Design of Experiments for early phase process development of Antibody Drug Conjugates

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06 May 2021

Piramal Pharma Solutions

SARTORIUS

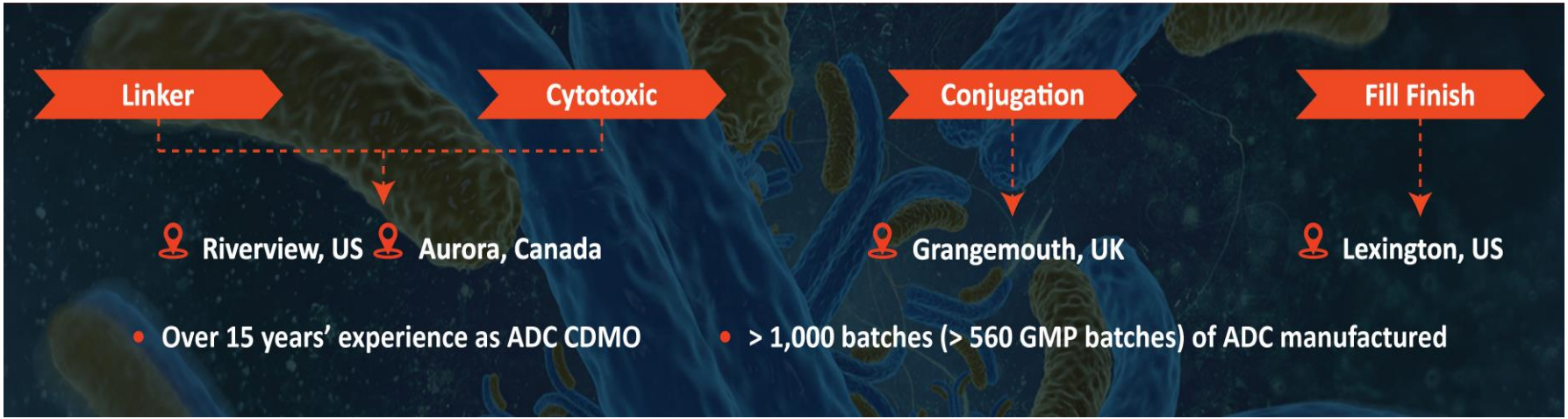
- Antibody
- DOE software MODDE expertise

- Drug Linker
- Bioconjugation expertise

- Introduction to Piramal bioconjugation services
- Introduction to ADC Development
- Case Study: Process development of an ADC Reactive stage
    - Process
    - Considerations for DOE
    - Parameters/responses
- Design Of Experiments

- Piramal Grangemouth is a world leading Bio-conjugation CDMO
- >15 years experience in Antibody Drug Conjugation and Bio-conjugation
- Support Proof of Concept (milligram scale) through to Bulk Drug Substance Commercial manufacturing
- Highly skilled workforce across Development, Manufacturing and Quality Units

- Supply chain simplification for ADCs & bioconjugates



- Piramal GMP manufacture:
  - 40 distinct conjugates
  - 2 commercial products

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Introduction to ADC Development

- Develop scientifically sound analytical methods suitable to support pre-clinical and ultimately, clinical release and stability testing of ADC
- Develop process conditions to meet key quality attributes for the ADC
- Have sufficient understanding of process robustness to enable safe scale-up
- Establish control strategy

