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LOW-ELECTROOSMOTIC-MOBILITY COATINGS FOR ASTP FREE-FLUID ELECTROPHORETIC SEPARATION

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INTRODUCTION

The Apollo 16 Electrophoresis Experiment demonstrated the need for a low-electroosmotic-mobility coating in a free-fluid electrophoretic separation[1]. The photographs of the experiment showed parabolic particle profiles that are characteristic of electroosmosis in the cell and which render a practical separation impossible. These parabolic particle profiles were in good agreement with theoretical profiles calculated assuming an electroosmotic mobility of the cell walls equal to -10μm cm/volt sec.[2] Therefore, a major effort was initiated to develop a coating that would reduce the zeta potential at the cell-wall/liquid interface to zero or near-zero, to eliminate the driving force responsible for electroosmotic flow in the presence of an applied electrical potential. The initial work was directed towards the electrokinetic behavior of different coatings on a variety of surfaces in the presence of different buffer systems because neither the buffer nor the electrophoresis cell material were decided upon until very late in the development of the ASTP experiment.

EXPERIMENTAL DETAILS

Electrophoresis Cell-Wall Materials

The cell-wall materials which were coated and investigated for their electrokinetic properties during the course of this study included glass, Plexiglas, and Lexan. The glass was available in small capillaries with an inside diameter of 1.0 ± 0.05 mm and a wall thickness of 0.15 mm. Lexan and Plexiglas were not available in small capillaries, but tubes were obtained with an inside diameter of 6 mm and a wall thickness of 1 mm. Sheets of Plexiglas and Lexan of 1.6 and 1.0 mm thickness, respectively, were also obtained.

Buffers

The electrokinetic properties of the coated materials were investigated under a variety of ionic conditions. The buffers generally used were: (i) the borate buffer which was identical to that used for the Apollo 16 electrophoresis experiment[1]; (ii) a phosphate buffer which contained 4.32% glucose, 4% glycerol, 0.18% Na_2HPO_4, and 0.02% KH_2PO_4; (iii) the A-1 buffer, a modification of the phosphate buffer, which contained 4.0% glucose, 0.025% Na_2HPO_4, 0.005% KH_2PO_4, 0.0375% NaCl, 0.0125% $Na_2EDTA \cdot 2H_2O$, 3.8% glycerol, which was used for the ASTP electrophoresis experiments.

Experimental Method of Measuring Electroosmotic Flow in Cylindrical and Rectangular Channels

Several methods are available for measuring electroosmosis which depend upon the physical configuration of the sample under investigation. The method developed in this study was to construct both cylindrical and rectangular microelectrophoresis cells from the materials under investigation and to measure directly the velocity of individual particles in an applied electric field as a function of position in the cell. Under these conditions, the observed electrophoretic velocity $\underline{V}_{obs}$ is equal to:

$$V_{obs} = V_e + V, \tag{1}$$

where V_e is the true electrophoretic velocity, which is constant, and V is the solvent velocity due to electroosmosis, which is a function of position in the channel. When the channel is cylindrical, the solvent velocity may be expressed as:

$$V = U\left(\frac{2r^2}{a^2} - 1\right), \tag{2}$$

where r is the distance from the center of the channel, a is the channel radius, and U is the electroosmotic velocity at the channel wall, i.e., at $r = a$. When the channel is rectangular, the solvent velocity may be expressed as[3]:

$$V/U = 1 - 3\left[1 - (y^2/b^2)\right] \Big/ 2\left[1 - (192/{}^5K)\right] \tag{3}$$

where V is solvent velocity at the center of the channel, $K = a/b$ the ratio of channel width/channel height, a is one-half the channel width, b is one-half the channel height, and y is the height measured from the center of the channel.

The experimental design for these electrophoresis cells requires that the channel be easily removable from the cell. Figure 1 shows a diagram of an electrophoresis cell designed for small capillaries constructed with threaded nylon caps and O-rings which seal the capillary channel into the cell and allow for quick disconnection and replacement of the capillary. The platinum electrodes are similarly sealed in place to give a completely closed system. Standard glass capillary tubes with an outside diameter of 1.0-1.5 mm are coated as desired and inserted in the cell for determination of the electroosmotic flow under standard conditions. A metal cell holder was constructed to clamp the electrode compartments in a fixed position

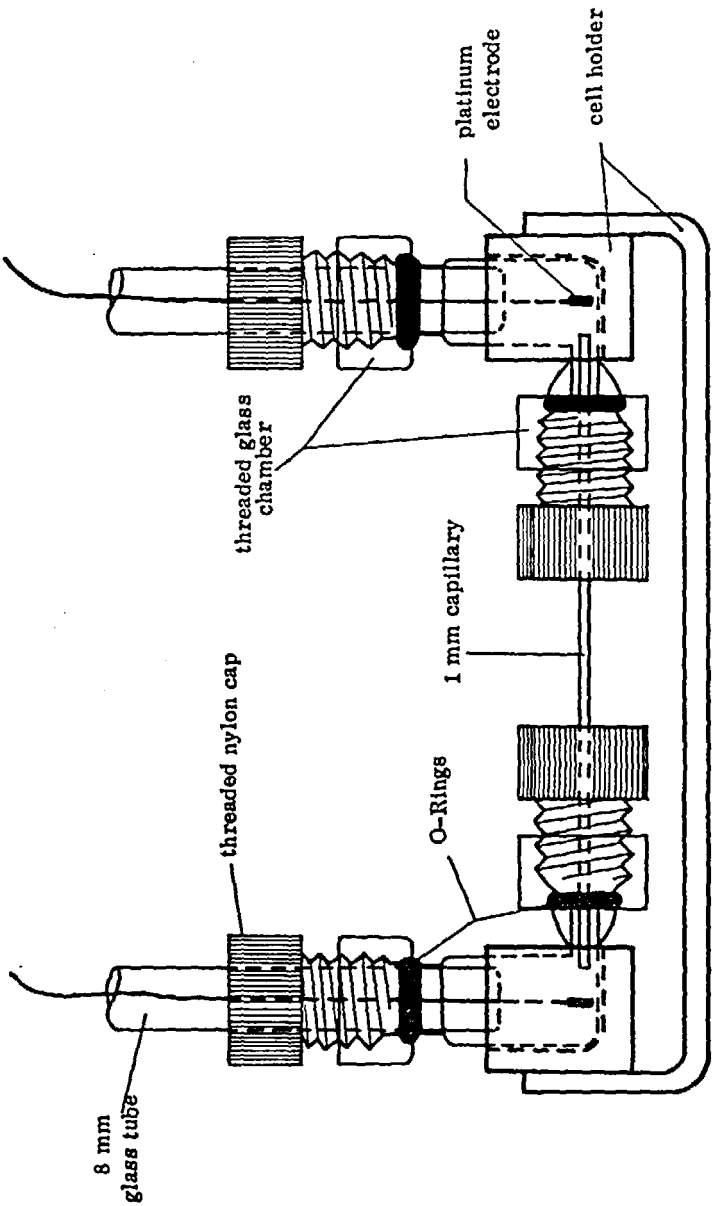

FIGURE 1

Electrophoresis Cell with Removable Capillary Channel.

