Exploring Optimum Storage Conditions of Biologics using Calorimetry
Biopharmaceuticals

- Any pharmaceutical drug product manufactured in, extracted from, or semi-synthesized from biological sources

- Biopharmaceuticals have been around for hundreds of years, what has changed?
  - Vaccines
  - Insulin
  - *Monoclonal antibodies (Various)*

**Traditional**

**Patient Self-Administration**
Requires High Concentration

% Sales

#1 Lipitor $121 B

14% sales from biologics:
#7 Enbrel
#10 Humira $32 B

Top 10 Drugs 2014

- Shift 4 years later
- 7 out of 10 Biopharmaceutical
- Still a place for Small molecules #2 was Solvadi (Hep C). List also included Crestor and Advair.
- 1 Year Sales Humira: 12.5 B USD
Biopharmaceutical Stability Assessment

- **Background**
  - Biopharmaceuticals are not orally bioavailable
  - Traditional delivery configuration: Intravenous infusion
  - Patient self-administration requires high concentrations (100-200 mg/mL)
  - High concentration Mab formulations concerns
    - Aggregation
    - Denaturation
    - High Viscosity
  - Primary Goal of effective formulation characterization – *Rapidly determine the best buffer and excipient conditions that maximize stability and minimize protein aggregation for at least one year at required high concentrations*
Advanced Microcalorimetric Techniques

- **Formulation Stability Testing - TAM**
  - Direct measurement of heat flow from dilute or high concentration formulations
  - Rapid estimation of formulation stability and shelf life

- Calorimetry quantifies the amount and rate of heat release in terms of heat flow, heat and heat capacity.
  - Non-specific, native, no immobilization
Isothermal Microcalorimetry

**Sensitive**

- **mW**
  - TAM IV & TAM 48
  - 1-48 – samples
  - Sample Flexibility
  - 1-20 mL sample Vol.
  - Temp Range: 4 – 150°C

**µW**

- Affinity ITC & Nano ITC
  - 1 – sample
  - Max Sensitivity
  - 1 mL or 190 µL cells
  - Temp Range: 2 – 80°C
  - Optional automated sample handling

**nW**

- More Sensitive
TAM Stability Testing

Heat Flow $\text{exo}$

Time

Least stable
Most stable
Oxidation of Meclofenoxate Hydrochloride Containing *dl*-α-Tocopherol

Biopharmaceutical Formulation Stability
Protein Thermodynamic Characteristics

Increasing Temperature
DSC of Irreversible Denaturation

\[ k_1 \quad \text{→} \quad k_2 \]
Two Different Mechanisms of Protein Denaturation/Aggregation

Denaturation Precedes Aggregation

\[ k_1 \gg k_2 \]

Denaturation, Aggregation Occur Simultaneously

\[ k_1 \ll k_2 \]
DSC Characterization of Proteins

**HEW Lysozyme at pH 8.2**

- **Endotherm Due to Denaturation**
- **Exotherm Due to Aggregation/Precipitation**

**Tm = 73.3°C**

**TAM Assay Temps**

**Cp (cal/(K*mol))**

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<th>C (cal/(K*mol))</th>
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Two Events HEWL

TAM of HEWL pH 9: Endotherm Occurs Before Exotherm

Denaturation Precedes Aggregation
Stable mAb5

TAM of mAb5: No Evidence of Separate Exothermic and Endothermic Processes

dQ/dt = k*H*exp(-k*t)
Heat Flow is Proportional to the Amount of Denatured and Aggregated Protein Formed

\[
Heat = v\Delta H_u[U] + v\Delta H_{agg}[Agg]
\]

\[
Heat = v\Delta H_u[P_T]F_u + v\Delta H_{agg}[U]F_{agg}
\]

\[
Heat = v\Delta H_u[P_T]F_u + v\Delta H_{agg}[P_T]F_uF_{agg}
\]

\[
Q = \frac{Heat}{v[P_T]} = \Delta H_uF_u + \Delta H_{agg}F_uF_{agg}
\]

\[
Q = \Delta H_u(1 - e^{-k_u t}) + \Delta H_{agg}(1 - e^{-k_u t})(1 - e^{-k_{agg} t})
\]

Quantity Measured by Calorimeter dQ/dt

\[
dQ/dt = R1*H1*exp(-R1*t) + R2*H2*exp(-R2*t)) + R1*H2*exp(-R1*t) -(R1 + R2)* H2*exp(-(R1+R2)*t) \tag{Eq 6}
\]

If R1 \ll R2 the above equation reduces to:

\[
dQ/dt = R1*(H1 + H2)*exp(-R1*t) \tag{Eq 7}
\]
Non-linear Least Squares Fit (Eq 6) of HEWL pH 9 Heat Flow
TAM Characterization of Proteins

Preliminary Results

Data from Non-linear Least Squares fit to Denaturation/Aggregation model

DSC Scans
Arrhenius Analysis of Rates for HEWL pH 9 obtained at 57, 58 and 59°C

\[ \ln k \text{ vs } \frac{1}{T} \]

At 25°C, 357 days
Correlation between the percent of mAb aggregates measured by size exclusion chromatography after ten weeks’ incubation at 25°C and the denaturation/aggregation rates measured by TAM (10 day test).
Early Adopters of IMC Shelf-life

Stability study of a monoclonal antibody: Abbott X in phosphate buffer

DSC trace

Lumry-Eyring:

\[
N \xleftarrow{k_1, k_2} U \xrightarrow{k_3} A
\]

rate = \( \sum \frac{1}{\Delta H_i} \cdot P_i \)

Isothermal calorimetry trace of a at 4 different pH

Power of TAM Measurements

The TAM IV is currently used for small molecules – could it fit this same niche with biopharmaceuticals?

- Can detect denaturation/aggregation kinetics at temperatures below the $T_m$
- Gives answer to when.
- Provides sensitivity to detect heat signal in volumes of $<1$ mL and high protein concentrations ($>100$ mg/mL)
Acknowledgement

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Biopharmaceutical Stability


Thank You

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In 5 years, >50% of top-selling drugs will be biologics.
MANAGED CARE  October 2013. Michael D. Dalzell