Validate your Clean-In-Place (CIP) Process with Uncle

Introduction

In pharmaceutical manufacturing, removal of drug product residues is an integral part of process development. Clean-in-Place (CIP) is a cleaning protocol designed for bioreactors and fermentation tanks, in which equipment is cleaned without the need to disassemble process components. CIP encompasses a combination of heat, force and chemical exposure to remove drug product residue as well as any lingering detergents or solutions from cleaning cycles. FDA requirements specify that CIP must be validated by demonstrating that there is no active residue carryover from a previous batch that can impact the next batch.[1] As such, complete denaturation of residual protein after each cleaning process must be established.

Figure 1: Uncle enables 12 stability applications in an all-in-one protein characterization platform.

A well-designed method to validate CIP must accomplish two things:

- Verify that a cleaning procedure inactivates and effectively removes residual drug product residues.
- Demonstrate reproducibility of the cleaning process and its ability to restore conditions to predetermined acceptable levels.

How do you know if you are following a cleaning regimen that minimizes the risk of contaminated products and consistently produces acceptable results? Current practices for validating CIP protocols rely on analytical methods such as SDS-PAGE, SEC-HPLC, immunoassays, or total organic carbon (TOC) detection.[2,3] However, these techniques can give false positives, have slow throughput or require specialized skillsets. Uncle is a protein characterization platform that can directly measure the structure of a protein (i.e. folded and unfolded states) via fluorescence. This method can be used to demonstrate denaturation and inactivation of a protein drug product, which is necessary for CIP validation.

Uncle is a one-stop platform for protein stability that uses three detection methods: full-spectrum fluorescence, static light scattering (SLS), and dynamic light scattering (DLS) to fully profile a protein "fingerprint" (Figure 1). Multiple measurements such as native fluorescence, aggregation, sizing, polydispersity, and thermal melting can be performed with the same samples in just one experiment, allowing you to obtain orthogonal information on protein stability. Uncle uses only 9 µL of sample and can measure up to 48 samples simultaneously, enabling greater flexibility for characterizing your biologics.

This application note describes how Uncle can be used to profile your drug products, optimize your CIP, and validate in-process runs.

Methods

Drug "fingerprint" profiling and denaturation validation on Uncle

A drug "fingerprint" profile is a combination of 5 stability metrics which characterize a protein. The NIST monoclonal antibody standard (reference 8671) was prepared at 0.5 mg/mL in 25 mM histidine buffer at pH 6.0. For CIP experiments, another monoclonal antibody, mAb 1, was formulated at 200 mg/mL. 9 µL of each sample was loaded in a Uni in triplicate and a T_m & T_{agg} experiment was run from 15 °C to 95 °C with a ramp rate of 0.5 °C/min. DLS was collected before and after the ramp using 4 acquisitions of 5 seconds each. The barycentric mean (BCM) of fluorescence intensity was selected as the analysis method for all experiments.

CIP optimization with Freeform mode

Along with 12 built-in applications, Uncle offers a customizable mode called Freeform. This mode allows users to build and model unique temperature profiles with specific time intervals. Freeform mode on Uncle was selected to model a process cycle, which reflects the temperatures and contact times used in the CIP procedure. The fluorescence and SLS of the samples were measured to monitor the stability of the protein during the CIP method. The samples were measured over several intervals, starting at 25 °C, while ramping to 80 °C, holding at 80 °C, and cooling back to 25 °C.

Results

Native and denatured protein characterization on Uncle

Uncle can confirm that a CIP protocol irreversibly denatures target proteins by directly detecting changes in protein folding and aggregation. First, Uncle can fully "fingerprint" your drug product using fluorescence, SLS, and DLS detection (Figure 2). Next, to evaluate product inactivation, comparisons can be made between these values for both an intact reference and a drug product after going through the CIP process. Protein characterization metrics that can be obtained from a single experiment on Uncle using a NIST antibody standard are shown in Figure 2.

Multimodal detection on Uncle provides 5 metrics with which to "fingerprint" your protein in a single experiment. This profile describes the drug product in its active form. After a drug product has been subjected to the CIP protocol, the profiling experiment can be performed again to demonstrate that the values are different from the active drug profile, indicating that the structure and activity of the drug product are irreversibly changed. This profiling experiment can be used to uniquely characterize a protein drug product, optimize a CIP process, and perform in-process validation of CIP cleaning.

