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# NASA TECHNICAL MEMORANDUM

NASA TM X-64807

## DEVELOPMENT OF COATINGS TO CONTROL ELECTROOSMOSIS IN ZERO GRAVITY ELECTROPHORESIS

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Astronautics Laboratory

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16. ABSTRACT  A major problem confronting the operation of free fluid electrophoresis in zero gravity is the control of electrokinetic phenomena and, in particular, electroosmosis. Due to the severity of counter flow, as a result of electroosmosis, the electrical potential ( $\zeta$ potential) developed at the surface of shear must be maintained at near, or as close to, zero millivolts as possible. Based upon this investigation, it has been found that the amount of bound water or the degree of hydroxylation plays a major role in the control of this phenomena. Of necessity, factors, e.g. adhesion, biocompatibility, protein adsorption, and insolubility were considered in this investigation because of the long buffer-coating exposure times required by present space operations. Based upon tests employing microcapillary electrophoresis, it has been found that $\gamma$ amino propyl trihydroxysilane produced a coating which provides the lowest $\zeta$ potential ( -3.86 mv ) at the surface of shear between the stationary and mobile layers. This coating has been soaked in both borate and saline buffers, up to three months, in a pH range of 6.5 to 10 without deleterious effects or a change in its ability to control electrokinetic effects.			
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## INTRODUCTION

Zone electrophoresis, as is usually done in earth gravity, involves the migration of charged particles supported on an inert, homogeneous solid, gel, or particulate framework to minimize convective disturbances which generally override perturbations resulting from other electrokinetic effects. These techniques, while quite innovative, are generally plagued by losses in resolving power, and contamination by the supporting media when used as a preparative technique. Therefore, electrophoresis, as a preparative technique, has not been found practical for commercial situations. In particular, as in the case of the separation of viable cells, no earth-based technique has been found which could separate these highly differentiated systems containing specific immunoresponsive moieties for use in preventive medicine. Nor have they been provided in such a condition as to allow for research into their in vivo biochemical specificities.

It has been considered by many researchers in the field of electrophoresis that fluid zonal cell electrophoresis, free of earth-bound thermal convective forces, could provide a separation bed equal to or exceeding the high resolving power of polyacrylamide gel systems. In addition, it is believed that the elimination of the gravitational force will eliminate or sharply reduce the denaturation of proteinaceous particles which occurs during sedimentation.

As a result of these conjectures, two static column free fluid electrophoresis demonstrations were flown on Apollo 14 and 16 to test the concept as a means of providing a technique for separation of delicate biological systems. Photographic evidence and data obtained from these demonstrations established that particulate separations in space are feasible and markedly different from those produced in earth gravity. (1) (2) (3)



Figure 1: Free Fluid Zonal Electrophoresis in 1 Gravity

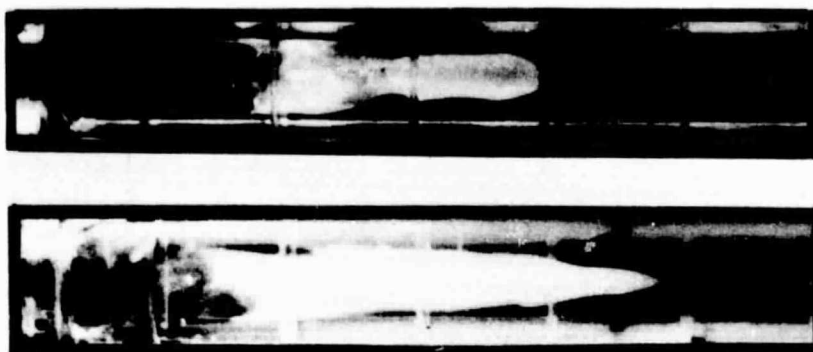


Figure 2: Free Fluid Zonal Electrophoresis at  $1 \times 10^{-4}$  Gravity

In addition, data also showed that electroosmosis was a major problem to be circumvented if free fluid zonal cell electrophoretic techniques were to be employed in space as a means of separating viable cells on a preparative scale.

As a result of these data, an investigation ensued to find or develop coatings which would control this electrokinetic phenomena.

