Aggregation study of mAbs using size exclusion chromatography coupled to multi angle light scattering

Application Note Biopharmaceutical Analysis

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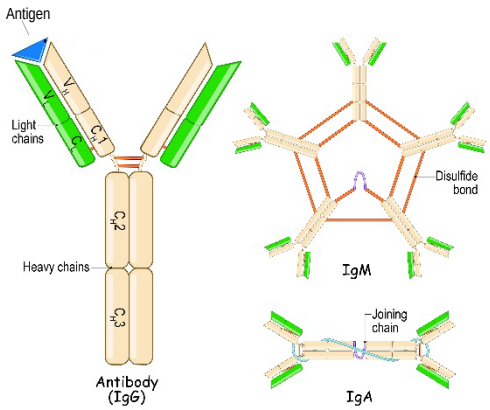
Size exclusion chromatography, SEC, equipped with UV, RI, and static light scattering detection is a powerful set of analytical tools to determine the content and size of mAb aggregates and fragments. Modern high-resolution SEC columns with an optimized separation range allow to make use of the full method potential.

Introduction

Biotherapeutics, such as monoclonal antibodies (mAbs), are of increasing interest due to their high efficacy and potential for the diagnosis and therapy of various chronical autoimmune or tumorous diseases.



The control of the critical quality attributes (CQAs) of mAbs is crucial to avoid a loss in pharmaceutical efficacy and to prevent from immunogenic reactions. One essential parameter to monitor is the content of aggregates (dimers, trimers, and higher aggregates). Aggregation can take place during manufacturing processes, shipping, or are the result of long-term storage.

Besides aggregation, antibody fragments, resulting from degradation of full-length antibodies, should be monitored.

GPC/SEC is the method of choice to monitor aggregation and fragmentation. Hence, a GPC/SEC method needs to be addressed, which allows for the simultaneous analysis of native antibodies, their aggregates, and fragments with superior resolution and highly sensitive detection techniques such as UV and light scattering detection.

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System Requirements

	Conditions
Pump	PSS SECcurity² GPC1260 isocratic pump • flow rate: 1.00 mL/min • mobile phase: aqueous, 34 mM phosphate buffer pH 6.4 + 0.3 M NaCl
Injection system	PSS SECcurity² GPC1260 Autosampler • variable injection volume
Columns	• PSS MAB 3µm precolumn (8*50 mm) • PSS MAB 3µm (8*300 mm)
Detectors	• SECcurity² MWD at λ=280 nm and/or λ=214 nm • SECcurity² RI • SECcurity² SLD2020 at λ=633 nm
Software	PSS WinGPC UniChrom with light scattering software module optional: Compliance Pack for enabling compliance with 21 CFR part 11 and modules for mass spectrometry, 2D, viscometry, end group analysis

Procedure, Results & Discussion

GPC/SEC hyphenation with multiple detection techniques including UV, RI, and LS is a powerful analytical tool for simultaneous determination of antibody aggregates and fragments. A huge advantage of the combination of these detection types is that they are complementary detection techniques. UV and RI are both concentration detectors, which are typically applied to determine the purity of the sample but can also be used to quantify aggregates and fragments, assuming the refractive index increment (dn/dc) and extinction coefficient (ε) are the same.

However, the molar mass can only be determined after a calibration with known calibration standards, hence, the hyphenation with an additional light scattering detector complements the analysis of mAbs and their aggregates as light scattering detectors are molar mass sensitive detectors. Two major advantages are that light scattering is an absolute method to determine the molar mass of a macromolecule, and it is highly sensitive for high molar masses. Hence, due to the molecular weight dependency, the PSS SLD2020 MALLS detector offers high sensitivity also for small quantities of mAb associates and higher aggregates.

Besides the detection, to gain highly resolved and compliant data, a successful GPC/SEC experiment requires a column or column set with a separation range suitable for the analyte of interest. The PSS MAB column is well-designed for protein and mAb applications. Covering the complete separation range required for mAb monomer, associate, aggregate, and fragment monitoring, the PSS MAB column also provides a long-term performance with high resolution for the determination of the CQAs of mAbs.
Moreover, the PSS MAB column is pre-equilibrated for the hyphenation with light scattering. The PSS SECcurity² GPC system, the PSS SLD2020, and the PSS MAB column are also available in bioinert fabrication to avoid contact with stainless steel during analysis.
Figure 1 shows an overlay of elugrams obtained for a native antibody (i.e., IgG from rabbit serum) and antibody aggregates, while in Figure 2 an overlay of elugrams obtained for a native mAb and antibody fragments is depicted. Both data sets result from applications using one PSS MAB 3µm column.

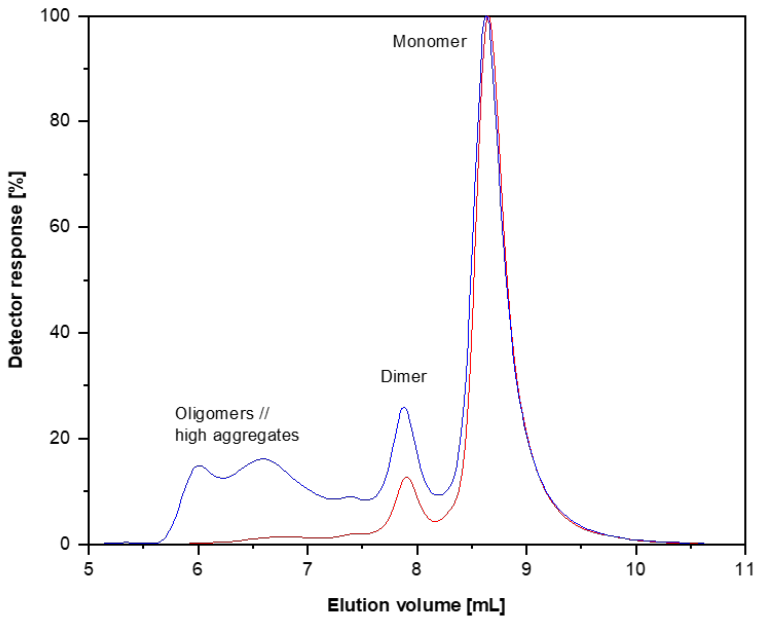

Figure 1: Separation range of the PSS MAB 3µm column. The red curve shows the UV signal of a native antibody and its associates plotted against the elution volume. The blue curve is the elugram of an aggregated antibody sample.

