Using BMI for High Protein and Excipient Concentration Particle Analysis

Introduction

Many self-administered subcutaneous protein parenteral therapeutics are manufactured at high concentration to allow lower dosing volumes and to increase the ease of self-administration. Monitoring subvisible particles in high protein concentrations and/or therapeutics that contain high concentrations of excipient added to prevent protein aggregation, however, is challenging due to their dramatically increased viscosity and complexity. This can add uncertainty about a formulation's stability.

The Aura™ system uses 100% sampling and high contrast Backgrounded Membrane Imaging (BMI) to deliver robust, reliable, and repeatable high-throughput and low volume particle analysis. This precise and reproducible analysis can be performed on high concentration formulations using a membrane rinsing methodology that builds on a commonly used USP 788 Method 2 (Membrane Microscopy) in addition to knowledge acquired by analyzing various high concentration, complex samples. The main objective is to use water washing to remove highly viscous, soluble elements that obscure membrane-based particle analysis.

In this technical note, we present two methods for optimally working with high concentration solutions using BMI. We define high protein concentrations as solutions exceeding 10% solids per unit volume. For example, a ≥100 mg/mL protein formulation or a 50 mg/mL protein solution with ≥5% sucrose or total excipient concentration would meet this criterion.

Method 1: Membrane Wash

This approach is the most straightforward and requires no additional method development. To analyze high concentration samples with this method:

1. Background the plate.
2. Membrane Pre-Wetting: Pre-wet the wells with 30 µL of water for injection (WFI). Do NOT blot dry at this step.
3. Invert the samples thoroughly right before plating to ensure good quality particle distribution on the membrane.
4. Pipette the desired volume of samples onto the wet membrane and apply vacuum for 1 minute or longer to ensure entire sample has been aspirated through.
5. Pipette 5 rounds with 40 µL WFI, applying vacuum for 30 seconds. A total of 200 µL of WFI will be aspirated through the membrane.
6. Dry the membrane plate on wicking paper.
7. Re-insert the plate and measure particles with the Aura instrument. If wells are flagged yellow or red in the software, determine whether more washing is appropriate.

Method 2: Optimize Wash Number

Some high concentration samples may require fewer or more rinse steps. To determine the optimal wash steps for your sample:

1. Background the plate.
2. Membrane Pre-Wetting: Pre-wet the wells with 30 µL of water for injection (WFI). Do NOT blot dry at this step.
3. Invert the samples thoroughly right before plating to ensure good quality particle distribution on the membrane.
4. Pipette the desired volume of samples onto the wet membrane and apply vacuum for 1 minute or longer to ensure the entire sample has aspirated through.
5. Re-insert the plate and measure particles using the Aura instrument.
6. Using Particle Vue software, clone the background of the plate. This will automatically create a new software plate.

NOTE: We recommend creating another condition called "Wash #" if you only have a single condition on the plate to easily track the impact of washing per wash run.

7. Pipette an additional 40 µL of WFI and applying vacuum for 30 seconds.
8. Dry the membrane plate on wicking paper.
9. Re-insert the plate and measure particles in the Aura instrument.
10. Repeat steps 6 thru 9 until the counts from the last two rounds of washing are within 10% of each other.

Conclusion

This washing protocol, which builds on USP 788 Membrane Microscopy best practices, enables proper particle analysis for high concentration protein formulations. Unlike other tools, Aura using BMI offers a simple approach for dealing with high concentration solutions and a straightforward process for precise method development.

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