Bioprocessing

Productivity optimization and process calculations for AAV affinity chromatography

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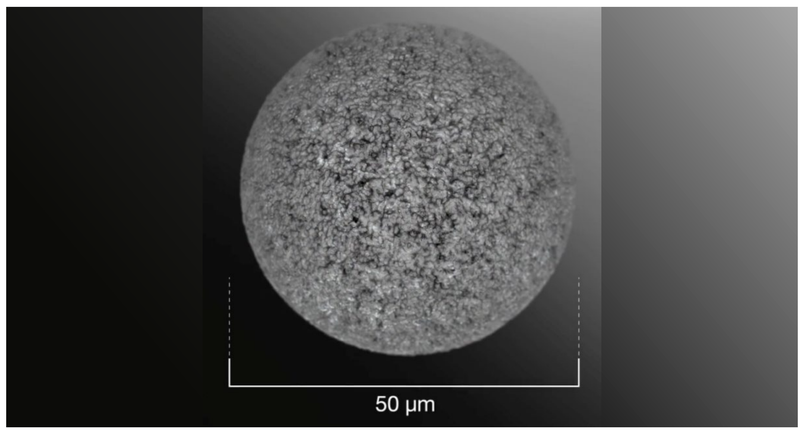
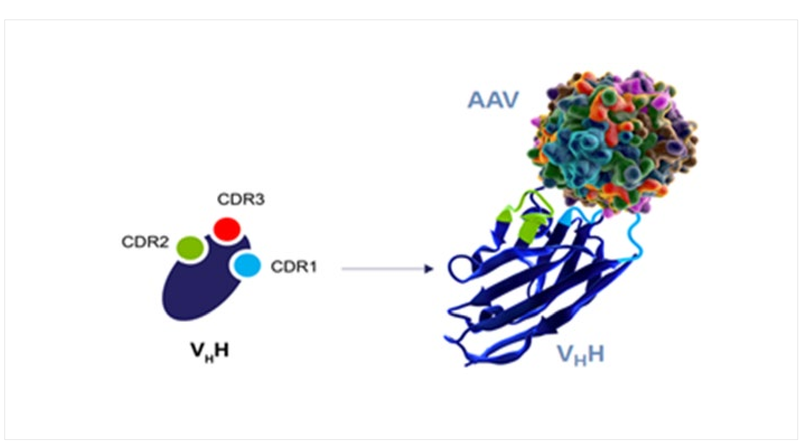
Introduction

The use of recombinant adeno-associated virus (rAAV) as a delivery method for gene therapies continues to be successful with hundreds of ongoing clinical trials and some recent approvals. The diversity of applications for rAAV ranges from rare diseases affecting small patient populations to more prevalent inherited ailments such as hemophilia. The doses required vary widely from ~4E11 vg/eye for subretinal administration to 3.5E14 vg for intrathecal applications [1].

From a manufacturing perspective the field has moved to common approaches for production and purification of rAAV. Upstream approaches typically use transfection of HEK293 cells and titers are routinely in the 1-2E10 vg/mL although higher titers of up to 6E11 vg/mL at a 2000 L scale were recently reported [2]. These high titers will be needed for large dose and/or patient populations to meet the demand of these therapies and reduce costs.

For rAAV purification the majority of the field has moved to scalable processes employing an affinity capture chromatography step [3] and commonly utilizing Thermo Scientific™ POROS™ CaptureSelect™ AAVX resin. In this work, dynamic binding capacity (DBC) data for multiple AAV serotypes were leveraged to estimate an optimal productivity of rAAV using the AAVX resin. An analysis of process conditions and column geometries that would fit maximum processing times and resin utilization was conducted for two case scenarios representing current titers for clinical manufacturing and high titers for commercial manufacturing scales.



