Development of a novel automated loading approach which significantly reduces processing time for enriching full AAV capsids using ultracentrifugation

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Introduction

Engineered adeno-associated virus using engineered capsids and optimized cargos (eAAV) is a promising technology for treating both rare and common genetic disorders. To support clinical trials, there is a growing need to manufacture more material with appropriate levels of enriched full capsids (capsids containing therapeutic gene). Challenges persist in the field related to processing time and equipment for enriching full capsids.

The current standard approach—ultracentrifugation using a cesium chloride (CsCl) density gradient—has drawbacks due to long processing times and capacity limitations. In this study, we evaluated the OptiMATE Gradient Maker (Beckman Coulter®), an automated tool that forms a linear CsCl gradient prior to ultracentrifugation. By using a pre-formed gradient and optimizing run conditions, spin times were significantly reduced. This allows faster separation of full and empty capsids compared to the standard bulk addition approach.

The product quality profile, assessed by ddPCR, HPLC-SEC, CE-SDS, and AUC, remained consistent between the standard bulk CsCl addition process and pre-formed gradient process. Overall processing time was reduced tenfold.

Methods and Materials

Engineered AAV (eAAV) was produced using a HEK293 suspension process followed by capture using affinity chromatography. The affinity elution pool was concentrated and used as the starting material for the ultracentrifugation (UC) studies. For control samples, the UC load followed the routine process: a homogenous suspension was generated, loaded into tubes, and spun for an established duration. For experiments involving a pre-formed gradient, the OptiMATE system (Beckman Coulter®) was used. Two different modes of gradients, step and continuous gradient, were evaluated. Operating parameters (density gradients, spin durations, and temperatures) were optimized. Full fractions were analyzed for recovery (ddPCR), aggregation (SEC-FLD), VP stoichiometry (CE-SDS), capsid content (AUC). The gradient work was performed with two different engineered capsids.

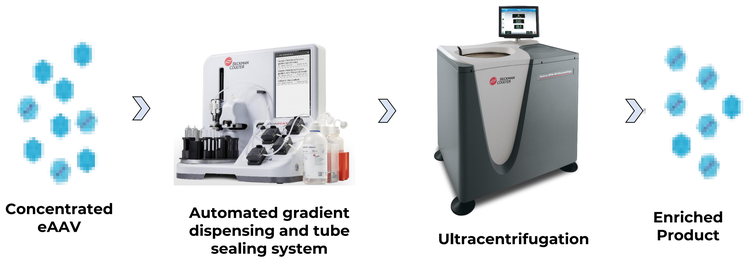


Figure 1: Ultracentrifugation Process Flow Overview

Results and Discussion

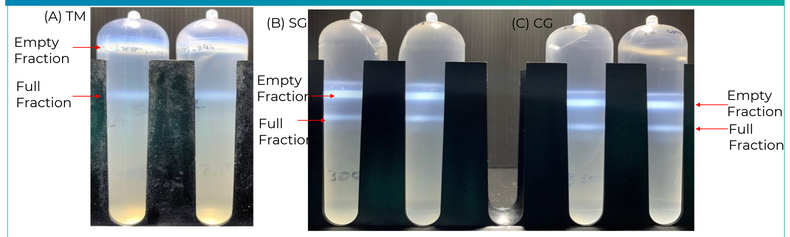


Figure 2: Comparing enrichment of full capsids using (A) homogenous suspension versus (B) and (C) automated pre-formed gradient

- **Typical Method (TM)**: Tubes show the separation of full and empty capsids using the typical method (TM). UC was performed using homogeneous suspension of purified eAAV in cesium chloride solution.
- **Step Gradient (SG)**: Tubes were filled in a step gradient by varying the addition of cesium chloride solution and eAAV. Tubes were spun for 4-fold shorter durations. The relative separation between the full and empty capsids was smaller as compared to the typical method.
- **Continuous Gradient (CG)**: A continuous gradient was formed using the automated tube filler and the spin time was the same as the step gradient process. Relative separation of the full and empty capsids was similar to the step gradient.
- The total cesium chloride content in each tube was the same. The formation of the gradient and spin times leads to small shifts in the band locations.

