PREPARATIVE ELECTROPHORESIS IN LIQUID COLUMNS STABILIZED BY ELECTROMAGNETIC ROTATION*

I. THE APPARATUS

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INTRODUCTION

In a previous publication[1] a method for continuous electrophoretic fractionation in horizontal liquid columns was described. The original apparatus did not permit continuous simultaneous withdrawal and collection of all of the separated fractions. The present paper describes a means for achieving this objective. The theory of operation of the apparatus is treated in two preceding publications[1,2]. This article describes the further development of the method and of the apparatus and presents illustrations of its performance.

A mixture of particles differing in electrophoretic mobility is fractionated into individual components by injection of a fine stream of the mixture into a horizontal fluid column shaped as an annular cylinder (Fig. 1). This column is enclosed between two plastic (lucite) cyclinders C_1 and C_2 of as thin a wall thickness as possible (preferably not over $^3/_4$ mm). It forms a fluid bridge between the two electrode compartments EC_1 and EC_2 which contain the stainless steel or platinum foil electrodes E_1 and E_2. This horizontal fluid column is stabilized against thermal convection by rotation about the cylinder axis. This rotational motion periodically inverts relative to the field of gravity any incipient vortices which may be generated in the fluid column as a result of thermal convection engendered by temperature gradients in the fluid. This periodic inversion of the fluid inhibits the development of vigorous turbulence as previously explained[2]. The fluid rotation is generated by interaction of the axial electric current (passing between the electrodes E_1 and E_2 through the annular column) with a radial magnetic field. This field is created by two bar magnets NS facing each other with like poles (N, N) with a soft-iron cylinder m sandwiched between them. Fig. 2 shows the magnetic field pattern for this configuration exhibiting a radial field component near the surface of m. An electrically neutral particle injected into the solution by the injector IN would go into a circular orbit about the cylinder axis due to entrainment in the revolving fluid. An electric charge on an injected particle (in the absence of electro-osmosis) would superimpose an axial migration upon the circular motion making the particle move in a helical path of a

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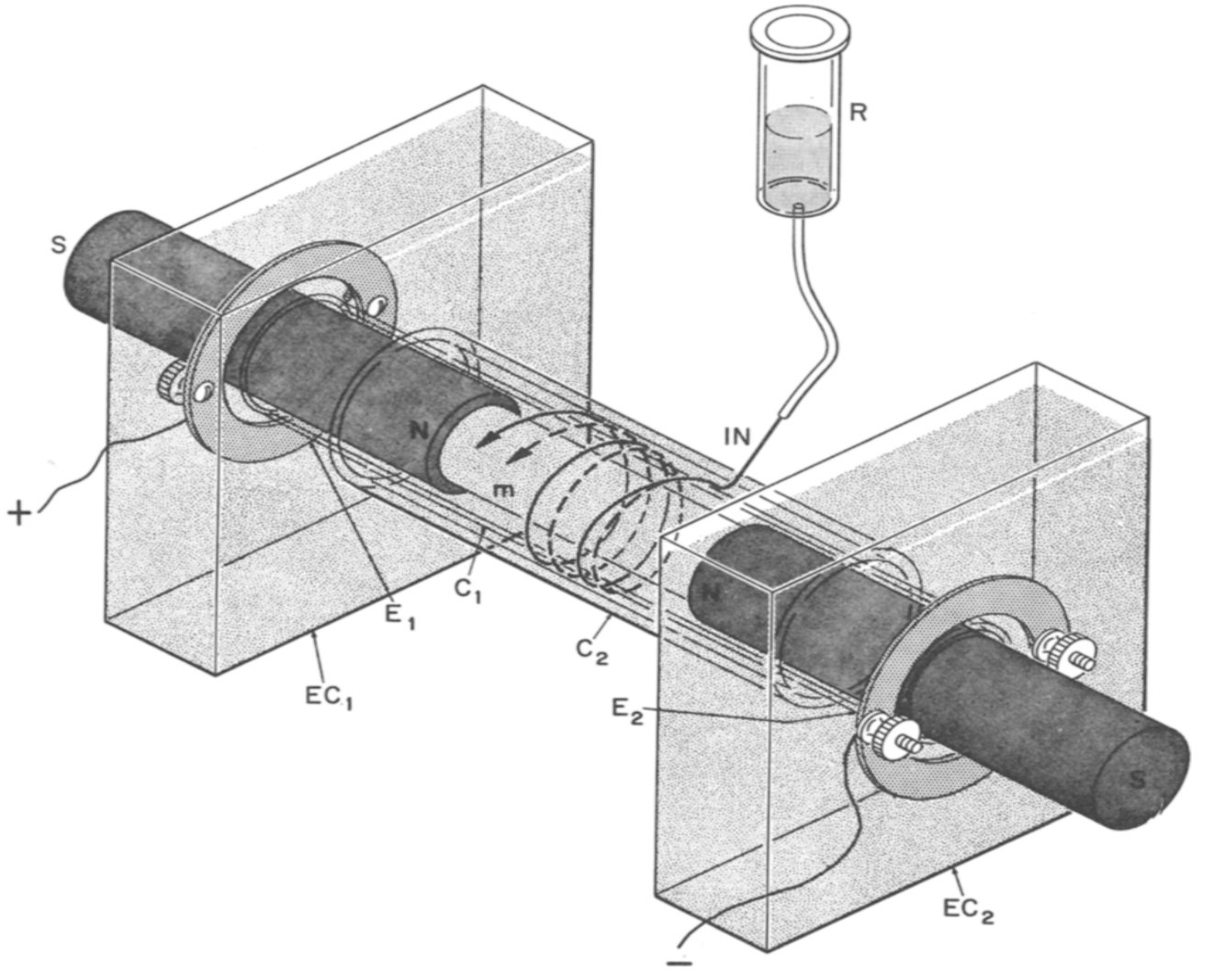


Fig. 1. Scheme of helical path electrophoresis. N-S = Bar magnets; m = soft-iron cylinder E_1, E_2 = electrodes; EC_1, EC_2 = electrode compartments; C_1 = inner lucite cylinder; C_2 = oute lucite cylinder; IN = injector; R = reservoir for mixture to be analyzed.

pitch determined by its electrophoretic mobility and by the period of revolution (see reference (1) for quantitative relations). Fig. 1 shows the helical paths for two negatively charged particle species differing in electrophoretic mobility. The following section describes how fractions thus separated can be visualized and collected simultaneously in a stable and continuous fashion.

THE APPARATUS

Fig. 3 shows a perspective view of the rotationally stabilized electrophoretic separator. Fig. 4 shows a schematic diagram to which one should refer concurrently for supplementary illustration during the following discussion of the apparatus. Fig. 5 shows a photograph of the instrument with lettering corresponding to Figs. 3 and 4.

