Taking advantage of the virus inactivating effect in caprylic acid-based impurity precipitation for downstream processes

Sabine Faust, Natalie Hörold, Sven Schubert, Anja Trapp, Alexander Faude
Rentschler Biotechnologie GmbH, Laupheim Germany, sabine.faust@rentschler.de

Introduction

Virus inactivation is an essential process step in the downstream processing of biopharmaceuticals. Inactivation methods as low pH and solvent/detergent (S/D) treatment are well known and described. However, these methods cannot be applied for pH-sensitive proteins or show drawbacks as practical handling or unfavourable detergent properties.

Background

Virus inactivation by caprylic acid (CA) represents an alternative method and is widely used in the plasma industry since decades (Steinbuch et al., 1969). It can be utilized in a moderate pH range and is easily removed by standard DSP purification methods. As proposed by Lundblad et al. (1991) the protonated form of CA can penetrate into the viral membrane disrupting the lipid coat of enveloped viruses.

Rentschler’s mAb DSP platform

Rentschler’s mAb DSP platform starts with a Protein A capture chromatography. Conventionally, the subsequent virus inactivation step is performed by low pH treatment. As this process step enables a simple integration of an impurity precipitation step, it can be improved by adding CA. Besides its virus inactivation capacity, CA provides efficient precipitation of host cell proteins (HCP) and DNA that can be simply removed by depth filtration. Consequently, the low content of the remaining process- and product-related impurities can than be easily removed by just one polishing chromatography step. Finally, our purification platform is completed with the process steps virus filtration, ultra/diafiltration and the final formulation.

Virus inactivation by caprylic acid

Virus inactivation kinetic:

CA concentrations of 6.2 mM and 15 mM were tested with Protein A purified mAb at 15 g L\(^{-1}\) in a citrate buffer at pH 5.1. At both CA concentrations, amphotrophic MuLV (A-MuLV) was spiked at 5 % (v/v) prior to caprylic acid addition. The mixtures were incubated at 17 ± 1.5 °C with constant stirring. The kinetics of virus inactivation over a period of 60 min was determined by sampling after 15 min, 30 min and 60 min. To distinguish CA-mediated virus inactivation from virus inactivation by possible low pH conditions, hold control samples at the same pH (5.1) and neutral pH (> 6) were included in the study. A TCID\(_{50}\) end-point titration (EPT) assay was used at all time points. To improve the detection limit of samples with low virus concentrations, large volume plating (LVP) was performed for the final time point. The inactivation kinetic is displayed in figure 2. Complete inactivation was achieved after 15 min. EPT showed no residual infectivity for both CA concentrations. LVP revealed log reduction values (LRV) of ≥ 5 log\(_{10}\), at 60 min incubation time. Exposure of the enveloped virus in a control assay only to pH 5.1 without CA resulted in a marginal inactivation (LRV<1).

Virus inactivation restrictions:

In a second approach the virus inactivation ability was determined at minimal CA concentration at two different pH values. Therefore, 3 mM and 6 mM CA were tested with Protein A purified mAb at pH 5.6 and pH 6.5. According to the CA pH titration curve, these conditions resulted in 0.1 – 1.0 mM protonated CA (Figure 3). Samples for virus titer determination were taken after 15 and 300 min. Other parameters and conditions were carried out as described above for the first study. LRVs < 1 log\(_{10}\) were evaluated for the 4 different conditions. Figure 3 summarizes the results of both studies. All virus related work was performed by Labor Dr. Merk & Kollegen GmbH, Ochsenhausen, Germany.

Figure 2: Inactivation kinetic of A-MuLV by caprylic acid

Figure 3: Correlation of pH and protonated caprylic acid concentration

Influence on virus retentive filtration:

To confirm solely size-related retention of the viruses, filtration of material containing residual CA was performed. Therefore, AEX purified mAb containing 5.2 mM CA at pH 5.6 was applied on a Virosart HF\(^{®}\) virus filter including an adsorptive Virosart Max\(^{®}\) pre-filter built in-line (both Sartorius). The test material was spiked with 1 % (v/v) 0.22 µm filtered A-MuLV. In total 400 L m\(^{-2}\) were filtered and filtrate fractions (each 100 L m\(^{-2}\)) were analyzed by quantitative real-time PCR (qPCR). Additionally, the pooled filtrate and post-wash fraction were evaluated with TCID\(_{50}\) EPT and LVP. qPCR analysis revealed no free virus genome in any of the observed samples. The LRVs determined by infectivity assay and qPCR were ≥ 4.59 log\(_{10}\) and ≥ 4.32 log\(_{10}\), respectively. As 5.2 mM CA at pH 5.6 corresponds to 0.9 mM of the in-activating protonated form it was proven that the residual CA did not affect virus retention.

Figure 4: Inactivation of A-MuLV related to concentration of protonated caprylic acid

Conclusion

CA provides efficient virus inactivation of enveloped viruses after short incubation times, representing an excellent alternative to conventional low pH as well as S/D treatment. The virus inactivation efficacy can be controlled by the CA concentration and the pH value, which determines the concentration of the inactivating CA protonated form. Resulting in either high virus reduction values or no inactivation capacity. Furthermore it could be shown, that low CA concentrations did not interfere the virus-retentive filtration of an enveloped virus. Further investigations should include the determination of the critical protonated CA concentration and the influence of CA on the polishing chromatography step.

Knowing the relevant parameters, CA-induced virus inactivation represents a simple, robust and economic process step in a mAb purification. CA treatment can be implemented after the Protein A capture step in a concentration range of usually 10 – 40 mM. In most cases it contributes additionally to a further host cell protein (HCP) reduction by precipitation which can easily be removed via depth filtration. Accordingly, CA treatment leads to a more robust downstream process with respect to residual HCP. Furthermore, the methodology may open the possibility to design a two chromatographic step-based DSP resulting in an intensified downstream process with robust virus clearance.

Lundblad et al. 1991. Vox Sang 60(2): 75-81

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