SINGLE-STAGE PREPARATIVE ELECTROPHORESIS WITH DISCONTINUOUS ELUTION

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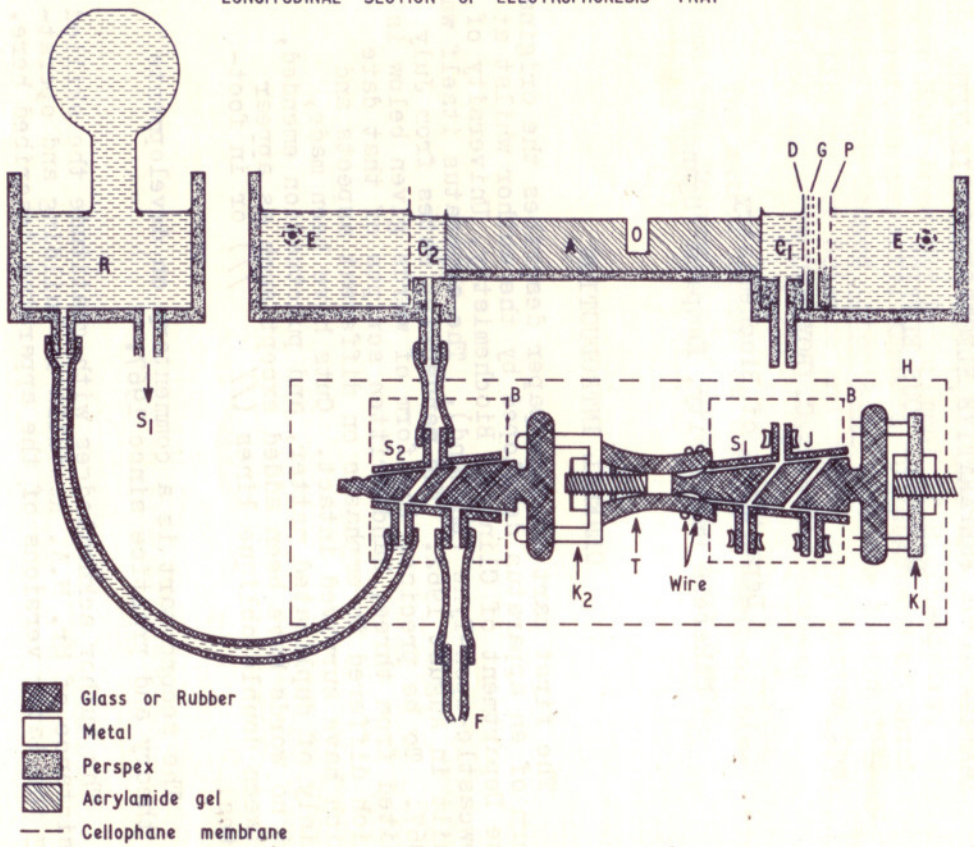
Fig. 1

LONGITUDINAL SECTION OF ELECTROPHORESIS TRAY



PART I "A NEW APPARATUS FOR PREPARATIVE ELECTROPHORESIS"

A. INTRODUCTION

In recent years, there have been a number of descriptions of apparatus for preparative electrophoresis. The simplicity and the excellence of the results of analytical zone electrophoresis have tempted many workers to become inventors and amateur mechanics. Yet in spite of the remarkable ingenuity of many of the designs, the results seem to have been rather disappointing. Preparative electrophoresis has not become a standard method as happened in a far shorter period with column chromatography on modified celluloses and on dextran gels. If the separations were comparable with what can be easily achieved on the analytical scale, acceptance would have been rapid and enthusiastic.

We describe here, in outline, an apparatus which differs in several important respects from any previously published, and discuss the theory and potential developments of the new approach.

B. PRINCIPLE

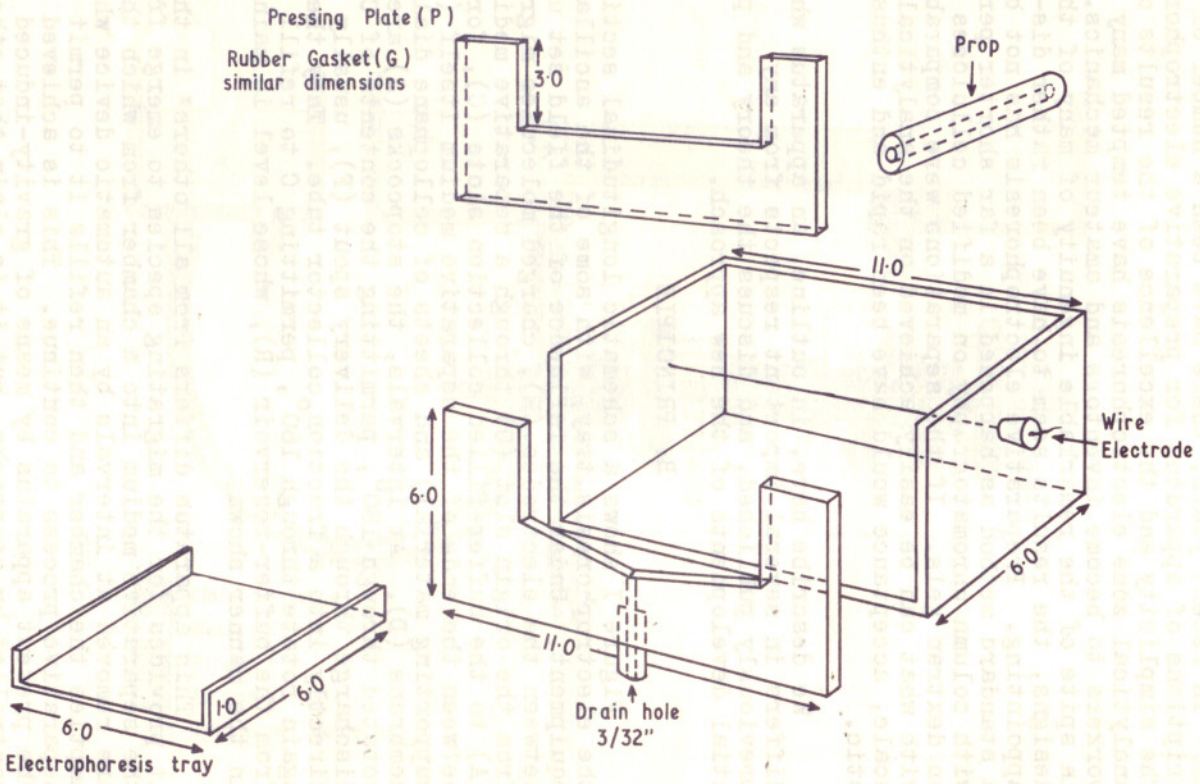
Figure I shows a schematic longitudinal section of the electrophoresis tray, with some of the ancillary equipment. Under the influence of the field set up between the electrodes (E), charged molecules migrate from the origin slot (O) through a separative medium (A) to the buffer-filled collection slots (C), formed between the ends of the separative medium itself (or supporting material) and sheets of cellophane dialysis membrane (D). At intervals, the stopcocks (S) are rotated through 180°, permitting the contents of C to discharge through the delivery spout (F), usually directly into a fraction collector tube. They then again rotate through 180°, permitting C to refill from the buffer-reservoir (R), whose level is maintained in the manner shown.

