Protein Formulation Screening and USP <787> Testing

AccuSizer® automated particle count analysis for protein formulation optimization to minimize aggregation and perform USP <787> subvisible particulate matter testing.

INTRODUCTION

Dynamic light scattering (DLS) is a standard tool for determining particle size of proteins, but this method does not provide concentration information. The AccuSizer® single particle optical sizing (SPOS) system is an ideal technique for determining aggregated protein size and concentration in particles/mL for sizes greater than 150 nm. Figure 1 shows Nicomp® DLS results before and after heat stressing a monoclonal antibody. The after stress result in blue shows the high resolution multimodal result including larger aggregate peaks beginning at ~150 nm. The AccuSizer cannot detect down to the 10 nm monomer size, but but can provide quantitative data indicating the degree of aggregation >150 nm. In this study the AccuSizer FX Nano was used to investigate how various formulation stabilization chemistries affected the resultant state of aggregation for a NIST standard protein. The same AccuSizer particle counter can also be used for USP <787> subvisible particulate matter testing.[1]

MATERIALS

The protein used was NIST reference material 8671, Lot 14HB-D-002, NIST mAb, Humanized IgG1k monoclonal antibody[2].

The primary size of the protein was measured on the Nicomp dynamic light scattering (DLS) system using a 35 mW laser diode source, APD detector. The protein aggregates were measured on the AccuSizer FX Nano SIS system equipped with two sensors:

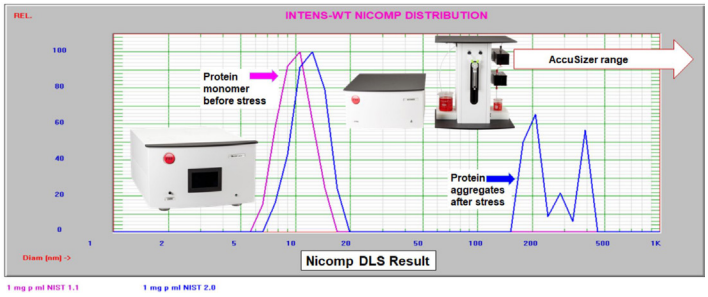


Figure 1. Nicomp DLS results before and after heat stress

LE-400; 0.5 – 400 µm and FX Nano; 0.15 – 10 µm. The SIS sampler provides accurate sample volume down to 100 µL with sample recovery.

iFormulate[3] (Figure 2) plates preloaded with formulations, designed by DoE for rational formulation development, were used to test aggregation levels as a function of formulation chemistry. These evaluate four major variables of protein formulation; pH, ionic strength, buffer, and stabilizer concentration using multivariate experimental design.

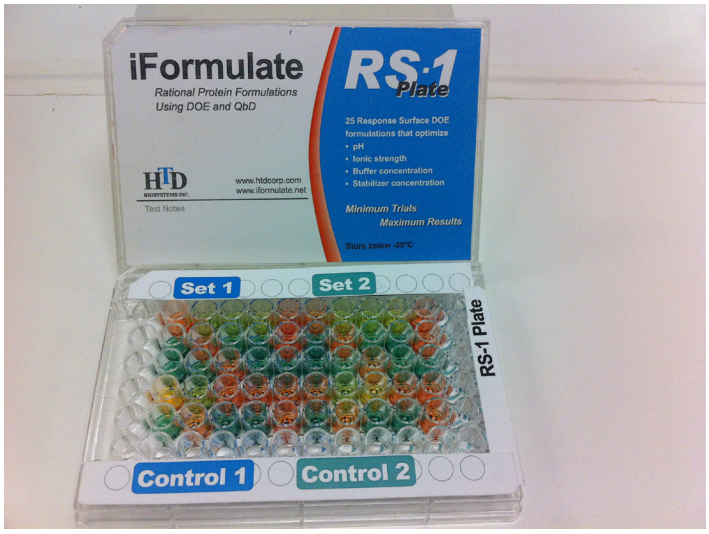


Figure 2. iFormulate plate

The iFormulate plates used tested the following variables:

- pH: 5 – 7.6
- Ionic strength: 0 – 200 mM NaCl
- trehalose stabilizer: 0 – 10 %
- Buffer concentration: 10 – 50 mM

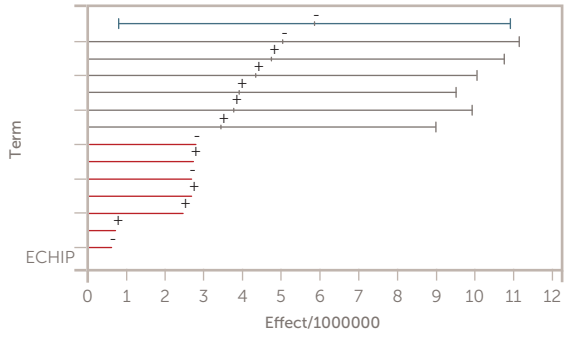
METHODOLOGY

The NIST mAb was thawed and diluted to 1 mg/mL. First 160 µL of filtered DI water was added to each well in the iFormulate plate. Next 40 µL of NIST mAb was added to each well. The plate was placed in an oven at 60°C (just below the protein melting point) for 24 hours. Each well was analyzed using the AccuSizer particle counter. The particle count data for >0.15 µm (total) and >0.53 µm was submitted to iFormulate, who then performed the data analysis and stability modeling.

