FINAL REPORT

on

DEFINITION OF PERFORMANCE SPECIFICATIONS FOR AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY (AAEF)

April 7, 1975 to February 29, 1976

Contract No. NAS8-31386

by D.E. Brooks

Prepared for:

National Aeronautics and Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, Alabama 35812

By:

Department of Neurology
University of Oregon Health Sciences Center
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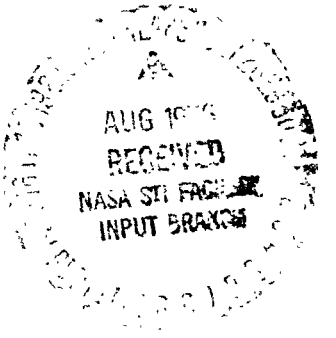

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1 SUMMARY

1.1 Background

As part of its Space Processing Program, NASA has expended considerable effort in developing preparative cell electrophoresis in zero g. It is to be used as a method of separating biological cells into subpopulations, the subsequent recovery of which will have significant benefit in biomedical applications. As the preparative electrophoresis program gained momentum and flight hardware came closer to being made available to a broadly-based medical and biological user community the need became apparent for an accurate and rapid method of assessing the possibility of separating candidate subpopulations on the basis of their electrokinetic properties. It was clear that accurate analytical data on the electrophoretic mobility distribution of a sample had to be available before a decision as to the feasibility of a separation could be made. Such data would be vital as well for determining which mode of preparative electrophoresis should be applied to a particular problem, and under what operating conditions.

At the time this contract was let the standard method for obtaining analytical electrophoretic data on cell populations was through the use of the microelectrophoresis method (1). The technique consists of visually observing the motion of individual cells through a microscope focussed at the stationary layer of the

sample chamber and timing their motion across a calibrated eye-piece graticule with a hand-actuated stopwatch. Knowing the interval over which each cell migrates in the measured time the cell velocity, and hence electrophoretic mobility (velocity per unit electric field strength applied) can be calculated. Individual cell mobilities are then accumulated from the same sample population and the data displayed as a histogram of cell number as a function of mobility.

The microelectrophoresis method suffers from three principle disadvantages as current employed:

- It is slow; under the best of conditions only 20 to 30 measurements can be made before the sample must be replaced and either the original sampled re-measured - with danger of subsequent cell damage due to multiple pipetting - or a new sample must be introduced, necessitating large numbers of cells being made available. Each filling takes $\sim$ 15 minutes, so only 80 to 120 points can be accumulated per hour, and this rate is seldom realized in practice for a variety of technical reasons.

- It is of limited accuracy. Timing displacements by eye introduces errors due to human reflexes, judgement of sharpness of focus and sometimes bias in cell selection

that limit reproducibility to approximately ± 3%.

- It is extremely wearing on those making the measurements. Eye strain is frequently severe and virtually nobody can work at the rate given above for a full day.

In a program of the magnitude and length of that being considered by NASA it seemed clear that a large number of analytical determinations on complex cell populations would be required and that manual mobility distribution determinations would therefore not be suitable. Furthermore, it seemed that with state-of-the-art technology it was feasible to automate analytical electrophoresis by any of several techniques in such a way as to alleviate all of the problems associated with the manual technique. In order to determine just what operating specifications should be required of the automatic apparatus, and to determine what technical approach might best be followed to meet such specifications the present contract was awarded.

1.2 Objectives

The present project has the following aims:

- To provide performance specifications for an Automated Analytical Electrophoresis Facility (AAEF);

- To identify which of the currently recognized techniques

for automating analytical electrophoresis may best be expected to satisfy these specifications;

- To provide a priority rating for the performance specifications.

1.3 Approach

In order to provide specifications for the AAEF that would satisfy the broadest variety of demands of a future user community, a survey was carried out of all those people who were identified as having published papers on cell electrophoresis in the past four years. These researchers were identified from surveys carried out to our specifications by the Institute for Scientific Information, Philadelphia. The Institute carried out a computer search of the relevant literature from which a list of 87 investigators was derived and defined as the user community for purposes of the mailing. A questionnaire was developed covering the areas of performance which required definition which was subsequently circulated to the user community. A copy of the questionnaire and the list of scientists to whom it was mailed is included as Appendix 1 to this report. Based on the response to this survey and on the personal experience of the PI as well as that of others in the Department of Neurology at the University of Oregon Health Sciences Center, the performance specifications given below were assembled.

The recommendation regarding which technique was felt to be most appropriate for the AAEF was based on an examination of the possible approaches to the problem, and on discussions with a series of experts in areas with possible application to the AAEF. The approaches, and the experts consulted, were as follows:

Technique	Investigator
Laser doppler spectroscopy	Dr. B.R. Ware Department of Chemistry Harvard University Cambridge, Mass. 02138
Laser doppler spectroscopy	Dr. E.E. Uzgiris General Electric Research and Development Center Schenectady, N.Y. 12301
Optical transduction of image of moving particle field	Mr. Phil Goetz Pen Kem Company P.O. Box 364 211 Cleveland Dr. Croton-on-Hudson, N.Y. 10520
Computer analysis of video image of moving particles	Dr. Peter H. Bartels Optical Sciences Center University of Arizona Tucson, Arizona 85721
Particle field holography	Dr. J.D. Trolinger Spectron Development Laboratory 3303 Harbor Blvd. Costa Mesa, Ca. 92626

As discussed in the Detailed Technical Section, there were good reasons for eliminating all but one of the above methods, resulting in the recommendation made below.

The priority rating for the performance specifications were derived, like the specifications themselves, from a consideration both of the user community survey results and of the PI's personal experience. In fact, it turns out that the recommended approach to implementing the AAEF is anticipated to be capable of satisfying virtually all of the performance specifications, and should provide additional capabilities as well so the priority rating will probably be of little utility.

1.4 Results

1.4.1 Performance Specifications for the AAEF

It is felt the AAEF should be capable of performing to the following specifications:

- The mobilities of approximately 500 cells should be obtained in 10 minutes from a sample of 10^5 cells or less. Separate mobility distributions should be able to be collected at a rate of 4 per hour.

- Excluding problems associated with cell sedimentation the AAEF should be capable of collecting mobility data on non-pigmented cells in the size range of 0.5 to 100 microns in diameter.

- Each individual mobility determination should be accurate to ± 0.3% over a mobility range of 0.2×10^{-4} cm^2 s^{-1} v^{-1}

to 8.0×10^{-4} cm^2 s^{-1} v^{-1}.

- The AAEF must be capable of operating with suspending media of specific conductivity <0.021 Ω^{-1} cm^{-1} over a temperature range of 4°C to 37°C and over a pH range of 2 to 11.

- The chamber and electrodes should be compatible with normal biological support media (containing proteins, carbohydrates, multivalent ions, etc.) in their operating configuration. Measurement conditions and chamber materials should be such that cells undergoing measurement retain the same viability and surface properties as an appropriate control suspension not exposed to the instrument.

- The applied electric field may be either DC, or AC to take advantage of the suppression of electroosmotic flow in the electrophoresis chamber, provided the applied field frequency is less than approximately 1.0 kHz.

- The AAEF should incorporate the capability to recognize and record other individual cell parameters besides electrophoretic mobility, as seem appropriate for the population under examination. In particular, the ability to distinquish between cells with and without a fluorescent label should be included in the AAEF.

- As well as providing hard copies of electrophoretic mobility distributions, the AAEF should include the capability for detailed statistical analysis of the mobility data. These programs should provide:

    - descriptive statistics for the data.

    - assessment of the unimodality of the mobility distribution via the computation of distribution-free statistics for goodness of fit to known mobility distributions obtained from calibration populations.

    - special locally most powerful test statistics to detect very small subpopulations of known mean and standard deviation, with errors of the first and second kind controlled to within preset limits.

1.4.2 Recommended Technique for Developing AAEF

It is recommended that the AAEF be based on computer analysis of a video image produced by a television camera linked to the microscope of a microelectrophoresis apparatus. The principle of this approach is described in detail in an unsolicited contract proposal from the Optical Sciences Center of the University of Arizona to Marshall Space Flight Center dated April 22, 1975. The approach

described in that submission should in general be followed except that:

- The electrophoresis chamber must be very well thermostatted to allow the potential accuracy of the technique to be realized. This will probably necessitate a change in the optics since the long working distance phase contrast objective originally suggested will no longer be appropriate.

- An alternating electric field should be used to drive electrophoretic migration since in this way a much greater depth of field may be utilized in the optics. As discussed in Section 2.1.6 use of alternating fields at frequencies $\geq$ 100 Hz suppresses electroosmotic flow over greater than 50% of the chamber cross-section resulting in a constant, near-zero flow velocity. If mobilities are determined anywhere in this region their values will be independent of position and their absolute values easily obtained. Provision should also be made for stepping the focal plane through a series of positions so that speed of measurement will not be limited by the time necessary for sedimentation of a new cell population into the microscopic field.

1.4.3 Priority Rating of Specifications

If it is necessary to compromise on any of the performance specifications the following list should be used as a guide in the choice of trade-offs or capability limitations. The characteristics required of the AAEF, in decreasing order of importance, may be grouped as follows; characteristics within a given group are considered to be of roughly equal importance.

Group I

- Determine individual mobilities of $\sim$ 500 cells of diameter 2 to 25 microns within 30 minutes from a sample of 10^7 cells or less.

- Determine individual mobilities to an accuracy of $\pm$ 1.0% over a mobility range of 0.5×10^{-4} to 3.0×10^{-4} $cm^2\ s^{-1}\ v^{-1}$.

- Operate at 25°C with suspending media of specific conductivity < $0.02\ \Omega^{-1}\ cm^{-1}$ over a pH range of 6.5 to 7.5.

- Use applied electric fields of frequency < 1000 Hz.

- Chamber and electrodes must be compatible with normal biological support media (containing proteins, carbohydrates, multivalent ions, etc.) in their operating configuration. Measurement conditions and chamber

materials shall be such that cells undergoing measurement retain the same viability and surface properties as an appropriate control suspension not exposed to the instrument.

- Provide hard copy of mobility distribution plus mean and standard deviation of total population.

Group II

- Determine individual mobilities of $\sim$ 500 cells within 10 minutes at a rate of four total determinations per hour.

- Provide a statistical assessment of the unimodality of the mobility distribution via the computation of distribution-free statistics for goodness of fit to known mobility distributions obtained from calibration populations.

Group III

- Determine mobilities on a total sample size of 10^5 cells.

- Excluding problems of sedimentation the apparatus should be capable of collecting mobility data on cells in the size range 0.5 to 100 microns in diameter.

- Determine individual mobilities to an accuracy of ± 0.3% over a mobility range of 0.5×10^{-4} to 3.0×10^{-4} cm^2 s^{-1} v^{-1}.

- Operate over a temperature range of 4°C to 37°C and a pH range of 2 to 11.

- Incorporate the capability of recognizing and recording other individual cell parameters besides electrophoretic mobility, as seems appropriate for the population under examination. In particular, provide the ability to distinguish between cells with and without a fluorescent label.

1.4.4 Comments on User Community Survey

Of the 87 surveys mailed out to the electrophoresis user community 31 replies were received, many from well known investigators in the electrophoresis field. The vast majority of respondents commented favorably on the concept of the AAEF (see Appendix 1) and many asked to be kept aware of its development. A number of different uses for the apparatus were suggested, many of which were clinical in nature. These included:

Medical research uses; suggests connecting AAEF to an automatic cell-separation device	Chris J. van Boxtel, M.D. Visiting Scientist Department of Pharmacology Vanderbilt University School of Medicine Nashville, Tennessee

Lympho yte electrophoresis	Dr. Chollet Centre Jean Perrin place Henri Dunant B.P. 392 63011 Clermont Ferrand Cedex France
Study large monocytes, trophoblasts in zero g	Dr. S.N.S. Hanjan Dept. of Biochemistry All India Institute of Medical Sciences New Delhi - 16, India
Work with cells from immuno-deficient babies, spleen cells and lymphoid cells	Dr. Johan N. Willig 5016 Haukeland Hospital Norway
Analysis of different fractions of platelets obtained by other means, separated according to mobility, and in fairly small amounts (~ 100 platelets/ fraction)	Dr. Donna J. Carty Dept. of Biochemistry Jordan MEB Univ. of Virginia Charlottesville, Va. 22901
Study of the mobility of cells as functions of ionic strength at a given pH to gain understanding of conformational change of the biopolymers at interfaces; microelectrophoretic study of adsorbed proteins and nucleic acids for genetic features of the adsorbed biopolymers	Dr. D.K. Chattoraj Dept. of Food Technology and Bio-chemical Engineering Jadavpur University Calcutta - 700032, India
AAEF would be useful in studying subcellular particles, as well as in developing an early detection of membrane systems alterations which could possibly act as pathogenetic factors	Dr. Gabriela Dinescu-Romalu Institutue Oncologie Dept. Immunology and Biochemistry of Cancer B-dul 1 Mai Nr. 11 POB 5916 Bucuresti 12, Romania
Electrophoretic fractionation of aseptic cells for subsequent cell culture to detect colony forming ability	Dr. Chikako Sato Dept. of Experimental Radiology Aichi Cancer Center Res. Inst. Chikusa-ku Nagoya, Japan

Electrokinetic characterization of lymphocyte subpopulations; documentation of electrophoretic behavior of blood platelets on exposure to a variety of pharmaceutical agents; kinetic studies of cellular growth rates, cell cycle changes and regeneration of modified cell surfaces of cultured or isolated biological cells by electrophoretic means	Dr. G.V.F. Seaman Dept. of Neurology Univ. of Oregon Health Sciences Center Portland, Ore. 97201
Use of AAEF in developing an accurate test for malignant disease	Dr. Alan W. Preece Principal Physicist Radiotherapy Centre Horfield Road Bristol, BS28ED, U.K.
Use of AAEF in platelet studies	Dr. J.R. Hampton Dept. of Medicine General Hospital Nottingham, England
Use of a high precision instrument in determination of E.M. in the area of cell membrane immunogenetics and immunochemistry and many other studies	Dr. P. Rubinstein New York Blood Center 310 E. 67th St. New York, N.Y. 10021
Detection of small electrophoretic subpopulations	Dr. Paul Todd 618 Life Sciences Bldg. University Park, Pa. 16802
Use of AAEF in cancer diagnosis emphasized by the MEM test (BMJ 2, 613 (1971)	Dr. Alan S. Coates Walter and Eliza Hau Instit. c/o P.O. Royal Melbourne Hospital Victoria 3050, Australia
Use of AAEF in detecting cancer in connection with the MEM test	Dr. John Moore Velendre Hospital Whitchurch Cardiff, U.K.

