Lentiviral vector capture using anion-exchange chromatography: removing an industry bottleneck through rational design

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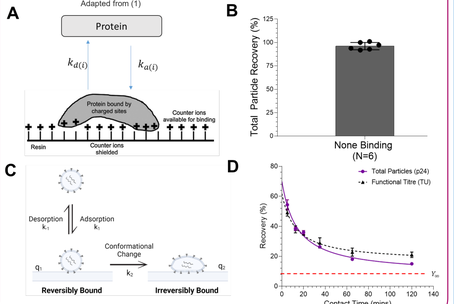
Overview

Charge-based separation using anion-exchange chromatography (AIEX) is near ubiquitously employed across the viral vector manufacturing industry for primary product capture of Lentiviral Vectors (LVs). Yet inconsistent performance is reported with most of the product loss in LV bioprocessing coming from this unit operation alone (LV recovery < 30%).

These poor recoveries, and inconsistent performance across LV products, stems from a poor understanding of the mechanisms underpinning charge-based separation of large vectors like LV. This has led not only to poor process recoveries but also to a largely "trial and error" approach to process development and a lack of industry wide consensus on optimal chromatography conditions. Furthermore, due to a lack of LV targeted chromatography products , old technologies not initially designed for viral purification must be retrofit fit into LV manufacturing processes.

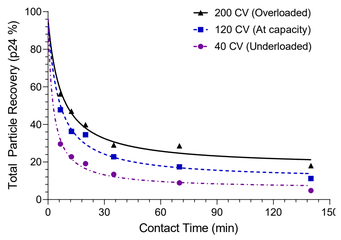
Here, we identify the major product loss mechanisms driving poor LV recovery on current commercial AIEX membranes. High recovery processes are demonstrated when using a novel AIEX membrane prototypes developed specifically for LV purification by Sartorius. Finally, the impact of LV product heterogeneity is assessed identifying at least two binding subpopulations. This description opens the door to mechanistic AIEX models and *in silico* process development that we aim to use to expedite process characterisation timelines.

① LV product loss due to irreversible binding and conformational change in the bound state

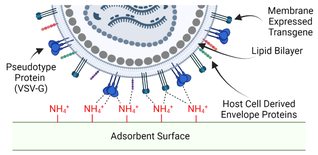


- Typical mechanistic descriptions of AIEX adsorption processes, (A), are not fit for viral systems making rational development difficult.
- None-Binding experiments demonstrate no loss of LV, indicating loss of product is linked to the binding process and not entrapment (B).
- We propose loss of both LV product increases the longer it spends in the bound state due to irreversible binding that results from a conformational change (C).
- Data confirms the hypothesis , with a rapid rate of total and functional vector loss with time in the adsorbed state ($t_{1/2}$ = 12.7-14.2 mins) (D).

② LV loss driven by excess adsorbent charge

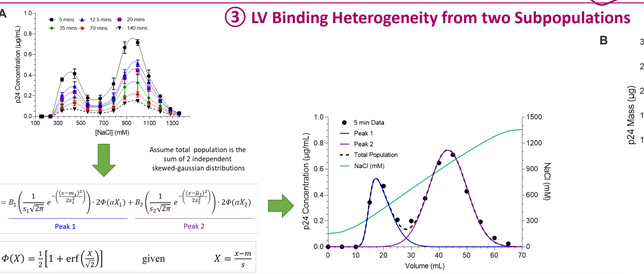


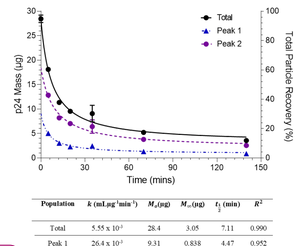
Load	k_2(min^{-1})	Y_m	Y_{ss}	$t_{Y1/2}$(min)	R^2
200 CV	$1.78x10^{-3}$		25.8	7.14	0.96
120 CV	$2.04x10^{-3}$	96	10.5	5.74	0.96
40 CV	$4.12x10^{-3}$		5.75	2.69	0.95



- Rate of vector loss is reduced at increased membrane loading.
- At increased adsorption times, free ligands on the adsorbent surface are able new attachments with charged species on the LV surface leading to increased multipoint attachment with time.
- Increasing membrane occupancy reduces the number of availability of free ligands to form new attachments, thus reducing the rate of material loss.
- Current membrane charges are likely too high for viral vector purification thus driving this loss process – Unsurprising as they were not designed for viral vectors.

③ LV Binding Heterogeneity from two Subpopulations

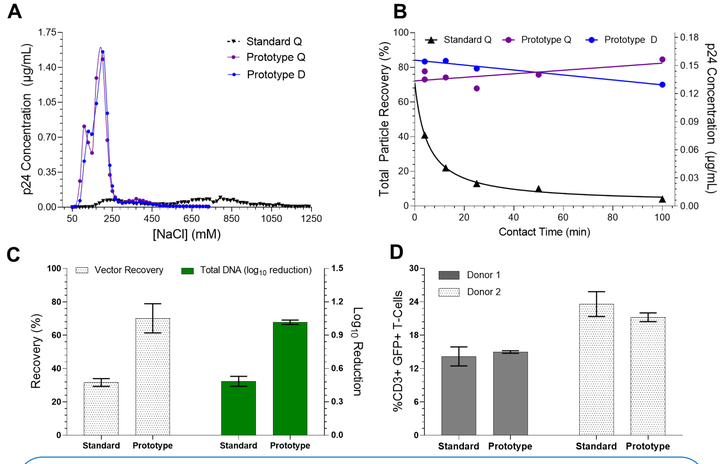




Population	k (mL.pg^{-1}min^{-1})	M_m(pg)	M_m(µg)	$t_{\frac{1}{2}}$ (min)	R^2
Total	5.55×10^{-3}	28.4	3.05	7.11	0.990
Peak 1	26.4×10^{-3}	9.31	0.838	4.47	0.952
Peak 2	6.53×10^{-3}	18.8	2.01	9.12	0.956

