Sophisticated antibody analysis by GPC/SEC multi-chrome light scattering

Application Note Pharmaceutical Analysis

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A multidetection GPC/SEC method including UV, RI and Light scattering can be used for the simultaneous determination of aggregate content of monoclonal antibodies and antibody fragments using a single column set.

Introduction
Monoclonal antibodies (mAB) are increasingly growing in importance for the diagnosis and therapy of various diseases, including cancer as well as autoimmune and inflammatory disorders.

One essential parameter to define their quality of is the content of aggregates (dimers, trimers and higher aggregates). These aggregates can be formed during processing and purification or are the result of long-term storage. Due to the aggregation antibodies lose their pharmaceutical efficacy and can facilitate immunology response.

Antibody fragments, which lack the Fc region, can be used for the treatment of diseases. Also they can be the result of degradation of full length antibodies. Therefore a GPC/SEC method which offers the opportunity to analyze antibodies and their aggregates as well as antibody fragments simultaneous with superior resolution is worthwhile, as well as a highly sensitive detection such as light scattering detection.

System Requirements

	Conditions
Pump	PSS SECcurity GPC1260 isocratic pump • flow rate [mL/min]: 1.00 • mobile phase: aqueous, 100 mM sodium phosphate pH 6.7 + 0.25 M NaCl
Injection system	PSS SECcurity GPC1260 Autosampler • variable injection volume
Columns	• PSS PROTEEMA precolumn (8*50mm) • PSS PROTEEMA 5µ 300 Å + 300 Å (8*300mm each)
Injected mass	• 60 to 80 µg
Detectors	• SECcurity 1260 RI • SECcurity 1260 MWD at λ=214 nm • SECcurity 1260 SLD1000 at λ=488 nm

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Software	PSS WinGPC UniChrom with light scattering software module • optional for 21CFR11 compliance: Compliance Pack • optional: modules for mass spectrometry, 2D, viscometry, end group analysis

Procedure, Results & Discussion

A multidetection GPC/SEC method including UV, RI and RALS can be used for the simultaneous determination of aggregate content of monoclonal antibodies and antibody fragments.

Special about light scattering detectors is that their signal intensity increases with increasing molar mass. This has the two major advantages that light scattering is an absolute method and that light scattering detectors are very sensitive for higher molar masses. Due to its molecular weight dependency, the PSS SLD1000 RALS detector offers high sensitivity also for small quantities of high aggregates and also allows the determination of the absolute molecular weight of the antibodies.

An unique advantage of the PSS SLD1000 multichrome light scattering detector is that it combines the ease-of-use of 90° light scattering detection with increased sensitivity. It is the only light scattering detector that offers users the possibility to change the wavelength and to profit from the higher sensitivity at lower wavelengths. For this application a wavelength of 488 nm has been selected.

The PROTEEMA column combination covers the complete separation range required for multimers, mAB monomers and dimers and fragments or low molecular weight impurities and nonetheless provides a high resolution for the determination of the dimer content.

Figure 1 shows an overlay of elugrams obtained for a full length antibody and antibody fragments analysed on one set of columns.

