Biophysical Tools for Reformulation of Biosimilar Therapeutics

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INTRODUCTION

Several biophysical tools can be utilized for rapid screening and development of biosimilar antibody formulations. The two major considerations in liquid antibody formulation development are conformational and colloidal stability. To evaluate the conformational stability, Differential Scanning Calorimetry (DSC) and Intrinsic Fluorescence are utilized, colloidal stability of the formulations is evaluated by Dynamic Light Scattering (DLS) and Static Light Scattering (SLS). These biophysical tools and related indicators like melting temperature (T_m), diffusion interaction parameter (k_D) and second virial coefficient (B_{22}) help in identifying alternate formulation compositions with improved or comparable stability. In addition to the biophysical techniques, the developed alternate formulations have been subjected to standard biochemical stability testing including sub-visible particle analysis (fluid imaging), size exclusion chromatography (SEC) and cation-exchange chromatography (CEX) which indicate that the developed formulation has an improved stability profile.

OBJECTIVES

The objective of this study was to develop an alternate formulation for a biosimilar antibody therapeutic. This alternate formulation required conformational and colloidal stability which was equivalent or better than the reference product biosimilar. Oncobiologics (ONS) employed the above mentioned techniques in a high throughput fashion to screen alternate formulation conditions.

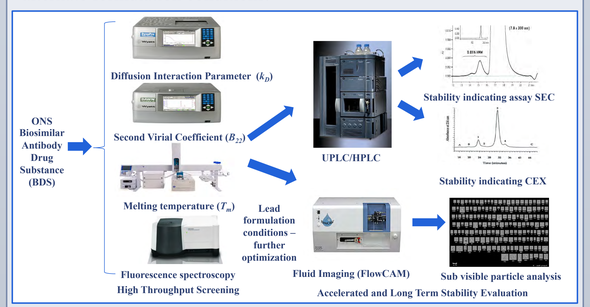


Figure 1: Biosimilar formulation screening and development process flow chart

MATERIALS & METHODS

- Excipients: Sugars, Polyols, buffers, surfactants, amino acids used for typical high throughput screening; all excipients used are GRAS approved and suitable for parenteral administration
- Differential Scanning Calorimetry (DSC): determine the conformational stability, report all thermal transitions (T_m °C)
- Intrinsic Fluorescence Spectroscopy: Determine the secondary structure of antibody in solution; hydrophobic residue distribution
- Dynamic Light Scattering (DLS): Determine the colloidal stability by evaluating the hydrodynamic radius (R_h), the Diffusion Interaction Parameter (k_D) and the Second Virial Coefficient (B_{22}) of the protein in the different formulations.
- Size Exclusion Chromatography (SEC): Separates high molecular weight species and low molecular weight species from monomer of protein.
- Cation-Exchange Chromatography (CEX): Separates acidic and basic species from the main species of the protein.
- Digital Fluid Imaging (FlowCAM): Measures sub-visible particles (size and shape, morphology and identity); characterizes particles from 2µm – 100µm; evaluate aggregation in protein formulations

Background Theory and Equations for Determination of Second Virial Coefficient (B_{22}) and Diffusion Interaction Parameter (k_D)

Diffusion Interaction Parameter (k_D)

Plot diffusion coefficient against protein concentration. Perform linear regression: slope = $k_D D_0$ and the *Y*-intercept indicates D_0[1].

$$D = D_0 + k_D c D_0$$

Slope = $k_D D_0$ Y-intercept = D_0

The measured diffusion coefficient is a rough approximation related to the hydrodynamic radius using the Stokes-Einstein equation.

$$D = kT/6\pi\eta R_h$$

D = Diffusion Coefficient k = Boltzmann Constant
T = Temperature η = Viscosity R_h = Hydrodynamic Radius

Second Virial Coefficient (B_{22})

Debye plot can be used to calculate the B_{22}

$$KC/R\theta = 1/MwP(\theta) + 2B22C$$

- K = Debye constant
- C = sample concentration
- $R\theta$ = Rayleigh ratio
- M_w = weight average molecular weight
- B_{22} = second virial coefficient
- $P(\theta)$ = Shape (or form) factor

