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Review : Field Flow Fractionation

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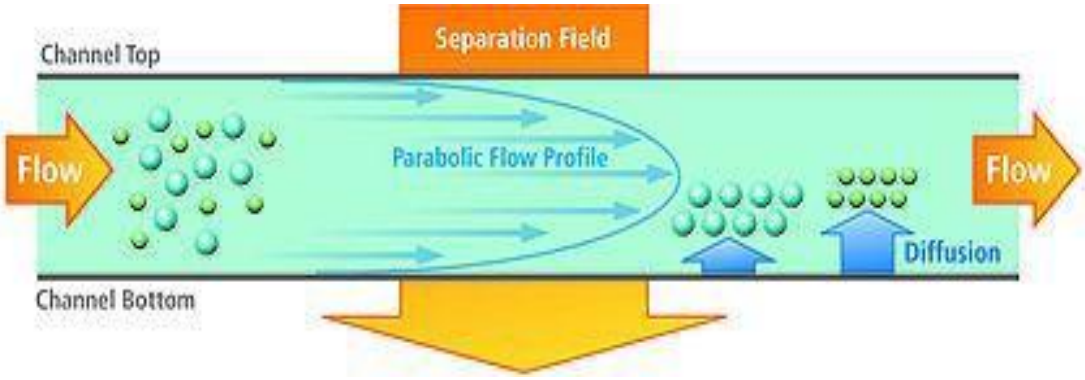
Abstract: *Field flow Fractionation is a technique which is used for the separation of colloidal or high molecular weight substances in liquid solution, flowing through the separation platform, which does not have a stationary phase. It is similar to liquid chromatography, as it works on dilute solutions or suspensions of the solute, carried by a flowing eluent.separation is done by applying a fields like hydraulic, centrifugal, thermal, electrical, magnetic, gravitational or cross flowing, perpendicular to the direction of the sample .Field flow fractionation (FFF) is a chromatography-like separation technique used to separate macromolecules, colloids, and particles. The idea is straightforward. A parabolic velocity profile is created by a laminar flowof carrier liquid between two walls separated by approximately 0.1* mm.

Keywords: Field flow Fractionation

I. INTRODUCTION

Prof. J. Calvin Giddings invented the separation technique field-flow fractionation [FFF]. The method is based on the separation of colloidal or high molecular weight substances in liquid solutions that flow through a separation platform that lacks a stationary phase. It is similar to liquid chromatography in that it works with dilute solute solutions or suspensions carried by aflowing eluent. Separation is accomplished by applying a field (hydraulic, centrifugal, thermal, electric, magnetic, or gravitational) or cross-flow perpendicular to the sample's transport direction, which is pumped through a long and narrow laminar channel. The field exerts a force on the sample components, directing them to one of the channel walls known as the accumulation wall.The force interacts with a sample property, causing separation; in other words, the components have different "mobilities" under the force exerted by the crossing field. The translational diffusion coefficient or the hydrodynamic size, for example, is the property driving separation in the hydraulic, or cross-flow, FFF method. It is the ratio of the thermal and translational diffusion coefficients in a thermal field (heating one wall and cooling the other).



THEORY

Distinction in A laminar channel is used for field flow fractionation. It is made up of a top and bottom block separated by a spacer. As the spacer is sealed between the blocks, a cut-out (rectangular or trapezoidal) void provides the channel volume. Alternatively, the channel canbe machined as a cavity into the top block. The channel is designed in such a way that the force field may be applied, which means that a specific channel is required for each FFF method. The sample is injected into the channel in a dilute solution or suspension and separated during migration from input to exit as the carrier solution is circulated through the channel. One or more detectors are installed downstream of the channel exit to analyze theeluting fractions.

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Giddings and colleagues created a theory that describes the general retention equation that is shared by all FFF approaches.