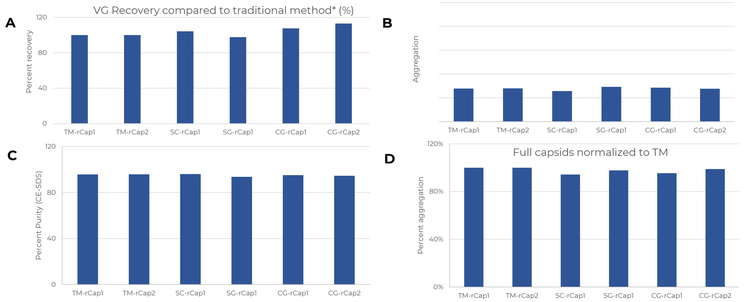


Figure 3: Comparing full capsid attributes for homogenous suspension (TM) versus automated pre-formed gradient system (SG, CG)

- Similar recovery (≥95%) for all methods. No product loss observed with gradient methods.
- No change in aggregation levels or purity across the different run conditions
- Similar enrichment of full capsids, measured via AUC, in the step and gradient systems versus the TM.

Results and Discussion

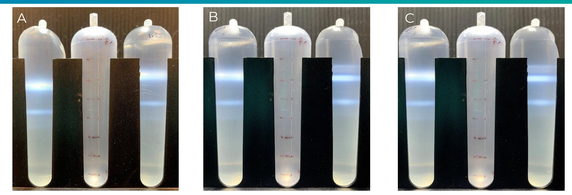
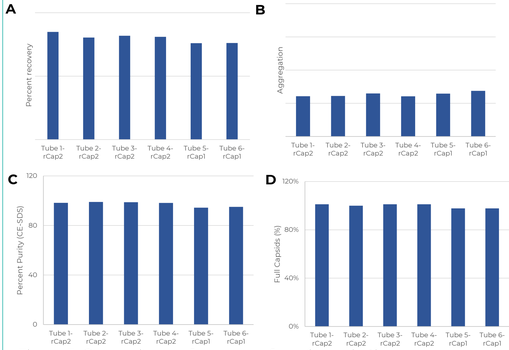


Figure 4: Varying spin times for continuous gradient method compared to TM (a) Tube 1 and Tube 2 - 5x shorter spin time(b) Tube 3 and Tube 4 - 6x shorter spin time (c) Tube 5 and Tube 6 - 10x shorter spin time



- Overall performance is unchanged with reduced spin time.
- No significant change in aggregation or purity observed when the total spin time was reduced 5x, 6x, and 10x as compared to the typical method.
- Enrichment of full capsids is similar in each run condition based on the AUC results
- Residual DNA levels were not affected with shorter spin durations (data not shown).

Figure 5: Comparing performance of varying spin times

Conclusions

- Using a pre-formed cesium chloride density gradient created with the OptiMATE Gradient Maker system, the time taken to achieve equilibrium in the tube was significantly reduced allowing for faster separation of full and empty capsids
- Based on the optimized run conditions, the spin time to achieve separation between full and empty capsids is 10-fold shorter as compared to the traditional method
- Recovery (>95%) and attribute profile was similar for shorter spin times with gradient loading conditions when compared to the traditional method.

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- rAAV Manufacturing Solutions: Strategic Designs of Engineered rAAV Two Plasmid Systems for Cost Effective Scaling and Product Safety, 5:30 – 7:00 PM CT, Abstract Number: 962, Presenter: Jenna Rodden, Senior Research Associate, Capsida

Presented on WEDNESDAY
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- Identification of Multiple Novel Blood-Brain-Barrier Receptors for CNS Gene Therapy and Other Drug Modalities via an Integrated AAV Capsid Engineering Platform, 2:45 – 3:00 PM CT, Abstract Number: 93, Presenter: Nick Goeden, Ph.D., Founder, Chief Technology Officer, Capsida
- Systemic Gene Therapy CAP-002 Demonstrates Potential for Disease-Modifying Treatment of Seizures and Motor and Cognitive Deficits of STXBP1-DEE Using an Engineered, CNS-Targeted AAV, 3:45-4:00 PM CT, Abstract Number: 123, Presenter: Nick Flytzanis, Ph.D., Founder, Chief Research and Innovation Officer, Capsida
- CAP-003, a CNS-Targeted IV-delivered AAV Gene Therapy, Safely Increases Brain GCase in NHPs to Level Supporting Potential Normalization of Activity in PD-GBA Patients, 5:30 – 7:00 PM CT, Abstract Number: 1435, Presenter: Kim McDowell, Ph.D., Director, Preclinical Research, Capsida
- Dual-Platform NGS for Comprehensive Characterization of Engineered rAAV Vector Integrity,5:30 – 7:00 PM CT, Abstract Number: 1326, Presenter: Zach Mason, Associate Scientist, Capsida

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