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VELOCITY GRADIENT STABILISED, CONTINUOUS, FREE FLOW ELECTROPHORESIS. A REVIEW.

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SUMMARY

A new technique for continuous preparative electrophoresis based on the concept of J. St.L. Philpot[1] is described, in which electrophoresis is carried out in free solution in an annulus, the voltage gradient being perpendicular to the direction of flow. Stable annular flow is maintained by a gradient of angular velocity created by rotation of the outer wall of the annulus while the inner wall is held stationary.

The free-flow conditions enable a wide range of biologically active materials, from small molecules to whole cells, to be fractionated with virtually all the injected material being recovered in up to 30 fractions which are collected simultaneously. Sample throughout can be varied up to at least 20ml/min in the system described allowing up to 0.4g/min protein or 10^9 cells/min to be processed with good resolution and excellent recovery of biological activity. The system is essentially adiabatic, and a temperature rise of 20-25^{o} occurs during passage through the separator. However, since the residence time is very short (30-60 seconds), loss of biological activity is minimised. The separator has been operated continuously for periods of up to at least 60

hours, and conditions remain stable with minimal attention. In addition, maintenance requirements are minimal. Operating conditions can be varied over a pH range of at least 3-11 at conductivities up to $\sim$2mS cm^{-1}.

A mathematical model of the electrophoretic system has been developed and describes well its performance and scale-up potential. Three separators with different dimensions have been constructed and operated successfully, and systematic studies of throughput and resolution show that the system has significant further development potential.

A number of applications ranging from the separation of small molecules to whole cells are described illustrating its applicability as a general laboratory fractionation system as well as for the production of biologically active materials on an industrial scale.

Further work is in progress aimed at improving resolution and throughput, developing applications (including operation under sterile conditions) and simplifying design.

1. INTRODUCTION

Cells contain very complex mixtures of macromolecules and sub-cellular particles with a wide range of sizes and properties. Even ostensibly homogeneous cell populations (e.g. non-synchronised cells of a single strain of micro organism) display different properties at different stages of the cell cycle, and animal cell populations as generally obtained are frequently heterogeneous (e.g. blood cells). The biologist has a wide range of techniques for separating proteins, nucleic acids etc., on the laboratory scale although there are fewer methods for fractionating cells and sub-cellular particles. The main problem is how to combine these techniques to achieve the maximal purity of a single component. However, on a production scale (> 10gm/day) the choice is much more limited, and even more so at the scale required for the commercial isolation of natural products from animal and plant tissues and

living organisms in culture. At this level, high recovery of biological activity is essential to minimise unit costs, and available techniques are limited largely to precipitation methods, though recent work has highlighted the possibility of solvent extraction.

However, the acceptability of procedures which rely on addition of 'foreign' materials such as polyelectrolytes, or potentially toxic ions is becoming increasingly doubtful, while operation under hygienic or sterile conditions where the products are to be used therapeutically or as foodstuffs, raised significant problems for some of the purification systems currently available.

Electrophoresis is used universally in research and analytical laboratories because of the very high resolution possible (when supporting media such as polyacrylamide gels are used[2]), and the relatively mild conditions generally required although the most highly resolving electrophoretic techniques such as isoelectric focusing[3] and polyacrylamide gel electrophoresis in the presence of detergents[4], generally cause inactivation.

Because of the obvious advantages of electrophoresis, intensive efforts have been made over the past three decades to scale up the technique for small scale production in the research and development laboratory, and for use on an even larger scale in industry. The main problem of scaling up electrophoresis is maintaining stability of free-flow systems against convective disturbances caused by Joule heating. A number of ways of overcoming this problem on a preparative scale have been investigated, including:-

(a) Free-flow electrophoresis, such as multi-membrane decantation[5] or electrophoresis-convection[6]. Electro-decantation has had success in the commercial production of rubber latex[7]. However, in general, this and other free-flow techniques are not widely used for the separation of biologically active materials because of operational problems and the low resolution obtainable for all but a few components[5].

(b) Separation in a thin free-flowing film in a system with very efficient cooling (Hannig system)[8]. This has found a useful place for separation of cells on a small scale (throughputs of 1-5ml/hour).

(c) Flow stabilisation by enhancing viscosity[9] or by using density gradients[10]. Neither of these approaches are used widely.

(d) Isoelectric focusing in a multi-chamber system with continuous flow[11]. An intermediate scale of operation has been investigated, but the ampholytes required to generate the pH gradient are expensive, and are difficult to remove from the products.

(e) Using hollow fibre membranes with an electrical field perpendicular to the fibre axis[12]. This has been investigated only on a very small scale so far.

To date, none of these systems has provided a fully adequate solution for use on a preparative scale.

In this paper we describe a high-throughput, continuous, zone electrophoresis system, in which laminar flow is stabilised by a gradient of angular velocity. The system has been operated successfully over a number of years at throughputs of up to 0.4g protein/min and 10^9 cells/min. Versions with throughputs one-third and three times that of the separator described have been built and operated successfully. Further scale up is being investigated. The system goes significantly further than previous techniques in providing a versatile production scale system and is being evaluated for use in the biological industries.

2. SEPARATOR DESIGN AND OPERATION

The continuous electrophoretic separator described in this review is designed to enable electrophoresis of mixtures to be carried out in free solution without the constraints imposed by the use of supporting anti-convective material. The usual convective turbulence effects caused by Joule heating during electrophoresis

in free solution have been minimised successfully by applying a gradient of angular velocity at right angles to the direction of carrier flow, the sample being layered on the carrier as a discrete zone. This arrangement is accomplished by carrying out electrophoresis in an annulus bounded on its inner wall by a stationary cylinder (stator) and on its outside by a rotating cylinder (rotor). A conducting buffer of low ionic strength flows continuously through the annulus, and the sample to be separated (the migrant) is introduced into the carrier stream as a thin discrete zone at the lower end of the stator. The voltage gradient, which is perpendicular to the direction of carrier flow, causes individual components in the migrant to move out into the carrier stream as discrete zones, to a distance dependent on their mobilities and the conditions selected. The carrier fluid containing the separated zones can be withdrawn from the annulus and collected separately and simultaneously. A prototype separator based on this concept has been operated for several years and enables the continuous separation of up to 25g of protein mixture/hour into about 20 fractions, each of which is collected simultaneously.

2.i. Design

The external appearance and main features of the design are shown in Figures 1 and 2. The overall dimensions of the separator module are ∿ 2m x 0.5m x 0.5m, the separator unit being mounted ∿ 1m above floor level to facilitate access and to enable the drive motor to be coupled directly with the central axis of the unit. The electrode section is 30.5cm long, the stator diameter is 8.0cm and the diameter of the inner wall of the rotor is 9.0cm, giving an annular width of 0.5cm. The rotor unit is coupled directly to the drive which is mounted beneath the metal base plate. The stator fits inside the rotor and is positioned accurately at its upper and lower ends by bearing and seal assemblies which allow free rotation of the rotor with minimal leakage of fluid between the various compartments.

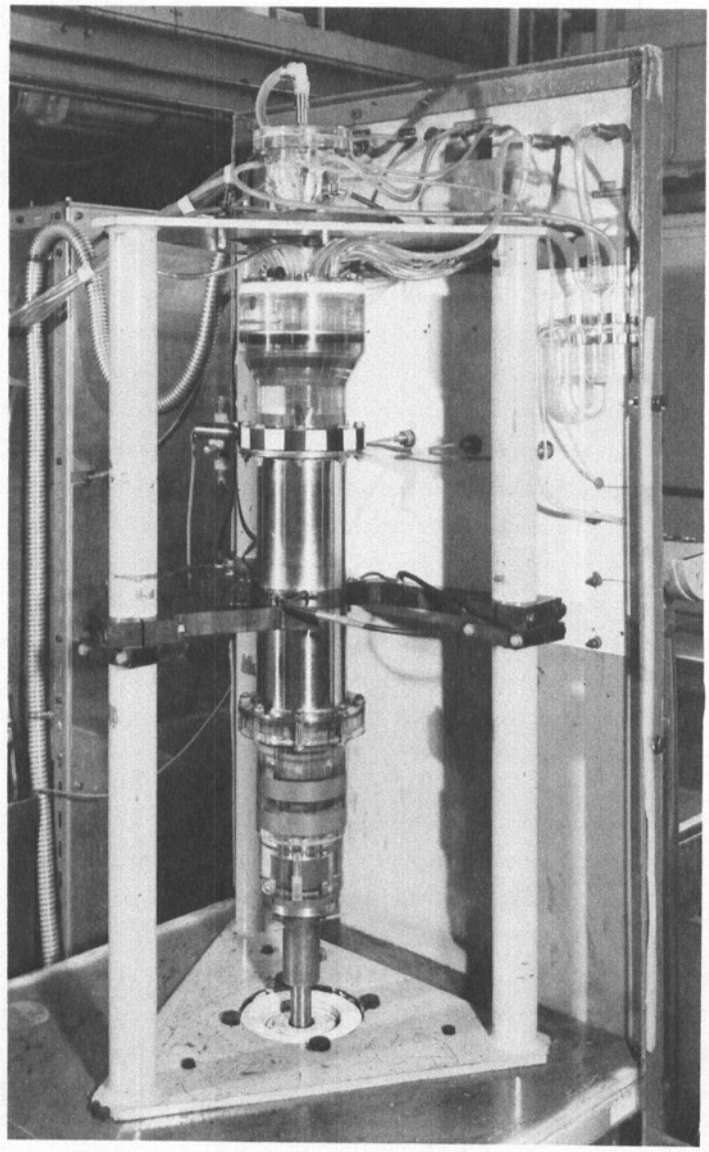

FIG. 1 View of the continuous flow electrophoretic separator showing the outer appearance of the system. The stainless steel outer electrode can be seen with carbon brushes making contact from supports on the frame. Inlet and outlet tubes can be seen entering the top of the separator. The height of the separator is ∿1m.

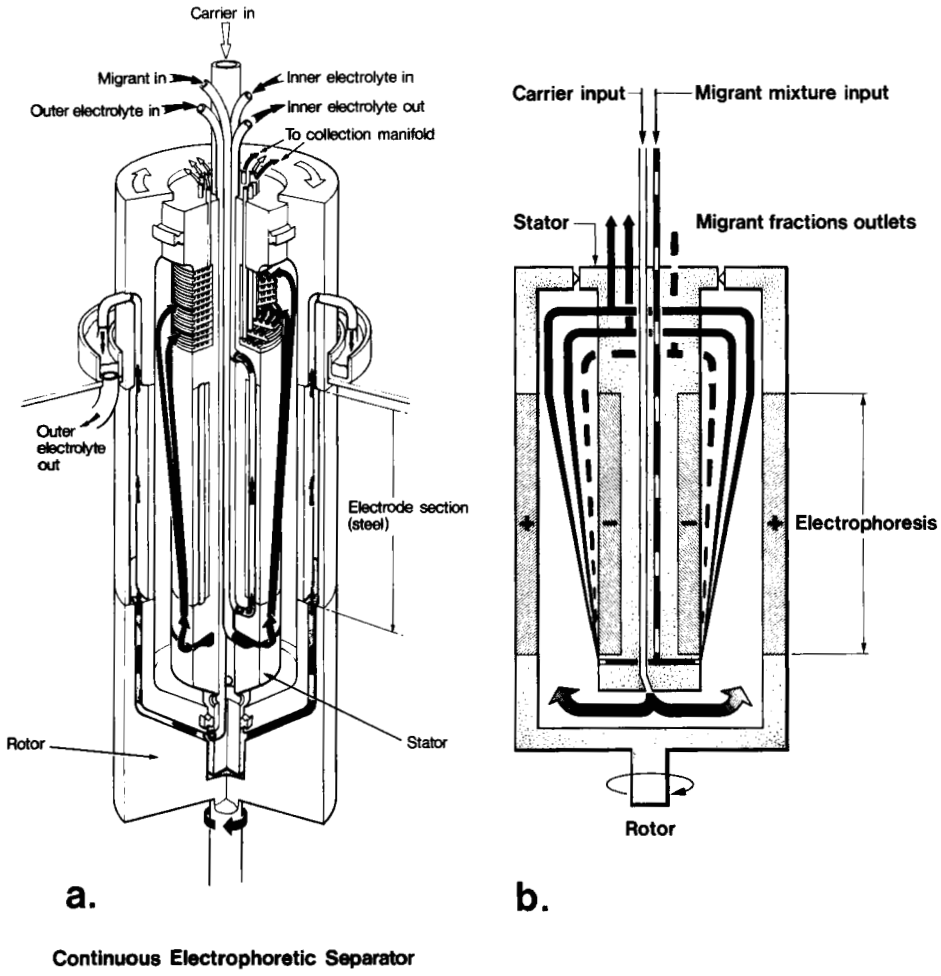

FIG. 2 Diagrammatic representation of the continuous flow electrophoretic separator. a. is a cutaway drawing of the system showing the construction of the rotor, stator and main flow channels. b. is a diagram of the system to demonstrate the separation of three components as they are carried up through the annulus.

The rotor consists of a central electrode section bounded above and below by perspex sections housing the bearings and seals, and the entry and exit systems for the electrolyte compartments. The electrode section of the rotor is made up of an outer stainless steel cylinder and a rigid porous inner cylinder which forms the outer wall of the annulus, the chamber between the two forming the outer electrolyte chamber. Through this chamber is pumped concentrated electrolyte solution. A semi-permeable regenerated cellulose membrane is mounted on the outside of the porous cylinder. This arrangement enables electrical continuity to be maintained across the annulus while electrolyte gases and electrolysis products are removed continuously, thus avoiding turbulence in the annulus due to gas bubbles and minimising inactivation of migrant species by electrolysis products. The lower perspex housing contains the lower drive bearing and a channel to supply buffer to the electrode chamber from tubes passing down the stator (see below). The upper perspex housing contains the exit tubes from the electrode chamber through which the used electrolyte and gas emerge, the former being collected in a stationary circumferential trough fitted round the top of the stator. In addition, an upper bearing and seal centralises the rotor on the stator.

The stator assembly is similar to that of the rotor but with an inner stainless steel electrode and an outer porous cylinder with semi-permeable membrane mounted on its outer surface which forms the inner wall of the annulus. The inner electrolyte compartment is supplied continuously with a suitable high conductivity electrolyte which, after use, flows out of the separator via an exit tube passing upwards through the centre of the stator body. It should be noted that since hydrogen and oxygen are generated in significant quantities during electrophoresis, it is necessary to take precautions to ensure their safe removal.

Below the electrode section in the stator, there is a thin (0.1mm) circumferential slit designed to enable the migrant

solution to be introduced continuously and at a uniform rate around the whole circumference of the stator assembly. At the bottom of the stator are inlets for the main carrier flow and below this are located the bearing and seals which allow free rotation of the rotor. The assembly for splitting the annular flow into fractions is mounted above the electrode section of the stator. It consists of a stack of 30 discs of equal diameter. The discs are made of perspex and into each is cut a series of concentric channels linked radially at intervals to create a maze-like channel system. Around the circumference of each disc is a continuous groove which communicates via the maze with a single hole near its inner edge. Into this hole is sealed a tube which passes directly out of the separator to the fraction collection system. Each disc is connected to a separate exit port. The maze arrangement in the collecting discs is designed to ensure that the path length from any point on the circumference of any disc to the central collection point in the disc is almost identical, so that equal pressure is maintained around the disc thus ensuring uniform collection of fluid around the whole annulus. Provided smooth laminar flow is maintained in the annulus, each take-off disc will "peel off" a thin layer of liquid from the annulus so that the lowest disc will collect the layer flowing immediately next to the stator wall, the next higher disc, the layer next to that, and so on. By this means, annular flow can be divided into 30 radial fractions with minimal mixing. The outlet tubes emerge through the top plate of the stator and are connected to the fraction collection system.

The upper bearing and seal assembly are located above the outlet disc stack, while the tubes through which carrier, migrant and inner and outer electrolyte are introduced into the separator are housed in a tube positioned in the centre of the stator. The carrier lubricates the upper and lower bearings.

The thin semi-permeable membranes are mounted on polymer-impregnated filter candles (Cuno), machined when dry to the

required dimensions in order to ensure sufficient rigidity and concentricity of the annulus. The filter candles are hydrophilic and highly porous yet sufficiently rigid when wet to define accurately the annular gap. The filter-candle-supported membrane units are clamped into the rotor and stator assemblies and are sealed at their ends to avoid cross-leakage of fluid.