Measurement of mobilities of micelles of surface active agents, overcoming difficulty in observation due to refractive index close to that of water	Dr. J.B. Kayes Pharmaceutics Res. Group Pharmacy Dept. Univ. of Aston Bosta Green Birmingham B4 7ET, U.K.
Use of AAEF in study of endothelial cells, blood platelets, erythrocytes and kidney cells	Dr. Reginald G. Mason Dept. of Pathology The Memorial Hospital Pawtucket, R.I. 02860
Use of AAEF with mixed populations of cells and cells used for cell culture	Dr. Richard M. Fike Dr. Carel J. Van Oss Dept. of Microbiology School of Medicine State Univ. of New York Buffalo, N.Y. 14214
Study of platelets, especially in presence of ADP or noradrenaline and other new compounds	Mr. E.G. Tomich Glaxo Research Ltd. Sefton Park Stoke Poges Buckinghamshire SL2 4DZ England

It would seem that the above investigators might usefully be contacted when the scientific community is invited to participate in the more advanced NASA electrophoresis flight opportunities.

2 DETAILED TECHNICAL SECTION

2.1 Performance Specifications for the AAEF

The specifications given in Section 1.4.1 are discussed in detail below with the rationale for each.

2.1.1 Sample Size and Rapidity of Measurement

The mobilities of approximately 500 cells should be obtained in 10 minutes from a sample of 10^5 cells or less. Separate mobility distributions should be able to be collected at a rate of 4 per hour.

These requirements satisfied the majority of the survey respondents. At least five hundred data points are generally required if curve-fitting is to be carried out statistically via computer (2), although 1,000 would be preferable. Also, it has been our experience that several hundred points are necessary to provide a reliable histogram even at low resolution for some cell populations, such as peripheral lymphocytes.

Some typical data on the number of timings possible using a cylindrical chamber microelectrophoresis apparatus are given in Table 2.1.1.1 for formaldehyde (CH_2O) fixed erythrocytes and

Concentration of cells (No. cells/cc)	No. timings taken before complete sedimentation (1 person with stopwatch)	Time for whole population to sediment (minutes)	No. cells in focus in central 10x10 graticules (∿150μ x 150μ) Start	After 5 min	Comment
CH_2O FIXED RBC					
1.2×10^6	5	9	0	1	Too few cells
2.0×10^6	8	-	1	0	Too few cells
2.9×10^6	14	10	3	2	Too few cells
3.0×10^6	13	-	1	1	Too few cells
5.8×10^6	19	11	5	4	OK
1.1×10^7	28	-	-	-	OK
1.5×10^7	26	-	-	-	OK
2.3×10^7	31	-	6	-	OK
3.1×10^7	33	-	3	-	Too opaque
3.8×10^7	-	-	5	-	Too opaque
4.6×10^7	-	-	4	-	Too opaque
5.8×10^7	-	13	0	3	Too opaque
LYMPHOCYTES					
1.0×10^6	10	8	1	1	
2.0×10^6	14	8	1	2	
5.0×10^6	12	7	3	1	
1.0×10^7	Av 20 (26-14)	8	3	2	

Table 2.1.1.1. Data on timing characteristics, number of cells in focus and visibility for fixed human red cells and human peripheral lymphocytes.

human peripheral lymphocytes in 0.154 M NaCl buffered to pH 7.4 with $NaHCO_3$.

It takes approximately 10 minutes for one sample to sediment out of the field of view. On the average approximately 5-6 cells are in focus at any one time at cell concentrations (6×10^6 to 2×10^7 cells/ml) for which the optical quality is high but at which enough cells are present to allow a measurement to be made. If a velocity measurement were to be made every time a t.v. line was scanned the measurement rate would be $\sim$ 15 kHz. In 10 min = 600 s about 100 fields would have to be scanned to measure 500 mobilities, and if 1 s is allowed for transfer from field to field via a stepping motor, then $(\frac{600\ s}{100} - 1) = 5$ s would be the measurement time per optical field. In this time $5 \times 1.5 \times 10^4 = 7.5 \times 10^4$ velocity estimates per cell would be made. Even if at this time resolution electrophoretic migration proceeds with a stochastic component the accuracy of the measurement would increase as $N^{1/2}$ for N estimates. Hence, the relative accuracy of the determination would be $N^{1/2}/N = N^{-1/2} = (7.5 \times 10^4)^{-1/2} \simeq 3 \times 10^{-3}$. From this calculation it would seem feasible to increase the sample size to 1000 cells without much loss in accuracy, but much depends on how long is required to change optical fields. The given figures seem a good compromise, therefore.

The presently used small volume chambers contain ~ 1 ml of suspension, and the table shows that at least 6 x 10^6 cells would be required to provide a sufficient cell density for measurement. It is possible, however, to inject the cell suspension via catheter tubing only in the region of the optical field and reduce the number of cells required by two orders of magnitude. Hence, it would appear feasible to achieve the sample size specification of 10^5 cells. Allowing 5 minutes for chamber rinsing and sample replacement the indicated rate of 4 samples/hr should be achieved.

2.1.2 Cell Size Requirements

Excluding problems associated with cell sedimentation the AAEF should be capable of collecting mobility data on non-pigmented cells in the size range of 0.5 to 100 microns in diameter.

This size range easily covers all conceivable mammalian cells which might be of interest. Two respondents to the survey specified the upper limit, but many cells of clinical interest would fall in the range 2 to 25 microns diameter.

2.1.3 Mobility Accuracy and Range

Each individual mobility determination should be accurate to ± 0.3% over a mobility range of 0.2 x 10^{-4} to 8.0 x 10^{-4} cm^2 s^{-1} v^{-1}.

The considerations discussed in Section 2.1.1 partially defined the ± 0.3% accuracy specification as being the best that could be expected for the number of cells specified. Since nothing is known about cell subpopulation distributions at this level of resolution, probably the more accurate the better. On the other hand an accuracy less favorable than ± 1% might be worse than some preparative electrophoresis techniques and the AAEF accuracy should be better than those to fulfill its purpose.

The mobility range specified covers all known native cell mobilities at physiological pH and allows for mobility determinations after a variety of chemical or enzymatic treatments as well.

2.1.4 Specific Conductivity, Temperature and pH Range

The AAEF must be capable of operating with suspending media of specific conductivity < 0.021 Ω^{-1} cm^{-1} over a temperature range of 4°C to 37°C and over a pH range of 2 to 11.

It is essential that the AAEF be able to operate with suspending media of physiological ionic strength (which determined the conductivity upper limit) since only under such conditions would an unknown cell population be reasonably sure to provide a mobility distribution which reflects properties of the native cell surface. In lower ionic strength media leakage of cell contents followed

by adsorption to the cell surface frequently occurs, leading to spurious electrokinetic characteristics (3). It is essential to have physiological ionic strength baseline data for cell populations whose surface charge properties haven't been completely explored.

The operating temperature and pH ranges included all those requested by respondents to the survey. They represent the usual limits for such parameters when viable cells are employed.

2.1.5 Chamber and Electrode Material

The chamber and electrodes should be compatible with normal biological support media (containing proteins, carbohydrates, multivalent ions, etc.) in their operating configuration. Measurement conditions and chamber materials should be such that cells undergoing measurement retain the same viability and surface properties as an appropriate control suspension not exposed to the instrument.

This requirement is self evident. Some cell types such as platelets and macrophages adhere to glass, the usual material of choice. However, this adherence need not interfere with the mobility determination unless any cell products leaked by adherent cells interfere with the cells being measured. This can in principle be a problem with platelets, but macrophages have

not been shown to be a problem in this sense.

2.1.6 Applied Field Specification

The applied electric field may be either DC, or AC to take advantage of the suppression of electroosmotic flow in the electrophoresis chamber, provided the applied field frequency is less than approximately 1.0 kHz.

It has been shown by Vorob'eva, Vlodavets and Dukhim (4) that the electroosmotic flow profile in a chamber with rectangular cross-section is significantly blunted if an alternating electric field is applied. This blunting provides a core region in the center of the chamber over which mobility determinations should be able to be made independent of position, provided only that the magnitude of the core velocity be known. The blunting is independent of the magnitude of the electroosmotic velocity at the chamber wall, and hence is independent of the chamber wall surface charge characteristics.

The interest in this phenomenon from the point of view of the AAEF is clear if the discussion in Section 2.1.1 is considered. In order to make accurate electrophoretic mobility measurements at more than one radial location in the chamber the background fluid flow should be constant throughout the region of interest.

With a DC appli d field this can only occur if either no electro-osmotic flow is present, implying a zero wall charge, or if plug flow occurs induced by the presence of porous plugs bearing the same capillary surface charge as the chamber walls (5). Both these procedures involve wall coating techniques which are not yet well worked out nd which are likely to be time-dependent. Using an AC field to flatten and reduce the core flow appears to be an excellent solution to the problem provided sufficiently rapid velocity measuring techniques are available. Techniques which rely on displacement and time measurements to calculate mobilities cannot easily utilize this innovation because of the small displacement amplitudes involved at the frequencies required.

Although an analytical solution for a flat chamber exists in the literature (4), no calculations have been made for AC electro-osmosis in cylindrical chambers. We therefore undertook an experimental and theoretical study of this problem, the results of which are described below.

2.1.6.1 Theoretical Investigation of Relaxation Effects

We consider the flow in a long closed cylindrical chamber of circular cross section, radius a, filled with a fluid of viscosity η and density ρ. Next to the chamber wall at the radial coordinate $r = a$ the fluid velocity due to electroosmosis is $v_o e^{i\omega t}$ due to

the application of a sinusoidally varying electric field. We assume we are far enough from the ends of the chamber that the flow is purely axial. The Reynolds Number is assumed such that the creeping flow equations apply. Since the chamber is closed a pressure gradient dP/dz exists in the axial direction (results for an open chamber are found by setting dP/dz = 0). We seek an expression for the time-dependent velocity profile v(r,t).

For axial flow, the Navier-Stokes equation in cylindrical coordinates is:

$$\rho \frac{\partial v}{\partial t} - \eta\left(\frac{1}{r}\frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial r^2}\right) + \frac{dP}{dz} = 0$$

or, setting $\nu = \eta/\rho$

and $K = \frac{1}{\rho}\frac{dP}{dz}$

$$\frac{\partial v}{\partial t} - \nu\left(\frac{1}{r}\frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial r^2}\right) + K = 0$$

Let $v(r,t) = u(r)e^{i\omega t}$, $u(r)$ complex, and write $K = K_o e^{i\omega t}$, K_o = complex constant. Using the fact that the flow at $r = 0$ must be finite, and the bounding conditions:

1) $u(a) = u_o$ = real

2) $\int_o^a u(r)\cdot 2\pi r\cdot dr = 0$ in a closed system, it can be shown that the following solution for u(r) applies:

$$u(r) = \frac{u_o}{\frac{\alpha a p}{4} - r_1}\left[\left\{\left(\left(\frac{\alpha a}{4}\,\mathrm{ber}\alpha a - \frac{1}{2}\,\mathrm{bei}'\alpha a\right)\mathrm{ber}\alpha r\right.\right.\right.$$

$$\left. + \left(\frac{\alpha a}{4}\,\mathrm{bei}\alpha a + \frac{1}{2}\,\mathrm{ber}'\alpha a\right)\mathrm{bei}\alpha r - r_1 + \frac{q}{2}\right\}$$

$$+ i\left\{\left(\left(\frac{\alpha a}{4}\,\mathrm{ber}\alpha a - \frac{1}{2}\,\mathrm{bei}'\alpha a\right)\mathrm{bei}\alpha r\right.\right.$$

$$\left.\left.\left. - \left(\frac{\alpha a}{4}\,\mathrm{bei}\alpha a + \frac{1}{2}\,\mathrm{ber}'\alpha a\right)\mathrm{ber}\alpha r + \frac{r_o}{2}\right\}\right]$$

where: berx, beix are zeroth order real and imaginary Kelvin functions of x (6)

ber'x, bei'x are their first derivatives ω.r.t.x.

$\alpha = (\omega/\nu)^{1/2}$

ω = radian frequency of applied field

$p = \mathrm{ber}^2\alpha a + \mathrm{bei}^2\alpha a$

$q = \mathrm{ber}\alpha a \cdot \mathrm{bei}'\alpha a - \mathrm{ber}'\alpha a \cdot \mathrm{bei}\alpha a$

$r_o = \mathrm{ber}\alpha a \cdot \mathrm{ber}'\alpha a + \mathrm{bei}\alpha a \cdot \mathrm{bei}'\alpha a$

$r_1 = q - p_1/\alpha a$

$p_1 = \mathrm{ber}^2\alpha a + \mathrm{bei}^2\alpha a$

Some typical plots of the fluid velocity amplitude at a point r, divided by its value at the wall, are given for a 2 mm diameter tube, both open and closed, in Figures 2.1.6.1.1 and 2.1.6.1.2 according to this expression. It is clear that in the cylindrical

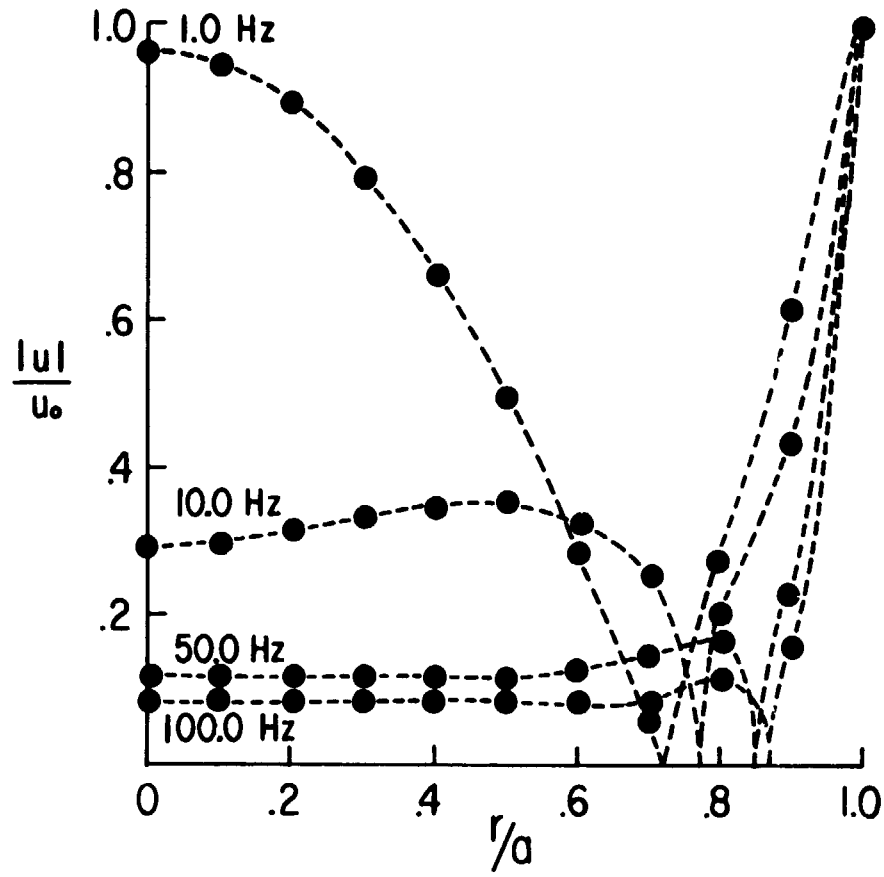

Figure 2.1.6.1.1. Amplitude profile of flow velocity in a closed cylindrical chamber induced by electroosmosis in alternating electric fields of the frequencies indicated; velocity amplitude expressed relative to amplitude of wall velocity u_0; chamber radius = 0.1 cm.

