Formulation optimization reduces cannula-associated vector loss during precision AAV brain delivery

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Introduction

Adeno-associated virus (AAV) vectors are a central platform for CNS-directed gene therapies, where stereotactic administration enables highly localized delivery to defined brain regions. However, the long, narrow-bore cannulas required for these procedures create unusually **high surface-area-to-volume ratios** and **prolonged material contact** with device interfaces, introducing substantial risk of **vector loss during administration**.
Under these conditions, surface adsorption, interfacial stress, and shear exposure can significantly **alter the administered dose** and may additionally compromise particle integrity through aggregation or other physicochemical destabilization mechanisms. For precision CNS applications, where administered **volumes are small** and **dosing margins** may be **narrow**, even modest formulation-dependent differences in cannula recovery can have meaningful consequences for preclinical interpretation and clinical performance. Importantly, improved titer recovery alone does not fully capture formulation performance if administration conditions also promote particle heterogeneity or aggregate formation.
In this study, we evaluated the **impact of surfactant optimization** on both AAV recovery and particle characteristics following passage through a clinically relevant stereotactic **cannula model**. By combining sensitive DNA quantification (dsDNA via Qubit) with DLS-based assessment of particle size and dispersity, we investigated how **formulation composition influences** both **delivered dose and resistance to administration-induced physicochemical stress**.
Our findings below support the need to consider in-use administration performance as a critical component of AAV formulation design.

***Localized delivery** to the **brain** with live infusion visualization via MRI requires a special setup using a long, non-ferric **cannula**:*
- *extremely **thin: 220 µm** inner diameter*
- *length: **3 meters/10 feet***
- *inner surface: deactivated **fused silica (glass)***

AAV9
2.5 E12 vg/mL
20 mM TRIS pH 8
200 mM NaCl
1 mM $MgCl_2$

MIND THE LOSS

Experimental Overview

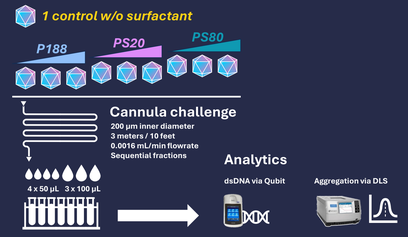
AAV serotype, formulation, concentration
Serotype: AAV9
Concentration: 2.5 E12 vg/mL
Practically **relevant low-to-mid dose range** and provides a sensitive model for **detecting absolute vector losses** during device passage.
Formulation: 20 mM TRIS pH 8, 200 mM NaCl, 1 mM $MgCl_2$
Employed in both Zolgensma and Itvisma formulation that use the same serotype.

Formulation screening strategy
10 formulation conditions were evaluated to assess the impact of surfactant type and concentration on cannula-associated recovery:
- Control **without surfactant**
- **Poloxamer 188** (P188) at 0.001%, 0.0025%, and 0.005% w/v
- **Polysorbate 20 (PS20) and 80 (PS80)** both at 0.001%, 0.005%, and 0.01% w/v

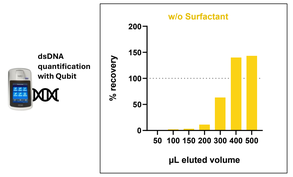
Cannula challenge model
Deactivated fused silica capillary, Agilent
Part No: 160-2205-10
Length: 3 m
Inner diameter: 200 µm
Outer diameter: 360 µm
Flow rate: 0.0016 mL/min

Sequential eluate fractions were collected to resolve recovery dynamics throughout the infusion process:
4 × 50 µL fractions
3 × 100 µL fractions

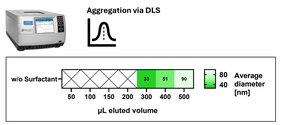
Analytical characterization
Recovered fractions were evaluated using complementary methods:
- **Genome titer quantification**
  Qubit dsDNA assay
  5 min heat treatment at 95°C prior to analysis
- **Particle size/distribution analysis**
  Dynamic light scattering (DLS)
  Wyatt Dynapro III plate reader