This apparatus differs from all others* in that it provides for the migrating species to emerge from the separative medium into a chamber from which they are removed at intervals by an automatic device which empties the chamber and then refills it to permit the separative process to continue. This is achieved in the present apparatus by means of gravity-induced flow, controlled by stopcocks, but it is plain that other systems could be used involving flows induced mechanically, (including via gas pressure or vacuum) and the emptying and/or the refilling process to be done otherwise than from below the chamber.

*Note subsequent appearance of papers by
Schenkein, Levy and Weis, Anal. Biochem., 25(1968)387
Brownstone, Anal. Biochem., 27(1969)25 and by
Hodson and Latner, Anal. Biochem., 41(1971)522
and c.f. Part II

Fig. 2

ISOMETRIC DIAGRAM SHOWING CONSTRUCTION OF ELECTROPHORESIS TRAY AND ELECTRODE COMPONENTS



Such would involve no change in principle, nor would operation of the apparatus in other than a horizontal mode.

This apparatus also differs from all others* in that arrangements are provided for collection on both anode and cathode sides of the origin. Thus, anionic and cationic species can be collected simultaneously. This object can be achieved with two-stage systems of preparative electrophoresis, and with curtain electrophoresis, but not with any existing apparatus of the kind distinguished below as type III (quasi-chromatographic) - although it may sometimes be possible to overcome this defect by a suitable choice of pH.

C. DESCRIPTION//AND OPERATION//OF APPARATUS

C i) Electrophoresis Tray Assembly

(see Figs. 1 and 2)

The whole of this part of the apparatus is made of 'perspex' - "glued" together to form a single piece. In some early versions the various parts were separable and//were//joined by machine screws and rubber gaskets. This was done precisely to permit the use of several different trays (of different lengths) with the same buffer trays and collection apparatus. It has been found more convenient in practice to make the whole apparatus over again when trays of different lengths were required chiefly because this avoids any trouble with leaks.

The tray proper is open ended, the dimensions of the most used tray being 6.0 cm wide, 1.0 cm deep and 6.0 cm long (internal dimensions). Pieces are glued outside the side walls, rising 0.3 cm above the level of the side walls proper. These permit the buffer level in the collection slots to rise slightly above the top surface of the electrophoresis slab without risk of spillage.

The collection slot is formed from a single piece of perspex. Most conveniently, the thickness of the piece determines the length of the slot in the electrophoretic axis - usually 0.6 cm. The lower part of the slot is formed into a V-shape, and at the apex of this a 0.24 cm drill-hole is made to emerge at the bottom edge of the perspex form. This is enlarged at its lower end to accommodate a small perspex tube, conveniently formed *in situ* by glueing in position a short length of perspex rod, turned to fit, allowing it to set thoroughly (48 hours) and drilling along the length of the rod, preferably from within the collection slot. On this fits the flexible tube leading to the stopcock.

*But see Hodson and Latner (op. cit.)

The next portion of the apparatus is the electrode compartment. This is, in effect, a box of which one side is the perspex "collection slot". Dimensions are not critical, but the box must be wider than the electrophoretic tray (11 cm is usual) and there is no reason to make it very large, so that 6 cm long and 6 cm deep is convenient. The box is drilled at one side on a level with the electrophoresis tray, to accept a rubber bung through which passes the platinum wire electrode. Further holes are made for perspex tubes conveying circulating buffer.

This completes the description of the permanent electrophoresis tray assembly, but it requires certain ancillary parts which are not glued on.

The collection slot itself has the separative gel as one wall. The opposite wall is a sheet of cellophane dialysis membrane which is held in position by a U-shaped rubber gasket (G) and a perspex pressing plate (P), the "U" being of dimensions corresponding to those of the cross-section of the electrophoresis tray. The pressing plate in turn must be held in position and this is most conveniently done by cutting lengths of rubber pressure tubing slightly longer than the distance between the pressing plate (when in position) and the opposite wall of the electrode compartment. These are used as props. The collection slot is assembled manually by applying in turn the dialysis membrane (cut to a convenient size), the rubber gasket and the pressing plate, and then forcing in the rubber props. They can be made stiffer, if necessary, by slipping the tubing over a slightly shorter length of glass rod.

C ii) Stopcock Assembly and Related Parts

(Fig. 1)

Each collection slot is emptied and refilled via a double-bore stopcock of the type used in conventional burettes. They are operated mechanically by a low geared electric motor, and are connected to it, and to each other, by a flexible mechanical train, allowing for considerable errors in alignment. In the prototype apparatus, the retaining spring and clip of the first stopcock (S1) are replaced by a short length of rubber pressure tubing (T), wired in place, gripping the groove in the glass which normally accepts the retaining clip. Obviously, this must be adjusted with care, as it serves the double function of retaining the stopcock plug without permitting leaks or causing it to jam, and connecting it to the next part. The other end of the rubber holds an "OBA" nut, ground down until it could just be forced in, and into this is screwed a short piece of 1/4 inch threaded brass rod (OBA thread, "studding"). The claw (K2) which turns the second stopcock (made of sheet aluminium) is fitted over this rod, and is held in place by an OBA nut.

In this prototype, the two stopcocks are secured each to a small wooden block (B), 5/8 inch thick, by spring clips (J), 3 to each stopcock. These blocks are, in turn, secured with bolts to a piece of perforated metal sheet (H) and this to the scaffolding of the assembled apparatus via a bolt and a laboratory boss-head. The moving parts of the assembly are clear of any obstructions to their rotation. Notwithstanding its apparent complexity, this whole unit can be taken down or assembled in a matter of seconds.

The original stopcock assembly was made with the upper tubes (connecting to the collection slots) exactly 6.6 cm apart. In turn, the delivery spout tips of the stopcocks were arranged exactly over fraction collector tubes. In this way, flexible connecting tubes were virtually eliminated, except for the formation of joints, but severe restrictions were placed upon the general arrangement of the stopcock assembly and thus in turn of the stopcock drive assembly and of the electrophoresis tray assembly. Some difficulties in handling also appeared. It has, therefore, been found desirable to connect the various parts with somewhat longer pieces of flexible tubing. It is not possible to use the narrow bore tubing conventional for chromatographic work, nor is there free choice as to the length of the tubing and its arrangement (its "path"). In general, the length, bore, and conformation of the flexible tubing employed must be such as to permit rapid emptying and refilling of the collection slots, avoid the formation of air-locks and minimize dead space (which causes unnecessary dilution of the fractions).

