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Monolithic Columns as Downstream Processing Solutions for Lentiviral Purification

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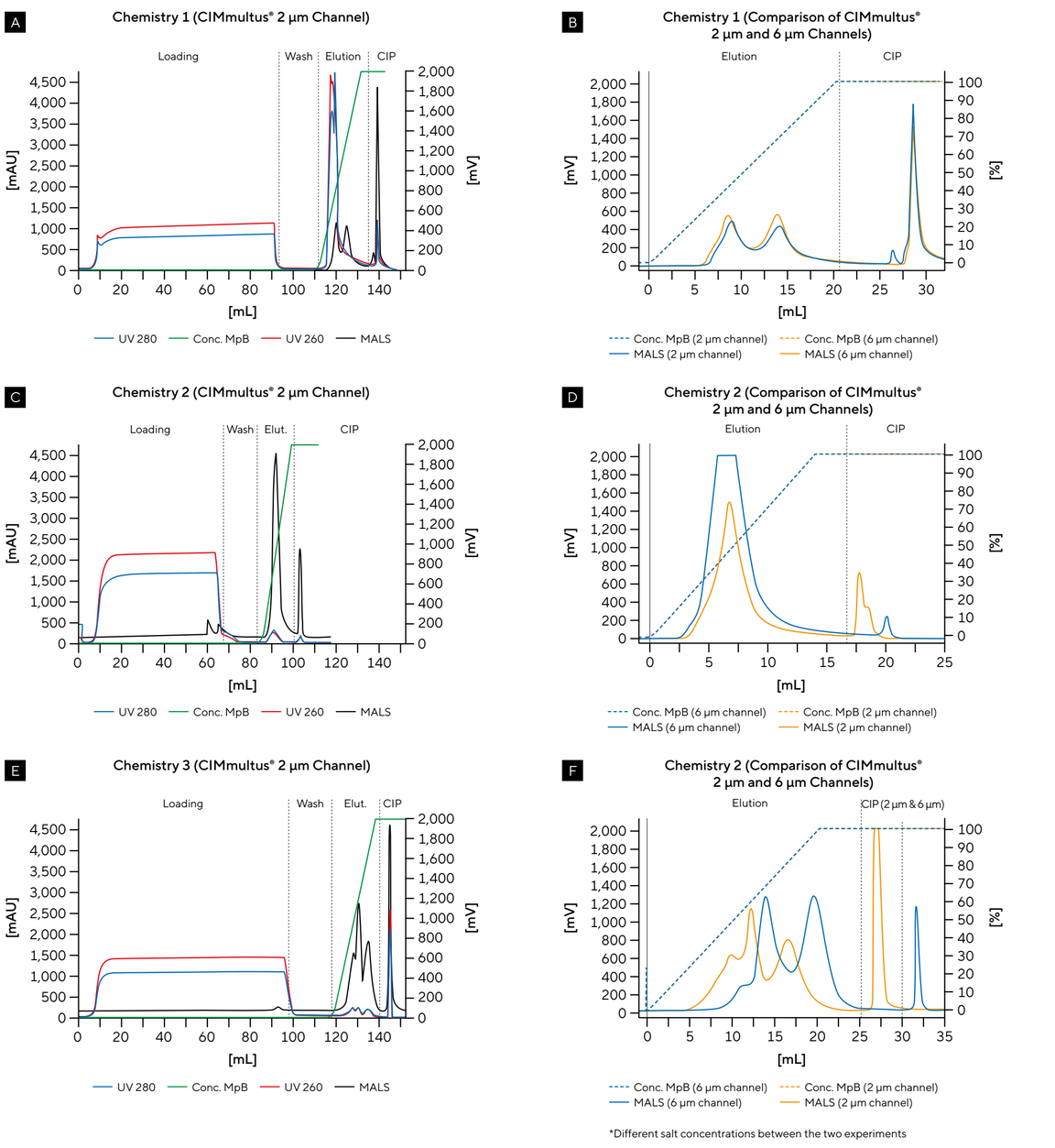
Introduction

Lentiviral vectors are efficient tools for the transfer and stable integration of large gene inserts into the genomes of both dividing and non-dividing cells. Third generation lentiviral vectors, which use a fractional set of HIV genes, is replication-incompetent and self-inactivating, offering a relatively safe tool for academic and industrial use, while delivering larger gene transfer capabilities compared to more commonly used adeno-associated viruses (AAVs). As a result, several therapies using lentiviral vectors are already approved or in clinical trials, with primarily ex vivo use. However, limitations in the downstream purification of lentiviruses have hindered their widespread use and the development of in vivo therapies.

