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Review

Field-flow fractionation in bioanalysis: A review of recent trends

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ABSTRACT

Field-flow fractionation (FFF) is a mature technique in bioanalysis, and the number of applications to proteins and protein complexes, viruses, derivatized nano- and micronsized beads, sub-cellular units, and whole cell separation is constantly increasing. This can be ascribed to the non-invasivity of FFF when directly applied to biosamples. FFF is carried out in an open-channel structure by a flow stream of a mobile phase of any composition, and it is solely based on the interaction of the analytes with a perpendicularly applied field. For these reasons, fractionation is developed without surface interaction of the analyte with packing or gel media and without using degrading mobile phases. The fractionation device can be also easily sterilized, and analytes can be maintained under a bio-friendly environment. This allows to maintain native conditions of the sample in solution.

In this review, FFF principles are briefly described, and some pioneering developments and applications in the bioanalytical field are tabled before detailed report of most recent FFF applications obtained also with the hyphenation of FFF with highly specific, sensitive characterization methods. Special focus is finally given to the emerging use of FFF as a pre-analytical step for mass-based identification and characterization of proteins and protein complexes in proteomics.

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1. Introduction

The explosive request of methods for the analysis of complex biological samples has involved a continuous development of improved separation techniques with a wide range of applications, adequate resolution, and versatility. Liquid chromatographic (LC) and capillary electromigration techniques are the separation methods most widely used and applied to a large variety of samples of biological interest. Field-flow fractionation (FFF) is also emerging in this field for its unique peculiarity to separate macromolecular, supramolecular and particulate analytes in a very broad molar mass range and under mild instrumental conditions [1]. The FFF principle does not rely on interaction of the analyte with a stationary phase but with an externally generated field, which is applied perpendicularly to the direction of the mobile phase flow. Under these conditions it is possible, for instance, to separate directly in a given biological fluid functional proteins like enzymes while keeping their activity, living cells, non-covalent aggregates or adducts. This opens a large number of analytical opportunities in functional proteomics, protein drug characterization, cell sorting and in biosciences in general.

The FFF mechanism and the elution modes have been exhaustively described in previous literatures [2,3]. Briefly, in FFF the separation is achieved within a capillary, empty channel in which a laminar flow of mobile phase sweeps sample components down the channel. The field is applied perpendicularly to the parabolic flow to drive the analytes into different laminar flows due to differences in their size, density, and surface properties, resulting in different retention times. In the normal elution mode, retention times are shorter for lower molar mass/size analytes. When analyte diffusion becomes negligible, as in the case of micronsized particles, the elution order is in fact reversed. That is, larger particles are eluted more rapidly than smaller particles. The elution mode is called steric/hyperlayer, and retention depends on size and other physical features of the sample particles, such as their shape, density, rigidity, and surface features. No matter the elution mode, the retention mechanism is always sufficiently "soft" to fractionate analytes in their native conformation, making FFF particularly interesting for applications in bioanalysis.

2. FFF technologies and devices

The basic configuration of most common FFF devices is based on a rectangular, flat-type channel obtained by cutting a plastic, thin foil that is sandwiched between two flat walls. Depending on the applied field, different technical implementations are required.

The use of a second flow stream as the hydrodynamic field to develop separation makes the flow field-flow fractionation (F4) technique. This is in general the most developed and applied FFF methodology, which can be found in the market as symmetrical F4 (SF4) [4] or asymmetrical F4 (AF4) [5] variants. The latter is characterized by only one permeable channel wall, which is an advantage in terms of channel simplicity and cost. AF4 uses only one pump to generate both the longitudinal and the cross-flow, and it allows for sample focusing before the elution, which is also an advantage in terms of separation efficiency. This variant has been finding the broadest application, and it is commercialized by Wyatt Technology Europe (http://www.wyatt.de/) and by Postnova Analytics (http://www.postnova.com/). The latter company also commercializes F4 technology to realize hydrodynamic (in-flow) sample relaxation (frit-inlet, FI F4 [6]) and outlet sample concentration [7] to increase sample detection. Application of a cross-flow has been found also possible using a cylindrical, porous channel. The variant, which employs a polymeric or ceramic hollow-fiber (HF) as fractionation channel (HF5) [8], shows promising features, though it is still at a prototype stage. HF5 has been showing a fractionation performance that is comparable to that of flat-type F4. It is also a micro-volume technique since the channel volume is about 10-fold lower than the volume of commercial flat-type channels [9]. The low-cost of the HF channel allows for possible disposable usage, and this is particularly relevant when used for biological samples to minimize biohazard, reduce sterility issues, and avoid run-to-run sample carry-over.

In centrifugal, sedimentation FFF (SdFFF) the channel is spooled inside a centrifuge bowl [10]. To avoid carrier liquid leakages, the SdFFF apparatus requires sealing parts spinning at high-speed, which involves some technical complexity. The SdFFF technology was implemented into an ultracentrifuge, and it was commercialized as SF^3 by DuPont. SF^3 allowed high-speed rotation and, therefore, the application of high sedimentation fields. This allowed to broad the limit of application from particles to high molar-mass bio-analytes such as nucleic acids [11–13]. However, likely because of the high investment and maintenance costs, SF^3 did not fulfill market expectations and commercialization was suspended by Dupont. A lower-intensity field, SdFFF machine is currently commercialized by Postnova Analytics.

Since early FFF developments, the application of Earth's gravity as sedimentation field (gravitational FFF; GrFFF) has been proposed [14]. Application of Earth's gravity makes GrFFF the simplest technique from a technical point of view. The channel is made of glass or plastic walls, and it can be inserted into a system for low-pressure LC or FIA [15,16]. GrFFF is still a prototype technology, whose application is limited to analyte particles that are sufficiently big and/or dense to sediment in the Earth's gravity.

Other variants using different fields such as thermal (thermal FFF; ThFFF) or electrical (electrical FFF; ElFFF) fields have been also applied to bioanalytes, with their microfluidic variants showing interesting peculiarities that are typical of microfluidic separation systems [17]. FFF-like systems using split-flow thin cells (SPLITT) for continuous, preparative-scale fractionation, of macromolecules and particles are also present in the market (from Postnova Analytics). SPLITT develops in a thin, rectangular channel where flow splitters are located at both ends of the channel. Samples are separated on a preparative scale into two size-based fractions [18,19].

3. From stand-alone to hyphenated FFF

Because of the intrinsic possibility to obtain a size/mass characterization of the analyte, pioneering applications in the bioanalytical field were in most cases based on stand-alone FFF, and oriented for both the separation and the biophysical characterization of the analytes [20]. A list of pioneering applications of FFF to bio-analytes ranging from proteins to whole cells is reported in Table 1. However, thanks to the unique peculiarity of FFF over conventional separation techniques to perform a non-invasive separation or enrichment of the analytes from complex biological systems like biological fluids or cell lysates, its integration with other analytical methodologies soon showed appealing perspectives. Hyphenation of FFF with high-sensitivity, orthogonal methods has recently shown to substantially amplify the