The buffer reservoir consists of a perspex box drilled at the back for two perspex tubes, to which are attached flexible tubes leading to the stopcocks. A large flat-bottomed flask is filled with the buffer, inverted in this box, and secured to the scaffolding by a clamp and boss-head. The box is fixed wherever may happen to be convenient and at approximately the right height, the precise level of the buffer in the collection slots is then determined by adjusting the level of the aperture of the reservoir flask.

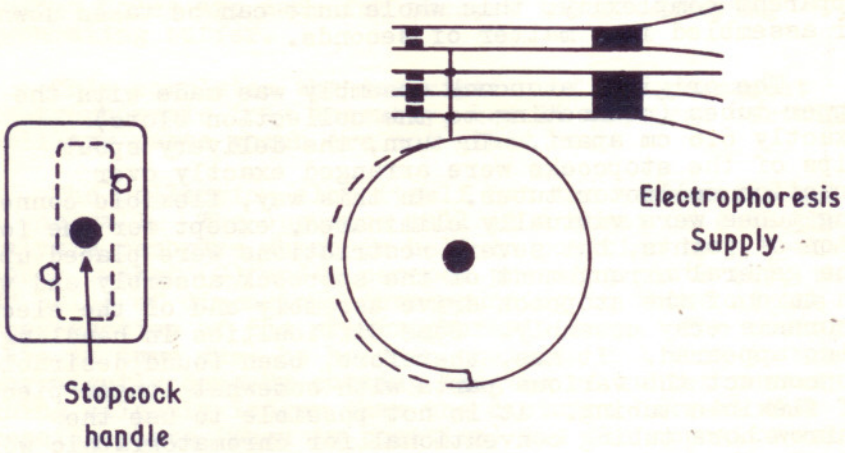
With the stopcock assembly described here, it is possible to arrange for "double-ended" collections with electrophoresis trays 1 - 10 cm long. For longer trays, if double-ended collection is required, it is better to use a new assembly with stopcocks further apart, because the alternative is to use inordinately long tubes between the collection slots and stopcocks.

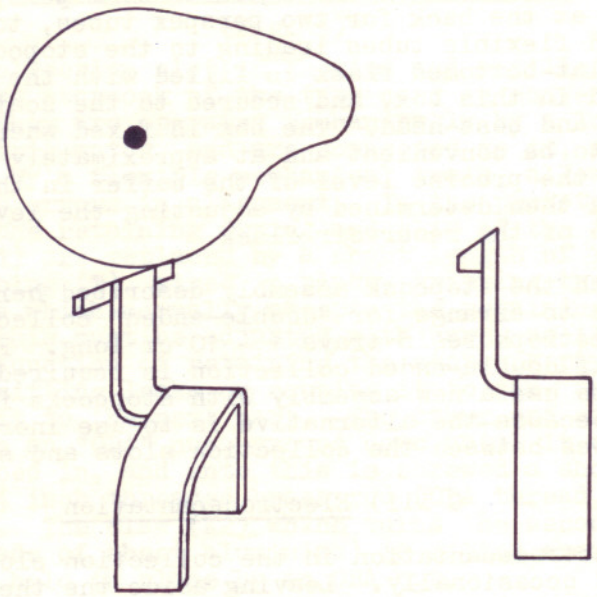
C iii) Electrodecantation

Electrodecantation in the collection slots has been observed occasionally. Leaving aside the theoretical possibility of zone-distortion due to this phenomenon, some loss of resolution may occur because of the denser protein-containing solution sinking out of the collection slot and coming to rest at a minimum point of the inter-

Fig. 3

ARRANGEMENT OF CAMS ON STOPCOCK DRIVE SHAFT
(REST POSITION LOOKING FROM MOTOR)





connecting channels. A glance at Fig. 1 will show that this is at, or beyond, the stopcock. Material in those situations will not be swept out when the slot empties, but is not lost forever, being swept into the slot with the next charge of buffer, and thus the bulk of it recovered only 1 fraction late. However, even this degree of//loss of resolution//is avoided at the expense of some further dilution of the fraction, by inserting an S-bend between the collection slot and the stopcock.*

C iv) Timing System

This consists simply of a mains-operated synchronous motor geared down to one revolution per 15 minutes (electric clock). The spindle bears a cam operating a two-way switch and there is another such switch operated by a cam on the stopcock drive shaft (Fig.3). Together they control the current to the stopcock drive motor in much the same way as 2 two-way switches may be used to control one lamp in domestic lighting systems. Starting from rest, the switch on the clock changes over from position 1 to position 2. The stopcock drive motor now starts up and continues operating until the stopcock drive shaft has turned through one half revolution and the appropriate cam changes the switch here from position 1 to position 2 (and also operates another switch to cut out the electrophoretic current). After an interval (determined by the cam) the switch on the clock spindle is changed back from position 2 to position 1 and again the stopcock drive motor operates until the cam on the stopcock drive shaft changes over this switch too from position 2 to position 1. The same cam switches the electrophoretic current back on just before reaching this point.

C v) Stopcock Drive Assembly

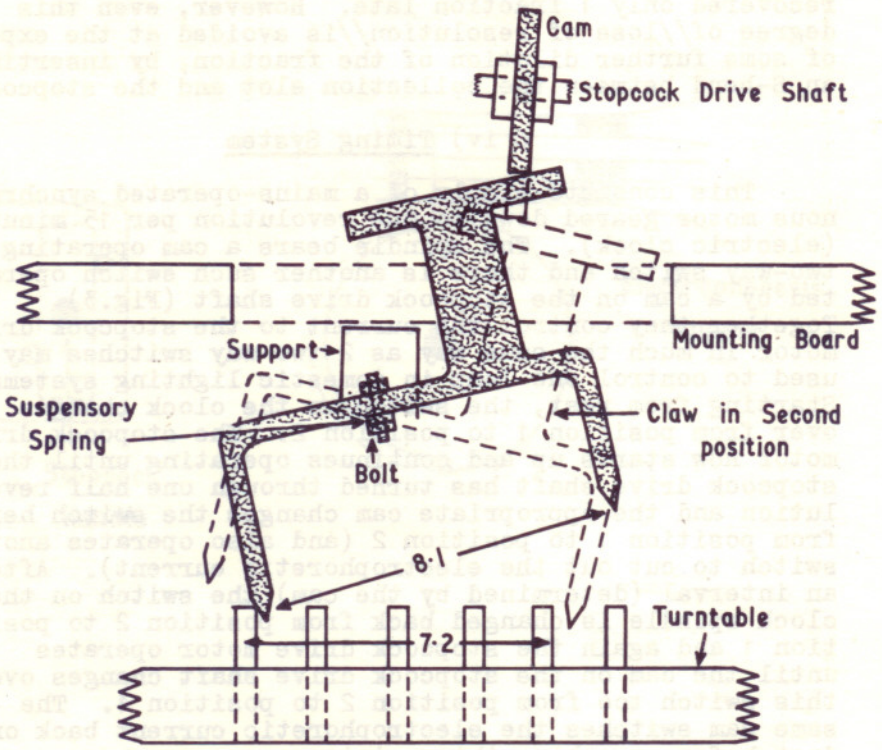
This is based on an electric motor geared down to 2 revolutions per minute. The final drive shaft is in line with the stopcocks and is made of 1/4 inch threaded rod (OBA thread, "studding"). On this shaft are mounted the timing cam described above, a cam operating the escapement mechanism, and the claw (K1), which turns the first stopcock (Fig. 3). In the present model this is a plate of perspex carrying two 1 inch long 6 BA brass bolts, which engage the projecting arms of the stopcock plug. All three are held in place by two locknuts, so that the longitudinal and angular position of each is fully adjustable.

