Identify Optimal Biologic Candidates: Low Volume, Early Stage Developability Assessment with Aura PTx

Introduction

Developability assessment is used during biotherapeutic development to evaluate the manufacturability of leading drug candidates.[1,2] Performing this assessment as early as possible identifies candidates with inherent stability risks, mitigating potential failures or expensive, time-consumig formulation efforts required for a suboptimal candidate. Finding the most stable candidates and buffer conditions to reduce subvisible biological aggregation, a critical quality attributed (CQA) linked to immunogenicity, drug efficacy, and product shelf life, is a key goal when assessing a candidate's developability.[3] However, the ability to perform this assessment is severely hampered since sample availability is extremely limited during early stages of the development process. Conducting subvisible particle analysis with traditional legacy techniques such as flow imaging and light obscuration has not been possible due to the high sample volume demands (>500 µL per run). As a result, researchers have turned to other low volume techniques for developability assessment, including size exclusion chromatography, dynamic light scattering and differential scanning fluorimetry, that do not predict subvisible particle formation.

In this application note, we share how Aura™ systems transform developability assessment by directly characterizing subvisible content using a high throughput, low volume screen of three different proteins against a platform of 14 industrially relevant buffers and excipients. The most stable protein was identified by quantifying the subvisible content across the different conditions using volumes as low as 5 µL in under three hours of total experimental time. In addition, high contrast images and Fluorescence Membrane Microscopy (FMM) analysis revealed key insights on the samples' stability profile.

Methods & Experimental Design

Three proteins and a platform of buffers (**Table 1**) that are commonly used as part of the commercial manufacturing process were assessed. The buffers were optimized to include excipients that reduce the stresses that occur during low pH viral clearance, elution, storage (air water interfaces), freeze/thaw, and ultrafiltration (UF)/diafiltration (DF)/ tangential flow filtrations (TFF) for buffer exchange of drug substance to drug product. This buffer platform screens the impact of ionic strength, buffer type, salt (NaCl), pH, and stabilizing additives. Each protein (A, B, and C) was formulated in each buffer from lyophilized powder to a final concentration of 0.1 mg/mL. Each sample condition was processed in quadruplicate using 40 µL per well on Aura PTx.

Buffer #	Buffer	pH	NaCl Conc. (mM)	Excipient	Excipient Conc.
1	Acetate (50 mM)	3.6	50	Sucrose	100 mM
2			0	—	—
3			100	—	—
4			150	—	—
5			200	—	—
6		4.6	50	PS80	0.05% v/v
7	Citrate (50 mM)	6.2	50	Arginine	100 mM
8				PS80	0.05% v/v
9				—	—
10				Sucrose	100 mM
11		3.6		—	—
12	Tris (50 mM)	7.5	50	—	—
13	Glycine (50 mM)	8.2		PS80	0.05% v/v
14		10.6		Arginine	100 mM

Table 1: Formulation conditions: buffer, pH, salinity, and excipient matrix.

Results

Protein Developability Assessment in Acetate Buffer

Proteins A, B, and C generated subvisible particles in acetate buffer as shown in Figure 1. Protein C displayed the most particle formation for these buffers compared to Proteins A and B, with all conditions exceeding 45,000 counts/mL, ≥2 µm in equivalent circular diameter (ECD). Protein A displayed counts very close to 35,000 counts/mL, ≥2 µm across all acetate buffer conditions while Protein B showed similar behavior in counts to Protein A, except when no NaCl was present. Protein B exhibited the most stable condition when formulated using buffer #2. %CVs across all measurements were generally below 10% for most acetate containing samples, demonstrating measurement robustness despite low sample volumes.

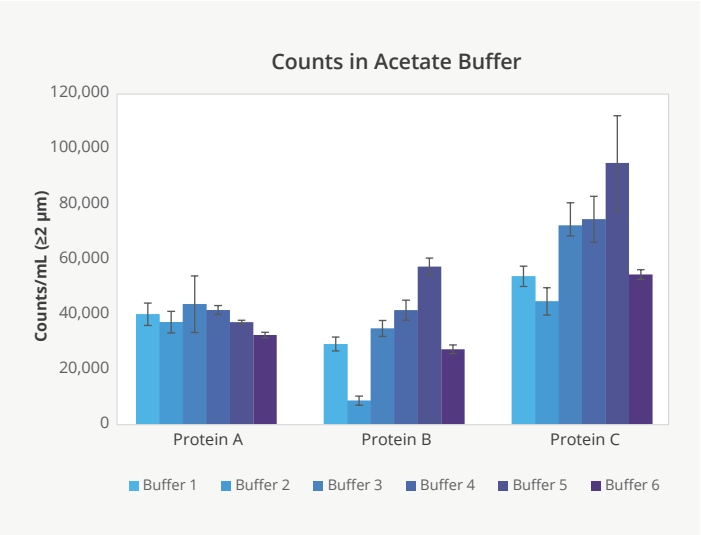

Figure 1: Developability assessment of Proteins A, B, and C in acetate buffer conditions. Counts/mL shown represent subvisible content with ECD ≥2 µm. Protein B was most stable when prepared in buffer #2 while Protein C displayed the most inherent instability.

