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Review

Asymmetrical flow field-flow fractionation technique for separation and characterization of biopolymers and bioparticles

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ABSTRACT

Field-flow fractionation (FFF) is one of the most versatile separation techniques in the field of analytical separation sciences, capable of separating macromolecules in the range 10^3–10^{15} g mol^{-1} and/or particles with 1 nm–100 μm in diameter. The most universal and most frequently used FFF technique, flow FFF, includes three types of techniques, namely symmetrical flow FFF, hollow fiber flow FFF, and asymmetrical flow FFF which is most established variant among them. This review provides a brief look at the theoretical background of analyte retention and separation efficiency in FFF, followed by a comprehensive overview of the current status of asymmetrical flow FFF with selected applications in the field of biopolymers and bioparticles.

Contents

1. Introduction

Field-flow fractionation (FFF) is one of the most versatile separation techniques in the field of analytical separation sciences, capable of separating macromolecular colloidal and particulate materials [1]. In 1966, Giddings first introduced the concept of field-flow fractionation (FFF) [2]. Since then, various subtechniques of FFF emerged. The major subtechniques are sedimentation FFF, thermal FFF, electric FFF, and flow FFF. In all the FFF subtechniques, sample separation is performed inside a narrow ribbon-like channel. The different techniques of FFF and their applications have been exhaustively described in general reviews [1,3–11]. Briefly, most of the FFF subtechniques have a channel with typical dimensions of 20–50 cm in length, 2–3 cm in width, and 0.01–0.05 cm in thickness. From the inlet, carrier liquid is pumped along the channel, establishing a parabolic flow profile (laminar Newtonian flow) moving the analytes towards the outlet. An external force field is

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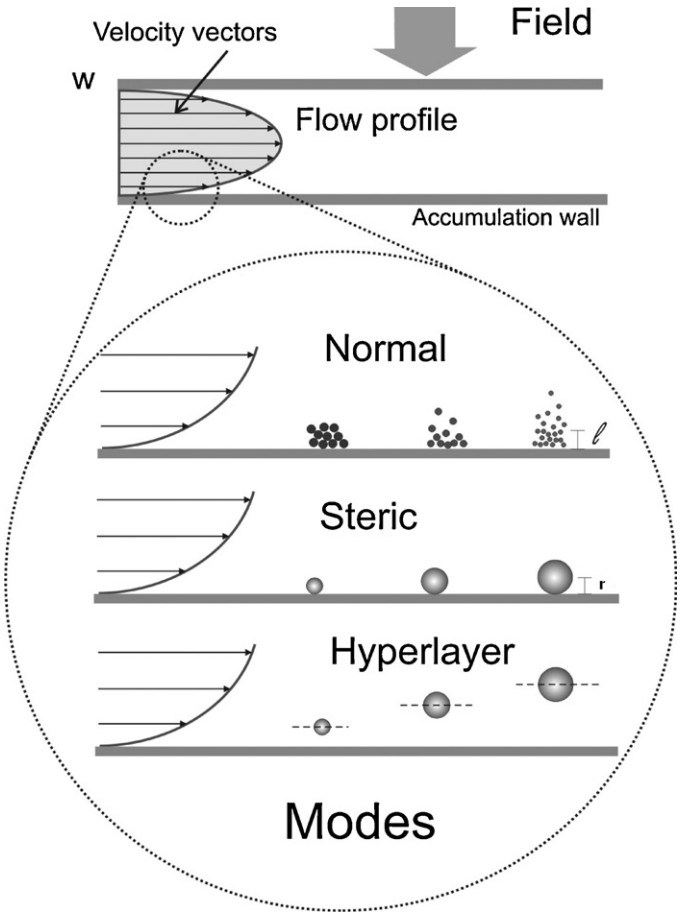

Fig. 1. Basic separation principle of field flow fractionation.

applied perpendicularly to the direction of the carrier liquid flow, forcing the sample components to accumulate towards the channel wall, termed accumulation wall (Fig. 1). Depending on the field applied (centrifugal, thermal, electrical, and hydrodynamic), each of the FFF subtechniques has different separation mechanism. Separation range for biopolymers is from 10^3 up to 10^{15} g mol^{-1} and that for bioparticles from 2 nm to 50 μm in diameter.