#### DISCUSSION OF ELECTROSMOTIC PHENOMENA

##### Rigid Layer

The motion of dissolved or suspended material under the influence of an applied electric field is dependent or affected by four electrokinetic phenomena; electrophoresis; electroosmosis, streaming potential; and sedimentation potential. Only two, electrophoresis and electroosmosis, are of concern here. All of these, however, are dependent on the nature of the electric double layer and relate to its properties at the surface of shear or slipping plane between the two parts of the double layer, i.e., the rigid and mobile parts. In essence, most substances acquire a surface electric charge when brought into contact with a polar medium. The charging mechanisms are thought to be ionization, ion adsorption, and ion dissolution. The surface charge, in turn, influences the distribution of ions in the medium and combined with the mixing tendency of thermal motion, at least in the earth gravity case, and leads to the formation of an electric double layer. Thus, electrokinetic behavior is thought to depend on the potential at the surface of shear between the charged surface and the electrolyte solution. Stern<sup>(4)</sup> proposed a simple model (Fig.3) in which the double layer is divided into two parts separated by a plane (Stern Layer) located about a hydrated ion radius from the surface. He also considered the possibility of specific ion adsorption. The ions are

attached to the surface by electrostatic and/or van der Waal's forces strongly enough to overcome agitation. The ions may be hydrated, at least, in the direction of the surface where the centers of these specifically adsorbed ions are located in the Stern layer ( $\delta$ ). Ions with centers located beyond the Stern plane form the diffuse part ( $1/\kappa$ ) of the double layer.

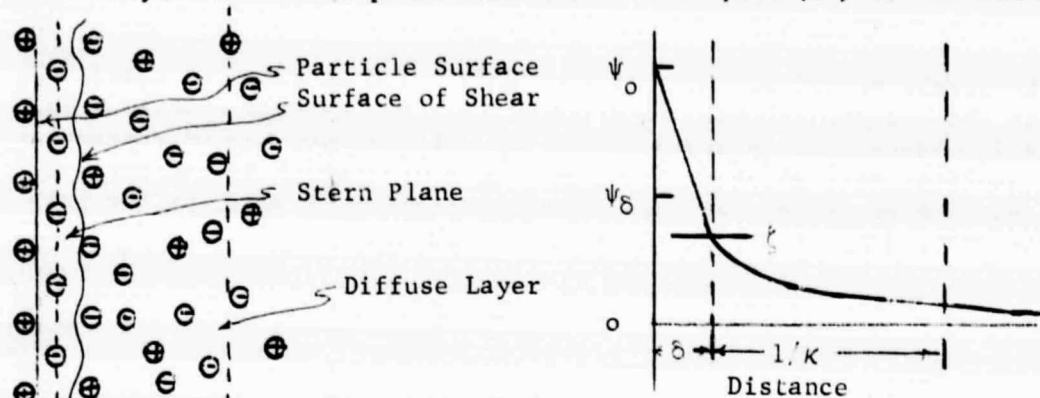


Figure 3: Electric Double Layer According to Stern

Stern also described the equilibrium between ions in the Stern layer and those in the diffuse part by a Langmuir type adsorption isotherm. Considering only the adsorption of counter ions, the surface charge density  $\sigma_1$ , of the Stern layer is given by the expression:

$$\sigma_1 = \frac{\sigma_m}{1 + \frac{N}{n_0 M} \exp \left[ \frac{Z_e \psi_\delta}{\kappa T} \right]} \quad (1.0)$$

Where  $\sigma_m$  is the surface charge density corresponding to a monolayer of counter ions,  $N$  is Avagadro's number,  $N_0$  is the bulk concentration of each ionic specie,  $M$  is the molecular weight of the solvent,  $\kappa$  is the Boltzman constant, and  $T$  is the temperature. The adsorption energy is divided between the electrical ( $Ze\psi_\delta$ ) and van der Waal ( $\phi$ ) terms where  $Ze\psi_\delta$  is the electric potential energy distribution between the wall surface charge ( $Ze$ ) and the Stern layer ( $\psi_\delta$ ). However, the Stern treatment assumes that the double layer is a uniformly charged surface and does not consider the effect of neighboring adsorbed counter ions which affect and rearrange the general surface charge atmosphere to create a two-dimensional analogue of the self atmosphere potential ( $\phi_B$ ) established by the well known Helmholtz and Debye-Huckel theories for electrolytes. The Stern model as refined by Grahame(5) incorporates this discreteness of charge (self atmosphere term) into the Stern-Langmuir expression, which now becomes:



$$\sigma_1 = \frac{\sigma_m}{1 + \frac{N}{n_0 M} \exp \left[ \frac{Ze(\psi_s + \phi_B)}{\kappa T} + \phi \right]} \quad (1.1)$$