2.ii. Operation

For electrophoresis, four solutions are required, viz. carrier buffer, migrant solution and inner and outer electrolytes. The carrier solution is prepared in bulk to minimise variations in buffer conductivity. The carrier pH can be varied within the range 3-11 while the conductivities used have been generally within the range 0.5 - 2mScm^{-1}. Constant carrier flow (usually $\sim$ 500ml/min) is achieved with a constant head device operating at about 3m water pressure.

The composition of the electrolytes pumped through the electrode chambers can be varied depending on the application, but are normally high conductivity solutions ($\sim$ 35-55mScm^{-1}) to minimise changes in hydrogen ion concentration caused by electrolysis. Their flow rates are not critical and are usually maintained at $\sim$ 50ml/min.

To minimise the temperature at the outlets, the input carrier and electrolyte solutions are passed through a cooler (at 2-3^{o}C). The carrier is fed into the separator through the central stator tube and enters the annulus at the bottom of the stator. It flows upwards through the annulus past the electrode section where electrophoretic separation takes place and leaves the separator through the 30 maze discs.

In order to maintain stabilised laminar flow in the annulus during electrophoresis, the rotor is spun at $\sim$ 150 r.p.m., which is sufficient to overcome any major turbulence caused by density changes due to Joule heating. At the top of the annulus the carrier flow is segmented into 30 separate fractions which are collected simultaneously via the disc stack system.

It should be borne in mind however, that flow through the annulus is essentially parabolic with the highest flow rate in the centre and the lowest at the annulus walls. As a result, residence time varies with position across the annulus and lies between ∿ 30 and 60 sec.

In order that the maze discs collect fluid from equal distances across the annulus, the outlet flows from each disc is adjusted to reflect this parabolic pattern. To achieve this each outer tube is provided with an adjustable valve which can be used to alter the distribution of flow from the outlets but without affecting flow within the annulus or the overall resolution of the separator. Because of this, the collection of particular fractions can be optimised (see Section 3.i.(b)).

The electrolyte solutions are fed into the separator via two tubes which pass down the centre of the stator. The inner electrolyte solution passes into the lower end of the stator chamber and emerges from the top to waste. The outer electrolyte solution flows into the rotor via a gallery at the base of the stator which is sealed off from the carrier flow. The electrolyte then flows up through the rotor electrode chamber and emerges from the top of the chamber into the circumferential waste collection trough. Power is supplied via carbon brushes to the rotor, the outer wall of which is stainless steel, and to the inner electrode via an appropriate contact passing down the centre of the stator. Once the system is equilibrated, a constant current of up to ∿ 30A can be applied giving a voltage of up to ∿ 30V. The exact values will obviously vary depending on many factors including the conductivity and flow rates used and the mobilities of the ions in the system. The carrier solution entering the separator at 2-3^{o}C is warmed by Joule heating to ∿ 20^{o}C during electrophoresis (∿ 30-60 seconds residence time).

Once a steady state has been reached with power on (usually 10-15 minutes), the migrant solution is injected through the migrant injection slit at the bottom of the stator. In the

absence of a voltage gradient, the migrant enters as a thin layer of liquid around the whole circumference of the stator and is carried upwards by the carrier flow to the lowest of the exit ports of the main stack, i.e. there is no migration into the carrier stream. With power applied and conditions adjusted so that the migrant components move away from the stator as they pass through the electrode section, individual components will migrate a distance which will depend on their particular mobilities. At the top of the electrode section, a cross section of the annulus will show the separated species as a series of fine concentric rings around the annulus. These are then collected in up to 30 fractions as they flow out through the disc collection system.

Normally, to obtain satisfactory separation, the density, viscosity and conductivity of the migrant stream should be as close as possible to that of the carrier, and at a suitable concentration (usually < 50 mg/ml protein). The most suitable injection rate is usually 10-20 ml/min (0.1-0.4 gm/min protein).

Electrophoresis in the system causes a 5-10-fold dilution which is similar to other fractionation techniques such as ion exchange chromatography. Normally, ultrafiltration, precipitation or adsorption methods are effective for re-concentrating the separated components, particularly since the fractions emerge in a low ionic strength buffer.

2.iii. Fraction Collection

Fractions are collected directly from outlet tubes in a variety of containers (from test tubes to 5L bottles), according to experimental requirements (Figure 3). Provision can be made for cooling fractions immediately after collection.

2.iv. Monitoring Fractions

Clearly, since the fractionation system has such a rapid throughput, it is necessary to monitor the separation as quickly as possible. With components which adsorb visible or ultra violet light, this can be done by pumping aliquots from each of the outlet tubes in turn through a flow cell in a spectophotometer, the output

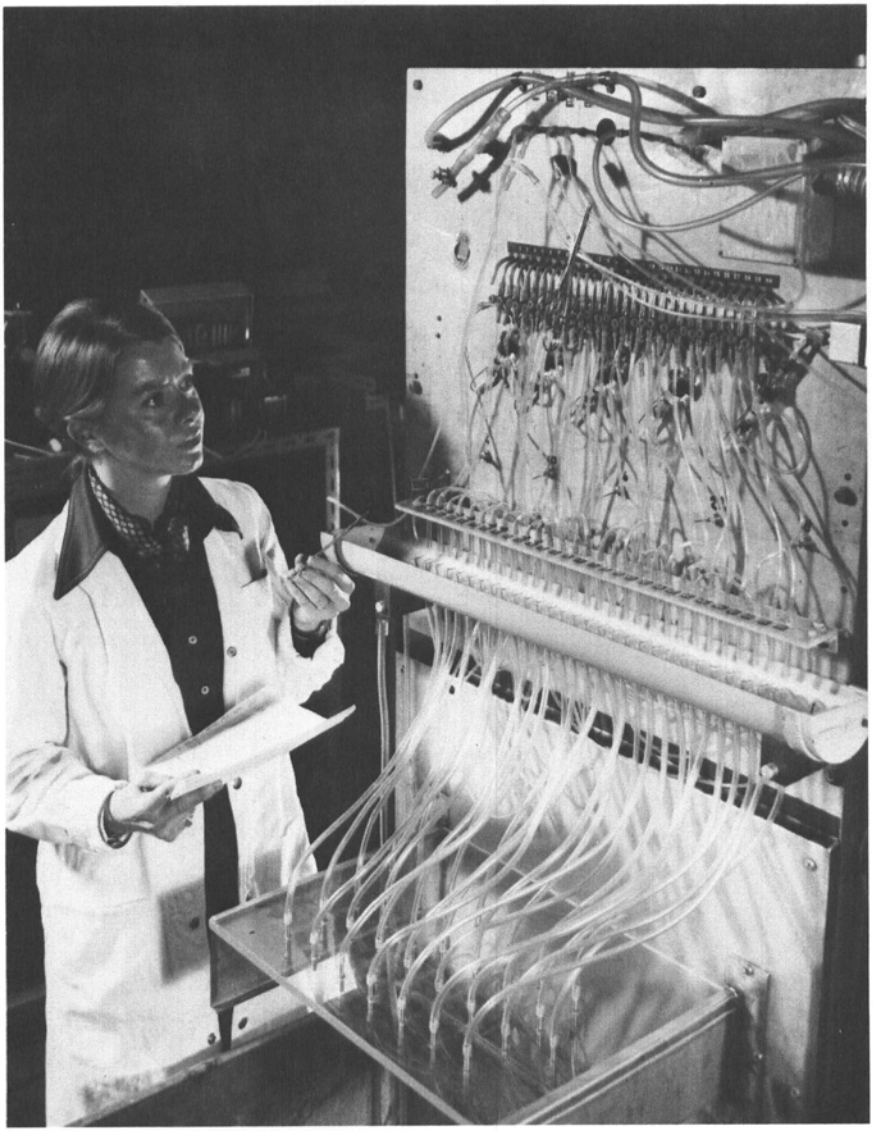

FIG. 3 View showing the fraction collection system of the separator. The 30 fractions exit via tubes the flow through which can be controlled by valves. The temperature and conductivity of individual fractions can be monitored also. The flows can be directed to waste via a trough or to collection either for small volumes or for litre scale volumes.

from which can be recorded. The total scan time for all 30 fractions is generally about 10 min.

3. THEORETICAL BACKGROUND

The electrophoresis system described is extremely complex in that many of the variables are strongly interactive. Detailed studies have been carried out on the effect of these variables on its performance in order to build up an adequate understanding of the processes occurring in the system and to enable the limits of resolution, stability and throughout to be determined. These have led to the construction of a theoretical model of the system which has enabled us to design and operate successfully equipment with different throughputs.

The factors controlling separation in the system fall mainly into two categories, viz.

(i) hydrodynamic factors influencing laminar flow stability;

(ii) electrophoretic factors associated with the separation process itself.

3.i. Hydrodynamic Factors

The resolving power of any free-flow system will depend to a major degree on how well stabilised laminar flow can be maintained. In the system described here, laminar flow is preserved effectively by creating an angular velocity gradient across the annulus. This is achieved by rotating the outer wall of the annulus while keeping the inner wall stationary. It should be noted also that the flow velocity through an annulus has an approximately parabolic profile and this imposes its own limitations on the resolution possible.

The sample to be injected, the migrant, is introduced into the stabilised carrier flow through a circumferential slit in the stator wall. The volume injected, or more precisely, the ratio of migrant to carrier flow rates, determines the initial width of the migrant zone and hence the degree of resolution obtainable after electrophoresis. Both the density and viscosity of the migrant have a marked effect on resolution and must be as close as possible to that of the carrier and preferably the viscosity should be near

that of water. Figure 4 shows the distribution of a typical monocomponent protein (bovine serum albumin, B.S.A.) during electrophoresis.

Furthermore, the disc stack must be designed to ensure minimal turbulence and loss of resolution in collecting fractions from the annulus. Some of these factors are considered below.

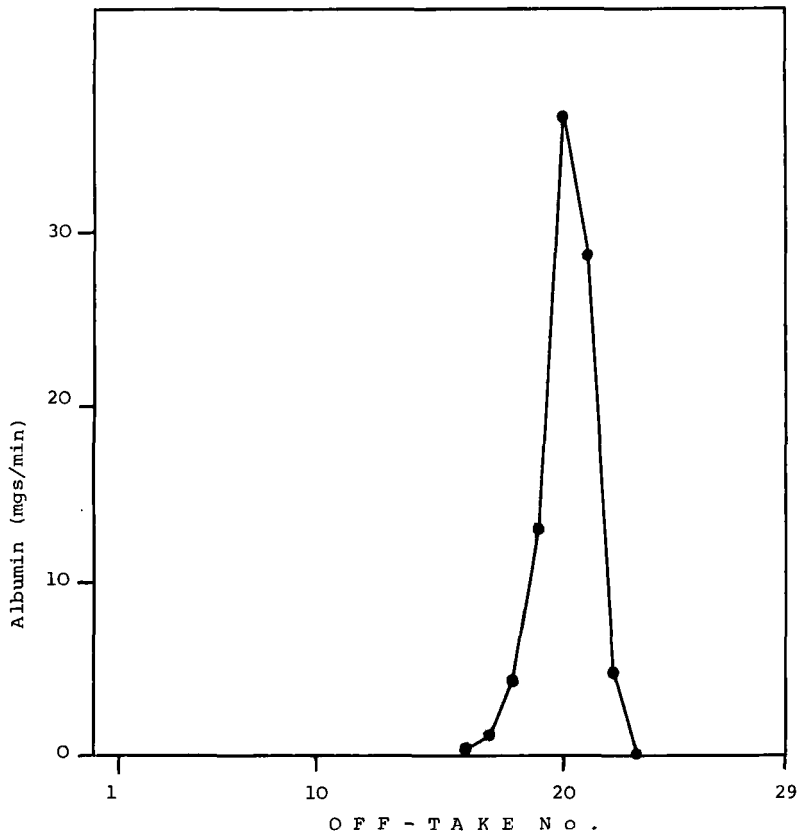

FIG. 4 Distribution of a monocomponent protein (BSA) in the fractions after electrophoresis in the continuous flow separator. Carrier - ammonium acetate pH 10.5, conductivity 1.2mScm^{-1} 600ml/min flow rate. Sample - 10mg/ml BSA, 10ml/min flow rate. 37A, 30V. Since the flow from individual offtakes reflects the parabolic flow in the annulus, the protein throughput has been expressed as mgs protein collected from each offtake/min.

(a) Rotational Stabilisation

The stabilising effect of rotation is due to inertial (Coriolis) forces and can be accounted for by the Taylor-Proudman theorem[13]. This states that all steady slow motions in a rotating inviscid fluid are necessarily two-dimensional.

Rayleigh's Criterion[14], that stratification of angular momentum for an inviscid fluid about an axis is stable only if it increases monotonically outward, is fulfilled by rotation of the outer wall of the annulus of the separator. Thus,

$$\frac{d}{dr} (r^2 \Omega)^2 > 0 \quad \text{where } r^2 \Omega \text{ is the angular momentum.}$$

The inverse condition, viz. rotation of the inner wall of the annulus (while keeping the outer stationary), produces a series of counter-rotating "Taylor vortices" which, although still well ordered and laminar, produce radial mixing and therefore is unlikely to be of use for zonal electrophoresis.

From experimental work by Taylor[15], a critical rotational Reynolds number can be associated with the ratio of annulus gap and outer radius in a system where only the outer wall rotates. It was later proved quite rigorously by Schultz-Grunow[16] that flow under such conditions was completely stable and that the turbulence often observed in practice was due to imperfections in concentricity and vibrations induced by imperfect balancing. We have detected some instability at rotational rates of about 5 r.p.s. which compares with the value of about 6.5 r.p.s. indicated by Taylor's work for an annulus of 5 mm and outer radius 4.5 cm.

The effect of rotation on zone width of a mono-component protein sample (B.S.A.) (expressed as the Standard Deviation (S.D.) is shown in Figure 5. It is apparent that a considerable improvement in S.D. is obtained even at low rotation rates while beyond $\sim$1 r.p.s. only a marginal improvement occurs. As a result we have used a rotation rate of $\sim$2.5 r.p.s. in most experiments. The centrifugal force generated ($\sim$ 1 g) has no significant effect on the electrophoretic separation of ions or small particles.

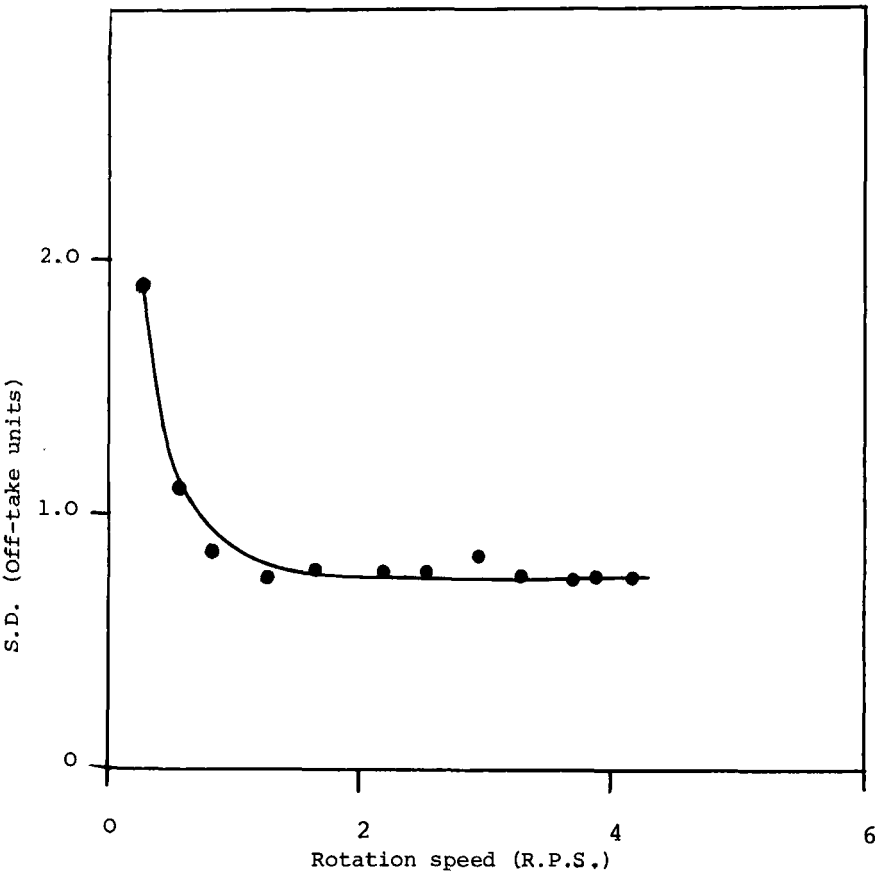

FIG. 5 The effect of rotation rate on the zone width of B.S.A. (expressed as S.D. in offtake units). Carrier - Tris citrate pH 8.5 $1mScm^{-1}$. 50A, 35V. Flow 1500ml/min. Sample - BSA 6mg/ml, 20ml/min.

