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What is field-flow fractionation?

Field-flow fractionation (FFF) is a family of analytical techniques that was developed specifically for the separation and characterization of materials ranging in size from macromolecules to particulates. Unlike chromatography, FFF separations are carried out in a single phase. The flow profile in an FFF channel can be described by a parabola with the highest flow velocity at the center of the channel and decreasing flow velocity with increasing proximity to the channel walls.

Separation in FFF is accomplished by establishing equilibrium layers for each sample component (l_X, l_Y in Figure 1) at unique positions across the parabolic flow profile. Sample components that on average spend more time further away

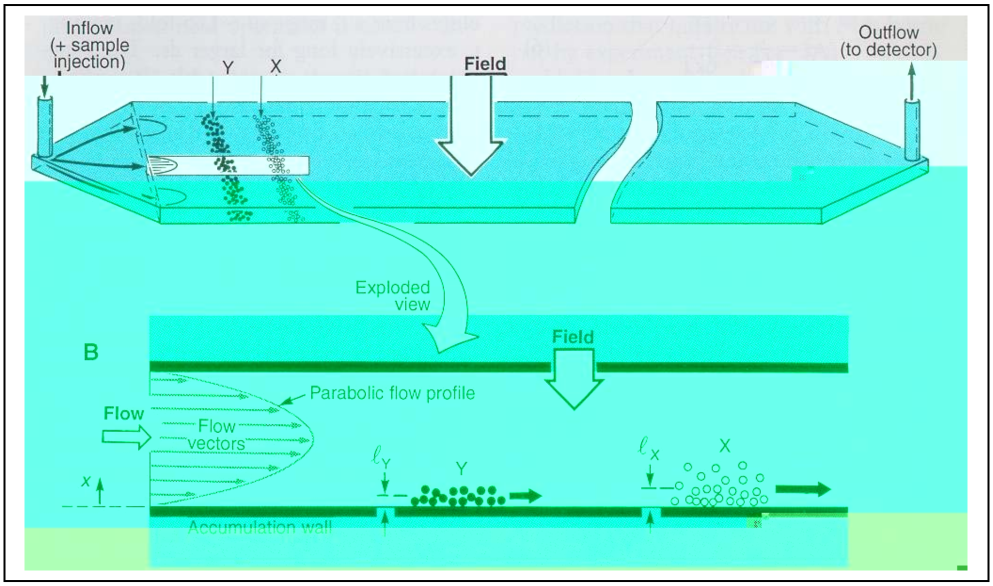
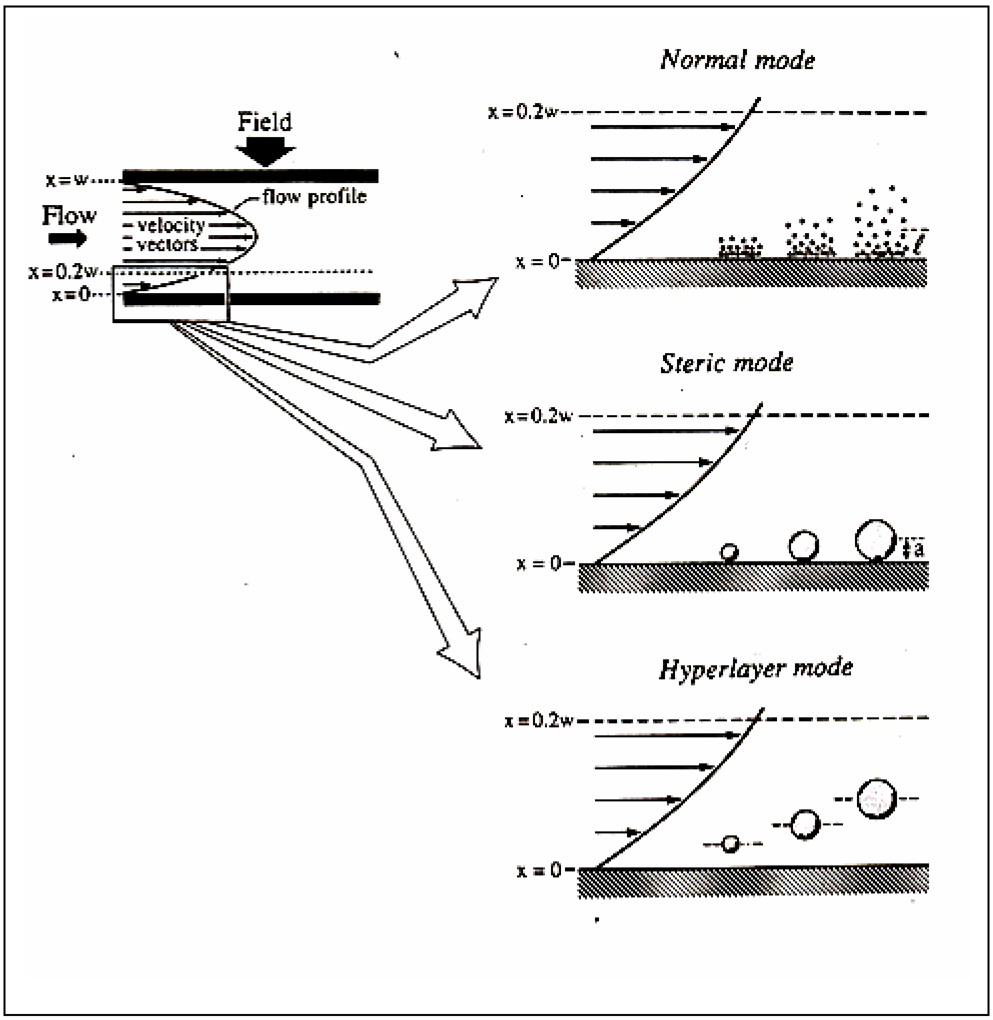


