296e Downstream Processing of Cell Culture Derived Influenza a Virus

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For the production of biologicals using microorganisms or cell cultures, downstream processing next to the actual biosynthesis in bioreactors is often the most expensive and time consuming step. Based on the physical and bio/chemical properties of the desired product and the level of contaminating molecules from the cultivation broth a broad range of separation methods can be applied. Here we report on the development and optimisation of downstream processing methods for the purification of cell culture derived influenza A virus for the production of inactivated vaccines. The process involves the following unit operations: depth filtration, chemical inactivation, ultrafiltration (UF) and gel filtration (GF). Hemagglutinin (HA) and neuraminidase (NA) are the two surface membrane proteins of the virus with HA as the major antigenic component. Monitoring of all unit operations is done by protein, DNA and HA assays. In addition, we determine NA activity of relevant intermediate steps [1]. Analysis of overall recovery for HA and NA shows that GF is the most crucial step in downstream processing. Typically, yields are about 40% and 60% for HA and NA, respectively while reported recovery of HA based on an ELISA assay for a similar commercial process was in the range of 60-70% [2]. Chromatography plots show a broad HA peak and sharp NA as well as protein peaks. This implies either the destruction of the intact virus particles due to high shear stress involved in the UF or is related to the release of HA molecules in the extracellular medium or HA bound to cellular membranes during virus replication in the bioreactor. In addition, non-specific interactions of the inactivated virus to the gel matrix are indicated by inconsistencies in the overall mass balance for HA and NA (70% and 69%) during gel filtration. After GF the level of contaminating proteins and DNA is reduced by at least 96% compared to the inactivated UF retentate. While the present combination of UF and GF steps is robust and reproducible enough for industrial applications, recovery and purity of the final product as well as overall process economics can be further improved by optimizing UF and GF operation conditions, careful selection of UF membranes and GF matrices and the integration of ion exchange chromatography for further reduction of contaminating DNA. Together with additional assays (mass spectrometry, capillary electrophoresis) which allow us to closely control the structure of the major antigenic antigens during downstream processing and the use of statistical methods to improve the design of experiments we eventually hope to develop robust methods to quantitatively characterize different downstream processing schemes and to evaluate recovery, purity and process efficiency for various processing options. [1] Nayak, D. P. et al. (2001) J. Chromatogr. A 922: 63-76 [2] Reichl, U. (2000): Proceedings of the 4th Internat. Congress on Biochem. Engineering; Ed. H. Brunner; Fraunhofer IRB Verlag, 314-318