RESULTS

The data was processed using a Pareto analysis approach, a formal technique useful where many possible courses of action are competing for attention. The Pareto analysis gives ranking of variables and interactions between variables. Figures 3 and 4 show the Pareto analysis for >0.15 µm and >0.53 µm, respectively. The colors indicate the relative importance of the formulation variable to increased particle count with blue = significant, gray = borderline, and red = less significant.

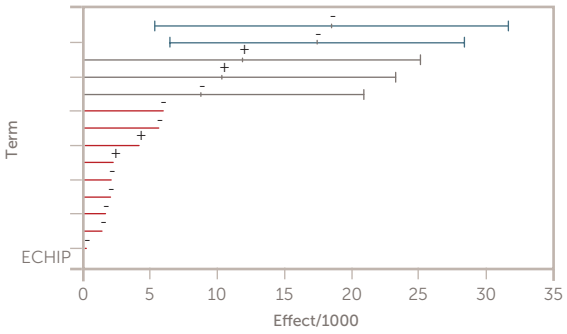
Pareto Effects for Total



12 Buffer_Cone^2
4 Trehalose
11 pH^2
14 Trehalose^2
6 pH*NaCl
10 NaCl*Trehalose
9 Buffer_Cone*Trehalose
1 pH
8 Buffer_Cone*NaCl
3 NaCl
2 Buffer_Conc
7 pH*Trehalose
5 pH*Buffer_Cone
13 NaCl^2

Figure 3. Pareto effects for >0.15 µm (Total)

Pareto Effects for MT 0.53 Micron



12 Buffer_Cone^2
4 Trehalose
11 pH^2
14 Trehalose^2
6 pH*NaCl
10 NaCl*Trehalose
9 Buffer_Cone*Trehalose
1 pH
8 Buffer_Cone*NaCl
3 NaCl
2 Buffer_Conc
7 pH*Trehalose
5 pH*Buffer_Cone
13 NaCl^2

Figure 4. Pareto effects for >0.53 µm

The next results, shown below present 3-D response surface plots showing aggregation effects under different conditions. This empirical view of design space provides optimization to identify formulations that minimize aggregation as indicated by higher particle counts at the two size ranges. Figures 5 and 6 show results for varying pH and stabilizer (trehalose) while keeping buffer and NaCl concentration constant.

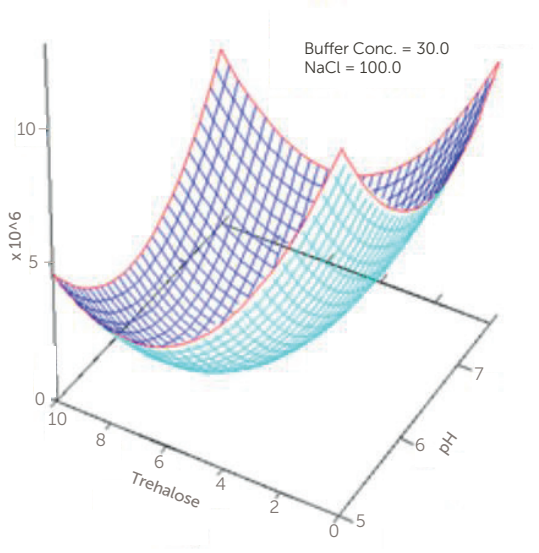


Figure 5. Effect of pH and stabilizer >0.15 µm

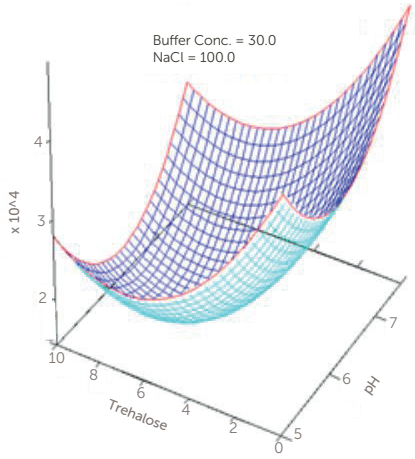


Figure 6. Effect of pH and stabilizer >0.53 µm

Figures 7 and 8 show results for varying NaCl and stabilizer (trehalose) concentration while keeping buffer concentration stable and pH varying slightly from 5.91 to 6.3.

