The Downstream Process: Playing A Crucial Role In Extracting Essence

The goal of downstream process (DSP) development for bispecific antibodies (BsAbs) is to develop a method for obtaining highly purified bsAbs with optimal recovery and quality. Like traditional antibodies, the concept of the chromatography platform can be applied to DSP development. However, bsAb DSP development is more challenging due to the higher complexity of product-related impurities. The principle of the bsAb purification strategy was mainly based on the difference between the product and the impurities, as illustrated in Figure 1. In addition, some additives could be used to improve resolution during chromatography.

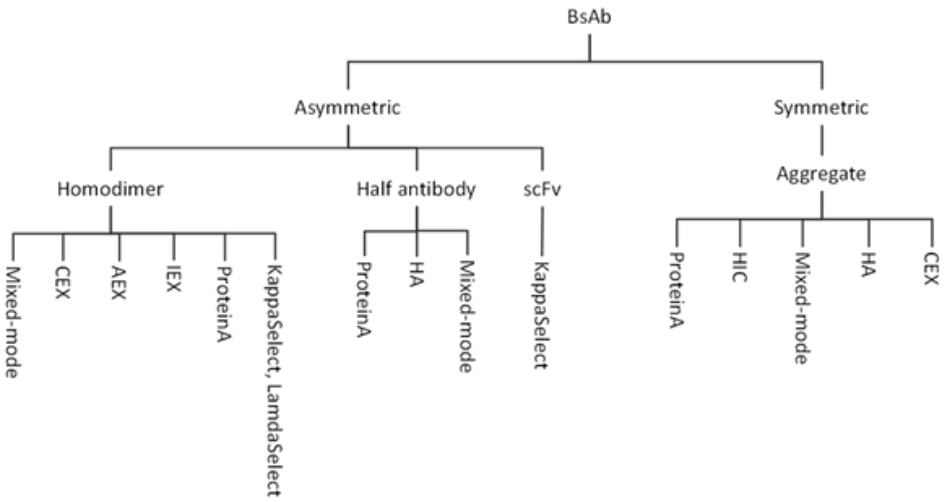

Figure 1: A roadmap for IgG-like bsAb purification.[1]

For better separation of monomer and by-product, different designs could be used in bsAb. The purification approaches for different formats of bsAb are listed in Table 1. Choosing the optimal resins for DSP development not only enhances the quality of the drug but also shortens the timeline.

Table 1.

BsAb design or format	Rational	Purification method
Unmodified bsAbs	Random pairing	IEX, HIC, HA
KiH	Different properties of hole-hole homodimer	MMC, HIC
Electrostatic interaction	Opposite charges in complementary CH3	IEX
pI engineered HC	pI difference between the bsAb and homodimer	IEX
Protein A-binding abolished HC	Protein A binding capacity is abolished	Protein A with gradient elution
Common HC bsAb -κλ-body	Two different LCs: one kappa and one lambda	KappaSelect and LambdaFabSelect
IgG with ScFv appended	Binding affinity between aggregates and monomer	IEX, HIC, HA, MMC

Purification Approaches of bsAb with Different Designs

Using appended IgG as an example, the addition of a domain (ex. a single-chain variable fragment; ScFv) typically results in reduced stability and an increased level of aggregates. A significant proportion of aggregate was also confirmed by analytical results (Figure 2). These aggregates can impact product efficacy and raise immunogenicity, necessitating their removal. Aggregates often exhibit proportionally greater surface charge or hydrophobicity properties, making them separable by ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), or mixed-mode chromatography. Among these methods, mixed-mode chromatography proves more efficient than others, offering higher selectivity and separation efficiency within fewer purification steps (Figure 3). Consequently, mixed-mode resins stand out as excellent candidates for selected BsAb purification.

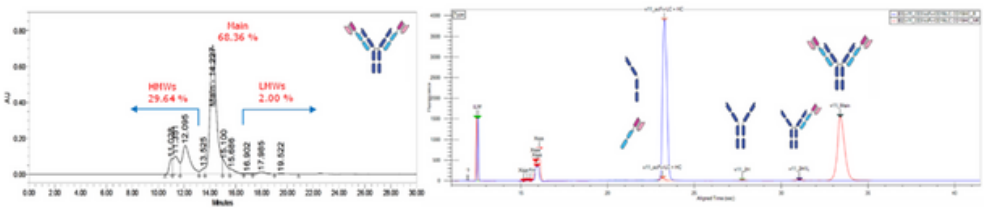

Figure 2: Composition of product-related impurities in the scFv-IgG format bispecific antibody**.**

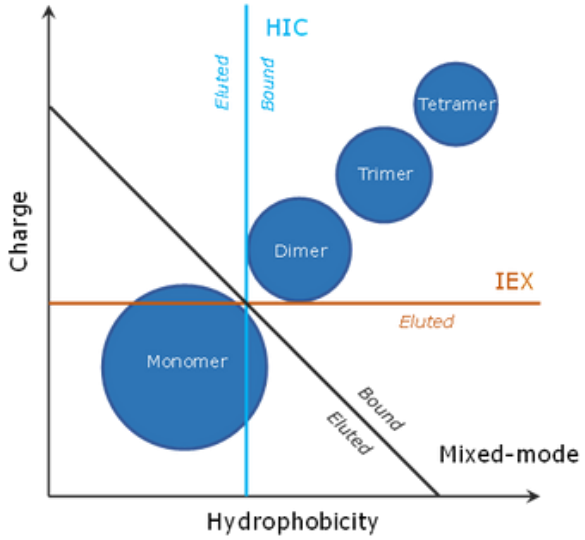

Figure 3: Strategy for aggregate removal with IEX, HIC, and mixed-mode chromatography.[2]

A Solution For BsAb Purification to Achieve High-Quality Drug Substance

Mycenax can apply a variety of strategies in the chromatography process to overcome the obstacles of BsAb purification. Based on the chosen appended IgG format, Mycenax proposed an appropriate DSP (Figure 4). In the first step of purification, rProteinA chromatography is usually applied to capture the Fc-containing product and initial removal of the process-related impurities. Many rProteinA candidates and purification conditions were screened to achieve high purity through effective impurity removal without compromising recovery.