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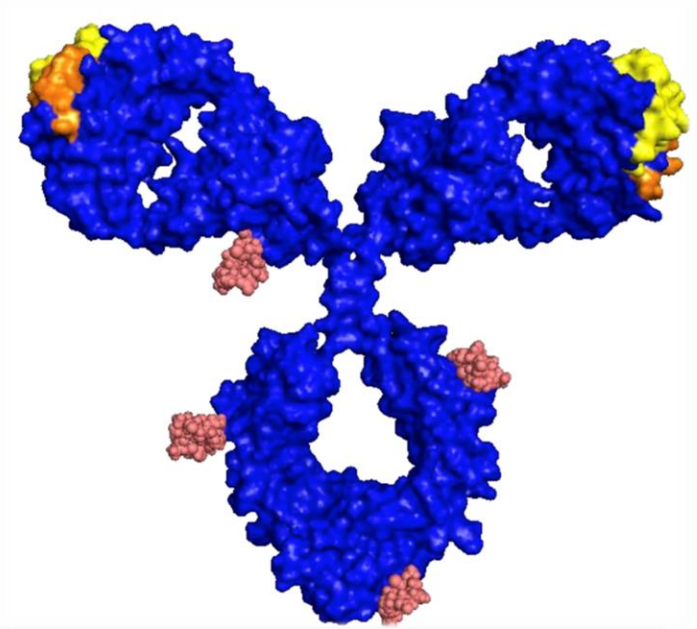
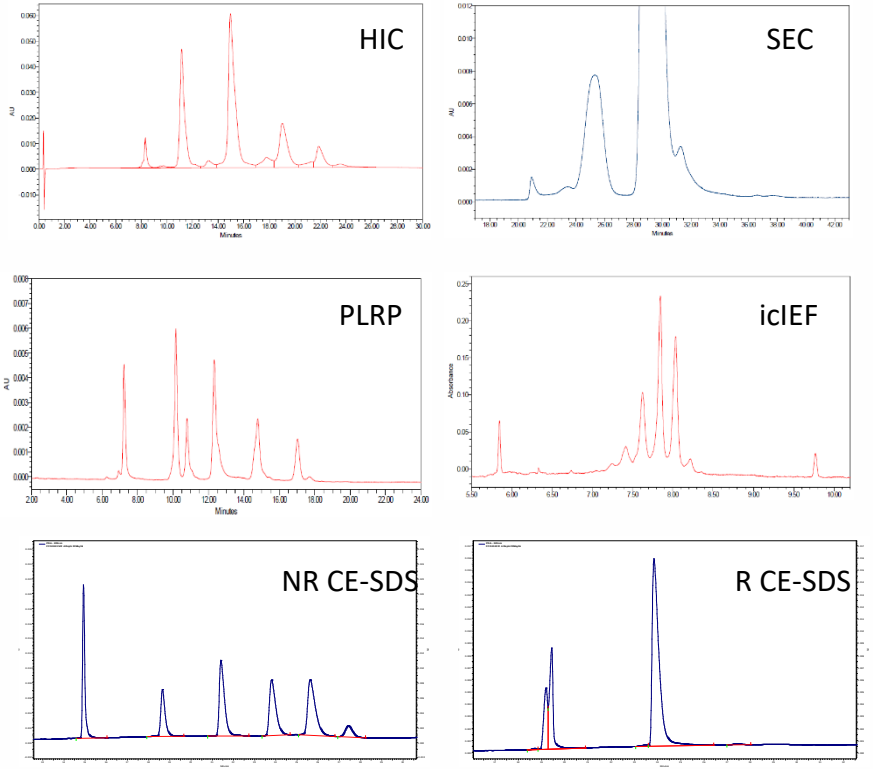
key components:

- Monoclonal antibody highly specific to target cell antigen
- Anticancer drug (payload) highly potent for cell killing activity
- Linker to covalently join payload to antibody



" ADCs Bring together the best features of
Antibodies &
Cytotoxic drugs "

- Analytical complexity: antibody + payload + conjugate
- Methods developed immediately for key quality attributes: SEC, DAR and distribution (HIC, PLRP) and icIEF to support quick Process Development start
- Free drug and CE-SDS (R+NR) also initiated at early stage