A low geared motor is required to ensure ample torque and instantaneous arrest when the current is switched off. In the prototype considerable force is required to operate the escapement, but it will always be desirable to have ample reserve torque for operating the stopcocks.

*And see also Part II, Section H ii.

Fig. 4

OPERATION OF ESCAPEMENT MECHANISM



C vi) Escapement and Fraction Collector

The fraction collector built as part of this apparatus consists of a circular turn-table of rigid plywood, mounted on a hollow spindle which fits over a vertical shaft on the base-board. Motive force is provided by a cord wrapped round the circumference of the turn-table (lying in a groove) and running thence through a fixed pulley to another which is weighted and free to move. Around its perimeter, the turn-table bears 72 pegs projecting from the top surface, equally spaced and 10 cm in from the edge. Outside each peg, on the same radius, are 2 holes to accept the 5/8 inch x 6 inch centrifuge tubes which are used to collect the fractions. There are, thus, two rows of fraction collector tubes, one for each of the collection slots at opposite ends of the gel. But when the stopcocks are switched on, the two slots do not empty into tubes lying on the same radius; that is, corresponding fraction collector tubes are displaced relative to each other, by several holes. This is because the axis of the electrophoretic tray and stopcock assembly, is arranged at an angle to the turn-table radius, an angle which depends upon the length of the electrophoretic tray and stopcock assembly in use. Obviously, this arrangement was adopted to allow for the use of electrophoretic trays of varying length.

It would be perfectly simple to make a turn-table to hold more tubes of smaller diameter, but that in use holds sufficient for 18 hours with the timing system described above.

The pegs mentioned above constitute the rachet of an escapement mechanism. The claw (Fig. 4) is a specially shaped piece of perspex bearing two knife edges, spaced $(n + \frac{1}{2})d$ apart, where d is the distance between two pegs and n is any small integer (4 in the case of the prototype). This is suspended between supports by means of a piece of flat spring steel and projects through a hole in the mounting board. In its rest position, the leading knife edge prevents rotation of the turn-table. When the device is rotated about its suspensory spring, the leading knife edge releases the turn-table after the rear knife edge has engaged between two pegs further back, and vice versa. During mechanical operation of the apparatus the rear knife edge is depressed by the action of a cam on the stopcock drive shaft forcing down the upper limb of the claw against the restoring force of the suspensory spring.

C vii) General Arrangement

The apparatus is built on a wooden base-board, which carries the turn-table shaft and scaffolding supports. To these latter are fixed girders (of angle-section aluminium) which support a mounting board, carrying all the moving parts of the apparatus with the exception of the turn-table itself.

The position of the mounting board is thus freely adjustable in all dimensions, yet it is rigidly fixed in operation. To the mounting board are fixed directly the stopcock drive motor and gear train, the clock, supports for the escapement claw and the switches operated by the stopcock drive shaft cam, and scaffolding supports. It is perforated for the escapement claw, for the stopcock delivery spouts, for numerous fixing screws and bolts, and for wires. Above the mounting board, further scaffolding carries the stopcock assembly and girders for the electrophoresis tray assembly.

C viii) Buffer Circulation

To avoid pH changes during electrophoresis, one must either use very large electrode tanks, or change the buffer frequently, or, as suggested by Raymond*, circulate the buffer from one electrode tank to the other. This is adopted here.

C ix) Power Supplies

Mains power is required for the electric clock and for the buffer circulation pump. In the prototype, the stopcock drive is from a 12V D.C. motor which is considerably over-run being supplied by a transformer and full wave selenium rectifier giving 50V on open circuit, though only 20V at the current drawn by the motor (2 amps). The electrophoretic potential is supplied by a variable transformer (Phillips) and a full-wave silicon rectifier. Although a stabilized voltage supply would plainly be desirable, this simple arrangement has proved quite satisfactory so far.

C x) Preparation of Gels

Gels are best cast in special trays, not in the electrophoretic tray proper. Thus, one such tray is required for each electrophoretic tray in use and their dimensions must exactly match. The cover is made of a single piece of perspex, and glued to this is another piece of perspex which forms the origin slot. If the cover is made considerably longer than the casting tray, one can vary the position of the origin slot at will without making a new cover each time, but if it is required to vary the dimensions of the origin slot, then one must have a new former. For a series of experiments with identical gels it is worth having a special cover, with some means of locating it positively on the casting tray.

*Raymond, Ann. N.Y. Acad. Sci., 121(1964)350

The acrylamide gels are made essentially by the technique of Raymond(1), but the gel strength (concentration of monomer) is varied to suit the problem in hand and it is customary to dialyse the polymerized gel to remove catalysts and unchanged monomer and to introduce the chosen buffer(2). Suitable large perspex trays are required for this purpose. An anticonvectant must be used in the origin slot to prevent electrodecantation(3); agarose(2) has proved useful but it is also possible to introduce the sample in an acrylamide solution which is polymerized in situ - if there is no objection to the means of doing it and to the occurrence of a free radical reaction in the sample solution. In "disc electrophoresis(4)" this is done, but the riboflavin-catalysed photopolymerisation procedure only works in dilute protein solutions.

C xi) Buffer Level in Collection Slots

To avoid distortion of the electrophoretic field, the buffer must be at least level with the top of the separative medium. In practice, it is best to arrange for the equilibrium level to be about 0.3mm above this, since the approach to equilibrium is gradual and the electrophoretic current is switched on before it is attained. The electrophoresis tray must be very carefully levelled: this was less important in early versions of the apparatus which had a separate buffer reservoir for each collection slot(5).

