Split Flow Thin Cell (SPLITT) Separation

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Introduction

SPLITT fractionation (SF) is a relatively new family of separation techniques primarily – but not exclusively – applicable to macromolecules and particles. The SF techniques utilize a thin ribbon-shaped flow cell and achieve fractionation by differential transport across the thin (transverse) axis of the cell. Since the cell is only a few hundred micrometres thick, the separation path – which may be less than the nominal channel thickness – is extremely short and the separative transport is correspondingly rapid. Separation is typically accomplished in only a few minutes. This is a particularly valuable feature for example for fragile species that must be fractionated rapidly to avoid degradation (e.g. biological samples). The fluid that carries dissolved or suspended components through the SPLITT cell is divided at both ends by thin flow splitter elements (see **Figure 1**). The inlet splitter element allows for the smooth merging of two incoming laminae, one carrying the suspended feed material and the other generally containing only the pure carrier liquid. Differential transport of feed components between the two laminae (after they are brought into contact) then occurs as a result of a transverse driving force or gradient. At the outlet end, the flowing liquid volume is divided at a predetermined position by a second splitter element, thus producing two sub-streams that are enriched or depleted in the desired components as a result of the differential transport.

Both preparative and analytical fractionation process can occur in the SPLITT cells, depending on the injection procedure. A continuous (CSF) process of feeding the cell is advantageous for preparative fractionation (gram, kilogram), offering rapid throughput, minimum holdup volumes, and a sharp separative cut off; examples of continuous fractionation can be found in the separation of mineralogical, industrial and food samples.

The analytical version of SPLITT fractionation (ASF) is often more practical to operate. The injection of discrete pulses can be made, if desired, to follow one another in close sequence. In this use, separation is performed for the measurement of quantitative properties of sample components and the fractionation is termed 'analytical SPLITT fractionation';

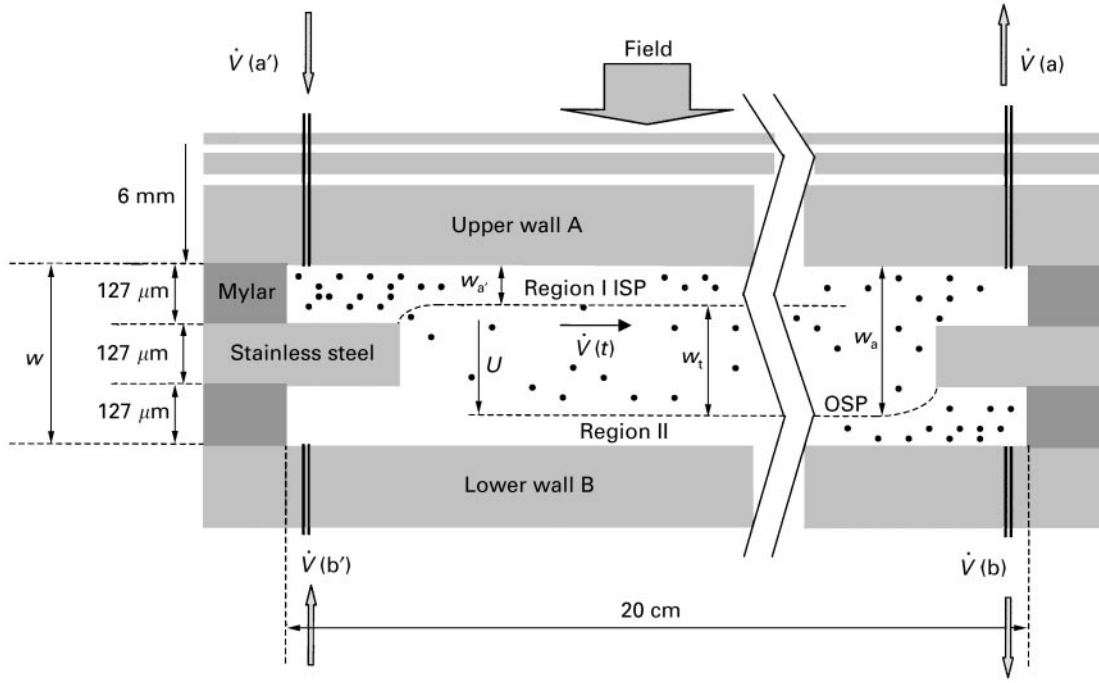

Figure 1 Side view of a generic SPLITT cell.

