by Robert L. Stevenson

When to Choose FFF: Interview With Dr. Christoph Johann of Wyatt Technology

Field-flow fractionation (FFF) has slowly evolved since its introduction in 1976. Its principal advantage is the robust, size-based separation of protein aggregates, large polymers, and subvisible particles. In this regard, FFF complements size exclusion chromatography (SEC), which also separates molecules by size, but starts to lose resolving power as one moves up in the mega-dalton (MDa) range. While FFF can readily separate particles from 1 nm and up, the sweet spot for FFF is MDa to about one micrometer.

Over the decades, several cross-channel force fields have been applied to accentuate selectivity in separations. These include centrifugal force created by a rapidly rotating channel, thermophoresis, and electrical charge. However, hydrodynamic cross-flow, called flow FFF, or F4, is the most useful today.

Despite the early invention of F4, it took decades for interest in nanoparticles to mature into a vibrant field of investigation to the point where F4 could be commercially viable. But look where we are today: particles, inorganic/organic hybrids, and biologicals are active research topics that all benefit from F4.

FFF is a potentially useful tool that should help research in these vertical silos. With this background, I was privileged to interview Dr. Christoph Johann, a world-recognized leader in FFF technology.

Dr. Christoph Johann has been manager for Eclipse® FFF products at Wyatt Technology since October 2018. He earned his Ph.D. in 1985 in Physical Chemistry at the University of Mainz. Dr. Johann has been active in polymer and biopolymer analysis for over 30 years and has several peer-reviewed publications in the field of macromolecular characterization. In 1991, he introduced the first commercial field-flow fractionation systems in Europe. In 1993, he founded Wyatt Technology's main European subsidiary. Some of his more recent innovations include the development of hollow-fiber flow-FFF and electrical/asymmetric-flow FFF.

RLS: You were involved in the development of Wyatt's Eclipse FFF instrumentation, introduced in 2002, and shepherded it through several generations of technological breakthroughs. Can you give some background?

CJ: The Eclipse FFF product was started in Europe and was produced by a spin-off from Wyatt Technology Europe called Superon. Wyatt Technology has recently decided to expand its FFF portfolio by bringing Eclipse development and production to Santa Barbara, CA. Further development of the Eclipse product line will profit from the know-how of the team in Santa Barbara and the production infrastructure, which has made Wyatt the industry leader in light scattering and macromolecular characterization.

RLS: You've been involved in FFF for decades. How did you get started?

CJ: Yes, as a representative for Wyatt's multi-angle light scattering (MALS) detectors that were typically used to characterize macromolecules for molar mass and size, the first question many visitors at our trade show booth would ask is, "Can you do particles?" I was intrigued with the capability of FFF to separate particles so that they can be characterized in detail by MALS, providing detailed size distributions well beyond the capabilities of dynamic light scattering. After attending one of the seminars that Cal Giddings did during the years 1989 to 1992, I got hooked on FFF and started to distribute the instrumentation from Giddings' spin-off company in Germany. Once we got started with FFF, we honestly could say, "Yes, we can."

RLS: Back then, what were the most interesting applications of FFF?

CJ: The ground was laid by Giddings' publications, which showed a comprehensive list of applications—mostly particles, which are too large to be separated by SEC. At that time, particle size was determined by elution time and calibration standards, similar to analytical SEC. Wyatt gave FFF an extra boost via the combination with MALS. This turned out to be a crucial step to make it valuable to customers, and in fact one of the first sales I got in Germany was for liposomes used in a drug-delivery application by Schering (now Bayer). Using light scattering, you could actually see that the separation was successful and simultaneously determine size in an independent, absolute manner.

RLS: I recall that early flow chambers were frustrating to use. How have they evolved?

CJ: The starting point was symmetrical-flow FFF. I used it for years in the 1990s and sold it to quite a few customers. Most gave up on it after struggling for some time to keep it up and running. It was just too tedious and unreliable. The breakthrough was AF4, the asymmetrical variation introduced by Karl-Gustav Wahlund. I jumped horses in 1995 for AF4 (working with a small company in Germany) before making the decision to build my own instrumentation and eventually rejoin forces with Wyatt Technology.

RLS: Tell me about the evolution of instrumentation for AF4.

CJ: I had experience in how customers new to FFF struggled to understand and operate it. The early systems required operating three pumps, which was clumsy and intimidating to many. So, I came up with the concept of using only one pump and splitting the flows using computer-controlled needle valves and flowmeters, which is far more elegant and robust. The advanced design integrates with a standard isocratic HPLC stack (the same as SEC) from leading LC suppliers such as Agilent, Shimadzu, and Thermo to generate a streamlined, reliable, and automated FFF system.

