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# Deploying DOE to Accelerate R&D for Biotech

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## Maximizing this educational opportunity

Welcome everyone! To make the most from this webinar:

- Attendees on mute
- Chat addressed afterward
- Send further questions to mark@statease.com

PS Presentation posted to www.statease.com/webinars/

*Please press the raise-hand button if you are with me.*



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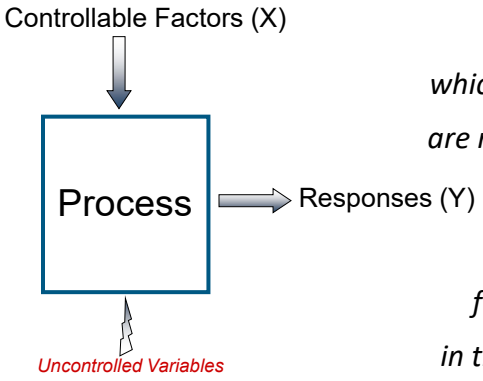
## DOE Works on Any Process




**DOE is**:

*"A series of tests, in which purposeful changes are made to input factors, to identify causes for significant changes in the output responses."*



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## Multi-Factorial (VS OFAT)
(life from accelerated test)




*Start point for One Factor at a Time (OFAT)*

*Relative efficiency = 16/8*

*2 to 1!*

*"To make knowledge work productive will be the great management task of this century."*
-- Peter Drucker



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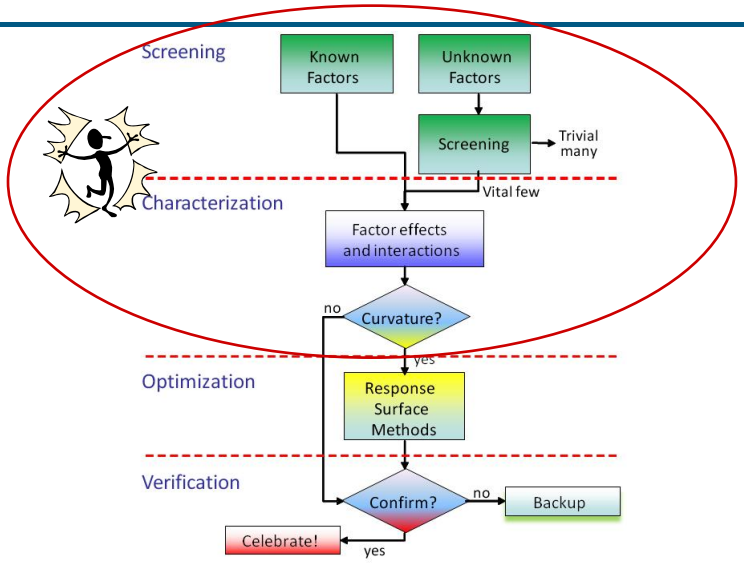
## Strategy of Experimentation

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## Screening/Characterization

Purpose: Quickly sift through a large number of potential factors to discard the trivial many. Then follow-up with an experiment that focuses on the vital few.

Tool: Two-level factorial designs:

1. Fractional for resolving main effects in minimal runs.
2. Full (or less fractional) to resolve two-factor interactions.

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## Screening Case Study

A research scientist at a biotechnology firm reports* the use of DOE in pilot studies on a pharmaceutical made via recombinant E. coli fermentation. He screened six factors:

A. Culture-medium temperature
B. Fermentation-medium pH
C. Dissolved oxygen concentration (DO)
D. Induction optical density (OD) – a measure of cell density
E. Feed rate of proprietary complex solution
F. Feed rate of inducer solution

The primary response was harvest OD – a measure of cell-growth.

*(Arun Tholudur, et al, "Using Design of Experiments to Assess Escherichia Coli Fermentation Robustness," *BioProcess* – posted at www.statease.com/pubs/fermentationrobustness.pdf.)



