BIOTECH

Technical Regulatory Topic

USTR 3825

Framework for QbD Assessment of AAV Processes
What Are the CPPs for a Typical AAV DSP Process?

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This Frequently Asked Question (FAQ) series is related to the white paper “Quality by Design (QbD) for Adeno-Associated Virus (AAV)” [1]. To access the white paper for more information visit www.qbdpaper.com.

1 Introduction

In recent years the development of Gene therapy drugs has seen a significant evolution. Recombinant adeno associated viruses are regarded as one of the most promising gene delivery vectors for the treatment of a wide range of diseases. Despite the fast development and numerous possible applications, gene therapies have faced setbacks: the implementation of a robust manufacturing process remains a hurdle as regulators have rejected or delayed multiple product submissions or clinical study requests. In several of the rejections, the pushback was due to lacking information in the Chemistry, Manufacturing, and Controls (CMC) part of the submitted documentation. The CMC documentation summarizes detailed process information and shows that the process is accurately understood and characterized to assure that the product is safe for patients and of consistent quality. A key part of the CMC documentation is the Quality by Design (QbD) principle where quality and safety are achieved through a defined manufacturing operation and control strategy. Critical Process Parameters (CPPs) are a part of the QbD framework and describe operating parameters in the different steps of drug manufacture which have an impact on drug quality or patient safety. Carefully evaluating, mapping and understanding the CPPs is critical to achieve a robust manufacturing process.

This FAQ gives an overview of a typical Adeno Associated Virus (AAV) drug substance platform process and dives into Critical Process Parameters (CPPs) of the different steps in Downstream Processing (DSP). This series is based on the white paper "Quality by Design (QbD) for Adeno-Associated Virus (AAV) [1].

2 What Are Critical Process Parameters (CPPs)?

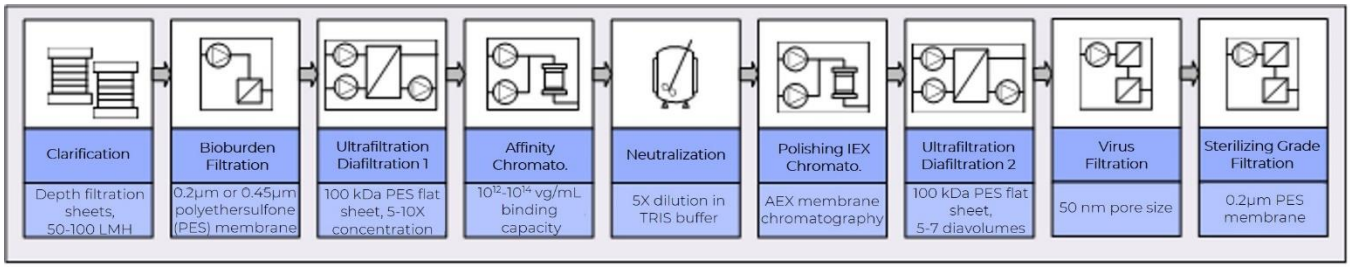
CPPs are a part of the QbD approach which ensures product quality and safety during the manufacturing process. Together with the Critical Material Attributes (CMAs) they describe operating parameters and material characteristics that have an influence on critical quality attributes of the AAV product. For a robust manufacturing process, it is therefore critical to carefully evaluate, understand, and monitor/control the CPPs and CMAs. In this framework, CPPs are defined as any process parameters that are controlled in the manufacturing process that have an impact on AAV quality or yield. CMAs include attributes of materials that are brought into the manufacturing suite whose variability can impact AAV quality or yield. Material attributes related to the material quality and chemical properties (such as material type, nominal rating of membranes or hydrophobicity) are excluded as it is expected that consumables such as filtration membranes or cassettes are integer and of consistent quality. More information on the QbD process can be found in 'USTR 3823 Framework for QbD Assessment of AAV Processes- How to Define CQAs for a Typical AAV Process?' and a description of how to evaluate the CPPs and CMAs is given in 'USTR 3824 Framework for QbD Assessment of AAV Processes- How to Define CPPs for a Typical AAV Upstream Process?', accessible via the Accelerator℠ Documentation Center.

3 What Does a Typical AAV Downstream Process Look Like?

In the past years, a platform approach has been established for the downstream steps of AAV manufacture [1] [5] [6]. To initiate the harvest, a cell lysis is often performed to release the AAV which, for most AAV serotypes, accumulates within the cell. This can be done either through adding a high salt solution or a detergent. At this point, endonuclease is added to digest host cell DNA and unpackaged virus DNA. In a first clarification step, the cellular debris is removed through depth and bioburden filtration. A first ultrafiltration/diafiltration (UFDF) step purifies and concentrates the AAV further. The purification process generally includes an immunoaffinity chromatography step where the AAV is captured in bind-elute mode. Since the elution is done at a low pH of ~3, the eluate is quickly neutralized to pH ~9 to reach favourable conditions for AAV stability. The following polishing chromatography is typically performed on an anion exchange membrane or resin where remaining host cell proteins, DNA or empty capsids can be reduced. In the subsequent second UFDF step, the AAV is concentrated, and the buffer is exchanged to the formulation buffer. A virus filtration step can be incorporated optionally either directly before or after the second UFDF, and aims at removing virus contaminants through a 50 nm nominally rated membrane. Whether to incorporate a virus reduction filtration depends on the desired final dose concentration and how the clearance of potential adventitious viruses is assured. The sterilizing grade filtration completes the DSP cascade of a typical AAV product.

