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A System Based on Split-Flow Lateral-Transport Thin (SPLITT) Separation Cells for Rapid and Continuous Particle Fractionation

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Abstract

In this paper we introduce a versatile separation system based on the operation of some rather simple separation cells termed SPLITT cells. These thin cells are capable of acting singly or in linked arrays depending on the problem at hand. Separation in the cells can be driven by sedimentation, electrical, and related forces, by diffusion, and by various gradients. SPLITT cells resemble FFF channels and are subject to similar methods of construction, except for the requirement for various split-flow elements in different parts of the SPLITT system. The separation path, across the narrow dimension of the thin SPLITT cells, is extremely short (often <1 mm), leading to rapid separation. The system is capable of continuous operation. The combination of continuous processing and rapid separation is anticipated to provide a relatively high throughput, despite the low volume of the cells. The optimization of throughput is discussed. Because separation takes place without the distortion accompanying some continuous methods, resolution is expected to be reasonably high (peak capacity ~2-20 or more) despite the short path and high speed.

INTRODUCTION

All separation processes require the differential transport of component species. Quite naturally, the speed of separation is closely linked to the speed with which the necessary transport step or steps can be completed (*1*). If the path length for basic separative transport can be made short, increased speed is generally possible.

One way to enhance separation speed is to carry out the separation in a

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thin channel where the essential transport occurs over the thin dimension. The length of the path is then the channel thickness or some fraction thereof. The above conclusion applies whether the transport required is simply a single displacement step or if it consists of multiple diffusional excursions over the transport path. The latter is required, for example, to maintain near-equilibrium conditions and high efficiency in chromatography.

Clearly, thin capillary tubes used in chromatography provide one example of a thin-channel separation technique. We note that ordinary packed-column chromatography is in many ways equivalent because the extended space in the column is effectively broken into thin channels by the packing particles (*2*). Field-flow fractionation (FFF) is another thin-channel method. In FFF, the usefulness of the thin-channel concept is further enhanced by the fact that, after a single relaxation step, transport is required only across a very thin particle cloud whose thickness is normally one or two orders of magnitude less than that of the channel itself.

In both chromatography and FFF, separation occurs as a batch process along the axis of the flow channel. However, this axial separation is based upon an enrichment whose direction is basically perpendicular to that of the channel axis (*1*). Both methods are designed to convert the basic lateral enrichment into powerful axial separation using axial flow to create a new separation coordinate at right angles to the old. Reasonable levels of resolution require many transport steps across the lateral region of enrichment, a process hastened by thin channels.

Here we propose to utilize the lateral separation or enrichment directly. Axial flow will be retained, but its function will change; rather than *integral* flow participating in separation, it will assume the role of *passive* flow which, in this case, will make continuous separation and fraction collection possible (*1*).

The conditions necessary for continuous separation have been outlined elsewhere (*3*). In general, two perpendicular displacement processes are needed, only one of which must be selective. The one nonselective displacement process allowed is often flow. Accordingly, many continuous separation techniques have evolved in which flow is oriented at right angles to a field, where the latter causes the selective (separative) displacement. Some of these techniques are discussed later.

Different means can be used to develop the separative displacement over the thin dimension of a flow channel. This will usually require the application of some lateral field or gradient. The nature of the separation will depend on whether components are driven by the field toward

steady-state concentration distributions, or simply reach transient distributions. These matters will be detailed later. In all cases, the speed with which the separation is developed will be high because of the rapid transport across the thin channels. While separation power will generally be less than that of chromatography or FFF, separation speed is potentially higher because only one lateral transport step is required rather than many.

Several powerful separation methods (e.g., electrophoresis and sedimentation) are based on a single transport step. The essential transport process is driven by some external field or gradient of strength S. The velocity U of transport is given by

$$U = mS \tag{1}$$

where m is a generalized mobility (e.g., electrical mobility or sedimentation coefficient). Different components have different m values; they thus migrate at different velocities and, in a given time, traverse paths of different lengths. This differential transport leads directly to separation.

Usually the transport described above is forced to occur over a rather long path (1–50 cm and beyond), a process requiring considerable time for completion. In many cases the degree of resolution required in the separation is not high enough to necessitate such an extended path; the long path is retained in order to realize the separation in a practical sense, i.e., to achieve sample detection and collection and to overcome the effects of broad initial zones and other resolution-degrading factors.

Our thesis is that a much shorter and more quickly traversed path, ranging typically from a few tens of micrometers to a few millimeters in extent, is adequate to execute many separations where an exceedingly high resolution is not needed. The requirement is that the separation proceed from a very thin sample zone without distortion. It is anticipated that such separations can be carried out over the short path between channel walls in relatively thin channels. We propose that the physical separation achieved over this short path can then be translated into experimentally meaningful separation—continuous if desired—by designing channels with various split-flow configurations. The split-flow approach, combined with the approximate control of various flow substreams, also provides a mechanism to obtain thin initial sample zones.

