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# Applications Of Space-Electrophoresis In Medicine

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## APPLICATIONS OF SPACE-ELECTROPHORESIS IN MEDICINE

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### ABSTRACT

The contributions of ground based electrophoresis to biology and medicine have been briefly reviewed and placed into context with further advances potentially realizable from a space electrophoresis facility. This has to be viewed primarily as a unique national research facility, which may eventually yield significant benefits for advancement of basic biomedical knowledge and its technological utilization. Primary objectives are increased resolution and throughput for critical separations of living cells and biomacromolecules.

### INTRODUCTION

The ability to escape from the confines of terrestrial gravity and reach for the moon and beyond has been a dream of mankind for centuries. It has been realized only recently, and our space capabilities will soon be greatly expanded with the development of the Shuttle System and its Spacelab. They will constitute a unique national resource, which should be exploited with the utmost care and concern for the greatest benefit of mankind. This can be achieved only with the participation of broadly based segments of scientific and technological communities; the present Congress and the newly organized National Space Institute are fulfilling an essential role in increasing our awareness of the challenge of space.

This presentation is an outgrowth of the current NASA efforts to develop advanced technologies and processing techniques utilizing the singular environmental conditions prevailing in orbiting spacecraft. Foremost among these is the virtual absence of gravity effects, a condition impossible to duplicate on earth. A potentially significant application of the zero gravity environment is the electrophoretic separation of living cells. The feasibility of this approach has been demonstrated in preliminary experiments carried out aboard Apollo 14 and 16 (1), the Skylab (2), and the recent joint Apollo-Soyuz mission (3). It is expected that a facility for cell separations will be incorporated in the early flights of the Shuttle. If so, it will be the result of the foresight of a number of NASA scientists who

organized a major R and D program in electrophoresis involving several leading academic and industrial laboratories in this country, as well as the European Space Research Organization. I am greatly privileged to be today an unofficial spokesman for this large group, to which all credit is due.

Electrophoresis (4) is the most useful single technique for the analysis and separation of complex biological mixtures, and it is used for this purpose in thousands of scientific and medical laboratories throughout the world. Its importance is evident from the more than 10,000 scientific publications on electrophoresis and its applications. Nevertheless, the development of electrophoresis was largely haphazard, usually the fortuitous byproduct of individual research efforts of biochemists or physicians, and in the past it has received only modest direct research support from various granting agencies. It has remained largely neglected by many disciplines, such as the engineering profession, physicists, hydrodynamicists, and surface scientists who might all have contributed to its advancement. The current NASA program is the first multidisciplinary effort to advance its state of art, and, as in so many other NASA sponsored activities, it has already had a significant fallout for earthbound applications.

### ELECTROPHORESIS

Electrophoresis (4) 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 of colloids, 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 electric 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:

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

A multitude of techniques and instruments have been developed for the exploitation of the basic phenomenon of electrophoresis. Their description or classification is outside the scope of the present paper. For some applications the minutest sample is sufficient, and microscopic techniques have been developed (5,6). At the other extreme, a few large industrial installations have been constructed (7), though such applications are generally lagging (8).

The most important applications of electrophoresis are in molecular biology and medicine. In biology, it has contributed to the advancement of our knowledge of proteins more than any other laboratory technique. Its impact originated with Tiselius (9), who first demonstrated the complexity of human blood proteins, and received for it the Nobel prize. More recently, electrophoresis led to a new branch of human genetics, the study of the inherited variabilities of blood proteins. In medicine, electrophoresis offered the first clue to the primary cause of sickle cell anemia by demonstrating the presence in sickled red blood cells of an abnormal hemoglobin, and has been widely used for the diagnosis of this disease. It is also the method of choice for the diagnosis of a variety of acquired and inherited other protein-linked diseases, such as multiple myeloma, agammaglobulinemia, Waldenström macroglobulinemia, etc. Electrophoresis is also used for the detection of lipoproteinemias, the earliest indicator of future development of atherosclerosis, which is a primary cause of heart disease. In short, electrophoresis is an essential tool of modern medicine, widely used in such diverse areas as nutrition, cancer research, immunology, etc.

