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FINAL REPORT

May, 1979

"DEVELOPMENT OF STABLE LOW-ELECTROOSMOTIC MOBILITY COATINGS"

NAS8-32406

Contract Number 1-7-ES-07591

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Prepared for

National Aeronautics and Space Administration
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This report was prepared by Lehigh University under NAS8-32406 "Development of Stable Low-Electroosmotic Mobility Coatings" for the George C. Marshall Space Flight Center of the National Aeronautics and Space Administration.

A. INTRODUCTION

The principal objective of this work is the development of a stable electroosmotic-mobility coating for use in electrophoresis systems in space. A low-electroosmotic-mobility coating was developed and used successfully on the ASTP MA-011 experiment (1,2). This coating consisted of Dow Corning Z6040-primed methylcellulose, and was effective in reducing the electroosmotic mobilities of the pyrex glass columns to near-zero in free-flow electrophoresis separation, (FFE). However, the permanency of this coating may be inadequate for flowing systems since it consisted of physically-and chemically-adsorbed methylcellulose, which was shown to desorb over a long period of time. Therefore, this type of coating probably is not suitable for Continuous Particle Electrophoresis, (CPE) for two reasons. The first reason is that the continuous flow of solvent in the CPE requires a coating of a higher degree of permanency than was necessary in the FFE. The second reason is that maximum resolution in the CPE is obtained when the electroosmotic flow approximately matches the electrophoretic mobility of the particles undergoing separation. The objective of this phase of the program, therefore, is to develop coatings which are stable under continuous fluid movement and which will exhibit finite and predictable electroosmotic mobility values. These coatings must also be effective on different types of surfaces, such as glass, various plastics, and ceramic alumina, which is currently being used as the electrophoresis channel of the GE-SPAR-CPE apparatus.

Two other phases of this program include the surface charge modification of polystyrene latex, especially by protein adsorption, to be used as model materials for ground-based electrophoresis experiments, and the preliminary ground-based work directed towards the seeded polymerization of large-particle-size monodisperse latexes in a micro-gravity environment. The surface modification of polystyrene latex was carried out as a feasibility study in order to demonstrate the potential of this method for preparing model particles, with a very narrow particle size distribution and a high degree of stability, as standards for biological separations in space. The work in this area was limited due to the complexity of the experimental approach involved in surface grafting of molecules on polymeric latexes. The monodisperse latex program involved the initial phases of the experimental approach to be used in an automated device for producing large-particle-size monodisperse latexes by seeded polymerization techniques. Although this program has recently been funded by a separate NASA contract, the initial work completed in this program has led to the elimination of one experimental approach in favor of another approach which currently is under development.

B. STABLE ELECTROOSMOTIC MOBILITY COATINGS

Two approaches have been used for the development of stable coatings
which would yield predictable electroosmotic mobility values over a useful range. The first approach is molecular in that molecules would be adsorbed at the surface. The Z6040-methylcellulose coating, for example, was a molecular coating and, as expected, was a sensitive function of the surface properties of the substrate to be coated. The other approach involves the formation of a continuous coating, with a thickness in the micrometer range, whose effectiveness in controlling electroosmosis would not be dependent upon the nature of the substrate; the nature of the substrate, however, could be critical for controlling the adhesive properties of the coating.

1. Experimental

The electrophoretic properties of the coatings developed in this program were measured by a technique developed in this laboratory (Final Report, "Electrophoresis Experiment for Space", NAS8-28654, April, 1976). The method was originally developed for both cylindrical and rectangular microelectrophoresis cells where the cell channels were constructed from the materials under investigation. An appreciation of this experimental approach requires a knowledge of electroosmotic flow which occurs in a closed system, such as a standard electrophoresis cell. The presence of an electric double layer at the cell wall/liquid interface results in electroosmotic flow in the presence of an electric field. For example, if the cell wall has a negative charge, the counterions in the liquid phase will be positive and the liquid at the cell wall interface will move toward the negative electrode in the presence of an electric field. Since the cell represents a closed system, the net cross sectional flow will be zero and liquid will thus be forced to move in the opposite direction in the center of the cell with a flow profile which is parabolic. Figure 1 is a schematic representation of this type of electroosmotic flow, where the channel wall is negatively charged with respect to the liquid and the shaded area marked A is equal to the sum of the two shaded areas marked B.

