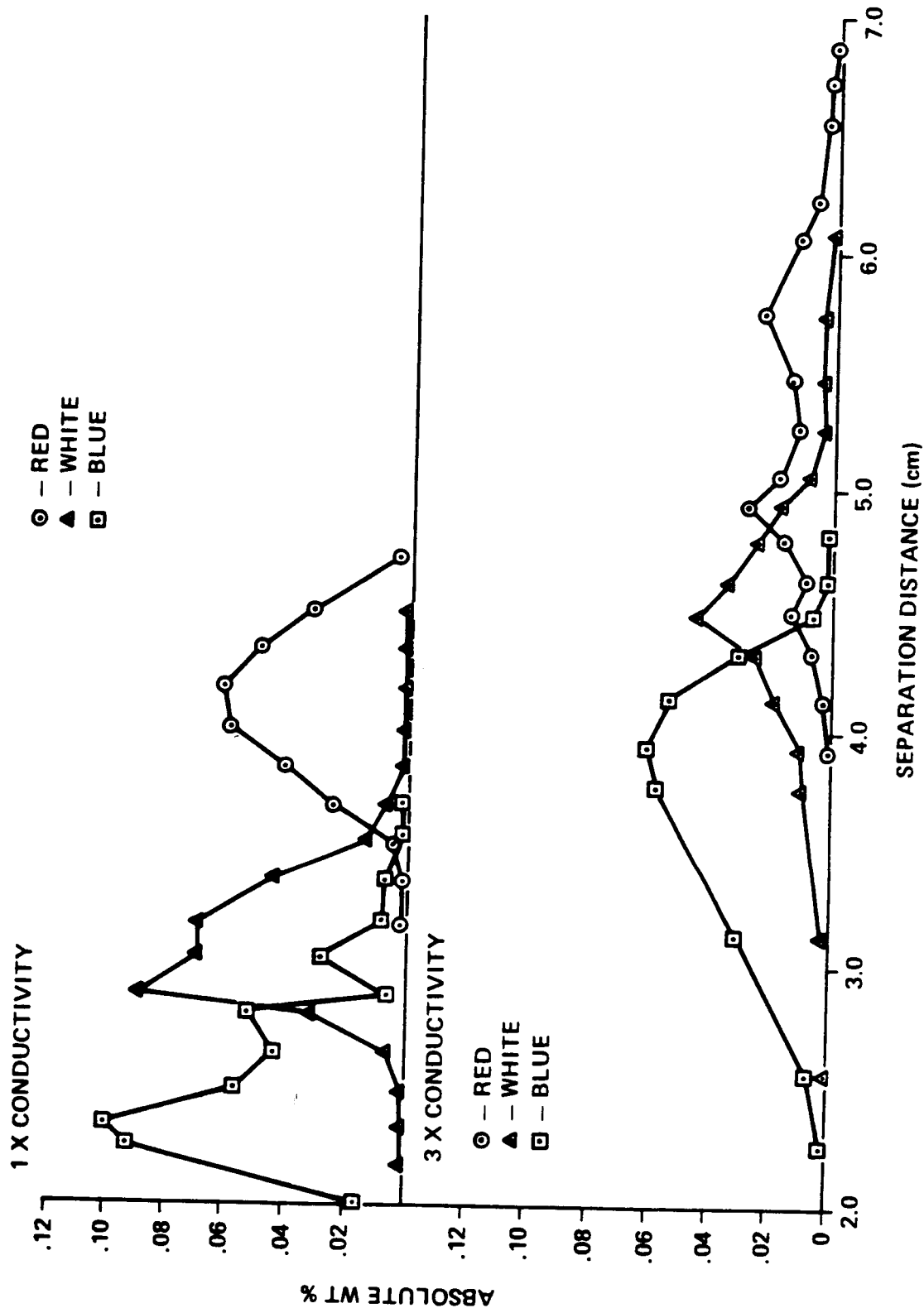
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Figure 6   Band Spread of Polystyrene Latex Samples

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filament configuration of continuous flow electrophoresis. Second, increasing the quantity of protein or latex particles added to the electrophoresis buffer to constitute the sample also proportionally changes the electrical properties of the sample and this has a significant impact on the subsequent electrophoresis of that sample by continuous flow electrophoresis.

The predisposition of the analysis made the interpretation of the two separate but complementary microgravity experiments more difficult. The hemoglobin samples were done first in space, but the pattern of the collected sample did not fit our model. Although the hemoglobin had a single electrophoretic mobility, this mobility could not be measured with precision in the barbital buffer. This made any estimate of electroosmosis subject to error and thus the band structure of the high concentration of hemoglobin was not understood.

During the past year, a more detailed analysis of the hemoglobin experiment has led to the following probable sequence of events. The 9% hemoglobin sample added to the 2 mM barbital buffer (pH 8.2) reduced the overall sample pH to 7.5. Since the pK of the barbituric ion is 7.8, less than half of those ions in the sample were ionized. As soon as the sample entered the chamber and the region of the electric field, the positive sodium ions had to carry most of the current so Na^+ rapidly built up at the rear of the sample. This led to a hemoglobin sample with reduced conductivity in the front of the band and higher conductivity in the rear. The significance of this conductivity

distribution in causing the dispersion of the hemoglobin in space was not determined until after the polystyrene latex distributions in space were analyzed.

Electrophoretic mobilities of the polystyrene latex were measured in a microscope electrophoresis instrument for the next flight experiment but the sample bands still did not fit the anticipated distribution. The sample with higher conductivity showed the expected broader spread but an attempt to fit the shape of the sample distribution into the nested crescents formed by electroosmosis was only partially successful.

The resulting sample geometry may be the product of a phenomena first reported by Sir Geoffrey Taylor [13] describing the distortion which occurs when an electric field is applied to a conducting drop suspended in a liquid of lower conductivity. The elongation of the drop parallel to the field is analogous to the spread of the high conductivity polystyrene latex sample in the center-plane of the chamber when the electric field was applied. Ground-based experiments and a theoretical analysis of continuous flow electrophoresis based upon Taylor's framework now appears to confirm this analogy. The polystyrene latex apparently did not reach the vicinity of the chamber walls where electroosmosis would dominate. Instead, the sample was smeared by the conductivity mismatch with the surrounding electrophoresis buffer.

A plausible model of the hemoglobin sample can also be obtained from Taylor's drop analysis. Since a low conductivity drop in a higher conductivity liquid elongates perpendicular to

an applied electric field, the low conductivity front of the hemoglobin sample spread perpendicular to the field and approached the chamber walls where electroosmosis carried the hemoglobin toward the cathode. At the rear of the hemoglobin sample, the higher conductivity caused a sample spread in the chamber center-plane similar to the polystyrene latex. Thus, the collected hemoglobin distribution shows some sample spread by electroosmosis and a broad but uniform concentration of hemoglobin near the front of the electrophoresis pattern.

This qualitative model of the space experiments is an attempt to explain the observed results of past experiments. When CFES is operated again on the Shuttle it will be important to conduct additional experiments to test the sample distortion predicted by Taylor.

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