(1) Raymond and Wang, Anal. Biochem., 1(1960)391

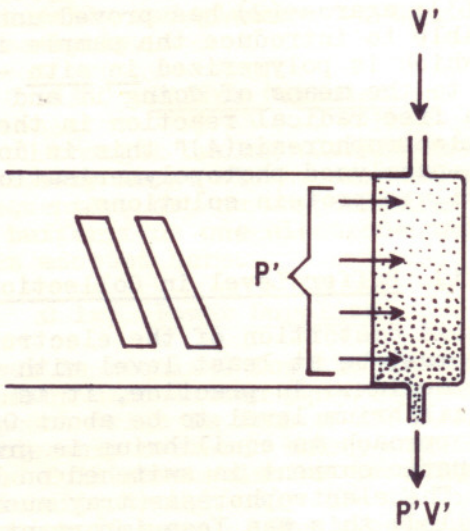
(2) Boyde, Z. Klin. Chem. 6(1968)431
Boyde, Biochem. J. 111(1969)59

(3) Gordon et al., Coll. Czech. Chem. Comm. 15(1950)

(4) Ornstein, Ann. N.Y. Acad. Sci., 121(1964)321
Davis, ibid., 121(1964)404

(5) See also Part II, Section H ii

Fig. 5



D. THEORY

D i) //Classification//*

Preparative electrophoresis procedures may be divided primarily into 3 classes. In type I, two stages are implicit: the electrophoretic separation is carried out and then in a separate operation the spread-out material is fractionated, (1) by dividing up the medium as is commonly done with free-zone electrophoresis or with zone electrophoresis on paper, starch block, starch gel, Pevikon, agar, agarose, Sephadex, sponge rubber, etc., or, (2) by draining the medium out of its container as with column electrophoresis on cellulose or in a sucrose density gradient, or, (3) by electroconvection**. Type II is 'curtain' electrophoresis, a method of tremendous potential because of the possibility of continuous operation - making the lack of universal commercial application even more surprising. Type III is the type most closely analogous to column chromatography, and, perhaps for that reason alone, the most popular in biochemical circles in recent years. Operation is discontinuous in that a single charge of material is applied to the electrophoretic medium, but the products are obtained by allowing them to migrate out of the medium into a special chamber or slot from which, in previous machines, they are removed by a continuous flow of buffer and carried to a conventional fraction collector.

D ii) //Discontinuous Elution//

The starting point of the present design was the realization that continuous flow was unnecessary, being a mental carry-over from chromatography, i.e., based on a false analogy with chromatography and with curtain electrophoresis, in both of which the flow of solvent is a necessary part of the process of separation. Here this is not the case. Intermittent emptying of the collection chambers should be just as good, and indeed it can be shown that continuous flow elution from a collection slot is potentially inimical to good resolution:

Consider a slot (Fig. 5), through which eluting buffer flows at a rate V'. A protein zone is in the process of elution, protein is entering the slot at a rate P', and cannot leave the slot except by being carried in the eluting buffer. We assume that for the moment a steady-state exists such that protein is leaving the slot as fast as it enters: in this case the concentration of protein in the buffer as it leaves the slot is P'/V'. Thus the concentration of protein in the bottom-most element of the slot is P'/V', whereas that in the top-most element is zero.

*See also Part II, Section H iv
**Raymond, Science 146(1964)406

This variation in protein concentration causes inhomogeneity of the electric field in the separative medium behind the slot, such that the lower parts of succeeding zones are accelerated relative to the top parts. Obviously, this can lead to overlapping of zones as indicated in Fig. 5.

The simple analysis given above confirms the subjective impression that a concentration gradient must build up between top and bottom of the slot and agrees with a more rigorous mathematical examination of the situation. It must be emphasized that the concentration difference between entrance and exit is independent of the dimensions or geometry of the slot. There is nothing to be gained by making the slot narrower*. Apart from some relief obtained by diffusional lateral mass transfer, the degree of zone distortion from this cause will be as bad in the shallowest gel as in the deeepest since the results predict a proportionately greater concentration gradient for the shallow gel. The only generally applicable means of reducing the concentration difference is to increase the rate of flow of eluting buffer.

It is not yet possible to say whether the predicted effect will prove serious in practice. Another serious impediment to resolution in the continuous-flow elution system is the occurrence of streaming in the elution slot. This is inevitable with most designs, but Jovin, Chrambach, and Naughton** may have succeeded in eliminating it by arranging for hydrostatic equilibrium throughout their system.

D iii) Electrodecantation

As a protein emerges from the gel into the collection slot it will form a narrow zone of increased density which will tend to sink towards the bottom of the slot. If the geometrical form of the slot permits, this will result in a region of increased protein concentration at the bottom of the slot, causing non-homogeneity of the electrophoretic field and hence impaired resolution just as discussed above. Electrodecantation in the origin slot can certainly produce very severe distortion.

We would not expect electrodecantation in the collection slot to produce as severe effects as this - the protein concentrations attainable are lower - and in fact there is no experimental evidence as yet of any zone distortion or impaired resolution from this cause.

* But see also Part II, Section H v.
** Anal. Biochem. 9(1964)351

It should be possible to eliminate electrodecantation by efficient stirring, and it is proposed to carry out comparative experiments, with and without stirring of the collection slots, to determine whether there is any difference in the resolution achieved.

If the geometry of the collection slot is such as to permit the concentrated protein solution formed to lie below the separative gel, out of the "path" of the electrophoretic field, its effects upon the field would be minimal. If the sedimentation of this concentrate were rapid and complete, there would then be no even transient field distortion. Such an arrangement could even be advantageous in permitting the bulk of the fluid in the collection slot to act as a rinse! It seems very likely that just this kind of situation occurs in practice with the prototype apparatus and may be responsible for the absence of conspicuous electrodecantation effects on resolution.

If the electrophoretic field were in a vertical axis, the electrodecantation effects discussed above would vanish - although it is possible that other more subtle disturbances might then arise from this cause.

D iv) Timing System

The obvious choice was to arrange for the collection slots to be emptied at fixed time intervals, but this is not ideal. Consider two proteins, identical in every respect except that one bears half the charge of the other, and accordingly migrates at half the velocity (neglecting the barrier effect of the supporting medium). Now, not only will this protein take twice as long to reach the collection slot, but its zone will take twice as long to elute when it does get there - without taking into consideration the effects of diffusional zone spreading. In order to make the two zones appear comparable on an elution histogram, it would be necessary to make the collection interval a function of time elapsed - probably the best choice would be direct proportionality.

E. ANTICIPATED DEVELOPMENTS

E.i) //Stirring in Elution Slots//

Experiments with the ICA* so far have been relatively crude and there certainly remains a possibility that resolution could be improved by eliminating electrodecantation.

* ICA = "intermittent collection apparatus", i.e. the apparatus discussed in this paper.

