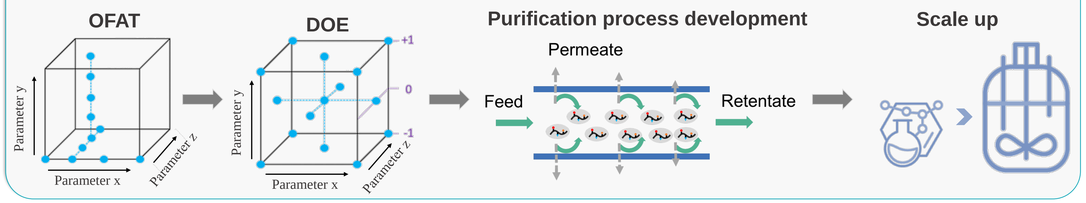
Developing Robust and Scalable Bioconjugation Processes for Accelerated ADC Development and Manufacturing

Jidong Zhao, Song Liang, Qiang Peng and Weibin Chen
Shanghai Asymchem Biotechnology Co., Ltd, 4th Floor, Building No. 1, 518 Haoye Road, Jinshan Industrial Park, Jinshan, Shanghai, China

Introduction

As a vast number of ADC candidates advance to clinical stages, the needs for streamlined and robust conjugation process to handle different designs of ADC molecules have become more prominent. However, significant challenges put forth by the inherent properties of ADC in heterogeneity, aggregation, impurity removal and batch-to-batch inconsistency still exist and would take a great deal of efforts & experiences to be addressed. As a global CDMO organization, AsymBio provides end-to-end ADC solutions to help address those CMC (chemistry manufacturing control) challenges. In this poster, we presented several case studies to highlight our efforts in addressing the challenges in ADC conjugation and purification process development. Our strategy includes comprehensive OFAT and DOE optimization of conjugation parameters, proven TFF and chromatography process and robust scale-up scheme. It enables the accelerated development of robust and scalable conjugation processes, minimizing the risk in future ADC manufacturing.

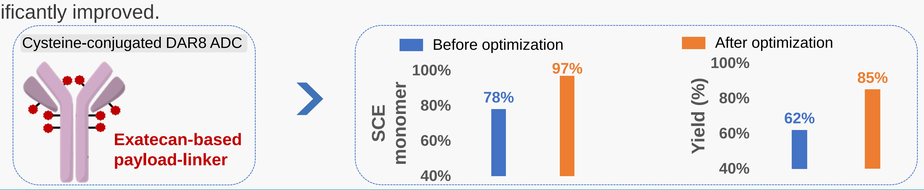


Case Study I: Process Optimization for More Reproducible & Homogeneous ADCs

In this case, the ADC is designed as a cysteine-conjugated nanobody-Fc fusion bispecific antibody with a targeted value of DAR 4. The payload is MMAE and the linker contains a hydrophilic moiety branch as an added modification to the molecule design. The BsAb bears a disulfide-containing knob-into-hole structure, which results in additional needs to control non-specific Cys-conjugation. We systematically optimized the reduction and conjugation process parameters to generate ADC products with DAR 4 species accounting for greater than 98%, and the DAR value remains highly consistent at different product scales.

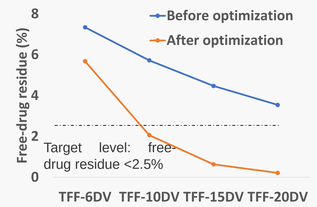
Case Study II: Optimization to Minimize the Level of ADC Aggregation

In In this case, the ADC is designed as a cysteine-conjugated DAR8 ADC. The exatecan-based payload-linker in this ADC is relatively hydrophobic and the conjugation resulted in severe ADC aggregation and low yield. We optimized the process by adjusting the reaction parameters, as well as using solubilizing reagents to improve the solubility of the payload-linker and ADC molecules. After optimization, the monomer ratio of the ADC (by SEC) and the reaction yield were significantly improved.

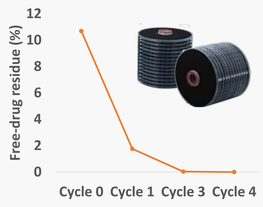


Case Study III: Optimization on the Removal of Free-drug Related Species

The hydrophilic payload-linker tends to absorb onto proteins. In this case, we developed a purification strategy using a co-solvent in the TFF-1 step to remove residual payload-linker to the target level, and then deploying a TFF-2 step to remove the added co-solvent.



In the second example, the hydrophobic and large size payload-linker was unable to be removed through the traditional TFF approach. We used activated charcoal filtration to remove the payload-linker to below the limit of quantitation (LOQ) levels.



Case Study IV: Robust Scale-up Strategy

AsymBio has established a comprehensive scale-up strategy by focusing on key process parameters. To ensure successful manufacturing, we confirm and lock the process parameters by performing a 20-50 g scale test run. In addition, a 2-5 gram scale of use test run using the same grade of materials as those used in manufacturing production is also carried out prior to the manufacturing kickoff.

Process Parameter Types	Examples of Process Parameters	Scale-up Strategy
Volume Dependent	PL/Reducing Reagent Feeding Speed	Maintain the Same Feeding Speed
Volume Independent	mAb Concentration, pH, Equivalent of PL/Reducing Reagent, Reaction Time, Temperature	Optimizing the Operation Space
Nonlinear	Agitation Speed	Mixing study at different scales

Process Development → **Process Scale-up** → **Process lock run** → **Use Test** → **Tech Transfer to MFG Dept** → **Manufacturing**

Conclusion

Case studies showcase the capability of AsymBio's bioconjugation platforms in addressing process development challenges such as ADC heterogeneity, aggregation, impurity removal and batch-to-batch inconsistency. Our well-developed conjugation process platforms are flexible to handle different ADC molecule designs and form a solid foundation to support the process development and process transfer for pre-IND, IND and BLA projects. The conjugation process development can be expedited with a compressed timeline, typically 2-3 months, for an IND project. Under the strict compliance to EHS regulation on OEB5 level materials, we aim to safely and efficiently deliver consistent and scalable bioconjugation processes for all our clients.

Web：www.asymbio.com E-mail：MKT@asymchem.com.cn