Viscosity & Pharmaceuticals – Introducing Viscosity Measurements with VROC® initium one plus

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Viscosity – Resistance to Flow

- Velocity gradient perpendicular to flow
- Shearing motion – adjacent fluid elements forced to slide past each other
- Shear viscosity \( \eta \equiv \frac{\text{shear stress}}{\text{shear rate}} = \frac{\sigma}{\dot{\gamma}} \)
- Reflects molecular level behavior
  - Size, shape, interactions
  - Microstructure

\[
\eta \equiv \frac{\text{shear stress}}{\text{shear rate}} = \frac{\sigma}{\dot{\gamma}}
\]

\[
\sigma = \eta(T, \dot{\gamma})\dot{\gamma}
\]
VROC® — Viscometer/Rheometer-on-a-Chip

Microfluidics and MEMS (Micro-Electro-Mechanical Systems)
- MEMS Sensors – Silicon (Si) Pressure Sensor Array
- Microfluidics – Precision Glass Micro-Channel
- “rectangular-slit method” (USP, chapter 914)

Control
\[ \dot{\gamma} \sim Q \]

Measure
\[ \tau \sim \frac{\partial P}{\partial x} \]

Dynamic Viscosity
\[ \eta = \frac{\tau}{\dot{\gamma}} \]

Graph of Pressure vs. Sensor Position (mm)
VROC® initium one plus— Features

- High Throughput Automatic Viscometer/Rheometer
- 26 µL of Sample (minimum) 50 µL Full Range
- 40 Vial Rack, 96 Well Plate
- Sample Retrieval and Recovery
- 4-70 °C with Built-in Peltier Temperature Control
- Sample Rack Temperature Control, 4-40 °C
- Shear Rate and Temperature Sweeps
- Advanced Measurement & Cleaning Protocols
VROC® initium one plus

- Instrument components now compatible with organic solvents
- More efficient cleaning protocols
  - Organics often requiring only one solvent
  - More samples between refills
- Low sample and cleaning solvent volumes – less waste generated
- Loading protocols compatible with new vials with improved seal – minimizes evaporation
VROC® initium

- VROC® Chip
- Auto Sampler Syringe Cleaning Port
- Pump
- 40 Vial Rack
- 96 Well Plate
- Custom Rack
- Temperature Controlled
- 100 μL Test Syringe
- 100 μL Injection Port
- Waste Port
Overview

Benefits of Viscosity Measurement

• Practical application – injectability, processing, blinking, topical........
  ➢ Predict performance & processability

• Reflects microscopic behavior – investigative tool
  • Individual molecules – size, shape
  • Pair interaction
  • Complex structure formation
  • Impact of environment on all above
  ➢ Intelligent formulation – work smart, not hard!

Topics

• Sample recovery details
• Injectability of concentrated protein solutions
• Storage stability – sample age
• Temperature variation
  • Concentrated proteins
• Shear rate dependence
  • Excipients
  • Protein solutions
Sample Recovery – Efficiency

Efficiency defined as
\[
\frac{\text{mass after recovery}}{\text{initial mass}} \times 100
\]

Dependent on
- Initial volume in vial
- Viscosity

- 3 cP
- 10 cP
- 50 cP
- 150 cP

efficiency (%) vs. sample volume (µL)
Sample Recovery – Impact on Viscosity (≤ 3%)

- thera tears®
- Murine Tears®
- Bovine γ-Globulin 70 mg/mL
- BSA 30 mg/mL
- PBS
Concentrated Proteins Storage Stability

- 100 mg/mL Bovine $\gamma$-Globulin in PBS
  - premium vs. elite quality grade
  - stored at 4°C
- Viscosity measured at 25°C over time
  - Identify stability window
  - Quantify rate & magnitude of change
Concentrated Proteins
Storage Stability

• 250 mg/mL Bovine γ-Globulin
  • PBS, PBS + NaCl, PBS + Arginine, PBS + Arginine + NaCl
  • stored at 4°C
• Viscosity measured at 25°C over time
  • Identify stability window
  • Quantify rate & magnitude of change

![Graph showing viscosity over time for different buffers.](image-url)
Concentrated Protein Solutions
Temperature Sweeps

- Relevant temperatures
  - Storage/processing – 4°C
  - Delivery/injection – 25°C
  - Body – 37°C
- Arrhenius behavior prior to denaturation
  \[ \eta = \eta_0 e^{E_a/kT} \]
- Activation energy of protein solutions
  - Varies with concentration
  - Concentrated solutions
    - \( E_a \) deviates from buffer
    - Cannot predict from buffer, must measure