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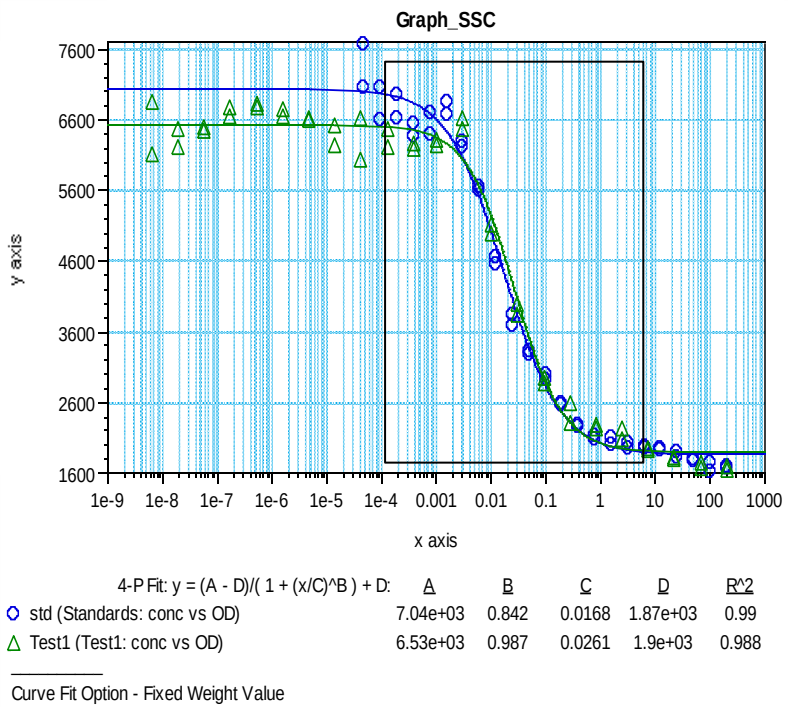
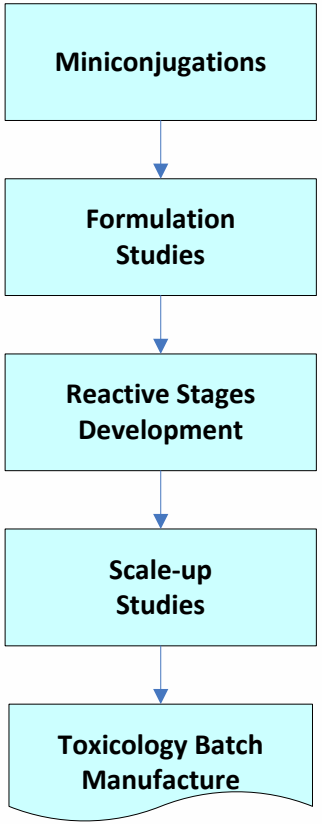
- Antibody Functional assays
  - ELISA (binding)
  - Potency (cell killing assay)
  - Effector functions

- Significant development required
  - Methods development starts early





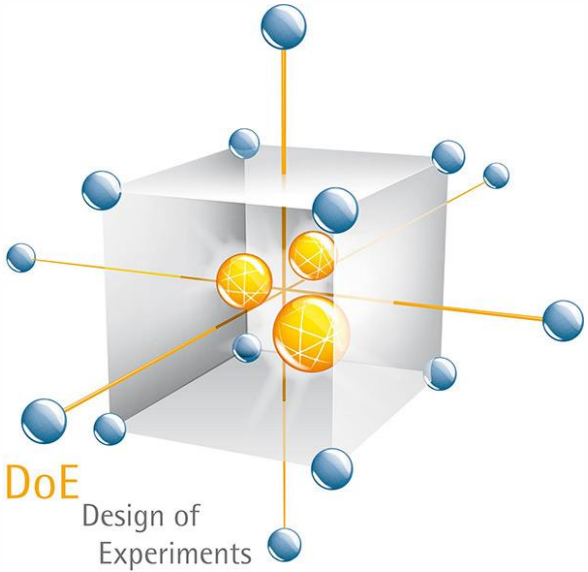
- 5 Stages of development to go to GMP
- Approach designed to speed up overall program
  - (Analytical Development throughout Process Development)
  - Formulation Studies & Reactive Stages run in parallel
- Specific set of activities for each stage
- Final process with purification tested at scale prior to GMP manufacture

Case Study :