The result of adding the self atmosphere term is that the model now predicts that, under suitable conditions,  $\psi_s$  goes through a maximum as  $\psi_0$  is increased. It should be noted that the discreteness of charge effect qualitatively shows that the zeta potential which is the potential at the surface of shear also goes through a maximum as well, as the surface potential ( $\psi_s$ ) of the Stern layer is increased.

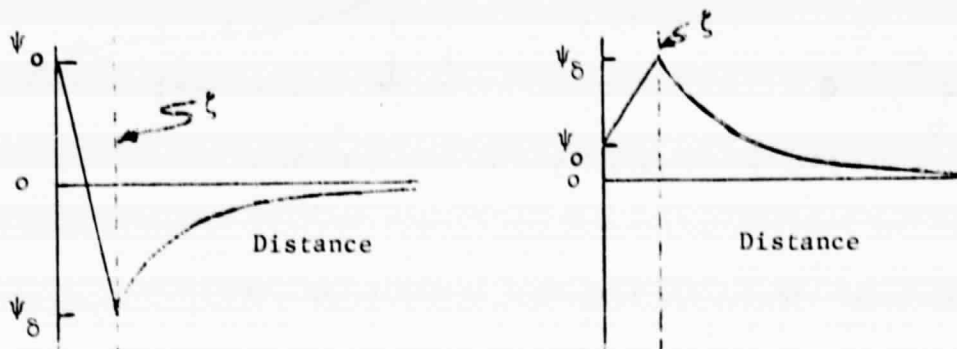


Figure 4: Reversal of Charge Due to Adsorption of Ions and Co-ions

#### Diffuse or Mobile Layer

Electrokinetic phenomena are thus directly related to the nature of the mobile part of the electric double layer and can be interpreted in terms of the charge density or potential ( $\zeta$ ) at the surface of shear. If one considers the double layer as two capacitors ( $C_2$ ) in series the diffuse part of the double layer can be calculated as

$$C_2 = \frac{\sigma_2}{\psi_s} = \frac{\epsilon \kappa}{4\pi}$$

where  $\sigma_2$  is the surface charge density of the diffuse part,  $\epsilon$  is the permittivity, and  $\kappa$  the Boltzman constant  $\psi_s$  can be estimated from electrokinetic measurements. The above mathematical statement is an over-

simplification since one must consider the motion of liquid in the diffuse part of the double layer relative to the nonconducting surface when an electric field is applied parallel to the surface. The liquid layer will attain a uniform velocity parallel to the surface when the electrical and viscous forces are in equilibrium. The electrical and viscosity forces on a liquid of unit area can be equated as,

$$\chi \rho dx = \left( \eta \frac{dv}{dx} \right)_{x+dx} - \left( \eta \frac{dv}{dx} \right)_x = \frac{d}{dx} \left( \eta \frac{dv}{dx} \right), \quad (2.0)$$

Where  $\chi$  is the electric field,  $dx$  is the liquid layer thickness,  $x$  the distance from the surface and  $\rho$  the bulk charge density. Inserting the Poisson equation,

$$\rho = -\frac{1}{4\pi} \frac{d}{dx} \left( \epsilon \frac{d\psi}{dx} \right) - \frac{\chi}{4\pi} \frac{d}{dx} \left( \epsilon \frac{d\psi}{dx} \right) = \frac{d}{dx} \left( \eta \frac{dv}{dx} \right),$$

$$\text{and integrating we obtain } -\frac{\chi\epsilon}{4\pi} \frac{d\psi}{dx} = \eta \frac{dv}{dx} + \text{constant}. \quad (2.1)$$