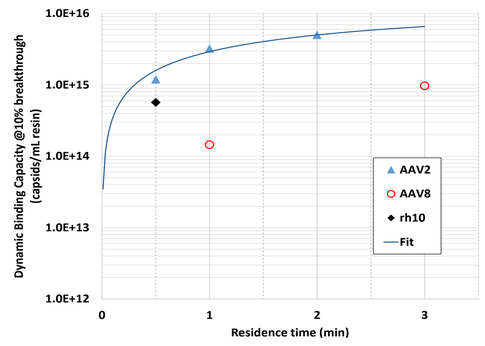


POROS™ base bead technology (polystyrene divinylbenzene, top) and CaptureSelect™ ligand technology (single-domain antibody, bottom) are combined in the manufacturing of AAVX resin

Methodology

- **Dynamic binding capacity:**
  - AAV2 breakthrough curves were generated using HEK293 clarified lysate to determine DBC at 10% breakthrough.
  - AAV8 and rh10 DBC data were obtained from references 4 and 5, respectively.
  - Equation I was fitted to the DBC data using a linear regression numerical method.
- **Productivity:**
  - Productivity curves were generated using equations I and II.
  - Column volumes and residence time for the non-loading steps were 25 CV and 2 min.
  - Column volumes and residence time for CIP steps were 10 CV and 3 min.
- **Scale-up and process considerations:**
  - GMP pre-packed column pressure limitations were based on literature from multiple vendors.
  - Pressure drop at 3 bar was based on pressure-flow curves for POROS CaptureSelect AAVX resin (internal pressure-flow data).
- **Case scenarios**
  - Processing time and resin utilization calculations were performed using Microsoft® Excel® assuming 20% full capsids and the results were further analyzed and plotted using MODDE® software.

Dynamic binding capacity

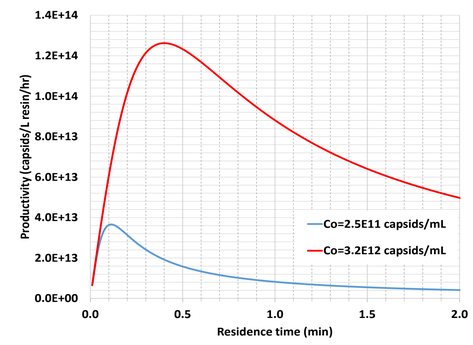


$$Q_d = \frac{Q_d^{max}\ RT}{\theta + RT} \quad (I)$$

Q_d = Dynamic Binding Capacity @10% breakthrough
Q_d^{max} = DBC at long residence times
RT = Load residence time
θ = Residence time constant

- Limited DBC data are available due to high capacity of AAVX resin, relatively low titers, and sample availability.
- DBC for AAV2 is relatively high (~1E15 capsids/mL resin) even at 30 sec residence time.
- Data fit to equation (I) approximates the dependence of DBC to residence time.

Productivity



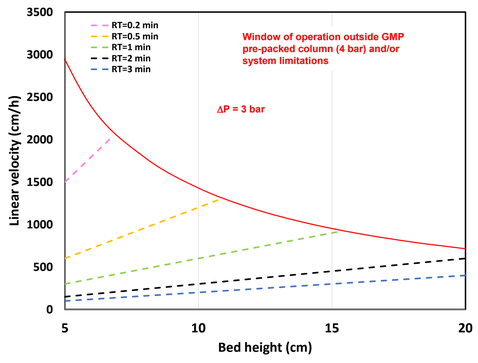
$$P = \frac{Amount\ of\ AAV\ capsids\ purified}{Unit\ resin\ volume\ \times Unit\ time}$$

$$P = \frac{\eta\, Q_d}{\frac{Q_d}{C_o} RT\ + CV_{non-load}\ RT_{non-load} + CV_{CIP}\ RT_{CIP}} \quad (II)$$

P = Productivity
η = Loading safety factor (% DBC)
C_0 = Load sample concentration
$CV_{non\text{-}load}$ = Column volumes for non-loading steps
$RT_{non\text{-}load}$ = Residence time for non-loading steps
CV_{CIP} = Column volumes for non-loading steps
RT_{CIP} = Residence time for non-loading steps

- Productivity maximum is achieved at residence times below 0.5 min.
- Productivity increases by ~3.5x with an increase in titer of ~12x.
- Increased titer shifts productivity maximum from ~7 to ~24 seconds RT for loading.

Scale-up and process considerations

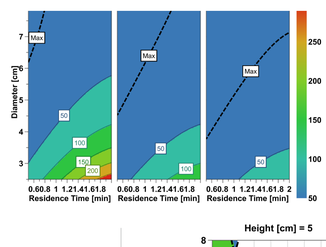


- Pre-packed columns are widely used in GMP manufacturing (4 bar limit).
- Owing to hardware limitations the optimal productivity can only be achieved with a 5 cm bed height and 30 sec residence time only with 10 cm bed heights.
- For larger columns (e.g. >25 cm i.d.) commonly used chromatography systems may limit operation to residence times >0.5 min.

