

Characterizing the Electrophoretic Mobility, Charge, And Zeta Potential of Biopolymers

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Abstract

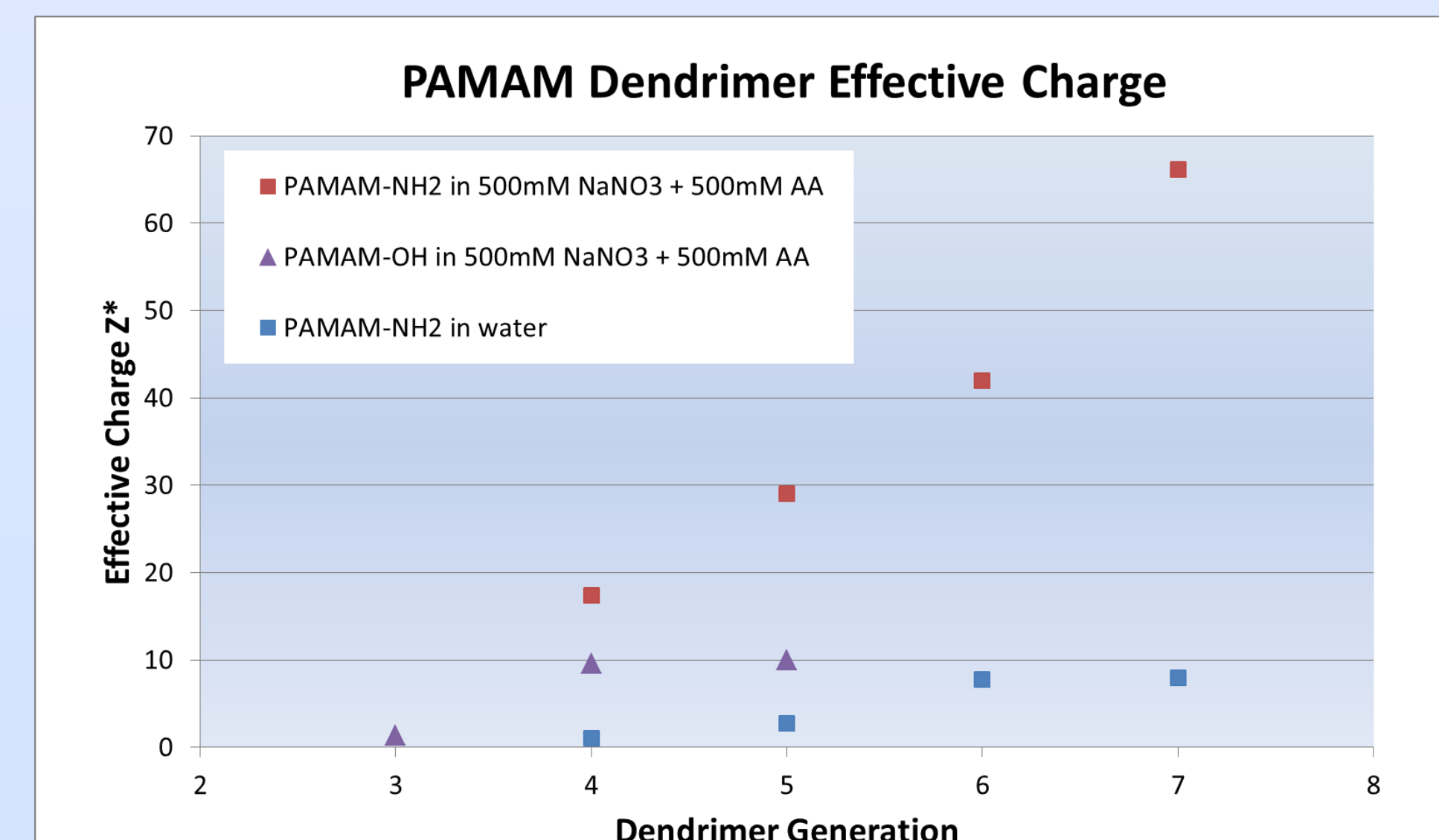
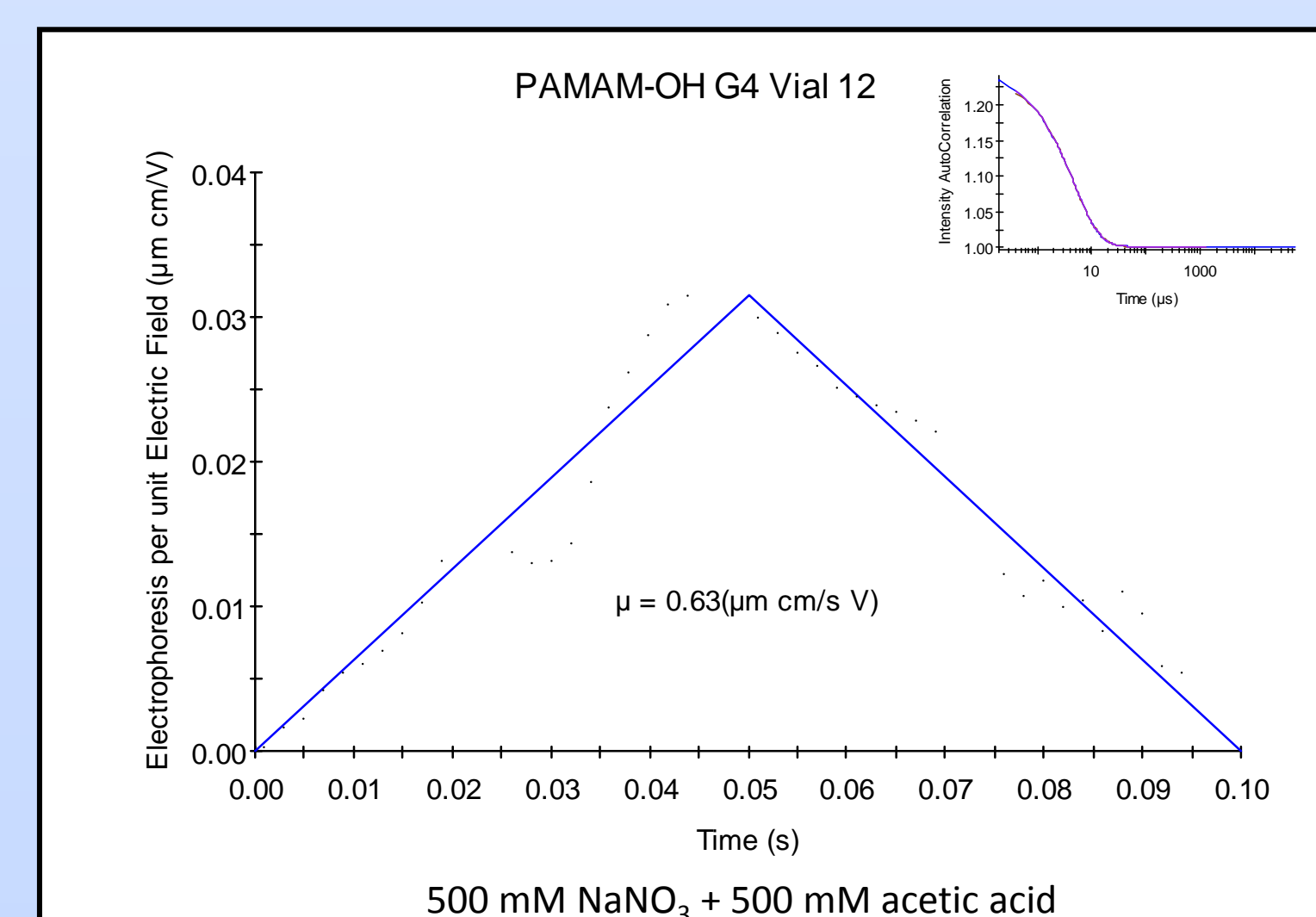
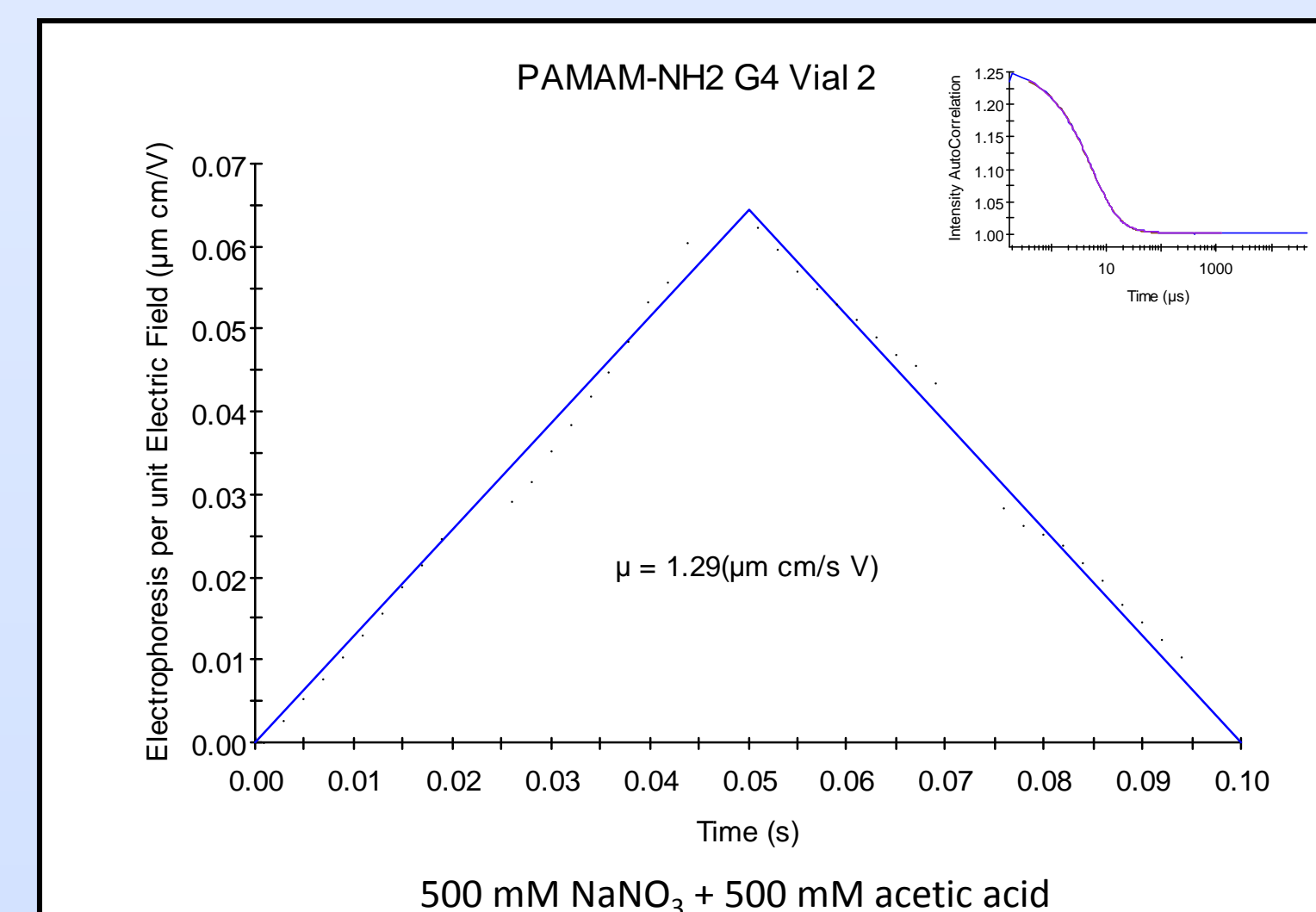
Batch measurement of electrophoretic mobility enables rapid, nondestructive characterization of the effective charge of biopolymers. While measuring electrophoretic mobility in solution has been a commonly applied to colloids for decades, improvements in solid-state electronics and electro-optics now enable this technique to be applied to fragile molecules, such as biopolymers, even at low concentrations. Additionally, the challenging electrolysis problem faced by researchers interested in measuring sample mobility in high-salt solutions has been overcome, such that samples with high ionicity (at or above physiological saline) can be measured easily. We applied the Wyatt Möbiuz and Atlas to measure the effective charge as a function of molecular weight and surface functionality of poly(amino amine) (PAMAM) dendrimers and heparin polymers. In the case of PAMAM, these dense star polymers have a narrow molar mass distribution and specific size and shape characteristics as well as a highly functionalized surface. The electrophoretic mobility and net effective charge was characterized for a series of amine terminated PAMAM dendrimers (G4-G7) and hydroxyl terminated PAMAM dendrimers (G3-G6) at neutral and acidic high salt concentrations. In the case of heparin, the charge:mass ratio of heparin standards with different molecular weights enabled the characterization of heparin from two different suppliers and the identification of a lot contaminated with super-sulfated material.

