

Preface

The collection of analytical techniques suitable for separation and characterization of fragile biopolymers contains, among many others, a group of methods collectively referred to as Field-Flow Fractionation (FFF). Common to these methods is that they are liquid phase elution techniques, in which the separation is executed in open channels unobstructed by solid packing materials, and that they offer a wide resolution range particularly well suited for macromolecules and particles. Recently, these techniques have had a strong upswing in use, especially due to the increased availability of convenient-to-handle commercial instrumentation. The FFF techniques differ from each other in terms of the field chosen to accomplish selectivity, e.g. thermal, gravitational, electrical, etc. Today, the hydrodynamic “flow field” is most commonly used, and hence the present collection of articles focuses extensively, although not exclusively, on a number of attractive applications of flow FFF to problem solving in the biomedical field. The growth of a technique brings with it nonuniformity in terminology. For example, asymmetrical flow FFF is commonly designated as AsFIFFF or AF4. This variation is apparent in the published literature and was purposefully maintained in this book.

Chapter 1 describes the theory of flow FFF, both in the symmetric and asymmetric channels presently in use. The evolution and fine-tuning of the technique is discussed in conjunction with the effects of channel dimensions and operating conditions on retention and resolution.

Chapter 2 discusses the choice of membrane to serve as sample accumulation wall in the flow FFF channel. The discussion leads to a scrutiny of sample recovery in relationship to membrane composition and zonal compression (retention).

Chapter 3 introduces the tubular, hollow fiber flow FFF channel which provides the advantage of being easy to replace, as one eliminates cross-over between runs. Through this approach sample volumes can be kept low to allow for MS-analysis on line.

Chapter 4 advances the technique into the 2D domain, where the first dimension is an isoelectric focusing and the second is a size-based separation accomplished by

asymmetric flow FFF. The system design is described and the technique is proven amply suited for problem-solving in proteomics.

Chapter 5 illustrates the use of flow FFF in pharmaceutical problem solving. Target identification and development of production processes are discussed in conjunction with process analytical technology formulation (PAT) and use in the discovery phase of protein therapeutic development.

Chapter 6 is another pharmaceutical application. It examines the analytical reliability of flow FFF and compares it to the performance of AUC and the work horse SEC in characterizing pharmaceutical proteins in terms of purity and aggregation.

Chapter 7 constitutes a detailed study on protein aggregate formation in the flow FFF channel, with or without crossflow.

Chapter 8 illustrates how the flow FFF technique, unlike the packed bed based SEC, can demonstrate weak protein interaction ($K_D > \mu\text{M}$) and analyze the components participating in complex formation under different conditions.

Chapter 9 examines the wide resolution range of the FFF techniques and demonstrates its particular value for particles produced for drug delivery and as an on-line sample clean-up tool to remove non-specific background molecules and enhance signal-to-noise ratio in immunoassays.

Chapter 10 demonstrates how highly complex protein structures, such as prions, can be purified and analyzed using flow FFF thus allowing correlation of protein aggregate size and structure to infectivity.

Chapter 11 presents the sedimentation FFF technique in its capacity as a sensitive mass balance which allows an exact and reproducible determination of the number of molecules – be it proteins or synthetic polymers- that are introduced to a nanoparticle surface during modification. This quantification allows a determination to be made e.g. of the specific binding of a protein to its substrate.

Chapter 12 gives a polymer chemist's use of the combination Flow FFF/MALS in the analysis of a range of starches and other polysaccharides in terms of e.g. molecular weight, size, and branching.

Chapter 13 addresses nanoparticles used for drug and gene delivery and the required evaluation of size as well as load. The AF4 is shown to be invaluable in determination of both size and size distribution, comparing favorably with DLS, AUC, and a number of microscopic techniques. The chapter contains an extensive literature review of FFF analyses of drug and gene delivery systems.

Chapter 14 discusses the studies of size and size distribution of liposomes, especially those intended for drug delivery purposes. The Flow FFF /MALS is shown to provide detailed insight into shifts in these parameters caused by shifts in fabrication conditions.

Chapter 15 demonstrates the ability of sedimentation FFF to sort populations of mammalian cells in terms of degree of maturation, differentiation and apoptosis. The cells remain undamaged by the sorting, which does not require binding of markers or specific identifiers to the cell surfaces.

Chapter 16 cells can be typed and enriched in miniaturized flow channels by dielectrophoretic FFF for which a theory is outlined in this chapter. The technique is

highly specific and does not require the binding of antibodies or other marker identifiers.

Chapter 17 reviews the use of flow and sedimentation FFF to determine size distributions of environmental and engineered nanoparticles. Nanoecotoxicity is an emerging field. Here size is an obvious characteristic of importance, as it relates to uptake and organ penetration. Hyphenation of the FFF channels with the element sensitive ICP-MS is shown to be of unique value in pinpointing environmental metal transport and understanding toxicity.

Golden, CO, USA
Uppsala, Sweden

S. Kim R. Williams
Karin D. Caldwell

Field-Flow Fractionation in Biopolymer Analysis

Williams, S.K.R.; Caldwell, K.D. (Eds.)

2012, X, 306 p., Hardcover

ISBN: 978-3-7091-0153-7