Of special relevance to this audience is the fact that development of the various electrophoretic techniques has been dominated by the need to circumvent - or utilize - the effects of gravity. In fluid media, gravity can cause random convection and mixing in presence of density instabilities, and density differences are

unavoidable in electrophoresis due to thermal and concentration gradients. Convection, of course, causes remixing of fractions separated by the electric field. To avoid this, gels or other supporting media are often utilized, and they completely circumvent the effects of gravity. In other techniques, artificially or naturally generated density gradients are utilized to stabilize the liquid system against smaller variations in density induced by the electrophoretic process. Finally, two electrophoretic techniques, forced-flow electrophoresis and electrodecantation utilize gravity-caused convection as part of the driving force inducing separation (7).

Gravity is thus not an unmitigated foe of electrophoresis, but under proper circumstances can be utilized in a constructive manner. The determining factor is the objective one seeks. For analytical or micropreparative work, i.e. fractionation and separation of products on a small, laboratory-scale operation, there is a profusion of excellent methods 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.

There are many potential applications for such instruments. Human blood is a unique national resource in short supply, to which a dollar value cannot be realistically applied (10). In the United States alone, about 9,000,000 donations are collected yearly, and about 2,000,000 liters of human plasma are fractionated into individual components such as clotting factors, serum albumin, and immunoglobulins (11). While electrophoresis is routinely used for quality control of the products obtained, these are actually prepared by rather archaic methods, including alcohol precipitation, and are obtained in poor yield and less than optimal purity. While the sheer bulk of total plasma would prevent its fractionation by electrophoresis at zero gravity, the situation is different for some of the minor components, such as clotting factors, desperately needed by hemophiliacs. A similar case might be made for other biologically active macromolecules, including a variety of enzymes, vaccines, nucleic acids, etc. As an example, in our laboratory we are currently engaged in collaborative efforts to electrophoretically purify two trace components of human serum: Somatomedin, a growth hormone, and the phagocytosis Recognition Factor, a potentially important anti-tumor agent.

## 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 (12). 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 (13), stable-flow electrophoresis (14), electromagnetophoresis (15), and rotationally stabilized instruments (16, 17), 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.

There are numerous areas of medicine and biology where cell separation would be desirable. Some data are already

available on peripheral blood cells, spermatozoa, bacteria, viruses, and cells derived from bone marrow, spleen, lymph nodes, kidneys and other organs (5,6,18). Major current interest is focused on lymphocytes, as these cells are directly involved in the immune mechanism and thereby control such diverse responses as resistance to cancer, rejection of transplanted kidneys and other organs, autoimmune diseases, lymphocyte neoplasias, etc.

Separation of T and B type lymphocytes has been reported in several species (19-21). Unlike other blood cells, which have a narrow spread of electrophoretic mobilities, lymphocytes are characterized by having wide mobility spectra. As yet we know little about changes in mobilities in various pathologic conditions, or the functional or immunological characteristics of subpopulations obtainable by electrophoresis, except for the difference in B and T markers.

There is ample evidence that such differences do indeed exist. I cite only a few. Wioland et al (22) have shown genetic influence on mouse lymphocyte mobilities. Zeiler and Hannig (23) have reported on specific differences in lymphoid populations derived from different organs, i.e. lymph nodes, spleen, lymph duct, bone marrow, and thymus. Droege et al (24) have reported on changes in cellular composition of thymus cells during early development of mice and the effect of hydrocortisone on the same.

A most interesting group of human diseases is characterized by abnormal production of specific and monoclonal immunoproteins, IgG, IgA, or IgM. Two such disease entities are recognized, multiple myeloma and Waldenström macroglobulinemia. These diseases have been particularly important for our understanding of immunology because of the monoclonal characteristics of the protein produced: all the abnormal immunoprotein seems to be derived from a single cell clone. They provide the most readily available source of immunologically homogenous proteins. The abnormality has been identified as a B type lymphocyte neoplasia (25), and Mellstedt et al (26) have demonstrated by immunological means the presence of a corresponding monoclonal lymphocyte population. No information is as yet available to show if this immunological differentiation results in a specific narrow band in the electrophoretic histogram, but such a finding would be highly significant for the diagnosis and possible treatment of this kind of cancer.

