Simplifying Progress

Lentiviral Downstream Process Optimization and Design of Experiments on CIM® QA 0.05 mL Monolithic 96-well Plates

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Introduction

Lentiviral vectors are being increasingly used as tools for stable integration of large gene inserts into the genomes of both dividing and non-dividing cells. With 3rd and 4th generation of lentiviral vectors being incompetent of replication, they also offer a relatively safe tool for more widespread use. Currently, lentiviral based therapies are primarily *ex-vivo*, such as CAR-T, however recent animal studies have successfully demonstrated their use *in-vivo* as well. To enable more widespread use, as well as development of *in-vivo* therapies in humans, better downstream processes for lentiviral purification will have to be developed.

In this work we present the results from initial buffer optimisation and following DoEs, through which we successfully increased infectious lentiviral recovery. Interestingly, salt concentration was determined as the most important factor in loading sample for high infectious recovery of lentivirus from CIM QA column. We also present results from PATfix® analytics, which we used to determine sample purity and composition.

1. Buffer optimisation with clarified harvest on CIM QA 0.05 mL Monolithic 96-well Plates

As lentiviruses are negatively charged at neutral pH, we performed initial testing on QA and DEAE columns. Of the two columns tested for lentiviral purification, QA showed better potential for further development (data not shown). To increase recoveries on the downstream process, buffer optimisation was performed. Clarified harvest material was used as a loading sample to simplify downstream process. The primary analytics for downstream process evaluation was infectious recovery. We performed the buffer optimisation and following DoE studies, using CIM QA 0.05 mL Monolithic 96-well Plates (6 µm channels). The results from optimization of buffers, salts and sugars determined that a selection of buffers and salts had a positive effect on recovery, however, overall recoveries were still below 25% (Figure 1A-C). The results indicated, that loading sample conditions need to be modified, to achieve better recoveries.

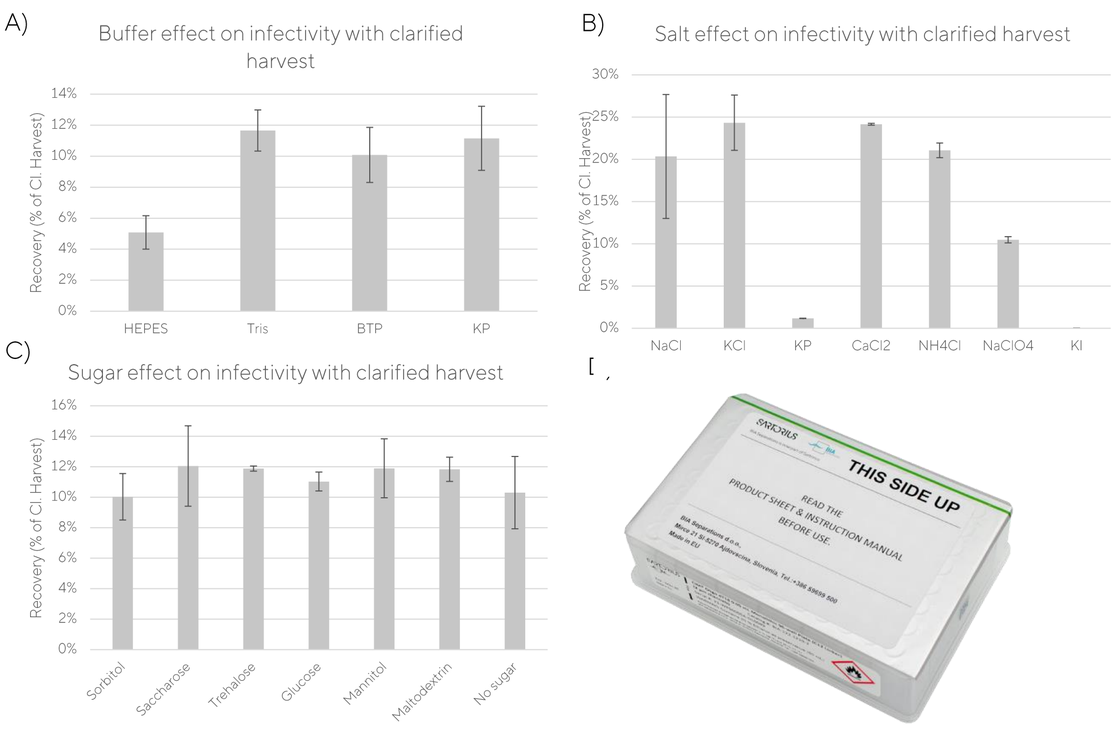

Figure 1: Results from buffer optimisation of process for lentiviral purification. Effects of different A) buffers, B) salts, and C) sugars were tested for buffer optimisation. D) Photo of CIM QA 0.05 mL Monolithic 96-well Plates (6 µm channels), which were used for the buffer optimization and DoE.