The integration constant is zero because when  $x = \infty$ ,  $d\psi/dx$  and  $dv/dx = 0$ . Also assuming that the permittivity ( $\epsilon$ ) and viscosity ( $\eta$ ) are constant and integrating again

$$\frac{\chi\epsilon}{4\pi} \psi = \eta v + \text{constant}. \quad (2.2)$$

Considering electrophoresis, the boundary conditions are  $\psi = 0$  and  $v = 0$  at  $x = \infty$  and  $\psi = \zeta$ ,  $v = -v_E$  at the surface of shear ( $v_E$  = electrophoretic velocity).

$$\text{Therefore } \frac{\chi\epsilon}{4\pi} \zeta = \eta v_E$$

or the electrophoretic mobility ( $U_E$ )

$$U_E = \frac{v_E}{\chi} = \frac{\zeta\epsilon}{4\pi\eta} \quad (2.3)$$

which is the Smoluchowski equation. (6)

Using boundary conditions of  $\psi = 0$ ,  $v = v_{E,0}$  at  $x = \infty$  and  $\psi = \zeta$ ,  $v = 0$  at the surface of shear, an identical expression can be derived for electroosmosis by adding specific conductance ( $K_s$ ) and current ( $I$ ) terms. Thus

$$\zeta = \frac{4\pi\eta U_E K_s}{\epsilon I} \quad (2.4)$$

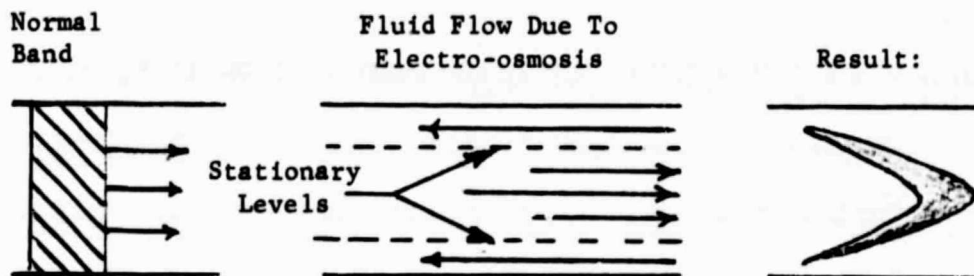
To convert from electrostatic units  $\zeta$  must be multiplied by 300. By doing this, the Smoluchowski equation at 25°C becomes

$$\zeta = 12.85 U_E \text{ millivolts} \quad (2.5)$$

for large  $K_s$ .

Since electroosmosis is the movement of a liquid relative to a fixed solid under the influence of an external field, there is essentially no difference between the results obtained by electrophoretic or electroosmotic measurements. The data presented by Ham and Douglas<sup>(7)</sup> for octadecanol supports this conclusion. Also, since the zeta potential represents the potential gradient across the mobile part of electric double layer existing from the solid-liquid interface out into the liquid phase, the zeta potential is an indication of the surface charge of the solid phase.

In free fluid zonal cell electrophoresis, electroosmosis must be controlled if you expect to obtain a worthwhile separation of particles or material since the fluid flow due to electroosmosis tends to disrupt the planar band shapes. Figure 4 shows the effects of electroosmosis and its effect on electrophoretic band dynamics.



Effects of Electro-osmosis  
Figure 5

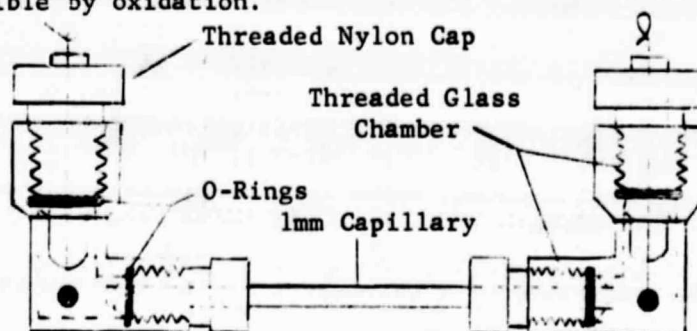
The effect of surface charge build up and the resulting electroosmotic flow can be verified experimentally in a microcapillary

electrophoresis apparatus. Therefore, this technique is an effective means for evaluating surface coatings to control these phenomena.