(b) The Fraction Collection System

The 30 off-take discs represent a novel method for collecting small successive fractions circumferentially from a narrow (2-5 mm) annulus width with minimal flow disturbance. This method of collecting discrete circumferential elements of the carrier flow is an example of a phenomenon reported by Rayleigh[17] who demonstrated that when a revolving fluid is drawn off near a point on the axis

of rotation it is removed always from along the axis and not symmetrically in all directions as happens with no rotation. Assuming the same pressure drop through each off-take, $^{1}/30$ of the carrier flow will be removed through each successive disc. However, by means of valves on the outlets, the flow from each can be adjusted to reflect the parabolic flow in the annulus so each off-take will then represent $^{1}/30$ of the annulus width. It follows also, that by varying the outlet flows, regions of the annulus in effect can be stretched or compressed without loss of resolution so that limits for collection of certain fractions can be carefully adjusted to optimise resolution and recovery. This has been verified experimentally by electrophoresing bovine serum albumin under standardised conditions and adjusting the flow from the off-takes for a variety of profiles. The B.S.A. content of each off-take was determined as well as the flow rates, and the actual distribution of B.S.A. in the annulus was calculated for parabolic flow. The results of two examples of extreme imposed profiles show that after correction for parabolic flow, nearly identical distributions were obtained (Table 1).

(c) Migrant Injection

Clearly the width of the migrant starting zone is an important factor influencing the optimum resolution obtained in the electrophoretic system. The factors influencing it have been studied by experiments measuring the distribution of the migrant components in the outlets from the separator operated under various conditions but in the absence of an applied field. Under these conditions the observed zone width is a complex function of the behaviour of the migrant at injection and exit from the annulus as well as the effects of molecular diffusion and any turbulence during its residence in the annulus.

Experimental results confirm the intuitive picture that the migrant remains adjacent to the stator on injection and flows upwards close to the wall of the stator in the absence of an applied field, its zone width dependent on diffusion, flow rates and

TABLE 1 The effect of the imposed outlet flow profiles on sample distribution across the annulus. The carrier buffer was tris citrate pH 8.5, conductivity $1mScm^{-1}$. Flow rate - 1500ml/min. The sample was BSA (10mg/ml) injected at 20ml/min. Electrophoresis was performed at 52A.

Off-Take Number	Experimental Results		Calculated Distribution for Annular Flow	
	Imposed Outlet Flow Profile		Imposed Outlet Flow Profile	
	Linear Increase % of total	Linear Decrease % of total	Linear Increase % of total	Linear Decrease % of total
6	0.8	-	-	-
7	3.9	-	-	-
8	15.0	-	-	-
9	29.8	-	0.1	-
10	39.5	-	0.7	0.5
11	10.1	-	3.7	3.4
12	0.9	-	14.2	17.7
13	-	-	31.7	37.9
14	-	-	40.3	32.6
15	-	-	8.7	7.4
16	-	-	0.6	0.4
17	-	0.2	-	-
18	-	1.0	-	-
19	-	5.1	-	-
20	-	22.9	-	-
21	-	40.7	-	-
22	-	28.7	-	-
23	-	1.3	-	-
24	-	0.1	-	-
Mean	9.4	21.0	13.4	13.3
S.D.	1.06	0.95	1.03	0.99

turbulence. A theoretical analysis[18] has shown that, when the ratio of migrant to carrier flow (F_m/F_c) is very small

$$S_o = \frac{2}{3} a \sqrt{\frac{Fm}{3Fc}} \quad \text{and} \quad \sigma_o = \sqrt{\frac{Fm}{54Fc}}$$

where a = annulus width

S_o = initial mean value of distribution

σ_o = initial standard deviation of distribution.

The band widths observed experimentally are somewhat greater than those predicted but at a low Fm/Fc ratio approach the theoretical figure to within a variance error of 0.2 off-take widths. The results suggest therefore that there is minimal turbulence at the migrant injection slit.

3.ii. Electrophoretic Factors

In addition to the hydrodynamic constraints discussed above, there are also limitations imposed by the electrophoretic process itself which must be taken into account also in the design of electrophoretic separators using stabilised laminar flow. In this section, some of these aspects are discussed.

(a) Ionic mobility in an annulus

The radial distance (s cm) travelled by the migrant is given by

$$s = UET$$

As $E = I/\bar{K}L\Pi(2R_1 + s)$

and $T = \Pi Ls(2R_1 + s)/F_s$

then $UI = \bar{K}F_s$ (1)

Substituting for $F_s = F_c \left(3(s/a)^2 - 2(s/a)^3\right)$ (see Appendix 1)

we obtain the equation

$$s^3 - \frac{3as^2}{2} + \frac{UIa^3}{2\bar{K}F_c} = 0$$

As shown in Figure 6, a linear relationship is obtained for the

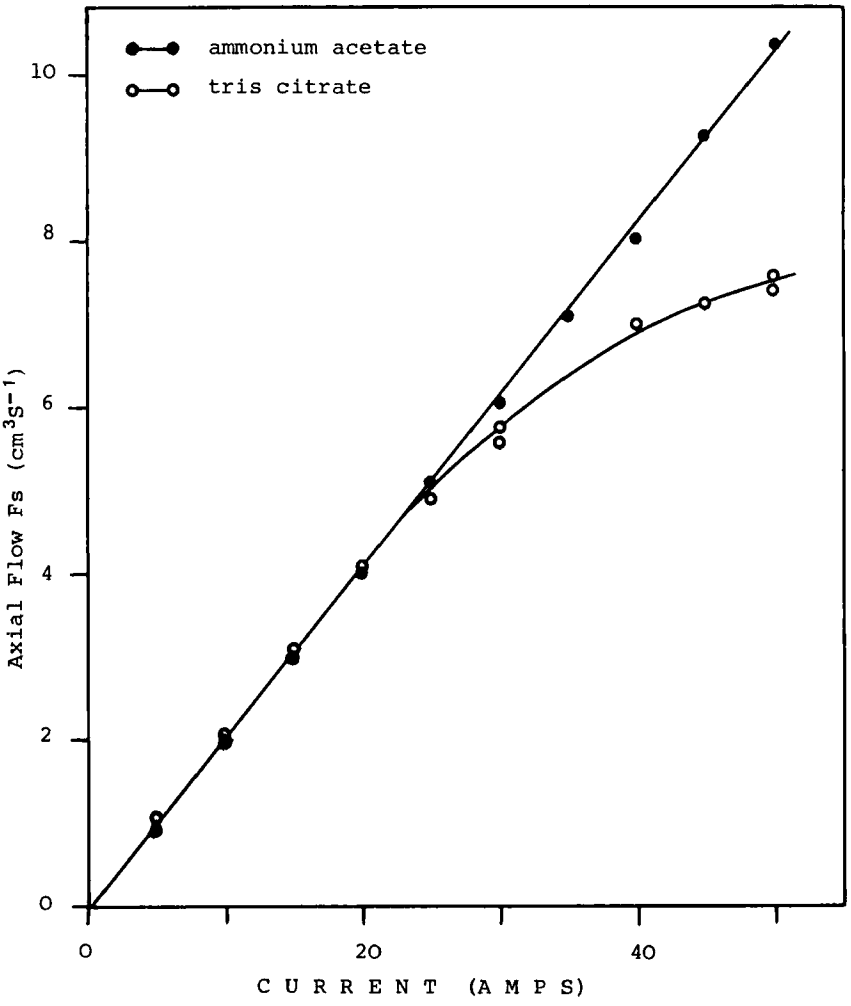

FIG. 6 Relationship between the position in the annulus of the distribution mean of a migrant (BSA) and the current applied for ammonium acetate and tris citrate carrier buffers. The position of the sample is plotted as proportion of the carrier flow between the stator and the mean value of the sample. Carrier - ammonium acetate pH 10.5, conductivity $1mScm^{-1}$; tris citrate pH 8.5, conductivity $1mScm^{-1}$. The flow rate was 900ml/min. The sample was BSA at 10mg/ml and a flow rate of 6ml/min.

plot of the axial flow (F_s) of the carrier against the current when the conductivity profile is relatively constant across most of the annulus. There is a greater increase in conductivity near the rotor when Tris buffer is used; this results in a departure from linearity.

(b) Migrant zone width

When a charged component is electrophoresed into the carrier stream, the initial zone width is modified both by molecular diffusion and by the parabolic velocity profile of the carrier since the axial velocity at the stator and rotor walls is essentially zero while the maximum velocity occurs close to the mid-point of the annulus. Consequently, the total residence time of any ion in the electric field will depend upon its position in the annulus. Thus, since the leading edge of the initial migrant zone will enter a faster flowing regime than the trailing edge, it will have a shorter residence time in the annulus and will be electrophoresed a shorter radial distance allowing the trailing edge to "catch up". This zone sharpening effect will occur up to near the mid-point of the annulus, beyond which zone spreading will then occur.

In the absence of diffusion, a migrant zone of initial width ma which is electrophoresed to a position s cm across the annulus (width a cm) will be compressed (or expanded) to a width $m_s a$ where

$$m_s = \frac{(3m^2 - 2m^3)a^2}{6s(a - s)}$$

Modular diffusion interacts in a complex way with this 'parabolic flow sharpening' to give a dispersion (measured as S.D.) after electrophoresis of

$$\sigma_{(cm)} = \frac{1}{s(a - s)} \left[\left(\sigma_o s_o (a - s_o)\right)^2 + \frac{DLa^2}{3V} \frac{Q_s - Q_o}{C_s - C_o}\right]^{\frac{1}{2}}$$

where $Q_s = a^2s^3/3 - as^4/2 + s^5/5$

$Q_o = a^2s_o^3/3 - as_o^4/2 + s_o^5/5$

$C_s = as^2/2 - s^3/3$

$C_o = as_o^2/2 - s_o^3/3$

Experiments have been carried out to compare observed S.D. values with those calculated using a protein with a relatively small diffusion coefficient (B.S.A., $D_{20} \sim 6 \times 10^{-7}$ cm^2s^{-1}) and a small ion with a significant diffusion coefficient (sodium fluorescein $D_{20} \sim 4 \times 10^{-6}$ cm^2s^{-1}) at different locations in the annulus after electrophoresis.

The results (Figures 7(a) and (b)) indicate that the sodium fluorescein data agree quite well with the calculated S.D. values whereas those for B.S.A. are substantially greater than theory, particularly near the rotor wall. Clearly there are other factors affecting the distribution in the case of B.S.A. One factor which has been considered is electrophoretic heterogeneity of the protein[19] (i.e. the existence in the purified protein of multiple forms due to ion-binding, aggregation, partial denaturation, partial deamidation, etc.). In the case of electrophoresis in an annulus, the contribution of heterogeneity to the variance of the distribution is given by

$$\sigma_h^2 = \left(\frac{h}{U} \cdot \frac{\frac{as}{2} - \frac{s^2}{3}}{a - s} \right)^2$$

where h is the electrophoretic heterogeneity constant measured in units of mobility.

It is apparent from this equation that if a heterogeneity of 3-5% in the B.S.A. is assumed, there is much closer agreement between calculated and experimentally observed data both in terms of the magnitude of the discrepancy and its variation with annular location (see Figure 7b).

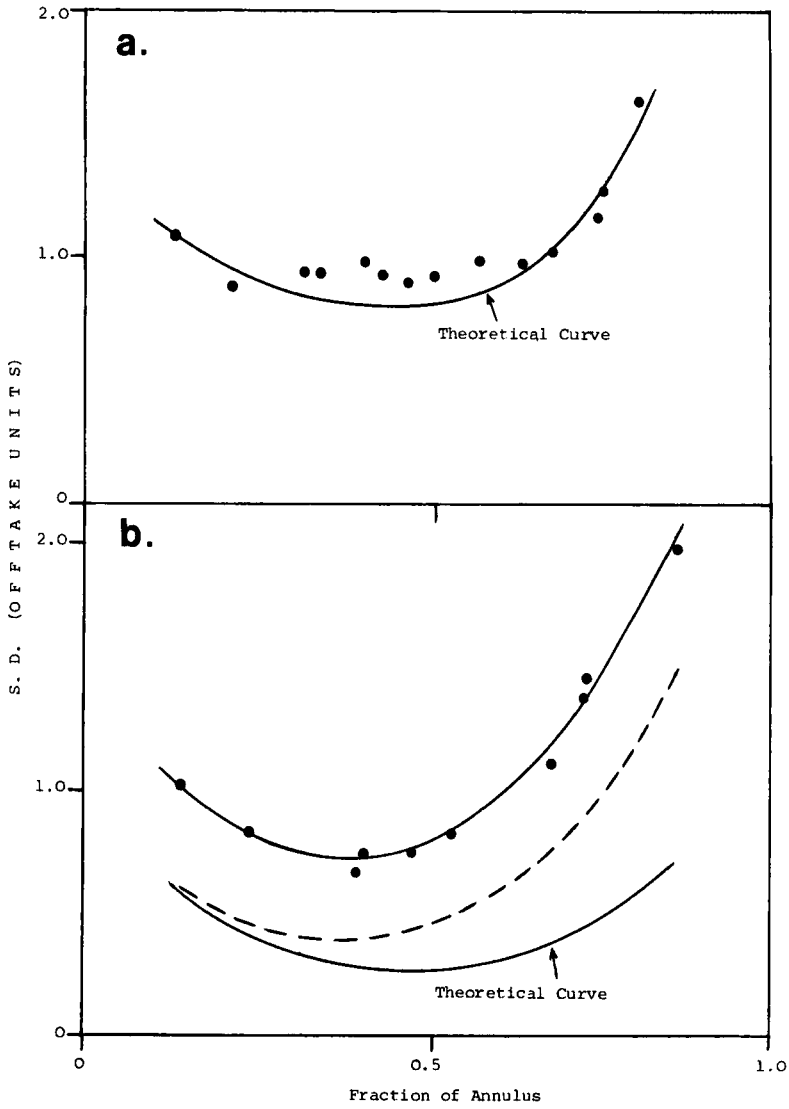

FIG. 7 Effect of location of the migrant in the annulus on its zone width (expressed as S.D.). Theoretical and experiment values are compared for (a) Sodium fluorescein and (b) BSA. (a) Carrier - ammonium acetate pH 10.5. Conductivity 1mScm^{-1}, flow rate 900 ml/min. Sample - sodium fluorescein 0.2 mg/ml. Flow rate 15 ml/min. (b) Carrier - ammonium acetate pH 10.5. Conductivity 1 mScm^{-1}. Flow rate - 800 ml/min. Sample BSA, 10 mg/ml. Flow rate 20 ml/min. --- represents the theoretical curve corrected for 4% electrophoretic heterogeneity of BSA.

(c) Resolution

The resolution of two species during electrophoresis depends upon their respective dispersions and the distance between peak maxima.

It can be shown that for two species with normal Gaussian distributions, at the same concentration and with standard deviations, σ_A and σ_B, the separation distance required to achieve a 95% recovery at 95% purity level is

$$d = 1.645\ (\sigma_A + \sigma_B)$$

Normally, therefore, for a mixture of ions with small differences in mobility the greater the distance migrated the greater the degree of separation that can be expected, as d varies linearly with time but the effect of diffusion on σ is proportional to the square root of time.

In the system described here the migrant is moving rapidly across a parabolic velocity profile and perpendicular to the carrier stream. Hence, the standard deviation of the migrant is influenced by parabolic flow sharpening and in theory is at a minimum when its peak maximum is near the mid-point of the annulus (Figures 7(a) and (b)).

Thus if s, the distance moved across the annulus, and $\hat{\sigma}$ the minimum standard deviation, are expressed as fractions of the annulus width, i.e. $s = ma$ and $\hat{\sigma} = x'a$, then it can be shown (see Appendix 2) that

$$\frac{\Delta U}{U} = 4.94x'/m^2 \quad (3-2m)$$

Resolution should therefore improve markedly up to about the middle of the annulus, with a continued but much smaller improvement in resolution towards the rotor.