![Diagram of electroosmotic flow](image)

When colloidal particles dispersed in a fluid medium are subjected to an electric field, they move with a velocity which is a function of their electrical double-layer properties. If there is no electroosmosis, the particles move with a constant velocity, independent of their position in the cell. When there is an electroosmotic flow, the total particle velocity \( v_p \) will be the sum of the electrophoretic velocity \( v_e \) and the solvent velocity \( v_s \) according to the equation:
\[ v_t = v_e + v_s \]  

(1)

where \( v_e \) is constant for a given applied potential and \( v_s \) is a function of position in the channel. When the channel is cylindrical, the solvent velocity is defined by the equation:

\[ v_s = v_o \left( \frac{(2r^2/a^2) - 1}{(2r^2/a^2)} \right) \]

(2)

where \( a \) is the channel radius, \( r \) the radial distance from the center of the channel, and \( v_o \) the solvent velocity at the channel wall/liquid interface, i.e., at \( r = a \). The condition for zero solvent flow, i.e., \( v_s = 0 \), which is important for analytical electrophoresis, is defined by the equation:

\[ r = (a/2)^{1/2} \]

(3)

The driving force for the solvent velocity is the potential of the electrical double-layer as expressed in the following equation derived by Helmholtz:

\[ v_o = \frac{Ec \zeta}{4\pi \eta} \]

(4)

where \( E \) is the strength of the applied electric field, \( \varepsilon \) the dielectric constant of the fluid, \( \eta \) the fluid viscosity, and \( \zeta \) the potential of the liquid layer which does not move relative to the channel wall, i.e., the potential at the slipping plane or the zeta potential.

Another type of cell consists of a channel with a rectangular cross section. The electroosmotic solvent profile for a cell with this geometry has been developed by Komagata (3), according to the following equation, which uses the nomenclature of Figure 2:

\[ \frac{v_s(x = 0)}{v_o} = 1 - \frac{3}{2} \left[ 1 - \frac{y^2}{b^2} \right] \left[ 1 - \frac{192}{5K} \right]^{-1} \]

(5)

where \( v_s(x = 0) \) is the solvent velocity measured as a function of height \( y \) from the center of the channel and at the mid-width, \( v_o \) the solvent velocity at the channel wall/liquid interface, i.e., at \( y = b \), \( b \) is one-half the channel height, and \( K \) the ratio of channel width to channel height.

Figure 3 shows the linear form of the calculated parabolic flow profiles, normalized with respect to the electroosmotic flow at the channel wall, for rectangular channels with different values of \( K \), and for cylindrical channels. A knowledge of the true electrophoretic particle velocity, \( v_e \), and of the solvent velocity, \( v_o \), at the cell wall/interface, as determined by measuring the total particle velocity, \( v_t \), as a function of position in the channel, enables the zeta potential at the interface to be calculated according to Equation 4.

The experimental design for these electrophoresis cells requires that the instrument have the channel be easily removable from the cell. Figure 4 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 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 He-Ne laser as the light source.

Figure 5 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 gluing 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 RTV. The channel can be replaced by simply stripping away the RTV rubber seal and separating the parts. Several replaceable channels were constructed and coated in various ways; each in turn was cemented into the cell and its electroosmotic mobility was measured.

2. Results

The initial approach was to investigate more extensively the Z6040-methylcellulose coating for residual electroosmotic mobility values after long-term rinsing. The rationale for this approach was that, if a certain amount of irreversibly adsorbed methylcellulose remains on the surface, then the electroosmotic mobility would tend towards a fixed value which would be intermediate between zero and the value of the uncoated substrate. Long rinsing times, however, suggested that the electroosmotic mobility was tending towards the uncoated value since constant values could not be obtained after several weeks of rinsing. This approach has since been dropped from consideration as a viable method for coating glass or plastic surfaces.

