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ELECTROPHORESIS FOR BIOLOGICAL PRODUCTION

Louis R. McCreight
General Electric Company
Space Sciences Laboratory

Abstract

Preparative electrophoresis may provide a unique method for meeting ever more stringent purity requirements. Prolonged near zero gravity in space may permit the operation of preparative electrophoresis equipment with 100 times greater throughput than is currently available. Some experiments with Influenza Virus Antigen, Erythropoietin and Antihemophaliac Factor, along with process and economic projections, will be briefly reviewed.

Introduction

The idea of preparing biologicals of improved purity and specificity in space has both great technical and economic basis. It could become a multi-billion dollar business and be an important use for the STS. There is however a great deal of research and development to perform first.

This paper reviews some of the early work; from the initiation of the idea through some flight demonstrations and on to the current development of a sounding rocket experimental unit and some ground based separations work. A brief concluding section then outlines some of the projections for possible future preparative electrophoresis in space.

Early History of Electrophoresis in Space

Electrophoresis has been widely used for several decades, primarily based on the work of Tiselius, for analysis of biological materials. There are now an estimated 30,000 research and analysis personnel who utilize the technique in the U.S. alone and several hundred technical papers are based on this work annually.

Unlike other analysis and process techniques it has not been possible however to scale up the electrophoretic analytical technique to provide a truly preparative scale of operation. This is primarily due to gravity induced convection and sedimentation which can be sufficiently counteracted by such approaches as the use of gels, orientation, cooling, and small dimensions in the case of analytical devices, but are too restrictive to permit scaling up to an economical preparative level.

Thus in a "brain storming" type of 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 among the several ideas at that meeting. Several possible product examples such as vaccines, hormones, enzymes, and cells were suggested by Wyeth as well as other organizations over the next year or so.

About a year later, specific R&D work was initiated on the idea and almost immediately an opportunity arose to have a flight demonstration on Apollo 14. In about four months, we then designed and developed a small flight demonstration shown in Figures 1 and 2.

Samples of salmon sperm DNA, hemoglobin, and a mixture of red and blue dye were chosen to represent a broad range of molecular weights and to demonstrate electrophoretic mobility under microgravity conditions. Only the red and blue dye were expected to be, and were, electrophoretically separated.

Although the results were not up to our high expectations, the red and blue dyes did separate but the photography did not provide clear pictures. The biologicals were destroyed, apparently by bacteria, during the four month flight and quarantine period, but the engineering aspects of the unit were excellent and were reused on later flights.

A second flight demonstration was then scheduled on Apollo 16 with again about four months to develop it. Steps were taken to overcome the problems of the Apollo 14 flight, namely: a tripod and lens extension tube system was provided to improve the photography (Figure 3) and PSL (polystyrene latex) was suggested by the USRA (University Space Research Association) as a more stable non-biological sample.

Along with the choice of PSL as the sample, ground based work using sucrose solution density gradients was suggested and used to indicate (Figure 4) and define the separation of the 0.2 and 0.8 micron PSL which was flown as a mixture in the upper tube and individually in the bottom and middle tubes, respectively, of the apparatus.

An example of the results of the Apollo 16 demonstration is shown in Figure 5 with a ground based view for comparison. A clear indication of the possible improvements in electrophoretic separation performed in space is indicated even though electroosmosis and some bubbles are also indicated. The latter two problems warrant some brief mention.

The bubbles were, we now believe, caused by the permeability of the silicone tubing - especially when it is subjected to a rapid external depressurization as was the custom on Apollo flights. During the development of the Apollo 16 unit there had been some indication of stress corrosion problems with the Lexan used to fabricate the electrophoresis cells. This was primarily due to the use of thin wall sections for greater transparency as compared to the more massive monolithic machined block used for Apollo 14. While the design was indeed demanding of the full capabilities of the Lexan, it was the best choice of transparent plastic and probably not the source of fluid leaks that permitted bubbles to form.

The electroosmosis is the result of the high zeta potential on the walls of the electrophoresis chambers. At that time there was no low zeta potential coating available which would adhere adequately to the chamber walls, and ground based tests in surcrose density gradients indicated no benefit from the use of a collodion coating as compared to leaving the Lexan uncoated. Since applying a coating may have been detrimental to the Lexan from the stress-corrosion standpoint, it was decided to leave it uncoated. The problem then is one of either not being able to translate the density gradient work to the flight demonstration unit or in not being able to obtain ground based results from the flight demonstration unit that would show the electroosmosis problem.

Happily, the remaining problems with these two flight demonstrations have been overcome in the more major experiment MA-011 (which on the other hand had some other difficulties which are being assessed and reported separately). The MA-011 also again made use of the phase separators and small peristaltic pump plus other technologies from Apollo 14 and 16. Thus it appears that while each flight has corrected the deficiencies of the previous flight, new problems have arisen by virtue of the changes made in

the demonstration materials, or design, or equipment, as each unit was being hurriedly developed for a singular flight opportunity.

It is therefore highly satisfying to see the sounding rocket and space shuttle flight schedules and the possibility for repeated flights of an experiment until it is satisfactory for as long as warranted.

Preparative Electrophoresis

Equipment - With the completion of the sufficiently satisfactory 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 the continuous flow type of unit as offering the greatest ease of inserting and removing samples and sample fractions. While the engineering of such a unit requires ingenuity, it is not extremely difficult and the basic idea for the electrophoresis cell is to simply make it thicker than the 0.5 to 1.5 mm commonly used for such units on earth. It was estimated that the cell in such a unit for space could be as much as 8-10 mm thick and provide an improvement by a factor of about 5-10 in resolution or an improvement of about 80-100 in throughput. This much greater performance is simply due to being able to scale up the thickness without gravity induced convection and to increase the sample concentration without sedimentation problems.