Use of DOE to develop the reactive stages of an ADC process

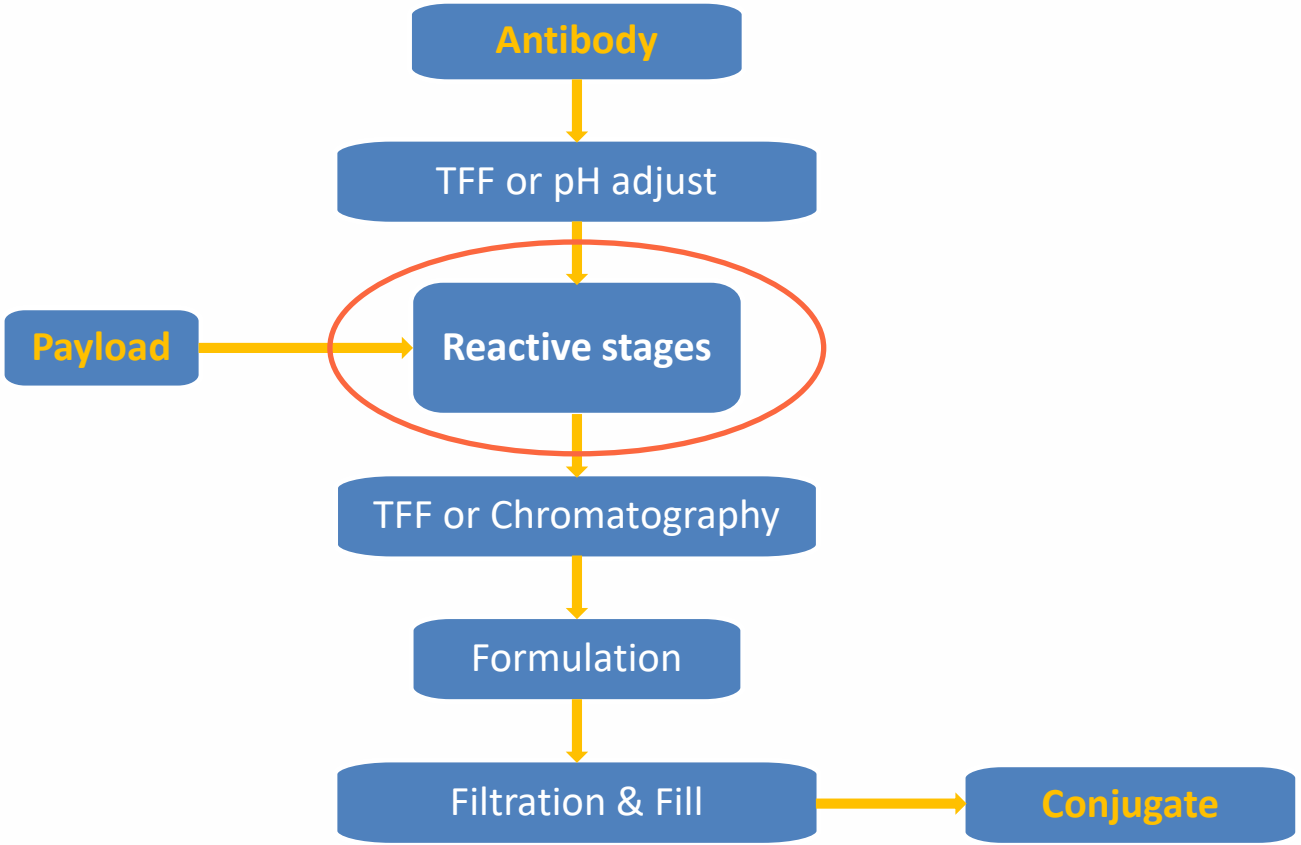
Why DoE?

- Maximizes the information content while keeping the number of experiments low
- Allows systematic assessment of effect of multiple Factors (and their interactions) on Responses
  Factor = Process Parameter
  Response = Critical Quality Attribute
- Allows definition of a “Design Space” – safe operating conditions with CQAs meeting targets/ranges.

