Viral Vector Platform Purification using Continuous Aqueous Two-Phase Systems

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Background & Motivation

Continuous processing has created major efficiency gains in monoclonal antibody manufacturing.

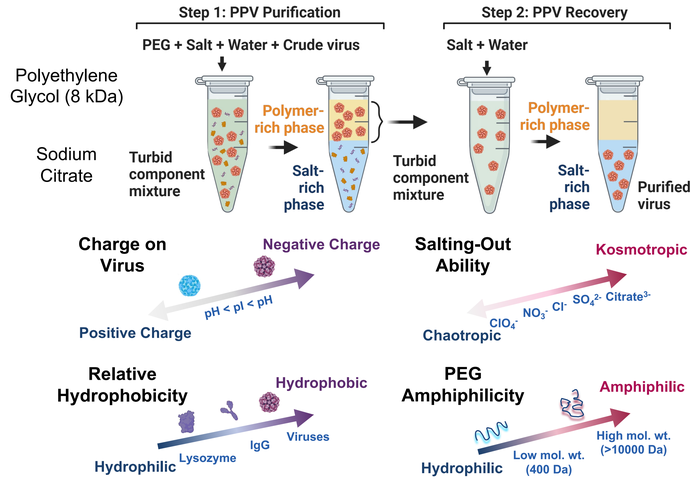
Transitioning from batch to continuous processing yielded:

⇩ **90%** Reduction in downtime[1]

⇩ **75%** Reduction in process footprint[1]

Goal: Lower cost per dose by creating a platform for **continuous purification** of viral vectors.

Aqueous Two-Phase Systems (ATPS):
raw materials-based liquid-liquid extractions.



Materials & Methods

Viral Products Tested with ATPS

Porcine Parvovirus (PPV)
Non-enveloped
pI = 4.8 – 5.1

Herpes Simplex Virus (HSV)
Enveloped
pI = 4.9

Adeno-Associated Virus 2 (AAV)
Non-enveloped
pI = 5.9

AAV 9
Non-enveloped
pI = 5.9

Lentivirus (LV)
Enveloped
pI = Acidic

Influenza A/B (IBV/IAV)
Enveloped
pI = Acidic

Analytical Methods

MTT Assay: Infectious virus titration PPV, HSV

Digital droplet PCR (ddPCR): Viral vector titration AAV, LV, IBV

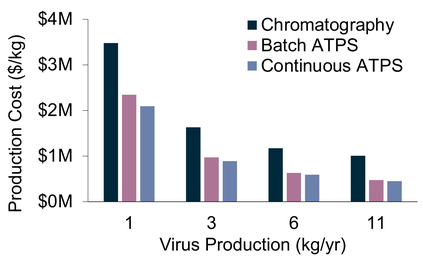
Gator Biolayer Interferometry: AAV capsid titration

Picogreen Assay: DNA quantitation

Bradford Assay: Protein quantitation

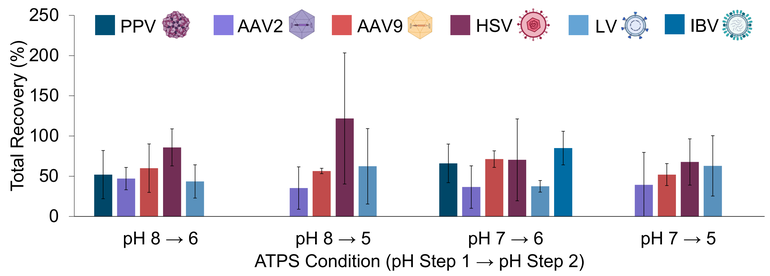
Results & Discussion

Economic Feasibility



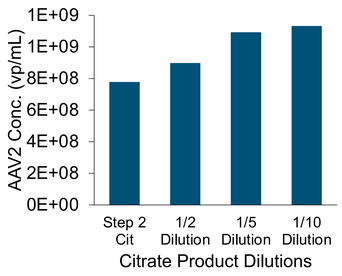
50% lower production costs compared to traditional, large-scale processes[4].

Vector Recovery



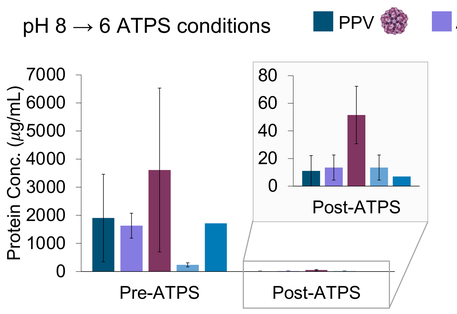
Overall viral vector recovery was **at least 62%** for all viral products for at least one of the pH shift conditions tested.

Capsid Detection

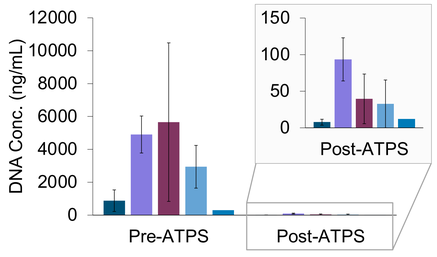


BLI can detect AAV2 in diluted citrate-rich product.

Host Cell Protein

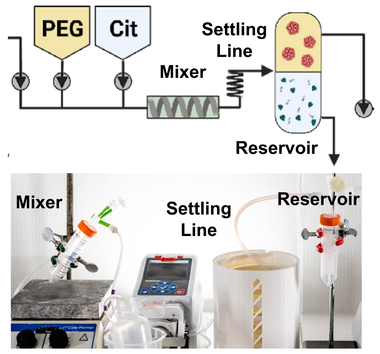


Host Cell DNA



Protein and DNA removal are generally **consistent regardless of the initial titers**.

Continuous ATPS



Continuous ATPS delivers statistically **similar PPV recovery** to batch ATPS.

Complete **mixing and settling** are key to continuous ATPS performance.

Conclusions & Acknowledgements

- PEG-Citrate ATPS delivers 62-100% recovery for all viruses and viral vectors tested.
- Final protein and DNA titers are generally consistent regardless of the starting titer.
- Continuous ATPS delivers similar PPV recovery to batch.

Acknowledgements:

Thanks to the James & Lorna Mack Chair in Continuous Processing, Michigan Translational Research and Commercialization, the Fredrick G. Cottrell Foundation, and the US Food and Drug Administration (1R01FD007461) for support. Thanks to Gator Bio for access to their Gator Plus BLI instrument.

Future Work & References

- Improve process understanding of impurity removal.
- Connect to upstream steps for end-to-end processing.
- Implement in-process monitoring and control.

References:

1. Ramos, I., et al. (2023) *Biotechnol & Bioeng.*
2. Joshi, P. U. et al. (2019). J. Chromatogr. B.
3. Heldt, C. L. and Nold, N. M. Purification of Viral Particles with Two Stage Aqueous Two-Phase Extraction. 63/563,789. Provisional patent filed March 11, 2024.
4. Nold, N. M., et al. (2024) *Biot Progress.*

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