Relating force (F) to retention time (tr) :

First principles can be used to derive the link between the separative force field and retention time. Take a look at two particle populations in the FFF channel. Both particle clouds are driven towards the lower "accumulation" wall by the cross field. The particle's natural diffusion, or Brownian motion, opposes this force field and creates a counteracting motion. When these two transport processes achieve equilibrium, particle concentration c approaches the exponential function of height x above the accumulation wall, as seen in the equation.

$$c = c_0 e^{\frac{-x}{\ell}}$$

eq..1

The characteristic elevation of the particle cloud is represented by ℓ.This relates to the average height that the particle cloud reaches within the channel and only when the value for ℓ is different for the particle populations separation will occur. The ℓ of each component can be related to the force applied on each individual particle or to the ratio of the diffusion coefficient D and the drift velocity U.

$$\ell = \frac{kT}{F} = \frac{D}{U}$$

eq..2

The Boltzmann constant is k, absolute temperature is T, and the force field exerts on a single particle is F. This demonstrates how the characteristic elevation value is inversely proportional to the applied force. As a result, F governs the separation procedure. As a result, the separation can be controlled to achieve optimal levels by varying the field strength.

A cloud's velocity V is simply the average velocity of an exponential distribution embedded in a parabolic flow profile Retention time, tr can be written as :

$$t_r = \frac{L}{V}$$ eq..3

Where L is the channel length.

The retention ratio in FFF is usually expressed as the void time t0 (emergence of a non-retained tracer) divided by the retention time tr. The retention equation is then:eq..4

$$R = \frac{t_0}{t_r} = 6\lambda[\coth(\frac{1}{2\lambda}) - 2\lambda]$$ eq..4

where ℓ is the channel thickness or height, and w is the channel width. The retentionratio with respect to the applied cross force is illustrated by substituting kT/F for ℓ.

$$R = 6\frac{kT}{Fw}[\coth(\frac{Fw}{2kT}) - 2\frac{kT}{Fw}]$$ eq..5

The channel thickness value w far exceeds ℓ for an efficient operation. When this occurs, the term in brackets approaches unity. As a result, equation 5 can be approximated as follows:

$$R = 6\lambda = 6\frac{kT}{Fw}$$ eq..6

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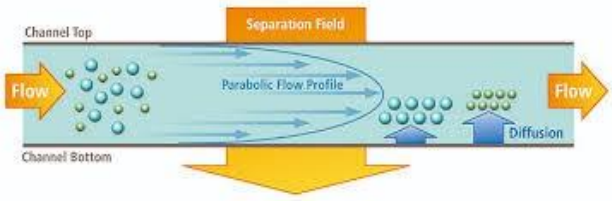
As a result, tr is roughly proportional to F. Separation of particle bands X and Y is achieved only if the force increment ΔF between them is sufficient. A force difference of only 10^{-16} N isrequired for this to be the case.
The magnitudes of F and ΔF are affected by particle properties, field strength, and field type.This allows for technique variations and adaptations. Many different types of FFF have evolved from this basic principle, with the nature of the separative force used and the molecule size range to which they are targeted varying.

II. DISCOVERY & GENERAL PRINCIPLE

J. Calvin Giddings invented and first published FFF in 1966 and 1976. Giddings had written numerous articles on Flow-FFF, the most important FFF technique today. Giddings, who iscredited with inventing FFF, was a chemistry professor and expert in chromatography and separation techniques at the University of Utah.
As previously stated, the field can be hydraulic (with a cross flow through a semi-permeable membrane as the accumulation wall), gravitational, centrifugal, thermal, electrical, or magnetic in field-flow fractionation. In all cases, the separation mechanism is caused by differences in particle mobility under field forces, in stationary equilibrium with diffusion forces:The field causes a downward drift velocity and concentration towards the accumulation wall, which the diffusion works against. After a certain amount of time (referredto as the relaxation time), the two forces equilibrate in a stationary equilibrium. This is best represented as a particle cloud, with all components in constant motion but an exponential decrease in average concentration as one moves away from the accumulation wall and up into the channel. The decrease in air pressure as it rises from sea level follows the same exponential pattern as described in the Barometric formula.When the channel flow is activated after relaxation, elution begins. A parabolic laminar-flow-velocity profile exists in the thin channel (typical height 250 to 350 m), which is characterized by a strong increase in flow velocity with increasing distance from the accumulation wall. This determines the velocity of a specific particle based on its equilibrium position from the channel wall. Particles closer to the accumulation wall will migrate more slowly than those higher up. The retention ratio R is the ratio of the velocity of a particle species to the average velocity of the fluid. R inFFF must be less than 0.2 for efficient separation; typical values range from 0.02 to 0.1.