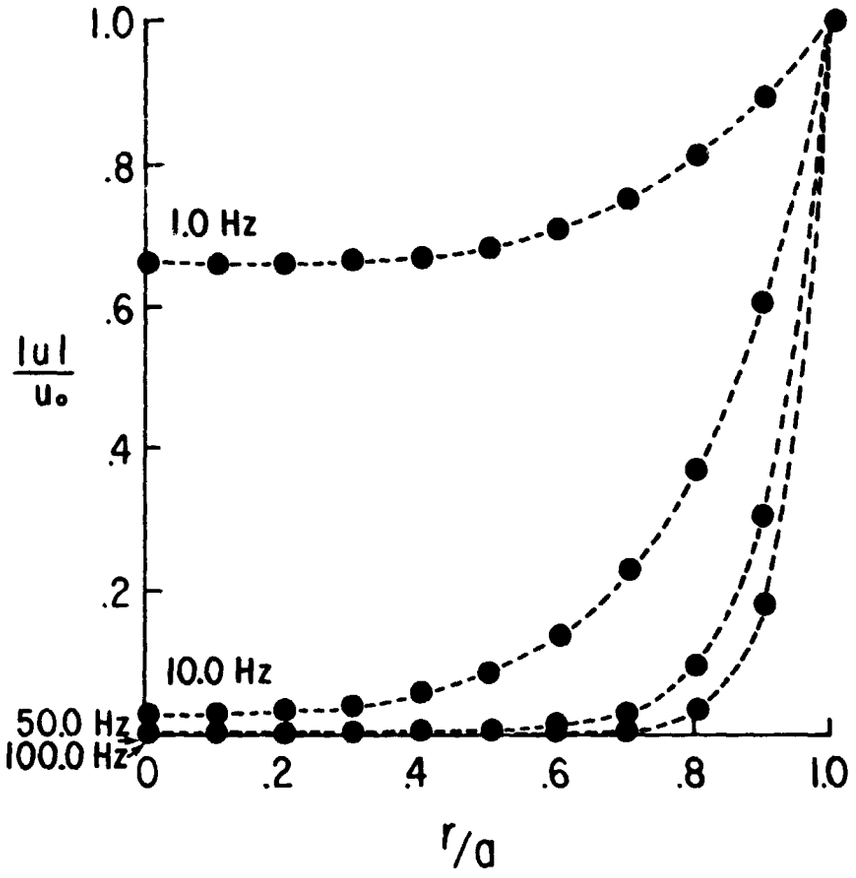

Figure 2.1.6.1.2. Amplitude profile of flow velocity in an open cylindrical chamber induced by electroosmosis in alternating electric fields of the frequencies indicated; velocity amplitude expressed relative to amplitude of wall velocity u_0; chamber radius = 0.1 cm.

geometry as well there is considerable blunting of the flow profile accompanied by a decrease in amplitude as the frequence is increased. The effect occurs at lower frequencies in the open than in the closed chamber. Figure 2.1.6.1.3 shows that the region over which position-independent flow occurs occupies greater than 90% of the cross-sectional area in a closed chamber at frequencies above 100 Hz. It should therefore be possible to make cell electrophoretic mobility determinations over a considerable depth of the chamber without variation in the electroosmotic component of the observed velocity, providing the cell mobility values themselves are not affected by fields of the frequency employed.

An estimate of the upper frequency limit beyond which AC fields should not be employed for accurate results may be obtained from a consideration of the relaxation behavior of a particle undergoing electrophoresis. For small particles in low ionic strength medium where the ionic double layer is expanded, the double layer itself becomes significantly polarized in the applied electric field. This polarization acts to retard the particle motion. The relaxation behavior of the retardation effect has been studied in some detail (7,8), and the relaxation frequency found to be very low for particles of the size of cells (5-50 Hz). Fortunately, however, the conditions under which electrophoretic retardation has any effect on cell mobilities are such that no retardation

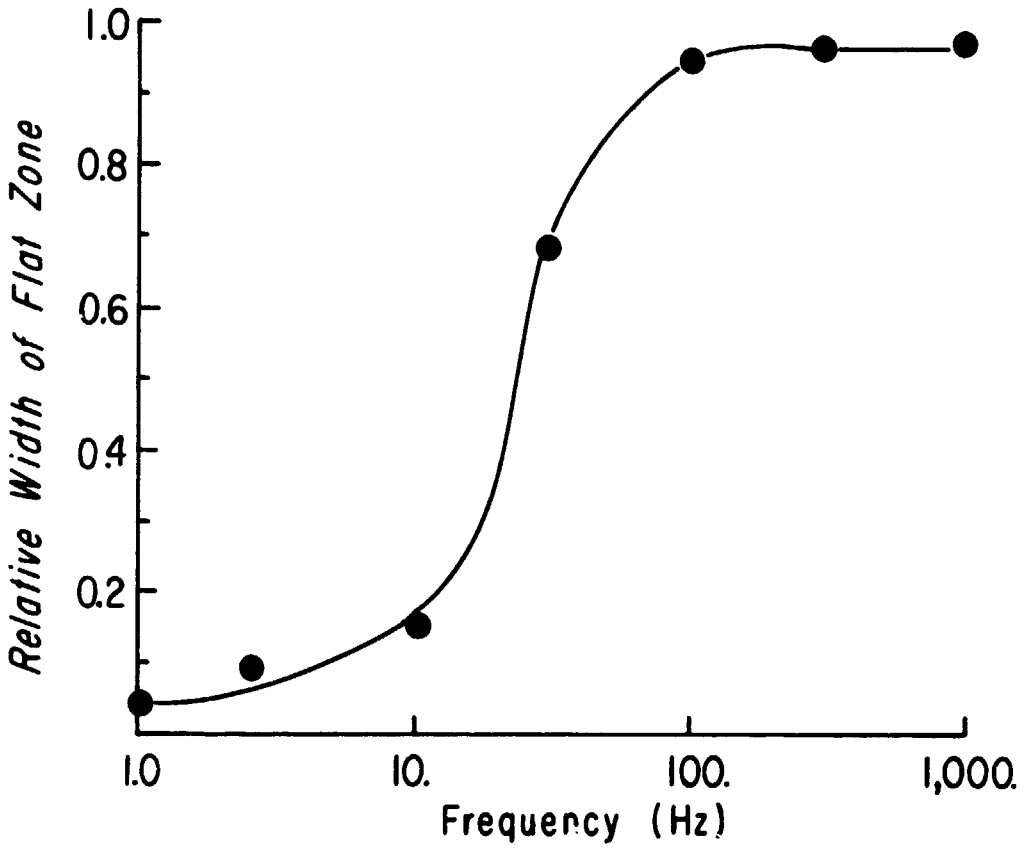

Figure 2.1.6.1.3. Relative width of core region over which flow velocity amplitude is independent of position in a closed cylindrical chamber of radius 0.1 cm.

occurs under the usual biological operating conditions. Retardation effects are only felt if

$$\kappa a > 300$$

where κ is the inverse of the double layer thickness, given by:

$$\kappa^2 = 8\pi N_A e^2 I/1000\ \epsilon kT$$

where N_A = Avogadro's number

e = electron charge

ϵ = medium dielectric constant

kT = Boltzmann's constant x absolute temperature

I = ionic strength $\equiv \frac{1}{2} \sum_i c_i z_i^2$

z_i = valence of ionic species i

c_i = molar concentration of ionic species i

Even in a lower ionic strength medium of $c = 10^{-3}$ M so long as $a > 2.5$ microns no retardation will occur, and hence relaxation of retardation will have no effect on cell mobility.

The other relaxation behavior which requires investigation is the frequency dependence of the relaxation due to cell inertia, that is, the time it takes for a cell to reach terminal velocity when an electric field is applied. The relaxation time may be obtained from a straight forward analysis of the second order differential force balance equation using Henry's (9) expressions for the

forces acting on a particle undergoing electrophoresis.

The differential equation is:

$$m\ddot{x} + 6\pi\eta a\dot{x} - \frac{3\varepsilon aE\zeta}{2} = 0$$

which leads to:

$$v(t) = v_o(1 - e^{-t/\tau})$$

where $\tau = \frac{2}{9}\frac{a^2}{\eta}\rho_c$ is the characteristic relaxation time for a spherical cell. Here:

E = magnitude of electric field applied as a step function at time t = 0

ζ = particle zeta potential

x = spatial coordinate; number of dots indicate first or second derivative

$v(t), v_o$ = time-dependent and steady state velocity respectively

m = mass of cell = $\frac{4}{3}\pi a^3\rho_c$

ρ_c = cell density

The relaxation frequency $\nu_r = \tau^{-1}$ for a cell of 5μ radius is therefore 1.5×10^5 Hz, and the cell velocity will be within 0.3% of v_o after a time $\tau' = 38$ μs, implying frequencies < 26 kHz would be acceptable. A frequency of 1.0 kHz would therefore be satisfactory for cells up to 50μ diameter, although the largest cells suggested by survey respondents (100μ diameter) would be

limited to measurement frequencies of $\lesssim$ 250 Hz. Since these very large cells probably could not be measured at one g, the 1 kHz upper limit was considered reasonable as a frequency specification.

2.1.6.2 Experimental Investigation of AC Electroosmosis

The only techniques available to us to test the theory presented in the previous section without major expenditure were displacement amplitude measurements of test particles undergoing electrophoresis. We were therefore limited in frequency to $\nu \lesssim 10$ Hz because in spite of maximizing the field strength, particle mobility and optical magnification the displacements were too small to measure visually at higher frequencies.

The test particles used were polyvinyl toluene (PVT) latex spheres of 2.02μ diameter suspended in 5×10^{-4} M $NaHCO_3$ plus 0.02% sodium dodecyl sulphate (SDS). The SDS was added to produce a high uniform surface charge by equilibrium adsorption that would be time independent and that would maximize particle displacements due to the sum of electroosmotic flow and electrophoretic motion. A high field chamber was constructed for the experiments with a short 2 mm i.d. capillary section (3.5 cm compared to the usual 10 cm) to maximize the field strength obtainable for a given applied voltage. The AC power supply was comprised of a modified Exact Model 251 Function Generator and an Exact Model 170

Amplifier which combination delivered a reasonably faithful sin and square wave of 80 v pk-pk at up to 0.5a. Field strength of 9.7 v cm^{-1} were applied via platinized-Pt electrodes at frequencies between 0 and 10 Hz to suspensions of the PVT spheres in $NaHCO_3$/SDS. Particle oscillations were recorded on a SONY AV 3600 video tape recorder hrough the microelectrophoresis apparatus microscope at ∿ X1000 optical magnification. Oscillation amplitudes were measured by a frame-by-frame analysis of the recordings using a calibrated grid on the screen of a high resolution Concord VM12 video monitor. Oscillations were recorded throughout one half of the depth of the capillary chamber, the position with respect to the wall being given by a dial gauge indicator gauged to the focussing adjustment of the microscope.

The PVT/SDS system had a narrow, reproducible mobility distribution with mean mobility (DC) measured at the stationary level of $-7.64 \pm 0.34\ \mu\ s^{-1}\ v^{-1}$ cm. Extrapolation of the DC parabola of apparent mobility as a function of distance from the wall gave the ratio of wall velocity to particle velocity f = 1.06.