Without surfactant: Severe titer loss



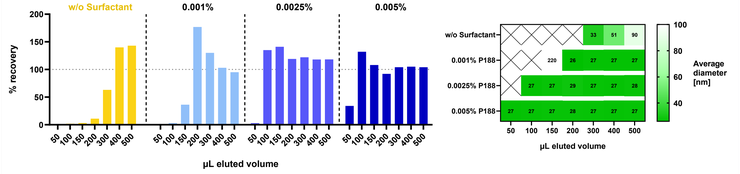
→Severe **titer loss** without surfactant, especially in early fractions
→Recovery improves only after **progressive surface saturation**
→Indicates **strong adsorption** to cannula surfaces
→**Highlights need for surfactant optimization to preserve delivered dose**



→First **200 µL were below DLS detection limits** due to near-complete nanoparticle depletion
→Mirrors Qubit dsDNA results showing essentially **no recoverable vector genomes**
→Indicates **severe adsorption within the cannula**
→Detectable particles emerged only after surface saturation

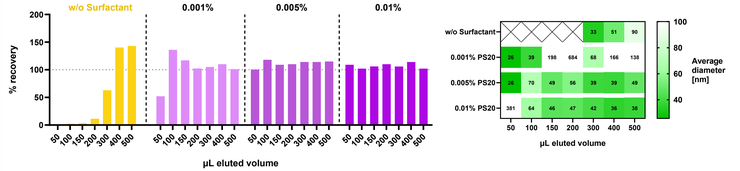
P188

Poloxamer 188: severe losses at 0.001%, acceptable at 0.005%



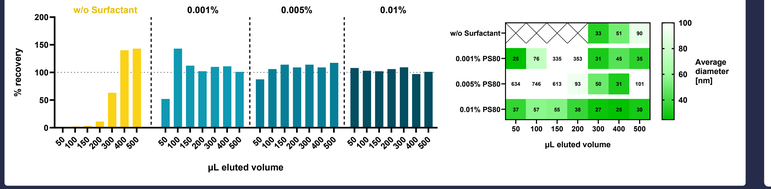
PS20

Polysorbate 20: acceptable recovery at 0.001%



PS80

Polysorbate 80: acceptable recovery at 0.001%



Results & Conclusion

Key findings:
- Cannula-associated **AAV loss** is highly formulation-dependent and can **severely compromise effective delivered dose**
- Inadequate surfactant protection may create substantial **underdosing risk**, particularly in low-volume CNS applications
- Early fraction losses are **especially critical**, with potential to **distort preclinical toxicology** interpretation through **false-negative** safety outcomes
- In clinical settings, inconsistent recovery may lead to **asymmetric or unpredictable tissue exposure**, reducing dosing precision

Surfactant-specific conclusions:
- **P188** required **higher concentrations** to adequately suppress losses, but provided **superior control of particle size stability and dispersity**
- **PS20 and PS80 improved recovery** at lower concentrations, but showed **less effective** maintenance of nanoparticle **uniformity**
- Formulation optimization must therefore balance:
  - Recovery efficiency
  - Particle integrity
  - Practical administration workflows
  - Product stability requirements
  - Safety/tolerability of excipient exposure

Translational implications:
- Sufficient surfactant levels are required to **prevent** clinically relevant **vector depletion**
- **Cannula flushing** strategies must balance dose recovery against unnecessary product waste
- CMC programs should incorporate **administration-phase performance, not solely storage stability**
- Toxicology programs might additionally evaluate surfactant tolerability within sensitive CNS tissues

Overall conclusion:
- Administration-compatible **formulation design** is essential to ensure **reliable dose delivery**, coherent preclinical interpretation, and reproducible **clinical translation for stereotactic AAV therapies**.

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