TYPES OF FIELD FLOW FRACTIONATION TECHNIQUES :SYMMETRICAL FLOW :

Flow FFF was the first of these techniques to be commercially available. Flow FFF separates particles by size, regardless of density, and can measure macromolecules in the 1 nm to 1 m range. It is the most versatile FFF sub-technique available in this regard. Flow FFF's cross flow enters the channel through a porous frit at the top and exits through a semipermeable membrane outlet frit on the accumulation wall (i.e. the bottom wall). In the last two decades, asymmetrical flow has largely replaced symmetrical flow.



ASYMMETRICAL FLOW

Flow that is asymmetric FFF (AF4), on the other hand, has only one semi-permeable membrane on the channel's bottom wall. As a result, the cross flow is caused by the carrier liquid exiting the channel's bottom. This provides a very gentle separation as well as a "ultra-broad" separation range. The vast majority of FFF instruments are AF4 systems.
Proteins, viruses and virus-like particles, and liposomes are the most common applications in pharmaceutical research and development. Because AF4 can be used in both aqueous and organic solvents, it can be used to separate organic polymers.
For the separation of high and ultra-high molar mass polymers soluble at temperaturesabove 150 C, High Temperature Asymmetric Flow Field-Flow Fractionation is available.

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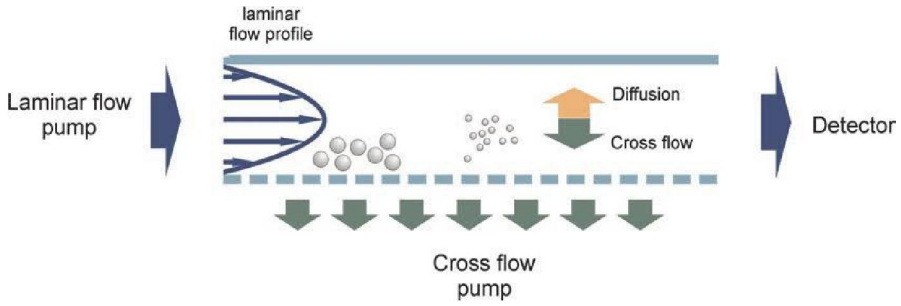
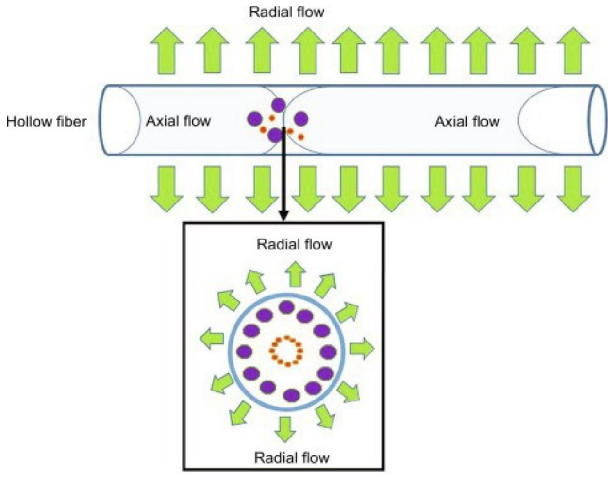


HOLLOW FIBER FLOW

Lee et al. (1974) invented hollow fiber flow FFF (HF5).HF5 has been used to investigate proteins and other macromolecules. In 1974, the first form of flow FFF, HF5, was developed. The benefit of HF5 is that it provides a disposable channel unit that can be easily replaced in routine applications. One disadvantage of HF5 is the limited selection of membrane materials available; only polyethersulfone (PES) membranes are available. Because of the lack of flexibility and sample load limitations, HF5 is not widely used at the moment.