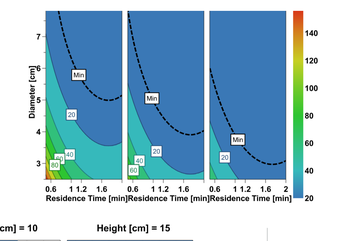
Scenario 1. Clinical manufacturing, 200 L, Co=2.5E11 capsids/mL

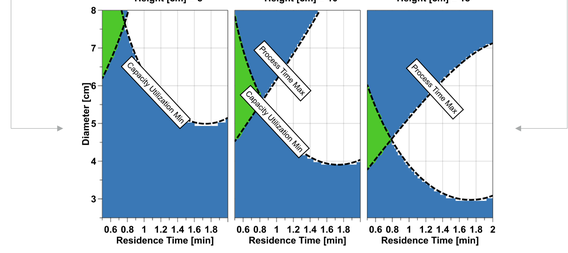
Analysis Criteria

Processing Time Maximum = 12 hours



Minimum Resin Capacity Utilization = 10%



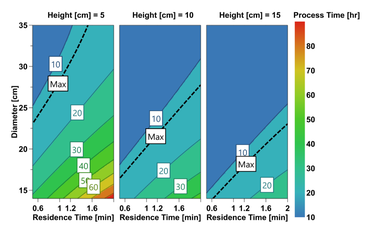


- Residence times <0.8 min meet acceptance criteria for processing time and capacity utilization.
- Capacity utilization is low but CV are <0.4 L resin, i.e. low contribution to overall process cost.
- All column configurations in acceptable space require only 1 process cycle (data not shown).

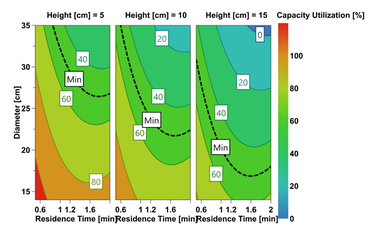
Scenario 2. Commercial manufacturing, 2000 L, Co=3.2E12 capsids/mL

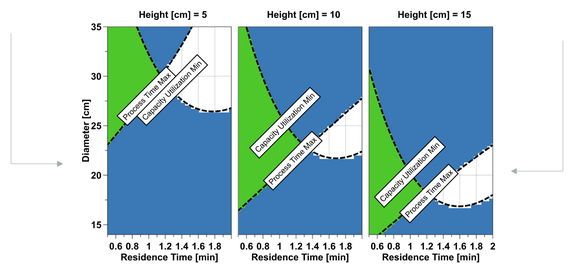
Analysis Criteria

Processing Time Maximum = 12 hours



Minimum Resin Capacity Utilization = 50%





- Broader window of operation bed heights of 10 and 15 cm max res. time at 1.1-1.2 min.
- Capacity utilizations of 60-80% for column configurations meeting acceptable criteria.
- Only some configurations require 1 process cycle (data not shown). Considering potential system pump limitations optimal configurations are 20 cmD x 15cmL or 25 cmD x 10cmL.

Conclusions

- The relatively high binding capacity of POROS CaptureSelect AAVX resin was confirmed to be >1E15 capsids/mL resin at residence times >= 0.5 min for AAV2.
- Productivity is maximized at load residence times <= 0.5 min depending on titer but hardware and/or system considerations limit operation closer to 1 min.
- For clinical manufacturing the high DBC allows for a range of process conditions and requires small column volumes.
- For large bioreactor volumes and high titers the model suggests columns 20-30 cm diameter to meet typical processing limits while maximizing resin utilization.

References

1.Burdett and Nuseibeh, Gene Therapy (2023).
2.Van Lieshout, et al. Molecular Therapy-Methods in Clinical Development (2023).
3.Adams et al., Biotechnology and Bioengineering (2020).
4.Ravault and Laroudie, Cell & Gene Therapy Insights (2022).
5.Hurwit and Morrison, ASGCT Meeting (2018).

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