The theory of Section 2.1.6.1 was utilized to calculate the theoretical particle displacement amplitude, D, by the following expression:

$$D = \frac{v_p}{\omega} [f^2A^2 - 2fu_R + 1]^{1/2}$$

where v_p = particle velocity due to electrophoresis only

$f = u_o/v_p$

u_o = electroosmotic flow velocity at chamber wall

$A^2 = u_R^2 + u_I^2$

u_R, u_I = real, imaginary parts of complex fluid velocity amplitude u(r)

ω = radian frequency of applied field

The results of both experiment and theory are presented in Figure 2.1.6.2.1. The arbitrary displacement amplitude units are in fact cm of displacement on the video monitor screen. There was an absolute systematic 17% underestimate of the theoretical prediction of the magnitude of the observed displacement which was probably an optical calibration error. The experimental values were therefore all corrected by this amount to allow a better comparison of radial dependence results to be made. The Figure shows that the theoretical predictions were borne out by the experimental results over the range tested. If the theory were not applied and the displacements calculated assuming $u(r)/u_o$ was independent of frequency the theoretical results at 5 Hz and 10 Hz would have fallen uniformly 2 to 3 standard deviations above the measured values near the center of the

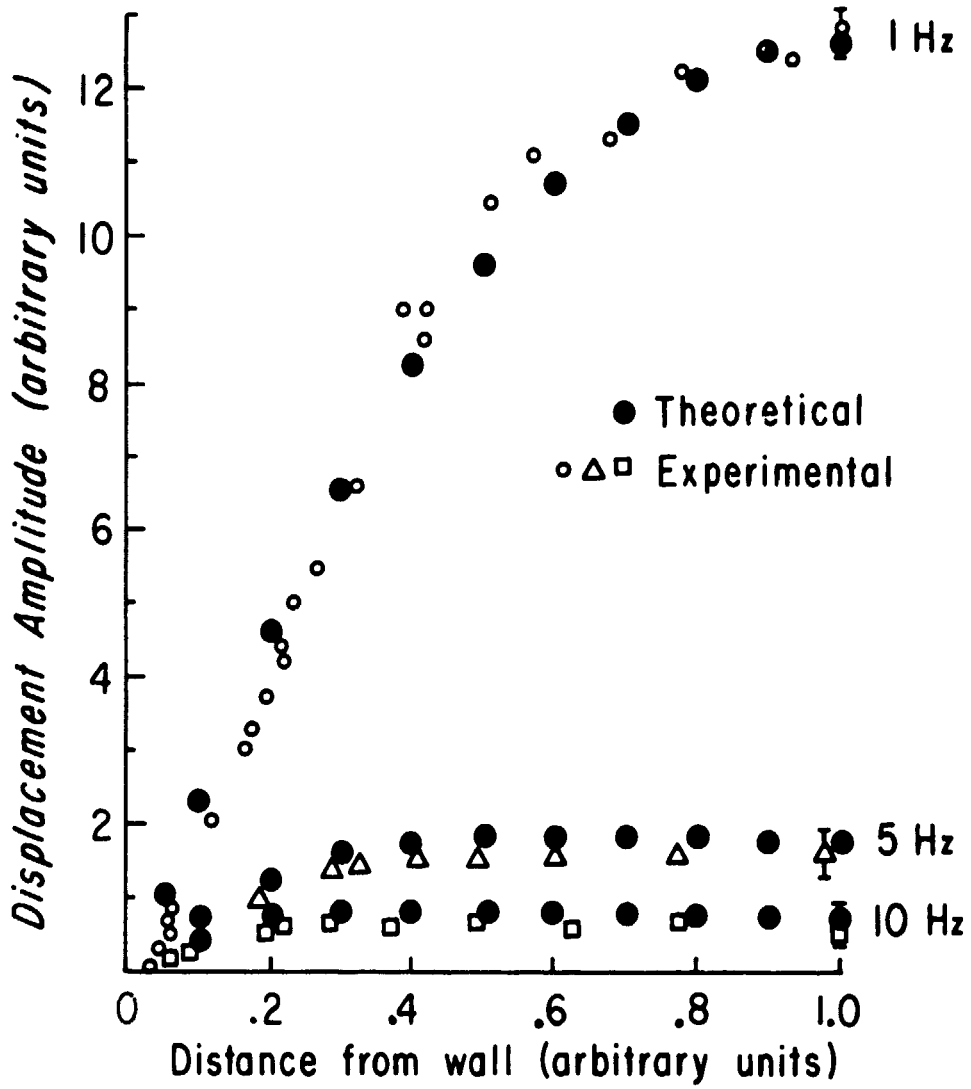

Figure 2.1.6.2.1. Comparison of theory with experimental values of particle displacement amplitudes as a function of radial position at the frequencies indicated; closed cylindrical chamber of radius 0.1 cm.

chamber. The theory is therefore probably correct, although much more work would have to be done to verify it exactly.

2.1.6.3 Implications for AAEF

The results described above strongly suggest that it would be advantageous to operate the AAEF in an AC mode. AC operation would allow cell velocity measurements to be made over most of the volume of the chamber without having to reduce the wall charge to zero. Such freedom is clearly required if a large number of mobility determinations are to be made on each sample If an open chamber were designed, the electroosmotic flow would be essentially zero over the core region. If a closed chamber were to be used at frequencies between 1.0 kHz and 0.1 kHz the magnitude of the core velocity would be 2% to 5% of the wall velocity. Apparent mobility histograms would therefore exhibit an internally consistent absolute error of this size. Absolute histograms could easily be derived by either measuring the core velocity with particles of known finite or zero mobility or by calculation applying the above theory.

2.1.7 Non-electrophoretic Characteristic Recognition

The AAEF should incorporate the capability to recognize and record other individual cell parameters besides electrophoretic mobility,

as seem appropriate for the population under examination. In particular, the ability to distinquish between cells with and without a fluorescent label should be included in the AAEF.

Optical examination of individual cells in the AAEF opens up the possibility of measurement of other parameters which may be correlated with electrophoretic mobility. The sedimentation rate of each cell could in principle be measured simultaneously with its velocity measurement, providing information on the geometry and density of the cell. It would also appear possible to incorporate the ability to detect the presence or absence of a fluorescent label on each cell. In this way very sensitive surface property correlations could be made since highly specific antibodies and lectins may easily be tagged with fluorescent labels. This capability would tremendously strengthen the AAEF since it would then include a sensitive and widely used recognition procedure which would allow subpopulation identification.

2.1.8 Data Analysis Software

As well as providing hard copies of electrophoretic mobility distributions, the AAEF should include the capability for detailed statistical analysis of the mobility data. These programs should provide:

- *descriptive statistics for the data.*

- *assessment of the unimodality of the mobility distribution via the computation of distribution-free statistics for goodness of fit to known mobility distributions obtained from calibration populations.*

- *special locally most powerful test statistics to detect very small subpopulations of known mean and standard deviation, with errors of the first and second kind controlled to within preset limits.*

The requirement for statistical calculations and tests of the data is clear. Particular attention should be paid to the identification and characterization of subpopulations since these are the objects of interest for preparative electrophoresis. The better characterized they are the better may be chosen the conditions for their separation. There exist in the literature special test statistics designed to detect very small subpopulations of known characteristics (10). These should be included in the AAEF software.

2.2 Recommended Automation Technique

As a result of reading and discussions with various concerned individuals four possible techniques for automating analytical electrophoresis were identified and considered. Each will be

described below and its merits discussed.

2.2.1 Laser Doppler Spectroscopy

The Doppler shift in laser light scattered by cells undergoing electrophoresis has recently been utilized independently by two investigators to estimate mobility distributions. Dr. Ben Ware of Harvard University and Dr. Ed Uzgiris of General Electric Research and Development Center have both developed apparatuses of comparable accuracy (3%-5%). The measurement technique consists basically of optically beating the light scattered from moving cells against an unshifted reference beam and detecting the mixed beam with a photocell. The frequency differences due to Doppler shifts in the beam scattered from cells undergoing electrophoresis are proportional to the cell velocity. Providing no electroosmotic or other flows are present in the system the beat frequency spectrum is proportional to the mobility distribution of the cell sample.

The laser Doppler approach has the following advantages:

- The mobility distribution is obtained rapidly; an acceptable spectrum from the cells present in the scattering volume can be collected in less than one minute.

- Sample volumes are small, well under 1 ml, and less than 10^5 cells could be used to provide a distribution.

- The technique is very versatile with respect to the dimensions of the cells or particles being examined; anything from the size of macromolecules on up may be used as the sample.

- Most of the basic apparatus designs, with the exception of a satisfactory chamber for physiological ionic strength work, have been worked out.

- An apparatus could be assembled for relatively low cost (< $20,000 excluding statistical analysis capability).

It is recommended that the AAEF not be developed around laser Doppler spectroscopy, however, for the following reasons:

- The technique would not be very accurate, particularly for cells suspended in media of physiological ionic strength where mobilities are relatively low. Typical Doppler frequency shifts recorded under these conditions would be 20 ± 0.5 Hz where the uncertainty represents the resolution of the spectrum analyzer used. Hence, even assuming no other errors were present the accuracy

and resolution would be limited to ± 2-3%. Since it is corceivable that preparative electrophoresis techniques could surpass this resolution, a more precise technique for the AAEF would be preferable.

- It would be difficult to apply the AC mode of electrophoresis in order to eliminate problems associated with electroosmosis. If frequencies much higher than the magnitude Doppler frequency shift are applied, the frequency spectrum cannot be analyzed within the period of one cycle of the applied electric field. Switching transients then appear in the scattered light spectrum at harmonics at the applied field frequency. While in principle the required true Doppler frequency spectrum can be extracted from the spectrum this could be a difficult problem when a complex mobility spectrum is superimposed on the transients which would probably reduce the accuracy of the derived mobility distribution somewhat. Alternative methods for eliminating electroosmosis such as keeping the electrodes away from the chamber walls or using chamber wall coatings are objectionable in that sample contact with electrode products is to be avoided and wall coatings are time dependent at best.

- There is no way to obtain non-electrophoretic information on individual cells or cell populations undergoing electrophoresis.

- No one has yet worked successfully with the technique in high ionic strength media; certainly the accuracy and resolution of distributions of low mobility populations would be very poor due to the resolution limit of the spectrum analyzer.

- The mobility spectrum produced is not a true mobility distribution. The output from the spectrum analyzer is in fact a plot of scattered light intensity as a function of beat frequency. While the beat frequency is directly proportional to cell velocity, the scattered light intensity is not proportional only to the number of cells in the scattering volume. The intensity of light scattered at a given angle will depend on cell size, shape, orientation and refractive index. Unless all these properties are known as a function of mobility, the true distribution of cell number vs mobility cannot in principle be obtained. In an unknown cell population these parameters could vary widely, producing apparent peaks in the distribution that could lead to misinterpretation of the spectrum and identification of sub-

populations that would not be separable by preparative electrophoresis.

For these reasons, particularly for that regarding the fidelity of the apparent mobility distribution produced, it would seem that laser Doppler spectroscopy would not be as suitable a technique as that recommended.

2.2.2 Pen Kem Automation Technique

Mr. Phil Goetz of the Pen Kem Corporation has invented a technique for transducing the focussed microscopic image of a field of moving particles into a signal of scattered light intensity as a function of migration velocity. The image of the particle field and a grid are focussed on a photocell. As the light beam scattered from each moving cell crosses the grid it generates a light intensity pulse. The pulse frequency for each cell is porportional to the velocity of the cell relative to the grid. Hence the frequency distribution associated with the population can be analyzed with a spectrum analyzer to produce an apparent mobility spectrum. This technique has the advantages that:

- In principle it can be applied directly to micro-electrophoresis as it is presently used.

- It is compatible with the application of an AC field of the frequencies required to obviate electroosmosis problems.

- It should be less sensitive to vibration than the laser Doppler method.

The Pen Kem approach suffers, however, from the following limitations:

- At the time Pen Kem was contacted no system had been developed that could be used for cells. A breadboard model was being put together but was being tested only on TiO_2 particles which scatter much more light than cells. It was not clear that a cell suspension would give a usable signal, nor that a sufficient number of cells could be analyzed.

- Development costs would probably be high.

- No individual cell information could be obtained by this technique.

- Typical frequencies for cells in media of physiological ionic strength would be < 50 Hz so the accuracy and resolution of the technique would then be > ± 1% due to the resolution of the spectrum analyzer.

- The output is a plot of scattered light intensity as a function of particle velocity. As discussed in Section 2.2.1 this is not necessarily the true mobility distribution and is therefore open to only restricted interpretation and analysis.

Principally for the last reason the Pen Kem approach was not recommended for the NASA AAEF.

2.2.3 Particle Field Holography

As one of the aims of developing the AAEF is to obtain information on a large number of cells the feasibility of using time lapse holography to store the positions of cells undergoing electrophoresis as a function of time was investigated. Subsequent analysis of the holograms would allow the determination of a large number of cell mobilities. Applying holography to the AAEF would have the following advantages:

- A large number of mobility determinations could be obtained on the same sample. Using available standard technology holograms of ~ 1 mm^3 of sample volume could be obtained with sufficient frequency to provide $> 10^4$ mobility determinations on a sample.

- Individual cell geometries would be available to correlate with mobilities.

- Small sample sizes could be employed of $\sim 10^5$ cells.

However, the technique has several disadvantages:

- A method for automatically analyzing holograms for cell geometry and position would have to be developed as none currently exists. Such a development would be quite expensive and take probably three years.

- The analysis would have to be done off-line which could be a disadvantage for some applications.

- Since the method employs displacement measurements over known intervals to determine cell mobilities the AC mode for electroosmosis suppression could not be used. The electroosmotic profile would have to be known if a standard chamber geometry were used, or another chamber with electrodes away from the walls developed.

- Individual mobility determinations might not be very accurate, depending on the amount of noise in the hologram. Holography at present has lower resolution than photography.

For these reasons it seems clear that at the present time it would be unwarranted to attempt to apply particle field holographic techniques to the automation of analytical electrophoresis.

2.2.4 Computer Analysis of Video Images of an Electrophoresis Field

This approach is discussed in detail in an unsolicated contract proposal to MSFC dated April 22, 1975 and in subsequent documents. Basically, a microelectrophoresis apparatus equipped with phase contrast optics would be used with a television camera replacing the eye of the operator. The video image would then be analyzed line-by-line to measure the position of every cell on each sweep. Measurements of cell position would therefore be made at the rate of $\sim$ 15 kHz. Phase contrast optics would ensure the sharp definition of cells in focus necessary for their identification. Automation using this approach would have the following advantages:

- Each mobility determination would be extremely accurate because of the large number of estimates that could be made on each cell in a few seconds; accuracy should be very good over the whole mobility range of interest.
- Electrophoresis could be carried out in alternating applied fields to suppress electroosmosis and provide a large volume in which mobilities would be independent

of radial position in the electrophoresis chamber. A large number of cells could therefore be measured without having to wait for sedimentation to replenish the optical field at the stationary level.

- Individual cell characteristics could be measured and correlated with mobilities. Fluorescent cells could also in principle be distinguished.

- The mobility data would be suitable for sophisticated statistical analysis because of its absolute and relative accuracy.

The principal disadvantages of using video image analysis for the AAEF would be that:

- The system will largely have to be developed from scratch and will be fairly expensive.

In spite of this difficulty, the advantages of this approach - particularly the accuracy, single cell recognition capability and the possibilities for statistical testing - make this the technique of choice for development of the AAEF.

