A SINGLE-USE CHROMATOGRAPHIC PURIFICATION PLATFORM FOR VIRAL GENE TRANSFER VECTORS & VIRAL VACCINES

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Key Words: Membrane chromatography; single-use; virus purification; downstream processing; platform technology

In steric exclusion chromatography (SXC), a crude sample is mixed with polyethylene glycol (PEG) and fed onto a single-use cellulose column. In this operation, selectivity is influenced strongly by the target species' size, so SXC is well suited for purification of virus particles. The purified product is recovered at physiological pH and conductivity. We have observed recoveries above 95% for several cell culture-based virus particles used as viral gene transfer vectors or as viral vaccines, including: adeno-associated virus (AAV), Modified Vaccinia Ankara (MVA) virus, influenza virus, and yellow fever virus. Preliminary data for purification of lentiviruses suggests recoveries exceeding 60%. Host cell DNA and protein depletion are typically above 90% and infectivity is not compromised thanks to the inert character of PEG towards biomolecules and the mild elution conditions.

Several AAV serotypes and display mutants were produced using HEK cells and purified with up to 95% recovery. Elution fractions had $\leq 2\times10^{14}$ viral genomes$\cdot L^{-1}$ and, depending on the specific AVV particle, the purified viruses successfully transduced or induced gene knockdown *in vitro*. Elution pools from MVA virus produced in continuous bioreactors with an avian cell line contained about 3.7×10^{9} infectious virions measured by $TCID_{50}$. For influenza virus, four strains were produced in MDCK cells. Full recovery of all strains was observed using identical SXC conditions for both infectious and chemically inactivated viruses. The column capacity in terms of the viral hemagglutinin antigen was > 50 mg$\cdot m^{-2}$. In the case of yellow fever virus, two attenuated strains were produced in Vero cells. Here, full recovery of infective titers was also achieved: the elution fraction was concentrated more than 100-fold to a titer of $>6\times10^{9}$ plaque forming units (≈100 000 doses).

In summary, SXC capture with PEG and unmodified cellulose membranes seems to perform very well for a broad range of viruses from different production processes. Thanks to the high degree of success in a relatively narrow operational range, SXC can drastically reduce process development. The high recoveries obtained so far, enable subsequent polishing operations with minimum risk to low overall process yields.