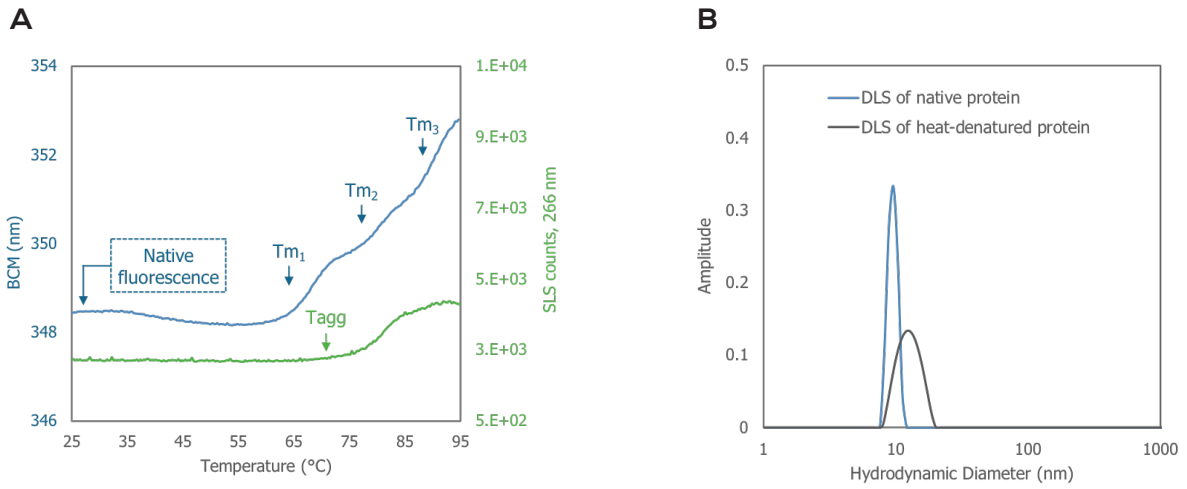

Figure 2: **(A)** Thermal ramp experiment showing intrinsic fluorescence (in blue) and SLS aggregation at 266 nm (in green) of a NIST monoclonal antibody. **(B)** DLS measurements of NIST mAb were collected on the native and thermally denatured protein.

Drug Product	T_m	T_{agg}	Initial BCM	Z-Ave. Diameter	PDI
Before CIP	70 °C	71 °C	339 nm	10 nm	0.5
After CIP	N/A (Denatured)	N/A (Aggregated)	368 nm	>500 nm	>2

Table 1: Uncle uses fluorescence, SLS, and DLS detection methods to characterize and compare protein unfolding and aggregation between a reference (before CIP) and the denatured protein (after CIP) to help optimize CIP procedures.

An antibody (mAb 1) was measured to create a reference profile and then a sample of mAb 1 after the CIP process was measured. Comparison between the reference profile and the samples indicates that mAb 1 unfolds and aggregates after cooling following completion of the CIP process (Table 1).

CIP optimization

Uncle can be used routinely to explore the design space for a CIP process by evaluating and optimizing parameters for protein inactivation or degradation. Many parameters, including temperature, chemical composition and concentration, exposure time, and number of cycles can be tuned to optimally denature therapeutic protein molecules. Freeform mode on Uncle can be used to recreate the time and temperature parameters of a CIP protocol for optimization. Alternatively, while holding the time and temperature profile constant, the concentration of chemical denaturants can be optimized.

For a CIP protocol created using Freeform mode on Uncle, in-process measurements of fluorescence show an increase in protein inactivation for mAb 1 as denaturant concentration is increased (Figure 3). While the CIP protocol with heat alone denatures the protein, when used in combination with chemical denaturation, the CIP protocol completely and irreversibly unfolds mAb 1. These results were used to determine the minimum effective concentration (MEC) of chemical denaturants in the CIP protocol required for complete denaturation.

In-process validation

Comparison of the "fingerprint" profiles of the active drug product and the post-CIP samples can be used to validate whether the protein is

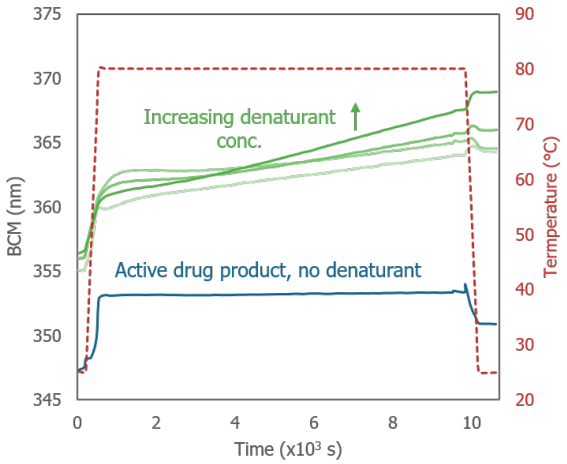

Figure 3: The freeform application on Uncle was used to model the CIP protocol for mAb 1 to optimize the denaturant concentration.

irreversibly denatured following the CIP process (Figure 4). Periodic QC testing of wash fluid can demonstrate that the protein fluorescence characteristics differ from that of active product and can confirm process residuals are within specifications (Figure 4A). As shown in Figure 4B, the initial and final BCM values of the test sample indicate the presence of fully denatured mAb 1 after CIP compared to the reference profile of mAb 1 before CIP. DLS measurements also showed an increase in the size and polydispersity values of the drug product after CIP with denaturants, indicating complete protein unfolding and subsequent aggregation (data not shown).

