Absolute Molar Mass of Proteins and Antibodies with ÄKTA-MALS

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Technology	FPLC-MALS
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Separation of Proteins and Antibodies with ÄKTA Fast Protein Liquid Chromatography

FPLC (Fast Protein Liquid Chromatography) is a form of liquid chromatography that is often used to analyze or purify mixtures of proteins. The most widely used FPLC systems are ÄKTA Systems™ from GE Healthcare. ÄKTA systems are used for the purification, separation and characterization of proteins, antibodies and other biomolecules in the pharmaceutical area and the field of biomolecular research. A modern FPLC system is the ÄKTA pure™ system (Fig. 1), which is usually equipped with just a UV detector.

The UV detector is a concentration detector that is very sensitive toward protein and antibody samples. It is not possible to derive the molar mass of a protein or antibody sample directly from the UV detector signal. If ÄKTA users are interested in the molar mass of their samples they have to measure a series of protein standards with known molar mass from which they are able to generate a calibration curve of molar mass vs. elution volume. However, this calibration does not take account of the protein conformation and can only be viewed as an estimate at best.

Absolute Characterization of Proteins and Antibodies with MALS

A more reliable, precise and absolute molar mass of a protein or antibody sample can be obtained from a Multi Angle Light Scattering detector (MALS) that can be easily coupled to an ÄKTA system. Additionally, a Refractive Index (RI) detector is often used as a second concentration detector together with the UV detector.

The MALS detector directly responds to the molar mass of a protein or antibody sample. Hence, information collected from the MALS detector is independent from the elution volume of a sample.

Fig. 1: ÄKTA pure™ system from GE Healthcare.

Fig. 2: Postnova PN3621 21-Angle MALS detector.

Run Conditions

- Sample: BSA (Bovine Serum Albumin)
- Solvent: PBS Buffer
- Column: GE Superdex™ 200 10/300 GL
- Eluent Flow: 0.5 mL/min
- Detectors: Postnova PN3621 MALS (Fig. 2)
  Postnova PN3211 UV
  Postnova PN3150 RI Detector

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Results

The MALS detector shows large peaks for protein aggregates (dimer and trimer) thereby revealing superior sensitivity compared to both concentration detectors (RI and UV). This is particularly useful for the detection of small amounts of high molar mass protein aggregates, which otherwise would not be accessible (Fig. 3).

Moreover, MALS detection enables the determination of the absolute molar mass of protein monomer, dimer and trimer (Tab. 1) thus delivering valuable additional information that cannot be assessed using UV-only detection (Fig. 4).

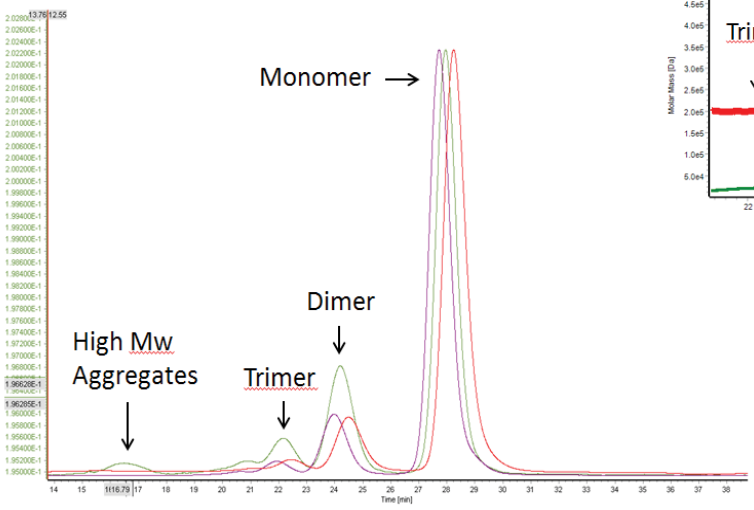

Fig. 3: Separation of BSA monomer, dimer, trimer and higher aggregates using Äkta-UV-MALS-RI (green line: MALS-signal; red line: RI-signal; magenta: UV-signal).

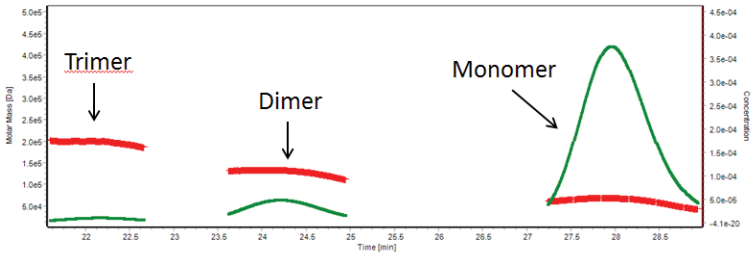

Fig. 4: Corresponding molar mass (red line) of BSA monomer, dimer and trimer obtained from MALS data (green line: concentration detector signal).

BSA	Molar Mass [g/mol]
Monomer	66000 +/- 500
Dimer	132000 +/- 1000
Trimer	199000 +/- 1000

Tab. 1: Molar mass for BSA monomer, dimer and trimer obtained from a Äkta-UV-MALS-RI measurement.

Conclusion

Adding Multi Angle Light Scattering (MALS) detection to FPLC separation systems such as the ÄKTA pure™ significantly increases the analytical capabilities of such chromatography systems as it not only facilitates the determination of the absolute molar mass of protein and antibody samples, but also enhances the detection sensitivity for high molar mass aggregates.

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