Möbiuζ Computation of *Protein Net Charge* from Electrophoretic Mobility

The Möbiuζ measures the mobility (electrophoretic) of macromolecules by means of Phase Analysis Light Scattering (PALS). In addition to a protein's mobility, most researchers are interested in computing the net charge Z^*e carried by the molecules.

It is well known that a protein's mobility is very much influenced by its environment and parameters such as the solution pH value, ionic strength and excipients—all of which can affect the measured mobility. As the ionic strength of the solution increases, more counter ions are present in the vicinity of the protein molecules and the mobility generally decreases due to the electrophoretic effect[1].

With the WyattQELS™ option, the macromolecules' hydrodynamic radius r_h can be simultaneously measured and the effective charge Z^*e is computed from the DHH (Debye-Henry-Hückel) formula:

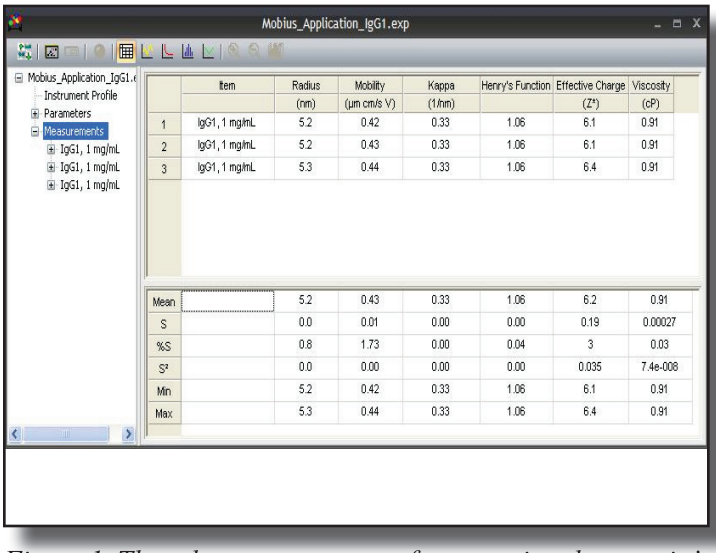
$$Z^*e = 6\pi\eta r_h \mu_E \frac{1+\kappa r_h}{f_1(\kappa r_h)},$$

where η is the solution viscosity, κ is the inverse Debye length and $f_1(\kappa r_h)$ is Henry's function. Dynamics™ has built into it the capability to calculate samples' net charge; once the user specifies the ionic strength of the sample, both the inverse Debye length and Henry's function are computed accordingly, as shown below in Figure 1.

In Figure 2, we present the computed net charge of an IgG1 antibody sample titrated to 9 different pH values.

There are numerous advantages associated with macromolecular characterization by laser light scattering. First of all, the Möbiuζ is a non-invasive instrument. In addition, Möbiuζ-based measurements facilitate the elimination of undesirable interactions (such as that between the proteins and the capillary walls), which so often renders other methods unreliable.

The unique and innovative optical design of the Möbiuζ boosts the sensitivity of mobility measurements, enabling protein charge characterization at much *lower* concentrations than traditional PALS instruments. Note that all measurements in Figure 2 have been carried out with a moderate antibody concentration of 1.0 mg/mL, which had been challenging for PALS-based instruments until the advent of Möbiuζ.



	Item	Radius (nm)	Mobility (µm cm/s V)	Kappa (1/nm)	Henry's Function	Effective Charge (Z*)	Viscosity (cP)
1	IgG1, 1 mg/mL	5.2	0.42	0.33	1.06	6.1	0.91
2	IgG1, 1 mg/mL	5.2	0.43	0.33	1.06	6.1	0.91
3	IgG1, 1 mg/mL	5.3	0.44	0.33	1.06	6.4	0.91
Mean		5.2	0.43	0.33	1.06	6.2	0.91
S		0.0	0.01	0.00	0.00	0.19	0.00027
%S		0.8	1.73	0.00	0.04	3	0.03
S²		0.0	0.00	0.00	0.00	0.035	7.4e-008
Min		5.2	0.42	0.33	1.06	6.1	0.91
Max		5.3	0.44	0.33	1.06	6.4	0.91

Figure 1. The relevant parameters for assessing the protein's net charge are computed and displayed in the data log grid of Dynamics™.

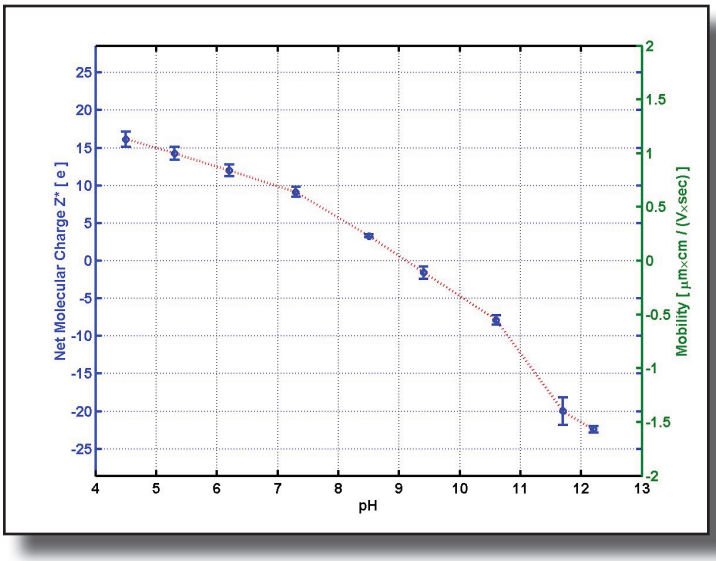


Figure 2. The net charge of an IgG1 antibody sample is computed and graphed along with its mobility at 9 pH values. As expected, the antibody carries a positive net charge below its pI and a negative one above the pI

[1] C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York, 1961.

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