Development of alternatives to Triton X-100 cell lysis for AAV2, AAV5 and AAV8 primary recovery

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INTRO

AAV primary recovery

At harvest the majority of AAV particles are retained within the cells after they are produced, requiring cell membrane disruption to release them.

➡ Historical lysis method

Triton X-100 (non-ionic surfactant)
Prohibited from use since 2021 due to environmental concerns (endocrine disruptor for aquatic life and strict limits in injectable drug products).

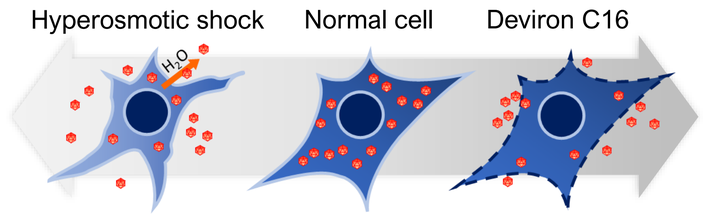
➡ Alternatives to Triton X-100

❶ TDAO (commercially Deviron® C16)
- Amphoteric detergent identified as most suitable in early phase screening compared to polysorbate based detergents.
- Not in REACH list & available for cGMP use.

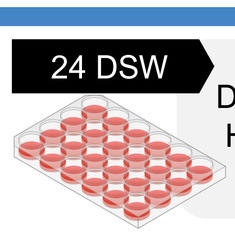
❷ Hyperosmotic shock AAV release method
Reports evidencing its effectiveness has furthered interest to remove detergent from the AAV primary recovery step altogether.

AIM

Optimization of AAV recovery methods using Deviron C16 detergent and hyperosmotic shock for AAV2, AAV5 and AAV8 serotypes



METHOD



24 DSW → DOE screening Hyperosmotic method → Successful? → Yes / No → 250mL PB → Scale-up of hyperosmotic method / Testing of Deviron C16 concentrations → 10L PB → Confirmation runs

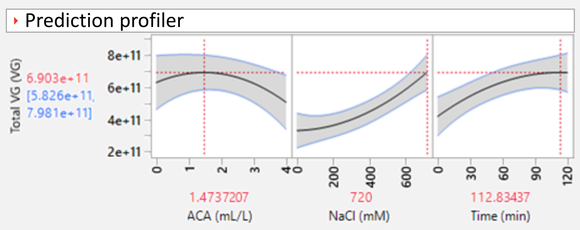
RESULTS

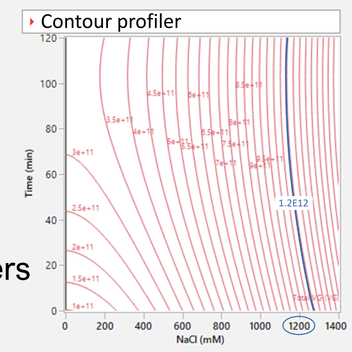
AAV5

Hyperosmotic shock in 24 DSW – DOE1

Effect summary

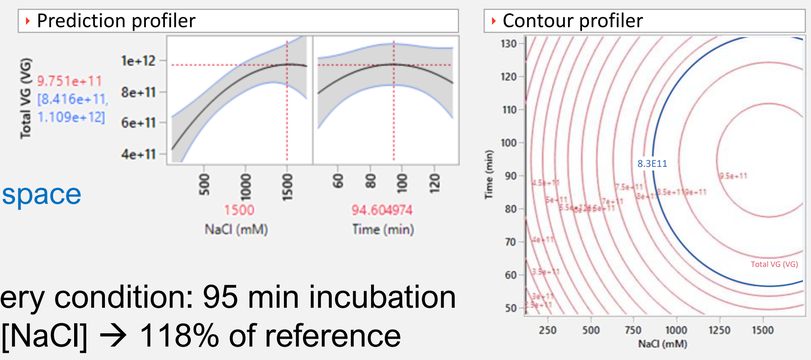
Source	Logworth	PValue
NaCl (mM)(180,540)	7.645	0.00000
Time (min)(30,90)	4.489	0.00003
ACA (mL/L)*ACA (mL/L)	2.126	0.00749
NaCl (mM)*NaCl (mM)	1.381	0.04156
Time (min)*Time (min)	1.201	0.06297
Ad1(mL/L)*Time (min)	1.099	0.07966
Ad1(mL/L)(1,3)	0.544	0.28590 ^





- [NaCl] and NaCl incubation time are the parameters with greatest impact during AAV release.
- Further benefit in ↑ [NaCl] suggested by profile predictor trend.

Hyperosmotic shock in 24 DSW – DOE2



Expanded design space towards ↑ [NaCl]

- Highest recovery condition: 95 min incubation and 1500mM [NaCl] → 118% of reference 0.1% Triton X-100 method.
- Hyperosmotic shock method deemed unsuitable due to volumes exceeding bioreactor capacity.

