# Calculating Particle Valence From Electrophoretic Mobility

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In the electrophoretic light scattering (ELS) technique utilized within the Malvern Panalytical Zetasizer series of products, an electric field (E) is applied across the sample and the velocity of the particles moving under the influence of the applied field is derived from the measured Doppler shift (Δv) in the light scattering from the particles. The electrophoretic mobility (μ_E) is then calculated according to the expression given here, where λ is the wavelength and θ is the scattering angle.

$$\mu_E = \frac{\Delta v\, \lambda}{2E\, \sin\left(\theta/2\right)}$$

When coupled with the particle size, the electrophoretic mobility can be used to calculate both the valence, also known as the Debye Hückel Henry charge (Z_{DHH}), and the effective charge (Z_{Eff}), or the apparent charge at the hydrodynamic boundary. The figure below can be used to schematically describe the difference between the effective and Debye Hückel Henry charges. The Debye Hückel Henry charge or the valence, represented by $-q_P$, is the surface charge due to hydrogen ion dissociation plus any tightly bound ions, such as Zn in the hexamer storage form of insulin. Territorially or loosely bound ions, such as those in the Stern layer are not included in the valence, but are a component of the diffuse double layer, represented by $+q_{DL}$. Historically, protein valence was measured via quantitative pH titration. The effective charge is the apparent charge at the hydrodynamic boundary, defined as the boundary within which all ions are diffusing with the protein, and takes into account screening effects arising from the diffuse double layer ions. The hydrodynamic boundary and subsequent location for the measurement of the effect charge resides somewhere between the hard particle surface, with a radius of a, and the outer limit of the diffuse layer, defined by the Debye screening distance. For low salt concentrations, the diffuse layer is thick, and it is difficult to define the location of the hydrodynamic boundary. But under moderate salt concentrations, *e.g.* > ~20 mM NaCl, the diffuse layer shrinks significantly, with the hydrodynamic boundary being very near the Stern or hydration layer.
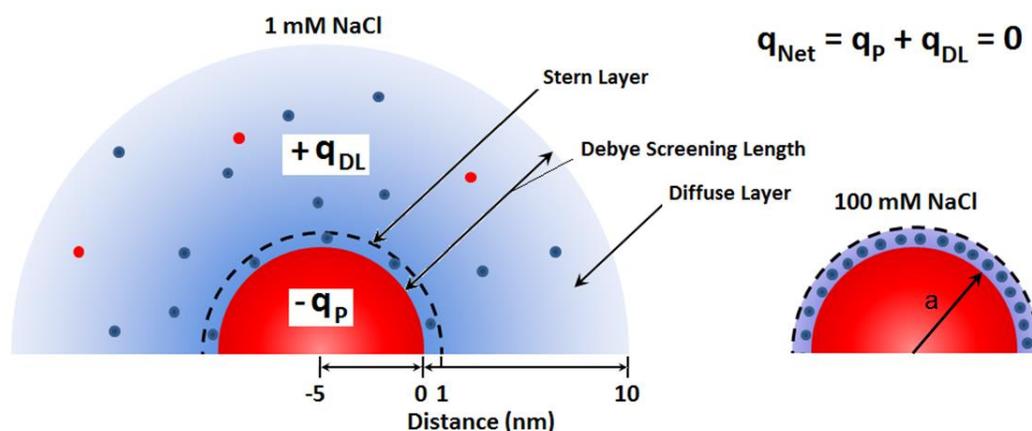
With regard to measurement, the simplest of the two types of charge to measure is the effective charge, which can be calculated from the electrophoretic mobility using the expression below, where η is the viscosity and R_S is the Stokes radius, or the hydrodynamic radius under ideal solution conditions, *i.e.* no particle interactions.

$$Z_{Eff} = \mu_E(6\pi\eta R_S)$$

The valence or Debye Hückel Henry charge can then be calculated from the 1st expression given here, where κ is the inverse Debye length, calculated from the ionic strength (I) using the 2nd expression, where ε_r is the dielectric constant, ε_o is the vacuum permittivity, k_B is the Boltzmann constant, T is the temperature, N_A is Avogadro's number, and e is the electronic charge, and $f(\kappa a)$ is the Henry equation, given in the 3rd expression.

$$Z_{DHH} = Z_{Eff}\left[\frac{1 + \kappa a}{f(\kappa a)}\right]$$

$$\frac{1}{\kappa} = \left(\frac{\varepsilon_r\varepsilon_o k_B T}{2N_A e^2 I}\right)^{1/2}$$

$$f(\kappa a) = 1 + \frac{1}{2}\left[1 + \left(\frac{2.5}{\kappa a[1 + 2\exp(-\kappa a)]}\right)\right]^{-3}$$

Note that a, the hard sphere radius of the particle, in the above expressions is not equivalent to the Stokes radius (R_S). The Stokes radius will include the hydration layer, where as the hard sphere radius will not. With that said, the thickness of the hydration layer is typically small in comparison to the hard sphere radius, with a often approximated by R_S.

As indicated earlier, the effective charge is the easiest to determine of the two types of protein charge, with three easily measurable parameters (μ_E, η, and R_S) and no approximations. In addition, the shielded effective is the charge experienced by other particles in close proximity, as opposed to the valence or surface charge. It is for these reasons, that the effective charge, rather than the valence, is often the type of protein charge of most interest to researchers working in the area of protein interactions.