## Full and Fractional Two-Level Designs

A 'happy medium' for screening 6 factors: adequate resolution with decent power (n=8 high vs low).

Factors

| Runs | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Full | 1/2 Fract. | | | | | | | | | | | | |
| 8 | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | | | | | | | | |
| 16 | | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | 1/32 Fract. | 1/64 Fract. | 1/128 Fract. | 1/256 Fract. | 1/512 Fract. | 1/1024 Fract. | 1/2048 Fract. |
| 32 | | | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | 1/32 Fract. | 1/64 Fract. | 1/128 Fract. | 1/256 Fract. | 1/512 Fract. | 1/1024 Fract. |
| 64 | | | | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | 1/32 Fract. | 1/64 Fract. | 1/128 Fract. | 1/256 Fract. | 1/512 Fract. |
| 128 | | | | | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | 1/32 Fract. | 1/64 Fract. | 1/128 Fract. | 1/256 Fract. |
| 256 | | | | | | | Full | 1/2 Fract. | 1/4 Fract. | 1/8 Fract. | 1/16 Fract. | 1/32 Fract. | 1/64 Fract. | 1/128 Fract. |

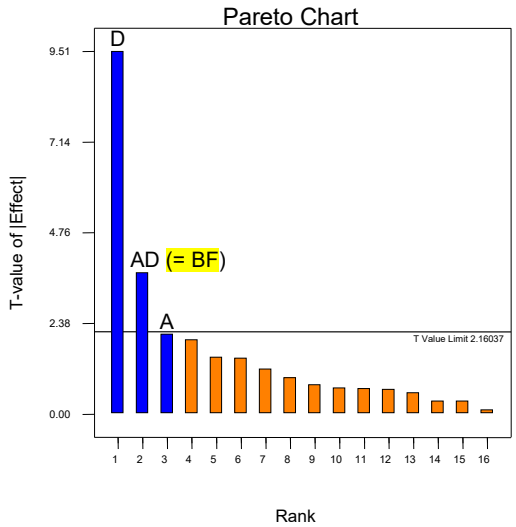
## Experimental design template

(1/4th fraction of $2^6$ via std design with E=ABC and F=ABD)

| Std | A: Temp. | B: pH | C: DO | D: In. OD | E: Soln 1 | F: Soln 2 |
|---|---|---|---|---|---|---|
| 1 | Lo (–) | Lo (–) | Lo (–) | Lo (–) | Lo (–) | Lo (–) |
| 2 | Hi (+) | Lo (–) | Lo (–) | Lo (–) | Hi (+) | Hi (+) |
| 3 | Lo (–) | Hi (+) | Lo (–) | Lo (–) | Hi (+) | Hi (+) |
| 4 | Hi (+) | Hi (+) | Lo (–) | Lo (–) | Lo (–) | Lo (–) |
| 5 | Lo (–) | Lo (–) | Hi (+) | Lo (–) | Hi (+) | Lo (–) |
| 6 | Hi (+) | Lo (–) | Hi (+) | Lo (–) | Lo (–) | Hi (+) |
| 7 | Lo (–) | Hi (+) | Hi (+) | Lo (–) | Lo (–) | Hi (+) |
| 8 | Hi (+) | Hi (+) | Hi (+) | Lo (–) | Hi (+) | Lo (–) |
| 9 | Lo (–) | Lo (–) | Lo (–) | Hi (+) | Lo (–) | Hi (+) |
| 10 | Hi (+) | Lo (–) | Lo (–) | Hi (+) | Hi (+) | Lo (–) |
| 11 | Lo (–) | Hi (+) | Lo (–) | Hi (+) | Hi (+) | Lo (–) |
| 12 | Hi (+) | Hi (+) | Lo (–) | Hi (+) | Lo (–) | Hi (+) |
| 13 | Lo (–) | Lo (–) | Hi (+) | Hi (+) | Hi (+) | Hi (+) |
| 14 | Hi (+) | Lo (–) | Hi (+) | Hi (+) | Lo (–) | Lo (–) |
| 15 | Lo (–) | Hi (+) | Hi (+) | Hi (+) | Lo (–) | Lo (–) |
| 16 | Hi (+) | Hi (+) | Hi (+) | Hi (+) | Hi (+) | Hi (+) |