to support the cell. This cell was designed to fit into the constant-temperature bath of the Rank Brothers electrophoresis apparatus, replacing the conventional microcapillary electrophoresis cell. This apparatus was also modified to observe the particles in the dark-field configuration using a 5 mW He-Ne laser as the light source.

Figure 2 shows a diagram of an analogous cell with a replaceable rectangular center channel constructed from 0.8 mm-thick Lexan or Plexiglas sheets. The replaceable channel was constructed by glueing strips of Lexan or Plexiglas sheets between two larger plates using ethylene dichloride as adhesive. The replaceable channel with a height of 0.8 mm and a width of 20 mm fits tightly into the two electrode compartments and is sealed into position with silicone rubber - G.E. Company. The channel can be replaced by simply stripping away the RTV rubber seal and separating the parts. Several

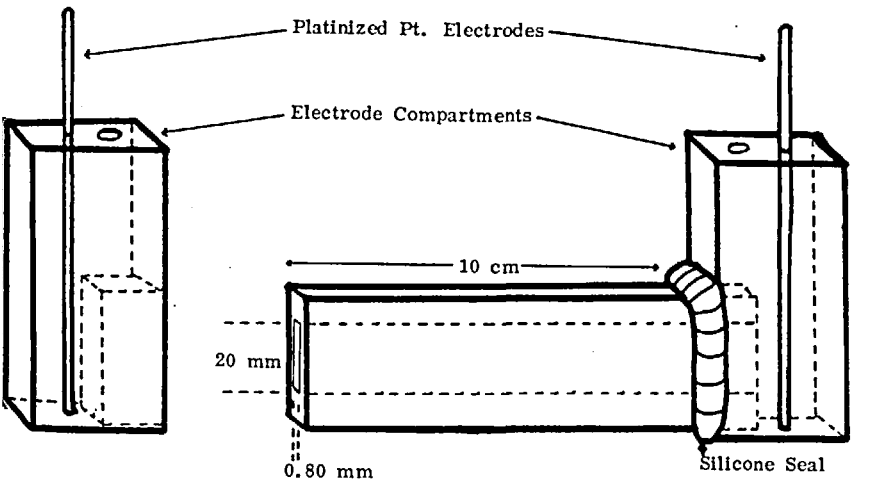

FIGURE 2

Electrophoresis Cell with Removable Rectangular Channel.

replaceable channels were constructed and coated in various ways; each in turn was cemented into the cell and its electroosmotic mobility was measured.

The electrophoresis capillary cell (Figure 1) was tested by measuring the velocity profile of 0.8μm-diameter monodisperse polystyrene particles (LS-1200-B; The Dow Chemical Company) in uncoated capillaries. Figure 3 shows the results for the latex particles dispersed in the borate buffer (pH = 8), the boric acid component of the buffer (pH = 4), and distilled water. The results are given as the velocity-distance^2 plot, which converts the parabolic velocity-distance relationship to a straight line. Only one half of the parabola is shown in Figure 3, although velocity measurements were made across the entire width of the channel. This method of plotting allows comparison of straight lines with an intercept at the ordinate equal to the electroosmotic flow velocity and an intersection with the stationary level (height = $\pm$ 0.35 mm) equal to the electrophoretic mobility of the latex particles.

Figure 4 shows the results obtained with latex particles of known electrophoretic mobility in an uncoated rectangular Lexan channel in the electrophoresis cell shown in Figure 2. Figures 3 and 4 show that the plots of the electrophoretic velocity vs. the square of the distance are linear, indicating that the solvent velocity flow profile is parabolic, in agreement with Equations 2 and 3.

EXPERIMENTAL RESULTS AND DISCUSSION

Glass Surfaces

The rationale for the pretreatment of the glass capillaries depends upon an assumption of a mechanism of electrical double layer formation at the capillary wall interface. Since the capillary wall

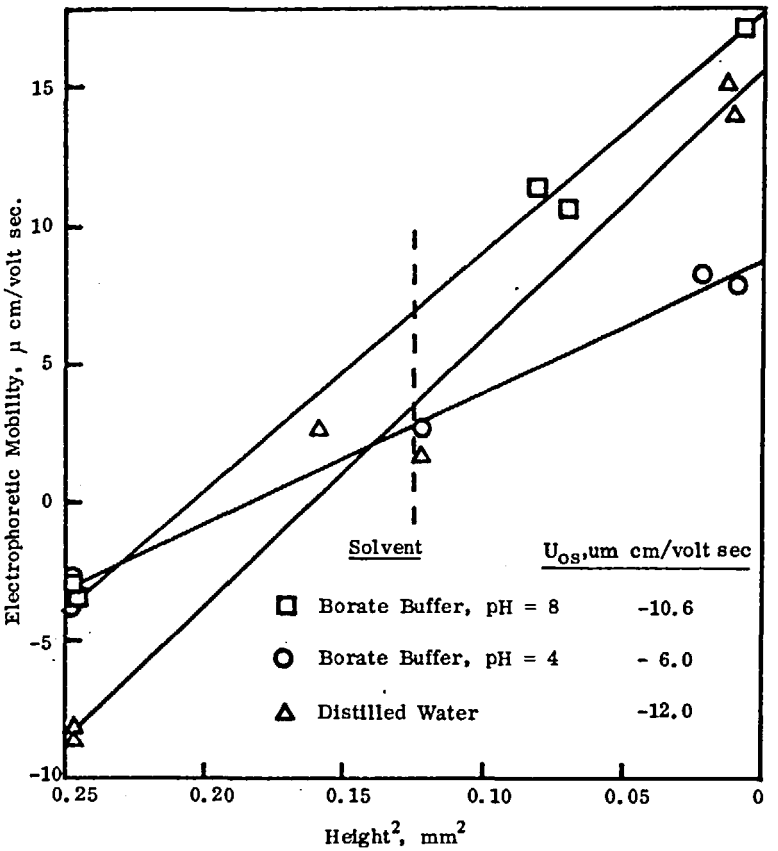

FIGURE 3

Observed Electrophoretic Mobility Parabolic Profile in Uncoated Glass Capillary.