#### METHOD

##### Microelectrophoresis

Macrotechniques cannot be used to adequately evaluate electrokinetic phenomena; therefore, cell electrophoresis and the determination of zeta potential (hence electroosmosis) was performed using the general procedure and apparatus described by Seaman<sup>(8)</sup> and Seaman and Heard<sup>(9)</sup>. For ease of operation, an electrophoresis cell with a replaceable glass capillary channel (Figure 5) was used. The electrodes were platinum made reversible by oxidation.



Electrophoresis Cell with Removable Capillary Tube  
Figure 6

Potentials between 12 to 100 volts were employed, which produced a field strength of  $1 \text{ Vcm}^{-1}$  to  $10 \text{ Vcm}^{-1}$ , using either borate, phosphate, or saline buffers. All measurements were made at  $25 \pm 0.2^\circ\text{C}$ . The particles employed were 1.01 micrometers diameter polystyrene latex spheres having a mean mobility of  $5.95 \mu\text{cm V}^{-1}\text{sec}^{-1}$ . The results are obtained as a velocity-distance plot which converts the parabolic velocity-distance relationship to a straight line. This allows comparison of straight lines which give the electroosmotic flow velocity as the intercept at the ordinate and the electrophoretic mobility of the latex particles at the stationary level within the electric double layer. The zeta potential is then calculated according to the Smoluchowski equation after making the necessary corrections for optical refraction described by van Gills<sup>(10)</sup>.

#### SURFACE INTERFACIAL PHENOMENA

To develop zero or near zero zeta potential interfaces one must not only consider bulk and surface properties, but must also consider the interaction or adhesion between the two phases and the particle e.g., the solid or coating surface, the fluid, and/or the mobile particle.<sup>(11)</sup>

In addition, the synthetic coating in question must be designed so that the bulk properties of the fluid and interfaces appear to be very similar in nature to that of biological systems. Unfortunately, the physical phenomena of biological systems are little understood. However, the picture is not totally impossible, since most biostructures contain around 90% water and there is mounting evidence that its role is not just as a passive media, but rather plays an important role in biological processes, particularly at the interfaces (13-15).

The interface of vascular systems of many biological organisms consists of a single layer of thin polygonal cells (generally one micron thick) separated by an intercellular junction (16). This intercellular junction contains negatively charged pores on the order of 20 to 50Å in diameter having a double electric layer ~10Å thick with a zeta potential ranging from 10 to 15 millivolts. It is believed that this surface charge is essentially neutralized at about 10Å into the fluid environment. Considering zeta potential, alone, would indicate that these biological interface surfaces cannot be biocompatible with other biological surfaces. However, if this were the case, no life form could exist. Indeed, since the interfacial tension for cells is very low (on the order of 1-3 dynes cm<sup>-1</sup>), there must be a buffer zone between the cell interface and the fluid. Recent studies (17)(18)(19) have shown the existence of a carbohydrate-rich coating approximately 300Å thick, covalently attached to the cell surface. Due to the nature of these carbohydrate moieties, it is very likely that they are gelatinous in nature and highly hydrated. In turn, this surface coating probably acts as the buffer zone between the high interface zeta potential and the fluid environment. It should be noted that the mere presence of water on the carbohydrate coating is not sufficient cause for the low interface tension observed on cells, nor would it necessarily lower the apparent zeta potential at the cell membrane surface. Therefore, the more important issue is to correlate how this water of hydration is oriented and structured in relation to other functional groups, e.g. CH<sub>3</sub> and OH, in the carbohydrate structure.

#### LOW ZETA POTENTIAL COATINGS

Initially, our study was directed toward the evaluation of hydrophilic polysaccharide gels containing high concentrations of water. Our reasoning for this selection was based on the contention that gels have biological significance and also, that they are used in gel column electrophoresis. However, it was found that polysaccharides such as gelatin, agar, and agarose tended to produce high negative potentials between 50 to 120 millivolts.

