LARGE SCALE PREPARATIVE ELECTROPHORESIS EQUIPMENT: The Current State-of-The-Art

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ABSTRACT

Progressive development in genetically-engineered biotechnological products in general and downstream processing in particular, has prompted the development of large scale electrophoresis equipment due to its capability and versatility to separate biochemical products. In this paper, large scale electrophoresis equipment and its important in downstream processing with respect to its development, current status and future prospect are reviewed.

INTRODUCTION

Downstream processing which involves separation and purification of biological products from a complex mixture is a field that is currently undergoing intensive study both in the academic and the industrial research laboratories. The research, mainly aimed at developing new separation process or modifying existing techniques to meet the challenges arise related to downstream processing which is fundamental to the success of biotechnology (1).

A particularly promising technique among the methods currently under investigation is the electrophoretic technique. It exploits the different electrophoretic separation velocity of charge spesies under the influence of an electrical field (2-3). Since practically all substances acquire a positive or negative charge as a result of either adsorption onto colloidal particles or by the dissociation of soluble spesies in an aqueous solvent, there is hardly a class of compound which has not been separated by electrophoresis (1). Thus electrophoresis is a good candidate for separation and purification of products from bioprocesses. Advantages include good resolution possible for separation of bioproducts and ability to maintain the bioactivity of these products (4).

Electrophoresis in its many formats widely adapted for analytical applications. The various techniques developed include moving boundary electrophoresis (MBE), zone electrophoresis (ZE), isotachophoresis (ITP) and isoelectric focussing (IEF). Each of these methods is well describes in a number of texts (5-11).

On the preparative scale, however, progress has been much slower because of some engineering problems face in scaling up (12-13). Recent years, there have been a renewed interest in electrophoresis due to its versatility for separation of biochemicals and some promising progresses have been achieved.

HISTORY AND DEVELOPMENT

The phenomenon of electrophoresis in liquid media was discovered by Reuss in 1890 (14). Half a century later, in 1930, electrophoresis began to be used as analytical and preparative tool. Tiselius had introduced his historical moving boundary electrophoresis apparatus for the separation of serum proteins (14). This technique , however, has serious limitation in terms of preparative capabilities . As a result, it has largely been

superceded by the use of supported media carried out either in colums packed with gel or filter paper in order to minimise convective disturbances (8).

For preparative applications it still has several problems including the limitation of cooling efficiency, the necessity of separating the sample and supporting medium, the potential for interaction between the samples and support and electroosmotic flows caused by residue charges which either inherent in the supporting medium or arise from adherence of sample component (8). In addition, it is limited to batch operation which is not suitable for large scale purifications.

It is nature that attempts to adapt batch electrophoresis to preparative large scale purifications would culminate in the design of a continuous electrophoresis. In order to increase throughput and overcome some of the problems caused by using the solid support, continuous free-flow electrophoresis was deloped by Philpot (15) and later on by Hannig (16) and Strickler (17). Both methods have eluent flowing through a narrow gap between flat parallel plats (Figure 1 & 2). The individual solute components are displaced in proportion to their electrophoretic mobilities and, when the resulting bands have been sufficiently separated, they are collected at the effluent ports.

The throughput of these instrument is rather modest, typically less than 10 ml/hr. Attempts to increase throughput in these devices have resulted in frustration due to two phenomena. First, the continuous flow electrophoresis exhibits a buoyancy instability (18-20) and second, device resolution suffer as a result of the intense convective and electroosmotic dispersion associated with ' cresent ' phenomenon (21-22). However, these problems can be eliminated, to some extent, by several innovative techniques including changes in design and operation of the device.

EQUIPMENT

Tremendous amount of research has been carried out in designing suitable equipment, not only for laboratory scale but also have importantly, for industrial scale. Some of the equipment which have potential to be scale up for large scale production are described:

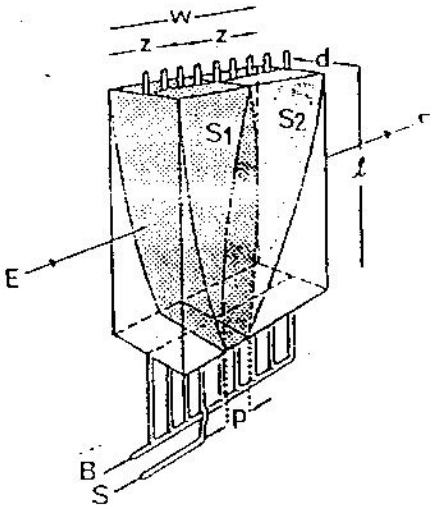

Figure 1: Schematic of the continuous free-flow zon electrophoresis according to Philpot (15). S, sample solution for separation; B, electrolyte; E, electric field; S1 and S2, separated sample components.