exhibits a negative charge over a wide pH range (as shown by experiments in this laboratory), the surface charge can arise from the adsorption of cations on the proton of silanol groups (SiOH) or desorption of the silanol proton. The initial approach, therefore, was to render the active surface silanols inactive by specific chemisorption

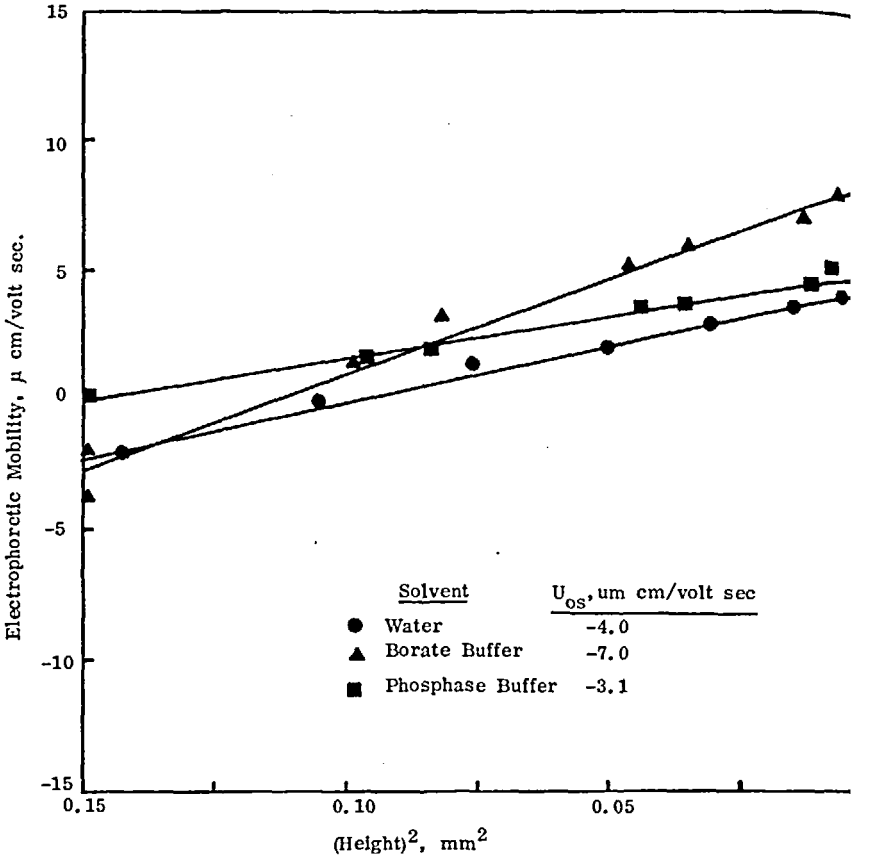

FIGURE 4

Observed Electrophoretic Mobility Parabolic Profile in Uncoated Rectangular Lexan Channel.

techniques, i.e., by alkylation of surface silanols according to the following reaction:

$$\rangle \text{SiOH} + \text{ROH} \rightarrow \rangle \text{SiOH} + H_2O$$

A number of experiments were performed with methanol (i.e., $R = CH_3$) by heating the glass capillary at 300°C in high vacuum and exposing the capillary to 30 mm of methanol vapor. Although the glass surface was rendered hydrophobic, the reaction appeared to be reversible in the presence of water, as evidenced by a steady decrease in hydrophobicity and an increase in surface charge.

Surface silanols can also be rendered hydrophobic by surface silylation. One of the more common silylating agents is hexamethyldisilazane (HMDS) which reacts with silanols according to the following mechanism:

$$2 \; \diagdown\!\!\diagup SiOH + (CH_3)_3\text{-}SI - \overset{H}{\underset{|}{N}} - Si - (CH_3)_3 \longrightarrow$$
$$2 \; \diagdown\!\!\diagup Si - O - Si - (CH_3)_3 + NH_3$$

This reaction is expected to be quantitative. A number of glass capillaries were treated with HMDS, and, although the surface charge was nominally reduced, the results were not consistent.

Another organo-silane coating which was evaluated was aminopropyltriethoxysilane (Union Carbide Co. A-1100; Pierce Chemicals). The coating procedure comprised rinsing the capillary with the A-1100 solution, drying, and curing at 150°C. The values of electroosmotic mobility for these A-1100-coated capillaries were erratic. Some capillaries showed extremely low mobility values, actually approaching zero, while others showed values corresponding to uncoated capillaries. This anomalous behavior was investigated further by taking two capillaries coated with the Union Carbide and the Pierce Chemicals coating materials, respectively, and sequentially measuring their electroosmotic flow profiles after exposure of the coatings to different ionic media. The results are shown in Figure 5, in which the order of measurement of each capillary is designated by the