Anion exchange chromatography (AEX) and mixed-mode anion exchange chromatography (MMA) are commonly employed for the removal of host cell proteins (HCP), host cell DNA (HCD), and viruses. The MMA was chosen for its higher efficiency and performed in flow-through mode to maximize the impurity removal capacity. The optimization was conducted based on empirical principles in the MMA process to achieve both efficient impurity removal and increased recovery. The results also demonstrate that challenging-to-remove high molecular weight species (HMWs) can be effectively removed under appropriate MMA conditions (Figure 5).

To remove product-related impurities, mixed-mode cation exchange chromatography (MMC) was employed for its enhanced selectivity and efficiency within fewer purification steps. The overall DSP achieves 37% recovery and 95% purity of drug substance.

Figure 4: One purification strategy for BsAbs in the Mycenax DSP.

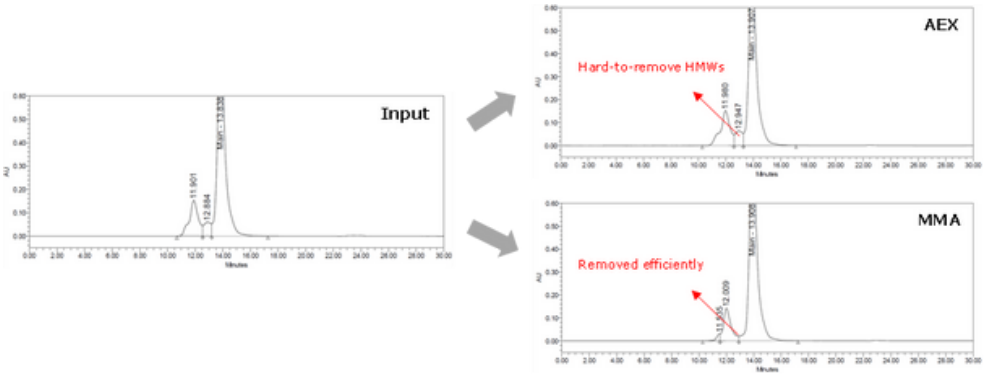

Figure 5: MMA chromatography efficiently removes a substantial portion of challenging-to-remove HMWs.

Improved BsAb DSP Performance via the Mycenax Toolbox

For product-related impurities removal, the additives and pH screening will help remove impurities effectively in the MMC step. In this case, the selected additive was introduced to boost both purity and recovery (Figure 6).

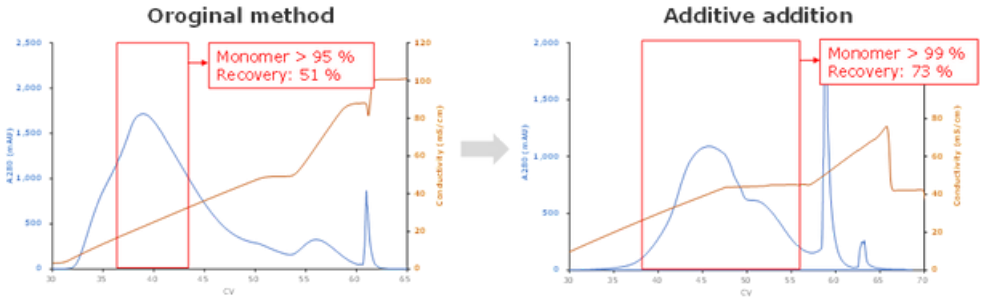

Figure 6: A specific additive was used in the MMC step for better performance.

For process-related impurities removal, the process can be further optimized to enhance the removal of HCP (Figure 7).

Purification step	Before optimization	Optimized
HCP residual after affinity capture	**3190 ppm**	**3190 ppm**
HCP residual after polishing	**1333 ppm**	**132 ppm**

Figure 7: HCP residual could be reduced after method optimization.

The final optimized condition achieved 64% yield and the aggregate level was lower than 1%. Also, the product-related impurities meet the drug substance requirements (Figure 8).

Step	Recovery (%)	
	Before optimization	**Optimized**
Affinity chromatography	91.6	93.5
Mixed-mode AEX	79.27	85.8
Mixed-mode CEX	51.2	79.9
Total recovery	**37.2**	**64.1**

Final Drug Substance	
Purity by SE-HPLC	99.91%
Purity by NR CE-SDS	97.39%
HCP residual	72 ppm
rPA residual	<LOQ(< 2.03 ppm)

Figure 8: Recovery of DSP and the quality of drug substance.

Combat Downstream Processing Obstacles

In conclusion, team experience plays a crucial role in addressing complex downstream processing challenges for BsAbs. Through our experienced practices and development, Mycenax can shorten the timeline of drug development and directly impact the quality, efficacy, and safety of the final pharmaceutical product, thereby holding a significant position in the drug development and manufacturing process.

References

1. Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics. 2020, Pages 167-179
2. Removing Aggregates in Monoclonal Antibody Purification. Pharmaceutical Technology, Pharmaceutical Technology-03-02-2015, Volume 39, Issue 3