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# Continuous Particle Separation in Split-Flow Thin (SPLITT) Cells Using Hydrodynamic Lift Forces

J. Calvin Giddings[a]
[a] DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH, SALT LAKE CITY, UTAH

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# Continuous Particle Separation in Split-Flow Thin (SPLITT) Cells Using Hydrodynamic Lift Forces

J. CALVIN GIDDINGS

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF UTAH
SALT LAKE CITY, UTAH 84112



**Abstract**

In this paper we discuss the relationship between field-flow fractionation and split-flow thin (SPLITT) cell methodology, both of which utilize transverse driving forces to establish different transverse concentration profiles for various suspended particle populations carried by flow down a ribbonlike channel. It is shown that hydrodynamic lift forces can assume a particularly important role among the stable of forces available; when combined with certain other forces the lift forces lead to the formation of thin hyperlayers of particles distributed within the channel. The conditions necessary to split the channel flow into substreams containing different particle populations by SPLITT techniques are discussed. It is shown that the SPLITT system can be operated in either an equilibrium or a transport mode, both benefiting by the use of an inlet as well as an outlet flow splitter in the cell.

## INTRODUCTION

There are two broad classes of techniques in which particle separation is achieved by controlling the transverse positions (or distributions) of particle populations within a thin (usually submillimeter) ribbonlike flow cell. In the first and best known of these classes, field-flow fractionation (FFF), differences in mean transverse particle positions are converted by the nonuniform (parabolic) flow in the channel into a differential migration rate along the longitudinal (flow) axis (*1*). A small injected sample pulse is thus separated along the flow axis and eluted as a sequence of component peaks.

In a more recently described class of techniques utilizing split-flow thin (SPLITT) cells (*2*, *3*), continuous separation is generated by taking direct advantage of the different transverse distributions (either equilibrium or nonequilibrium) of different particles across the thin dimension of the cell. In this case separation is realized along the transverse axis rather than the flow axis as in FFF. The different particle components, each contained in its own flow stratum, are then divided by one or more flow splitters at the end of the cell and collected in different outlet substreams.

Several kinds of transverse particle distributions can be established to implement these techniques. For FFF, where equilibrium distributions are generally employed, an exponential distribution at one wall (the accumulation wall) is most commonly used (*1*). Separation is achieved by taking advantage of the different thicknesses of the exponential layers for different particles. However, since the exponential distributions strongly overlap along the transverse axis, the separation of such distributions by SPLITT methodology is limited.



Among several other possible approaches, different particle populations can be focused into individual thin bands or layers between the channel walls (*4*). These differentially elevated layers are termed hyperlayers. Although highly promising, hyperlayers have been used very little in thin cell methods because of the difficulty of finding the proper combination of forces to focus the particle populations tightly into appropriate hyperlayers within the thin space available.

For both of the thin-cell methodologies, a wide variety of forces can be mobilized to manipulate, within certain limits, the transverse particle distributions. Many of the same primary (externally applied) forces can be used in the two classes of techniques, including sedimentation, electrical, temperature gradient, and crossflow forces. In relatively recent work we have utilized forces of a substantially different nature, namely hydrodynamic (inertial) lift forces, to help control the migration velocities of particles through field-flow fractionation (FFF) channels (*5*, *6*). The object of this work is to demonstrate that these lift forces can play a major role in achieving separation in SPLITT cells as well.

## FORMATION OF HYPERLAYERS USING LIFT FORCES

Lift forces, first comprehensively described by Segre and Silberberg in 1961–1962 (*7–9*), act in such a way that they drive entrained particles away from nearby elements of stationary wall (*7–15*). These forces differ in two major ways from the primary forces (sedimentation, etc.)

commonly applied across thin cells. First, the lift forces are highly nonuniform, exerting their greatest strength when particles are near the wall and dropping off rapidly as the particles penetrate more deeply into the interior of the channel. Second, the magnitude of the lift forces varies with the flow rate. These two unusual features lead to different operating requirements and some unique opportunities in the application of these forces in thin cell methods.

The nonuniformity of the lift forces makes it possible to combine these forces with uniform (or near-uniform) primary forces in order to develop component hyperlayers distributed at different transverse locations within the channel. This is illustrated in Fig. 1, which shows the force $F_L$ due to lift effects dropping off rapidly with distance $x$ from the wall. A uniform force $F_1$ is applied in opposition to $F_L$; $F_1$ is shown as a negative quantity because it is directed along the negative $x$ axis toward the accumulation wall. The sum of the two forces vanishes at position $x_{eq}$, which becomes the focusing plane of the hyperlayer.