This has now been demonstrated with a 4 mm thick cell, at least partially, on the ASTP-MA-014 experiment by Hannig. A similar unit has also been developed in our laboratory for sounding rocket usage. It is shown in Figure 6 as currently equipped with a camera for data acquisition. Other work is now underway on modifications to permit collecting up to 50 fractions of sample and to detect them by a U. V. scanner system. These are being done on a schedule to permit a flight test in late 1976. The cell is 5 mm thick by 5 cm wide and has a 10 cm long electrode section. It is supplied with approximately $4^{o}C$ buffer, coolant, and samples by the use of a passive refrigerant system. The possible operating conditions such as flow rates, volts/cm across the cell, etc. are very broad and can be adjusted over several decades by a choice of gear ratios on the pumps and by plug-in power supplies, as well as by "fine tuning" electrically up to a short time before flight.

Math Modeling and Computer Simulation - Prior to designing the current sounding rocket unit, a math model was prepared both to aid the design and to predict the performance of thick cell electrophoretic separators in space. A separate publication is in preparation on this work so it will only be briefly reviewed here. Figure 7 shows the factors considered in the math model along with indications of which are controllable. As compared to previous efforts to describe the operation of a continuous electrophoresis cell, the parameters in our model are allowed to interact and are calculated primarily as to their effect on resolution and secondarily, throughput. Some typical results for a realistic although hypothetical separation of four samples (as defined by zeta potential and other conditions in each of three different thickness flow cells) are shown in Figures 8, 9, and 10.

Extensive ground based testing in prototype electrophoresis cells built for the previously described sounding rocket, as well as with other electrophoresis units, has generally corroborated the calculations for at least low levels of power. A plot of the power versus resident time (as a measure of flow rate) and thickness for stable and unstable conditions (hydraulically) is presented in Figure 11. Unfortunately, gravitational convection induced by the joule heating in these thick cells occurs at levels of power (10-15 watts) which are an order of magnitude below the power levels useful for separation.

Therefore, while the calculations and ground based tests at low levels are in good agreement, the more realistic level tests will have to be accomplished in space under microgravity conditions.

Biological Tests - Ground based tests for both the development of equipment and for establishing operating conditions for biologicals are being accomplished in a commercially available unit shown in Figure 12. It is a Beckman CPE II which is well designed for model studies and to which we are making additions and changes for more easily handling biologicals. These include fused silica windows and a U. V. scanner, for example. Studies ranging from single experiments, so far, to about 20 experiments, plus numerous calibration runs with PSL, have been undertaken with each of various biologicals including:

Hepatitis Vaccine
Sperm
Lymphocytes
AHF
Erythropoietin
Influenza Virus Antigen

The results are generally encouraging but not necessarily easily achieved nor sufficiently complete. Considerably more effort has to be expended in this area before flight tests since it seems unlikely that a space flight test will accomplish a separation that has not at least been shown to be feasible on earth. Examples of some of the separations studied and results obtained are shown in Figures 13-15.

Projections for the Future - Contacts in numerous pharmaceutical houses indicate that, while indeed cells of various types and sources are an intriguing problem for separation science, numerous hormones, enzymes, blood and urinary source materials, and vaccines need or would be benefited by a great deal of improvement in purity. The practical limitation on the use of electrophoresis to prepare these products in sufficient purity to be of value is throughput efficiency. While absolute purity is required in certain cases, many products are only needed in more concentrated form and therefore resolution is often a subjective parameter which can perhaps be traded off against throughput. An estimate of the throughput in grams/hour versus cell thickness for two cases is shown in Figure 16. The upper right hand area of the figure depicts a high throughput case, i. e. for a case where sufficient resolution is easily obtainable and the extra capacity of the equipment can be utilized for throughput. The lower portion of the figure depicts a situation where resolution is to be stressed. In each case a range of 1-10% sample concentration is shown which should be compared with typical ground based practice of using about 0. 1 to 0. 5%. In any case, some 2 to 3 orders of magnitude improvement in throughput are predictable. This is far beyond the degree of improvement for which one might consider simply duplicating the ground based facilities when greater throughput is needed even if resolution were satisfactory.

Economic Predictions - Three general areas of potential payoff for this work are foreseen. First is the possibility of the research and development being beneficial to ground based electrophoresis equipment and techniques. Secondly is the possibility of preparing more specific strains or products in space which can then be used to culture and produce greater quantities of particular products on earth. Thirdly, when the first two or other approaches are insufficient, products may actually be produced in space.

Examples of the first two approaches already being productive are available. Improved electrophoresis equipment and coatings with nearly zero zeta potential are now available

and are examples of the first area of benefits. Increased yield of Urokinase through the improved separation of fetal kidney cells on the Apollo Soyus Test Project flight in the summer of 1976 is an early indication of a potential benefit in the second area. Preliminary examples of the third area must await further work but may well come from current projects for the sounding rocket and early shuttle flights.

Several products could potentially benefit from these capabilities and further work is recommended to establish the necessary protocols and reference data on which to base flight tests.

The human value of more effective biologicals is of course impossible to measure. The preparation of purer erythropoietin could free some 15,000 U.S. renal failure patients from repeated blood transfusions. Thus humanitarian and societal motivation in this area is unusually high, and even greater than the basic economic value. Some simple projections for space processing of biologicals can be made based on certain assumptions.

It is presumed first that for efficiency and economy, as much of the processing as possible will be done here on Earth. Then, only a reasonably pure concentrate will be taken to space for one more, or perhaps a few, processing steps. In addition, the large quantities of water normally used in biological processing are presumed to be recoverable and reusable in space so that this commodity will not need to be completely resupplied from Earth for each product. Finally, however, the general rule that each biological product should be prepared in isolation from other products in order to avoid cross contamination is likely to be necessary. This may necessitate some special scheduling, but should not create any insurmountable problems.