In Figure 8 resolution (expressed as $a\Delta U/\sigma_o U$) is plotted against location in the annulus thus defining the variation of resolution with location. The predominant features of this are that resolution is independent of machine dimensions for a given

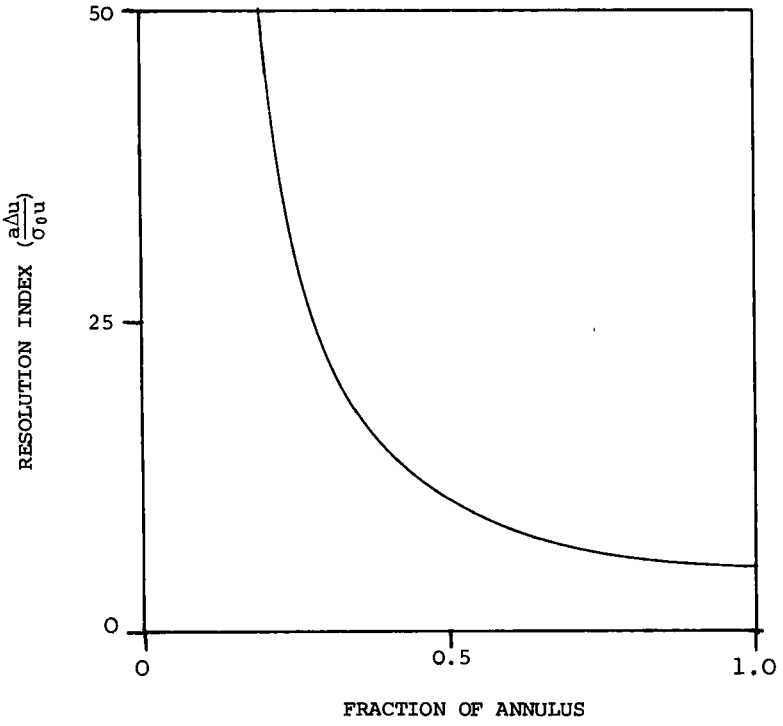

FIG. 8 Effect of locations of two migrant species in the annulus on resolution measured as a non-dimensional index. The lower the value of the resolution index, the greater separation. Thus, past about 0.75 of the annulus no significant improvement in resolution might be expected.

temperature rise and that there is negligible advantage in moving the highest mobility species more than ∿ 75% of the annulus width.

(d) Joule Heating

Probably the most important factor in maintaining stability during electrophoresis is the control of convective turbulence caused by Joule heating in the annulus. In the electrophoretic system, no attempt is made to remove heat but solutions entering the system are cooled to ∿ 2°C and conditions are selected to ensure that the exit temperature (usually ∿ 20°C) is acceptable for biological work.

Essentially all the Joule heat causing the temperature rise is retained in the carrier and is given by

$$\Delta\theta = I^2 a/\rho Cp\bar{K}\, F_c 2\Pi\bar{R}L \qquad (2)$$

The conductivity is temperature dependent according to

$$K_\theta = K_{20}\left[1 + \alpha(\theta - 20)\right]$$

and substitution for the mean conductivity ($\bar{K}$) results in a quadratic equation with a solution

$$\Delta\theta = \left[(\theta_i - 20 + \frac{1}{\alpha})^2 + \frac{I^2 a}{\alpha\rho Cp F_c K_{20} L\Pi\bar{R}}\right]^{0.5} - (\theta_i - 20 + \frac{1}{\alpha})$$

A series of measurements were made of the temperature rises for different conductivity buffers and these have been compared with calculated values. The results, summarised in Figure 9, indicate that the equation to a large extent describes the behaviour of the system, particularly when the carrier buffer has a conductivity of 1.5 - 2.0 $mS.cm^{-1}$. The conductivity of the carrier leaving the annulus is usually higher than the value calculated from the temperature rise and is probably due to strong electrolyte passing into the annulus through the semi-permeable membranes. Thus, the calculated temperature rise tends to over-estimate that actually observed. Obviously this effect is more important when the buffer has a low initial conductivity.

4. SCALE-UP OF THE ELECTROPHORETIC SEPARATOR

The amount of migrant sample that can be processed in the separator is dependent principally on the carrier flow used and the temperature rise. Both the ratio of migrant to carrier flows and the migrant concentration (which is interactive with migrant flow) have significant effects on zone width, which in turn effects resolving power.

The effect of migrant throughput on zone width is illustrated in Figure 10, where it can be seen that quadrupling input (from

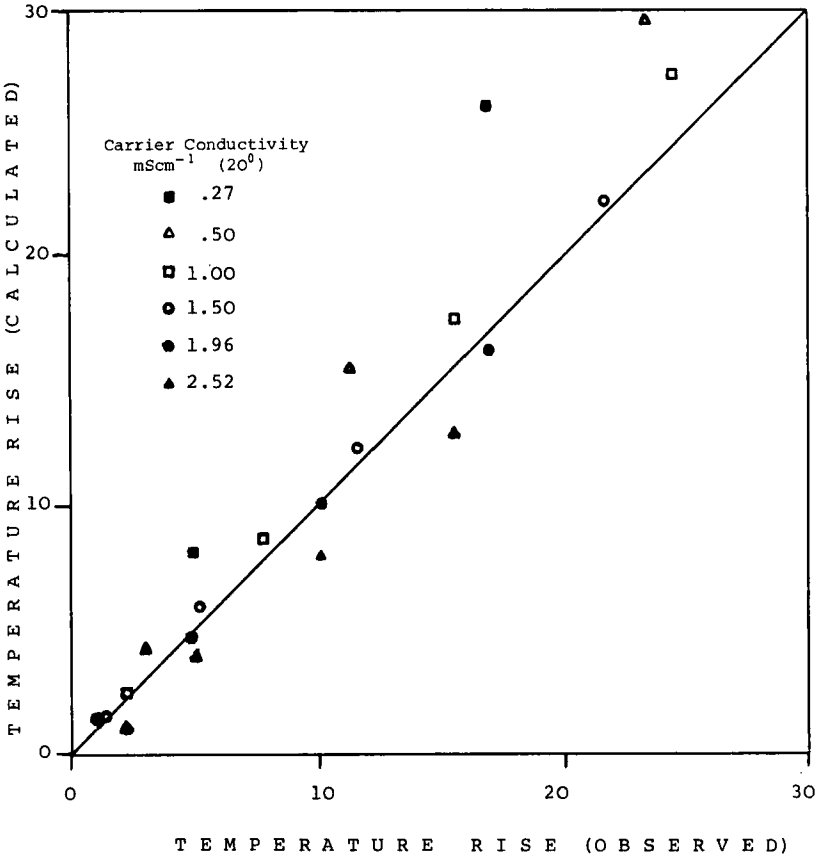

FIG. 9 A comparison of the observed with the calculated temperature increase of the carrier buffer during electrophoresis for different carrier conductivities. Carrier buffer - ammonium acetate pH 10.5, flow rate 500ml/min.

0.2 to 0.8 mgs/min) only increases the S.D. by about 60%. Clearly, with reasonably well resolved peaks, major increases in throughput can be achieved without too great a loss in resolution. In addition, the greater the differences in mobilities of the components of a migrant mixture, the easier they are to resolve and this permits a larger migrant/carrier ratio to be used. For the

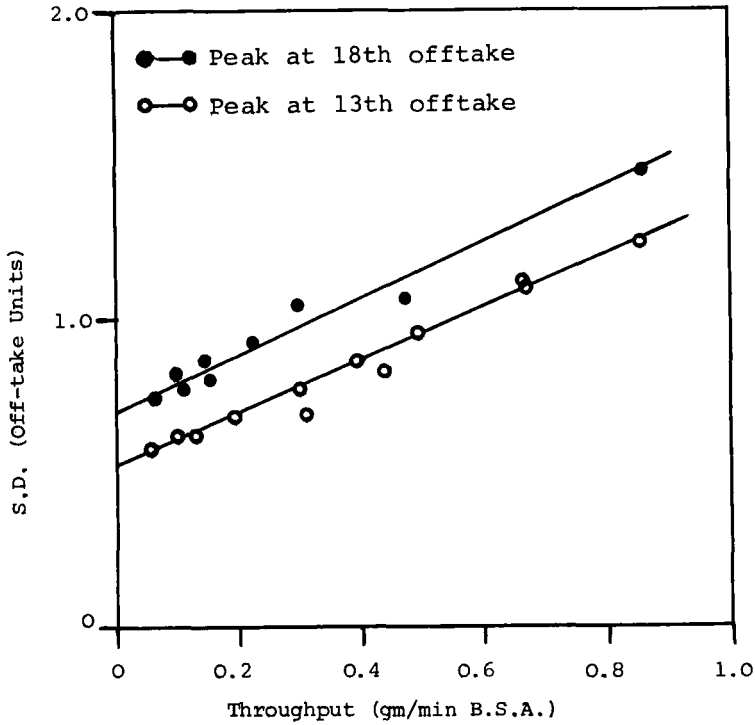

FIG. 10 Effect of sample loading on the zone width of bovine serum albumin (measured as S.D.) at two positions in the annulus. Carrier buffer - ammonium acetate pH 10.5, conductivity $1mScm^{-1}$, flow rate 1800ml/min. The uppper curve shows the S.D. with the protein peak at the 18th offtake (90A, 58V) and the lower with the protein peak at the 13th offtake (54A, 41V).

purposes of the following discussion it is assumed that a separator designed for a higher throughput entails a corresponding increase in carrier flow to maintain a given migrant/carrier flow ratio. From the equation (1)

$$UI = \overline{K}F_s$$

the current required for a given carrier flow is determined by U and $\overline{K}$. Using a maximum value for $\Delta\theta$ as the primary constraint, a

value for the ratio $a/L\bar{R}$ can be obtained for given values of I, F_s and $\bar{K}$ (equation (2)).

The axial flow bulk Reynolds number for flow through an annulus is obtained from the equation

$$R_e = \frac{V\rho}{\eta} \frac{2}{R_2} (R_2^2 - R_m^2) \quad \text{where } R_m^2 = \frac{R_2^2 - R_1^2}{2Ln(R_2/R_1)}$$

It has been shown[20] that the first indication of flow instability occurs at $R_e = 700 \pm 50$.

As the simpler expression $R_e = \frac{Vp.2a}{\eta}$ is a close approximation when $a \ll R$ and, as this can be expressed as

$$R_e = \frac{\rho}{\eta} \frac{Fc}{\Pi\bar{R}}$$

we can therefore introduce a further constraint on acceptable values for the mean radius ($\bar{R}$) of the annulus.

The rotational Reynolds number, if this is accepted as a practical constraint (which seems judicious), provides further limitations in the choice of annulus width and outer radius.

Using this basis for calculations to determine suitable dimensions a machine has recently been constructed with a nominal 3-fold increase in throughput. Early operating experience with this separator is satisfactory to date and suggests that the mathematical model describes the major features of the separator.

5. APPLICATIONS

The electrophoretic separator described above has been operated for several years and considerable experience has been gained in developing conditions which enables satisfactory separations to be obtained for a wide variety of materials[21,22]. In general, it has been found that the system is extremely flexible, allowing separations of small ions, macromolecules, particles and cells to be carried out under a wide variety of separation conditions. Retention of biological activity has been high (70-100%) and this permits separation of some materials which might otherwise

be inactivated by other purification methods, particularly those using adsorption, or precipitation with organic solvents.

A number of production scale applications are being developed but it is not possible to describe these in detail for reasons of confidentiality. However, the applications described below illustrate the flexibility of the system, the range of conditions possible for successful separations and above all the good yields and recoveries of biological activity generally achieved.

5.i. Macromolecules

The initial motivation for developing the system was to extend the applicability of electrophoresis to a much larger scale, particularly for the separation of proteins. The performance of the system has therefore been investigated in detail with typical biological feedstocks.

(a) Muscle Extract

The 45-60% $(NH_4)_2SO_4$ fraction from an aqueous extract of rabbit muscle was prepared for electrophoresis by dialysis against $\sim$ 5 mM Tris-citrate buffer pH 8.5, 1 $mScm^{-1}$. It was then centrifuged (5000 x g) to remove any large aggregates. The extract (K - 3.4 $mScm^{-1}$) was then electrophoresed at an input flow rate of 10 ml/min using Tris-citrate pH 8.5 (K = 1 $mScm^{-1}$) as carrier (flow rate 490 ml/min) with a rotational speed of 150 r.p.m. Electrophoresis was carried out at 35A (26V) leading to a final exit temperature of the fractions of $\sim 21^{o}C$. Fractions collected were assayed for pyruvate kinase and phosphoglucomutase enzyme activities and protein distribution was also determined (A_{280}). The results are shown in Figure 11. Both enzyme activities were recovered almost quantitatively and were resolved virtually completely. The bulk of each activity was recovered in 4-5 off-take tubes, thus pooling the appropriate fractions would provide a good recovery of enzyme activity with a substantial degree of purification (3-4 fold over starting material). Obviously the recovered enzyme pools are not pure at this stage but since they are in low ionic strength buffer, it would be feasible to purify

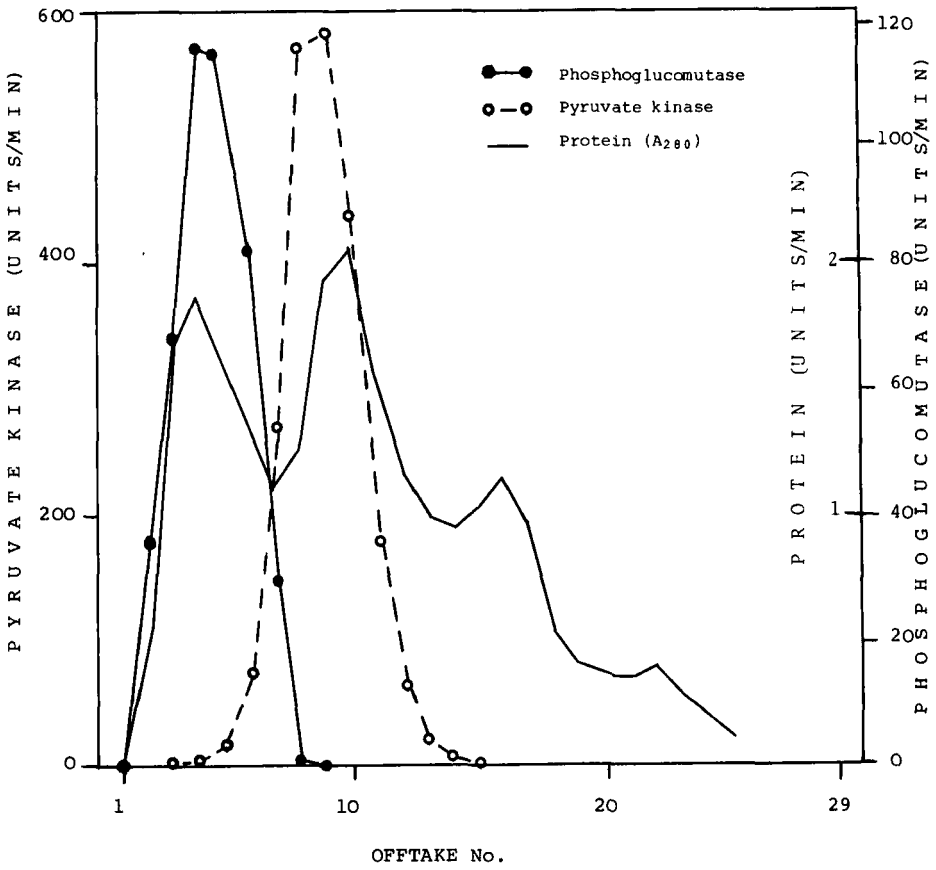

FIG. 11 Fractionation of rabbit muscle enzymes by continuous electrophoresis. The distribution of the two enzymes are shown, both of which were recovered in quantitative yield. Carrier buffer - tris citrate pH 8.5. Conductivity $1mScm^{-1}$. Flow rate 490ml/min. Sample was a 45-60% saturated ammonium sulphate fraction of rabbit muscle extract. Injection rate - 10ml/min at ~10mg/ml protein concentration. Electrophoresis was performed at 35A (26V).

and concentrate them further by ion exchange chromatography. It will be appreciated that in this example, the separate conditions were not optimised and it is likely that improved resolution could be obtained using different conditions.

