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Corresponding Author:

* Sana Ahmed

Department Of Pharmaceutical Analysis & QA, Deccan School Of Pharmacy, Darus Salam, Aghapura, Nampally,
Hyderabad, Andhra Pradesh, India

QR Code AJPTI

*Email Id- sana_aziz2006@yahoo.com

Field Flow Fractionation

Iffath Rizwana[1], Sana Ahmed*[2], M.Anitha Vani[3], Syed Abdul Azeez Basha[4]

ABSTRACT

Field flow fractionation (FFF) is a separation technique conceived by J. Calvin Giddings. FFF is a separation technique where a field is applied to a solution which is pumped through a long narrow channel which is perpendicular to the direction of flow in order to cause separation of particles present in the fluid, dependent on their differing mobility's under the force exerted by field. Analytes can be separated by different mechanisms. The mode of operation determines the elution order of analytes, along with other separation characteristics such as selectivity and resolution. Three widely used modes that can be implemented in any FFF technique are normal, steric and hyperlayer modes It uses most of the ancillary equipment employed in chromatography such as injector valves, pumps for the carrier liquid delivery, detectors, and some data acquisition devices such as chart recorders or more conveniently computers. It is particularly suitable for macro-molecules, colloidal and particulate materials extending from a few hundred to 10 Da.

Key-words: FFF, macromolecules, colloids, Steric and hyperlayer.

1.

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1. *Research Scholar, School Of Pharmaceutical Sciences, JNTU-K, Kakinada, A.P. India. email id: iffathriz@gmail.com*
2. *Department Of Pharmaceutical Analysis & QA, Deccan School Of Pharmacy, Darus Salam, Aghapura, Nampally, Hyderabad, Andhra Pradesh. email id: sana_aziz2006@yahoo.com*
3. *Department Of Pharmaceutical Analysis & QA, Deccan School Of Pharmacy, Darus Salam, Aghapura, Nampally, Hyderabad, Andhra Pradesh. email id: anitha.vani2@gmail.com*
4. *Deccan School of Pharmacy, Hyderabad, Andhra Pradesh, India*

Introduction :

Field flow fractionation (FFF) is a separation method conceived by J. Calvin Giddings in 1966.[1] It is particularly suitable for macro-molecules, colloidal and particulate materials extending from a few hundred [2 3] to 10 Da.[4] It is an elution technique and is often referred to as a chromatography –like technique. In chromatography, the solute's rate of migration is determined by its portioning between a mobile phase and a stationary phase in a column. In FFF, an externally applied field induces selective distribution of solutes in a fluid laminae flowing at a different velocities in a single phase inside a channel. The difference in the type of forces used in chromatography and in FFF defines the range of applicability of the techniques. While forces in chromatography are localized at interfaces and very selective, those used in FFF and electrophoresis are more diffuse and weaker. Consequently, the mass transfer phenomena occurring in a chromatographic technique, such as high performance liquid chromatography (HPLC), tend to be slower as solute molecular weight increases. When the molecule's energy of interface with the interface is significantly greater than the thermal energy kT , adsorption becomes irreversible.[5] However, even before irreversible adsorption appears, structural disruption and denaturation of the macro-molecular component may occur because of the strong shear forces present in the irregular flow in the tightly packed chromatography column.[6] By contrast, the FFF separation is carried out in the absence of a stationary phase within an open channel. This channel is obtained by removing a geometrical portion from a Teflon , Mylar, or polyimide spacer and then clamping the spacer between two flat parallel plates. As shown in Figure 1 .

Principle :

Field flow fractionation (FFF) is a separation technique where a field is applied to a solution (sample) which is pumped through a long narrow channel which is perpendicular to the direction of flow in order to cause separation of particles present in the fluid, dependent on their differing mobility's under the force exerted by field.

