OmniSEC

Analytical size exclusion chromatography (SEC)

Size exclusion chromatography is a method that separates molecules based on their size. It is based on a different retention of the molecules on a column with inert filling at a steady flow. When coupled to an array of detectors, it enables also a detailed analysis of composition of individual elution peaks. In the case of OmniSEC instrument, the detected features include refractive index, UV-VIS spectrum, static light scattering and intrinsic viscosity. This enables analysis of various types of samples, such as proteins and their complexes, nucleic acids, polysaccharides and nanoparticles. High pressure chromatography (1-20 MPa) ensures good separation of individual species, e.g. protein oligomers.

SEC can be used for:

- Determination of absolute molecular mass
- Analysis of the homogeneity of the sample – presence of microaggregates
- Oligomeric state determination
- Formation of complexes detection – for stable complexes, individual species can be detected and analyzed including the estimation of protein/nucleic acid content
- Quantification of individual species in the sample – if the species can be separated on the column
- Concentration determination – concentration is determined based on refractive index (or absorption) using an external standard molecule of known concentration
- Sample characterization at elevated temperature (e.g. 37°C)

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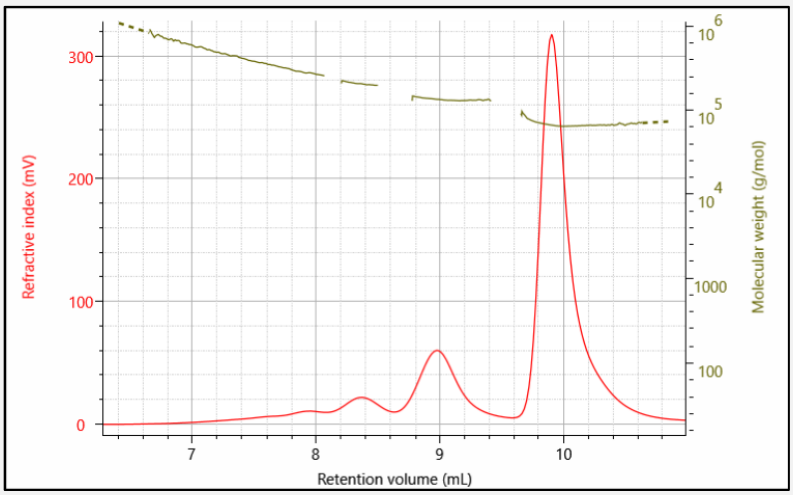
Technical specifications

Instrument: **OmniSEC** (Malvern)



Features:

- Autosampler for up to 2x96 samples in vials or microtiter plates
- 1-300 µl injection volume
- Temperature range: 20 - 65 °C (column and detectors), 4 - 60 °C (autosampler)
- Differential refractometer with ±2.5 x 10^{-4} RIU dynamic range
- Diode-array-based UV/Vis spectrometer with a range of 190 - 900 nm
- Two light scattering detectors (RALS at 90° angle, LALS at 7° angle) for accurate molecular mass determination over a broad range (200 - 10^7 g/mol)
- 4-capillary Wheatstone bridge viscometer

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Instrument operation

Assemblies:

- The instrument is equipped with a PLS3030 silica-based column (exclusion limit 1.25 MDa). A different column may be used if required and supplied by the user.

Data evaluation SW:

- OMNISEC v 11.21 SW is used for instrument control and data evaluation. The SW can be downloaded from the producer web page upon registration.

Operational mode:

The measurement is performed typically by CF staff. In case of high utilization of the technique by a single user, the user can be trained and perform the measurements himself/herself.

Provided services:

- Standard sample analysis (homogeneity, molecular weight, size)
- Instrument user training
- Basic SEC-MALS data evaluation training
- Consulting/assistance

Sample requirements

- Various aqueous running buffers can be used as long as they do have pH of 3-7.5. Aqueous buffers with organic modifiers such as acetonitrile, methanol and ethanol are also acceptable.
- Running buffer shall be supplied by the user. It has to be of high purity and has to be filtered by **0.1 µm filter** and degassed prior to use. A minimum of 500 ml of buffer is required for a single analysis, with 1000 ml of buffer, up to 20 sample injections can be performed.
- Sample volume depends of the concentration and the type of analysis. A typical injection of 50 µl is performed. For recommended analysis of triplicate, a total volume of 200 µL is needed (300+ µl preferred). The minimum volume is app 20 µl for a 1 µl injection.
- Instrument sensitivity depends on the size and properties of analyzed molecules. For a typical protein (BSA), as low as 50 µl of 0.02 mg/ml can be analyzed for homogeneity and MW. The precision of molecular mass, size and especially intrinsic viscosity calculation increases with higher sample concentration.
- Sample shall be filtered by **0.1 µm filter** or **centrifuged** (14,000 g/10 min) prior to injection.

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

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Instrument Location:

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