Figure 1

Downstream unit operations of a typical AAV process from clarification to sterilizing grade filtration of drug substance.



4 What Are the CPPs of a Typical AAV Downstream Process?

The CPPs and CMAs of a typical DSP process have been evaluated using literature, industry and in-house data and knowledge from process scientists at Pall Corporation [1]. They are summarized in Table 1.

4.1 Clarification

A depth filter, often followed directly by a bioburden control filtration step, can remove the bulk cell mass, and bind residual host cell DNA, residual host cell protein (HCP) and, in case positively charged filters are used, endotoxin. Pressure and flux are the CPPs of the filtration step and balance the impurity reduction and AAV yield as well as the throughput that can be achieved in the depth filtration stage.

4.2 Ultrafiltration/Diafiltration

UFDF is performed in one or two stages within an AAV platform, typically using a 100 kDa ultrafiltration membrane for concentration, followed by 5-7 diavolumes of buffer in diafiltration to provide stable conditions for the AAV. The UFDF can remove small-molecular weight impurities such as residual host cell DNA and HCP while retaining the larger AAV product. Critical process parameters aim at concentrating the AAV while preventing aggregate formation. This is achieved by:

1) Keeping the transmembrane pressure low (typically limited to 689-1034 mbar [10-15 psi]) resulting in a volume concentration factor of roughly 5-10 times

2) By limiting the permeate flux to 50-100 LMH. The diafiltration volume represents the fourth CPP of UFDF.

4.3 Affinity Chromatography

In this capture chromatography step, the AAV is bound to the immunoaffinity resin and purified from residual HCPs, host cell DNA and other serum protein impurities. Product-related impurities such as non-infectious AAV, empty capsids or AAV with wrong DNA inserts (encapsidated host cell or helper DNA) typically bind the resin together with the target AAV. The degree of binding and co-elution of these impurities may however be impacted by the operating parameters.

Critical parameters are the flow rate during binding and elution (typically between 100-450 cm/h), as well as the load density. The factors are expected to impact the extent with which impurities bind and co-elute with the AAV product. In addition, the conductivity and pH of both the wash and elution buffer are expected to impact the AAV yield and the impurity clearance [5].

4.4 Polishing Chromatography

An anion exchange membrane or resin is typically used for AAV polishing. The step reduces several process-related impurities such as HCP, residual host-cell DNA as well as potentially leached ligands. In addition, it is the most powerful step in separating full capsids from aggregated, degraded, or empty capsid variants. Recent results have shown more than 4 times enrichment, of full capsids in anion exchange membrane chromatography [7].

The operating parameter design spaces depend on which impurities are to be targeted during the polishing. Critical parameters are the load density, the elution volume, and the flow rate. The pH and conductivity of the load material (typically pH 9, < 5 mS/cm) as well as the load density (typically 10^{10}-10^{13} vg/mL) determine the efficiency of impurity binding. The flow rate is generally set to 3-7 MV/min to ensure sufficient mixing in the chromatography capsule, while optimizing residence time for high impurity binding. The wash and elution conditions are the final CMAs that impact the AAV yield, the reduction of impurities, and co-elution of unwanted AAV- variants.

4.5 Virus Filtration

Virus filtration is an optional step that is implemented in some manufacturing platforms either before or after UFDF 2. Nanofilters with a removal rating for 50 nm viruses can reduce adventitious viruses through depth size exclusion effects. CPPs are the differential pressure, the throughput and flux decay, the duration of the filtration, and possible process interruptions. These parameters can influence the retention of adventitious viruses and the resulting AAV yield, but also the degree of potential aggregate formation and removal.

4.6 Sterile Filtration

Sterilizing grade filtration using a 0.2 µm polyethersulfone membrane filter is a key step in assuring patient safety by removing any possible bioburden in the drug substance. The process and its CPPs are very well understood and have been studied for AAV in detail [6]. Critical process parameters are the flux, which typically controls the filtration, and the resulting differential pressure of typically 345-2068 mbar (5-30 psi.). The throughput as well as the filtration duration complete the CPP assessment. The quality and performance of the filter such as; successful pre-and post-use integrity tests, low leachables and particulates release, sufficient bacterial retention or pre- and post-use integrity, are CMAs summarized under "filter performance".

Table 1

Critical Process Parameters (CPPs) of downstream unit operations of a typical AAV process [1].

Process Step	Critical Process Parameter	Critical Material Attribute
	Pressure	Filter capacity
Clarification	Flux	Conductivity of flush buffer
	Permeate flux	
	Transmembrane pressure	
	Volume concentration factor	
Ultrafiltration/Diafiltration	Diafiltration volume	N/A
		Wash buffer pH/conductivity
	Flow rate	Elution buffer pH/conductivity
Affinity Chromatography **Polishing Chromatography**	Load density	Load pH/conductivity* * polishing chromatography only
	Differential pressure	
	Throughput/flux decay	
	Duration	Filter performance
Virus Reduction Filtration **Sterile Filtration**	Process interruption* ** virus filtration only*	Filter robustness

References

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