5-hour density gradient purification of Adeno-Associated Virus (AAV) using a novel automation platform – the OptiMATE Gradient Maker

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INTRODUCING THE OPTIMATE GRADIENT MAKER

A novel automation platform for density gradient ultracentrifugation

Adeno-associated viruses (AAVs) are powerful delivery vectors for gene therapy.[1] Enrichment of viral genome-loaded particles is a critical step in downstream purification workflows and is best accomplished by using density gradient ultracentrifugation (DGUC).[2] A typical AAV density gradient workflow comprises a 15-60% w/v iodixanol (IDX) step gradient (for purifying AAV capsids from other cellular contaminants) followed by cesium chloride (CsCl) gradient ultracentrifugation (for separating full capsids from empty capsids).

This process is not without challenges. Setting up the iodixanol step gradient is manual and can lead to variability depending on operator skill level. While setting up a CsCl gradient is less skill-intensive, ultracentrifugation run times are often 16 hours or more, which limits throughput.

To address these fundamental challenges, we developed an automated solution called the OptiMATE Gradient Maker. This system eliminates hands-on steps in gradient preparation including stock reagent preparation, dispensing gradients, and sealing tubes. It dispenses both step and linear gradients—or combinations of the two. Because lengthy DGUC run times are primarily due to the slow process of gradient formation, pre-formed linear gradients offer much faster separation. In the case of pre-formed gradients, run time is dictated largely by sample particle migration (and slight alteration in gradient profile based on run conditions). The OptiMATE Gradient Maker can be used to run established protocols with higher consistency, accuracy and ease of use.

Key Features	Benefits
Automated dispense and tube sealing	Significantly reduce errors and improve consistency
Pre-formed gradients	Dramatically shorter run times (75+% reduction)
Simple GUI and method creation	Train new operators in just hours, not weeks
Small footprint	Easily fits in BSC or on benchtop
Published methods	Simplify method selection and operation

Figure 1: OptiMATE Gradient Maker system components and features

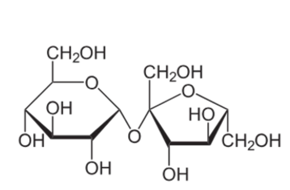
COMPATIBLE WITH WIDELY USED ROTORS, TUBES AND REAGENTS

A)

Rotor	Rotor Configuration	Nominal Rotor Capacity (mL)	Max RCF (x g)
Type 90 Ti	8 x 13.5	108	694,000
Type 70 Ti	8 x 39	312	504,000
Type 70.1 Ti	12 x 13.5	162	450,000
Type 50.2 Ti	12 x 39	468	302,000
Type 45 Ti	6 x 94	564	235,000
NVT 65	8 x 13.5	108	402,000
VTi 65.1	8 x 13.5	108	402,000
VTi 50.1	12 x 39	468	251,000
SW 32 Ti	6 x 38.5	231	175,000

B)

Density Gradient Materials



Include solutions from Beckman Coulter Life Sciences or your own stock solutions

Additives

- L-Histidine
- Glucose
- Sucrose
- Polysorbate 80
- Pluronic F-68
- Sodium citrate
- Sodium bicarbonate
- Sodium chloride
- Glycerol

Figure 2: (a) Rotors and tube types supported by the OptiMATE Gradient Maker. (b) Density gradient materials and chemicals compatible with the OptiMATE Gradient Maker.

