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Continuous monitoring of aggregation of biomacromolecules

"Using BRAVE technologies in combination with other cutting-edge biophysical techniques has opened up exciting new possibilities to monitor the aggregation behavior of biological nanoassemblies."

Investigating the aggregation of biomacromolecules using OF2i®-SLS coupled with SEC

To further medical research, e.g. in the context of atherosclerosis, researchers want to know the exact structure and composition of LDL molecules and better understand how and when they aggregate.

Challenge

In a feasability study with the Medical University of Graz researchers around Dr. Karin Kornmüller coupled OF2i® to SEC (Size Exclusion Chromatography) to investigate the dynamic aggregation behavior of LDL (low-density lipoprotein) molecules. A number of methods can be used to access information about nanoassemblies, such as LDL, i.e. SEC, (cryo-)EM, gel electrophoresis, SAXS, spectroscopic techniques (CD, IR, fluorescence) or calorimetric techniques (DSC).

Application highlights

The OF2i® device with its static light scattering (SLS) functionality (BRAVE B-Aware module) continuously measured the SLS signal as well as particle concentration in the fractions that passed through the SEC column. The first step was to work on a possible correlation between the SEC-UV 280 nm peaks, the peaks in the particle concentration measured using OF2i® and the corresponding signals from the SLS module BRAVE B-Aware. The goal was to discover particle aggregation fractions which might not be detectable via the UV signals alone.

A high static light scattering intensity correlates well with monodisperse LDL (time span t2 to t3 throughout Figure 1). This fraction represents the purest LDL sample with the lowest risk of aggregations present. Here, the unmatched sensitivity of OF2i®-SLS helps to select the purest fractions (i.e. without any detectable aggregates) – samples, which then qualify for being used in cryo-EM investigations on high-end instruments in order to retrieve the atomic structure of the biomacromolecule.

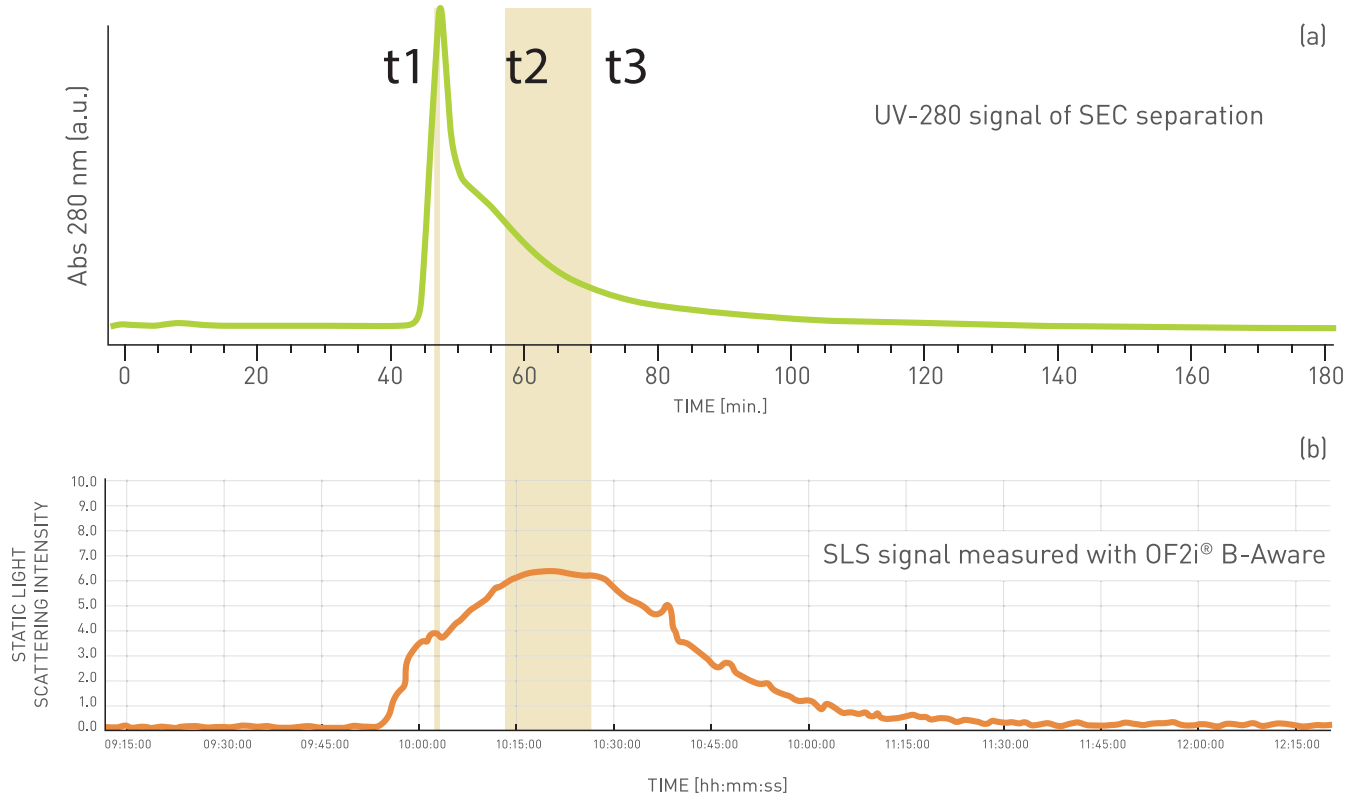

Figure 1(a) shows the fractions as recorded by the UV-280 detector within the SEC setup. Figure 1(b) represents the static light scattering recorded with the OF2i® detector B-Aware module. The overlay helps to select the purest fractions of non-aggregated fractions within the setup for further cryo-EM studies.

