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ANALYSIS OF THE APOLLO-SOYUZ TEST PROJECT EXPERIMENT ON
THE ELECTROPHORETIC SEPARATION OF FIXED RED BLOOD CELLS

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INTRODUCTION

The Apollo-Soyuz Test Project (ASTP) carried out in July, 1975 included an electrophoretic separation experiment of biological cells (MA-011). The nature of these experiments was to determine if biological separations could be carried out in space under microgravity conditions and with better resolution than obtainable on earth. The experiments consisted of a series of static and isotachoelectrophoresis experiments in which mixtures of aldehyde-fixed red blood cells, B and T lymphocytes, and urokinase producing kidney cells were separated. The separation results of the aldehyde-fixed rabbit, human and horse red blood cells, which were taken in the form of photographs taken at three-minute intervals in columns 1 and 5, are the subject of this report.

Previously, static electrophoresis experiments had been carried out on Apollo 14 with a mixture of colored dyes (1), and on Apollo 16 with a mixture of two monodisperse polystyrene latexes (2,3), but the resolution of these experiments was poor due to the electroosmotic effect on the fluid velocity. With the development of a suitable coating, the electroosmosis could be considerably reduced, and the ASTP electrophoresis experiments would justify and provide valuable information for further experiments to be carried out on the space shuttle and future programs.

There are various advantages to performing such experiments in space under microgravity conditions. The primary advantage is the elimination of the thermal convection mixing due to Joule heating of the liquid. In a gravity environment this fluid mixing due to density gradients obviates any possible separation. To perform a similar experiment on earth, a much lower voltage would be required resulting in less Joule heating, but also a much slower separation and longer separation time, leading to further problems. As the separation time increases, diffusion due to Brownian motion increases, thus decreasing the resolution. A second advantage is the elimination of particle sedimentation due to a density differential between the suspending fluid and the particles. If the particles settle the bands will become distorted decreasing the resolution. Therefore, these two advantages lead to a much improved separation resolution than is possible on earth. The obvious disadvantages are cost, scheduling limitations, and only small quantities may be sent and returned.

Experimental

The development of a low electroosmotic mobility coating greatly improved the resolution of the electrophoretic separations. A complete description of the coating development and theory may be found in previous work (4, 5), so only a brief discussion will follow. The coating for glass channels consisted of a precoat of Dow Corning ZG040, γ -glycidoxypolytrimethoxysilane, which bound the methylcellulose to the glass surface. The surface was then repeatedly washed or rinsed to remove unbound methylcellulose. Such a coating generally reduced the value of electroosmosis from values of -4.00 to $-0.20 \mu\text{m cm/volt sec}$. This reduction in electroosmosis results in sample migration by bands rather than elongated cones which can't be resolved. Therefore, this coating played a significant role in improving the results and resolution for the ASTP electrophoresis experiments.

Many species of fixed red blood cells were considered as candidates for the space experiment. Among these were chicken, dog, horse, human, turkey, cow, pig and rabbit red blood cells fixed with both formaldehyde and glutaraldehyde solutions. The species finally chosen to give the broadest range of electrophoretic mobilities and the best chance of separating were horse, human, and rabbit fixed red blood cells. Mobility distributions were determined by microcapillary electrophoresis techniques and are shown for the three candidate species in Figure 1. Measurements were done by both Dr. Robert Knox of the University of Oregon (6) and

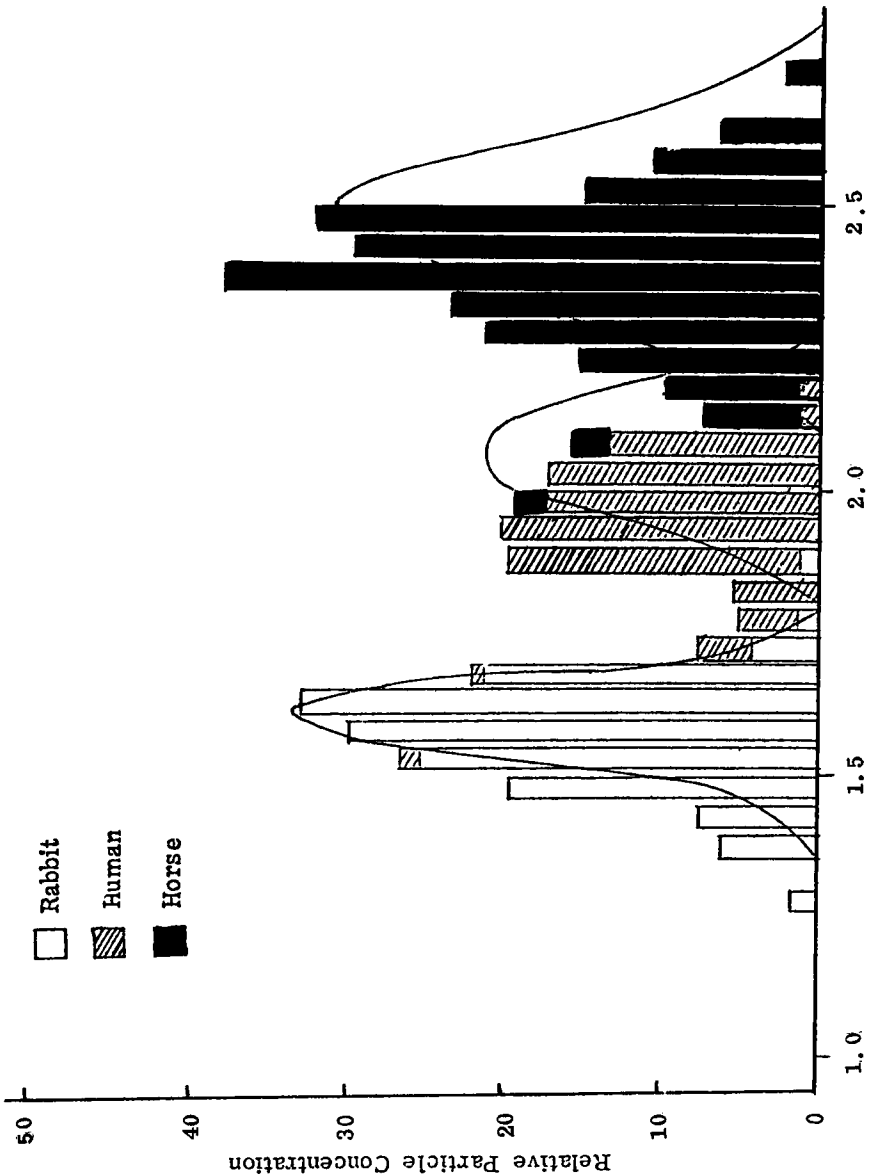


Figure 1. Electrophoretic mobility distribution of rabbit, human and horse fixed red blood cells.

ourselves and are displayed on the graph in two forms. The data of Dr. Knox is represented in bar graph form and represents 100 measurements per each cell species with the area under each curve proportional to the relative concentrations of the space samples. Our data is presented in a smoothed form and represents 150 measurements per each cell species. The measurements were performed in the A-1 buffer formulated for these experiments and agree well with respect to average mobility and range for each cell type.