ENHANCES EFFICIENCY OF STANDARD AAV PURIFICATION WORKFLOWS

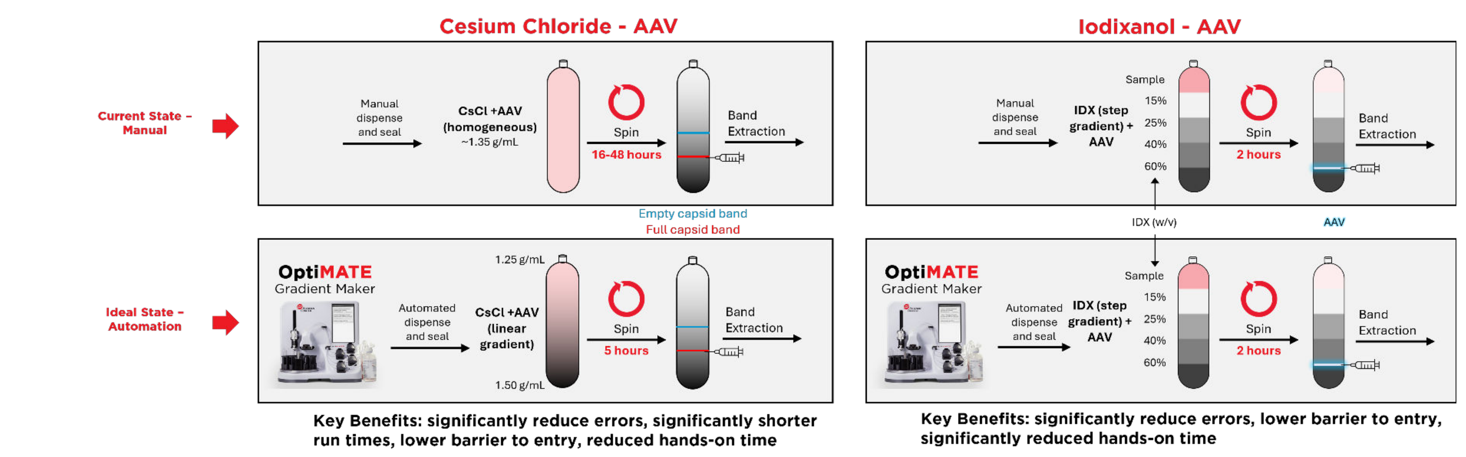
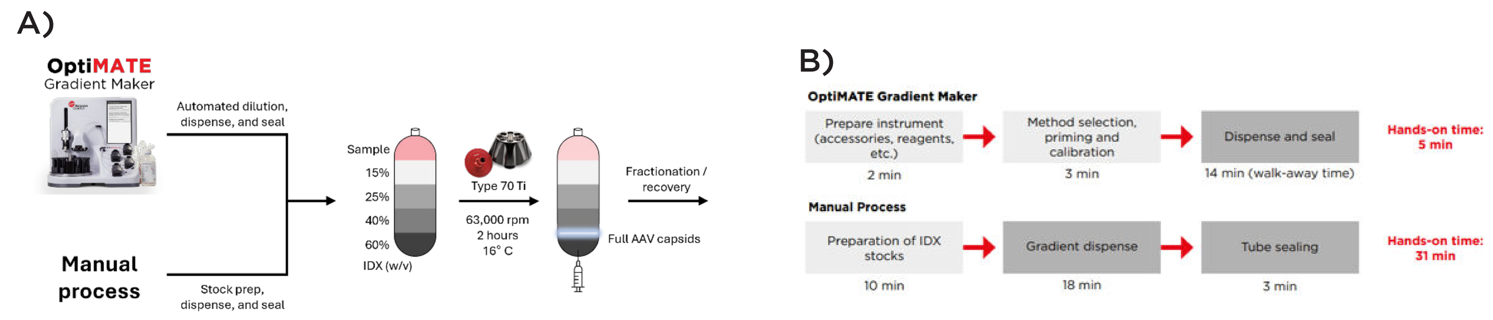
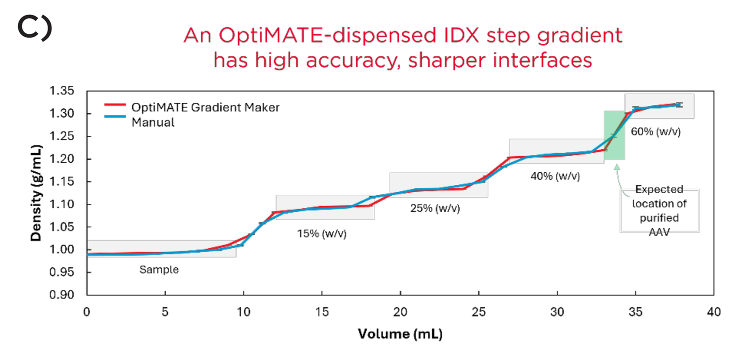


Figure 3: Schematics on how the OptiMATE Gradient Maker fits within existing AAV DGUC purification workflows.

