Simplifying Progress

Advancements in Lentiviral Purification: From High Throughput Screening to Scalable Solutions

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Introduction

Lentiviral vectors are increasingly utilized for the stable integration of large gene inserts into the genomes of both dividing and non-dividing cells. The 3rd and 4th generation lentiviral vectors, which are replication-incompetent, provide a relatively safe tool for broader applications. Currently, lentiviral-based therapies are primarily ex-vivo, such as CAR-T treatments. However, recent animal studies have successfully demonstrated their potential for in-vivo applications. To facilitate more widespread use and the development of in-vivo therapies for humans, enhanced downstream processes for lentiviral purification are essential.

In this study, we present the results of optimizing lentiviral downstream processes using CIM® QA monolith. Through Design of Experiments (DoE), we achieved a 70% recovery rate of infectious lentivirus, marking a threefold increase compared to initial results. Notably, the salt concentration in the loading sample emerged as the most critical factor for achieving high infectious recovery from the CIM QA column.

1. Buffer optimisation on CIM® QA 0.05 mL Monolithic 96-well Plates

Given that lentiviruses are negatively charged at neutral pH, we initiated testing with QA and DEAE columns. Among these, the QA column demonstrated superior potential for further development. The primary analytic for evaluating the downstream process was the determination of transducing units (TU). To enhance recovery, buffer optimization was conducted using clarified harvest material as the loading sample. However, buffer optimization alone did not result in significant improvements, with overall recoveries remaining below 25% (data not shown). To boost yields, adjustments to the loading conditions were necessary. By mixing clarified harvest with mobile phase A (MpA), we were able to control the loading conditions for the downstream process by altering the MpA composition. DoE studies on MpA were conducted using CIM QA 0.05 mL Monolithic 96-well Plates (6 µm channels) (Figure 1A). Initial optimization focused on pH, NaCl, and saccharose concentration, revealing a strong positive effect of NaCl addition on downstream recovery (Figure 1B-C).

