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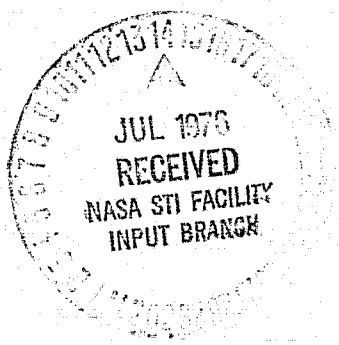
DEVELOPMENT OF POLYMERIC COATINGS FOR CONTROL OF ELECTRO-OSMOTIC FLOW IN ASTP MA-011 ELECTROPHORESIS TECHNOLOGY EXPERIMENT

By William J. Patterson
Materials and Processes Laboratory

May 1976



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16. ABSTRACT

This report describes the development of a methyl cellulose-based coating system for control of electro-osmotic flow (μ_{os}) at the walls of electrophoresis cells. The coating was developed to provide a μ_{os} near zero in experimental cells. Flight electrophoresis columns for the ASTP MA-011 Electrophoresis Technology Experiment were coated with this system, resulting in a flight set of six columns with μ_{os} values between 0 and -0.18 μ-cm-V^{-1}-s^{-1}. In-flight photography of MA-011 electrophoretic separations verified control of electro-osmotic flow.

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LIST OF SYMBOLS

Symbol	Definition
a	cylindrical cell radius
D_m	reference point for column axis
d	deviation
f	microscope micrometer reading at cell front wall (mm)
H_i	depth into cell at point of particle time measurement
$h_{e\phi}$	stationary level
h_i	distance of particle from axis of flight column
L	interelectrode distance (cm)
$\bar{M}_w$	weight-average molecular weight
ml	milliliter
mm	millimeter
N	normal
n_H	total number of data points
r	distance of observed particle from cylindrical cell axis
S_{a_i}	standard deviation of regression coefficient
$S_{(y \cdot x)}$	standard deviation of mean mobilities from regression
$S_{\mu_{os}}$	standard deviation of electro-osmotic flow

LIST OF SYMBOLS (Concluded)

Symbol	Distribution
t_n	individual time reading
V_e	particle electrophoretic velocity
V_o	observed particle velocity
V_{os}	electro-osmotic velocity at cell wall
V_w	solvent flow due to electro-osmosis
v	interelectrode potential difference (V)
v_s	standard variation of slope
μ_e	electrophoretic mobility (μ-cm-V^{-1}-s^{-1})
$\bar{\mu}_{H_i}$	particle mean mobility
μ_i	particle mobility value
μ_{os}	electro-osmotic flow (μ-cm-V^{-1}-s^{-1})
σ_μ	standard deviation

TECHNICAL MEMORANDUM X-73311

DEVELOPMENT OF POLYMERIC COATINGS FOR CONTROL OF ELECTRO-OSMOTIC FLOW IN ASTP MA-011 ELECTROPHORESIS TECHNOLOGY EXPERIMENT

I. INTRODUCTION

The Apollo 14 and 16 electrophoresis experiments demonstrated the feasibility of electrophoretic separations of molecular species and colloidal particles in a free fluid cell. However, a distinct disadvantage, particularly in the case of the Apollo 16 experiment, was the presence of significant electro-osmotic flow which distorted the separating band fronts into paraboloidal spikes (Fig. 1).

The potential for cleanly collecting separated species with such distorted band geometries is minimal, and one of the basic requirements for the later ASTP electrophoresis demonstration (which included sample collection) was adequate control of electro-osmotic flow in the columns. In contemporary electrophoretic work, this control is attempted utilizing polysaccharide coatings having a low residual charge.

This report describes the theoretical and experimental considerations which guided the evolution of a successful specific coating system for control of electro-osmotic flow on the ASTP Experiment MA-011.

II. THEORETICAL CONSIDERATIONS

The nature of electro-osmotic flow (electro-osmosis) will be considered from a basic electrokinetic standpoint to develop an understanding of its effects on electrophoretic migration of particles.

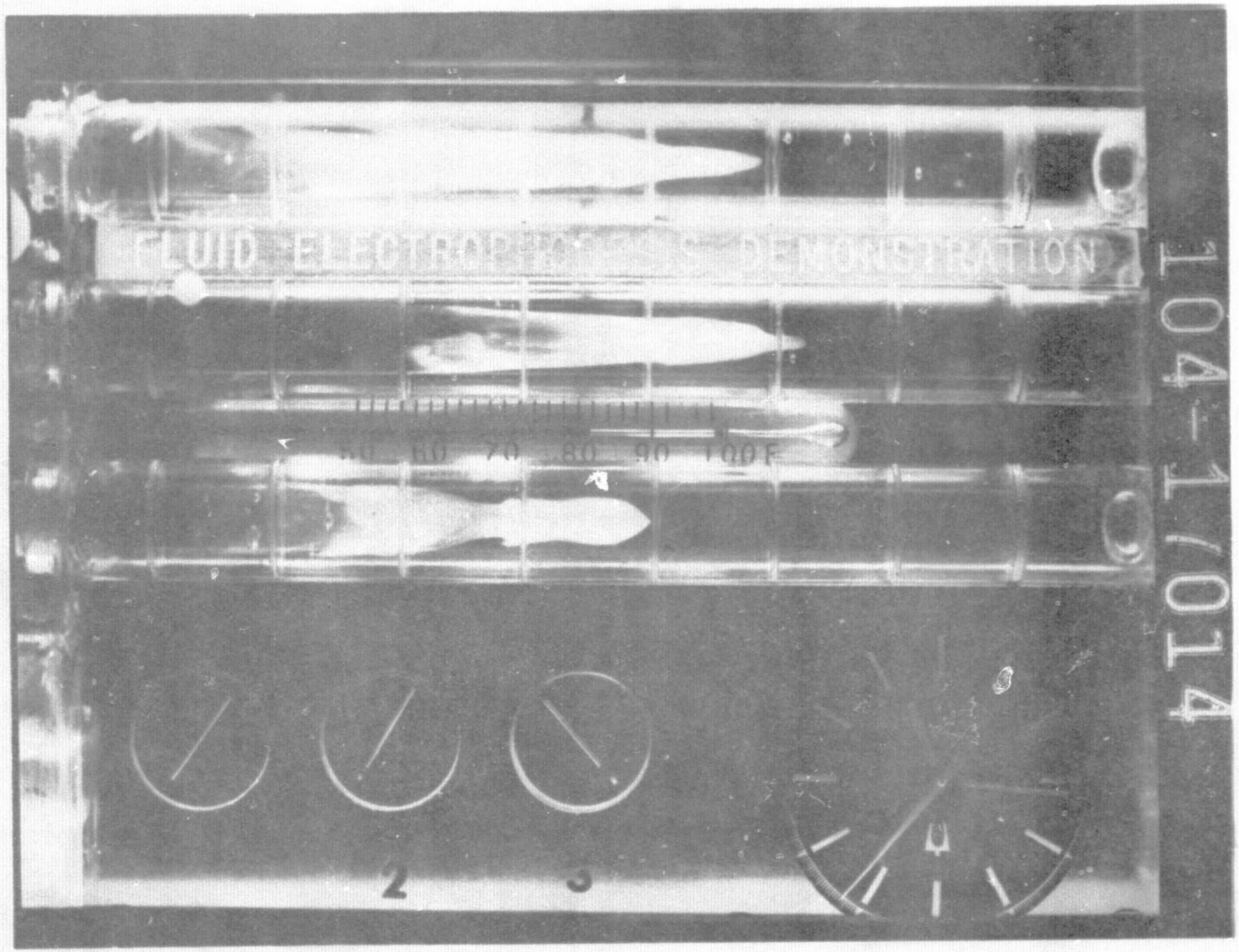


Figure 1. Effects of electro-osmosis in Apollo 16 electrophoretic separation.

A. Theoretical Origins on Electro-Osmosis

Electrophoresis involves the migration of dissolved or suspended particles relative to a stationary fluid under the influence of an applied electric field. If one considers a glass capillary wall as a substrate, a negative charge is induced on this surface when in contact with an aqueous electrolyte medium, possibly via ionization or ion adsorption [1]. This charge upsets the ion/counterion distribution in the medium by attracting ions of opposite charge toward the glass surface. Thus an electrical double layer is formed, consisting of an inner region rich in cations and a diffuse region relatively rich in anions. The electrolyte ions concentrated near the glass wall are characterized by a net migration toward the appropriate electrode, while the preponderance of counterions in the diffuse layer away from the surface undergo a net migration in the opposite direction. The aqueous medium solvates the electrolyte ions such that some liquid flow occurs in one direction in the center of the glass capillary and in the opposite direction at the walls (Fig. 2). A circulation effect within a closed cell can be seen. The implications of this effect for electrophoretic separations are significant, as shown in Figure 3. As a separating band traverses the electrophoresis cell, the countercurrent electro-osmotic flow at the walls tends to sweep back the band periphery resulting in a parabolic band front. The severity of this distortion is a function of the magnitude of the electro-osmotic flow.

B. Stationary Level Measurements

At this point it is necessary to define an important concept known as the stationary level. A parabolic distribution of liquid velocities normally exists in an electrophoresis cell. At a certain depth into the cell, the liquid strata flowing in opposing directions due to electro-osmosis produce a region of zero electrolyte flow (Fig. 3). The observed particle velocity (V_o) during electrophoresis can be defined as

$$V_o = V_e + V_w \qquad (1)$$

where V_e is the particle electrophoretic velocity and V_w is the electro-osmotically induced solvent flow. The solvent velocity is then predicted [2] mathematically for a closed cylindrical tube as

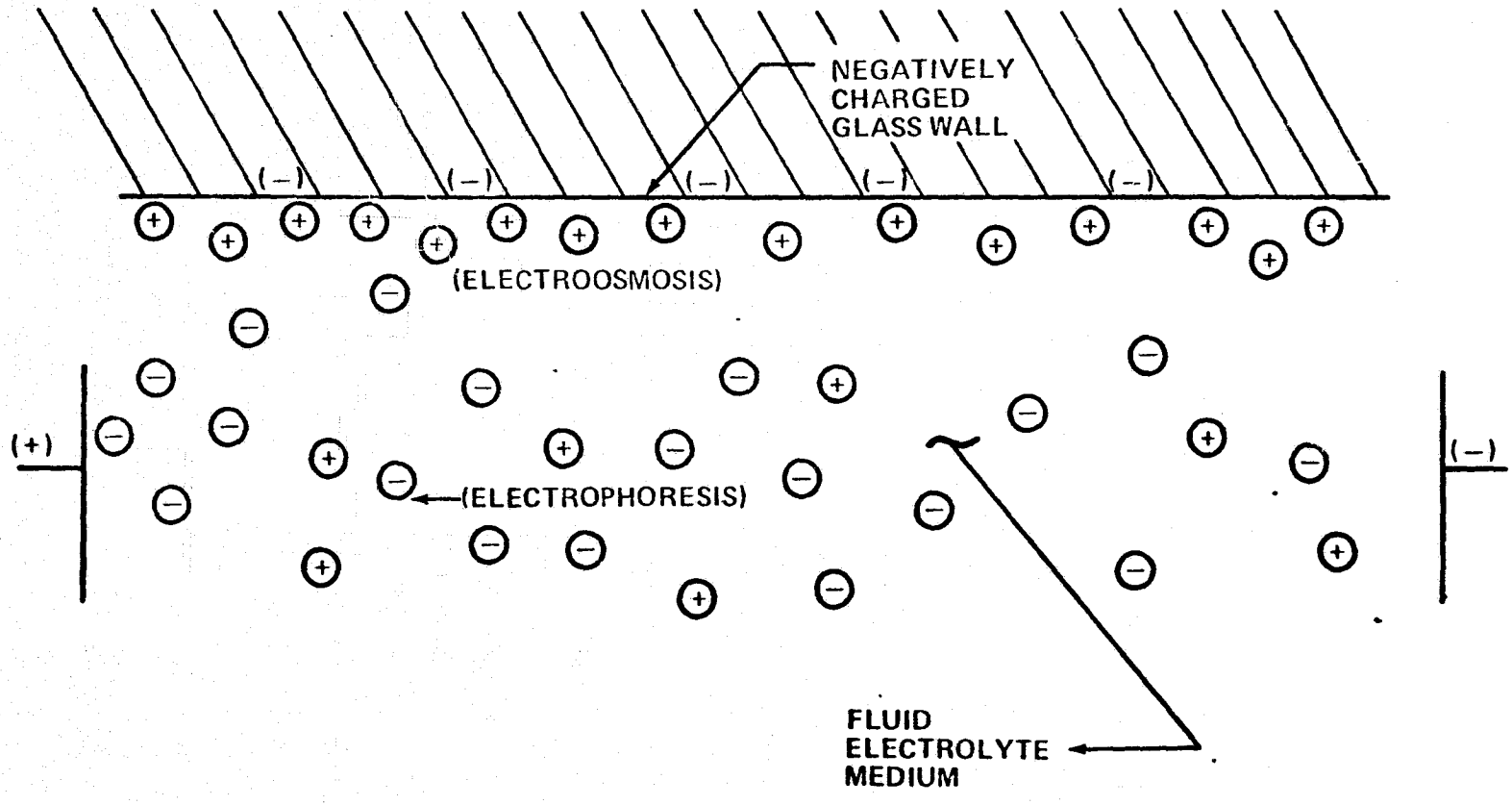


Figure 2. Electrolyte flow due to electro-osmosis.