FFF separation & mechanism & modes of separation :

FFF separation & mechanism: The most commonly used channel dimensions are 27-87 cm in length L, 1-2 cm in breadth b and 0.0075 -0.05 cm in thickness w. The typical structure of channel is shown in figure 6. Because of the very high aspect ratio of the FFF channel and the frictional drag at the walls, the velocity of the liquid carrier moving in the longitudinal direction has a parabolic profile with a maximum in the centre and minima, virtually zero, at the walls. In the normal mode of operation the field applied perpendicular to the flow direction drives sample components towards one wall, referred to as " accumulation wall",[7] with a velocity determined by the particle- field interaction . this filed-induced displacement, optimized in the absence of longitudinal flow, is always counteracted by the diffusive flux that originates from the concentration gradient across the channel. The combination of these effects results in the combination of non-uniform distribution of components across the channel, those with a higher rate of back –diffusion being driven further away from the accumulation wall than those with the lower diffusion rate. Because of the parabolic flow velocity profile in the channel, the faster diffusing component C shown in figure Figure1 will be displaced along the channel more rapidly than the component B, which has a lower diffusitivity. The output signal, collected by a detector sensitive to some solute property, will thus register the elution profile of distinct peaks. [8]

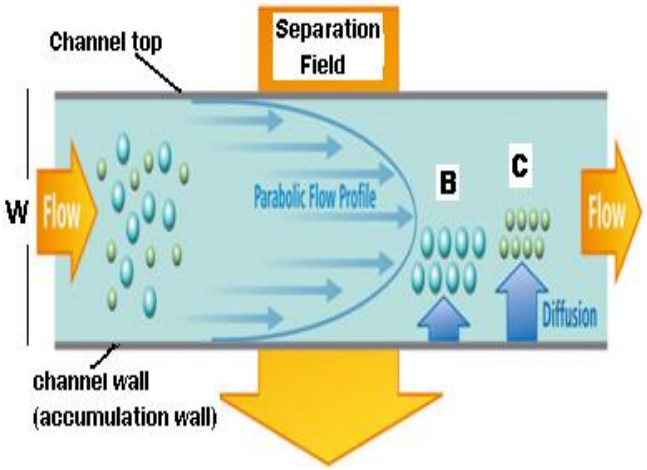

Fig 1 :FFF Principle

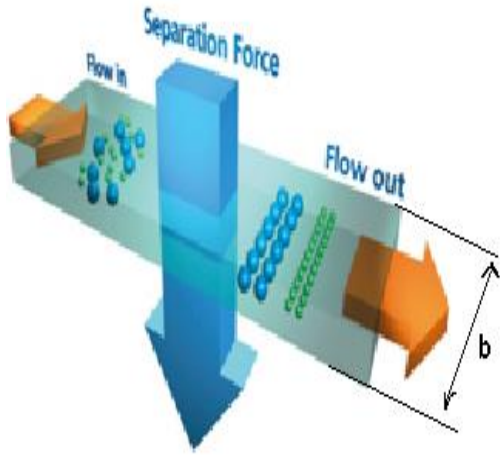

Fig 2 : 3D View

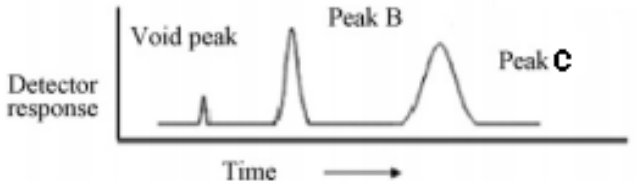

Fig 3: A typical FFF fractrogram

Modes Of Operation : Analytes can be separated by different mechanisms (modes of operation) in FFF that arise from different opposing forces. The mode of operation determines the elution order of analytes, along with other separation characteristics such as selectivity and resolution. Three widely used modes that can be implemented in any FFF technique are normal, steric and hyperlayer modes [9] which are shown in figure 4.