Conclusions

- Simultaneous measurements of electrophoretic mobility and hydrodynamic radius enable rapid, nondestructive characterization of effective molecular charge, even at high salinity.
- Amine-terminated PAMAM dendrimers show a pronounced increase of molecular charge at low pH while hydroxyl terminated PAMAM dendrimers are only slightly positively charged.
- The effective charge:mass ratio clearly distinguishes between unmodified and super-sulfated heparin.

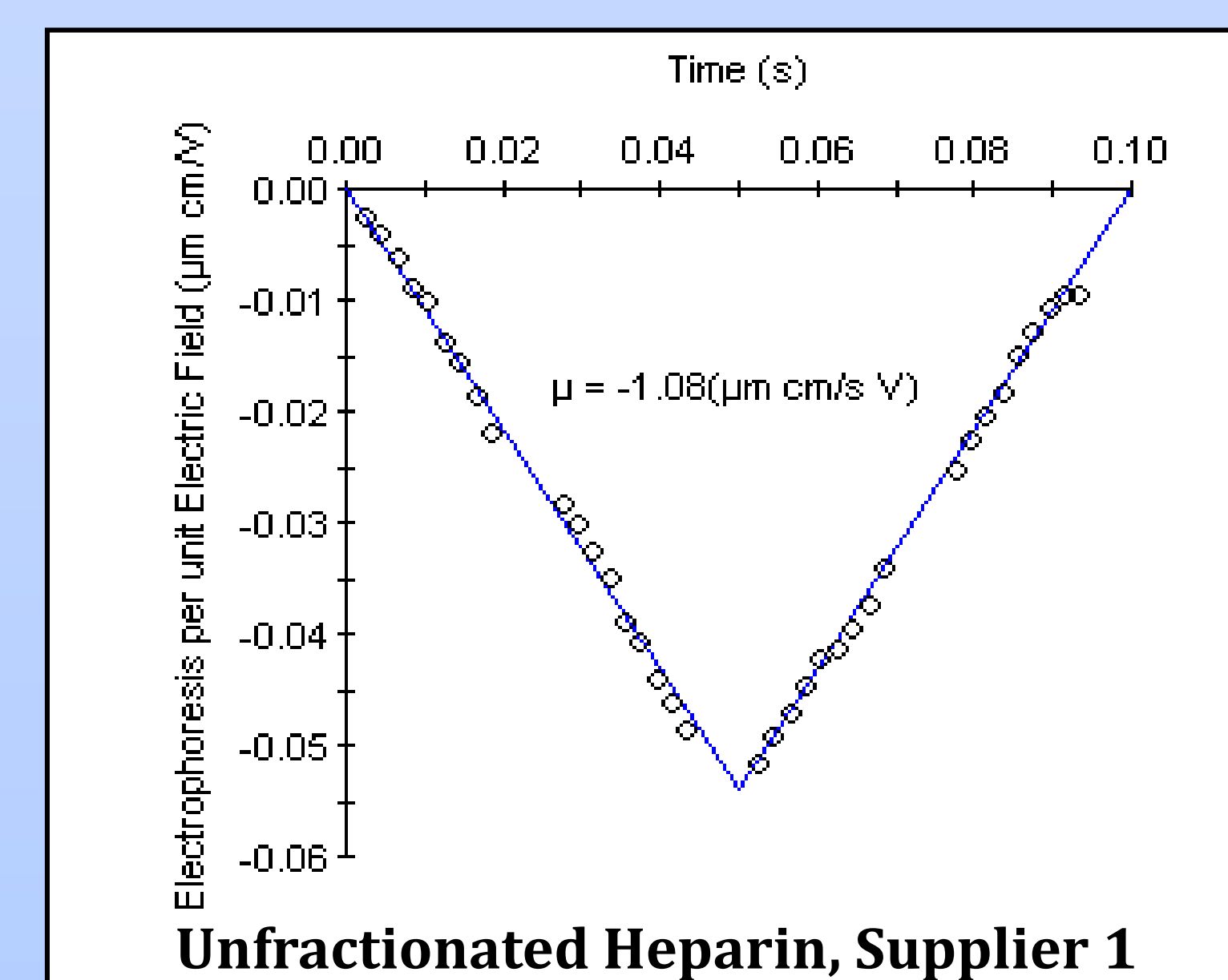
Results

PAMAM Dendrimers: Effective Charge vs. Generation



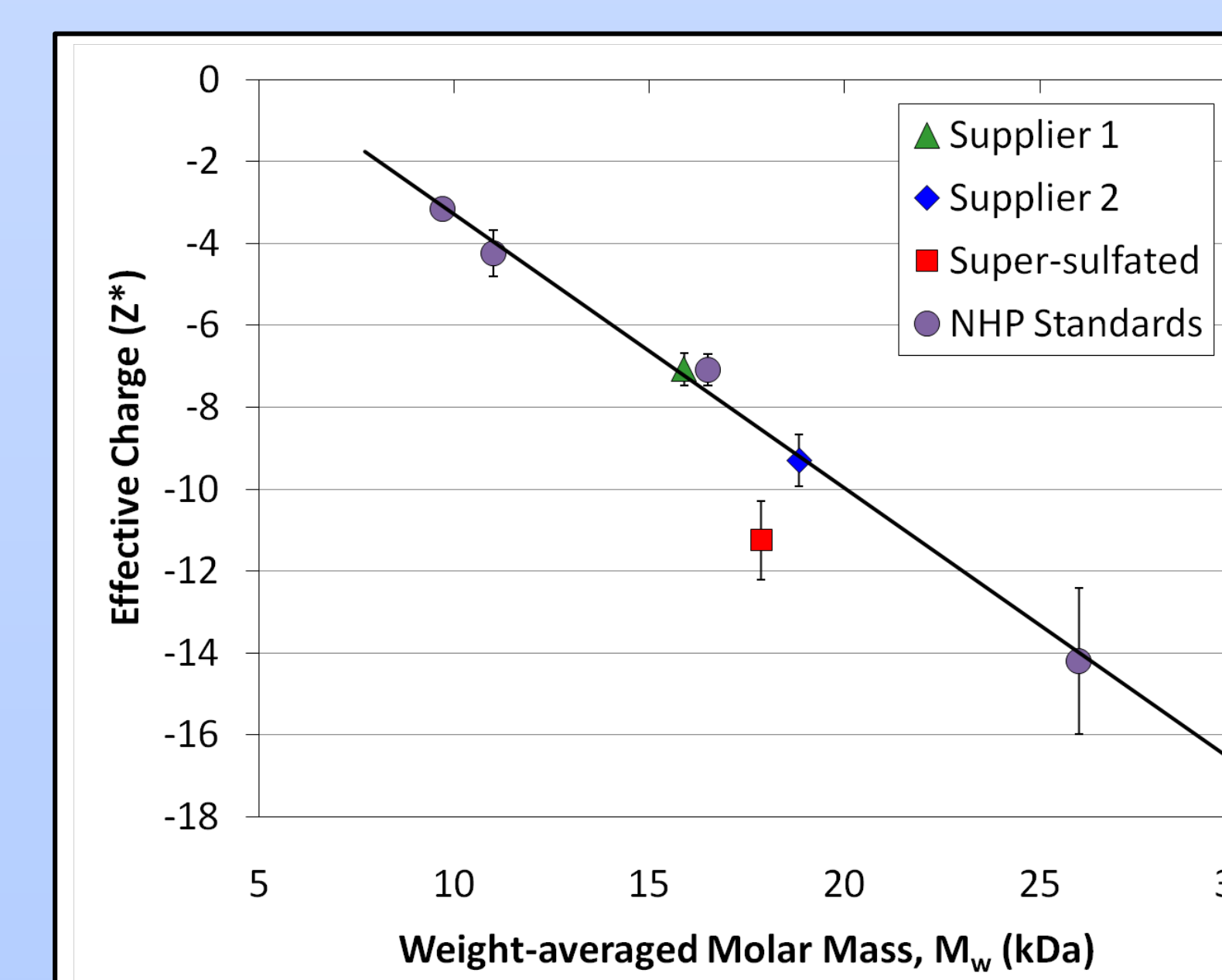
- Samples were measured in water as well as in 500 mM NaNO₃ + 500 mM acetic acid, conductivity 34 mS/cm.
- Sample concentration of 2 mg/mL.
- PALS and DLS collection period 30 s, 3V.
- Autosampler with Atlas was used to collect measurements automatically at high salt concentrations.