The basic procedure for the electrophoresis experiments entailed the preparation of the columns and sample slides on earth. Each column was thoroughly cleaned then coated with the Z6040-MC coating and rinsed for three days, after which they were separately sealed in plastic bags and stored for travel into space. The red blood cells were aldehyde fixed and measured for electrophoretic mobility. A mixture of 32.8% rabbit, 21.6% human, and 45.6% horse cells with a total concentration of 2.52×10^7 red blood cells per slide was prepared by freezing the cells in a small disc shape, 0.478 cm in diameter by 0.312 cm thick. These sample discs were then placed in a freezer for storage until time for the experiment. All electrophoresis experiments were done in duplicate on two separate days. Columns preloaded with A-1 buffer were placed in the apparatus. A sample disc was removed and inserted into one end of the column and allowed to thaw and the current turned on. The samples were allowed to electrophoresis for about 60 minutes with photographs taken every 3 minutes to record data. After 60 minutes the current was turned off and the freezing cycle begun. The frozen columns were then removed and placed in storage for return to earth for further analysis. Postflight analysis consisted of removing the frozen core from each column and slicing it into 5 mm. slices which were analyzed for red blood cell count and mobility.

Two problems occurred which marred the experiment and collection of important data. First, column 5 probably developed an air bubble which migrated to the far electrode causing the voltage to saturate. For this reason, the voltage was turned off temporarily and restarted but migration did not proceed down the entire column and some swirling was observed. The second problem occurred in the postflight slicing of column 1. Upon removal of the core, it broke and only very large slices were able to be made, which reduced the amount of information obtainable. As luck would have it, this was the column that performed well in space which should have provided the best information. Therefore, only

a fraction of the data was obtained, from which conclusions could be drawn as to the degree of success. Further information and analysis was extracted from the photographs which will be covered in detail later in this report.

Theoretical Computer Modeling

A large amount of effort was expended in developing a theoretical computer model of a static electrophoresis column (4, 7). With such a model many parameters can be varied very easily to determine the specific effect of each one, and this information can then lead to a better design or choice of operating conditions by which the resolution can be maximized. Also, before an experiment is performed, the model can be used to predict separations, or afterwards to compare results to theory. Therefore, the development of such a model is very useful as an analytical tool in studying static electrophoresis.

The model which has been used is rather simple in that it only accounts for effects on a macroscopic scale, ignoring particle interactions, diffusion, relaxation effects, and electric field distortion effects on individual particles. Of prime interest is the displacement and distortion of each particle band and the relative particle concentration along the column axis. This information is best expressed with the equation:

$$d = \left[U_e E \frac{\epsilon_2 \eta_{298}}{\eta_2 \epsilon_{298}} - U_{os} E \frac{\epsilon_1 \eta_{298}}{\eta_1 \epsilon_{298}} \left(\frac{r^2}{a^2} - 1 \right) \right] t \quad (1)$$

where d = displacement distance (um)

U_e = electrophoretic mobility (um cm/volt sec)

U_{os} = electroosmotic mobility (um cm/volt sec)

r = radial distance (cm)

a = channel radius (cm)

E = applied potential gradient (volts/cm)

t = time (sec)

ϵ = dielectric constant of suspending fluid - f (T)

η = viscosity of suspending fluid - f (T)

The subscripts 1, 2, and 3 refer to temperatures at the wall, at a distance r , and at the center respectively, while 298 refers to the reference temperature in degrees Kelvin at which the electrophoretic and electroosmotic mobilities were measured.

Equation 1 describes the displacement of a discrete element as a function of radial position in the channel. It also shows that the radial dependence can be reduced but not eliminated if the electroosmotic mobility is reduced to zero since the second term then becomes zero and the first term is a function of r through ϵ_2 and η_2 .

Equation 1 may be used to define the band shape and particle concentration for each species as a function of time. First, a least squares algorithm is used to fit a smoothed curve of up to a fifth order polynomial through a set of points from the bar graph mobility distribution of each particle species. These computed mobility distributions are shown in Figure 2 where the area under the curves is proportional to the actual concentration of cells. Next, the initial sample disc is divided up into a series of small finite elements, and Equation 1 is applied to each element as a function of radial position. Each element is assumed to have the same mobility distribution which is used to determine the spread and concentration profile for each element. Then the contribution from each element is summed up to determine the total concentration profile for each species and the band as a whole. With a computer,

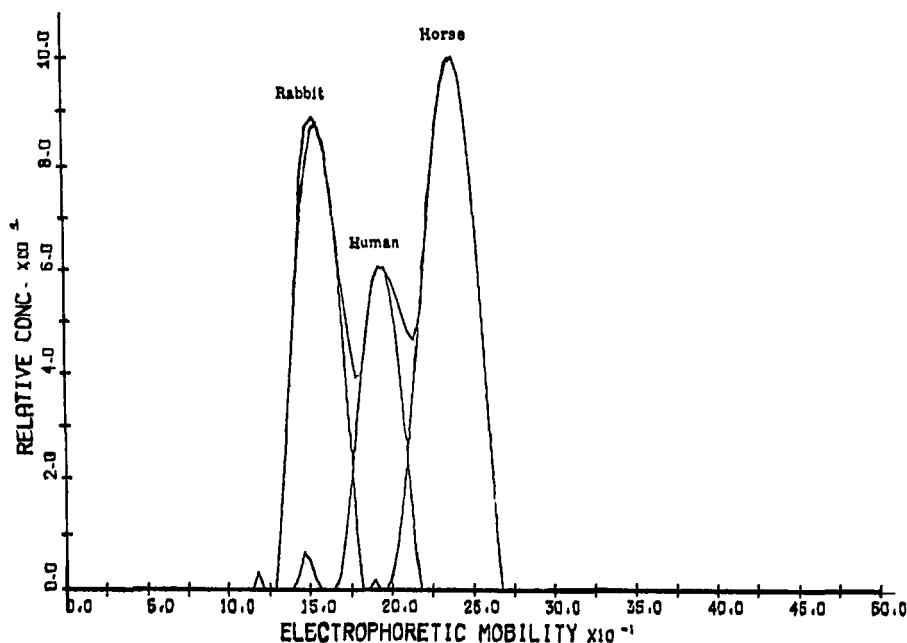


Figure 2. Computer electrophoretic mobility distributions of fixed red blood cells.

this summing process can be done very quickly and accurately for a large number of elements so as to allow a very high degree of confidence in the results. This is essentially how the computerized model was constructed so as to allow determination of band displacement, shape, and concentration for species exhibiting a broad mobility distribution. It could also be used to determine where each mobility fraction from a bar graph should be found. A listing of the actual program may be found in Appendix A.

With the help of this program, it was then possible to compare the theoretical resolution for a number of experimental conditions using the mobility distribution and relative concentration of the fixed red blood cells used for the space experiment where the location of the cells, from left to right, are always rabbit, human and horse. Figures 3 through 6 show the predicted separations and the effects of four important parameters on the resolution. In each figure, one parameter is compared at three values maintaining all other conditions constant, and the separation time is 60 minutes. The central figure in each case corresponds most closely to the actual experimental conditions used on ASTP and is, therefore, the same in all four figures. Figure 3 shows the effect of electroosmosis, U_{os} , on resolution for values of -0.40 , -0.20 and 0.00 $\mu\text{m cm/volt sec}$. It is readily apparent that this parameter can have a profound effect on the resolution, with best results expected for values as close to zero as possible. It should be noted that even for a value of 0.00 $\mu\text{m cm/volt sec}$, there is still some curvature to the band boundaries and this results from the 2°C temperature gradient predicted from Joule heating in the channel. Temperature is important because both dielectric constant and viscosity are functions of temperature and affect electrophoretic and electroosmotic mobility. Figure 4 shows the effect when this temperature gradient, ΔT , is the variable. The three cases correspond to gradients of 10°C , 2°C and -6°C from channel center to channel wall. Obviously if it were possible to maintain the center at a cooler temperature than the walls, then conditions would be improved, but such a condition is physically impossible for this system where Joule heating predominates. Figure 5 shows the effect of decreasing the ratio of sample plug radius to the channel radius, R , for values of 1.00 , 0.75 , and 0.50 . Smaller values of this ratio can improve the resolution especially if U_{os} is high, but at a cost of less sample volume which might be more important. Another factor effecting the volume is the initial sample plug thickness, Θ , and this effect is shown in Figure 6. This parameter, for values of 0.5 , 0.3 and 0.1 cm , is found to have only a small effect on the resolution, but

