ABSTRACT

A basic premise of continuous flow electrophoresis is that removal of buoyancy-induced thermal convection caused by axial and lateral temperature gradients will result in ideal performance of these instruments in space. Although these gravity-dependent phenomena disturb the rectilinear flow in the separation chamber when high voltage gradients and/or thick chambers are used, distortion of the injected sample stream due to electro-hydrodynamic effects causes major broadening of the separated bands.

Although the electrophoresis separation process is simple in concept, flows local to the sample filament produced by the applied electric field have not been considered. These electro-hydrodynamical flows, formulated by G. I. Taylor in 1965 for drops suspended in various liquids, distort the sample stream and limit the separation. In addition, electroosmosis and viscous flow, which are inherent in the continuous flow electrophoresis device, combine to further disturb the process. Electroosmosis causes a flow in the chamber cross section which directly distorts the sample stream, while viscous flow causes a parabolic profile to develop in the flow plane. This flow profile in turn distorts migration by causing a varying residence time across the thickness of the chamber. Thus, sample constituents at the center plane will be in the electric field a shorter time and hence move less than comparable constituents closer to the chamber wall.

A moving wall concept is being proposed for space which will eliminate and/or control all of the above-mentioned disturbances. The moving wall will entrain the fluid to move as a rigid body and hence produce a constant residence time for all sample distributed across the chamber thickness. By aligning the moving wall at an angle to the chamber axis, a component of the moving wall motion can be made to oppose and hence cancel the electro-osmotic flow. In the absence of electrokinetic effects, i.e. electroosmosis, the electro-hydrodynamical effect manifests itself as a ribbon, being either vertical (perpendicular to the electric field) or horizontal (aligned with the electric field) depending on the ratio of conductivity of the sample to that of the buffer. Therefore, by using low conductivity sample solutions to provide a vertical ribbon, the moving wall concept...
should produce distortion-free separations.

The moving wall electrophoresis chamber can only be operated in space because there is no viscous flow in the chamber to stabilize against thermal convection. Laboratory prototype instruments have been built which confirm the sensitivity of their operation. These prototypes have also identified engineering problems such as liquid seals. However, the moving wall electrophoresis system is a concept designed for space which should permit preparative electrophoresis to attain its potential.
Introduction

The major goal of the electrophoresis program at the Marshall Space Flight Center (MSFC) is to obtain high resolution separation of viable cells in space that is not attainable on Earth. Although the requirement for the separation and collection of viable cells is not as extensive as it was in the 1970's when cells were cultured principally for their protein production, purification of cells is still of interest for research. Continuous flow electrophoresis (CFE) remains the major technique which can fractionate viable unmodified biological cells in excess of $10^8$ cells/hour. However, the resolution of separation for this technique is limited by inherent extraneous flows and sample/electric field interactions which have not been recognized or fully understood.

At the beginning of the NASA electrophoresis program it was proposed that electrophoresis of cells in free fluid in space should achieve an optimum separation because the main detractors to resolution, thermal convection and sedimentation, were removed. This rationale was based on the fact that electrophoresis in porous gels had become the standard technique to separate and identify the various protein constituents in a complex mixture. Experiments in our laboratory established that the limiting pore size for agarose, an extremely large pore size gel, was $0.5 \mu m$. The first experiments in the Apollo spacecraft in 1972 and Apollo-Soyuz Test Project in 1975 attempted "gel-
free" electrophoresis of latex particles and viable cells in cylindrical columns of buffer. Photographs showed bands of polystyrene latex distorted by electroosmosis and separated cells that were too few in number to analyze for secreted products. These experiments did show, however, that for free zone electrophoresis of particles in microgravity, only electrophoresis and electroosmosis determined the migration.

The pause in space flight between the Apollo and Space Shuttle and the encouraging results from the zone electrophoresis experiments led to the development of the Continuous Flow Electrophoresis System (CFES) by the McDonnell Douglas Astronautics Company (MDAC). Commercial continuous flow electrophoresis instruments are not efficient because of the major design requirement to compensate for gravity-induced convection and sedimentation. Their chambers are thin (typically 0.5 mm) to minimize temperature gradients and, consequently, sample input is restricted and detrimental wall effects (to be discussed later) are enhanced. McDonnell Douglas increased their flight electrophoresis chamber dimensions by a minimum factor of two over their ground-based chamber to increase the sample throughput; however, the ratio of chamber thickness to sample bore diameter remained 3:1 which is typical of some commercial instruments.

Recent experiments and analysis at MSFC indicate that electrohydrodynamic considerations are the major factors limiting
the resolution of separation in continuous flow electrophoresis. A new electrophoresis instrument has been designed to take advantage of both the reduced gravity environment and electrohydrodynamic (EHD) effects. In order to determine the effect of EHD on continuous flow electrophoresis and how this effect may be used to enhance CFE performance in space, sample stream distortion and its resulting degradation of performance must be understood.

