Data-Rich Experimentation with Mechanistic Modeling
for Improved Process Development and Scale-up in ADCs

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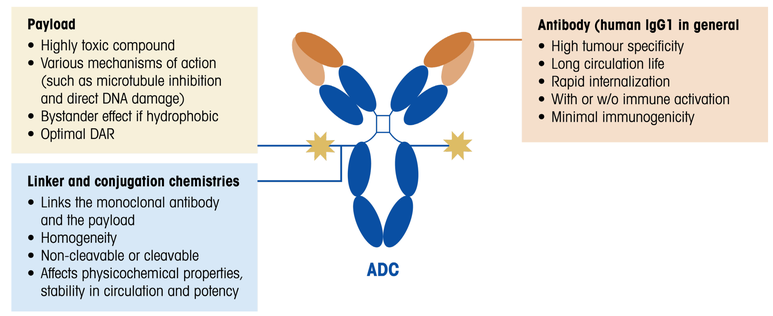
Introduction

Antibody-drug conjugates (ADCs) are powerful pharmaceuticals that are increasingly important in oncology treatments. To accelerate development of these drugs towards clinical trials and market launch, pharmaceutical companies are leveraging the powerful combination of data-rich experimentation with mechanistic modeling in both upstream and downstream operations. This integrated approach is crucial for optimizing each unit operation to consistently maintain Critical Quality Attributes (CQA) and Drug-to-Antibody Ratio (DAR) during scale-up and technology transfer from laboratory development to commercial manufacturing.

About Antibody Drug Conjugates

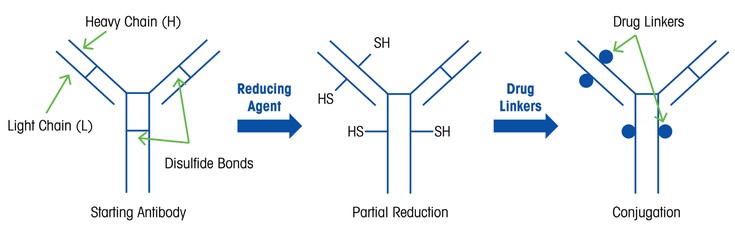
ADCs are composed of a highly selective monoclonal antibody (mAb) attached to a cytotoxic drug via a stable linker. The antibody directs the payload specifically to the target, ensuring effective treatment while reducing off-target toxicity. The payload of the ADC is defined by the DAR. A balance is sought between the drug loading level and the toxicity of the drug by establishing a desired DAR.



Example of Kinetic Studies for Optimization of the Partial Reduction and Conjugation Reaction

Kinetic studies are a powerful method to elucidate main reaction mechanisms by fitting time-course experimental data to a proposed reaction mechanism. The mechanistic insights gained can be used to predict how different process parameters influence product distribution and to identify optimal conditions, e.g. to maximize the yield of the desired DAR species. Leveraging results from a few data-rich experiments in kinetic studies is particularly effective in early development, when materials are scarce and extreme care must be taken in handling of the potent cytotoxic agents in each experiment run.

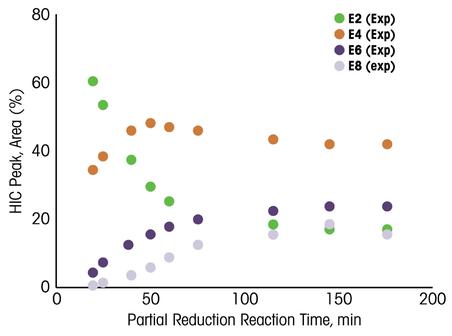
This approach has been published by Nayak and Richter[1] and is summarized below.



The reduction conjugation process creates a diverse population of antibodies with different numbers of drug per antibody

Data-Rich Experimentation and Experimental Analysis

Completely reduced IgG1 antibodies were placed in a neutral buffer solution in a well-mixed batch reactor containing an excess of the vc-MMAE drug-linker, with periodic samples taken for analysis using HIC (Hydrophobic Interaction Chromatography) and RP-HPLC techniques.



E2, E4, E6 and E8 represent conjugates with the number of drug-linkers per antibody of 2, 4, 6, and 8 respectively. Some of the experimental results are summarized in the graph provided.

Developing a Kinetic Model to Describe the Experimental Data

An irreversible kinetic pathway was proposed to interpret the temporal HIC data from the partial reduction of disulfide bonds in the antibody.

all disulfide bond intact (E0) + R →

1 disulfide bond broken (E2) + R →

2 disulfide bonds broken (E4) + R →

3 disulfide bonds broken (E6) + R →

all 4 disulfide bonds broken (E8)

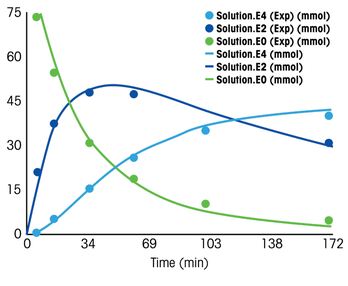
Where R represents the reducing agent, TCEP.