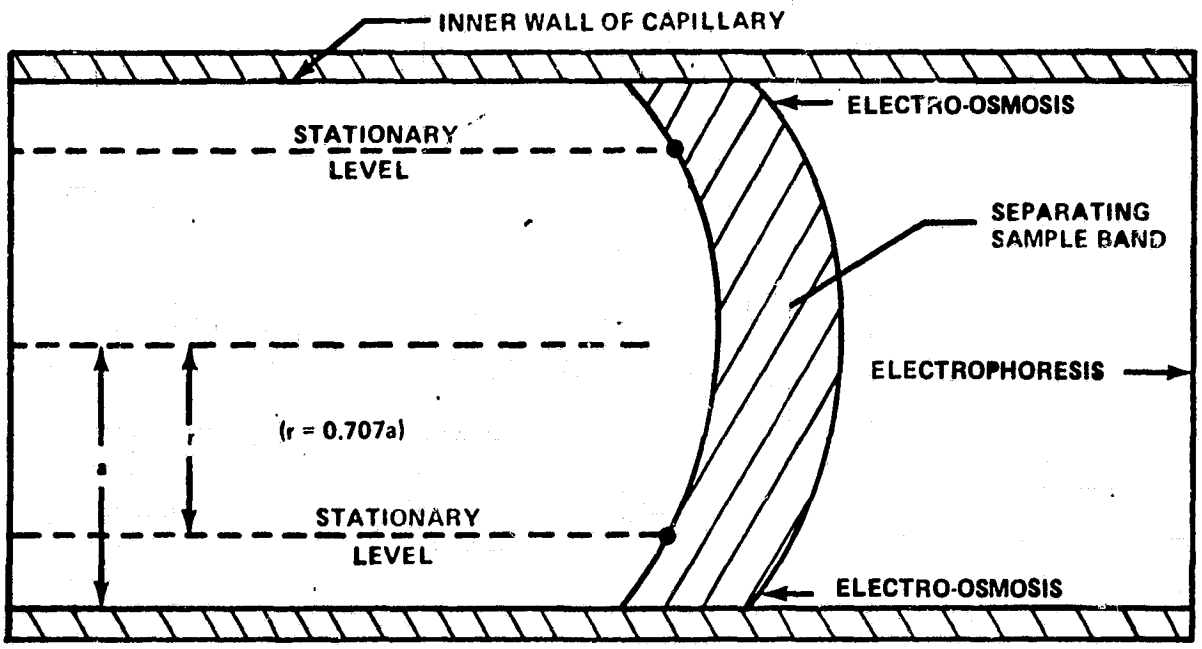


Figure 3. Illustration of the zero solvent flow level.

$$V_w = V_{os}\left(\frac{2r^2}{a^2} - 1\right) \qquad (2)$$

where V_{os} is now the electro-osmotic velocity at the cell wall, and r is the distance from the axis of the tube with radius a. The observed velocity is then the sum of its electrophoretic velocity and that of the surrounding fluid:

$$V_o = V_w + V_e = V_e + V_{os}\left(\frac{2r^2}{a^2} - 1\right) \quad . \qquad (3)$$

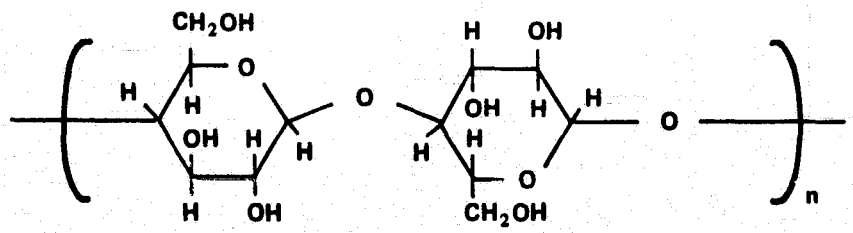
At the stationary level, $V_w = 0$ and $V_o = V_e$, so that $2r^2 = a^2$ and $r = \frac{a}{\sqrt{2}}$ or 0.707a. At this point the net fluid flow in the cell is zero (Fig. 3) and the observed particle velocities represent their true velocities, unbiased by the effects of electro-osmosis. The experimental measurement of electro-osmotic flow across the diameter of a cylindrical cell must be related to the stationary

level as a reference point. Due to the parabolic velocity distribution across the cell, the observed particle velocity is a linear function only of the square of the particle distance from the axis, and this relationship is utilized in experimental calculations of electrophoretic mobility, μ_e, and μ_{os}. It becomes evident that control of electro-osmotic flow at the cell walls would theoretically result in a constant particle velocity across the cell. Consequently, during an electrophoretic separation with a minimal value of μ_{os}, the migrating sample band edges would remain virtually planar and allow distinct fraction separation.

C. Low Charge Polymeric Coatings

Historically, attempts have been made to control electro-osmotic flow in electrophoresis cells by use of polysaccharide coatings with low charge. Dextrans as well as agarose and cellulose derivatives have shown some promise in this area. Ponder [3] first observed specific changes in residual cell charge of erythrocytes exposed to aqueous dextran solutions. More recently [4,5], agar has been used to limit endo-osmotic flow in zone electrophoresis. Van Oss et al [6] have utilized agarose-coated capillaries for microelectrophoretic measurements with significant reduction in electro-osmotic flow.

The dextrans, like agarose and cellulose derivatives, are polysaccharides, consisting of long chain, ether-linked saccharide residues. Dextran and cellulose are polymeric glucose units, while agarose results from polymerization of D-galactose and 3,6-anhydro-L-galacto-pyranose units. It is instructive to examine the structure of cellulose in some detail to understand the role it plays in reducing the wall charge. Cellulose is a largely crystalline material composed of anhydroglucose (glucopyranose) rings connected by 1 → 4 ether (glucosidic) linkages as follows:



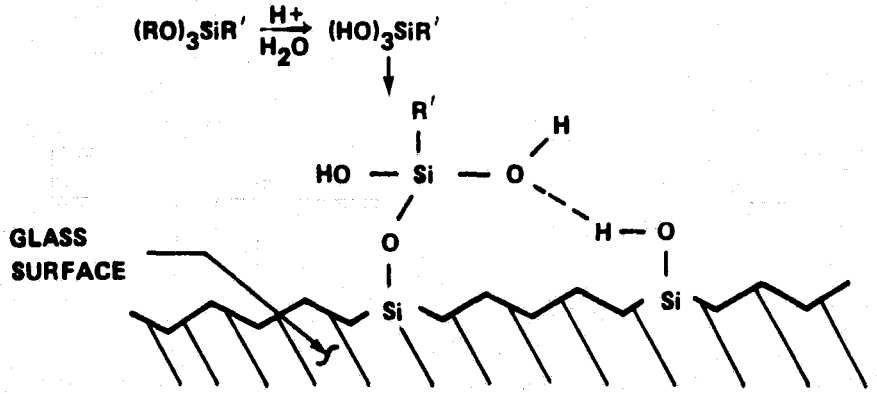
The high proportion of hydroxyl groups renders the polymer somewhat water soluble, with high intermolecular forces contributed by hydrogen bonding. Methylation of the basic cellulose structure is carried out in a typical S_N2 nucleophilic displacement with methyl chloride as the methyl donor. This substitution disrupts crystallinity of the polymer and allows more solvation of remaining hydroxyl groups by water. Also, the reduction in free hydroxyl groups along the polymer chain presumably lowers the magnitude of induced charge on contact with aqueous solutions. In any event, the residual charge of such substituted methyl cellulose derivatives is quite low and probably is controlled primarily by the presence or absence of residual ionizable carboxylate groups which occur at the chain ends and occasionally as pendant groups via oxidation of hydroxymethyl substituents. The carboxylate anion would provide a ready source of negative charge, and the methyl cellulose would require processing that would minimize formation of this group.

The successful utilization of polymeric coatings of this type to reduce cell electro-osmotic flow depends significantly upon their adsorption or binding characteristics relative to the wall material (in this case, pyrex glass). Adsorption in its simplist terms (such as gas adsorption on a solid) arises through the absence of an equilibrium of intermolecular forces beneath and above the surface, resulting in a net attraction of adsorbing species. When considering adsorption from solution onto a solid substrate the situation immediately becomes much more complicated with the three-way interaction of surface, solute, and solvent. In examining adsorption isotherms of various polymers, it is evident that the amount of adsorbed polymer is quite independent of initial polymer concentration or available adsorbing surface. Thus, adsorption equilibria are established at the adsorbing surface and cannot be influenced by large initial concentrations. The magnitude of the attractive forces between polymer and, in the present case, a glass wall must be partially influenced by the presence or absence of strong dipoles or hydrogen bonding sites along the polymer chain. Obviously, materials such as methyl cellulose, containing regularly repeating hydroxyl groups, have the capacity for such interaction. It has been shown [7] that the introduction of 10-15 percent of hydroxyl groups on polyvinylacetate resulted in more than twice the amount of adsorbed polymer than was initially observed. The molecular weight of the polymer as well as solvating power of the solvent also play significant roles in the nature and quality of the adsorbed polymer layer. Glass as a substrate offers its share of polarizable groups, since a limited number of Si - O - H linkages are available at the surface. Recent studies [8] have demonstrated the utility of amphoteric silane coupling agents in anchoring polymeric species to glass substrates. The agent most beneficial in this study has the following structure:

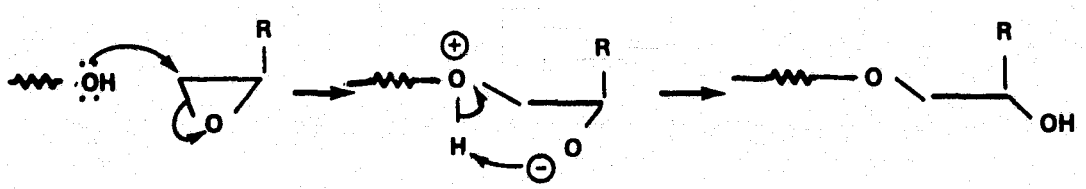
$(CH_3O)_3SiCH_2CH_2CH_2OCH_2CHCH_2$ (with O)

(gamma - Glycidoxypropyltrimethoxysilane) .

The mechanism of attachment of the silane to the substrate is very likely hydrogen bonding plus some covalent bonding:



When polymeric materials such as methyl cellulose are present in solution at the glass surface, the now altered surface morphology and/or availability of additional hydroxyl groups results in enhanced bonding to the surface. It may be postulated that the silane structure, although anchored to the surface, extends the range of interaction of polarizable groups between the methyl cellulose polymer and the surface. Alternately, the R' group contains the oxirane ring which is susceptible to nucleophilic attack yielding a covalent bond between polymer and surface. This is the more satisfying possibility but has not been substantiated experimentally. The nucleophilic attack by the aliphatic alcohol (a weak nucleophile) of methyl cellulose



is not favorable under neutral conditions at modest temperatures. For this to be a facile process under these conditions, the intermediate anion would require stabilization (perhaps through charge delocalization) to lower the transition state energy, and this is not allowed by the derived ionic intermediate. However, some low level covalent bonding may be effected; Figure 4 summarizes the potential reactions which could contribute to binding the coating to the glass substrate.

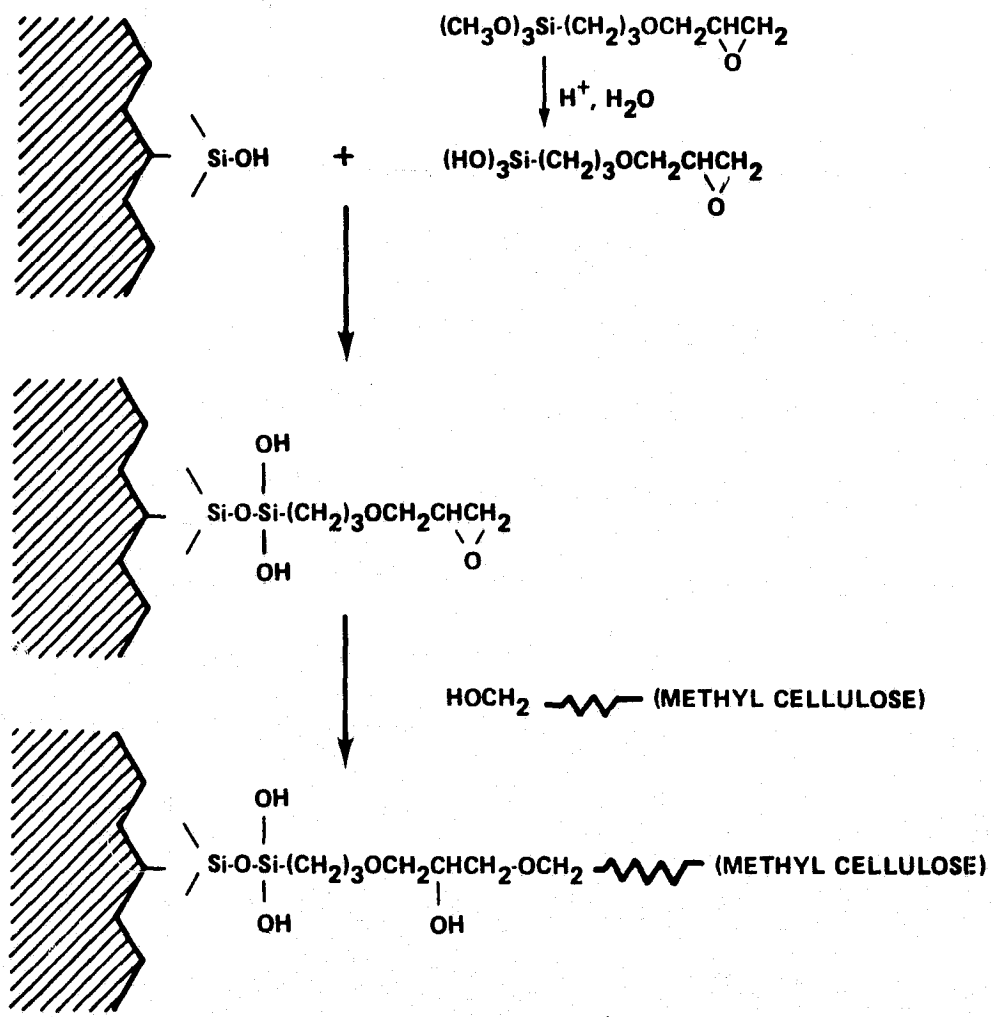


Figure 4. Potential chemical reactions at the substrate surface.

III. EXPERIMENTAL

A. Electrophoresis Measurement Techniques

All experimental data for this study were collected using the Rank Mark II microelectrophoresis instrument. The basic instrument is shown in Figure 5. It consists of a microscope with a calibrated graticule in the field of vision. The microscope can be moved vertically with respect to the test capillary by means

Figure 5. Rank microelectrophoresis instrument.

of a micrometer dial calibrated to 0.002 mm. The fixture supporting the capillary can be displaced horizontally. This fixture was contained in a constant temperature circulating bath maintained at 25° ± 1°C. The test capillary was mounted in a special holder which incorporated glass chambers at each end of the capillary. Each chamber was joined to the capillary by a threaded nut and o-ring to provide a liquid seal. This assembly constituted the microelectrophoresis cell shown in Figure 6. Platinum electrodes were extended into each end chamber and connected to an external dc power supply. The assembled cell was filled (completely excluding air bubbles) with a suspension of monodisperse polystyrene latex particles (0.8 μ diameter) in the selected electrophoresis medium, a phosphate-buffered, physiological saline solution known as A-1 buffer. The cell was placed in the constant temperature bath and aligned visually so that the beam from the light source was incident on the capillary. A number of adjustments were then made to locate the top of the inside wall of the capillary (or the front wall) with the microscope. This process was aided by adsorption of latex particles on the capillary walls. These particles did not move under influence of the applied voltage gradient and the microscope was focused on them.