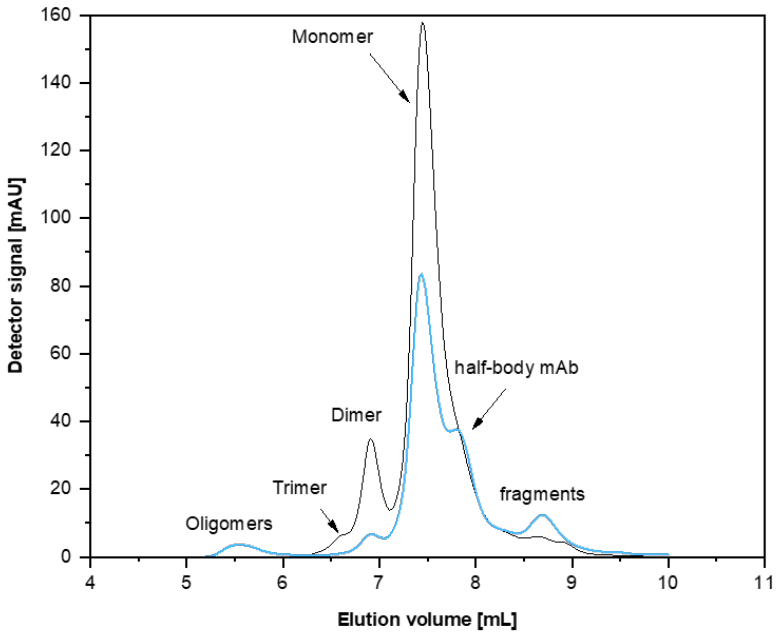

Figure 2: Separation range of the PSS MAB 3µm column. The black curve shows the UV signal of a native antibody and its associates plotted against the elution volume. The blue curve is the elugram of fragmented antibody sample by reduction.

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All three detector signals for the analysis of monoclonal antibodies are shown in Figure 3. The light scattering signal shows enhanced sensitivity for aggregates with high molar masses. The latter does not mean that light scattering is used for quantitation, but that monitoring of CQAs and the investigation of mAb aggregation processes is simplified.

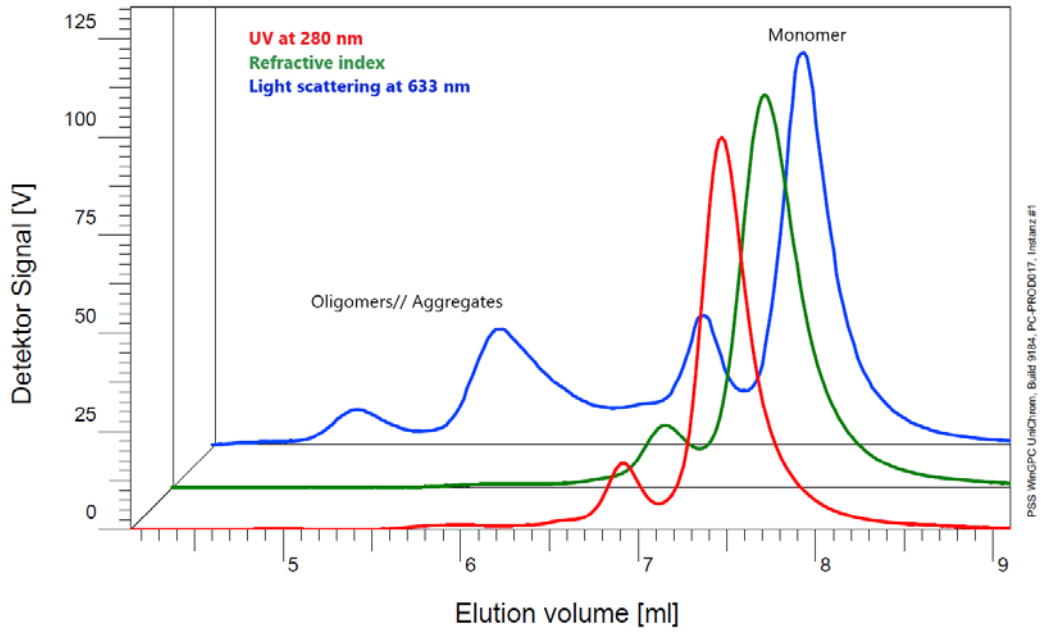

Figure 3: Sensitive analysis of antibody aggregates. The light scattering signal for the antibody associates and higher aggregates is relatively high compared to monomer signal due to molar mass dependency and provides enhanced sensitivity for the detection of high aggregates.

The following information can be obtained from data of the multi detection setup:

- absolute molar mass and radius of gyration (R_g) of monomer, dimer, trimer (PSS SECcurity^2 SLD2020)
- molar mass of fragments by relative method (calibration, PSS SECcurity^2 1260MWD/RI)
- Purity: quantity of high aggregates, associates, monomer, and fragments (PSS SECcurity^2 1260MWD/RI)

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