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BIOPROCESSING: PROSPECTS FOR SPACE ELECTROPHORESIS

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ABSTRACT

The basic principles of electrophoresis will be reviewed in light of its past contributions to biology and medicine. Preliminary experiments aboard of Apollo 14 and 16, Skylab and the recent Apollo-Soyuz Mission have confirmed the feasibility and advantages of a possible space electrophoresis facility. This has to be viewed primarily as a unique national research resource, which may eventually yield significant benefits for the advancement of biomedical knowledge and its technological utilization. Primary objectives of the facility should be the increase of resolution and throughput for critical electrophoretic fractionation of living cells and biologically active macromolecules.

INTRODUCTION

The technology of bioprocessing comprises two distinct categories of activity; on the one hand, there is need to grow and propagate various organisms through bacterial, mold or tissue culture and much work has been dedicated to optimize techniques and isolate strains possessing maximal activity. On the other hand, specific products have to be isolated from the biomass, often a difficult problem in view of the complexity of composition of living matter, and the similarity of the species to be separated. As a result, separation processes have acquired a unique importance in biotechnology, reflecting their importance in basic biochemistry.

Both of these aspects of bioprocessing may benefit from space research, and the last two speakers have discussed specialized aspects of space effects on growth and reproduction. In a later presentation, Dr. Barlow will show an example of where cell activity may have been enriched by separation in space. My present paper will focus on possible contribution of space electrophoresis, a separation process which has been identified as being most likely to benefit from the near-zero gravity environment prevailing in orbiting spacecraft.

The possible contribution of NASA's space capabilities to bioprocessing should be evaluated not only in terms of the economic importance of this industry, but primarily in terms of its social impact, through advances in medicine and its contributions to the quality of our lives.

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ELECTROPHORESIS

Electrophoresis (1) is defined as the transport of electrically charged species under the influence of a direct current electrical field. Most materials in aqueous solution or suspension acquire an electrical charge due to ionization of their functional groups, ion absorption, or other more complex phenomena, and are therefore attracted by electrodes of opposite polarity. The charged species may be simple ions, complex macromolecules or even particles, such as living cells, emulsion droplets, clay, etc. Their migration velocity in unit electrical field is referred to as their electrophoretic mobility, and is a complex function not only of their electrical charge, but also of their molecular size, shape and hydration, as well as the dielectric characteristics of the solvent. As a result, electrophoresis is capable of providing a high degree of characterization of individual ionized species, which is most important for macromolecular systems and living cells, where structural parameters are difficult to determine.

Based on this uniqueness of information provided by electrophoresis, a number of applications have been developed. To categorize them in their broadest outlines, these are as follows:

- (a) Identification and characterization of an ionized species.
- (b) Determination of the quantitative composition of a complex mixture.
- (c) Actual isolation of components of a mixture, separation being achieved on the basis of differences in transport rates.

Originally, electrophoresis was carried out in free solutions but it was soon recognized that problems arise due to convective disturbances in the bulk of fluid. We can categorize several major causes of these disturbances:

(a) The solute to be separated, if present in significant concentration, adds to the density of the supporting electrolyte. This difference in density between solution and pure solvent causes gravity-caused convective flow, unless means are found to prevent it.

(b) In some instances the particles may be sufficiently large to sediment noticeably. While there are techniques which utilize differential sedimentation to accomplish meaningful separations, within the context of electrophoresis such sedimentation is usually undesirable, especially as it is superimposed on the convective flow of the suspension as a whole, described above.

(c) The passage of electric current causes heating of the solution. As the vessels are externally cooled, a radial temperature gradient arises, again causing gravity-conditioned convection.

(d) The electric charge exhibited by the vessel walls within which electrophoresis is carried out causes an electroosmotic streaming of the fluid. This disturbance is independent of gravity and is a consequence of the electrical properties of the system as a whole.

In order to eliminate some or all of the above problems, a variety of techniques has been evolved, and a systematic classification is next to impossible. Thus, the techniques are differentiated according to their primary purpose (preparative or analytical), the mode of operation

(batchwise or continuous flow), shape of vessel (cylindrical, flat, annular, etc.), and other operational parameters. In the context of this paper, the most important classification is based on the anticonvective means employed to circumvent the effects of gravity. Three basic approaches were taken:

- (a) migration can be carried out in gels, where all convective flow is prevented,
- (b) fine porous structures of packed granules, or the interstitial spaces of filters and various specially developed membranes are also effective in preventing gross fluid motion without interfering in molecular transport, and
- (c) a density gradient can be artificially created within the liquid by using a non-migrating solute such as sucrose, of sufficient steepness to overcome the density gradients caused by the electrophoretic process.