(b) Plasma Fractionation

One of the most important applications of continuous electrophoresis to be identified to date is the fractionation of human blood plasma. At present, almost all plasma for medical use is fractionated by an ethanol precipitation method developed by Cohn[23]. Although this technique provides a limited number of well characterised and safe fractions for clinical use, including immunoglobulin and albumin, it has a number of disadvantages, particularly the resolution possible and the limited range of fractions that can be obtained. The low temperature necessary (down to $-7^{\circ}C$) and the large quantities of ethanol used pose processing problems. In addition, ethanol at the high concentrations and over the extended contact times used (up to 40%v/v, and 3-5 days respectively) can cause denaturation, and in general gives relatively low yields (50-70%). Further, ethanol precipitation cannot be used for the isolation of a number of labile proteins used in therapy (e.g. clotting factors). There could be, therefore, major advantages in using electrophoresis, particularly since it is non-denaturing and high yields might be anticipated, particularly if the technique provides higher resolution in the preparation of specific products.

Factor VIII, the anti-haemophilic factor is probably one of the most important components required from fresh frozen human plasma. The general technique used at present is to produce cryoprecipitate by cooling plasma to $0^{\circ}C$. This contains factor VIII but only in $\sim$ 30-40% yield, and contaminated with most other plasma components[24]. After further purification, recoveries are even lower. Electrophoresis of plasma has been studied in considerable detail by us, in an attempt to provide an acceptable factor VIII fraction in greater yield.

Fresh frozen plasma was dialysed against the carrier buffer for 18 hours before electrophoresis, and was then diluted to 1.5 x its original volume. Electrophoresis was carried out in Tris-citrate pH 7.5, 0.75 $mScm^{-1}$ as carrier buffer at a migrant flow of 10 ml/min. Using 30A (30V) ($\Delta\theta$, 20-21$^{\circ}C$), plasma proteins were

distributed across three-quarters of the annulus. Fractions were collected and analysed for the various plasma components by electrophoresis and immunological methods. Figure 12 shows the distribution of factor VIII, fibrinogen and albumin. As can be seen, factor VIII forms a fairly broad peak presumably due to its

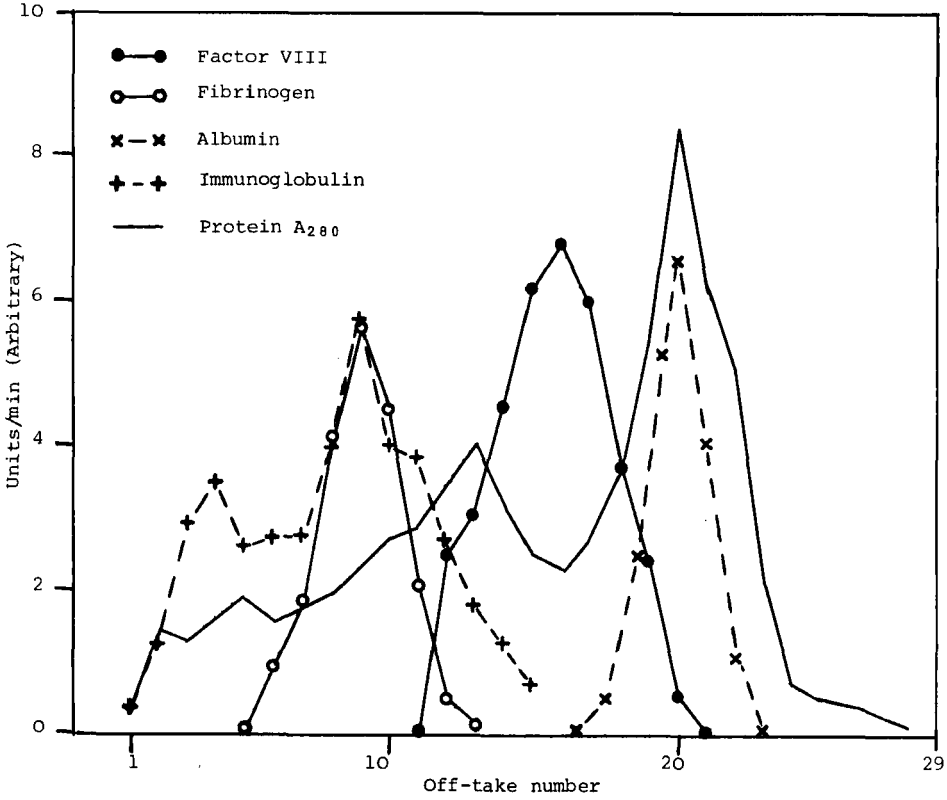

FIG. 12 Fractionation of human blood plasma by continuous electrophoresis. The distrution of the components of major interest, albumin, fibrinogen, immunoglobulins and factor VIII are shown. Carrier buffer - tris citrate pH 7.5, conductivity 0.75mScm^{-1}. Flow rate 500ml/min. The sample was fresh frozen plasma dialysed overnight (at 4^{0}C) against the carrier buffer and diluting 1.5 fold before electrophoresis injection. Electrophoresis was performed at 32A (28V)

heterogeneity. On the other hand, the distribution of albumin is more compact and is largely separated from the factor VIII. More important is the fact that fibrinogen, the major contaminant of factor VIII prepared by cryoprecipitation, is virtually completely separated. The recovery of factor VIII procoagulant activity is nearly quantitative, making it an attractive starting material for further purification.

Development of this technique may provide fibrinogen - free factor VIII preparations in higher yield from plasma or some fraction derived from plasma, thus enabling more efficient use to be made of the available plasma. It should be noted that other plasma components such as albumin and immunoglobulin can be prepared simultaneously making possible the isolation of a number of therapeutically used fractions at the same time and in good yield.

Cryoprecipitate also has been electrophoresed in the system. In this case the precipitate as usually prepared was dissolved and then dialysed against the carrier buffer, and was electrophoresed under the same conditions as above. Again, virtually complete separation of factor VIII from fibrinogen was achieved (Figure 13). Electrophoresis of cryoprecipitate does allow a much greater throughput to be achieved since it is up to 5-fold concentrated over starting plasma, depending on the method used to prepare the cryoprecipitate. The upper limits of throughput have yet to be determined but since the resolution of factor VIII from the major contaminant, fibrinogen, is good, significantly higher migrant injection rates may be possible without reducing significantly the purity of the factor VIII fraction.

5.ii. Separation of Cells and Particles

The limited range of techniques available for fractionating cell populations makes the possibility of separation by electrophoresis very attractive, particularly since the technique uses a different property (viz. charge) from those usually used for fractionating particulates (particle size or density). Clearly,

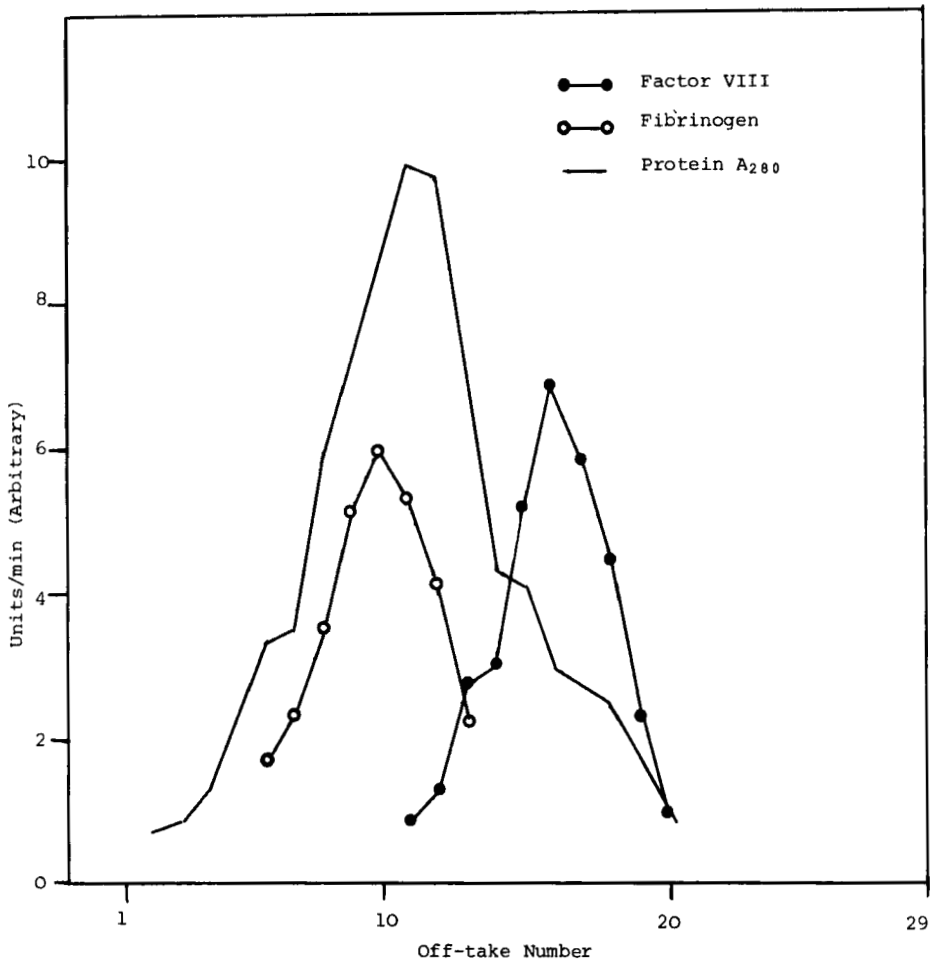

FIG. 13 Fractionation of cryoprecipitate by continuous electrophoresis. The distribution of factor VIII and fibrinogen are shown. Carrier buffer - tris citrate pH 7.5. Conductivity 0.75mScm^{-1}. Flow rate 500ml/min. The sample was cryoprecipitate dialysed overnight (at 4^{0}C) against carrier buffer and then electrophoresed directly at 10ml/min (32A, 28V).

electrophoresis in the presence of stabilising gels is not possible and considerable work has been done on the separation of whole cells and particularly lymphocytes using the thin film free-flow system[25,26]. This has demonstrated quite clearly that different cell types can be separated by electrophoresis while retaining their viability. The limitation of the thin film system (Hannig design) is that only relatively small numbers of cells may be processed ($\sim 10^8$ cells/hour). In an attempt to obtain greater throughputs using thicker films of liquid, experiments have been mounted in space to overcome the convective turbulence caused by density changes in electrophoresis buffers[27]. At g values approaching zero, density changes will have minimal disturbing effect on the separation.

Therefore, we are investigating the use of the velocity gradient stabilised system for the separation of cells and subcellular particles on a larger scale. As a test system, red blood cells from various species have been electrophoresed in a carrier buffer containing 9.6mM sodium potassium phosphate, 2.5mM EDTA, 10% sucrose (to maintain osmotic balance), at pH 7.3 and ~ 1 mScm^{-1}. Approximately 10^{10} cells/hour could be electrophoresed at 15A (20V). The results, shown in Figure 14, are in general agreement with published data[27], with the exception of human cells, bearing in mind the low ionic strength used. One important point to note is that S.D. values of some of the zones are significantly lower than those obtained for macromolecules as would be expected from the relative diffusion coefficients, and indicate that particles with significantly lower differences in mobility than macromolecules should be separable in this system. The higher S.D. values obtained with cells of some species probably indicate heterogeneity.

To demonstrate the feasibility of separating different cell types, a mixture of human and rabbit red cells was electrophoresed at various power inputs. The results (shown in Figure 15) show that even at relatively low currents, the separation of the two components is almost complete.

SPECIES	MEAN POSITION	S.D.	MOBILITY ($cm^2 S^{-1} V^{-1} \times 10^4$)	
			Observed	Published
RABBIT	7.4	1.16	0.84	0.60
CHICKEN	8.6	0.83	1.12	0.82
GUINEA PIG	10.5	0.57	1.56	1.21
SHEEP	10.7	0.60	1.61	1.44
HUMAN	11.2	0.47	1.75	1.16
RAT	11.8	0.70	1.90	1.45

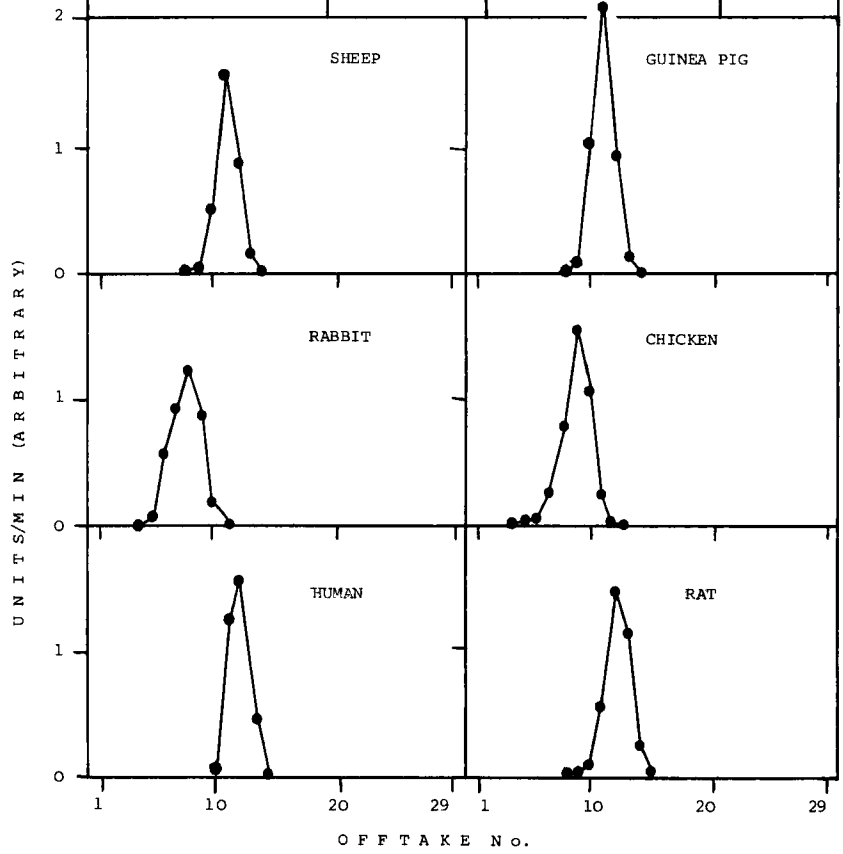

FIG. 14 Electrophoresis of red blood cell preparation from different species. Carrier buffer - 9.6 mM sodium potassium phosphate, 2.5 mM EDTA, 10% sucrose pH 7.3. Conductivity 1 $mScm^{-1}$. Flow rate 500 ml/min. Samples were washed preparations of packed red cells diluted ~1:100 with carrier buffer before use. Injection rate was 5 ml/min ($\equiv 10^8$ cells/min) and electrophoresis was performed at 15A (20V). The table inset shows the mobility of red cells obtained by continuous electrophoresis compared with published values.

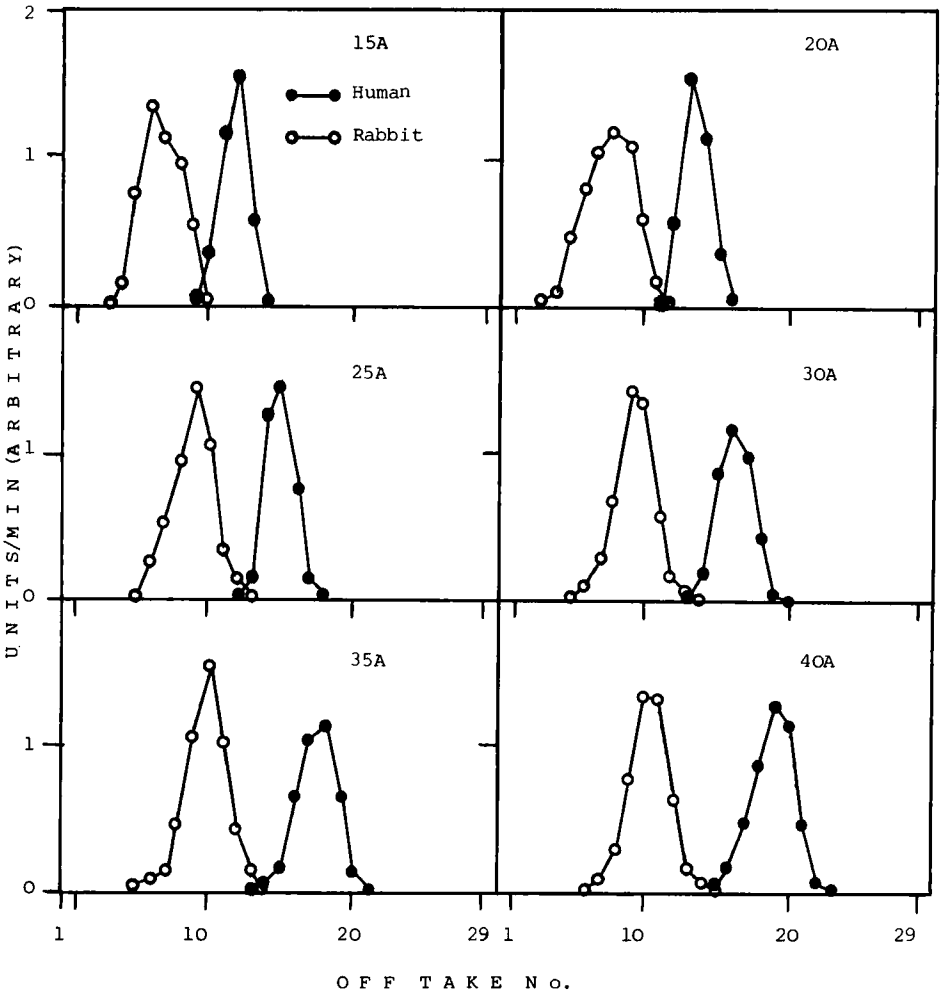

FIG. 15 Electrophoresis of a mixture of rabbit and human red blood cell preparations at different currents. Conditions were as desceibed in Fig. 14.

