

Online MALS-QELS (Quasi-Elastic Light Scattering)

While online multi-angle light scattering (MALS) is one of the most important techniques for macromolecular characterization, it can be made even more versatile with the addition of a quasi-elastic light scattering (QELS, a.k.a. dynamic light scattering) module for determination of hydrodynamic radius. QELS can be added to a Wyatt MALS system as a WyattQELS™ module embedded in the MALS instrument, or by connecting the MALS flow cell to a batch DLS instrument such as a DynaPro® NanoStar® or Mobius® via optical fiber. The QELS instruments can be used to determine the hydrodynamic radius, r_h , for a variety of samples in a continuous-flow mode. The combined MALS-QELS system will measure simultaneously r_g , r_h , and the absolute molar mass.

Since r_g (the root mean square radius) is determined directly from the *angular dependence* of the scattered light intensity, at least three angles are required to make a reliable measurement. On the other hand, r_h is derived from the *fluctuations* of light scattering intensity due to diffusion of the molecules (*aka* Brownian motion) and a single measurement angle suffices.

The QELS measurement is performed *on-line* in the DAWN flow cell. State-of-the-art optical design, high sensitivity, minimal dead volume, and ease-of-use are the hallmarks of the DAWN detectors. These characteristics, among others, enable them to produce superior signal-to-noise, stability and sensitivity.

An optical fiber receiver is mounted in the read head of the MALS detector at *any angular location*. The fiber is, in turn, coupled to an avalanche photodiode in an autocorrelator that has been specially modified to accept the signal from the DAWN instrument.

This application note illustrates the results obtained for bovine serum albumin, BSA, and a glycoprotein which were separated using a size exclusion chromatograph (SEC). The MALS-QELS detector determined the hydrodynamic radius versus elution time for the two proteins completely independent of the elution time. Figure 1 shows these results with the 90° LS signal superimposed. For these particular samples, r_g was below the MALS r_h measurement limit of 10 nm. However, simultaneous measurements of MALS and QELS in the integrated system provide complete results for r_h and molar mass (even though the small radius cannot be measured by MALS, molar mass measurements are not impacted). Figure 2 shows the molar mass results determined by the DAWN and the RI detector.

The MALS-QELS combination allows the simultaneous determination of absolute molar mass, root-mean-square radius (r_g from about 10-500 nm), and hydrodynamic radius (r_h from about 0.5-300 nm). Conformation results can now be obtained for molecules ranging from 200 g/mol to hundreds of millions of g/mol.

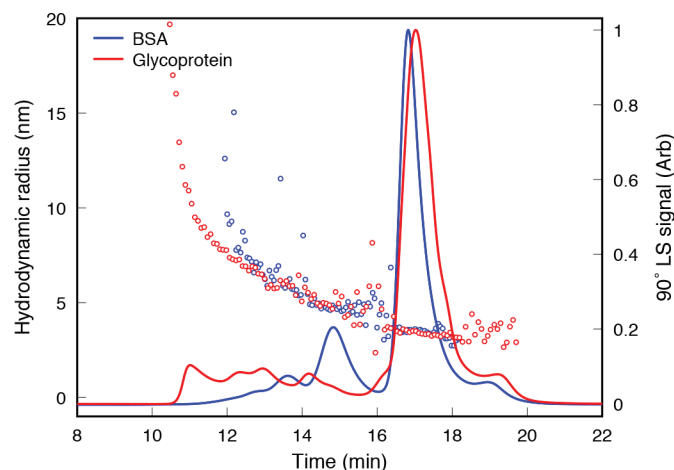


Figure 1. The hydrodynamic radius versus time for BSA and a glycoprotein, obtained from SEC coupled to on-line MALS-QELS detection. The measurements of size (shown here) and molar mass (shown below) can be combined to learn about molecular conformation.

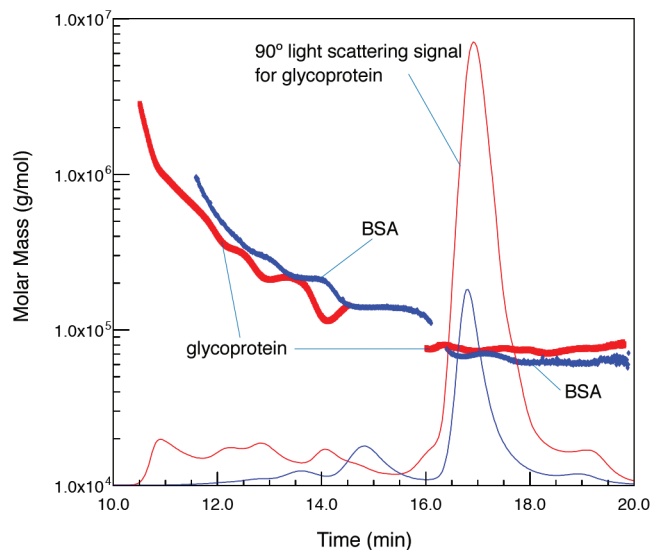


Figure 2. Absolute molar mass versus time for BSA and glycoprotein samples superimposed with the signals from 90° LS detector obtained from size-exclusion chromatography with MALS detection. Glycoprotein aggregates with similar molar mass as BSA aggregates elute earlier due to their extended hydrodynamic size. MALS provides accurate molar mass, regardless of elution time.