This could be done by stirring the fluid in the collection slots. Perhaps the most advantageous way of doing this would be by circulating the fluid through the cell of a recording photometric absorptiometer or fluorimeter so that, in addition, the concentration of eluted material was continuously monitored.

E ii) Timing System

This timing system has proved very robust but is inflexible. Probably future models will be built to operate in response to signals from external sources - timing devices which can be set to various intervals, or devices responding to indications of the amount of material collecting in the slot. An internal timing system* will still be required to allow the proper intervals for turning stop-cocks, draining, filling, etc.

One unsatisfactory feature of the present system is that electrophoretic current is switched on before the collection slot is completely refilled. Some improvement could be achieved with specially shaped stop-cock plugs, but it would be preferable to incorporate suitable controls in the internal timing system in future models.

E iii) Ideal Systems for Fraction Cutting

Time Based Systems

As discussed//in Section D iv//there is some reason to suggest that the interval between fractions should be proportional to the time elapsed since the 'run' was started, obviously with a minimum period at the beginning of the run (or, in practice, an exceptionally long initial period could be used covering the time up until the first fraction was anticipated). Four systems may be proposed for achieving this desideratum.

1) A cam is cut with several 'blips' on it at intervals, such as to give the desired time intervals. As it would probably be impracticable to cut sufficient blips on a single circular cam, this would be driven by a gearbox which would change automatically at the end of each revolution so that the speed of rotation of the cam was reduced by a suitable factor.

2) Related to (1), and perhaps the most likely to produce a generally useful and thoroughly dependable system.

*Referred to in Part II, Section H ii, as a "Secondary timing chain".

The cam is replaced by a rod (the 'index rod') on which is cut a helical groove, and in this are placed the 'blips', at intervals as described above. The switch is operated by a pointer which follows this helical groove and responds to the 'blips'. Probably, but not necessarily, the pointer would itself be carried along by being mounted on a 'nut' threaded on a helical screw of the same pitch (but not necessarily of the same diameter) as the first rod, and rotated at the same speed by the same mechanism. It might be worth while combining this system with a gearbox as proposed above, changing down at the end of each 'scan' of the helical groove. Automatic operation of the gearbox would be of less pressing necessity. A great advantage of this system would be the degree of control which could be exercised by simply changing the index rod for another with the 'blips' differently set. The intervals to be used would not necessarily be proportionate to time elapsed but could be specified individually - of particular importance for repetitive preparative work. It would not be very difficult to devise a 'do-it-yourself' kit in which the 'blips' could be moved and set wherever was desirable.

This system may be likened to the Edison 'phonograph' (with affiliations to the musical box): obviously an analogous system resembling the 'gramophone' would be equally practicable.

3) A shaft driven at constant speed carries a wheel which in turn drives (by friction) a cone or disc mounted on a second shaft. The wheel advances in the direction of increasing diameter of the secondary disc or cone which, therefore, rotates progressively slower and slower. The secondary shaft bears a cam operating the switch once in every revolution.

4) Oil or mercury drops into a conical vessel in which 'floats' a metal disc suspended over a pulley wheel. As it rises (progressively slower and slower) the floating disc permits the pulley wheel to rotate and a switch is actuated every time the wheel moves through a given angle, returning to a neutral position after being actuated.

With any system of fraction cutting the electrophoretic current will be interrupted whilst the collection slot is emptying and refilling. Clearly this period should not be 'counted' in any time-based fraction cutting system, and it is proposed to arrange for the internal timing system to stop the external timing device for as long as the electrophoretic current is switched off.

E iv) Other Fraction-Cutting Systems

If arrangements are made to circulate fluid from the collection slot through a recording spectrophotometer or fluorimeter, it will then be possible to arrange for the slot to be emptied only when sufficient of the material of interest has collected.

E v) Monitoring Eluted Material

The operation of a monitoring system would differ somewhat from what one is accustomed to in column chromatographic work. One might allow the effluent from the collection slot to pass through the optical cell of a recording spectrophotometer or UV 'colorimeter' or other detector - giving widely separated spikes, the height of which would indicate the content of the particular fraction. Although the simplest to provide for with conventional apparatus, this scheme would give results difficult to interpret quantitatively unless the composition of the fluid in the collection slot was strictly uniform. There might also be practical difficulties in avoiding any impediment to the flow of effluent, but this system might become practical if inserted into the effluent pathway after an intermediate reservoir.

The alternative is to record the build-up of the material in the slot itself, and three ways of doing this may be invisaged:

1) The side walls of the slot may be made with windows of optical glass or silica to allow readings of absorbance, refractive index, fluorescence, etc., by means of an instrument which would almost certainly require to be specially built or modified for the purpose.

2) The optical path might be provided by two J-shaped rods of glass or perspex dipping into the slot, allowing crude absorbance measurements by a specially made photometer. Perhaps more promising would be measurements of conductivity or permittivity by means of two electrodes instead of the rods of transparent material. Any such device might possibly serve also to stir the contents of the slot. If this was physically impossible some other means of stirring would probably be required.

3) The contents of the slot may be circulated to an external detecting and recording device. Existing conventional equipment could be used and the stirring would be provided automatically.

In all three cases the record would consist of a series of peaks building up gradually and declining suddenly. The height of each peak would indicate the final concentration achieved in the corresponding fraction.

E vi) Scale

The most obvious limitation to the scale of electrophoretic experiments is ohmic heating.

First, means must be provided for elimination of the heat generated and the actual temperature achieved in the centre of the gel must be kept low enough to prevent damage to the material of interest. This has always been interpreted as demanding a large surface area (parallel with the electrophoretic axis) in relation to the cross-sectional area of the gel.

Second, there is inevitably a temperature gradient between the surface and the interior of the gel: to a first approximation the temperature difference will be independent of the actual temperature. Since electrophoretic mobility is temperature dependent, there is a very strong interest in minimizing temperature differentials - again pointing to thin layers of electrophoretic medium.

However, if the temperature gradient is parallel to the electrophoretic axis, difficulties of the second kind are eliminated. It is proposed in a large scale apparatus to provide cooling only by refrigeration of the circulating buffer of the electrode tanks. It is to be expected that with cooling only through the ends of the gel, it will be possible to use only short gels, and/or low ionic strengths, and/or low potential gradients; but given a specified set of conditions there should be no loss of resolution because of the large cross-sectional area of the gel, provided the electrophoretic field is uniform.

Regrettably, there are other factors tending to worsen resolution as the cross-sectional area of any separative medium is increased. It remains to be seen whether these will prove serious in practice.

E vii) Alternative Methods of Fraction Collection

For small scale operations, the stop-cock device described here has been proved quite satisfactory. There would be no great difficulty, and no change in principle, in arranging instead to aspirate the collected fraction from above and replace it from above also, using the same or another pipette. Existing automatic dispensing and transferring devices could certainly be used in this way: the chief objections would be cost, cumbersome ancillary equipment, and liability to mechanical breakdown owing to the use of more moving parts.