A further illustration of the resolving capacity of the system is given by the fractionation of an abnormal red cell population (which shows hyper-agglutination), from normal red cells obtained from an otherwise normal donor. After washing the red cells with the phosphate/EDTA/sucrose buffer, described previously,

they were electrophoresed using the conditions described above. As can be seen from Figure 16, two almost completely resolved peaks were obtained, the less mobile major peak consisted of the abnormal hyper-agglutinating cells while the minor peak showed a mobility identical to that of normal cells. The separation provided enough material for detailed analysis of the surface differences between the two populations.

It should be noted that there was no sign of haemolysis during the separations indicating that the conditions chosen were suitable for maintaining osmotic balance. Extensive work using the thin film system also indicates that buffer systems can be chosen which will enable retention of cell viability so that the high throughput system described here looks promising for separating cells on a substantial scale.

The problem of dilute fractions produced by the separator can be overcome conveniently where cells are concerned simply by centrifugation. Thus, the system described here has the potential of a convenient high throughput and rapid method for preparing purified cell lines which may have applications in many areas of biology.

5.iii. Small molecules

Although the system was designed with fractionation of macromolecules in mind, it can also be used for separating mixtures of small ions (< 1000 MWt.) since the effect of diffusion on zone width is minimised by the very short residence time.

The fractionation of a magenta dye consisting of three isomers, illustrates the applicability of the electrophoretic system to small molecules. The separation of the isomers which had proved very difficult by conventional methods (solvent extraction, fractional crystallisation), was needed to enable the properties of each of the isomers to be investigated for specific applications. The isomers are ionic compounds and a pH was selected to give a

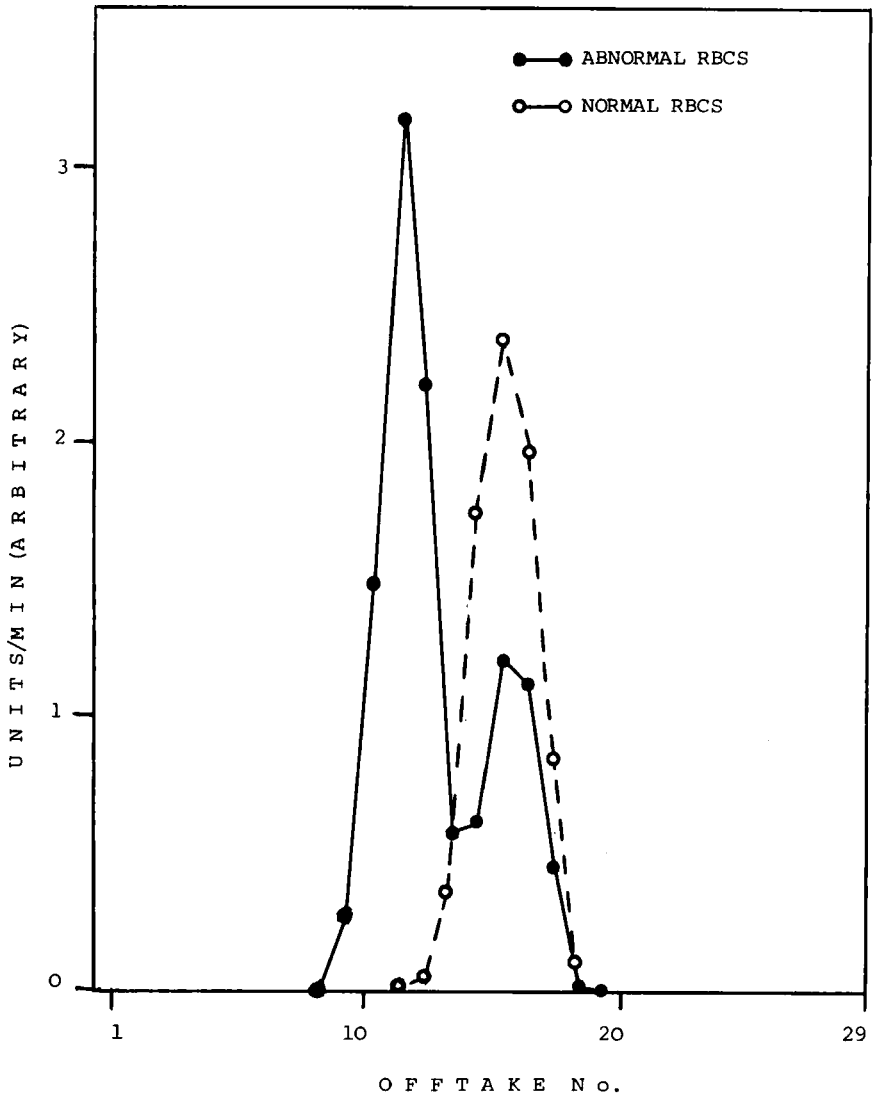

FIG. 16 Electrophoresis of a population of human red blood cells with hyperagglutinating characteristics and showing the difference in mobility between normal and abnormal cells. Conditions were as described in Fig. 14.

mixture of anionic, cationic and zwitterionic species. A severe problem was the low solubility of the dyes in aqueous solutions. This was overcome by dissolving the dye mixture in 20% v/v aqueous ethanol, and by using ethanolic carrier buffer (20% v/v ethanol). Because of their low molecular weight, the dyes could penetrate the semipermeable membranes, and separation was carried out in two stages using reversed polarity in the second stage.

The sample was dissolved to 150 μg/ml in 20% ethanol and was injected at 18 ml/min. Electrophoresis was carried out in Tris-citrate carrier buffer pH 7.5, 0.35 $mScm^{-1}$, containing 20% ethanol (v/v) at 500 ml/min with the rotor as the anode. At 15A (21V), the most anodic component migrated two thirds across the annulus (peak maximum at outlet 18). Fractions were collected and monitored for dye by absorbance at 542 mμ. Figure 17 (a) shows the distribution of the dye components after collection. The anionic component was well resolved from the neutral isomer while the positively charged dye passed through the semipermeable membrane into the stator electrode compartment. In order to isolate the cationic isomer, a second electrophoretic run was carried out as before but with electrode polarity reversed so that the rotor was now the cathode. In this case, the positively charged component was well separated from the neutral component, with loss of the anionic isomer (Figure 17(b)). It was possible to recover the separated isomers in a concentrated form from the various pools by solvent extraction.

6. DISCUSSION

6.i General

The two main obstacles to scaling up free-flow electrophoresis are convection caused by the generation of Joule heat, and flow hydrodynamics and are interrelated. On a small scale, these problems can be largely overcome by using supporting gels which also enable stacking and molecular sieving to be used to increase resolving power[2].

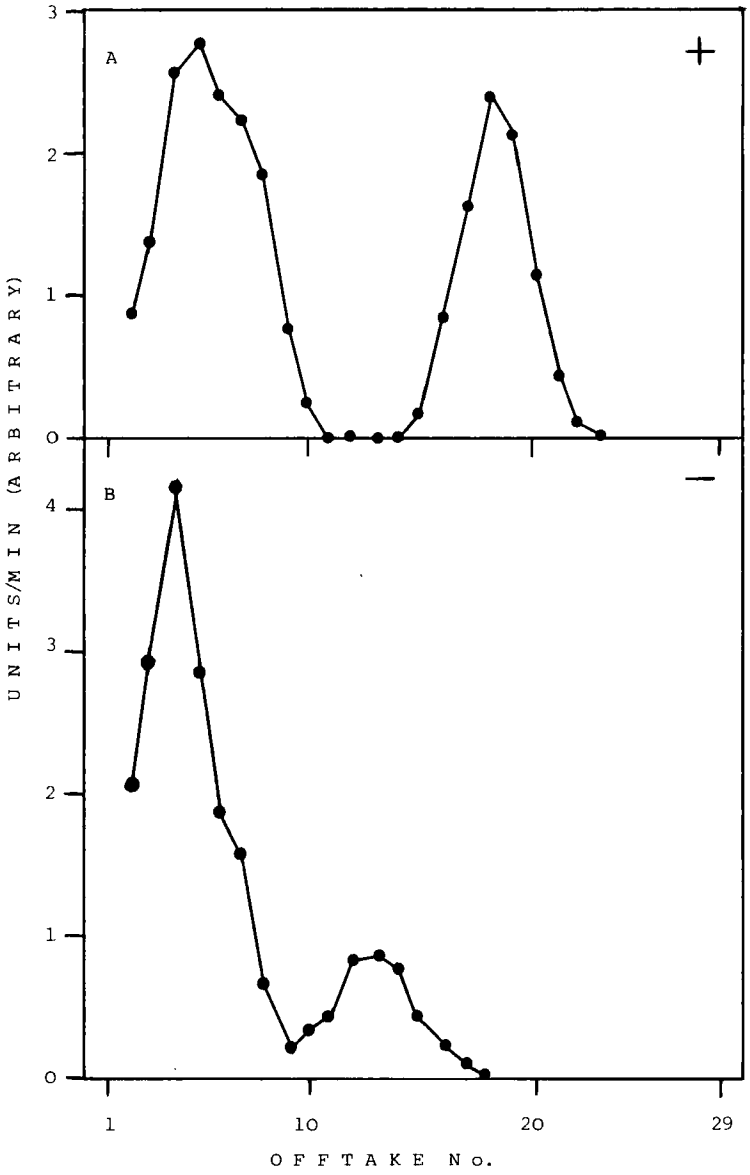

FIG. 17 Fractionation of isomers of a magenta dye mixture. Carrier buffer - Tris citrate pH 7.5, conductivity 0.35 $mScm^{-1}$ with 20% (v/v) ethanol, flow rate 500ml/min. Sample was the dye (150µg/ml) dissolved in carrier buffer injected at 18ml/min. Electrophoresis was performed at 15A (21V).

A. Electrophoretic pattern of the anionic components.
B. Electrophoretic pattern of the cationic components.

Iso-electric focussing in gels enables an unrivalled degree of resolution to be achieved (ΔpI differences of 0.01 can be detected[3]). More recently, these techniques have been combined in two dimensions in which over 1,000 protein spots have been observed[28].

However, attempts at scaling up these high resolving methods to process more than milligram quantities of material have met with limited success. The gel which stabilises so effectively for analytical purposes is a major draw-back to scale up, since heat generated in thick gels cannot be removed easily. Although iso-electric focussing has been carried out in density gradients on a reasonable scale, the cost of the ampholytes required is high[29]. In addition, methods which may cause denaturation, such as gel electrophoresis in the presence of detergents (e.g. sodium dodecyl sulphate (SDS)[4]), although acceptable on an analytical scale, are generally unsuitable where retention of biological activity is essential as in most preparative work.

On an analytical scale several alternatives to the use of supporting media have been investigated. These have included electrophoresis in a narrow bore tube which is rotated slowly in a horizontal plane to eliminate density effects[30], and electrophoresis in a conducting fluid rotated in an annulus by a magnetic field at right angles to the electric field[31]. These techniques do not lend themselves to preparative scale use however.

Electrophoresis has been used on a production scale in the form of multimembrane decantation and electrophoresis convection systems[5,6] only to a limited extent. For instance, electrophoresis decantation has been used for the large scale purification of rubber latex[7]. In both techniques, iso-electric separation is used, consequently inactivation of biological materials and fouling of membrane surfaces is a problem. Recently, the early stages of development of an ingenious technique has been described in which separation is carried out in a carrier solution flowing through hollow fibre membranes with an electric field maintained at right angles to the direction of carrier flow[12]. Large scale use will

require hollow fibre membranes with very reproducible dimensions and properties, but the technique may suffer also from wall effects since the separation depends on concentration of separated species against the inner membrane surfaces in a slow flowing region of the lumen.

The most widely used free-flow system is that devised by Hannig[8]. In this, separation is carried out in a thin film (0.5 or 1.0 mm) between two glass plates (generally 50 x 50 cm, or 50 x 10 cm). Laminar flow is maintained in a vertical direction by very efficient cooling, the voltage gradient being at right angles to the direction of flow. The system has proved its utility especially for separating mixtures of cells, but throughput is limited $\sim$ 5 ml/hr (or 10^8 cells/hr) and this has limited its use particularly for protein separations. However, it has shown its value for cell separations where fewer alternative methods are available.

The system described in this paper is a development of the original invention of J. St.L. Philpot[1] and overcomes in the main, many of the problems encountered in the more conventional electrophoresis systems. Convective disturbance caused by Joule heating is minimised by the creation of a gradient of angular velocity in the annulus, and the temperature rise produced is normally modest ($20^{\circ}C$) causing few problems of stability with labile biological materials due to the short exposure time (< 1 min). In addition, the parabolic flow profile existing across the annulus is harnessed to advantage, at least in part, because of the "parabolic flow sharpening" effect. Nevertheless, the new system described here although relatively simple to operate and stable in operation, has been quite complicated to design effectively and considerable chemical engineering and theoretical modelling has been necessary.

6.ii. Design Considerations

In designing the separator, a number of major mechanical and hydrodynamic problems had to be solved including

- the choice of dimensions,
- maintenance of annular concentricity over extended periods,
- maintenance of laminar flow conditions in the annulus,
- injection of migrant and collection of separated fractions with minimal loss of resolution,
- minimising any adverse effects in the annulus due to gassing at the electrodes or due to electrolysis products,
- maintenance of maximum resolution at g/hour throughputs.

In general, satisfactory solutions have been found to all of these problems so that the system has been operated successfully over a number of years.

(a) Membrane and support system

One important aspect of the design has been the accurate machining of the constituent parts to ensure concentricity, leak free joints and smooth rotation. In addition, the electrode compartments must be separated from the annulus by a membrane and support system which has to meet certain criteria viz:- it must have minimal electrical resistance; it must be relatively impermeable to large molecules and particles; it must have sufficient mechanical strength when wet to define accurately the walls of the annulus while being sufficiently robust under running conditions to require infrequent replacement; the cost of the system should also be reasonable.

Of a number of materials investigated, regenerated cellulose membranes (Visking) mounted on a machined filter support (Cuno) proved to be the most satisfactory and has been used in most of the work. The Cuno candle can be machined when dry to the dimensions required by conventional techniques and is sufficiently strong when wet to support the membrane without distortion for long periods. In our experience, the regenerated cellulose membrane is very robust in use and in general, requires replacement only at $\sim$ 6-month intervals, unless particularly severe conditions have been used in the separator, e.g. extreme conditions of pH in the electrolyte compartments. It is possible that substitution with

ion-exchange membranes as used in the Hannig system, would give improved pH and conductivity control. However, the advantages of using the readily available seamless Visking membrane which can be mounted directly on the filter candle support outweighs the advantages anticipated using flat ion-exchange membranes which would have to be "welded" sufficiently strongly and evenly to survive under operating conditions without creating turbulence. This is also much simpler to make than impregnating porous polymer sheets with membrane dopes. It should be noted, however, that due to the limited size of the filter candles available, the system will probably have to be modified for use with separators with significantly larger dimensions.