2. Buffer and loading conditions optimisation using DoE

To connect the loading conditions with infectious recovery, we performed DoE using MODDE, with three variables – pH, starting NaCl and saccharose concentration (Figure 2). Starting NaCl concentration indicates the NaCl concentration in buffer A, while the concentration in elution buffer was 1.5M. Saccharose concentration and pH were the same in buffer A and B. Clarified harvest was diluted 1:1 with buffer A before loading to the plate. The results from DoE study indicated that starting NaCl concentration was the most important factor for increasing infectious recoveries, with higher concentrations leading to better recoveries (Figure 2B-C). The pH and saccharose concentration effects on recovery were inconclusive (Figure 2B).

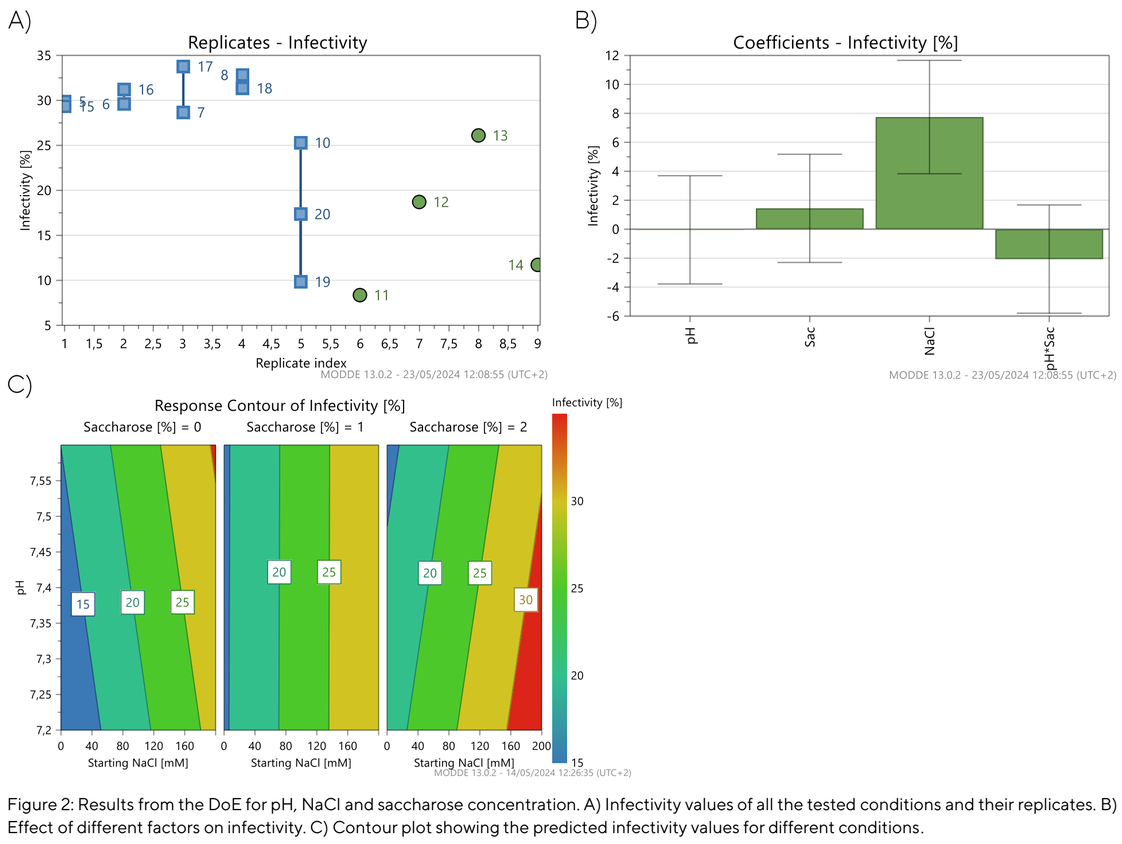

Figure 2: Results from the DoE for pH, NaCl and saccharose concentration. A) Infectivity values of all the tested conditions and their replicates. B) Effect of different factors on infectivity. C) Contour plot showing the predicted infectivity values for different conditions.