We have shown in FFF experiments (*4*) that it is possible to split thin-channel (305 μm) flow into at least two laminar streams containing different particle concentrations. The different particle content of the two

streams illustrates a simple particle-fluid enrichment executed over the channel thickness.

In this paper we propose to develop various split-flow strategies using thin channels to realize the rapid separation potential of the short lateral transport path. The object is to achieve particle/particle enrichment and separation, not merely particle-fluid enrichment. Continuous separation is possible, as opposed to the discrete (batch) nature of FFF separation.

The process can be illustrated by reference to FFF channels. Here, the flat channel walls are generally clamped over a thin (usually 50–500 μm) film of spacer material (Mylar, Teflon, stainless steel, etc.) from which the channel volume has been cut and removed (*5*). For the split-outlet FFF experiments described above (*4*), the single spacer layer was replaced by a sandwich of three layers. With the layers subject to cut-outs of different geometries, a binary splitting of the outlet stream was provided. This approach has also been suggested for inlet stream splitting to reduce separation time in FFF systems (*6*). In neither of these cases was it proposed to utilize stream splitting in conjunction with the particle separation power of lateral transport.

It is anticipated that lateral separations can be realized in channels resembling those used in FFF. Most such channels are rectangular with tapered ends. For sedimentation in a centrifuge, the channel is formed into a circle and layered at the inside wall of a centrifuge basket. For transport induced by electrical and cross-flow effects, the channel walls are semipermeable membranes.

The FFF-type channels proposed for use here could be fabricated using sufficient layers of spacer material to provide the desired splitting characteristics (to be described) at both ends and occasionally at intermediate points. Continuous operation is possible. The channel could be made up of one or more distinct cells in each of which an elementary lateral separation process would take place. We will begin by discussing some single-cell separation strategies.

SINGLE-CELL SYSTEMS

A simple application of the above concept entails the sorting of particulate material into two fractions according to differences in sedimentation coefficient. We anticipate that for particles of fixed density, a reasonably sharp size cut-off between the particles in the two fraction streams can be obtained by splitting both entering and exiting flow streams into upper and lower substreams. Importantly, the ratio of flow

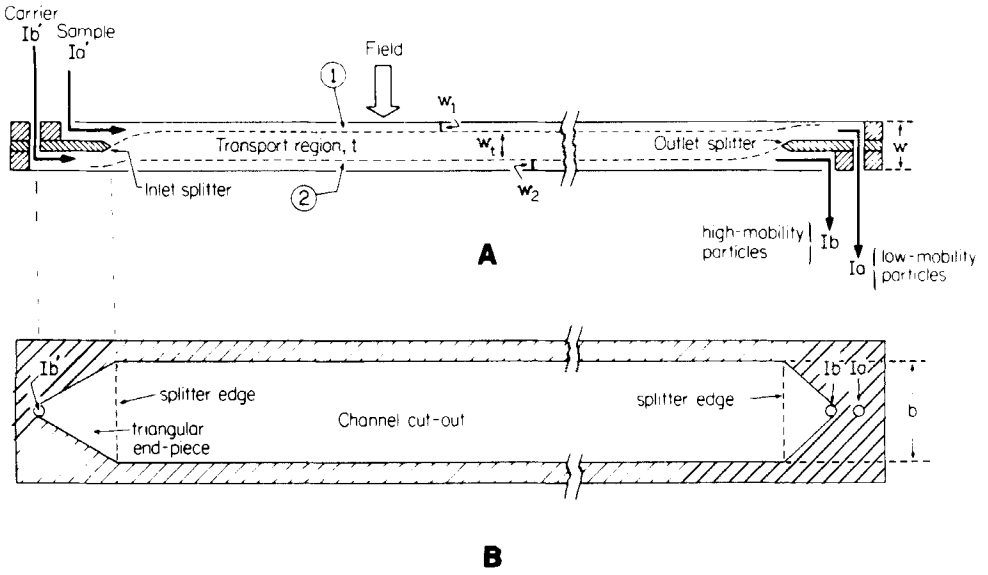

FIG. 1. Schematic diagram of simple single-cell SPLITT system with crossover flow. (A) Side view showing distinct transport region created by crossover flow. (B) Top view of bottom spacer, showing cut-outs for channel and for indicated substreams.

rates in the two substreams must be varied from inlet to outlet for reasonably high resolution (see below).

A diagram of the proposed separation cell (termed a SPLITT cell) is shown in Fig. 1. Figure 1(A) shows an edge view of the channel system and indicates in schematic form the placement of the two flow splitters at the two ends of the cell. (This configuration is realized by using three spacer layers.) The sample is introduced into flow substream Ia′ entering above the inlet splitter; the bulk of carrier is introduced into substream Ib′ emerging from beneath the inlet splitter. As the sample particles are carried the length of the channel, a field applied in a direction perpendicular to the flow axis forces them to migrate from region 1, the *input region*, toward region 2, the *collection region*. If the applied field is gravitational or centrifugal, the larger (and/or denser) particles will successfully realize this transfer. Smaller particles will not. This is the basis of separation.