To these three approaches, we now must add the radically new concept to avoid gravity altogether, by using orbiting spacecraft. The soundness of this approach has been confirmed in pilot experiments conducted aboard Apollo 14 and 16 (2), Skylab (3), and the recent Apollo-Soyuz Mission (4).

As defined above, electrophoresis is a separation process occurring within the bulk of the liquid phase (and not at the electrodes) and is based on the differences in electrical transport rates. Electrophoresis alone, however, does not provide for the ultimate separation of various molecular species of proteins present, as their mobilities may be overlapping. Highest resolution is obtained if a second separation parameter is employed by introducing an element of discontinuity into the liquid phase. Two methods are most often used. In high density gel electrophoresis an element of molecular sieving is superimposed on the electrical separation process by progressively increasing the density of the supporting gel matrix. In isoelectric focusing a continuous pH gradient is established and the proteins become immobilized at the pH corresponding to their characteristic isoelectric point (mobility of proteins is pH dependent - the narrow pH zone of zero mobility is the isoelectric point). The separation obtainable by isoelectric focusing is comparable to that in high density gels, and both are much superior in resolution to plain electrophoresis.

ELECTROPHORESIS OF LIVING CELLS

Because of its nondestructive nature, electrophoresis is one of the few separative methods applicable to living cells. Nevertheless, in comparison to proteins, cell electrophoresis is only in its infancy. Most of the techniques and instruments developed for protein electrophoresis are not applicable, and cell electrophoresis has remained the province of a few highly specialized laboratories. One of the great merits of the NASA program is that it has focused the attention of a number of scientists here and abroad on this long neglected field. At present, cell separation is the main objective of NASA's space electrophoresis facility.

Basic knowledge in this field is sorely needed. While there is a multitude of analytical electrophoretic methods applicable to proteins, until quite recently there was only one method suitable for cell electrophoresis. This technique involves direct visual microscopic measurement of electrophoretic migration velocity of individual cells, and has remained essentially unchanged for over 50 years (5,6). It is an inherently slow and unreliable method, burdensome and tedious

for the observer. As a result, while there is adequate information on some normal cell populations, such as red blood cells and lymphocytes, there are almost no reliable data on changes of cell properties in most clinical or pathologic conditions. This situation is intolerable, since the present state of the art would readily permit computer assisted automation of the microscopic method, resulting in rapid accumulation of important basic data on cell mobilities in health and disease. It is hoped that through NASA sponsorship, such an instrument will soon become available. Other alternatives to more rapid accumulation of data involve the measurement of the Doppler Effect caused by migrating particles under laser illumination (7). Both of these two types of instruments are operable in presence of gravity, but at zero gravity their scope of application would be extended to larger cells, characterized by rapid sedimentation in a normal gravity field. Moreover, such instruments will be essential for the space facility, to provide real time information on the quality of separation achieved in space in the preparative instruments.

Similar considerations prevail in preparative electrophoresis. Several techniques have been developed, including thin film free-flow electrophoresis (8), stable-flow electrophoresis (9), electromagnetophoresis (10), and rotationally stabilized instruments (11, 12), but most have remained almost exclusively in the hands of their original developers. This is largely due to their complexity and the paucity of basic analytical data, which are indispensable in pinpointing the most important areas of preparative application. Moreover, the throughput of the instruments is limited, and their resolution less than optimal.

It was previously emphasized that highest resolution of proteins is obtained only when a second discriminating parameter is superimposed on electrophoresis, as in high density gel electrophoresis. The same situation may prevail with cells, and we are presently developing a system where electrophoretic separation is followed by an in-line discrimination according to cell size. Other secondary discrimination factors may be usable, such as presence of fluorescent markers. Such systems bear the promise of much higher resolution than that obtainable by electrophoresis only.

SPACE ELECTROPHORESIS

Gravity is not an unmitigated enemy of electrophoresis, and, to the contrary, in numerous techniques it is utilized to great advantage. The determining factor is the objective one seeks and one has to consider in different light separation of proteins and that of living cells. With proteins, there is a profusion of excellent methods for analytical or micropreparative work, i.e. fractionation and separation of products on a small, laboratory scale operation, and no foreseeable advantage is to be gained from a zero gravity facility.