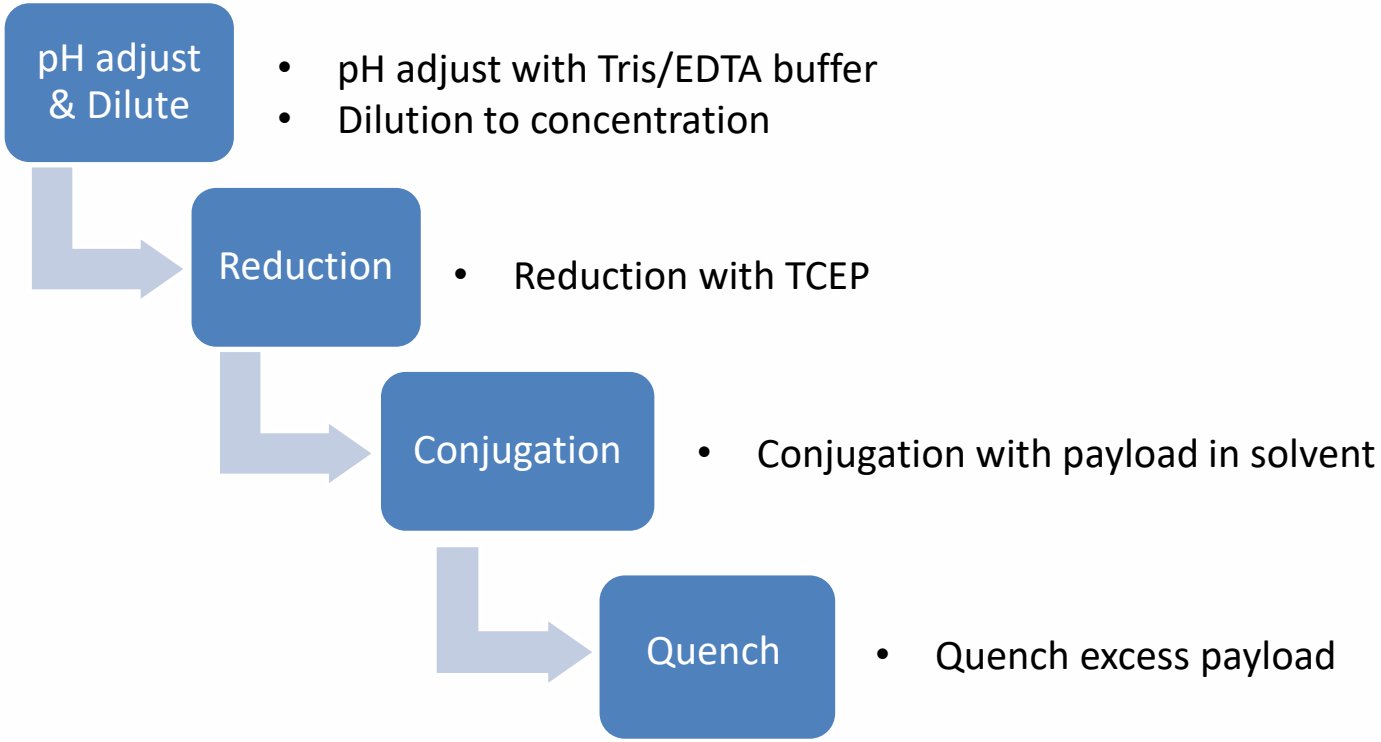


When and where ?

- DoE as an early Development tool to support ADC analytical & process development
- DOE for later phases to define the Design Space for parameters during pre-commercial Process Characterisation studies


Antibody
TFF or pH adjust
Payload
Reactive stages
TFF or Chromatography
Formulation
Filtration & Fill
Conjugate