Various other materials, such as parylene-N and SnCl4, have been used to coat glass capillaries, and Lexan and Plexiglas plates. The parylene-N treatment resulted in an increase in electroosmosis, while the SnCl4 coating increased the surface conductivity of the electrophoresis cell to the point where the electrodes were effectively shorted. The SnCl4 coating, however, is still interesting in that the surface conductivity could be controlled by the coating thickness. Apparently this method, which was originally thought to be molecular, results in a continuous coating. The possibility of preparing a coating of limited, but finite, conductivity leads to the possibility of actually controlling electroosmosis at the interface by the magnitude of the applied potential.

The concept of a continuous coating involves the utilization of a film-forming polymeric latex and a non-film-forming latex where the electrophoretic mobility of either one could be altered by prior treatment of the surface. The initial study involved an investigation of the electrophoretic mobility of two non-film forming latexes, polystyrene and polyvinyltoluene, and one film-forming latex, styrene-butadiene copolymer, as a function of exposure to different concentrations of methylcellulose (MC). Table I shows that the electrophoretic mobility of both non-film-forming latexes decreases with increasing MC concentration, with the polystyrene varying more sensitively with MC concentration. The results with the styrene-butadiene copolymer latexes were negative in that very little
decrease in electrophoretic mobility was observed with increasing MC concentration up to 0.167%. The degree of reversibility of MC adsorption on the latexes investigated was determined by means of a solvent exchange technique where the solvent was continuously replaced by deionized water at a rate of 100% per hour. The electrophoretic mobility was found to increase with increasing solvent exchange time for both polystyrene and polyvinyltoluene latexes with the mobility still increasing after one week of exchange. Although MC adsorbed strongly on these latexes, the adsorption process is apparently reversible with a very slow rate of desorption.

Table I

Electrophoretic Mobility of Polymeric Latexes as a Function of Methylcellulose (MC) Concentration

<table>
<thead>
<tr>
<th>Sample</th>
<th>MC Conc. %</th>
<th>% Solids</th>
<th>$U_e$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>0.0</td>
<td>10.0</td>
<td>-1.90</td>
</tr>
<tr>
<td>(0.46µm)</td>
<td>0.045</td>
<td>8.1</td>
<td>-1.40</td>
</tr>
<tr>
<td></td>
<td>0.083</td>
<td>8.3</td>
<td>-1.05</td>
</tr>
<tr>
<td></td>
<td>0.143</td>
<td>7.1</td>
<td>-0.70</td>
</tr>
<tr>
<td></td>
<td>0.167</td>
<td>6.7</td>
<td>-0.30</td>
</tr>
<tr>
<td>Polyvinyltoluene</td>
<td>0.0</td>
<td>3.9</td>
<td>-1.10</td>
</tr>
<tr>
<td>(0.23µm)</td>
<td>0.556</td>
<td>3.4</td>
<td>-1.00</td>
</tr>
<tr>
<td></td>
<td>0.167</td>
<td>2.6</td>
<td>-0.80</td>
</tr>
</tbody>
</table>

*Units: µm cm/volt sec.

A commercial film-forming latex XX210 (polyvinyl acetate; Air Products and Chemicals) was found to have a relatively low electrophoretic mobility in a variety of solvents. The measured electrophoretic mobilities in deionized water, A-1 buffer, and R-1 buffer were all less than 1.1 µm cm/volt sec. The XX210 latex was subsequently used to coat glass capillaries for electroosmotic mobility measurements. The electroosmotic mobility results obtained from these measurements, Table II, were 1.1, 0.7 and 0.3 µm cm/volt sec for deionized water, A-1 buffer, and R-1 buffer, respectively. Some problems however, were encountered in forming a continuous coating with the pure XX210 latex. The results shown in Table II are reported for measurements taken after rinsing the coated capillaries for a minimum time, two hours, and after extensive rinsing, at least two days. The rinsing procedure involves allowing deionized water to flow through the treated capillaries at the rate of 0.5 l/hr.

The results show that the XX210 is effective for reducing electroosmotic flow in A-1 and R-1 buffers, and the coating appears to be stable after extensive rinsing. Visual observation of this coating, however, does reveal some nonuniform layers which could be due to discontinuities
in the coating. The polyvinyltoluene, PVT, latex has a measured electrophoretic mobility in water of $6.0 \mu m \text{ cm/volt sec}$. A 50% addition of this latex to the XX210 is shown in Table II to result in an increase in electroosmotic mobility which appears to increase further after extensive rinsing. This latter effect appears to be due to the further breakdown in the quality of the coating as a result of the formulation problems encountered by the addition of a non-film forming latex.

