New Advances in the Zeta Potential Measurements of Proteins

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Introduction

Stability of protein formulations is of critical importance for the rapidly emerging areas of protein therapeutics and vaccine production from recombinant antigens. Zeta potential, or the surface charge density at the shear plane, is one of the parameters used in predicting the stability of these preparations. Zeta potential has also been used as an indication of conformational changes in response to ligand addition. Traditional means of zeta potential measurements have employed classical laser Doppler velocimetry to derive the electrophoretic mobility of suspended proteins. Here, results from the phase analysis technique, employing an electrode design that eliminates electro-osmotic artifacts, are presented. Using this technique, it is possible to measure zeta potential under an extraordinary range of solvent conditions, including conditions of near-physiological ionic strength. This method vastly expands the range of experimental conditions under which zeta potential can be determined.

EXPERIMENTAL

Zeta potential measurements were performed with a Brookhaven Instruments ZetaPALS.

For enhanced sensitivity, the motion of the charged protein is probed with so-called phase analysis light scattering (PALS). In traditional laser Doppler electrophoresis, the frequency shift in the scattered light that arises due to the motion of the scatterers is used to determine the motion of the proteins. However, by recognizing measuring the phase shift in the scattered light, a much more sensitive measurement of particle motion can be made[1]. The optical arrangement is shown in Figure 1.

For additional sensitivity, an Uzgiris type electrode assembly, shown in Figure 2, was used for these measurements. This design suppresses the electro-osmosis effect found in capillary type cells. Electro-osmosis is the bulk motion of a fluid due to an applied field and arises due to wall effects. Such bulk motion needs to be minimized as it interferes with instrument sensitivity. The charged protein moves in response to the applied field.

Measurements were performed at 25 ^{o}C.

Proteins were obtained from Sigma Aldrich and used as received.

Figure 1: Optical Arrangement for Zeta Potential Determination

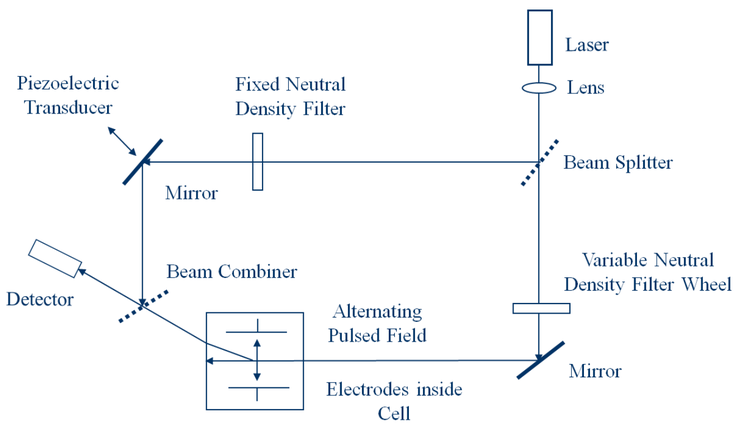
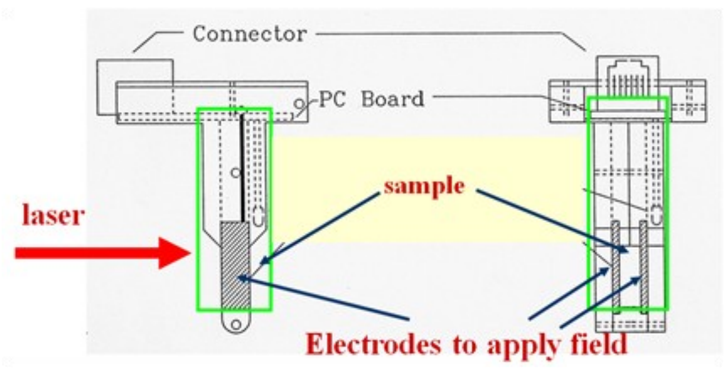


Figure 2: Uzgiris Cell for Zeta Potential Determination



Theory

Since it corresponds to the charge on the hyrdrodynamic unit, zeta potential, ζ, is the most intuitively appealing manner of discussing the surface charge . However, the quantity measured by electrophoretic light scattering is electrophoretic mobility, μ_{ep}.