The basic cell configuration described above (and expanded on below) will work in conjunction with other fields in much the same way. The other fields (such as electrical) will lead to particle fractionation based on

the different mobility levels corresponding to that type of field. Combinations of fields can be used if desired.

A key element of this single-cell system is the adjustment of substream flows such that the ratio of flow rates in the upper substream relative to the lower substream is greater at the outlet than at the inlet. This adjustment creates a crossover flow, in which part of the carrier introduced into the lower inlet substream Ib′ finds its way into the upper outlet substream Ia. This fluid transfer creates a critical buffer zone which we call the *transport region*, shown as region t in Fig. 1. With this region established by the unequal flow ratio, particles are not able to reach region 2 successfully and thus exit in the lower outlet substream Ib unless transport is completed across the thin transport zone. Without the unequal inlet/outlet splitting ratio, crossover flow and thus transport region t would disappear. In this case, particles entering at the bottom of input region 1 would easily and almost instantaneously transfer into the adjacent collection region 2 and thus exit from Ib. Particles of the same kind entering at the top of region 1, however, would need a much greater time to reach region 2 because they would require transport the full distance w_1 across region 1. The significant difference in the time required for identical particles, entering at different positions within region 1, to transport into region 2 would lead to a major loss of selectivity. The interposition of transport region t, by contrast, would establish a more or less equal transport path across which all particles would need to pass in order to reach region 2 and exit Ib.

The principle upon which transport region t is established in the channel is illustrated by the dashed boundary lines (actually, boundary planes) between the labeled regions. These boundary lines coincide with very specific streamlines (or streamplanes) of fluid flow. The upper boundary line follows the streamlines dividing the upper from the lower inlet flow. Thus, if entering sample particles underwent no transport relative to the fluid but simply followed their respective flow lines, all the incoming particles would stay in region 1, remaining above the upper boundary plane.

Note that the boundary planes may swerve up or down near the active edges of the channel splitters. These deflections are transient, resulting from the brief transition from one steady-state flow condition to another near the splitter edges. These transients will normally have little effect on separative transport. The principle transport occurs in the body of the channel, where the streamplanes maintain a stationary position which depends upon the ratio of flow rates in upper and lower substreams. Normally we would use a higher flow rate through entry port Ib′ than Ia′ in order to force the upper boundary plane to curve upward to a

stationary position closer to the top than to the bottom wall. This compresses the input region, which provides the narrow initial sample zone required for satisfactory resolution.

The lower boundary plane, by contrast, divides the channel into two regions according to where the fluid elements exit rather than enter. Because of the unequal flow rate ratios noted above, a substantial fraction of the pure carrier introduced into the bottom inlet substream Ib′ will emerge in the upper outlet substream Ia. This layer of carrier occupies region *t* of the diagram. Any particle which is to reach region 2, and thus be carried out of exit Ib, must traverse this layer of carrier, which therefore constitutes a critical transport region within the channel. By the proper adjustment of flow rates, this transport region can be made considerably thicker than region 1, thus making the particle's starting position from within region 1 of little consequence. This strategy is expected to enhance the sharpness of the separation. This sharpness will be somewhat eroded by the fact that sample particles entering region 1 near the upper wall will have a somewhat longer transport path to negotiate (across region 1 as well as region *t*) than those entering at the bottom of region 1 (which must traverse only region *t*). However, the particles near the wall will be carried downstream very slowly due to the parabolic flow profile; they will thus have more transport time and will partially catch up to those particles having a more advantageous initial position within region 1.

In well-designed cells, the boundary streamplanes should lie parallel to the axis of the channel throughout all of the channel except in the very narrow transient zone in the immediate vicinity of the splitting edges of the inlet and outlet splitters. The transport region is thus well defined by these two parallel boundary planes, and the above transport processes should be well-behaved, reproducible, and calculable.

The use of a thin channel in the above configuration has several advantages. First of all, the thickness w_t of the transport region is scaled to the channel thickness w and is therefore smaller in thin channels. This leads to rapid transport across region *t* as suggested earlier. Second, separative transport across the thin dimension is relatively free of distortion, as will be explained later. Third, the thin-channel configuration is a stabilizing influence against convection. Clearly, convection must be guarded against, especially in the case where a concentrated stream of dense sample particles is introduced into sample stream Ia. However, additional stabilization against convection can be provided by adding to the carrier stream some unobtrusive solute (such as sucrose) that will increase the fluid density in the lower part (or other selected parts) of the flow channel. Fluid stabilization by density gradients is a

well-known technique and has been applied widely to other types of cells (*7–9*).

The one-cell SPLITT system described above, requiring binary inlet and outlet splitting, will normally be constructed with three thin films of spacer material, one film for each of the two entering and exiting flow conduits and another for the stream-splitting element between them. (Other means, however, might be found for introducing thin splitting elements into the ends of the channel.) The upper and lower split-flow conduits will normally converge to a point (Fig. 1B) for convenient introduction and withdrawal of the various substreams. Triangle-like end pieces are used in FFF for the same purpose.

