Latex Nanoparticle Analysis by Flow FFF - DLS coupling

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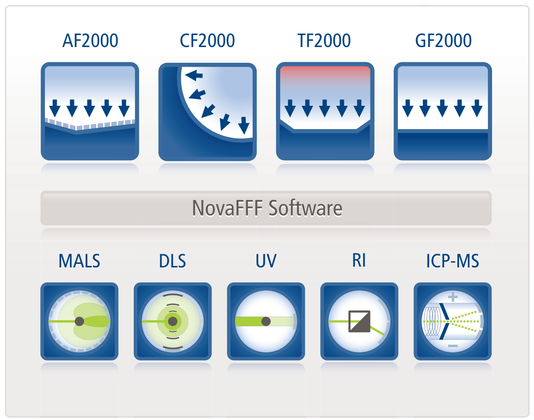
Introduction

Field-Flow Fractionation (FFF) technology has developed to a powerful separation technique capable for the analysis of various nano- and macro-sized sample types. Depending on the technique applied, FFF covers an entirely sample size range between 1 nm up to several microns. This enables the scientist to analyze particles, polymers and biomacromolecules, determine size- and molecular weight distributions of several magnitudes (10^3 Da - 10^{12} kDa, 1 nm - 100 µm, respectively). In particular, Flow FFF (AF4) and Centrifugal FFF (CF3) have become the state-of-the-art technique perfectly fitting to light scattering detection. Consequently, FFF online-coupling of suitable light scattering detectors (DLS, MALS) features integration of real-time separation and analysis. This is highly recommended for any scientist interested in highest resolution and reproducibility.

The FFF Platform

- Asym. Field-Flow Fractionation (AF2000)
- Centrifugal Field-Flow Fractionation (CF2000)
- Thermal Field-Flow Fractionation (TF2000)
- Gravitational Field-Flow Fractionation (GF2000)



FFF-Application range

Analyte Size and Weight

Analysis of nano- and micro sized particles, oligomers, high- and ultra high molecular weight polymers with state-of-the-art FFF-Technologies.

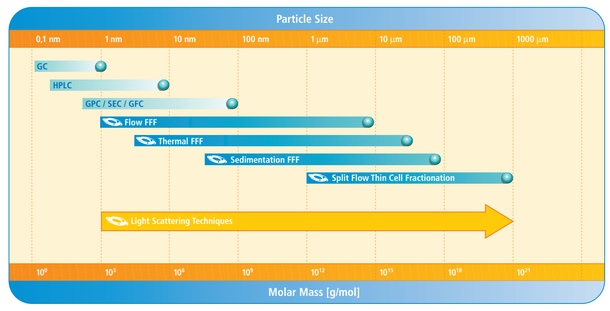


Figure 1: FFF-Platform and esteablished chromatographic techologies

Investigation of Latex Nanoparticle Standards

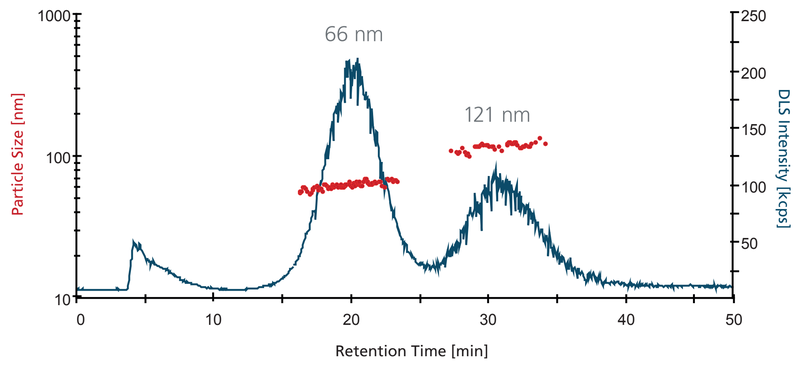
Online-coupling of AF4 and DLS for real-time resolved analysis

Flow FFF (AF2000)

Centrifugal FFF (CF2000)

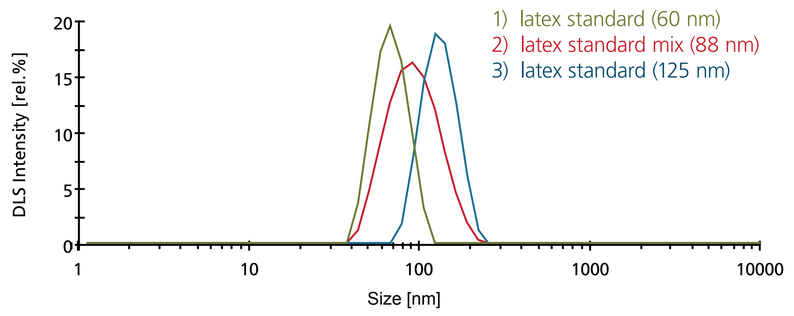
Malvern Zetasizer Nano ZS®

Replacement of cell for change between online and batch DLS analysis mode



Retention Time (R_T)	Hydrodynamic Diameter (D_h)
16.5 - 24.1 min	66 nm ± 4 nm
27.5 - 34.5 min	121 nm ± 5 nm

Figure 2: AF4 Fractogram and size of latex particle.mix using monodisperse standards



#	Hydrodynamic Diameter (D_h)	Polydispersity Index (PDI)
1	66 nm	0.008
2	88 nm	0.113
3	125 nm	0.024

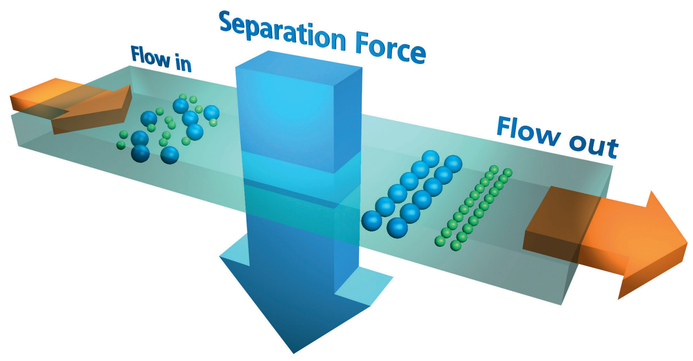
Figure 3: Traditional batch DLS measurements of.monodisperse and mixed latex standards

➡ Traditional batch measurements provide accurate data for mono disperse standards
➡ Batch mode is not applicable for sample analysis of polydisperse and broad samples due to:

- Calculation of average size distributions
- Discrimination of small particles due to strong scattering caused by large particles
- limited DLS resolution

Field-Flow Fractionation - DLS Benefits

➡ Total sample characterization using different FFF variants
➡ Integration of Separation and Detection by FFF-DLS online-coupling within a single run
➡ Highest reproducibility and resolution by real-time resolved measurements („true" size distribution, no averaging effect, no small particle discrimination)
➡ The FFF-Platform allows selection of the most appropriate FFF-technique coupled to various detectors of choice, e.g. MALS, DLS, UV, RI, MS
➡ Large size range of sample (10^3 Da - 10^{12} kDa, resp. 1 nm - 100 µm)



Conclusions

The FFF-Platform using different FFF variants in combination with appropriate detectors, such as DLS or MALS is a highly valuable tool for accurate analysis and reliable results. It was shown that FFF-DLS online-coupling is a powerful tool for analysis of mixed latex nanoparticles as traditional batch DLS measurements suffer from a „size-averaging" effect generating missleading data! Therefore, the integration of separation and detection shown here represents the method of choice for any scientist to obtain best results for mixed, polydisperse and broad distributed nano-sized samples.