Here, we showcase a process development approach for the use of CIM® monolithic columns in the downstream purification of lentiviruses. Several CIM® monolithic chemistries were tested to determine their performance for lentiviral purification, with three chemistries showing potential for further use (Figure 1). Of the three chemistries, two were selected for further development. Several modifications of the two chemistries were prepared and tested on CIMmic® columns with promising initial results (Figure 2). The chemistries were successfully upscaled to CIMmultus® monoliths, and the results from the initial findings were confirmed (Figure 3). Additional experiments are required to confirm the findings and perform optimization. The full downstream process will be developed once the most suitable chemistry is selected. During preparative chromatography, multi-angle light scatter (MALS) was used as an indicator of viral presence, and ddPCR and infectivity tests were used as analytics.

1. Testing Available CIMmultus® Monolith Chemistries

Initial experiments were performed with CIMmultus® monolith chemistries already available on the market. Three of the chemistries performed well and were tested on CIMmultus® monoliths with 2 and 6 µm channels. Interestingly, for chemistry 2 and 3, we observed that CIMmultus® monoliths with 6 µm channels generally performed better than CIMmultus® monoliths with 2 µm channels (Figure 1).



*Different salt concentrations between the two experiments

G

Non-Modified Chemistries	Elution ddPCR Recovery [%]	Elution Infectivity Recovery [%]
Chemistry 1 (2 µm)	67	42
Chemistry 1 (6 µm)	63	NA
Chemistry 2 (2 µm)	56	26
Chemistry 2 (6 µm)	82	91
Chemistry 3 (2 µm)	57	16
Chemistry 3 (6 µm)	96	56

Figure 1: *Results From Testing Available CIMmultus® Monolith Chemistries for Lentiviral Purification. Graphs Are Presented for Runs on 2 µm CIMmultus® Columns on Three Separate Chemistries (A, C and E) and Comparing Elution Profile on 2 µm and 6 µm CIMmultus® Columns (B, D and F). Results From ddPCR and Infectivity Recovery on Both 2 µm and 6 µm CIMmultus® Columns Are Presented in Table G*

2. Development of Modified CIMmic® Monolith Disks

In order to improve the recovery, CIMmic® disks adapted with chemical modifications 1 and 2 (Figure 1) were prepared. The disks were screened under the same conditions and the results compared to standard chemical modifications (Figure 2). MALS signal was used as indicator of lentiviral elution. The adaptations led to a shift in elution profile, caused by the earlier elution of bound lentivirus. The adaptations also caused elution in a single viral peak in disks with modified chemistry 1 (V1.1, V1.2, V2.1 and V2.2), rather than a split peak observed with regular chemical modification (V1 and V2). A slight shift in elution can also be observed with 2 µm disks with adapted chemistry 2 as well (V3-V3.2). However, on 6 µm disks with adapted chemistry 2 (V4.1 and V4.2), only a slight reduction in elution profile height was observed. V4.1 also had and improved resolution between impurity and lentiviral elution (data not shown). All disks with adapted chemical modifications had a lower cleaning-in-place (CIP) peak.