(b) Establishment and maintenance of laminar flow

The carrier is introduced into the annulus through 4 inlet orifices at the base of the stator, and the separator has a sufficiently long "smoothing" region for stabilised laminar flow to be established before the migrant injection level is reached. The length of the smoothing section was determined partly on a theoretical basis, and partly empirically following work on an early prototype not equipped with electrodes[32]. Without rotation, complete mixing occurs during electrophoresis, and it has been found that stable conditions can be maintained for prolonged periods at between 60 and 250 r.p.m., in which range zone dispersion is at its minimum. As expected, at higher rotational speeds, turbulence is generated due to small errors in concentricity of the rotor and stator walls. This rotational stabilisation is affected surprisingly little by imposed discontinuities in the annulus, for instance, by placing rubber bands around the stator surface. However, severe turbulence can be caused by any air bubbles in the annulus (hence the importance of degassing the carrier buffer), and by any irregularities such as particulate matter in the flow channel near the migrant injection slit or the outlets. In the separator described, power inputs > 2-2.5 kW, representing a $\Delta\theta$ of $\sim$ 45-50^{o}C, can also lead to turbulence although these conditions are not normally used.

(c) Migrant injection

The migrant injection system has been designed to allow the sample to be fed into the annulus evenly around the whole circumference of the stator. A mathematical model of the injection of a radially flowing migrant into a vertically flowing carrier stream has been developed, and the width of the migrant zone found in practice agrees closely with this model, suggesting that there is minimal turbulence due to migrant injection under normal conditions[18]. However, other factors such as the rate of migrant injection, concentration and the density of the migrant can effect smooth injection.

(d) Fraction collection system

Collection of the fractions separated by electrophoresis via the maze-plate system is very much more difficult to model and hence the design is essentially intuitive and empirical, although based on application of a phenomenon reported by Rayleigh[17]. The arrangement devised for the separator works well, but may not yet be fully optimised. It is not clear to what extent eddies occur at the top of the annulus due to the existence of what is essentially a dead space, and it is difficult to decide on the optimum position of the rotor/stator junction.

That fluid is collected reasonably evenly around the circumference of the annulus by the off-take disc is indicated strongly by the normal distribution of ionic species from outlet 5 and above after electrophoresis. Skewing would be expected if the collection was not even around the annulus. Nevertheless, the dispersion of the collected fractions is greater than predicted from theory and greater for those fractions emerging close to the rotor. This effect could be due to turbulence at the off-takes because of the non-ideal design, to mixing caused by eddies in the dead space near the upper part of the off-take assembly, or due to the precise location of the rotor/stator junction which will determine the extent and direction of eddies. Other reasons for loss of resolution are dealt with later. Attempts have been made

to observe directly any turbulence in the off-take region by observing the behaviour of dye solutions injected into the annulus through the off-take tubes by means of a hollow probe inserted into the appropriate take-off tubes. In these experiments, it was found generally that the dye emerged through the next 1-2 discs higher in the off-take assembly, indicating minimal turbulence in all but the upper region of the stack.

(e) Flow Profile

Flow velocity in the annulus is parabolic, i.e. there is minimum flow at the stator and rotor walls and maximum flow at the centre of the annulus. If the 30 outlet tubes are unrestricted, there will be equal flow through each outlet since all are at the same external pressure (atmospheric). In this case, fractions will represent equal volumetric elements of the annulus (viz. 1-30th of the total carrier plus migrant volume will emerge/ fraction). Thus, the lower and higher numbered fractions collected will represent a greater proportion of the distance across the annulus than those obtained from the centre. Conversely, if the flows through the outlet tubes are adjusted to give a parabolic out-flow profile, each fraction represents a fixed proportion of the annulus (e.g. in the separator described with an annulus of 0.5 cm and 30 off-take discs, 1/30th of 0.5 cm = 0.017 cm). We have elected to use the latter arrangement since the migration distance of any particular zone is then approximately proportional to the current and hence the fraction number is related linearly to mobility. Nevertheless, as shown in Table 1, the stabilising effect of rotation on the annular flow is such that a range of smooth outlet flow patterns can be imposed by means of the outlet valves, without perturbing laminar stability. Although we have operated the system generally with an imposed parabolic outlet profile, variation of the outlet flows could have an advantage in allowing the ionic species of interest to be spread over a greater number of fractions so that recovery or purification can be maximised.

6.iii. Electrophoretic Considerations

In order to optimise the electrophoresis system in terms of resolution and throughput it has been necessary to understand the hydrodynamics of the system and to determine how far its observed resolving power departs from that expected from theory. The model of the system presented here describes reasonably well the electrophoretic behaviour of ions in the system, bearing in mind the complex hydrodynamics involved, particularly at the migrant injection slot and the fraction collection discs, and the variation of conductivity across the annulus during electrophoresis. However, the zone distribution observed for BSA (see Figure 7 (b)) indicates that other factors should be considered. For example, it has been shown that 3-5% heterogeneity of the sample due to contaminants or aggregation could account for most of the increased dispersion of BSA observed, particularly near the rotor.

(a) Resolution

In addition to hydrodynamic constraints mentioned above, the resolving power of the system is limited by other factors, including temperature rise, migrant to carrier ratio and residence time in the separator. Normally, the resolving power of zone electrophoresis depends on the path length available for separation. However, the path length (in this case, annular width) which can be incorporated in a separator of the type described here, is severely constrained by the dimensions compatible with hydrodynamic flow stability. These are themselves a complex function of diameter, annular length, annular width, $\Delta\theta$ and rotational speed.

For the separation of most biologically active materials, there is a limit to the $\Delta\theta$ which can be tolerated and the maximum outlet temperature is probably about 30-40^{o}C. Bearing this in mind, the model predicts that for relatively high throughputs the annular widths must be relatively small (0.2 - 0.5 cm has been used successfully). In theory and by analogy with most zone migration systems, separation between components should be maximal at the maximum path length available, i.e. when the most mobile component

is close to the rotor wall. However, we normally operate so that the peak position of the most mobile component is about outlet 21 (i.e. 0.7 of the annulus, see Figure 8). The rationale behind this is that beyond off-take 24, loss of resolution occurs probably due to some mixing at the off-take discs in this region as well as to the presence of a high conductivity region near the rotor, probably caused by back flow of concentrated electrolyte through the membrane into the annulus. In addition, it has been shown (Figure 8), that in fact little advantage in resolution would be gained past about off-take 24.

Although it is unlikely that further major improvements in the resolving power of the system can be achieved by refining machine design, there is scope for enhancing resolution significantly by a number of techniques and modifications including

- zone sharpening (e.g. using isotachophoresis[33]),
- iso-electric focusing,
- specific mobility modification (affinity electrophoresis[34]).

These are being investigated at the present time.

(b) Electroendosmosis

Normally with electrophoresis systems, electroendosmosis occurs particularly in free solution, causing serious loss of resolution. This effect is due to charges present or accumulating on the surfaces of the electrophoresis cells used, and is particularly pronounced in the thin film system of Hannig which has large surface areas in relation to the path length. The problem has proved difficult to overcome although coating the walls with suitable polymers (e.g. methyl cellulose) or even with the ions being electrophoresed (e.g. albumin) significantly reduces the endosmotic flow[35]. The problem can be overcome partly by injecting the sample into the centre of the thin film, away from the walls, which reduces zone distortion. Nevertheless, it is still a major limitation to thin film systems.

Although there are large membrane areas in the system described here, these are in a plane at right angles to the

direction of current and movement of ions, and hence cause little endosmotic interference.

(c) Carrier Buffer

In general, a low conductivity must be maintained in the annulus to provide a sufficient voltage gradient to effect useful separations of most biological molecules and particles, without generating excessive heat. Thus, the effective operating conductivity range has been found to be $\sim$ 0.5-2.0 $mScm^{-1}$. Further, it is vital to maintain as flat a conductivity profile as possible across the annulus so that the maximum migration distance can be used for separation. A variety of buffers have been described in the literature within this conductivity range which also provide adequate buffering power and a number of these have been used in the electrophoresis system. These have included Tris-citrate for the physiological range ($\sim$ 7-9), ammonium acetate (9.6-11.5), Tris-acetate or ammonium acetate within the 4-6 pH range and ammonium formate down to pH 3.0. Generally speaking, adequate buffering can be maintained over the whole annulus at low conductivities with relatively inexpensive electrolytes. Figure 18 illustrates pH and conductivity profiles in a typical experiment using Tris-citrate. Although a reasonably flat conductivity profile is generally achieved, there is always an increase in conductivity towards the anode (normally the rotor). The reason for this is not clear but could be due to inward movement of ions from the outer electrode compartment which contains high concentrations of electrolyte, since the effect increases with current. In addition, a wide range of buffers have been described for use with cells to maintain buffering and osmolarity, some of which have been modified for use with the present system.

It is essential to exercise care in buffer make-up to maintain the conductivity ± 5% from batch to batch, in order to ensure stable zone positions during extended runs. It is worth noting that at the low ionic strengths used in the system, the mobilities of dissolved macromolecules are considerably enhanced ($\sim$ 3x) above

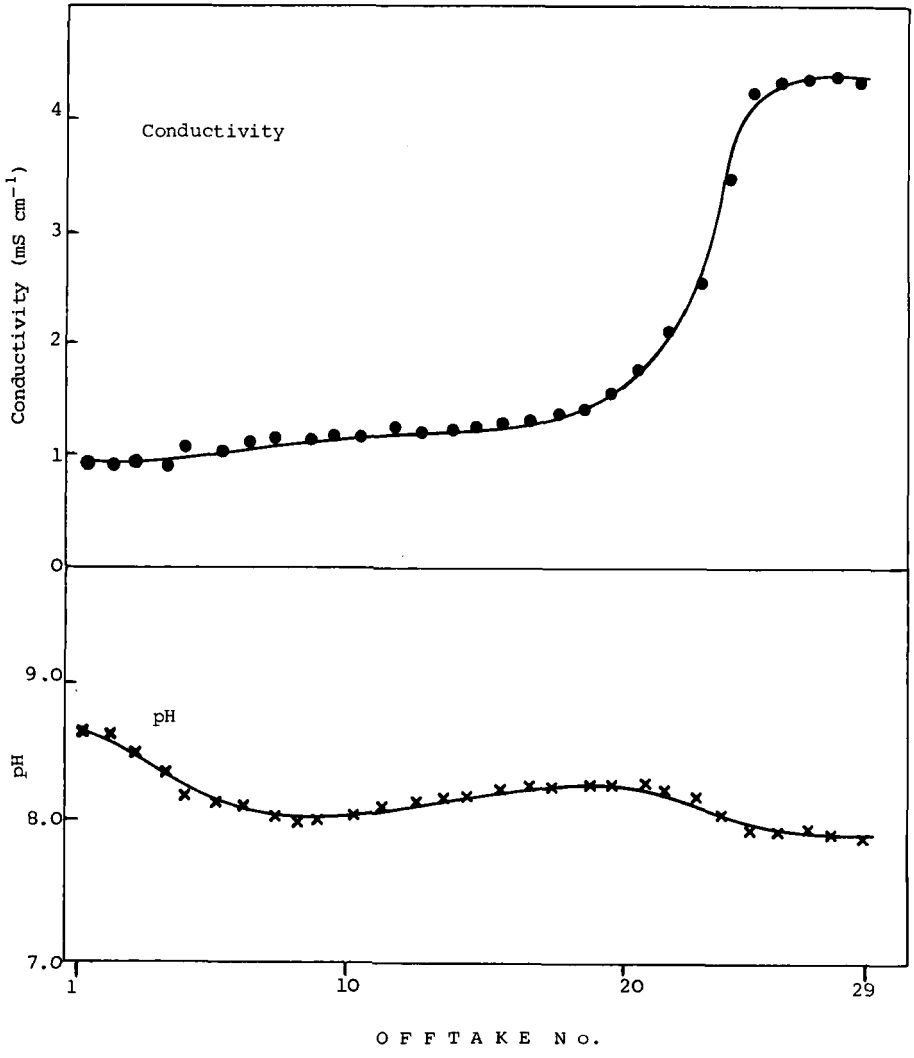

FIG. 18 Typical conductivity and pH profiles across the annulus during electrophoresis. Carrier buffer Tris citrate pH 7.5, conductivity 0.75mScm^{-1}. Flow rate 500ml/min. Electrophoresis was performed at 32.5A (28V).

those normally reported (Note $U \simeq 1/\sqrt{K}$). It should be remembered that the absolute mobility values quoted in the literature have been determined normally by analytical electrophoresis (e.g. with the Tiselius moving boundary system), generally using relatively high ionic strength buffers. Therefore, these values cannot be used directly to predict mobilities in the velocity gradient stabilised system. Mobility values derived from gel electrophoresis where molecular sieving has a major effect cannot be used directly. However, pI data are generally of greater value in indicating the likely relative mobilities, although even here the relationship between pH and mobility is not reliable.

6.iv Scale and Throughput

Throughput of the system is determined by the separator dimensions, flow rates and temperature rise. The relationship between these and other parameters is complex and has already been discussed. In addition, the resolution required in any one application will determine the migrant loading which in turn is affected by density and viscosity.

However, for any particular separator of fixed dimensions, the carrier flow rate and temperature rise for a particular power input can readily be determined. The mobilities of the components to be separated will determine the power input, remembering that the resolution will be improved the greater the distance migrated and the greater the difference in individual mobilities. The migrant load affects the resolution, and the optimum conditions will be determined by the resolution required and the density and viscosity of the sample. As a rule of thumb, density, viscosity and conductivity of the sample should match carrier conditions as closely as possible for maximum resolution. A sufficiently large density difference, as may be found with a concentrated protein solution, will cause the migrant initially to flow down the annulus instead of being carried upwards and can cause mixing. This effect is less pronounced as the linear velocity of the carrier flow is increased. In addition, an increase in either migrant flow or

concentration will reduce resolution by increasing the zone width of the electrophoresed components.

To overcome these problems, a compromise must be reached between these different factors although some have been solved by modifying the composition of the sample. For example, with highly concentrated protein solutions (> 50 mg/ml), the density can be reduced by addition of ethanol to the migrant stream. To date, the highest practical throughput has been achieved with migrant/carrier ratios of ∿ 1:50 in the existing intermediate scale separator, (i.e. ∿ 25 g/hr for plasma) although for components which are well resolved (e.g. IgG and albumin), considerable "overloading" may well be possible. Thus, the gain in throughput can often compensate in part for any yield reduction due to resolution.

To date, three different separators have been constructed with annuli of 2mm, 5mm and variable 3-7mm, with electrode lengths varying by a factor of 3. Table 2 shows the results from two of these separators with a nominal 3-fold difference in throughput. The data obtained so far indicate that at least a further 3-fold increase in throughput may be achieved. However, because of the highly interactive nature of the variables affecting the performance and operational stability of this type of separator, further work is needed to determine the upper practical limits of scale up. Nevertheless, we can envisage a family of separators with at least a 50/100-fold range of throughputs covering applications from analysis to full scale production.

6.v. Continuous Operation

The system is very stable in operation and has been operated continuously for periods of up to 60 hours with only relatively minor drifts in peak position or band width which have been restored readily by adjustment of the current (Figure 19). The build-up of protein on membranes which greatly affects the performance and usefulness of some other preparative electrophoresis systems, is not evident in the separator described here. At first

TABLE 2 This table compares the performance of two separators with a 3-4 difference in throughput and shows the differences in certain critical parameters.

