Streamlined pre-formulation screening with minimal sample requirements & 2-day turnaround to enable high efficiency AAV formulation development

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P0157

Abstract

Material constraints are a limiting factor in early stage AAV gene therapy development programs. This is especially true for initial, broad formulation studies, that consume substantial amounts of material and time.

We have established a low-volume, high-throughput pre-formulation screening approach using a simple proprietary buffer matrix that spans a broad pH range (5 – 8) and ionic strength levels (using 100 – 310 mM NaCl).

We show that rebuffering of AAV (exemplary serotype AAV3B/8 hybrid) using a commercially available 96-well spin-plate loaded with size-exclusion resin provides a rapid and reliable means of rebuffering. **With only 200 µL per condition, the material from this rebuffering campaign was used to generate thermal (nanoDSF) and colloidal (DLS) stability data along with assessing subvisible/visible particle content (BMI), DNA ejection (using SYBR GOLD) and titer recovery (Stunner).**

Since all methods are compatible with the 96-well format, results from this screening approach are available within hours of the rebuffering procedure with minimal manual handling.

For the exemplary serotype used in this study, **a buffer at pH 8 (20 mM TRIS, >190 mM NaCl) initially provided satisfactory results**, which is in line with published data. However, **DNA ejection progressed steadily at the tested temperature (37°C)** over 2 days compared to all other tested buffers, which exhibited a much less pronounced payload release. **Thus, development of a liquid formulation for the serotype in question should focus on buffers at lower pH levels to mitigate loss of encapsulated DNA during storage.**

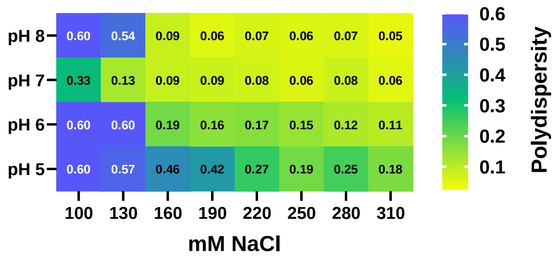
Overall, the wealth of data generated from this method with just 6.5 mL of sample AAV (≥2E12 vg/mL) can provide meaningful insights towards buffer and ionic strength preferences of a given serotypes and further inform rational formulation development. The approach is capsid agnostic and can be applied to any AAV serotype or other modalities based on protein nanoparticles.

Colloidal stability

Dynamic Light Scattering

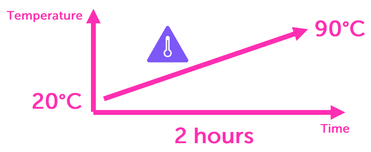
Low-salt conditions lead to aggregation after rebuffering, which is evident as an increase in dispersity of the sample (yellow fields).

pH levels of 7 or 8 resulted in less polydispersity after rebuffering compared **to pH 5 or 6, potentially hinting at better resistance** against aggregation during downstream processing and storage.

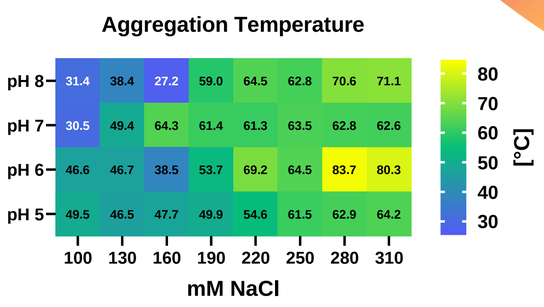


Identical 384-well plate used for evaluation of AAV aggregation upon heat-stress & simultaneous DLS assessment

Heat stress resistance:
20°C → 90°C over 2 h



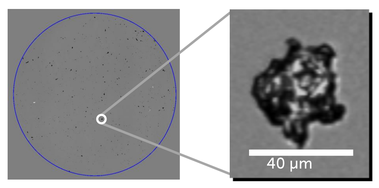
Monitoring temperature-induced onset of AAV aggregation (T_{onset}) showed that ionic strength in addition to pH are main contributors to colloidal stability upon heat stress (right). This result confirms published data[1], validates our platform, and is in line with nanoDSF data (shown below).



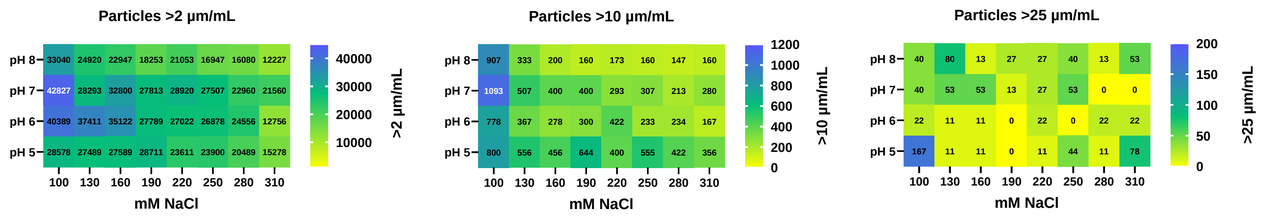
[1] JF Wright et al. *"Identification of Factors that Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence during Vector Purification and Formulation"* Molecular Therapy, Vol. 12, No. 1, 2005

Subvisible particles

Background Membrane Imaging



Evaluation of subvisible particle content is usually constrained to high-volume formulation studies at later stages due to high volume requirements of e.g. light obscuration. Background membrane imaging requires only microliters of sample and provides an insight into size & shape of particles. High-salt conditions lead to **reduced particle formation** due to rebuffering across pH levels (blue fields).