Various film forming latexes have been investigated as potential additives for the XX210. The most promising latex studied was Acrysol (Rohm & Haas Co.) which has a high surface charge in deionized water. Capillaries coated with Acrysol, Table II, exhibited electroosmotic mobilities of 8.0 and $8.9 \mu m \text{ cm/volt sec}$ in R-1 buffer and water respectively. Combination coatings of 75/25 and 50/50 XX210 in Acrysol showed a pronounced decrease in the electroosmotic mobility where, especially in water, the concentration of Acrysol in the coating was proportional to the magnitude of the Zeta potential.

TABLE II
Electroosmotic Mobility Results
in Coated Glass Capillaries

<table>
<thead>
<tr>
<th>Coating</th>
<th>Medium</th>
<th>Electroosmotic Mobility $\mu m \text{ cm/volt sec}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Water</td>
<td>8.8</td>
</tr>
<tr>
<td>Untreated</td>
<td>R-1</td>
<td>3.7</td>
</tr>
<tr>
<td>XX210</td>
<td>Water</td>
<td>1.1</td>
</tr>
<tr>
<td>XX210</td>
<td>A-1</td>
<td>0.7</td>
</tr>
<tr>
<td>XX210</td>
<td>R-1</td>
<td>0.3</td>
</tr>
<tr>
<td>XX210-PVT (50/50)</td>
<td>Water</td>
<td>4.9</td>
</tr>
<tr>
<td>XX210-PVT (50/50)</td>
<td>R-1</td>
<td>2.4</td>
</tr>
<tr>
<td>Acrysol</td>
<td>Water</td>
<td>8.9</td>
</tr>
<tr>
<td>Acrysol</td>
<td>R-1</td>
<td>8.0</td>
</tr>
<tr>
<td>XX210 + Acrysol (50/50)</td>
<td>Water</td>
<td>6.2</td>
</tr>
<tr>
<td>XX210 + Acrysol (50/50)</td>
<td>R-1</td>
<td>2.5</td>
</tr>
<tr>
<td>XX210 + Acrysol (75/25)</td>
<td>Water</td>
<td>3.0</td>
</tr>
<tr>
<td>XX210 + Acrysol (75/25)</td>
<td>R-1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The work on combination coatings has demonstrated the feasibility of this approach for preparing coatings of predictable electroosmotic flow properties under different ionic conditions. The stability of these coatings, both in
terms of surface electrical double layer properties and physical properties, has yet to be demonstrated in terms of temperature and ionic conditions of the solvent system. Further work is required to develop the proper coating formulations for improving adhesion to different substrates as well as the physical properties of the coatings.

C. EMULSION POLYMERIZATION FOR SPACE APPLICATION

Preliminary experiments have been carried out to evaluate various designs of polymerization reaction chambers for the production of large-particle-size monodisperse latexes in space. The initial concepts included a bellows or a diaphragm, which would make up the walls and the top of the reaction chamber, respectively. Two diaphragms, stainless steel and teflon, were ordered but never received. A two-inch diameter reaction chamber, however, had been constructed from a stainless steel bellows, with flat plates secured by spring-loaded bolts forming the top and the bottom and with provision for stirring using a magnetic stirrer. The fabricated bellows polymerization vessel, Figure 6, had a nominal capacity of 200 cc. Two seeded emulsion polymerizations have been carried out in this bellows reaction chamber, each using the following polymerization recipe:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>70.46</td>
</tr>
<tr>
<td>31% solids polystyrene latex seed (0.357μm)</td>
<td>13.54</td>
</tr>
<tr>
<td>Styrene monomer</td>
<td>15.74</td>
</tr>
<tr>
<td>K₂S₂O₈ initiator</td>
<td>0.11</td>
</tr>
<tr>
<td>NaHCO₃ buffer</td>
<td>0.11</td>
</tr>
<tr>
<td>Aerosol MA (80%) surfactant</td>
<td>0.04</td>
</tr>
</tbody>
</table>

All of the ingredients except the initiator were injected into the chamber, which was placed in a 60°C constant-temperature bath for two hours with stirring, so that the swelling of the particles with monomer could occur prior to initiation of polymerization. The initiator solution was then injected and the polymerization was carried out for 12 hours at 60°C.