If desired, a larger number of fractions could be simultaneously and continuously collected by using a multisplit outlet. A SPLITT cell with this type of outlet is illustrated in Fig. 2. Crossover flow, with a resulting transport (*t*) zone, is used to advantage here as well as in the binary-split system. Crossover flow, as before, reduces the thickness of the initial sample zone and thus improves resolution. The transport zone greatly sharpens the resolution between the particle populations collected in substreams Ia and Ib. While the multisplit system could, if desired, be used without crossover flow, the resolving power would be reduced considerably, corresponding to the loss of approximately one outlet unit.

A SPLITT cell with the outlet split into n channels for the collection of n fractions would require $2n - 1$ spacer elements, as suggested by Fig. 2. A multisplit inlet with up to n flow elements could be simultaneously used to introduce density or pH gradients, if desired. Such gradients could be used for quasi-steady-state operation, yielding continuous and rapid separation based on the same underlying steady-state mechanism as used for isopycnic sedimentation and isoelectric focusing. This approach will be discussed in a subsequent section.

The dimensions of cells with multisplit outlets and/or inlets might vary widely. However, it is important that the channel thickness w remain small for rapid mass transport and laminar flow; it is also essential that

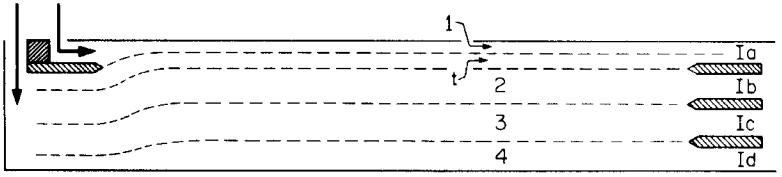

FIG. 2. Side view of a cell with multisplit outlet.

$w \ll b$ (b = channel breadth) to avoid excessive edge effects. (Edge effects could be eliminated altogether by some form of sheath flow, as proposed for FFF (*10*).) An example of a channel with a relatively large w would be one with flow split into $n = 10$ outlet streams, requiring $2n - 1 = 19$ spacer layers. If each layer were 100 μm (~0.004 in.) thick, w would be 1.9 mm. This w would work best with a breadth b of 2 cm or more (see later) to satisfy the condition $w \ll b$. Length L would probably lie in the range 2–50 cm, but might be longer. Cells thinner than 1.9 mm could be made with thinner spacer layers (although flow-space and splitter uniformity would be harder to maintain) or with smaller n, or both. Binary-split cells ($n = 2$) would tend to be thinner (e.g., 3×100 μm = 300 μm) than multisplit cells.

The "plumbing" necessary to handle the various streams flowing out of (or into) a multisplit end should not be excessively difficult to develop. One scheme would be that illustrated by Fig. 3. Here the individual flow streams would exit (or enter) the channel via narrow conduits or slits passing through the other spacer layers and out one of the two walls. Normally each stream would converge to the apex of a triangular end piece (Fig. 1B) before exiting for convenience in "plumbing." Once outside the channel, each stream might flow through a segment of narrow tube or other flow restrictor for flow control. By varying the relative resistance of the restrictors, different fractions could be shifted up and down the array of exit ports to optimize fraction purity.

The throughput of SPLITT cells should be relatively high despite the inherently small cell volumes because of the short transit times and the continuous operation. Throughput could be increased further by using banks of such cells working in parallel or by increasing channel breadth. Excessive channel breadth (for example, $b > L$), however, might lead to

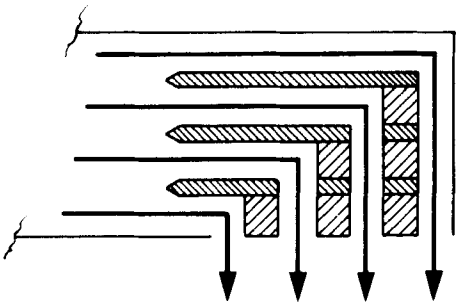

FIG. 3. Illustration of one type of internal "plumbing" for connecting the substreams in a multisplit cell with external "plumbing."

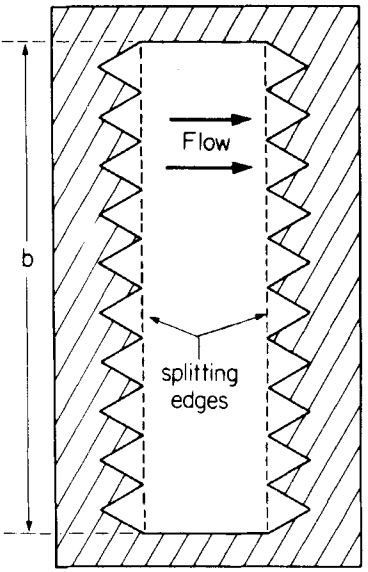

FIG. 4. Top view of channel of very large breadth b having a series of triangular outlets and inlets.

nonuniform flow and thus nonconstant transit times (and mobility cutoff values) because of disturbances originating in the correspondingly broad triangular end pieces. This problem could be remedied by using a series of triangular inlets and outlets, each of moderate breadth, for a single cell of considerable breadth, as illustrated in Fig. 4.