The situation is different when scaling up of these techniques to larger volumes is attempted. In this realm, ground-based electrophoresis has failed completely and all attempts to scale up high resolution micropreparative procedures have been unsuccessful. The zero gravity facility may provide the hoped-for breakthrough by allowing the use of novel instruments specifically designed for the weightless environment. As an example, in our laboratory we are currently engaged in collaborative efforts to purify two trace components of human serum, Somatomedin, a growth promoting polypeptide, and Phagocytosis Recognition Factor, a potential antitumor agent. The technique utilized is isotachophoresis, a relatively new variant among the many electrophoretic techniques (13), and we have designed a novel type of instrument (14) particularly suited for space use. A diagram of this instrument is shown in Fig. 1. It is constructed from a parallel array of feeder spacers and knife edge separators, assuring laminarity of liquid flow. Time does not per-

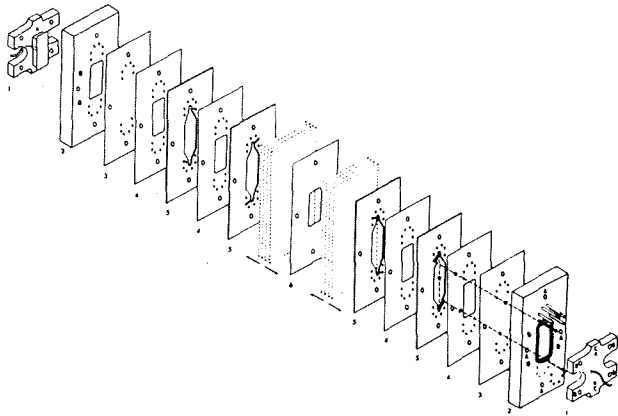


Fig. 1. Schematic presentation of a miniaturized flow electrophoresis apparatus designed for space electrophoresis. The parallel array of spacers and knife edge separators provide for laminar flow within the cell. The apparatus is envisioned for rapid flow-through with minimal migration distance, and may be particularly suited for isotachopheresis.

mit to go into the rationale why this apparatus promises a higher throughput in space operation than that of other similar continuous flow instruments available on ground. There are obviously a great number of other proteins which may benefit from space processing, including clotting factors, enzymes, and other protein hormones.

If the prospects of space electrophoresis were to be realized for proteins, there would be an immediate widespread usage for the products. Nevertheless, the primary emphasis of the current NASA program is centered on separations of living cells. The reason for it is that most techniques to circumvent gravity effects developed for protein electrophoresis are not applicable to cells. Moreover, cell electrophoresis is only in its infancy, and the development of better techniques may result in significant advancement of our knowledge of cell biology. This is the most opportune time for such a development, as it is only in recent years that cell biology has come into its own, with the recognition that apparently similar cells may have a variety of distinct functions. This is particularly true of lymphocytes, the mediators of immune reactions, which are of direct and immediate importance in such diverse areas of medicine as allergies, autoimmune diseases, leukemia and other forms of lymphocyte neoplasias, resistance to cancer, etc. Thus, there is a widespread current interest in cell separations by any and all means. Reliance on electrophoresis is based on the as yet fragmentary but significant evidence that functional, pathologic, genetic and environmental factors affect the electrophoretic behavior of lymphocytes (15).

The recent Apollo-Soyuz Mission provided an opportunity to test two prototypes of instruments suitable for zero gravity operation. The NASA prepared flight module was similar to those previously flown in Apollo 14 and 16 (2), though more ambitious in its aims. The essential part of the instrument was the electrophoresis column, reproduced schematically in Fig. 2. The two electrode compartments were detachable from the main body of the column, permitting a total of eight different columns to be tested. The columns were preloaded with sterile buffer, and the samples to be electrophoresed were frozen in liquid nitrogen till immediately before use by the astronauts. The samples contained kidney cells, lymphocytes and fresh and fixed lymphocytes. Photographs could be taken during the run to record the migration of the cells, while at the end of the run, the

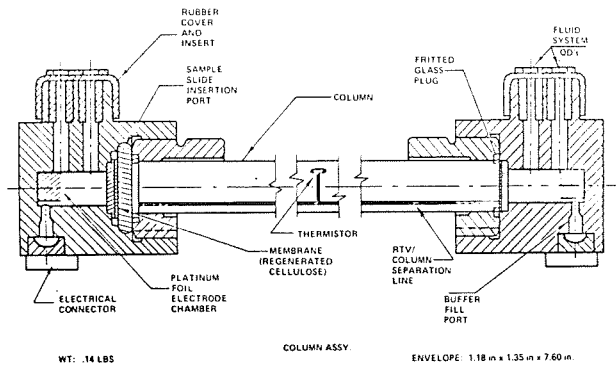


Fig. 2. Schematic diagram of the column assembly for the static electrophoresis experiment carried out as part of the Apollo-Soyuz Mission. The center part of the column is detachable from the electrode compartments on both ends.