Hyperlayers cannot in general be formed by the superposition of uniform forces because the slope of the $F_1$ versus $x$ plot, or the sum of



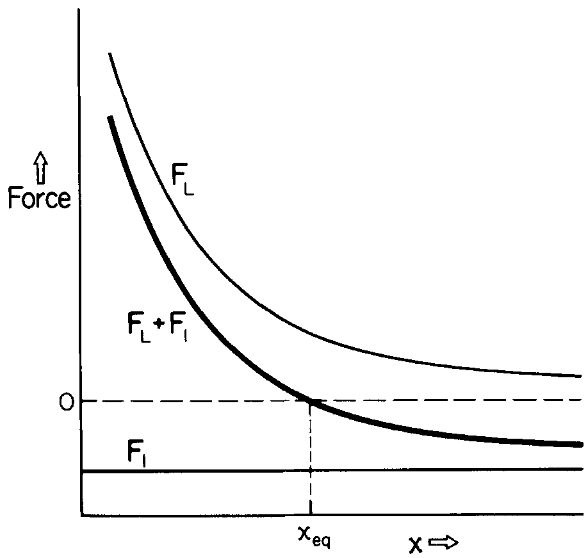


FIG. 1. A plot of the forces exerted on a particle along the positive $x$-axis versus $x$, the distance from the channel wall. Since the force $F_L$ due to lift effects drops off rapidly with distance $x$, it can be combined with an opposing uniform force $F_1$ to establish a position $x_{eq}$ of zero net force, around which a hyperlayer will accumulate.

such slopes, is essentially zero and cannot produce a zero force at a unique point. With few exceptions hyperlayers require at least one nonuniform force or a combination of a primary force (such as electrical) and a secondary force or gradient (e.g., in pH), as in isoelectric focusing. Some practical difficulties in developing differentially located hyperlayers in thin cells have been noted (*16*).

With regard to the second feature, the flow rate dependence of lift forces is, in principle, disadvantageous because one loses the versatility of adjusting independently the flow rate and the forces acting on the particles. This has been noted particularly for a class of nonuniform shear forces proposed for use in FFF (*17*). However, as a consequence of the fact that the lift forces are strongly nonuniform and lend themselves to a coupling arrangement with other forces to form hyperlayers (see Fig. 1), the lift forces exerted on any given component can be controlled by the magnitude of the nonlift force applied.

Although the fundamental separation mechanisms of FFF and of split-flow thin (SPLITT) cells are different, the resolution of both techniques operated in the hyperlayer mode is greatly improved if we impose two conditions. First, the equilibrium distance $x_{eq}$ of the hyperlayer from the wall must assume substantially different values for unlike particle species. Second, the band or hyperlayer of each particle population should be tightly focused around the position $x_{eq}$.

In order to drive different kinds of particles to different equilibrium positions (first criterion above), one or both of the applied forces (primary and lift) must differ from one particle type to another. Based on recent FFF work it now appears that both forces can be manipulated in order to increase the separation between hyperlayers (*18*).

The second condition requires that the focusing forces be relatively large so that Brownian motion or other fluctuations away from the equilibrium position are quickly subdued by strong restoring forces. High flow rates in thin channels generate strong lift forces near the channel walls. However, if no other forces are applied, particles are driven away from nearby wall elements where the lift forces weaken (Fig. 1) and eventually lose much of their effectiveness. In order to maintain the strength of the lift forces, it is necessary to apply a conventional driving force to the system which acts in a direction opposite to that of the lift forces. With such a counteracting force the particles are driven vigorously toward the well-defined transverse equilibrium position $x_{eq}$ shown in Fig. 1. With strong forces, particles of a given type will focus tightly around this equilibrium position to form a thin hyperlayer within the channel.

More specifically, the equilibrium position $x_{eq}$ of the above hyperlayer will be determined by the balance-of-forces condition

$$F_{\mathrm{I}} + F_L = 0 \qquad (1)$$

where, as indicated in Fig. 1, $F_{\mathrm{I}}$ is the externally applied primary force(s) and $F_L$ is the force due to lift effects. Since $F_{\mathrm{I}}$ is generally subject to independent control, it can be increased to a relatively high absolute value which drives the particles closer to the wall. The magnitude of $F_L$ increases correspondingly, as indicated by Eq. (1). Thus, within limits, the forces on a particle population can be controlled by external means and can be strengthened enough to ensure a tightly focused hyperlayer by the manipulation of this external control.