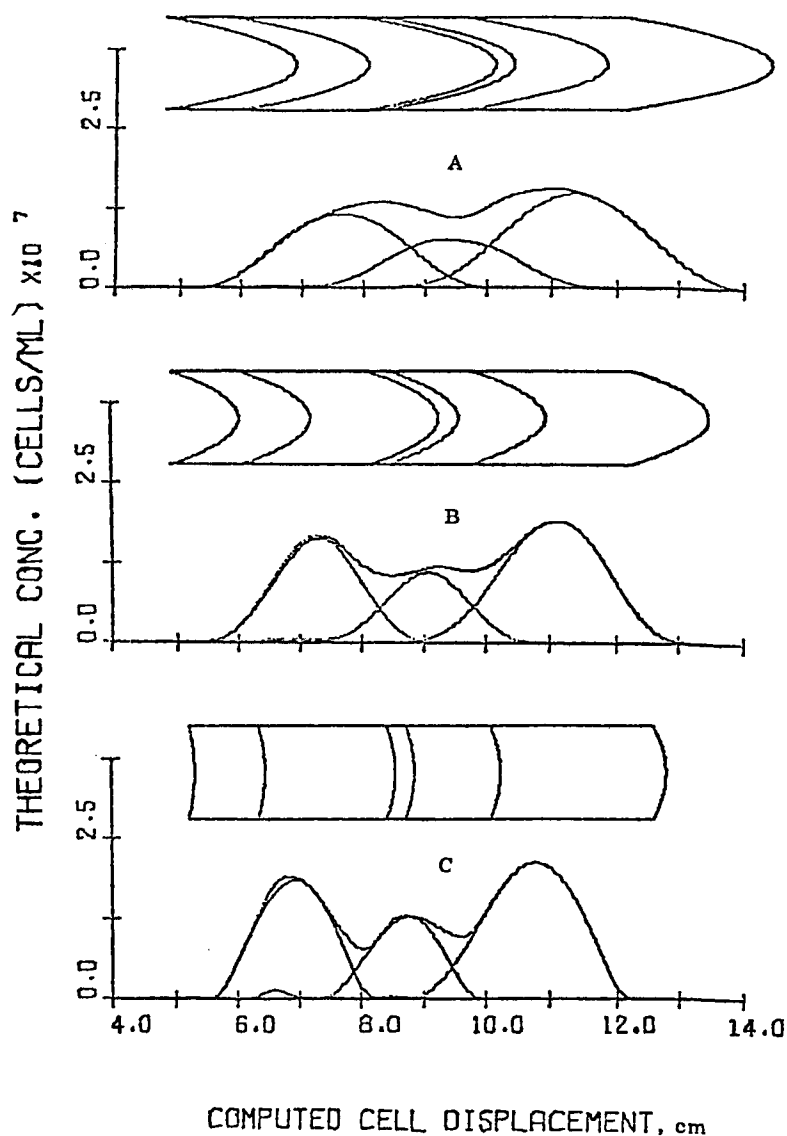


Figure 3. Effect of electroosmosis on cell separation with $\Delta T=2^\circ\text{C}$, $R=0.75$ and $\Theta=0.3\text{cm}$. A, $U_{os}=-0.4\text{ }\mu\text{m cm/volt sec}$; B, $U_{os}=-0.2\text{ }\mu\text{m cm/volt sec}$; C, $U_{os}=0.0\text{ }\mu\text{m cm/volt sec}$.

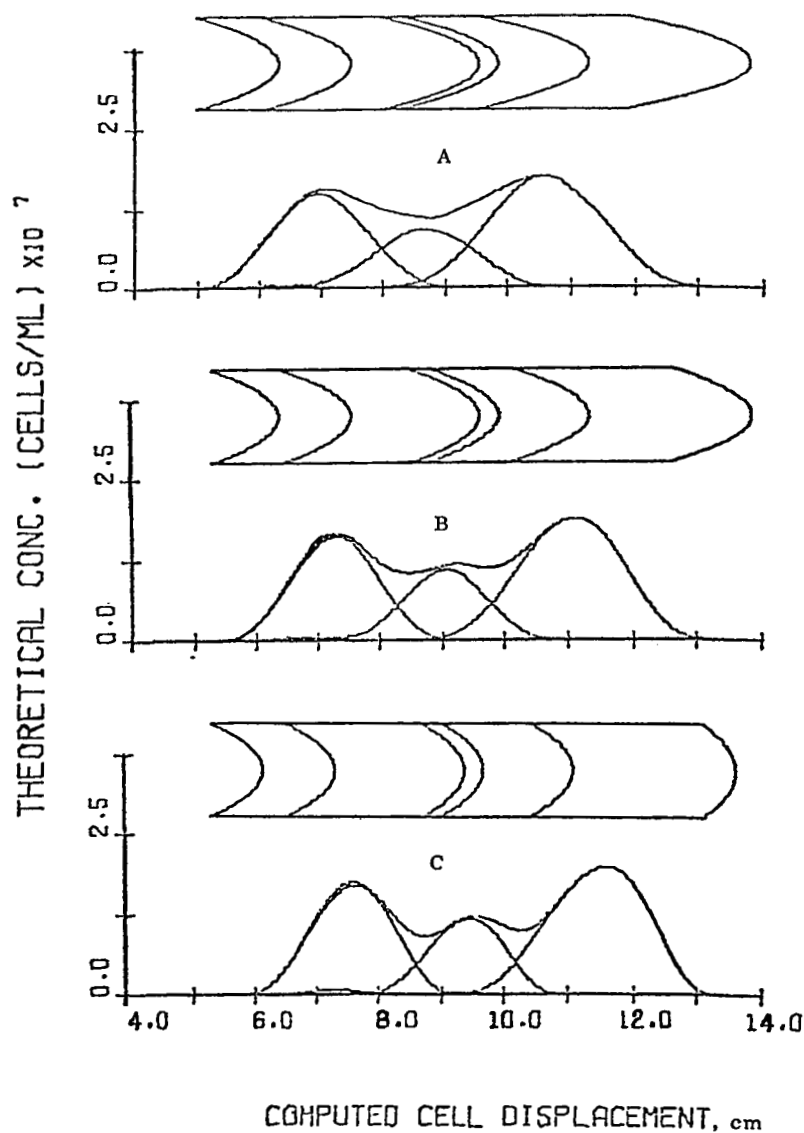


Figure 4. Effect of temperature gradient, ΔT , on cell separation with $U_{ps} = -0.2 \mu\text{m cm/volt sec.}$, $R = 0.75$ and $\Theta = 0.3 \text{ cm}$; A, $\Delta T = 10^\circ\text{C}$; B, $\Delta T = 2^\circ\text{C}$; C, $\Delta T = -6^\circ\text{C}$.