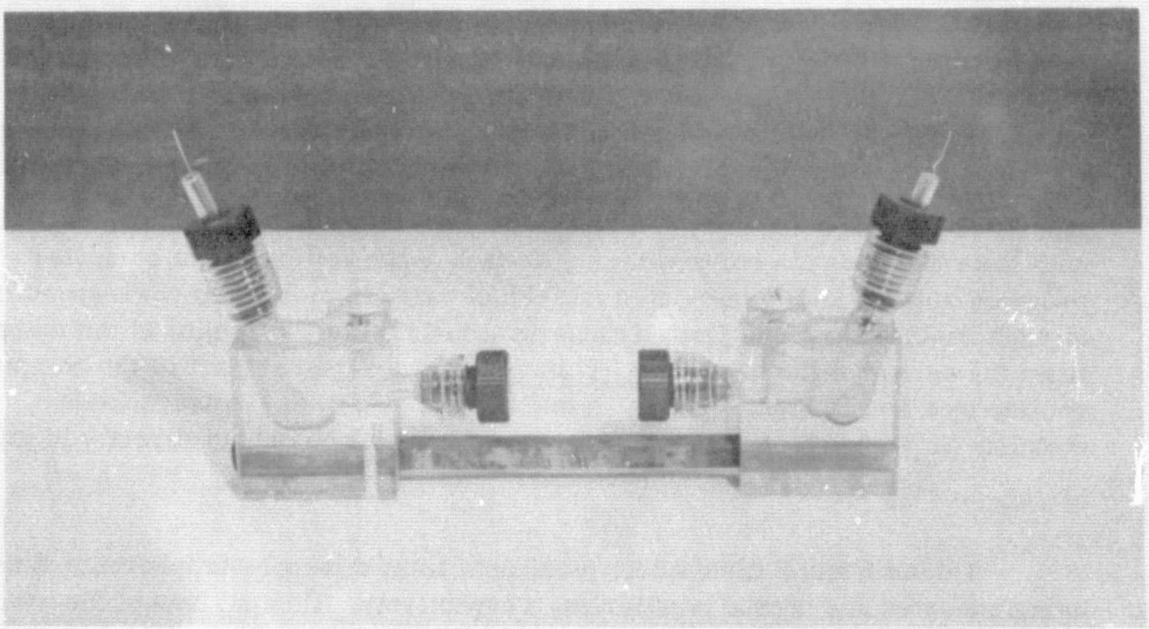


Figure 6. Experimental capillary electrophoresis cell.

The micrometer position was noted and the process was repeated to locate the back wall. This procedure provided an optical measurement of the capillary diameter. The equivalent electrical length of the cell was also required in the data reduction. Ideally, this is obtained by calculations involving the buffer conductivity, but can be approximated to good accuracy by a measurement of the distance between electrodes in the assembled cell. The dc voltage was then applied across the cell to cause movement of the particles in the capillary. Minimum voltages were used in all experiments to minimize joule heating of the electrolyte. The microscope was again focused on the front capillary wall and advanced into the capillary until particle movement was observed in response to the applied voltage. Particle drift during a "voltage off" condition indicated leakage in the cell which was remedied before continuing the measurement. A series of particles was then timed using the graticule (one graticule division corresponded to 30 μ). Five particles were timed (to the nearest 0.01 s) across the graticule in each direction, using a polarity reversal switch, so that a total of 10 time measurements was collected for the first depth level. Generally, 7 or 8 depth settings were utilized, terminating at the back wall of the capillary.

The constants required in data reduction were voltage (volts), graticule calibration (30 μ/division), equivalent electrical length of the cell (cm), and capillary diameter. The experimental data were reduced using a Hewlett-Packard Model 9100B programmable calculator and a computer program entitled "Electrophoretic/Electro-osmotic Mobilities Program #EØØ2." This program was subsequently modified to accommodate the electrophoresis flight column μ_{os} measurements (Appendix A). Input to the computer consisted of the previously described constants, followed by the series of depth settings, together with the raw time data corresponding to each depth setting. The computer program automatically calculated individual particle mobilities corresponding to each depth setting and plotted these as a function of the square of the distance from the center of the capillary. This plot was refined as part of the computer routine by a linear least squares line fit. The values of electrophoretic mobility (μ_e) and electro-osmotic flow (μ_{os}) were printed out directly in units of μ-cm-V^{-1}-s^{-1}.

Tables 1 and 2 illustrate typical data from microelectrophoresis runs using uncoated and coated capillaries, respectively. Comparison of the average times in the two cases reveals the experimental manifestation of the electro-osmotic flow effect. In the case of the uncoated capillary, slow moving particles were encountered at each wall with much faster particles near the center. This effect is virtually eliminated in the case of the coated capillary. The experimental particle velocity distribution observed in Table 1 correlates closely with parabolic flow profiles predicted in the theoretical discussion and shown previously in Figure 3.

TABLE 1. RAW DATA FROM MICROELECTROPHORESIS RUN OF UNCOATED CAPILLARY

Depth Setting (mm)	Individual Particles Traverse Time (s)					Average
10.825	—	—	—	—	—	
10.900	7.12	6.30	6.44	5.84	6.50	(6.56)
	6.12	6.98	6.56	7.34	6.38	
10.970	5.66	6.70	5.46	5.74	4.32	(5.64)
	6.22	5.50	5.78	4.98	6.08	
11.070	5.76	4.58	5.10	4.36	4.98	(4.97)
	4.38	5.24	4.64	5.60	5.00	
11.270	3.84	3.34	4.12	3.38	4.06	(3.82)
	3.94	4.04	3.86	4.32	3.82	
11.470	3.56	3.38	3.84	3.46	3.74	(3.61)
	3.64	3.78	3.48	3.74	3.48	
11.570	4.62	4.16	4.06	3.92	4.44	(4.35)
	4.44	4.32	4.44	4.60	4.46	
11.670	6.38	7.42	6.06	6.40	7.20	(6.97)
	6.82	6.92	6.88	7.70	7.92	
11.715 (Back Wall)	—	—	—	—	—	

(μ_{os} calculated from these data = -2.8)

Test Parameters and Constants

Travel Distance:	30 μ
Voltage:	40 V
Electrical Length:	13.6 cm
Temperature:	25° ± 1°C
Medium:	Latex Particles/A-1 Buffer
Capillary Condition:	Cleaned, Uncoated

TABLE 2. RAW DATA FROM MICROELECTROPHORESIS RUN OF COATED CAPILLARY

Depth Setting (mm)	Individual Particle Traverse Time (s)					Average
10.280 (Front Wall)	—	—	—	—	—	Average
10.320	3.18 3.42	3.06 3.18	3.26 3.28	3.28 3.08	3.66 3.66	(3.20)
10.400	2.98 3.10	3.16 3.12	3.20 3.10	3.12 3.13	3.16 3.12	(3.12)
10.500	2.84 3.20	2.98 3.02	3.10 3.84	3.08 2.86	2.89 2.90	(2.97)
10.700	3.04 3.08	3.14 3.06	3.02 3.02	3.06 3.16	 3.04	(3.04)
10.900	2.98 3.12	2.90 2.84	3.02 2.86	2.82 3.02	2.98 2.80	(2.93)
11.000	2.84 3.16	3.06 2.88	3.00 3.00	2.92 3.04	3.06 2.94	(2.90)
11.100	3.20 3.12	3.18 3.12	2.80 3.14	3.14 3.30	3.14 3.22	(3.09)
11.165 (Back Wall)	—	—	—	—	—	

(μ_{os} calculated from these data = -0.08)

Test Parameters and Constants

Traverse Distance:	1 μ
Voltage:	40 V
Electrical Length:	13.6 cm
Temperature:	25°± 1°C
Medium:	Latex Particles/A-1 Buffer
Capillary Condition:	Cleaned, Coated

B. Experimental Cleaning/Coating Procedures

Considering the fundamental importance of adsorption of the coating to the pyrex surface, a study of glass cleaning procedures seemed advisable as a prelude to coating development. A hydrofluoric acid etch (1N HF) of pyrex glass slides was carried out for time intervals from 30 s to 30 min. Even in the shortest exposure time, some opacity was observed in the glass. In view of the requirement for maximum optical clarity of the flight-configured electrophoresis columns, this approach was discarded in favor of a strong base/acid sequential cleaning. The experimental substrates used for the initial phase of this study were pyrex capillaries approximately 14 cm long by 1 mm inside diameter. The capillaries were first sonicated 5 min in distilled water with several drops of liquid detergent. They were sequentially soaked in alcoholic NaOH for 1 h; aqua regia for 1 h, and high resistivity distilled water for 16 h (see Appendix B for details of this procedure). The water purity for this experiment was considered critical, since ionic charge accumulation at the capillary walls was to be minimized. The rinse water for experimental capillaries had a specific resistance greater than 0.75 MΩ -cm. A series of capillaries which had been washed via this procedure was subjected to a second 16 h soak in triple distilled water the resistance of which had been carefully determined. No significant drop in resistance of this water was observed after the second 16 h soak, indicating that the initial water soak was effective in leaching any ionic contaminants from the capillary walls.

The initial coating experiments were based on methyl cellulose (Dow premium grade Methocel $\overline{M}_w$ = 110 000). This material had the form of a fine white powder, sparingly soluble in water at room temperature. Based on preliminary studies [9] by Micale and Vanderhoff, dilute aqueous solutions of methyl cellulose of 110 000 weight-average molecular weight were found effective in reducing the wall electro-osmotic flow by roughly a factor of 10. In the present investigation, an amphoteric silane coupling agent was used to modify the glass surface and render it more receptive to subsequent, irreversible binding of the methyl cellulose. Cleaned capillaries were typically treated with a 2-3 percent aqueous/alcoholic solution of gamma-glycidoxypropyltrimethoxysilane (Dow Corning Z-6040) followed by a vacuum bake. The treated capillaries were then soaked in 0.1 percent aqueous methyl cellulose solutions and vacuum baked a second time by the procedures described in Appendix C.

IV. RESULTS AND DISCUSSION

A. Methyl Cellulose Coating Optimization

Several parameters throughout this phase were common to all experiments. Pyrex capillaries were used exclusively in the initial work. They were uniformly cleaned by the procedure described earlier, and stored in deionized water until use. Either 0.8 or 2.0 μ diameter latex particles were used as the test particles in freshly prepared A-1 buffer.

The time of exposure of the silane-treated capillaries to methyl cellulose solutions was studied in some detail. Exposure times in 0.1 percent methyl cellulose varied from 30 s to 1 h. The specific exposure periods were always followed by a vacuum bake for 1 h/60°C and a prolonged water soak. The electro-osmotic flow as a function of methyl cellulose exposure is shown in Figure 7. It was concluded that the methyl cellulose-glass adsorption equilibrium was essentially established within 10 min exposure time.

The question of reversible versus irreversible adsorption of methyl cellulose was investigated in a similar fashion. Throughout the initial studies with latex particles, it was observed that particle electrophoretic mobility was often reduced to zero in the case of coated but unrinsed microelectrophoresis cells. The establishment of an irreversibly adsorbed coating was considered critical in view of the potential reduction in mobility of biological cells in the flight hardware due to possible preferential adsorption of the methyl cellulose. The latex particles proved to be extremely sensitive indicators of the presence of reversibly adsorbed cell wall coating, and this feature was exploited in developing post-coating water rinse procedures. The rinse conditions were varied with respect to time as well as static versus dynamic rinse. The initial experiments on dynamic rinsing involved forcing a slow stream of water through freshly coated capillaries for various time intervals. This condition was compared with a static soak over an equivalent time interval in which the water in the capillaries was replaced periodically.

There was a slight advantage in the static soak procedure, as inferred from recovery of particle μ_e to nominal values. It was necessary to verify these parameters on flight configured electrophoresis cells, and an experimental cell was constructed as shown in Figure 8.

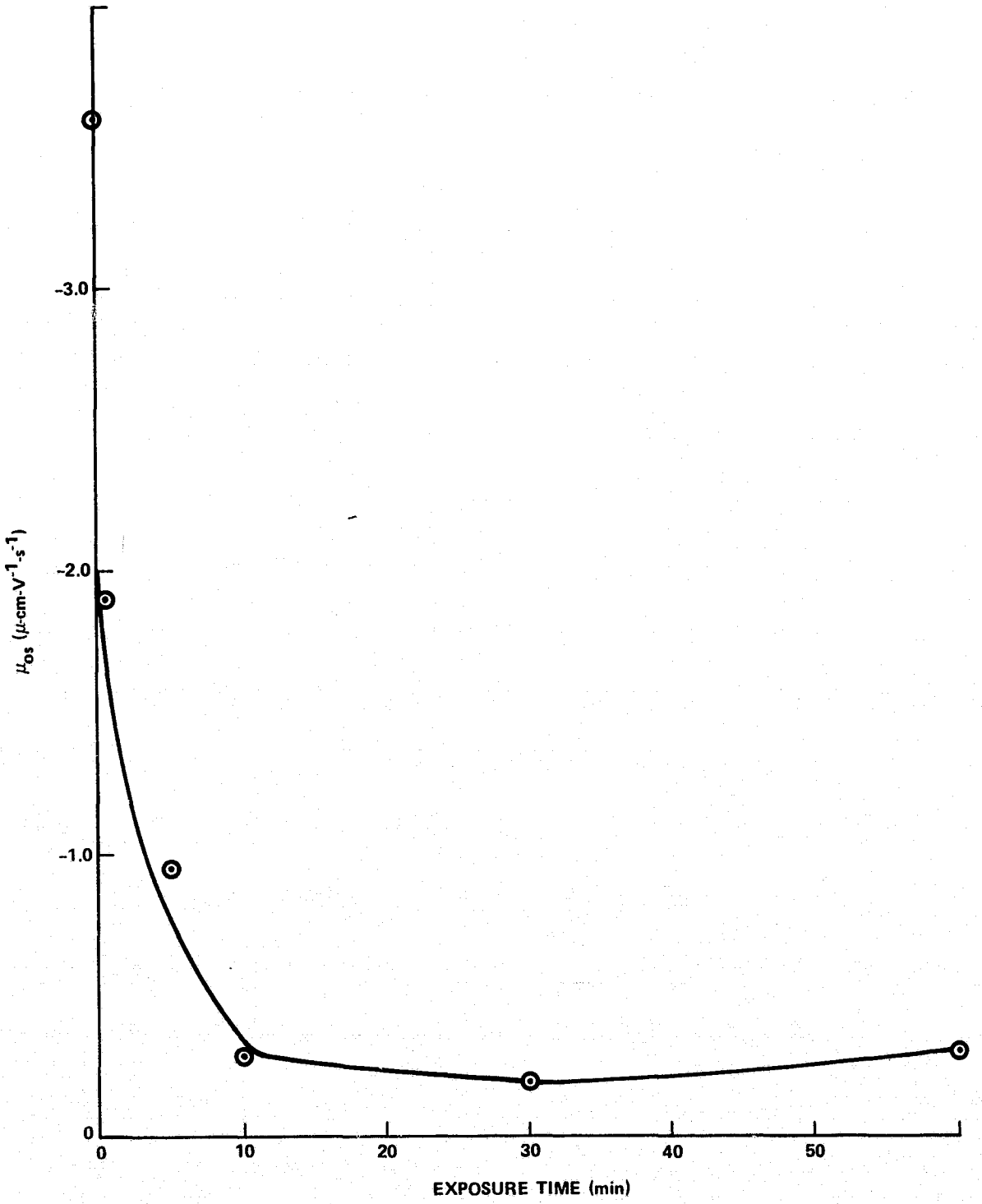


Figure 7. Electro-osmotic flow versus methyl cellulose exposure for pyrex capillaries.

