Combination of BMI, DLS and Visual Inspection to resolve particle formation issues in formulations commonly used in gene therapy

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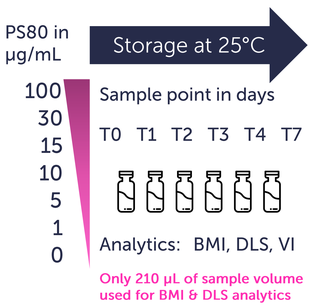
Abstract 878

Abstract

The development of gene therapies necessitates a rigorous approach to ensure product safety and efficacy. Historically, consideration of subvisible particles as a parameter in formulation development was **deferred to later stages** due to **resource-intensive methods like light obscuration (LO) and micro flow imaging (MFI)**. The scarcity of material in early-stage gene therapy requires the exploration of **low-volume, high-throughput methods** for early detection of both subvisible and visible particles. This study highlights the **effectiveness of Backgrounded Membrane Imaging (BMI) as a low-volume method** to identify optimal buffer conditions that **mitigate subvisible and visible particle formation early in formulation development**. Starting from a standard PBS AAV formulation containing a protein excipient, we **screened a range of excipient conditions** at room temperature over the course of 7 days to discover the optimal formulation for **prevention of particle formation**. The low volume requirement of BMI enabled us to perform this study with **only 150 µL of sample per condition**.

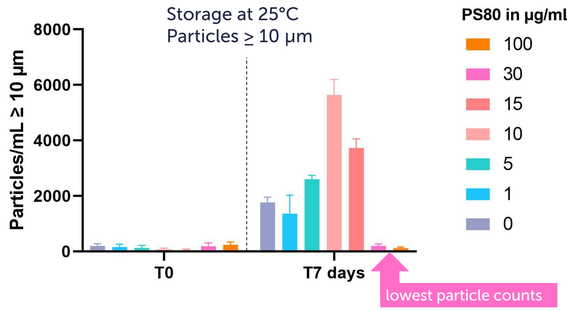
Complemented by Dynamic Light Scattering (DLS) and Visual Inspection (VI), our findings showcase the successful resolution of particle aggregation by introducing a common surfactant to the formulation buffer and determining the optimal concentration. The use of **BMI allows particles in both the subvisible & visible range (2 µm - 5 mm) to be characterized** at low volumes and complements DLS data, which captures smaller aggregates (less than 1 µm) and VI, which primarily identifies larger particles (> 150 µm). Nonetheless, a potential **blind spot exists for particles between 1 µm and 2 µm**, posing a challenge to achieving thorough characterization of the entire particle size range. However, the **combination of all three methods** represents a comprehensive and synergistic approach to mitigate the challenges associated with particle formation in formulation development for gene therapies.

Study outline

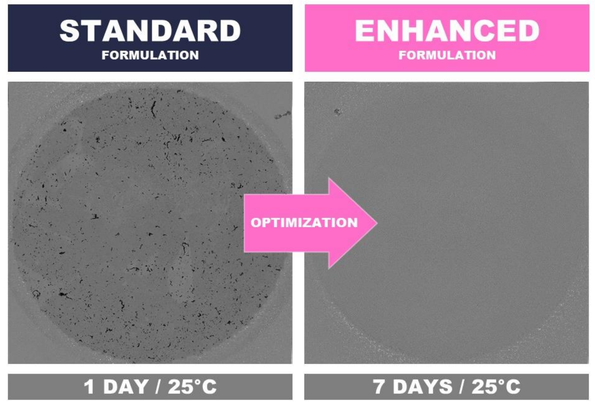


- Evaluation of particle formation as a function of polysorbate 80 (PS80) concentration in a standard PBS AAV formulation containing a protein excipient as stabilizer
- Storage of samples in 10 mL CZ® vials at 25°C for one week
- BMI, DLS and VI were used to monitor particle formation and aggregation

Low-volume BMI analysis reveals a clear increase in particles with low PS80 content

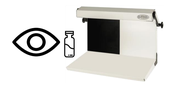


A concentration of ≥ 30 µg/mL PS80 is sufficient to prevent the formation of particles ≥ 10 µm after storage at 25°C for 7 days.



