Application Note

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Charge and Interaction Analysis for Predicting Antibody Formulation Stability

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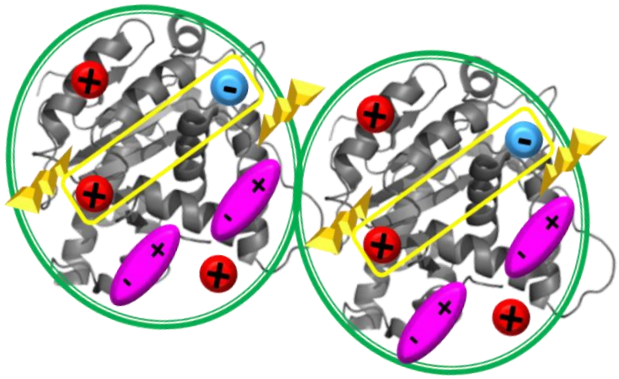
Summary

Colloidal stability is a key solution property of a biotherapeutic formulations, indicative of long-term stability. This property is indicative of the propensity for aggregation arising from the sum total of weak surface interactions due to surface charges, hydrophobic patches and related phenomena. Because it is readily measured over much shorter time scales than long-term stability testing or even accelerated testing regimens, measurements of colloidal stability are often utilized in the course of formulation work to screen out poorly behaved conditions.

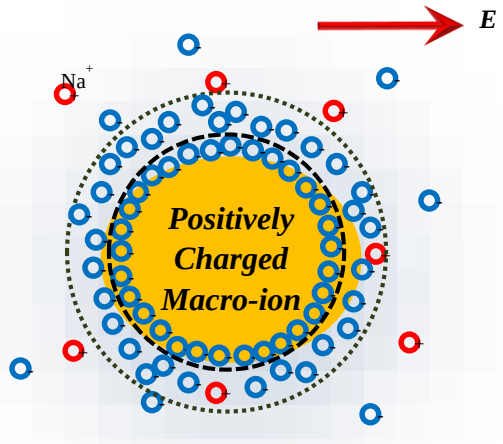
Net surface charge may be characterized readily by means of electrophoretic light scattering, implemented in the Möbius® as massively-parallel phase-analysis light scattering (MP-PALS), combined with simultaneous dynamic light scattering (DLS) for hydrodynamic radius. In some cases (high charge, moderate concentration) the net charge is sufficient to predict aggregation propensity. However, when the charge is relatively weak, additional information is necessary.

The overall magnitude of non-specific interactions at moderate protein concentrations is described well by the diffusion interaction parameter, k_D. This quantity is measured via the concentration dependence of the diffusion coefficient, also determined by DLS. Since it can be plumbed to an autosampler, the Möbius facilitates automated k_D measurement in parallel with charge determination.

In this note we present an example of combined measurements of net charge and k_D in order to understand the difference between two formulations with different stability behavior. Even though they have approximately the same net charge, its value is relatively low and so stability is dominated by the secondary effects of asymmetric charge distributions and hydrophobic residues.



The overall colloidal stability is influenced by the sum total of net charge and other weak interactions.



Charge is determined from electrophoretic mobility, the motion of a charged molecule subject to an electric field, and the hydrodynamic radius.

I. Introduction

Colloidal aggregation of proteins is driven by a complex arrangement of relatively weak electrostatic interactions. Characterizing a protein's net charge and non-ideality (non-specific interactions) is essential in predicting its propensity to form aggregates, as well as understanding its solubility and viscosity characteristics in a particular formulation. The Möbius can measure a protein's charge and non-ideality in a single, automated experiment. Charge is determined from electrophoretic mobility, and non-ideality can be determined from k_D, the diffusion interaction parameter. The Möbius uses Phase Analysis Light Scattering (PALS) to measure the electrophoretic mobility of macromolecules, and, simultaneously, Dynamic Light Scattering (DLS) to measure k_D.