examples of quantitative determinations include diffusion coefficients of proteins, settling velocity and the relative content of oversized particles above a cutoff diameter in a particulate material. Moreover because of its ease of theoretical interpretation, the SPLITT cell can be used for the rapid measurement of transport-related properties such as particle size and particle size distribution. The throughput of SF is proportional to many variables such as the sample concentration in the feed stream, the volumetric flow rate of the sample stream, the applied field strength and the SPLITT cell area. For preparative applications there is obviously a trade-off between the resolution and throughput in the operation of SF: maximizing the throughput and maintaining an acceptable resolution is the common choice.

The effectiveness of the SPLITT process can be modulated by simply varying the flow rates of the inlet and outlet substreams, which determine the position of the inlet splitting plane (ISP) and the outlet splitting plane (OSP) and controlling the thickness of the transport region; sometimes, unwanted displacements of a few tens of micrometres may be difficult to discern but they are sufficient to interfere with effective separation. In some cases, the efficiency of the SPLITT process can be controlled by altering the strength of the field or gradient driving the separative transport.

The efficacy of SF separation depends instead, on the hydrodynamic integrity of the SPLITT cell. Effective separation is based in fact, on two central requirements:

- there must be no hydrodynamic mixing across stream planes; and
- the splitters must be absolutely perfectly aligned so that they are capable of splitting the film of flowing liquid evenly along the stream plane.

Success in fulfilling these requirements is not easy to judge because of the thinness of the cell and the shortness of the transport path.

The selectivity of SPLITT fractionation comes from the applied force. The principal transverse driving forces used include gravity, centrifugation, diffusion, electrical potential gradients, magnetic gradients and hydrodynamic lift forces. The geometry of all the different cells is similar to that depicted in Figure 1, except for the curvature characteristic of the centrifugal SPLITT cell.

The simplicity of the SPLITT cell leads to rather rigorous theoretical guidelines on the conditions necessary to achieve a given level of separation.

Theory

The theory of separation by SPLITT cell was formulated by J. C. Giddings in terms of experimentally controllable flow rates in the inlet and outlet substreams and it has been developed and implemented through the years; SPLITT fractionation theory can now be found in numerous publications. The separation is performed inside a thin channel, where the behaviour of a sample particle depends on the balance between the external force field and frictional forces (as for field flow fractionation techniques (FFF)), combined with the action of the fluxes operative within the cell.

In Figure 1 the sample, suspended in a suitable carrier fluid, is introduced through the top inlet a′ at a predetermined volumetric flow rate $\dot{V}(a')$. At the same time, pure carrier fluid enters through the bottom inlet b′ at a flow rate $\dot{V}(b')$; where the two inlet streams join to form a single stream we have the ISP. When the fluid stream reaches the end of the channel, it is mechanically divided into two fractions by the outlet splitter.

The differential displacement of the particles occurs towards wall B, based on the driving force exerted on each type of particle by the applied field and the frictional resistance offered by the carrier fluid to particle motion. Thus different types of particle occupy different laminae while the flow through the channel displaces them axially towards the outlet end.

The total volumetric flow rate $\dot{V}$ in the channel can be written both in terms of inlet flow rates or outlet flow rates

$$\dot{V} = \dot{V}(a') + \dot{V}(b') = \dot{V}(a) + \dot{V}(b) \qquad [1]$$

and since the walls A and B are parallel and their dimensions much larger than w (thickness) and b (width), the velocity profile is essentially two-dimensional (see **Figure 2**). The mean fluid velocity $\bar{v}$ can be computed as $\dot{V}/bw$, and the velocity parabolic profile across the cell thickness is described by the equation:

$$v\left(\frac{x}{w}\right) = 6\bar{v}\frac{x}{w}\left(1 - \frac{x}{w}\right) = 4v_{\max}\frac{x}{w}\left(1 - \frac{x}{w}\right) \qquad [2]$$

where $v_{\max}$ is the maximum fluid velocity found at the midplane ($x = w/2$) of the cell.