Continuous Flow Electrophoresis (CFE) takes place within a flowing curtain of aqueous electrolyte contained in a long rectangular chamber of high aspect ratio as shown schematically in Figure 1. The narrow gap between the broad faces of the chamber confines the flow to a thin curtain. The sample is continuously injected into this curtain as a finely-drawn stream (filament) and fractionated under the influence of a lateral electric field produced by flanking electrodes. The fractionated sample bands are subsequently collected in a uniform array of collection ports situated along the exit end of the chamber. The no-slip condition at the chamber wall causes a parabolic profile to develop in the direction of buffer flow. In addition, a lateral flow, across the width of the chamber (perpendicular to buffer flow), called electroosmosis, exists when charged walls are present. These independent phenomena are inherent in the CFE process and they combine to produce crescent-shaped distortions, the curvature of which is determined by the flow that predominates,
Figure 1. Schematic of typical CFE separation chamber.
either laminar flow or electroosmosis. This mechanism of sample stream distortion has always been thought to be a major cause of resolution degradation in CFE devices. The term "wall effects" has frequently been used to describe the phenomenon.

These "wall effects" were first noted and recorded by Strickler\textsuperscript{6} in 1967 through the use of his cross-section illuminator. Figure 2 shows six different samples of varying electrophoretic mobility and their distorted bands. The crescents arising from viscous flow and electroosmosis influence the observed shapes in two ways. First, the no-slip condition at the chamber wall causes the parabolic profile to develop in the direction of buffer flow causing sample residence time to vary locally in the thickness of the chamber. Therefore, sample near the walls remains in the electric field longer than identical sample near the center plane and is deflected further by the electric field. This causes a crescent with the nose toward the negative electrode on the left. Electroosmosis acts to produce the opposite effect. Positive charges induced in the buffer at the wall as a result of its negatively charged surface causes the buffer at the wall to flow to the left toward the negative electrode and return to the right along the chamber center plane. This effect causes crescent formations with noses toward the positive electrode on the right. It is important to note that these are competing effects, so that one must either balance or eliminate them. Until recently, this so-called "wall effect" was
all that was known about sample filament distortion, i.e., it was not known what caused the sample to deviate from the center-plane and approach the wall. Although a correlation between sample stream spreading and sample conductivity was observed by Strickler\textsuperscript{7}, no explanation of sample movement toward the walls was given. Therefore, sample stream spreading has primarily been considered sample migration away from the center plane region into marginal zones near the wall, with spreading of the band induced by combined action of viscous flow and electroosmosis.

It has been determined, however, that the actual initial sample stream distortion is an electrohydrodynamic effect whose shape is determined by ratios of dielectric constant and conductivity between the sample and buffer, and which has a magnitude proportional to the square of the voltage gradient. It is this distortion mechanism which moves the sample toward the walls and produces the spreading previously mentioned. However, under certain conditions the sample stream can also spread laterally and migrate toward the chamber center plane. This lateral mode of sample stream distortion could have a more adverse effect on resolution than the case of transverse sample migration.

2. ANALYSIS

The electrohydrodynamic relationships are developed along the lines of Taylor\textsuperscript{8}. Taylor showed the elongation of a drop of one dielectric fluid in another depended on the ratios of the conductivity, viscosity and dielectric constant for the drop.
and the surrounding fluid. He determined a discriminating function to predict whether the drop would become prolate or oblate.

His arguments can be used to calculate the distortion of a sample stream during electrophoresis. Consider a cylindrical fluid sample filament in a rectangular flow section. It is assumed that the Reynolds number is small so that it is permissible to use the linear (Stokes) equation of motion; and that the cylindrical sample stream is small compared to the thickness of the chamber. If a typical cross-section is analyzed, the sample will appear as a circle in an infinite expanse of buffer. Taking the subscripts of 1 for the sample and 2 for the buffer, the conductivities, dielectric constants and viscosities are $\sigma_1$, $k_1$ and $\eta_1$, respectively. The sample filament has a radius $a$ and $E$ is the uniform electrical field far from the filament. Note that the field is such that a negatively charged particle moves from left to right. Following the arguments of Taylor an expression for the surface stress can be derived:

$$C = \frac{E^2 K}{4\pi (R^2 + 1)} \left[ S (R^2 + R + 1) - 3 \right] \cos 2\theta$$

where: $C =$ surface stress

$E =$ Electric field strength
R = Ratio of Conductivity of Sample to that of Buffer
K = Dielectric constant of buffer
S = Ratio of dielectric constant of buffer to that of sample - $K_2/K_1$

The term inside the brackets can be considered a discriminating factor since it determines the sign of the surface stress at a specific angle, $\theta$. The ratio of dielectric constants, S, will be near unity for experiments with dilute polystyrene latex samples in buffers made of the same constituents as the flowing curtain. When the conductivity of the sample equals that of the buffer, $R = 1$, and the surface stress is zero signifying an undistorted sample stream. When $R$ is greater than one (high conductivity sample), the discriminating factor and surface stress is positive and the surface stress varies with $\theta$ as shown in Figure 3. When a relatively low conductivity sample is used, $R$ is less than one and the surface stress vectors shown in the Figure 3 are reversed.