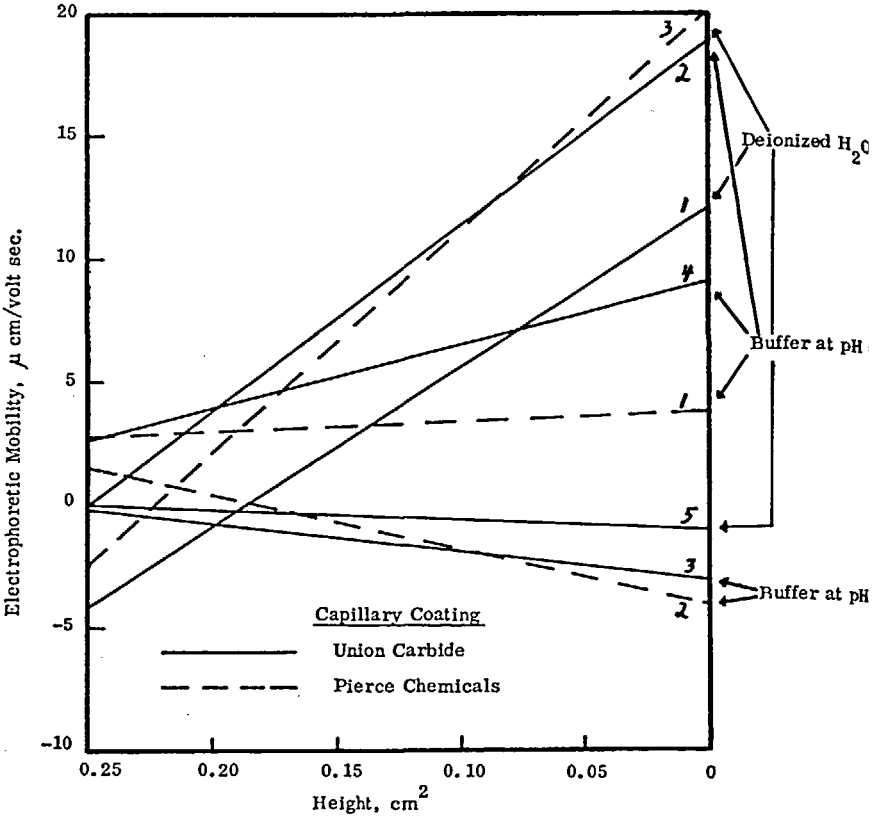

FIGURE 5

Observed Electrophoretic Mobility Parabolic Profile in A-1100 Silane Coated Capillaries.

appropriate number. A negative slope indicates a reversal of the parabola velocity profile, which means that the surface has changed from negative to positive values. These results show that the zeta potential is dependent upon the pH of the media and the pH history of the sample surface. The uncertainties involved in the use of this coating made it unacceptable for a low-electroosmotic-mobility coating.

Glass capillaries were coated with methylcellulose (Dow Methocel MOC) with molecular weights in the range 11 x 10^4 by drying an aqueous solution of Methocel and baking for 15 hours at 150°C to reduce its solubility (Methocel is insoluble in hot water and soluble in cold water). The media used were distilled water, borate buffer with and without sodium lauryl sulfate (pH = 8), and borate buffer without sodium borate, i.e., boric acid (pH = 4).

Samples of 0.80μm-diameter monodisperse polystyrene latex (LS-1200-B) showed little or no movement at the stationary level in the microelectrophoresis cell, independent of voltage gradient, solvent composition or pH, or the type of Methocel used for coating (e.g., see Figure 6). However, the diluted latexes were usually allowed to stand in the coated capillary for about 30 minutes before the measurements were made. The zero or near-zero electrophoretic mobilities, along with the 30 minutes elapsed time between loading and measuring, suggests that the methylcellulose molecules desorb from the capillary walls and adsorb on the surface of the latex particles (the adsorption of nonionic or steric stabilizers is known to reduce the electrophoretic mobility).

Therefore, a technique was developed to begin the measurements within five minutes after loading the cell with the diluted latex. Figure 7a shows that the electrophoretic mobility of the latex particles was initially unaffected by the presence of the methylcellulose coated capillary walls, but gradually decreased to essentially zero upon standing in the capillary. Thus, the methylcellulose is very effective in reducing the zeta potential of the capillary wall-liquid solvent interface and, hence, the electroosmotic flow within the capillary; however, it must be crosslinked or otherwise chemically-bound to the capillary wall so that it cannot desorb and adsorb on the surfaces of the particles in the dispersion. In comparison, Figure 7b

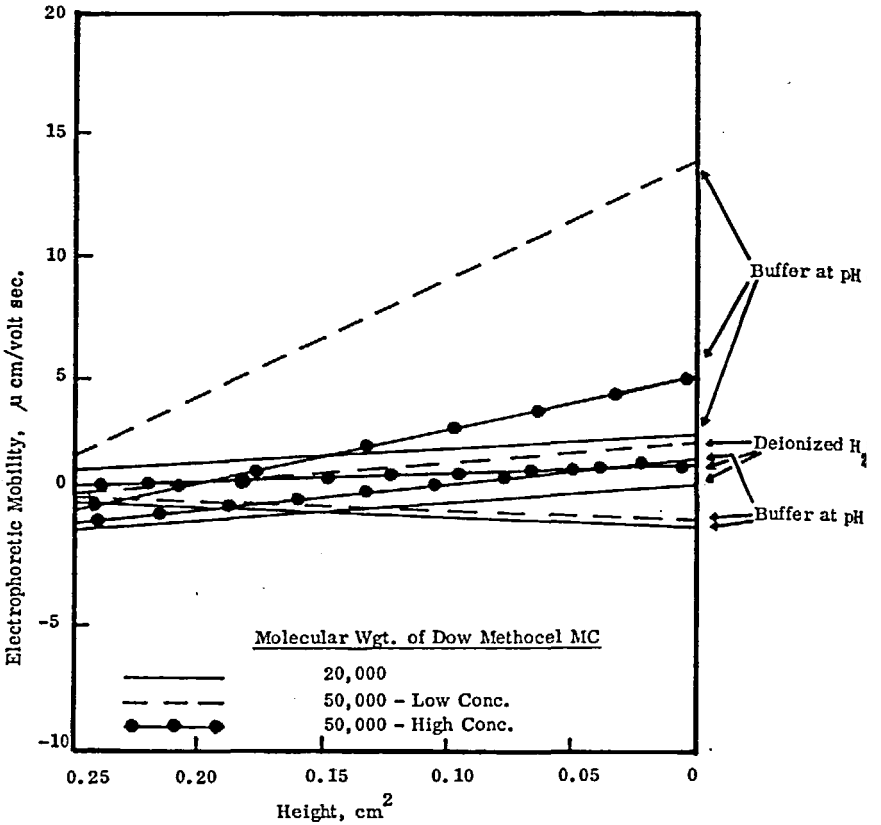

FIGURE 6

Observed Electrophoretic Mobility Parabolic Profile in Dow Methocel, MOC, Coated Capillaries.

shows the results of allowing the latex particles to stand in a capillary treated with the Union Carbide A-1100 coating. The results after 15 hours are comparable to the initial results. In this case the A-1100 coating is irreversibly adsorbed onto the glass surface.