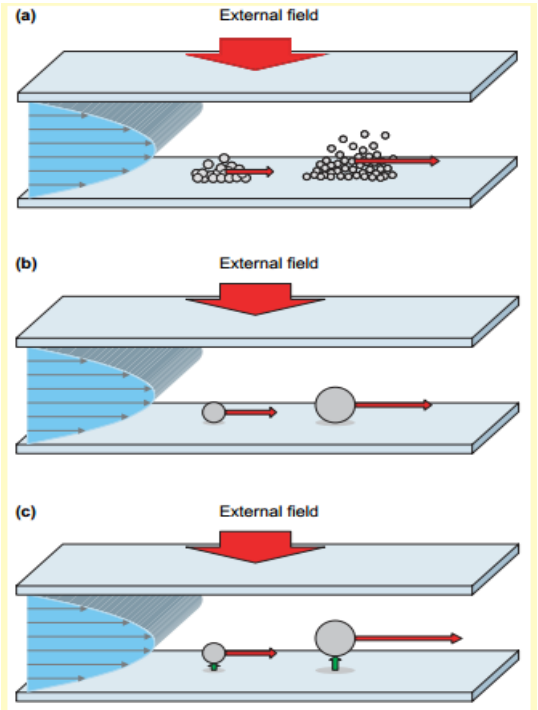

Fig 4 : Schematic representation of different modes of operation that can occurs in FFF (a) Normal (b) Steric and (c) Hyperlayer mode.

The normal mode (based on Brownian motion of the analyte in the channel) is usually used for the analyte sizes smaller than ~1µm. smaller component populations accumulate in regions of faster streams of the parabolic velocity profile and elute earlier than larger components. Steric mode is applicable for components larger than ~1µm where diffusion becomes negligible and retention is governed by the distance of closest approach to the accumulation wall. Small particles can approach the accumulation wall more closely than larger particles and thus the former's centre of mass is in the slower flowing stream lines. The elution order in steric mode is from largest to the smallest. Finally, lift or hyperlayer mode is one in which lift forces drive sample components to higher velocity streams located more than one particle radius from the accumulation wall. These hydrodynamic lift forces occur when high velocities are used. The elution order is the same as in the steric mode. Most polymers are separated by the normal mode mechanism because their dimensions are less than ~1µm. [10]

Instrumentation

An FFF system is generally assembled in a manner similar to that of a chromatographic apparatus. It uses most of the ancillary equipment employed in chromatography such as injector valves, pumps for the carrier liquid delivery, detectors, and some data acquisition devices such as chart recorders or more conveniently computers. A generalized FFF system is shown in figure 5.

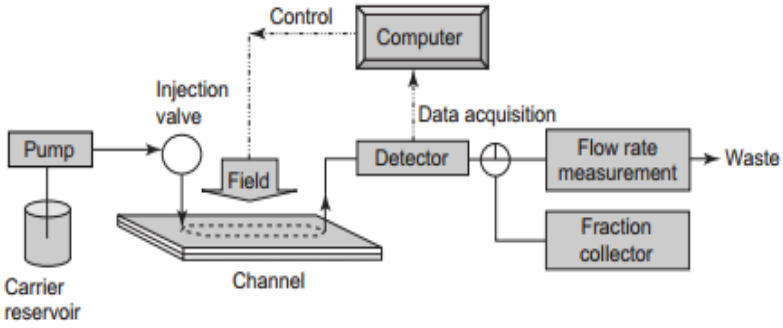

Fig 5 : FFF system assembled with the ancillary equipment

Channel Structure

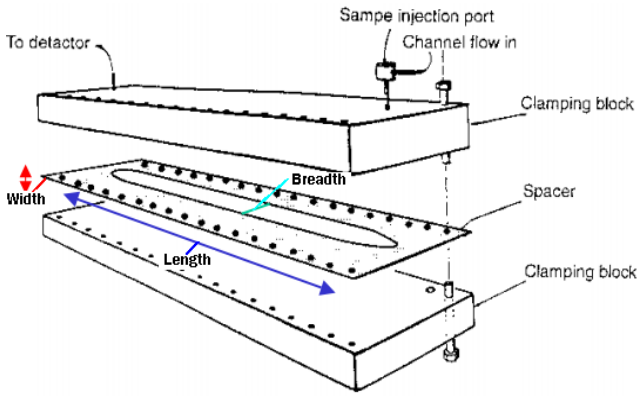

Fig 6 : Structure of channel in field flow fractionation.

Types of FFF : Each type of field requires a separate FFF channel. Depending upon field the FFF is divided in to following types

1. Sedimentation FFF (SdFFF)
2. Electrical FFF (EIFFF)
3. Thermal FFF (ThFFF)
4. Centrifugal FFF

5. Flow FFF (FIFFF)
    a) Symmetrical flow FFF
    b) Asymmetrical flow FFF.
    c) Hollow Fiber flow FFF.

Sedimentation FFF (SdFFF) : Sedimentation Field flow fractionation is a one phase separation technique that is particularly well suited for the fractionation and size determination of colloidal materials.[11 12] The one phase nature of the separation minimizes interfacial adsorption, and the open and well defined channel geometry does not restrict the size of analyzable particles in the same manner as a gel permeation column.