THERMAL

Thermal FFF, as the name implies, creates a separation force in the channel by applying a temperature gradient. Thermal diffusion drives polymers and particles towards the cold wallby heating the top channel wall and cooling the bottom wall. Thermal FFF was created as amethod of separating synthetic polymers in organic solvents. Thermal FFF is unique among FFF techniques in that it can separate macromolecules by molar mass as well as chemical composition, enabling the separation of polymer fractions with the same molecular weight. Today, this technique is ideal for characterizing polymers, gels, and nanoparticles.

The simple and well-defined dimensions of the separation channel make inter-lab or inter-instrument Universal Calibration possible because the Thermal FFF calibration

constants closely describe the ratio of ordinary (molecular) diffusion coefficient D to thermal diffusion coefficient (or, thermophoretic mobility) DT, which is only polymer dependent. As a result, the ThFFF Universal Calibration is instrument and lab transferable, whereas the well-known size exclusion chromatography Universal Calibration is only polymer-transferable on the same instrument.

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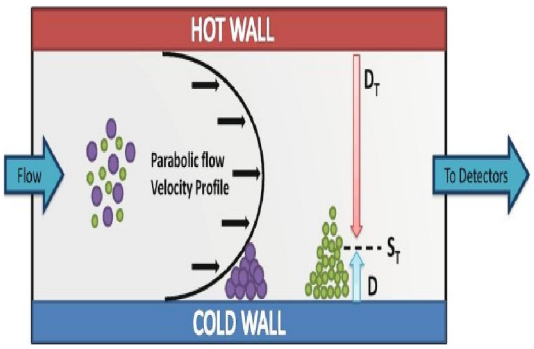
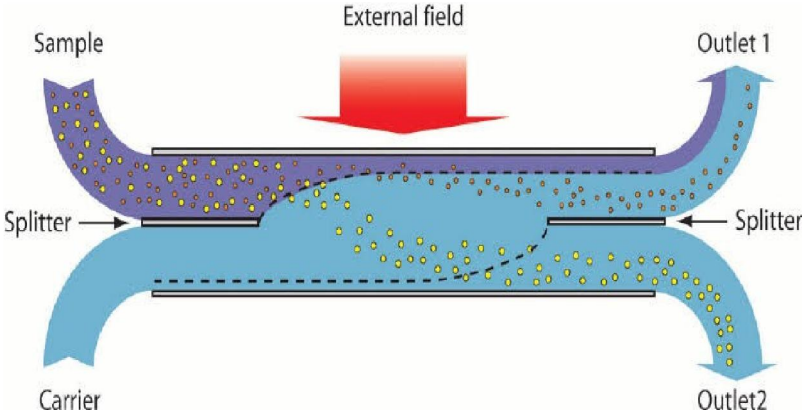


SPLIT FLOW THIN - CELL FRACTIONATION

Split flow thin-cell fractionation (SPLITT) is a special preparative FFF technique that uses gravity or electric or diffusion differences for continuous separation of particles larger than min size. The SPLITT system includes two inlets and two outlets. It is accomplished by pumping a sample immersed in a liquid into one of the channel's inlets at a low flow rate while simultaneously pumping a carrier liquid into the second inlet at a much higher flow rate. The separation can be controlled by varying the flow rate ratios of the two inlet streams and two outlet streams, and the sample components are separated into two distinct sized fractions. SPLITT is the least sensitive FFF technique because it relies solely on gravity as a separating force, and it is limited to particles larger than 1µm.

CENTRIFUGAL

The separation field in centrifugal FFF is generated by a centrifugal force. The channel is shaped like a ring and spins at rotation speeds that can be programmed during the run. The flow and sample are pumped into the channel and centrifuged, allowing the operator to determine particle size and density by mass. Because particle size is proportional to particlemass to the third power, the advantage of centrifugal FFF is the high size resolution that can be achieved by varying the force applied.



The ability of centrifugal FFF to achieve high resolution given sufficient buoyant density is its distinguishing feature. This enables the separation of particles with only a 5% size difference.