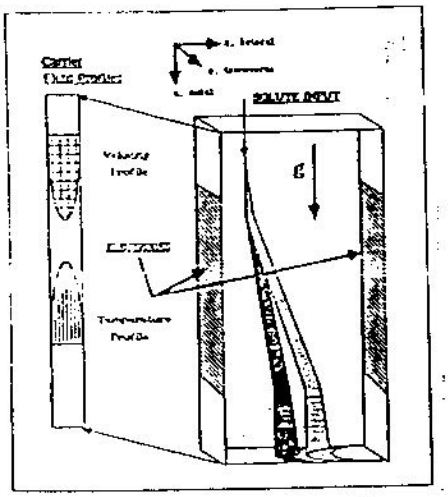

Figure 2: Schematic of the continuous free-flow zon electrophoresis according to Hannig (16).

The BIOSTREAM Separator

The technique was originally proposed by Philpot (17) and recently developed by researchers at the Harwell Laboratory of the U.K Atomic Energy Authority (23-26).

The electrophoresis process occurs in upwards vertical laminar flow of buffer solution whithin a vertical annulus, of 3 mm thickness, formed by two coaxial cylinders (figure 3 & 4). The laminar flow conditions are maintained by rotation of the outer wall of the annulus at about 150 rpm while keeping the inner cylinder stationary. Stack of disks are employed to achieve fractionation at the top of the cylinder and are capable of recovering 29 cylindrical layers of fluid. The residence time in this equipment is of the order of 20-60 seconds. This apparatus is designed and built for processing in the zone mode and has the largest protein processing capacity of any electrophoretic equipment (100 g/hr).

The Recycling IEF (RIEF) Apparatus (27-31)

The operation of this apparatus is based on continuous recycling of the fluid until full separation is achieved. The operation mode is applied to IEF due to the independent of the final steady state distribution of the initial or transient condition.

The apparatus consist of a compartment containing focussing cell with monofilament nylon screens between each channel. The segmentation of the seperation cell is essential for fluid stabilization during ground-based operation but this imposes a pH step-gradient.

The solution to be fractionated is recycled between a seperation cell and a set of heat exchange reservoirs (figure 5). In each pass there is a migration of the proteins towards their equilibrium positions, until the final distribution is achieved.

The RIEF apparatus can be operated either as a continuous (ZE mode) or batch (IEF mode) process. The continuous process used the technique which has been called " feed and bleed " (FAB).

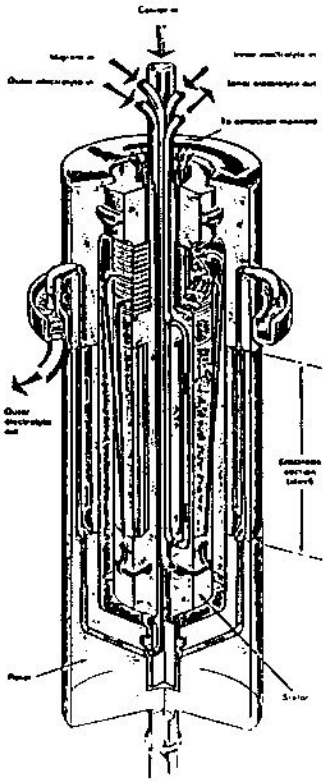

Figure 3: The continuous electrophoretic separator (26).

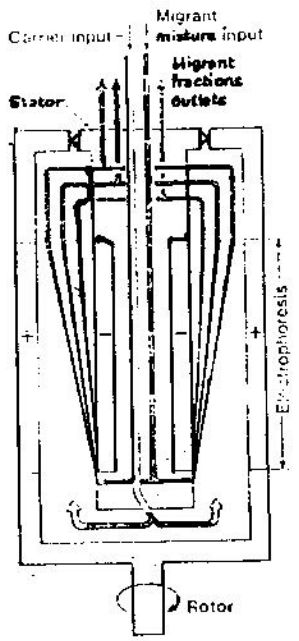

Figure 4: Diagramatic representation of the continuous electrophoretic separator(26).

