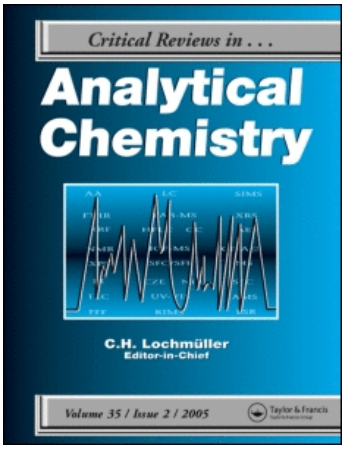

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Field-Flow Fractionation: Theory, Techniques, Applications and the Challenges

T. Kowalkowski[a]; B. Buszewski[a]; C. Cantado[b]; F. Dondi[b]
[a] Department of Environmental Chemistry and Ecoanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland [b] Department of Chemistry, University of Ferrara, Ferrara, Italy

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Field-Flow Fractionation: Theory, Techniques, Applications and the Challenges

T. Kowalkowski and B. Buszewski
Department of Environmental Chemistry and Ecoanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland

C. Cantado and F. Dondi
Department of Chemistry, University of Ferrara, Ferrara, Italy

In this review, field-flow fractionation (FFF) is presented as a versatile and powerful analytical technique for separation and characterization of different sample types. The underlying principles of FFF separation theory including sources of the obstacles and the difficulties as well as the new approaches is also introduced. This paper describes FFF sub-techniques, presents its exemplary application and the future trends in development of FFF techniques.

Keywords field flow fractionation, SPLITT, theory of FFF, FFF sub-techniques

INTRODUCTION

Field-flow fractionation (FFF) encompasses a large variety of separation methods and characterization of supramolecular compounds (macromolecules, colloids, particles, cells). Although a few studies using a methodological approach resembling that of FFF were previously (1, 2) or independently (3–5) performed, the general concept of FFF was invented in the mid-1960s by J. Calvin Giddings. He envisioned the wide potential of this new separation method in terms of applications and system variants (6, 7). Prof Giddings is, by far, the person who has most contributed to the development of FFF, authoring or coauthoring almost 25% of the more than 1100 papers already published on FFF theory, instrumentation or applications (source of data: field-flow fractionation references web site—⟨http://www.rohmhaas.com/fff/⟩. Figure 1 shows the number of papers in the field of FFF. It indicates the increase of implementation of field-flow fractionation techniques in the last two decades, mainly due to development of new, flexible sub-techniques.

According to the classification of separation methods, FFF belongs, as chromatography, to the F(+) class, as both are flow-assisted methods in which the chemical potential gradient responsible for retention occurs essentially in directions perpendicular to the main direction of the flow. Consequently, FFF can be considered as a hybrid of chromatography and field-driven methods such as electrophoresis and ultracentrifugation. Like chromatography, FFF is an elution technique with inherent differential flow displacement phenomena. On the other hand, like ultracentrifugation, FFF separation is based on an applied gradient or field of force. The comparison of separation ranges of different analytical techniques in respect to the mass of the analyte is presented in Figure 2.

Separation effect in field-flow fractionation (FFF) is reached by a combined action of the non-uniform flow velocity profile of a carrier liquid and a transverse physical field applied perpendicularly to this carrier. Carrier liquid flowing along the channel forms a nearly parabolic Newtonian flow velocity profile (as in capillary tube) across the channel. The substance to be investigated is dissolved or suspended in a carrier fluid and is pumped through a 50–500 μm thin, not filled, channel (Figure 3). FFF is applicable to supramolecular species (macromolecules, colloids, gels, emulsions, particles, cells).

