Continuous Flow Electrophoretic Separator for Biologicals

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In the near absence of gravity, a free-flow electrophoretic separator can be operated with a much thicker separation chamber than is possible under 1 *g* conditions. This should permit either better resolution or shorter separation time per unit of sample. An apparatus to perform experiments on sounding rockets is described. The electrophoresis cell is 5-mm thick by 5-cm wide with 10-cm-long electrodes. It is supplied with buffer, sample, and coolant at about 4°C through the use of a passive refrigerant system. Ultraviolet sample detection and recovery and cold storage of up to 50 sample fractions are provided. A wide range of operating conditions is electronically programmable into the unit, even up to a short time before flight, and a further range of some parameters can be achieved by exchanging power supplies and gears in the motor-drive units of the pumps.

Introduction

ELECTROPHORESIS is an analytical technique widely used in the biological and biomedical fields for analysis of complex natural products. As is the case with many analytical techniques, there has long been a desire to scale up the analytical procedure to a means of isolating pure substances on a useful scale. At present this is done generally by scaling up the analytical apparatus and using a block or slab of gel, a paper curtain down which the sample and electrolyte solution seep, or a column filled with glass or gel beads. The objective of all of these methods is the elimination of thermal convection problems. For a truly preparative process, however, it is almost necessary to have a continual flow of sample into the apparatus and a continual flow of isolated product out. Such an apparatus was developed first by Barrolier,[1] and later improved by Hannig[2] in Germany and Strickler[3] in the United States. In order to avoid convective problems, the free-flow apparatus used in normal gravity generally has a thin layer (0.5-1.5 mm) of fluid. Various orientations of flow with respect to gravity have been used, including horizontal in the early Elphor machines, vertically downward in present-day machines, vertically upward,[4] and spiralling around either a vertical[5] or horizontal[6] axis. However, the best way to avoid thermal convection is to eliminate the gravitational driving force, as can be done in an orbiting spacecraft.

Thus, in a "brain-storming" discussion on space processing ideas among staff members of the Wyeth Laboratories and General Electric Space Sciences Laboratory in the spring of 1969, preparative scale electrophoresis was suggested along with several other ideas. Possible product examples, such as vaccines, hormones, enzymes, and cells, were also suggested by Wyeth staff members, as well as other organizations over the next year or so.

About a year later research and development work on the idea was initiated under NASA sponsorship. This led to small demonstration units on Apollo 14 and 16, and the more sophisticated MA-011 experiment on the Apollo-Soyuz mission.

Presented as Paper 77-234 at the AIAA 15th Aerospace Sciences Meeting, Los Angeles, Calif., Jan. 24-26, 1977; submitted Feb. 1, 1977; revision received Nov. 22, 1977.

Index category: Space Processing.

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With the completion of the Apollo 14 and 16 flights, our attention was turned toward meeting the original, and still desirable, goal of developing a truly preparative unit for space experiments. We chose a continuous flow unit as offering the greatest ease of inserting and removing samples and sample fractions. The basic change that is made possible by the absence of gravity is simply to make the electrophoresis cell thicker than the 0.5-1.5 mm commonly used for such units on Earth. It was estimated that the cell could be 5 mm or more thick and provide a fivefold improvement in resolution or a large improvement in throughput. These predictions have been at least partially demonstrated by Hanning with a 3.8-mm-thick cell in the ASTP-MA-014 experiment.[7]

In this paper we describe an automated free-flow electrophoresis device built for use on sounding rockets according to guidelines developed from a mathematical model, along with some background and reference work being performed in a ground-based unit.

Mathematical Model of Free-Flow Electrophoresis

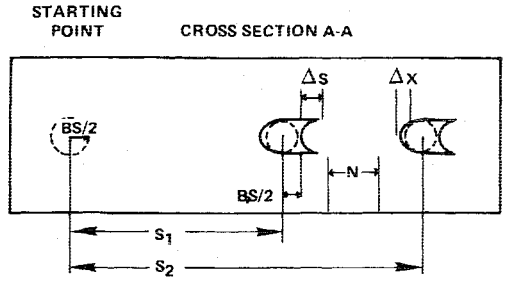
A mathematical model of a convectionless, sedimentation-free electrophoresis cell has been constructed and used in the design of an electrophoretic separator experiment for the Advanced Applications Flight Experiment (AAFE) program.