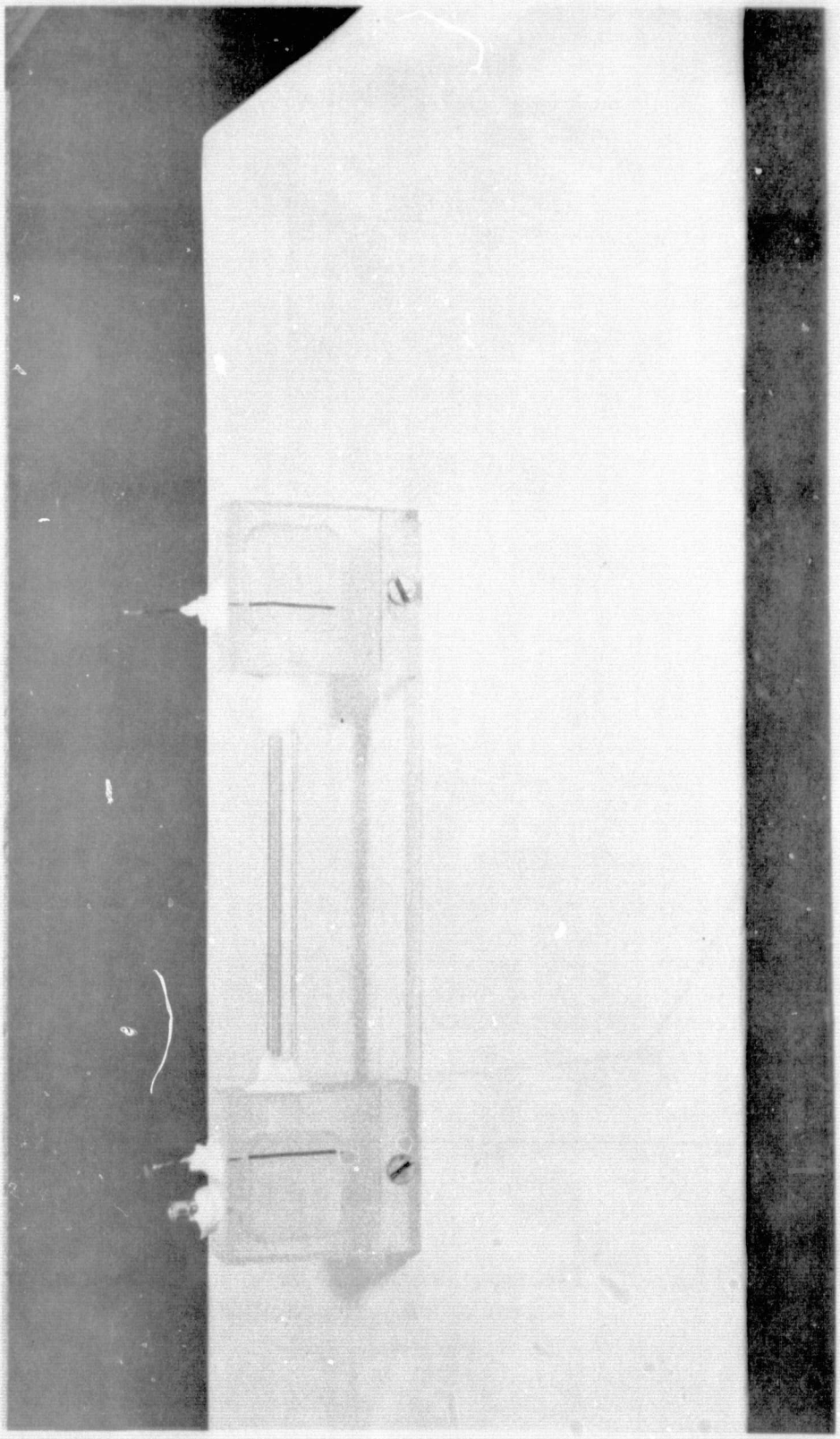


Figure 8. Flight configured electrophoresis cell.

This cell utilized pyrex glass of the same length and inner diameter as the ASTP flight columns, with plexiglas end housings bonded with RTV 731 silicone. Optical flats were ground on the tube wall to reduce wall thickness and improve visibility into the tube. It was recognized that the plano-concave column wall would introduce some refractive errors relative to the optical depth measurements. Using the original optical corrections developed by Henry [10] and modified by Brooks[1], an appropriate correction factor was derived and introduced into the computer program for mobility computation. Experimental error was further reduced in the case of the flight configured cells by utilizing the water immersion microscope objective which extended through a water-tight rubber gland into the water bath. This eliminated any refractive errors which would have been present had the optical path traversed a distance in air, through the water bath wall, and finally through the water to the cell wall.

The post-coating, static rinse procedure was repeated for a series of three cells with the results shown in Figure 9. Each data point represents an average from three cells. The three day interval represented a reasonable compromise between the approach to nominal mobility and rinse duration. The rinse water in the cells was changed eight times during the first day, four times during the second day, and twice during the third day. After studying numerous variations of this procedure, it was observed that time and, to a lesser extent, rinse water replacement controlled the rate of removal of reversibly adsorbed methyl cellulose.

After establishing the conditions necessary for removal of the reversibly adsorbed coating, the effectiveness of the remaining, more adherent portion of the coating was evaluated. A set of three flight-configured experimental cells were cleaned, coated, and rinsed according to the previously optimized procedures. The resulting values of μ_{os} were -0.12, -0.06, and 0.02 μ-cm-V^{-1}-s^{-1}. These desirably small values demonstrated that the coating materials and procedures, developed to this point, could reduce electro-osmotic flow in flight-configured cells to an exceptionally low level.

At this point, the experimental cells were modified to incorporate the RTV silicone seams which joined together the two column halves in the actual flight columns. Thus the test column halves were cleaned as before and joined with a composite RTV 140/RTV 560 silicone seam. The chemically cleaned inner surfaces of the column halves were kept scrupulously clean and free of contamination from fingerprints, dust, primer, or silicone. Cells were

1. Private communication with D. E. Brooks.

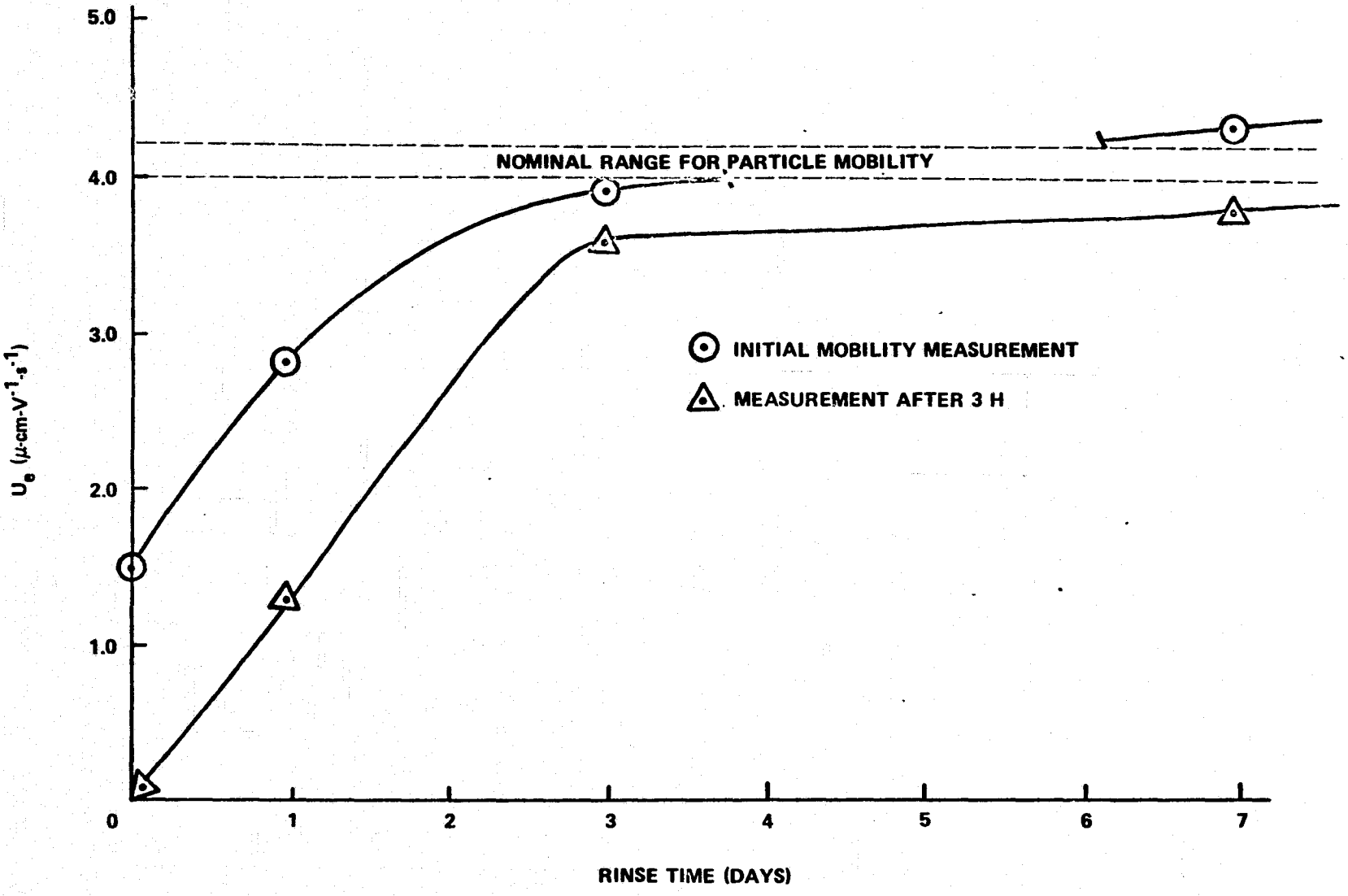


Figure 9. Electrophoretic mobility versus rinse time for flight configured cells.

assembled from these split construction columns and were appropriately coated. Mobility measurements on three of these cells resulted in μ_{os} values of -0.16, -0.15, and -0.04 μ-cm-V^{-1}-s^{-1}. This verified that the presence of the small RTV beads along the column did not result in any anomalous effect on μ_{os}.

The requirement for ethylene oxide sterilization and extended buffer exposure times of the flight electrophoresis columns necessitated assessment of the effects of sterilization conditions on μ_{os}. A group of 20 cleaned, coated capillaries was sterilized under the exact conditions specified for the electrophoresis flight columns. A set of five capillaries was removed for control μ_{os} measurements. Additional sets of five capillaries were then immersed in separate sterile A-1 buffer containers under sterile conditions and held for periods up to 28 days. The variation in μ_{os} is shown in Figure 10. Each data point is an average of μ_{os} measurements on at least three different capillaries. The flight columns were expected to be exposed to sterile buffer for 3-4 weeks before carrying out the MA-011 experiment, and the small variations in μ_{os} reflected in Figure 10 over this time frame were considered acceptable. The upper limit for μ_{os} was set somewhat arbitrarily at -0.25, since theoretical predictions by Knox [11] indicated that parabolic distortion of band geometries became severe above that value.

An additional modification of the mobility measurement protocol consisted of replacing the latex particles with human, aldehyde-fixed red blood cells (RBC). While these cells did not have the narrow mobility distribution of the latex particles, they were much larger and better suited to the flight configured columns where visibility in the field of view of the electrophoresis microscope was poor. These cells were characterized by a mean value of μ_e of -1.8 ± 0.06 μ-cm-V^{-1}-s^{-1} at 25°C in A-1 buffer. Appropriately coated, flight configured cells were filled with dilute RBC/A-1 buffer suspensions, and μ_{os} measurements were made. Values of -1.8 to -2.0 for μ_e and 0.02 to -0.08 for μ_{os} were obtained.

These measurements served not only to verify the excellent control of μ_{os} but also to confirm the accuracy of the experimental technique of measuring μ_e (1.8-2.0) by comparison with data obtained during precise stationary level measurements (1.8 ± 0.06).