RESULTS

Differential Scanning Calorimetry & Intrinsic Fluorescence Spectroscopy

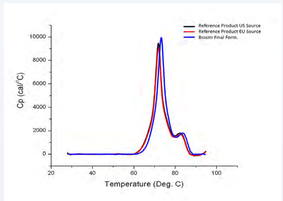


Figure 2: DSC profile of the Final ONS formulation overlay with Reference Product, note the T_m being slightly increased

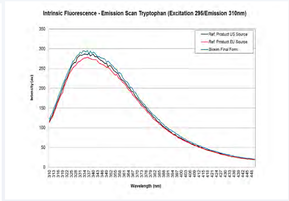


Figure 3: Intrinsic fluorescence spectra of the Final ONS formulation, overlay with Reference Product

Dynamic Light Scattering (DLS) Protein Concentration Study

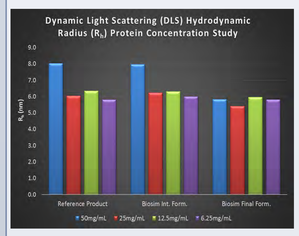


Figure 4: DLS results of protein conc. study; ONS final formulation has smaller R_h at 50mg/mL

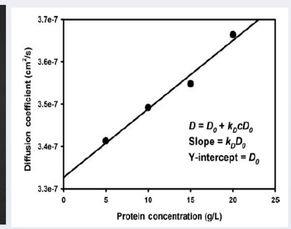


Figure 5: An example of k_D calculation based on DLS measurements. The diffusion coefficient measured by DLS is plotted against protein concentration. Linear regression was employed to determine the slope as $k_D D_0$ and the *Y*-intercept indicate D_0.[1]

Parameters	ONS Final Formulation	Reference Product
k_D (ml/g)	1.67	-4.30
B_{22} (mol ml/g^2) x 10^4	0.634	0.244
DLS/SLS conditions	Repulsive	Repulsive
Viscosity (cP)	1.266	1.277

Table 1: Biophysical properties of ONS Final Formulation compared to Reference Product

Accelerated Stability Analysis (55°C 7 Day)

Size Exclusion Chromatography (SEC)

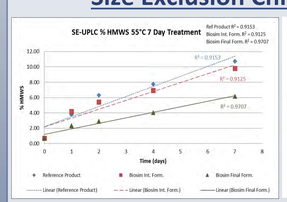


Figure 6: SEC plots for comparing formulations of treated samples at 55°C 7 days

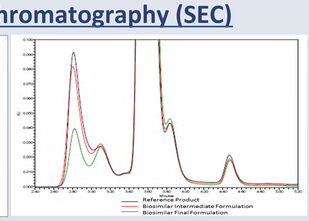
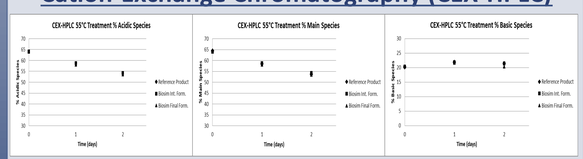


Figure 7: SEC Chromatogram – overlay of different formulations treated 55°C 7 days

Cation-Exchange Chromatography (CEX-HPLC)



Figures 8, 9 and 10: CEX-HPLC plots describing the Acidic, Main and Basic Species for comparing formulations of treated samples at 55°C