Centrifugal FFF has the advantage of separating particles and macromolecules based on particle density rather than particle size. In this case, two identically sized gold and silver nanoparticles can be separated into two peaks based on density differences in the gold and silver nanoparticles.

The mass-to-time ratio in AF4 separations is 1:1. The addition of the third density parameterto centrifugal FFF results in a ratio more akin to mass:time to the power of three. This results in a significantly larger difference between peaks and a significantly improved resolution.

This is especially useful for novel products like composite materials and coated polymers containing nanoparticles, which are particles that vary in density but not size. If the density is different, two identically sized particles can still be separated into two peaks.

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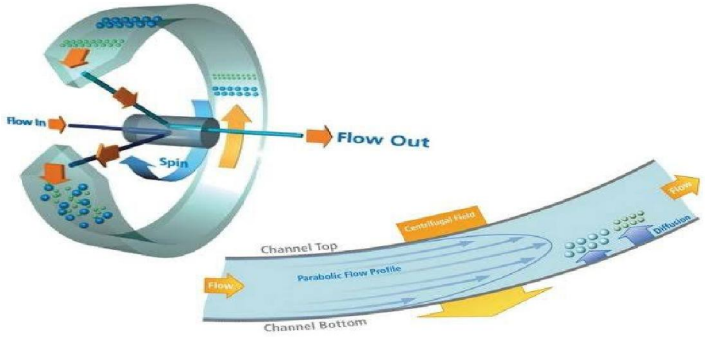
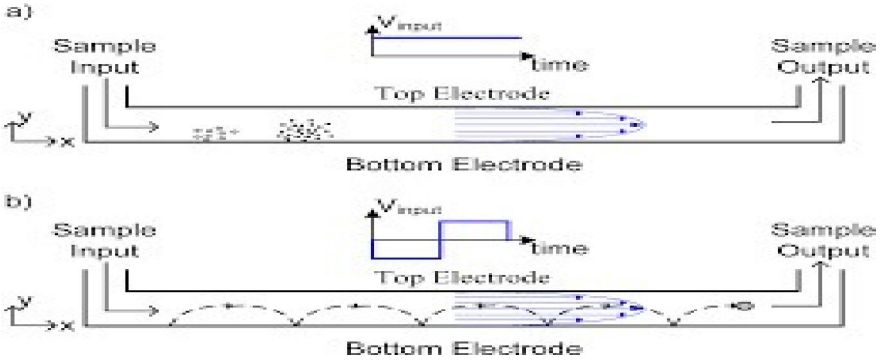
The method's limitation is its lower size limit, which is determined by the density of the sample. The limit for biological samples is in the range of 20 to 50 nm in diameter.



ELECTRICAL

A transverse electrical current (DC) is used to create an electric field in electrical FFF. An electrophoretic drift velocity is induced depending on the charge of sample components, which is counteracted by diffusion from Brownian motion, so separation depends on the ration of electrophoretic mobility and size. Electrical FFF application has been limited and is now rarely used. Other modifications have been created, such as cyclical electrical FFF, which uses a special alternating current. It enables separation based on electrophoretic mobility. Electrical asymmetrical flow FFF (EAF4) is another variant in which an electrical field is applied in addition to a cross flow field.EAF4 overcomes the limitations of pure electrical FFF, which has low resolution and is prone to electrolysis products and bubbles contaminating the channel outflow and compromising detector signals



ADVANTAGES

- Size-Based Separation: FFF separates particles based on their size, making it ideal for polydisperse samples where components vary in size, such as polymers, nanoparticles, and biomolecules.
- Versatility: It's applicable to a wide range of materials, from macromolecules to colloids and nanoparticles, without extensive modification.
- Gentle Separation Conditions: FFF operates under mild conditions (low shear, no solid stationary phase), preserving the integrity of delicate or sensitive samples, like proteins or biological molecules.
- High Resolution: it offers high resolution and excellent separation efficiency due to the interplay between the applied field and the carrier fluid, allowing fine differentiation between species with small size differences.
- Fraction Collection: FFF allows for fraction collection, enabling the isolation and analysis of individual components for further study or application.
- Compatibility with Various Detection Methods: Compatible with various detection techniques (UV, MS, etc.), allowing simultaneous characterization of separated components.
- Sample Recovery: FFF usually allows for the recovery of separated components,minimizing sample loss and enabling downstream analysis or use.