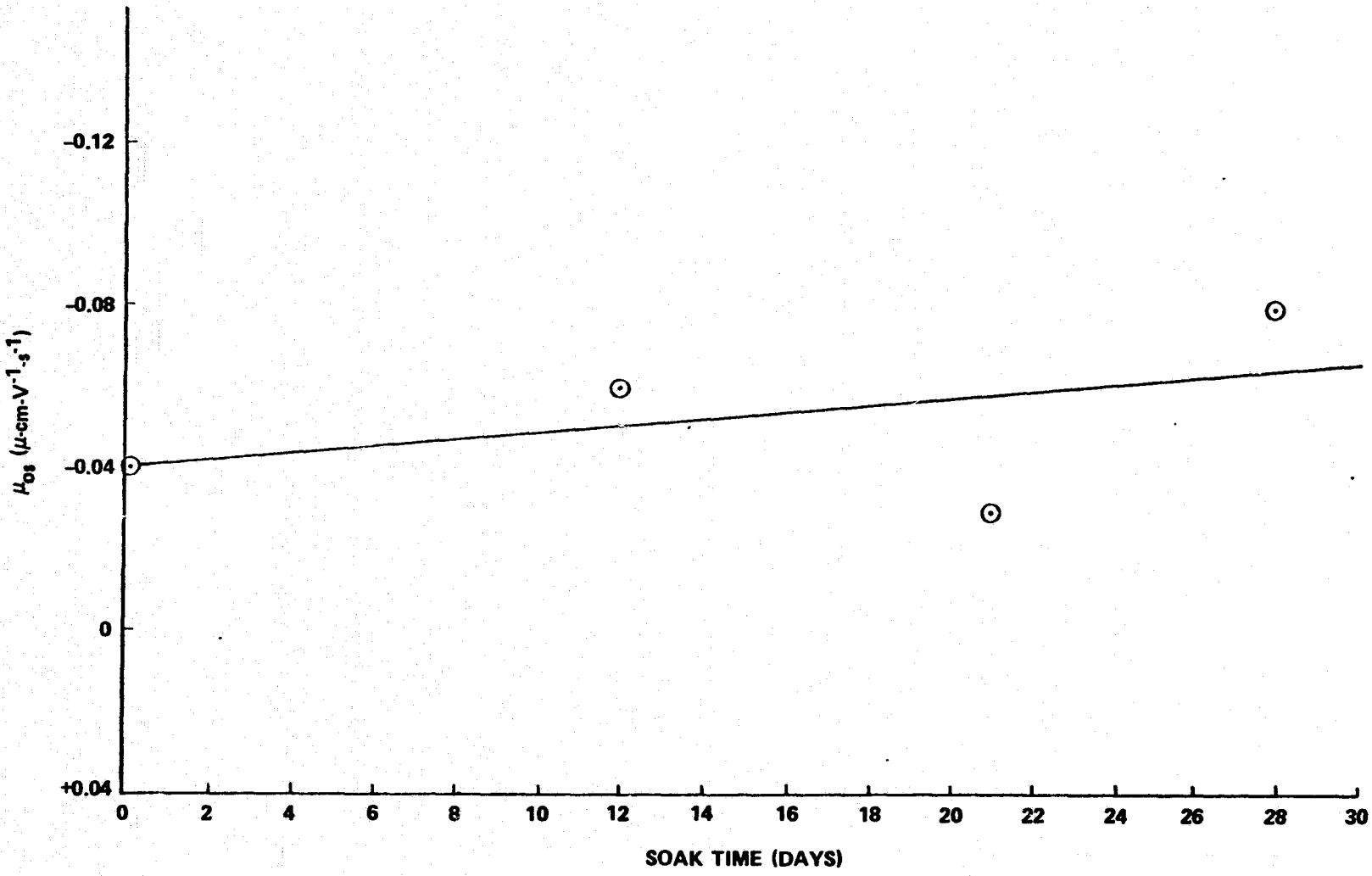


Figure 10. Effect of soak time on μ_{os} for flight configured cells.

B. Methyl Cellulose Modification Studies

Concurrent with the coating optimization studies, effort was directed toward chemical modification of the methyl cellulose polymer with appropriate positively charged groups to result in a zero or slightly positive value of electro-osmotic flow. The chemical modification of methyl cellulose was carried out under contract NAS8-30887 by Dr. R. J. Knox, University of Oregon. The methyl cellulose was alkylated under alkaline conditions with 2-chlorotriethylamine hydrochloride via nucleophilic displacement at the beta-carbon:

$$\text{(Methyl Cellulose)----OH} \xrightarrow[(2)\ \ClCH_2CH_2(CH_3CH_2)_2N\text{-H CL}]{(1)\ \text{NaOH}} \text{(Methyl Cellulose)----}OCH_2CH_2N(CH_2CH_3)_2 \ .$$

This diethylamino derivative was further alkylated at nitrogen via ethyl bromide to yield the positively charged, quaternary ammonium derivative:

$$\text{(Methyl Cellulose)----}OCH_2CH_2N(CH_2CH_3)_2 \xrightarrow{CH_3CH_2Br} \text{(Methyl Cellulose)----}OCH_2CH_2N^{(+)}(CH_2CH_3)_3 \ .$$

The random incorporation of the quaternary nitrogen along the methyl cellulose polymer chain was expected to result in a slightly positive residual wall charge with a corresponding positive value of electro-osmotic flow. A sample of this derivatized methyl cellulose was made into 0.1 percent aqueous solutions. This material was extremely water soluble as compared to very sparing solubility of the unmodified polymer. Test capillaries were coated with pure derivatized methyl cellulose, derivatized dextran, and 90/10 mixtures of methyl cellulose and derivatized methyl cellulose. The effects of silane surface modification were assessed in each case. The effects of these coatings on electro-osmotic flow at the capillary walls are summarized in Table 3. The triethylaminoethyl (TEAE) and diethylaminoethyl (DEAE) derivatives of methyl cellulose generally were characterized by a low initial μ_{os}, but the coatings rapidly desorbed from the capillary walls as evidenced by the large μ_{os} after a 72 h soak. This is interpreted as a shift in the adsorption equilibrium due to the substantially greater solubility of the derivatized materials. This observation also mitigates against direct chemical covalent bonding of substrate to coating as the primary mechanism, since a chemically bound coating should have been much less sensitive to solubility effects.

TABLE 3. RESULTS OF μ_{os} STUDIES ON MODIFIED COATINGS

Modified Coating Type	Silane	Coating Soak Time in Water (h)	μ_{os} (μ-cm-V^{-1}-s^{-1})
100% DEAE[a]	No	24	- 0.19
100% DEAE	No	72	- 1.0
100% DEAE	Yes	24	- 0.25
100% DEAE	Yes	72	- 0.45
100% TEAE[b]	No	24	- 0.05
100% TEAE	Yes	24	- 0.13
100% TEAE	Yes	72	- 0.43
90/10 MC/TEAE[c]	Yes	72	- 0.12
100% TEAE/Dextran[d]	No	24	+ 0.10
100% TEAE/Dextran	No	72	- 0.35
100% TEAE/Dextran	Yes	24	- 0.20

a. DEAE = Diethylaminoethyl — Modified Methyl Cellulose

b. TEAE = Triethylaminoethyl — Modified Methyl Cellulose

c. 90/10 MC/TEAE = 90% Methyl Cellulose, 10% TEAE — Modified Methyl Cellulose

d. TEAE/Dextran = TEAE-Modified Dextran

In the case of TEAE/dextran, an inversion of the flow parabola was observed initially in the experimental data, and this was manifested in the positive value of μ_{os}. However, this coating quickly desorbed also, and was unsuitable for any prolonged buffer exposure times. The mixtures of modified and unmodified coatings showed no noticeable advantage over the conventional coating system. It was concluded that substantial work on chemically anchoring the derivatized coatings would be required to acquire long term stability in aqueous buffer systems.

C. MA-011 Electrophoresis Test Column Coating Parameters

The methyl cellulose coating system was originally integrated into the flight electrophoresis hardware buildup as shown in Figure 11. When this phase of the program was started, the effects of column wall contamination before and subsequent to coating had not been defined. To assess this potential problem, a series of flight configured test electrophoresis columns was fabricated and processed with the prescribed cleaning/coating procedures. Figure 12 shows the coating configuration for flight columns. Five of the coated columns were selected for μ_{os} measurement, and values of -0.32, -0.30, -0.35, -0.41, and -2.1 μ-cm-V^{-1}-s^{-1} were obtained using the μ_{os} measurement configuration for flight columns depicted in Figure 13. It had been demonstrated earlier on the flight-sized experimental cells that excellent control of μ_{os} was obtainable with rigorously clean substrates, and the anomalous data on the test columns suggested that (1) either the coating was not binding effectively and had rapidly desorbed or (2) pre-coating and post-coating contamination of the column walls was increasing the electro-osmotic flow.

It was considered that contamination of the glass wall surfaces during the many post-coating column fabrications steps was the most likely suspect, and a study was carried out to assess glow discharge cleaning techniques that could be performed on the fabricated columns.

Some preliminary studies supporting this technique were performed by Dr. J. Andrade, University of Utah, under Contract NAS8-30253 [12]. The original rigorous chemical cleaning could not be considered at this point in the column fabrication due to the presence of non-metallic materials that would have been degraded by the base/acid cleaning sequence.

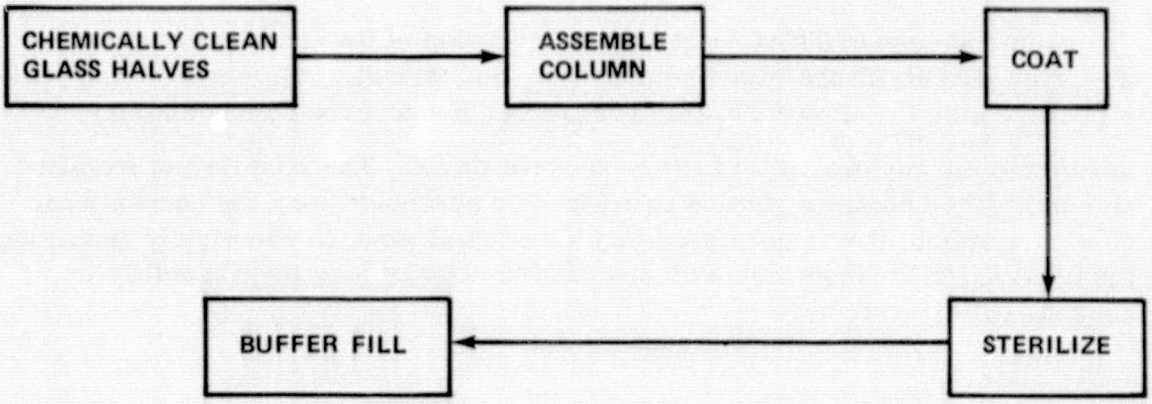
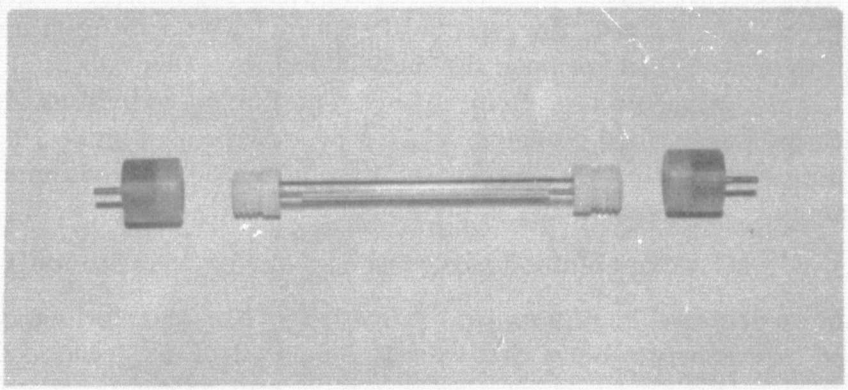
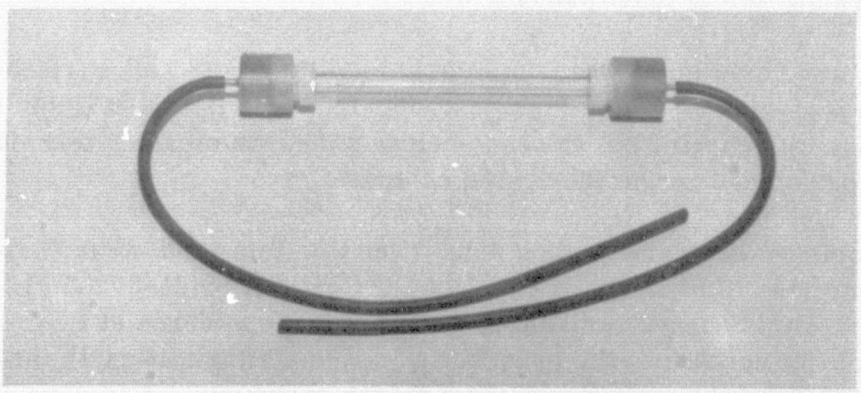


Figure 11. Original flow diagram for coating flight electrophoresis columns.

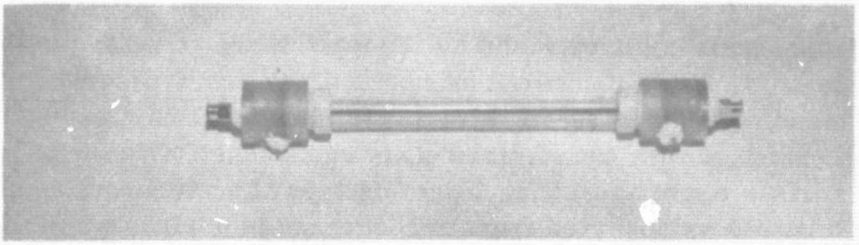


a. Lexan end blocks to provide liquid-tight system.

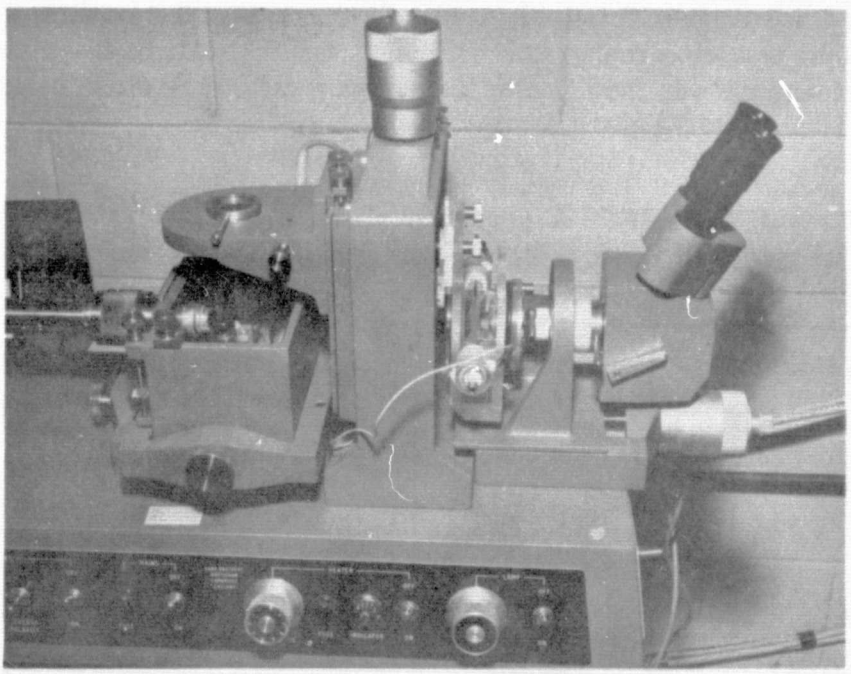


b. Ready for methyl cellulose coating.

Figure 12. Test column coating configuration.



a. Lexan electrode housings with platinum electrodes.



b. Mounted in rank instrument for measurement.

Figure 13. μ_{os} measurement configuration.