Modified methyl cellulose (methocel) products were investigated as well. Cellulose derivative gels were tested in the molecular weight range between 20 to 110K. The negative zeta potentials were found to vary between 10 to 30 millivolts with increasing molecular weight. However, during soaking in the electrophoresis buffer, it was found that these methocels tended to dissolve, and in turn, coat the polystyrene latex spheres which were used in the mobility measurements. As a consequence, it was found that the mobilities dropped to near zero rendering the data useless. Attempts were made to control the solubility of these methyl cellulose derivatives by cross linking with formalin and glutaraldehyde according to the procedure described by Hjerten<sup>(20)</sup>. The result of cross linking methocel (M. W. 110K) increased the negative zeta potential to 98 millivolts. While cross linking was found to improve insolubility of the coating, it became more hydrophobic. The degree of cross linking was found to be directly related to the increase in zeta potential and hydrophobicity. The data for Methocel MC (M. W. 110K) is shown in Figure 7.

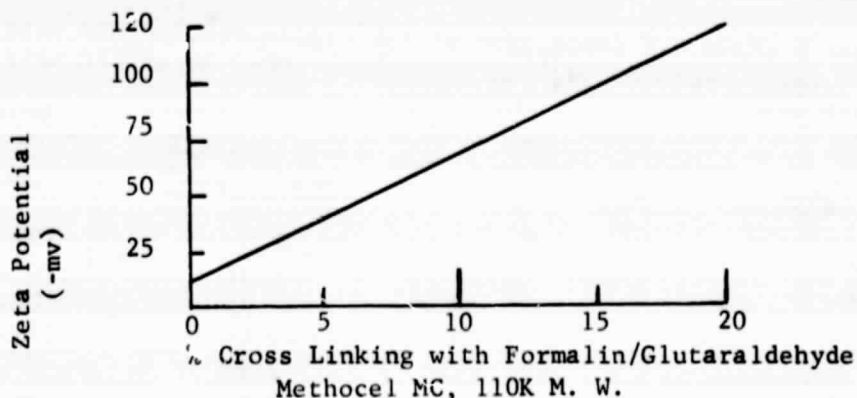


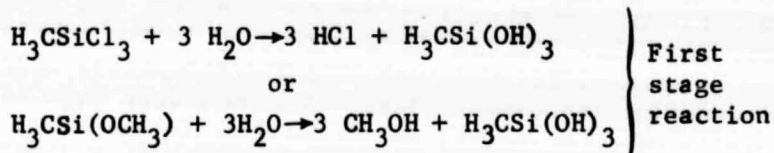
Figure 7

It should be noted that lower molecular weight cross linked methocels gave similar characteristic curves. While cross linking of the cellulose derivatives did not show the interfacial condition we wished to achieve, it may be possible to covalently bond them to subbing layers such as the alkoxy or glycidylsilanes to decrease their inherent solubility without the need to cross link. Our future work will investigate this possibility.

As based on the data obtained from our studies on the polysaccharide and cellulose derivative gels, it was believed that perhaps control of surface electrokinetic phenomena could be achieved by the use of simpler molecules containing a high degree of bound water or hydroxy groups. As a result, we decided to investigate the electrokinetic properties of silane monomers, because many are easily hydrolyzed and their chemical structures can be manipulated with relative ease. These monomers generally show good adhesion qualities with numerous substrates and in some cases have been found to be biocompatible.



Nine silane monomers were initially selected for testing. These were trichloromethylsilane, triethoxymethylsilane, chlorobutyltrimethoxysilane,  $\gamma$  aminopropyltriethoxysilane, and 3-(2-aminoethylamino) propyltrimethoxysilane. These silanes will react with water, in air, in the following manner:



These moities were made as a 10% solution by volume with a suitable solvent. Capillary tubes ( $1.0 \pm 0.1$  mm dia.) coated by exposing the interior of the tubes to the solution for a few minutes were allowed to drain. The remaining excess liquid was removed by touching the end of the tube with an absorbent towel. The coatings were then permitted to hydrolyze in air for 24 hours. Zeta potential measurements were made by the previously indicated method.

Figure 8 shows the comparison of the electroosmotic and zeta potential measurements on various silanes to other materials used in electrophoresis.