3. NaCl and pH have significant effect on infectious recovery

Following the first DoE study, a second DoE study was performed with increased NaCl concentrations and a wider range of pH and saccharose concentration (Figure 3). The study confirmed the findings from the first DoE, with NaCl concentration again being the main factor in determining infectious recovery. This time, however, pH was also a significant factor, with lower pH being beneficial for lentiviral recovery. Saccharose concentration effect was, again, insignificant (Figure 3B).

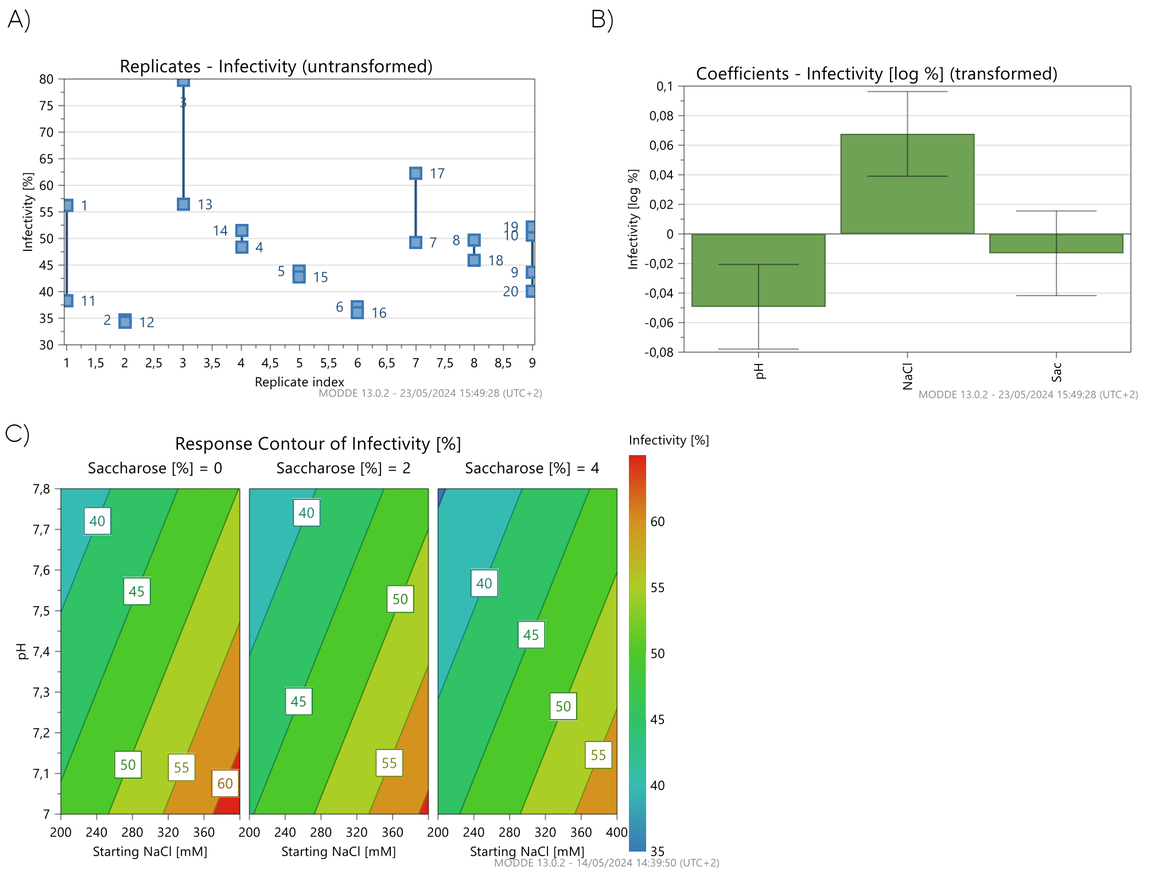

Figure 3: Results from the second DoE study with adjusted pH, NaCl and saccharose concentrations. A) Infectivity values of all the tested conditions and their replicates. B) Effect of different factors on infectivity. C) Contour plot showing the predicted infectivity values for different conditions.