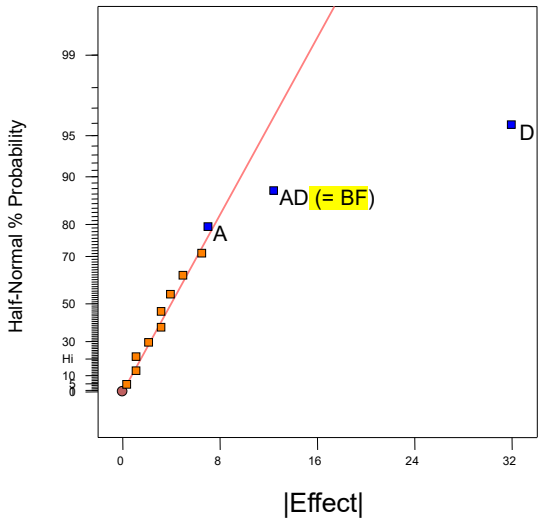
## Effects: Ranked





## Effects: Half-Normal Plot

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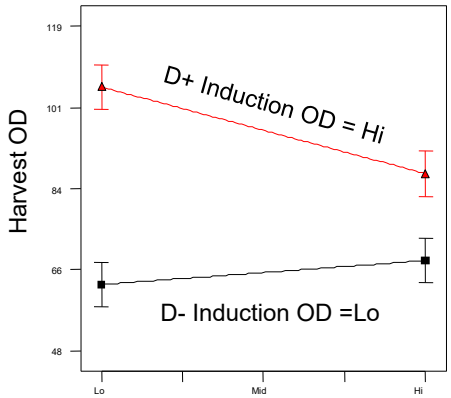
## Interaction Effect
*Breakthrough!**




**After follow-up DOE confirmed AD as the interaction—not BF. This was the price paid for using a screening design.*

*Fermentation*
*Rebuild, re-open & analyze*

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## Minimum-Run Designs (up to 50 factors)

*Considerable Savings Over Standard Fractions*

Characterization

| Factors | Std Res V | MR5* |
|---|---|---|
| 6 | 32 | 22 |
| 7 | 64 | 30 |
| 8 | 64 | 38 |
| 9 | 128 | 46 |
| 10 | 128 | 56 |
| 11 | 128 | 68 |
| 12 | 256 | 80 |
| 13 | 256 | 92 |
| 14 | 256 | 106 |

Screening

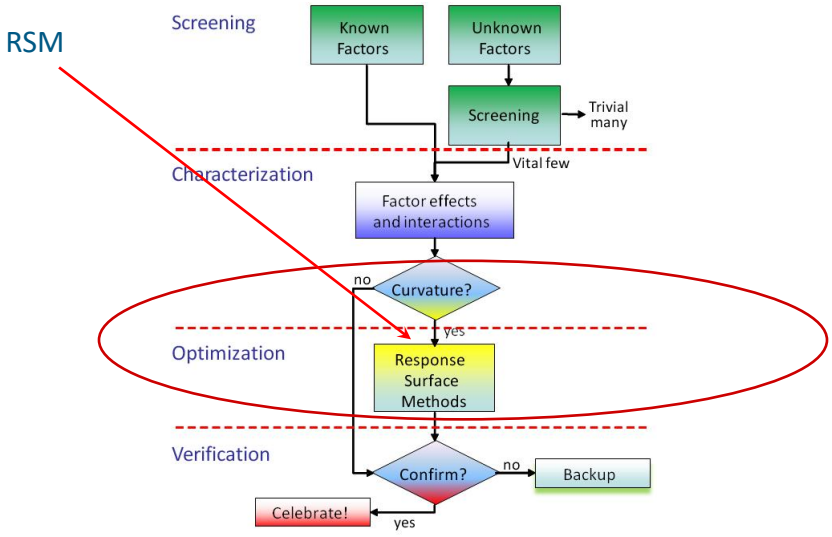
| Factors | Std Res IV | MR4** |
|---|---|---|
| 9 | 32 | 18 |
| 10 | 32 | 20 |
| 11 | 32 | 22 |
| 12 | 32 | 24 |
| 13 | 32 | 26 |
| 14 | 32 | 28 |
| 15 | 32 | 24 |
| 16 | 32 | 26 |
| 17 | 64 | 28 |