60 µL

40 µL

30 µL

10 µL

10 µL

Your AAV

+ Rebuffering spin plate
= Results within 2 days

Only 7 mL of > 2E12 vg/mL required
Exemplary buffer set: 20 mM TRIS/Phosphate/Histidine/Acetate

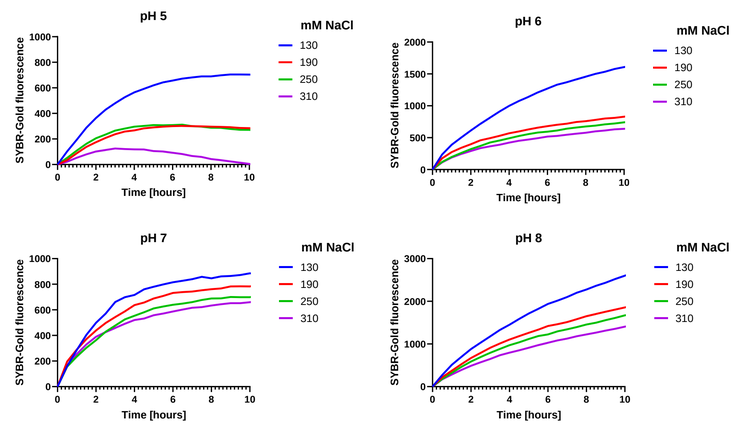
1) Equilibrate spin-plate with buffers
2) Apply AAV, spin down using centrifuge
3) Distribute rebuffered AAVs using multi-tip pipette

DNA-Ejection

SYBR-Gold Fluorescence

Measurement of SYBR-Gold fluorescence as an indicator of ejected DNA at 37 °C over 24 hours is **performed in simple thermocyclers – no need for specialized equipment.**

For all buffer levels, rapid DNA ejection is observed in the first 10 hours of incubation. Subsequently, there is only minor additional payload release evident at that temperature except for the condition at pH 8, which exhibits a steady increase in SYBR-Gold fluorescence. Overall, the extent of DNA ejection is dependent on NaCl concentration in each buffer level tested, with **higher salt concentrations leading to less ejection**.



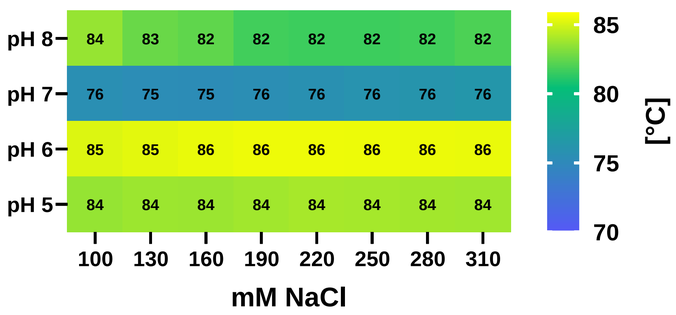
Capsid stability

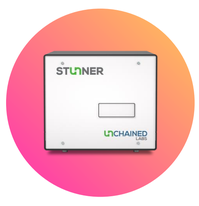
nanoDSF

Capsid unfolding temperatures are pH-depended and not influenced by ionic strength.

A pH level of 6 resulted in the highest **capsid stability** during heat stress. Formulations at pH 7 exhibited the lowest resistance to heat with melting temperatures at 76°C, **irrespective of ionic strength**.

In line with previously reported data, a drop in pH from 7 to 6 leads to a marked **increase in capsid stability of 10°C.**





Recovery after rebuffering

DLS/UV-Vis using Stunner

Assessment of capsid recovery after rebuffering using Stunner's DLS/UV-Vis combination revealed a trong dependency on **ionic strength at pH 8**.

Downstream processing should thus avoid "salt valleys" especially at this pH level – our **DSP-poster P0031** shows how we applied this knowledge to get to >50% vector-recovery in the purification process.

NaCl-levels >190 mM **did not further enhance** the recovery at pH 5/6/7.

Summary

The poster outlines how a wealth of formulation data can be generated within only 2 days from minimal amounts of sample.

Using a rebuffering approach via 96 well spin-plates enables sample handling with either a pipetting robot or (as in our case) or a multi-channel pipette.

Compared to other methods of rebuffering (Slidealyzer cassettes/cups, spin-filters "amicons", PD-10 columns), the presented workflow is fast and tailored to the low-volume 96-well-compatible methods that are already in place in most laboratories.

Overall, the workflow is capsid agnostic and can be applied to any AAV serotype or other modalities based on protein nanoparticles.

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