Another approach (suggested by H. Burrell, Inmont Corp.) used to reduce the electroosmotic mobility was to coat the glass with a solution of polyvinyl acetate, allow the solvent to evaporate, leaving a thin film of strongly-adherent polymer, then surface-hydrolyze the polymer film to form a thin layer of polyvinyl alcohol (uncharged and normally water-soluble, but, in this case, chemically-bound to the underlying polyvinyl acetate film). A 4% solution of polyvinyl acetate in methyl ethyl ketone was prepared, and the glass capillary was dipped into this solution for one half hour and then dried in air. The polyvinyl acetate-coated glass surface was then hydrolyzed by heating at 110°C for 30 minutes and exposing the surface to a 5% sodium hydroxide solution for 10 minutes. Three different molecular weight grades of polyvinyl acetate (specified only as "high, intermediate, and low", obtained from H. Burrell, Inmont Corp.) were applied and hydrolyzed with 5% sodium hydroxide. The results summarized in Table I show that the low-molecular-weight polyvinyl acetate coating (LMW-PCAc) reduced the electroosmotic mobility only slightly; the medium-molecular-weight coating (MMW-PVAc) reduced the electroosmotic mobility to a greater extent; the high-molecular-weight coating (HMW-PVAc) was less effective than the MMW-PVAc. In general, the polyvinyl acetate coating was not sufficiently effective in reducing the electroosmotic flow in glass capillaries.

The most effective material for reducing electroosmosis was found to be methylcellulose, which was shown, however, to desorb from the surface in less than an hour (Figure 7). Therefore, the methylcellulose was more strongly adsorbed to the glass surface by treating the surface with γ-glycidoxypropyltrimethoxysilane (Dow Corning Corp. Z-6040) which binds to the glass, then using the epoxide group to link with the hydroxyl groups of the methylcellulose

TABLE I

Electroosmotic Flow in Coated Glass Capillaries

Capillary Coating*	Solvent	U_{os}, μm-cm/volt-sec	U_{os} Ratio $\frac{\text{Coated}}{\text{Uncoated}}$
LMW-PVAc	H_2O	5.8	0.6
MMW-PVAc	H_2O	2.0 (3.1)	0.2 (0.3)**
HMW-PVAc	H_2O	4.8	0.5
LMW-PVAc	Borate Buffer	5.1	1.0
MMW-PVAc	Borate Buffer	1.8	0.4
HMW-PVAc	Borate Buffer	2.9	0.6
LMW-PVAc	Phosphate Buffer	3.8	1.0
MMW-PVAc	Phosphate Buffer	1.5 (1.8)	0.4 (0.5)**
HMW-PVAc	Phosphate Buffer	1.8	0.5

*Coating Identification:

LMW-PVAc Low-molecular-weight polyvinyl acetate
MMW-PVAc Medium-molecular-weight polyvinyl acetate
HMW-PVAc High-molecular weight polyvinyl acetate
(all three PVAc's surface-hydrolyzed to PVA)

**Duplicate Coating Measurements

molecules. The proposed mechanism[4] for the binding of this and other trialkoxysilanes to glass surfaces is as follows:

1. hydrolysis of the trialkoxysilane to the corresponding silanetriol;
2. chemisorption of the silanetriol on the glass surface (interaction between a hydroxyl group of the glass with one of the hydroxyl groups of the silanetriol);

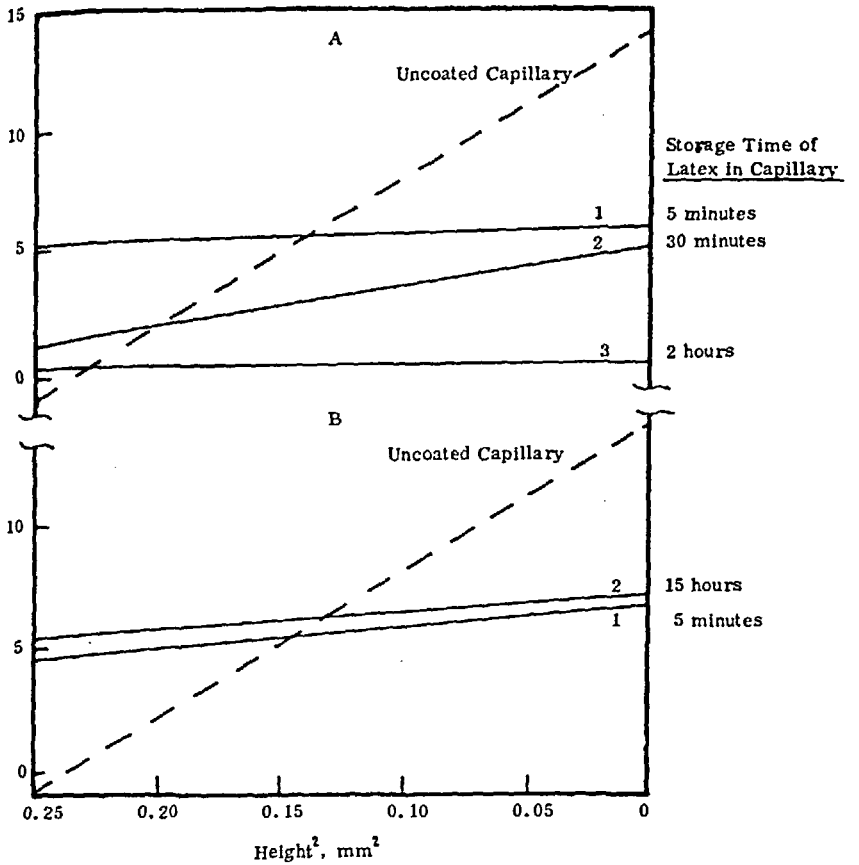

FIGURE 7

Observed Electrophoretic Mobility Parabolic Profile in Coated Capillaries: A. Dow Methocel; B. Union Carbide Co., A-1100.

3. formation of hydrogen bonds between hydroxyl groups of adjacent chemisorbed silanetriol molecules;

4. polymerization of adjacent silanetriol molecules during drying by splitting out water from hydrogen-bonded hydroxyl groups.

