Automated Electrophoretic Mobility Measurements of High Salt Solutions

The electrophoretic mobility of a solute is very much influenced by the solvent environment and, in particular, on parameters such as the solute concentration, solution pH, temperature, and ionic strength or formulation excipients. Data suggest that at low ionic strengths, solute charge is predominant and causes repulsion through long range interactions. Under high ionic strength conditions, shielding of these ionic interactions by counter-ions is increased, and repulsion of solute molecules is generally decreased. This suggests that ion shielding reduces long-range charge–charge interactions and favors mid- and short-range interactions. The more the ionic strength of the solution increases, the more counter ions are present in the vicinity of the solute molecules and the mobility decreases. The goal of this study is to show the effect of decreasing mobility due to increasing sodium chloride concentration for the protein bovine serum albumin (BSA).

For aqueous samples with conductivities near or above physiological saline conditions, mobility measurements have historically been difficult—or impossible. For a solution which conducts electricity, creating the electric field required for measuring mobility results in current traveling through the solution. As current passes through the solution, electrochemical redox reactions bring about electrolysis on the surface of the electrodes, evolving gas bubbles which distort the driving electric field and compromise the light scattering signal used for mobility measurements.

Wyatt Technology's Atlas™ accessory pressurizes the Möbiuζ cell for mobility measurements at high conductivities to supress and reduce the total volume of the existing gas bubbles. The Möbiuζ flow cell is a closed-system that enables automation and high-throughput simultaneous measurement of electrophoretic mobility and hydrodynamics radius. A pre-programmed HPLC sequence delivers 500 µL injections of the sample thereby making automated measurements of multiple samples completely straightforward.

The samples were formulated at 2 mg/mL into 50 mM phosphate buffered saline where the total sodium chloride concentration varied from 50 to 2000 mM, covering a conductivity range from 5.6 mS/cm for the 50 mM Phosphate ; 50 mM NaCl up to 86.2 mS/cm for the 50 mM phosphate; and 2000 mM NaCl. The total time for six measurements of each sample was 3 minutes plus 5-12 minutes required to deliver sample, stop flow, equilibrate, and then flush spent sample. Each sample was injected three times for a total of 18 measurements. The low applied voltage (3.5 volts) and low sample temperature (4°C) coupled with short measurement times minimized electrolysis and sample degradation.

The unique combination of the Möbiuζ with the Atlas™ and auto sampler allows, perhaps for the first time, automated analyses of high salinity samples. The versatility of the Möbiuζ hardware makes it more practical than ever to measure the electrophoretic mobility and hydrodynamic radius for solutions and formulations at high ionicities. Charge characterization of samples prepared in high-salt buffers is no longer a daunting task.
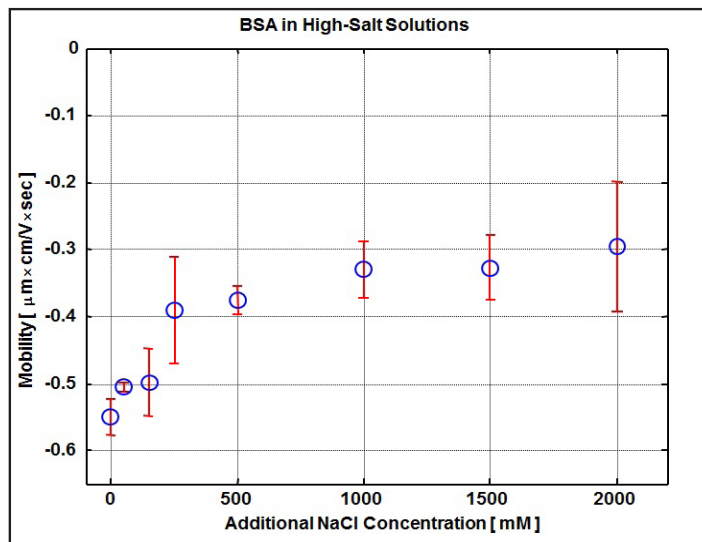

Figure 1. Plot of electrophoretic mobility vs. increasing salt concentration for 2mg/mL BSA injections measured at 4°C. At the lowest NaCl concentration (50mM) the measured mobility has the greatest magnitude, with a value of -0.51 mm cm/Vsec. As the NaCl concentration is increased, the BSA mobility magnitude decreases and eventually plateaus at a value of approximately -0.33 mm cm/Vsec. The mobility remains near this value as salt is added up to 2000 mM NaCl (corresponding to a conductivity of 86 mS/cm). As the ionic strength increases, there are more counter ions associated with the solute molecule; this has the effect of screening the molecular charge, resulting in a decrease of the electrophoretic mobility.

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