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Continuous purification of hepatitis C virus-like particles by multi-column chromatography

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Introduction

- VLPs present challenges for DSP, thus alternative strategies are desired;
- We evaluated semi-continuous, multicolumn chromatography techniques, that have the potential to improve process economics;
- A simple serial connection of two chromatographic columns directs the effluent of one column to an adjacent one, capturing the breakthrough from the previous column and avoiding product loss;
- This simple setup modification overcomes the limitations of single-column processes by achieving saturation of the first column;
- It also benefits from the countercurrent flow between the mobile and the stationary phases, which optimizes the driving force for mass transfer throughout the overall trajectory of the two phases;

AIM: Evaluate continuous multicolumn chromatography for hepatitis C VLP purification

Materials & Methods

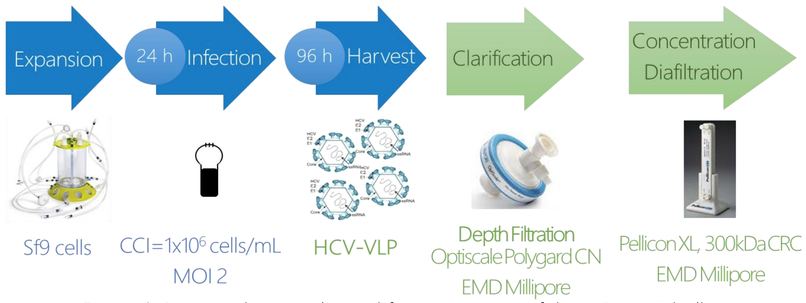


Figure 1: Steps and materials used for preparation of the HCV-VLP bulk

Anion exchange resins: two commercially available matrices (Fractogel® TMAE and DMAE) and three Eshmuno® Q prototypes (1, 2, 3) with different ligand densities (Merck Millipore)

Buffers: 50 mM HEPES at a pH of 7.4 with 0-1 M NaCl

DBC determination for single-column experiments using 1 mL scout columns on ÄKTA Explorer 10s system. This system was modified to support the multicolumn process operation

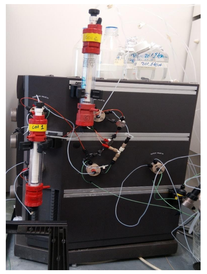
Columns used in the multicolumn process were slurry packed with Eshmuno® prototype 1 to a final bed volume of 4 mL

HCV-VLP quantification: ELISA kit targeting the GAG p30 protein of the VLPs capsid (QuickTiter™ MuLV, Cell Biolabs, Inc.)

Total Protein quantification: BCA protein assay kit (Pierce Biotechnology)

Total double stranded DNA quantification: PicoGreen™ dsDNA Assay kit (Life Technologies)

Total host cell protein quantification: two-site immunoenzymetric assay (Cygnus Technologies, Inc.)



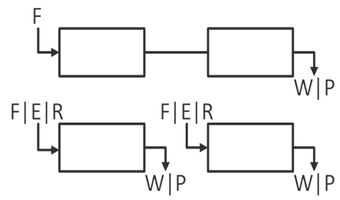


Figure 2: Picture of modified ÄKTA Explorer 10s (left) for implementation of the multicolumn flow schemes (top)

Resin screening

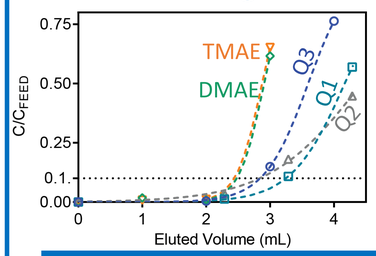


Table 1: DBC (ng p30/mL packed resin) for evaluated AEX resins and relation to ligand density

AEX Resin	Q1	Q2	Q3	TMAE	DMAE
$DBC_{10\%}$	1310	1168	1175	923	917
LD	+++++	++	+++	+	+

Figure 3: Frontal experiments for DBC determination using scout columns (1mL)

Eshmuno 1 was selected due to its higher capacity

Single Column Batch Experiments

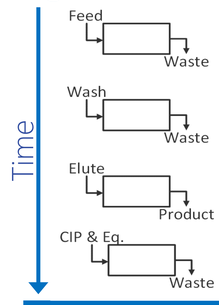


Table 2: Summary of experiments to determine optimal buffer elution conditions; Load up to 90% of column breakthrough

Elution step (mM NaCl)	400	550	700
VLPs Recov.	19 %	35 %	37 %
1 M (NaCl) Elution step Recov.	11 %	5 %	5 %
DNA Remov.	67 %	60 %	53 %
Host cell Prot. Remov.	42 %	33 %	27 %
Total Prot. Remov.	92 %	63 %	46 %

Figure 3: Sequence of steps in Bind & Elute chromatography

- ✓ A step elution with 550 mM of NaCl displayed best compromise between recovery and impurity removal;
- ✓ Loading below 10% of column breakthrough resulted in lower VLP recoveries (< 19%, data not shown);
- ✓ Loads of 90% of column breakthrough lead to a loss of VLPs in the flowthrough fraction, since the mass transfer zone (MTZ) exited the column.