In the formulation buffer lacking sufficient PS80, particle formation and aggregation of the protein excipient occurs after 1 day of storage at 25°C. Conversely, an improved formulation buffer remains particle-free even after 7 days at the same temperature.

Particle analysis combining BMI, DLS and VI



VI: using the Apollo II Liquid Viewer (Adelphi)
10 mL CZ vials with 5 mL fill volume (n = 6)

Particle size
> 150 µm

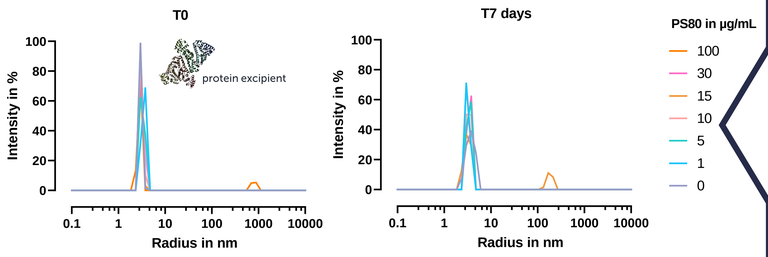
BMI: using the Aura® (Halo Labs)
150 µL sample volume (n = 3)

> 2 µm

DLS: using the DynaPro® Plate Reader III (Wyatt)
60 µL sample volume (n = 2)

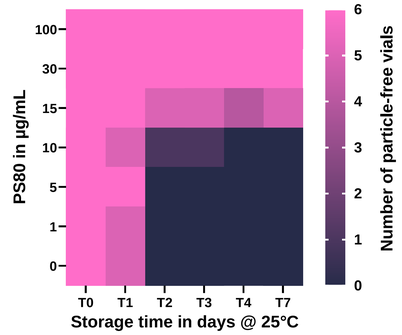
< 1 µm

DLS: no aggregation is detected



DLS analysis indicates that particle size and size distribution of the protein excipient in the formulation buffer does not change with different PS80 concentrations over a period of 7 days at 25°C. In addition, no aggregate peaks in the 100 - 1000 nm range are visible in most formulations.

VI: large particles detected by eye



- 6 vials for each PS80 condition
- 5 mL fill volume
- VI at T0 and at the respective timepoints
- Number of particle free vials for each PS80 condition is counted

A level of 30 µg/mL PS80 is sufficient to prevent visible particle formation. However, this method is highly operator dependent and requires careful and regular training.

Summary

We have **successfully determined** that a **PS80 concentration of 30 µg/mL effectively prevents the formation of visible particles** in a standard PBS AAV formulation with a protein excipient. These findings were corroborated by three methods: BMI, DLS, and VI, **with BMI providing the primary confirmation**. Specifically, **BMI revealed that the number of particles larger than 10 µm remained at low levels** during the 7-day storage at room temperature. VI directly demonstrated the formation of visible particles at insufficient PS80 concentrations during storage at 25°C. Importantly, **DLS completely failed to detect the degree of aggregation** observed by BMI & VI. This implies that **DLS alone does not fully capture the aggregation dynamics**. Sole reliance on DLS for confirmation could have led to a misleading assessment of sample condition, as it might have deemed the samples acceptable despite the presence of aggregates. By exclusively employing the BMI method, we could accurately conclude and even quantify particle count, facilitating the ranking of formulations based on aggregation and storage conditions. Moving forward, we will **leverage the combined strengths of BMI and DLS** to interrogate particle formation in the nanometer to millimeter range. **Replacing VI with BMI during early development** leads to **significant reduction** in sample volume requirements and allows a **more objective quantification of particles.** Thus, it will allow us to proactively tackle aggregation concerns in the early stages of development.

Aim higher

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