REDUCES HANDS-ON TIME AND IMPROVES CONSISTENCY FOR IDX PURIFICATION OF AAV





D)

Method	Concentration (vg/ml)	Amount recovered (vg)	% vg recovery
OptiMATE Gradient Maker	1.57E+12	8.16E+11	39.24%
Manual	1.76E+12	8.08E+11	38.84%

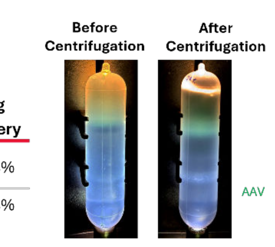


Figure 4: The use of the OptiMATE Gradient Maker to automate step-gradient generation. (a) Purification scheme for IDX density gradient experiment comparing automated dispense (top) and manual dispense (bottom) (b) Times taken for IDX step density gradient setup manually or using the OptiMATE Gradient Maker. (c) Comparison of gradient profiles from OptiMATE Gradient Maker (red) and manual (blue) (d) IDX step gradient tubes before and after ultracentrifugation with qPCR analysis results of recovered AAV after purification.

REDUCES RUN TIMES BY 75% WITHOUT SACRIFICING RESOLUTION FOR CSCL GRADIENTS

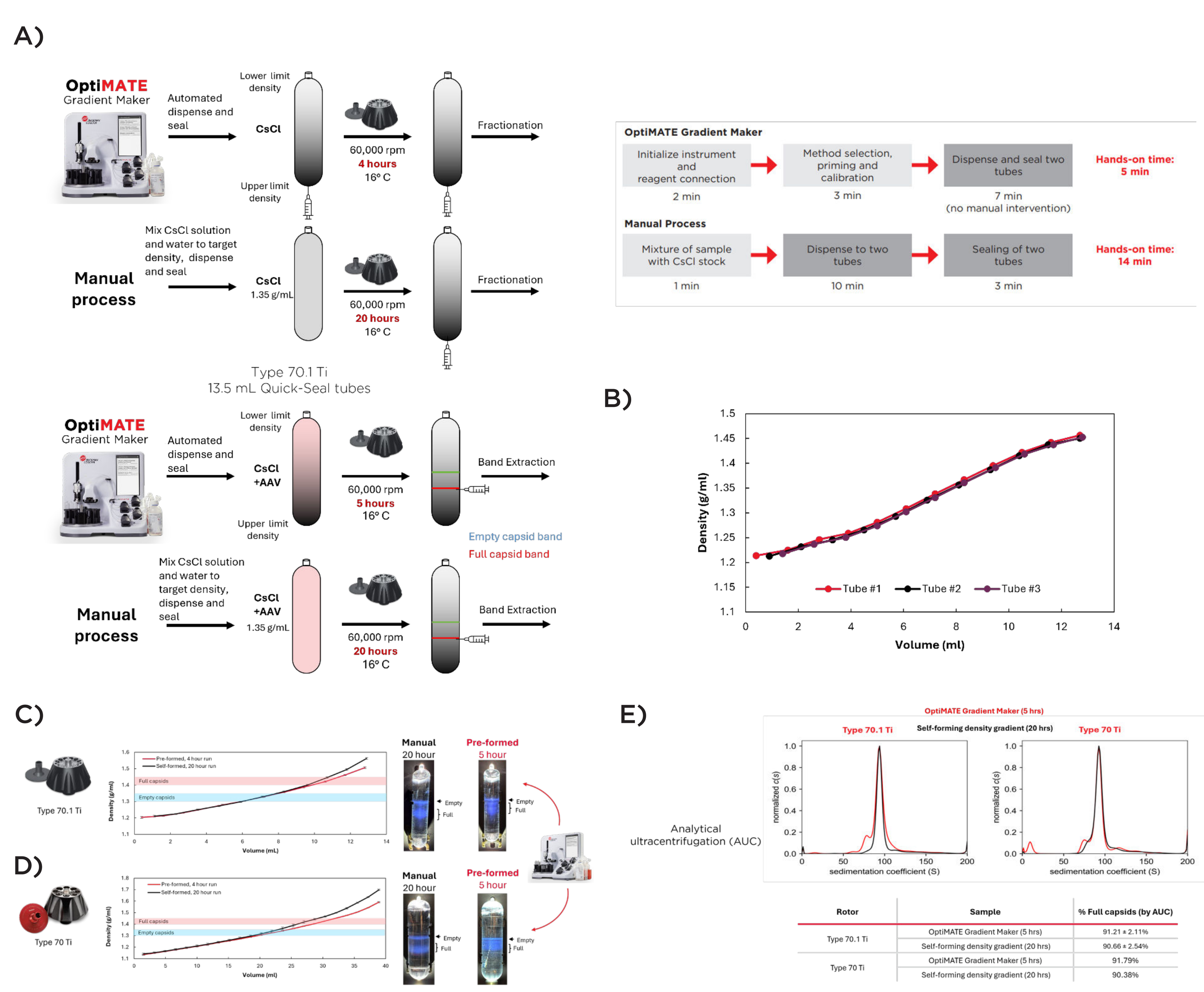
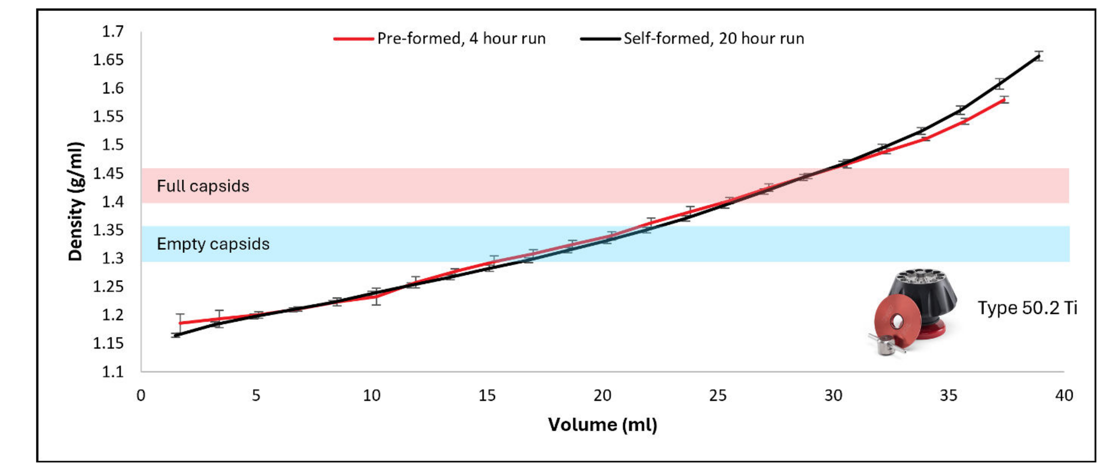