Figure 1. FFF separation mechanism – normal mode

from the channel wall will be transported out of the FFF channel faster than their counterparts near the wall. The equilibrium layer thickness (which governs the average displacement velocity) for each sample component is formed as the result of two opposing transport processes 1.) migration to the accumulation wall as a result of interaction with an applied field and 2.) diffusion from regions of high concentration at the accumulation wall to regions of lower concentration away from the wall. (This field-induced transport and diffusion occur continuously throughout the separation.) In this so-called normal mode of operation, retention times increase with decreasing equilibrium layer thickness. Strong interactions with the applied field and/or slow diffusion will cause a sample species to form a compact equilibrium layer and high retention times will result. In the normal mode, small particles tend to elute first.

The main criterion for a useable field is that it must generate sufficient force to drive different particle populations into distinctly different positions across the parabolic flow profile. Applied 'fields' that have been used in FFF include centrifugal (giving rise to sedimentation FFF or SdFFF), electrical (electrical FFF or ElFFF), temperature gradient (thermal FFF or ThFFF), and crossflow (flow FFF or FlFFF). Although many other types of fields have been used (e.g., magnetic, dielectric), equipment is commercially available only for these four. For the normal mode separation described above, equations have been derived for each specific field to relate the retention time to physicochemical properties of the sample and the experimental conditions.

As the particle size increases, the diffusion coefficient decreases until it becomes a relatively insignificant transport process. For micron size particles, the extent of protrusion into the channel becomes the decisive factor in determining the order of elution. As shown in Figure 2, the field drives the particles to the accumulation wall. The distance of closest approach of the particle center to the wall is one particle radius. The larger the particle, the further is its center of mass from the wall, the faster it is propelled down the



length of the channel, and the shorter is its retention time. In this steric mode of operation, large particles elute first.

When high flow velocities are used, hydrodynamic forces are generated which lift particles away from the wall. The applied field and lift forces act in opposing directions and focus particles into narrow bands giving rise to the hyperlayer mode. The magnitude of the lift forces is related to flow velocity and diameter. Consequently, high flow rates and field strengths can be used to yield rapidly eluting narrow sample bands. Separations of 49, 30, and 20 μm polystyrene latex beads have been accomplished in 6 seconds using flow FFF. The use of two opposing forces to focus sample gives rise to the hyperlayer mode of separation.

The normal mode of separation is active for sample species with diameters < ~1 μm and the steric and hyperlayer modes are applicable to particles >~ 1μm.

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