In the process of increasing the focusing forces, we note that the lift forces must be strong enough to maintain a force balance without the particle being driven into the wall or so close to the wall that wall interactions cause undue perturbations. Since the lift forces increase with flow velocity, substantial flow rates are necessary to maintain a functioning hyperlayer in the presence of large $F_{\mathrm{I}}$ forces. Consequently, both high flow and high force conditions are necessary to optimize the resolution. However, in requiring high flow conditions we automatically establish favorable circumstances for high speed separation. For the SPLITT system, the high flow rates yield increased throughput.

The FFF methodology designed to use elevated equilibrium layers is termed hyperlayer FFF (*4*, *18*). Like all FFF techniques this approach uses the increase in flow velocity with distance from the channel wall, which is effective out to the channel center. Species with the largest values of $x_{eq}$ are carried by flow at the highest velocities and thus emerge rapidly, separated from slower species having lower $x_{eq}$ values. Thus a small sample pulse injected into an FFF channel divides into zones traveling at different velocities down the channel axis and emerging at different times. Such a separation is illustrated in Fig. 2(A).

It should also be possible to obtain separation in thin rectangular SPLITT cells by allowing particles to approach different transverse equilibrium positions, that is, different values of $x_{eq}$. Two such populations can, in theory, be separated around the outlet flow splitter as shown in Fig. 2(B). The particles are then collected from separate outlets.

Below we examine in more detail the anticipated requirements for the implementation of hyperlayer SPLITT operation, particularly for hyperlayer systems based on lift forces.

## OPERATIONAL CONSIDERATIONS

We begin by making it clear that hyperlayer SPLITT operation is not limited to the special conditions suggested by Fig. 2(B). The figure shows

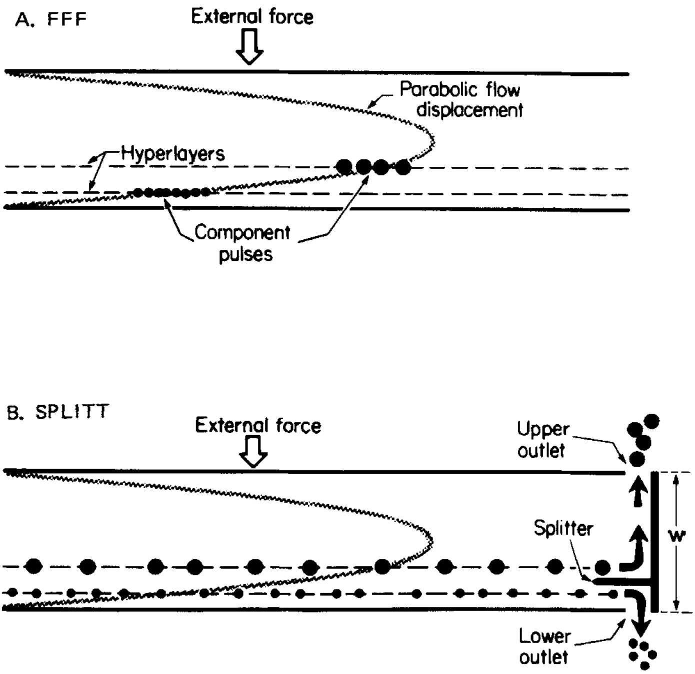


FIG. 2. Contrast between hyperlayer separations in FFF systems and in SPLITT cells. In hyperlayer FFF a sample pulse first divides into hyperlayers and then separates along the flow axis by virtue of the parabolic flow profile. In hyperlayer SPLITT operation, a continuous sample stream divides into component hyperlayers which are then separated along the transverse axis by a stream splitter.



the separation of two particle populations, but the methodology is not intrinsically limited to only two components. In parallel with other (nonhyperlayer) forms of SPLITT cell operation, one can increase the number of fractions separated either by using multiple outlet splitters to divide the flow into a number of outlet substreams, each with its own component or fraction, or by linking cells together in such a way that the outlet substreams from the first cell enter subsequent cells for additional fractionation steps (*2*). Both approaches should be applicable to hyperlayer operation whether lift forces are utilized or not.