Figure 5: The use of the OptiMATE Gradient Maker generated pre-formed gradients to reduce CsCl DGUC run-time. (a) Schematics of the experimental plan and steps involved. Preparation steps are in light gray while dispense and seal steps are in dark gray. (b) Comparison of gradient profiles from three tubes dispensed by the OptiMATE Gradient Maker. (c) Comparison of gradient profiles from 13.5 mL Quick-Seal tubes and (d) 39 mL QuickSeal tubes. (e) Analytical Ultracentrifugation analysis of recovered AAV purity after centrifugation.

Run your established protocols, only better

RAPID, HIGHER-SCALE PURIFICATION OF AAV WITH PRE-FORMED GRADIENTS

A)



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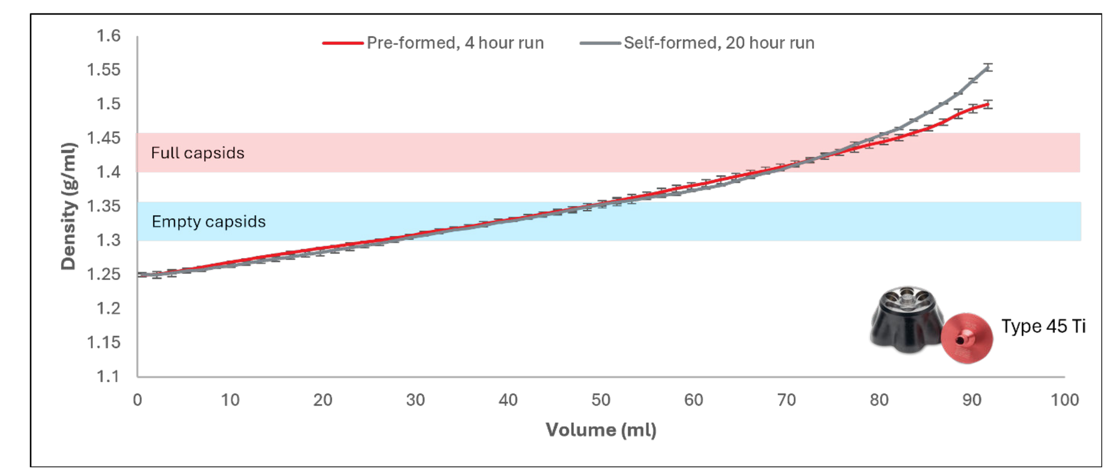