Reactive stages



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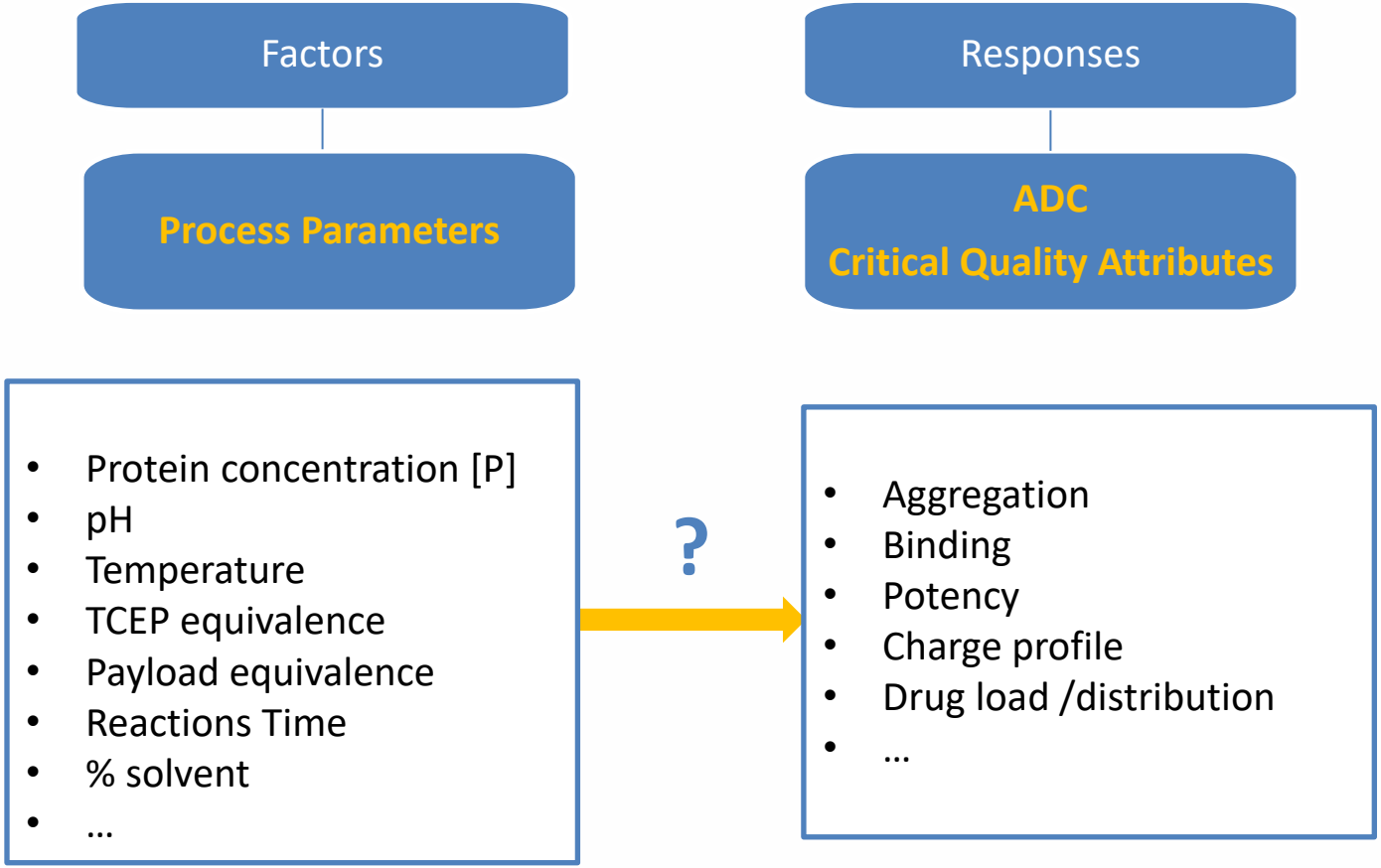
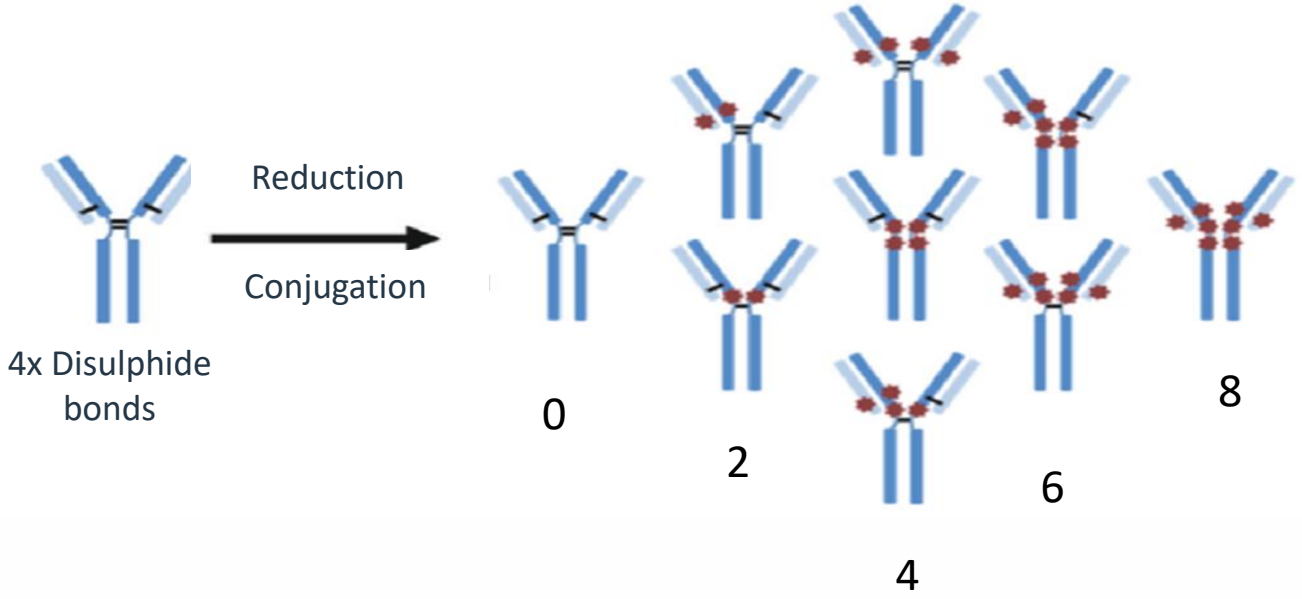