![Diagram](image)

Fig. 3. Case of high conductivity sample showing surface stress acting on a cylindrical sample filament in an electric field.
The major difference between Taylor's case of the immiscible fluid drop and this case of the sample filament in a buffer solution is that no restoring force such as surface tension is present to limit the distortion of the sample filament. Therefore, it follows that the deformation will continue until a ribbon is formed. This ribbon formation is confirmed by the following experiments. Figure 4 shows a view of the buffer collection end of a horizontal electrophoresis chamber. A single species of white polystyrene latex was inserted into the input end of the chamber (not in view) and the thin cylindrical sample can be seen. The electric field is produced by electrodes that extend the length of the six-inch long chamber. Figure 5 shows an example of the low conductivity latex sample distorted into a vertical (transverse) ribbon in the high conductivity buffer. Figure 6 shows a horizontal (lateral) ribbon obtained when a high conductivity sample is inserted into a low conductivity curtain buffer. These distortions resulted from the application of a 400 Hz A.C. field. Since the equation for surface stress contains $E^2$, a steady component along with the varying component of 400 Hz can be expected. Of course, in this instance, only the steady component is evident and, thus, only the phenomena described by the analysis is observed. The use of an A.C. field also serves to eliminate all electrokinetic effects, i.e. viscous flow effects and electroosmosis, which otherwise mask the electrohydrodynamic phenomena.
Since there is no practical way to exactly satisfy conditions with the discriminating function equal to unity, the distortions must then necessarily be controlled. Also, since it is apparent that none of these phenomena are gravity-related, then for any continuous flow device to operate efficiently in space, the candidate apparatus must control these effects.

**Moving Wall Electrophoresis**

The moving wall electrophoresis instrument disclosed in a previous publication\(^9\) and patent\(^10\) will theoretically take care of these distortions (Figure 7). The moving wall will entrain the buffer to flow as a rigid body, eliminating the parabolic profile and providing constant residence time for all sample in the chamber thickness. Also, by rotating the chamber axis at an angle to the direction of motion of the moving walls, a component of belt motion can be made to cancel the electroosmotic flow at the wall. Therefore, with the "wall effects" eliminated, sample suspensions of lower conductivity than the curtain buffer can be utilized to produce the vertical ribbon formation that focuses the sample filament. Since the apparatus will be operated in space, curtain buffers of higher ionic strengths can be used without producing the convective disturbances generated by equivalent voltage gradients at 1-g. This arrangement will not only give optimum resolution, but also will increase the throughput of the device by utilizing the entire thickness of the chamber.

To effect the elimination of electroosmosis, the two paral-
Figure 7. Moving wall chamber prototype.
lel flanking electrodes which bound the separation chamber are moved at a slight angle to the direction of belt motion. This produces a component of belt motion which can be used to compensate for the electroosmotic velocity. The natural wall charge will then determine the amount of electroosmosis present; also, using a material with a high zeta potential will minimize the affects of any contamination. The compensation is thus achieved mechanically through the use of a stepper motor which, in turn, is controlled by a computer. Thus, the output in the collection tubes can be monitored and the resulting potential information fed into the computer to give precise control of electroosmosis.

The three main subassemblies of the device are shown in Figure 8. The upper moving wall assembly contains a belt which is shown being transported from one roller and collected on another roller. The gear synchronizes the belt motion. Channels allow a slight vacuum to be applied to ensure close contact of the moving film to the subsurface. The schematic of the electrophoresis chamber shows a buffer insertion port, a sample insertion port, the two electrode channels, and the multi-tube collection array. Semi-circular protrusions fit into the guides of the lower moving wall assembly. This arrangement permits the electrophoresis chamber to rotate relative to the moving film belts in both the upper and lower assemblies. The lower assembly is similar to upper one and functions to transport the thin, polymeric film slowly from one roller to the other. The speeds
Figure 8. Schematic of moving wall electrophoresis.
of both belts are controlled precisely by the synchronization gear. The film is kept flat on the surface using the same method of vacuum application.

Conclusion

It is clear from flight experiments using the MDAC CFES that the initial argument, the removal of thermal convection and sedimentation in space would result in optimized operation, is wrong. These same space experiments, recent literature reviews, and laboratory experiments have shown that electrohydrodynamic factors significantly degrade CFE in space as well as in 1-g. At present, only a device such as the moving wall electrophoresis system containing a high conductivity buffer with a large applied electric field can give high resolution separations in space. This device is designed exclusively for space and can only be operated there with optimum efficiency. Although the feasibility of this moving wall concept has not yet been established, it does not seem practical to fly devices which are defeated in space by the same phenomena that limit their efficiency on the ground.
References


7. Strickler, A., personal communication.