By looking at the flow stream components (see Figure 1) it is possible to find a relation which takes into account the fluid flow proceeding in the transport layer $\dot{V}(t)$:

$$\dot{V} = \dot{V}(a') + \dot{V}(t) + \dot{V}(b) \qquad [3]$$

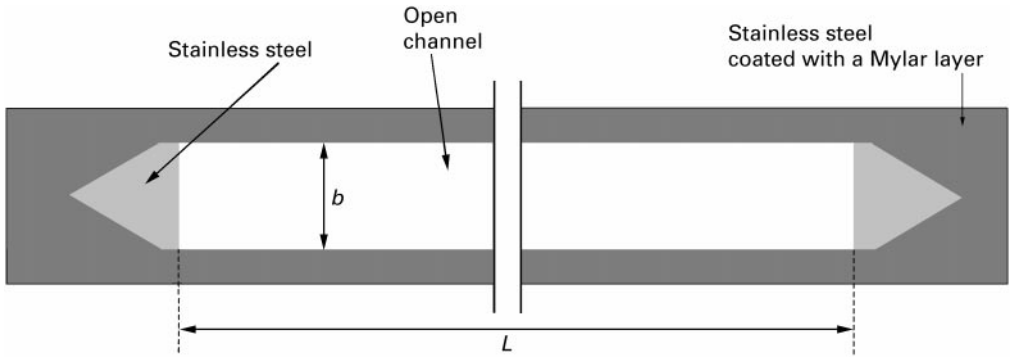

Figure 2 Upper view of the 'Splitter' used in the gravitational SPLITT cell.

$\dot{V}(t)$ can be obtained by combining eqns [1] and [3] as:

$$\dot{V}(t) = \dot{V}(\mathrm{a}) - \dot{V}(\mathrm{a}') = \dot{V}(\mathrm{b}') - \dot{V}(\mathrm{b}) \qquad [4]$$

The separation of sample components is achieved, as previously described, by their different rates of transport toward the opposite wall B under the influence of the transverse field. It is important to be able to calculate the distance that a component has to travel. The ratio of sample flow rate $\dot{V}(\mathrm{a}')$ to the total flow rate $\dot{V}$ defines the position of the ISP as distance w_{a}, from wall A:

$$\frac{\dot{V}(\mathrm{a}')}{\dot{V}} = 3\frac{w_{\mathrm{a}'}^2}{w^2} - 2\frac{w_{\mathrm{a}'}^3}{w^3} \qquad [5]$$

The feed stream is then confined to the lamina thickness $w_{\mathrm{a}'}$, between wall A and the ISP.

By assuming that, during their residence in the SPLITT cell, the particles are driven from wall A to wall B at constant velocity U, the volumetric flow rate $\Delta\dot{V}$ of the lamina traversed by a particle is simply given by

$$\Delta\dot{V} = bLU \qquad [6]$$

which contains the physical dimensions of the channel b, and L (length); U is the velocity of the induced transverse transport. In fact, a number of different driving forces have been utilized to implement CSF: gravitational, centrifugal, magnetic, electrical. U assumes different expressions depending on the field inducing the transverse transport, i.e.

$$U_{\mathrm{gravitational}} = \frac{\mathrm{g}d^2|\Delta\rho|}{18\eta_{\mathrm{o}}} \qquad [7]$$

$$U_{\mathrm{centrifugal}} = \frac{\omega^2 r d^2 \Delta\rho}{18\eta_{\mathrm{o}}} \qquad [8]$$

$$U_{\mathrm{magnetic}} = \frac{\Delta\chi\Delta H^2 d}{48\eta_{\mathrm{o}}} \qquad [9]$$

$$U_{\mathrm{electric}} = \mu E \qquad [10]$$

where d is the particle diameter (assumed spherical), $\Delta\rho$ is the difference between particle density ρ_{s} (compact particles) and carrier density ρ_{l}, η_{o} is the viscosity of the carrier, g is the acceleration of gravity (earth field eqn [7]); ω is the angular velocity (rad s^{-1}) and r is the radius of the rotation (centrifugal acceleration eqn [8]); $\Delta\chi$ is the difference between the magnetic susceptibilities of the particles, χ_{p}, and the carrier, χ_{l}, and ΔH is the drop in magnetic field strength (eqn [9]); μ is the electrophoretic mobility and E is the electric field strength (eqn [10]). In some cases, experimental studies have been based on diffusive transport or by using hydrodynamic lift forces (which can be used in unique ways because of their nonuniformity).