3 PRIORITY RATING OF SPECIFICATIONS

The specifications were formed into three groups in diminishing order of priority as follows:

Group I

- *Determine individual mobilities of ~ 500 cells of diameter 2 to 25 microns within 30 minutes from a sample of 10^7 cells or less.*
- *Determine individual mobilities to an accuracy of ± 1.0% over a mobility range of 0.5×10^{-4} to 3.0×10^{-4} $cm^2\ s^{-1}\ v^{-1}$.*
- *Operate at 25°C with suspending media of specific conductivity < $0.02\ \Omega^{-1}\ cm^{-1}$ over a pH range of 6.5 to 7.5.*
- *Use applied electric fields of frequency < 1000 Hz.*
- *Chamber and electrodes must be compatible with normal biological support media (containing proteins, carbohydrates, multivalent ions, etc.) in their operating configuration. Measurement conditions and chamber materials shall be such that cells undergoing measurement retain the same viability and surface properties*

as an appropriate control suspension not exposed to the instrument.

- *Provide hard copy of mobility distribution plus mean and standard deviation of total population.*

These were considered the minimum requirements for the production of a facility useful to the NASA electrophoresis program. They would overcome the problems of using the manual technique without providing too severe an imposition on the developer. It would accept cells in the normal size range in a number commonly required at present. A 30 minute measurement time is compatible with most cell samples. Accuracy to ± 1% would probably equal the best preparative electrophoresis procedures currently conceived. It would operate under physiological conditions in the AC mode and would provide enough data for a reliable reproducible mobility histogram.

Group II

- *Determine individual mobilities of ∿ 500 cells within 10 minutes at a rate of four total determinations per hour.*

- *Provide a statistical assessment of the unimodality of the mobility distribution via the computation of*

distribution-free statistics for goodness of fit to known mobility distributions obtained from calibration populations. Provide special locally most powerful test statistics to detect very small sub-populations of known mean and standard deviation, with errors of the first and second kind controlled to within preset limits.

These additional requirements would allow a large number of samples to be examined and analyzed statistically for the presence of subpopulations that may not be obvious from the appearance of the histogram. Completion to this level would allow the AAEF to be applied to most samples of preparative interest.

Group III

- *Determine mobilities on a total sample size of 10^5 cells.*
- *Excluding problems of sedimentation the apparatus should be capable of collecting mobility data on cells in the size range 0.5 to 100 microns in diameter.*
- *Determine individual mobilities to an accuracy of ± 0.3% over a mobility range of 0.5×10^{-4} to 8.0×10^{-4} cm^2 s^{-1} v^{-1}.*

- *Operate over a temperature range of 4°C to 37°C and a pH range of 2 to 11.*

- *Incorporate the capability of recognizing and recording other individual cell parameters besides electrophoretic mobility, as seems appropriate for the population under examination. In particular, provide the ability to distinguish between cells with and without a fluorescent label.*

Completion to the final level would provide the ultimate instrument capable of examining the small sample sizes requested by many respondents to the user community survey. It would provide the most accurate data consistent with the number of measurements required. It would provide additional diagnostic capabilities that would describe the sample more thoroughly than simply by its mobility distribution. It would therefore have a very powerful capability for sample characterization that would have diagnostic applications as well as applications in determining separability. These diagnostic applications were identified by many of those surveyed as prime possibilities for use of the AAEF in clinical situations.

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Appendix 1

User Community Survey

This Appendix is comprised of:

- the names and addresses to whom the user community survey was distributed,
- the letter and form which was mailed out,
- the responses received.

Statements of particular interest to the NASA electrophoresis program have been underlined.

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Division of Pathology
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Dr. S.E. Zalik
University of Alberta
Department of Zoology
Edmonton, Alb., Canada

UNIVERSITY OF OREGON
HEALTH SCIENCES CENTER

DEPARTMENT OF NEUROLOGY
Area Code 503 225-7711
Portland, Oregon 97201

As you may know, the National Aeronautics and Space Administration of the United States is currently involved in an extensive program designed to develop facilities for preparative electrophoretic separations of biological materials under zero gravity conditions. By taking advantage of the lack of cell sedimentation and thermal convection in such an environment more effective separations can in principle be achieved. As part of this program, NASA has found a need to obtain an automated analytical electrophoresis facility (AAEF) that will allow rapid, accurate determinations of electrophoretic mobility distributions on a variety of complex mixtures of cells. The AAEF will be used primarily for ground-based examination of cell populations which are considered candidates for zero gravity separations.

In order to make the development of a fully automatic analytical electrophoresis apparatus maximally useful to scientists outside NASA, I have been asked to survey the electrophoresis user community to determine the performance levels to which such an instrument should operate. In this way it is hoped that in subsequent availability the instrument will fulfill the requirements of as many investigators as possible. I would therefore greatly appreciate it if you would fill out the attached form as fully as possible. Keep in mind that the AAEF, as presently envisaged, will be completely automatic and should provide data on a large number of cells, so please indicate the specifications you would like to see satisfied without direct regard for the current state of the art.

To satisfy NASA's schedule, I will need the completed specification forms returned by December 19, 1975. Please address them to:

Dr. D.E. Brooks
Department of Neurology
University of Oregon Health Sciences Center
Portland, Oregon 97201

May I thank you in anticipation of your assistance in compiling the operating specifications for the AAEF. Your cooperation will help produce an instrument which I am sure will prove to be a real advancement in electrophoretic investigations.

Yours sincerely,

D.E. Brooks, Ph.D.

DEB:jc

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   _____

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   _____

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   _____

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   _____

   _____

5. What are the highest and lowest absolute mobility values you would wish to measure?

   _____

6. What should the absolute accuracy of each mobility determination be?

   _____

7. What is the minimum mobility difference the AAEF should be able to resolve?

   _____

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   _____

9. Over what temperature range should the AAEF be capable of operating?

   _____

10. Over what range of pH and ionic strength would you like to operate?

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   $10^4 - 10^5$

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   500 - 1000

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   0.5 to 25 μ

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   Primarily accurate histograms, second: comparison between histograms of different samples - computer storage of all data and analysis of deviance, with particular emphasis to differences in mobility of small proportion of cells in sample.

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

   0.3 to 6 M.U.

6. **What should the absolute accuracy of each mobility determination be?**

   1%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   .02 M.U.

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   Speed of measurement is not very critical - 10-15 minutes is sufficiently fast. Main limitation is not speed of analysis, but number of specimens available.

9. **Over what temperature range should the AAEF be capable of operating?**

   5°C to 25°C

10. **Over what range of pH and ionic strength would you like to operate?**

pH 3 to 9 < .15 Γ

11. **Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?**

no

12. **Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?**

As specified above speed is of no particular consequence- no one can prepare that many different cell specimens as the apparatus will be able to process even at 15 min/sample. Accuracy, on the other hand, is of paramount importance.

13. **Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?**

The development of AAEF is highly desireable, provided it can give really accurate data in terms of actual mobility distribution of individual cells, counted one by one. Average mobility data, or data giving only indirect information on mobility as a function of scattering, or other cell functions (except mobility) are of more limited usefulness. I must reemphasize that speed of each measurement is much less important than accuracy of data.

Milan Bier
Veterans Adm. Hospital
Tucson, Az.
Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   see question 13

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   see question 13 ~100

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   1.5 λ — 3.5 λ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   fraction of cells with mobility in a given 100 cm/hr/volt range would suffice

5. What are the highest and lowest absolute mobility values you would wish to measure?

   2400 cm/hr/volt — 200 cm/hr/volt

6. What should the absolute accuracy of each mobility determination be?

   not important to me

7. What is the minimum mobility difference the AAEF should be able to resolve?

   100 cm/hr/volt would be sufficient for my purposes

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   all electrophoresis should be done within 5 hrs of platelet collection

9. Over what temperature range should the AAEF be capable of operating?

   0 - 40° C.

10. Over what range of pH and ionic strength would you like to operate?

pH 5-8, ionic strength

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

platelets should not be exposed to glass

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

one that was slower + more accurate

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

We have been using electrophoresis for preparation purposes. Therefore we have used 10^{10}-10^{11} platelets at a time and have collected fractions of 10^{8}-10^{9} platelets homogeneous in ~~mobility~~ mobility. We could use it for analysis of fractions obtained by other means in which case we could use the electrophoretic analysis of as few as 100 platelets of each fraction. I am not sure whether you plan a preparative or analytical instrument. From these questions I think probably an analytical one and such an instrument should be worthwhile for a variety of research projects.

Donna J. Carty
Name and address
Dept. of Biochemistry
Jordan MEB
Univ. of Virginia
Charlottesville, Va.
22901

JADAVPUR UNIVERSITY
CALCUTTA-700032, INDIA
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FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF FOOD TECHNOLOGY & BIO-CHEMICAL ENGINEERING

File No. J.1/FT/242/TKC/75

Dated: December 12, 1975.

From:
Dr. D. K. Chattoraj, D.Sc., Ph.D.,
Reader.

To:
Dr. D. E. Brooks,
Department of Neurology,
University Oregon,
Health Sciences Centre,
Portland,
Oregon 97201.

Dear Dr. Brooks,

Thanks for your letter of November 24, addressed to Dr. S. N. Upadhyay in which you have shown interest in our works and experience in Cell Electrophoresis. Since Dr.Upadhyay left Calcutta after finishing his Ph.D. in 1971, I am writing the reply for your enquiry in the attached sheets.

I shall be glad to receive the results of your research on the microelectrophoretic study in space which will be a very interesting proposal, I believe.

With regards,

Sincerely yours,

D. K. Chattoraj

(D. K. Chattoraj)

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   50

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   20

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   20μ , 0·5μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

5. What are the highest and lowest absolute mobility values you would wish to measure?

   20 μ ; 0·1 μ/sec/volt/cm.

6. What should the absolute accuracy of each mobility determination be?

   within 1%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0·2 μ/sec/volt/cm.

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   10 sec ; at ~ 10 sec. interval ; Yes

9. Over what temperature range should the AAEF be capable of operating?

   5 to 50°C .

10. Over what range of pH and ionic strength would you like to operate?

2 to 11

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Radiation leakage may affect the biological systems to be used.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Second one may be preferred

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

(a) Study of the mobility of the cells as functions of ionic strength at a given pH may be investigated to understand conformational changes of the biopolymer at interfaces.

[our ref: D.K. Chatterjee & coworkers, Indian J. Biochem & Biophys. 9, 12, 17 (1972); ibid 11, 123 (1974)]

(b) Microelectrophoretic study of adsorbed proteins and nucleic acids for genetic features of the adsorbed biopolymers should also be investigated. (our Ref; Ref. Chatterjee et al; Biopolymers 5, 173 (1967) ibid. 5, 173 (1967) Brotel (1968); Ind. J. Biochem. 7, 199 (1970), Indian J. Biochem. 7, 82 (1970). ibid

D. Kr. Chatterjee.

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed ; please include them in response to question I3.

I - What is the smallest total sample size (number of cells) that you would wish to examine ?

We would be satisfied if 10^6 cells could provide 100 determinations.

2 - How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal ?

500 determinations.

3 - What are the diameters of the largest and smallest particles you would like to examine electrophoretically ?

5 and 20 μm

4 - What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations ?

A mobility distribution in the form of an histogram as a function of cell frequency for each mobility class.

5 - What are the highest and lowest absolute mobility values you would wish to measure ?

In NaCl 0.145 M 0.50-1.50 $\mu m/v^{-1}/sec^{-1}/cm^{-1}$

6 - What should the absolute accuracy of each mobility determination be ?

2 percent

7 - What is the minimum mobility difference the AAEF should be able to resolve ?

0.025 $\mu m/v^{-1}/sec^{-1}/cm^{-1}$

8 - How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out ? Is speed of measurement important to you ?

The speed is not very important ; if possible 30 minutes with a a small interval.

9 - Over what temperature range should the AAEF be capable of operating ?

20 - 37°C

IO - Over what range of pH and ionic strength would you like to operate ?

pH 7.2 - 7.5 in NaCl 0.I45 M

II - Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis ?

non-toxic material for cell use

I2 - Would you rather have an apparatus that produced rapid (I per minute) but approximate ($\pm$ 5%) mobility spectra or one that was slower (I5 minutes per determination) but more accurate ($\pm$ 0.I%)?

We would prefer a slower but more accurate apparatus.

I3 - Do you have any other requirements that should be considered in providing specifications of the AAEF ? Do you think the development of such an instrument is worthwhile ? Do you have any additional comments ?

We are very interested by such an apparatus. We have been working on lymphocyte electrophoresis for three years now, and have several papers to be published in Biomedicine, J. Immunol. Methods and Europ. J. of Cancer.
This form was given to us by Dr SAUVEZIE, which is a member of our laboratory. Unfortunately, he has been for some time in England, so your letter will have a rather slow reply.
Could you write us for any development of this question ?

Dr CHOLLET
Centre Jean PERRIN
place Henri Dunant
B.P. 392
630II CLERMONT FERRAND CEDEX
FRANCE.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL
"LECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   1×10^7

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   Prefer total analysis. (4-4 types)

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   6 - 40 microns.

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Mean ± standard error mobility of each major peak/sub-population

5. What are the highest and lowest absolute mobility values you would wish to measure?

   $0 - 1.3\ \mu\ sec^{-1}\ V^{-1}\ cm$

6. What should the absolute accuracy of each mobility determination be?

   ±2%.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   ± 6%

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   No

9. Over what temperature range should the AAEF be capable of operating?

   $+4 \rightarrow +45^{\circ} C$

10. Over what range of pH and ionic strength would you like to operate?

pH 7.0-7.4; human ionic strength

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Pb^{++} apparently toxic in trace amounts

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

RAPID option

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Automation will probably be more easily achieved on the perpendicular principle of continuous flow of medium with electrophoretic deflection into a range of channels, each of which would then be counted on the Coulter principle.

Failing this, time exposure photography (or an electronic equivalent) of a given field of particles may work, with an instrument conceptually similar to Zeiss Cytopherometer

The potential of AAEF in: cancer diagnosis is emphasized by the MCH test (BMJ, 1971, 2: 613 etc)

Name and address

DR ALAN S. COATES MD FRACP
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INSTITUTUL ONCOLOGIC
B-dul 1 Mai nr. 11 P.B. 5916
BUCUREȘTI 12

Telefon: 13.22.70 Centrala
16.48.28 Direcțiunea

10. XII. 1975

Dr. D.E.Brooks
Department of Neurology
University of Oregon Health Sciences Center
Portland, Oregon 97201, SUA

Dear Dr. Brooks,

Thank you for your letter concerning AAEF, which I received in 8 December; I include the completed specification forms and I hope they will reach you till 19 December.