Cell electrophoresis has also been explored as a diagnostic tool for other forms of cancer (27). Bone marrow transplantation might be greatly aided by availability of pure cell lines (28). The same situation prevails in numerous areas of tissue culture, used in growth of various viruses and preparation of biologicals. As an example, electrophoretic separation of kidney cells is currently being investigated by Dr. Barlow of Abbott Scientific Laboratories for the isolation of the cells producing the enzyme urokinase. If suitable enrichment were to be achieved,

It may open the possibility of large scale production of this enzyme, which is of considerable potential importance for the intravenous dissolution of thrombi. Dr. Barlow's cells have been included in the recent NASA space experiment in electrophoresis (3), which will be discussed further on.

## SPACE INSTRUMENTATION

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 (1), though more ambitious in its aims. The essential part of the instrument was the electrophoresis column, reproduced schematically in Fig. 1. 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 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 (3). Time does not permit to discuss in greater details these 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. 2, 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 (13), 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. One of these is presented schematically in Fig. 3. It was conceived as a rapid continuous-flow instrument for the fractionation of concentrated solutions in a high intensity electric field. Miniaturization of the apparatus permits the dissipation of the electrically

generated heat by the thermal capacity of the fluid itself, thus decreasing the need for external cooling. Laminarity of flow within the instrument is obtained by the parallel arrays of feeder-spacers and knife edge separators. Such an apparatus is characterized by the presence of large density gradients caused by, both, electrically induced heating and high solute concentration, and its operation is conceivable only in absence of zero gravity (29).

These three diagrams illustrate prototypes of eventual space electrophoretic instruments. It is obvious that there is as yet no consensus as to what apparatus is best suited for space, and flexibility in planning is essential. It is probable that no single instrument will answer all the objectives of the facility, and that several instruments will be developed, reflecting the diversity of ground-based equipment.

## CONCLUSIONS

The term electrophoresis comprises a great variety of techniques, having in common the principle of molecular or particle separation on the basis of their electrical properties. The applications of the phenomenon have been manifold, and have been of importance in diverse areas of modern biology and medicine. While eminently successful in ground based work, electrophoresis is limited in quantities of samples it can separate, due to problems related directly or indirectly to gravity. Its limitations are most pronounced when applied to living cells, but it is just this application which is currently of greatest interest. Biologists have only begun to look into functions of specific cells, and have recognized the importance of cell subpopulations in many diverse areas.

A space facility for electrophoresis will overcome the limitations imposed by terrestrial gravity, but will not overcome all the 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.

We have to consider therefore the space electrophoresis facility as primarily a unique research tool, which eventually may evolve into a processing plant. Such a facility may conceivably acquire significant economic potential, but it will certainly have an impact on our understanding and applications of cell biology. Its main importance will be in extending the scope of electrophoresis to living cells, but separation of proteins and other biologically active macromolecules should not be neglected.

In all separation processes there is always a trade-off between resolution and throughput for basic immunology, for instance, highest resolution is essential, while for clinical or industrial usage, some resolution may have to be sacrificed to obtain the needed throughput. This is particularly important for proteins, where the space facility would be justified only if it would result in at least an order of magnitude greater throughput than achievable on earth. Continued ground based research is needed not only on instrument design, but also on basics in electrophoresis. Some of the novel principles may have to be tested in a zero gravity environment, and until the Shuttle becomes available, unmanned rockets will be the only available vehicle. These provide about 5 minutes of near-zero gravity, insufficient for any full-scale operation, but adequate for testing of many parameters. Ground based research should also pinpoint the most promising applications for the early Shuttle flights, and should evaluate their relative scientific, medical and economic benefits. Detailed cost analysis of space processing should be provided, and compared with possible alternate ground-based techniques.

Space electrophoresis is only one example of possible benefits of space research in the broad area of life sciences. Two biosatellites have already been flown by NASA, and several more by USSR. These have only scratched the surface of potential applications of outer space. From the broadest point of view, man has learned to modify his environment to suit particular scientific and technological requirements, and has found numerous uses for the extremes of temperature and pressure. Many thousandfold increase of gravity is readily achievable by centrifugation. Thus, it is hardly conceivable that the converse condition, the elimination of gravity, would not find its own scientific and technological applications.

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