In order to settle whether particles exit the channel through outlet a or b, the relative values of $\Delta\dot{V}$ and $\dot{V}(t)$ are of critical importance. For a sample introduced close to the ISP, particles exit from outlet a if:

$$\Delta\dot{V} \leqslant \dot{V}(t) \qquad [11]$$

and from outlet b if:

$$\Delta\dot{V} > \dot{V}(t) \qquad [12]$$

In the case of a SPLITT cell operating under a gravitational field, the relation which allows one to set the diameter cutoff of a spherical solid particle can be obtained by combining eqns [4], [6] and [7]. In fact, if $\dot{V}(t) = \Delta\dot{V}$ and:

$$\Delta\dot{V} = \frac{bL\mathrm{g}d^2|\Delta\rho|}{18\eta_{\mathrm{o}}} \qquad [13]$$

then the diameter at which 50% of the particles exit outlet b is called the cutoff diameter d_c, expressed as:

$$d_c = \sqrt{\frac{18\eta_o(\dot{V}(a) - 0.5\dot{V}(a'))}{bL\Delta\rho g}} \qquad [14]$$

in which only half of $\dot{V}(a')$ is considered (cf. eqn [4]). Once d_c has been chosen for a given channel, the difference between $\dot{V}(a)$ and $0.5\dot{V}(a')$ is set according to eqn [14]. However, the two constituents flow rates $\dot{V}(a)$ and $\dot{V}(a')$ are not uniquely defined by this equation, but some criteria useful for setting the flow rates are available in the optimization of SPLITT operations literature. In general, in order to obtain a separation with a good resolution, the practical rule which states the following necessary but not sufficient condition:

$$\dot{V}(b') \gg \dot{V}(a') \quad \text{or} \quad \dot{V}(a) \gg \dot{V}(b) \qquad [15]$$

can be followed. This condition ensures that regions I and II will be narrow, which automatically increases the transport region, i.e. the cell space in which the separation process occurs. For maximum resolution, typical experimental conditions, are chosen to obtain a $\dot{V}(a')/\dot{V}(t)$ ratio within 0.1–0.3. From a theoretical point of view, the high resolution in the operative *transport mode* is, as already stated, contingent on compression of the feed sub-stream, a′, into a thin lamina near wall A, and the sharpness of the separation, as in chromatography, can be judged by the number N of the theoretical plates generated during transport. Generally, for field (gravitational) driven migrations, the effective N is given by the ratio of two energies:

$$N = \frac{Fw_t}{2kT} = \frac{\pi d^3 \Delta\rho g w_t}{12kT} \qquad [16]$$

where F is the force on the particle inducing its transport, w_t the length of the transport path, and kT the thermal energy. Generally values of $N \geqslant 10^2$ are required to assure achievable resolution, and each type of macromolecule or particle should be checked against this criterion.

Resolution in SF can be related to channel flow rates, defining an index which measures the relative breadth of the unresolved region. It has been demonstrated that for sedimentation particles the equation of the resolving power has the following form:

$$\frac{d_1}{\Delta d} = \frac{d_1}{d_1 - d_0} \cong 2\,\frac{\dot{V}(a)}{\dot{V}(a')} \qquad [17]$$

where d_0 and d_1 are respectively the diameters of the particles exiting from a and b. The particles falling between these two sizes (d_1 and d_0), which are not fully resolved, exit from both outlets in different proportions, so the difference should be small. According to eqn [17] the ratio of $\dot{V}(a)$ to $\dot{V}(a')$ allows control of the range of unresolved particles which exit both outlets a and b.