Heparin: Effective Charge vs. Molar Mass



Electrophoretic Mobility:

- Data represents average of 300 electric field oscillations, multiplexed across 30 detectors.
- Electrophoretic mobility (μ) indicative of negative charge.
- Effective molecular charge and zeta potential calculated from electrophoretic mobility and hydrodynamic radius (r_h).

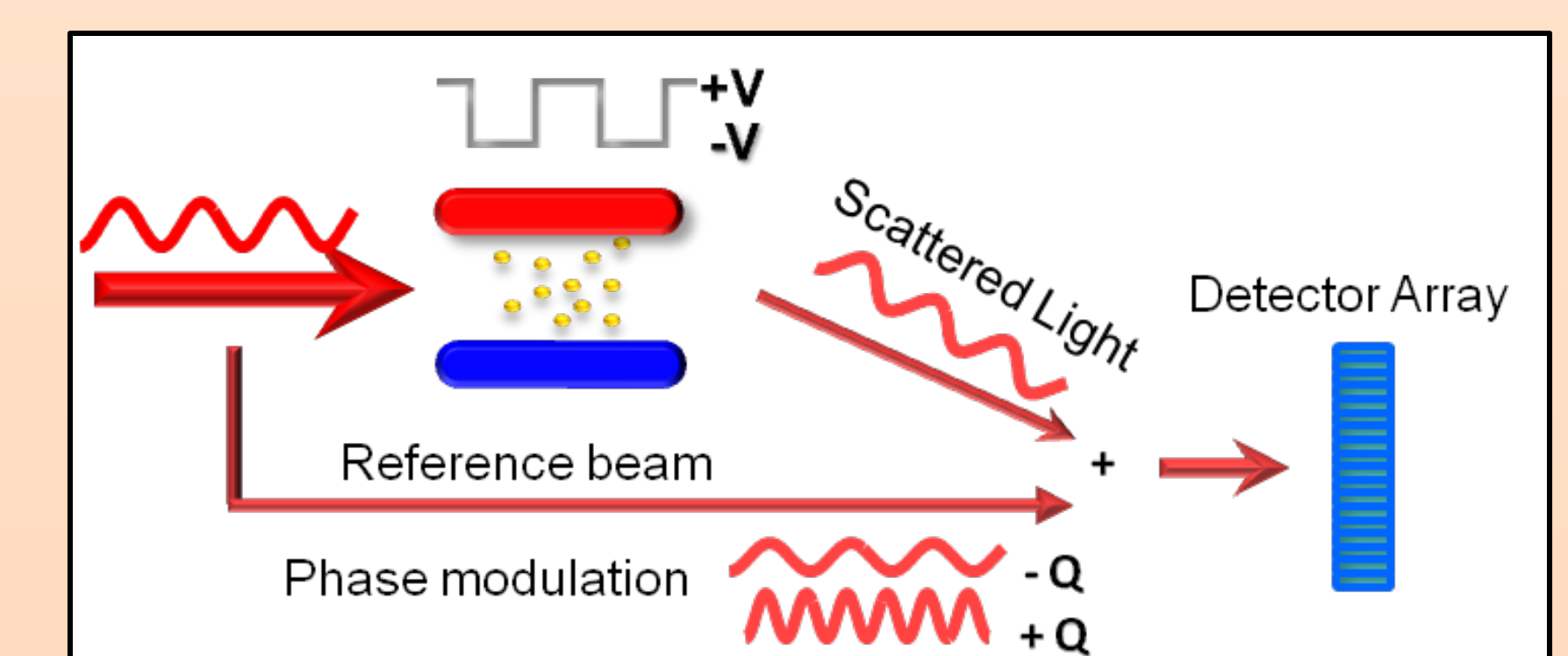


- Trendline drawn through Neoparin heparin-derived polysaccharide standards shows constant slope (charge:mass ratio).
- The charge:mass ratios for Suppliers 1 and 2 were consistent with the NHP samples.