Large scale operation is as yet unexplored. It may be that it will take too long to drain and refill the collection slots by gravity alone. Aspiration from above will probably not be satisfactory, but one may anticipate the possible need for mechanically assisted drainage of the slot followed by mechanically assisted refilling.

Perhaps the most likely arrangement will be free drainage followed by mechanical refilling either from above or below, since the refilling cycle is the slowest by far if left to nature.

E viii) Nature of Separative Medium

Although discussion has centred round polyacrylamide gel, the apparatus has been used for agar and agarose gels, and was originally designed and built for use with starch gel. This does not exhaust the list of possibilities. Given a rigid permeable (sintered glass) screen between the separative medium and the collection slot there is no reason why powdered, pulpy or viscous fluid media should not be used. Free zone electrophoresis in aqueous media must probably be excluded because it is hard to see how vibration effects are excluded. The only other medium known to be unsatisfactory is in fact starch gel, which swells or contracts very markedly during the run, either blocking the collection slot completely or making it leaky.

F. DISCUSSION

The apparatus outlined above has given excellent results in a number of applications. Where comparison is possible, the performance is decidedly better than has been claimed for other preparative electrophoresis devices. However, there is yet no conclusive evidence that the differences are due to factors concerned with the design of the machine. Before a valid comparison can be made, experiments will be required in which //ionic strength, length of gel, gel strength, temperature and potential gradient//are identical in the two systems under test.

From the above theoretic analysis one would expect that the resolution of continuous elution devices would always be poorer than that of the intermittent collection apparatus, but would approach it at small loads and high rates of buffer flow. Whether or not this expectation is realized, the apparatus is worthy of consideration as a preparative tool because of the simplicity and speed with which many separations can be made.

Difficulties arise only when the species of interest are slow-moving in the electrophoretic field. One can meet this problem by shifting the pH to a more suitable value, or by using a very short electrophoretic bed (which usually implies a second run to separate the fast-moving species).

It may be worth calling attention to a very general method of protein purification involving two runs on the machine in polyacrylamide gels of widely differing strength - say 8% and 3.5%. Materials contaminating a protein after this procedure are closely similar to it in charge and size, or are bound to it.

The present apparatus could obviously be scaled up 2 to 5 fold with little change in layout or operation, and would then be capable of handling loads as big as any which are customarily applied to laboratory chromatographic columns. If the proposed 'face-cooling' system proves satisfactory in operation, large scale machines could be built to handle separations on the industrial scale.

G. SUMMARY

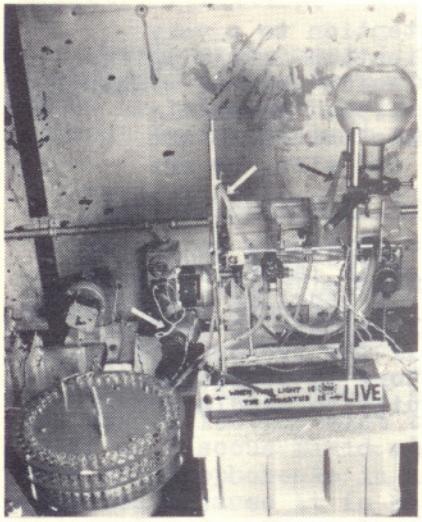
1) The apparatus described provides for automatic, intermittent collection of materials migrating out of both ends of a separative medium, arranged horizontally.

2) Electrodecantation in the origin slot is prevented by suspending the sample in agarose.

3) It is shown that the continuous flow of buffer through an elution slot (as in several earlier designs) is likely to impair resolution.

4) Future developments of the apparatus are discussed with particular reference to the possibility of operation on a very large scale.

FIGURES 6,7 AND 8, ARE PHOTOGRAPHS OF A RECENT VERSION OF THE APPARATUS, EQUIPPED FOR SINGLE-ENDED COLLECTION.



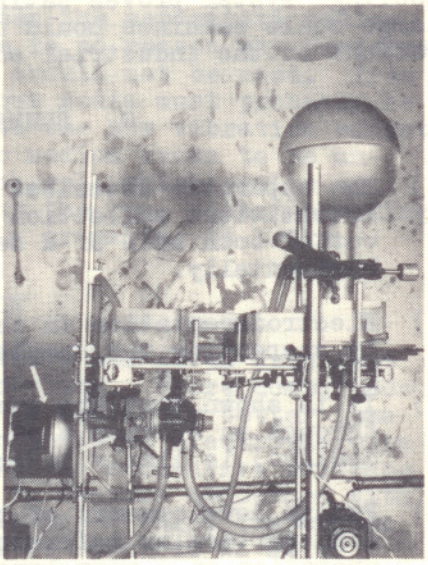
← Fig. 6

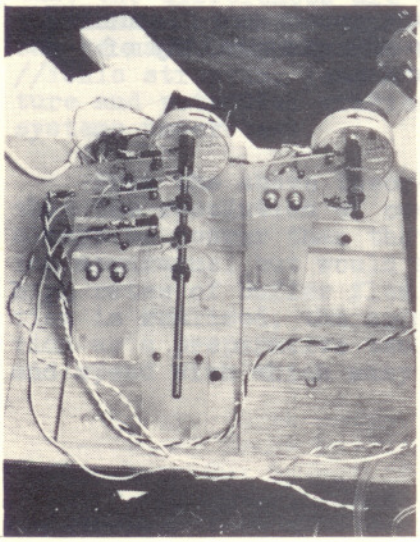
General view, showing fraction collector and (arrows) the buffer circulation pump and tubing.

The separation gel, origin slot and collection slots are seen from above. The electrophoresis circuit is disconnected - the run was completed some 24 h earlier.

Fig. 7 →

Detail of electrophoresis tray and stopcock drive assembly. Arrows indicate:- stopcock drive motor; control cam on stopcock drive shaft; perspex claw gripping stopcock handle. The microswitch operated by the cam is away from the camera, and, therefore, hidden from view.





← Fig. 8

Detail of control "box". On the right is the primary timing device - with a single cam on its shaft. On the left is the secondary timing system with (front to back) cams for:- signal to fraction collector; self-operated switch for secondary timing chain; stopcock drive control (interacting with cam on stopcock drive shaft). Cam for electrophoresis current control has been dismounted and is not shown.

PART II DEVELOPMENTS SINCE 1967

H. SPECIFIC CONSIDERATIONS

H i) Cooling

This is an obvious requirement and may be obtained by forced circulation of coolant around the separative medium. More simply, with a small apparatus such as this, the whole may stand in a cold room and adequate cooling is obtained with a fan directed at the electrophoresis tray. Alternatively, the electrophoresis tray may be surrounded with ice. It is not difficult to provide enough ice for an overnight run if one insulates the walls, and there is the peculiar advantage of working at 0° C, at which temperature the swelling of acrylamide gels appears to be completely eliminated.