For the Dow Corning Z-6040, such a process would give a polysilanetriol chemisorbed to the glass, with an exposed surface of epoxide groups, which are free to form bonds with compounds containing active hydrogen, such as methylcellulose. The methylcellulose (Dow Methocel MOC) is applied over the Z6040-treated surface and heated to increase the interaction between the epoxide groups of the Z6040 and the hydroxyl groups of the methylcellulose.

Part of this methylcellulose coating can be removed by rigorous washing -- this is referred to as "strongly bound". This distinction is important because the "physically adsorbed" methylcellulose apparently is governed by an adsorption-desorption equilibrium and thus, when a colloidal sol with partially covered surfaces is added to the cell, the methylcellulose may desorb from the cell wall and readsorb on the colloidal particles, reducing their electrophoretic mobility to that of methylcellulose (zero). Such desorption and readsorption would obviate the electrophoretic separation. Fortunately, the "physically adsorbed" methylcellulose can be removed by rigorous washing, leaving the "strongly bound" methylcellulose, which does not desorb under these conditions.

The methylcellulose forms a layer of uncharged hydrated polymer on the surfaces of both the colloidal particles and the cell walls, and thus effectively reduces the zeta potential to zero. The mechanism proposed for this reduction in zeta potential comprises an increase in viscosity at the distance δ from the surface (usually the distance of the slipping plane) such that the slipping plane is moved outward from the surface to a distance where the potential is close to zero.

This paper presents results for this Z6040-MOC coating system which show that the electroosmotic mobility of glass capillaries (used in microcapillary electrophoresis cells) is reduced from the usual 3.5μm-cm/volt sec to about 0.1μm cm/volt sec.

The procedure used to coat the glass capillaries with the Z6040-MOC combination was as follows:

1. prepare the Z6040 solution by adding 80 gm of methanol to 20 gm of water, then adding 3 gm of Z6040 (as received) and one drop of glacial acetic acid;
2. immerse clean glass capillary tubes in this Z6040 solution for one hour;
3. remove glass capillary tubes from Z6040 solution, position vertically with lower end in contact with an absorbent paper towel, and dry under vacuum at 60°C for one hour;
4. prepare MOC solution by adding 0.175 gm of Dow Methocel HG (8000 cps) to 100 ml of distilled water, stirring for 5 hours, allowing to stand for one hour, and then decanting the clear supernatant layer;
5. immerse Z6040-coated glass capillary tubes in MOC solution;
6. remove glass capillary tubes from MOC solution, position vertically with lower end in contact with an absorbent paper towel, and dry under vacuum at 60°C for three hours;
7. rinse coated glass capillary tubes with distilled water until "physically adsorbed" methylcellulose is removed and only the "strongly bound" methylcellulose remains.

The glass capillary tubes coated with different variations of the foregoing procedure were inserted in the electrophoresis cell shown in Figure 1 and their electroosmotic mobilities were determined from the parabolic electrophoretic velocity profiles. Table II shows the results for glass capillary tubes coated with different variations of the Z6040-MOC combination and tested using 0.80μm-diameter monodisperse polystyrene latex particles (LS-1117-B; the Dow Chemical Co.). Although many more measurements were made than are listed in Table II, the results given are representative and reproducible to within + 0.2μm cm/volt sec. The charges on the glass capillary tube walls and on the latex particles were always negative.

These results show that uncoated pyrex glass capillary tube walls (Run No. 1) have a strong negative charge (U_{OS} = 3.5μm cm/volt sec),

TABLE II

Electroosmotic Mobility of Coated Glass Capillary Tubes

Run No.	Coating	Buffer Medium	Treatment	Storage Time of Latex, Min.	U_e*	U_{os}**
1	none	A-1	pyrex control	10	2.50	3.50
2	Z6040	phosphate	pyrex; no rinse	10	3.40	1.80
3	MOC	phosphate	pyrex; 2 hrs in 1% latex; 1-hr. rinse	10	1.80	3.20
			4-hr. rinse	10	2.20	4.80
4	MOC	phosphate	pyrex; 16-hr. rinse	10	1.30	0.10
				120	0.80	0.10
5	Z6040-MOC	phosphate	pyrex; 30-hr. rinse	10	1.60	0.05
				240	1.60	0.05
				1320	1.40	0.10
6	Z6040-MOC	A-1	pyrex; 1-hr. rinse;	10	2.15	0.35
			in A-1 buffer;	10	2.00	0.40
			3-wk.	120	1.85	0.20
7	Z6040-MOC	phosphate	pyrex; 30-hr. rinse; 7-month storage	10	2.05	0.75
8	Z6040-MOC	A-1	pyrex; 30-hr. rinse; 7-month storage	10	2.35	0.80
9	Z6040-MOC	A-1	THF***-cleaned	10	2.10	0.50
			Kimax; 2-hr. rinse	10	2.15	0.20
10	Z6040-MOC	A-1	THF-cleaned Kimax;	10	1.90	0.10
			3-wk. storage in A-1	240	1.50	0.10

* electrophoretic mobility (μm cm/volt sec)
** electroosmotic mobility (μm cm/volt sec)
*** tetrahydrofuran

which is halved by coating the surface with Z6040 (Run No. 2). Coating the glass surface with methylcellulose but omitting the Z6040 undercoat (Run No.'s 3 and 4) gave a low electroosmotic mobility initially, but the

introduction of a 1% dispersion of the monodisperse polystyrene latex resulted in complete removal of the methylcellulose from the wall within 2 hours. (These experiments were carried out to determine the feasibility of using a sacrificial polystyrene latex to decrease the rinse time required to remove the excess methylcellulose from the cell; although the experiments were successful, this approach was not developed further because the rinsing procedure was effective and there was a possibility that some polystyrene particles might adhere to the coated glass capillary tube walls).

The pyrex glass capillary tubes that were coated with the Z6040-MOC combination according to the foregoing procedure (Run No.'s 5 and 6) showed after extensive rinsing stable low-electroosmotic-mobility coatings that did not lose methylcellulose by desorption, as indicated by the unchanged electrophoretic mobility of polystyrene latex particles in phosphate and A-1 buffers stored in the tube for long periods of time. Similar results were observed when distilled water was stored in the coated capillary tubes for 7 months (Run No.'s 7 and 8); although the electroosmotic mobility was increased somewhat, it did not exceed 1μm cm/volt sec. This Z6040-MOC coating was as effective for Kimax glass capillary tubes (Run No.'s 9 and 10) as it was for the pyrex capillary tubes.