The first reaction was carried out with the chamber half-filled (ca. 100cc) with the polymerization recipe; stirring was accomplished by a magnetic stirrer mounted ca. 1.5cm from the bottom of the chamber on a teflon shaft, approximately in the center of the polymerization mixture. Inspection of the bellows after polymerization revealed the presence of some coagulum within the "fins" of the bellows' and on the stirrer and post. It is believed that the stirring stopped during polymerization because of this coagulum. The conversion of monomer to polymer based on the total monomer was 61%. This is equivalent to a particle size increase from 0.357μm to 0.530μm if no new particles were generated.
Two changes were made for the second reaction. First, the stirrer was replaced with a regular magnetic stirrer placed on the bottom of the cell and, second, the cell was filled completely (ca. 200cc). This gave more effective stirring and more complete polymerization with much less coagulum. The conversion was 84% corresponding to a particle size increase from 0.357μm to 0.572μm. Although the presence of particles were detectable on the surface of the bellows in the form of a thin layer, the loss was considered small and, more importantly, the coagulum did not appear to be brought about as a result of the surface coating.

A number of seeded emulsion polymerization reactions were carried out with the above recipe and varying experimental conditions. The results showed that both the rate of heating and the degree of stirring were critical experimental parameters which had to be controlled in order to prevent flocculation of latex in the hot zone regions of the immersion heater. For example, with 45 watts of power into the heater, and with the stirrer at 120 RPM, a 2-mm thick coating of coagulated latex particles was observed on the heater after completion of the experiment. Reducing the power to 40 watts and increasing the stirrer speed to 150 RPM resulted in no coating on the heater. Subsequent experiments have also revealed that stirrer design and the presence of baffles in the reaction chamber, which have the effect of increasing turbulence, can be varied to reduce the danger of flocculation of latex particles at the surface of the heater.

A typical polymerization utilizing the above recipe after 12 hours at 60°C, gave a conversion of 84% as determined gravimetrically which corresponds to an increase in particle size from 0.357μm to 0.572μm. The transmission electron micrograph for the final latex (Figure 7b) may be compared to that for the seed latex (Figure 7a). A preliminary estimate of the particle size of the polymerized seed latex shows that the results agree with the calculated value of 0.572μm to within 5%. These results demonstrate that the bellows reactor design can be used to produce monodisperse latexes by seeded emulsion polymerization techniques.

D. SURFACE MODIFICATION OF POLYSTYRENE LATEXES

The surface modification of polystyrene latexes has potential application for the electrophoretic separation of biological cells in space because the surfaces of the latexes can be modified to match the surfaces of the biological cells to be separated and subsequently used for instrument evaluation. In an independent research project at our laboratories, an attempt is being made to bind protein to the surface of polystyrene latex particles. Carboxylated and crosslinked polystyrene latex particles with a diameter of 0.19μm were used as a substrate for binding γ-globulin. The γ-globulin was covalently bonded to the carboxyl on the surface using carbodiimide. The electrophoretic mobility of the γ-globulin treated particles in deionized water was 2.73μm cm/volt sec as compared with 3.29μm cm/volt sec for the untreated latex. The electrophoretic mobility of the treated and untreated latex were also measured as a function of pH and constant conductivity. The results showed that the γ-globulin treated latex had a lower mobility over the pH range of 3 to 11. The conclusion is that carboxylated polystyrene latexes, which may be prepared in a very monodispersed state, can be used as an active surface to adsorb protein molecules, and hence can be used as model particles for biological systems.
E. REFERENCES


Figure 2: Cross Section of Rectangular Electrophoresis Channel.

Figure 3: Linearized Parabolic Flow Profiles for Rectangular Electrophoresis Channels with Different Values of $K$, Cylindrical Channel, and Double-Cylindrical Channels.
Figure 4  Electrophoresis Cell with Removable Capillary Channel.
Figure 5  Electrophoresis Cell with Removable Rectangular Channel.
Figure 6  Bellows Polymerization Vessel - side view
Figure 7  Transmission Electron Micrographs of a.

Seed Latex; and b. the Polymerized Seed Latex.