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DISADVANTAGES:

- Complexity and Technique Sensitivity: FFF requires a deeper understanding of the instrument and its operation, making it more complex compared to some other separation techniques. It's sensitive to variations in flow rates, detector sensitivity, and particle interactions, which can affect the accuracy and reproducibility of results.
- Sample Compatibility: Not all samples are easily compatible with FFF. High salt concentrations or complex matrices can interfere with the separation process, impacting the efficiency and resolution of the separation
- Instrumentation Costs: Setting up and maintaining FFF instruments can be expensive. The necessary specialized equipment and columns can require significant investments, making it less accessible compared to some other separation methods.
- Limited Commercial Availability: Availability of commercial FFF instruments and consumables might be limited compared to more established techniques like chromatography, restricting access for researchers or labs.
- Analysis Time: FFF can be time-consuming, especially for complex samples or when high resolution is needed. Separation processes might take longer, affecting the overall throughput of sample analysis.
- Particle Retention and Loss: Some particles might adhere to the membrane or column used in FFF, leading to retention or loss during the separation process. This can affect the accuracy of results, particularly for low-abundance particles.

LIMITATIONS

- Because of their rapid diffusion, small molecules do not respond to FFF. To achieve an effective separation, the sample must be concentrated very close to the accumulation wall (less than 10 m), which necessitates a drift velocity caused by the force field that is two orders of magnitude greater than the diffusion coefficient. The lower size range of separation is determined by the maximum field strength that can be generated in an FFF channel. This is about 1 nm for current instrumentation.
- Although FFF is a highly adaptable technique, there is no "one-size-fits-all" solution for all applications. Different FFF methods necessitate the use of specialized equipment. Only asymmetric flow field-flow fractionation (AF4) is currently in widespread use. Other methods, such as centrifugal, thermal, or electrical FFF, are still in use.
- FFF behaves differently than column chromatography, which can be confusing for HPLC or SEC users. Understanding the FFF operating principle is critical for successful application of the method.

III. CONCLUSION

FFF should be a useful tool for nanomaterial separation. Despite the introduction of only two major FFF systems, flow FFF and centrifugal FFF, the selection of the appropriate force field allows material fractionation by size or density. Furthermore, the hyphenated flow FFF and centrifugal FFF system allows for the separation of mixed materials (of varying size and density), which one-dimensional FFF separation alone does not allow for. FFF can be used to prepare samples for electron microscopy as well as to precisely determine the size distribution of nanomaterials.

REFERENCES

[1]. Giddings, J.C. (1993) Field-flow fractionation: analysis of macromol- ecular, colloidal and particulate materials. Science 260, 1456–1465
[2]. Schimpf, M.E. et al., eds (2000) Field-Flow Fractionation Handbook, Wiley-Interscience
[3]. Caldwell, K.D. et al. (1972) Electrical field-flow fractionation of proteins. Science 176, 296–298
[4]. Giddings, J.C. et al. (1977) Flow field-flow fractionation as a methodology for protein separation and characterization. Anal. Biochem. 81, 394–407
[5]. Stevenson, S.G. et al. (1999) Automated frit inlet/frit outlet flow field- flow fractionation for protein characterization with emphasis on polymeric wheat proteins. Anal. Chem. 71, 8–14

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[6]. An, J.H. et al. (1999) Properties of malonyl-CoA decarboxylase from Rhizobium trifolii. J. Biochem. Mol. Biol. 32, 414–418

[7]. Park, I. et al. (2002) Separation and selective detection of lipoprotein particles of patients with coronary artery disease by frit-inlet asymmetrical flow field-flow fractionation. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 780, 415–422

