

Rapid, Automated Molecular Characterization Using a Binary HPLC pump

A standard binary pump, such as those used for HPLC systems, may be used to prepare, mix, and inject multiple sample concentrations required for a standard Zimm plot. Continuous flow and automation result in an easier experiment, cleaner light scattering data, and expedited results.

Historically, this type of experiment has been performed with a syringe pump and five or more concentrations of the sample. The solutions were made prior to the experiment, and a syringe had to be filled with each solution. A syringe pump was then used to inject the solutions sequentially. This required an intensive, manual approach to running the experiment. Additionally, air often was inadvertently injected into the detectors, resulting in large noisy spikes in the data. Concentrations had to be known *a priori* and entered by hand.

Now, in addition to automated mixing and delivery by the binary HPLC pump, an Optilab rEX can be used to automatically determine the concentrations during the experiment. The differential refractive index values corresponding to the concentrations of the solutions are collected by ASTRA, eliminating the need for the user to determine the concentration of the solution before the experiment.

In the example shown, a ~545kDa Dextran, a natural polymer, was dissolved at 2.5 mg/mL in buffer and put in reservoir "A". Buffer alone was put in reservoir "B". A low volume, 0.45 mm filter was installed between the pump and the light scattering detector. The Optilab rEX was installed downstream of the light scattering detector. The flow rate was set to 1 mL/min, and the pump was set to deliver a concentration series 0% A to 100% A, and then back to 0% A.

Each concentration was run for three minutes, for a total of 3 mL per step. This volume ensured that a plateau was attained in both the static light scattering and the concentration detector. With plateauing, identical concentrations were present in both detectors.

Data were collected and processed using ASTRA software. For the experiment shown, measured values were a molecular weight of $(547,000 \pm 1,900)$ g/mol, a rms radius of (23.1 ± 0.5) nm, and a second virial coefficient (A_2) of $(1.27E-4 \pm 0.02E-4)$ mol mL/g².

As can be seen clearly in the two graphs (at right), automated sample dispensing and concentration detection results in clean data acquisition, enabling accurate detection of the molar mass, size, and second virial coefficient, in fewer than 30 minutes.

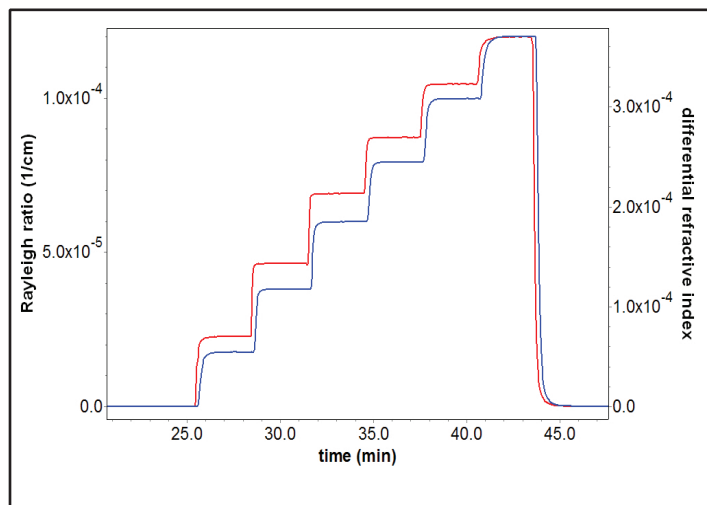


Figure 1. Preparation, mixing and injection of dextran sample with binary pump. Static light scattering (red) detection by DAWN HELEOS. Concentration (blue) detection by Optilab rEX. Experiment time ~30min.

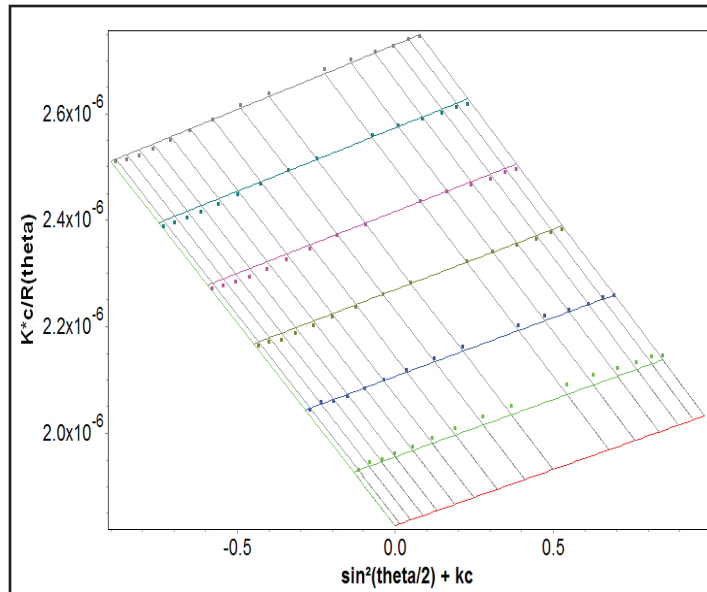
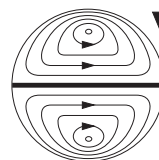


Figure 2. Zimm plot from binary pump preparation and injection. Data were collected and analyzed in ASTRA. Data quality meets or exceeds data quality of standard syringe pump runs.



**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>