- Super-sulfated material exhibited ~30% increased charge:mass ratio, as compared to unaltered material.

Methodology:

Electrophoretic Mobility

Z-averaged electrophoretic mobility was measured by MP-PALS (massively parallel phase-analysis light scattering) using a Wyatt Möbiuz. Simultaneously, z-averaged hydrodynamic radius (r_h) was determined by dynamic light scattering. Both were used to calculate effective molecular charge.



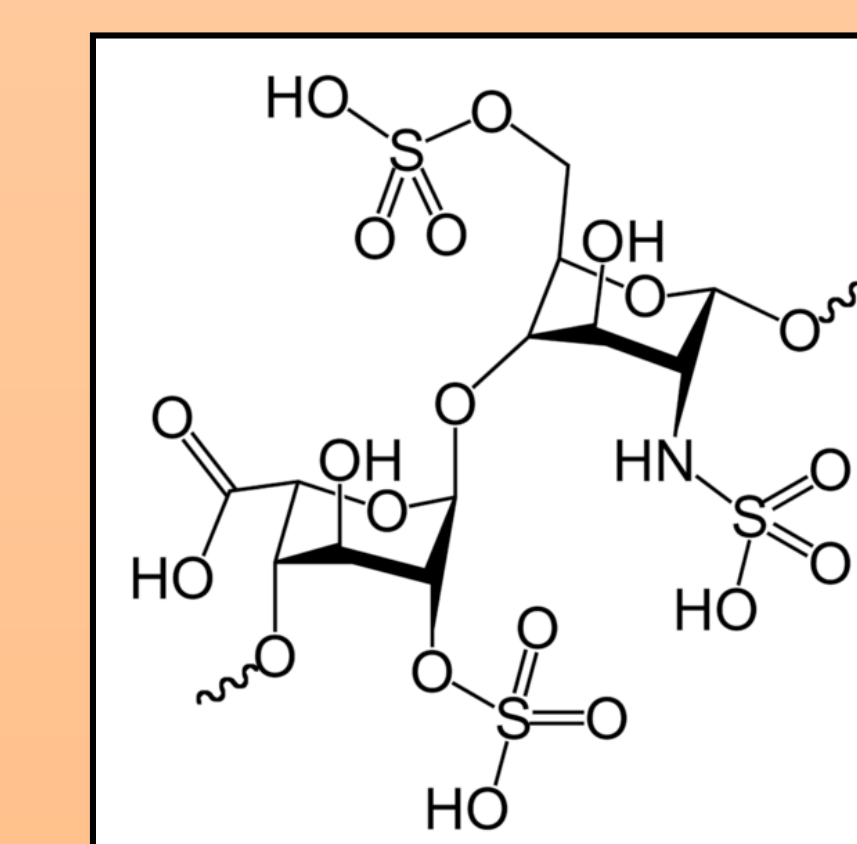
Massively Parallel Phase Analysis Light Scattering (MP-PALS)

