WuXi XDC's Development Platforms Deliver Consistent & Scalable Conjugation Processes

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Introduction

As of 2023, 14 innovative antibody-drug conjugates (ADC) based on Lys or interchain Cys conjugation have been commercialized globally while more than 500 modalities using a variety of newer conjugation chemistries are in research pipelines. As more ADC modalities advance from the preclinical candidate (PCC) stage into the Chemistry Manufacturing Control (CMC) development stage, process development (PD) scientists inevitably face tremendous challenges. As microgram quantities of ADC conjugates are needed at the discovery stage and produced with preliminary conjugation protocols that may not necessarily deliver multi-batch consistency in physical chemical properties, the challenge then becomes developing the larger-scale and more consistent CMC processes generating ADC product for toxicology studies and clinical trials. These CMC processes will have to evolve from vigorous PD studies focused on the specific conjugation chemistry using approaches like design of experiment (DoE).

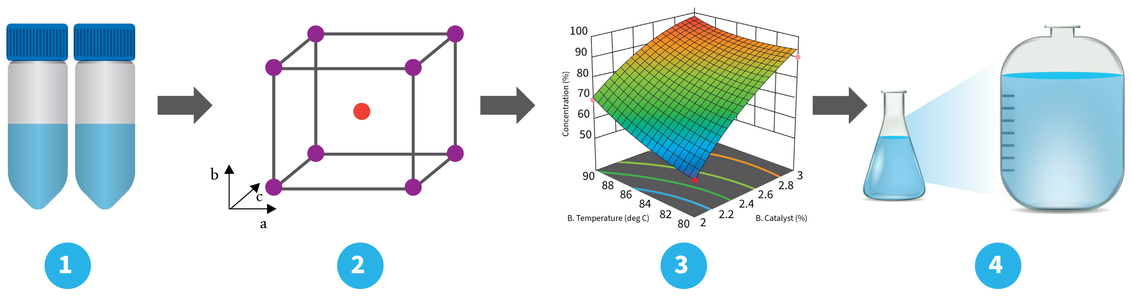
For example, at the discovery stage inconsistent and heterogenous drug antibody ratio (DAR) often results from a difficult-to-control feed ratio of the reducing agent and/or linker-payload intermediate. The same can be true in the control of other parameters including reaction temperature, time, and mixing speed. Meanwhile, small scale purification by gravity columns or ultrafiltration cassettes, dialysis or gel filtration chromatography (GFC) to enrich the target molecules may well be suitable for PCC generation, whereas more reproducible and scalable purification processes must be developed to produce grams to kilograms of ADCs.

At the Bioconjugation Process Development (BCPD) department of WuXi XCD, experienced experts specialized in consistent and scalable conjugation and purification have delivered more than a hundred processes within aggressive timelines to support IND filing or to conduct process characterization (PC) activities for programs working towards a BLA submission. This poster will present case studies of the solutions established for challenging process development projects.

Methods/Discussion

Our typical CMC PD project starts with using the client's mAb and linker-payload intermediates at milligram to gram scales and/or generating these intermediates and other required reagents or enzymes at WuXi Biologics and WuXi XDC. Laboratory conjugation protocols and early reference materials are critical at this stage and WuXi XDC can quickly produce these ADC materials for the initiation of PD. A complete PD program consists of 4 segments of experiments as depicted in **Fig. 1**. Firstly, assessment of the initial set of process parameters using one-factor-at-a-time (OFAT) experiments is undertaken. Secondly, a variety of process parameters are investigated via details in the DoE setup. Thirdly, further experiments and statistical assessment of the complicated effects and responses from multiple factors on the critical quality attributes (CQAs) are performed through various tools and software. Lastly, generating the data on process robustness at different process scales and performing the process lock and production runs to generate material for toxicology studies is conducted. From these activities the process is streamlined to generate the technology transfer protocol (TTP) for GMP unit operation.