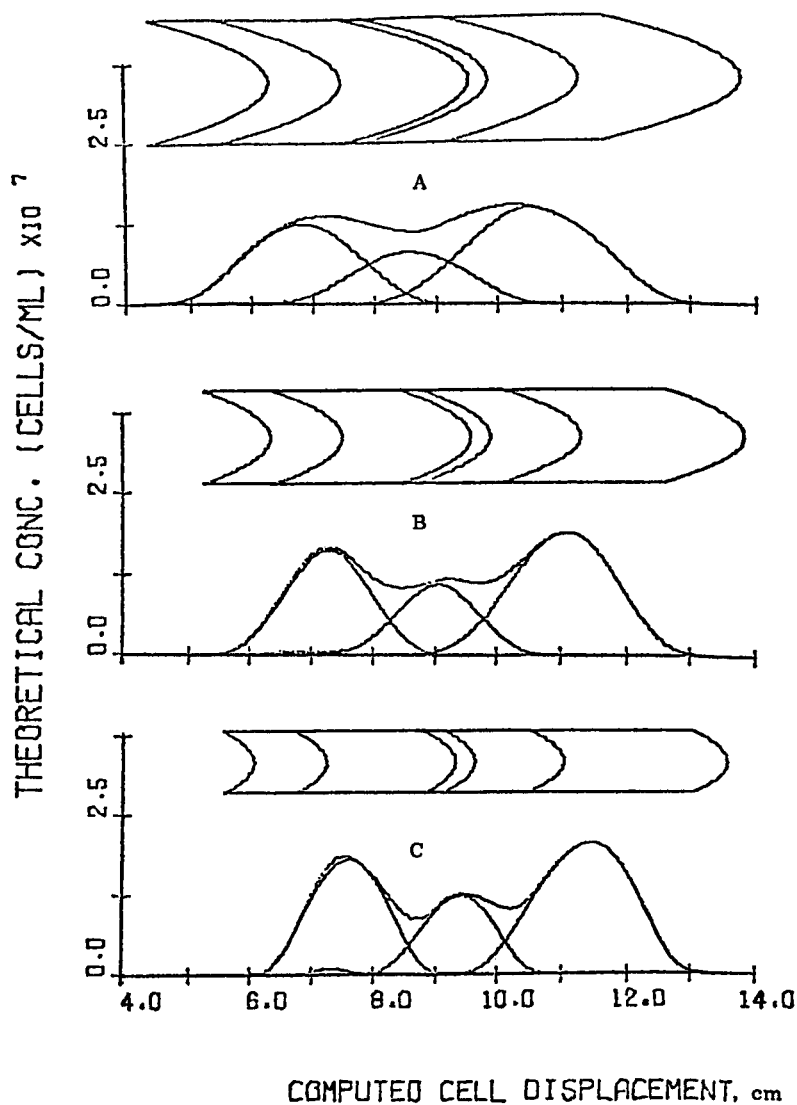


Figure 5. Effect of ratio of sample plug radius to channel radius, R , on cell separation with $U_{os} = -0.2 \mu\text{m cm/volt sec}$, $\Delta T = 2^\circ\text{C}$ and $\Theta = 0.3 \text{ cm}$; A, $R = 1.0$; B, $R = 0.75$; and C, $R = 0.50$.

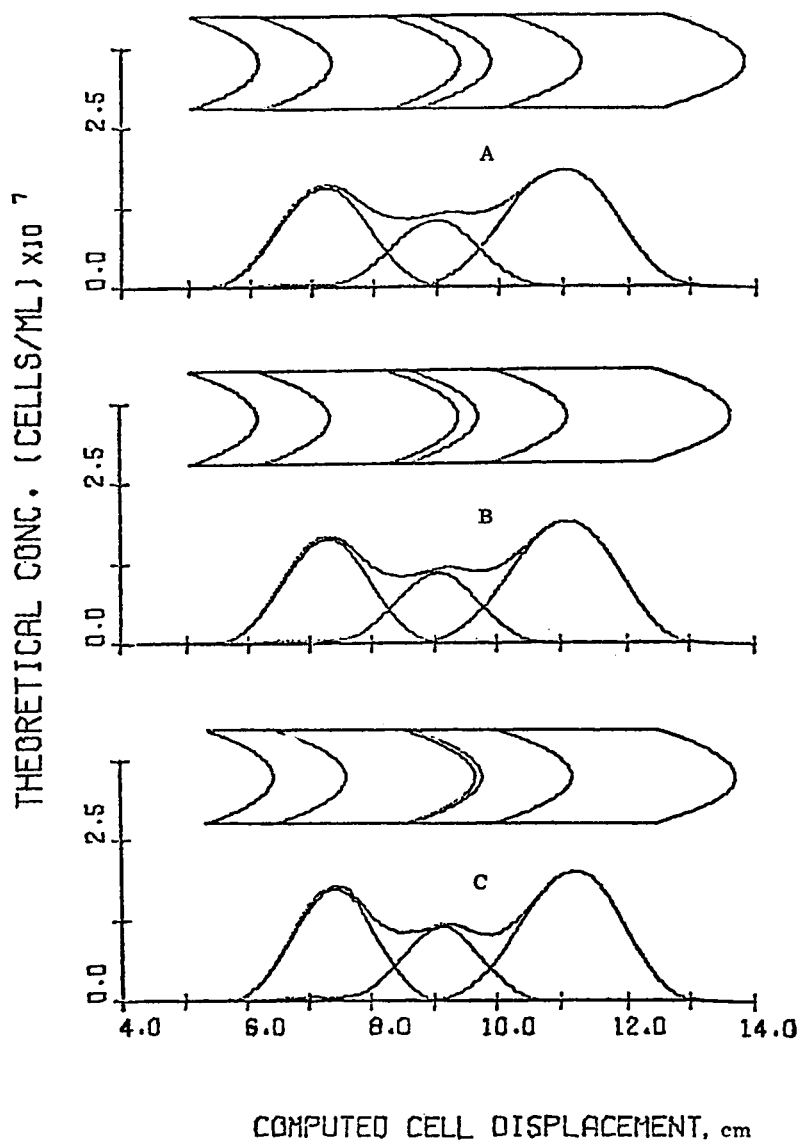


Figure 6. Effect of sample plug thickness, Θ , on cell separation with $U_{0S} = -0.2 \mu\text{m cm/volt sec}$, $\Delta T = 2^\circ\text{C}$ and $R = 0.75$: A, $\Theta = 0.5 \text{ cm}$; B, $\Theta = 0.3 \text{ cm}$; and C, $\Theta = 0.1 \text{ cm}$.

this is because the migration distance is large in relation to Θ , making it negligible. For cases with a large migration distance, R could be reduced and Θ increased to maintain the desired volume but with improved resolution. It should be noted that even under optimum conditions, these particles could not be completely resolved into separate fractions because the mobility distributions overlap; however, they could be resolved according to their mobilities.

Photographic Analysis

As mentioned before, photographs were taken at 3 minute intervals, providing a pictorial account of the progress of the experiments. These photographs were able to provide valuable information as to the success of the low electroosmotic coating, validity of the theory and the results of the separation.

Initially, only the second generation film was available for observation and, therefore, only information on the apparent band boundaries could be extracted by visual examination. Figures 7 and 8 show the discernible boundaries and displacements versus time for the two columns containing fixed red blood cells. Of course, the exactness of these measurements depends entirely on the photographic quality and the judgement of the observer, but they are representative of the separation that took place. Figure 7 shows the results from Column #1, in which two bands were clearly discernible as the experiment progressed. At first, only one band of a lighter and darker region followed by another lighter region was visible. Then, at about 25 minutes into the experiment, the leading lighter region appeared to separate from the main portion as a separate band with apparently few fixed red blood cells in between. At about 42 minutes, the darker and lighter regions of the second band were no longer discernible. This data shows that the migration of the band boundaries with respect to time proceeded at a linear rate which is as expected, and the rate corresponds to the mobility values that were determined for the fixed red blood cells and the electroosmosis with an applied gradient of 10.6 volts/cm. Also, the sharp planar boundaries attest to the low value of electroosmosis due to the methylcellulose coating. However, it seemed distressing that very few blood cells could be detected in the region between the two bands when the mobility distributions of the three species clearly overlapped. It could be possible that the concentration was low enough that it would not appear on the second generation film.