Factors
Process Parameters
Responses
ADC
Critical Quality Attributes
• Protein concentration [P]
• pH
• Temperature
• TCEP equivalence
• Payload equivalence
• Reactions Time
• % solvent
• …
?
• Aggregation
• Binding
• Potency
• Charge profile
• Drug load /distribution
• …



- Heterogeneous mixture of species
- Different number of “Drug or Payload” per molecule of Antibody

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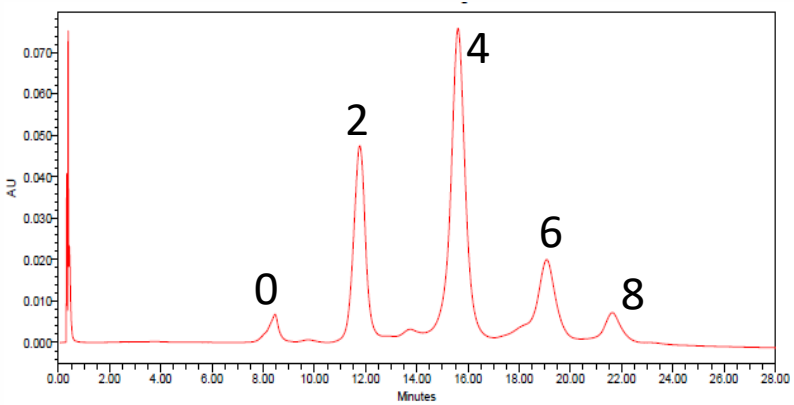
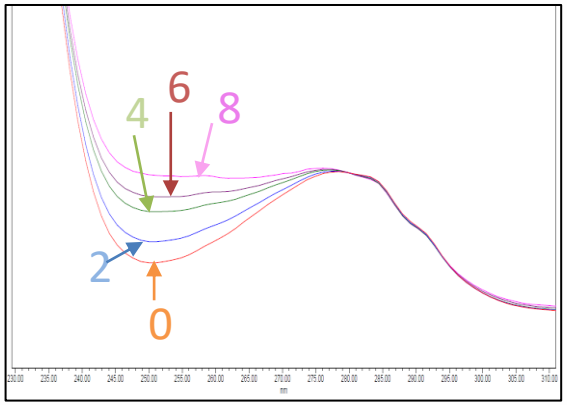
- Hydrophobic interaction (HIC) spectrum :
  - Distribution
  - Unconjugated antibody
  - Average DAR