Assembly of the Model

A useful electrophoresis system, designed to operate in a 0-*g* environment, should be flexible enough to handle some very different biological materials which remain unseparated by present terrestrial methods. The resolution necessary to obtain useful material will vary with each species. This implies a unit with considerable operational latitude in selected parameters.

The model is based on a criterion called the separation resolution and defined as $\Delta\mu$, the minimum difference in sample component mobility which will result in the complete separation of two adjacent sample components by an amount equal to the spacing of the product fraction collection tubes. This is illustrated in Fig. 1.

The term ΔS, the sample distortion, is the crux of the matter. This term is calculated by taking a sample particle at two locations on the outer edge of the sample stream and calculating net displacements at those points. Figure 2 illustrates the concept. The net displacement is directly proportional to the net velocity (sum of electro-osmosis and electrophoresis). In the free-flow method of electrophoresis, electro-osmosis is the flow of fluid in the direction of the applied field that results from charge groups on the surface of the cell walls.[8] The velocity, in turn, has a viscosity dependence which is ultimately temperature-dependent. Thus,



DEFINITION OF SEPARATION RESOLUTION $\Delta\mu$

$$\Delta\mu = S_2 - S_1$$

$$S_2 = S_1 + BS + \Delta S + N + \Delta X;\ \Delta X = (6\ DTR)^{1/2}$$

$$\Delta\mu = \Delta S + N + \Delta X + BS$$

WHERE, S IS THE SAMPLE DISTORTION, N IS THE COLLECTION TUBE SPACING, BS IS THE ORIGINAL SAMPLE STREAM DIAMETER, D IS THE DIFFUSION COEFFICIENT AND TR IS THE RESIDENCE TIME

Fig. 1 Definition of separation resolution.

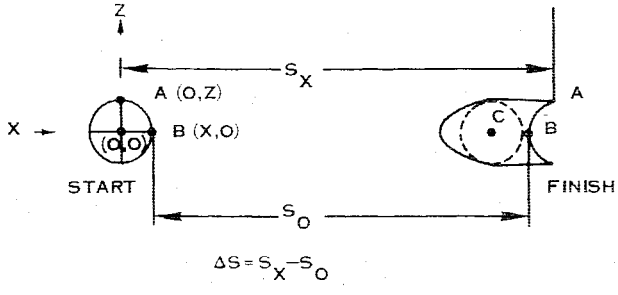


Fig. 2 Sample distortion.

temperature is the most important parameter in the model for separation resolution.

An overview of the system will illustrate the interdependence of the cell variables, as shown in Fig. 3. The steps in the model are: calculate temperature distribution; hydrodynamic profile; electro-osmotic profile; diffusion effects; residence time; distortion ΔS of sample bands; and minimum resolution $\Delta\mu$.

Applications to Design and Results

Several realistic, yet hypothetical, cases were examined with the model. A sample containing four components was theorized. These components had mobilities corresponding to those measured for the fixed red blood cells of a chicken, human (A), human (B) and a dog; the dog being most mobile and the chicken the least. In each case, the active cell width and length were 5×10 cm. The thickness was varied. The flow rate through each cell was adjusted so that a particle at the centerline would have a residence time comparable to the other cases. Figures 4-6 are the results of these calculations for cells of 0.07, 0.16, and 0.5 cm, respectively.

The first case, a 0.07-cm-thick cell (Fig. 4), is very close to the thickness of an Elphor cell. The effects of electro-osmosis and the hydrodynamic profile are profound. The very long residence time at the outer edge of the sample stream has caused the sample to diffuse to the walls even though a very small, 10^{-9}, diffusion constant was used. As can be seen from the collection graph, no separated material can be collected in any large amount.