The phase shift of the scattered light, Φ, is determined by measurement. It can then be related to the mobility via the following equation[1].

$$\Phi = \langle A \rangle \int \vec{q} \bullet \left(\mu_{ep} \vec{E}\right) dt$$

Here, <A> is the average scattered amplitude, q is the scattering vector the magnitude of which is $(4\pi n/\lambda)\sin(\theta/2)$ where n is the refractive index of the medium, λ is the wavelength of the light used to probe the sample, and θ is the scattering angle. E is the applied electric field. The integration is performed over the duration of the measurement.

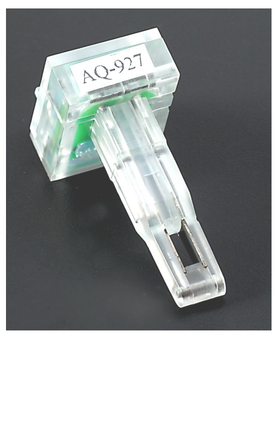
Mobility is converted to zeta potential using a model. The choice of model depends on two parameters, the particle size and the double layer thickness. Typically, the Smoluchovski model is appropriate for aqueous solutions and the Huckel model for nonaqueous. Here, the Smoluchovski model, shown below is used to convert determined mobility values to zeta potentials.

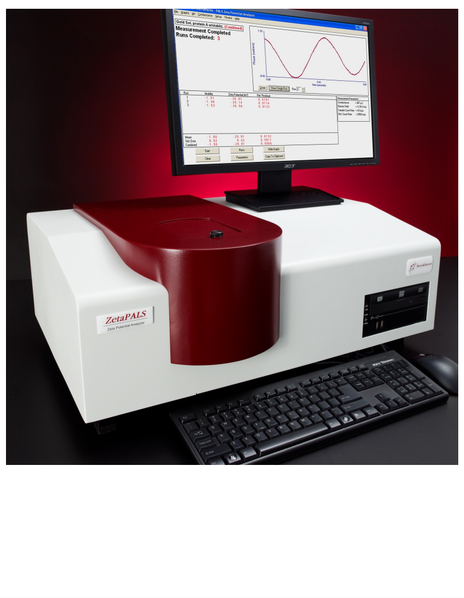
$$\mu_{ep} = \frac{\varepsilon_l \varepsilon_0 \zeta}{\eta}$$

Here, ε_l is the permittivity of the liquid, ε_0 is the permittivity of vacuum, and η is liquid viscosity.

References

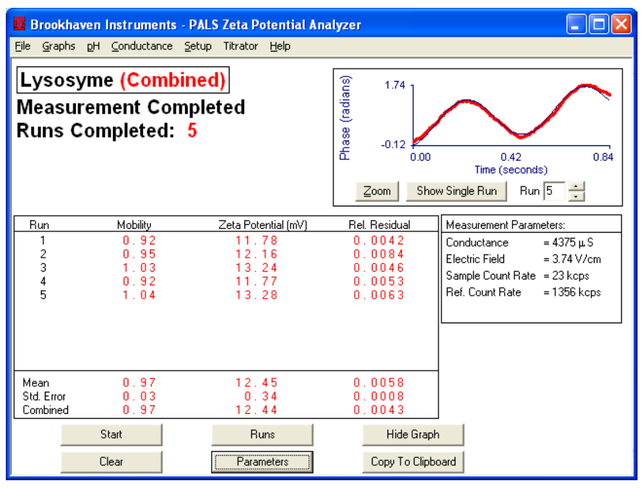
[1]Mobility Measurements by Phase Analysis, Walther W. Tscharnuter, *Applied Optics*, 40(24), August 2001, 3995-4003





Results

Typical Plot of phase vs. time for Phase Analysis.



Zeta Potential of Various Proteins

Protein (in PBS)	Measured Zeta Potential (mV)
Lysozyme from Chicken Egg White	5.91
Apoferritin from Horse Spleen	-13.70
Bovine serum albumin, initial fraction	-14.27

Effect of salt content on Lysozyme zeta potential. Note that decreased salt content leads to higher zeta potential values

PBS concentration	Measured Zeta Potential (mV) of Lyzsozyme
1x	5.91
0.1x	12.45

CONCLUSION

The ZetaPALS can be used to determine the zeta potential of proteins in high salt suspension.

Decreased salt content leads to higher zeta potential values due to the decreased impact of electrostatic shielding.