We note that increases in channel thickness w will not normally increase throughput because the increased channel volume is offset by the more sluggish transport and the corresponding increase in transit time through the cell. Increases in cell length L, however, will lead to corresponding increases in throughput, but the associated increase in flow velocity may lead to flow disturbances if carried too far. Similarly, flow velocity and throughput will increase in proportion to field strength S, but the heightened S might tend to induce unwanted convection. In each case, limits will need to be established by experimentation.

MULTIPLE-CELL SYSTEMS

The single-cell systems described above can be expanded in another way to achieve the separation of multiple fractions instead of just two.

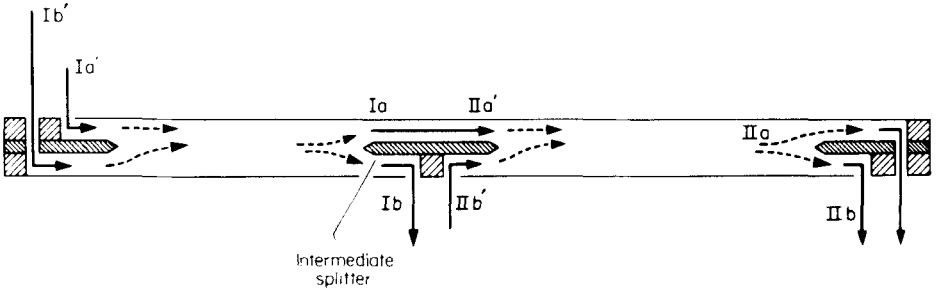

FIG. 5. The linkage of two SPLITT cells in series by means of an intermediate splitting unit.

Instead of using a multisplit outlet, one can add additional binary-split (or simply binary) separation cells to form a serial array of cells along the main flow axis. This is illustrated by Fig. 5 in which the flow stream encounters an intermediate (rather than terminal) splitter after the first binary separation cell. The intermediate splitter shunts the lower (high-mobility) stream Ib out of the channel for collection but retains the upper (low-mobility) stream Ia, simply transferring it through the splitter region to emerge as sample input stream IIa' for the second binary separation unit. This stream is then joined by a new carrier stream IIb' to establish the conditions (including adequate crossover flow) necessary for another binary separation. The length of the second separation cell and the flow rates of carrier streams IIb', and to some extent IIa, and IIb, can be adjusted to achieve separation around another critical value of mobility, different from that utilized to divide the particle population in the first flow cell. If the cutoff value m_c of the generalized mobility (e.g., sedimentation coefficient in the case of a sedimentation field) in the second unit is adjusted to be slightly below that in the first, a narrow

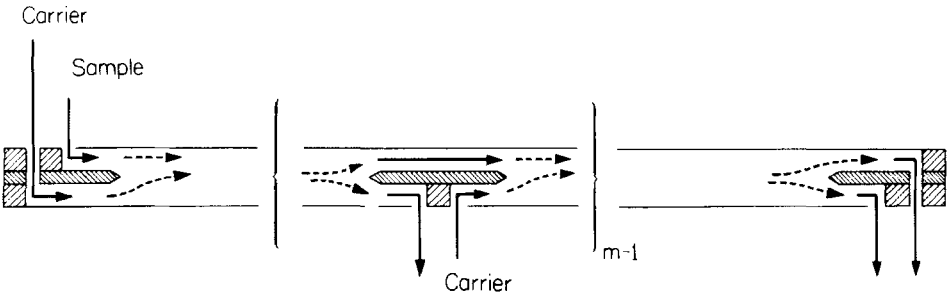

FIG. 6. A series of m SPLITT cells linked by $m - 1$ intermediate splitters.

fraction of particles having mobilities between the two cutoff values should emerge in stream IIb. Streams Ib and IIa would contain the high-mobility and low-mobility tails, respectively. With further adjustments, the original sample would be divided into different mobility ranges among the three outlet streams Ib, IIb, and IIa.

Separations of higher order than ternary could be achieved by adding additional intermediate splitters as suggested by Fig. 6. With $m - 1$ intermediate splitters partitioned between m binary cells, the sample could be split into $m + 1$ distinct fractions. These fractions would be defined by the m successive mobility cutoff values $m_c(\mathrm{I}), m_c(\mathrm{II}), \ldots, m_c(m)$. In each succeeding cell, the m_c value must be smaller than the previous one in order to "bleed" off a new lower-mobility fraction, i.e.,

$$m_c(i + 1) < m_c(i) \tag{2}$$

Unfortunately, this condition becomes increasingly difficult to maintain as the cell number increases. The problem is that the crossover flow in each cell augments the flow rate of the sample-containing stream passing into the succeeding cell, causing a more rapid transit of sample through the succeeding cell and, without compensating adjustments, increasing rather than reducing m_c. One form of compensation would be to increase the length × breadth product for each succeeding cell sufficiently to increase overall cell transit times. However, to utilize the crossover effect optimally, substantial crossover flows are needed. Thus flow rates and cell size would become unmanageably large beyond a few cell units.