Vaccines are the best defined available product on which to base projections for the future. In the U.S., some 60 million doses of vaccine are used annually. If we utilize the World Health Organization's estimates of World population in 1990-2000 as 5 billion and assume the same rate of vaccine applications world-wide as is now current in the U.S., we project the need for about 1.5 billion doses of vaccine per year. Using a conservative average number of 100,000 doses per gram of active ingredient, we calculate the need for 15,000 grams of active ingredients per year. Many currently used and very fine biological products are at best however quite dilute or impure (but not necessarily with harmful impurities). The purity may range from less than 1% to about 50%. This is assumed to be the starting material for a space purification operation. Therefore, the weight of starting material could range from 2 to 100 times the 15,000 gram final product weight derived above. Assuming a conservative average of 50, it is expected that some 750 Kg of partially purified vaccines might be used as the starting materials. In addition, some several hundred kilograms of water would be required. While vaccines generally cost about 20¢ per unit to produce, some examples of higher costs for greater specificity indicate that $1.00 per unit may be an acceptable value. This then indicates a $1.5 billion dollar activity in vaccines alone, a fraction of which may require space operations.

The processing of some other biological products such as cells and the blood derivatives in space while less specifically calcuable could easily exceed the estimates for vaccines by up to an order of magnitude in volume and value.

Acknowledgement

The support and encouragement for much of this work by NASA through several contracts is appreciated and acknowledged. In addition, major roles in various apsects of this work have been filled by several associates especially Dr. R. N. Griffin, R. J. Locker, Dr. J. Giannovario, and Frank Cosmi, as well as others too numerous to mention. It is a pleasure to acknowledge the work of all of them.

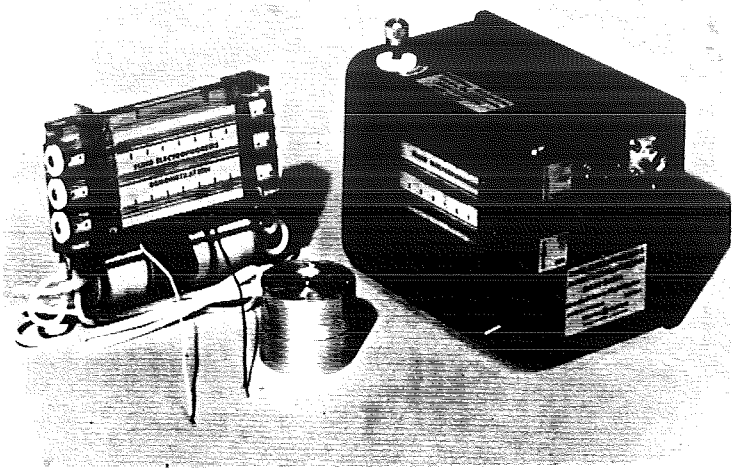


Figure 1.- Apollo 14 fluid electrophoresis demonstration unit on right with some of the major components at left. These include the three electrophoresis cells machined in a monolithic block of Lexan, with the phase separators below and the peristaltic pump at center. The overall dimensions of the experiment are approximately 4 by 5 by 7 in. plus appurtenances.

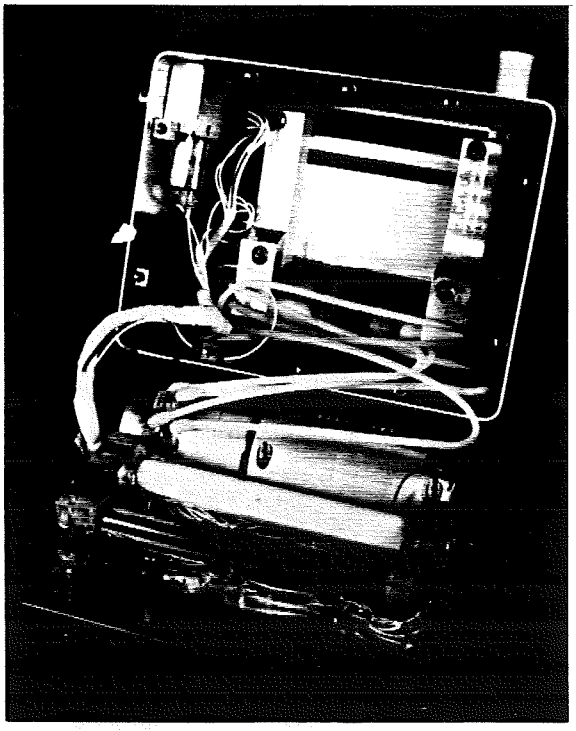


Figure 2.- Apollo 14 fluid electrophoresis demonstration unit in opened configuration showing back view of electrophoresis cell in upper portion of box with phase separators and peristaltic pump in middle and fluorescent lamps and potted electronics in lower area.