The main innovation in the present apparatus is the introduction of the collector C which serves the purpose of collecting simultaneously all of the separated

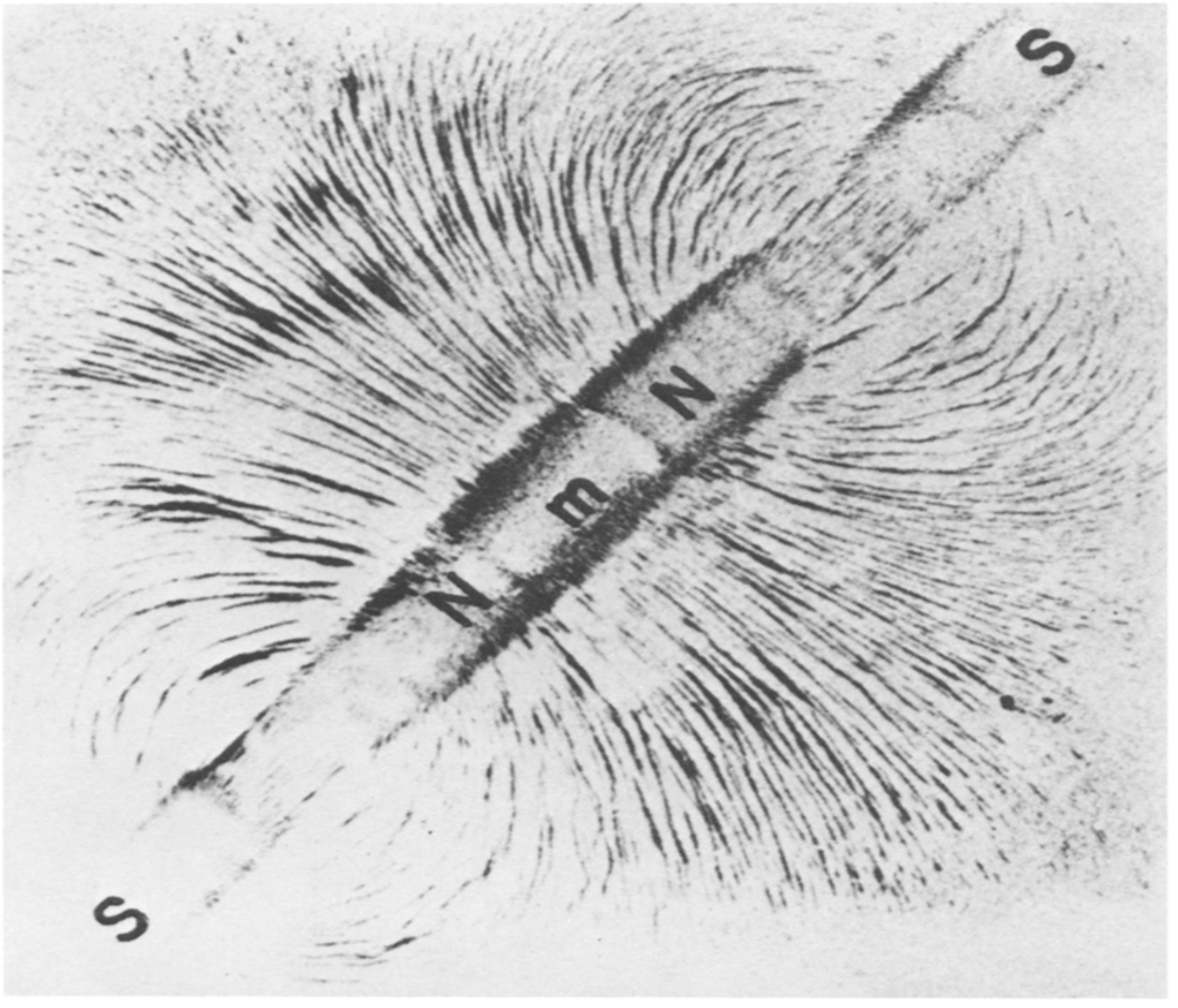


Fig. 2. Magnetic field surrounding the configuration of two bar magnets NS with an intermediate soft-iron cylinder m.

fractions. It consists, in its simplest form, of flexible polyvinyl chloride tubes ($^3/_4$ mm I.D.) cemented with Tygon cement to form parallel channels which can be inserted tightly into the 2 mm wide annulus passing through the left buffer compartment B, as shown in Fig. 3. The tubes are flexible enough to adapt themselves to the shape of the annulus as the collector is slipped into it and are thick enough (the thickness is adjusted by repeated applications of the plastic cement) to completely fill the space between the cylinders C_1 and C_2 (*cf.* also Fig. 1). Fig. 6 illustrates different forms of flexible collectors, that in Fig. 6a being the simplest to make and that in Fig. 6c being more advantageous having the maximum number of tubes per unit length of the collector entry. The latter collector is made by pre-cutting the tubing to form the shown configuration and inserting L-shaped lengths of teflon-insulated wire to avoid sealing the tubes by the cement which is applied to the "elbow" to attach to each other the two mutually perpendicular tube sections shown in Fig. 6c. In Fig. 6b, the tubes are placed next to each other in staircase fashion and cemented together by Tygon cement as described above.