A portable Plasmod glow discharge instrument with a 13.56 MHz rf generator was utilized. This process had previously been studied to prepare substrates for adhesive bonding [13, 14] and to prepare ultraclean substrates for semiconductor research [15]. Preliminary plasma exposure tests of pyrex slides indicated substantially increased surface wettability as compared to non-exposed slides. This condition is generally associated with an ultraclean, high-energy surface, and it was judged that the similarly treated surfaces of

the electrophoresis columns would be rendered more receptive to the coatings. The parameters of glow discharge exposure time, gas pressure, and time between cleaning and coating were varied to allow minimum exposure of the columns consistent with satisfactory glass surface activation. It was found that the high surface energy resulting from this type of activation favored rapid recontamination, as observed from the decrease in surface wettability of slides with time, and from the increase in contact angle of water in pyrex capillaries with time. The optimum conditions were found to be a 3 min exposure time at 250 ± 10 μ Hg followed by immediate initiation of the coating procedure. (See Appendix C for further details.) Table 4 summarizes the results of these treatments on the test electrophoresis columns. On the basis of these studies, the coating procedure was modified to include glow discharge cleaning of flight columns immediately before coating.

TABLE 4. EFFECTS OF GLOW-DISCHARGE CLEANING TEST ON μ_{os} OF COLUMNS

Test Column	Initial μ_{os}	μ_{os} Following Glow Discharge[a]
S/N 023	-0.32	-0.25
S/N 024	-0.35	-0.22
S/N 025	-0.41	-0.17
S/N 027	-0.30	-0.22
S/N 043	-2.1	-0.21

a. Columns were glow discharge cleaned and recoated per standard procedure prior to making these measurements.

D. MA-011 Electrophoresis Flight Column Coating Protocol

Based on the nonuniform and prohibitively high μ_{os} values obtained for the test electrophoresis columns, and the large degree of uncertainty concerning ultimate levels of contamination in finished column assemblies, it was decided to not only glow discharge clean all flight columns before coating, but also to measure, using experimental microelectrophoresis procedures, the values of μ_{os} for each column.

Fixed RBC were chosen as the test particle for these measurements. The details of the measurement procedure are given in Appendix D. The initial measurements were carried out on 18 columns designated as flight, flight-backup, and spares. The resulting μ_{os} values for these columns were a critical factor in selecting the six columns for the actual flight experiment. Columns which did not fall within the μ_{os} range of 0 to -0.25 μ-cm-V^{-1}-s^{-1} were eligible for up to two additional recycles through the coating sequence as illustrated in Figure 14. By this procedure the entire complement of 18 columns was coated and determined to have reasonably low values of μ_{os}. Table 5 summarizes the actual results of this coating protocol. At least six columns with μ_{os} of -0.18 or less were available from this hardware for a flight set.

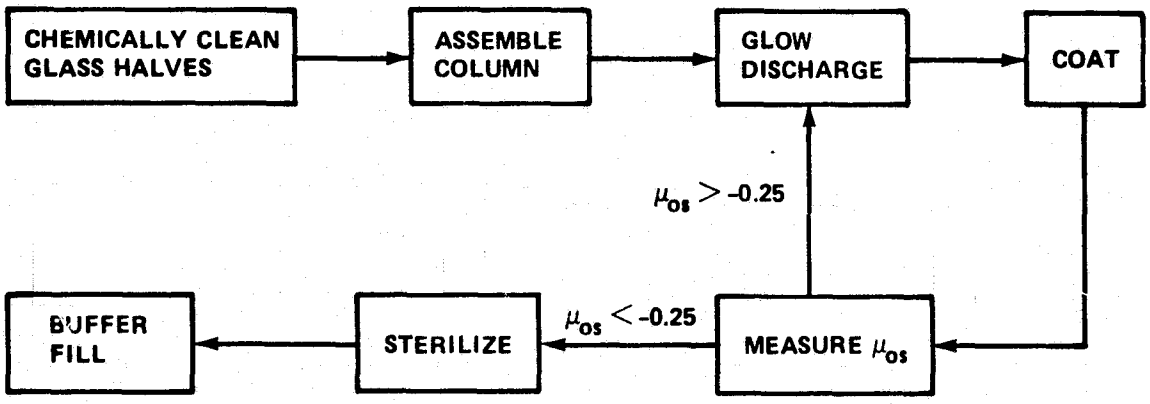


Figure 14. Flow diagram for final coating assessment of flight electrophoresis columns.

Figures 15 and 16 illustrate two of the ASTP MA-011 separations in progress, where the advancing band fronts are virtually free of parabolic distortion. This provides direct visual verification of the effectiveness of this coating in minimizing electro-osmotic flow in the flight columns.

TABLE 5. FINAL ELECTRO-OSMOTIC FLOW VALUES FOR FLIGHT ELECTROPHORESIS COLUMNS

Column S/N	μ_{os} (μ-cm-V^{-1}-s^{-1})
045	-0.22
046	-0.13
047	-0.11
048	-0.21
049	-0.29
050	-0.25
051	-0.13
052	-0.17
053	-0.19
064	-0.22
065	-0.17
066	-0.24
067	-0.34
068	-0.18
069	-0.26
071	-0.38
072	-0.24
073	-0.28

V. CONCLUSIONS

It has been demonstrated that the experimental coating systems based on methyl cellulose can effectively control electro-osmotic flow (μ_{os}) in experimental cells and in flight electrophoresis columns. This reduction of μ_{os} at the column walls to near zero, with the resulting distortion-free band geometries, provides optimum conditions for zero-gravity separation and collection of biological fractions in static electrophoresis columns.

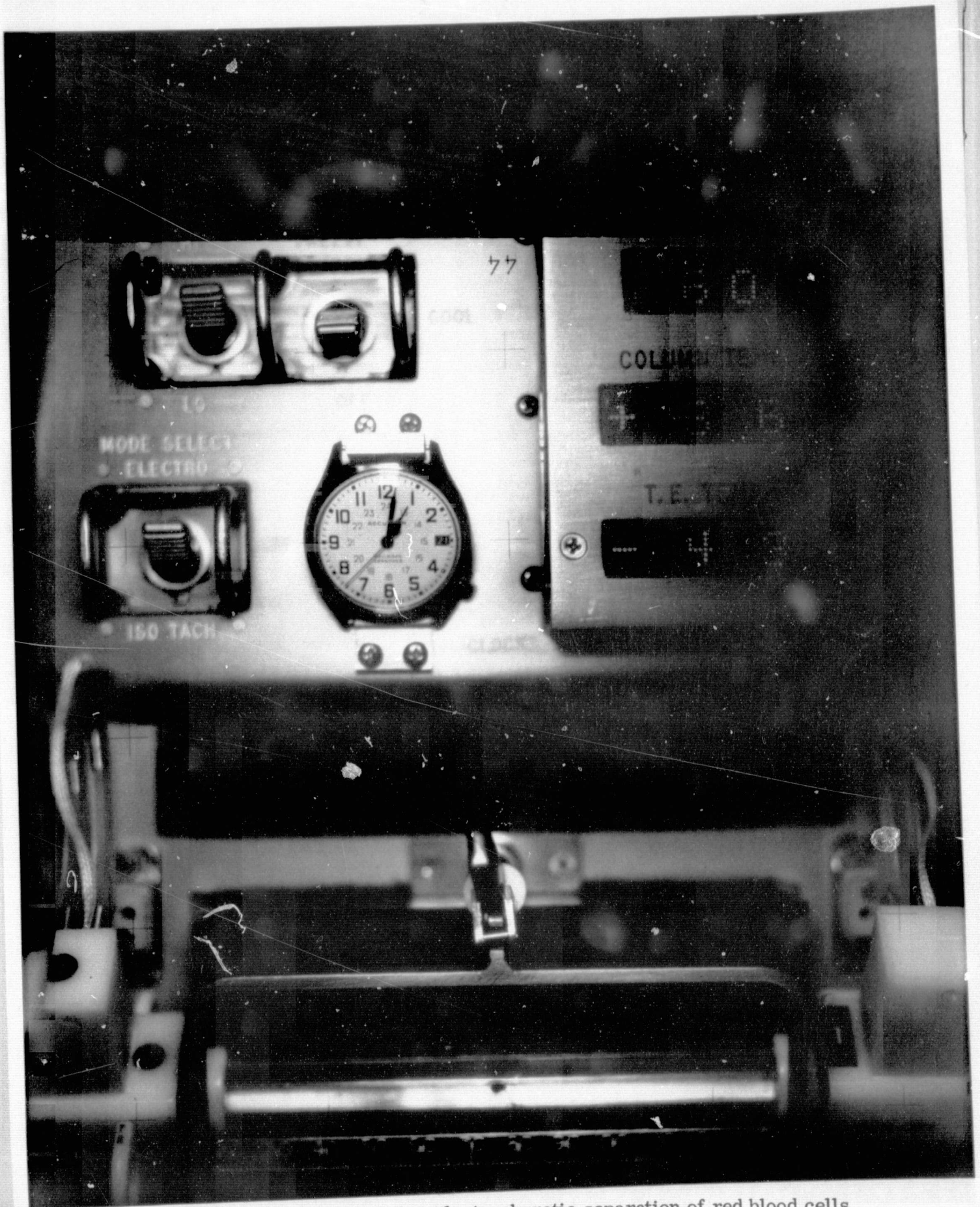


Figure 15. ASTP MA-011 electrophoretic separation of red blood cells.

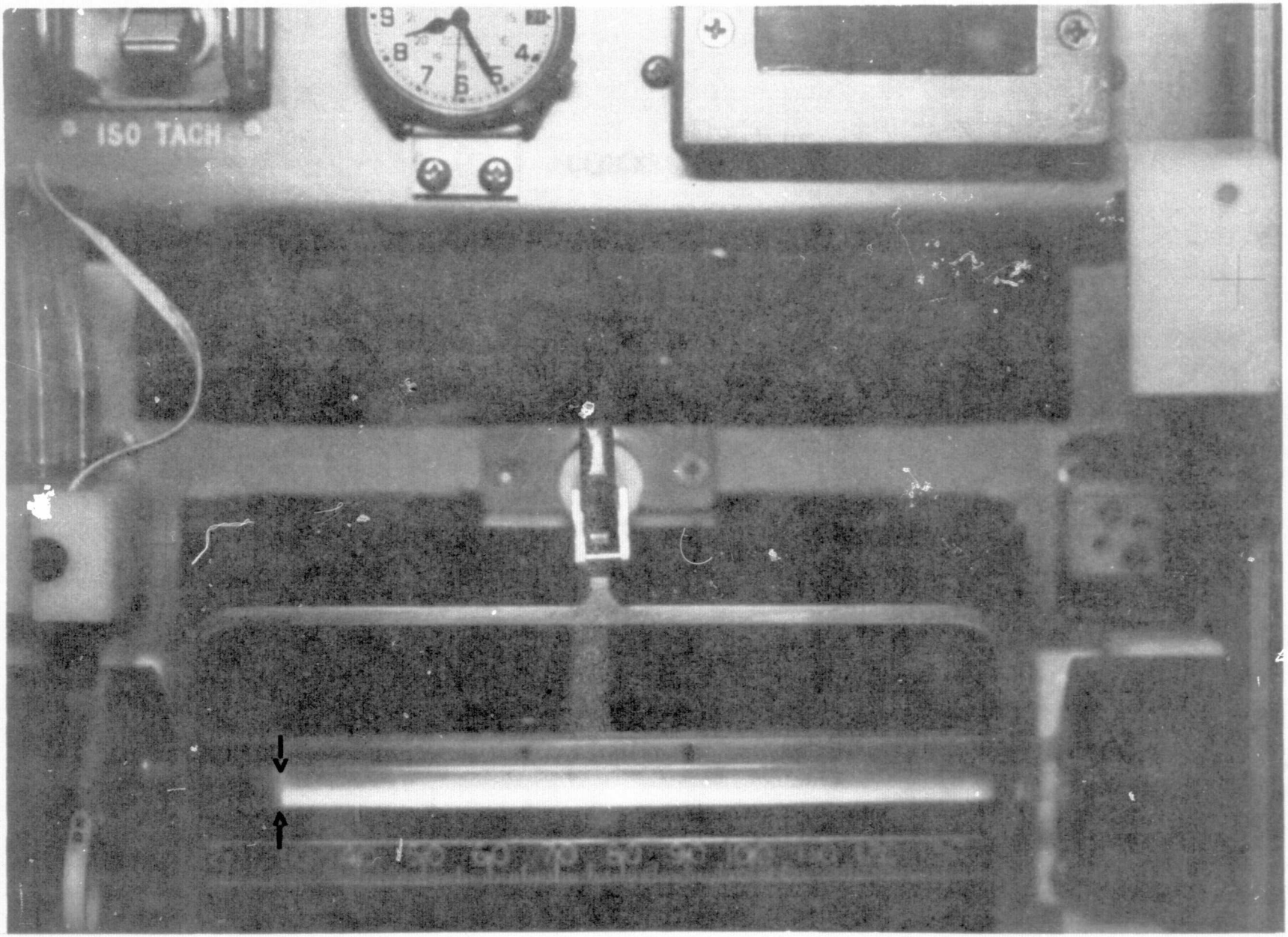


Figure 16. ASTP MA-011 isotachophoretic separation of red blood cells.

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APPENDIX A

ELECTROKINETIC MOBILITY COMPUTATION PROGRAM E004 FOR CYLINDRICAL FLIGHT COLUMNS

This program computes, prints, applies correction factors, performs statistical error analysis, and plots all desired mobility and statistical information from basic inputs of fixed and stored experimental parameters, direct microscope micrometer readings, and raw particle traverse time data for cylindrical flight columns. The program was prepared for the HP2100B programmable calculator with extended memory, printer and x-y plotter. It first initializes its registers and stores self-generated constants, then accepts the following parameters from which additional operational constants are computed and stored: f, v, and L, where

f is the actual microscope micrometer reading at the front wall of the cell

v is the interelectrode potential difference (in V).

L is the interelectrode distance (in cm).

Program constants calculated with these inputs include:

D_m, a reference figure representing the column axis

K, a value which includes v, L, and a correction factor for particle traverse times taken with either 60 Hz AC time or 50 Hz AC time

$h_{e\phi}$, the stationary level.

Self-generated constants used by the program are relatively unvarying and are not requested by the calculator as keyboard inputs, for the sake of minimizing required operator action. These include graphic constants, indices of refraction, the flight column radius, and similar parameters.