All of the foregoing measurements were made in glass capillaries with an ID of one mm because the measurements of the parabolic particle velocity profiles are more precise for small capillaries. The glass columns used on the ASTP electrophoresis experiment, however, had an ID of 6.3 mm. Therefore, a series of measurements were run in large glass columns in order to verify the coating procedure and especially the rinse procedure developed for the capillaries.

The major problems associated with the measurement of electrophoretic velocities in these relatively large glass channels result from

their optical characteristics: 1. the wall thickness is relatively great (about 2 mm), which makes it difficult to determine the exact position of measurement in the channel; 2. the electrophoretic velocity of the particles can be measured down only to the center of the channel because of the limited working distance of the objective. Accurate microcapillary electrophoretic velocity measurements require that the measurement of particle velocities as a function of distance across the capillary be in a plane that intersects the longitudinal axis of the capillary, i.e., the measurements must be made through the exact center of the channel. Since the refractive index of glass is different from that of water, the optical path is displaced as it passes through the glass. Microcapillary electrophoresis cells usually have walls so thin (i.e., less than 100μm) that this optical displacement is small enough to be neglected.

The geometry and wall thickness of the glass electrophoresis columns used in these measurements were not known accurately enough to make precise corrections for the optical displacement. Moreover, the particle velocities could not be measured from one wall to the other. Therefore, the procedure used was to measure the particle velocities from the wall to the approximate center of the channel, where the fastest moving particles were selected for measurement (the fastest moving particles should be in the exact center of the channel). The uncertainty in the values of the electroosmotic mobilities was estimated to be ± 0.3μm cm/volt sec.

Table III gives the electroosmotic mobility results for pyrex glass columns coated with the Z6040-MOC combination according to the procedure described above and filled with A-1 buffer containing monodisperse polystyrene latex particles. As expected, the uncoated control column (Run No. 1) showed a high electroosmotic mobility and a high electrophoretic mobility of the polystyrene particles placed in it. The coated column which had been subjected to a 4-day static rinse with no water

TABLE III

Electroosmotic Mobility in A-1 Buffer of Z6040-MOC Coated Glass Columns

Run No.	Column O.D., mm	Treatment	Storage Time of Latex, min.	U_e*	U_{os}**
1	9	uncoated control	10	3.8	4.8
2	7	4-day static rinse with no water change	10	1.4	0.8
			1440	0.3	0.1
3	7	7-day static rinse with several water changes	10	1.1	0.3
			1440	1.0	0.1
4	7	4-day static rinse plus 1-day dynamic rinse	10	2.7	0.3
			1440	2.1	0.5
5	7	1-day dynamic rinse 3-week in A-1 buffer	10	1.0	0.5
6	9.5	2-day dynamic rinse	10	2.1	0.5
7	9.5	30-hour dynamic rinse	10	2.6	0.5
			210	1.6	0.5

change (Run No. 2) showed a relatively low electroosmotic mobility (o.8μm cm/volt sec), but also the presence of "physically adsorbed" methylcellulose, which at first gave erratic electrophoretic mobilities of the polystyrene particles stored in it and, after 24 hours, a greatly decreased electrophoretic mobility.

In comparison, all the columns subjected to the other rinsing procedures (Run No.'s 3-7) showed electroosmotic mobilities of 0.5μm cm/volt sec or less. A 7-day static rinse with daily water changes (Run No. 3) removed the "physically adsorbed" methylcellulose, while the 4-day static rinse with no water change (Run No. 2) did not. The combination

of a dynamic rinse (a continuous flow of water through the column at a rate of one liter/hour) with a static rinse (Run No.'s 4 and 5) also removed the "physically adsorbed" methylcellulose effectively. The dynamic rinse alone (Run No.'s 6 and 7) was effective only if it was continued for a period of at least two days (e.g., a 30-hour dynamic rinse (Run No. 7) still left some "physically adsorbed" methylcellulose).

These results show that a period of at least 3 days is necessary to remove the "physically adsorbed" methylcellulose. The rinsing may be dynamic or static provided that the water is changed frequently, especially in the early stages of the rinse. In these early stages, 1-2 hours is sufficient for the solvent to become saturated with desorbed methylcellulose. After one day of efficient rinsing, several hours are required for the desorbed methylcellulose to attain an equilibrium concentration, which is lower than that of the earlier stages.

The concentration of methylcellulose which desorbs from coated and extensively-rinsed columns filled with A-1 buffer was determined as follows. Two sets of three coated columns were filled with A-1 buffer, sealed, and stored for periods of 3 and 4 weeks, respectively. The A-1 buffer in each set of columns was collected, a small concentration of polystyrene particles was dispersed in each solution, and after a time their electrophoretic mobilities were measured in the Rank microcapillary electrophoresis cell. No significant decrease in electrophoretic mobility was found, which indicates that the concentration of methylcellulose in the A-1 buffer was very small.

Plastic Surfaces

Plexiglas and Lexan were considered by NASA as possible materials of construction for electrophoresis cells in space. Therefore, preliminary experiments were carried out to develop a low-electroosmotic-mobility coating similar to those developed for glass. The binding of molecules such as methylcellulose to Plexiglas substrates in-

volves different chemical reactions than the corresponding binding to glass or Lexan. Plexiglas is essentially polymethyl methacrylate, sometimes with small proportions of other methacrylate or acrylate esters, or acrylic or methacrylic acid in the case for the high-heat-distortion grades. The obvious method of chemical binding is the partial hydrolysis of surface methacrylate ester groups to form the carboxylate salt, followed by neutralization to the carboxyl form; this would give a random distribution of carboxyl groups over the Plexiglas surface. Possible reactions of these carboxyl groups (and the functional group on the molecule to be chemically-bound) include: (1) esterification with elimination of water (hydroxyl group); (2) hydrogen-bonding (carboxyl group); (3) anhydride formation by heating of hydrogen-bonded carboxyls to eliminate water; (4) salt formation with a di- or trivalent metal ion (carboxyl group); amide formation (amine group).