A more elaborate collector system shown in Fig. 6d has the advantage of

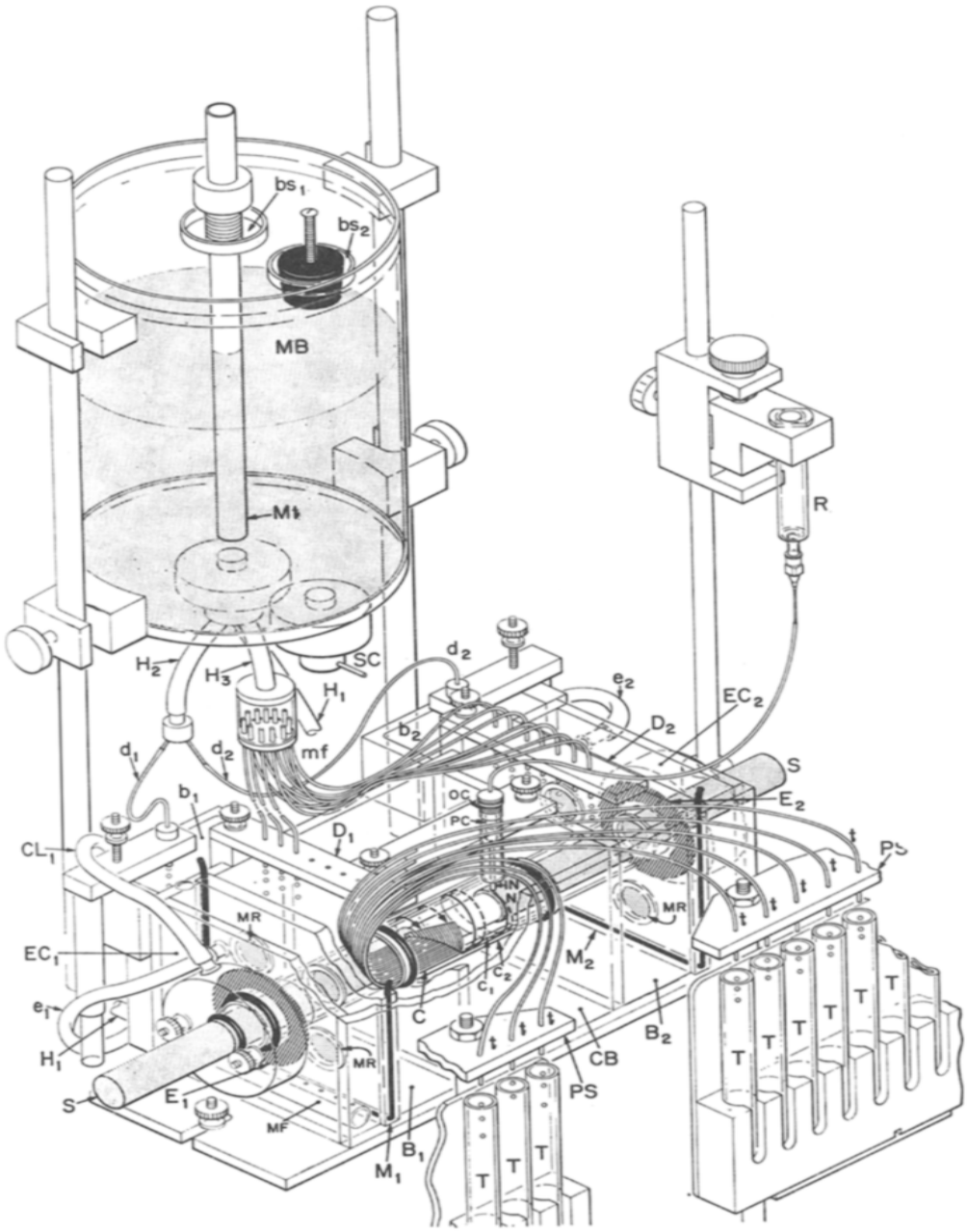


Fig. 3. Perspective view of the apparatus. MB = Mariotte bottle; bs_1, bs_2 = basins filled with water to avoid air leakage; Mt = tube communicating with atmosphere, capable of up and down movement, which allows fine-adjustment of pressure head delivered by Mariotte bottle; NS = bar magnets; C_1, C_2 = inner and outer lucite cylinders; IN = injector; C = collector; t = collector tubes; T = test tubes; PS = plastic tube holders; EC_1, EC_2 = electrode compartments; B_1, B_2 = buffer compartments; CB = chamber for cooling water; M_1, M_2 = membrane plates; MR = circular dialyzing membranes in plates M_1, M_2; MF = manifolds conveying buffer to the electrode compartments; e_1, e_2 = tubes permitting escape of buffer from electrode compartments; H_1 = tube conveying buffer to the apparatus; H_2 = tube delivering buffer to the "balconies" b_1, b_2 of the electrode compartments *via* the thin tubes d_1, d_2; H_3 = tube conveying buffer to the manifold mf which distributes the buffer flow between the two buffer compartments by means of thin tubes held in plastic holders D_1, D_2; R = reservoir for mixture to be analyzed; PC = plastic cylinder containing the injector tube IN; OC = outer cylinder into which PC fits; SC = stopcock. The heavy dark lines are rubber gaskets.

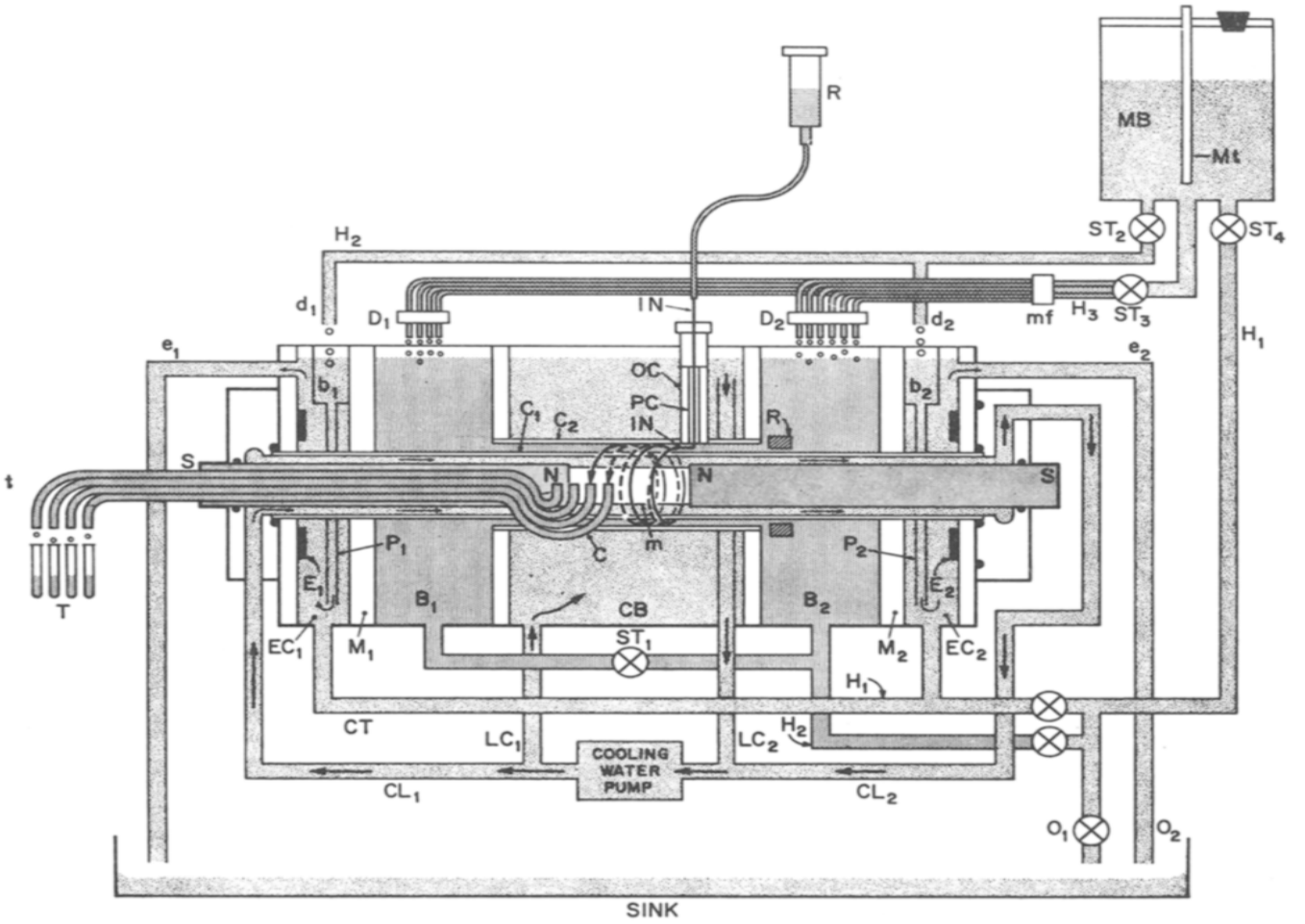


Fig. 4. Schematic plan of apparatus. MB = Mariotte bottle; Mt = Mariotte tube; ST = stopcocks; R = reservoir for mixture to be analyzed; H_1, H_2 = tubes conveying buffer solution; O_1, O_2 = outlets; ⊗ = symbol for stopcock; d_1, d_2 = buffer delivery tubes to "balconies" b_1, b_2 of the electrode compartments; EC_1, EC_2 = electrode compartments; e_1, e_2 = buffer exit tubes; E_1, E_2 = electrodes; P_1, P_2 = plastic tubes; B_1, B_2 = buffer compartments; CB = cooling water bath; CL_1, CL_2, LC_1, LC_2 = tubes carrying cooling water; NS = bar magnets; m = soft-iron cylinder; C = collector; t = collector tubing; T = test tubes; C_1, C_2 = inner and outer lucite cylinders; IN = injector; PC = plastic cylinder containing injector tubing; OC = outer cylinder into which PC fits; M_1, M_2 = membrane plates; R = (optional) ring sliding over C_1 which inhibits thermal convection near injector when placed close to the left wall of compartment B_2.

minimizing the obstruction of the current entering the annulus created by the plastic collectors inserted laterally into the annulus. A plastic (lucite) plate C shown in side view has 20 L-shaped drilled channels oriented as shown in Fig. 6d so as to receive the fluid rotating in the annulus as indicated by the arrow. The outer lucite cylinder C_2 is provided with a rectangular slot to admit the collector C. The end surface of the collector is pressed against the inner cylinder C_1 (a gasket of soft intermediate layer of rubber or soft plastic material is recommended). The collector body is then permanently cemented to the outer lucite cylinder C_2. The cemented joints are indicated in the diagram (CJ). Thin flexible tubes, t, convey the fluid entering the collector to the test tubes T (*cf.* Fig. 3).