Coating Material	Electroosmosis	Zeta
	$\mu\text{cm V}^{-1} \text{ sec}^{-1}$	Potential -mv
Glass	-8.55	109.87
Collodian	-8.37	107.55
Agar	-8.28	106.45
Agarose	-9.95	127.92
Methocell (110K M. W.)	-2.94	37.78
Dihydroxymethylsiloxane	-3.61	46.39
$\gamma$ Aminopropyl dihydroxysiloxane	-0.30	3.86
Allylcarboxypropyl dihydroxysiloxane	-8.60	110.51
3-(2-aminoethylamino)propyl dihydroxysiloxane	-3.57	45.87
Dry Film SC-87	-6.23	80.06
Propyl dihydroxysiloxane	-0.53	122.46
Chlorobutyl dihydroxysiloxane	-4.28	55.00
Methoxyethoxypropyl dihydroxysiloxane	-8.85	113.72

Figure 8

The  $\gamma$  aminopropyl dihydroxysiloxane was also covalently bonded to glutaraldehyde and egg albumin and produced progressively higher negative zeta potentials of 82.2 and 56.54 mv respectively as did the cellulose derivative gels. In addition, the  $\gamma$  aminopropyl dihydroxysiloxane was also exposed to different curing temperatures and time durations. Figure 9 shows a comparison of temperature and cure time on the electro-osmosis and zeta potential.

<u>Curing Temperature and Time</u>	<u>Electroosmosis <math>\mu\text{cm V}^{-1} \text{sec}^{-1}</math></u>	<u>Zeta Potential -mv</u>
100°C for 3 hr.	- 0.30	3.8
100°C for 8 hr.	- 0.40	5.1
100°C for 24 hr.	- 0.61	7.8
125°C for 3 hr.	- 0.30	3.8
125°C for 8 hr.	- 0.70	9.0
125°C for 24 hr.	- 3.29	42.3
200°C for 3 hr.	- 2.33	30.0
200°C for 8 hr.	- 2.60	33.4
200°C for 24 hr.	- 2.00	25.7

Effects of Cure Time and Temperature on Some Electrokinetic  
Properties of  $\gamma$  Aminopropyldihydroxysiloxane

Figure 9

The apparent discrepancy in the electrokinetic data on curing at 200°C for 24 hours was due to decomposition of the silane, principally caused by the loss of amine.

The stability of  $\gamma$  aminopropyldihydroxysiloxane when exposed to saline and phosphate buffers for periods up to three months and repeated electrophoresis shows no loss in the coatings ability to control electroosmosis and does not require anything other than drying at room temperature.

#### CONCLUSIONS

It has been found that simple organosilanols can be used to control electrokinetic phenomena in microcapillary electrophoresis and can be expected to work well in zero gravity electrophoresis where macro systems will be used.

At present, the underlying mechanism by which  $\gamma$  aminopropyldihydroxysiloxane controls electroosmosis is not understood. Intuitively it is believed that this mechanism is very similar in nature as that due to the polysaccharide gel interface around biological cells.

Further elucidation of this particular molecule and its interaction between the fluid and stationary layers in electrophoresis may aid in the understanding of biological interfaces since these interface phenomena appear to be perturbed by the orientation and structuring of the hydroxy and alkane radicals between the proteinaceous cell membrane interface and the polysaccharide buffer zone.



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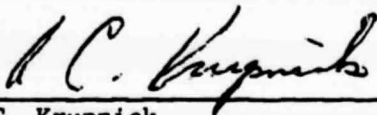
APPROVAL

DEVELOPMENT OF COATINGS TO CONTROL ELECTROOSMOSIS  
IN ZERO GRAVITY ELECTROPHORESIS

By

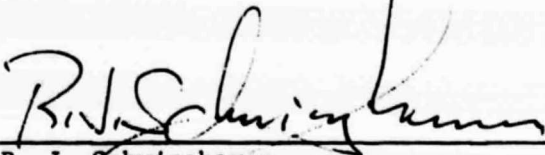
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The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the MSFC Security Classification Officer. This report, in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



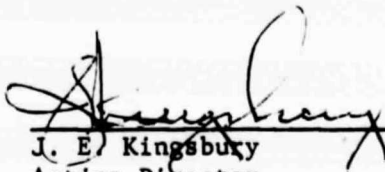
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