Rectangular electrophoresis cells of the same configuration shown in Figure 2 were constructed of 1.6 mm-thick Plexiglas G sheet (Rohm & Haas Co.). These cells have an interchangeable center section that can be used to evaluate the electrokinetic properties of different coatings on the Plexiglas and Lexan as well as other plastic materials which are available only in sheet form.

The interchangeable center sections were treated in the following ways:

1. the cell section was merely rinsed with distilled water and dried, to establish the electroosmotic mobility of the untreated Plexiglas surface;
2. the cell section was filled with the coating solution, allowed to stand for 30 minutes, drained, and dried for a few hours in air at 50°C.

Table IV gives the measurements of electroosmotic mobility for the variously-treated Plexiglas surfaces in distilled water, borate buffer, or phosphate buffer. The Z6040 coating had very little effect on the electro-

TABLE IV

Electroosmotic Flow in Coated Plexiglas Cells

Cell Coating*	Solvent	U_e**	U_{os}**	U_{os} Ratio $\left(\frac{\text{coated}}{\text{uncoated}}\right)$
None	Water	2.60	3.50	----
MOC	Water	4.05	1.50	0.43
Z6040	Water	4.10	6.90	1.95
Z6040 + MOC	Water	2.65	1.95	0.56
S.T. + MOC	Water	2.60	2.65	0.76
H.T. + MOC	Water	3.00	0.50	0.14
None	Borate Buffer	5.00	-5.80	----
MOC	Borate Buffer	5.25	-1.80	0.31
Z6040	Borate Buffer	5.40	-5.60	0.97
Z6040+MOC	Borate Buffer	4.45	-3.85	0.66
S.T. + MOC	Borate Buffer	5.10	-3.20	0.55
H.T. + MOC	Borate Buffer	5.40	-2.10	0.36
None	Phosphate Buffer	3.15	-3.25	----
MOC	Phosphate Buffer	3.20	-1.00	0.31
Z6040	Phosphate Buffer	2.75	-2.55	0.78
Z6040 + MOC	Phosphate Buffer	2.50	-0.75	0.23
S.T. + MOC	Phosphate Buffer	2.50	-0.60	0.18
H.T. + MOC	Phosphate Buffer	2.80	-1.00	0.31

*Coating Identification:

MOC - 0.175% solution of methylcellulose (Dow; 110,000 M.W.)
Z6040 - γ-glycidoxypropyltrimethoxysilane (Dow Corning)
S.T. - surface treated with 0.1M NaOH, then 0.1M HCl
H.T. - heat treated in H_2O at 70°C for 30 minutes

**Negative charge; units: μm cm/volt sec.

osmotic mobility indicating that little or no reaction with the Plexiglas surface had occurred. All Methocel coatings showed significant decreases in electroosmotic mobility. The best results in phosphate buffer (electroosmotic mobility of 0.6μm cm/volt sec) were obtained by hydroly-

sis of the Plexiglas surface with sodium hydroxide, followed by neutralization and reaction with the Methocel. The degree of irreversible physical adsorption of Methocel observed with glass surfaces was not observed with Plexiglas, indicating that, in those cases where a significant reduction in electroosmotic mobility was observed, there was some degree of chemical binding of the Methocel to the Plexiglas.

The electroosmotic mobility results on coated Lexan, Table V, indicate that methylcellulose could not be strongly bound to the surface. Neither the Z6040 coating nor heating in a vacuum oven to high temperatures, which was intended to induce a bond between the surface carbonate group and the methylcellulose, was sufficient to strongly bind the methylcellulose to the surface. This conclusion is based on the erratic decrease in electrophoretic mobility of the latex particles due to the desorption of methylcellulose and to the increase in electroosmosis with standing time after initial filling of the cell. Apparently the polycarbonate chains present on the Lexan surface are unreactive to this type of coating procedure.

Conclusions

The following conclusions can be drawn from this work:

1. The Z6040-MOC coating on pyrex glass columns or Plexiglas surfaces decreases their electroosmotic mobilities to small negative values.

2. This coating gives both "physically adsorbed" and "strongly bound" methylcellulose;

3. The "physically adsorbed" methylcellulose must be removed because otherwise it may desorb from the cell wall and readsorb on the particles to be subjected to electrophoretic separation;

4. Extensive rinsing of the coated columns for a period of at least 3 days is required to remove the "physically adsorbed" methylcellulose from the surface;

TABLE V

Electroosmotic Flow in Coated Lexan Cells

Cell Coating*	Solvent	U_e**	U_{os}**	U_{os} Ratio coated / uncoated
None	Water	4.40	7.50	----
Z6040	Water	6.80	7.40	0.99
Z6040 + MOC	Water	3.20	0.25	0.03
H.T. (110°C) + MOC	Water	3.80	0.55	0.07
H.T. (155°C) + MOC	Water	0.85	0.15	0.02
None	Borate Buffer	6.40	8.10	----
Z6040	Borate Buffer	5.90	5.90	0.73
Z6040 + MOC	Borate Buffer	2.75	0.35	0.04
H.T. (110°C) + MOC	Borate Buffer	3.80	1.80	0.22
H.T. (155°C) + MOC	Borate Buffer	1.70	1.70	0.21
None	Phosphate Buffer	3.80	4.00	----
Z6040	Phosphate Buffer	4.30	3.30	0.83
Z6040 + MOC	Phosphate Buffer	3.10	0.80	0.20
H.T. (110°C) + MOC	Phosphate Buffer	2.70	0.90	0.23
H.T. (155°C) + MOC	Phosphate Buffer	2.10	1.40	0.35

*Coating Identification:

MOC - 0.175% solution of methylcellulose (Dow; 110,000 M.W.)
Z6040 - γ-glycidoxypropyltrimethoxysilane (Dow Corning)
H.T. - heat treated in vacuum oven

**Negative charge; units: μm cm/volt sec.

5. Rinsing of the coated columns over much longer periods of time eventually results in the removal of part of the "strongly bound" methylcellulose, resulting in an increase in the electroosmotic mobility.

6. The permanency of the Z6040-MOC coating is suitable for free-fluid electrophoretic separations such as the ASTP experiment, but its suitability for continuous particle electrophoretic separations, e.g., the Beckman CPE, is very questionable.

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