Arrhenius Plot

- 250 mg/mL Bovine \( \gamma \)-Globulin (PBS, pH 7.4)
  - 430 cP (4°C)
  - 82 cP (25°C)
  - 39 cP (37°C)

\begin{align*}
\text{predicted from buffer}
\end{align*}

Arrhenius Plot

- 250 mg/mL BgG
  - \( \gamma = -2127.8x \)
  - \( R^2 = 0.9984 \)
- PBS
  - \( \gamma = -6346.6x \)
  - \( R^2 = 0.9997 \)

\begin{align*}
\ln(\eta/\eta_{\text{ref}}) &= \gamma (1/T_{\text{ref}} - 1/T) \\
\gamma &= \frac{E_a}{R}
\end{align*}
Bovine $\gamma$-Globulin (250 mg/mL) pH 7.2 (pI~7), vary salt type & concentration

- Near pI
  - Initially salt increases solubility
  - Further addition decreases solubility
- Interaction potential
  - Salt initially decreases attraction
  - Additional salt increases attraction
- Viscosity increase due to reversible cluster formation
- Shear rate dependence to probe characteristic time/length scales

Hofmeister series

$\text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^-$

$\text{K}^+ > \text{Na}^+$

Salting out → Salting in
Shear Rate Sweeps
Na$_2$SO$_4$ ionic strength

Cross (dashed)
\[ \eta_o = \eta_\infty + \frac{(\eta_o - \eta_\infty)}{[1 + (\lambda \dot{\gamma})^m]} \]

Carreau Yasuda (solid)
\[ \frac{\eta - \eta_\infty}{\eta_o - \eta_\infty} = [1 + (\lambda \dot{\gamma})^a]^{(n-1)/a} \]

<table>
<thead>
<tr>
<th>[IS] (mM)</th>
<th>Cross</th>
<th>C-Y (a=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \lambda ) (msec)</td>
<td>( m )</td>
</tr>
<tr>
<td>75</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>1200</td>
<td>0.15</td>
<td>0.94</td>
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\( \lambda \) – characteristic relaxation time scale
\( m \) – indicates increasing polydispersity <1

Increase in \( \lambda \), polydispersity consistent with cluster formation
Concentration Dependence – Full Range

- Ross-Minton equation
  \[ \frac{\eta}{\mu} = \eta_{rel} = \exp \left( \frac{[\eta]c}{1 - \frac{k}{\nu}[\eta]c} \right) \]
  - \( k \) – crowding factor
  - \( \nu \) – Simha shape parameter
- Analogous to colloidal hard sphere behavior
- Divergence at maximum packing
- Introduce \( \nu \) – proteins not spherical

NaCl (mM) | Ross-Minton parameters
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([\eta]) (mL/g)*</td>
</tr>
<tr>
<td>150</td>
<td>8.4</td>
</tr>
<tr>
<td>1000</td>
<td>8.4</td>
</tr>
</tbody>
</table>

*Determined from single point intrinsic viscosity.
Injection Force Estimation from Viscosity

Force required to sustain viscous flow & overcome friction

Newtonian Case

\[ F = F_{\text{viscous}} + F_{\text{friction}} \]

where

\[ F_v = P_1 \times \pi R^2 \]

\[ \Delta P \times \pi R^2 = \sigma \times 2\pi R l \]

\[ \sigma = \eta \dot{\gamma} \quad \text{and} \quad \dot{\gamma} = \frac{4Q}{\pi R^3} \]

\[ \Delta P = \frac{8\eta lQ}{\pi R^4} \]

\[ P_1 = \Delta P_1 + \Delta P_2 + \Delta P_3 \]

\[ P_1 \sim \Delta P_3 \]

resistance in needle dominates

\[ F_v \approx 8\eta l n Q \frac{R_p^2}{R_n^4} \]

\[ I_n = \text{needle length} \]

\[ R_p = \text{piston radius} \]

\[ R_n = \text{needle radius} \]

\[ Q = \text{volumetric flow rate} \]

\[ \gamma \approx 100,000 \text{ s}^{-1} \]
Injection Force Predictions
Aqueous Glycerol Solutions - Newtonian

Variable flow rate $Q$, fixed 27G NW needle

Variable needle gauge ($R_n$), fixed $Q = 0.1$ mL/sec

- prediction slightly underestimates measured values
- data includes friction
  - literature values suggests $F_f \sim 2 – 4$ N (glass), 2 – 6 N (plastic)