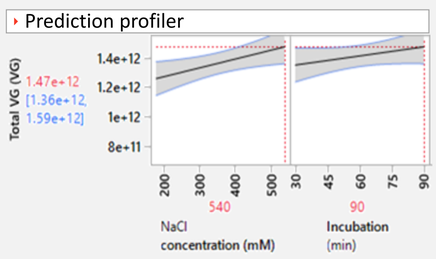
Deviron C16 optimization in STR

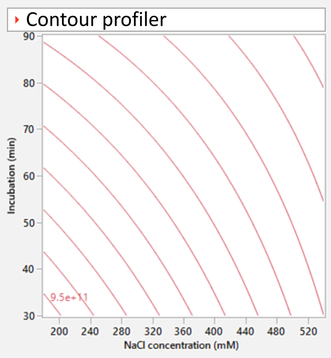
	VG recovery in CL (%)
AAV5-transgene A | 250mL | 0.1% Triton X-100 (n=1)	102%
AAV5-transgene A | 250mL | 0.1% Deviron C16 (n=1)	93%
AAV5-transgene A | 250mL | 0.3% Deviron C16 (n=1)	103%
AAV5-transgene A | 250mL | 0.5% Deviron C16 (n=1)	99%
AAV5-transgene B | 10L | 0.1% Triton X-100 (n=3)	82%
AAV5-transgene B | 10L | 0.5% Deviron C16 (n=1)	78%

- VG recovery through harvest and clarification for 0.5% Deviron C16 comparable to the recovery with reference 0.1% Triton X-100 method.

AAV8

Hyperosmotic shock in 24 DSW – DOE





- To match Triton X-100 lysis performance, model extrapolation predicted incubation duration >200 min and/or [NaCl] >700 mM.
- Due to volume constraints 400 mM [NaCl] further scaled up at different incubation durations.

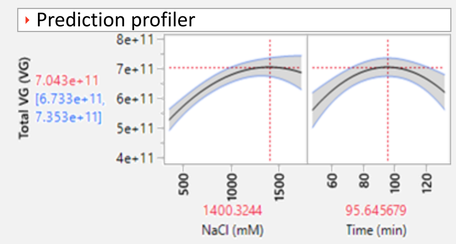
Hyperosmotic shock scale-up in STR

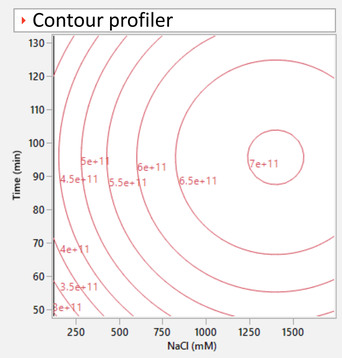
	VG recovery in CL (%)	Full Capsids (AUC) (%)	res pDNA (% of VG)	res hcDNA (ng/dose)	res HCP (ng/dose)
AAV8| 250mL| 0.1% Triton 120min (n=4)	88%	50%	6%	2.8	13.7
AAV8| 10L| NaCl 400mM 75min (n=2)	57%	47%	5%	2.4	28.5
AAV8| 10L| NaCl 400mM 90min (n=2)	67%	47%	3%	1.0	30.4
AAV8| 10L | NaCl 400mM 120min (n=2)	60%	-	3%	-	-

- VG recovery ≥ 60% of the 0.1% Triton X-100 lysis method at 90-120min incubation time.
- No negative impact of hyperosmotic shock on % full capsids and DNA or protein residuals after affinity chromatography.

AAV2

Hyperosmotic shock in 24 DSW – DOE





- Model predicting 1400 mM [NaCl] and 90-100min incubation time for a maximum of 67% recovery from the Triton X-100 method.
- Hyperosmotic shock method deemed unsuitable due to volumes exceeding bioreactor capacity.

Deviron C16 optimization in STR

	VG recovery in CL (%)	Full Capsids (AUC) (%)
AAV2-transgene A| 250mL | 0.1% Deviron C16 (n=2)	83%	36%
AAV2-transgene B| 250mL | 0.1% Deviron C16 (n=2)	80%	38%
AAV2-transgene A| 10L | 0.1% Deviron C16 (n=3)	71%	33%
AAV2-transgene B| 10L | 0.1% Deviron C16 (n=3)	69%	32%
AAV2-transgene C| 10L | 0.1% Deviron C16 (n=3)	68%	44%
AAV2-transgene C| 10L | 0.1% Triton X-100 (n=2)	87%	-
AAV2-transgene A| 10L | 0.5% Deviron C16 (n=2)	88%	36%
AAV2-transgene B| 10L | 0.5% Deviron C16 (n=1)	80%	31%
AAV2-transgene C| 10L | 0.5% Deviron C16 (n=2)	102%	46%

- 0.1% Deviron C16 VG recovery equivalent to 0.1% Triton X-100 at 250mL scale but reduced upon scale up.
- Lysis with 0.5% Deviron C16 yielded comparable VG recovery to 0.1% Triton X-100 at 10L scale, and no impact of % full capsids.

CONCLUSION

- Different optimized AAV recovery methods depending on serotype.
- Successful Triton X-100 replacement from AAV upstream manufacturing with comparable results.

0.5% Deviron C16 2h incubation
- AAV5 96%
- AAV2 104%

400 mM [NaCl] 2h incubation
- AAV8 68%

of 0.1% Triton X-100 VG recovery & No negative impact on % full capsids, DNA and protein residuals