Process Characterization for New Modalities Using Design of Experiments (DoE)

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What is the Goal of Process Characterization

The typical goal of a Process Characterization Strategy is to identify process parameters that impact product quality

- Identifying interactions between process parameters and critical quality attributes
- Justifying or adjusting manufacturing operating ranges and acceptance criteria
- Ensuring that the process delivers a product with reproducible yields and quality
- Detection of manufacturing issues using an established control strategy and knowledge gained during process

Successful Process Characterization – A How-to-Guide in 7 Steps. Accessed 9/20/2022. https://ispe.org/pharmaceutical-engineering/ispeak/successful-process-characterization-how-guide-7-steps

What is the Goal (continued)

- Efficient experimentation
- Risk-based decision making
- Establishing a model-based control strategy
- Process knowledge and assurance of quality--quality built in!

Image Adapted from Freepik

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What are the Steps in Process Characterization?

1. Perform scale down model (SDM) qualification to detect offsets between scales
2. Gather Subject Matter Experts (SMEs)
3. Conduct a risk assessment (FMEA) to incorporate prior knowledge
4. In parallel to FMEA it is possible to start investigating scale down model qualification to be ready for experiments
5. Plan and conduct efficient experiments to identify impact of process parameters (PPs) on critical quality attributes (CQAs)
6. Model Based definition of a control strategy (e.g. PAR) for individual unit operations
7. Results of purposefully planned experiments can be used to construct overall, integrated process models and set up optimal control strategies (PARs and design space, if applicable) with least possible restriction for manufacturing

Successful Process Characterization – A How-to-Guide in 7 Steps. Accessed 9/20/2022. https://ispe.org/pharmaceutical-engineering/ispeak/successful-process-characterization-how-guide-7-steps

Gather Subject Matter Experts

- Upstream Process Development
- Downstream Process Development
- Analytical/QC
- Manufacturing
- CMC Statistics
- Regulatory/QA

Image Adapted from Freepik

Conduct Risk Assessments--Upstream Risk Assessment

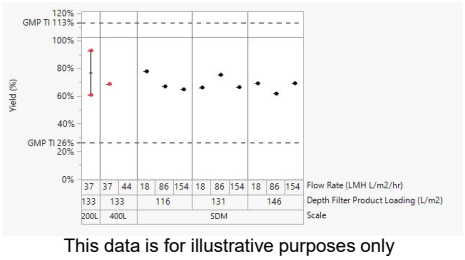
CQA	Unit Operations 1	Unit Operations 2	Unit Operations 3	Unit Operations 4	Unit Operations 5
Cell Health Measure 1	Low	Medium	High	Low	Low
Cell Health Measure 2	Low	Medium	High	Low	Low
Yield	Low	Medium	High	High	High
Growth Time	Low	Medium	High	Low	Low
Process Impurity 1	Low	Low	Low	Medium	Medium
Identity	Low	Low	Low	Medium	Low
Process Impurity 2	Low	Low	Low	Medium	Medium
Purity	Low	Low	Low	Medium	Medium
Potency	Low	Low	Low	Medium	Medium
Process Impurity 3	Low	Low	Low	Low	Medium
Process Impurity 4	Low	Low	Low	Low	Medium
Process Impurity 5	Low	Low	Low	Low	Medium
Process Impurity 6	Low	Low	Low	Low	Medium