My best wishes for the realisation of very good AAEF

Yours sincerely
Dinescu-Romalo
Dr. Gabriela Dinescu-Romalo

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   Mitochondria: 1 mg/ml; Cells: 30/ml

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   20 timings in both directions for one sample.

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   Cells: 20 μ; Nucleus: 4-6 μ; Mitochondria: 0.5-3 μ; Lysosomes: 0.25-0.5 μ

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   U=mobility; =average net surface charge density; N=nr. elementary charges per square μ.

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

6. **What should the absolute accuracy of each mobility determination be?**

   $\pm$ 0.5 - 1%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   Yes, for cells which sediment quickly in usual conditions

9. **Over what temperature range should the AAEF be capable of operating?**

   4°C - 25°C - 37°C

10. **Over what range of pH and ionic strength would you like to operate?**

pH: 6 - 8.5; Ionic strength: 0.05 - 0.005

11. **Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?**

12. **Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?**

Response to question 13.

13. **Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?**

a) Response to 12: If sedimentation does not interfere (zero gravity conditions) the more accurate apparatus is prefered; otherwise, for cells which sediment very quickly (f.i. tumor ascites cells) a rapid determination is to be chosen to ensure cell stability at the stationary level.

b). AAEF may help in early detection of membrane systems alterations which can possibly act as important pathogenetic factors.

c). Could it be possible to visualize the electrophoretic behaviour of subcellular particles (nucleus, mitochondria, lysosomes) within the cell?

10. XII. 1975

Dr. Gabriela Dinescu - Romalo
Name and address
INSTITUTUL ONCOLOGIC, Dept. Immunology and Biochemistry of Cancer,
B-dul 1 Mai Nr. 11, POB 5916,
Bucuresti-12, ROMANIA

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^7

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   $10^2 - 10^3$

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   15μ — 5μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   MODE, MEAN, STANDARD DEVIATION, HALF-MODE, QUARTER-MODE and TENTH-MODE POINTS.

5. What are the highest and lowest absolute mobility values you would wish to measure?

   3.0 → 0.3 μsec^{-1} per volt cm^{-1}.

6. What should the absolute accuracy of each mobility determination be?

   ± 2%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   2%

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   AS QUICKLY AS POSSIBLE. YES (see Q11.) (electrode by-products).

9. Over what temperature range should the AAEF be capable of operating?

   20-37°C

10. Over what range of pH and ionic strength would you like to operate?

pH 7.0-7.6 $\mu = 0.25 - 0.40$

11. Are there any unusual limitations on the types of materials to which your samples can be exposed durirg electrophoresis?

DETERGENTS; ELECTROLYTE BY-PRODUCTS. BACTERIAL, ... other ANTIGENS. MOST PLASTICS

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

± 2% in 5 mins.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

i) —

ii) Could be!

iii) —

DR. J. P DICKINSON.
UNIVERSITY DEPARTMENT OF RADIOTHERAPY
REGIONAL RADIOTHERAPY CENTRE
COOKRIDGE HOSPITAL
Name and address
LEEDS, LS16 6QB
ENGLAND.

With the Compliments of
Professor C. A. F. Joslin

UNITÉ DE CANCÉROLOGIE EXPÉRIMENTALE
ET DE RADIOBIOLOGIE U 95
(I. N. S. E. R. M.)

Plateau de Brabois
54500 VANDŒUVRE LÈS NANCY
(FRANCE)
TÉL. 53.52.91

Vandœuvre, le December 10, 1975

Doctor D.E. BROOKS
Department of Neurology
University of Oregon Health
Science Center
PORTLAND, OREGON 97201

U.S.A.

Dear Doctor Brooks,

Your name was given to me by my colleague F. DUMONT in connection with your inquiry about the performances of a fully automatic analytical cell electrophoresis apparatus. From my point of view, the display of an automatic apparatus is of crucial importance. I think a lot of hospitals will be interested in this technique if NASA brings out an automatic equipment. Furthermore, it will be useful for workers who study variations of cell surface charge within a short interval time.

Using this technique for two years, I take the liberty of answering your questionaire.

Yours faithfully

Mrs Mireille DONNER
Chargée de Recherche I.N.S.E.R.M.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   50 cells

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   500 cells

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   4 microns - 20 microns

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Deviation standard or standard error

5. What are the highest and lowest absolute mobility values you would wish to measure?
   They are dependent on the ionic strength of suspending medium. With a 0.145 ionic strength medium, these values are about 0.30 - -2.0 micron/sec/V.cm

6. What should the absolute accuracy of each mobility determination be?

   2%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0.02 micron/sec / V.cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   50 cells should be scored within 4 minutes

9. Over what temperature range should the AAEF be capable of operating?

   4°C - 25°C

10. Over what range of pH and ionic strength would you like to operate?

pH - 6 - 8

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Plastic materials should be prohibited

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?
The apparatus should allow these both possibilities.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

a) It is difficult to indicate the range of ionic strength of media. It is dependent on the voltage used in the apparatus. If Nasa develops an apparatus which measures speed of cells by laser Doppler spectroscopy, it is likely that media with a weak ionic strength ($\simeq$ 0,005) will have to be used. Otherwise, it would be better to use physiological ionic strength ($\simeq$ 0,15).

b) It would be very interesting to have mobility spectra on an oscilloscope or an external recorder. For other applications, the display of the electrophoretic mobility for each cell would be necessary.

Mme Mireille DONNER
U 95 - INSERM -
Plateau de Brabois

Name and address

54500 - Vandoeuvre les Nancy (France)

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^4 cells

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   300

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   5 - 15μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Histogram showing numbers of cells grouped together in 0.1 μ sec^{-1} V^{-1} cm differences.

5. What are the highest and lowest absolute mobility values you would wish to measure?

   at ionic strength μ = 0.15,
   0.50 - 2.00 μ sec^{-1} V^{-1} cm

6. What should the absolute accuracy of each mobility determination be?

   ± 0.01 μ sec^{-1} V^{-1} cm

7. What is the minimum mobility difference the AAEF should be able to resolve?

   ± 0.01 μ sec^{-1} V^{-1} cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   Manually, ≈ 2 hours per 300 readings. Only so as not to result in deterioration of the sample.

9. Over what temperature range should the AAEF be capable of operating?

   20° - 40°C

10. Over what range of pH and ionic strength would you like to operate?

pH 6.0 - pH 8.0; μ = 0.15 - 0.01

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

The requirements of aseptic, non-pyrogenic cell culture handling techniques.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

The slower but more accurate apparatus would be preferable.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

With mixed populations of cells, such readings by traditional apparatus can be tedious; an AAEF would help.

Dr. Richard M. Fike
Dr. Carel J. van Oss
Department of Microbiology
School of Medicine
State University of New York
at Buffalo
Buffalo, N.Y. 14214

Name and address

DEPARTMENT OF BIOCHEMISTRY
ALL INDIA INSTITUTE OF MEDICAL SCIENCES
NEW DELHI-16 INDIA

CABLE: MEDINST

Dr. D.E. Brooks,
Department of Neurology,
University of Oregon Health Science Center,
Portland, Oregon 97201,
U.S.A.

15th January, 1976.

Dear Dr. Brooks,

Enclosed, please findThe specification form for the Automatic Analytical Electrophoresis duly filled by S.N.S. Hanjan. Unfortunately due to his preoccupation he could not complete th form earlier, but he hopes that this information will help you acquaint with the needs of our department. Please let us know if you need any other information.

With best wishes.

Yours sincerely,

Indira Nath

(Indira Nath, MD)
Asst. Professor,
Dept. of Biochem.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   100,000

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   200

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   2μ – 100 μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Till now we have been comparing the mobilities of our samples with reference to human R.B.C (1·07 ± ·01 μ/sec/V/cm). This is not ideal, some standard charged particles of app. 7·6 μ should be designed with mobility equal to 1·00 ± ·01 μ/sec/V/cm.

5. What are the highest and lowest absolute mobility values you would wish to measure?

   0·25 μ – 2·0 μ/sec/V/cm

6. What should the absolute accuracy of each mobility determination be?

   Accurate upto ·01 μ/sec/V/cm.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0·1 μ/sec/V/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   In 10 minutes, Speed of measurement is very important.

9. Over what temperature range should the AAEF be capable of operating?

   0°c – 37°c.

10. Over what range of pH and ionic strength would you like to operate?

pH 5.5 — 9.5 in Isotonic Saline

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

1. Some times there are unusually large cells like Trophoblast they should not be allowed to fall under gravity &
2. Then there are sometimes cells like macrophages.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

An intermediate say in 10 min an accuracy of 1%

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

The development of this apparatus is very essential. The instruments currently in use are very cumbersome to use. Therefore such an instrument as envisaged by you would be welcome.

Comments.

1. Normally in the biological system, when studing the effect of "effector agents" it is always desirous to take the mobility measurements in shortest possible time, so that any biological activity taking place as a result of the binding of the "effector agent" with the receptor on the cell membrane should not interefere with the alleviation in the charge caused by the receptor binding. This would be only possible if the instrument is capable of taking the

Name and address

P. T. O.

mobility measurements in a short time and also at low temp.

2. The instrument should be free from the gravitational forces. Then it would be possible to measure the mobility of large cells (like trophoblast, large monocytes etc.).

3. The capillary of such an instrument should be such that "sticky" cells like macrophages do not stick with surface.

4. The instrument should have an XY recorder and a plotter attached, so that the electrophoretic mobility of each individual cell is recorded and plotted. Also if possible the instrument should be able to resolve the resultant histograms thus obtained into the total constituting gaussian populations.

5. Last but not the least, the instrument designed should have a reasonable price. Too expensive instrument will not become popular.

Thanks for consulting us, and hope that you will keep us informed about the developments.

S.N. Hanjan
(S.N.S. HANJAN)
Deptt. of Biochemistry
All India Institute of
Medical Sciences,
New Delhi - 16

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^6

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   [illegible]: always 100 could go to 10^4

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   0.5 to 3.0 µm

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Standard deviation   Confidence limits for single mean at $P = 0.05$

5. What are the highest and lowest absolute mobility values you would wish to measure?

   $+2.0$ to $-3.0 \times 10^{-8}\ m^2 s^{-1} V^{-1}$

6. What should the absolute accuracy of each mobility determination be?

   $\pm 0.02 \times 10^{-8}\ m^2 s^{-1} V^{-1}$

7. What is the minimum mobility difference the AAEF should be able to resolve?

   $\pm 0.02 \times 10^{-8}\ m^2 s^{-1} V^{-1}$

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   (a) 100 readings in 6 min; (b) intervals of 30 min (c) Not really important

9. Over what temperature range should the AAEF be capable of operating?

   5–40 °C

ORIGINAL PAGE IS
OF POOR QUALITY

10. Over what range of pH and ionic strength would you like to operate?

pH 3–11, $\bar{I} = 5 \times 10^{-2} - 0.5$ mol dm^{-3}

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

No contact with ~~wit~~ or polyvalent electrolytes (Ca^{2+}, Ba^{2+}), grease, surfactants

12. Would you rather have an apparatus that produced rapid (1 per minute) but approx'mate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Slower more accurate results preferred

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

I doubt if the development of such an instrument is really worthwhile. Ten years ago when I investigated a similar project I felt that it would be possible to make 10^5 readings on individual particles in 10–15 minutes by an adaptation of our own home made equipment. The cost for development then was about £20000.
Since surfaces are so easily subject to slight contamination I cannot see much advantage in improving the accuracy of the instruments which are available at the present time. More work should be put into the preparation of pure samples. The only advantage of such an instrument would be to increase the number of readings on a sample which provide more statistically reliable results. Who is likely to be able to afford such an instrument; after all the technique has not even been shown to be of diagnostic use.

Do it intended to have a facility to photograph the particle as well as measure its mobility, & will it be possible to measure mobility values of the different blood cells in a mixed suspension (cf. Zeiss cytophoresometer)?

Name and address

Prof. A. M. James
Bedford College
London NW1 4NS

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   No difficulties with sample size

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   Say 10.

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   From 250 Å to 5μ or above.

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

5. What are the highest and lowest absolute mobility values you would wish to measure?

   → 0 to ~~3~~ 7.

6. What should the absolute accuracy of each mobility determination be?

   ± 2%.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0.01.

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   No

9. Over what temperature range should the AAEF be capable of operating?

   → 0°C to say 50°C

10. Over what range of pH and ionic strength would you like to operate?

pH 0-14, μ = 10^{-7} to 10^{-1} M

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Samples likely to be actual drugs or biological cells.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Median ± 2%

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

1) Low temperature control important. Measurements tend to be inaccurate at temperatures above 25-30°C

2) Would like to measure mobilities of micelles of surface active agents, difficulties in observing due to Refractive Index being close to that of water.

DR. J. B. KAYES
PHARMACEUTICS RESEARCH GROUP
PHARMACY DEPARTMENT
UNIVERSITY OF ASTON IN BIRMINGHAM

Name and address

GOSTA BREEN
BIRMINGHAM B4 7ET
UNITED KINGDOM

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   not ours

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

5. What are the highest and lowest absolute mobility values you would wish to measure?

6. What should the absolute accuracy of each mobility determination be?

7. What is the minimum mobility difference the AAEF should be able to resolve?

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

9. Over what temperature range should the AAEF be capable of operating?

C.2

10. Over what range of pH and ionic strength would you like to operate?

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

We do not use EM measurements for these purposes but to study homogeneous colloids. A device such as described could be used but would be unnecessary for our purposes. Your questions [illegible]

M. Litt
M A Khan
Univ of Pa
Dept Chemical & Biochemical Eng.
Name and address

Pharmaceutical Sciences Laboratories
DEPARTMENT OF PHARMACY
NINEWELLS HOSPITAL
DUNDEE DD2 1UB
Tel. 0382 60111

Our Ref:TMM/AR

AIRMAIL

8 December 1975

Dr D E Brooks
Department of Neurology
University of Oregon Health
Sciences Center
PORTLAND
Oregon 97201
USA

Dear Dr Brooks

Thank you for your letter of 24 November to our research student Mr Donald.

We have discussed your questionnaire with Dr Hutchinson and have pleasure in enclosing our replies.