Fig 1. PD from Lab-scale (mg) Conjugate Preparation to Pilot-scale (kg) Manufacturing



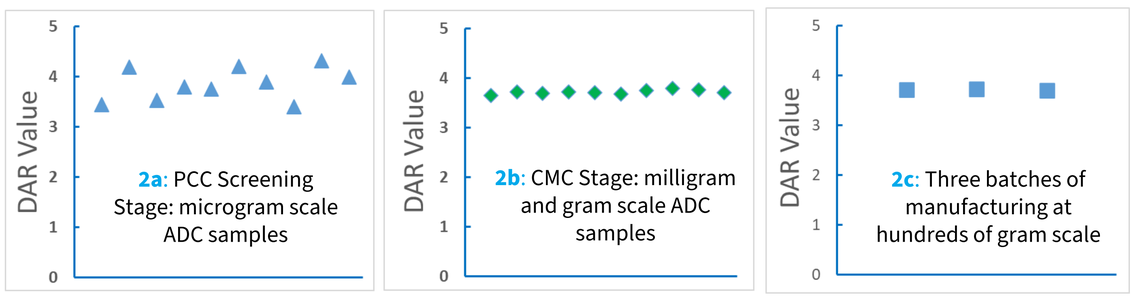
1. **OFAT:** One-factor-at-a-time experiments to assess initial set of process parameters;
2. **DoE:** Design-of-experiments setup in fractional/full factorial design to investigate a variety of process parameters;
3. **DoE response surface methodology:** Statistical assessment of complicated effect of multiple factors on responses of critical quality attributes (CQAs);
4. **Scale-down & scale-up:** Multiple runs at different scales of ADC preparation including process lock and toxicology lot runs to generate technology transfer protocol for GMP production

Results

Case Study 1: Optimization of Feeding Ratio & Consistency in DAR

This PD study was carried out in a proper scale-down model with fixed volume in order to increase the accuracy of feeding. During the conjugation reaction, feeding ratio and amount of raw materials including TCEP reducing agent and linker-payload intermediate were in strict control and in a manner scalable to GMP production. As a result, DAR values for multiple batches and different scales of ADC preparations are centered around 3.8 within +/- 0.05 during CMC development (**Fig. 2b**) and GMP production (**Fig. 2c**) as compared to 3.8 +/- 0.6 (**Fig. 2a**) during PCC screening stage. Control of feeding ratio is not trivial as excess or insufficient addition of any raw material will not only affect the final product quality but also yield and cost of goods (COG) during GMP production.

Fig 2. Multiple Batches of ADC Targeting DAR 4.0 Produced in PCC, CMC and GMP Stages



2a: PCC Screening Stage: microgram scale ADC samples

2b: CMC Stage: milligram and gram scale ADC samples

2c: Three batches of manufacturing at hundreds of gram scale

Case Study 2: Fractional/ Full Factorial Design: Temperature & Conjugation Time

In this case study, the reduction and conjugation was carried out simultaneously with different conjugation duration in a time course and with temperatures at 22°C and 10°C, respectively. It was found that a lower temperature is advantageous in maintaining antibody stability and steady progress toward a DAR of 8. However, a longer reaction time thus a longer shift was necessary (**Fig. 3b**)

