Application Brief

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Aggregation Study of mAbs

Using size exclusion chromatography coupled to multi-angle light scattering

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Abstract

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 monoclonal antibody (mAb) aggregates and fragments. Modern high-resolution SEC columns with an optimized separation range exploit the full potential of the method.

Introduction

Biotherapeutics, such as mAbs, are of increasing interest due to their high efficacy and potential for the diagnosis and therapy of various chronic autoimmune or tumorous diseases.[1]

The control of the critical quality attributes (CQAs) of mAbs is crucial to avoid a loss in pharmaceutical efficacy and to prevent immunogenic reactions. One essential parameter to monitor is the content of aggregates (dimers, trimers, and higher aggregates). Aggregation can take place during manufacturing processes or shipping, or is 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. There is a need for a GPC/SEC method that allows the simultaneous analysis of native antibodies, their aggregates, and fragments with high resolution and highly sensitive detection techniques such as UV and light scattering detection.

Experimental

See Table 1.

Table 1. Instrument parameters.

	Conditions
Pump	Isocratic pump Flow rate: 1.00 mL/min Mobile phase: aqueous 34 mM phosphate buffer pH 6.4, 0.3 M NaCl
Injection System	Autosampler Variable injection volume
Columns	Agilent MAB 3 µm precolumn, 8 × 50 mm (p/n MAA080503) Agilent MAB 3 µm, 8 × 300 mm (p/n MAA083003MC)
Detectors	Multiple wavelength UV-Vis detector (MWD) at λ = 280 nm and/or λ = 214 nm Refractive index (RI) detector Multi-angle light scattering detector (MALLS) at λ = 660 nm
Software	Agilent WinGPC UniChrom

Results and 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. UV and RI are both concentration detectors, which are typically applied to determine the purity of the sample. These detectors 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. Therefore, due to the dependence on molecular weight, the MALLS detector also offers high sensitivity for small quantities of mAb associates and higher aggregates.

Besides 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 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 MAB column also provides long-term performance with high resolution for the determination of the CQAs of mAbs.

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 MAB 3 µm column.

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.

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

- Absolute molar mass and radius of gyration (Rg) of monomer, dimer, and trimer (MALLS detector)
- Molar mass of fragments by relative method (calibration, RI/UV detector)
- Purity: quantity of high aggregates, associates, monomer, and fragments (RI/UV detector)

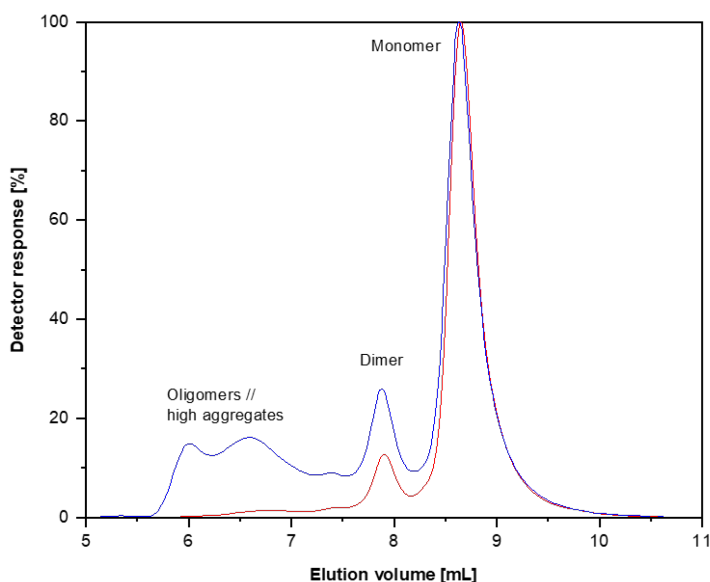

Figure 1. Separation range of the Agilent MAB 3 µm column. The red curve shows the UV signal of a native antibody and its associates plotted against the elution volume. The blue curve is the elugram of an aggregated antibody sample.