LDL particles have a diameter of approx. 22 nm, thus monodisperse LDL is monitored using the OF2i® static light scattering signal as described in Figure 1. However, as soon as aggregates are present (exceeding sizes of 50 nm to 100 nm, which corresponds to clusters of only a few LDL particles) they can be monitored with OF2i® with single-particle accuracy. The peak of particle distributions at t1 in Figure 2(b) correlates with the peak detected by the UV-detector at t1 in Figure 2(a). The presence of aggregates in this fraction was supported by gel electrophoresis and TEM imaging. This fraction is of high interest as it is further investigated to understand more about how aggregated LDL differs from non-aggregated LDL and what consequences this has for health.

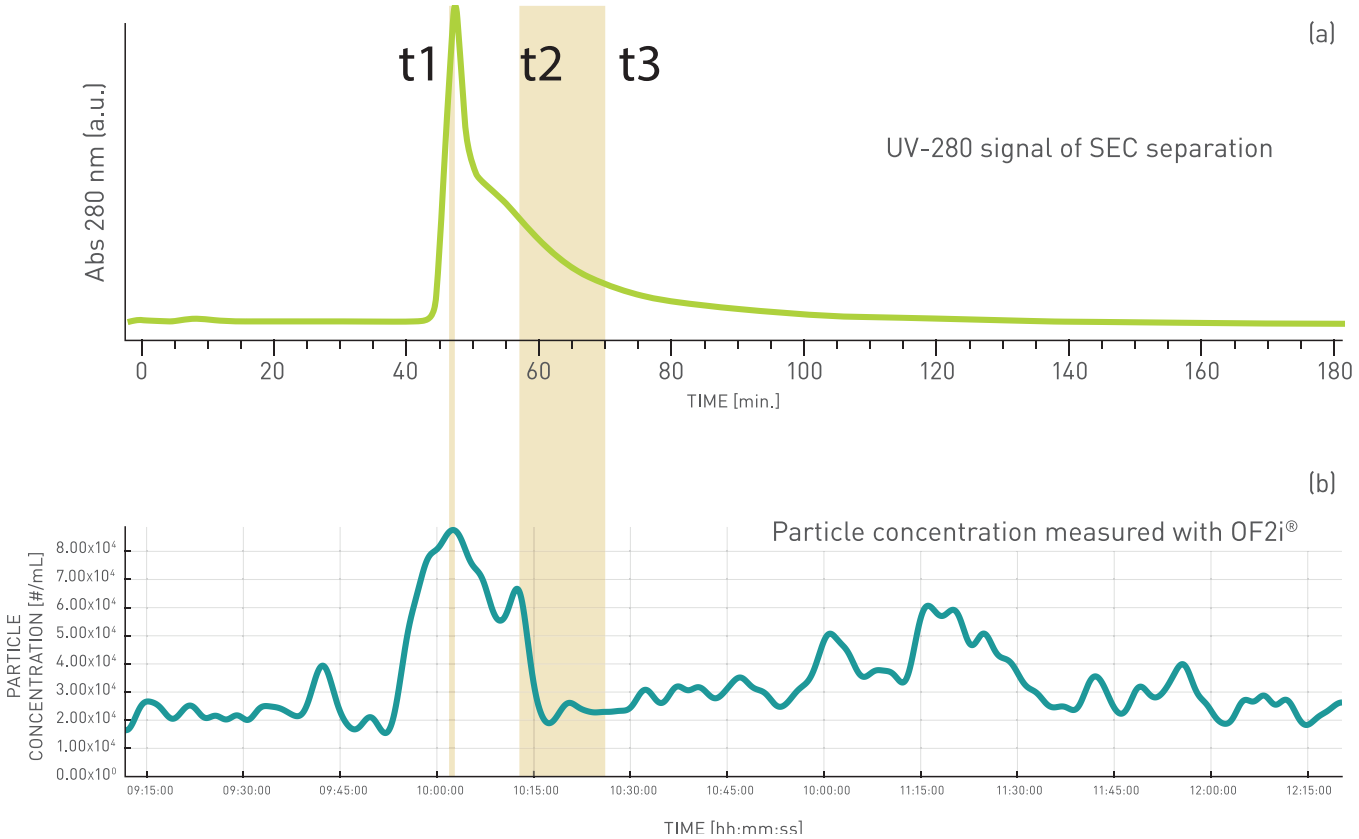

Figure 2(a) shows the fractions as recorded by the UV-280 detector within the SEC setup, comparing these to the concentration of aggregates detected by OF2i® (Figure 2(b). The shoulder at time t2 represents a very low concentration of aggregates, also supported by the gel electrophoresis and TEM findings. It is interesting to see additional peaks in the 120 min SEC area not detected by the UV detector. Further investigation is needed.

Benefits & Outlook

Unlock next-level insights into aggregation behavior with the powerful combination of continuous OF2i® particle-based characterization and complementary static light scattering (SLS) data. This innovative synergy offers a range of compelling advantages for real-time aggregation monitoring:

- Simultaneous results with SEC: analyze your sample in parallel with SEC workflows for seamless integration and greater efficiency.
- High-resolution particle identification: distinguish between non-aggregated and aggregated fractions on a single-particle level, ensuring only the purest samples are selected for high-value applications like cryo-EM.
- No additional sample prep: measurements are performed directly in flow, eliminating unnecessary steps and saving valuable time.
- Real-time reaction monitoring: track molecular interactions as they happen within the flow cell, allowing you to assess sample purity dynamically and with confidence.
- Combined OF2i® and SLS with BRAVE B-Curious and the BRAVE B-Aware software module gives you a smarter, faster and more precise way to monitor aggregation and ensure sample integrity.