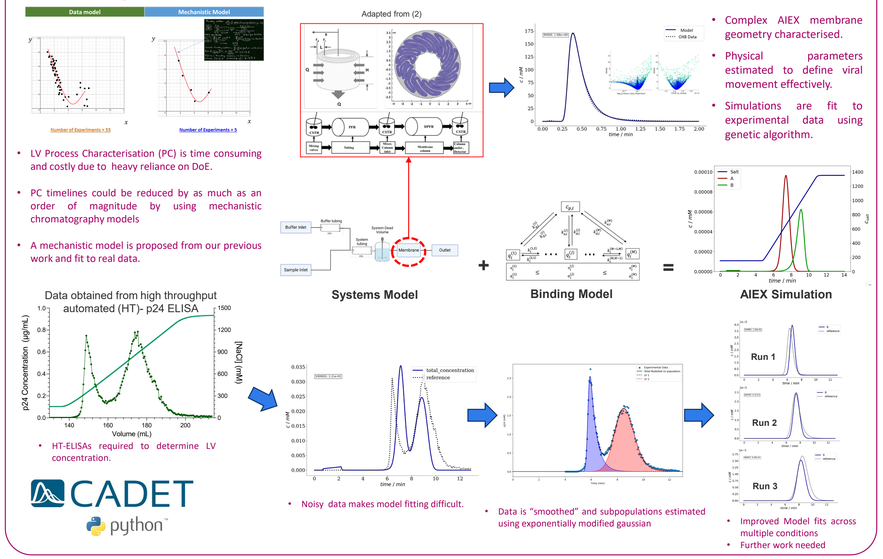
- LV structural heterogeneity gives rise to a bimodal elution profile indicating two separate and distinct binding subpopulations (A).
- Statistical modelling of elution profiles demonstrates both peak are susceptible to the time dependent loss mechanisms (B).
- Data shows in terms of charged-based purification , LV cannot be considered as a homogenous product pool.

④ Sartorius LV Tailored Membrane Prototypes Give Step Change in LV Recovery



- Novel membrane prototypes, systematically screened for the most suitable pore size and binding properties for LV purification by Sartorius, are evaluated against current commercially available Sartobind® Q membranes.
- Distinctive broad 150-1350mM NaCl Sartobind Q elution profile is replaced by a sharp elution peak at less than 500 mM NaCl (A).
- Recovery is now largely or completely decoupled from contact time loss mechanism (B).
- Unprecedentedly high LV recoveries of 60-80% with enhanced DNA removal (C).
- Activity of purified material is confirmed on T-Cells (D).

⑤ Moving Towards Rapid *In Silico* Process Development With Mechanistic LV AIEX Model



- LV Process Characterisation (PC) is time consuming and costly due to heavy reliance on DoE.
- PC timelines could be reduced by as much as an order of magnitude by using mechanistic chromatography models
- A mechanistic model is proposed from our previous work and fit to real data.
- Complex AIEX membrane geometry characterised.
- Physical parameters estimated to define viral movement effectively.
- Simulations are fit to experimental data using genetic algorithm.
- HT-ELISAs required to determine LV concentration.
- Noisy data makes model fitting difficult.
- Data is "smoothed" and subpopulations estimated using exponentially modified gaussian
- Improved Model fits across multiple conditions
- Further work needed

CADET
python

Summary

- Purification with Anion-Exchange accounts for most of the product loss in LV bioprocessing (~80%).
- Despite near ubiquitous use across the industry, AIEX is poorly understood for large vector targets.
- A key mechanism causing loss of LV product, time-dependent irreversible binding, is presented.
- This mechanism is linked to excess charge present in current commercial AIEX membranes not designed for purpose.
- Mitigating this mechanism in current chromatography materials gives 3-fold increase in recovery but is impractical in a GMP manufacturing setting.
- The presence of two binding subpopulations is identified highlighting the need to consider multiple target LV "species".
- Sartorius scalable prototype LV membranes mitigate time dependent loss giving unprecedently high recoveries of up to 80%.
- We look to a future mechanistic model of the AIEX process to enable *in silico* process development and accelerate PC timelines.

References

1) Diedrich, et al (2017). Multi-state steric mass action model and case study on complex high loading behavior of mAb on ion exchange tentacle resin, https://doi.org/10.1016/j.chroma.2017.09.039

2) Qu et al (2024), Application of mechanistic modelling in membrane and fiber chromatography for purification of biotherapeutics — A review, https://doi.org/10.1016/j.chroma.2023.464588

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