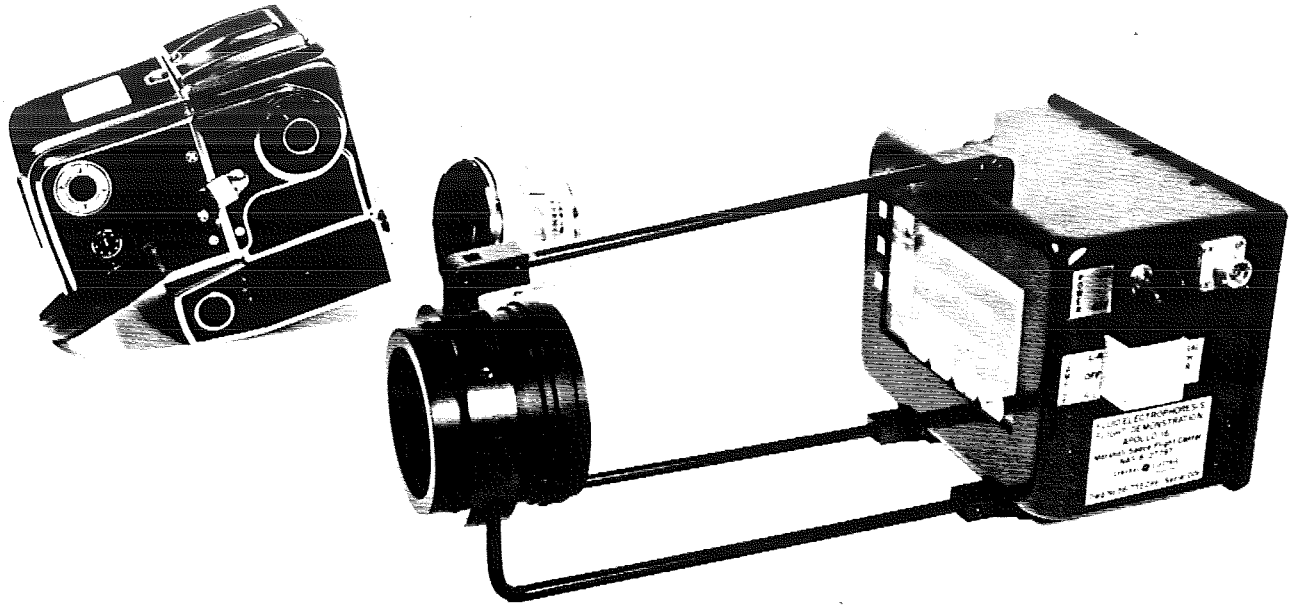


Figure 3.- Apollo 16 fluid electrophoresis demonstration mockup. Larger window, instruments, and camera-tripod arrangement improve data acquisition.

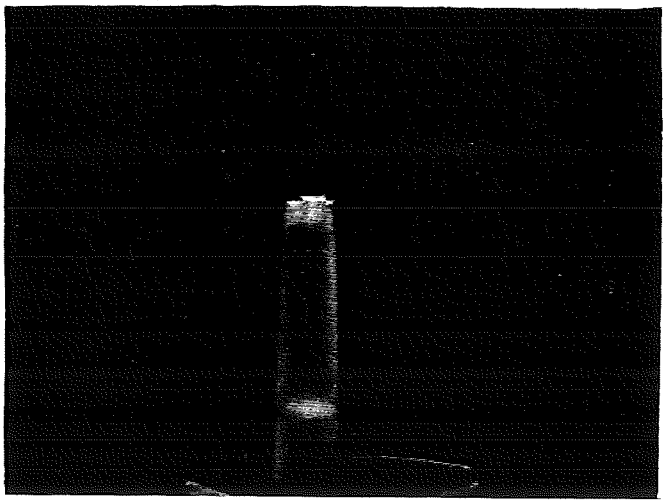
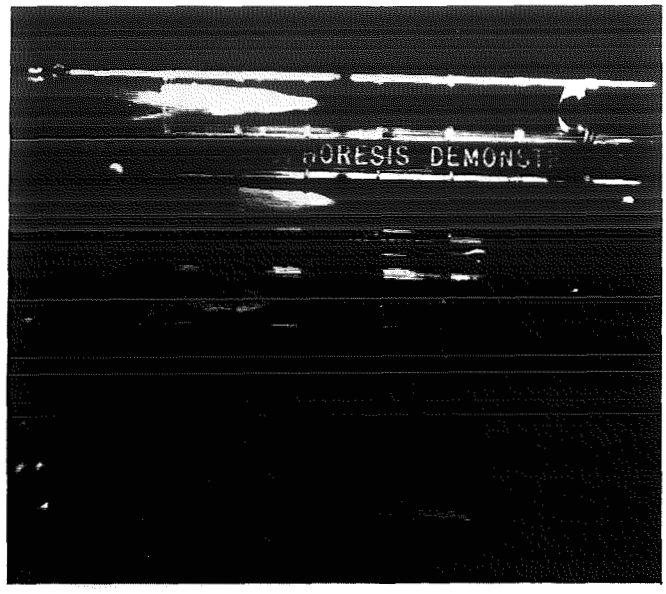
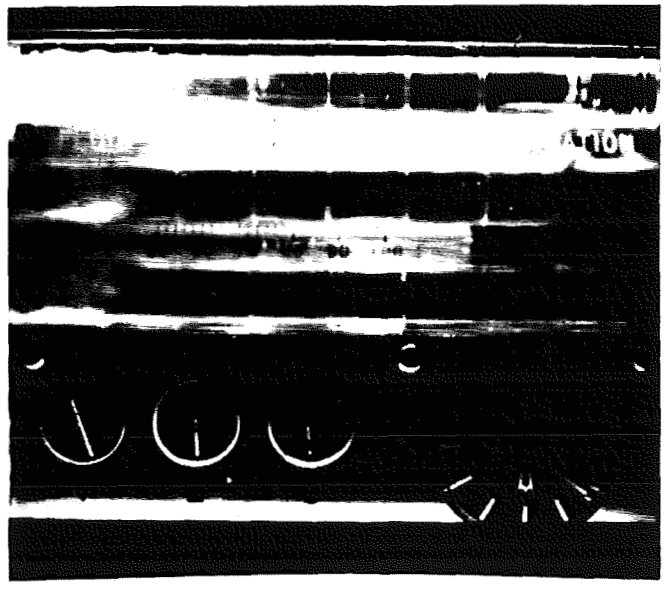


Figure 4.- Electrophoretic separation of 0.2- and 0.8-micrometer polystyrene latex in a sucrose density gradient after 40 minutes during which time the leading band (0.8 micrometer) traveled 8 centimeters and the trailing band 5.6 centimeters using a 0.085 M borate buffer of pH 8.5.



(a) Flight results.



(b) Ground results.

Figure 5.- Results of Apollo 16 fluid electrophoresis demonstration. The flight results clearly show the benefit of reduced gravity on the electrophoretic mobility at 30 V/cm in borate buffer of a mixture of 0.2- and 0.8-micrometer polystyrene latex (PSL) in the upper tube, 0.8-micrometer PSL in the middle tube, and 0.2-micrometer PSL in the lower tube. Equivalent samples in the ground-based results show the detrimental effects of gravity-induced convection and sedimentation.

