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PUBLICATION HIGHLIGHTS

PH6001: Charge-Mediated Colloidal Stability of mAbs Formulation

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Introduction

The colloidal stability of proteins in solution is driven by many factors, including electrostatic interactions, short-range non-electrostatic interactions (e.g., hydrophobic and van der Waals forces), and steric repulsion.

Charge can induce electrostatic repulsion, preventing aggregation. However, higher concentrations of buffering agents, salts, or other ionic species reduce repulsion due to ionic shielding. In addition, charge distribution around the protein also impact its tendency to self-associate.

Attractive interactions including reversible self-association, can lead to high viscosity and aggregation. To counteract this, excipients and surfactants are often used to shield hydrophobic residues, enhancing stability through steric repulsion and reducing hydrophobic interactions.

Dynamic Light Scattering (DLS), Static Light Scattering (SLS), and Electrophoretic Light Scattering (ELS) provide complementary techniques for assessing colloidal stability. DLS and SLS can be used to assess irreversible aggregation as well as net attraction and repulsion among molecules by measuring the change in size and molar mass as a function of concentration. DLS measures the diffusion interaction parameter (k_D) while SLS determines the second virial coefficient (A_2), both of which quantify net attractive or repulsive interactions. ELS, on the other hand, measures zeta potential (ζ) and the Debye-Hückel-Henry effective charge (Z_{DHH}), specifically capturing the electrostatic contributions to stability.

This collection of publications highlights studies investigating the impact of charge, pH, and ionic strength on the colloidal stability of monoclonal antibodies (mAbs). These works feature key metrics such as k_D, A_2, zeta potential, and Z_{DHH}, measured using the Wyatt DynaPro™ DLS/SLS/ELS instrument line.

Publication Highlights

The Role of Electrostatics in Protein–Protein Interactions of a Monoclonal Antibody

Roberts, D. et al. Mol. Pharm. 11(7), 2475–89 (2014)
https://doi.org/10.1021/mp5002334

Roberts et al. combined simulation, theoretical, and experimental approaches, using data obtained with a DynaPro DLS/ELS instrument, and examined the influence of pH and ionic strength on protein-protein interactions, emphasizing the role of electrostatics and their relationship to key interaction parameters.

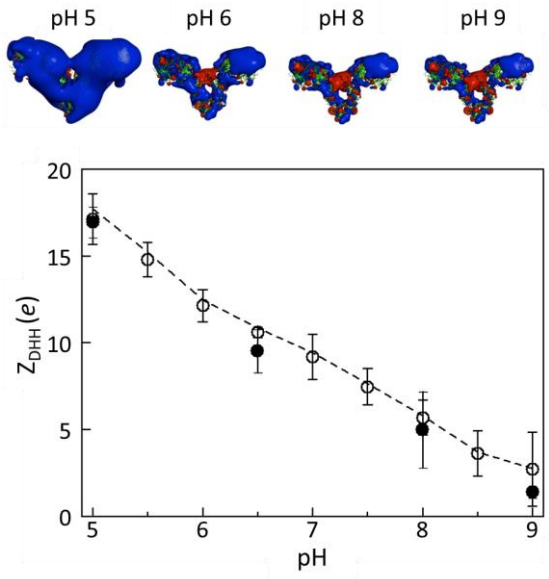

Figure 2: (A) Simulated mAb surface potential vs. pH; blue indicates a positive charge. (B) Effective charge (Z_{DHH}) is calculated from zeta potential.
Reprinted (adapted) from Roberts et al. Mol. Pharm. (11)7, 2475-89 (2014): with permission. Copyright 2014 American Chemical Society.

The researchers found that at lower pH, mAbs exhibited a positive effective charge, which decreased as pH increased. At higher pH, a region of negative charge

emerges in the hinge area of the protein and further increases in pH expose more neutral and positive charges, reducing the protein's overall effective charge.

They also investigated the effects of ionic strength at different pH values, demonstrating that small changes could shift interactions from repulsive to attractive. These findings underscore the delicate balance governing colloidal stability in protein solutions.

Solubility Challenges in High Concentration Monoclonal Antibody Formulations: Relationship with Amino Acid Sequence and Intermolecular Interactions

Pindrus, M. et al. Mol. Pharm 12(11) 3896-3907 (2015) https://doi.org/10.1021/acs.molpharmaceut.5b00336

Pindrus et al. investigated two mAbs, mAb-G and mAb-R, which bind the same target. The team provided series of images of mAbs in dialysis cassettes with varying levels of turbidity, depending on buffer conditions.