* Oehlert & Whitcomb, "Small, Efficient, Equireplicated Resolution V Fractions of $2^k$ designs …", Fall Technical Conference, 2002: www.statease.com/pubs/small5.pdf

** Anderson & Whitcomb, "Screening Process Factors In the Presence of Interactions," Annual Quality Congress, American Society of Quality, Toronto, 2004: www.statease.com/pubs/aqc2004.pdf



## Strategy of Experimentation





## Response Surface Methods (RSM)
### When to Apply It (Strategy of Experimentation)

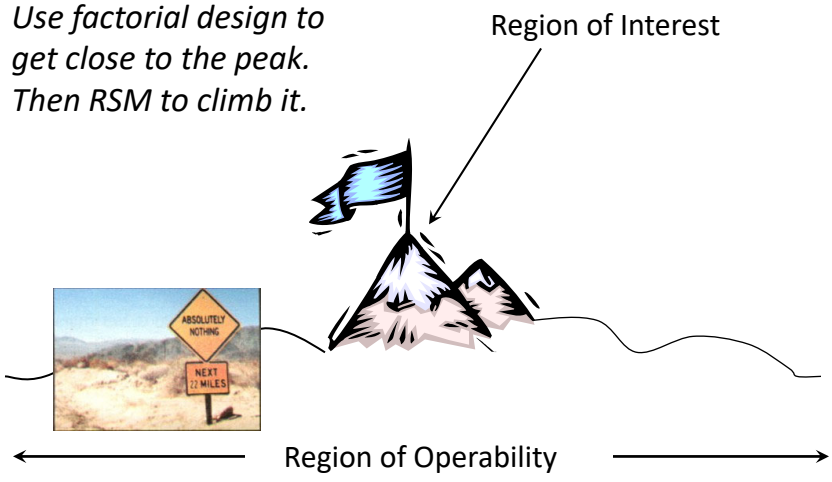
1. Fractional factorials for screening such as MR4
2. High-resolution fractional, such as MR5, or full factorial for characterization of interactions *(add center points at this stage to test for curvature)*
3. Response surface methods (RSM) for optimization

*Contour maps (2D) and 3D surfaces guide you to the peak.*



## RSM: When to Apply It

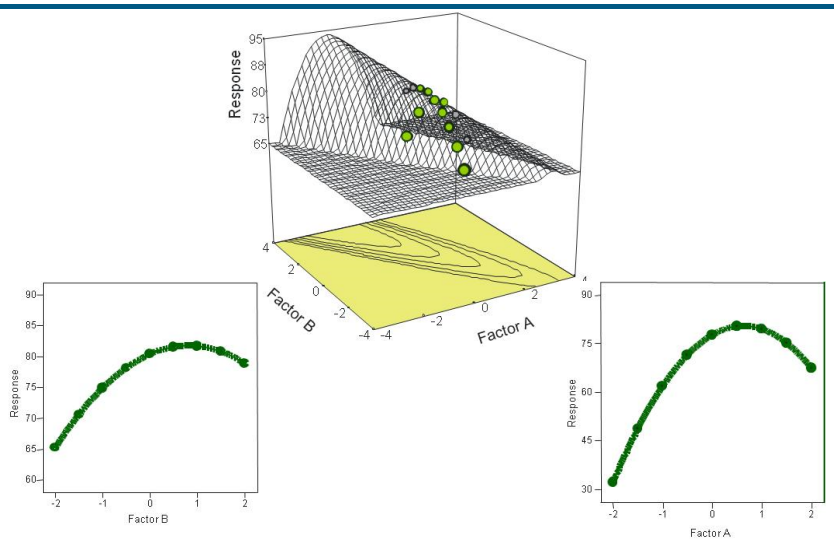
*Use factorial design to get close to the peak. Then RSM to climb it.*