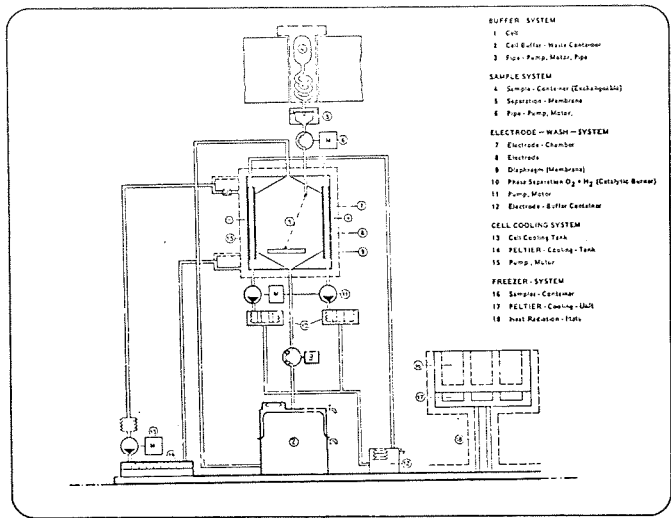


Fig. 3. Schematic drawing of the continuous flow electrophoresis apparatus used in the Apollo-Soyuz Mission. The cell itself is in the center. Accessories provide for frozen sample storage, sample insertion, and buffer circulation.

columns were frozen in situ, and returned to earth in liquid nitrogen. The kidney cells were subsequently grown in tissue culture, demonstrating the viability of the cells so recovered and we will hear more about it later on. Time does not permit to discuss in greater details the other experiments, or the apparatus.

A second apparatus was also included in this Mission. It was designed by Hannig of the Max Planck Institute for Biochemistry in Munich, Germany, and was constructed by the Messerschmitt-Bolkow-Blohm consortium, and financed by the German Government. The diagram of this apparatus is presented in Fig. 3, and it can be readily seen that it is far more complex than the first apparatus. It is an automated and miniaturized version of the well known continuous flow instrument of Hannig (8), and contained three samples: a mixture of human and rabbit erythrocytes, a mixture of B and T lymphocytes, and a suspension of bone marrow cells. Their migration was followed photometrically, and no recovery of fractions was intended.

Both of above instruments were space adaptations of ground based equipment, and represent prototypes of two basic concepts in electrophoresis: stationary fluid versus continuous flow operations. Their designs were kept within the narrow constraints inherent in experiments aboard manned rockets. The availability of the Shuttle will probably eliminate most of these constraints, and there have been already several proposals within the NASA program of instruments more specifically designed for the space application.

CONCLUSIONS

The near-zero gravity environment of orbiting spacecraft may present some unique advantages for a variety of processes, by abolishing the major source of convection in fluids. As the ground-based development of electrophoresis was heavily influenced by the need to circumvent the effects of gravity, this process should be a prime candidate for space operation. Nevertheless, while a space facility for electrophoresis may overcome the limitations imposed by gravity, it will not necessarily overcome all problems inherent in electrophoresis. These are, mainly, electroosmosis and the dissipation of the heat generated by the electric field. The NASA program has already led to excellent coatings to prevent electroosmosis, while the need for heat dissipation will continue to impose limits on the actual size of equipment. It is also not excluded that, once the dominant force of gravity is eliminated, disturbances in fluid stability may originate from weaker forces, such as surface tension.

There is as yet no consensus on the best apparatus for space electrophoresis. Reflecting the diversity of ground-based electrophoresis instruments, it is likely that more than one instrument will be needed for the space facility as well. It is important to consider the nature of the possible advantages to be derived from space electrophoresis: these are only a matter of degree. In all separation processes, one has to consider the factors of resolution and throughput, there being usually a trade-off between the two. For cell separation in space, both may be of importance, while for proteins, only a quantum increase of throughput would constitute a definitive advantage. To achieve either or both of these advantages, optimization of the design of the space instruments is essential. Less than the best designed instruments may well jeopardize the whole program, particularly when one considers that its operators in space may not have the skills of principal investigators or highly skilled technicians. The interdisciplinary expertise available to NASA offers a unique opportunity not only to optimize the space but also ground-based equipment.

The space facility for electrophoresis has to be considered primarily as a unique research tool for the advancement of our knowledge of cell biology, though its potential technological applications should not be overlooked. In view of the cost of space experimentation, greatest care should be given to the selection of candidate materials for space processing. To accomplish this, it would be desirable if the NASA program were to be integrated or correlated with the work of other agencies or organizations having a more primary interest in the health field. The process of selection should encompass a thorough evaluation of the material by ground-based electrophoresis and the consideration of alternatives. As we are dealing with a rapidly advancing area of science, maximum flexibility in planning is essential for both, instrument design and their application.

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