This study aimed to elucidate the molecular interactions underlying mAbs self-association and precipitation, using biophysical measurements to predict solubility behavior at high protein concentrations. Additionally, the researchers investigated site-specific modifications to reduce exposed hydrophobic regions and assessed their impact on aggregation.

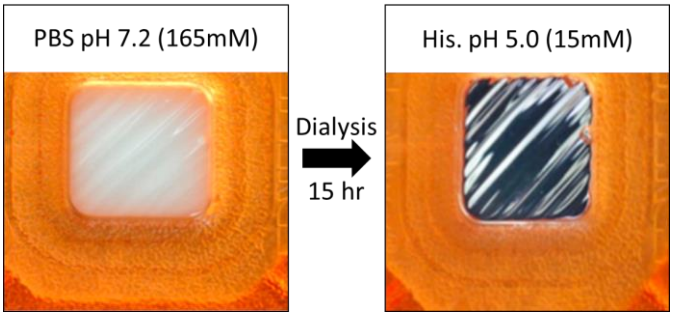

Figure 3. mAb-G dialyzed in PBS for 24 h; same dialysis cassette dialyzed in pH 5.0, 15 mM ionic strength buffer for 15 h (note: although image was taken after 15 h of dialysis, the solution became clear after only 2 h).
Reprinted (adapted) from Pindrus et al. Mol. Pharm 12(11), 3896-3907 (2015) with permission. Copyright 2015 American Chemical Society.

The team found that mAb-G exhibited significant aggregation, particularly under high ionic strength conditions. They demonstrated that buffer exchange of mAb-G from PBS (pH 7, 165 mM ionic strength) to a histidine buffer with a lower pH and lower ionic strength (pH 5, 15 mM ionic strength) reversed the aggregate formation observed in their dialysis cassette.

Using DLS and ELS data from a Wyatt DynaPro ELS instrument, the team measured k_D and Z_{DHH} to assess protein-protein interactions under varying pH and ionic strength conditions and check if they correlate with the observed aggregates.

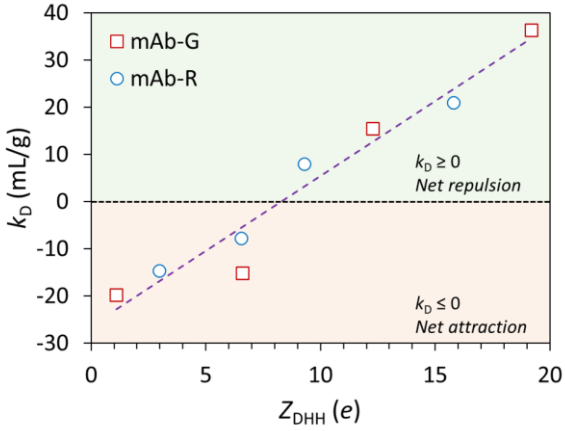

Figure 4. mAbs with measured effective charge (Z_{DHH}) greater than 6 shows a net repulsive protein-protein interaction, as indicated by k_D greater than zero. Data adapted from Pindrus et al. Mol. Pharm 12(11), 3896-3907 (2015)

Their findings showed that mAb-G displayed net attractive interactions (k_D = −23 mL/g) in PBS (ionic strength 165 mM), correlating with its aggregation behavior. Transitioning to a low-salt histidine buffer (ionic strength 15 mM) hindered this behavior, resulting in net repulsive interactions (k_D = +36 mL/g) and a positive charge (+19). This highlighted the critical role of pH and ionic strength in stabilizing mAb formulations.

The team further explored site-specific mutations to reduce hydrophobicity. Remarkably, substituting or removing just a few hydrophobic residues was sufficient to prevent precipitation, demonstrating how minor sequence changes can significantly impact protein properties.

Impact of Glycosylation on Protein–Protein Self-Interactions of Monoclonal Antibodies

Palakollu, V. et al. Mol. Pharm. 21(3) 141423 (2024) https://doi.org/10.1021/acs.molpharmaceut.3c01069

Using a Wyatt DynaPro DLS/SLS instrument, Palakollu et al. measured key metrics, such as k_D and A_2, of both native and deglycosylated mAbs under varying pH and ionic strength conditions to understand their impact on colloidal stability.