## RSM vs OFAT

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## RSM: Process Flowchart

Subject Matter Knowledge
(Plus, Factorial Screening)

↓

Vital Few Factors (x's)

↓

Process → Measured Response(s) (y(s))

↓

Fitting*

↓

Polynomial Model

↓

Response Surface

*"All models are wrong, but some are useful."* - George Box

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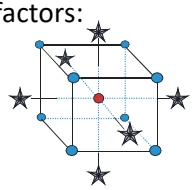
## RSM Case Study *(1/3)*
### Fermentation Optimization

A bioengineer ran a central composite design (CCD)—a tried-and-true RSM—to maximize output of an antibiotic from fermentation of *Streptomyces coelicolor* by systematically varying two factors:

A. Perfluorodecalin (PFC) – an oxygen carrier
B. Glucose (Glc)



The responses were:

1. Actinorhodin (ACT) – indicator of antibiotic
2. Biomass (Bio)
3. Oxygen uptake rate (OUR)
4. Glucose uptake rate (GUR)

*("Response surface methodological approach for inclusion of perfluorocarbon in actinorhodin fermentation medium," *Process Biochemistry* 38 (2002) 667-673.)

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## RSM Case Study *(2/3)*
### CCD Template and Fermentation Results

| Std | Type | A: PFC %, v/v | B: Glc g/l | ACT mg/l | Bio g/l | OUR $mgO_2$/l h | GUR g/l h |
|---|---|---|---|---|---|---|---|
| 1 | Factorial | 20.00 | 8.75 | 18 | 1.346 | 86 | 0.082 |
| 2 | Factorial | 60.00 | 8.75 | 30 | 1.450 | 84 | 0.031 |
| 3 | Factorial | 20.00 | 16.25 | 19 | 2.900 | 96 | 0.085 |
| 4 | Factorial | 60.00 | 16.25 | 24 | 1.780 | 82 | 0.032 |
| 5 | Axial | 11.72 | 12.50 | 19 | 2.308 | 43 | 0.048 |
| 6 | Axial | 68.28 | 12.50 | 22 | 1.600 | 59 | 0.012 |
| 7 | Axial | 40.00 | 7.20 | 32 | 1.100 | 125 | 0.110 |
| 8 | Axial | 40.00 | 17.80 | 32 | 2.300 | 128 | 0.120 |
| 9 | Center | 40.00 | 12.50 | 54 | 1.985 | 176 | 0.108 |
| 10 | Center | 40.00 | 12.50 | 52 | 1.889 | 168 | 0.109 |
| 11 | Center | 40.00 | 12.50 | 55 | 2.100 | 184 | 0.110 |

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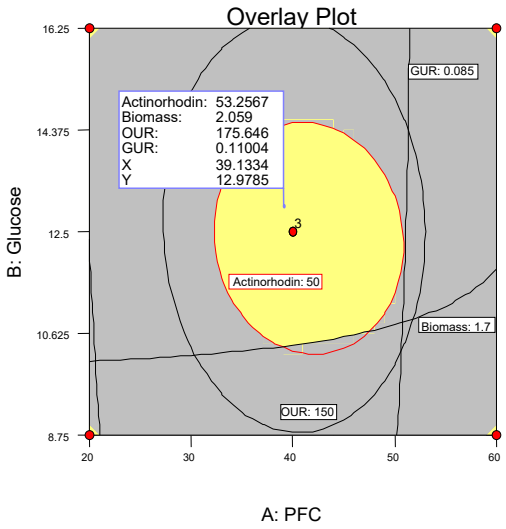
## RSM Case Study *(3/3)*
## The Sweet Spot! (Add CI for safer QbD)