$Z_{effective}$	Z_{DHH}	Colloidal Stability
0 to 0.5	0 to 1.5	Coagulation/Flocculation
1 to 3	3 to 9	Incipient Instability
3 to 4	9 to 12	Moderate Stability
4 to 6	12 to 18	Good Stability
> 6	>18	Excellent Stability

Table 1. Effective charge and Debye-Henry-Huckel charge and corresponding predicted protein stability. Courtesy of Dr. Tom Laue, Biomolecular Interactions Technology Center, University of New Hampshire.

DLS also provides information on the radius (r_h) such that the net charge can be computed from the mobility and the radius using the 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. The so-called Debye-Henry-Hückel charge is often indicative of colloidal stability, as shown in Table 1.

While charge can be sometimes be used as a sole predictor of a protein's solubility, stability, and viscosity (Table 1), a second indicator of protein stability, the diffusion interaction parameter (k_D) is used to understand the residual interactions due to other phenomena occurring on the surface of the protein. The diffusion interaction parameter is measured with DLS, and correlates well with the second virial coefficient, B_{22}, or A_2 measured with multi-angle Static Light Scattering (1, 2). The second virial coefficient is a measurement of non-specific solute-solute interactions (3). These interactions include dipole-dipole interactions, van der Waals interactions, and hydrophobic effects. A negative A_2 or k_D value indicates attractive interactions; a positive A_2 or k_D value indicates repulsive interactions. Attractive interactions are typically indicative of poor protein stability, as aggregation is likely due to these interactions. These interactions are affected by buffer salinity, pH, and excipients so their measurement is a valuable tool for formulations development. k_D is calculated from a linear fit of diffusion coefficient (D) vs. concentration (c) as follows: $D = D_0 + k_D c D_0$. The Möbius performs simultaneous, independent DLS and PALS measurements, making it the ideal tool for protein characterization.

In this study, we compare charge and k_D for two antibodies: one which is known to be stable under a variety of conditions and a second with poor stability.

II. Materials and Methods

We used an Agilent 1260 HPLC interfaced with a Wyatt Atlas™ and Möbius for complete automation of these experiments. The sample was introduced into the Mobius flow cell using the Autosampler. The sample chamber was then pressurized using the Atlas accessory, preventing formation of electrolysis bubbles during mobility measurements. All data were collected with the DYNAMICS software.

Samples were dialyzed into formulation buffer with 10 mM NaCl, 10 mM Histidine, pH 6.7. Antibody concentrations ranged from approximately 2 to 10 mg/mL for both Protein 1 and Protein 2. Using DYNAMICS, we made automated measurements of DLS only, followed by a simultaneous DLS and PALS measurement for each concentration. DLS was measured prior to application of current to ensure that the current did not affect the sample's behavior. Diffusion coefficients vs. concentration were fitted to find k_D for both antibodies, and the average charge was calculated as explained above.

III. Results and Discussion

The average charge was similar for both antibodies: 8.4 for protein 1 and 6.1 for protein 2, indicating that we would expect both antibodies to exhibit 'Incipient Instability' (*Table 1*).

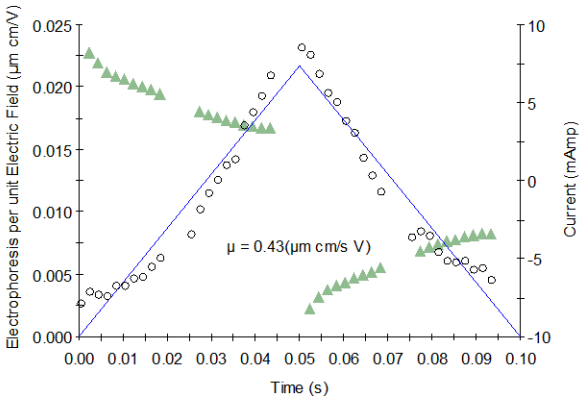


Figure 1. Representative mobility graph for Protein 1 showing mobility data (○), fit, and current (Δ).