The collector blocks the rotational motion of the fluid in the annulus when the collector is not "activated". The intent is to avoid the obstruction of this rotational

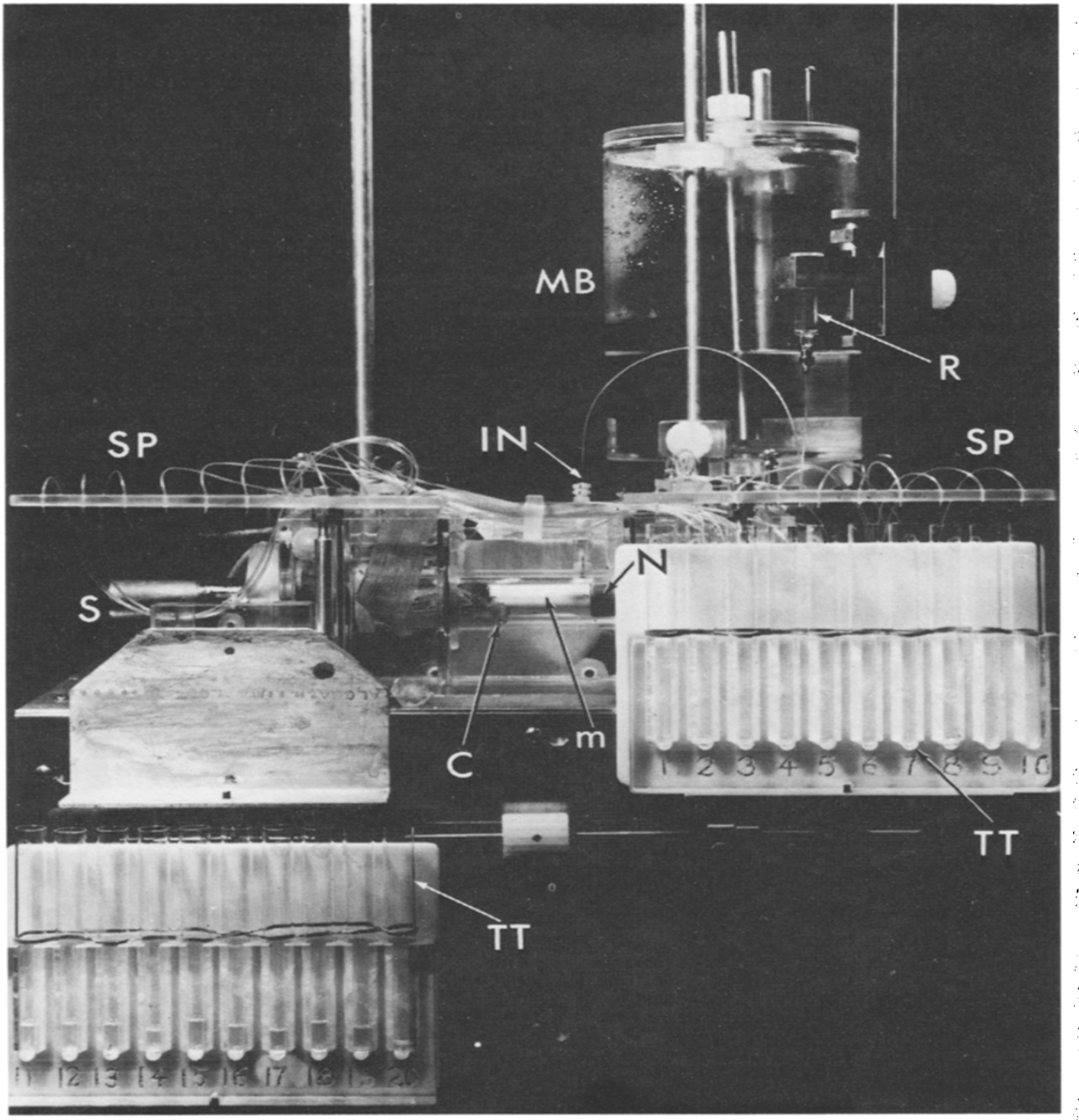


Fig. 5. Photograph of instrument. (The left test tube stand is removed from its shelf to avoid obstruction of the structures behind it.) MB = Mariotte bottle; R = reservoir for mixture to be analyzed; IN = injector; SP = plastic shelves holding the thin collector tubes; T = test tubes; (both shelves holding the test tubes can be raised and lowered simultaneously by turning the cylindrical knob seen at the bottom between them); S = the south pole of the left bar magnet; N = the north pole of the right bar magnet; C = collector; m = soft-iron cylinder; IN = injector.

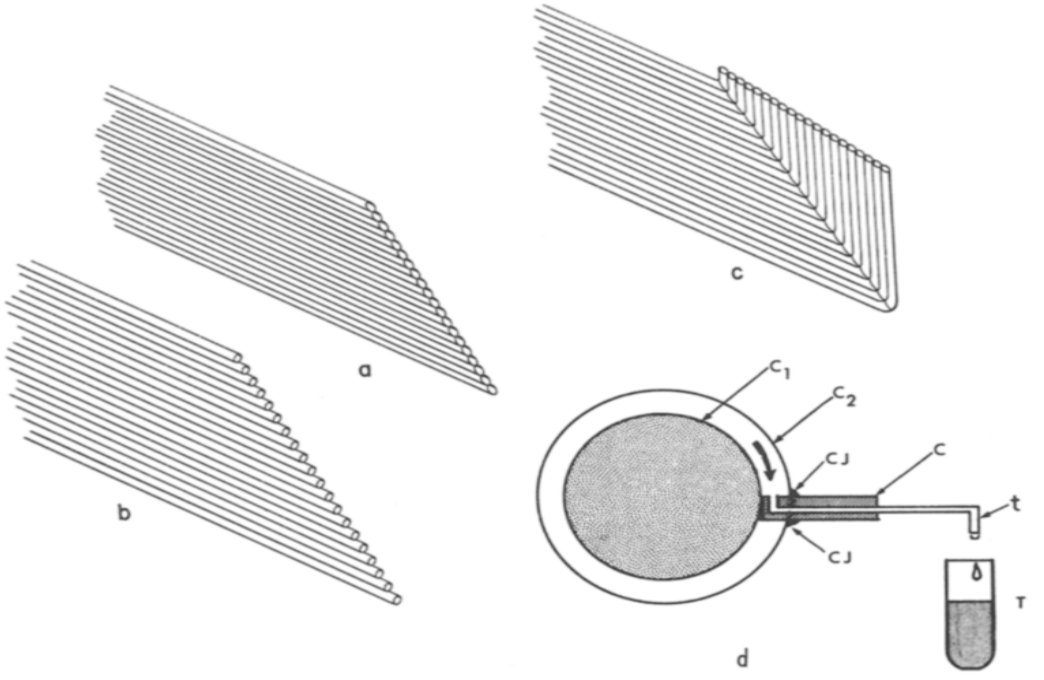
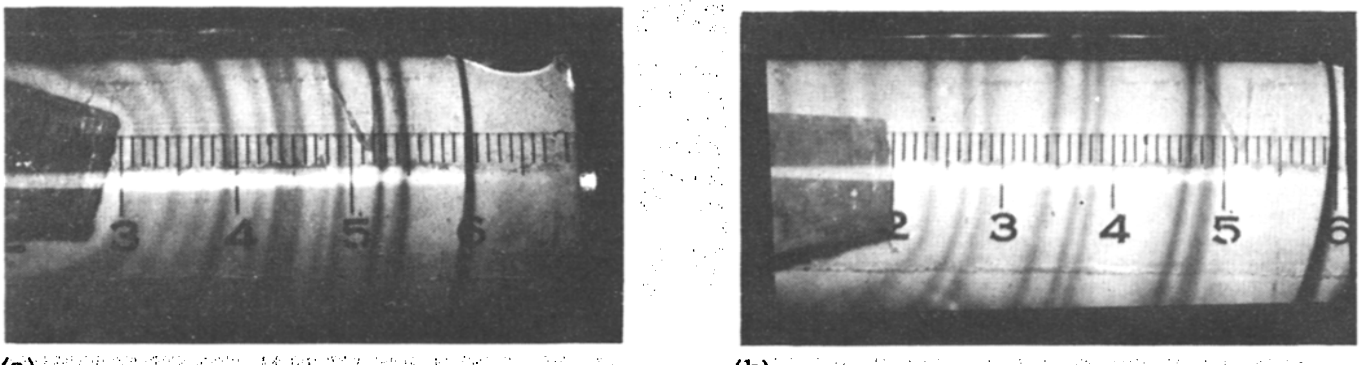