Concentrated Proteins
Viscosity vs. Co-solute & Injection Force

Bovine Gamma Globulin (BγG) **250 mg/mL (pH 7.4)**

- Viscosity threshold – 20 to 50 cP
- Individual amino acids to reduce viscosity
  - Arginine & Histidine HCl
  - Screen electrostatic & hydrophobic interactions
- Minimize additional testing with force estimate

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Viscosity (cP)</th>
<th>Injection Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>55.6</td>
<td>50</td>
</tr>
<tr>
<td>250 mM HisHCl</td>
<td>41.5</td>
<td>37</td>
</tr>
<tr>
<td>150 mM ArgHCl</td>
<td>35.2</td>
<td>31</td>
</tr>
</tbody>
</table>

(Q = 62.5 µL/sec, l_n = 12.7 mm, R_n = 0.092 mm, R_p = 3.175 mm, neglect friction)
Ageing’s impact on injection

**Assumptions**
- Needle Length: 17 mm
- Needle Radius: 0.135 mm
- Piston Radius: 4.325 mm

<table>
<thead>
<tr>
<th></th>
<th>Shear Rate (sec(^{-1}))</th>
<th>Flow Rate (mm/sec)</th>
<th>Injection Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
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<tr>
<td>3500.00</td>
<td>6.76</td>
<td>1.92</td>
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<tr>
<td>25000.00</td>
<td>48.31</td>
<td>13.00</td>
<td></td>
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<tr>
<td><strong>Day 92</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3500.00</td>
<td>6.76</td>
<td>2.29</td>
<td></td>
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<tr>
<td>25000.00</td>
<td>48.31</td>
<td>15.09</td>
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<tr>
<th></th>
<th>Shear Rate (sec(^{-1}))</th>
<th>Flow Rate (mm/sec)</th>
<th>Injection Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2500.00</td>
<td>4.83</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>26000.00</td>
<td>50.24</td>
<td>20.41</td>
<td></td>
</tr>
<tr>
<td><strong>Day 55</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2500.00</td>
<td>4.83</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>26000.00</td>
<td>50.24</td>
<td>24.69</td>
<td></td>
</tr>
</tbody>
</table>
Summary

• Viscosity is more than a single number under one set of conditions
• Powerful tool when fully utilized
  • Select appropriate conditions to correlate to performance – predictive tool
    • Injectability
    • Processing/manufacturing
  • Probe interactions and microstructure – investigative tool to guide formulation
    • Formation of complex microstructure
    • Stability/denaturation of proteins with temperature
VROC® — Viscometer/Rheometer-on-a-Chip
Microfluidics and MEMS

\[ \dot{\gamma} = \frac{\partial u_x}{\partial y} \]

\[ u_x(y = 0, h) = 0 \]

\[ u_x = u_x(y) \]

\[ \eta \equiv \frac{\text{shear stress}}{\text{shear rate}} = \frac{\sigma}{\dot{\gamma}_w} \]

\[ \dot{\gamma}_w = \frac{6Q}{wh^2} \]

\[ \sigma = -\frac{\Delta P}{\Delta L} \frac{wh}{2(w + h)} \]

Where
- \( Q = \text{volumetric flow rate} \)
- \( w = \text{flow channel width} \)
- \( h = \text{flow channel height or depth} \)
- \( \Delta P = \text{pressure drop} \)
- \( \Delta L = \text{length of flow path} \)
VROC® initium – Advantages

- Micron scale slit flow
  - Small volume
  - High shear rates without instability

- Flow through channel design
  - Eliminates air-fluid interface
    - No evaporation
    - No interfacial viscosity contribution

- Sample retrieval – unlimited measurement with single loaded volume
  - Confirm repeatability
  - Temperature sweeps
  - Shear rate sweeps
  - Control sample history

- Advanced software features – operation & data analysis (Clariti™)
Protein Denaturation/Stability

Temperature Sweeps

• Detect loss of higher order structure – unfolding & association

• Alternative method avoiding interfacial artifacts

• Buffer – Arrhenius behavior & reversible

• BSA solution (pH 6.30 & pH 7.42)
  • Initially consistent with buffer
  • Abruptly deviates at ~62°C
  • Forward ≠ Reverse
  • Irreversible change dependent on buffer/stabilization
Shear Rate Dependence

• Multiple flow channel options now available (interchangeable) – achieve >100,000 sec\(^{-1}\)
  • Mimic application shear rates – injection or blink cycle
• Single sample volume for each flow channel
• Combine with temperature variation

