



Live Webinar Q&A Sheet:

Breakthroughs in FFF-MALS – new levels of performance and usability

The recorded webinar may be viewed from the [FFF](#) webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, CG-MALS, RT-MALS and FFF may be found on the Wyatt web [Library](#) under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding [Product page](#) and [Solutions](#) page of our web site.

Please contact info@wyatt.com with any additional questions.

EAF4

Q: How do zeta potential results compare between EAF4 and batch zeta potential measurements using light scattering?

A: Validation experiments using polystyrene particles have shown consistent values between EAF4 and PALS (Phase Analysis Light Scattering). But there can be differences depending on the sample type, because the solvation conditions are different in the EAF4 channel, where the sample is close to the membrane, versus batch light scattering measurements, where the sample is confined in a low-volume cell. Zeta potential depends on the ionic strength in the direct vicinity of the particles, and this can be different between the two methods even if the bulk ionic strength of the solution is the same.

Q: How can I determine an inhomogeneous charge distribution within a sample using EAF4?

A: This is detected by comparing the shape of the fractograms of experiments with increasing electrical field strength (current). If the shape stays the same, in other words the fractogram is shifted in retention time without changing shape, then the charge is homogeneous. If it changes, that indicates fractions within the sample have different charge. In the presentation an example was shown of a liposome which developed a shoulder with higher fields; the mobility and zeta potential could be calculated for both populations within the sample.

Q: For which types of samples is EAF4 applicable? Are there size limitations?

A: EAF4 can be applied to any sample for which the FFF separation is possible. But the shift in retention time is proportional to both the charge and size. This means that the measured effect is smaller for smaller sizes and this can be a limiting factor for proteins or peptides, where a higher current must be applied to create a significant shift in retention time. EAF4 is most suitable for particles larger than 10 nm in radius.



Dilution control module

- Q: If we already have a previous model of FFF instrument and channel, do we need to buy new ones in order to have the DCM feature?*
- A: The additional fluidic control units are installed inside the Eclipse controller, so both the new channel and the new Eclipse are needed for DCM.
- Q: Can DCM flow be monitored for any analyte?*
- A: Yes, the DCM functionality is not dependent on analyte properties, or solvent properties for that matter.
- Q: What is the range of DCM control?*
- A: DCM can control split ratios of up to 20:1 with respect to the channel flow. To avoid excessive secondary band broadening of the sample, the detector flow should not be less than 0.1 mL/min.

Computer-aided method development

- Q: What can I expect from the computer-aided method development? Is the same as SCOUT DPS™?*
- A: Computer-aided method development (CAM) uses FFF theory to calculate a fractogram based on the assumed particle sizes, a flow program and a channel geometry. The result is the theoretical limit of what can be expected of the separation. The user can immediately confirm that the result is satisfactory for the given task, e.g. a dimer is to be separated from a monomer species, then the calculation will confirm that this is possible under the entered conditions. CAM helps to avoid extraneous experiments, because the real experiment cannot be better than the calculation.

After optimizing the method via CAM, a real experiment will generally exhibit lower resolution or different retention times. If that is the case, there could be several reasons including a different channel height than assumed, self-interaction of the sample or non-ideal sample-membrane interactions. A correct channel height can be fed back into the simulations, but for the other causes, the task then is to optimize the carrier solution composition, type of membrane, sample prep, etc. However, there is no need for more experiments to test different flow programs.

Computer-aided method development for FFF was previously available in Wyatt's SCOUT software. It has been extended and the new features of the Eclipse controller have been added to what we now call VISION DESIGN.

- Q: Does Wyatt Technology have an introductory course or webinar on how to use VISION DESIGN or SCOUT for analysis?*
- A: Yes, this is part of the curriculum of the LSU/FFF training courses. Thanks to COVID-19 we have online seminars on this topic.



Solvent conditions

Q: Is the FFF system compatible with organic solvents? Have you ever used organic solvents like hexene, etc.?

A: Yes, the Eclipse FFF system is compatible with both aqueous and organic solvents like THF, toluene, hexane, alcohols and others. Hexane, hexene or other non-polar solvents should work well as long as the sample is well soluble in the respective solvent. Strongly polar organic solvents like DMAC, DMF and such are problematic because the membranes provided do not withstand these solvents. However, you can prepare a membrane from a compatible material such as PTFE using the membrane template kit.

Q: Can you use gradient flow for FFF?

A: Running a solvent gradient by changing the composition is not done in FFF, since the solvent composition does not change the separation in a meaningful way. 'Gradients' in FFF usually refer to decreasing the cross-flow rate during elution, which makes the separation more effective for samples with a broad size distribution.

Q: What is the range of solvent viscosity that the Eclipse can support?

A: There are no fixed specs on the viscosity. Since channel pressure increases with increasing viscosity, the maximum pressure limit will be reached at a lower overall flow rate with a more viscous solvent. The new channel heating capability helps reduce solvent viscosity.

Q: If we use different compositions of carrier solution, standard retention might change, so how can we confirm the target sample's hydrodynamic size even standards' size also changed?