The program then computes and plots in succession each set of particle mean mobility (μ_{H_i}) versus the square of the distance (h_i^2) from the axis of

the column[1] using raw micrometer and time data inputs. The Henry correction for the nonlinear displacement of the apparent focal position relative to the real position along the column radius of the cylindrical columns is computed and applied to each micrometer setting. The calculated values are plotted on an x-y recorder and the plus and minus standard deviation (σ_μ) is drawn from the mobility data contributing to each point (as a delimited vertical pen stroke through the point).

The relations used for this program are

$$h_i^2 = (H_i - D_m)^2 \quad ,$$

where H_i is the radial position in the cell where the traverse time data are taken (expressed in mm); then each

$$\mu_i = \frac{K}{t_n} \quad ,$$

and

$$\bar{\mu}_{H_i} = \sum^{\mu} \frac{i}{n_t} \quad ,$$

where t_n is one time reading (in seconds) from the set of time readings associated with one H_i data set. The total number of time readings in that data set is n_t, and μ_i is a particle mobility value computed for each t_n time reading in an H_i data set. $\pm\sigma_\mu$ is then computed as

$$\pm \sqrt{\frac{1}{(n_t - 1)} \left[\sum \mu_i^2 - \frac{\left(\sum \mu_i\right)^2}{n_t} \right]} \quad .$$

1. The square of the distance is used to linearize the parabola.

Following this, the program fits a least squares computed straight line through the plotted data points, then computes and plots two additional significant data points where $x_i = h_w^2$, which is the square of the radius, and $x_i = h_{e\phi}^2$, which is the stationary level.

Finally the program computes and prints (a) the electro-osmotic flow μ_{os}; (b) the standard deviation of the mean mobilities from regression, $s_{(y \cdot x)}$; (c) the standard deviation of the electro-osmotic flow, $s_{(\mu_{os})}$; (d) the standard deviation of the regression coefficient, $s_{(a_1)}$; and (e) the standard variation of the slope, v_s. The latter two figures are measures of "goodness of fit" and of confidence in the least squares straight line with respect to the overall data inputs contributing to the least squares computation.

In fitting the least squares straight line to the data points (each point being represented by $x_i = h_i^2$ and $y_i = \mu_{H_i}$), the equation

$$y = a_o + a_1 x$$

is used where the slope

$$a_1 = \frac{\sum x_i \sum y_i - n_{H_i} (\sum x_i y_i)}{\sum x_i \sum x_i - n_{H_i} \sum x_i^2}$$

and

$$a_o = \frac{\sum y_i - a_1 \sum x_i}{n_{H_i}} \quad .$$

Here, n_{H_i} is used to denote the total number of H_i data sets entered into the program (the total number of data points). The variables x_i and y_i take on the specific values of h_i^2 and $\bar{\mu}_{H_i}$ for the several points.

As previously stated, the two significant points, (h_w^2, μ_w and h_{eo}^2, μ_{eo}), are plotted where $h_w^2 = r^2$ and $h_{eo}^2 = r_{eo}^2$. The respective mobility values are used in the equation $\mu_{os} = \mu_w - \mu_{eo}$, where μ_{os} is the electro-osmotic flow. These two points are determined by direct substitution in the least squares equation, after a_1 and a_o are determined (i.e., $\mu_w = a_o + a_1$, and $\mu_{eo} = a_o + a_1 r_{eo}^2$).

Few investigators would pursue the tedious task of calculating goodness of fit indicators by hand. The programmable calculator makes it a natural and easy concluding operation. The sum of squares of deviations from the mean at all data points is defined as $\sum d^2_{(y \cdot x)}$. This is read as the sum of the squares of deviations from the regression of y on x, and is the basis for an estimate of error or goodness of fit in fitting the least squares line to the given data points, the total number of which is n_H. The number of "degrees of freedom" in fitting a straight line is $(n_H - 2)$, because it takes any two points to define a straight line absolutely. Therefore, the mean square deviation from regression is given as:

$$s^2_{(y \cdot x)} = \sum \frac{d^2_{(y \cdot x)}}{(n_H - 2)} .$$

The square root of this expression is the standard deviation from regression of the data points. The standard deviation from regression of μ_{os}, given the symbol $s_{\mu_{os}}$, is then $2s_{(y \cdot x)}$. The standard deviation of the regression coefficient, or slope term, is

$$sa_1 = \frac{s_{(y \cdot x)}}{\sqrt{\sum (x_i - x)^2}}$$

The standard variation of the slope of the line at any point can be defined as $V_s = sa_1/a_1$. Referring to the previous equation for sa_1, it can be seen that the various sums required for the analysis of fit reflect a mean x and y (written as $\bar{x}$ and $\bar{y}$). Such means are determined either by the least squares final equation, or by summing all x' s and y's for all the data points and dividing by n_{H_i}.

These means have to be subtracted from each individual x_i and y_i. Such a method requires information on each individual data point which is not retained in the computer. Due to the nature of the program, the points lose their individual identities throughout the subroutines. Therefore, some "short cut" identities are employed which do not require point by point information, but need only accumulated sums of ordinate and abscissa values, their squares, and their products. $\sum d^2_{(y \cdot x)}$, from page , is defined as

$$\frac{\sum y^2 - (\sum xy)^2}{\sum x^2}$$

where

$$y = (y_i - \bar{y}_i)$$

and

$$x = (x_i - \bar{x}_i)$$

The substituted identities are as follows:

$$\sum x^2 = \frac{\sum x_i^2 - \left(\sum x_i\right)^2}{n_{H_i}}$$

$$\sum y^2 = \frac{\sum y_i^2 - \left(\sum y_i\right)^2}{n_{H_i}}$$

$$\sum xy = \frac{\sum x_i y_i - \left(\sum x_i\right)\left(\sum y_i\right)}{n_{H_i}}$$

where x_i and y_i represent, as before, computed h_i^2 and μ_i values. All these accumulated sums of squares and products are available from program memory where they were stored for use by the least squares computations.

As a summary of the use of these reliability indicators, recall that μ_{os} was defined and calculated arithmetically as $(\mu_w - \mu_{eo})$. It was decided that a worst case condition for the standard deviation (from regression) of μ_{os} can be represented as $2s_{(y \cdot x)}$. The rationale for this follows: The standard deviation from regression at each of the two points (h_w^2, μ_w) and $(h_{e\phi}^2, \mu_{eo})$ is $s_{(y \cdot x)}$. The arithmetic operation of subtracting μ_{eo} from μ_w yields a value for μ_{os} which must be considered as no more reliable than $\pm 2s_{(y \cdot x)}$.

In considering V_s, it should be remembered that $d_{(y \cdot x)}$ alone is a measure of the failure of the line to fit the data at any single point, and s_{al} is the standard deviation of the regression coefficient of the least squares line. From the beginning, the standard variation of the slope (or regression), V_s, was computed and printed out in decimal form. Multiplying the figure by 100 would yield a percent "standard variation" in the slope to be expected all along the fitted least squares line as a result of the data point spread.

This program replaces a laborious manual data reduction and plotting technique which used to take several hours including deliberate manual repetitions of all calculations to assure error-free results. The program performs its calculations, printing, and curve plotting in a few seconds, during and immediately following data input. Also, uniformity and direct comparability of the curves generated for each run are guaranteed due to the standardized least squares technique.

APPENDIX B

CLEANING PROCEDURE

1.0 PURPOSE

The purpose of this procedure is to insure a clean and uniform glass surface on the inner wall of electrophoresis column assemblies for use in ASTP MA-011.

2.0 MATERIALS REQUIRED

Item	Quantity	Purity	Source
Nitric acid, concentrated	1 gal	Reagent grade	Fisher Chemicals Co., Atlanta, GA
Hydrochloric acid, concentrated	1 gal	Reagent grade	Fisher Chemicals Co., Atlanta, GA
Sodium hydroxide	1 lb	Reagent grade	Fisher Chemicals Co., Atlanta, GA
Ethyl alcohol	1 gal	Reagent grade	Fisher Chemicals Co., Atlanta, GA
Magnetic stirrer	1	n/a	M& P Lab
Teflon stirring jar	1	n/a	M& P Lab
Ultrasonic bath	1	n/a	M& P Lab
Pyres tubes	50 ea	n/a	M& P Lab
Deionized water	as required	greater than 0.75 megohm - cm specific resistance	M& P Lab
Glass stir. rod	4	n/a	M& P Lab

Item	Quantity	Purity	Source
Erlenmeyer flask	1	2 liter vol.	M&P Lab
Erlenmeyer flask	1	1 liter vol.	M&P Lab

NOTE: All of the cleaning operations shall be carried out in a laminar flow bench with the exception of those of paragraphs 3.5 and 3.6, dealing with the use of aqua regia as a cleaning agent. These specific operations shall be performed in a clean fume hood to exhaust the potentially toxic fumes generated by the aqua regia and maintain safe working conditions. The aqua regia shall be handled only when wearing rubber gloves, rubber aprons, and eye protection. These operations shall be carried out in an area which has eye wash and acid shower facilities.

3.0 CLEANING OF COLUMNS

3.1 The individual column halves are each sonicated for 2 minutes in a deionized water/soap solution (use liquid detergent, such as Joy), using 10 drops of detergent per liter of water.

3.2 Each part is then thoroughly rinsed in dionized water from a squeeze bottle, and inspected to assure that all glass grindings have been removed from the column surface.

3.3 Each column half is then placed in an individual tube and a previously prepared alcoholic sodium hydroxide solution (see note) is carefully poured into the tubes, until the column half is covered. The parts are allowed to soak in this solution for 1 hour.

NOTE: The ethyl alcohol/sodium hydroxide solution is prepared using a 2-liter Erlenmeyer flask, teflon stirring bar, and magnetic stirrer. Add 120 gm of sodium hydroxide to the flask. Then add a mixture of 120 ml deionized water and 700 ml ethyl alcohol. Stir for 1 hour. This solution may darken on standing, but its cleaning capacity will not be affected.

CAUTION: This is a strong caustic solution; use rubber gloves, safety glasses, and protective clothing.

3.4 The cleaning solution is then decanted from the tubes containing the column halves by pouring it back into the cleaning solution flask. While pouring off the solution, carefully hold the column half in place with a glass stirring rod. The individual column halves are thoroughly rinsed in deionized water and laid on a paper towel.

3.5 The column halves are then carefully placed back into separate clean glass tubes and aqua regia (see note) is added carefully to the tubes until the column halves are covered. The parts are then allowed to soak for 1 hour.

NOTE: Aqua regia is prepared by pouring 410 ml of concentrated hydrochloric acid and 90 ml of concentrated nitric acid into a clean, dry 1-liter Erlenmeyer flask and by swirling the flask gently until the acids are thoroughly mixed. This mixture will turn yellow on standing, but its cleaning capacity is not affected.

CAUTION — STRONG ACID: Use in a fume hood, with appropriate protective equipment.

3.6 Carefully pour off aqua regia from each tube back into the acid container, while holding each column half in place with a glass stirring rod. Refill each tube with deionized water and pour this rinse water out, again holding the column half in place with the rod. Repeat this procedure once more, remove the column half and rinse each column half thoroughly with deionized water from the squeeze bottle (use at least 1/2 the volume of the squeeze bottle on each column half).

3.7 Place each column half in a separate suitable glass container or tube (use gloves) and cover the column half with deionized water. Allow the column half to soak in the water at least 16 hours.

3.8 Carefully remove the column halves from the containers (use gloves, do not touch column halves with bare hands) and flush each part once more with water. Allow excess water to drain off, and carefully place the parts on a rack in a vacuum oven. Two layers of paper towels are laid over the column halves so that all glass surfaces are completely covered. Vacuum dry the parts at 60° ± 5° C, at 5 torr pressure or lower, for a minimum of 4 hours. Very slowly bleed the oven back to atmospheric pressure, taking care not to dislodge the paper towels. Using gloves, transfer each column half to a separate polyethylene bag (with zip lock top) which has been marked to allow identification and traceability of the parts, and includes all quality control documentation. Satisfactory completion of the cleaning operation shall be appropriately noted on this documentation by Q& RA personnel.

APPENDIX C

COATING PROCEDURE

1.0 PURPOSE

The purpose of this procedure is to assure proper and uniform coating of the MA-011 columns with a polymeric material to result in minimum electro-osmotic flow within the columns. This procedure is to be implemented for MA-011 column assemblies in the ASTP MA-011 Flight Experiment during July 1975.

2.0 MATERIALS REQUIRED

Item	Quantity	Purity	Source
White cotton gloves	20 pr	n/a	M& P Laboratory
Clamps	6 ea	n/a	M& P Laboratory
Glacial acetic acid	1 pt	Reagent grade	M& P Laboratory
Isopropyl alcohol	1 pt	Reagent grade	M& P Laboratory
Z-6040 silane coating	1 pt	Reagent grade	Peninsular Chemical Research, Inc. Gainesville, Florida
Silicone tubing	30 ft	n/a	M& P Laboratory
Methyl cellulose, 110 000 molecular wt.	5 gm	purified	LeHigh University Bethlehem, PA
Gray teflon tape	1 roll (1 in. wide)		M& P Laboratory

Item	Quantity	Purity	Source
50 ml glass syringes	4	n/a	M& P Laboratory
250 ml polypropylene disposal beakers	50	n/a	M& P Laboratory
Methyl alcohol	1 gal	Reagent grade	M& P Laboratory
200 ml Erlenmeyer flask	1	n/a	M& P Laboratory
Clamps for ring stand	6	n/a	M& P Laboratory
Magnetic stirrer	1	n/a	M& P Laboratory
Teflon stirring bar	1	n/a	M& P Laboratory
Ring stands	6 ea	n/a	M& P Laboratory
O-rings, viton rubber	100	n/a	M& P Laboratory
Column end-blocks	12	Lexan	M& P Laboratory
Plasmod Glow Discharge Apparatus	1		M& P Laboratory

NOTE: All operations shall be performed in a laminar flow bench, with the exception of weighing operations involved in preparation of the two coating solutions described in paragraphs 4.4 and 4.1. All handling of the column shall be with disposable white cloth gloves.

3.0 GLOW DISCHARGE PRECLEANING OF COLUMNS

3.1 Connect the plasmod glow discharge apparatus to a vacuum pump and pump the vacuum lines and gauge down to at least 50 microns Hg pressure, with the vacuum inlet solenoid switch off.

3.2 Place an electrophoresis column (without electrode housings or O-rings) on an appropriate glass tray inside the removable vacuum chamber jar. Mount the column such that it is centered horizontally and the column end extends 8 ± 2 mm beyond the open end of the chamber jar.

3.3 Carefully slide the jar with the column inside the fixed vacuum chamber until the red RTV seal contacts the lip of the chamber. Position the jar relative to the chamber lip so that the silicone seal extends past the lip equally around its circumference.