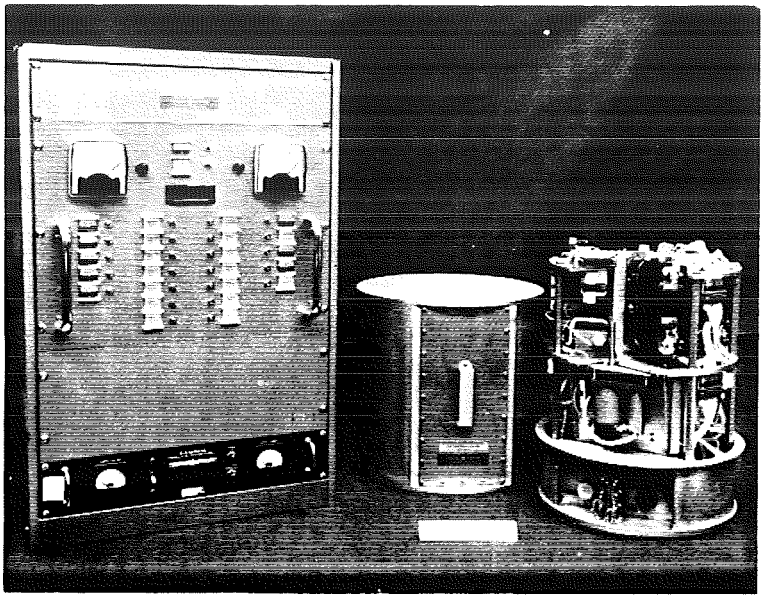


Figure 6.- Advanced applications flight experiment (AAFE) continuous-flow electrophoretic separator (right) under development for use on a sounding rocket. The upper enclosure (middle) with an access door for installing the sample, servicing the camera, and setting experiment conditions before flight; and the test and control panel including power supply (left) are also shown.

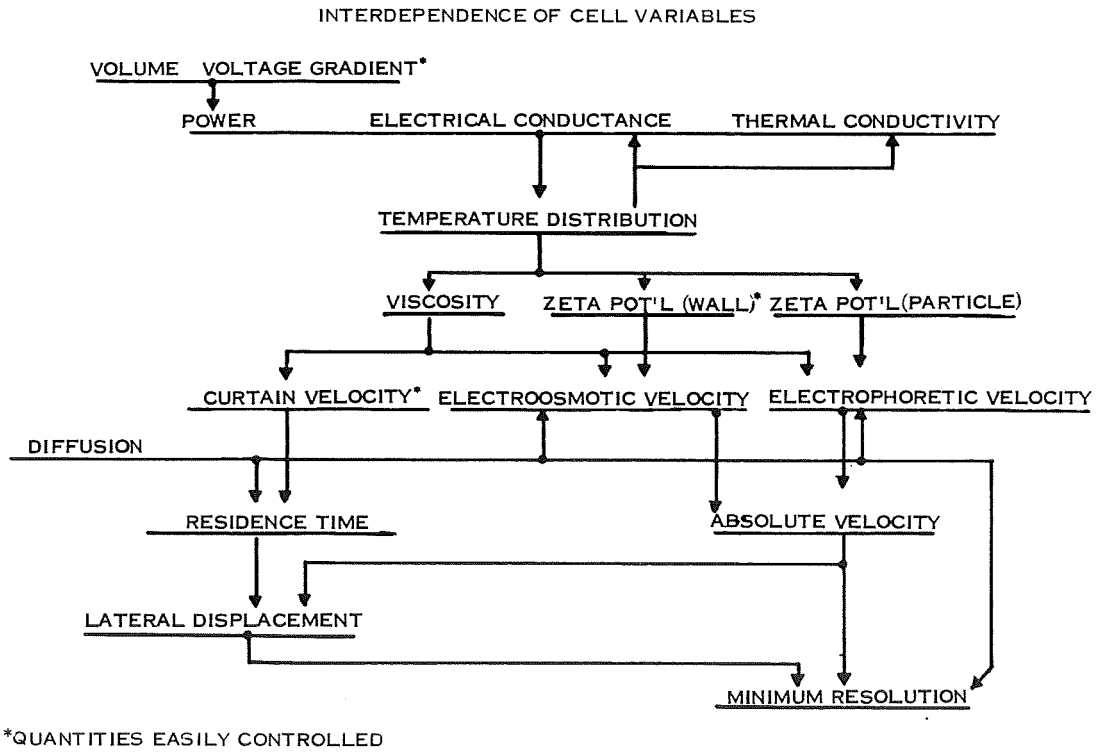


Figure 7.- Major features of a computerized mathematical model of a continuous-flow electrophoretic separator. (Controllable variables are indicated by asterisks.)

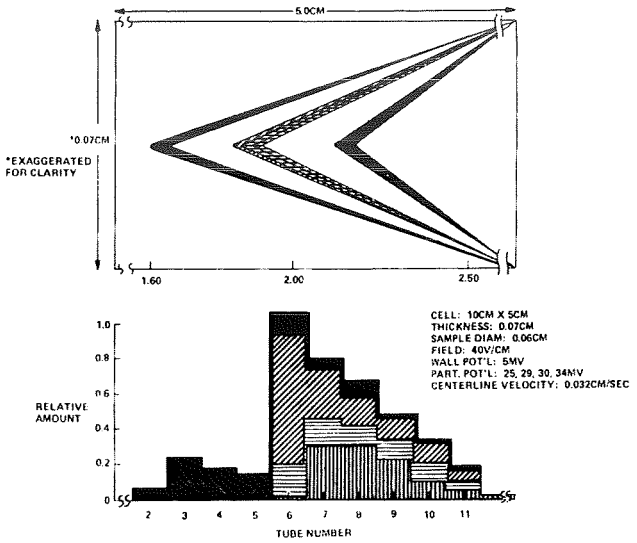


Figure 8.- Illustration of calculations for a four-component separation in a 0.5-mm-thick continuous-flow electrophoresis cell. (Note expanded scale; other assumed conditions as indicated.)