Conduct Risk Assessments--Downstream Risk Assessment

CQA	Unit Operations 6	Unit Operations 7	Unit Operations 8	Unit Operations 9	Unit Operations 10
Yield	High	High	High	High	High
Titer	Low	Low	Low	High	Medium
Appearance	Medium	Medium	Low	Low	Low
pH	Low	Low	Low	High	Low
Osmolality	Low	Low	Low	High	Low
Impurity Concentration	Low	Low	Low	High	High
Process Impurity 1	Medium	Medium	Medium	High	Medium
Process Impurity 2	N/A	N/A	N/A	N/A	N/A
Process Impurity 3	Low	Medium	High	Low	Low
Purity	Low	High	Low	Low	Low
Process Impurity 4	Low	High	Low	Low	Low
Process Impurity 5	Low	High	Low	Low	Low
Process Impurity 6	Low	High	Low	Low	Low
Process Impurity 7	Low	High	Medium	Low	Low
Process Impurity 8	Low	High	Low	Low	Low
Process Impurity 9	Low	High	High	Medium	Low
Process Impurity 10	Low	High	Medium	Medium	Low
Process Impurity 11	Low	High	Low	Low	Low
Process Impurity 12	Low	High	Low	Low	Low
Process Impurity 13	Low	High	Low	Low	Low
Potency	Low	Medium	Medium	Low	Low

Investigate Scale Down Models (SDM)

Because the Process Characterization will be done in low/small volume (AMBR reactors, high thru-put filter tubes) and not at scale, a scale down model should be well understood.
This includes having data at manufacturing scale and running a small design at small scale and comparing them either graphically or statistically.



This data is for illustrative purposes only

Development, Qualification, and Application of a Bioreactor Scale-Down Process. Accessed 9/20/2022. https://bioprocessintl.com/upstream-processing/expression-platforms/development-qualification-and-application-of-a-bioreactor-scale-down-process-modeling-large-scale-microcarrier-perfusion-cell-culture

Scale Down Model Qualification

- To develop SDMs for all unit operations:
  - Engineering principles were used to scale down material volumes, equipment and scale-dependent process parameters
  - DOEs were carried out varying, primarily, scale-dependent process parameters to investigate scale effects

- SDM Qualification
  - Goal was to leverage SDM models to predict performance at target conditions and compare that prediction to at-scale data
  - Multiple quality assessment criteria were discussed
    - TOST (two one-sided t-tests) for equivalence of scales
    - SDM (near) target conditions falling within MFG Data Tolerance Intervals (95/90)
    - Consideration of Practically Significant Difference (PSD) between MFG and SDM (near) target data
      - PSD = 2 x Standard Deviations of MFG data for each response

- Obstacles
  - Need for partial understanding of CQA/success criteria at SDM development phase along with investigating scale effects; qualifying the model on too few success criteria could impact PC success. Changes in scale impacted PC timelines

Upstream SDM Objective: Achieve a representative model of large-scale performance in a small-scale bioreactor

- Unit Operations 1
  - Parameter 1
  - Parameter 2
- Unit Operations 2
  - Parameter 1
  - Parameter 2

Image: Accessed 9/20/2022.https://www.sartorius.com/en/products/fermentation-bioreactors/ambr-multi-parallel-bioreactors/ambr-250-high-throughput

Downstream Scale Down Model Development

- Unit Operations 3
  - Parameter 1
  - Parameter 2
  - Parameter 3
- Unit Operations 4
  - Parameter 1
  - Parameter 2
  - Parameter 3

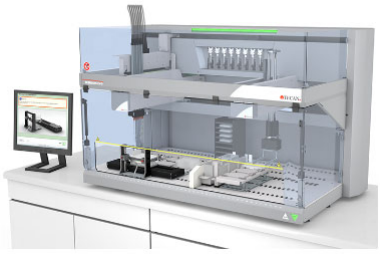
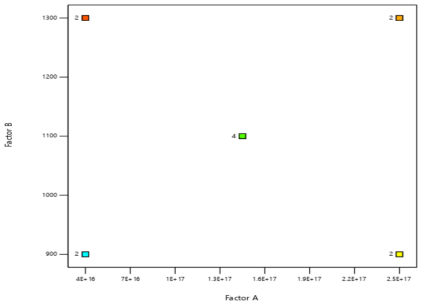
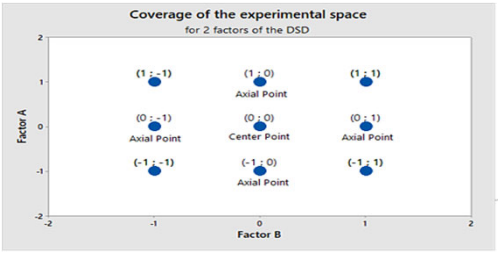