THEORY

The sample components can be considered as non-interacting point masses, with their center of gravity near to the accumulation wall. Due to the established concentration gradient, a diffusion flux in reverse direction is induced according to Fick's law. As a consequence of the two rival parameters—the exerted field of force and the opponent diffusion flux—a steady-state profile is generated, and the equilibrium distributions of the sample components across the channel can be expressed by a mean layer thickness. Depending on the strength and uniformity of the external force different theoretical approaches can describe the separation process in FFF. In general, the classical FFF theory,

Address correspondence to T. Kowalkowski, Department of Environmental Chemistry and Ecoanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarina 7, 87-100 Toruń, Poland. E-mail: pinez@chem.uni.torun.pl

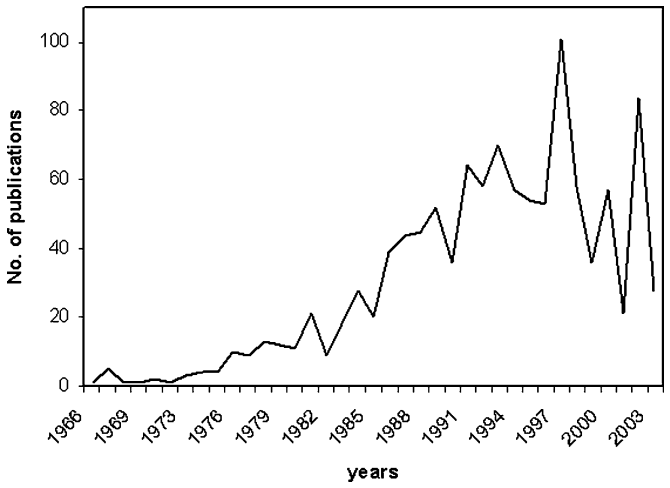

FIG. 1. Number of FFF papers published (source of data: field-flow fractionation references web site—⟨http://www.rohmhaas.com/fff/⟩).

which combines the retention with the force exerted by the external field on the analyte molecules or particles, is based on few assumptions related to the magnitude and the course of this field, to the properties of the analytes and, and in some cases, to the flow rate of the carrier liquid. The theory assumes three major elution modes in FFF separation processes (8).

The first one is the *Brownian* elution mode, where the field-induced velocity of analyte in the separation channel is constant and comparable with its diffusive motion:

$$U_x = const., U_x * t \approx \sqrt{2 * Dt} \quad [1]$$

where U_x is velocity induced by external field at distance x from the accumulation wall and t is the time. The resulting concentration profile of the analyte is given by well-known exponential relationship:

$$\frac{c}{c_o} = \exp\left(-\frac{x}{l}\right) \quad [2]$$

where c_o is the maximum concentration at the accumulation channel wall, c is the concentration at distance x from the accumulation wall and l is the mean thickness of the analyte layer and can be expressed as the following ratio:

$$l = \frac{D}{[U_x]} \quad [3]$$

where D is diffusion coefficient of the analyte.

Two main factors are influencing behavior of analytes in Brownian elution mode: the properties of the analytes (characterized by diffusion coefficient) and the strength of the field applied. The general expression for retention in which the retention ratio R can be presented as an approximate form:

$$R = \frac{6 * k * T}{F * w} \quad [4]$$

where k is Boltzmann constant, T is the absolute temperature, F is the external force strength acting on the analyte inside the channel, and w is the thickness of the channel.

In the second elution mode, the velocity induced by the force field in the separation channel is constant and much higher than the velocity caused by diffusive motion of the analyte.

$$U_x = const., U_x {}^{*}t \gg \sqrt{2^{*}Dt} \quad [5]$$

In this case, the analyte forms a layer on the accumulation channel wall and its concentration in any other position inside the channel equals zero. The particle radius r_p describes the distance of the particle center from the accumulation wall:

$$c(r_p) = c_0 \quad \text{and } c(x \neq r_p) = 0 \quad [6]$$

This elution mode is called *steric*. The retention ratio can be expressed in the form:

$$R = \frac{6^{*}r_p}{w} \quad [7]$$

It shows that the retention ratio is independent both of the field applied and of the flow rate, and it is dependent only on the particle radius and the channel thickness.