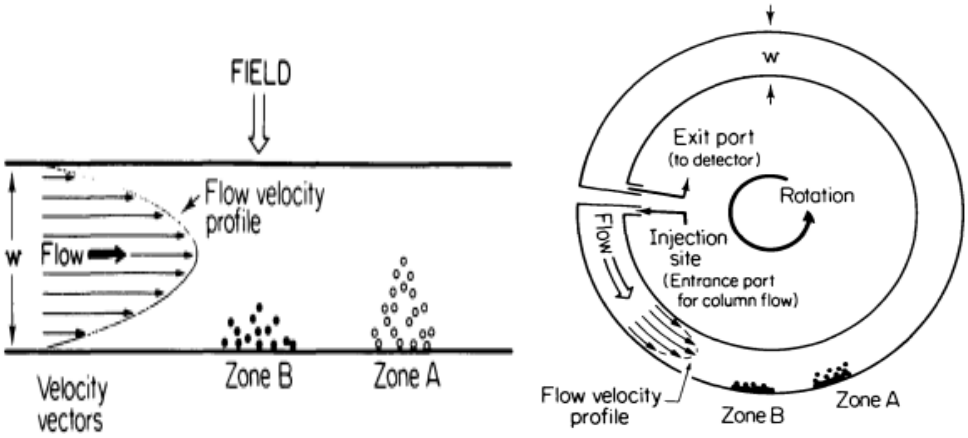

Figure 7: Sedimentation field flow fractionation

In Sedimentation FFF the sample is injected in to a thin ribbon-like channel through which a liquid is flowing laminarly, as shown in Figure 7. The channel, coiled to fit inside a rotor, can be spun so as to generate a field perpendicular to the direction of flow. Under the influence of the field particles in the channel will migrate towards the outer wall, provided they are denser than liquid, or towards inner wall if they are less dense than the carrier.[13] The tendency to concentrate at the wall is opposed by diffusion, and at equilibrium, more or less compact layers will be formed in the wall region where the fluid motion is the slowest. Particle separation thus occurs, and the different sized particles emerge one by one at the channel exit where they are led through a special seal in to a detector. Centrifugal FFF has the advantage that molecules can be separated by particle density, rather than just particle size. [15]

Applications: This is applied in sizing monodisperse [11] and polydisperse latex samples, virus particles, [12] bead polymerized serum albumin,[13] emulsions for intravenous nutrition and milk in various stages of aging.[14 16]

Thermal field flow fractionation :

Thermal FFF, as the name suggests, establishes a separation force by applying a temperature gradient to the channel. The top channel wall is heated and the bottom wall is cooled driving polymers and particles towards the cold wall by thermal diffusion. Thermal FFF was developed as a technique for separating synthetic polymers in organic solvents. Thermal FFF is unique amongst FFF techniques in that it can separate macromolecules by both molar mass and chemical composition, allowing for the separation of polymer fractions with the same molecular weight. Today this technique is ideally suited for the characterization of polymers, gels and nanoparticles. [17]

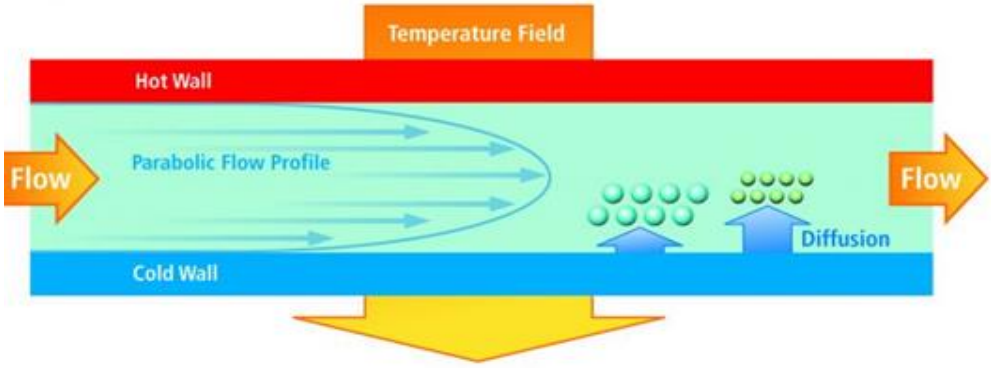

Figure 8: Thermal filed flow fractionation

Split flow thin cell fractionation (SPLITT)

