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**CAPILLARY ELECTROPHORESIS: IMAGING OF ELECTROSMOTIC AND
PRESSURE DRIVEN FLOW PROFILES IN FUSED SILICA CAPILLARIES**

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Introduction

This study is a continuation of the summer of 1994 NASA/ASEE Summer Faculty Fellowship Program. This effort is a portion of the ongoing work by the Biophysics Branch of the Marshall Space Flight Center. The work has focused recently on the separation of macromolecules using capillary electrophoresis (CE). Two primary goals were established for the effort this summer. First, we wanted to use capillary electrophoresis to study the electrohydrodynamics of a sample stream. Secondly, there was a need to develop a methodology for using CE for separation of DNA molecules of various sizes.

In order to achieve these goals we needed to establish a procedure for detection of a sample plug under the influence of an electric field. Detection of the sample with the microscope and image analysis system would be helpful in studying the electrohydrodynamics of this stream under load. Videotaping this process under the influence of an electric field in real time would also be useful. Imaging and photography of the sample/background electrolyte interface would be vital to this study. Finally, detection and imaging of electroosmotic flow and pressure driven flow must be accomplished.

Experimental Section

Experiments to accomplish the following were designed and implemented this summer.

1. capillary electrophoresis chamber design and construction
2. sample materials selected and prepared for CE experiments
3. background electrolytes or buffer systems were prepared
4. experiments with sample injection methods
5. power requirements for CE were determined
6. systems interfacing, monitoring and safety requirements
7. methods for detection of sample
8. fluorescence and imaging in real time during CE runs
9. documentation by way of videotape
10. data analysis

A small CE chamber ~ 36 cm long was built using two pieces of fused silica with polyamide coating, O.D. = 356 μm , I.D. = 246 μm . Each was sealed with silicone adhesive sealant inside the ends of an uncoated square glass capillary, 400 μm I.D. Total volume for this system is ~ 163 μL . This assembly was placed on a small plastic block, 75 mm X 25 mm, and sealed into place with silicone adhesive sealant. The capillary ends of the chamber were placed into small 25 mL buffer wells of glass with copper bottoms for electrical connection. The inlet and outlet buffer wells were placed on to a small copper electrode attached to the top of a small lab jack with double sided tape.

This system allowed for the leveling of the background electrolytes and elimination of pressure driven flow.

Detection and imaging of the sample fluid required a material which could be seen with the light microscope. We decided to use a fluorescent sample material which could be intensified, videotaped, and analyzed using the Zeiss Axioplan and Hamamatsu image analysis system. The buffer of choice was a phosphate buffer with a conductivity of $\sim 320 \mu\text{mhos}$. This electrolyte was seeded with quinine bisulfate, $100 \mu\text{g/mL}$, and the sample material was a preparation of 0.1 N sulfuric acid with quinine bisulfate at a concentration of $100 \mu\text{g/ml}$ (1). A volume of $200 \mu\text{L}$ of dilute $5.4 \mu\text{m}$ diameter polystyrene latex microspheres was placed into the outlet and inlet buffer chambers. The leveling jacks were adjusted while viewing the movement of these particles until all particles had completely stopped moving in the square capillary. This was done to eliminate pressure driven flow within this CE system. Quinine bisulfate is excited by ultraviolet radiation at 360 nm , and since the background electrolyte and the sample both contained this compound these experiments were conducted in a darkened laboratory. The ultraviolet light was turned on and the emission was observed with the naked eye, brought into view on a tv monitor, and videotaped.

Samples were injected by electroosmotic flow for twenty seconds (2) and the inlet buffer capillary tip was replaced in the background electrolyte. Optimum power requirements were $\sim 2 \text{ kv}$, at $\sim 8.0 - 9.0 \mu\text{A}$. A dc power supply was used to provide power and an in-line meter was used for monitoring the current. The CE runs were accomplished with two persons for safety. Detection of the sample/buffer interface was easily achieved since the emission spectrum was slight for the buffer and much brighter for the sample. The tube assembly was placed on a Zeiss Axioplan research microscope and the fluid observed and videotaped using the Hamamatsu/Argus-10 system. Averaging of the raw image of four frames per second provided optimum image enhancement.

Another test chamber was constructed with a longer square glass capillary in the center. This unit was placed on top of a $75\text{mm} \times 25 \text{ mm}$ copper block which was covered with black electrical tape. This unit allowed us to place it on a specially designed and custom built motorized stage which has a speed control on the horizontal axis as viewed in the microscope. After the image was seen on the tv monitor the CE unit was set to the speed synchronized to the electroosmotic flow of materials inside the capillary. This system enabled us to observe and videotape the sample/buffer interface for several minutes.

Results and Discussion

The CE system was ideal for observing the interface. The fluorescent material provides good visibility, and the image analysis system with image enhancement provides a smooth, stable sample image for observation and online experimentation such as the changing of parameters during CE runs under load. Time requirements for movement of this sample materials vary with voltage. We used much higher voltages, about 55.5 v/cm, for sample injection and movement to the detection area on the capillary. Once the sample stream was in view, voltages were reduced to ~ 12 v/cm and the electroosmotic flow was drastically reduced.

Electroosmotic flow (EOF) was first identified in the late 1800's when Helmholtz worked with an applied electric field in a horizontal glass tube containing an aqueous salt solution. He pointed out that the silica imparts a layer of negative charge to the inner surface of the tube, which, under an electric field, led to the net movement of fluid toward the cathode (3). EOF or bulk flow acts as a pumping mechanism to propel all molecules (cationic, neutral, and anionic) toward the detector with separation ultimately being determined by differences in the electrophoretic migration of the individual analytes. The magnitude of EOF is dependent on a number of parameters including pH and ionic strength of the analytes. The inclusion of EOF in the calculation of velocity is essential (4).

During some of our experiments we were able to induce movement of the sample slug by reversing the polarity during the run. This resulted in a flow in the opposite direction. Also, pressure driven flow profiles were easily induced by either elevating or lowering buffer wells located beside the microscope stage on lab jacks. Overall, this portion of the summer work went extremely well and we are pleased with our results.

We are also working with a fluorescent material which binds to cells. We would like to be able to detect and videotape the separation of proteins using CE. Finally, we are continuing the work with DNA using fluorescence, gels for sieving, and capillary electrophoresis for the separation of various sizes of these macromolecules.

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