- 30 independent and simultaneous measurements
- Reduced measurement time: < 30 seconds per sample
- Improved statistical averaging
- More efficient detection

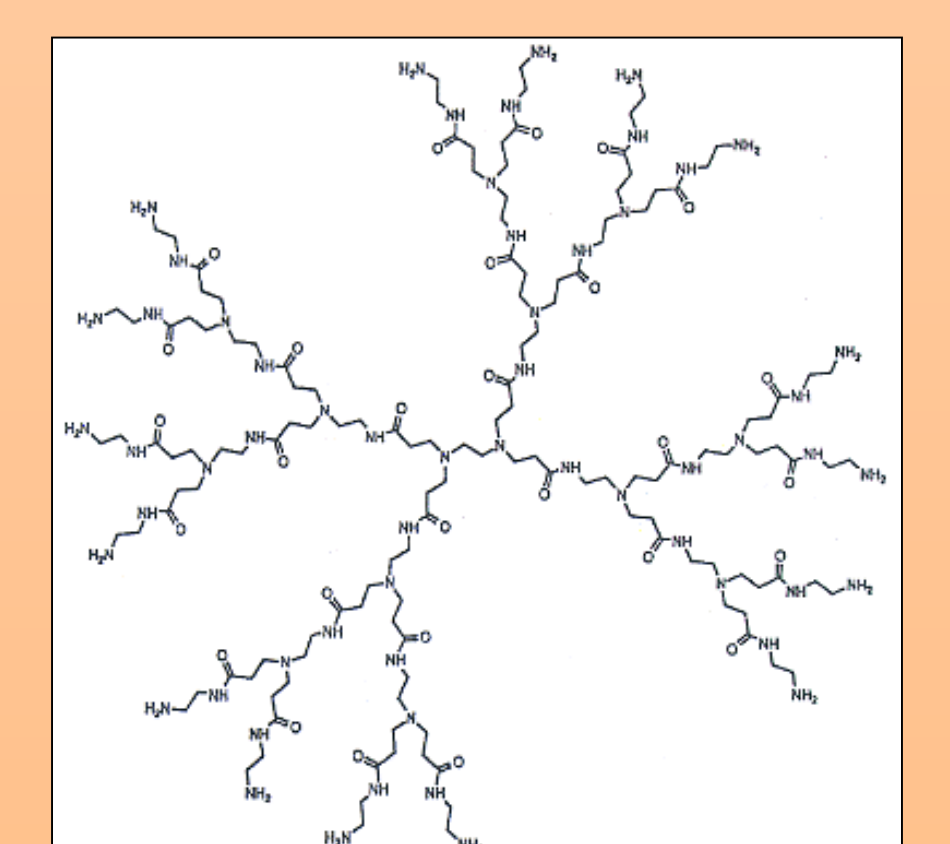
$$\text{Electrophoretic Mobility: } \mu_E = \frac{v}{E}$$

$$\text{Zeta Potential: } \zeta = \frac{3\eta}{2\epsilon f(\kappa R_h)} \mu_E$$

$$\text{Effective Charge: } Z^* = 6\pi\eta R_h \mu_E \frac{1 + \kappa R_h}{f(\kappa R_h)}$$



Heparin



PAMAM Dendrimer (G3)