Fig. 6. Different types of collector. (a) A row of plastic cemented tubes cut diagonally; (b) a row of plastic cemented tubes cut in staircase fashion; (c) collector with L-shaped plastic tubes; (d) section through rigid collector introduced through slot in cylinder C_2. CJ = cemented joint; C_1 = inner cylinder; C_2 = outer cylinder; C = collector body; t = collector tube; T = test tube.

motion when the collector is activated. This is accomplished by adjusting the collection system to make the collector withdrawn the fluid at the same rate at which it would have been traversing the annulus in the absence of an obstruction by virtue of its electromagnetic rotation. Fig. 7a shows how the streaks of 3 separated dyes (from left to right: Evans blue, rose Bengal, green "Brush" recording ink) stream around the inactivated collector which blocks their path. Fig. 7b shows how the streaks land in the collector at nearly perpendicular incidence when fluid is allowed to escape through it at a proper rate. Since buffer is withdrawn along with the separated fractions, the fractions are being diluted in the process of separation but their subsequent concentration by evaporation or by precipitation presents no problem. If the buffer escapes too rapidly through the collector, the streaks tend to become thinner and to converge as they enter it. This can be unfavorable for good resolution if two

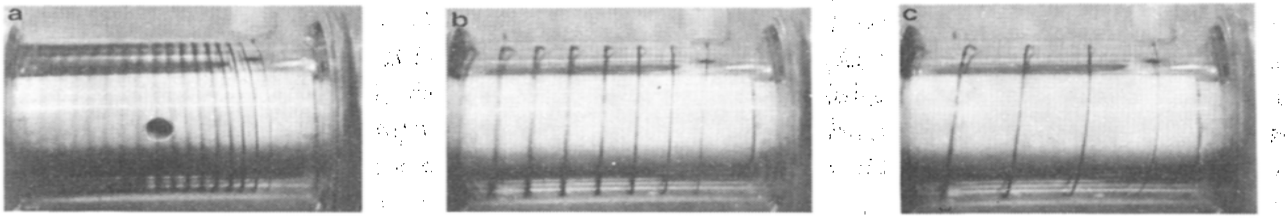


(a) (b)

Fig. 7. Pattern of three separated dyes (Evans blue, rose Bengal and "Brush" green recording ink). (a) Streaks bending around inactive collector. (b) Streaks entering activated collector.

streaks would get close enough to each other to enter the same collector tube. On the other hand, if the fluid escapes too slowly, the streaks tend to become wider and to diverge before entering the collector. This can improve collector resolution despite the widening of the streaks if the interval between the closest adjacent streaks becomes wide enough to avoid simultaneous collection of these two components in the same collector tube. The approximate adjustment for parallelism of streaks shown in Fig. 7b is recommended as the normal condition. It is, of course, important to maintain the collection pattern so that the streaks do not shift to different collector entrance tubes in the course of time. A stabilized constant-current power supply and temperature control are essential for stable operation over long periods of time. (An Electronic Measurements Company Model C 636 power supply was used in our apparatus. The temperature control was accomplished by circulation of water derived from the ice-water bath.)

The entry of the separation pattern into the collector is controlled by the pitch of the helical paths of its components. The pitch of the helical path of an ion species depends on several factors in the present apparatus. To begin with, it depends on the speed of the axial flow which is deliberately established by a slight difference in surface level in the two buffer compartments in order to avoid overlapping between the fastest component of the n-th turn of the helix and the slowest component of the $(n+1)$-th turn. Fig. 8 shows how this superposition of axial flow upon rotational



(a) (b) (c)

Fig. 8. Effect of axial streaming on helical pitch. The axial flow is toward the left and is increasing as we progress from a to c. The injector is seen at the top on the right in each photograph.

motion in the annular electrophoretic column allows the pitch of the helix to be varied over a wide range. The axial flow can thus also be used to adjust in desired fashion the entry of the separation pattern into the collector (*cf.* Fig. 7b). For instance, one need not intercept the three components at the same turn of their helices as shown in Fig. 7b. One could, for instance, catch the blue dye (fastest component) in its fourth turn at the proximal edge of the collector allowing the red and green dyes to complete their fifth turn before interception by the collector. The collection pattern will then show the color sequence (from left to right) red, green, blue which is different from the normal collection pattern (blue, red, green) shown in Fig. 7b which agrees with the separation pattern.

The second factor controlling the pitch of the helix is the electric current. As we increase it, we augment the rotational speed and shorten the period of revolution thus decreasing the helical pitch*.

* This is not true in the absence of axial streaming when electrophoretic migration and electro-osmosis are the only factors determining axial ion migration, as previously shown[1]. The above statement is correct only in the presence of superimposed axial movement originating in causes other than electrokinetic or electromagnetic effects.

The third factor determining the pitch is the strength of the radial magnetic field. Since it is derived from permanent magnets it is perfectly constant. It is not varied in normal operation. The magnetic field can be altered by changing the magnets NS for a pair of different strength, by removing one of these magnets or by changing the length of the central soft-iron cylinder. A cylindrically symmetrical magnetic field is not a necessary requirement for successful operation of the instrument. The cross-section of the bar magnets or of the soft-iron piece m need not be circular.

The axial flow is affected by the rate of buffer outflow through the collector which generates axial streaming directed toward electrode E_1. This augments the helical pitch and constitutes thus the fourth factor affecting it. The pitch can be reduced or increased further independently of the collector drainage by altering the distribution of the inflow tubes passing through the plates D_1 and D_2 conveying buffer to the buffer compartments B_1 and B_2. By increasing the number of tubes in D_2 emptying into compartment B_2, the rate of axial flow can be increased, whereas increase in the number of tubes in D_1 discharging into compartment B_1 will decrease the axial flow. In fact, one can even generate a retrograde axial flow in this fashion. This procedure to modify the axial flow is the main factor utilized for the control of the helical pitch.

The rate of buffer supply must remain constant and equal to the rate of buffer drainage through the collector. The number of the buffer supply tubes in the plates D_1 and D_2 is kept constant and the axial flow is varied merely by redistributing these tubes among the buffer compartments B_1 and B_2. These tubes are held in drill holes provided in the plastic plates D_1 and D_2 of Figs. 3 and 4. The tube inside diameter is $^3/_4$ mm. Buffer solution escaping from the Mariotte bottle MB through the twenty tubes distributed among D_1 and D_2 enters the buffer compartments B_1 and B_2 at a rate determined by the length and inside diameter of the equally long plastic tubes linking D_1 and D_2 to the Mariotte bottle, by the viscosity and density of the liquid and by the difference in elevation between the bottoms of the Mariotte bottle tube Mt and of the tubes in D_1 and D_2. The latter tubes are shown as emptying dropwise into the buffer compartments since it is convenient to adjust and estimate the rate of buffer flow by timing the interval between consecutive drops. In actual operation, however, the tubes are dipped under the buffer surface after completion of adjustment to avoid minute fluctuations in axial flow which may be caused by dropwise entry.