The above problem could be remedied by redesigning the intermediate splitters (or arranging other equivalent "plumbing") as shown in Fig. 7. In this case the low-mobility fraction is bled off at the end of each cell and the high-mobility fraction is passed on to the next cell for another separation step. With this strategy, each succeeding mobility cutoff value is adjusted to a higher level, i.e.,

$$m_c(i + 1) > m_c(i) \tag{3}$$

This condition could be arranged rather easily by adjustments in flow rates or cell volumes, or both.

The above examples barely begin to illustrate the numerous possibilities with linked-cell systems. Different SPLITT cells could be distributed as desired over an extended three-dimensional matrix. The matrix, by way of example, could be formed from a large number of thin spacer layers, each with sections cut out in such a manner that the interconnected cells are formed in proper relationship to one another.

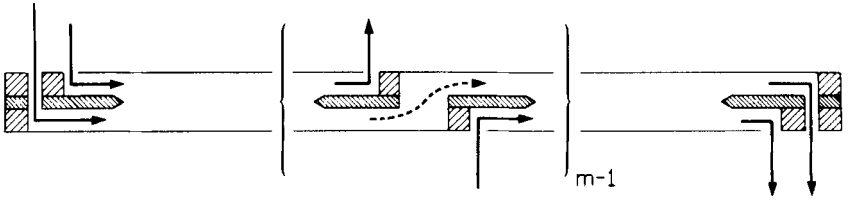

FIG. 7. An alternative form of intermediate splitter for linking SPLITT cells in series.

Different substreams from one cell could, for example, be diverted sideways to different locations, each to enter its own cell or series of cells specifically designed for processing that substream. Thus various sample-containing streams and substreams could be divided, merged with other streams, recirculated, and/or introduced at different levels of either multisplit or binary-split cells. In short, the sample-containing streams could be directed through mazes of cells of arbitrary structure and complexity. This design freedom would be supported by the natural versatility of "plumbing" in thin-cell systems composed of multilayers of thin spacer material. Substreams could cross one another at different levels and they could pass through small apertures in other layers to reach different lateral positions. In general, they could conduct fractions around rather freely over the three-dimensional structure containing the various SPLITT cells.

OPERATION IN DIFFUSIVE MODE

In the cases described above, differential lateral migration induced by an applied field serves to fractionate samples along the (short) lateral coordinate. However, simple diffusion can also lead to rapid fractionation along the lateral coordinate; this too can be converted into high-throughput continuous separation. A third mechanism for inducing usable fractionation over this coordinate will be described in the next section.

Lateral diffusion is incapable of separating a large number of distinct components because it is a dissipative rather than a structuring process (*1*). At best, it can provide limited separation by acting on an ordered distribution and dissipating its components at different rates. Thus the sample stream flowing into region 1 of Fig. 1(A) introduces, ideally at least, a step-function concentration profile for the various components along the lateral coordinate. In the absence of an external field, this profile is flattened by diffusion, but at different rates for the different

components. With appropriate adjustments in flow rates, substantial quantities of rapidly-diffusing components will reach collection region 2 while very small amounts of slowly diffusing components will enter that region before reaching the outlet stream splitter. Thus, a dialysis-like separation of high and low molecular weight species can be anticipated in a (membrane-free) SPLITT cell. The membrane would, effectively, be replaced by transport region t. If relative flow rates were adjusted to give the highest flow rate in region 2 and the lowest in the transport region, a large percentage of low molecular weight material could be removed without excessive dilution of the high molecular weight materials. More complete removal could be achieved in successive SPLITT cells, but at the expense of additional dilution. However, the process has the potential for considerable speed (and thus throughput), even in multiple-cell systems. For a transport distance ($\sim w_t$) of 50 μm, a significant fraction of a component with $D = 10^{-5}$ cm would complete its transfer in time $t > 1$ s. Thus several successive cells could be traversed in times ranging from a few seconds to a few tens of seconds.

STEADY-STATE AND QUASI-STEADY-STATE OPERATION

It is possible to establish conditions such that different components will approach different steady-state distributions across the lateral coordinate. These distributions—or distributions approaching the steady-state—can be converted into continuous separation using split-flow cells much as before. Again, the short lateral coordinate will greatly hasten the approach to steady-state, thus increasing fractionation speed and throughput.

There are several possible classes and subclasses of steady-state (or more generally, quasi-steady-state) operation in SPLITT cells. We start with the equilibrium-gradient approach. This approach underlies such important techniques as isopycnic sedimentation and isoelectric focusing. In these techniques, transport paths are relatively long and operation is generally noncontinuous (*9*). In isopycnic sedimentation, fractions of different densities, driven by sedimentation forces, come to equilibrium as narrow bands centered at different points (corresponding to the respective sample densities) along an imposed density gradient. Isoelectric focusing works similarly with charged species driven by electrical forces toward their respective isoelectric points in a pH gradient (*1, 9*).