Flow field-flow fractionation (FlFFF), introduced in 1976 has proven to be the most universal and most frequently used of all FFF techniques [12]. The universality comes from the fact that the technique employs a hydrodynamic field applied by means of a secondary flow (cross flow) of carrier liquid perpendicular to the main flow [13]. Specifically, pumping a bulk liquid into the channel through one of the porous walls creates a convective flux. The liquid then exits the channel through the opposite wall, the so called "accumulation wall", that consists of a membrane placed on the top of a porous wall. For this reason, retention time in FlFFF is, in principle, dependent on diffusive flux, and the separation of macromolecules or particles occurs solely on the basis of differences in diffusion coefficients [12]. There are two main types of flow FFF: symmetrical flow FFF and asymmetrical flow FFF. In addition, some variant techniques exist, such as hollow fiber FlFFF (HFFlFFF). In the case of HFFlFFF, the field is radial with the cross flow radiating outwards over the entire inner surface of the tube while the rest of the liquid makes the channel flow.

The symmetrical flow FFF (FlFFFF) channel consists of upper and lower semi-permeable porous frits within the external blocks. When sample materials are introduced to a symmetrical FlFFF channel, they are pushed towards one side of channel wall (accumulation wall) by the applied field (cross flow). The main channel flow is stopped during the relaxation period for the time needed for the cross flow pump to deliver about one channel volume. During this short period of time, sample components find equilibrium positions where the field force and the diffusion are counterbalanced, and the sample components are differentially distributed along the channel cross section/thickness according to their sizes.

In the asymmetrical flow field-flow fractionation (AsFlFFF) the upper wall of the symmetrical flow FFF channel is replaced by an impermeable glass plate. The bottom channel plate is permeable, and made of a porous frit material (Fig. 1). AsFlFFF was first introduced by Wahlund and Giddings [14]. It was optimized further in the late eighties and the beginning of nineties [15–19]. In AsFlFFF, the cross flow is generated directly inside the channel, where the main flow coming from the inlet is divided to generate a cross flow through a semi-permeable membrane that is located in the bottom wall, while the rest of the longitudinal flow stream is directed to the detector(s). The membrane pore size in AsFlFFF is selected in such a way that only the solvent can pass through while the sample particles are retained (Fig. 2). In AsFlFFF, the relaxation process is called relaxation-focusing, i.e. the carrier liquid is allowed to flow in from both the inlet and outlet of the channel and meet at one point, the so-called focusing point. During this relaxation-focusing period sample migration is temporarily halted for achieving equilibrium after sample injection.

The focus of this review is on recent advances in asymmetrical flow field-flow fractionation. Selected examples that illustrate the benefits of AsFlFFF in the separation and the characterization of biopolymers and bioparticles hopefully accelerate future the exploitation of the technique.

2. Theoretical background in analyte retention

The theory of FlFFF applies almost directly to AsFlFFF, and it has been described in detail in many publications [6,12–14]. Hence, it will be described only briefly here. Two or three types of retention modes can be utilized within the same channel, namely normal (Brownian) and steric/hyperlayer (Figs. 1 and 2). After sample injection, the sample molecules are distributed homogeneously across the channel thickness (w), and are being pushed towards the bottom of the channel by the applied external, hydrodynamic force field. Finally, an exponential concentration distribution at the accumulation wall is built up. Since the accumulation wall acts as a barrier to the particles, the net movement of the sample species towards the external field is caused by diffusion from an area of high concentration at the accumulation wall to an area with lower concentration. After a certain relaxation period, a dynamic steady state is reached. A dimensionless retention parameter λ is defined as a ratio of l (the average distance between the sample particle and the channel wall) to w. For FlFFF λ is related to diffusion coefficient D of the sample particle, channel void volume V^0, cross flow rate $\dot{V}_c$, and w by:

$$\lambda = \frac{l}{w} = \frac{DV^0}{w^2\dot{V}_c} \quad (1)$$

In Eq. (1), V^0 and w are constants from the physical geometry of the channel, and $\dot{V}_c$ is the measurable volumetric flow rate. Separation of different particle zones in the channel is therefore based solely on the differences in diffusion coefficients of the particles [12]. Due to the parabolic flow profile, particles will migrate through the channel differentially according to their distance (l) from the accumulation wall. Smaller particles, which are located in the middle of the channel where the flow is faster, are eluted earlier. Larger particles are relatively closer to the accumulation wall and thus are eluted later. The retention ratio R, which is the ratio of the retention time t^0 of an unretained solute to the retention time