Automated Measurements of Electrophoretic Mobility with the Möbiuζ

An HPLC system equipped with an autosampler enables unattended, automated light scattering measurements of multiple samples for SEC-MALS analysis. The same system can be implemented to perform high-throughput measurements of electrophoretic mobility and hydrodynamic radius with the Möbiuζ. 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.

In this study, 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 analyzed using an autosampler sequence with simultaneous data collection performed via the Dynamics software Event Schedule. After each sample injection (500 µL), the flow was stopped to allow five measurements of electrophoretic mobility and hydrodynamic radius. Buffer was then injected to flush the flow cell and avoid 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.

As shown in Figures 1 and 2, each injection of the same sample produced a similar hydrodynamic radius and electrophoretic mobility, despite differences in size of over an order of magnitude (polystyrene, Figure 1) and changes in net charge from positive to negative to neutral (proteins, Figure 2). Moreover, the Möbiuζ retains 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 larger size and opposite charge (Figure 2). The low applied voltage (2.5 V) coupled with short measurement times (about 25 seconds) ensure no electrolysis occurred and that the samples were not degraded during the measurement.

The unique combination of a Möbiuζ plus an existing HPLC to automate sample delivery creates a powerful, reproducible characterization system. In short, the versatility of the Möbiuζ hardware (coupled with the Dynamics software) makes it simple to measure the electrophoretic mobility and hydrodynamic radius for a variety of samples in solution—virtually unattended.

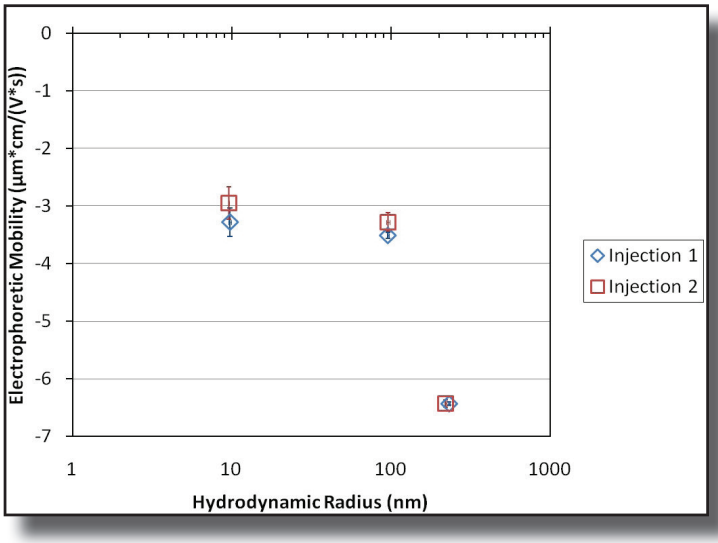

Figure 1: Measured hydrodynamic radius and electrophoretic mobility for polystyrene spheres. Dynamics software collected 5 measurements for each injection (average and standard deviation shown), and each 25-second measurement consisted of mobility data collected across 30 detectors and 5 DLS acquisitions.

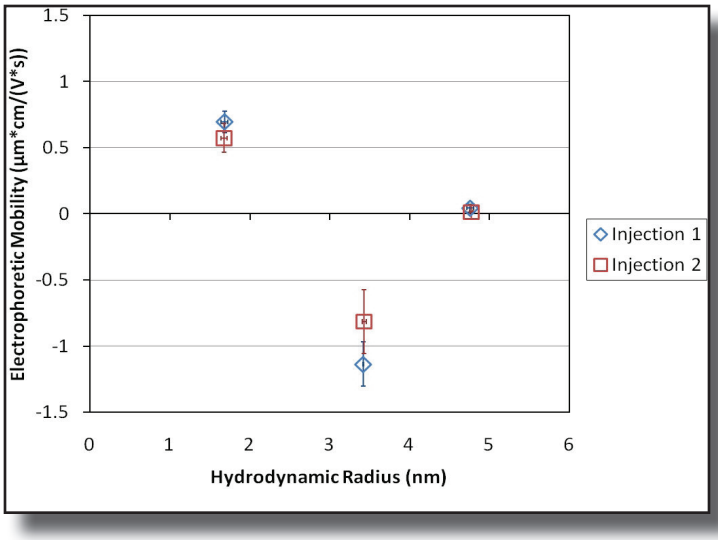

Figure 2: Measured hydrodynamic radius and electrophoretic mobility for lysozyme (5 mg/mL, r_h~1.7 nm), BSA (2 mg/mL, r_h~3.4 nm), and a monoclonal antibody (1 mg/mL, r_h~4.8 nm). Average and standard deviation of five measurements are shown for each injection, as for Figure 1.

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