To start the instrument, the collector tubes ttt... are filled with buffer (by a vacuum suction device), to start syphoning action, and their ends are lowered below the buffer surface level in the compartments B_1, B_2 so as to remove buffer solution through the collector openings (located near the left end of the annular electrophoretic column) at a rate equal to the rate of buffer supply from the Mariotte bottle. This supply rate is regulated by adjusting the height of the Mariotte bottle. The equality of the rates of buffer inflow and outflow is estimated roughly by timing the interval between consecutive drops escaping from the tubes in D_1, D_2 and the tubes t, t... For simplicity of comparison, the racks D_1, D_2 contain the same number of tubes of the same gauge as the collector C.

For stability of separation, the constancy of the buffer level in the buffer compartments and the equality between gain and loss of buffer solution by the separator must be exact. This equality is established automatically as follows: Let us assume that the influx of buffer exceeds the rate of buffer flow to the test tubes

TT... Then the liquid level in compartments B_1, B_2 will be rising, thus increasing the rate of outflow, until the latter has become equal to the rate of influx of the buffer from the Mariotte bottle. Conversely, an excessive rate of buffer escape through the collector C will lead to a drop of the liquid level in compartments B_1, B_2 and, hence, of the buffer outflow rate until it has become equal to the inflow rate from the Mariotte bottle. The rough pre-adjustment by timing the intervals between consecutive drops, shortens the time for attainment of the steady state.

The next step consists of adjusting the axial flow to the desired value by transferring some of the tubes from D_1 to D_2 or *vice versa*. The total rate of buffer inflow is, of course, not altered thereby.

The Mariotte bottle is a very convenient device for constant rate of delivery of fluid but it is not ideal in this application because of pressure fluctuations created by periodic entry of air bubbles through the tube Mt. This creates slight flow pulsations which may affect the sharpness of the collection pattern by undulating the course of the streak injected through IN. The fluctuation in axial streaming thus produced may be minimized by using long and thin tubes leading to D_1 and D_2 so as to increase the hydrostatic pressure head required for buffer delivery. This will minimize the effect of the small pressure fluctuations caused by discontinuous entry of air into the Mariotte bottle.

The buffer compartments B_1 and B_2 are hydraulically isolated by membranes M_1 and M_2 from the electrode compartments EC_1 and EC_2 which contain the ring-shaped stainless steel electrodes E_1 and E_2 (comp. Figs. 3 and 4). The electrolyte in the electrode compartments EC_1 and EC_2 is constantly renewed by buffer entering from the tubes d_1 and d_2 through the small basins b_1 and b_2 via the channels P_1 and P_2 and escaping through the exits e_1 and e_2 (see Figs. 3 and 4). The membranes M_1 and M_2 seen in Fig. 3 consist of lucite plates perforated by four round windows (MR of Fig. 3) made of dialyzing sheeting. The hydrostatic pressure is equalized between compartments EC_1 and EC_2 by the thin connecting tubing CT. (The waste of electric current passing through CT is negligible)*.

The annular electrophoretic column is cooled with water from the inside as well as the outside. A small circulating pump pumps the cooling liquid (derived from a reservoir filled with a mixture of crushed ice and water) through the tube CL_1 into a gap between the magnets and the inner plastic cylinder C_1 returning it to the reservoir *via* CL_2. The outer cylinder C_2 is surrounded by a cooling fluid bath CB. One can connect it to the cooling pump as shown by means of tubes LC_1 and LC_2.

The injector IN consists of a hypodermic needle (gauge 24 to 22) which is encased in a nylon cylinder PC which can be moved up and down in an outer lucite

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* A simpler alternative to the circular membranes in the membrane plates M_1 and M_2 (Figs. 3 and 4) can be used to convey the current to the buffer compartments. The plates M_1 and M_2 as well as the ring electrodes E_1 and E_2 can be omitted. The compartments EC and B (Fig. 4) thus become one single compartment. A rectangular "window" of as large a size as possible is then cut into a side wall of such a compartment facing the reader and is covered with a dialyzing membrane. A "balcony" of plexiglass of dimensions to match the "window" is pressed against the plastic wall surrounding the "window" with an intermediate rubber gasket. The "balconies" are filled with buffer solution and rectangular stainless steel electrodes are inserted into the "balconies" which thus become equivalent to the buffer compartments B_1 and B_2 of the design shown in Figs. 3 and 4. It goes without saying that the buffer influx tubes d_1 and d_2 are now redirected into these compartments which are also equipped with drainage tubes corresponding to e_1 and e_2 shown for the design of Figs. 3 and 4.

cylinder OC. PC is held by friction in the selected position. The upper end of the needle is connected to the reservoir R (a syringe cylinder) containing the mixture to be analyzed. The lower end of the needle is insulated by a layer of "Duco" cement diluted with acetone in which basic fuchsin is dissolved to make the insulating layer better visible. Several injectors may be mounted side by side to carry out simultaneous separations of mixtures derived from separate reservoirs R_1, R_2 as described for serpentine separators[3]. Only one collector is necessary.

ADJUSTMENT FOR PREPARATIVE SEPARATIONS

We shall summarize now the steps taken in preparing the apparatus for prolonged runs involving collection of separated fractions.

(1) The various compartments are filled with buffer solution derived from the Mariotte bottle MB of Fig. 4 through plastic tubing H_1 and H_2. Pinch clamps are indicated by the symbol ⊗. The electrode compartments EC_1 and EC_2 are filled through the tubing H_1. The lower portion of the same tubing is used to empty them through the outlet O_1. The buffer compartments B_1 and B_2 are filled through the tubing H_2 and emptied also *via* outlet O_1. The clamp ST_1 is closed as soon as the buffer compartments are filled to permit the establishment of axial flow through the annular cylinder as described below.

The buffers used were prepared from "Hydrion" buffer tablets. The buffer solutions were diluted to obtain resistivities varying from 1,000 to 1,500 ohm-cm. In working with suspended particles, one may add sucrose to the buffer to increase the density of the buffer in order to retard the rate of sedimentation. In this case, the conductivity can be re-adjusted to above values by increasing the buffer concentration above the values used in the absence of sucrose. Another way of suppressing sedimentation is to increase the rate of electromagnetic rotation by increasing the magnetic field strength or the electric current.

As soon as the clamp ST_2 is opened, the buffer begins to drip onto the compartments EC *via* the basins b. The excess escapes *via* channels e into the sink. The rate of buffer supply to the electrode compartments is not critical; it merely has to be rapid enough to avoid excessive changes due to electrolysis in the electrolyte compartments EC_1 and EC_2 during operation. The rate of about two drops per second was used in our experiments. The compartments remain in hydraulic communication *via* the horizontal section CT of tube H_1. When the instrument is not in operation, the buffer is allowed to remain in compartments B_1, B_2 and EC_1, EC_2 to keep the membranes wet.

(2) The next step is activation of the collector by lowering the end points of the collector tubes (t, t...) which move together with the test tubes TT... shown in Figs. 3 and 4 and which are mounted on racks above the test tubes by means of horizontal plastic strips SP of Fig. 3 which are also seen in Fig. 5. The strips SP have holes centered above the test tubes through which the ends of the collector tubes t pass. When the ends of these tubes are lowered below the buffer level in compartments B_1, B_2, buffer solution is syphoned out of the electrophoretic column through the collector openings at a rate set by the level of the tube exits. To fill the plastic tubes with buffer and thus start the syphoning, a rectangular tub lined by a rubber gasket running parallel to the edges of the strip SP and connected to a vacuum line is applied to the bottom surface of SP.