C)

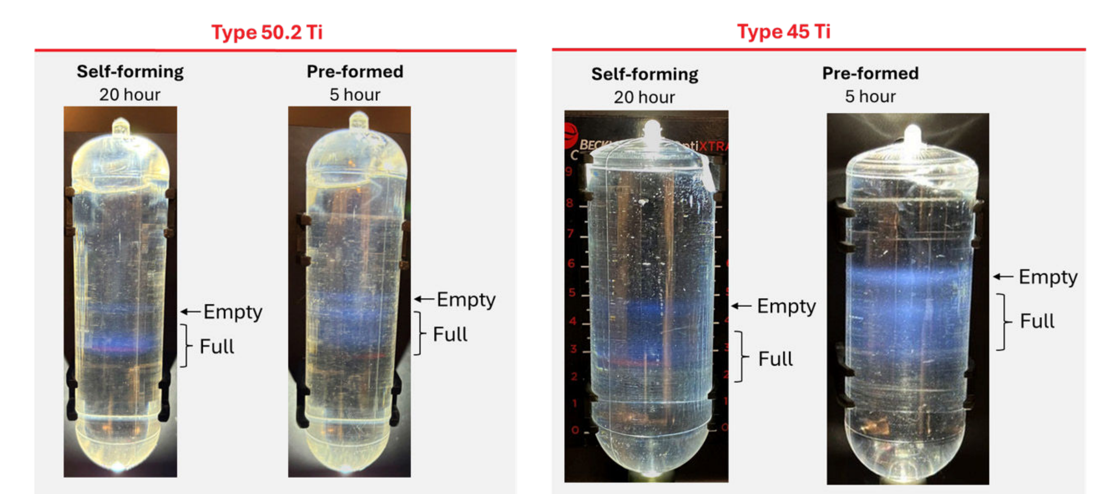

Figure 6: The use of the OptiMATE Gradient Maker for CsCl DGUC at higher rotor capacities. (a) Comparison of gradient profiles from 39 mL Quick-Seal tubes from a Type 50.2 rotor ultracentrifugation and (b) 94 mL Quick-Seal tubes from a Type 45 Ti rotor ultracentrifugation. (c) Representative images of AAV bands after ultracentrifugation from self-forming density gradients run for 20 hours and pre-formed density gradients run for 5 hours in 39 mL tubes (left) and 94 mL tubes (right).

CONCLUSIONS

The OptiMATE Gradient Maker precisely dispenses density gradients (volume accuracy ± 4%), addressing three major challenges in DGUC: effort/skill, variability and long ultracentrifugation run times for CsCl gradients. This is evidenced by the consistent density profiles and expected purity and recovery of AAV post-purification. This reduction in ultracentrifugation run time for CsCl gradients can potentially shorten a 2-3 day purification process to a single-day operation, facilitating better scale-up of the purification process. We have also shown that this improvement is consistent even in higher-capacity tubes and rotors.

The instrument enables user-friendly method creation via automation, and its precision allows even new users to begin purifying samples on day one. The rapid setup and minimal hands-on time streamline the workflow while reducing operator exposure to chemicals and biological materials. Furthermore, this instrument integrates seamlessly into existing purification processes, lowering skill barriers for creating high-quality density gradients and enhancing overall process efficiency.

In these studies, we have demonstrated equivalence of AAV purity in a fraction of the time using the OptiMATE Gradient Maker. However, users are encouraged to conduct their own optimization studies, as we believe the time savings realized can be greater in some cases with different OptiMATE methods that are specific to individual hardware and consumable configurations.

REFERENCES

1. Wang, Jiang-Hui, et al. "Adeno-Associated Virus as a Delivery Vector for Gene Therapy of Human Diseases." Signal Transduction and Targeted Therapy, vol. 9, no. 1, Apr. 2024, pp. 1–33

2. Zolotukhin, S., et al. "Recombinant Adeno-Associated Virus Purification Using Novel Methods Improves Infectious Titer and Yield." Gene Therapy, vol. 6, no. 6, June 1999, pp. 973–85

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