Split Flow Thin Cell Fractionation (SPLITT) is a special preparative FFF technique, using gravity for separation of µm-sized particles on a continuous basis. SPLITT is performed by pumping the sample containing liquid into the top inlet at the start of the channel, whilst simultaneously pumping a carrier liquid into the bottom inlet. By controlling the flow rate ratios of the two inlet streams and two outlet streams, the separation can be controlled and the sample separated into two distinct sized fractions. The use of gravity alone as the separating force makes SPLITT the least sensitive FFF technique, limited to particles above 1 µm .

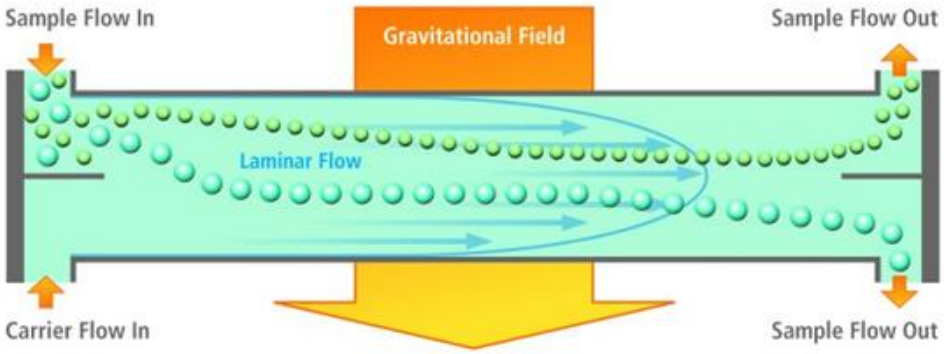

Figure 9: Split flow thin cell fractionation

Applications : Split Flow Thin Cell Fractionation (SPLITT) has applications in

- the biomedical field (cell separations),
- environmental area (diatoms, sediments, algae, soil particles)
- chemical sector (polymer and silica particles).

Flow field flow fractionation (FIFFF) :

Flow field flow fractionation is of two types symmetric and asymmetric flow field flow fractionation. [18]

Symmetric FlFFF was introduced in 1976 by Giddings. In Symmetric FIFFF the channel spacer is clamped between two parallel plastic blocks fitted with porous ceramic frits (2-5µm pores) in each wall. A cross-flow is applied as a 'field' perpendicular to the channel flow. Cross-flow enters the channel through the porous frit on the top wall and exits the channel through an ultra-filtration membrane overlaying a second porous frit at the bottom of wall (the accumulation wall).[20]

The asymmetrical version, AsFlFFF, was first introduced in 1987. In asymmetric flow field flow fractionation (AsFIFFF), a membrane is also used here as accumulation wall. However, AsFIFFF differs from FIFFF in that the channel has only a single permeable wall (accumulation wall). The upper porous wall is replaced by solid wall that is impermeable to the carrier liquid. A single channel inlet flow is split in to the channel flow and the cross-flow. The ratio between the two depends on operator controlled in-line flow resistances. AsFIFFF has the following advantages over FIFFF : simpler construction and the ability to visualize the sample through a transparent upper wall. [21] A schematic representation comparing FIFFF and AsFIFFF is shown in Figure 10