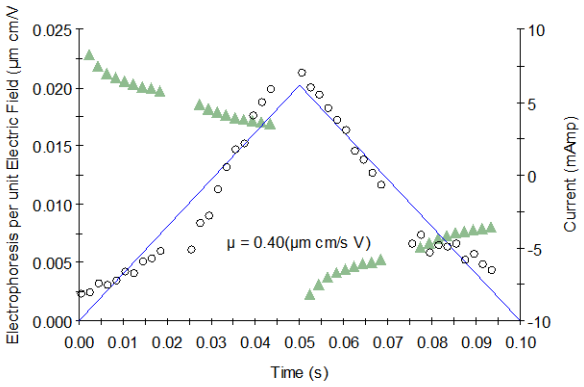


Figure 2. Representative mobility graph for Protein 2 showing mobility data (○), fit, and current (Δ).

However, protein 1 exhibits poor stability while protein 2 has excellent stability, so it is surprising that the charge is similar. In this case, the k_D measurement adds valuable information about the solution behavior of these antibodies. Protein 1 has a negative k_D (*Figure 3*), while protein 2 has a positive k_D (*Figure 4*); protein 1's negative k_D explains its tendency to form aggregates.

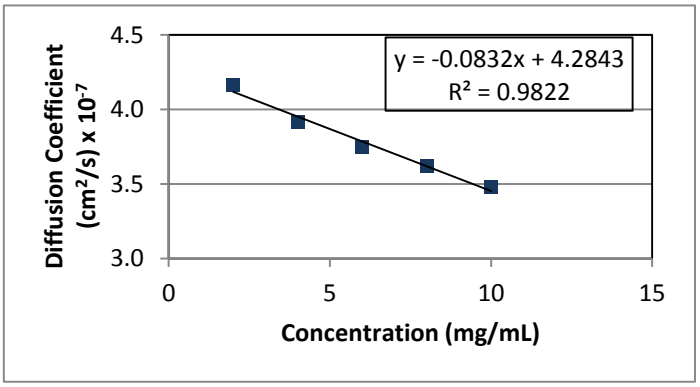


Figure 3. Diffusion coefficient as a function of concentration for Protein 1. The slope divided by the y-intercept yields a k_D value of -1.9 x 10^{-2} ml/mg.

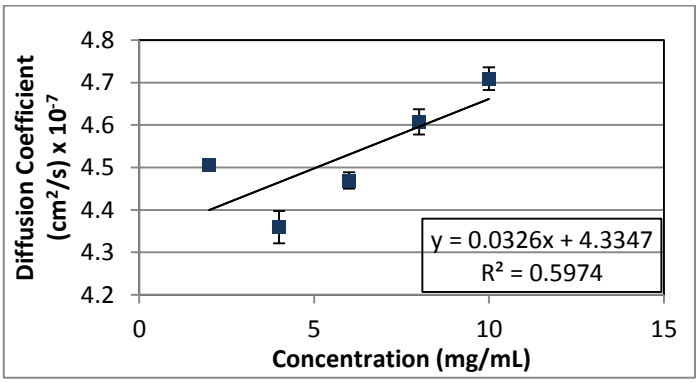


Figure 4. Diffusion coefficient as a function of concentration for Protein 2. The slope divided by the y-intercept yields a k_D value of 7.5 x 10^{-2} ml/mg.

IV. Conclusions

Both charge and diffusion interaction parameter are important parameters indicative of colloidal stability. However, charge alone may not provide a complete picture of the factors affecting stability. While the presence of a net charge provides stability, a molecule can have a favorable net charge but be de-stabilized by localized electrostatics such as a dipole moment as well as hydrophobic residues. The Wyatt Möbius has the unique ability to measure charge and k_D in one automated experiment. This capability provides a more complete picture of factors affecting a protein's stability than either individual measurement.

V. References

1. He et. al; *High Throughput Assessment of Thermal and Colloidal Stability Parameters for Monoclonal Antibody Formulations*. J. Pharm. Sci. **100**(12), 5126-5141 (2011).

2. C. Lehermayr et al. *Assessment of Net Charge and Protein-Protein Interactions of Different Monoclonal Antibodies*, J. Pharm. Sci. **100**(7), 2551-2562, (2011)

3. Charles Tanford. *The physical chemistry of macromolecules* - Wiley (1961).