The use of small angle X-ray scattering for studying excipient modulated physical stability and viscosity of monoclonal antibody formulations

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Challenges of monoclonal antibody (mAb) formulation

• High concentration is required to achieve therapeutic dosage

• High concentration leads to increased non-specific protein-protein interactions (PPI) that could lead to self-association and solution viscosity

• Excipients are used to improve protein colloidal stability (tendency to remain monomeric form)

• Selection of excipients involves laborious empirical screening due to limited knowledge of the effects of excipients on PPI

*Commonly used excipients*

*Image cited from: https://www.youtube.com/watch?v=LMG07v2wIvQ*
Aims of this study

- To characterize the effects of excipients on a particular monoclonal antibody (NISTmAb)

- To evaluate different techniques for studying excipient modulated PPI in concentrated mAb formulations
  - Physical stability: Dynamic Light Scattering (DLS) vs Small Angle X-ray Scattering (SAXS)
  - Solution viscosity: DLS, SAXS (predicted) vs Viscosity measurements (experimental)

<table>
<thead>
<tr>
<th>Excipient Class</th>
<th>Excipients</th>
<th>Buffer</th>
<th>Ionic Concentration (mM)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>300 mM Glucose</td>
<td>25 mM Histidine</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>300 mM Sucrose</td>
<td>25 mM Histidine</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>300 mM Trehalose</td>
<td>25 mM Histidine</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>300 mM Mannitol</td>
<td>25 mM Histidine</td>
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<td>6</td>
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<tr>
<td>Amino Acids</td>
<td>171 mM Arginine</td>
<td>25 mM Histidine</td>
<td>196</td>
<td>6</td>
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<tr>
<td></td>
<td>200 mM Proline</td>
<td>25 mM Histidine</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>200 mM Glycine</td>
<td>25 mM Histidine</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>200 mM Alanine</td>
<td>25 mM Histidine</td>
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<td>6</td>
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<tr>
<td>Non-ionic Surfactants</td>
<td>0.06 mM Polysorbate 20</td>
<td>25 mM Histidine</td>
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<tr>
<td></td>
<td>0.12 mM Polysorbate 80</td>
<td>25 mM Histidine</td>
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<tr>
<td>Salts</td>
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<td>25 mM Histidine</td>
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<tr>
<td></td>
<td>150 mM Na₂SO₄</td>
<td>25 mM Histidine</td>
<td>312.5</td>
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<tr>
<td></td>
<td>150 mM NaCl</td>
<td>25 mM Histidine</td>
<td>162.5</td>
<td>6</td>
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<td>150 mM NaClO₄</td>
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<td>162.5</td>
<td>6</td>
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<tr>
<td>pH</td>
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<td>67 mM Phosphate</td>
<td>82.8</td>
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<tr>
<td></td>
<td>-</td>
<td>67 mM Phosphate</td>
<td>148.3</td>
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</tr>
<tr>
<td></td>
<td>-</td>
<td>67 mM Phosphate</td>
<td>196</td>
<td>8</td>
</tr>
</tbody>
</table>

mAbs in 25mM histidine buffer (without excipient) is used as control sample
NIST monoclonal antibody reference material (NISTmAb)

- First mAb (IgG1) reference material, representative of the largest class of biological therapeutics

- Standard reference material for analytical characterization of biopharmaceutical products, facilitates the assessment of existing analytical methods and promotes faster adoption of new technologies

- Used as representative mAb for this study

Small Angle Scattering

The scattered intensity is expressed as:

\[ I(q) = (\Delta \rho^2 \phi V) \ast P(q) \ast S(q) \]

Where \( \Delta \rho \) is the difference in scattering length density, \( \phi \) is the volume fraction, \( V \) is the volume of the scattered objects, \( P(q) \) is the form factor and \( S(q) \) is the structure factor.
Small Angle Scattering

**Form Factor \( P(q) \)**
- Measured from dilute solution, where intermolecular interactions are negligible
- Contains information on the size and shape of scattering objects

**Structure Factor \( P(q) \)**
- Arise due to intermolecular interactions with increasing concentration
- Contains information on the relative position/spatial correlation of scattering objects
Protein colloidal stability: DLS vs SAXS

- Dynamic light scattering (DLS)
  - Measured at low concentrations, (<10mg/ml), but used to predict properties of concentrated formulations
  - Interaction parameter $k_D$ is obtained from DLS measurements:
    \[
    k_D = 2B_{22}M_W - (k_f + 2v)
    \]
    Where $B_{22}M_W$ is the thermodynamic component, $k_f + 2v$ is the hydrodynamic component
  - $k_D > -8 \text{ ml/g}^* : \text{Net Repulsive PPI}$
  - $k_D < -8 \text{ ml/g}^* : \text{Net Attractive PPI}$