The RF Apparatus (29)

The recycling focussing apparatus (RF 3) is designed and constructed in which the fluid flow through a narrow channel (0.75 mm) between two parallel plates. The device is oriented vertically with fluid flows from bottom to top. The fluid residence time in the apparatus is only a few seconds with the characteristic Reynolds number of approximately 600. This assured a remarkable stability of the laminar flow.

The anticipated throughput of macromolecular materialis somewhat lower than the RIEF but higher than Elphor VaP 21 apparatus. Figure 6 is a schematic is representation of this apparatus set-up for use in a continuous feed and zone electrophoresis mode called " feed and bleed ".

The Rotofor (30-31)

The Rotofor employs an annular focusing chamber which is dividing into 20 subcompartment with microfilament nylon screen material. A cooling tube runs through the centre along the focusing axis. The entire assembly is rotated around the cylindrical axis by means of synchronous motor and gear assembly at 6.6 rpm to provide fluid stability and promote efficient cooling. Samples are collected by means of a vacuum device. The volume processed is a maximum of 40 ml.

The Elphor VaP 21 (32-33)

The Elphor VaP 21 apparatus was developed for analytical and preparative separations. The separation cell of this apparatus consist of a small gap, between two parallel plates, which laminar flow conditions are maintained. A glass front plate is held apart from the cooled back plate by a thin spacer of typically, 0.5 mm thickness. A thermostat cooling system dissipates the heat generated by the current. This apparatus is further equipped with a regulated transverve electrolyte counterflow and on-line detection at the end of the separation chamber (Figure 7). The Elphor VaP 21 system is potentially applicable to industrially meaningful quantities.

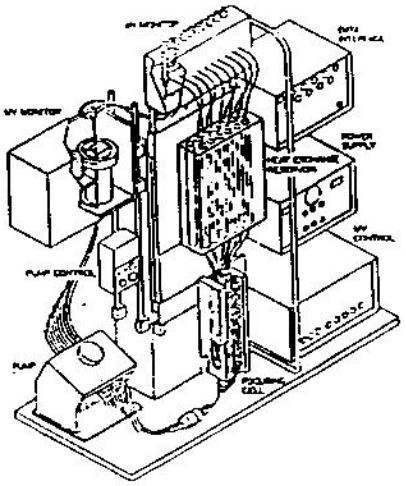

Figure 5: The recycling isoelectric focusing (RIEF) device (27).

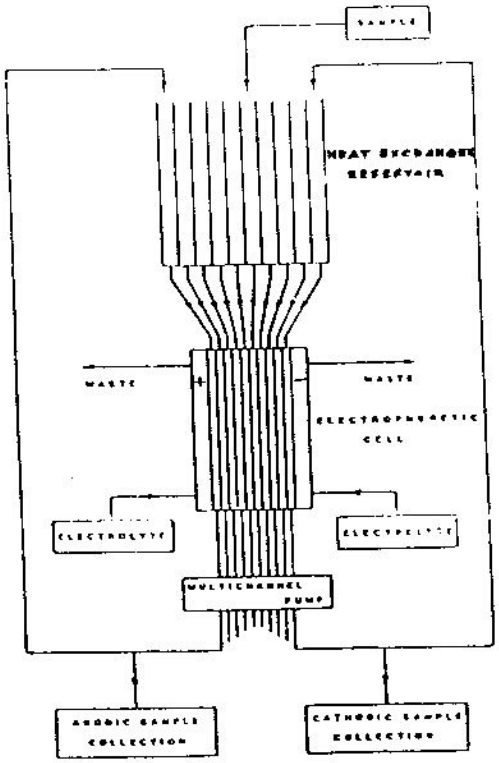

Figure 6: The RF 3 apparatus set up for use in a continuous feed and zon electrophoretic mode called feed and bleed (29).