In the isopycnic case, density gradients may or may not stem from sedimentation equilibrium. Because of the short path over which density

gradients must be established in SPLITT cells, equilibrium would require that the densifying medium contain relatively high-mass particles, even in strong sedimentation fields.

The short path would also appear to work against the use of dynamic gradients because the density profile under nonequilibrium conditions would change rapidly as it approached equilibrium in the narrowly confined space. However, the steady-state distribution of sample components, although shifting around with the changing density gradient, would also be approached rapidly, offering hope of a quasi-steady-state condition, i.e., components approaching (but not quite reaching) a steadily shifting steady-state distribution. The attainability of the quasi-steady-state distribution requires essentially that the relaxation time for component redistribution in the channel be less than the relaxation time for redistribution of the gradient former. The validity of this condition would need to be justified in each case. If the condition did not hold, the distribution of components upon reaching the exit splitter(s) would be determined more by transport coefficients, as described earlier, than by steady-state conditions.

Dynamic density gradients would normally be developed with the highest density at the bottom (or outside) to control convection. One could introduce the density-forming medium entirely through the bottom entry substream and allow it to diffuse up into the channel. In this case, channel thickness, cell length, and flow rate would be adjusted to provide the desired density gradient at the end of the cell, developed over sufficient time to allow the components to approach their steady-state distribution as noted above. (The distribution would preferably consist of distinct bands for each component, as in normal isopycnic sedimentation.) The particle size or molecular weight of the densifier could also be chosen to aid optimization through control of the densifier diffusion coefficient and thus of its relaxation time.

The density gradient could be more finely tuned (e.g., a nearly-linear gradient could be formed) by introducing different concentrations of densifier into the various substreams of a multisplit inlet. In this way, component bands could be spaced in different ways with respect to the outlet ports. Here, too, careful optimization would be required.

Similar considerations would apply to the use of isoelectric focusing in a SPLITT cell. However, it may prove easier to form and use steady "equilibrium" pH gradients than to make use of dynamic gradients.

In both the sedimentation and electrical cases noted above, the sample could be introduced through any desired entry substream. Generally, an

entry position would be chosen that would provide the most rapid approach to steady-state banding for the largest number of components.

Another promising form of steady-state operation would result from allowing components under the influence of an applied field to form thin steady-state distributions or "layers" next to one channel wall, the so-called accumulation wall. The steady-state distribution in this case is normally exponential, as in FFF systems. Components with widely different layer thicknesses could be separated at reasonable resolution levels by withdrawing material concentrated in the "thinnest" layer (say component A) through the bottom exit substream (Ib in Fig. 1) operated at a low flow rate. Most of the material (component B) forming the much thicker layer would then be withdrawn through the higher flow rate upper substream (Ia). Thus relatively pure B in substream Ia would be separated from partially purified A in substream Ib. If a more complete purification of A were desired, several serially linked cells, much like those shown in Fig. 6, could be used. However, the pass-through flow substream of the intermediate splitters would be located so as to exit next to the accumulation wall. If the latter were the bottom wall, the intermediate splitters would resemble those shown in Fig. 6, but would be turned upside down to allow pass-through flow at the bottom wall. Crossover flow would not be needed to confine the sample stream to a thin layer near the wall; the role of crossover flow would be taken over by the external field, which would confine the desired components near the accumulation wall. Consequently, none of the dilution of the sample input substream that normally results from crossover flow would be necessary; some concentration could be introduced, if desired.

The above system should prove effective as a dialysis substitute, using as many serially linked cells (generally with binary split) as necessary to achieve the desired removal of low molecular weight impurities. Fluids with high molecular weight or particulate impurities (e.g., processing fluids subject to microcontamination) could also be effectively and rapidly decontaminated, usually in a single binary SPLITT cell.

Multisplit outlets (or inlets) would usually not contribute to multicomponent separation in single SPLITT cells using the wall-based steady state because of the lack of clearly separated lateral zones. However, multicomponent separation might be achieved by successive "skimming" operations carried out in a series of binary cells, each operated under different conditions. Here, components would be skimmed off one at a time, starting with those forming the thickest steady-state layers. However, this approach is limited because several skims (cells) would be required for each component, the number depending upon the purity desired.

It is worth noting that with the overlapping exponential distributions formed in the wall-based steady-state, the component with the thickest layer becomes increasingly pure as one moves away from the wall (*11*). Therefore, small amounts of very pure component can be skimmed off in each SPLITT cell, even if the components involved have similar properties and thus similar layer thicknesses. Large amounts of the referred-to component could be obtained (although in dilute form) through a series of skims executed in successive cells.

Many of the above procedures could be operated in a batch mode by recirculating the sample stream enough times through a simple SPLITT cell to purify A or B to the desired level.

A number of kinds of fields or gradients are possible to establish a wall-based steady state, as has been emphasized in the literature of FFF (*5*). Among the choices is a cross-flow of the carrier fluid, established by means of an external carrier source pumped through semipermeable channel walls. A SPLITT cell with lateral cross-flow would allow all the approaches noted above (with separation based on differences in diffusion coefficients), and it would also provide additional flow control by virtue of the additional flow streams entering and exiting the cell. The semipermeable membrane at the accumulation wall could also transmit low molecular weight impurities, thus aiding dialysis-type separations (*12*).