Specific Applications

Determination of the Diffusion Coefficient *D*

CSF and ASF can be successfully applied to the separation of macromolecules such as proteins and liposomes. The transport region in this case is seen as a diffusion barrier, which acts as a dialysis membrane. In order to be able to determine the diffusion coefficient D, an important parameter useful for characterization of the sample components, the theory has to be looked at in a deeper way, with equations which explicitly contain D.

If a component enters inlet a′ as a steady stream and its transport through the SPLITT system is governed by the simultaneous displacements of diffusion and parabolic flow, mass conservation requires that:

$$\frac{\partial c}{\partial t} = -v(x)\frac{\partial c}{\partial z} + D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial z^2}\right) \qquad [18]$$

$\partial c/\partial t$ is the rate of change of concentration at an arbitrary point within the channel, x is the transverse distance into the cell measured from wall A, z is the distance into the channel along the length measured from the inlet splitter and v is the local stream velocity. In order to simplify eqn [18], it can be observed that all transverse transport in the channel occurs by diffusion and that all the components are transported along the cell (z-direction) by convention, so that the diffusion term ($D\partial^2 c/\partial z^2$) can be neglected.

Moreover, if the system operates in CSF mode, the component concentrations throughout the cell will attain a steady state, i.e. $\partial c/\partial t = 0$, so that eqn [18] reduces to:

$$\frac{\partial c}{\partial z} = \frac{D}{v(x)}\left(\frac{\partial^2 c}{\partial x^2}\right) \qquad [19]$$

where $c(x, z)$ is subject to the boundary conditions:

$$\begin{aligned} &c(x, 0) = c_o && \text{for } 0 \leqslant x \leqslant w_{a'} \\ &c(x, 0) = 0 && \text{for } w_{a'} \leqslant x \leqslant w \\ &\partial c/\partial x = 0 && \text{for } x = 0 \text{ and } x = w \text{ at all } z \end{aligned}$$

In order to calculate D, a dimensionless diffusion time $\tau_D = Dt^o/w^2$ has been developed. The parameters t^o and w^2 are known because w is fixed by the geometry of the channel and t^o is the elution time of a species dispersed in the total volume of the channel and is related to the total flow rate $\dot{V}$ by:

$$t^o = \frac{V^o}{\dot{V}} = \frac{bLw}{\dot{V}} \qquad [20]$$

where V^o is the cell void volume expressed as a product of channel dimensions b, L and w. The dimensionless time parameter t^o is thus related to D, $\dot{V}$, and the channel dimensions by:

$$\tau_D = \frac{Dt^o}{w^2} = \frac{DbL}{w\dot{V}} \qquad [21]$$

Consequently D is given by:

$$D = \frac{w\dot{V}\tau_D}{bL} \qquad [22]$$

This equation is used to obtain experimental D values, once τ_D is found. The total procedure requires the following steps: (i) to compute theoretically the concentration profile $c(x, L)$ over the lateral coordinate x at outlet ($z = L$) by using the Crank–Nicolson numerical method. In this way, the retrieval of a component at each outlet substream F_a (in i) and F_b (in ii) can be calculated from $c(x, L)$; (ii) to construct a graph of F_a versus τ_D for different $\dot{V}(a)/\dot{V}$ ratios; (iii) to determine experimentally the retrieval factor at outlet sub-stream a (F_a) from the relative strength of the detector signal (this value depends on the flow ratio $\dot{V}(a)/\dot{V}$ used in the experiment); (iv) to compute the correspondent τ_D value from the graph F_a versus τ_D by finding the correspondence between the experimental and theoretical F_a values; (v) to use eqn [22] to calculate D using the τ_D value, and the geometrical dimensions b, L, w and $\dot{V}$.

Effect of Particle Shape and Density in the Gravitational SPLITT

The SPLITT cell has been largely applied for the separation of environmental samples. Since the natural matter particles have different properties (porosity, density, shape), the basic SPLITT equations have to be revised to fit the relevant particle properties.