We also note that if the hyperlayers are crowded into a limited fraction of the channel cross section, as illustrated in Fig. 1(B), the splitter need not be located in that limited region in order to divide the hyperlayers. Instead, as we have made clear in earlier publications (*2*, *3*), the splitter can be placed at some more convenient location, often at a position halfway across the channel. To split the flow at some off-center position, we need only adjust the flow rates (specifically in this case the two outlet flow rates) so that each outlet stream carries a specified fraction of the total flow. This is explained as follows.

For any given ratio of outlet flows, an outlet splitting plane can be identified running back from the edge of the physical splitter to the cell inlet region (see Fig. 3). We define a splitting plane as a plane dividing two adjacent flow laminae in the cell; it is thus a plane across which no fluid is transported by flow. Providing the flow conditions remain laminar, all the fluid above the outlet splitting plane will exit outlet *b* and all that below, outlet *a*. While the downstream edge of the outlet splitting plane will, by definition, always be anchored to the outlet splitter as shown in Fig. 3, the steady-state position of the splitting plane through most of the channel will be determined by the ratio of the volumetric flow rates above and below the splitting plane, a ratio controlled by the two outlet flow rates. Thus the splitting plane can be moved up and down with changes in relative flow rates. Over a very short distance (corresponding to about one channel thickness, typically less than 1% of the cell length) near the outlet splitter, it will swerve up or down from its steady-state position (at $x = x_s'$) to intercept the splitter edge (see Fig. 3). The position of component particles relative to the splitting plane, which establishes the exit stream they will occupy, will be determined by steady-state conditions in the body of the cell and not by the actual position of the physical splitter. (In extreme conditions there may be some particle transport across the splitting plane by inertial forces where the plane curves up or down from its steady-state position to the splitter position.) Thus the position of the splitter(s) can be fixed independently of the position of the hyperlayers requiring separation.

For a splitting plane located at steady-state position $x = x_s$, the fraction



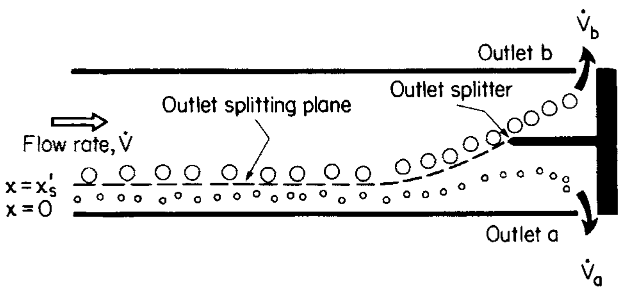


FIG. 3. Relationship of outlet splitting plane and outlet splitter.

of fluid flowing beneath that streamplane (and thus exiting outlet *a*) is given by

$$F(x_s) = \frac{\dot{V}_a}{\dot{V}_a + \dot{V}_b} = \frac{\int_0^{x_s} v(x)dx}{\int_0^{w} v(x)dx} \tag{2}$$

where $\dot{V}_a$ and $\dot{V}_b$ are the flow rates through outlets *a* and *b*, respectively, *w* is the cell thickness, and *x* is the distance across the cell measured from the lower wall. If we use the expression for parabolic flow

$$v(x) = 6\langle v\rangle\left(\frac{x}{w} - \frac{x^2}{w^2}\right) \tag{3}$$

then the integrals of Eq. (2) can be evaluated to yield

$$F(x_s) = 3\frac{x_s^2}{w^2} - 2\frac{x_s^3}{w^3} \tag{4}$$

where $\langle v\rangle$ is equal to the mean cross-sectional flow velocity. We observe that $\langle v\rangle$ has dropped out of the final expression because only relative flow rates and velocities are relevant in fixing the position of the splitting plane. Equation (4) provides the means for calculating the steady-state position of that plane, $x_s$, as a function of the fractional flow rate emerging from outlet *a*. Thus $x_s$ can be adjusted to whatever position is necessary to fractionate the sample appropriately simply by controlling the relative flow rates according to Eq. (4).

The above considerations are particularly relevant for hyperlayers created with the aid of lift forces. There is evidence based on FFF retention experiments that for vanishing external forces, particles tend to accumulate at an equilibrium position for which $x/w \simeq 0.2$ and, by symmetry, 0.8. Ignoring for the moment the possible accumulation of particles at $x/w \simeq 0.8$, we observe that any application of an external force as illustrated in Fig. 2(B) will drive particles to new equilibrium positions somewhere below $x/w = 0.2$. With optimally adjusted external forces and flow rates, the particle hyperlayers will be distributed as widely as possible over the limited region between the accumulation wall and $x/w \simeq 0.2$. The splitting plane will also require location at some selected position in this region in order to divide the particle populations appropriately. However, a physical splitter would be difficult to position precisely this close to a wall. Fortunately, for any splitter position, the outlet flow rates can be adjusted in accordance with Eq. (4) to yield any desired position $x_s$ of the splitting plane. Thus if it is desired to position the splitting plane one-tenth of the distance across the channel ($x_s$ = 0.1$w$), Eq. (4) indicates that this can be achieved, no matter what the splitter position, by allowing only 2.8% of the total flow to exit from $a$.