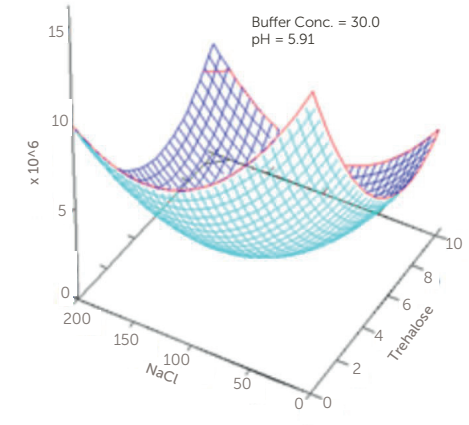


Figure 7. Effect of NaCl and stabilizer >0.15 µm

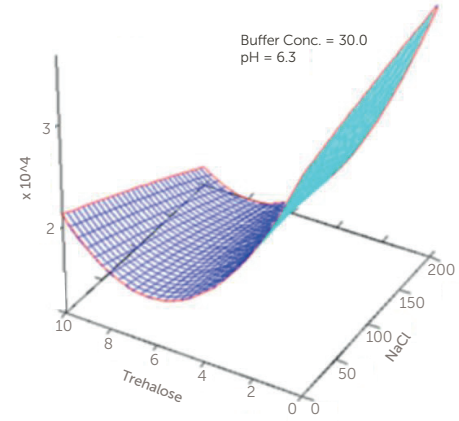


Figure 8. Effect of NaCl and stabilizer >0.53 µm

These, and other results not shown here, were then compiled to create the minimum particle count optimization plots shown in Figures 9 and 10.

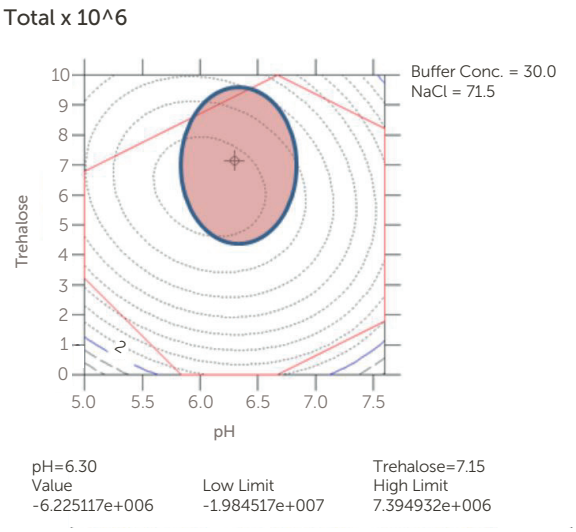


Figure 9. Particle minimization plot >0.15 µm

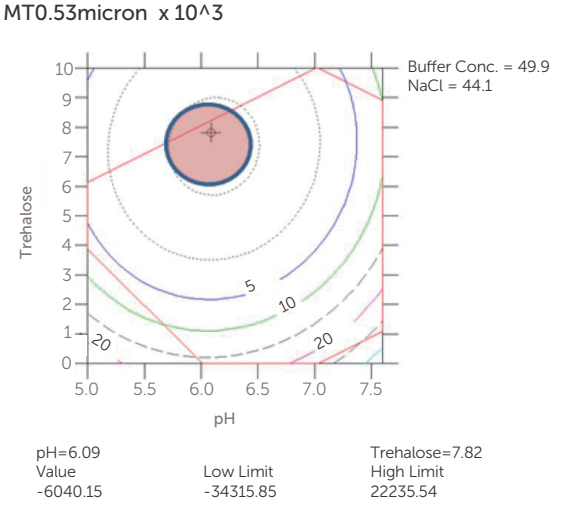


Figure 10. Particle minimization plot >0.53 µm

CONCLUSIONS

The results shown here indicated that the optimum formulation chemistries to minimize particle count, and therefore protein aggregation, were:

pH = 6.1 – 6.3
NaCl = 40 – 70 mM
trehalose = 7 – 8%
Buffer = 50 mM

The AccuSizer can also be used to perform USP <787> testing at ≥10 and 25 µm, and can be automated using the A2000 MPA Microplate Analyzer 4.

References

[1] USP <787>, *Subvisible Particulate Matter in Therapeutic Protein Injections,* see usp.org

[2] NIST reference material 8671, Lot 14HB-D-002, NIST mAb, see https://www.nist.gov/programs-projects/nist-monoclonal-antibody-reference-material-8671

[3] iformulate, see https://iformulate.net/

[4] AccuSizer A2000MPA, see https://www.youtube.com/watch?v=aMXRwg88 Ro&t=278s

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