The basic relationship for the SPLITT cell, previously derived (see eqn [6]) can be written as:

$$\Delta\dot{V} = \dot{V}(t) = bLU_{\text{gravitational}} \qquad [23]$$

By recalling the basic properties of the sedimentation process different expressions can be obtained, which contain the density parameter. During the SPLITT fractionation, under a gravitational field, the sample components are subject to two forces: the gravitational force $F_g = m_{\text{eff}}\,g$ and the frictional force $F_f = fU$ (m_{eff} is the effective mass, and f the friction coefficient). Usually, the stationary state is established very rapidly and the two forces balance each other out and thus:

$$U = m_{\text{eff}}\frac{g}{f} \qquad [24]$$

Spherical particles In the case of compact spherical particles, by assuming that the particles do not undergo any shrinking or swelling $m_{\text{eff}} = m - m_b$ or $m_{\text{eff}} = V_s\rho_s - V_s\rho_l$, where m is the real mass and m_b the buoyant mass while V_s and ρ_s are, respectively, the volume and density of the particle and ρ_l the density of the liquid. More explicitly

$$m_{\text{eff}} = \tfrac{1}{6}\pi d^3|\Delta\rho| \qquad [25]$$

The $||$ accounts for positive or negative mass values in eqn [25] corresponding, respectively, to a falling or a floating particle. The friction coefficient f can be expressed by Stokes law $f = 3\pi\eta d$, where η is the viscosity of the suspension fluid, which can be approximated by the carrier viscosity, η_0. By combining eqns [23], [24] and [25] one obtains the classical expression (see eqn [13]):

$$\dot{V}(t) = \frac{bLgd^2|\Delta\rho|}{18\eta_o} \qquad [26]$$

In the case of porous particles, porosity is defined as:

$$\varepsilon = \frac{V_p}{V_s + V_p} = \frac{V_p}{V_{\text{tot}}^p} \qquad [27]$$

where V_p is the volume of the pore, V_s the volume of the solid and V_{tot}^p the total volume of the particle. Then eqn [25] changes into:

$$m_{\text{eff}} = \tfrac{1}{6}\pi d^3(1 - \varepsilon)|\Delta\rho| \qquad [28]$$

and the correspondent eqn [26] into:

$$\dot{V}(t) = \frac{bLgd^2(1 - \varepsilon)|\Delta\rho|}{18\eta_o} \qquad [30]$$

The porosity can be expressed also in terms of 'apparent density':

$$\rho_{app} = (1 - \varepsilon)\rho_s + \varepsilon\rho_l \quad [31]$$

from which the differential apparent density is defined as:

$$\Delta\rho_{app} = \rho_{app} - \rho_l \quad [32]$$

By combining $\Delta\rho = \rho_s - \rho_l$ with eqns [31] and [32] one has:

$$\Delta\rho_{app} = (1 - \varepsilon)\Delta\rho \quad [33]$$

which can be substituted in eqn [30].

When the 'mass porosity' is available:

$$p = \frac{V_p}{m} \quad [34]$$

where m is the real mass of the particle, i.e. $m = V_s\rho_s$ and by using eqn [27] one can show that:

$$\left(\frac{1}{p\rho_s + 1}\right) = (1 - \varepsilon) \quad [35]$$

which gives:

$$\dot{V}(t) = \frac{bLgd^2|\Delta\rho|}{18\eta_o}\left(\frac{1}{p\rho_s + 1}\right) \quad [36]$$

Alternatively, one can employ the 'bulk density', which is the ratio between the amount of the porous material, m_{tot}, and the total volume occupied by the packed particles V_{tot}, including both the inter-particle volume, V_{ex}, the particle volumes, V_p^{tot} (total volume of the pores) and V_s^{tot} (total solid volume):

$$\rho_{bulk} = \frac{m_{tot}}{V_p^{tot} + V_s^{tot} + V_{ex}} = \frac{m_{tot}}{V_{tot}} \quad [37]$$

In this instance, both the ρ_{bulk} and ε_{ex} depend on the degree of packing. The relative apparent density, $\Delta\rho_{app}$ can be obtained by combining eqns [33] and [37]:

$$\Delta\rho_{app} = \frac{\rho_{bulk}}{\rho_s(1 - \varepsilon_{ex})}\Delta\rho \quad [38]$$

From eqn [33] it is apparent that, in this case, one must know the value of ε_{ex} under the same experimental conditions which ρ_{bulk} was determined. Lacking this information, it is only possible to make a rough estimate of $\Delta\rho_{app}$.