Industrial Autofocusing Apparatus (34-35)

Industrial Autofocusing operates on the IEF mode without using ampholytes. It has been developed for large scale isoelecric focusing in industry. The production capacity of the system may be as high as 250 kg/hr. The apparatus is a simple box made from electrically insulating material, such as acrylate, plastics or ceramics. The open top of the box is provided with a cover made from the same material. At the side of the cover there are two holes of 0.3 cm in diameter, through which the electrodes are introduced. The autofocusing is always carried out at a power of 3 watt at variable field strength from 200 to 100 v/m.

Continuous Rotating Annular-Bed Electrop.(CRAE) Column (36-37)

This device is designed to overcome the major disadvantages of the thick-bed column such as Vermeulen column which is capable being to scale up and to minimise temperature rises in the bed.

An electric current is imposed axially in a packed bed annular column which is in the same direction as the eluent flow. The annular bed is rotated very slowly about its axis. Sample and eluent introduction ports at the top of the column and multiple product collection ports which ring the circumference at the bottom are fixed in space. As a result of this configuration, separated fractions appear as helical bands as shown in Figure 8 with each component leaving the column at a characteristic angular position.

Since separation takes place in the angular direction the bed may be kept relatively thin to reduce the temperature gradients and the flow stability due to buoyancy effects. The anticonvectant packing used may be either inert or adsorptive because of opposite charge or surface activity.

The CRAE column offers several advantages such as continuous operating, ease of scaling and capability to produce high resolution of products. Thus this device appears to have considerable potential to industrial application for the separation of biochemicals.

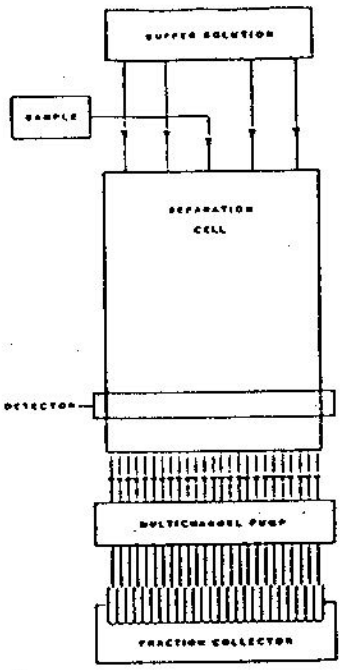

Figure 7: Schematic representation of the Elphor VaP 21 apparatus (29).

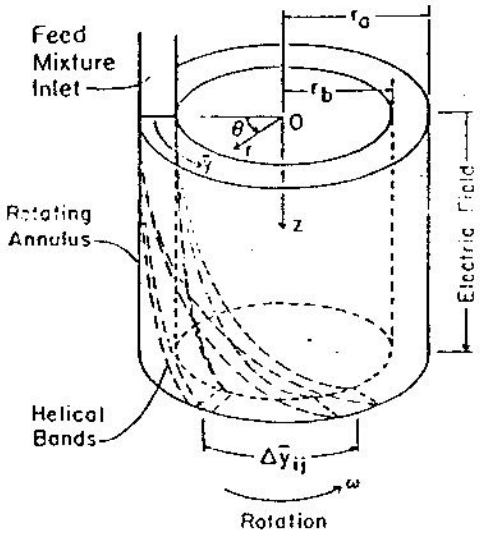

Figure 8: A schematic of the continuous rotating annular-bed electrophoresis column (36).

Large Scale Preparative Membrane Immobilized pH Gradient Apparatus (38-39)

This device is a scale up version (figure 9) from experimental apparatus which has several features:

1. the immobilise gels facing the flow chamber have been reduced to membranes only 2-3 mm thick, with a fixed pH value.
2. the apparatus is horizontal.
3. the electrodes are movable and can be positioned as close as 5 cm apart.
4. the immobilized pH gel membranes and the two Pt electrodes have a larger diameter (9 cm) to ensure even current flow and high transport rates of non-isoelectric proteins.

Separation is achieved by recycling the sample continuously onto the electric field. Proteins will migrate to pH which is correspond to its isoelectric point. This separation method is a batch operation.

The largest version of the segmented IEF recycling chamber has the potential to process > 60 g/hr.

Recycling Continous Flow Electrophoresis (RCFE)(40-43)

This device has been developed based on the classical free-flow electrophoresis formely developed by Hannig which its resolution is limited by deleterious effect of diffusion, electroosmotic dispersion and convective dispersion. These problems can be overcome by continuous recycling effluent through out the chamber in the manner which allows complete recovery of undiluted product at arbitrarily high purities. Figure 10 shows the schematic presentation of this technique. The processing capacity of this device is at least as high as 100-500 ml/hr of feed.