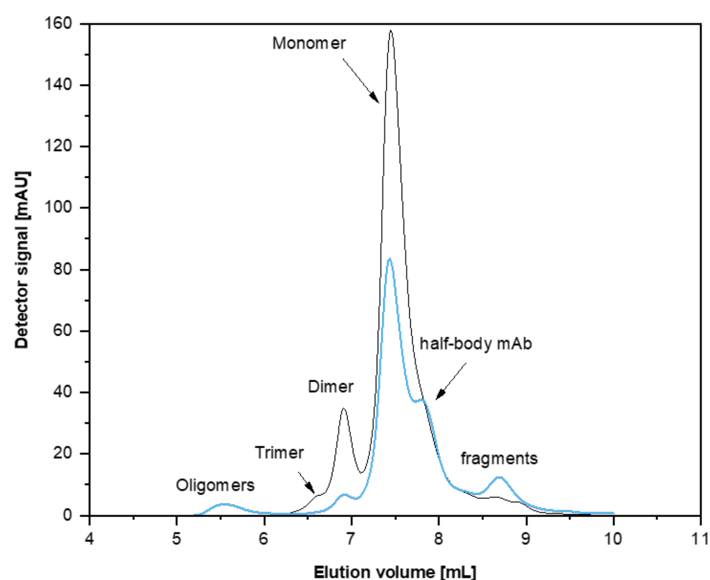

Figure 2. Separation range of the Agilent MAB 3 µm column. The black curve shows the UV signal of a native antibody and its associates plotted against the elution volume. The blue curve is the elugram of fragmented antibody sample by reduction.

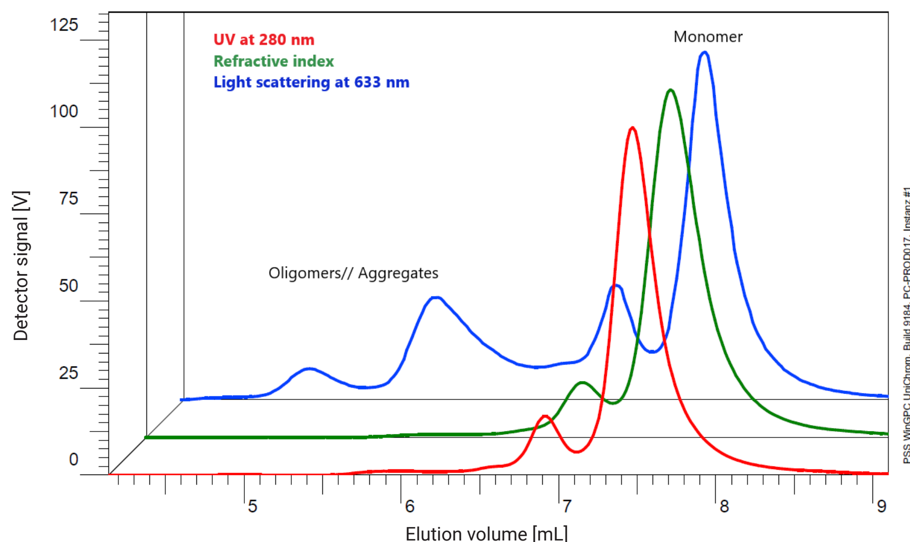

Figure 3. Sensitive analysis of antibody aggregates. The light scattering signal for the antibody associates and higher aggregates is relatively high compared to monomer signal due to dependence on molar mass, and provides enhanced sensitivity for the detection of high aggregates.

Conclusion

GPC/SEC in combination with MALLS detection is a powerful tool for the aggregation analysis of mAbs, which is one aspect of the CQAs. Besides this, the absolute molar mass and dimensional information such as the radius of gyration (Rg) are also available with the described measurement setup. In addition, the Agilent MAB column is already pre-equilibrated for the hyphenation with light scattering. The GPC/SEC system, the MALLS detector, and the MAB column are also available as bio-inert versions to avoid contact with stainless steel during analysis.

Reference

1. Klein, C. Monoclonal Antibodies, MDPI books, **2018**.

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