PURIFYING VIRUSES WITH A SHEET OF PAPER: SINGLE-USE STERIC EXCLUSION CHROMATOGRAPHY AS A CAPTURE PLATFORM FOR VACCINE CANDIDATES

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Steric exclusion chromatography (SXC) is a method in which a crude sample is mixed with polyethylene glycol (PEG) and fed to a hydrophilic stationary phase. Selectivity in SXC is strongly influenced by the target species' size, so it is particularly well suited for purification of large biomolecules such as viruses and virus-like-particles. The product is captured without a direct chemical interaction thanks to the mutual steric exclusion of PEG between the product and the stationary phase (cellulose membranes with micron-sized pores). Product elution is achieved by removing the PEG from solution, and can theoretically be made in any buffer system. The low cost of the cellulose membranes allows this operation to be single-use.

Using SXC, we have achieved virtually full recovery of several viruses produced in serum-free mammalian cell culture: influenza virus, yellow fever virus, and Modified Vaccinia Ankara (MVA) virus. For influenza virus, four different strains were produced separately in MDCK cell suspension cultures using either chemically defined medium or serum-free medium. Full recovery of all strains was observed using identical SXC conditions (loading with 8% PEG-6000) for both infectious and chemically inactivated virus particles. Coupling a nuclease treatment for DNA digestion prior to SXC, dsDNA was depleted >99.98%. The column capacity in terms of the viral hemagglutinin antigen was at least 50 mg m^{-2}. In the case of yellow fever virus, two attenuated strains used for commercial manufacture were produced separately in adherent Vero cells grown in serum-free medium. Full recovery of infective virus titer for both strains was attained using 10% PEG-6000 for sample load. The elution fraction was concentrated >100-fold compared to the feed with the very high titer of 6×10^9 plaque forming units, equivalent to ≈100 000 doses. Total recovery was also observed for MVA virus loaded at 4% PEG-6000; produced in an avian cell line in chemically defined medium, the SXC elution pools contained $\approx3.7\times10^9$ virions as estimated by $TCID_{50}$ assay.

In conclusion, SXC can drastically reduce process development in terms of time and equipment requirements. The convenience of purifying different virus strains using similar chromatography conditions is almost impossible to match by other methods, as are the high product recoveries typically achieved with SXC. The latter gives space to include additional polishing operations without risking low overall process yields. We deem membrane-based SXC as a promising platform technology for capturing viruses and virus-like particles in vaccine manufacturing.