A Q&A

A New Approach to Light Scattering Detection for the Characterization of Proteins and Polymers

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Traditional light scattering detectors have their own innate limitations. However, an updated instrument design can help alleviate measurement setbacks. *LCGC* spoke with Dr. Sébastien Rouzeau, Product Manager for Gel Permeation / Size Exclusion Chromatography (GPC/SEC) Systems and Columns at Tosoh Bioscience, about a new approach to light scattering and how it can benefit protein and polymer characterization.

LCGC: What is light scattering?

Rouzeau: The basis of light scattering can be described using a situation familiar to most people: driving in the fog at night. A car's headlights illuminate droplets in the fog, which re-emits light in all directions. As a result, we can see the fog from wherever we stand, even from behind the car.

In analytical instrumentation, a laser beam is used to illuminate a vial or a cell that contains a sample in solution. The theory and equations of light scattering were established back in the 19th century by Lord Rayleigh and are still used today by all light scattering instruments. According to the Rayleigh equation, the intensity of scattered light is related to the molecular weight of the sample. However, that intensity is not equal in all directions; it changes with the angle of observation, and this scattering pattern is related to the size of the sample. So essentially, light scattering relates to both molecular weight and size of the molecules.

LCGC: Why has light scattering become so popular for polymer and protein characterization?

Rouzeau: The light scattering phenomenon is primarily used to determine the true or absolute molecular weight of a sample. And in some cases, size information can be obtained in the form of the sample's radius of gyration (R_g), which is truly related to the shape and the structure of the molecule.

Light scattering detection is most often coupled with SEC so that different species in the sample are separated by size first before being measured by light scattering in order to obtain their molecular weight and size. This is known as SEC-multiangle light scattering (SEC-MALS).

Typically for protein applications, monomers, oligomers, or higher aggregates and fragments can be easily identified by their molecular weight both in their native or denatured state, regardless of their shape or whether or not they are globular. So in that regard, SEC-MALS differs from high-performance liquid chromatography (HPLC) with mass spectrometry in that the analysis will denature the protein during the process. With liquid chromatography–mass spectrometry (LC–MS), it is actually impossible to identify the protein oligomers that are present in the sample.

For polymer characterization, light scattering is mostly used to look into the molecular weight distribution of polymers without referring to standards, which is a major advantage over conventional SEC. And it is also possible to obtain valuable information on the structure or the confirmation of the sample (e.g., branching).

LCGC: How do traditional light scattering detectors work?

Rouzeau: Essentially, light scattering instruments either measure as close to the incident beam as possible to obtain molecular weight, or measure at multiple angles to observe how the intensity changes and extrapolate back to 0°

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to estimate molecular weight. This is the well-known MALS approach.

Typically, a MALS detector would consist of multiple photodiodes that are arranged around a circular or cylindrical flow cell that is usually made of quartz glass. These photodiodes collect the scattered light simultaneously and continuously while the sample is flowing through the cell. All the signals are recorded, and the intensity of the scattered light is plotted against the angle of observation to obtain molecular weight and R_g via an extrapolation model.

LCGC: Are there any limitations to MALS measurements?

Rouzeau: Like any analytical technique, MALS has its limitations, some of which are because of the instrumentation itself. Traditional MALS detectors have one major and purely technical limitation due to their lowest and highest measurable angles. This is a result of the geometry of the flow cell as well as because of the limited space available to physically fit the detectors around it. The lowest and highest positions that can be measured are relatively far from 0° and 180°.

Additionally, because of the proximity of the incident laser beam and stray light, those angles are subject to higher background noise. This affects the quality of the signal to the point where the highest and lowest angles sometimes become unusable and must be discarded for data processing.

One might ask why this is an issue considering the presence of multiple angles. The issue arises from the fact that the lowest angle is extremely critical to obtain an accurate molecular weight from the extrapolation. The highest angle is also very important when it comes to detecting very small differences in scattering for small samples in size. This is basically the reason why the lower limit for R_g measurements by a traditional MALS detector is approximately 10–12 nm, because below that, no angular dissymmetry can be accurately detected.

LCGC: How does the LenS3 MALS from Tosoh address such limitations?

Rouzeau: The LenS3 MALS was specifically designed to address the previously mentioned limitations. In this device, the traditional flow cell is replaced with a completely new concept of "flow channel". This new channel is an elongated flow path with a dual-cone shape that is machined directly in a black polymeric material. The block is sealed with two lenses—one at each end—which will let the laser beam go through the channel to illuminate the sample while ensuring the collection of the scattered light at both an ultralow and ultrahigh angle. There is also a third angle that collects light at 90° to form a three-angle measurement.

This new design features two major benefits over traditional flow cells. The first is that the black polymeric material eliminates any stray light that could interfere with the scattered light and generate noise. And second, the elongated flow path maximizes the interaction with the sample and significantly increases the amount of light that can be collected.

In addition to the new flow channel, the LenS3 also uses a green laser that produces about three times higher intensity of scattered light than a regular red laser, which increases the signal. And lastly, the device's optical bench eliminates the incident beam at the low and high angle position so that both provide a clean signal.

Overall, what sets this instrument apart from others is twofold: the position of the angles—with a true usable low angle for direct molecular weight measurement, combined with an extreme high angle for R_g measurement—and the new flow channel and optics that maximize the signal-to-noise to increase the overall sensitivity of the detector.

LCGC: From a practical standpoint, what does all this mean for SEC-MALS users?

Rouzeau: The instrument's higher sensitivity brings about various benefits for users. For example, the LenS3 requires smaller quantities to characterize samples, which is critical for protein applications where sample quantity can be a major limiting factor when it comes to physico-chemical characterization.

Another notable benefit is the ability to detect the presence of aggregates and fragments of an antibody down to a much lower level when compared with conventional detectors. For polymers, a higher sensitivity means samples can be measured with a very low molecular weight, a very low dn/dc, and a broad distribution, or all of the above.

Additionally, the high sensitivity combined with the position of the extreme angles opens a whole new field of application for small macromolecules with an R_g below 10 nm because the angular dissymmetry can be measured for such small sizes, which was not possible before.

LCGC: Can you give us a few concrete examples of what users will gain from this technology?

Rouzeau: For instance, because the LenS3 is fully compatible with UHPLC systems, we injected decreasing concentrations and volumes of a monoclonal antibody (mAb) in our applications laboratory. We were able to detect the mAb down to as low as only 2 ng of sample loading, which is extremely low.

We also achieved the complete molecular weight profiling of an unpurified and purified oligonucleotide despite the relatively low molecular weight and sample loading used. For polymers, we successfully determined the R_g of a series of polystyrene standards down to only 2 nm in radius. To our surprise, the R_g values agreed perfectly with the R_g measured by small angle X-ray scattering reported in the literature (1).

Reference

1. F. Abe, Y. Fumiaki, T. Yoshizaki, and H. Yamakawa, Macromolecules, 26(8), 1884-1890 (1994)