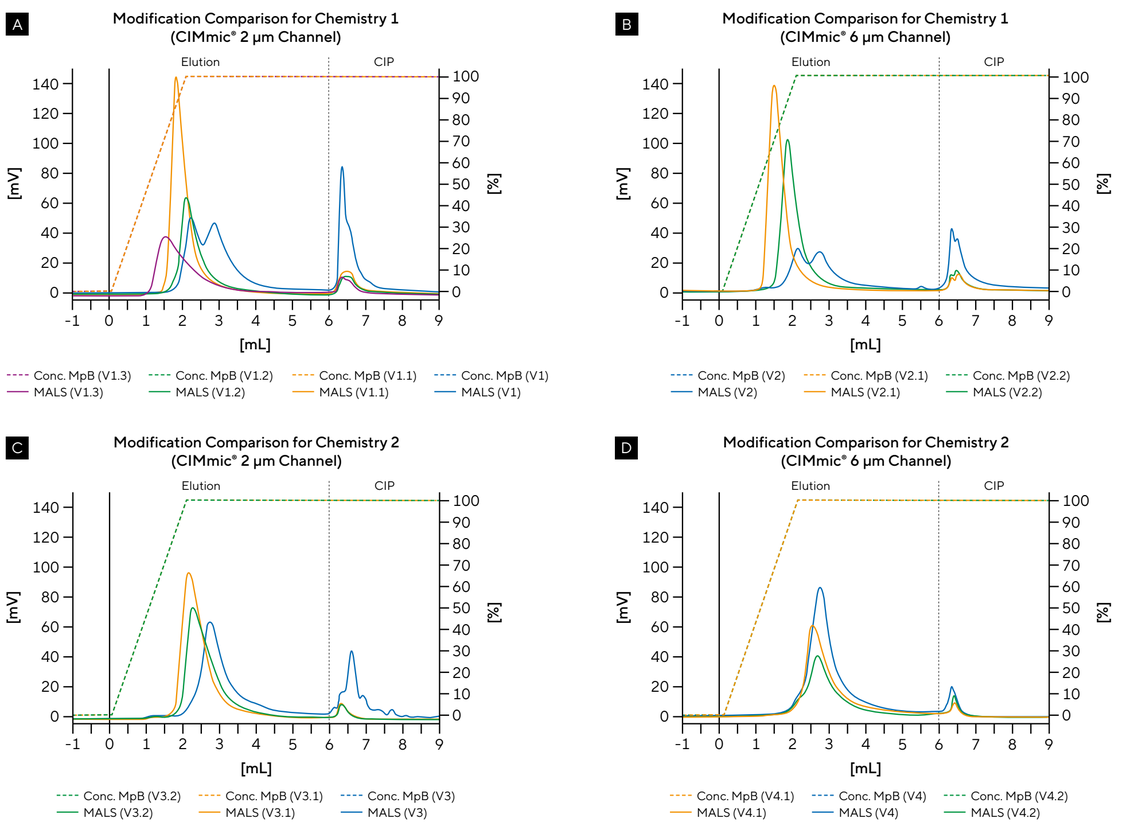
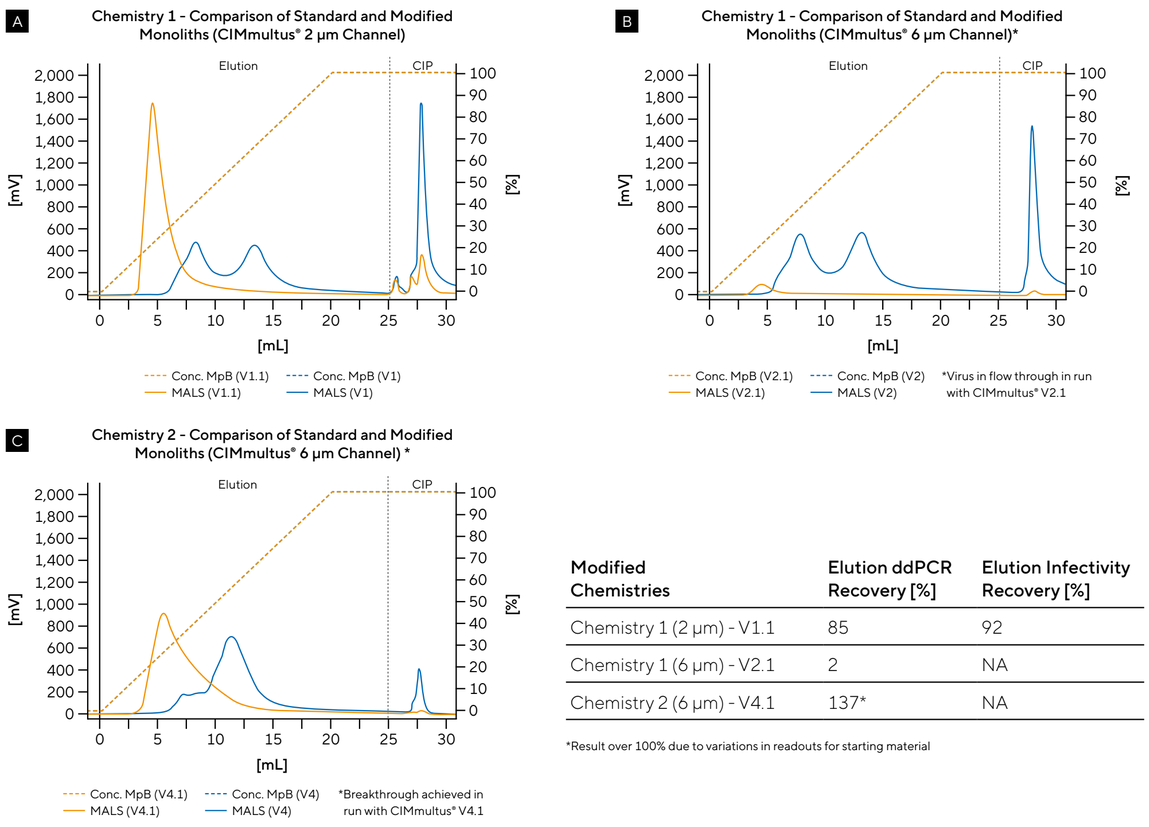