If there are equilibrium positions both at approximately 0.2$w$ and 0.8$w$, as indicated above, one would normally attempt to establish conditions such that only one equilibrium position would be occupied. Otherwise, dissimilar fractions from the vicinities of these two positions might be collected at the same outlet, nullifying the resolving power. It is likely that particles would be forced out of the upper equilibrium position if substantial external forces were applied. Alternately, an inlet splitter could be used to assure that particles were introduced only on one side of the centerline. An inlet splitter would also speed up the process of relaxation to the equilibrium hyperlayer position, as explained later.



If the fluid stream enters the SPLITT cell from a single inlet, the particles suspended in the stream will normally assume an initial distribution spread widely over the flow cross section. Under the influence of the steady flow and forces acting within the cell, the particles will be driven toward their respective equilibrium positions. This focusing process requires a finite time to become essentially complete. Normally the SPLITT cell must be adequately long to allow this particle relaxation (focusing) to approach completion, at least for some of the particles. The time and distance necessary for relaxation is not known exactly but evidence from FFF suggests that relaxation for larger particles occurs rapidly and may require only a few centimeters of cell length. Furthermore, it is likely that the relaxation distance for a given particle type will be relatively independent of flow rate.

Two limiting modes of operation can now be distinguished for lift-modulated SPLITT cells. In the equilibrium mode, particles approach their equilibrium hyperlayer positions under the combined influence of primary and lift forces and are separated on the basis of the differences in their hyperlayer positions. In the transport mode, particle separation is accomplished by virtue of differential transport velocities as particles undergo relaxation toward the equilibrium positions. Mixed operation, where some particles approach equilibrium and others do not, is also anticipated. The state of operation relative to the equilibrium/transport limiting cases will depend largely on cell length, cell thickness, particle size range, and the magnitude and type of primary force applied.

For the transport mode of operation, the stream of suspended particles should enter the SPLITT channel in such a way that it quickly forms a very thin flow stratum. This gives all particles a common starting position from which they can separate by differential transport. The transport would be driven by the lift forces combined with any other applied forces. Since lift forces are strongest near the wall, the best approach would likely be that of introducing the particle stream as a thin film close to the accumulation wall, following which, under the influence of strong lift forces, components would move at different rates away from the wall toward their ultimate hyperlayer positions.

The dominance of lift forces near the wall suggests that these forces would generally control initial transport rates. There is no obvious benefit to be gained by applying opposing forces, as found desirable in the equilibrium mode. Therefore SPLITT separation based on differential transport should be achievable in exceedingly simple systems without meeting the special requirements for applying adjustable primary forces.

The introduction of particles into the separation cell as a thin lamina close to the wall would not only serve the purposes of transport-based SPLITT separation, but would speed up the equilibrium-based SPLITT separation by reducing the relaxation time, as suggested earlier. Relaxation enhancement would occur because the maximum transport distance of entering particles necessary to reach the particle equilibrium positions would be substantially reduced. Also, transport would be hastened by the strong forces acting near the wall where the particles are introduced.

The introduction of particles as a thin ribbonlike stream occupying only a fraction of the channel thickness can be achieved by using an inlet splitter. By introducing the particle stream on the side of the splitter adjacent to the accumulation wall, the entering layer of particles can be substantially localized. The layer can be further compressed by adjusting the ratio of inlet flow rates. This is illustrated in Fig. 4. The key element of this figure is the identification of another splitting plane in the cell, the inlet splitting plane. This plane, originating at the edge of the inlet rather than the outlet splitter, is again defined as a plane extending into the cell across which no flow transport occurs.