4. PATfix analytics of DoE samples

We have also tested the samples from DoE using PATfix (Figure 4). The column used for PATfix analysis was CIMac Adeno 0.1 mL Analytical Column (2 µm). The analysis indicates how elution purity changes with increasing NaCl concentration, which was determined as the most important factor in the two DoE studies. PATfix analysis indicates better removal of protein and DNA impurities, as indicated by fluorescence and UV signals, as well as removal of additional larger particles, as observed with first MALS peak. As such, PATfix analysis enables quick analysis of sample purity and composition, with viral peak on MALS also serving as indicator of viral particle recovery.

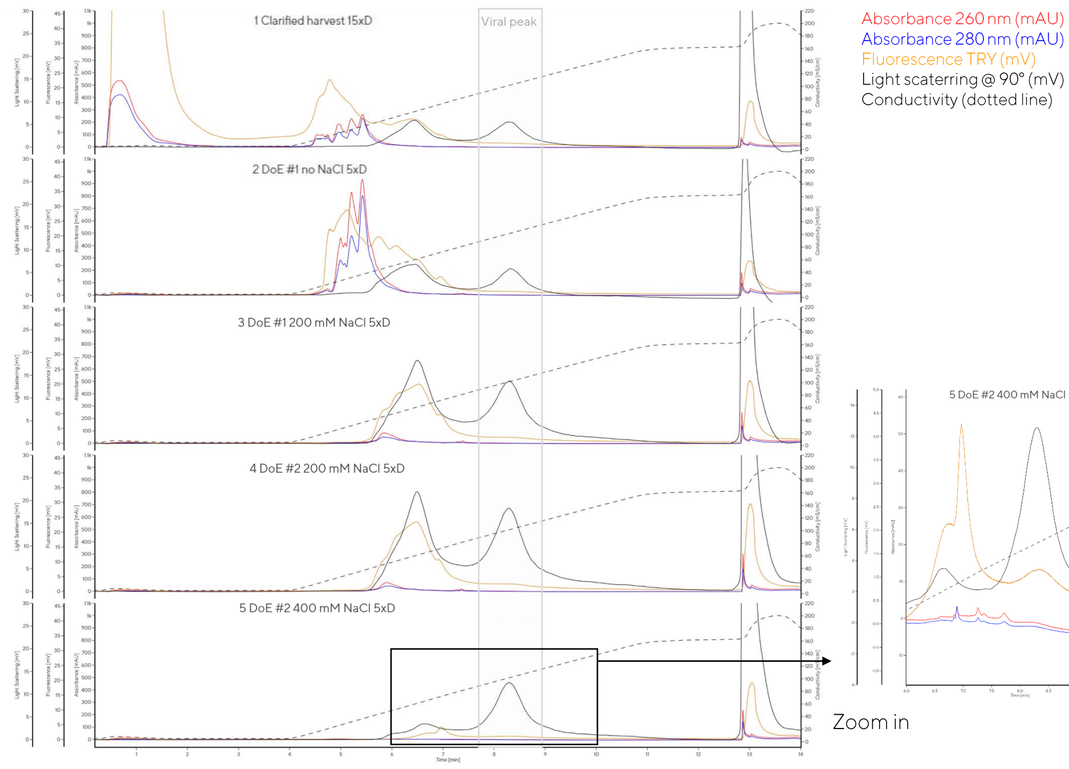

Figure 4: Left: PATfix analytical chromatograms of samples from two DoEs with different NaCl concentrations. Samples have been diluted 5x, while clarified harvest was diluted 15x. Right: Zoom in of elution peaks of DoE #2 400 mM NaCl sample.

5. Conclusions

We have successfully demonstrated that, using DoE studies, conditions for lentiviral downstream can be optimised by modifying pH and starting NaCl concentration. Using QA monolithic columns, over 60% recovery of infectious lentivirus can be achieved. Experiments are planned to further improve downstream conditions and recoveries, as well as to confirm the conditions on 1 mL columns.

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