The kinetic constants and activation energies of these individual reaction steps were obtained by fitting the temporal HIC data at various temperatures in Dynochem to obtain the best fits for the model as shown in the table below.

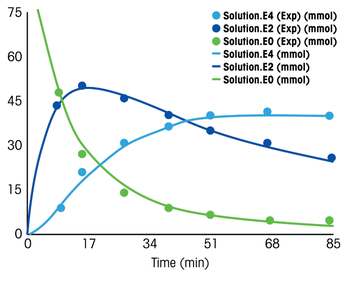
Reactions	Kinetic Constant L/mol.s (k @298 K)	Activation Energy, kJ/mol
E0 + R → E2	6.08E-03	43
E2 + R → E4	3.32E-03	49
E4 + R → E6	2.25E-03	55
E6 + R → E8	1.33E-03	70

The fit between experimental data and simulation predictions was found to be good, as shown in the simulation results pictured below.

2.7 eq tcep at 10 °C



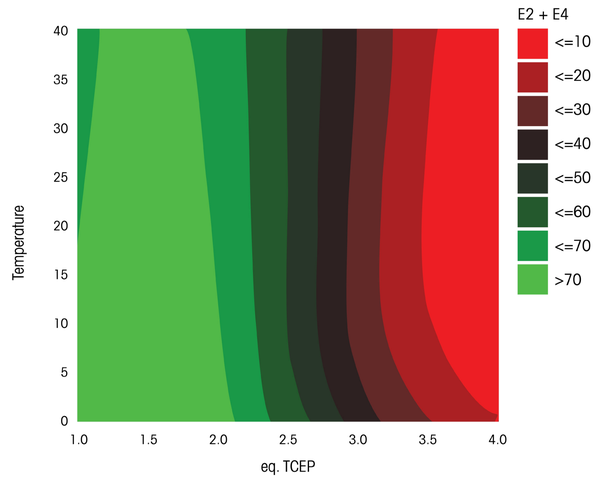
2.7 eq tcep at 25 °C



Developing Process Understanding, Optimization, and Robustness Studies from a Model

The kinetic model showed that the partial reduction reactions are slower than the conjugation reactions and act as the rate-limiting step in ADC synthesis. This can give rise to a wide product distribution.

The kinetic model was employed in prediction mode to investigate the influence of various process parameters on product distribution and to identify the optimal design space for ADC synthesis that maximizes the yield of the desired DAR. Subsequent experiments were conducted to validate the model's predictions.



Benefits of Using a Kinetic Model in ADC Development

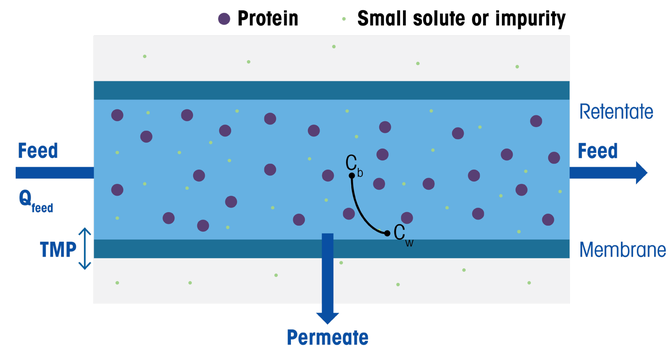
Model-guided process development leveraging data-rich experimentation enabled an understanding of the rate-limiting steps within the kinetic pathway and provided a platform for identifying conditions that maximize yield of the desired species, thereby significantly reducing time and resources required for experimental exploration of process parameters.

Applying a Modeling Approach to Downstream Operations

As noted previously, once the synthesis is complete there is a downstream step required to remove unreacted cytotoxins and impurities, while maintaining Critical Quality Attributes and DAR. This step is most often carried out in Tangential Flow Filtration (TFF), with buffer exchange.

Data-Rich Experimentation and Modeling Approach

Providing properties of the TFF unit operation equipment (membrane area, channel diameter, channel length) along with experimental conditions (e.g., initial amounts per phase, feed rates) and physical properties (density, viscosity) allows addition of experimental data to the model to regress parameters describing membrane resistance, mass transfer coefficient, and maximum gel layer concentration.



Trans-membrane pressure (TMP)