[8]. Arfvidsson, C. and Wahlund, K-G. (2003) Mass overloading in the flow field-flow fractionation channel studied by the behavior of the ultra-large wheat protein glutenin. J. Chromatogr. A. 1011, 99–109

[9]. Yohannes, G. et al. (2004) Cytochrome C-dimyristoylphosphatidyl- glycerol interactions studied by asymmetrical flow field-flow fraction- ation. Anal. Bioanal. Chem. 380, 757–766

[10]. Schallinger, L.E. et al. (1984) Sedimentation field flow fractionation of DNA's. Science 225, 434–437

[11]. Litzen, A. and Wahlund, K-G. (1989) Improved separation speed and efficiency for proteins, nucleic acids and viruses in asymmetrical flow field flow fractionation. J. Chromatogr. 476, 413–421

[12]. Liu, M.K. and Giddings, J.C. (1993) Separation and measurement of diffusion coefficients of linear and circular DNAs by flow field-flow fractionation. Macromolecules 26, 3576–3588

[13]. Nilsson, M. et al. (1999) Expression of Vitreoscilla hemoglobin in Escherichia coli enhances ribosome and tRNA levels: a flow field-flow fractionation study. Biotechnol. Prog. 15, 158–163

[14]. Arfvidsson, C. and Wahlund, K-G. (2003) Time-minimized determi- nation of ribosome and tRNA levels in bacterial cells using flow field- flow fractionation. Anal. Biochem. 313, 76–85

[15]. Lee, H. et al. (2001) Analysis of self-assembled cationic lipid-DNA gene carrier complexes using flow field-flow fractionation and light scattering. Anal. Chem. 73, 837–843

[16]. Mozersky, S.M. et al. (1988) Sedimentation field flow fractionation of mitochondrial and microsomal membranes from corn roots. Anal. Biochem. 172, 113–123

[17]. Nilsson, M. et al. (1996) Determination of relative amounts of ribosome and subunits in Escherichia coli using asymmetrical flow field-flow fractionation. J. Biochem. Biophys. Methods 33, 9–23

[18]. Nilsson, M. et al. (1997) Use of flow field-flow fractionation for the rapid quantitation of ribosome and ribosomal subunits in Escherichia coli at different protein production conditions. Biotechnol. Bioeng. 54, 461–467

[19]. Nilsson, M. et al. (1998) Monitoring of ribosomes and subunits in Escherichia coli during production of glucose isomerase using flow field-flow fractionation. Biotechnol. Tech. 12, 477–480

[20]. Lu, H. et al. (2004) A Microfabricated device for subcellular organelle sorting. Anal. Chem. 76, 5705–5712

[21]. Giddings, J.C. et al. (1975) Application of sedimentation field-flow fractionation to biological particles. Molecular weights and separ- ation. Sep. Sci. 10, 133–149

[22]. Caldwell, K.D. et al. (1980) Field-flow fractionation of alkali-liberated nuclear polyhedrosis virus from gypsy moth Lymantria dispar Linnaeus. J. Virol. Methods 1, 241–256

[23]. Caldwell, K.D. et al. (1981) Characterization of T4D virus by sedimentation field-flow fractionation. J. Chromatogr. 215, 323–332

[24]. Yonker, C.R. et al. (1985) Physical characterization of PBCV virus by sedimentation field flow fractionation. J. Virol. Methods 11, 145–160

[25]. Giddings, J.C. et al. (1977) Flow field-flow fractionation: new method for separating, purifying, and characterizing the diffusivity of viruses. J. Virol. 21, 131–138

[26]. Thielking, H. and Kulicke, W-M. (1998) Determination of the structural parameters of aqueous polymer solutions in the molecular, partially aggregated, and particulate states by means of FFFF/MALLS. J. Microcolumn Sep. 10, 51–56

[27]. Berg, H.C. and Turner, L. (1991) Selection of motile nonchemotactic mutants of Escherichia coli by field-flow fractionation. Proc. Natl. Acad. Sci. U. S. A. 88, 8145–8148 482 Review TRENDS in Biotechnology Vol.23 No.9 September 2005.