Nonspherical particles There are two main effects to be accounted for with nonspherical particles: the first is related to the particle volume expression and the second to Stokes Law. The parameter d contained in eqn [7] has to be changed depending on the kind of data available.

The first effect is accounted for by using the volume equivalent diameter, i.e. the diameter of a sphere having the same particle volume V_{Sp}, i.e. $d_v = \sqrt[3]{(6V_{Sp}/\pi)}$ instead of the sphere diameter, d. This quantity sometimes can be related to true geometrical dimension of the particle if its geometry is known.

An irregular shape affects the behaviour of the particle while it is moving within the fluid. Stokes Law takes account of this by substituting the diameter d with the 'drag diameter', i.e. the diameter of a sphere having the same resistance to the motion within the fluid

$$f = 3\pi\eta_o d_d \quad [39]$$

In order to conclude this section, an appropriate combination of all the above cases is necessary for irregular porous particles.

List of Symbols

a = aspect ratio
b = width of the SPLITT channel
d = diameter of the sphere
d_c = cutoff diameter
d_d = drag diameter
d_v = diameter of an equivalent sphere
D = diffusion coefficient
E = electrical field
f = frictional coefficient
F_a = retrieval of a component from outlet a
F_b = retrieval of a component from outlet b
F_g = gravitational force
F_f = frictional force
g = gravity acceleration
h = thickness of the transport layer
h_c = high of a cylindrical particle
L = length of the SPLITT channel
m = real mass of a particle
m_b = mass corrected for buoyancy
m_{eff} = effective mass
m_{tot} = total mass of all particles present in the container
p = mass porosity
r = radius of rotation
t_r = crossing time, i.e. the time a particle takes to pass through the cross sectional area bh in the h-direction.

U = particle migration velocity
$\bar{v}$ = mean fluid velocity
v_{max} = maximum fluid velocity
$\dot{V}$ = total volumetric flow rate through cell
$\dot{V}(a')$ = volumetric flow rate at outlet a′
$\dot{V}(b')$ = volumetric flow rate at outlet b′
$\dot{V}(a)$ = volumetric flow rate at inlet a
$\dot{V}(b)$ = volumetric flow rate at inlet b
$\dot{V}(t)$ = volumetric flow rate of the transport region
V_{ex} = external volume between particles
V_p = pore volume of a particle
V_p^{tot} = total pore volume for all the particles
V_s = volume of the solid part of a particle
V_s^{tot} = total solid volume for all the particles
V_{Sp} = volume of a sphere
V_{tot} = total volume occupied by all particles present in the container
V_{tot}^p = total volume of a particle
w = thickness of the SPLITT channel
$w_{a'}$ = thickness of the fluid lamina between wall A and ISP
w_t = thickness of the transport region
χ_l = magnetic susceptibility of the carrier
χ_p = magnetic susceptibility of a particle
ε = internal porosity
η = suspension viscosity
η_o = carrier viscosity
μ = electrophoretic mobility
ρ_{app} = apparent density
ρ_{bulk} = bulk density
ρ_l = density of the liquid
ρ_s = density of the spherical particle
ω = angular velocity
τ_D = dimensionless diffusion time

See also: **II/Particle Size Separation:** Field Flow Fractionation: Electric Fields; Theory and Instrumentation of Field Flow Fractionation. **III/Polymers:** Field Flow Fractionation.

Further Reading

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Theory and Instrumentation of Field Flow Fractionation

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Principle

Field-flow fractionation (FFF) is one of the important analytical methodologies, suitable for the separation and characterization of particles in the submicron and micron ranges. The effective field generates the flux of the separated particles and forms a concentration gradient of each particular species across the ribbon-shaped separation channel. The concentration gradients are counter-balanced by a diffusion flux. At equilibrium, a stable concentration distribution of each particular species is established in the direction across the channel. Simultaneously, a flow velocity profile is formed across the channel due to the viscous