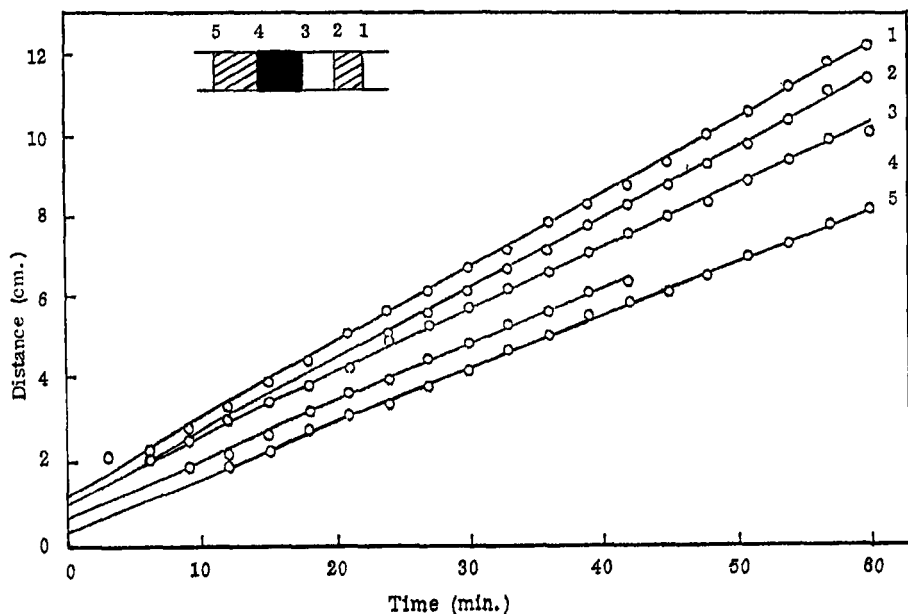


Figure 7. Band displacements as a function of time in Column 1.

Figure 8 shows the results from Column #5 which incurred technical problems when an air bubble resulting from a leak migrated to the far electrode and probably caused the voltage to saturate. Some information may still be obtained from the two regions in which the migration proceeded normally. As in Column #1, there was a lighter and darker region as the bands emerged from the column end. Also the migration rate of the bands seemed to be very close to that in Column #1, confirming those results.

In an effort to compare these photographic results to the theoretical concentration, the computer was supplied with the best known values for all the parameters and allowed to compute the expected concentration profile which was divided up into four concentration regions. These results are shown in Figure 9 along with the experimental band appearances for Column #1. The experimental and theoretical concentrations are shown every six minutes versus migration distance. What this shows is that the fixed red blood cells should have been observed over a much larger

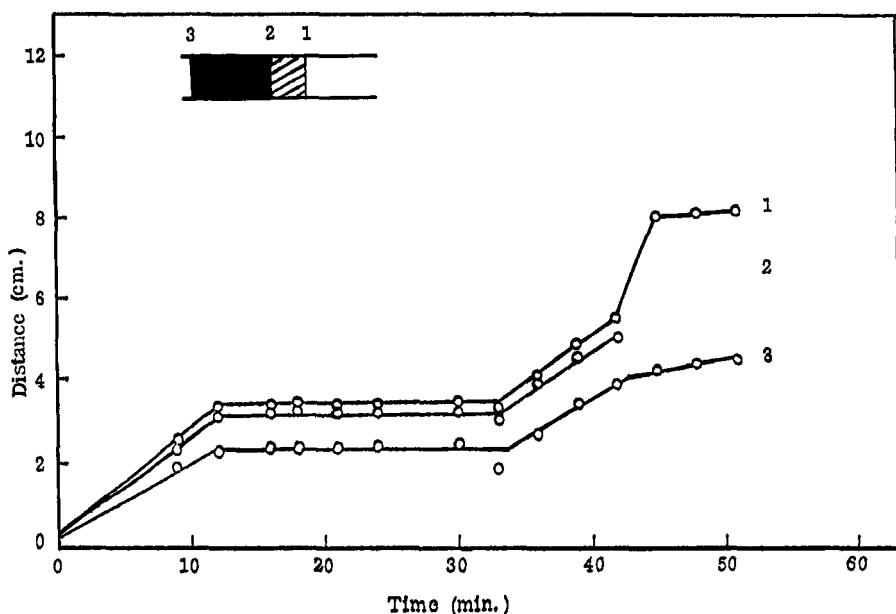


Figure 8. Band displacements as a function of time in Column 5.

portion of the column and in between the two bands. However, the agreement is acceptable, assuming that the concentration of cells was not sufficient to show up on the second generation film. There does seem to be some disagreement between experimental and theoretical in the displacement of the second band. This could be explained if there was some clumping of the cells due to the freezing and thawing process on such a high concentration of fixed red blood cells.

More recently, micro-densitometer scans of the first generation film, which shows much more detail, have been provided by NASA. The scans were taken by measuring the optical density of the separation column while traversing from one end of the column to the other end with a very small orifice in the direction of the traverse. Figure 10 shows two graphs obtained by this technique. Figure 10A shows a reproduction of scans obtained from frame 1 and frame 10, in which frame 1 is used as a base line with no cells yet present in the column. It should be noted that the two traces agree quite closely with only a small deviation in the background at distance greater than 8 cm. This is because the cells in frame 10, or 30 minutes into the

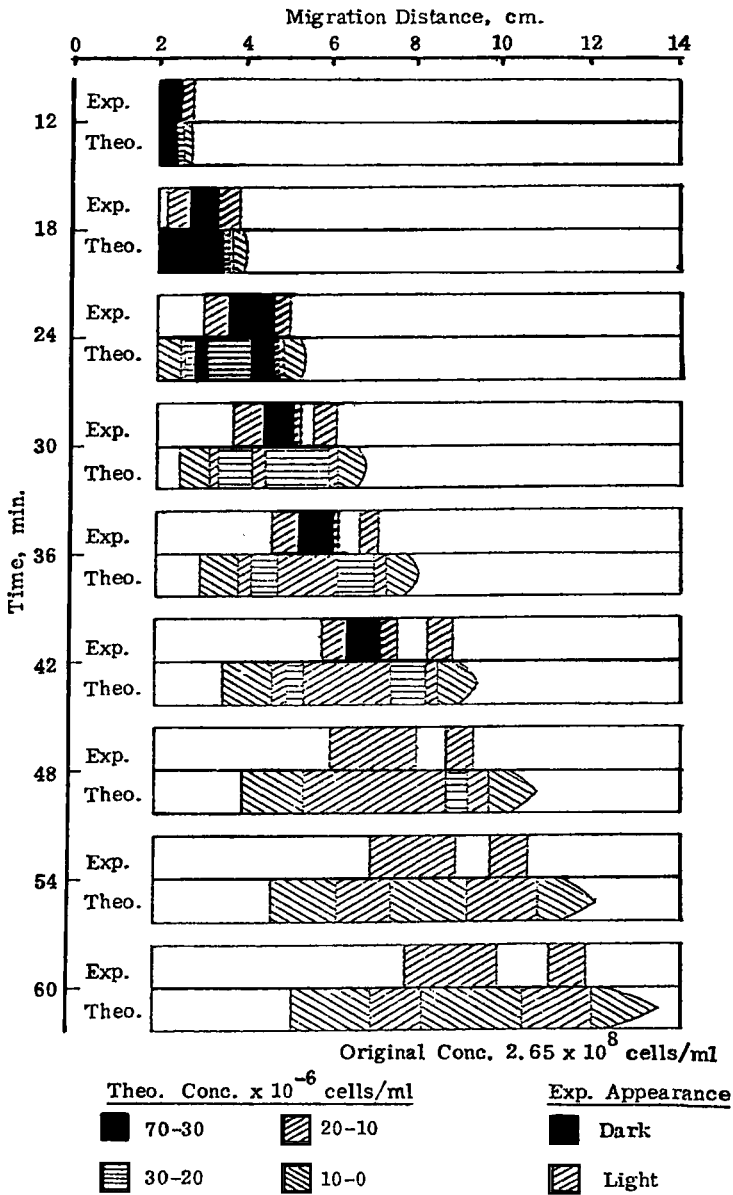


Figure 9. Position of bands as determined from visual observation of flight film and theory.