Protein Developability Assessment in Citrate Buffer

Proteins A, B, and C also generated subvisible particles in citrate buffer. However, Protein B, which was nominally the most stable along with Protein A in acetate buffer, was far more unstable than both Proteins A and C as shown in Figure 2. Protein B exhibited 4– to 425–fold more subvisible aggregate formation than Protein A across the citrate buffer conditions, and 2– to 30–fold more subvisible aggregate formation than Protein C. Protein C in turn produced 2 to 14 times as many protein aggregates than Protein A in this buffer, with Protein A clearly emerging as the most stable in citrate buffer. %CVs across all measurements were generally below 10% for most acetate containing samples, showing measurement robustness despite low sample volumes.

Protein Developability Assessment in Tris and Glycine Buffer

Proteins A, B, and C also generated subvisible particles in tris and glycine buffers. Like in citrate buffer, Protein B was also the least stable, exhibiting over 2 million counts/mL, ≥2 µm across all conditions as shown in Figure 3. For this buffer, Protein B exhibited a 76– to 166–fold increase in aggregate formation over Protein A, and 16– to 58–fold more aggregate formation than Protein C. Protein C was also more unstable than Protein A, displaying 3– to 5–fold more subvisible aggregates across these buffer conditions. Protein A also emerges as the most stable protein for this buffer condition. %CVs across all measurements were generally below 10% for most acetate containing samples, showing measurement robustness despite low sample volumes.

Developability Assessement Summary

Table 2 shows the subvisible count summary for the entire sweep and is presented in conditional formatting to easily rank the samples by stability. Protein B is clearly the least stable, showing the most counts particularly in citrate, tris, and glycine buffer conditions. Protein C was in the middle of the pack with comparable stability in acetate buffer, but Protein A exhibited significantly higher stability in citrate, tris, and glycine buffer conditions than Protein B.

If one only evaluated protein developability in acetate buffer conditions, it would have been tempting to pick Protein B as the most stable candidate. However it produced up to 420-fold higher formation of subvisible particles in the more aqueous and alkaline buffer

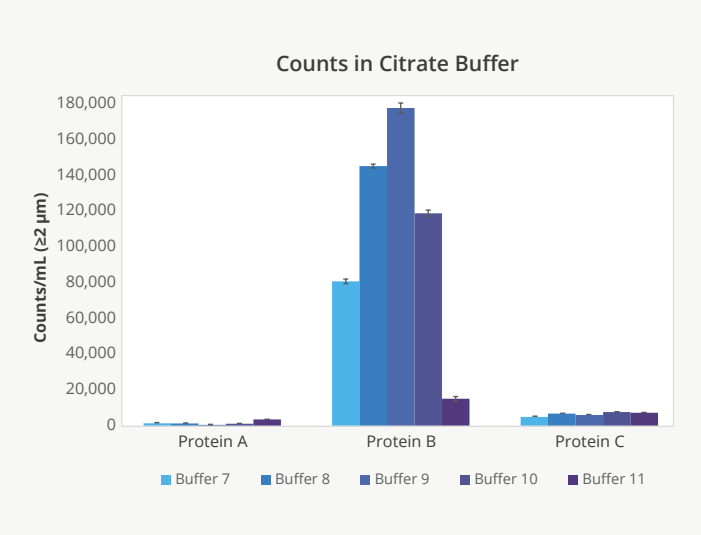

Figure 2: Developability assessment of Proteins A, B, and C in citrate buffer conditions. Counts/mL shown represent subvisible content with ECD ≥2 µm. Protein A was most stable, while Protein B displayed the most inherent instability.

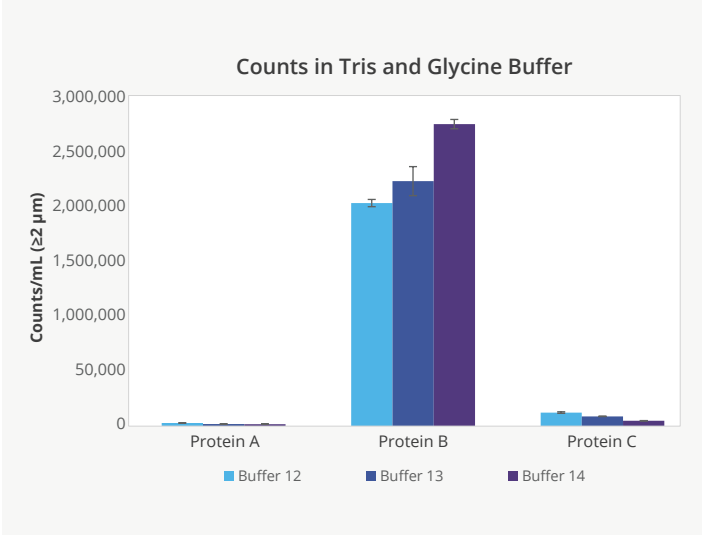

Figure 3: Developability assessment of Proteins A, B, and C in citrate buffer conditions. Counts/mL shown represent subvisible content with ECD ≥2 µm. Protein A was most stable, while Protein B displayed the most inherent instability.