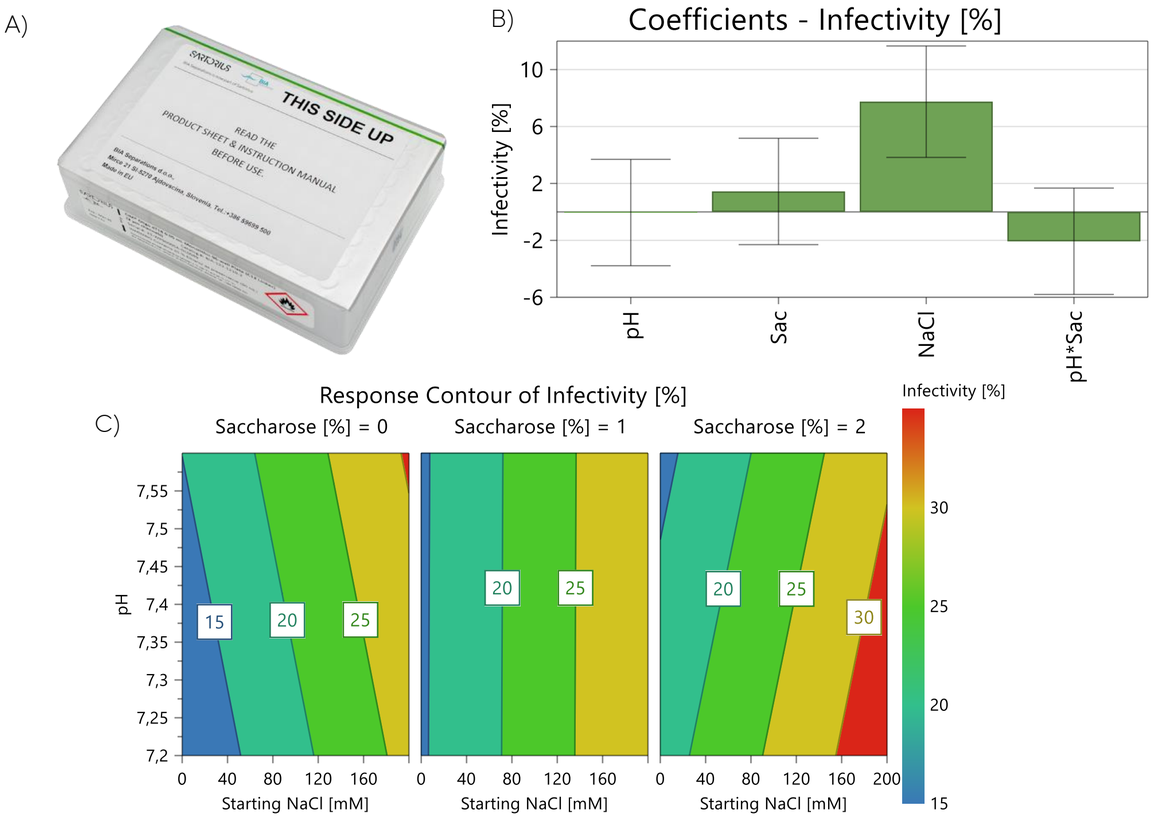

Figure 1: Effect of mobile phase A composition on infective LV recovery. A) Photo of CIM QA 0.05 mL Monolithic 96-well Plates, B) Plot of factors tested in DoE and their effect on infective recovery (Sac - saccharose, pH*Sac – pH and saccharose interaction), C) Contour plot of predicted infective recovery in response to different conditions.

2. NaCl and pH have significant effect on infectious recovery

Building on initial study, a follow-up DoE was performed with increased NaCl concentrations and a wider range of pH and saccharose concentration (Figure 2). In this study, both NaCl concentration and pH emerged as key factors influencing infectious recovery. The findings corroborated the results from the first DoE regarding NaCl concentration, while also revealing that a lower pH is advantageous for lentiviral recovery. The effect of saccharose concentration remained insignificant (Figure 2A). Additional experiments were carried out to finalize the optimal pH and NaCl concentration for maximum recovery.

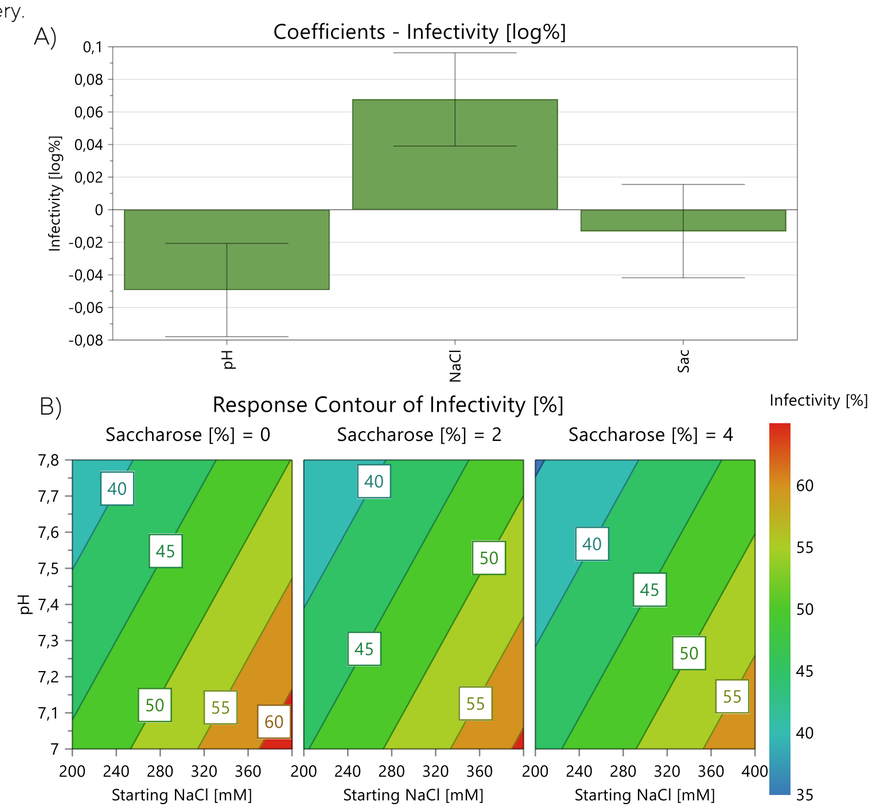

Figure 2: Results from the follow-up DoE study with adjusted pH, NaCl and saccharose concentrations in MpA. A) Effect of different factors on infectivity. B) Contour plot showing the predicted infectivity values for different conditions.