If we utilize a much higher flow rate for inlet substream $b'$ than for the particle-containing substream $a'$, the high flow from $b'$ forces the splitting plane to swerve downward and compress (but not concentrate) the contents of stream $a'$ into a thin lamina. The thickness of the lamina can be adjusted by varying the ratio of flow rates in the same way that the position of the outlet splitting plane is adjusted by outlet flow rate changes. More specifically, following Eq. (4), the fraction $F(x_s')$ of the total channel flow contributed by the particle-laden stream (inlet $a'$) is related to inlet splitting plane position $x_s'$ by

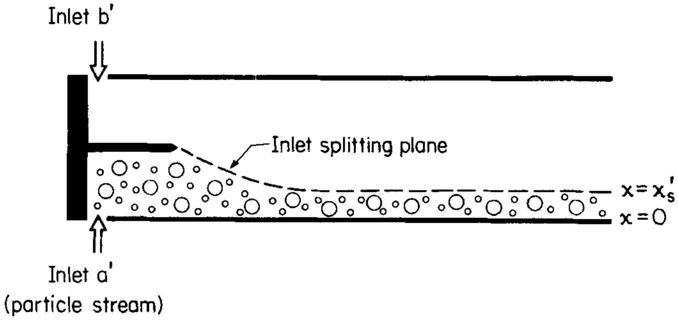


FIG. 4. Use of inlet splitter and controlled inlet flow rates to focus entering particle stream $a'$ into a thin stratum.



$$F(x_s') = 3\frac{x_s'^2}{w^2} - 2\frac{x_s'^3}{w^3} \tag{5}$$

Generally, $F(x_s') \ll 1$ in order to maintain a tightly focused particle layer characterized by a small $x_s'/w$. Earlier we showed that for the outlet, the constraint $F(x_s) \ll 1$ would normally apply to capture the particle components differentially. For optimal performance in the transport mode, the fractional flow rates at the inlet and outlet will be related by

$$F(x_s') \leqslant F(x_s) \tag{6}$$

and thus

$$x_s' \leqslant x_s \tag{7}$$

These conditions will generate a thin transport zone, analogous to that used with other transport-based SPLITT operations, that particles must cross in order to exit from outlet $a$ (*2*, *3*). (Even when the flows are equal, an effective but very thin transport zone will exist because of finite particle size; the particle centers must all begin from a position below the plane at $x = x_s'$ but must cross $x = x_s$ for collection in $b$. This effect will be accentuated by lift forces acting to push particles away from the splitter element upon entrance, as noted below.)

For optimal operation in the equilibrium mode, the flow rate ratios at the inlet and outlet will be related to one another in just the opposite way as expressed by

$$F(x_s') \geqslant F(x_s) \tag{8}$$

in which case

$$x_s' \geqslant x_s \tag{9}$$

While the latter two conditions will not necessarily improve resolution, they will improve the throughput by allowing the maximum possible flow rate of the particle stream through inlet $b'$.

We note that the above conditions may be modified somewhat by lift forces operating in the region beneath the inlet splitter. These forces, repelling particles from both the splitter surface and the accumulation wall, will serve to further focus the particle population within the lamina between $x = 0$ and $x = x_s'$. This may remove the constraints of Eqs. (6) and (7).



Lift forces within the splitter regions have another crucial role. For large particles, which tend to adhere to adjacent surfaces, the lift forces will act to keep particles entrained in the fluid stream. Thus these forces may have an important role within the splitting regions (as well as in the channel itself) in maintaining desired particle motion, position, and throughput.

While we have focused here on inertially based lift forces of the type described by Segre and Silberberg (*7–9*), we note for completeness that other forces of hydrodynamic origin can be similarly used. For example, entropic-driven forces appear to act on random-coil macromolecules in a manner similar to that of inertial forces acting on rigid particles (*19*). In the former case the equilibrium position in the absence of primary forces lies at the center of the channel where the shear rate is zero. This position is much more convenient than that at $x \simeq 0.2w$ because the extreme splitting condition implied by $F(x_s) \ll 1$ could be avoided.

While the above approaches are likely to be most easily implemented with particles suspended in liquids where the lift forces are substantial, the possibility exists that particles suspended in air could also be separated by these techniques at sufficiently high flow rates.

For separation in the equilibrium mode, virtually any kind of external (primary) force can be used as long as the magnitude of the force is great enough to focus the equilibrium hyperlayers tightly, as noted earlier. Candidate forces include those associated with sedimentation, electrical fields, crossflow, temperature gradients, magnetic fields, and others. Any of the above forces might also be used for the transport mode, but in this case, as noted above, one might do away altogether with the primary force, relying solely on the lift forces to generate the differential transport needed for separation.

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