Image: Accessed 9/20/2022. https://www.tecan.com/protein-purification-package-offer

Strategy for Process Characterization Design





Fractional Factorial designs (FF)
- Resolution IV or higher
- Main effects are confounded with higher level interactions
- Easy to interpret
- Can accommodate up to 9 factors in less than 20 runs with center points
- Easy to augment to tease out curvature

Definitive Screening designs (DSD)
- Resolution IV like designs
- Main effects are not confounded
- Can be more difficult to interpret
- Can accommodate more than 9 factors in not too many runs
- Not as easy to augment to tease out important effects
  - Especially when 2 factor interactions are present
- Sparsity of effects is assumed

A general design strategy was to use FF unless the number of factors under study was prohibitive

This data is for illustrative purposes only

Voelkel J. *Technometrics*. 2005; 47(4):488-494.
Center and Axial Points. Accessed 9/20/2022. https://blog.minitab.com/en/bruno-scibilia/definitive-screening-designs-for-products-and-processes-optimization
Factorial and Fractional Factorial Designs. Accessed 9/20/2022. https://support.minitab.com/en-us/minitab/21/help-and-how-to/statistical-modeling/doe/supporting-topics/factorial-and-screening-designs/factorial-and-fractional-factorial-designs/

Plan and Conduct Efficient Experiments

For the unit operations under study, the final designs were determined.

Unit Op	1	2	3	4	5	6	7	8	9
No. Experiments	8	20	22	20	5-10	20	20	12	3-5
No. Factors	3	5	10	6	3	6	5	5	2
Design Type	2^3 Full Factorial	2^{5-1} Res V Frac Factorial	10 Factor DSD	2^{5-1} Res V Frac Factorial	2^3 Full Factorial	2^{6-2} Res IV Frac Factorial	2^{5-1} Res V Frac Factorial	2^{5-1} Res V Frac Factorial	Worst Case

Unit Operations 1

Std	Block	Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
11	{ 1 }	1	1	1	-1	1	-1	1
8	{ 1 }	2	1	-1	1	-1	-1	1
14	{ 1 }	3	-1	-1	1	1	1	1
1	{ 1 }	4	-1	-1	-1	-1	-1	-1
18	{ 1 }	5	0	0	0	0	0	0
7	{ 1 }	6	1	-1	-1	1	1	-1
13	{ 1 }	7	-1	1	1	1	-1	-1
17	{ 1 }	8	1	1	1	-1	1	-1
12	{ 1 }	9	-1	1	-1	-1	1	1
2	{ 1 }	10	0	0	0	0	0	0
6	{ -1 }	11	1	1	1	1	1	1
16	{ -1 }	12	-1	-1	1	-1	1	-1
4	{ -1 }	13	-1	1	-1	1	1	-1
20	{ -1 }	14	-1	1	1	-1	-1	1
5	{ -1 }	15	1	1	-1	-1	-1	-1
3	{ -1 }	16	1	-1	1	1	-1	-1
19	{ -1 }	17	0	0	0	0	0	0
9	{ -1 }	18	0	0	0	0	0	0
15	{ -1 }	19	-1	-1	-1	1	-1	1
10	{ -1 }	20	0	-1	-1	-1	1	1