The second case, 0.16 cm (Fig. 5), is similar to equipment used in this laboratory to conduct ground-based studies. The crescent effect is still quite pronounced, and it is still not

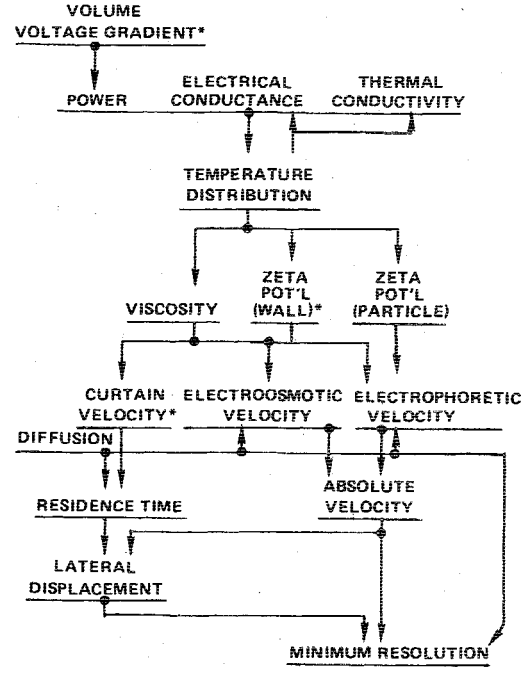


Fig. 3 Interdependence of cell variables.

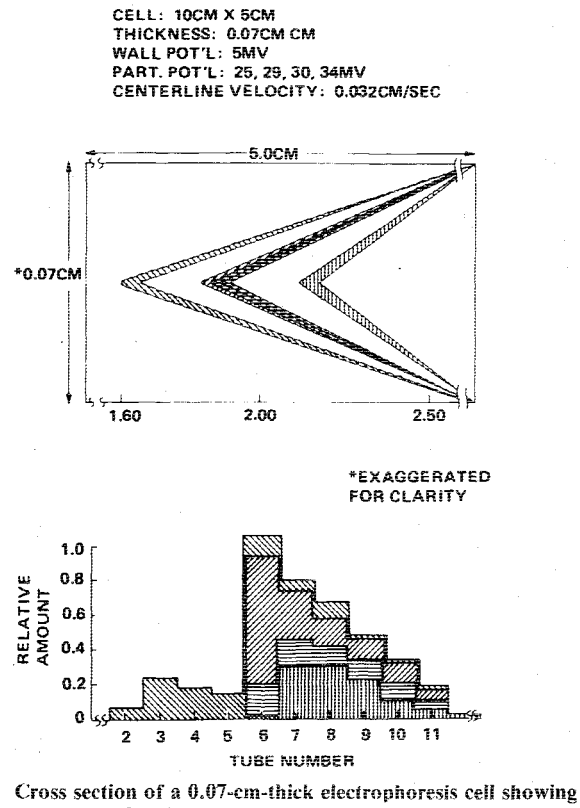


Fig. 4 Cross section of a 0.07-cm-thick electrophoresis cell showing hypothetical sample bands.

possible to obtain a complete separation between any of the components.

The last case, 0.5-cm (Fig. 6) is the proposed "thick-cell" 0-g experiment cell. A cell of this dimension cannot sustain the resultant temperature gradient in a 1-g environment without convection setting in. Although the crescent effect is still present, it is greatly reduced and it now becomes possible to collect two components of 100% purity.

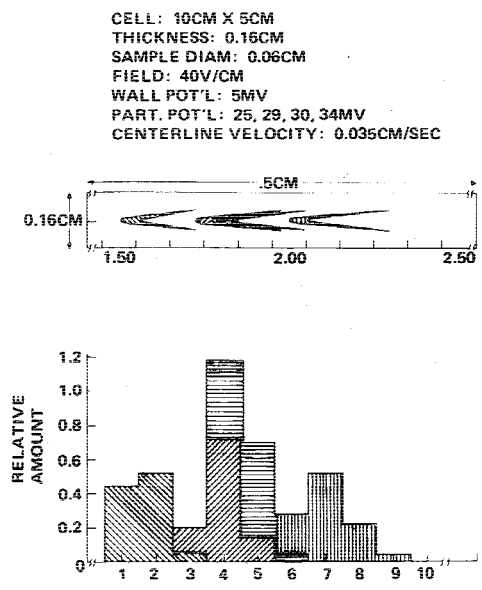


Fig. 5 Cross section of a 0.16-cm-thick electrophoresis cell showing hypothetical sample bands.