Figure 2: *MALS Elution Profiles of Lentiviral Purification Using Monolith Disks With Standard and Adapted Chemical Modifications: **A)** Chemistry 1, 2 µm Disks, **B)** Chemistry 1, 6 µm Disks, **C)** Chemistry 2, 2 µm Disks and **D)** Chemistry 2, 6 µm Disks. Disks Marked as V1, V2, V3 and V4 Have the Basic Modifications Already Available on the Market, While the Rest Have Adapted Modifications*

3. Upscaling of Adapted Chemical Modifications to CIMmultus® Monoliths

In order to determine if adapted chemical modifications can be upscaled, we prepared and tested CIMmultus® monoliths with the best performing adaptations – V1.1, V2.1 and V4.1. V4.1. The results were compared to the results obtained with CIMmultus® columns with standard chemical modifications (Figure 3). The V1.1 adaptation performed similarly to the CIMmic® disk. In contrast, V2.1 did not perform well, as the virus did not successfully bind to the column. CIMmultus® V4.1 also performed well, but we observed a shift in the elution profile which was not seen with the CIMmic® disk. Compared to standard chemical modifications, the V1.1 adaptation led to higher recovery on the infectivity test (Figure 3D). Other versions are currently being analyzed for infective viral recoveries.



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Modified Chemistries	Elution ddPCR Recovery [%]	Elution Infectivity Recovery [%]
Chemistry 1 (2 µm) - V1.1	85	92
Chemistry 1 (6 µm) - V2.1	2	NA
Chemistry 2 (6 µm) - V4.1	137*	NA

*Result over 100% due to variations in readouts for starting material

Figure 3: *Comparison of Lentiviral Purification Using CIMmultus® Columns With Standard and Modified Chemistries. **A)** Chemistry 1, 2 µm Disks, **B)** Chemistry 1, 6 µm Disks, **C)** Chemistry 2, 6 µm Disks and **D)** Table of Recovery Results With ddPCR and Infectivity Test*

4. Conclusion

In this work, we have showcased the development of CIM® monoliths for downstream processing of lentiviruses. We demonstrated the application of already available chemistries for lentivirus purification. Screening results from the novel chemical modifications of CIMmic® disks, and their subsequent upscaling to CIMmultus® monoliths, suggest that recovery can be improved. However, further experiments will have to be conducted to confirm the performance of newly developed chemical modifications and to build full downstream process for lentiviral purification.

Acknowledgments

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