3.4 While maintaining gentle hand pressure on the sealing surfaces, turn on the vacuum inlet solenoid switch, close the air bleed line, and close the instrument door.

3.5 Allow the system to pump down to approximately 250 microns, and open the air bleed valve until the pressure stabilizes at 250 ± 10 microns.

3.6 Note the time and turn on the RF discharge switch, set the power at 50 watts, and tune the instrument with the tuning knob until the pink color of the glow discharge is at maximum intensity. The tuning operation may cause a power setting change. If so, reset the power to 50 watts and retune. An initial pressure increase will be observed, which will slowly drop during the run.

3.7 Continue this process for 3 minutes, then turn off the RF switch and the solenoid switch.

NOTE: Immediately after closing the solenoid switch open the instrument door and hold the chamber jar manually to prevent movement as the chamber pressurizes.

3.8 Carefully remove the chamber jar and remove the column using gloves.

3.9 Quickly proceed to Section 4. Do not allow more than 2 minutes between removal of column from the glow discharge chamber and application of the Z-6040 solution.

4.0 Z-6040 COATING OF COLUMNS

4.1 Prepare the Z-6040 solution (paragraph 4.5) before the first column is glow discharged. Fit the column with O-rings available for this purpose.

NOTE: Do not use flight O-rings supplied with column.

4.2 Carefully screw one of the lexan end blocks available for this operation onto each end of the first column until the O-ring seats firmly in the end block.

NOTE: Do not apply torque to column. Hold the delrin sleeve on the end being capped while threading on the lexan end block.

4.3 Position the first column vertically with padded clamps attached to ring stands. Each end block is equipped with flexible tubing.

4.4 Fill a 50 ml syringe with 50 ml of deionized water and connect to the tubing on the lower lexan and block. Force the water through the column and allow it to flow into a waste container. Then remove the syringe, allow the column to empty into a waste container, and quickly proceed to fill the first column with Z-6040 solution.

NOTE: Carefully observe the wetting characteristics of the column wall during this procedure. If any areas of the glass (excepting the RTV seam) appear to be nonwetting, this condition shall be noted in the column log book.

4.5 Prepare Z-6040 coating solution as follows:

Measure 100 ml of methyl alcohol in a graduate cylinder and add to a clean beaker. Measure 25 ml deionized water and add to a clean beaker. Stir thoroughly. Place the beaker on a balance (which will weigh to the nearest 0.1 gm) and add 3.1 gm Z-6040 coating agent. Stir thoroughly.

NOTE: Keep Z-6040 tightly capped when not in use and carry out weighing process quickly to minimize exposure of Z-6040 to air.

Add 1 drop of glacial acetic acid from an eye dropper to the beaker. Stir thoroughly and cover tightly with aluminum foil. The solution is now ready to apply.

NOTE: This solution must be made up fresh for each batch of columns to be coated and cannot be used if allowed to stand more than 45 minutes.

4.6 Draw the solution into a clean 50 ml syringe and connect it to the tubing extending from the lower end block. Slowly force the solution up through the column assembly until the liquid level extends into the tubing attached to the upper end block. Close the tubing between lower end block and the syringe with a hose clamp. Remove the syringe and initiate the glow discharge procedure on the second column. Continue this sequence until all columns have been glow discharged.

4.7 Allow the solution to remain in each column for 10 minutes, then open the clamps and drain into a waste container. Reattach the syringe, refill the column as before, and close the tubing with the hose clamps. Continue this process until each column has been exposed to three consecutive 10-minute soak periods, each time using fresh solution.

4.8 Allow the columns to drain following the last soak period. Attach a dry syringe to the lower end block tubing and apply suction for several seconds to pull out residual solution. Remove column assembly from the clamp, place it horizontally on a paper towel, and carefully remove the end blocks, again holding the appropriate delrin sleeve (not the glass portion) to allow end block removal.

4.9 Carefully remove the O-rings from the column ends and blot the ends with a paper towel. Direct a jet of low pressure, pure helium gas through the column for several seconds (maximum 5 seconds to aid in removal of residual droplets). Inspect the delrin sleeve and assure that no solution has leaked onto the threaded surface.

4.10 Place the columns horizontally on a rack and dry in a vacuum oven at 60 ± 5°C and 5 torr pressure (or lower) for a minimum of 1 hour.

4.11 Remove the columns from the vacuum oven and allow them to return to room temperature.

5.0 METHYL CELLULOSE COATING OF COLUMNS

5.1 Replace the O-rings in the column ends and reassemble the lexan end blocks as previously described in paragraphs 4.1, 4.2, and 4.3.

5.2 The methyl cellulose solution is prepared in advance and can be stored for periods long enough to coat several column batches coming through the fabrication sequence. A 2-liter Erlenmeyer flask is carefully cleaned and rinsed several times with deionized water. Methyl cellulose, 1.0 gm, is weighed and transferred to the flask. Then 1 liter of deionized water is added. The mixture is stirred with a magnetic stirrer for 24 hours or longer if the methyl cellulose is not completely in solution. The flask is capped with a ground glass stopper.

5.3 Expose the inside walls of the columns to the methyl cellulose solution and vacuum dry as described in paragraphs 4.6, 4.7, 4.8, 4.9, 4.10, and 4.11, with the following deviation:

When carrying out the procedures described in paragraph 4.7, expose the columns to only one 10 minute soak with methyl cellulose.

6.0 WATER FLUSH OF COATED COLUMNS

6.1 Replace the O-rings in the column ends and reassemble the lexan end blocks as described in paragraphs 4.1, 4.2, and 4.3.

6.2 Replace the rubber tubing on the top and bottom lexan end blocks and mount the columns as described in paragraph 4.2. Using a 50 ml syringe, add deionized water through the bottom end block until it is full. Allow the filled columns to remain undisturbed for 1 hour.

6.3 Drain the columns, reclamp the bottom hose, refill the columns with fresh deionized water and allow to soak for a second 1 hour period. Continue this fill and drain cycle every hour for 8 hours. Then fill the column and allow to soak for 16 hours. This constitutes one 24 hour cycle. The first 24 hour cycle following the coating procedure must contain at least 8 of the fill, 1 hour soak, and drain sequences. It is desirable, but not mandatory, that the first 24 hour rinse cycle be carried out as follows:

8 am:	Fill
9 am:	Drain and Refill
10 am:	Drain and Refill
11 am:	Drain and Refill
12 noon:	Drain and Refill
1 pm:	Drain and Refill
2 pm:	Drain and Refill
3 pm:	Drain and Refill
4 pm:	Drain and Refill
8 am:	Drain (Following Day)

6.4 The second 24 hour rinse cycle shall be performed similarly, but with 2 hour soak periods between water changes, which would then occur at 10 am, 12 noon, 2 pm, 4 pm, and at 8 am the following morning.

6.5 Third 24 hour rinse cycle shall be performed similarly but with 4-hour soak times between water changes, which would then occur at 12 noon, 4 pm, and at 8 am the following morning.

NOTE: The time sequences outlined in paragraphs 6.2, 6.3, and 6.4 are desirable guides. If necessary, the soak sequences may be varied as long as the following criteria are met:

First 4 hour cycle: Eight water changes, with eight 1 hour soak periods.

Second 24 hour cycle: Four water changes, with four 2 hour soak periods.

Third 24 hour cycle: Two water changes, with two 4 hour soak periods.

If necessary, the columns may be allowed to soak for a maximum of 5 days, but in no case shall the soak time be less than the previously described, three 24 hour cycles.

6.6 Perform the operations described in paragraphs 4.8, 4.9, 4.10, and 4.11, with the deletion of the gas blowing step in paragraph 4.9.

NOTE: Small, random spots from irregular layering of the coating materials (not to exceed 1 mm in diameter) do not affect the coating performance and shall not be cause for rejection of the coated columns.

6.7 Transfer each column to a separate sealable polyethylene bag which has been marked to allow identification and traceability of the parts and includes all quality control documentation.

Satisfactory completion of the coating operation shall be appropriately noted on this documentation by Q& RA personnel. These bagged columns shall not be exposed to environmental stresses more than 50 to 70 percent humidity, 70 to 80°F, and subsequent handling shall be as required for flight hardware.

APPENDIX D

PROCEDURE FOR MEASUREMENT OF ELECTRO-OSMOTIC FLOW (μ_{os}) ON MA-011 FLIGHT ELECTROPHORESIS COLUMNS

1.0 SCOPE

This appendix describes the procedures to be used in measurement of electro-osmotic flow in electrophoresis columns on ASTP Electrophoresis Technology Experiment MA-011. The test program is intended to determine the ability of the column coatings to minimize electro-osmotic flow at the column walls.

2.0 TEST SPECIMENS

The hardware consists of appropriately coated electrophoresis flight columns.

3.0 MEASUREMENT OF ELECTRO-OSMOTIC FLOW (μ_{os})

3.1 Prepare a dispersion of fixed red blood cells (RBC) from the stock solution, designated as (B^+) Human RBC prepared August 8, 1972, with identifying number 21F21460C. The mean electrophoretic mobility (μ_e) of these cells is -1.81 ± 0.06 μ-cm-V-s in A-1 buffer at 25°C. Shake the stock solution of fixed RBC thoroughly to uniformly suspend the cells. Withdraw approximately a 1 mil aliquot of the suspension and place into a clean centrifuge tube. Add A-1 buffer to give a total approximate volume of 15 ml. Centrifuge the dispersion for 5 minutes to pack the cells. Decant the clear supernatant, add approximately 15 ml fresh A-1 buffer, shake until all cells are resuspended and centrifuge again for 5 minutes. Repeat the suspension-centrifuge process a total of five times. After the last decanting step, add approximately 1 ml of A-1 buffer to the cells and shake until all cells are resuspended. To prepare the final test dispersion, add approximately 0.1 ml of the concentrated cell dispersion to 250 ml of fresh A-1 buffer and shake thoroughly.

3.2 The columns are filled with the RBC/buffer suspension by first equipping the column with the test electrode end housings supplied for this purpose. Then attach a syringe valve to each housing. Draw 30 ml of well

shaken test suspension into a glass syringe and fill the column in the vertical position via the bottom syringe valve with gentle tapping of the column/syringe assembly to dislodge air bubbles from the lower housing. Continue to fill until solution flows out of the upper syringe valve. Close the lower valve first, then the top valve and remove the syringe.

NOTE: (1) Do not fill but one column at a time. This column is to be carried through the μ_{os} measurement before subsequent columns are filled.

(2) The RBC will settle out of the buffer with time. The procedure must move quickly to the μ_{os} measurement step, paragraph 3.3.

3.3 The electro-osmotic flow measurements are performed on a Rank Mark II microelectrophoresis apparatus. Power up the instrument by turning on the main power switch and the electrode voltage switch (with voltage dial on zero). Place one of the column assemblies in the 11 cm long plexiglas temperature bath and assemble the bath ends around the column. Insure that the RTV seam is not in the optical path of the microscope.

3.3.2 Remove microscope objective and mount the column/bath assembly on the Rank instrument by means of two retaining screws.

3.3.3 Reinstall objective, connect the tygon tubing from the pump to the plexiglass water bath and turn on the heater and pump on the Rank instrument, allowing the bath to fill.

3.3.4 Verify that the bath has come to temperature equilibrium at 25° ± 1°C. Connect the leads from the dc power supply to the electrode in each electrode housing.

3.3.5 Turn on the light source slowly to full intensity and adjust the horizontal vernier until the microscope field is illuminated.

3.3.6 Move the microscope objective until the RBC are clearly visible. Determine if there is any drift to the particles. If the particles are moving horizontally, wait an additional 5 minutes and recheck the drift. If the particles continue to drift, there is probably a leak in the assembly and it must be removed and checked for leaks.

3.3.7 Adjust horizontal vernier to focus the microscope approximately one-third of the distance from front wall to back wall of the column. Adjust the

power supply voltage so that the particles which are moving horizontally in the microscope field of view will traverse one grid division (the grid is located in one of the microscope eye pieces) within 3-6 seconds. Record the voltage on the data sheet and maintain the voltage constant throughout the test.

3.3.8 Adjust the horizontal and vertical verniers to find the vertical center of the front wall of the column. Enter this value (read to the nearest 0.002 mm) on the test data sheet.

3.3.9 Advance the microscope from the front center wall position to a position approximately 0.20 mm further toward the column.

3.3.10 Adjust the light source as necessary to maintain an illuminated field of view. Record the depth setting on the test data sheet. At this depth setting, apply voltage to the column by means of the polarity reversal switch, and determine the time (measured to the nearest 0.01 second) necessary for particle to traverse an integral number of grid divisions using the instrument timer. Record the time on the test data sheet.

3.3.11 Using the polarity reversal switch, now reverse the polarity and time another particle across one grid division. Record the time on the test data sheet.

3.3.12 Continue to record times for individual particles, reversing the polarity after each time measurement. Carry out a minimum of 10 time readings at this depth setting.

3.3.13 Advance the microscope an additional 0.20 mm toward the column, record the new position on the test data sheet, collect time data for at least 10 particles, and record the time data.

3.3.14 Continue advancing the microscope in increments of 0.20 mm, measuring particle times at each depth setting, until lack of resolution of individual particles precludes additional depth settings.

3.3.15 Turn off the pump, allow water to drain back into the circulating system, and clamp off the water input line.

3.3.16 Disassemble the plexiglas bath and remove the test column assembly.

3.3.17 Turn off the light source by slowly turning the control knob to the full counterclockwise position. Turn off the dc voltage power supply and turn off the main instrument power switch.

3.3.18 Enter the remaining test parameters of interelectrode distance (17.50 cm), microscope grid calibration (30 microns/division), column diameter (0.250) and the column back wall setting on the test data sheet.

3.4 Hold the column assembly in a vertical position and drain the contents into a waste container.

3.5 Flush the column twice with deionized water from a clean syringe and allow to dry in a laminar flow bench.

4.0 COMPUTER CALCULATIONS

Using the Hewlett-Packard Model 9100B programmable calculator with extended memory and x-y recorder and the computer program "Electrokinetic Mobility Computation Program E004 for Cylindrical Flight Columns," calculate the values of electrophoretic mobility (μ_e) and electro-osmotic flow (μ_{os}) for each of the columns. Enter these results on the appropriate test data sheets. Carefully mark each computer printout to identify it relative to the columns and attach these data to the test data sheet.

APPROVAL

DEVELOPMENT OF POLYMERIC COATINGS FOR CONTROL OF ELECTRO-OSMOTIC FLOW IN ASTP MA-011 ELECTRO-PHORESIS TECHNOLOGY EXPERIMENT

By William J. Patterson

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

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

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