

Application Note: Automated 2nd virial coefficients with Calypso

The 2nd virial coefficient (A_2) is a general measure of weak, non-specific protein-protein interactions. Characterization of A_2 vs. buffer properties, e.g. ionic strength or pH, is useful in optimizing a variety of processes, including purification, formulation and protein crystallization.

Multi-angle Light Scattering (MALS) batch measurements of protein solutions over a series of concentrations can determine A_2 effectively and nondestructively. However, the standard technique of carefully preparing well-determined concentrations, and introducing each in succession via either scintillation vials or microbatch injections, is tedious and labor-intensive. As such it is prone to human error, and suffers from poor reproducibility.

With the Calypso system, a MALS detector (e.g. DAWN-HELEOS or miniDAWN-TREOS) and an on-line concentration detector (usually a dRI or UV absorption detector), A_2 measurements are automated for rapid turnover, minimal operator effort and high reproducibility. The user need only supply a single stock solution and buffer. Dilutions and injections into the detectors are carried out by the Calypso triple-syringe-pump hardware accessory, under the control of the Calypso software. The software also acquires and analyzes the data via a user-friendly graphical interface.

Figure 1 shows the hardware arrangement. The syringe pumps draw only the amount of sample necessary for each injection. Upon injection into the detectors, concentration steps are produced by varying the ratio of flow rates of sample and buffer. The injected volume must be sufficient to fill the flow cells of both the MALS and concentration detector, in this case an Optilab rEX; typical injection volumes are 2 mL (the sample may of course be recovered). The flow is stopped at the end of each injection, to allow for equilibration or to collect additional data points for noise reduction.

The data and analysis graphs for bovine serum albumin (BSA) in phosphate buffered saline solution (PBS) are shown in Figure 2. A portion of each plateau is selected for analysis, and the data fit to the Zimm equation to obtain weight-averaged molar mass and A_2 . For larger molecules, mean square radius may also be determined. Reproducibility of the data is typically better than 1% in molar mass and $0.2 \cdot 10^{-4} \text{ mol} \cdot \text{mL} / \text{g}^2$ in A_2 . The results will be less precise if lower protein concentrations are used.

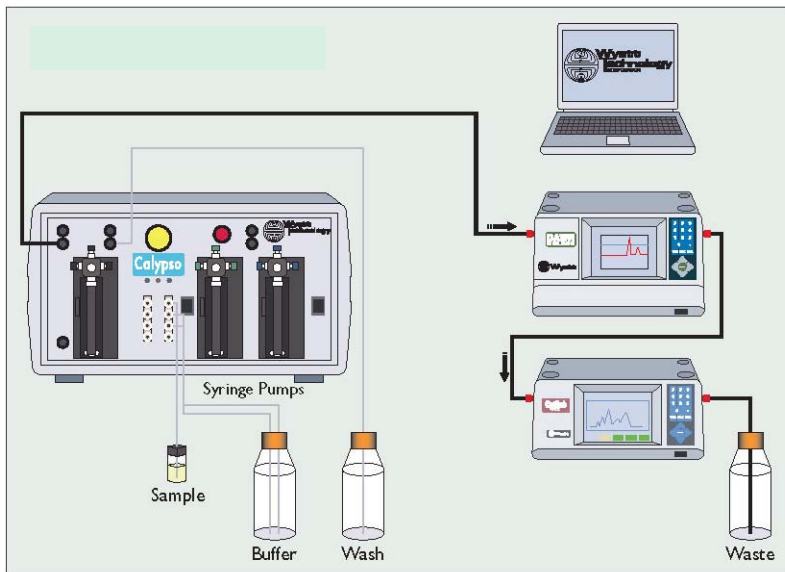


Figure 1. The Calypso syringe pumps automate sample preparation and injection to the detectors.

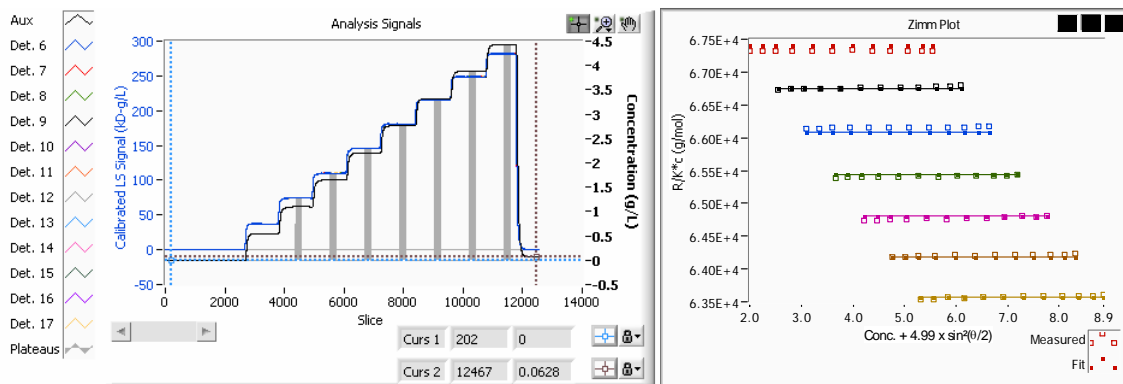


Figure 2. Multi-angle light scattering data overlaid with Optilab concentration data, for the BSA step-wise concentration gradient (left) and final Zimm plot (right), resulting in a weight-averaged molar mass of 68.8 kD and A_2 of $1.34 \cdot 10^{-4} \text{ mol} \cdot \text{mL} / \text{g}^2$.