CONTINUOUS PURIFICATION OF CELL CULTURE-DERIVED INFLUENZA A VIRUS PARTICLES THROUGH PSEUDO-AFFINITY MEMBRANE CHROMATOGRAPHY

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Continuous manufacturing is a relevant trend in biopharmaceutical production to reduce the process footprint and to improve the process economy. Vaccines against world-spread diseases, such as influenza, should benefit in particular from such an approach, given the increasing demand for seasonal vaccines and the need for a fast response in case of a pandemic outbreak. Upstream processing of viral vaccines has seen important progress in continuous production of viral vaccines [1], which further supports the development of hybrid or fully continuous flow-schemes for downstream processing.

In this work, we implemented a multi-column strategy for the chromatographic purification of a continuous feed stream of cell culture-derived influenza A virus particles using sulfated cellulose membrane adsorbers (SCMA). The use of SCMA for batch purification of influenza A virus particles is well described [2] as well as the process conditions required for a successful separation [3]. This facilitated the transfer of this chromatography technique from batch to continuous-mode. Using a 3-device set-up, we reproducibly purified cell-culture derived influenza A/Puerto Rico/8/1934 virus particles during 10 cycles. Each cycle comprised load, wash, elution and re-equilibration of all three devices. The virus hemagglutinin activity (HA) average yield obtained was 67% ± 11%, with contaminant removals above 70% for total protein and 99.8% for DNA, respectively. Moreover, the contaminant content relative to the eluted HA were 1.0 ± 0.1 $\mu g_{total\ protein}$/kHAU and 3.5 ± 0.7 ng_{DNA}/kHAU. These are similar to those achieved for comparable batch runs. In addition, based on the breakthrough curves [Fig. 1], the SCMA were challenged to about 69% of their estimated static binding capacity. Compared to traditional batch operation (capacity challenge at 70% of the dynamic binding capacity, $DBC_{10\%}$), continuous operation of the SCMA saves at least 10% of the processing time.
Overall, the implementation of this continuous chromatography approach for the purification of viral particles would result in a considerable reduction of plant footprint, buffer consumption, and operating costs. Yields and contamination levels achieved support the future use of membrane chromatography as a platform solution, especially suited for low-cost vaccine manufacturing.

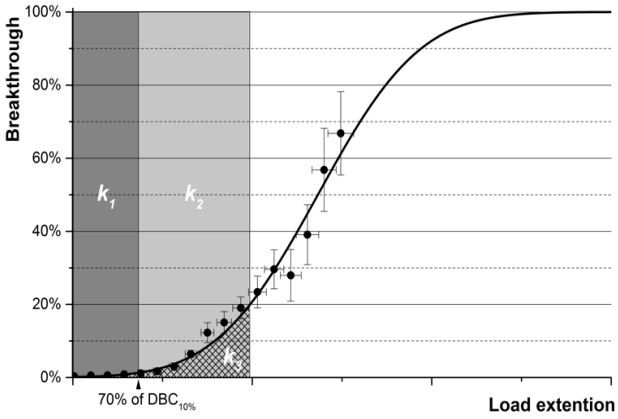

Figure 1 – A continuous multi-column strategy for the purification of influenza A virus using SCMA increases to overall capacity used per device (k_2), in comparison to the traditional capacity challenge used in batch mode (k_1). The virus particles loss on the flow through of the main load device are caught by the following device (k_3).

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