In the last mode introduced by Janĉa (9), the velocity of analyte transport induced by a force field in the separation channel is dependent on the position across the channel (U_x is not constant). The non-constant transport velocity can be described as:

$$U_x = -a(x - s) \quad [8]$$

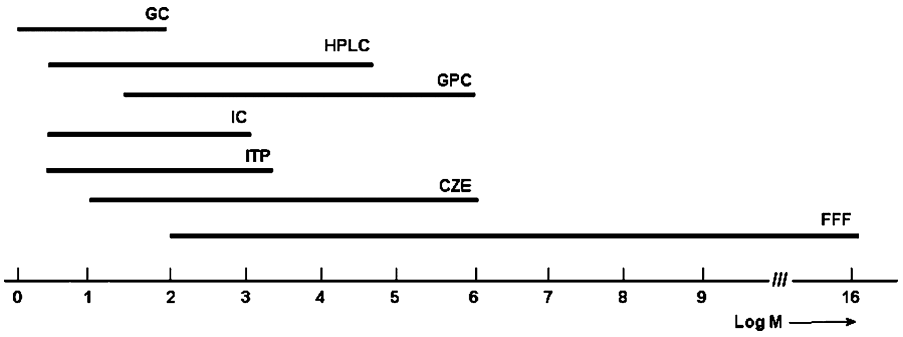

FIG. 2. Working mass ranges for different separation techniques.

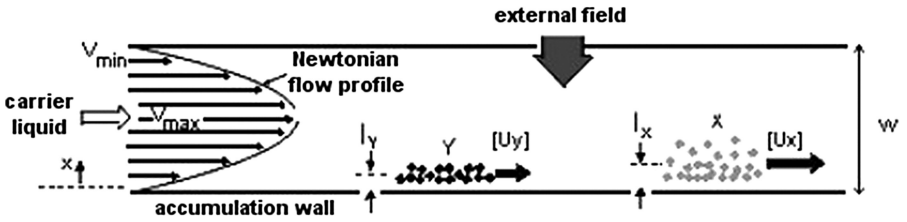

FIG. 3. The idea of separation process in FFF.

where s is the distance of the center of the focused zone from the channel wall. It is affecting the concentration profile of the analyte:

$$\frac{c}{c_o} = \exp\left(-\frac{a}{2^*D} * (x-s)^2\right) \qquad [9]$$

The concentration profile of the analyte across the channel thickness, in this simplest case, is Gaussian. The elution mode is called *focusing* (or *hyperlayer*).

These equations able to predict retention and optimize the separation as well as to characterize an unknown analyte from its retention time. Sometimes, however, the experimental operating conditions do not fit to the classical retention equations and the other assumptions should be strictly fulfilled (10). According to Giddings (11), factors of errors, beside those arising from the experimental measurements of retention time and caused by FFF system are, due to the neglect of:

- steric exclusion;
- lift forces;
- particle slip;
- van der Waals forces;
- electrostatic forces;
- non-parabolic flow (edge effects and viscosity gradients);
- non-uniformity of the field across channel thickness.

There is still a need for fundamental fluid mechanics studies for understanding and predicting the behavior of colloidal materials flowing near a wall. Some of the new assumptions related to different FFF sub-techniques are listed or described next.

FIELD-FLOW FRACTIONATION SUB-TECHNIQUES FAMILY AND ITS EXEMPLARY APPLICATIONS

At the present time, FFF comprises a family of separation devices with a great number of sub-techniques used mainly for the separation and characterization of macromolecular, colloidal and particulate species in the size range from 10^{-3} to 10^2 μm. The comparison between separation ranges and exemplary applications of the classical sub-techniques is resented in the Figure 4.