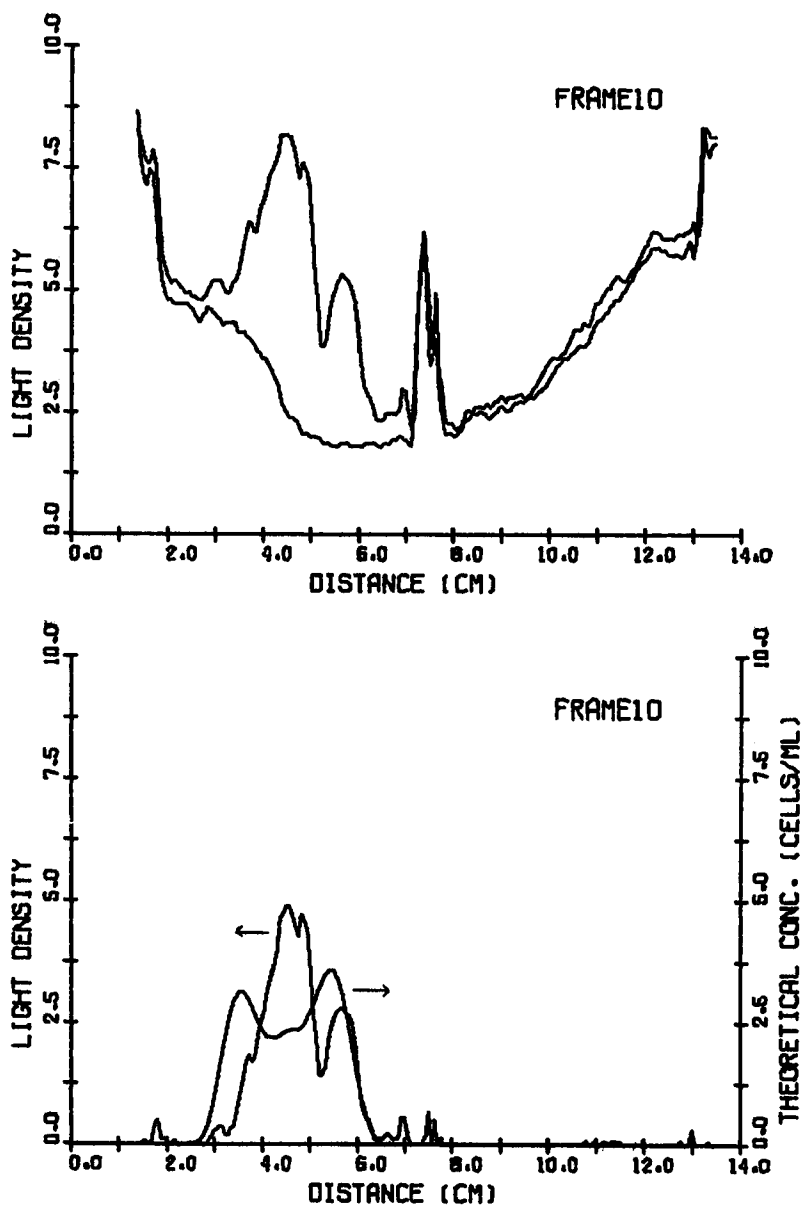


Figure 10. Micro-densitometer scan and computed displacement.

A. Raw data micro-densitometer scan for frames 1 and 10.

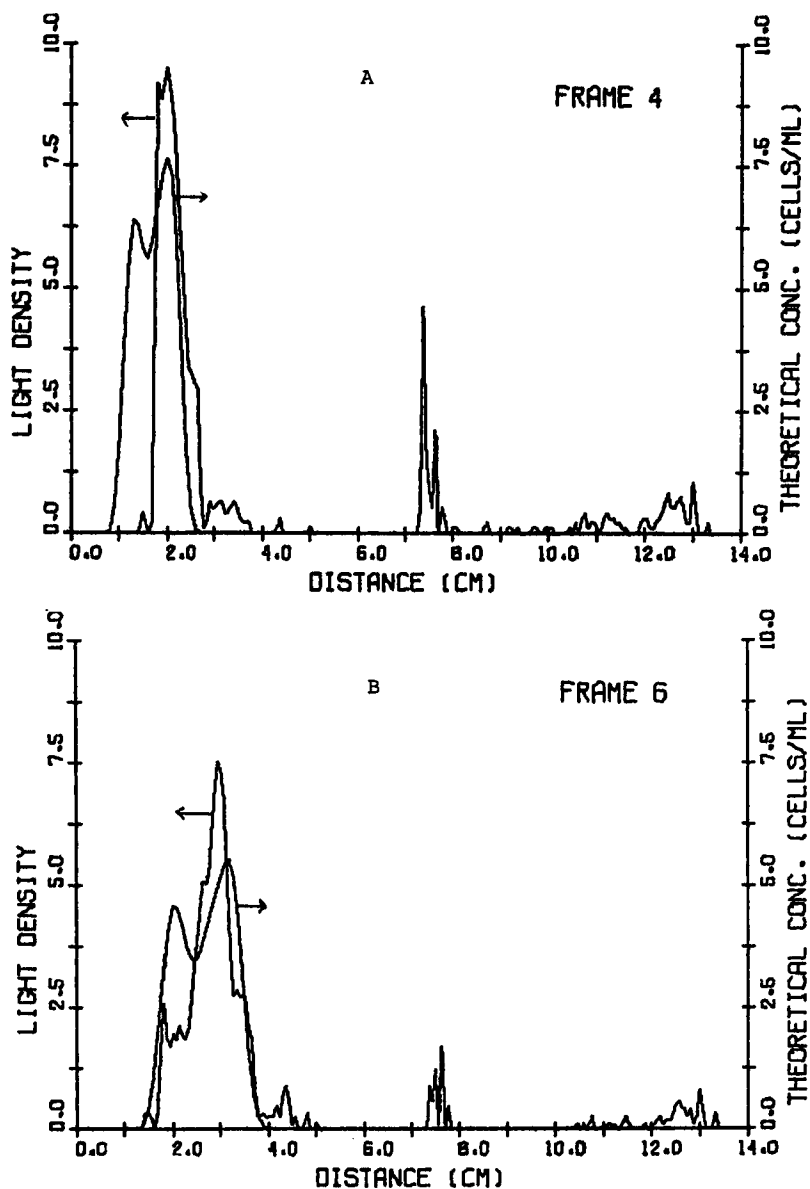
B. Net micro-densitometer scan and computed displacement.

separation, have not yet proceeded beyond this distance. The large deflection at about 7.5 cm is from the thermocouple present in the column at that position for monitoring temperature. Figure 10B shows two curves, one of the subtracted difference between the micro-densitometer scans corresponding to the left hand axis and the second of the computed cell concentration as evaluated from the theoretical model corresponding to the right hand axis. The theoretical model for these computations assumes an applied potential gradient of 10.6 volt/sec and U_{os} of $-0.2 \mu\text{m cm/volt sec}$. Both the micro-densitometer and theoretical curves are generated by the computer and the areas under each curve equated for comparison.