SUMMARY AND CONCLUSIONS

By way of summary, SPLITT systems take advantage of 1) the rapid development of separation over the short lateral dimension of a thin cell, 2) the distortion-free development of that separation, as will be explained shortly, 3) the combination of axial channel flow and stream-splitting elements to realize the separation, 4) the use of crossover flow in many situations to sharpen the resolution, and 5) the use of perpendicular flow to generate continuous separation. The combination of some of these important elements yields the awkward name "split-flow lateral-transport thin" (continuous) separation cells, from which we have condensed the simple acronym, SPLITT cells.

Various other systems have been developed over the years to aid separation by combining flow with sedimentation and electrophoresis. In many cases the flow and field directions are perpendicular, yielding continuous separation, as in the SPLITT cells. Unfortunately, despite the common basic principles underlying sedimentation and electrophoretic separations with perpendicular flow (*1*), very little effort has been made to

translate gains made in one discipline to the other. Thus perpendicular flow systems for the two techniques have evolved rather independently. In this context, the SPLITT cells described here are unusual in that they have been conceptualized from the start as being applicable to both sedimentation and electrophoretic processes, as well as to other forms of separative transport.

A number of systems combining sedimentation and perpendicular flow have been described as horizontal elutriators (*13*). Some of these devices use relatively thin channels, but fractions are generally deposited along one of the channel walls running parallel to the flow stream rather than being collected in the substreams of a split-flow system. Fraction recovery from wall-deposited material is awkward, interrupting continuous operation.

The electrophoretic literature, especially, is replete with references describing combinations of electrophoresis with perpendicular flow. Most of these fall in the category variously described as continuous flow electrophoresis, deflection electrophoresis, curtain electrophoresis, etc. (*9*). These methods originally used paper sheets hanging from an edge with a curtain of buffer draining down the vertical coordinate and an electrical field applied horizontally from one side to the other. Continuous separation was realized by collecting components, deflected by electrophoresis at different angles, at different points along the bottom edge of the sheet. The nonuniformity of the pore space in paper, however, degraded resolution. The complications of the supporting medium (paper) were eliminated by using enclosed rectangular channels (*14–16*). These channels were made very thin to avoid convective effects. The uniform geometry of the channel and the lack of convection would suggest that maximum theoretical resolution is achievable in such a system. However, both parabolic flow and electroosmotic flow degrade resolution.

We can explain the degradation process in terms of the behavior of a thin line of sample (or sample line) spanning the short distance between channel walls. In general, the sample line, representing a short burst of introduced sample, extends along the third coordinate of the system, perpendicular both to flow and field axes. In an ideal system of maximum resolution, the sample line will undergo translation without bending or distortion (except by diffusion) under the influence of flow and field. (However, distortion can be tolerated if it is aligned along the component trajectory.)

In the "conventional" thin rectangular channel system discussed above, the sample line will be immediately distorted by parabolic flow. The result is the formation of crescent-shaped zones, commonly observed in such systems (*16*). The distortion is further fueled by electroosmotic

flow occurring at right angles to the main flow. While these two distortions can be adjusted to compensate for one another under special circumstances for a limited number of components, they remain a major impediment to high resolution.

In the SPLITT system, a thin line of introduced sample will translate through the cell without distortion, except for a narrow (and generally negligible) region where it bends back near the cell edges. The parabolic flow between channel walls will not distort the sample line because the line (at any point) follows a contour of the parabolic flow plane rather than cutting across the parabolic maximum. Joule heating in the system will distort the parabola but not the sample line, which will still lie along a contour of the flow plane. Since the field is applied uniformly from one wall to another (where for electrophoresis the walls will generally be semipermeable membranes), electroosmotic flow through the membranes will simply contribute uniformly to the lateral motion of the sample line. Therefore, SPLITT cells should come close to the maximum theoretical levels of resolution consistent with the short separation path.

We note that the small distortion of the sample line near the cell edges extends into the channel a distance of $\sim w$. Since sample capacity, as noted earlier, is proportional to cell breadth b (where b is also the initial length of the sample line), and w is best kept small, the effectiveness of separation will clearly be greatest for large b/w ratios.

Another approach to avoiding the zone distortions commonly found in continuous flow electrophoresis involves using a relatively thick ($\sim$5 mm) annular channel stabilized by differential wall rotation. This system, while experimentally complicated and unusually cumbersome, has proven successful in the continuous separation of proteins and cells (*17*). It is likely to be far less versatile and more costly than readily-constructed arrays of SPLITT cells such as those discussed above.

We conclude that SPLITT cells, although lacking intrinsically high levels of resolution, should come close to realizing their full resolution potential. Importantly, by avoiding major forms of resolution degradation, separation is achieved rapidly. With this and the high capacity accompanying the long sample line, high throughputs should be feasible. This, combined with the simplicity and versatility of SPLITT systems, should lead to many important applications in sedimentation and electrophoresis.

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