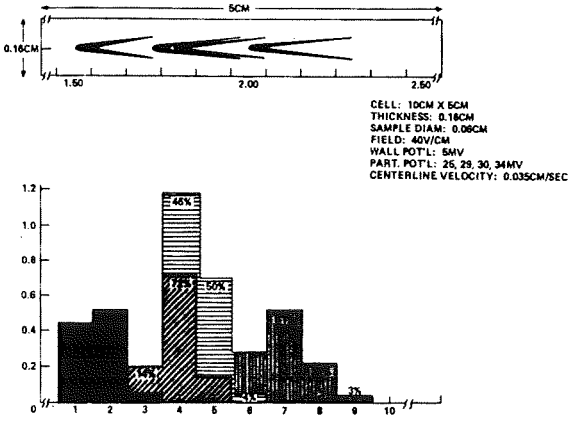


Figure 9.- Illustration of a calculated resolution for the same four-component separation as figure 8 in a 1.5-mm-thick cell.

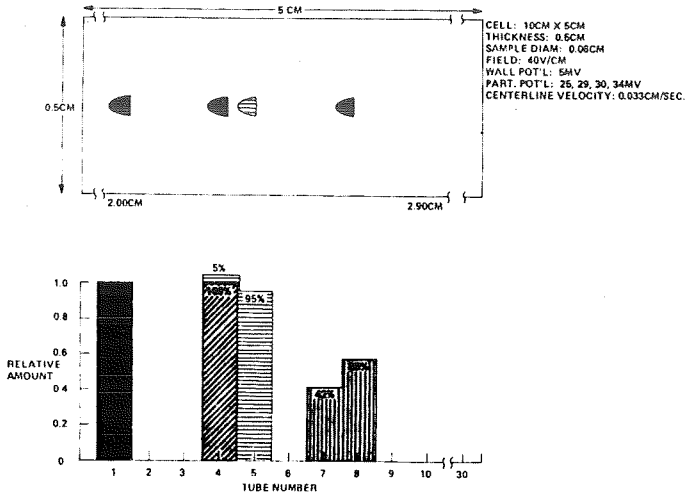


Figure 10.- Illustration of calculated resolution for the same four-component separation as figures 8 and 9 in a 5-mm-thick cell.

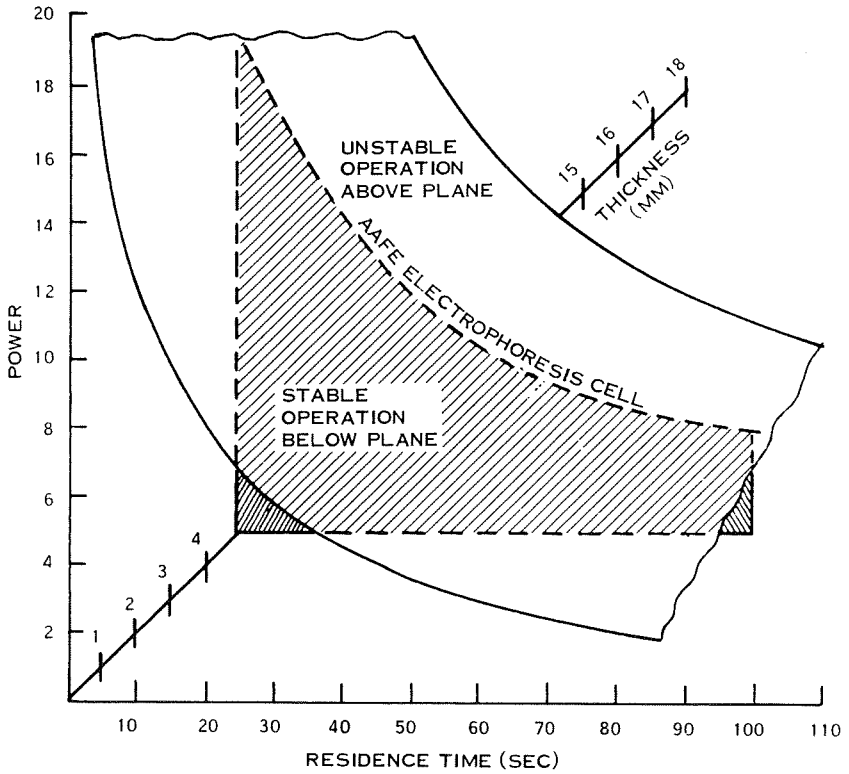


Figure 11.- Experimentally determined regions of stable and unstable operation of the AAFE (fig. 6) electrophoresis cell as a function of power and residence time for a 5-mm-thick cell with other conditions estimated.