(3) The desired current is turned on and a dilute test mixture of two dyes (*e.g.* rose Bengal and Evans blue) is introduced *via* the injector IN to adjust the rate of syphoning to about $^1/_6$ of a drop per second per tube. Simultaneously the clamp ST_3 (Fig. 4) is released allowing the buffer to drip from D_1 and D_2 into the apparatus. All of the tube outlets are lowered below the buffer surface in B_1 and B_2. One of the tubes from D_1 or D_2 is removed and held outside the instrument with its outlet on the level of the buffer surface to measure the time interval between two consecutive drops. The Mariotte bottle is raised or lowered until this interval is as nearly as possible equal to the corresponding interval for the collector tubes in the racks SP. The precise equalization is now achieved automatically by allowing the system to run for several minutes, the time depending on the closeness of the preliminary adjustment.

(4) While the balance between the in and outflow rates of the buffer is being established, the pumping system circulating the cooling water (indicated in Fig. 4) is turned on. Adequate results are obtained by using water at room temperature as a coolant but considerable improvement in sharpness of the streaks is secured by filling the tank containing the coolant (see Fig. 4) with an ice-water mixture.

(5) The two test streaks into which the injected streak containing a mixture of two dyes is split are watched until the delivery of the two dyes to the collector is stabilized so that each dye continues to enter the same test tube without a drift. Once this adjustment is found and maintained, it becomes unnecessary to repeat the above test before each separation run. When the collection is finished, the test tube rack is raised until the ends of the tubes tt... are level with the buffer surface in the buffer chambers to stop the buffer flow through the collector. The position of the test tube rack in which the collector flow has the desired value is marked by a stop. This obviates the necessity to re-adjust the level of the collector tubes tt... for successive runs. As soon as the rack is lowered as far as the stop will permit, the apparatus is ready for a preparative fractionation run.

(6) To replace the test dyes by the mixture to be analyzed, the injector IN is removed and the syringe reservoir R and injector are flushed by filling R with the cleaning solution and expelling it with a syringe piston. The piston is left in the lowest position and the injector is re-inserted. The piston is then slowly withdrawn allowing the buffer to enter and partly fill R whereupon the piston is removed. The buffer in R can now be removed with a small syringe equipped with a long needle and replaced by the mixture to be fractionated. The rate of injection of this mixture into the apparatus is regulated by changing the level of R.

(7) The rate of axial flow is now adjusted by redistributing the buffer delivery tubes among the racks D_1 and D_2. Fine adjustment is made by current regulation or by slight displacements of the collector position. With colorless mixtures, it is advisable to add a marker dye in order to adjust for an adequately fine and sharp streak as well as to detect possible drifts and to correct for them by adjustments which safeguard continued delivery of the dye to the same collecting tube.

For best sharpness of the separation pattern, the injector exit opening should be carefully centered in the gap of the annulus. The optimum injection is obtained with a very fine injector needle releasing its content at the center* of the annular gap. The best results are obtained when the density of the injected streak equals the density

* The optimal location for the streak is more precisely defined not as the center of the annulus but, rather, as the location of the maximum velocity in the velocity profile of the rotational flow.

of the buffer. The greater the disparity in these densities, the thinner should be the injected streak. The injected material spirals then centrally between the inner and outer cylinders of the annulus until its offspring spirals reach the collector.

MODES OF OPERATION

There are several different modes in which separations can be carried out.

(*a*) *Single-order collection*

Continuous separations can be adjusted so that the components of the n-th helical turn enter the collector in the same order in which they appear in the separation pattern. The decision on how many turns of the helix to allow before interception by the collector is to be based on the two least clearly resolved components. The separation between two adjacent streaks should be at least equal to the distance between two neighboring collector tubes to minimize the hazard of re-uniting the split components by collection in the same collector tube. The optimum condition for collection of two separated components is achieved when component A is located midway between two consecutive turns of component B near the approach to the collector. Any deviation from the central location during the preceding or subsequent helical turns will diminish the distance of the streak of component A from one of the adjacent streaks of component B.

(*b*) *Split-order collection*

The continuous helical pattern can also be intercepted by the collector so as to collect the desired number of the fastest components in the n-th turn while permitting the slower components to complete an additional turn before collection. This may be advantageous in cases where the fastest components are clearly resolved while the slower ones require additional time for adequate resolution.

(*c*) *Isoelectric accumulation*

Isoelectric accumulation can be achieved in the absence of electro-osmotic streaming at zero axial flow in a buffer corresponding to the isoelectric pH of the component in question. In this case, the pitch of the helical path is zero *i.e.* the injected material accumulates in a circular orbit while ionized components of either sign wander away in both directions in right handed and left handed helical paths. When desired, the accumulated substances can be transported to the collector by establishing axial streaming. Fig. 9 shows human hemoglobin A in a helix of nearly zero pitch at pH 7.0.

(*d*) *Non-isoelectric accumulation*

The accumulation method described in (c) is not limited to ampholytes at their isoelectric point. One can compensate the electrophoretic migration of any species of charged particles by an axial counter-flow so as to accumulate them in a circular orbit. This makes it possible to detect and accumulate materials present in low concentration. Fig. 10a shows an accumulation of india ink thus obtained within 3 min.

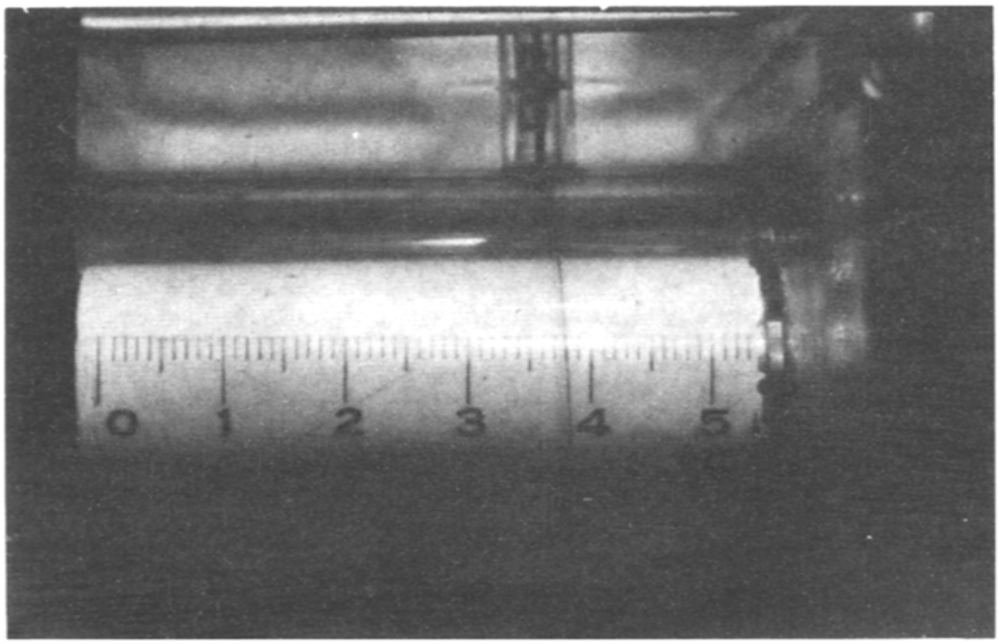

Fig. 9. Hemoglobin streak at pH 7.0.

