Protein Solutions™

Automated, Online Second Virial Coefficient (A_2) Measurements

Measurement of weak protein-solvent interactions is essential in a wide array of processes, such as stabilization of therapeutic protein formulations, purification of protein mixtures, and crystallization of proteins. Both self and cross-association can be quantified by the second virial coefficient, (A_2).

A_2 values are dependent on both the protein and its solvent: Temperature, salinity, pH, chemical excipients, etc., can induce dramatic shifts in A_2. Positive A_2 corresponds to repulsive intermolecular interaction, whereas negative A_2 corresponds to attractive intermolecular interaction. Rapid determination of the buffer conditions yielding an optimal A_2, such as a positive A_2 for formulations, or a slightly negative A_2 for crystallization, is highly desirable.

In the past, such measurements were labor intensive, time consuming, and expensive in terms of time and protein: For each buffer condition tested, extensive dialysis and preparation of multiple concentrations were required. Additionally, each concentration had to be injected manually. Wyatt Technology has overcome these issues, and developed an accurate, automated method for measuring A_2 online. In our online A_2 method, only a single protein stock solution is needed for an entire series of measurements.

In addition to complete automation and online dialysis, the online A_2 method has the added benefit of flushing the light scattering flow cell between each injection, helpful for researchers working with "sticky" proteins.

The Trainoff-Wyatt Online A_2 method is as follows: A sample delivery unit, such as a standard autosampler, delivers a sequence of 5 or more different volumes (such as 20µ to 200µL) through an optional size exclusion chromatography column (SEC) or a desalting column, to a DAWN or miniDAWN light scattering instrument and an Optilab RI. The Optilab RI serves as a concentration detector and also records the complete dialysis of each injection.

The Online A_2 method will enable researchers to fully utilize the second viral coefficient as an important indicator of protein-protein interaction. The historic impediments to this type of measurement, lengthy sample preparation and experimental labor, have been overcome. Now, using standard chromatography equipment and Wyatt detectors, an entire series of A_2 values can be determined in less then a day!

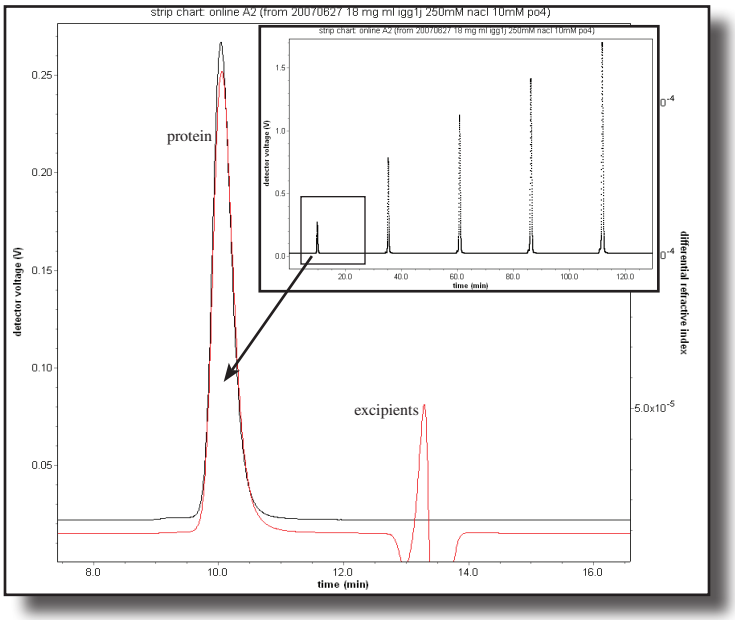

Figure 1: Upper right graph (foreground) shows the light scattering signal (Detector 11) of the 20µL to 200µL antibody solution injection series. The expanded (box) region shows both the light scattering signal in black, and differential refractive index signal in red, demonstrating complete dialysis of injected antibody with the mobile phase by the SEC column. Only the protein peaks are used in the Online A_2 analysis.

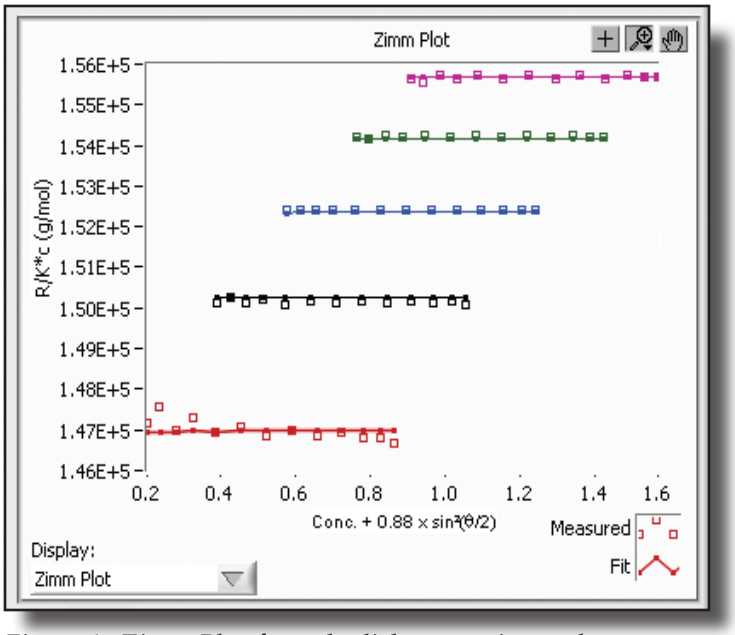

Figure 1: Zimm Plot from the light scattering and concentration data of the protein peaks. Each color represents a different peak. Note the excellent agreement of the measured data to the fitted data. This Zimm plot reports the antibody in PBS has an A_2 of –5.46E-5 mol mL/g2.

Wyatt Technology CORPORATION
6300 Hollister Avenue • Santa Barbara, CA 93117
TEL (805) 681-9009 • FAX (805) 681-0123
E-mail: info@wyatt.com • URL http://www.wyatt.com