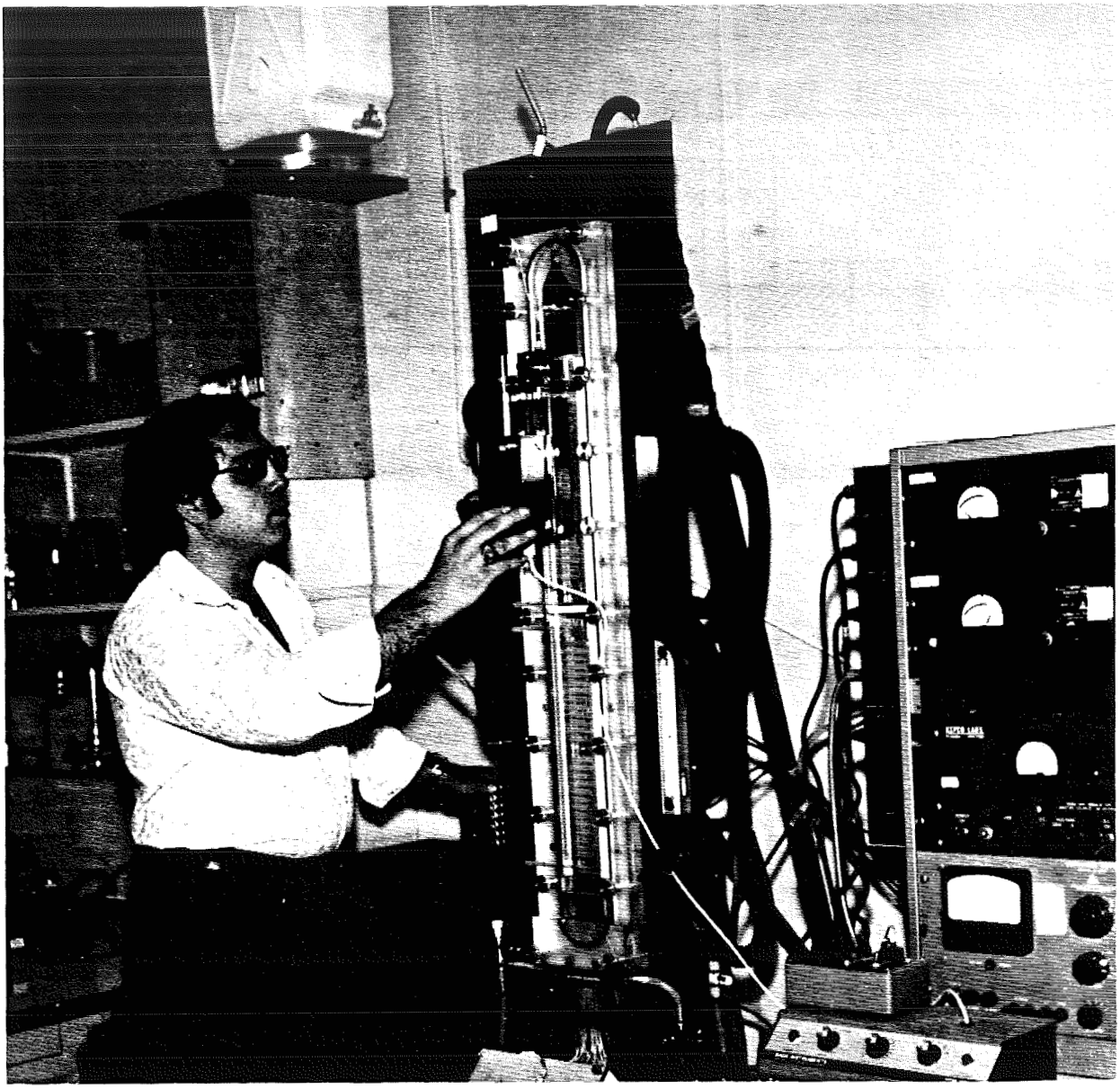


Figure 12.- Modern laboratory continuous-flow electrophoretic separator.

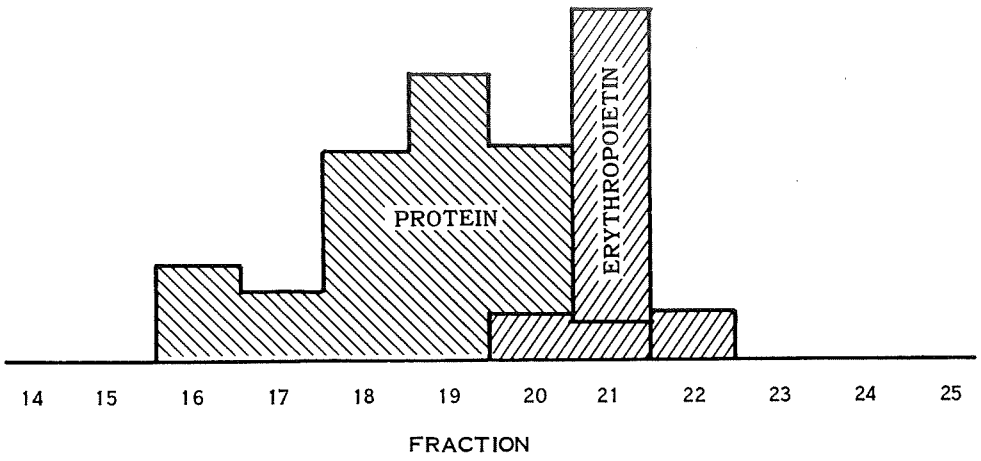


Figure 13.- Partial concentration of Erythropoietin from protein by electrophoresis.

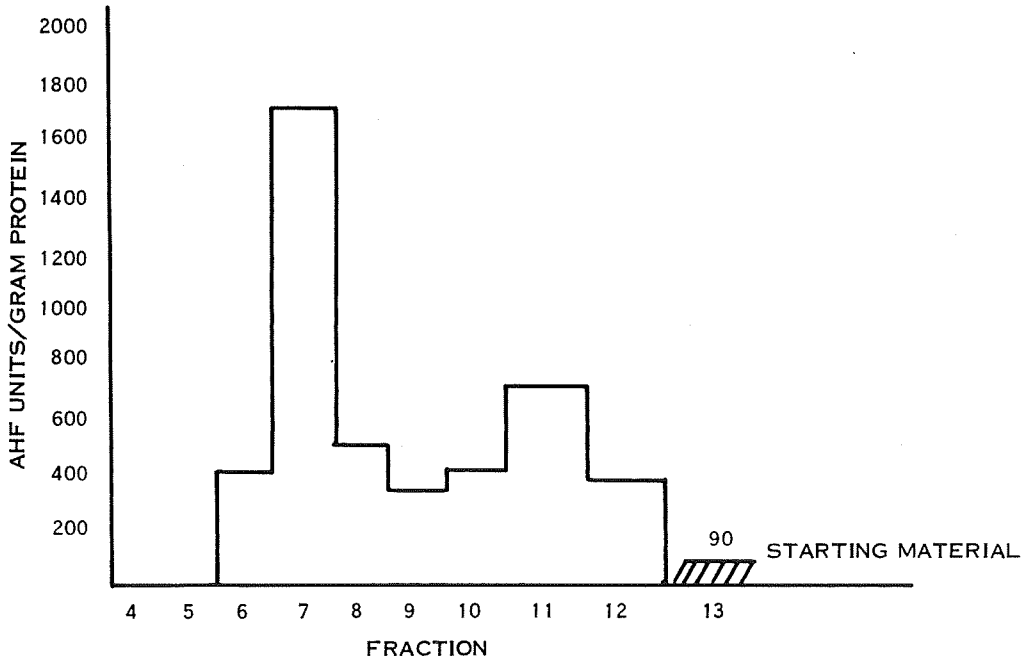


Figure 14.- Concentration of antihemophiliac factor VIII by continuous-flow electrophoresis.