The simplest sub-technique of FFF is a gravitational field-flow fractionation (GrFFF). It employs the Earth's gravitational field to fractionate a variety of particulate analytes in the micrometer size range. This technique has been shown to be successful for the separation and further characterization of cells, microorganisms and the large particles (12). Very similar sub-technique is the sedimentation FFF (SdFFF), which uses either centrifugal force (multiseparation gravitational FFF) to separate and/or for purification of micron-sized species of any origin. The first two experimental studies using FFF techniques were the fractionation of polystyrenes (18) and the fractionation of *E. coli* bacteriophages and particles exactly by sedimentation/gravitational-FFF (3–5).

Separation in GFFF and SdFFF takes place inside a ribbon-like capillary channel and is induced by a field applied perpendicular to flow (sedimentation field). It was established that the analytes are eluted according to the *lift-hyperlayer* mechanism (13). This mode assumes no attractive or repulsive particle–wall interactions occurred. The species are submitted to the balance of two opposite forces: one produced by the external field and the second, of hydrodynamic origin, described as *hydrodynamic lift forces* (14). Such interactions focus the particles into an equilibrium position in the channel thickness, according either to particle characteristics like size and density (15), either to the operating conditions like external field intensity and flow velocity (16). Retention ratio is related to the particle average equilibrium position and the channel thickness. However, it was

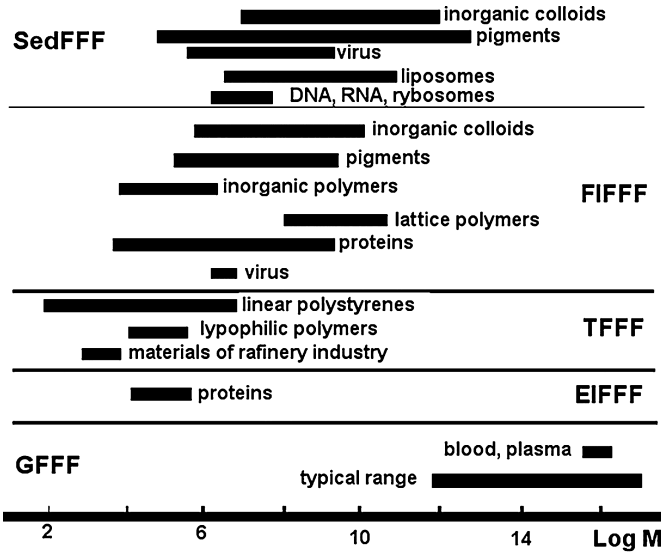

FIG. 4. Comparison between different FFF sub-techniques.

assumed also that the *steric* elution mode is a limit condition of the *lift-hyperlayer* model, when effects of the hydraulic lift forces are reduced by the external field, to a negligible extent. This led to particle retention being size dependent and independent of flow velocity. In several cases other channel dimensions, length and breadth, should be also taken into consideration (17).

The oldest of all FFF method is thermal FFF. TFFF was at first applied for the separation of polystyrenes (18), and its universal applicability for the fractionation of various species was broadly demonstrated in the literature (19–28 and other). In thermal FFF, the temperature difference across the channel, is the field force. One side of the channel is heated and the other cooled. A relatively small temperature difference applied between top and bottom walls of the channel produces high thermal gradient. One of the disadvantages of the thermal sub-technique is the possible flocculation of the dispersed system. Other problem is the calculation of the void time t_o. The precise assign of t_o is complex because of the temperature dependence of the carrier liquid density and directly proportional to the average carrier density in the channel. The estimation of t_o should take into account these influences (29). The classical macro-scale TFFF instrument requires the long analysis time, a big volume of sample and high power consumption are its disadvantages. The miniaturization of a TFFF system has been introduced in 2002 and presented in the literature (30–33). Such μ-TFFF improves the quality of separation, cut the time and dead volume of analysis. Thanks to miniaturization, the temperature gradient may be increased in order of magnitudes in comparison with the standard TFFF system.