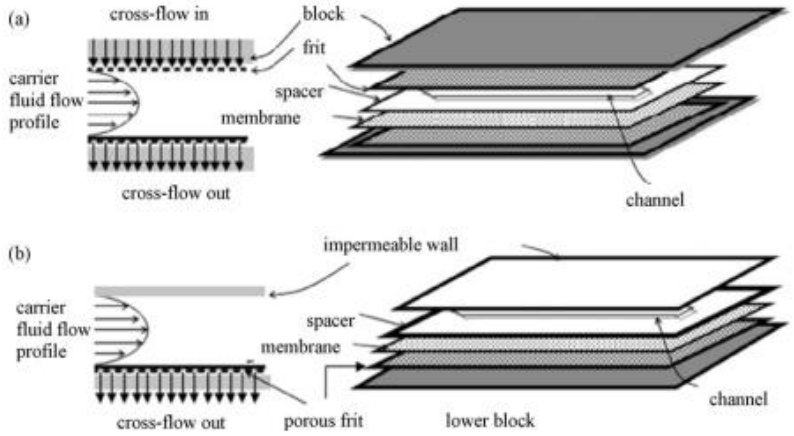

Figure 10 : A schematic representation of (a) symmetric (FIFFF) (b) Asymmetric (AsFIFFF) channel structures

Hollow-Fiber Flow-Field-Flow-Fractionation (HF5)

Hollow-Fiber Flow-FFF (HF5) is known since 1976, almost as long as flat channel Flow-FFF. HF5 uses a completely different channel geometry based on a polymeric or ceramic hollow-fiber with porous walls as a cylindrical channel. The fibers are produced in large quantities for water purification. They have ideal properties for FFF as they allow a high flux, are mechanically stable, and have been shown not to interact with most types of samples, specifically proteins.

Sensitivity of HF5

HF5 has a main advantage: high efficiency of separation. This benefit is based on specific properties of the HF5 separation mechanism. Plate numbers in Flow-FFF increase with the cross-flow velocity and retention time. Higher efficiency is achieved by using more cross-flow, which concentrates the sample closer to the membrane. From FFF theory it is known that retention ratio is directly related to the concentration distribution above the membrane. In HF5 the same retention results at 33% lower concentration level compared to AF4. This helps to prevent overloading and sample aggregation or absorption. Peak dilution depends on the channel volume and the detector flow rate.

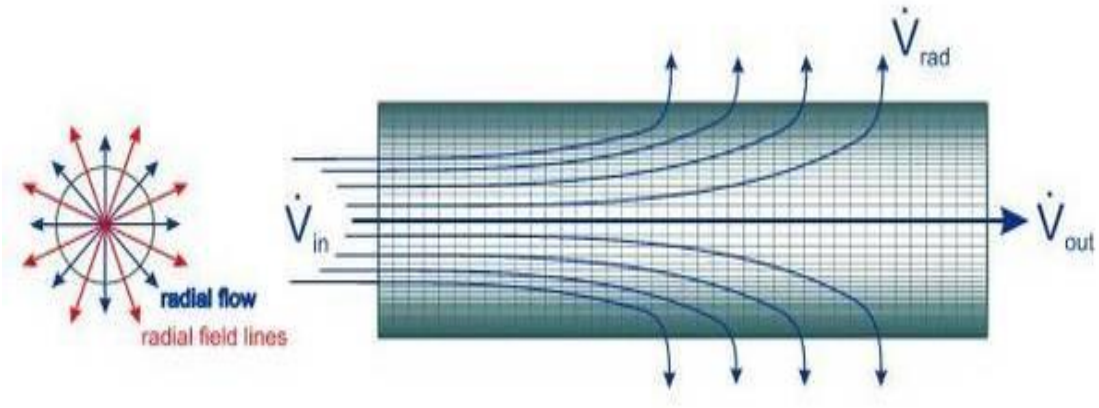

Figure 11: Hollow-Fiber Flow-Field-Flow-Fractionation

Productivity and flexibility of Hf5

HF5 has a 10 times lower channel volume compared to a typical AF4 channel and detector flow rates can also be reduced leading to lower dilution factors. Both narrow peaks and low dilution contribute to increased detectability and sensitivity. Short analysis time and high throughput, ease of use and minimum downtime characterize a productive analytical tool. Run time is typically shorter compared to other FFF methods primarily because of the small channel volume. Consumption of carrier solvent is lower for the same reason, reducing time and effort to prepare buffers plus the hassle to dispose of the waste.