Figures 11 through 15 show the results at six minute intervals or every second frame from 4 through 22. The theoretical results indicate the presence of two peaks as early as twelve minutes into the separation whereas the micro-densitometer results show the initiation of two peaks in frame 8 or 24 minutes into the separation. The breaking up of the band into two bands by visual observation of the flight film, Figure 9, occurs after 30 minutes of separation, frame 10. The micro-densitometer results, however, show that although the advancing peak increases in definition, it does not split off from the main band, Figures 12 through 15. Furthermore, while the position of the band, as it proceeds through the separation, agrees extremely well with the theoretical prediction, the magnitude and position of the second peak does not agree with theory. This discrepancy in band shape can be attributed to a number of reasons, including photographic quality, the complex function between light density and cell concentration and type, and last of all the possibility of clumping by the cells. Although the specific effect of these considerations cannot be determined, the agreement between the micro-densitometer results and the theoretical predictions seems quite reasonable and would support both the theoretical model and the assigned experimental parameters.

Conclusions

The electrophoretic separation of a mixture of three species of fixed red blood cells in space was successful in that fractionation according to mobility did occur and was found in the sliced samples although technical problems occurred in the operation of column 1 which was the subject of this analysis. Photographic evidence indicates that the low electroosmotic methylcellulose coating was successful in reducing the electroosmosis to a near zero value. Also the flight film shows that



MICRO-DENSITOMETER SCAN AND COMPUTED DISPLACEMENT

Figure 11. Micro-densitometer scan and computed displacement.

A. Frame 4, 12 minutes separation;

B. Frame 6, 18 minutes separation.

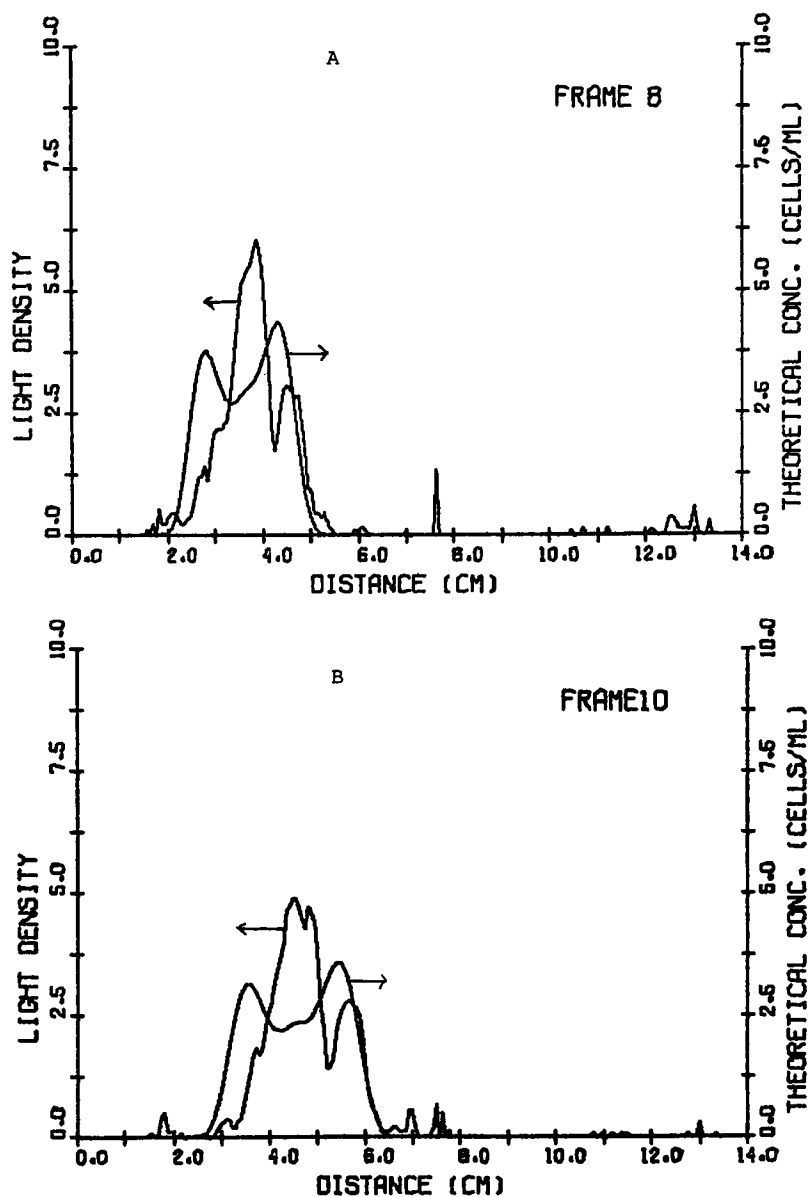


Figure 12. Micro-densitometer scan and computed displacement.

A. Frame 8, 24 minutes separation;

B. Frame 10, 30 minutes separation.

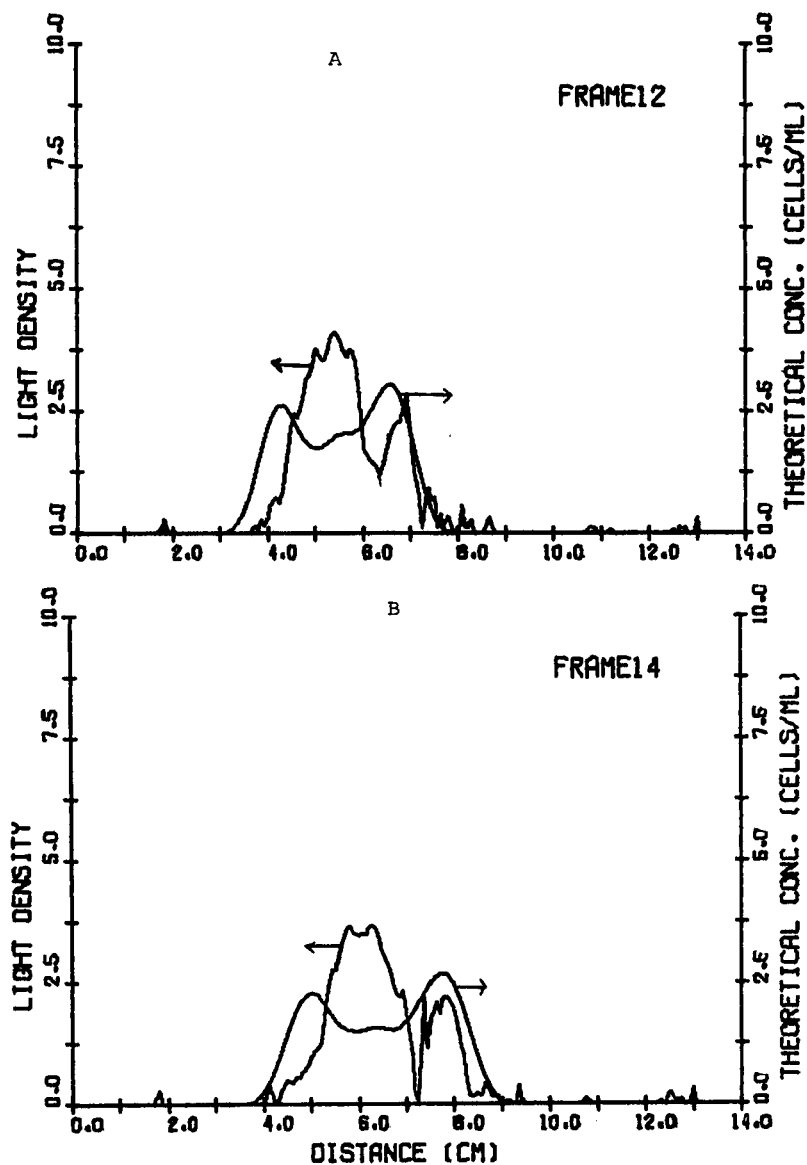


Figure 13. Micro-densitometer scan and computed displacement,
A. Frame 12, 36 minutes separation;
B. Frame 14, 42 minutes separation.