Average DAR – response for our study

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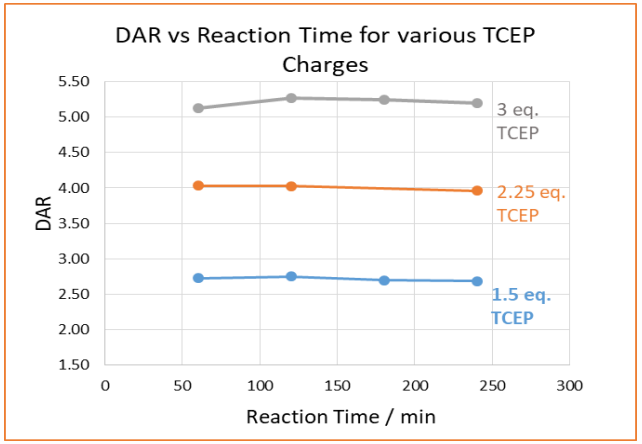
Considerations prior to DOE

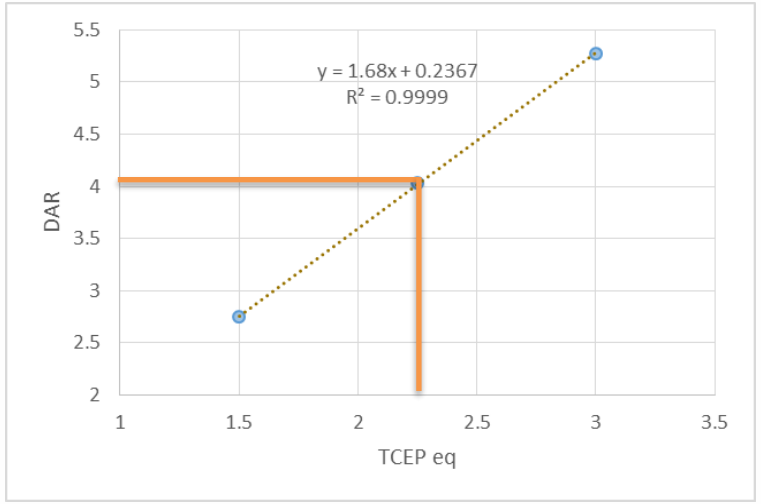
- Good analytics
- Parameters selection and range
  - Prior knowledge
  - Scouting experiments
  - Manufacture fit
- Statistical Design selection
- Preparation of input materials to design (eg pH)
- Use of scale-down model

TCEP equ.	Time (h)	DAR	Time (h)	DAR
1.5	1	2.73	2	2.75
	3	2.70	4	2.69
2.25	1	4.03	2	4.03
	3	2.21*	4	3.96
3	1	5.12	2	5.27
	3	5.25	4	5.19

* undercharge of payload



- Factor evaluation :
  - TCEP
  - time



TCEP-DAR relationships

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- Proposed DOE Factors and ranges :

Factors				
Name	**Units**	**Low**	**High**	**Control Range (±)**
Protein Conc.	mg/mL	5	15	1
Temperature	°C	16	26	2
pH		6.8	7.8	0.2
Reduction Time	min	60	180	30

Response				
Name	**Abbrev.**	**Min**	**Target**	**Max**
Drug Antibody Ratio	DAR	3.4	3.9	4.4

- Checked ‘extremes’ of proposed ranges :
  - All ‘LOW’ versus All ‘HIGH’
  - Significant DAR variation 2.9 & 4.1 ☺