Introduction to the Design and Analysis of Order-of-Addition Experiments

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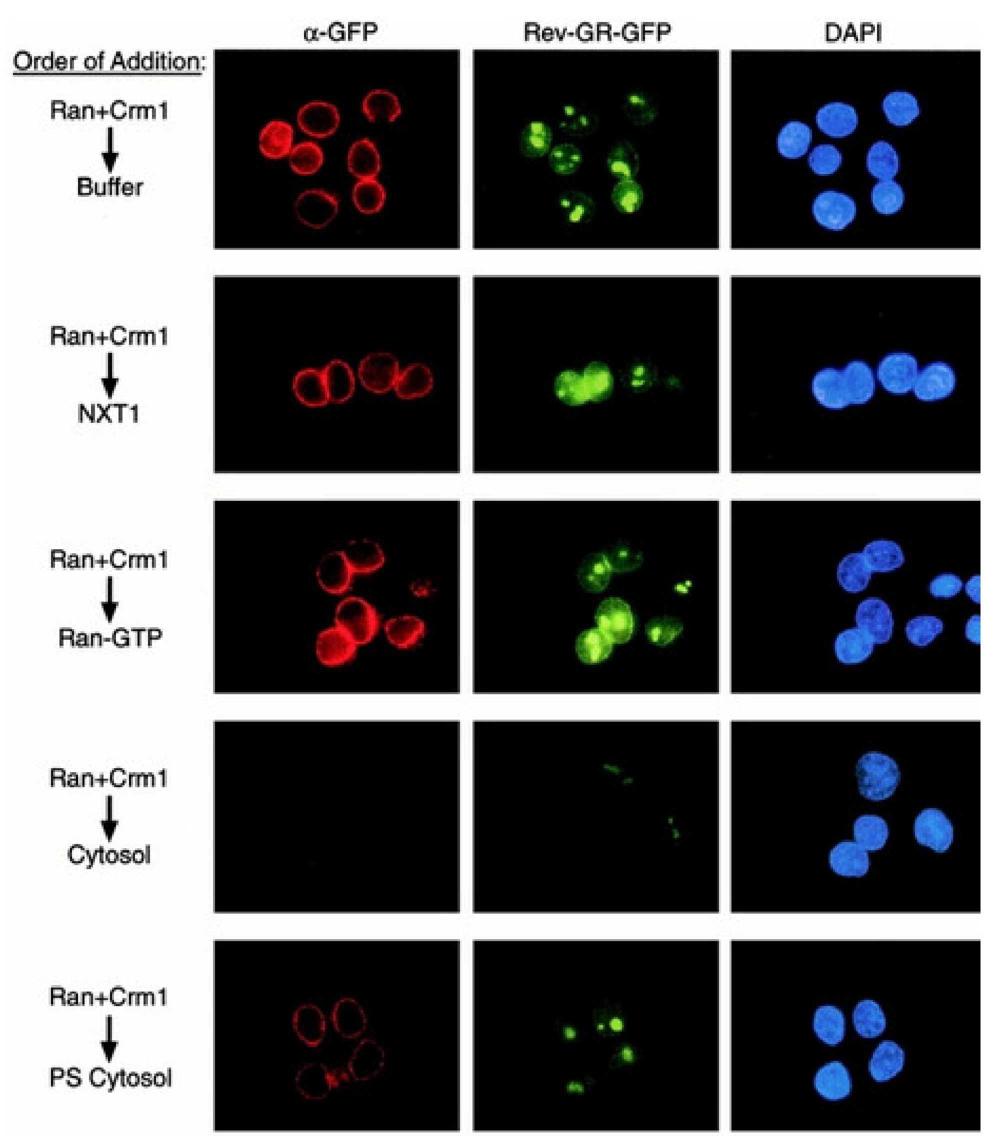
Fisher's tea-tasting experiment, with Muriel Bristol
$m = 2$ components, 4 replications.

Order of addition experiment indicating the requirement for Ran, Crm1, and NXT1 early in the export pathway.

J of Cell Biology, 2001



short (1 hr) exposures to PDGF are sufficient to stimulate DNA synthesis, provided the cultures are incubated with forskolin after PDGF treatment (PF $\rightarrow$ F, P $\rightarrow$ F). Unlike PDGF, forskolin is required continually throughout the 20 hr lag period for growth to occur. Other permutations of the PDGF/forskolin order of addition (PF $\rightarrow$ P, F $\rightarrow$ P) do not result in Schwann cell growth.