Multicolumn Process Shortcut Design Approach

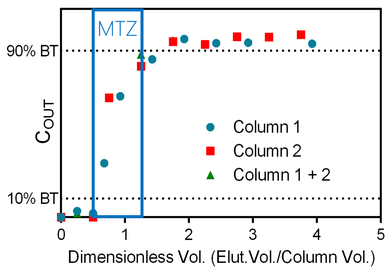
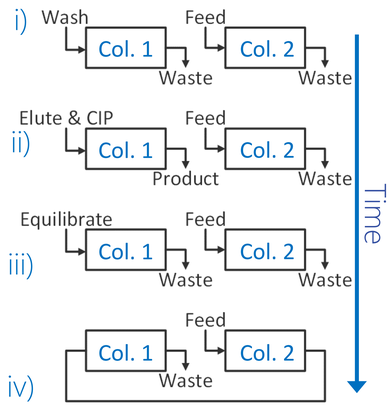


Figure 4: Frontal experiments performed in the chromatographic beds used for the multicolumn process (left); Proposed step sequence for the first half cycle of the multicolumn process (right); In the second half-cycle columns exchange position.



- ✓ In steps i) to iii) the downstream column is loaded up to 10% of breakthrough, while the upstream column, loaded in the previous operation cycle follows a sequence of steps similar to a batch operation;
- ✓ The effluent of the downstream column is connected to the previous column while loading is maintained, thus avoiding the exit of the MTZ and allowing a 90% of breakthrough column load;

Experimental Run

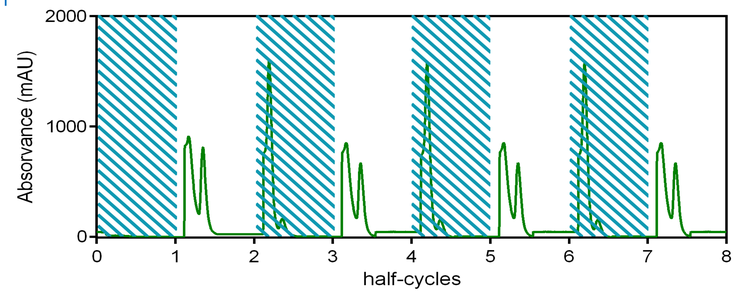


Figure 5: Experimental run of the proposed multicolumn process for continuous purification of HCV-VLPs; Shaded areas correspond to product collection in column 1; non-shaded areas correspond to the product collection in column 2;

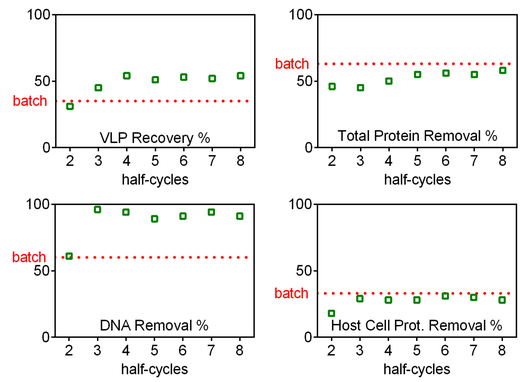


Figure 6: Comparison between batch and multicolumn runs

- ✓ Similar HCP and total protein clearance was achieved;
- ✓ Increased clearance of DNA;
- ✓ Increased recovery of HCV-VLPs

Final Remarks & Future Work

- ✓ Successful implementation of a fully continuous multicolumn chromatographic step was achieved;
- ✓ The used shortcut design method allowed to increase HCV-VLPs recovery from 35% to 55% without cycle optimization;
- ✓ By maintaining the MTZ inside the column loop it was possible to improve the resin capacity utilization;
- ✓ Due to the cyclic exchange of the columns position, the multicolumn process benefits from the advantageous simulated countercurrent movement of fluid and solid phases
- ✓ Mathematical modelling can be used to further improve cycle design and optimize both step sequence and duration;

Acknowledgements

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