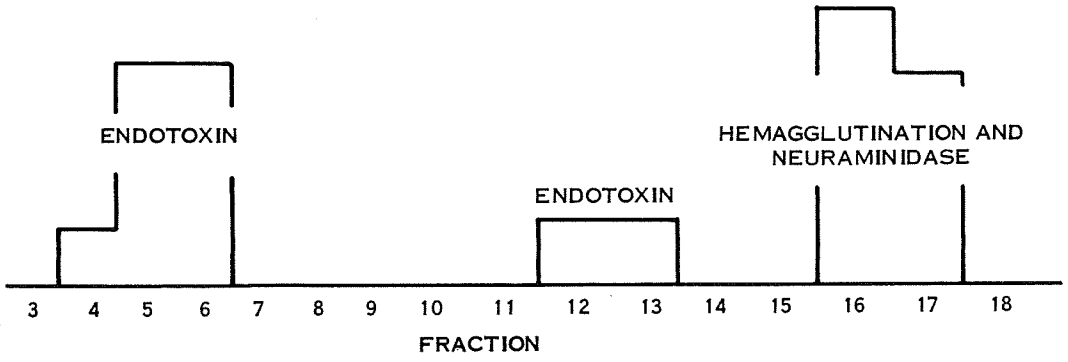


Figure 15.- Separation of influenza virus antigen from endotoxins by continuous-flow electrophoresis.

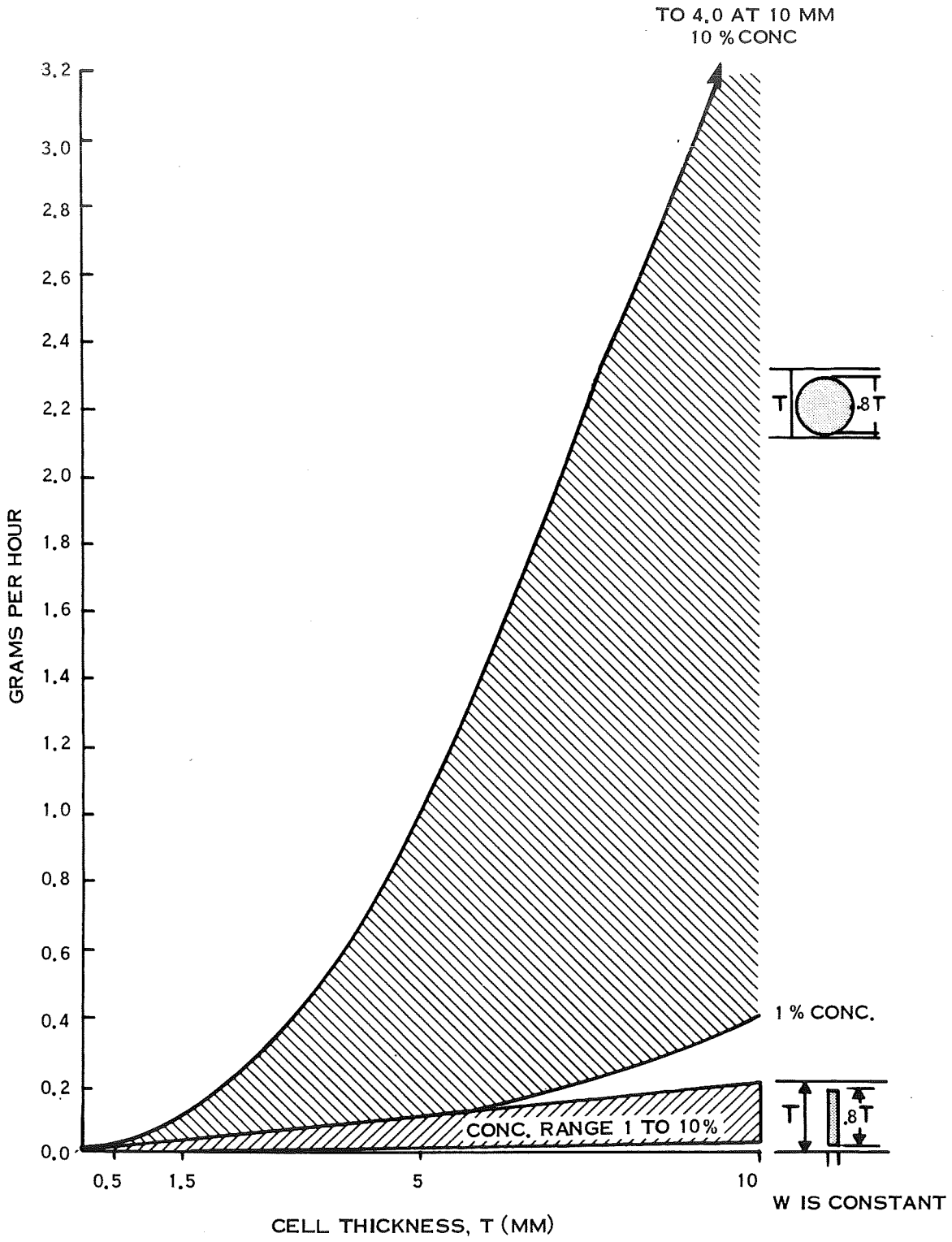


Figure 16.- Calculated throughput for continuous electrophoretic separators as a function of cell thickness and sample concentration for high-resolution (lower curves) and high-throughput examples.