Later on we developed hollow-fiber flow-FFF (HF5), which requires just nanograms of sample, and have just recently released a new technology, the Eclipse Mobility® electrical/asymmetric-flow FFF (EAF4) system, which performs 2-D separation of macromolecules and nanoparticles by size and electrical charge. In terms of user interface, we originally provided Eclipse drivers for use with the native LC software, but recently we introduced the VISION® software platform, which is specialized for FFF and controls the HPLC components.

RLS: What applications were empowered by AF4?

CJ: The highlights are analysis of complex samples consisting of soluble macromolecules, insoluble particles, and/or aggregates. The large size range necessary to separate these samples in one run is the unique capability of AF4, while MALS provides the desired details of size, molecular weight, and conformation. We have seen the widest adoption and most promising applications in biopharmaceuticals, especially monoclonal antibodies; nanomedicines including liposomal/polymersomal drug delivery nanoparticles; environmental tracer nanoparticles; cosmetic nanoparticles and emulsions; and food applications such as polysaccharides and starches, which form very large polymers, as well as nanoparticle contaminants. Separation and characterization of the proteins and polysaccharides in beer was particularly popular with some of our application scientists!

With the explosive expansion of gene-delivery drugs, we will see an equally rising interest in FFF for gene-vector and protein-nucleic acid complex analysis in the next couple of years. An exciting new application is the isolation and characterization of exosomes and similar bionanoparticles, where FFF has been shown to be a very powerful research platform.

RLS: Reproducibility results are a major concern today. How reproducible are FFF results?

CJ: FFF has come a long way in that respect. Starting from full manual control, even turning needle valves by hand, now we are speaking of sophisticated, high-precision systems. In our software, we collect all the system traces in addition to the detector signals. You can overlay these traces for a series of runs and convince yourself and others of the perfect reproducibility of the cross-flow rates, pressure in the channel, injection flow rate, etc. The variation in terms of retention time is comparable to SEC, though of course with MALS detection this is not such an important factor.

RLS: Can FFF be quantitative?

CJ: Very similar to SEC, if you do it right, FFF is just as quantitative. You have to choose the right conditions to avoid absorption or aggregation of your sample, and then the analysis is quantitative and precise.

RLS: What is the state of support software for FFF control, data analysis, and report generation?

CJ: In our VISION software platform, we have a comprehensive, integrated software suite to perform instrument control and data acquisition (VOYAGER CDS®) and data processing (SCOUT DPS®). VOYAGER allows you to run sample sequences in one place, controlling the HPLC system and all Eclipse modules, with a user interface optimized for FFF-MALS. ASTRA® software, which carries out light-scattering data acquisition and analysis, is controlled in the background through an automation interface.

SCOUT uniquely performs two critical tasks: virtual method development, via numerical simulations of the hydrodynamic fractionation physics, and data analysis to determine hydrodynamic radius and (if running EAF4) zeta potential. Though in the past FFF method development has been a tedious, time-consuming task, SCOUT's virtual method development really unleashes the power of AF4—it empowers users to analyze samples rather than develop methods. For analysis, SCOUT has automatic baseline and peak detection, and allows you to enter elution-time calibrations based on first-principles fractionation physics, or with a series of standards (similar to SEC).

Report generation is quite flexible. All the data can be compiled for output in a Word-compatible file, from which it can be copied or exported to Excel or other applications.

RLS: What are the most interesting applications of electrical-flow FFF?

CJ: Colloidal stability and interaction of macromolecules and nanoparticles depend on charge. With electrical-flow FFF (EAF4), it is possible to determine the charge of multiple components in a mixture, which is not possible with standard zeta potential analyzers. The charge is calculated based on a shift in retention time when an electrical field is applied. Just by looking at the fractograms, you can understand if the sample is charged and what the polarity is. SCOUT will provide a fully quantitative data analysis and generate mobility, zeta potential, and charge values for each particle size present.

RLS: What are the most interesting applications of hollow-fiber FFF?

CJ: I see coupling to mass spectrometry as one application. We have published data on coupling to ICP/MS for gold particles. There are also publications on using hollow-fiber FFF coupled to MS for protein characterization.

RLS: You are a visionary in separations. What do you expect in the next decade?

CJ: I see the development of more robust and error-tolerant systems. Expert software will give guidelines to users on how to improve the separation. With that, we will see that FFF becomes an accepted method in release assays and QC applications.

RLS: From my catbird seat in separation science, I've enjoyed watching the growth of various techniques such as SFC, HPLC, and FFF. The key driver of growth is always driven by the underlying need of the science. Small-molecule pharmaceuticals pulled HPLC along, petrochemicals were responsible for GC and GC/MS, and proteomics would not have happened without LC-MS/MS. Today, FFF is enabling rapid progress in material science and nanomaterials. The need has finally caught up to the capability of FFF.

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