### Channel Details

<table>
<thead>
<tr>
<th>Channel</th>
<th>Max Pressure (kPa)</th>
<th>Depth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B05</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>C05</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>E02</td>
<td>1800</td>
<td>20</td>
</tr>
</tbody>
</table>

### Viscosity Chart

- **25°C**
  - Boving γ-Globulin (BγG) in PBS

- **37°C**

### Temperature Chart

- sodium carboxymethyl cellulose (eye drop formulation)

- **25°C**
- **37°C**

- **B05 (filled)**
- **E02 (open)**
Viscosity vs. Shear Rate Scaling

Peclet Number

\[
Pe = \frac{\frac{L^2 \dot{\gamma}}{D_s^0}}{\frac{1}{\dot{\gamma}}} = \frac{\tau_B}{\tau_S} = \frac{L^2 \dot{\gamma}}{D_s^0 \frac{6\pi \eta_o L^3 \dot{\gamma}}{kT}}
\]

Adjust characteristic length scale L
(r_h ~ 5 nm from [\eta])

<table>
<thead>
<tr>
<th>[IS] (mM)</th>
<th>L (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>11.5</td>
</tr>
<tr>
<td>1200</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Increase in L consistent with cluster formation
Focus in on dilute regime – intrinsic viscosity ([η])

- Distinguish small viscosity increments
- Molecular characterization
  - Individual molecules
  - Pair or protein-protein interactions
Viscosity vs. Concentration

- Viscosity increases with concentration of macromolecules
  - Limit \( c \to 0 \)
    - Molecules far separated
    - Individual contribution – size, shape
  - ↑\( c \) further (dilute)
    - Molecular spacing decreases
    - Pair interactions become relevant
Viscosity ($\eta$) vs. Concentration ($c$) – Dilute Limit

- **1st order** – individual molecules ($[\eta]$)
  - MW, Intramolecular interactions/structure
- **2nd order** – pair interactions ($k_H$)
  - Hydrodynamic
  - Thermodynamic – repulsive & attractive

$$\eta = \frac{[\eta]}{\eta_s} = [1 + [\eta]c + k_H[\eta]^2c^2 + \ldots]$$

$$\eta = \frac{\eta}{\eta_s} = [1 + 2.5\phi + 6.0\phi^2 + \ldots]$$

Hydrodynamic radius:

$$r_H = \left(\frac{3[\eta]M_w}{10\pi N_A}\right)^{1/3}$$

$\eta_s \equiv$ solvent viscosity

$\phi \equiv$ effective hard sphere volume fraction

$[\eta] \equiv$ intrinsic viscosity

$k_H \equiv$ Huggins constant
Impact of Molecular Weight – BSA & BγG

Scaling viscosity data for analysis

\[
\frac{\eta_r - 1}{c} = [\eta] + k_H \eta^2 c
\]

\[
[\eta] = \lim_{c \to 0} \frac{\eta_r - 1}{c}
\]

<table>
<thead>
<tr>
<th></th>
<th>MW</th>
<th>[\eta] (mL/g)</th>
<th>(r_h) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (140mM NaCl)</td>
<td>66K</td>
<td>4.6</td>
<td>3.6</td>
</tr>
<tr>
<td>BγG (PBS)</td>
<td>158K</td>
<td>6.0</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Higher MW → larger [\eta] & \(r_h\)
Impact of pH & Ionic Strength – BSA

Isoelectric Point = pI ~ 5

- pH = 5.4
  - Minimal negative charge near pI
  - Minimal impact of ionic strength
- pH = 8.4
  - More negatively charged
  - Increasing ionic strength
    - Screens electrostatic repulsion
    - Reduces $k_H$ and PPI

<table>
<thead>
<tr>
<th>pH</th>
<th>Ionic Strength (mM)</th>
<th>$[\eta]$ (mL/g)</th>
<th>$r_h$ (nm)</th>
<th>$k_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4</td>
<td>20</td>
<td>4.5</td>
<td>3.60</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>4.4</td>
<td>3.58</td>
<td>1.15</td>
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<tr>
<td>8.4</td>
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<td>4.3</td>
<td>3.55</td>
<td>2.42</td>
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<tr>
<td></td>
<td>160</td>
<td>4.2</td>
<td>3.54</td>
<td>1.32</td>
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</table>
Get Clariti™: Intrinsic Viscosity
Get Clariti™: Injectability