Fig 3. Time-course Study at Different Conjugation Temperatures

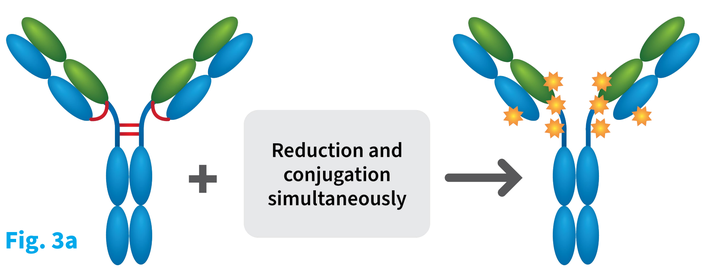


Fig. 3a

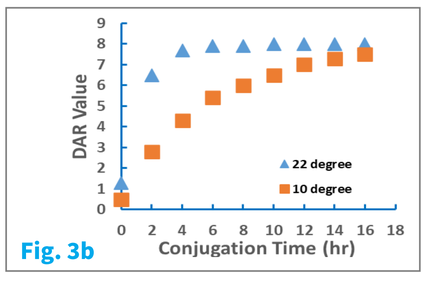


Fig. 3b

Case Study 3: *in situ* Synthesis & Mixing Speed

A novel and complex conjugation technology is the subject of this case study. The linker-payload is synthesized ***in situ***, and conjugated to the antibody carrier in a one-pot-chemistry fashion (**Fig. 4a**). Due to the strong tendency for hydrolysis, the *in situ* synthesized linker-payload is not stable in an aqueous solution. Thus, two opposite processes are competing to consume linker-payload in solution: one is the conjugation to form the ADC and the other is hydrolysis to generate free small molecules. Probably due to this two-way reaction kinetics, stirring was found to be critical as the intended DAR of 2 was achievable only through an optimized mixing speed and reaction duration (**Fig. 4b**).

Fig 4. Conjugation with *in situ* Synthesized Linker-Payload & Mixing Speed Study

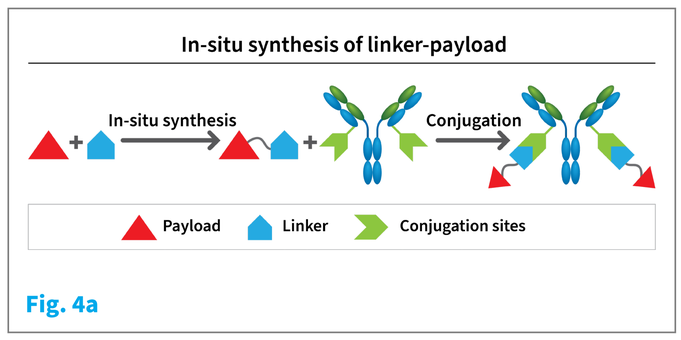


Fig. 4a

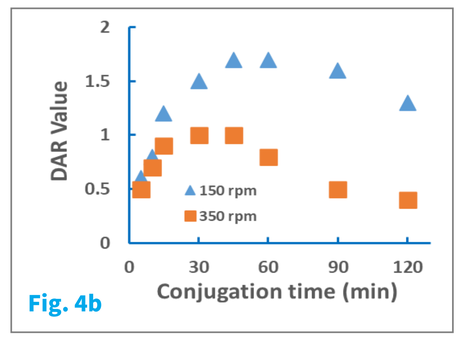
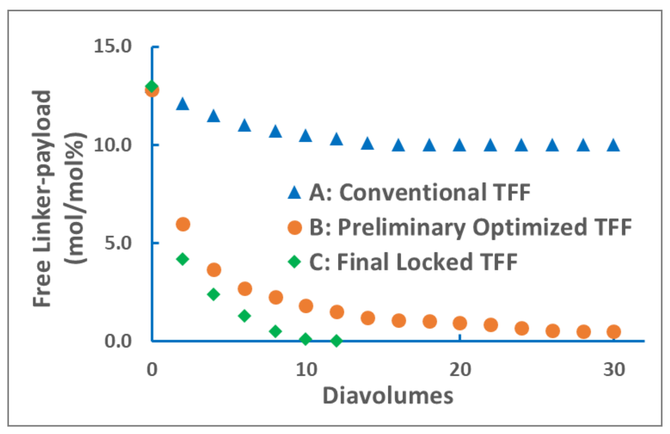


Fig. 4b

Case Study 4: Optimization of Free-drug Removal by TFF

Usually free mcMMAE/MMAF linker-payload can be successfully removed by tangential flow filtration (TFF). However, many conjugation processes that either use enzymatic catalysts or novel linker-payloads may render the free drug removal more difficult post conjugation. Specifically, some Exatecan-like small molecules may have strong non-covalent interactions with either naked antibody or ADC conjugates, leaving higher-than-specification levels of free drug post TFF. For example, in **Fig. 5 (A)**, after 30 diavolumes (DVs) of conventional TFF, the residual free drug level was persistently high at 10% molar-to-molar values relative to total linker-payload in ADC drug substance.