*Antibiotic*
*Rebuild, re-open & analyze*
*Optimize and overlay*
*Add CI on ACT & set flag*





## Mixture Design*

Considerations:

- Factors are ingredients of a mixture.
- The response is a function of proportions, not amounts.
- Given these two conditions, fixing the total (an equality constraint) facilitates mixture modeling as a function of component proportions.

*Let's try forcing a factorial design onto a mixture.*

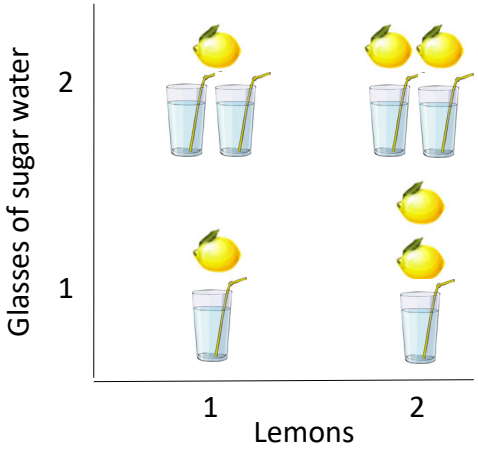
*(Pioneered by Henry Scheffé, U Cal., 1957)





## Forcing (squeezing?) factorial design on a mixture: Lemonade



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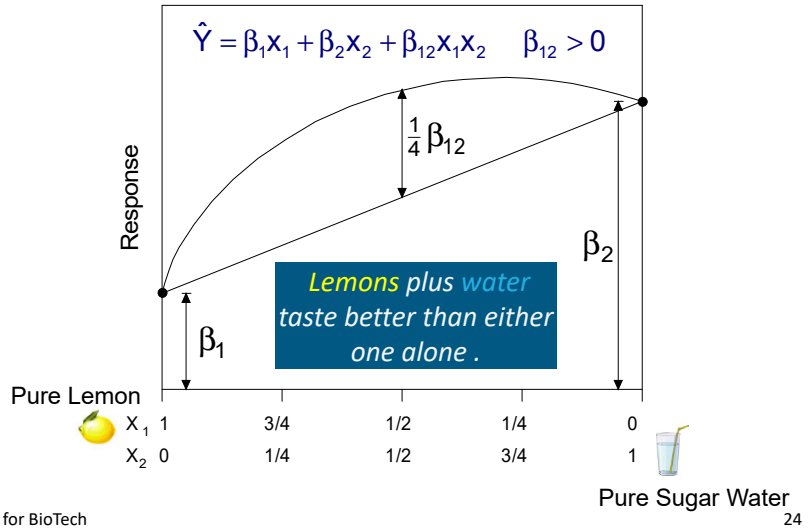
## Mixture Design and Modeling (sweet!)

Two components: Quadratic (synergistic)



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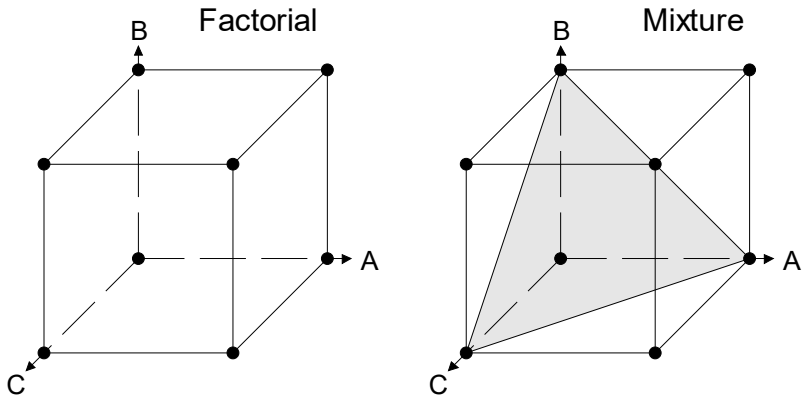
## Three-Component Mixture

