STERIC EXCLUSION CHROMATOGRAPHY FOR THE PURIFICATION OF RECOMBINANT BACULOVIRUS

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Steric exclusion chromatography (SXC) has already proven to be a valuable tool in the purification of proteins and virus particles. An important benefit of the method is the fast and simple procedure at mild chromatography conditions as no harsh binding and elution buffers are needed. The sample is initially mixed with a polyethylene glycol (PEG) containing buffer of choice. The steric exclusion of a macromolecule from the polyethylene glycol and the stationary phase allows a selective retention of the product, depending, among others, mainly on its size as well as on the molecular weight and concentration of the PEG. Here, SXC was set up in order that smaller process contaminants, i.e. host cell proteins and DNA, did not bind to the stationary phase, in contrast to the targeted larger virus particles. These were subsequently eluted reducing the PEG concentration in the mobile phase. Regenerated cellulose was used as stationary phase to purify VSV-G pseudotyped *Ac*MNPV baculoviruses derived from *Spodoptera frugiperda* cells (Sf9 cells) by SXC. The purified virus particles are used as gene transfer tools for human mesenchymal stroma cells. For this purpose, the baculovirus was clarified prior to the SXC by sequential centrifugation (4700 g_{max}). The SXC conditions were optimized in terms of yield and purity by a design of experiment approach considering the PEG molecular weight, its concentration and the ionic strength of the elution buffer as critical process parameters. Within the design space virus recovery was ≥70%. Without further nuclease treatment the depletion of double-stranded DNA was >90% and the amount of host cell proteins were reduced >90% in the virus fraction.

In conclusion, SXC can drastically reduce the process development in terms of time and equipment requirements for the purification of recombinant baculoviruses, as well as for the achieved purity which is superior over classical methods.