RESULTS

Long Term Stability

Sub-visible Particle Formation – 6 months at 5°C

Reference Product: Sub-visible Particles

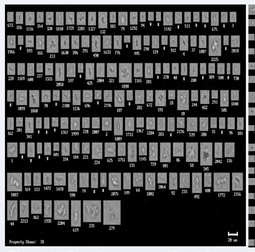


Figure 11: Protein Aggregate Particles

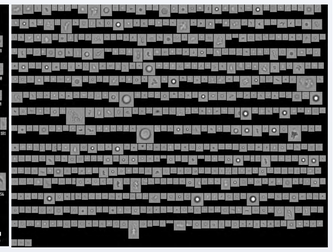


Figure 12: Protein Particles and Silicone Oil Droplets silicone oil air bubble

FlowCAM Sub-visible Particles in Biosimilar Final Formulation

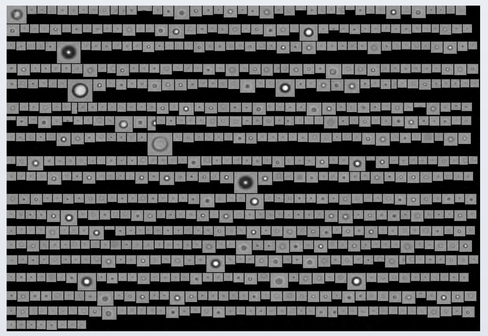


Figure 13: Biosimilar Final Formulation – No protein particles detected

DISCUSSION

The Biosimilar Final Formulation developed by Oncobiologics showed an increase in conformational and colloidal stability compared to the Reference Product formulation. An increase in conformational stability was observed by an increase in T_m. Intrinsic Fluorescence testing showed comparable secondary structure of the different formulations. The DLS and SLS provided information on R_h, k_D and B_{22} parameters. The Biosimilar Final Formulation had a smaller R_h and positive k_D and B_{22} values. The smaller R_h value indicates repulsive conditions and lesser potential to aggregate. The positive k_D and B_{22} values are indicative of increased colloidal stability and a stable liquid formulation long term. Size exclusion data supported the DLS data of lower aggregation present at stressed conditions. Charge data showed comparable results between the formulations. The FlowCam data also showed no sub-visible protein particles in the Biosimilar Final Formulation pre-filled syringe (PFS) compared to the reference product PFS. The data presented in this poster illustrates the enhanced conformational and colloidal stability of the Biosimilar Final Formulation developed at Oncobiologics.

DISCUSSION

Mechanism of Antibody Aggregation and Particle Formation

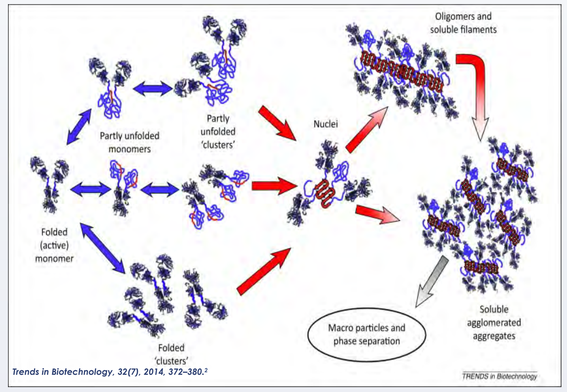


Figure 14: Mechanism of Action for Aggregation and Particle Formation

Antibody Particle Characterization Methodologies

Methods for the Analysis of:						
	Soluble Aggregates		Insoluble Aggregates/Particles			
Monomers	Oligomers	Submicron	Subvisible		Visible	
1 nm	10 nm	100 nm	1 µm	10 µm	100 µm	1 mm
Size Exclusion Chromatography						
SDS-PAGE						
	Dynamic Light Scattering					
	Nanoparticle Tracking Analysis					
Analytical UltraCentrifugation						
	Asymetrical Flow Field-Flow Fractionation					
	qNano					
		Archimedes				
		Flow cytometry				
		Light obscuration				
		Fluid Imaging				
		Optical/Fluorescence Microscopy				
		Coulter Counter				
		Turbidimetry				
					Visual inspection	

Journal of Pharmaceutical Sciences. 2012. 101(3), 914–935.[3]

Figure 15: Antibody Particle Characterization Methodologies

None of the available analytical methods can measure the entire spectrum of particulates (monomers - oligomers - sub-visible and visible particles). Oncobiologics uses orthogonal methods to understand protein aggregation and particle formation in antibody solutions during formulation screening, development and stability evaluation.

CONCLUSIONS

Biophysical tools such as the DSC, DLS and SLS were utilized to evaluate different protein formulations. The size exclusion data and charge data from CEX-HPLC also supported the biophysical data. The FlowCam played an important role in determining particle size and type in final container (PFS). The Biosimilar Final Formulation developed showed no sub-visible protein particles, only silicone oil droplets and air bubbles, compared to the reference product that contained aggregates, silicone oil droplets and air bubbles. This is an indication of better stability in the final container (PFS) of the Biosimilar Final Formulation drug product compared to the reference product. The data presented in this poster illustrates the increased conformational and colloidal stability of the Biosimilar Final Formulation developed at Oncobiologics.

REFERENCES

[1]Journal of Pharmaceutical Sciences 2011. 100 (12), 5126-5141.
[2]Trends in Biotechnology. 2014. 32 (7), 372-380.
[3]Journal of Pharmaceutical Sciences. 2012. 101 (3), 914–935.