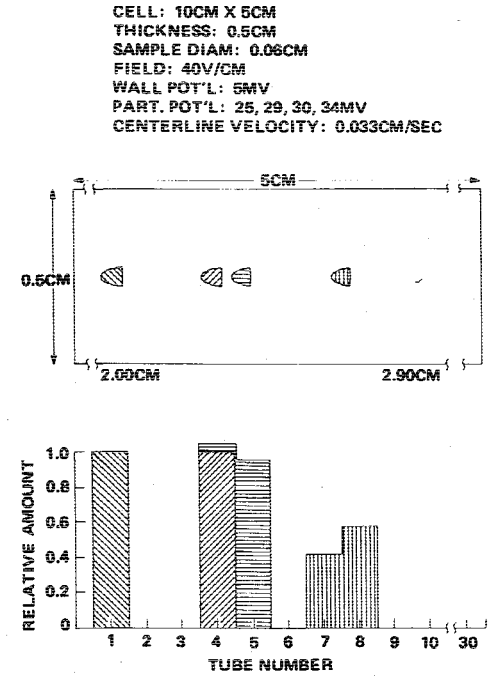
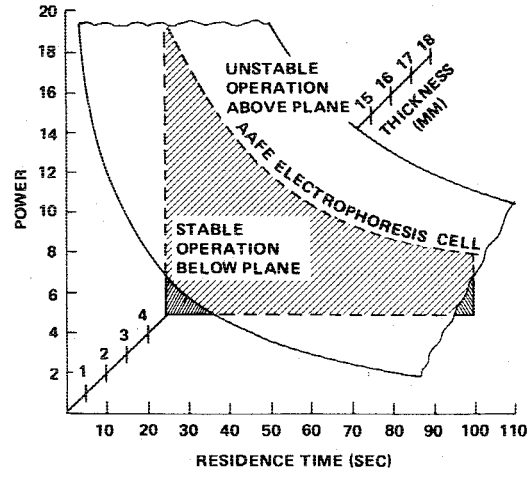


Fig. 6 Cross section of a 0.5-cm-thick electrophoresis cell showing hypothetical sample bands.



ACTIVE AREA 5CM X 10 CM
SAMPLE: NEUTRAL DENSITY POLYSTYRENE LATEX
ORIENTATION 20° FROM VERTICAL

Fig. 7 Stability limits of free-flow electrophoresis.

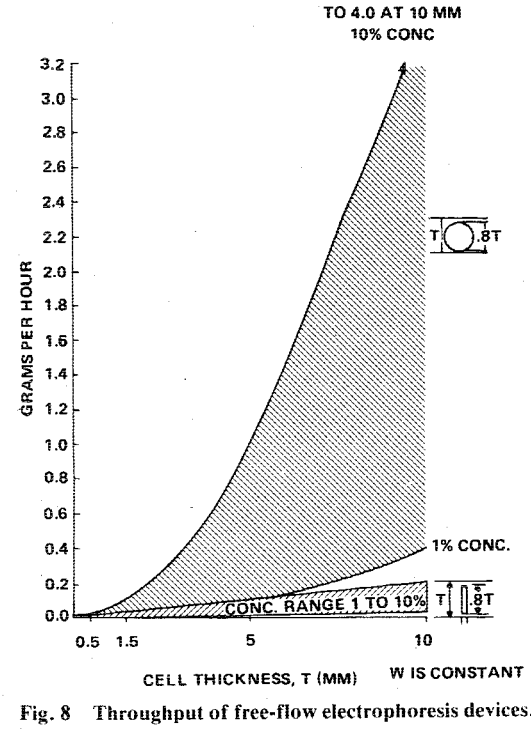


Fig. 8 Throughput of free-flow electrophoresis devices.

The first two examples were modeled after existing equipment to check the reliability of the predictions made with the math model. The last case is presently not able to be verified, since it requires a 0-*g* environment. However, an electrophoresis cell of the dimensions used in the third case has been built, and ground-based data were collected. These data were then compared to data for thinner cells and extrapolated to thicker cells. Figure 7 relates power density, residence time, and cell thickness to an arbitrary stability standard. This standard was defined as an undisturbed flow of neutral density polymer latex for a minimum of 3 min with the field applied. In a 1-*g* environment, stable operation of the system occurs only for points lying below the surface of the plane. Operation at or above the surface suggests a reduction in the gravity field to decrease convection.

