WYATT TECHNOLOGY

APPLICATION NOTE

AN6002: Automated Measurements of Electrophoretic Mobility

Introduction

An HPLC system equipped with an autosampler enables unattended, automated SEC-MALS measurements of multiple samples for analysis of molar mass and size. The same concept can be implemented to perform automated measurements of electrophoretic mobility μ_E and hydrodynamic radius R_h with the Mobius™ instrument for DLS and MP-PALS. A pre-programmed sequence controls the timing of injections, while the auto-inject signal triggers an Event Schedule in Wyatt's DYNAMICS® software to collect data automatically.

Materials and Methods

The Mobius was connected to an autosampler, HPLC pump and degasser, all from Agilent. The HPLC modules were controlled by DYNAMICS. Automated injections of three samples of different sizes and mobilities—either polystyrene spheres (20 nm, 200 nm, and 500 nm in diameter) or proteins (lysozyme, BSA, and a monoclonal antibody)—were performed using an autosampler sequence. Synchronous data collection was achieved via the DYNAMICS software Event Schedule. After each injection, consisting of 500 µL of sample solution, the flow was stopped to allow measurements of electrophoretic mobility and hydrodynamic radius.

Mobius MP-PALS electrophoretic mobility data were acquired across 30 photodiodes at adjacent scattering angles, simultaneously with DLS acquisition. During each 25-second MP-PALS acquisition, five 5-second DLS acquisitions were performed. Five complete sets of such acquisitions were acquired sequentially for each sample in order to calculate averages and standard deviations.

Buffer was then injected to flush the flow cell, preventing contamination of the next sample. The total time for five measurements of each injection was less than 3 minutes, with an additional 10 minutes required to deliver the sample, stop the flow, and flush out the flow cell after each injection.

After the first series of measurements was completed, each sample was injected a second time to demonstrate that there was no carry-over.

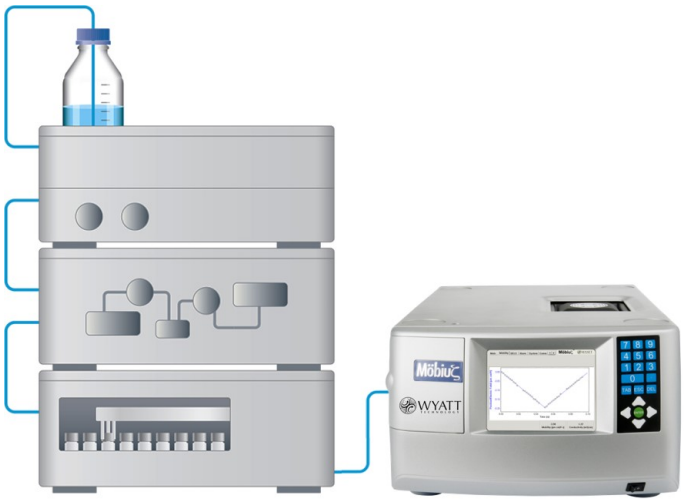

Figure 1: HPLC and Mobius System

Results

As shown in Figures 2 and 3, each injection of the same sample produced similar hydrodynamic radius and electrophoretic mobility results, despite having cycled through a series of samples with differences in size of over an order of magnitude (polystyrene, Figure 2) and changes in net charge from positive to negative to neutral (proteins, Figure 3).

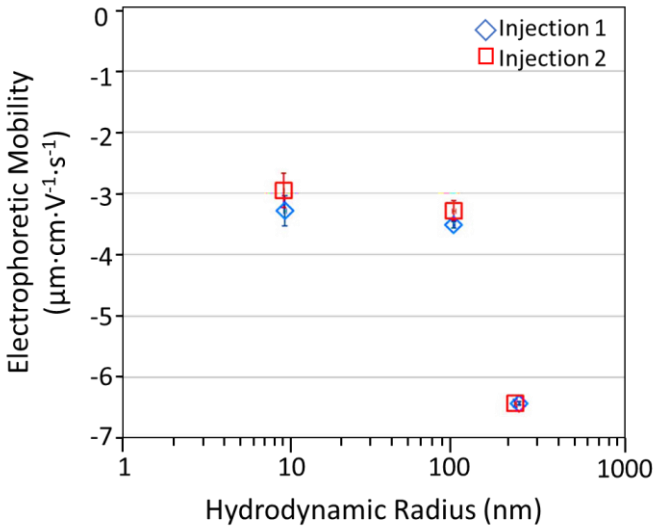

Figure 2: Measured hydrodynamic radius and electrophoretic mobility for polystyrene spheres. Average and standard deviation of five measurements are shown for each injection.

Moreover, the Mobius retained its unparalleled sensitivity and precision after multiple sample injections and was able to characterize lysozyme (R_h < 2 nm) despite exposing the flow cell to samples of much larger size and opposite charge (Figure 3). The low applied voltage—2.5 V—coupled with short measurement times—about 25 seconds—ensure that no electrolysis occurs and that the samples are not degraded during the measurement.

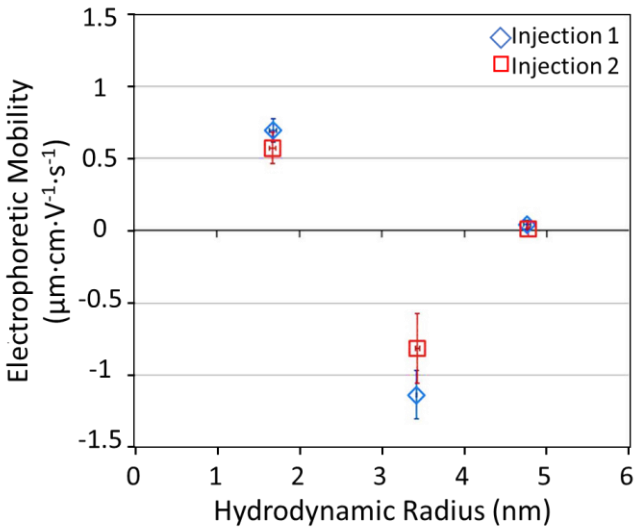

Figure 3: Measured hydrodynamic radius and electrophoretic mobility for lysozyme (5 mg/mL, R_h ~ 1.7 nm), BSA (2 mg/mL, R_h ~ 3.4 nm), and a monoclonal antibody (1 mg/mL, R_h ~ 4.8 nm). Average and standard deviation of five measurements are shown for each injection.

Conclusions

The unique combination of a Mobius with HPLC modules to automate sample delivery creates a powerful, reproducible characterization system. The versatility of the Mobius hardware and DYNAMICS software makes it simple to measure the electrophoretic mobility and hydrodynamic radius for a variety of samples in solution—virtually unattended.