A: The best way is to measure the hydrodynamic radius in FFF is by means of online dynamic light scattering. This becomes feasible even for challenging samples with the DCM function, where we can increase sample concentration and reduce the detector flow rate.

Applications

Q: Are you working on collecting fractions for spotting onto cryo-EM grids?

A: FFF fractions may be collected for use with TEM, and the new DCM feature will also concentrate collected fractions. However, we do not have any automated means of spotting on cryo-EM grids.

Q: Can magnetic nanoparticles be analyzed by AF4-MALS-DLS?

A: Magnetic nanoparticles can be analyzed in the same way as other particles. In Flow FFF the magnetic property does not influence the separation; only the diffusion coefficient determines the retention time.



Q: You mentioned using an Agilent HPLC for solvent delivery. How about other vendors e.g. Thermo, Waters? Or HPLC versus UHPLC?

A: The new Eclipse system only works with the Agilent 1260 Infinity II pump and autosampler as front-end. Generally UHPLC equipment is not suitable, because the narrow tubing is prone to plugging.

Q: Can you use EAF4 to separate polyelectrolyte complexes?

A: Certainly polyelectrolytes can be separated; we have examples of analyzing pectines. It would certainly be worth a try to work with complexes.

Q: Can EAF4 separate empty from full AAV capsids?

A: This still needs to be determined as we have not yet performed the experiment to separate full and empty. This is on our to-do list and the results will be published on our website.

Q: Can FFF separate charged branched polymers from charged linear polymers?

A: This sounds like a really complex sample. It can be expected that the sample is polydisperse not only in molar mass and branching characteristics, but in charge as well. This would lead to a polydisperse mixture eluting at any point in time. A way to get a handle on this, could be to use a semi-preparative channel to generate fractions and then re-inject those fractions to the EAF4 channel.

FFF general questions

Q: How difficult is it to learn FFF compared to SEC?

A: The main difference in FFF compared to SEC lies in the flexibility of different flow programs and channel geometry combinations. Most of the complexity involved in this plethora of parameters is removed using VISION™ DESIGN™'s computer-aided method development which reduces the time needed to familiarize oneself with the different separation properties of FFF. Furthermore, Wyatt offers documents and scientific support to aid in initial method development. The experimental handling is very similar to SEC: the same pump, autosampler and detectors are used in exact the same way.

Q: How long does a typical FFF experiment take?

A: Typical analysis time is between 10 minutes and one hour. The majority of methods are around 30 minutes, which is very similar to SEC using 8 mm i.d. columns. The difference here with respect to SEC is, in FFF the analysis time can be adjusted over a wide range without having to change the hardware.



- Q: What makes FFF a more gentle technique than SEC? Is not the pressure similar in both techniques?*
- A: Although the pressure may be similar, the difference is in the shear force and surface area of the packed columns compared to the open FFF channel. As the sample permeates through the SEC column's interstitial volume and diffuses into the pores, large shear forces are exerted on the macromolecules. This is not the case in FFF. Also, the few square cm of membrane are minimal compared to hundreds of square meters of surface of the column packing.
- Q: Can you run an on-line viscometer with the DCM connected?*
- A: At this point we have not done validation experiments, but we expect that this will be possible and the results far more satisfactory than in other FFF models since the DCM maintains very precise flow rates.
- Q: What are the major differences between new Eclipse and the previous DualTec model?*
- A: The new Eclipse improves analytical performance with features like the Dilution Control Module (DCM), channel heater, and better flow control. The new instrument also makes it easier to monitor your experiment and be alerted to any issues thanks to the front panel display. Finally, the SEC switching option has been upgraded through improvements in the channel, software, and hardware.
- Q: Does the software track the number of injections across a specific membrane, much like can be done for columns?*
- A: Yes, this is exactly how it is done. When a new membrane is installed, the counter is restarted (manually, since there is no bar code reader for membranes).
- Q: What type of membranes are available for charged polymers?*
- A: Currently we have regenerated cellulose and polyethersulfone membranes in various molecular-weight cut-offs. We do not offer membranes specific to charged polymers.
- Q: What is the regular maintenance for FFF (daily, monthly, annual)? What is the cost of this maintenance?*
- A: Daily maintenance mainly involves maintaining clean buffers and preventing organic growth in aqueous systems. Care should also be taken to minimize sample interaction with the system. Changing the membrane and cleaning the channel is done once or twice a month. With the new Eclipse controller, annual maintenance is reduced compared to the previous models since there are no more needle valves which needed to be cleaned or replaced. Wyatt offers preventative maintenance contracts which cover all the required maintenance work.



Q: What is the pH range of the channel?

A: The channel itself is made of stainless steel and ceramic, so the pH range only depends on the membrane. The range of the regenerated cellulose membrane is pH 3 to 12. Using a polyethersulfone membrane, the range is pH 1 to 14. There are also limitations on the detector side; for extended durations, the MALS flow cell should only be exposed to pH 1 - 10.