Asymmetric-Flow Field-Flow Fractionation AF4

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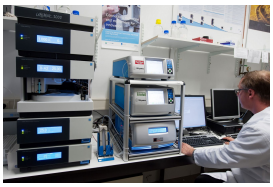

Brand

Wyatt/Dionex

Type

Contact

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Organisation

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Description

Asymmetric flow field flow fractionation (AF4) an analytical technique to separate larger molecules, aggregates and particles based on their size. It is an interesting alternative for size-exclusion chromatography (SEC).
The asymmetric flow field flow fractionation (AF4) is a technique to separate larger molecules (e.g. proteins, polysaccharides), aggregates (e.g. heat induced protein aggregates) and particles based on their size. This separation is obtained by the difference in mobility in the flow field induced by the liquid flow over the membrane and across the channel. AF4 can thus be used for separation of various sizes in a mixutre and obtaining their solution of physical properties. The set-up has a number of different detectors, together allowing the determination of:

• molar mass
• root mean squared radius (of gyration)
• second virial coefficient of the eluting compounds

Information obtained by AF4 analysis provides information on the diffusion coefficients, hydrodynamic radius and the shape of the aggregates.
Benefits of the AF4 over size-exclusion column chromatography are:
(1) the ability to separate both soluble and colloidal components over a wide size range, and (2) that in AF4 there is little or no specific interaction that can affect the elution of the samples (as no column material is used).

Technical Details

The AF4 system consists of multi angle laser light scattering (MALLS from Wyatt, Dawn Heleos-II), differential refractive index cell (dRI from Wyatt, Optilab T-rex) and UV absorbance cell (DAD, Diode Array Detector from Dionex). The AF4 system can be used with both the flat plate and hollow fibre cylindrical channel type flow cells. The flow conditions and data collection is controlled by Wyatt Eclipse separation system- Dual Tec. Eclipse is coupled with Dionex ultiMate 3000 UHPLC, which contains pump, auto sampler, column compartments and DAD.
AF4 results have been shown for the elution of single, monomeric proteins (e.g. bovine serum albumin, 66 kDa), larger proteins (Keyhole Limpet Hemocyanin 8.3 MDa), as well as silica particles (41-400 nm), illustrating the broad range of molecular weight / particle size that can (simultaneously) be analysed.

Applications

• Examples of selected systems which can be analysed with AF4 (but not limited to) are:
synthetic or natural (bio) polymers such as proteins, polysaccharides and liposomes
• various biological materials such as:

• vaccines

• viruses (virus like particles)
• aggregates (e.g. heat induced or covalently cross-linked proteins)
• complexes (e.g. protein-polysaccharide electrostatic complexes)
• conjugates (e.g. antibody-antigen conjugates)
• environmental samples such as colloidal soil suspensions
• nano/micro (colloidal) particles such as:

• chemical mechanical polishing slurries
• polymer latex particle
Sample requirements Samples should be prepared in filtered solutions (<= 0.2 mm filter) Typical injection volumes 10-50 mL Typical sample concentrations 1 - 5 mg / ml Particle size range 1 nm < R < 10 m (molecular mass ~ 3 kDa - 3*1012 kDa) Flow rates: total flow rate 0.2 - 10 mL/min typical channel flow rate 0.2 - 3 mL/min Typical run time per sample 20-40 minutes Typical solvents Water (millipore Low concentration buffers e.g. phosphate, 50 mM $NaNO_3$ Compatibility Ethanol, organic solvents, SDS pH range 2-12 Temperature range 10-70 °C Autosampler Up to 120 samples