3. $MgCl_2$ and Arginine addition improves LV downstream recovery

After establishing the optimal concentrations of pH, NaCl and saccharose concentrations, further DoE studies were conducted to assess the impact of various additives on the downstream process. Initially, the addition of $MgCl_2$ and Poloxamer188 was tested, with results indicating a positive effect of $MgCl_2$ on recovery (Figure 3A). Subsequently, a similar study was carried out to evaluate the addition of Histidine and Arginine, with Arginine showing a beneficial effect on downstream recovery (Figure 3B). Based on these DoE studies, the final buffer formulation was selected and tested using the CIMmultus® QA 1 mL Monolithic Column (6 µm).

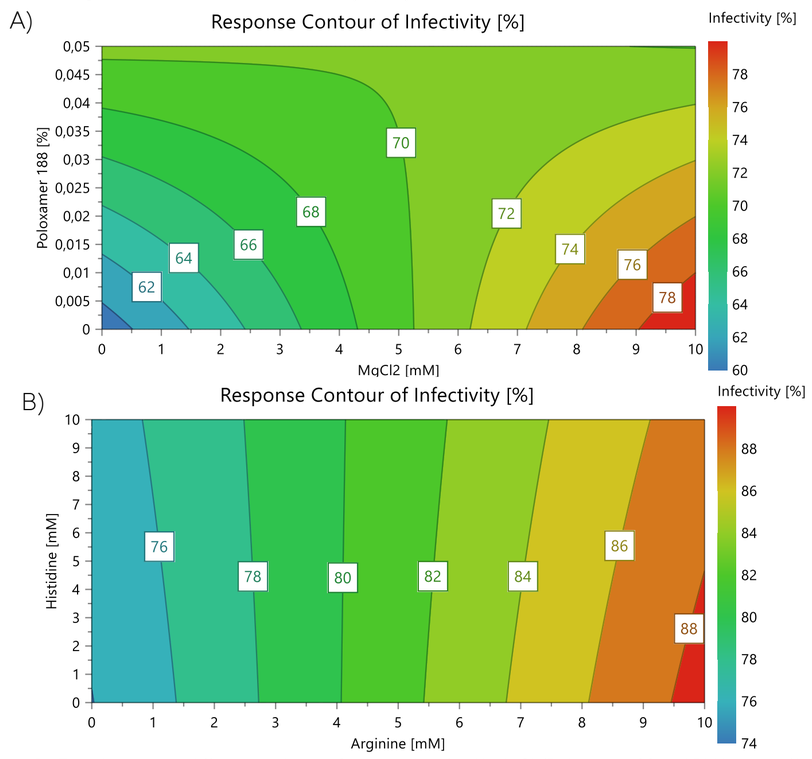

Figure 3: Results from DoE studies testing different additives. Contour plots for A) $MgCl_2$/Poloxamer188 and B) Hisitidine/Arginine addition.

4. Confirmation of optimised buffer using CIMmultus QA 1 mL Monolithic Column (6 µm)

After selecting the final buffer conditions, two confirmation runs were conducted with the CIMmultus QA 1 mL Monolithic Column (6 µm). The results validated the successful optimization achieved through the 96-well plate format and the DoE approach. An average infectivity recovery of 70% was attained, along with a reduction in host-cell protein (HCP) impurities by over 2000-fold. The final average HCP and host-cell DNA (hcDNA) contents were calculated to be 490 ng of HCP and 230 ng of hcDNA per dose of 10^9 TU. The results also indicated significant removal of non-infective particles during the downstream process, confirmed through particle composition analysis using Leprechaun (Unchained Labs) (Figure 4B). The capacity was tested and determined to be 3E+12 viral particles per mL of QA monolith (6 µm).