Yours sincerely

Dr T M MacLeod

M 03 809 75

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   50

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   100

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   5μ - 20μ

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   Modality i.e. Histogram.

   Mean $\pm$ SD of each mode.

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

   0.6×10^{-4} cm^2 v^{-1} sec^{-1} (5.6 secs)

   2.4×10^{-4} cm^2 v^{-1} sec^{-1} (1.4 secs)

6. **What should the absolute accuracy of each mobility determination be?**

   Mean $\pm$ 0.03×10^{-4} cm^2 v^{-1} sec^{-1}

   i.e. = 3%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   0.01×10^{-4} cm^2 v^{-1} sec^{-1}

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   $\pm$ 30 min. Preferably 15 min. Yes.

9. **Over what temperature range should the AAEF be capable of operating?**

   18°C - 25°C

10. Over what range of pH and ionic strength would you like to operate?

pH 7.0 - 7.4. Physiological.

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

No

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Slower and more accurate.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Possibility of video recording.
Histogram display.
Use of the instrument as a means of separating cell populations.

Dr T M MacLeod
Pharmaceutical Sciences Laboratories
Ninewells Hospital

DUNDEE, Scotland.

8 December 1975.

Name and address

DEPARTMENT OF PATHOLOGY
The Memorial Hospital
Pawtucket, Rhode Island 02860 • 401-722-6000

R.G. Mason, M.D., Ph.D. J.W. Jhung, M.D.
T.S. Micolonghi, M.D. F.P. Roland, M.D.
J.T. Kurtis, M.D. J. Katz, Ph.D.

A BROWN UNIVERSITY AFFILIATED HOSPITAL

December 3, 1975

D.E. Brooks, Ph.D.
Department of Neurology
University of Oregon
Health Sciences Center
Portland, Oregon 97201

Dear Dr. Brooks:

Although I am a member of the USRA committee, I do not anticipate conducting electrophoretic experiments in space. Nevertheless, if I were to generate research projects in the future that might be carried out in space, I imagine they would involve electrophoresis of endothelial cells or blood platelets. For my own needs, I would imagine an electrophoresis apparatus that would measure electrophoretic mobility of erythrocytes and kidney cells would certainly be adequate for my needs. I find it difficult to answer most of the other questions that you have posed, since I do not have specific experiments in mind. The slower but more accurate apparatus would appear desirable to me. It would likely be desirable to be able to measure pH over a range of 6 to 11.

I am afraid that I am not going to be of much help to you in your work, but this is the best I can do.

Could you or Geoff send me a brochure on the Rank brothers electrophoretic apparatus with laser light source? Our research group will likely wish to purchase one of these in the near future. I understand that they are available only from Rank and must be ordered from them in England. Is this correct?

Sincerely yours,

Reginald G. Mason, M.D., Ph.D.
Pathologist-in-Chief

RGM/emg

ACADEMISCH ZIEKENHUIS GRONINGEN

NEUROLOGISCHE KLINIEK
Toestel 2410

Prof. Dr. J. M. Minderhoud
toestel 2430

Prof. Dr. J. Droogleever Fortuyn
toestel 2400

Dr. J. P. W. F. Lakke
toestel 2433

Drs. A. E. J. de Jager
toestel 2410 en 2176

POLIKLINIEK volwassenen

Drs. H. Leenstra-Borsje
toestel 2447 en 2449

Nieuwe patiënten
volgens afspraak
toestel 2447

Contrôle patiënten
volgens afspraak
toestel 2446

KINDERNEUROLOGIE

Drs. R. le Coultre
toestel 2172

Drs. J. H. Begeer
toestel 2172

Polikliniek volgens afspraak
toestel 2445

E.E.G./E.M.G.

Dr. S. Boonstra
toestel 2425 en 2428

Drs. T. W. van Weerden
toestel 2599

NEURO - RADIOLOGIE

Drs. J. M. Rodermond
toestel 2631 en 2578

NEURO - PSYCHOLOGIE

Dr. B. G. Deelman
toestel 2408

NEURO - BIOCHEMIE

Dr. A. W. Teelken
toestel 2647

TELEFOON 050-139123

GRONINGEN, Oostersingel 59

December 15, 1975.

Dr. D.E. Brooks
Department of Neurology
University of Oregon Health Sciences
Center
Portland, Oregon 9720[illegible]
U.S.A.

Dear Dr. Brooks,

In answer to your letter of November 24, 1975, about the electrophoretic separation of biological materials: We worked with the Zeiss cytopherometer for some time in Newcastle, but now, about three years afterwards, we stopped this research because this method proved to be too difficult and too complex for the immunological research. So I am sorry to say that I can not give you answers to your question.

Yours sincerely,

Prof. Dr. J.M. Minderhoud.

The University of Nottingham

Department of Medicine
General Hospital
Nottingham
NG1 6HA

From PROFESSOR J. R. A. MITCHELL
Telephone 46161 (STD 0602)
Extension 544

JRAM/MHJ

1st December, 1975.

Dear Doctor Brooks,

Thank you for your letter about the possibility of developing a machine to provide accurate automated electrophoretic mobility measurements. My group would indeed be very interested in these developments and my co-worker in this field, Doctor J. R. Hampton, has completed your questionnaire. He will reply for both of us but I felt that you would wish to know that we are very interested in the outcome of your programme.

Yours sincerely,

J. R. A. Mitchell,
Professor of Medicine.

Dr. D. E. Brooks,
Department of Neurology,
University of Oregon Health Sciences Centre,
Portland,
Oregon 97201,
USA.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   Hospital facility - normal preparation
   contains > 200,000 per cu. mm.

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   100

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   1 - 10 μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Mean & SD

5. What are the highest and lowest absolute mobility values you would wish to measure?

   1 μ/sec/v/cm ± 50%

6. What should the absolute accuracy of each mobility determination be?

   1%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   3%

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you? YES.

   Measurement within 2 mins, repeated every 5-10 mins

9. Over what temperature range should the AAEF be capable of operating?

   25 - 37° C.

10. Over what range of pH and ionic strength would you like to operate?

pH 6 – 8

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Blood platelets are difficult to immobilise; & must not come into contact with detergents etc.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Neither would do! – but quicker measurement with.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Yes - definitely worthwhile

Fast separation platelets or

blood more difficult to immobilise

than stone-rigid particles of this

type, & must be suspended in

protein-containing medium.

J. K. HAMPTON

Name and address

ART MEDICINE
GENERAL HOSPITAL
NETTLEHAM
ENGLAND

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   AS FEW AS POSSIBLE

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   100's

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   1 – 20μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   TWO POPULATIONS WITH 15-20% DIFFERENCE IN MOBILITY COULD BE SIGNIFICANTLY SEPARATED

5. What are the highest and lowest absolute mobility values you would wish to measure?

   INTERESTED ONLY IN MAMMALIAN CELLS

6. What should the absolute accuracy of each mobility determination be?

   AS ACCURATE AS POSS.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   10% OR BETTER number of measurements important

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   YES.

9. Over what temperature range should the AAEF be capable of operating?

   RT to 37°C.

10. Over what range of pH and ionic strength would you like to operate?

0·9% Cl : P^H : 7·0
Only small variation allowed.

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

THOSE LIMITATIONS THAT APPLY TO BIOLOGICAL MATERIAL THAT MUST REMAIN ACTIVE.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

IMPOSSIBLE TO ANSWER. COMPROMISE

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

IN CONNECTION WITH MEM TEST FOR DETECTING CANCER SUCH A MACHINE WOULD BE AN ASSET.

John L. Moore

J. L. Moore.

Name and address

Velindre Hosp.
Whitchurch
CARDIFF
UK.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^6 cells

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   Minimum of 250

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   8 μM to 16 μM.

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   height, σ

5. What are the highest and lowest absolute mobility values you would wish to measure?

   0.75 to 1.45 $\mu M\ s^{-1} V^{-1} cm^{-1}$

6. What should the absolute accuracy of each mobility determination be?

   ± 1%.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   better than 4%.

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   250 measurements in 15 minutes (ideally)

9. Over what temperature range should the AAEF be capable of operating?

   25°C — 37°C.

10. **Over what range of pH and ionic strength would you like to operate?**

7.2 pH ± 0.2 - Normal saline equivalent -

11. **Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?**

No, provided that the conditions are physiologically acceptable.

12. **Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?**

Slower and more accurate.

13. **Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?**

What is indicated is far in advance of any technique at present employed. We would have an application immediately for such an instrument - since all the biological requisites for an accurate test for malignant disease exist in this laboratory, but it cannot be used since an instrument capable of rapid accurate electrophoretic determinations, free from subjective bias, does not exist.

I fully support the principle.

Alan W. Preece.
Principal Physicist
Name and address

Radiotherapy Centre.
Horfield Road.
BRISTOL BS2 8ED
U.K.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   10^3

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   3

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   5 - 25 μm

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   Histogram; means, standard errors and standard deviations; if more than one mode, the significance (?) of the difference.

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

   ~ 0.2 to 2.5 $\mu m^2 \times sec \times V^{-1}$

6. **What should the absolute accuracy of each mobility determination be?**

   ± 3%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   5%

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   Not as much as accuracy.

9. **Over what temperature range should the AAEF be capable of operating?**

   20 - 30°C

10. Over what range of pH and ionic strength would you like to operate?

pH ~ 7; 0.01M to 0.15

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

No: they are cells (erythrocyte - leukocyte - tumor cells)

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

The more accurate one.

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Your questions bear on all essential aspects; I do believe an instrument capable of providing high precision in the determination of E.M. is fundamental in the area of cell membrane immunogenetics and immunochemistry. (There are my personal interests; other objectives are equally worthwhile). - Would you please keep me informed as the project progresses?

Sincerely

P. Rubinstein, MD

Name and address

New York Blood Center
310 E 67th St.
N.Y.C., N.Y.
10021

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   10^5

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   (300)

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   5 μ - 50 μ

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   Cumulative histograms analyzed by chanel analyzer giving the number of cells, mean electrophoretic mobility of each population (as defined by Gaussian distribution)

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

   expressed in $\mu m.sec.^{-1}V^{-1}cm$ 0.20 - 1.70 $\mu m.sec.^{-1}V^{-1}cm$

6. **What should the absolute accuracy of each mobility determination be?**

   The mobility of human red cells is taken as control reference test

   99 %

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   ± 3 %

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   5' intervals, Yes

9. **Over what temperature range should the AAEF be capable of operating?**

   25 ± 0.01°C

10. Over what range of pH and ionic strength would you like to operate?

pH 7.2 NaCl 0.145 M and/or 0.168 M

11. Are there any unusual limitations on the types of materials to which your sample can be exposed during electrophoresis?

No

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Both possibilities

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

I would be very obliged to you if you could give me more informations about the apparatus, his price and availability.

Dr. D. SABOLOVIC

I. N. S. E. R. M.
Unité de Cancérologie Expérimentale
et de Radiobiologie - U 95
Plateau de Brabois
54500 VANDŒUVRE (France)
Tél. 55.52.01

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^5 cells

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   3000 cells

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   50μ ~ 0.1μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   standard deviation, frequency distribution (percentage ± 95% confidence limit)

5. What are the highest and lowest absolute mobility values you would wish to measure?

   $-0.3 \sim -1.3\ \mu \cdot sec^{-1} \cdot V^{-1} \cdot cm$

6. What should the absolute accuracy of each mobility determination be?

   ± 5% ~ ± 1%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   $0.001\ \mu \cdot sec^{-1} \cdot V^{-1} \cdot cm$

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   about 5 minutes. higher speed is not important for me.

9. Over what temperature range should the AAEF be capable of operating?

   0°C ~ 42°C

10. Over what range of pH and ionic strength would you like to operate?

7.0 ~ 8.0

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

-nil-

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

5 minutes and ± 1% (~ ± 5%) is most desirable

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

I hope to get electrophoretically fractionated aseptic cells for the subsequent cell culture to detect colony forming ability.

Chikako Sato

CHIKAKO SATO, M.D.

DEPARTMENT OF EXPERIMENTAL RADIOLOGY
AICHI CANCER CENTER
RESEARCH INSTITUTE
CHIKUSA KU NAGOYA, JAPAN

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   $\sim 10^5$ cells should be available in the sample

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   500-1000 individual cell mobility determinations

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   0.5 μm to 30 μm diameter

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   mean, mode, standard deviation, no. of individual measurements, histogram plotting capability

5. What are the highest and lowest absolute mobility values you would wish to measure?

   0.1 μm/sec/v/cm to 5.0 μm/sec/v/cm

6. What should the absolute accuracy of each mobility determination be?

   0.03 μm/sec/v/cm

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0.10 μm/sec/v/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you? As fast as possible say $\sim$ 50 individual cell mobility determinations per minute. Speed of measurement is important because of time dependent changes in mobility.

9. Over what temperature range should the AAEF be capable of operating?

   0° - 40°C

10. Over what range of pH and ionic strength would you like to operate?

pH 1.0-12.0 uni-univalent ion concentration range 1.0 M - 0.001 M

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Surface should be non-adhesive to biological cells and not produce activation of biological systems eg. platelets.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

Slower but more accurate

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

The development of such an instrument would be invaluable in several areas including (1) the electrokinetic characterization of lymphocyte subpopulations, (2) documentation of the electrophoretic behavior of blood platelets on exposure to a variety of pharmaceutical agents. Studies involving platelets have been difficult to impossible because of time dependent changes in their electrophoretic mobility, and (3) kinetic studies of cellular growth rates, cell cycle changes and regeneration of modified cell surfaces of cultured or isolated biological cells by electrophoretic means.

G.V.F. Seaman

Name and address

G.V.F. Seaman
Department of Neurology
University of Oregon Health Sciences Center
Portland, Oregon 97201

At the moment I am no longer engaged in investigations using cell electrophoresis. Therefore I can only make suggestions from my previous experiences with preparative electrophoretic separation using the apparatus of Hannig and Zeiller. From these I would like to make the following remarks:

The main question is whether or not electrophoretic mobility values can be correlated to biological properties. Therefore the use of analytical cell electrophoresis seems to be only worthwhile when well defined cell suspensions or cells separated by other separation methods are checked whether they are homogenous or not. The effort should be to develop further the preparative facilities. Analytical methods could then be used to decide whether or not a preparative electrophoretic separation would be worthwhile or not.

