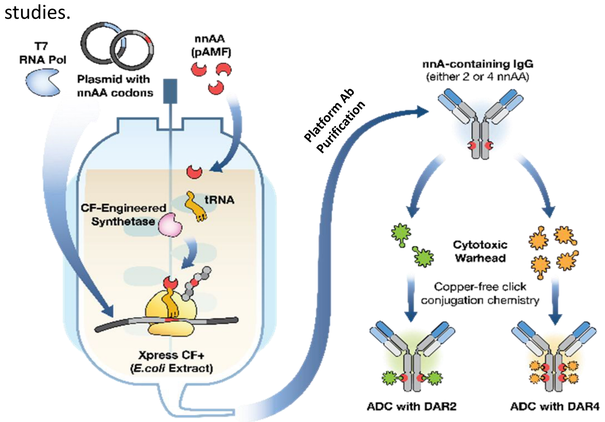
Process Development and Scale up for Site-Specific Bioconjugate Conjugation and Purification

Dharti Kothari, Karl Wessendorf, and Bob Kiss

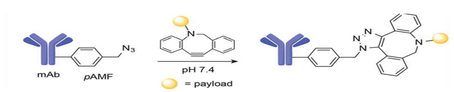
Downstream Process Development, Sutro Biopharma, Inc. South San Francisco, CA 94080

Abstract

Sutro's cell-free expression system based on *E. coli* extract has allowed production of antibodies and other proteins with non-natural amino acid incorporation (pAMF – para-azidomethyl-L-phenylalanine). This technology has allowed conjugation of linker warheads to pAMF, with nearly full site occupancy at only the specified sites. Evaluation of conjugation process parameters for 'Fast Kinetics' were performed for ADCs. Optimization of linker warhead to antibody molar ratio, protein concentration, pH, solvent and reaction time were performed to maximize drug to antibody ratio and minimize aggregation. The site-specific conjugation has enabled one-step purification by standard TFF. The TFF process was optimized for efficient flux, high yield, with various buffers for removal of free drug, solvent, and formulation. Rapid conjugation and high yield TFF processes were scaled up from bench scale to manufacturing scale for 100 – 350 g batches with successful tech transfer to a CMO for production of ADCs for GLP-Tox/phase 1 studies.



Expression of non-natural amino acid containing IgG by XpressCF+™ (XCF+ ™)



Scale Comparisons Conjugation → Formulation & Free Drug Removal (TFF)

PD Lab

Conjugation

Antibody Solution

T= 0 hr T = 4 hr

Reaction Mix (Ab + DL)

TFF – 30 KD Membrane

Spectrum KrosFlo Research Iii TFF System

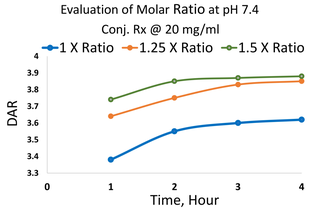
Manufacturing Scale

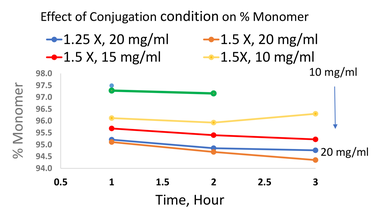
Conjugation

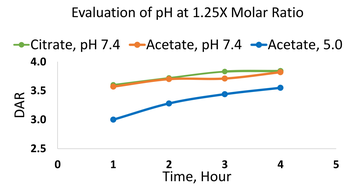
TFF – 30 KD Membrane

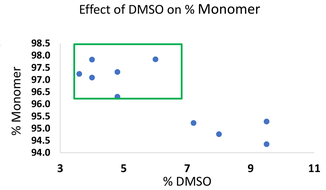
Millipore Mobius TFF System used at CMO

Evaluation of Conjugation Parameter – ADC 1



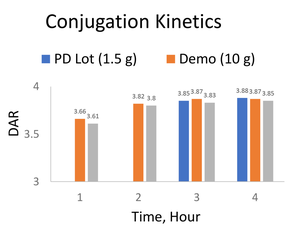


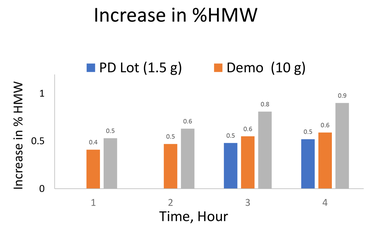




- 1.25 X Molar Ratio can give completion of reaction by 4 hr
- At pH 7.4 conjugation is completed by 4 hr with both Citrate & Acetate
- Reduction in % Monomer/Increase in % HMW observed
  - % DMSO
  - Protein Conc.
  - Reaction Time