It is possible to operate a thick cell in such a manner as to maximize either throughput or resolution. Figure 8 shows the throughput in g/h vs cell thickness for two kinds of sample streams. The first kind is thin in width (less than the i.d. of a collection tube), but the depth is varied as 80% of the cell thickness. This kind of stream might be used to maximize resolution. The second kind is round and its diameter varies as 80% of the cell thickness. This type of input might be used when high throughput is the objective. Comparing the two, at a 10% sample concentration, in a hypothetical 10 mm cell in 0-*g*, the increase in throughput from rectangular to circular cross section is greater than 20-fold.

Equipment

A free-flow electrophoretic separator was designed and built under the Advanced Applications Flight Experiment

Program to perform experiments on sounding rockets. The interior dimensions of the active portion of the electrophoresis cell are 5 cm wide, 0.5 cm thick, and 10 cm long.

The system elements are housed in a two-part assembly consisting of a pan and shroud 36.5 cm × 48 cm (14.4 in. diam × 19 in. high). The interior structure, rods, and plates are anchored to the pan and snubbed at the top by the shroud. The top plate is also an optical bench to which the electrophoresis cell, optics, and detector are mounted. Other components are distributed throughout the midplate and pan. An access door for sample loading and removal is provided.

The electrophoresis cell is mounted to the top plate and has two fused silica windows at the collection end to allow sample detection by uv absorption. After passing through the detector area, the separated sample fractions are pumped into individual collection tubes (50) by a multichannel peristaltic pump. The speed of the pump can be varied to give flow rates in the range of 5-15 cm^3/min. The pump output is connected to a removable collection device by a unique fluid connector. The collection box is refrigerated to maintain the viability of biological samples. The peristaltic pump is the prime mover for the curtain buffer and also the electrode rinse fluid; these being drawn from a cooled storage container below the midplate. The sample is introduced to the cell via a motor-driven, cooled syringe which has the ability to stir heavy samples to prevent sedimentation before and during flight. Startup of the sample pump is automatic, as is the system shutdown. During electrophoresis, joule heating of the buffer will occur, and it is desirable to keep the maximum temperature as low as possible. For biological materials, a limit of 37°C has been imposed and, for this reason, the electrophoresis cell is actively cooled by a circulating fluid during operation. This is a closed-loop system with the heat being absorbed in the buffer storage area by a eutectic coolant. The present material has a eutectic point of 4°C, but other mixtures may be substituted for other temperatures.

The voltage gradient can be varied along with the pump speed to suit the sample of interest. Fields of 13-130 V/cm can be applied with a corresponding change in buffer conductance from 10^{-2}-10^{-4} ohm^{-1} cm^{-1}. The decrease in conductance with increasing field is required in order to remain below the maximum temperature limitation of 37°C.

The unit carries enough fluids (and eutectic coolant) to operate during a 15 min "0-g" period and can be adapted for longer periods. The automatic start-up and turn-off features are adjustable at the launch site, and may be adjusted to actuate the unit for periods from 2-25 min.

While many of the dimensions and capabilities of the present equipment reflect the constraints of sounding rocket experiments, the equipment can serve as the foundation for more advanced separators which could be used in the Shuttle/Spacelab or other orbiting vehicles. Improvements which can be readily visualized include a larger supply of buffer and electrolyte to permit experiments of longer duration, a wider cell to improve the ultimate resolution, and a feedback control system to insure stability of the separation pattern during relatively long duration experiments, in spite of changes in ambient conditions or a gradual change in some operating parameter.

In spite of the short 0-g time available on a sounding rocket, calculations show that it should be possible on such a flight to process enough material for subsequent testing. For example, in a buffer with a specific conductivity of 7.3×10^{-4} ohm^{-1} cm^{-1}, the centerline temperature will not exceed 37°C if a gradient of 69 V/cm is applied. A sample with a mobility of 2.3 μcm/V s will be deflected 1 cm in 63 s, giving a theoretical resolution in this equipment of ± 0.23 mobility unit. The dimensions of the unit are such that in a flight with a 0-g window of 200 s, 5×10^6 cells could be processed at normal sample concentrations or 10 mg of protein at a reasonable concentration. We believe, however, that the real value of sounding rocket flights for electrophoresis experiments lies not in the isolation of a small amount of material, but in providing an experimental basis for design of free-flow separators for use on the Shuttle.

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