	Acetate						Citrate					Tris	Glycine	
Buffer #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Protein A	40094	37194	43738	41581	37188	32469	13238	12925	4169	19356	34231	26596	16769	16463
Protein B	29213	8513	34856	41538	57469	27306	805825	1449013	1773125	1185706	149813	2019306	2217694	2734419
Protein C	53906	44763	72400	74713	95238	54559	48950	66913	59525	75669	71906	120906	86744	46338

Table 2: Developability assessment summary for Protein A, B, and C in various buffers. Counts/mL shown represent subvisible content with ECD >2 µm. ■ – low counts, ■ – medium counts, ■ – high counts.

conditions. Aura PTx easily ranked three different proteins under a comprehensive buffer platform and elucidated the most stable candidate – Protein A. The entire sweep involved running 168 wells, used only 40 µL of sample per condition, and took less than three hours to produce comprehensive measurements in quadruplicate. The data generated was extremely robust, yielding results with low %CVs.

Analysis at the Single-Particle Level

In addition to providing high-level insights during candidate selection, Aura systems enable a deeper understanding of protein aggregation since data can be analyzed at the single-particle level. To demonstrate this, we analyzed single aggregates formed by Protein B in glycine buffer #14 using the image gallery shown in Figure 4. The observed images are subvisible particles stained with the fluorescent dye Thioflavin T (ThT), a well-characterized dye specific for protein aggregates. The red color indicates a strong fluorescence acquired with Aura platform's proprietary Fluorescence Membrane Microscopy (FMM) technology. Since the particles are round and ThT binds to small fibrils in proteins, it can be deduced that these are highly hydrophobic subvisible aggregates based on the strong ThT fluorescence and particle morphology that minimizes surface area to volume ratio. While high-level information is very important, the granular information possible when single particles are analyzed using FMM technology can help researchers better understand the mechanisms and degradation pathways that influence the biologic's instability.

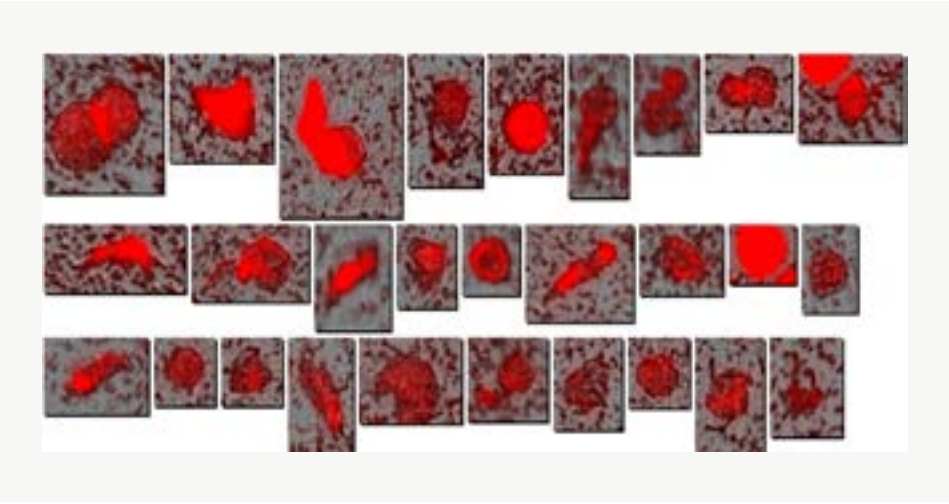

Figure 4: Particle Vue software image gallery enables analysis at the single-particle level. Subvisible particle formation of Protein B in glycine buffer. The red color indicates that dyed proteins fluoresced with Thioflavin T excitation, indicating their proteinaceous nature.

Conclusion

Proteins A, B and C, despite having a strong affinity for their target, have drastically different manufacturability potential. While affinity and pharmacokinetic characteristics are important, considerations being able to manufacture large molecules with good stability profiles are equally important. Determining this before scaling up production saves considerable time and reduces risk, and subvisible particles are a critical measurement at this stage.

For the first time, Aura PTx and Aura+ transforms the sample volume limited developability assessment stage by enabling subvisible particle characterization - the most important stability CQA. Its high throughput, low volume modality enables high level ranking decision making, and uncovers the most granular insights of stability and particle formation in a single platform.

References

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halolabs.com
sales@halolabs.com