Journal of Neuroscience, February 15, 2001

3.3 Order of addition

Order of addition can influence the signal generated to a large extent. The optimal order in which assay components interact should always be determined empirically. Keep in mind that some binding partners may interfere with the association of other binding partners if allowed to interact in the wrong order.

PerkinElmer
User's Guide to Alpha Assays:
Protein-Protein Interactions

Order-of-Addition Experiments

- Lady Tasting Tea: $m = 2$ components

$c_1 \rightarrow c_2$ 1: 1 2
$c_2 \rightarrow c_1$ 2: 2 1

- Consider $m = 3$ components
- $m!$ permutations
- An *Order-of-Addition* (*OofA*) experiment.

1:	1	2	3
2:	1	3	2
3:	2	1	3
4:	2	3	1
5:	3	1	2
6:	3	2	1

1: 1 2 3
2: 1 3 2
3: 2 1 3
4: 2 3 1
5: 3 1 2
6: 3 2 1

Some questions *(the talk!)*

1. What are the factors for an m-component OofA experiment? Levels?
2. Say m = 4–7 components: reasonable way to select a fraction of all $m!$ runs?
3. Can we add process variables to the experiment in a natural way?
4. How can we analyze such experiments?

1:	1	2	3
2:	1	3	2
3:	2	1	3
4:	2	3	1
5:	3	1	2
6:	3	2	1

Q1: Factors?

1. What are the factors for an m-component OofA experiment? Levels?

Factors: Van Nostrand (1995)

- Factors for the design?
- Idea: consider $\binom{m}{2}$ *Pseudo-factors (PF's)*
- *Here: pair-wise ordering factors (PWOF's)*
- $m = 3$ example, all runs

		F1<2	F1<3	F2<3
1:	1 2 3	1	1	1
2:	1 3 2	1	1	0
3:	2 1 3	0	1	1
4:	2 3 1	0	0	1
5:	3 1 2	1	0	0
6:	3 2 1	0	0	0

- Note: not all possible combinations of PWOF's are possible
- Transitive property
If 1<2 & 2<3 ⇒ 1<3

	F1<2	F1<3	F2<3	
1 2 3	1	1	1	✓
1 2 3	1	**0**	1	✗

Q2: Fraction?

2. If there are m = 4–7 components, say: a reasonable way to select a fraction of all $m!$ runs?...

Our approach

- First: what was Van Nostrand's approach?
  - Used 2^{k-p} ideas. (Was not very useful…)
- Our approach
  - Start with full $m!$ runs ($m = 5 \rightarrow n = 120$)
  - Generate corresponding PWOF combinations ($m = 5 \rightarrow \binom{5}{2} = 10$ PWOF's: F1<2, F1<3, … F4<5)
  - Find optimal N-run design using χ^2 (balance) or D-criterion as goodness measure
    - Balance??...

Q2a: Fraction?

2. If there are m = 4–7 components, say: a reasonable way to select a fraction of all $m!$ runs?

Balance?

To do this, first consider *Orthogonal Arrays.*

OA of strength t

- An $N \times k$ array A with k factors each at s levels is an OA (Orthogonal Array) with strength t if every $N \times t$ sub-array of A contains all possible t-tuples the same number of times
- OA with $t = 2$ often simply called an OA

- 2^{5-2} : $\mathrm{N} = 8, k = 5, s = 2$
  - OA with $t = 2$
  - Res III
- 2^{4-1} : OA with $t = 3$
  - Res IV.

	A	B	C	D	E
1	0	0	0	0	0
2	1	0	0	1	1
3	0	1	0	1	0
4	1	1	0	0	1
5	0	0	1	0	1
6	1	0	1	1	0
7	0	1	1	1	1
8	1	1	1	0	0

A	D	Freq
0	0	2
1	0	2
0	1	2
1	1	2

OofA OA of strength $t = 2$?

- $m = 3$ example:

		F1<2	F1<3	F2<3
1:	1 2 3	1	1	1
2:	1 3 2	1	1	0
3:	2 1 3	0	1	1
4:	2 3 1	0	0	1
5:	3 1 2	1	0	0
6:	3 2 1	0	0	0

- Recall: we will use the *PWOF's as the factors* in the OofA design

- Problem: even *full* design not balanced

F1<2	F1<3	Freq
1	1	2
0	1	1
0	0	2
1	0	1

- So even full design not a (standard) OA of strength 2.

OofA OA's of strength t

Definition

An $N \times k$ array A with $k = \binom{m}{2}$ PWOF's is an OofA OA with strength t if every $\mathrm{N} \times t$ sub-array of A contains all possible t-tuples *in the same proportions as the full array with $m!$ runs*.