Unit Operations 2

Std	Block	Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
1	AR1	1	0	-1	1	1	-1	1	-1	-1	-1
9	AR1	2	-1	-1	-1	1	-1	-1	-1	1	0
8	AR1	3	-1	-1	1	0	1	1	1	1	-1
3	AR1	4	1	1	1	-1	1	1	1	-1	0
6	AR1	5	0	0	0	0	0	0	0	0	0
2	AR1	6	-1	1	-1	-1	-1	1	0	1	-1
4	AR1	7	1	1	-1	0	-1	-1	-1	-1	1
11	AR1	8	0	1	-1	-1	1	-1	1	1	1
5	AR1	9	1	-1	-1	1	0	1	1	1	1
10	AR1	10	1	-1	1	1	1	-1	0	-1	1
7	AR1	11	-1	1	1	-1	0	-1	-1	-1	-1
23	AR1	12	0	0	0	0	0	0	0	0	0
22	AR2	13	1	0	1	-1	-1	1	-1	1	1
21	AR2	14	-1	1	0	1	-1	1	1	-1	1
18	AR2	15	-1	-1	-1	-1	1	1	-1	-1	1
15	AR2	16	-1	1	1	1	1	0	-1	1	1
19	AR2	17	1	-1	-1	-1	-1	0	1	-1	-1
13	AR2	18	1	-1	0	-1	1	-1	-1	1	-1
14	AR2	19	-1	-1	1	-1	-1	-1	1	0	1
20	AR2	20	1	1	-1	1	1	1	-1	0	-1
17	AR2	21	0	0	0	0	0	0	0	0	0
12	AR2	22	-1	0	-1	1	1	-1	1	-1	-1
24	AR2	23	0	0	0	0	0	0	0	0	0
16	AR2	24	1	1	1	1	-1	-1	1	1	-1

Unit Operations 3

Std	Block	Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
18	Amber Run 1	1	0	0	0	0	0	0
17	Amber Run 1	2	0	0	0	0	0	0
10	Amber Run 1	3	1	-1	-1	1	1	-1
12	Amber Run 1	4	1	1	-1	1	-1	1
8	Amber Run 1	5	1	1	1	-1	1	-1
3	Amber Run 1	6	-1	1	-1	-1	1	1
15	Amber Run 1	7	-1	1	1	1	-1	-1
13	Amber Run 1	8	-1	-1	1	1	1	1
6	Amber Run 1	9	1	-1	1	-1	-1	1
1	Amber Run 1	10	-1	-1	-1	-1	-1	-1
21	Amber Run 1	11	0	0	0	0	0	0
14	Amber Run 2	12	1	-1	1	1	-1	-1
11	Amber Run 2	13	-1	1	-1	1	1	-1
19	Amber Run 2	14	0	0	0	0	0	0
20	Amber Run 2	15	0	0	0	0	0	0
2	Amber Run 2	16	1	-1	-1	-1	1	1
5	Amber Run 2	17	-1	-1	1	-1	1	-1
9	Amber Run 2	18	-1	-1	-1	1	-1	1
7	Amber Run 2	19	-1	1	1	-1	-1	1
16	Amber Run 2	20	1	1	1	1	1	1
4	Amber Run 2	21	1	1	-1	-1	-1	-1
22	Amber Run 2	22	0	0	0	0	0	0

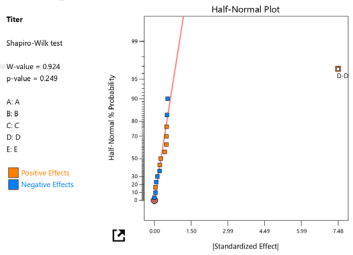
Unit Operations 4

Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
1	-1	-1	-1	-1	-1	-1
2	0	0	0	0	0	0
3	1	1	-1	-1	1	1
4	0	0	0	0	0	0
5	-1	1	1	-1	-1	1
6	1	-1	-1	1	1	-1
7	-1	-1	1	-1	1	1
8	1	1	-1	1	-1	-1
9	-1	1	-1	-1	1	-1
10	-1	1	-1	1	-1	1
11	-1	1	1	1	1	-1
12	1	-1	-1	-1	-1	1
13	-1	-1	-1	1	1	1
14	-1	-1	1	1	-1	-1
15	1	1	1	1	1	1
16	1	-1	1	1	-1	1
17	1	1	1	-1	-1	-1
18	1	-1	1	-1	1	-1