Separator	Stator Radius (cm)	Rotor Radius (cm)	Electrode Length (cm)	Throughput (Nominal) (mg/minute)	Current (Amps)	Carrier Flow ($cm^3 s^{-1}$)	Distribution Location (Fraction of Annulus)	S.D. (Off-take units)
Intermediate	4.0	4.5	30.5	100	25	8.3	0.56	0.94
Large	3.8	4.1	64.0	300 - 400	78	28.2	0.57	0.84

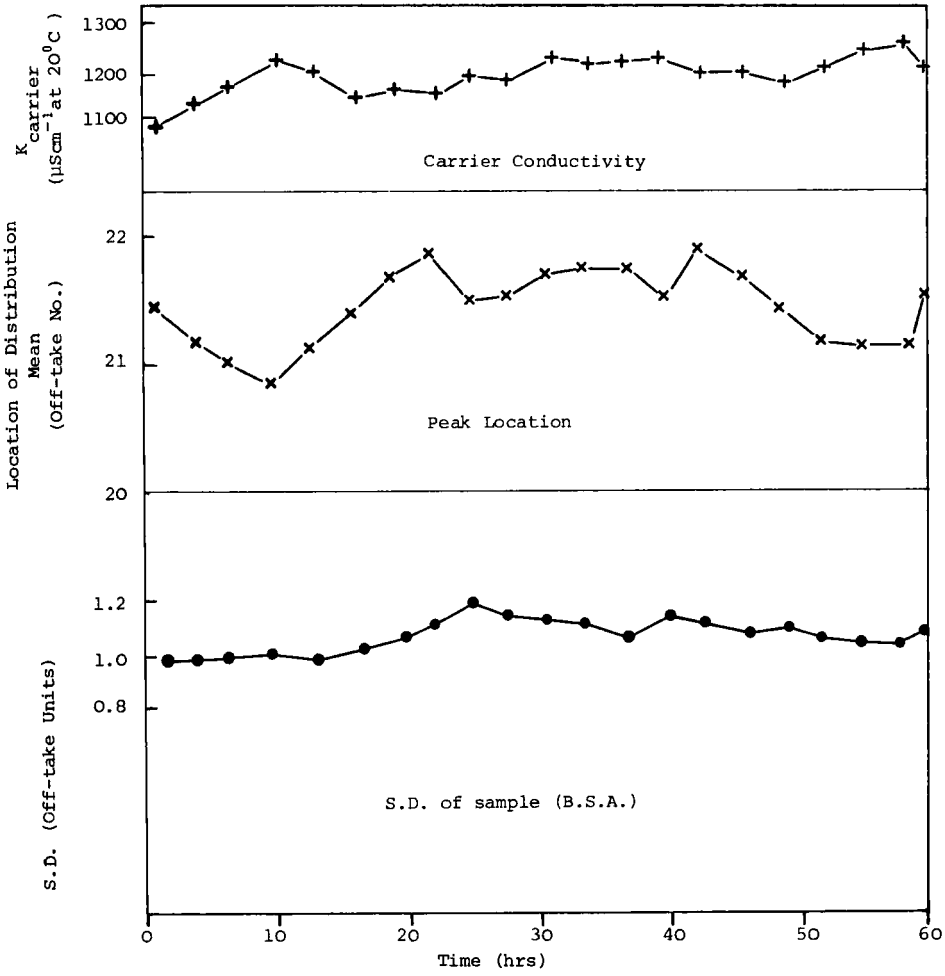

FIG. 19 Results of operating the electrophoresis system continuously over 60hrs. The carrier conductivity, location of the BSA sample and the S.D. of the sample were monitored routinely during the run. The carrier buffer was ammonium acetate pH 10.5 conductivity 1.2mScm^{-1} flow rate 600ml/min. The sample was BSA at 10mg/ml and an injection rate of 10ml/min. Electrophoresis was performed at 37A (30V).

sight it would appear that since the migrant is introduced into the annulus immediately adjacent to the rotor, all migrant species must possess the same charge sign in order to migrate away from the stator and towards the rotor. Any oppositely charged ions should migrate into the stator membrane and possibly cause build up which would affect permeability and voltage gradient stability. This is not found in practice and may be due partly to the existence of some flow into the annulus from the electrode compartments. In addition, if a build up of material on the stator membrane is suspected, the pH in the annulus immediately adjacent to the stator membrane can be changed. Thus, by altering the pH of the electrolyte in the stator compartment, conditions can be selected to impart an opposite charge on ions as they near the stator, reducing the possibility of deposition. This effect is shown by the behaviour of the low mobility proteins in plasma (mainly IgG) for, although a proportion are cathodic at pH 7.5, there is no sign of a build up at the stator (Figure 12).

It is possible that some problems of this nature will be experienced with proteins more basic than the immunoglobulins, or during electrophoresis at lower pH's, where cathodic mobilities of many proteins will be greater. However, it is unlikely that many separations of physiological molecules will need to be carried out under those conditions because of their instability.

The possibility of membrane fouling has been anticipated in the separator design which provides for an additional inlet approximately 1 cm above the normal migrant inlet. This can be used to provide a "buffer zone" which flows up between the stator wall and the migrant to keep anodic components away from the stator membrane.

In addition to stability over extended periods of operation, separations can be reproduced readily even after significant intervals of time. No special precautions are required other than to ensure the pH, flow rates and particularly the conductivity conditions are reproduced. Thus, separation conditions derived

from short term experiments can be reproduced easily for preparative runs.

6.vi. Applications

The separation system described here satisfies many of the requirements for biological work. It has a very high throughput when compared with other electrophoretic systems and compares favourably with other fractionation techniques such as ion-exchange. The system can be operated over an adequately wide pH range (3-11) and can be used with most materials of interest to biologists, viz. small molecules up to whole cells, with very good recoveries of biological activity. Resolution is good considering the throughputs possible and the system has proved relatively straightforward and reliable in operation. It should be noted that there are relatively few techniques for separating viable whole cells on a large scale, and free-flow electrophoresis utilises a different property (surface charge) to those usually used (e.g. density and size). Apart from the surfaces of inlet and outlet tubing, contact of samples with the separator is negligible during fractionation, hence it can be anticipated that relatively hydrophobic solutes (e.g. some proteins, lipoproteins, membrane-bound components), which tend to adsorb strongly to polymeric surfaces, can be fractionated successfully.

To date, the electrophoretic separator has been used to fractionate a wide range of materials and several important applications have been identified and developed. In most cases the fractions obtained are still impure, depending on the composition of the starting material, but it should also be pointed out that with most mixtures as complex as biological tissues (e.g. plasma), seldom is a single technique capable of resolving a particular component from a mixture in one step. Normally, recourse has to be made to the sequential use of a number of techniques which depend on different properties. In this regard, the continuous flow electrophoretic separator is attractive since it can be used at virtually any stage of a preparation. Thus,

crude extracts can be processed at substantial throughputs to give partially-purified fractions which can then be processed further by more highly resolving techniques usually at lower throughputs. It can also be used at later stages of a preparation to give a final purification. Operation of the system at the relatively low ionic strength necessary has not proved a severe problem even up to the maximum throughput investigated. However, this consideration necessitates reduction of the ionic strength of many samples before electrophoresis, and is readily effected on the litre scale by conventional dialysis, by diafiltration or gel filtration (e.g. Sephadex). This has been carried out routinely and successfully with little difficulty with a wide range of samples including blood plasma, and with negligible problems of precipitation or loss of biological activity. Clearly, however, on a large industrial scale, desalting will present more of a problem, but we feel that it is by no means insurmountable since precipitation and/or ultrafiltration are now frequently used to concentrate crude extracts, and to achieve desalting at the same time.

With cells and sub-cellular particulates, the problem of concentration can be overcome by centrifugation and re-suspension in an appropriate buffer, particularly since zonal centrifugation in a density gradient might well be a preliminary fractionation step used in the purification.

Location of components in the 30 fractions during electrophoresis requires a method of monitoring fractions in order to establish as quickly as possible whether the conditions used are optimal. The simple manual spectrophotometric method described enables this to be achieved relatively simply and a complete scan of fractions can be carried out in 5-10 minutes. Furthermore, this system can be used to monitor the stability of the separator by monitoring continuously a suitable outlet tube (e.g. the ascending or descending limb of a peak) and recording any minor fluctuations in the position of the peak. Once stable conditions have been established for a desirable separation, collection of bulk samples

then follows with periodic monitoring to ensure that minimum peak drift has occurred during collection.

The dilution occurring in the separator (5-10 fold) during electrophoresis presents no great difficulty, particularly with the advent of compact and effective ultrafiltration systems. Also, since the products emerge at low ionic strength, the conditions are particularly suitable for concentration or further purification by adsorption on ion-exchangers. We have successfully used Amicon hollow fibre units to concentrate fractions up to 10-fold after electrophoresis and before further processing, with negligible loss of biological activity, at room temperature and also at $4^{\circ}C$, provided care is taken to adjust pH and ionic strength before ultrafiltration. Also, we have used ultrafiltration to concentrate larger volumes (> 10 litres) of outlet fractions prior to freeze drying. It should be noted that many current fractionation procedures, (e.g. column chromatography and gel filtration), cause a similar dilution and a subsequent concentration stage is necessary.

6.vii. Operation under sterile conditions

For many production scale applications, it will be necessary to operate the electrophoretic system under hygienic or sterile conditions. Clearly, steam-sterilisation, although the method of choice, will present problems. We are investigating modes of operation aimed initially at minimising contamination with micro-organisms. Thus we have found that by the use of conventional bacteriocidal fluids such as hypochlorite and bacteriocidal detergents, microbial contamination can be reduced to very low levels which can be maintained by using pre-filtered (0.2μ) carrier buffers. Further, there is minimal re-contamination even on standing if the system is thoroughly washed with sterile water (0.2μ filter) after use. The semi-permeable membranes used can withstand these treatments, and appear to be very resistant to biodegradation over prolonged periods of use in the separator under non-sterile conditions. This aspect is being investigated in more

detail, with the aim of designing a system suitable for production of pharmaceutical compounds.

7. CONCLUSIONS

We see the continuous electrophoretic separator as offering a new and versatile general technique for separating and purifying biologically active materials and in particular, labile compounds, in high yield and with good recovery of biological activity. It can be used to fractionate crude extracts (even containing colloidal or slowly sedimenting material), at high throughputs giving fractions enriched with selected components which can be purified by other complementary techniques (such as ion-exchange chromatography) or by re-cycling through the separator using different operating conditions. Because of its operational stability and reproducibility, day-long or continuous operation can be used readily. In addition, since all components present in the input stream are recovered in the outlet fractions, the collection of several components simultaneously from a single feedstock could be of major importance for a starting material in short supply (e.g. human plasma), or one which is costly to produce (e.g. some tissue culture or fermentation products). Continuous electrophoresis could also have particular advantages for cell fractionation, already demonstrated to be feasible in the system described here, and already explored in more detail in thin film systems.

The theoretical hydrodynamic model of the separator describes well its performance, has high-lighted its development potential, and has enabled a 3-fold scaled up version to be constructed and operated successfully besides indicating a level of further scale up.

Work is in progress aimed at identifying specific production-scale applications, as well as improving resolution, developing on-line monitoring and control systems, and modifying the design for use under sterile conditions.

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NOMENCLATURE

R_1 = STATOR RADIUS - INNER BOUNDARY OF ANNULUS (cm)

R_2 = ROTOR RADIUS - OUTER BOUNDARY OF ANNULUS (cm)

a = ANNULUS WIDTH ($R_2 - R_1$) (cm)

$\bar{R}$ = MEAN RADIUS (cm)

L = ELECTRODE LENGTH (cm)

s = A RADIAL DISTANCE ACROSS THE ANNULUS - MEASURED FROM STATOR (cm)

s_o = INITIAL MEAN VALUE OF MIGRANT DISTRIBUTION AT ZERO POWER (cm)

σ_o = INITIAL STANDARD DEVIATION OF MIGRANT DISTRIBUTION AT ZERO POWER (cm)

σ = STANDARD DEVIATION OF MIGRANT AFTER ELECTROPHORESIS (cm)

σ_h^2 = VARIANCE DUE TO ELECTROPHORETIC HETEROGENEITY (cm^2)

F_c = MEAN AXIAL FLOW OF CARRIER ($cm^3 s^{-1}$)

F_m = MIGRANT FLOW ($cm^3 s^{-1}$)

F_s = TOTAL AXIAL FLOW OF CARRIER BETWEEN R_1 AND ($R_1 + s$) ($cm^3 s^{-1}$)

I = CURRENT (AMPS)

E = VOLTAGE GRADIENT ACROSS ANNULUS

T = TIME (s)

θ = TEMPERATURE (^oC)

θ_i = TEMPERATURE OF CARRIER AT INLET TO ANNULUS (^oC)

K_{20} = CONDUCTIVITY OF CARRIER AT 20^oC (Scm^{-1})

K_i = CONDUCTIVITY OF CARRIER AT INLET TEMPERATURE (Scm^{-1})

$\bar{K}$ = CONDUCTIVITY OF CARRIER - MEAN VALUE (Scm^{-1})

V = MEAN AXIAL VELOCITY OF CARRIER (cms^{-1})

N = ROTATION SPEED (s^{-1})

U = ELECTROPHORETIC MOBILITY ($cm^2 s^{-1} V^{-1}$)

η = DYNAMIC VISCOSITY (POISE)

α = TEMPERATURE COEFFICIENT FOR CONDUCTIVITY (θ^{-1})

ρ = DENSITY OF CARRIER SOLUTION (gcm^{-3})

D = MOLECULAR DIFFUSION COEFFICIENT ($cm^2 s^{-1}$)

h = ELECTROPHORETIC HETEROGENEITY COEFFICIENT ($cm^2 s^{-1} V^{-1}$)

Cp = SPECIFIC HEAT CAPACITY ($J\ g^{-1}\ \theta^{-1}$)

R_e = BULK REYNOLDS NUMBER

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APPENDIX 1

Annular Flow

Laminar axial flow through an annulus of outer radius R_2 and inner radius R_1 is given by the equation[36]

$$F = \frac{-\pi}{8\eta}\frac{dP}{dL}\left(R_2^2 + R_1^2 - \frac{R_2^2 - R_1^2}{\mathrm{Ln}\ R_2/R_1}\right)\left(R_2^2 - R_1^2\right)$$

where dP/dL is the pressure gradient.

The following equation can be derived for the volume flow between radii A and B ($R_1 \ll A < B \ll R_2$)

$$F_{AB} = \frac{-\pi}{4\eta}\frac{dP}{dL}\left[\left(R_2^2B^2 - \frac{B^4}{2} + \frac{R_2^2 - R_1^2}{\mathrm{Ln}\ R_2/R_1}\cdot B^2\left(\mathrm{Ln}\frac{B}{R_2} - 0.5\right)\right) - \left(R_2^2A^2 - \frac{A^4}{2} + \frac{R_2^2 - R_1^2}{\mathrm{Ln}\ R_2/R_1}\cdot A^2\left(\mathrm{Ln}\frac{A}{R_2} - 0.5\right)\right)\right]$$

However, when the annulus width is much smaller than R the flow profile is approximately parabolic and the simple expression

$$F_s = F_c \left(3(s/a)^2 - 2(s/a)^3 \right)$$

is a good approximation and adequate for practical purposes.

APPENDIX 2

Resolution

The extent of resolution of two normally distributed species of the same concentration depends upon their respective standard deviations (σ_A,σ_B) and their separation distance ($U_A - U_B$). For example, to recover 95% of species A at a purity level of 95%, the 'cut' is made at the ordinate which is distance y from U_A and where $U_A - U_B = d$

$$\text{then} \quad \left(1 + \text{erf} \, (y/\sigma_A\sqrt{2}) \right) /2 = 0.95$$

$$y = 1.645\sigma_A$$

$$\text{and} \quad \left(1 - \text{erf} \, (d-y/\sigma_B\sqrt{2}) \right) /2 = 1 - 0.95$$

$$d = 1.645 \, (\sigma_A + \sigma_B) \qquad (1)$$

To move component A (mobility U_A) a distance s cm requires a current I_s amps

$$\text{where} \quad 3as^2/2 - s^3 = U_A I_s a^3/2\bar{K}F_c$$

For component B (mobility U_B) to be separated from A by d cm

$$3a(s + d)^2/2 - (s + d)^3 = U_B I_s a^3/2\bar{K}F_c$$

where $U_B > U_A$

When $d < a$

$$3asd - 3s^2d = I_s s d^3 \Delta U/2\bar{K}F_c$$

hence $\Delta U = 6sdKF_c(a - s)/I_s a^3$

As $F_s = UI_s/\overline{K}$

$$\frac{\Delta U}{U} = 6sdF_c (a - s)/a^3 F_s$$

Substituting $F_s/F_c = 3(s/a)^2 - 2(s/a)^3$

$$\frac{\Delta U}{U} = 6d(a - s)/s(3a - 2s) \qquad (2)$$

When σ is due to molecular diffusion, it can be shown that σ/a is essentially independent of machine dimensions for a given $\Delta\theta$. Therefore we can express the standard deviation as a fraction of the annulus, i.e. $\sigma = xa$.

When $\sigma_A = \sigma_B$, from equation (1)

$$d = 3.29\ xa$$

and substituting in equation (2)

$$\frac{\Delta U}{U} = 19.74\ xa(a - s)/s(3a - 2s)$$

The effect of parabolic flow on the standard deviation is approximately

$$\hat{\sigma}\ a^2/4s(a - s)$$

where $\hat{\sigma}$ is the minimum S.D(= x'a)

Substituting for xa and expressing s as a fraction of a(= ma)

$$\frac{\Delta U}{U} = 4.94\ x'/m^2(3 - 2m)$$

or alternatively

$$\frac{\Delta U}{U} = 4.94\ x' F_c/F_s$$