The separation process in μ-TFFF can be described by the steric elution mechanism at the low flow rates only. However, this mode is not accurate at higher flow rates where the focusing phenomenon appears (34). In 1972, electrical-FFF (El-FFF) was introduced as a technique for the separation of proteins (35, 36). The major disadvantage of electrode polarization limits the aqueous carrier solutions that can be applied and, as a consequence, the sample materials that can be characterized (37). Additionally, conductivity differences between the analytes and the carrier liquid influence the retention, which means that sample concentration affects the data obtained (38–41).

The most versatile of all the FFF techniques is flow field-flow fractionation (Fl-FFF or F4) because of the nonspecific, hydrodynamic field across the channel, which is generated by application of a secondary mobile phase flow that drives sample components toward the accumulation channel surface. This accumulation wall is the permeable membrane. Selection of an appropriate membrane depends on the macromolecules or particles being separated, and the pore size should be small enough to retain the analytes but large enough to allow the carrier solution to pass through it. At the present time, there are many different types of membranes available with varying molecular mass cutoff points. However, it is essential that the membrane is flat and smooth because any flaws will affect the separation process (42). The proper selection of membranes is still the major disadvantage of Fl-FFF due to possible interactions with the solute and the danger of a membrane-induced non-uniformity in the channel thickness.

The position of individual species in the laminar carrier profile corresponds to their diffusion coefficient. In Fl-FFF separation relies on differences in the diffusion coefficients only. Thus, it nicely complements SFFF or Th-FFF with respect to size distribution analysis. Flow FFF is an absolute method and needs no calibration (43). In principle, the analytes do not experience intense contact with a separation medium. However, in practice, several investigators show that also in the case of FlFFF the interaction of colloids with equipment components or their permeation through the ultrafiltration membrane can influence the measured size distribution (44).

In general, there are two major Fl-FFF techniques. In the symmetrical Fl-FFF sub-technique, the cross-flow is achieved by pumping the carrier liquid directly across the channel through porous frits. In this mode both channel walls are permeable. The accumulation wall is covered by an ultrafiltration membrane, impermeable to the sample components. Such a classical system was introduced in the mid 1970s by Giddings et al. (45) and after developing of stop flow technique for the focusing of sample components (46) has been used broadly. In the second setup, in asymmetrical flow, field-flow fractionation (FI-AFlFFF or AF4) only one wall is permeable. The trapezoid shape of the channel has been introduced in A4F to avoid constant loss of axial flow occurring with the transport of carrier liquid along the channel (47). Asymmetrical flow field-flow fractionation is one of the favored systems today (48, 49). The third, helic FlFFF, is still under development, and only few papers describing this three-dimensional technique (50, 51) are published. The separation occurs in the annular space between two rotating concentric circular cylinders (Taylor–Couette device).

The focusing FFF theory is used also for continuous preparative fractionation used by Giddings (52) and named as "split-flow lateral-transport thin" (SPLITT) separation system. Split-flow thin-cell (SPLITT) fractionation is a technique similar to FFF, except that it has the ability to separate relatively large quantities of sample (mg or g) in a reasonable amount of time. The SPLITT system has two inlets and two outlets with controlled flow-rates separated by physical stream splitters (Figure 5). Analyzed sample is introduced continuously into the SPLITT channel through inlet a' with a flow rate of V(a'). Sample-free carrier solution is introduced into the channel through inlet b' with a flow rate of V(b'). The flow rates are usually adjusted such that V(b') is much greater than V(a') so that the sample particles are driven to the thin region above the inlet splitting plane (ISP) as the two inlet flow streams merge at the edge of the inlet splitter.

The difference in velocity for particles of a certain size is the driving force for separation. If this velocity is high enough, particles will cross the outlet splitting plane (OSP) and will exit outlet b. Smaller particles that cannot pass the OSP will be collected from outlet a (53). ISP and OSP positions are determined by the relative flow rates of the two inlet and two outlet substreams.