Key advantages

- Separates both soluble and colloidal components over a wide size range; especially unmatched powers in sub-micrometer range
- Large dynamic range: from a few nanometers to a few micrometers
- Rapid analysis: typically 10 to 30 minutes
- Absence of shearing forces (no stationary phase; separation takes part in separation channel)
- Ultra-high resolution separations, comparable to ultra centrifugation
- Simplifies analysis by eliminating many time-consuming sample preparation steps
- Adjustable and flexible separation ability: very efficient for complex samples
- Direct injection: minimal sample preparation necessary
- Interfaces with modern analytical methods like MALS, MALDI-TOF, etc.
- Fractions can be collected and used for off-line analysis (with electron microscopy, MS, ICP-MS, ELISA etc.)

Emergence and evolution of FFF in life sciences and biotechnology

Proteins

The first application of FFF to proteins was reported in 1972 . The authors used electrical FFF (ElFFF), a variant of FFF in which an electric field is used. ElFFF revealed several advantages with respect to protein electrophoresis, such as low required voltage, lack of adverse heating and support effects, and the existence of a mobile phase flow to amplify separation. Flow FFF (FlFFF), which instead uses a mobile

phase crossflow as the applied field, has been the most widely applied FFF technique for protein fractionation. The ability of FlFFF to measure the diffusivity of intact proteins, within a mass range of w105 in a single run, was first shown in 1977. This technique was used to characterize intact proteins of different origin, from wheat proteins and enzyme mutants to lipoproteins screened in patients with coronary artery disease.[19]

Nucleic acids

Sedimentation field-flow fractionation (SdFFF), which uses a sedimentation field generated by a centrifuge, has been demonstrated to be sufficiently gentle to fractionate λ DNA and smaller supercoiled plasmids, without altering the conformations during fractionation. FlFFF was applied to the fractionation of plasmid fragments, to elucidate DNA conformation and measure the different diffusion coefficients of linear and circular DNA . The ribosomal composition of recombinant proteins can be monitored by evaluating the cell translation capacity that produces the proteins. FlFFF was used to separate and quantify tRNA in recombinant Escherichia coli cells and, subsequently, to relate protein production levels to tRNA levels. [20]

Viruses :

The main advantage of FFF over convential LC and CZE techniques can be seen when separating very high molar-mass bioanalytes. The first example of the application of FFF to viruses dates back to 1975, when SdFFF as applied to the separation and molar mass determination of T2 phage. SdFFF fractionated oligomeric aggregates of rod-shaped viral particles of nuclear polyhedrosis virus form a complex mixture of enveloped aggregated forms and monomers. SdFFF was shown to be sufficiently gentle that passage through the system had a very little effect in the infectivity of the T4D virus. Accurate and precise determination of the molar mass and density of viruses, such as the *Paramecium bursaria* chlorella virus (PBCV), was also possible using SdFFF. As in the case of lower molar-mass analytes discussed in previos sections, FlFFFis also effective and rapid for virus analysis. Determination of virus diffusitivity from FIFFF retention times were reported, and a clean, quick separation of viruses as observed. The use of a multiangle laser-scattering detector coupled with FIFFF was also explored to separate and elucidate the size of tobacco mosaic virus. [21]

The role of FFF in modern biotechnology

Stem cells

The first application of FFF to stem cell separation was reported in 1996. It employed GrFFF for the micropreparation of stem cells from mouse bone marrow. The effectiveness of SdFFF to provide selective, immature cell isolation without inducing cell differentiation was further shown by fast purification (in less than 15 minutes) of an immature neural cell fraction from a human neuroblastic cell line. Among immature cells embryonic stem cells (ESs) are an important biotechnological tool. ESs are used as a vehicle for transgenesis and can be cultured *in vitro* onto a layer of embryonic fibroblasts, and collected by using time consuming and different methods. A cell suspension (~ 10^6 cells/ml) of ESs at various stages of proliferation was selectively fractionated within few minutes using SdFFF, and collected cell fractions with *in vivo* potential development were used to derive transgenic mice by the generation of chimeras. The hybrid DEP-GrFFF variant was used for cancer cell purging from normal T lymphocytes and from CD34 + hematopoietic stem cell, for the separation of the major leukocyte subpopulations, and for the enrichment of leukocytes. [22 23]

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