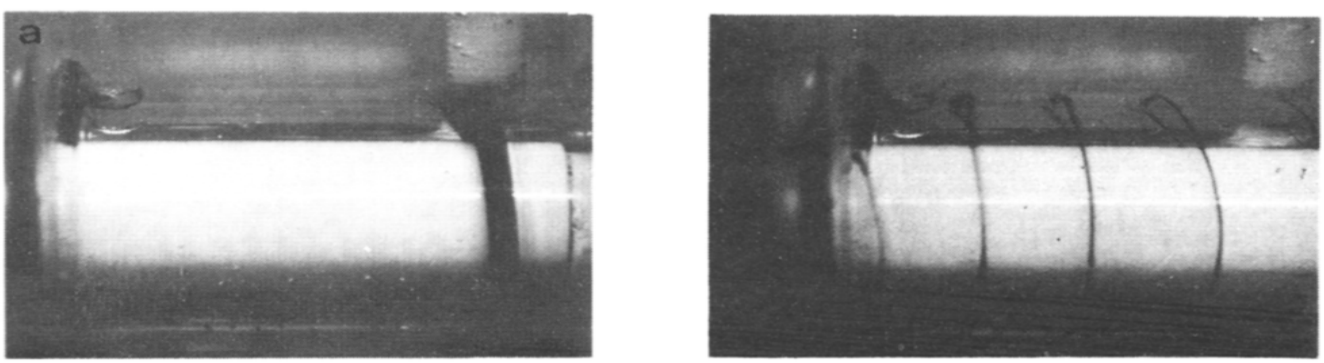

Fig. 10. (a) Condensation of a substance in circular orbit (india ink); (b) India ink streak prior to adjustment of axial flow for condensation in circular orbit.

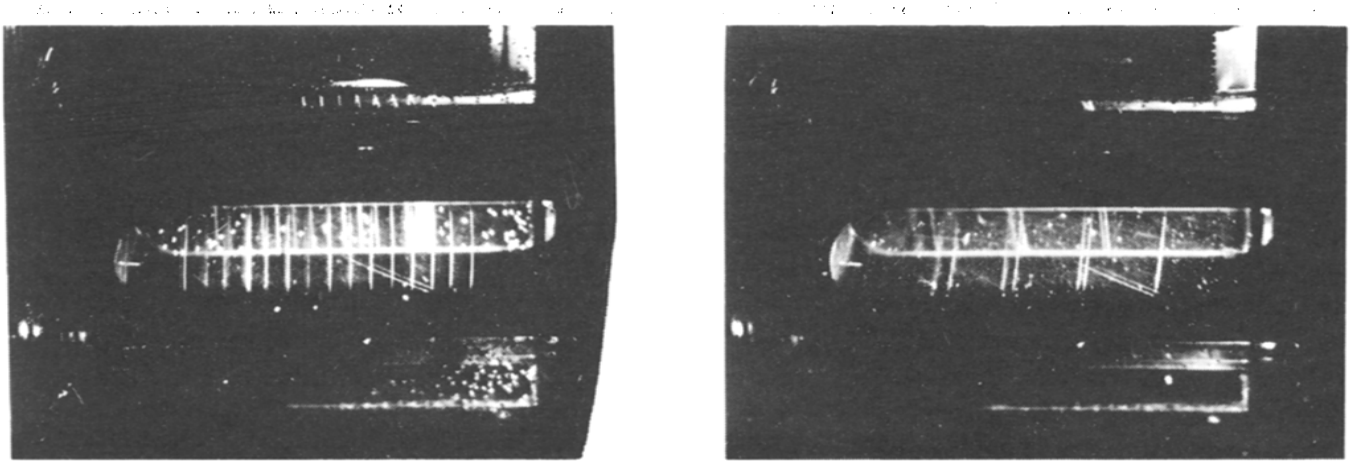

Fig. 11. Visualization of streaks of microbiological particles. (a) A helix of red blood cells suspended in physiological saline; (b) separation of two types of fungi: *Saccharomyces cerevisiae* and *Rhodotorula* in dilute buffer solution.

Fig. 10b shows the original helical streak which was compressed into the circular accumulation of Fig. 10a by axial counter-flow.

(*e*) *Zonal separation*

Zonal separation of very minute amounts of suspended or dissolved material injected in volumes of the order of a few ten thousands of a milliliter can be accomplished by simply injecting a very fine streak about 1 cm long permitting it to revolve about the inner cylinder on a helical path. In the course of this motion, the short streak breaks up into separate component streaks which land in different collector tubes. One can adjust axial flow so as to place the slowest component into a stationary circular orbit so that the full length of the migration path becomes available for the separation pattern.

(*f*) *Multiple separations*

The separation scale of continuous fractionation can be increased by mounting several injectors side by side to inject the same mixture whose components can be collected by one collector. If the mixtures introduced by the injectors are not identical, the same arrangement can be used for simultaneous analysis of several different mixtures (*cf.* ref. 3).

(*g*) *Multi-stage separations*

Inadequately purified fractions can be fed directly into the reservoir R of a second separator operated in series with the first one or they can be re-injected into the same separator for reprocessing.

(*h*) *Scanning of a separation pattern*

Even though injection of tracer dyes makes it possible to adjust the apparatus when working with colorless components, it is desirable to visualize the latter directly before collection in order to obtain preliminary analytical information. It has proved possible to guide the separation pattern into a space between two flat quartz plates outside the annular cylinder prior to entry of the separation pattern into the collector tubes. This offers the possibility of detecting colorless streaks of ultraviolet absorbing biological materials by scanning with a fine beam of ultraviolet light whose intensity is monitored photoelectrically. This arrangement will be discussed in a later publication.

(*i*) *Visualization of particulate components by light scattering*

Suspended colorless particles can be detected with ease prior to collection by light scattering. For this purpose, a "window" can be milled into the iron cylinder m (*cf.* Figs. 3 and 4) so that the cell can be transilluminated by a horizontal beam of light. A camera is mounted in front of the apparatus so that the illuminating light does not enter the lens directly. As a result of light scattering, the tracks of separated particles appear as white streaks on a black background as shown in Fig. 11a for human red blood cells suspended in 0.9 % NaCl solution. A separation of two species of fungi: *Saccharomyces cerevisiae* (slower component) and *Rhodotorula* suspended in a 0.02 molar pH 10 Hydrion buffer is shown in Fig. 11b. The scanning method described in (h) for U.V. absorption could be adapted to detect particle streaks by photoelectric recording of scattered light.

The magnetorotationally stabilized separator possesses an important advantage over other related solutions of the separation problem[2,3]. A small current is sufficient to maintain the necessary electric field for a long migration path. For instance, in the apparatus described here the circumference of the annulus is about 10 cm. A particle, after completion of 20 turns has covered a distance of 2 meters. In a linear cell 2 meters long and 2 mm wide, the cross-sectional area transverse to the electric current will be 40 cm^2. It will have to carry the same current density as the present apparatus to provide the same electric field intensity. The cross-section traversed by the current in the present instrument is about 2 cm^2. It requires thus $^1/_{20}$ of the current needed by the linear cell.

SUMMARY

A horizontal buffer column shaped as an annular cylinder serves as an electrophoretic column. It is stabilized against thermal convection by electromagnetically maintained rotation. The solution to be analyzed is injected as a thin streak which follows a helical path of different pitch for each electrophoretically distinct component. The separated components can be collected separately in a continuous fashion. Separation can be based on differences in electrophoretic mobilities or in isoelectric points. Discontinuous zonal separation is also possible on a micro-scale. The method is effective for separation of molecular components as well as of microbiological particles.

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