21 CFR part 11 compatibility

To meet GMP requirements, maintaining proper electronic records and data security is a must. Uncle has you covered with 21 CFR Part 11 software tools, providing full sample tracking, data authenticity, and user accountability. This add-on provides tools to help make your system compliant with 21 CFR Part 11 regulations and includes features such as system and experiment-specific

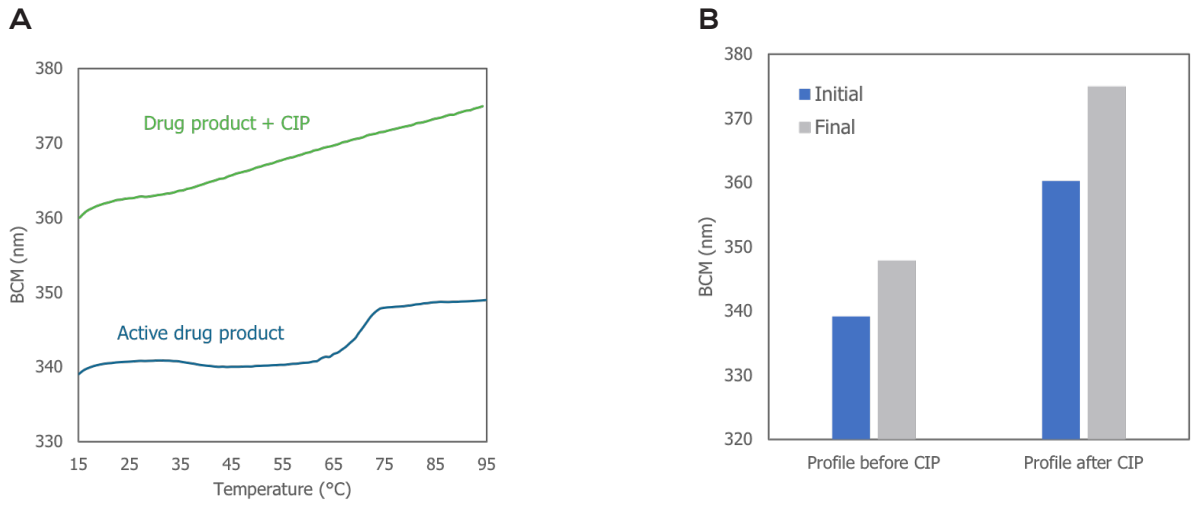

Figure 4: A thermal unfolding experiment on Uncle shows that the CIP process fully denatures mAb 1. **(A)** Fluorescence melting profile of an active drug product and denatured product after a CIP protocol. **(B)** Initial and final BCM values of a drug product before and after CIP.

audit trails, electronic signatures, and data integrity checks.

Conclusion

For CIP process validation, Uncle uses a combination of fluorescence, static, and dynamic light scattering to characterize the "fingerprint" profile of your drug product. Metrics including T_m, T_{agg}, initial fluorescence, and sizing and polydispersity values can be collected in a single experiment and can be used to compare an active drug product reference to the product after CIP. To validate whether process wash cycles are complete, you can assay wash fluids on Uncle to test if predetermined limits are met. For optimizing your CIP protocol, the flexible experimental setup in Freeform mode enables you to model CIP process cycles by adjusting temperature, interval duration, and ramp rates. You can also rest assured with a 21 CFR Part 11 compliance package if you need it. As described in this application note, Uncle provides wide applicability for ensuring your CIP meets requirements.

Acknowledgements

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References

1. Equipment cleaning and maintenance, 21 C.F.R. §211.67 (2018).

2. Rizwan S, Spencer A, Romero J, Runkle S, Carolan C, Hayes R, Mott A, Clark ME, Wyman E, Rasmi M, Donat S, Bellorado K. 2012. Methodology for assessing product inactivation during cleaning Part I: Experimental approach and analytical methods. Journal of Validation Technology. 18(4).

3. Mott A, Henry B, Wyman E, Bellorado K, Blümel M, Parks M, Hayes R, Runkle S, Luo W. 2013. Methodology for assessing product inactivation during cleaning Part II: Setting acceptable limits of biopharmaceutical product carryover for equipment cleaning. Journal of Validation Technology. 19(4).

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