CURRENT STATUS AND FUTURE PROSPECTS

The development of large scale preparative electrophoresis equipment has been very slow due to two major obstacles (44-45):

- -heat generated on passage of an electric current through the conducting solution.

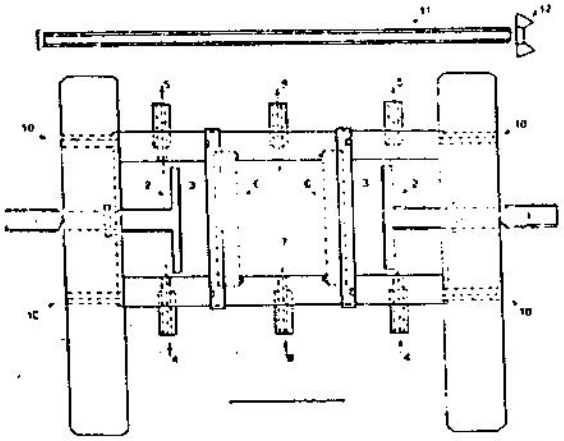

Figure 9: Large-scale preparative membrane immobilized pH gradient apparatus (38). 1, connection to power supply; 2, Pt electrode disks; 3, electrodic chambers; 4, inlet and 5, outlet of electrodic chamber; 6, housing for immobilized pH gradient isoelectric membranes; 7, sample flow chamber, with inlet (8) and outlet (9; 10, perforations housing for threaded metal rods (11) for bolting together the electrophoresis unit; 12, Winged nut.

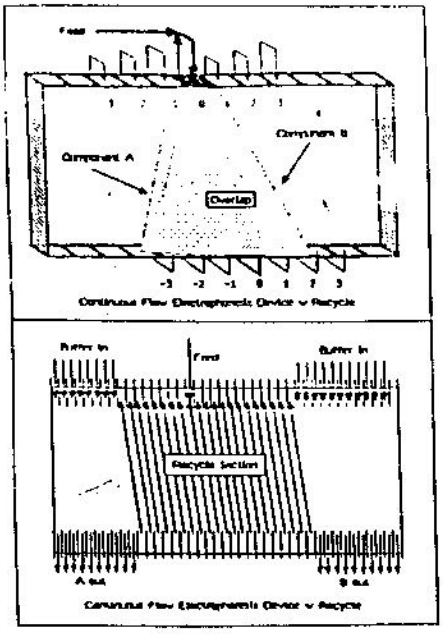

Figure 10: Schematic representation of the recycling continuous flow electrophoresis (40).

Excessive temperature rise could damage the products.

-hydrodynamics effect including turbulent and nature convective which could affect the resolution.

A wide range of technical solutions have been investigated and some of these problems have been overcome. These include increasing viscosity of the eluent, application of density gradients and a velocity gradient. Even though a number of devices have been reported as having potential for scale-up, presently, there are only three which are commercially available for production scale ;

-the BIOSTREAM separator from CJB Development Limited, U.K.

-the Elphor VaP 21 from Bender & Hobien (F.R.G)

-the Rotofor from BioRad, Richmond, USA.

others are still undergoing further development.

The future prospects of this technique for large scale downstream processing still depend upon the ability of the engineer to design and scale up either based on the existing or through the development of the new devices. Based on current progress in research it is of no doubt that electrophoretic techniques will appear to be one of the most important separation techniques in biotechnology near future.

CONCLUSIONS

The electrophoretic separation techniques have come of age. This can be proven through the availibility of several potential large scale electrophoresis equipment. It has been under scrutiny of either biochemist or process engineer for application to a very large scale protein (or enzyme) fractionation due to its unique capabilities over other methods such as chromatographic techniques, liquid-liquid extraction, precipitation and crystalisation, membrane based technology and centrifugation. In addition, the recent introduction of technologies capable of producing mass quantities of protein of commercial important and a very high purity of product is essential for clinical used, it has potential to replace existing process such as coventional affinity chromatography and high performance liquid chromatography (HPLC) which has several drawbacks.

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