Proteins/Aggregation Detection

Absolute characterization of proteins *in solution* is critical—and desirable—in a variety of industries. In pharmaceutical or biotechnology applications, for example, protein-based products must be made precisely and without the presence of aggregates. Multi-angle light scattering (MALS) is an ideal means by which to measure the molar mass of proteins *in solution*. It is also an extremely sensitive method for detecting whether—and how much of—any aggregates have formed, because the light scattering response is directly proportional to the weight-averaged molar mass (M_W) of the sample being measured, multiplied by the concentration.

When used as an on-line detector for an HPLC system, the miniDAWN (or DAWN) can measure the molar mass of an eluting protein *without* dependence on column calibration or reference standards. An additional benefit of this calculation lies in the fact that it is the molar mass of the protein *in solution*. This can reveal whether the protein exists as a monomer, dimer, or at a higher aggregation state.

Figure 1 shows one of the miniDAWN's detector outputs, as well as the refractive index signal for BSA (bovine serum albumin). The HPLC conditions were 0.05 M phosphate buffer with 0.1 M NaCl. An Optilab DSP Interferometric Refractometer was used as the RI detector. The flow rate was 1.0 ml/min using an HP 1050 liquid chromatograph. Two Shodex® columns (KW-803 and KW-804) were used for the separation. Interestingly, even though the BSA is a "monomer" standard, the miniDAWN clearly detects aggregates (dimer and trimer) that are almost invisible to the RI detector.

Figure 2 shows a Debye plot for a sample of human serum albumin (HSA), and the power of the multi-angle detector can be seen clearly because of its ability to determine the *size* of the aggregate—not only its mass. Here, a selected slice has been chosen that indicates that the aggregate has a molar mass of 1.5 x 10^6 g/mol, and an *rms* radius of 23nm. This contrasts markedly with the monomer which has a weight average molar mass of 7.5 x 10^4 g/mol and an *rms* radius below the limits of measurement.

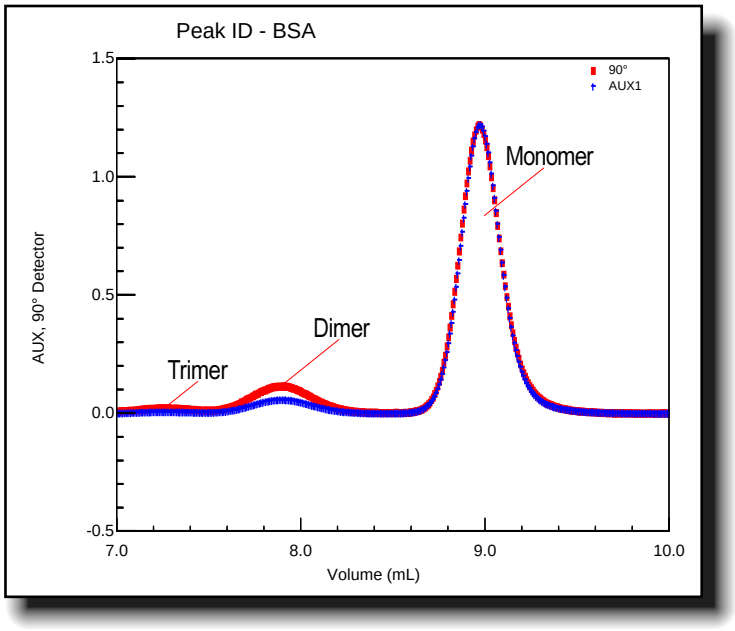

Figure 1. BSA clearly shows the effects of sample aggregation. The aggregate molar mass can be quantified precisely by the miniDAWN.

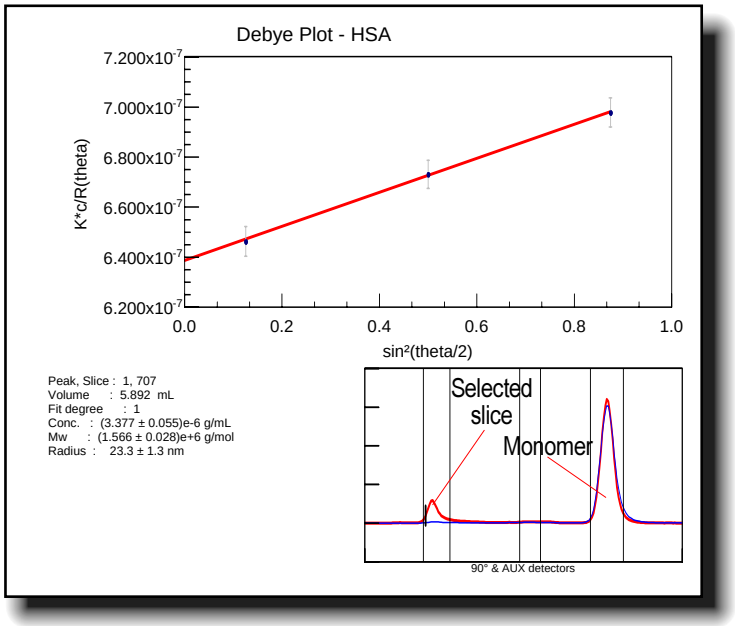

Figure 2. HSA's aggregate can be quantified not only by its molar mass, but by its molecular size *which, in this case, has a radius of approximately 53 nm.*

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