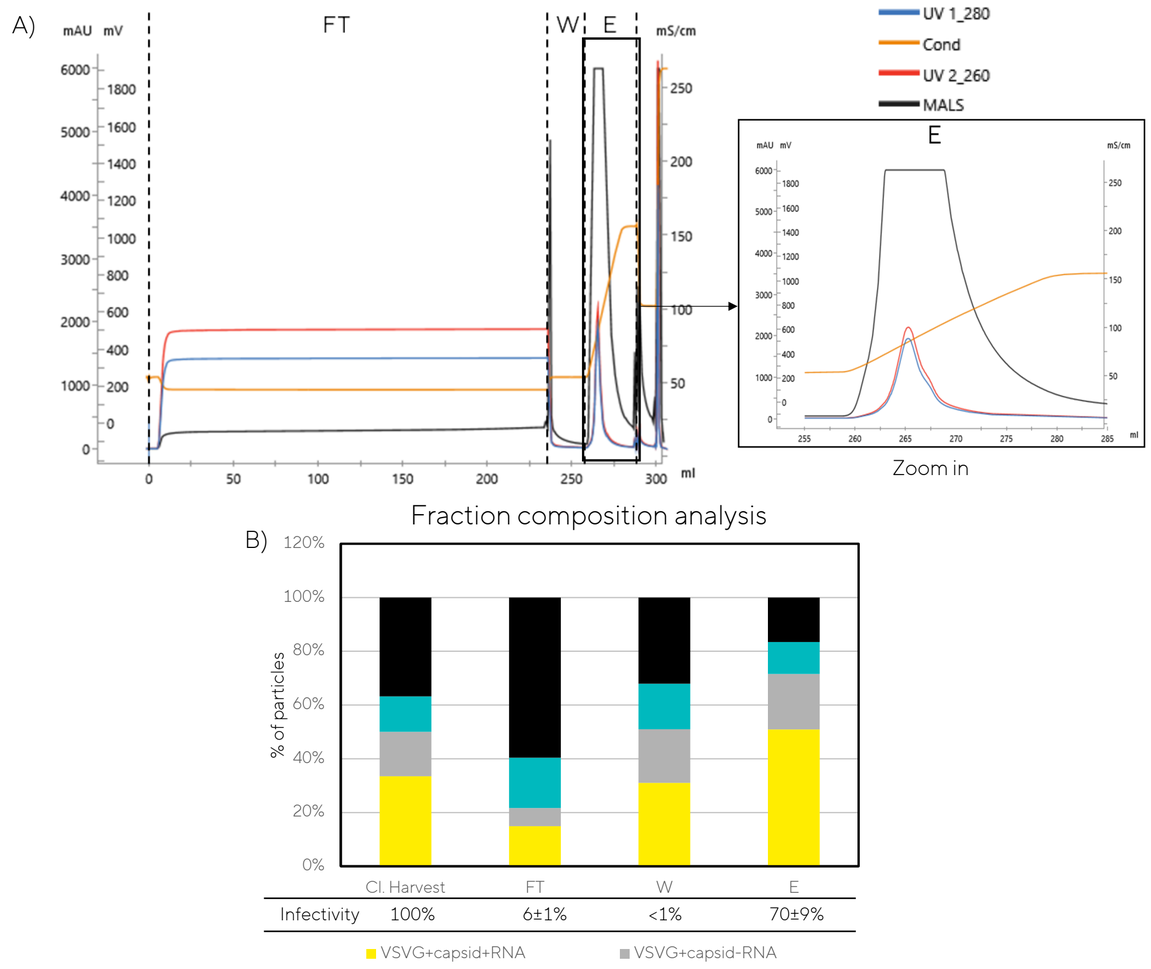

Figure 4: A) Chromatogram of one of the two confirmation runs, with the zoom in of the elution peak. B) Compositional analysis of collected fractions based on presence of VSVG, p24 capsid and RNA and corresponding infectivity recovery. Fractions: FT – flow through, W – wash, E – elution.

5. Conclusions

We have successfully demonstrated that by modifying pH, NaCl, $MgCl_2$ and Arginine concentrations, using 96-well format and DoE, purification conditions can be optimised. Optimised conditions from QA monolithic 96-well plates were successfully transferred to CIMmultus QA 1 mL Monolithic Column, where 70% average infectious recovery was achieved with over 2000-fold HCP removal as well as reduction of particle impurities.

Acknowledgements

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