Dr.med. Günther Stein
Univ. Ulm
Center of Basic Clinical Research
Dept. Clinical Physiologie
Parstr. 10-11
79 U l m , West Germany

new address

Dr.med. Günther Stein
II. Frauenklinik der
Universität München
Lindwurmstrasse 2
8 München, Germany

centre régional de transfusion sanguine et d'hématologie

DIRECTEUR PROFESSEUR F STREIFF

N.R.: CG/75518

Dr D.E. BROOKS
Department of Neurology
University of Oregon Health Sciences Center
Portland, Oregon 97201

December 06, 1975

Dear Doctor BROOKS,

Thank you for your letter of November 24th concerning liquid phase electrophoresis. Your idea seems very interesting to me and I send you back the form duly filled.

I shall very interested by the result of your inquiry and should like to know the position of the NASA.

Remaining at your disposal for any questions you would like to ask me,

Sincerely Yours.

Dr J.F. STOLTZ

9 - 11, RUE LIONNOIS - 54 - NANCY - TÉLÉPHONE 52.80.84 - 52.80.87 - 52.80.07 - TÉLEX 850-20

CENTRE HOSPITALIER REGIONAL DE NANCY

29, Avenue du Maréchal de Lattre de Tassigny, 29
Case officielle N° 134 54037 Nancy Cedex

SERVICE
DE RÉANIMATION
PROFESSEUR A. LARCAN

TÉL. : 24.69.89 - 24.00.34
(lignes groupées)
Poste 492

Nancy, 8 Décembre 1975

Dr D.E. BROOKS
Department of Neurology
University of Oregon Health Sciences Center
PORTLAND, Oregon 97201

Dear Doctor Brooks,

I received your very interesting letter concerning the apparatus for the study of electrophoretic mobility of blood components.

You had sent the same letter to my coworker M. STOLTZ. I quite agree with the terms of his answer. However, if you desire that I also complete the specification forms, please tell it to me, but I shall answer strictly in the same way as M. STOLTZ. Therefore, will you consider that Dr Stoltz's answer has been made in his name and in my own name.

Yours sincerely

Professeur A. LARCAN

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   volume 0,5 to 1 cc N = 5 000 cells/ml

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal? a) 100 for suspension of one ty... cell
   b) 500 to 1000 if several popul...

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   1 μ - 20 μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Mobility distribution curve

5. What are the highest and lowest absolute mobility values you would wish to measure?

   0,4 μ /s/V/cm - 3 μ /s/V/cm

6. What should the absolute accuracy of each mobility determination be?

   0,01 - 0,02 μ/s/V/cm

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0,05 μ /s/V/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   Rapidity (10 ...) for case a) and 1' for case b)
   The speed is important for kinetic measurements

9. Over what temperature range should the AAEF be capable of operating?

   0° - 40°

10. Over what range of pH and ionic strength would you like to operate?

2 < pH < 11
0,01 < I < 0,2

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

Nô

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

between both !! Precision ± 1 % - Time 3 to 5 minuts

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Dr J.F. STOLTZ
Chairman of Hemorheology research
Laboratory
Centre Régional de Transfusion Sanguine
Bureau de Poste de Brabois
Route Nationale 74
54500 - VANDOEUVRE-LES-NANCY France

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?**

   10^5

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?**

   10^4

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   1.0 μm - 50 μm

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   If microscopic method: complete parabola fit with S.D.s of all coefficients
   If laser-doppler method: frequency peak heights, widths, and areas plus graphics
   If bulk method optical scan transformed to cell number or mass, peak widths, heights, areas, graphics.

5. **What are the highest and lowest absolute mobility values you would wish to measure?**

   0.00 - 4.00 μm-cm/V-sec

6. **What should the absolute accuracy of each mobility determination be?**

   Accuracy 5%
   Precision 1%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   3%- 5%

   or about 0.04 μm-cm/V-sec

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?**

   15 min or less per measurement. Sometimes this is important.

9. **Over what temperature range should the AAEF be capable of operating?**

   1°C - 45°C

10. **Over what range of pH and ionic strength would you like to operate?**

pH 1.0 - 10.0, $\Gamma/2$ = 0.001 - 0.20

11. **Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?**

Exposure to neutral polymers in solution should be optional and unnecessary.

Polyvalent electrolysis products should be avoided.

12. **Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?**

Both options should be available, depending upon the application.

13. **Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?**

The problem of detecting small electrophoretic subpopulations of particles is an important one; therefore, it is important to rapidly determine the mobilities of large numbers of particles. There must be low noise and population distribution widths that do not for any technical reason exceed those that are determined by nature. If the facility will be very expensive to build an early start should be made on the development of a broadly-based user program with clearly-defined user policies and some indication from the granting agencies that travel funds will be available to investigators whose projects include use of the facility. I'll be glad to contribute whatever knowledge I can from my experience with users groups.

Paul Todd

Paul Todd
618 Life Sciences Building
University Park, Pennsylvania 16802

Name and address

Glaxo

Glaxo Research Ltd, Sefton Park, Stoke Poges, SL2 4DZ

Telephone: Fulmer 2121
Registered Offices:
Greenford, Middlesex

5th December 1975

Please address reply to:

Mr E.G. Tomich

Dr D.E. Brooks
Department of Neurology
University of Oregon Health Sciences Center
Portland
Oregon 97201
USA

Dear Dr Brooks,

Thank you for your letter of November 24.

I have answered your questionnaire as fully as possible, but you will see that my experience in electrophoretic mobility studies is limited to mammalian platelets. However, because of the leading role played by platelets in the initial stage of the aetiology of arterial thrombogenesis, interest in platelet behaviour is world-wide and increasing very rapidly.

I firmly believe that, when an effective antithrombotic agent is found and unequivocably confirmed clinically, its mode of action will be found to depend on the nature and degree of the electric charges it produces on the platelet membrane (direct or mediated through some blood component) and/or on the damaged endothelium.

Hoping that my answers will be of some assistance to you,

I am,

Yours sincerely,

E.G. Tomich

E.G. Tomich, B.Sc., F.R.I.C.,
F.I.Biol.

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   see under Q 13

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   The more the better but not less than 10

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   1-5 μ

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   Standard Deviation + Error ± Comp Mean

5. What are the highest and lowest absolute mobility values you would wish to measure?

   5- 30 μ/sec/V/cm

6. What should the absolute accuracy of each mobility determination be?

   see under Q.13.

7. What is the minimum mobility difference the AAEF should be able to resolve?

   ca 0.3 μ/sec/V/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   see under Q13

9. Over what temperature range should the AAEF be capable of operating?

   25- 40°C

10. Over what range of pH and ionic strength would you like to operate?

pH 7.4 ionic strength of human platelet-rich plasma

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

I restrict my samples to exposure to glass and platinum only. Other workers have used silver and copper electrodes

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

definitely the latter

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Q1 My experience in this field has been limited to the study of the effects of voltage gradient on the electrophoretic mobility of human platelets in plasma containing various concentrations of adenosine diphosphate or noradrenaline, and to the effects of new compounds on rabbit platelet electrophoretic mobility and on the changes produced by various concentrations of ADP and noradrenaline.
I have used the Rank apparatus which requires about 5 ml of sample (platelet-rich plasma is diluted 1→10 with platelet poor plasma giving PRP-PPP) to fill the micro electrophoresis cell. This is a large volume and severely restricts the number of experiments that can be done on a given volume of plasma, usually about 40 ml (obtained from 100 ml blood from one individual)

Q5. Absolute accuracy is not of prime importance, as altered mobilities are expressed as percentages of the control mobility, but reproducibility is of paramount importance. Determination and comparison of electrophoretic mobilities of a given cell type in different species would require absolute accuracy.

Q8. Determination of the mobilities of say 20 individual platelets in one sample of PRP-PPP takes about 15 min and the interval between tests is about 5 min. Reductions in these times would be very welcome. Zero gravity would greatly facilitate and expedite the conducting of the type of experiments I have described. An automated apparatus would possess the great advantages of eliminating person error and eye strain.

Name and address

E. G. Tomich
Glaxo Research Ltd.
Sefton Park
Stoke Poges, Buckinghamshire, England

I hope that this meagre information may be of some use to you!

VANDERBILT UNIVERSITY

NASHVILLE, TENNESSEE 37232 TELEPHONE (615) 322-7311

School of Medicine • Direct phone 322-3304

February 24, 1976

Dr. D.E. Brooks
Department of Neurology
University of Oregan Health
Sciences Center
Portland, Oregan 97201

Dear Dr. Brooks:

As your form was sent to Amsterdam I did not receive it before February 23.

I would be interested if you could keep me informed about the progress of this project.

Yours truly,

Chris J. van Boxtel, M.D.

CJvB:pc
Enclosure

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   10^4

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   200 or more

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   0,5 - 100 μ diameter

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   medium, mean, standard deviation, standard error

5. What are the highest and lowest absolute mobility values you would wish to measure?

   0.5 - 20 μ/S/V/cm

6. What should the absolute accuracy of each mobility determination be?

   ± 2%

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0.01 μ/S/V/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   No

9. Over what temperature range should the AAEF be capable of operating?

   Most systems appeared not to be temperature dependent

10. Over what range of pH and ionic strength would you like to operate?

Physiologic

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

---

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

slow and more accurate

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

Provisions for connecting the AAEF to an automatic all-separation device could be useful.

I feel that there are many applications for this instrument in medical research.

Chris J. van Boxtel, M.D.
Visiting Scientist
Dept. Pharmacology
Vanderbilt University
School of Medicine
Nashville, Tennessee 37232
U.S.A.

Name and address

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. **What is the smallest total sample size (number of cells) that you would wish to examine?** Some hundred cells. This would allow serial determinations in immunodeficient babies.

2. **How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?** For the mixed population of the spleen at least two hundred cells, depending of the accuracy of the apparatus

3. **What are the diameters of the largest and smallest particles you would like to examine electrophoretically?**

   4- 25 µm

4. **What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?**

   Student's t-test, Wilcoxons test for paired distributions. A test for the standard deviation of the mean.

5. **What are the highest and lowest absolute mobility values you would wish to measure?** Depends on the suspension medium. In saline about 1,5-0.3 µm/sec/V/cm

6. **What should the absolute accuracy of each mobility determination be?**

   1-5%

7. **What is the minimum mobility difference the AAEF should be able to resolve?**

   1-5%

8. **How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?** Hundred determinations in max. 10 min. Interval of max. 2 min for changing of cell suspensions. The interval of changing [illegible] speed of measurement.

9. **Over what temperature range should the AAEF be capable of operating?**

   + 4°C - + 37°C

10. **Over what range of pH and ionic strength would you like to operate?**

pH 5 - pH 10

11. **Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?**

12. **Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?** If determinations means the scoring of the labeled cells I would rather prefer the slower and more accurate type, if it means the scoring of one cell I would prefer the latter.

13. **Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?**

EPM scoring with the Zeiss Cytopherometer has several disadvantages:

Need for a great number of cells. Time consuming. Low reproducibility. Difficult to obtain stable conditions for scorring.

While the latter is seeing taken care of by the zero gravity I also think the former disadvantages must be dealt with if the technique shou d be any useful.

As for the EPM determinations of lymphoid cells I think an accuracy of at least 1% must be obtained if the AAEF should be able to provide new informations.

Yours sincerely

Johan N. Wiig
5016 Haukeland Hospital
Norway

P.S. Sorry for my late answering.

Name and address

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SERVICE DE BIOPHYSIQUE

PR. G. MILHAUD
PR. AGR. B. MENSCH

Paris, 12th December 1975

Dr. D.E. BROOKS
Department of Neurology
University of Oregon Health
Sciences Center

PORTLAND OREGON 97201

Dear Dr. Brooks,

Many thanks for including my name in the electrophoresis user community. I am very interested in the development of the AAEF, I hope you will succeed in the setting up of such an apparatus.

You will find here enclosed the comments you requested.

Sincerely yours,

M. Wioland

Dr. M. WIOLAND

OPERATING SPECIFICATIONS FOR AN AUTOMATED ANALYTICAL ELECTROPHORESIS FACILITY

Please provide the values for the parameters given below which would best suit your applications. Any comments or additional requirements are welcomed; please include them in response to question 13.

1. What is the smallest total sample size (number of cells) that you would wish to examine?

   150 cells

2. How many individual cell mobility determinations would be required in any one suspending medium to electrophoretically characterize the most complex population with which you would like to deal?

   250 cells

3. What are the diameters of the largest and smallest particles you would like to examine electrophoretically?

   20 to 3 μm

4. What statistical parameters should be provided by the AAEF to adequately describe the mobility distribution of your cell populations?

   The complete histogram. Otherwise, the modal values and the mean value ± 1 S.D. of the cell mobilities

5. What are the highest and lowest absolute mobility values you would wish to measure?

   2,50 to 0.30 μm/sec/V/cm

6. What should the absolute accuracy of each mobility determination be?

   0.05 μm/sec/V/cm

7. What is the minimum mobility difference the AAEF should be able to resolve?

   0.20 μm/sec/V/cm

8. How rapidly, and at what intervals, should the electrophoretic characterization described in question 2 be carried out? Is speed of measurement important to you?

   See comments

9. Over what temperature range should the AAEF be capable of operating?

   4°C → 40°C

10. Over what range of pH and ionic strength would you like to operate?

pH 7.2 $0.005 < I < 0.145$

11. Are there any unusual limitations on the types of materials to which your samples can be exposed during electrophoresis?

No.

12. Would you rather have an apparatus that produced rapid (1 per minute) but approximate (± 5%) mobility spectra or one that was slower (15 minutes per determination) but more accurate (± 0.1%)?

The second apparatus

13. Do you have any other requirements that should be considered in providing specifications for the AAEF? Do you think the development of such an instrument is worthwhile? Do you have any additional comments?

The differences in the cell mobility occurring immediately the treatment of living cells will be small. Therefore, a slow apparatus giving accurate data may be perhaps more useful, even if the very rapid kinetic of the phenomenon is lost.

The development of such an apparatus is worthwhile. I would appreciate being aware of its future.

May I ask you to include my name in your mailing list!

Sincerely yours

M. Wioland

M. WIOLAND
Name and address
LABORATOIRE DE BIOPHYSIQUE
CENTRE HOSPITALO-UNIVERSITAIRE SAINT-ANTOINE
27 RUE CHALIGNY
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My new address.