$\text{Concentration (mg/ml)}$

$D_M = D_0 (1 + k_D C)$

Dynamic light scattering (DLS)

- Measured at low concentrations, (<10mg/ml), but used to predict properties of concentrated formulations
- 2\textsuperscript{nd} virial coefficient \(B_{22}\) is obtained from DLS measurements:

\[
\frac{KC}{R_\theta} = \frac{1}{M_W} + 2B_{22}C
\]

Where \(K\) is an optical constant, \(R_\theta\) is the Rayleigh ratio of scattered to incident light intensity, \(M_W\) is the weight average molecular weight

- \(B_{22} > 0 \text{ mol ml/g}^2\): Net Repulsive PPI
- \(B_{22} < 0 \text{ mol ml/g}^2\): Net Attractive PPI
Protein colloidal stability: DLS vs SAXS

SAXS spectra and $S(q)$ measured from NISTmAb in Alanine solution as a function of protein concentration

- Small Angle X-ray Scattering (SAXS)
  - Measured at both low and high concentrations

$$I(q) \propto P(q)S(q)$$

$P(q)$ is measured from dilute solutions
$S(q)$ is measured from concentrated solutions
Protein colloidal stability: DLS vs SAXS

- Small Angle X-ray Scattering (SAXS)

- $S(q)$ at $q \rightarrow 0$, i.e. $S(0)$ is obtained from fitting $S(q)$ profile, it is used to study nature of PPI

$S(0) < 1$ : Net Repulsive PPI
$S(0) > 1$ : Net Attractive PPI

SAXS spectra and $S(q)$ measured from NISTmAb in Alanine solution as a function of protein concentration
Comparison between $k_D / B_{22}$ and $S(0)$

- $S(0)$ value less than 1 was measured from all excipient conditions, suggesting the net PPI was of repulsive nature
- Close agreement was found between $S(0)$ and $k_D$ values
Analysis of $S(q)$ reveals various energetic components towards the net PPI

- **Compared to DLS, more information on PPI is revealed by SAXS**

  - **Excluded Volume Effect** (hard sphere model)
  - **Additional Repulsive Interactions** (More stable)
  - **Additional Attractive Interactions** (Less stable)

Different contributors toward net PPI can be resolved by fitting $S(q)$ profile to different models
Analysis of $S(q)$ reveals various energetic components towards the net PPI

- Compared to DLS, more information on PPI is revealed by SAXS

Excluded Volume Effect (hard sphere model)

Additional repulsive forces lead to smaller $S(0)$

Different contributors toward net PPI can be resolved by fitting $S(q)$ profile to different models

Hayter-Penfold model
Analysis of $S(q)$ reveals various energetic components towards the net PPI

- **Compared to DLS, more information on PPI is revealed by SAXS**

**Excluded Volume Effect (hard sphere model)**

Different contributors toward net PPI can be resolved by fitting $S(q)$ profile to different models

**Hayter-Penfold model**

**Two Yukawa model**

Additional attractive forces lead to larger $S(0)$

NISTmAb in D2O salt solution
- $C = 130$ mg/ml
Analysis of $S(q)$ reveals various energetic components towards the net PPI

• $S(0)_{\text{exp}}/S(0)_{\text{HS}}$
  
  $\text{< 1: improved colloidal stability}$
  
  $\text{>1: reduced colloidal stability}$

• Further analysis of $S(q)$ reveals the presence of attractive intermolecular interactions even though the net PPI is repulsive

Summary of $S(0)_{\text{exp}}/S(0)_{\text{HS}}$. Each point represents the ratio obtained for a particular protein concentration in given excipient condition.
Measurements were made to obtain the viscosity ($\eta$) of concentrated NISTmAb formulations (170mg/ml), whereas $k_D$, $B_{22}$ and $S(0)$ were used to predict the viscosity ($\eta$) of concentrated NISTmAb formulations.

Shaded area highlights samples from which a decrease in $k_D/B_{22}$ or an increase in $S(0)$ is correlated with an increase in $\eta$, and vice versa.
Conclusions

- NISTmAb is colloidally stable in all of the examined excipient conditions. Although the net PPI is repulsive, elevated solution viscosity was measured with the presence of excipients.

- The close agreement between $k_D$ and $S(0)$ results suggests DLS could be used to provide reliable information on the colloidal stability of mAbs in concentrated formulations.

- Detailed analysis of $S(q)$ reveals various energetic components towards the net PPI, hence provides valuable insights in guiding the excipient selections.

- $B_{22}$ and $S(0)$ appeared to be better viscosity predictors than $k_D$. Disagreement between predicted and measured results suggests other factors apart from PPI contribute to the bulk rheological properties of concentrated protein solutions.
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