$$TMP = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate}$$

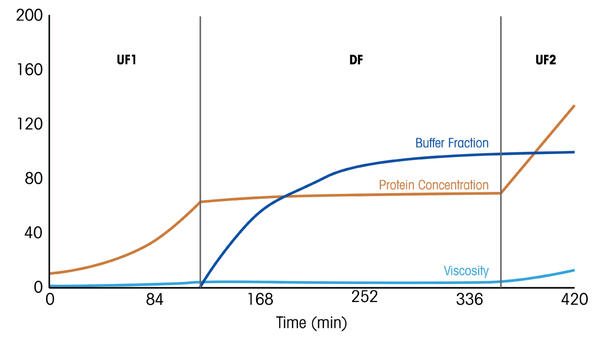
Flux across the membrane

$$J_{wall} = \frac{TMP}{\mu(R_m + R_c)}$$

Predictions Using TFF model

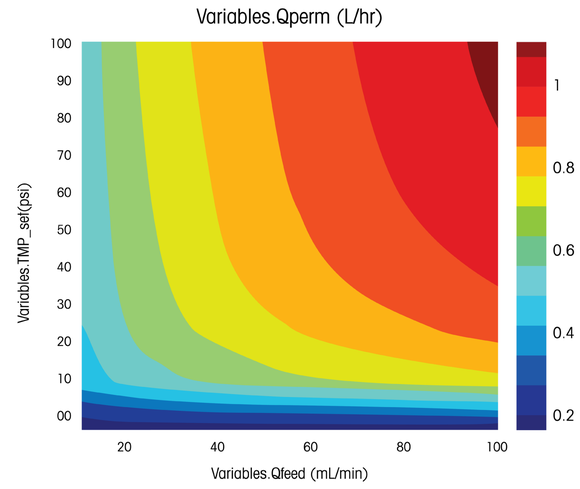
The TFF model can predict cycle time, protein concentrations, buffer fraction, optimum diavolumes (DVs), and pH drift (Gibbs-Donnan effect). This can be used to reduce the experimentation required at small scale, give optimal operating conditions, and predict scale-up behavior.

Diafiltration



Optimizing ADC TFF Process

The model can run virtual experiments in Dynochem's "Full Factorial" mode to find the optimum "knee point" TMP and feed flow rate, and similarly to find optimum concentration point to start diafiltration in UF1 DF UF2 mode. The optimized process can be scaled up to predict pressure profiles and run time.



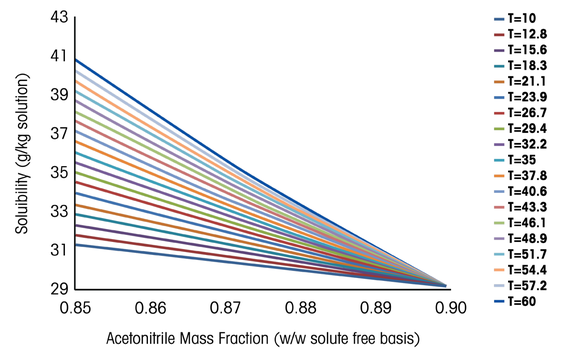
Using Modeling on Other Unit Operations

ADC synthesis offers numerous opportunities to leverage modeling for right-first-time scale-up of several unit operations. For example, by integrating knowledge of reactor and impeller geometries at both laboratory and production scale, scale-up can be achieved using the widely accepted principle of mixing equivalence, ensuring consistent power per unit mass from lab to full scale. Furthermore, incorporating insights into the reactor's heat transfer capabilities enables precise determination of feed rates and jacket temperatures, effectively managing adiabatic temperature rise during scale-up.

Bennett et al.[2] demonstrated simulation-based extrapolation of mixing parameters to ensure consistent multiphase mixing across scales, equipment, and reactor geometries. They quantified micro- and mesomixing—key to selectivity in sensitive reactions—governed by feed location, flow rate, and notably, local energy dissipation at the feed point. This energy dissipation was modeled at production scale and validated via scale-down experiments assessing feed time effects on selectivity.

Additionally, modeling has been effectively employed to analyze solubility dynamics in crystallization processes; by integrating experimental data within Dynochem, solubility models were constructed to guide compositional and process control decisions. Such mechanistic insight informs seeding strategies and antisolvent addition ratios, optimizing the formation of the target crystal during scale-up.

Improved Process Development and Scale-up Success



The combined kinetic and TFF modeling, with the use of simulation in crystallization and other unit operations, significantly reduces experimental burden while enhancing process understanding and yield outcomes. In the context of ADC processes, minimizing experimental runs is particularly critical to limit operator exposure to toxic materials inherent in these processes. To date, 15 ADC drugs have received regulatory approval, with a substantial pipeline of candidates advancing towards authorization, underscoring the growing importance of these methodologies in accelerating safe and efficient drug development.[3]

1. Nayak, S., & Richter, S. M. (2023). Kinetic Studies of the Partial Reduction and Conjugation Reactions in an Antibody-Drug Conjugate (ADC) Synthesis. Organic Process Research & Development, 27(11), 2091–2099. https://doi.org/10.1021/acs.oprd.3c00264
2. Bennett, N. B., Shen, X., Gates, B. D., Welch, D. S., Servos, M. A., Simanis, J. A., Ellis, R. G., Qiu, H., Moschetta, E. G., Feng, J., Boukerche, M., Rasmussen, M., McKee, L. A., Mlinar, L. B., Chen, S., & Wang, Z. (2024). Development and Execution of a Scalable Route to an Immunology ADC Drug-Linker for ABBV-154. Organic Process Research & Development, 28(7), 2499–2517. https://doi.org/10.1021/acs.oprd.4c00142
3. Biopharma PEG. (2019). FDA Approved Antibody-Drug Conjugates (ADCs) by 2025. Retrieved June 3, 2025, from https://www.biochempeg.com/article/74.html