Unit Operations 5

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
1	1	-1	-1	-1	-1	1
7	2	-1	1	1	-1	1
6	3	1	-1	1	-1	1
11	4	-1	1	-1	1	1
13	5	-1	-1	1	1	1
4	6	1	1	-1	-1	1
16	7	1	1	1	1	1
8	8	1	1	1	-1	-1
15	9	-1	1	1	1	-1
18	10	0	0	0	0	0
14	11	1	-1	1	1	-1
10	12	1	-1	-1	1	1
17	13	0	0	0	0	0
20	14	0	0	0	0	0
3	15	-1	1	-1	-1	-1
2	16	1	-1	-1	-1	-1
19	17	0	0	0	0	0
5	18	-1	-1	1	-1	-1
12	19	1	1	-1	1	-1
9	20	-1	-1	-1	1	-1

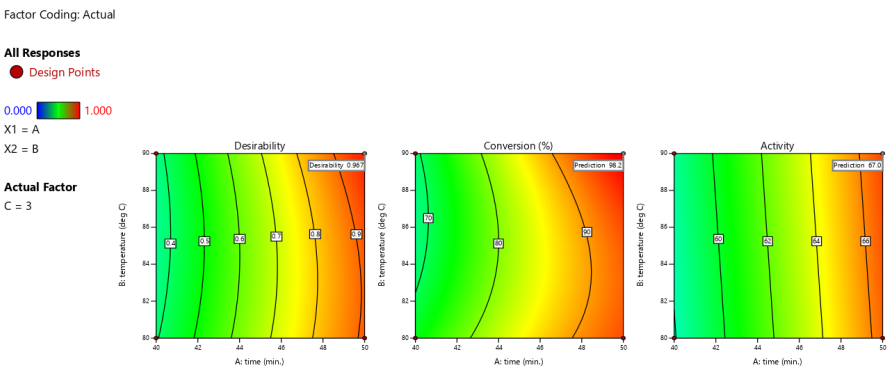
Build Models to Understand Which Factors Effect Quality Measures



Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.54	1	3.54	140.05	< 0.0001	significant
D	3.54	1	3.54	140.05	< 0.0001	
Residual	0.4549	18	0.0253			
Lack of Fit	0.4316	15	0.0288	3.71	0.1535	not significant
Pure Error	0.0232	3	0.0077			
Cor Total	3.99	19				

Hypothetical data. These are for illustrative purposes only.

Graph Optimized Settings for Each Unit Operation



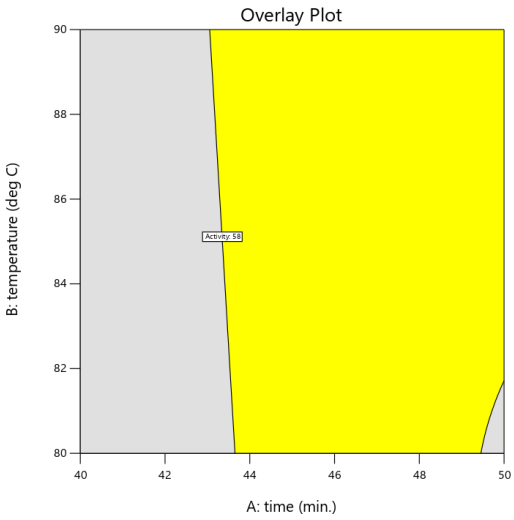
Hypothetical data. These are for illustrative purposes only.

Understand Operating Ranges

Factor Coding: Actual

Overlay Plot
Conversion
Activity
X1 = A
X2 = B

Actual Factor
C = 2.32



Hypothetical data. These are for illustrative purposes only.

What Worked Well

- Excellent collaborations between Manufacturing, Process Development, Analytical, QC and Statistics
- Comprehensive training program that included FMEA and Design
- Planning and executing of designs
- Triaging priorities for analytical testing
- New modalities benefit from DoE just as well as any other

What Could Be Improved

- Early development work to inform Process Characterization work
- Training in DoE (Design Expert is a great tool!)
- Understanding the science better for new modalities

Take Home Message

- A strong commitment to QBD can aid process development.
- Process Characterization is where development reaches a crescendo, but without QBD the symphony is a mess of different parts.

Acknowledgements

- Laura Adams
- Julian Peters
- George Atkins

References

www.statease.com
https://www.jmp.com/en_us/home.html