Fig 5. Optimization of Free-drug Removal by TFF



With preliminary optimization at WuXi XDC, the free drug was removed to below 1% level with only 20 DV's (**Fig. 5, B**). The number of diavolumes was finally shortened to 10 DVs and residual free drug was successfully removed to below limit of quantitation (LOQ) levels as demonstrated in **Fig. 5 (C)**. Below LOQ level of residual free drug is important for ADC drug substance used for toxicology studies and ultimately critical for GMP production prior to clinical trials.

Platform Beyond PD: Process Characterization (PC)

PC must be completed with scale-down models and GMP materials prior to process performance qualification (PPQ) manufacturing as part of the validation package in a BLA. WuXi XDC's PD team has experience in launching antibody and ADC projects to the BLA stage and currently more than 10 conjugate projects are going through late-phase development and PC studies. Typical high-level PC timelines and workflow are depicted in **Fig. 6**, even though detailed data summary will be the subject of future publications.

Fig 6. Process Characterization Timeline & Workflow

Fig. 6a, High-level PC Time Lines

PC: Process Characterization
SSMD/Q: Small-scale Model Development/Qualification
EPOR: Early Phase Operating Range
FMEA: Failure Modes and Effects Analysis
OFAT: One Factor At a Time
DoE: Design of Experiments

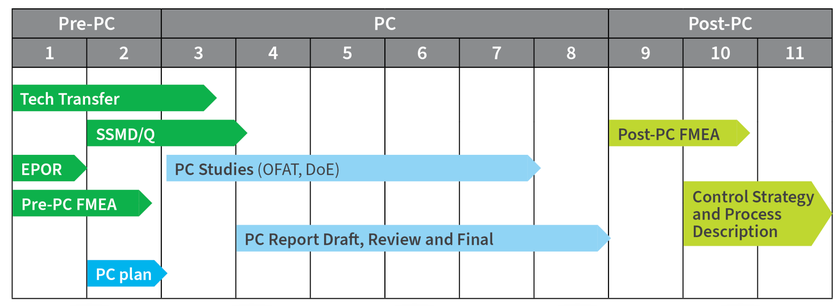
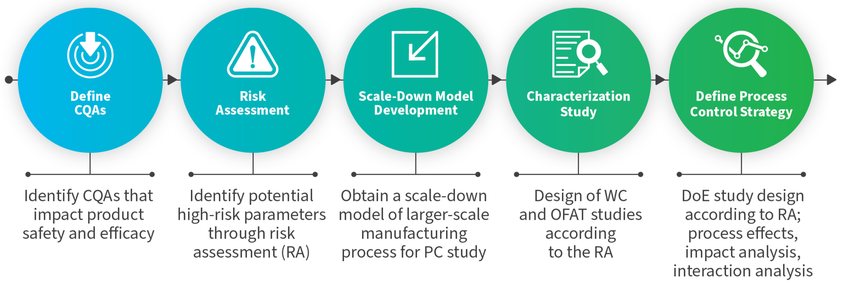


Fig. 6b, PC Workflow

WC: Worst Case
RA: Risk Assessment
CQAs: Critical Quality Attributes



Conclusion

In summary, case studies have demonstrated that robust and scalable conjugation processes have been consistently developed using WuXi XDC's PD platform. Trivial parameters at discovery stage can often be critical to batch consistency during CMC development, thus, these processes must be optimized during PD with approaches including DoE and scale-up for toxicology and GMP lot production. Our PD platform also extends to PC studies for late-phase ADCs as the complex moieties advance toward BLA.

About WuXi XDC

WuXi XDC, a WuXi Biologics subsidiary, is a joint venture between WuXi Biologics and the WuXi AppTec subsidiary, WuXi STA, providing end-to-end contract development and manufacturing of bioconjugates including antibody-drug conjugates (ADCs).

For more information, visit us at: wuxixdc.com

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