H ii) Control Circuits, Components, etc.

One would hardly recommend a worker with sufficient funds to make his own fraction collector as was done in this case. Nor would one advise him to employ the same control system*. It is best to have a secondary timing chain consisting of a slow-rotating motor (say, 1 turn in 3 minutes) driving a shaft bearing a series of cams. These operate microswitches which function to:
1) Switch "on" the secondary timing chain motor (thus making it independent of the primary timing device), and switch it off when all the operations 2-4 are complete.
2) Give a signal to the fraction collector.
3) Reverse the electrophoresis potential, then switch it off, then switch it on again after (4).
4) a) Switch on the stopcock drive motor. The motor is switched off by a cam on the stopcock drive shaft when it has turned to the 'drain' position. b) After an interval the first cam again switches the motor on until the stopcock reaches the "refill" position when it is again stopped by the cam on its own shaft. c) After a further interval, the cam again switches the motor on and the stopcock is brought to rest in an intermediate position. (This is done to eliminate the ill effects of electrodecantation of proteins out of the collection slot, noted in Part I, C iii and D iii. However, the time available for refilling the slot is now limited and it may be necessary to raise the reservoir level to compensate for this).

*Further constructional details will gladly be supplied on request. Suitable components are:-
Primary timing device: Crouzet type F10 motor, (1 rev/h)
Secondary timing motor: Crouzet type A10, (1 rev/3min)
Stopcock drive motor: Crouzet type 82.185 (2rev/min)
(Obtainable from F.R. Holford Ltd., 6, Imperial Sq., Cheltenham, U.K.)
Microswitches, Roller type, Radiospares Ltd., 4 Maple St., London W.1.

TABLE I

CLASSIFICATION OF ELECTROPHORETIC PROCEDURES

TYPE \ MEDIUM			FREE	SUPPORTING MEDIUM	
				No Molecular Sieving	Molecular Sieving
MICROSCOPIC (Analytical)			Ellis Abramson		
BOUNDARY (Analytical)			Franklin, Tiselius	Lodge	
ZONE ELECTROPHORESIS	ANALYTICAL			Cramer and Tiselius, etc.	Smithies, Raymond, etc.
	PREPARATIVE	2-stage	Tiselius, Column procedures	Kunkel and Wallenius	*Raymond (Science 1964)
		1-stage a) Flow Elution			*Murray *Jovin
		1-stage b) Intermittent Elution			*Brownstone *Hodson *this paper
		Continuous	Grassmann (ii)	Grassmann (i)	

Notes:

*Indicates a technique described with reference to one particular medium but which could well be applied to others, including media showing no molecular sieving.

References in table are exemplary only.

References: (Others cited in text.)

Abramson, J.Phys. Chem., 36(1932)1454
Cramer and Tiselius, Biochem. Z., 320(1950)273
Ellis, Z. Phys. Chem. 78(1912)321
Franklin and Cady, J.A.C.S., 26(1904)499
Grassmann (i), Brit. Pat. No. 679,278, (1949)
Grassmann (ii), Brit. Pat. No. 1,098,307, (1964)
Lodge, Report of British Association for the Advancement of Science, Birmingham (1886)389
(Perhaps should not be included within present definition of electrophoresis)
Kunkel and Wallenius, Science, 122(1955)285
Murray, Anal. Biochem., 3(1962)415
Smithies, Nature, 175(1955)307
Tiselius, Saartruck ur Svensk Kemisk Tidskrift 50(1938)58

H iii) Double-ended Collection

Double-ended collection is not often required and when it is, can best be provided for by a second stopcock drive motor and assembly - conveniently controlled by the same timing chain.

H iv) Classification of Electrophoretic Processes

The classification of preparative electrophoresis procedures given in Part I is simple but incomplete. The opportunity is taken here of giving a fairly widely applicable scheme, covering both preparative and analytical work (Table I). It is proposed to restrict the term electrophoresis, when unqualified, to measurements or separations based specifically on migration velocity in an electric field. This excludes a variety of techniques which employ similar apparatus but slightly different principles (isoelectric focussing, isotachophoresis*) or additional physical principles (electrodecantation, field-flow cataphoresis**, etc.), but includes electrophoretic separation techniques in which the supporting medium plays a significant part. The deliberate restriction does not imply that the excluded techniques are thought to be inferior.

Classification should obviously be multidimensional and a two-dimensional table with subdivisions is an unhappy compromise. Some important features are not indicated in the table and must be considered independently in every case, for example, electroendosmosis and "special" medium effects***.

Preparative electrophoresis procedures, then, fall operationally into 3 classes, continuous, 1-stage, and 2-stage. The 1-stage type is the closest in operation to column chromatography and perhaps for that reason alone has attracted most attention. Operation is discontinuous in that a discrete charge of material is applied to the electrophoresis medium, but in most designs, the separated products are obtained by allowing them to migrate into a chamber or slot from which they are removed by a continuous flow of buffer, and carried to a conventional fraction collector.

* Haglund, Science Tools, 17(1970)2
** Rony, Separation Science, 5(1970)121
*** e.g. Robinson et al., J. Lab. Clin. Med., 50(1957)745

H v) Disadvantages of Continuous Elution

It is shown in Part I that with continuous elution the concentration difference between inlet and outlet of the elution slot is independent of the geometry of the slot. One may point out, however, that in a narrower slot there will be less total protein present in the slot and we may presume that there will be some relief from distortion because of this.

H vi) Large Scale Apparatus

A large-scale machine based on the principles of Section E vi has been built and is in operation. The results so far have been somewhat disappointing- reminiscent of the early days of the apparatus described above - but there are prospects for improvement.

J GENERAL COMMENT

It is the fate of scientific work, and still more of dilatory scientists, to be overtaken by events. The apparatus of Brownstone was developed completely independently of that described here and agrees only in the use of the principle of discontinuous elution. With slight modifications, the actual physical piece of apparatus described here was used by a colleague, with permission and under supervision. The design of Hodson and Latner was modified from this, in the same laboratory, and is published without due acknowledgement. Their system is far more complex but differs not at all in principle, and no evidence is given that it yields superior results. As mentioned above, the important inventive step in this case was the realization that discontinuous elution may be expected, in principle, to give better resolution* than that of continuous elution machines. The theory of this effect has never been published before nor is any allusion made to the theory by Hodson and Latner.

The form of words of Part I would not have been used for publication, but for the recent turn of events. This commentary has had for its object to select some aspects of the design of the apparatus, and some aspects of the theory, so that the description as a whole is brought up-to-date, as well as to put the record straight.

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Kampala.

December, 1971

*Boyde, Separation Science 6(1971)771

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