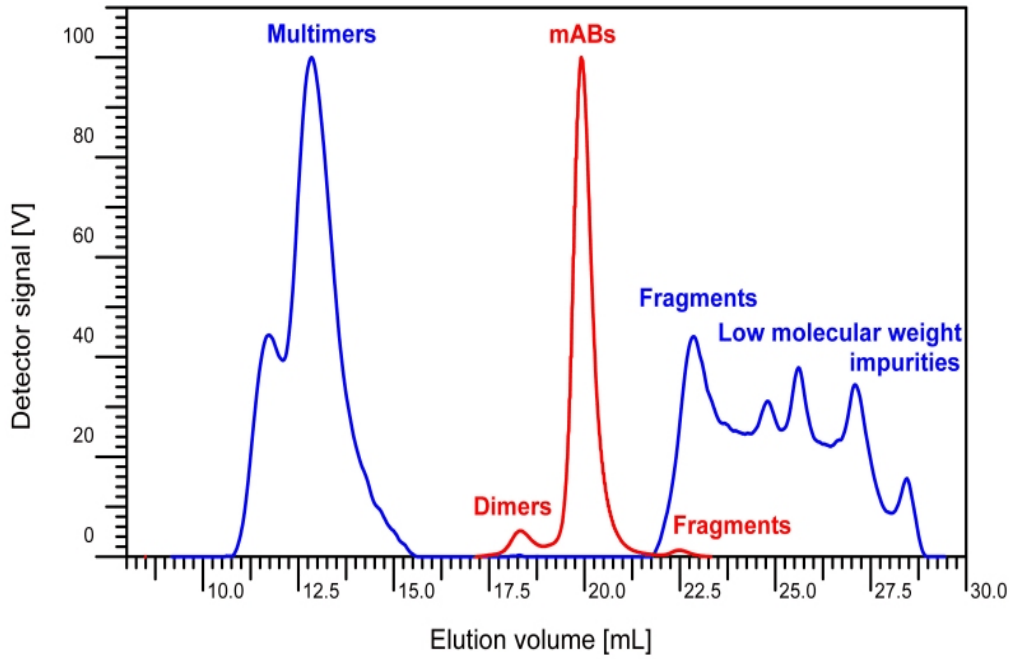

Fig. 1:
Separation range of the column combination. The red curve shows the UV signal of a full length antibody and its dimers plotted against the elution volume. The blue curve is the elugram of antibody fragments and their high level aggregates.

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All three detector signals for the analysis of a monoclonal antibody are shown in figure 2. The light scattering signal shows improved sensitivity for high aggregates

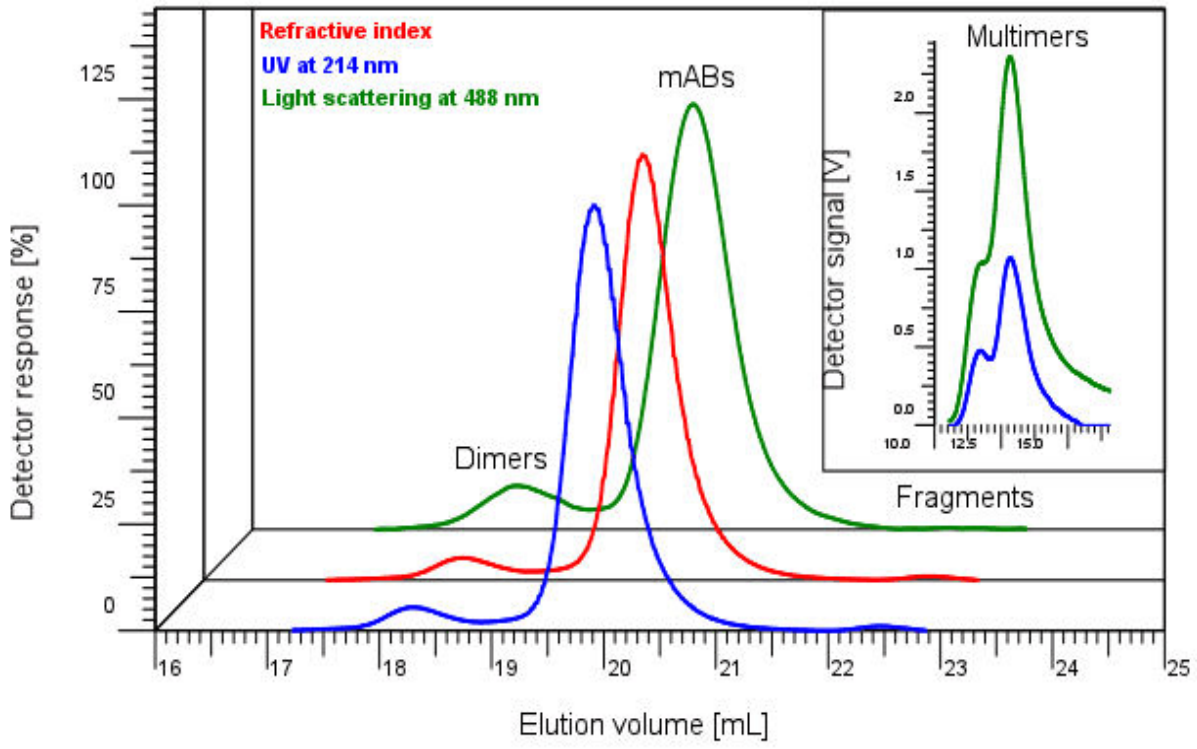

Fig. 2: Sensitive analysis of antibody aggregates. The light scattering signal for the dimer is relatively high compared to that of the mABs due to molar mass dependency and provides improved sensitivity for the detection of high aggregates (inset).

compared to the other signals.

The following results can be obtained from the data of the multidetection setup:
- Absolute molar mass of Monomer and Dimer
- Purity (amount of Multimers, Dimer, Monomer and Fragments)

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