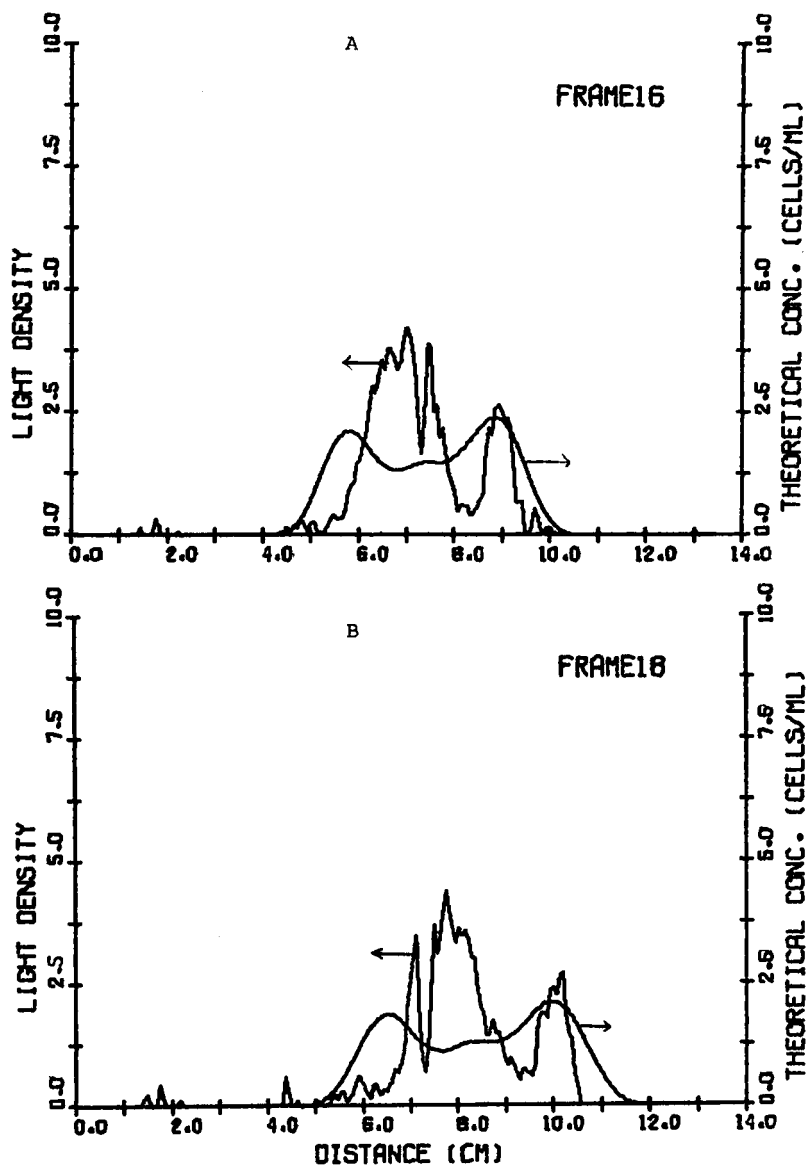


Figure 14. Micro-densitometer scan and computed displacement.

A. Frame 16, 48 minutes separation;

B. Frame 18, 52 minutes separation.

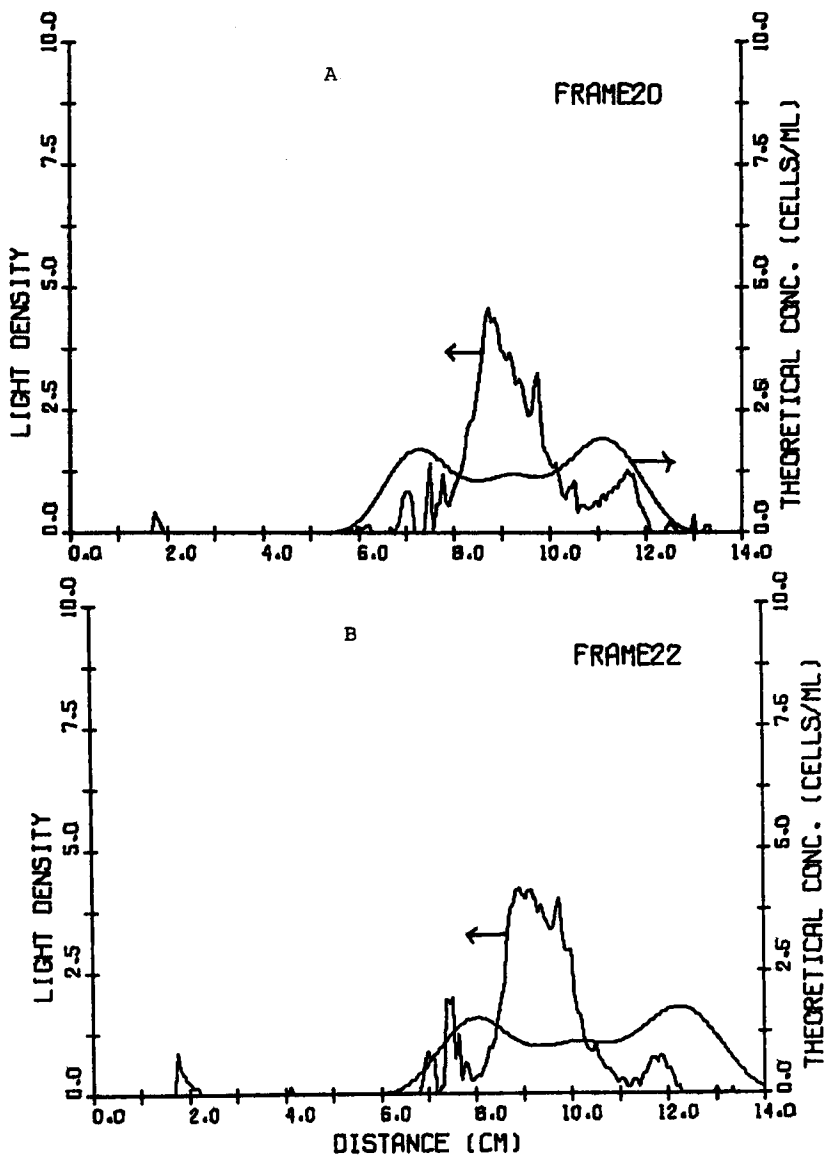


Figure 15. Micro-densitometer scan and computed displacement.
A. Frame 20, 60 minutes separation;
B. Frame 22, 66 minutes separation.

the bands migrated down the column as theory would predict, producing two bands of high cell concentration separated and surrounded by regions of lower cell concentration. However, most likely some clumping of the cells occurred to cause the trailing band to be larger than expected from theory.

The theoretical computer model gave good agreement with the experimental results, and was useful in defining the effect of the various experimental parameters. The best resolution can be obtained by reducing U_{os} to near zero with as small a temperature gradient between channel center and wall as possible. The effect of U_{os} can be minimized by reducing the ratio of the sample plug radius to the channel radius but at the expense of sample volume. This can be compensated with little loss in resolution by increasing the sample plug thickness as long as this is negligible in relation to the total separation distance.

Overall, the experiment was a success in demonstrating a static electrophoresis separation under microgravity conditions with a resolution not possible on earth.

ACKNOWLEDGMENT

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