The study revealed significant differences in k_D and A_2 between native and deglycosylated mAbs, particularly at low ionic strength where electrostatic interactions dominate. While these differences diminished at high ionic strengths (where electrostatics are screened), qualitative variations persisted, suggesting a role for steric effects mediated by glycosylation. These findings highlight the importance of accounting for glycans in understanding protein self-interactions.

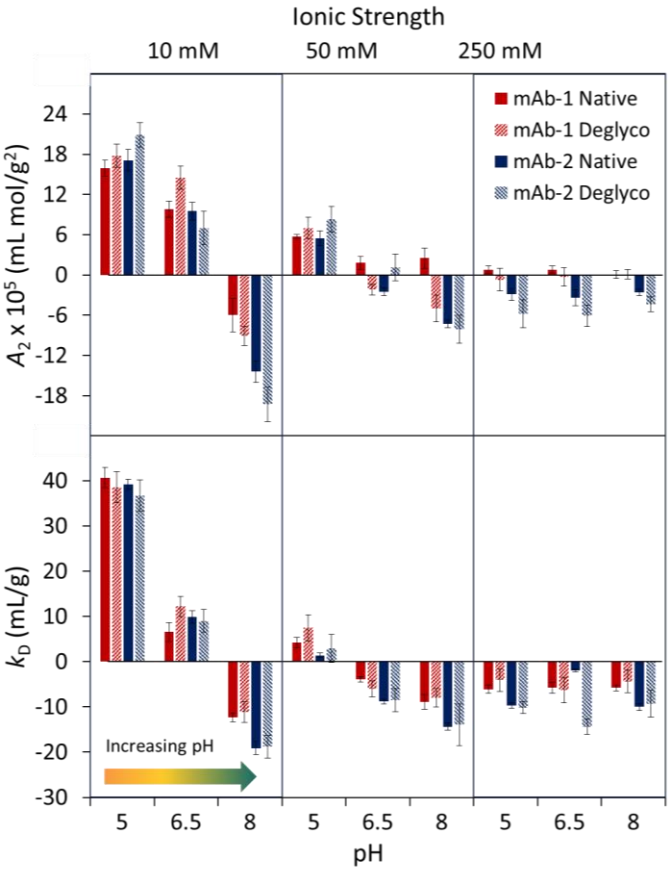

Figure 5. Second osmotic virial coefficient (A_2) and diffusion interaction parameter (k_D) values for native and glycosylated mAb1 and mAb2 as a function of pH and total ionic strength.
Data adapted from Palakollu et al., Mol. Pharmaceutics 21.3 (2024): 1414-1423.

Furthermore, the study demonstrated that increasing ionic strength led to a decrease in both k_D and A_2. At pH 5, the effect is the most dramatic, shifting interactions from strongly repulsive at low ionic strength to net attractive at higher ionic strengths. This trend underscores the role of ionic strength in modulating electrostatic contributions to mAb self-association.

Colloidal and Formulation Stability Solutions

Figure 6. (Left) The DynaPro ZetaStar™ automation-compatible DLS/ELS/SLS instrument and (Right) DynaPro Plate Reader high-throughput DLS/SLS instrument.

The DynaPro ZetaStar DLS/SLS/ELS instrument offers a versatile and efficient solution for assessing key colloidal stability metrics, including zeta potential, effective charge, k_D, and A_2. With its low-volume ELS cuvette, the DynaPro ZetaStar's dual detectors delivers rapid, simultaneous measurements of size and zeta potential in under a minute. Its seamless integration with the Arc pump and autosampler automates sample handling, reducing manual intervention while maximizing productivity. This makes the ZetaStar instrument an ideal choice for both routine tasks and complex analytical challenges.

For high-throughput screening, the DynaPro Plate Reader instrument provides advanced DLS and SLS measurements across 96, 384, and 1536 well-plate formats. With minimal sample requirements, it generates extensive data efficiently and can function as a standalone instrument or as part of an automated formulation workflow. Its ability to analyze multiple biotherapeutic candidates under diverse formulation and environmental conditions—completely unattended—makes it an indispensable tool for large-scale studies.

For more information on the technology and applications of ELS, please visit www.wyatt.com/zetapotential.

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