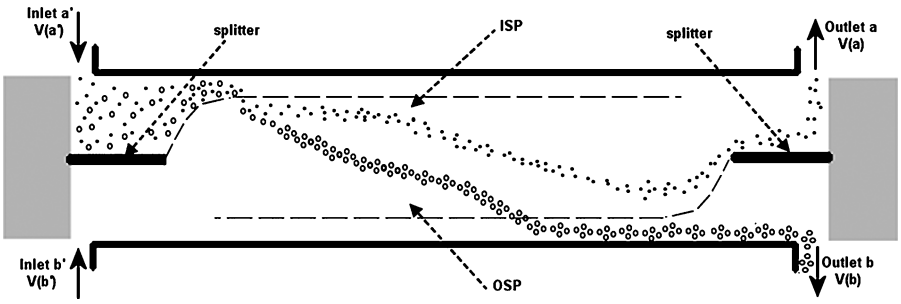

FIG. 5. SPLITT channel setup and illustration of separation process.

General theory of separation in SPLITT is described by Williams et al. (54). However, this theory is not sufficient in the case of a small ratio between length and thickness of the channel when velocity field cannot be described by parabolic profile. Theory for such systems has been modified by Zhang et al. (55).

Other FFF sub-techniques are more specific and can be used for limited amount of sample's types. The broad description of other FFF techniques such as: Magnetic-FFF, Dielectrophoresis-FFF, Pressure-FFF, Acoustic-FFF, Photophoretic-FFF, as well as some of the theoretically proposed only FFF techniques, one can find in (56). Presently, the flexibility of FFF techniques allow their application to a number of scientific domains, mainly to blood and other analysis (17), genomics (57) bacteria (58) and cell separation (59), materials characterization (60–62), assessments of colloids in freshwater and seawater (42), characterization of dissolved organic material fractionation (63), including fulvic and humic acids (64), and colloidally associated trace elements in natural and effluent waters. Very broad applications of FFF have been broadly presented for environmental samples by Gimbert et al. (65), and for life science by Fraunhofer and Winter (66).

FUTURE TRENDS IN FFF

A number of developments are required for the FFF techniques to be more widely applied in area of the particle size analysis. The FFF needs also to establish a broader user base with appropriate practical support. Coupling of FFF with other detectors (e.g., matrix assisted laser desorption/ionization (MALDI), time of flight MS (TOF) (67, 68), multi-angle laser light scattering MALLS (69), flow cytometer (70)) and hyphenating with other techniques (e.g., with HPLC (64), AAS (71), ICP-MS (72)) will generate novel multidimensional information. Miniaturization of FFF channel (e.g., described above μ-TFFF) or chip technology (73) enlarge the separation range and enhance the selectivity of FFF and are useful tools for screening analysis. There is also need for investigating the new features and obstacles in the FFF separation theory and for the fundamental fluid mechanics studies for understanding and predicting the behavior of colloidal materials flowing near a wall. Other trends to enhance the selectivity of the separation in FFF are programming of the external field strength (74) (other than the SdFFF) and the application of flow rate gradient.

CONCLUSIONS

Following Provder (75), one can list the major benefits of FFF techniques are:

- the large representative sample can be used (10^9 particles);
- samples can be measured in dispersion without particle shape change during the analysis;
- particle density can be $>$ or $<$ mobile phase density;
- apparent particle size distribution is obtained directly;
- retention is predictable theoretically;
- wide dynamic particle size range is achievable (0.01–1 μm for SdFFF, F1FFF, ThFFF, = 1–100 μm for StFFF);
- particle size distribution calculations are simple.

Despite that fact, that at present times, FFF techniques family are commonly used for number of different samples, successors of Prof. Giddings and the developers of FFF still have to deal with the difficulties of constructing the FFF channel with precise control over geometry, with the detection problems (e.g., correction for UV detector response-Mie-Scattering Correction), with upper limits to the particle size range = 1 μ, unless the Steric FFF Mode is involved and with spherical shape of particles for accurate data analysis.

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