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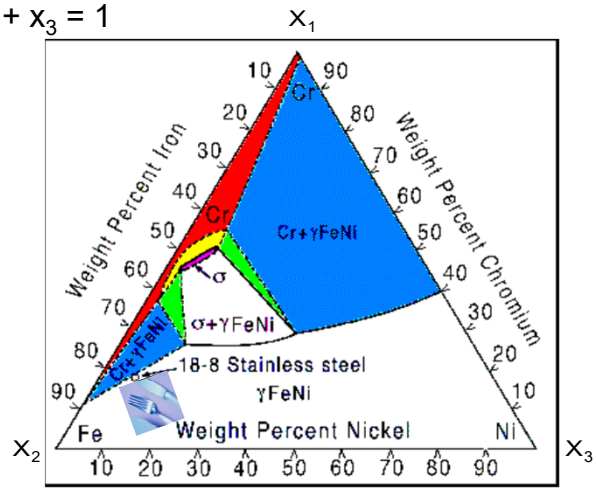
## Ternary Diagram for Mixture Composition
(for example, stainless steel flatware)

$x_1 + x_2 + x_3 = 1$

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## Mixture Case Study

In this hypothetical case, microbiologists at a pharmaceutical firm suspect that a blend of three commercially-available medias will lead to optimal (maximized) peak viable cell density (VCD). To put their theory to the test, they set up a simplex mixture design.

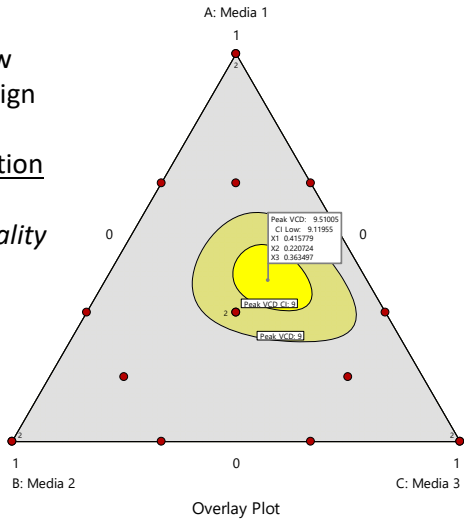
*Media*
*Rebuild, Run, Analyze*
*Maximize 9-10*





## Framing the Sweet Spot for QbD

- Graphical optimization now frames the functional "design space" where all modeled responses for a unit operation fall within confidence intervals: *Ideal tool for quality by design (QbD).*



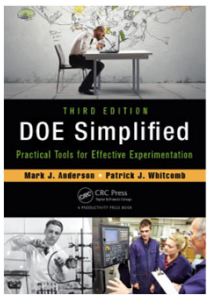

Overlay Plot

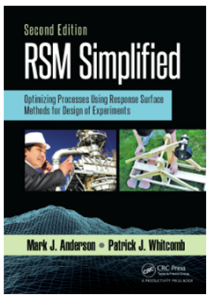


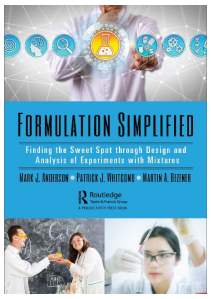


## References
*DOE/RSM/Formulation Simplified Series**




**Anderson, et al, Taylor & Francis, Productivity Press, New York, NY.*





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*Stay on for some chat if you like.*

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