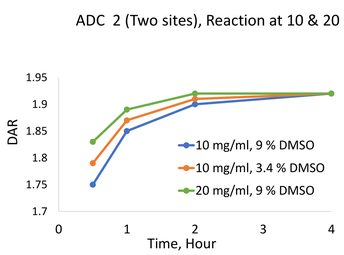
Scale up of Conjugation Process – ADC1

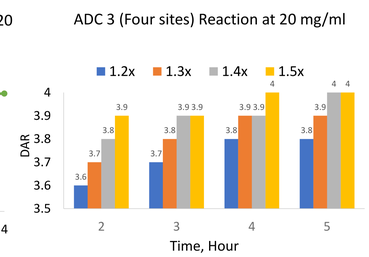




- Fast Conjugation Kinetics at all scale – DAR > 3.5 after 1st hour
- Moderate increase (0.4 to 0.9) in % HMW after 4 hour

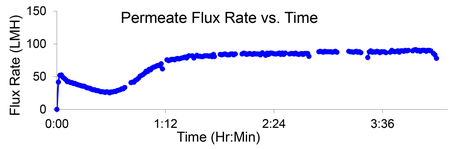
Conjugation Kinetics – ADC 2 and ADC 3

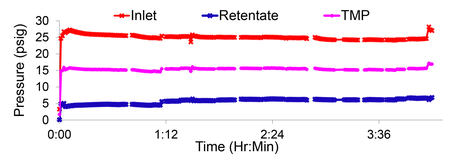




- Fast Conjugation Kinetics:
  - ADC 2 – DAR > 1.8 after 1st hour
  - ADC 3 – DAR > 3.5 after 2nd hour

Single Step Purification Free Drug and Solvent Removal TFF (30 KD) Process Performance

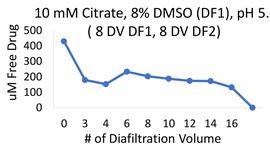


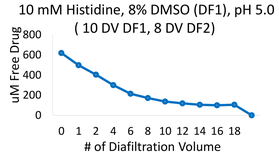


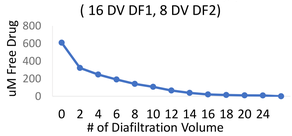
- TFF at 15 - 20 psi TMP and 360 LMH Feed flowrate
- Free drug removal (Diafiltration 1) and formulation (Diafiltration 2) in less than 5 hrs

Free Drug Removal - Buffer Evaluation

Scale Down (88 cm^2 membrane) ADC 1



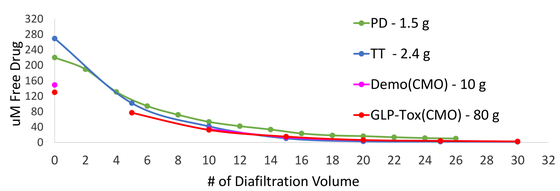




- Efficient removal of free drug in Histidine and Acetate buffers (DF1) with 10 -16 DV
- Formulation and residual DMSO removal in 8 DV of DF2 buffer

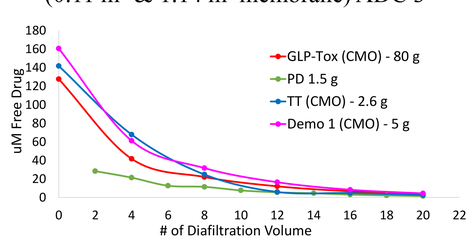
Free Drug Removal from Scale Up

(0.11 m^2 to 1.14 m^2 membrane) ADC 1

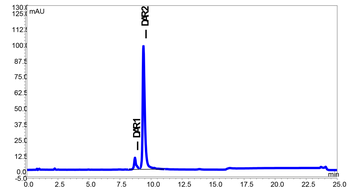


Free Drug Removal from Scale Up

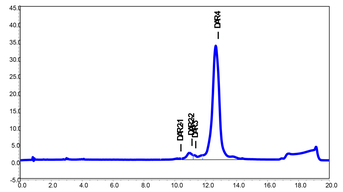
(0.11 m^2 & 1.14 m^2 membrane) ADC 3



DAR Profile (RP-HPLC) Purified ADC 2 and ADC 3



ADC with 2 sites - DAR 1.9



ADC with 4 sites - DAR 3.9

Product Quality and Yield at various Scale

	ADC 1 (4 sites)		ADC 2 (2 sites)			ADC 3 (4 sites)		
Batch Size, g	9	80	38	95	350	5.2	78	92
Overall Process Yield	85%	87%	97%	97%	100%	100%	81%	84%
DAR	3.87	3.9	1.9	1.9	1.9	3.9	3.8	3.8
% Monomer (SEC)	98.4	97	99.5	99.4	99.7	97.9	96.2	97.8
Residual Free Drug, (% Free to Bound)	0.7%	0.7%	0.3%	0.3%	0.3%	0.5%	0.4%	0.2%
Residual DMSO, ppm	1077	452	36	1644	82	371	138	20

Summary

- Fast Conjugation Kinetics with multiple products
- Site specific conjugation allows production of ADCs with desired DAR species, which eliminates need for chromatography for removal unwanted DAR species
- Single step purification (TFF) performed to remove residual free drug and formulate Drug Substance
- Successful scale up at CMO with larger batch sizes for process parameters developed in PD lab for both conjugation and TFF with acceptable product quality and high process yield

Acknowledgement

- Upstream Process Development, Sutro Biopharma Inc.
- Downstream Process Development, Sutro Biopharma Inc.
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- CMO for ADC production

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