OofA patterns for $t = 2$

- Ex: $m = 4 \Rightarrow \binom{4}{2} = 6$ PWOF's

  $\Rightarrow \binom{6}{2} = 15$ pairs of PWOF's.

		F1<2	F1<3	F1<4	F2<3	F2<4	F3<4
1:	1 2 3 4	1	1	1	1	1	1
2:	1 2 4 3	1	1	1	1	1	0
3:	1 3 2 4	1	1	1	0	1	1
4:	1 3 4 2	1	1	1	0	0	1
...							
21:	4 2 1 3	0	1	0	1	0	0
22:	4 2 3 1	0	0	0	1	0	0
23:	4 3 1 2	1	0	0	0	0	0
24:	4 3 2 1	0	0	0	0	0	0

OofA patterns for $t = 2$

- Ex:
  $m = 4 \Rightarrow \binom{4}{2} = 6$ PWO's $\Rightarrow \binom{6}{2} = 15$ pairs of PWO's
- 2 non-isomorphic patterns ($m = 4$; 24 runs)

Synergistic: 12

		F1<2	
		0	1
F1<3	0	**8**	**4**
	1	**4**	**8**

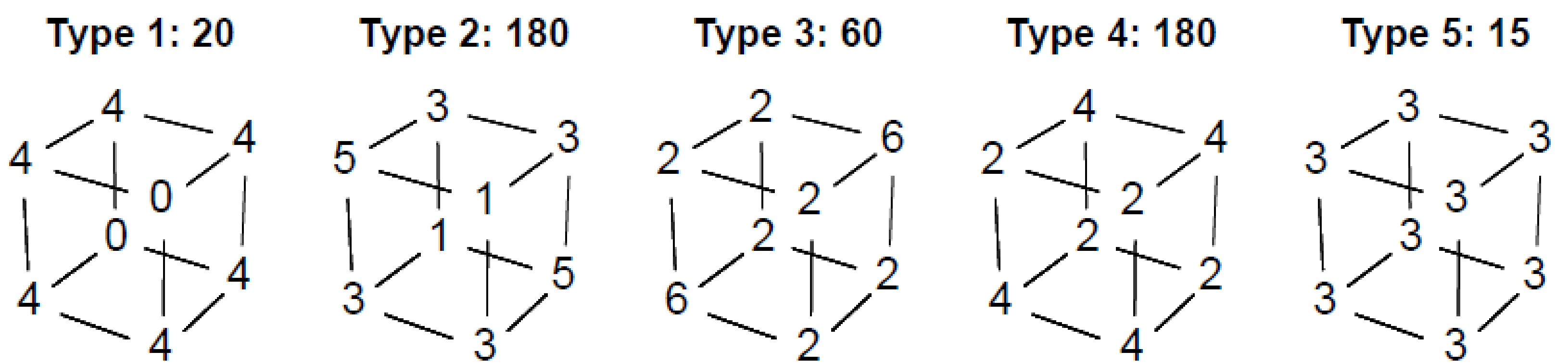
Independent: 3

		F1<2	
		0	1
F3<4	0	**6**	**6**
	1	**6**	**6**

- So, different pairs of PWOF's may have different proportions in the full array.

OofA patterns for $t = 3$

- Can have up to 5 non-isomorphic patterns
- Ex: $m = 6 \Rightarrow \binom{6}{2} = 15$ PWOF's $\Rightarrow \binom{15}{3} = 455$ 3-tuple PWOF's
  - An example of a 3-tuple: F1<2, F1<3, F2<3
- Patterns, on a 24-run (not 720-run) basis:



- Ex of Type 1: F1<2, F1<3, F2<3 (3 compon's—3 in 2 PWOF's)
- Ex of Type 2: F1<2, F1<3, F2<4. (4 compon's—2 in 2 PWOF's).
- Ex of Type 5: F1<2, F3<4, F5<6 (6 compon's)

Two simple OofA-OA results

- OofA OA of $t = 2$
  - for $m > 3$,
    need $N = 0 \; mod \; 12$
- OofA OA of $t = 3$
  - for $m > 3$,
    need $N = 0 \; mod \; 24$.

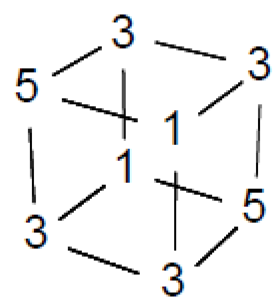
Synergistic: 12

		F1<2	
		0	1
F1<3	0	**8**	**4**
	1	**4**	**8**

Independent: 3

		F1<2	
		0	1
F3<4	0	**6**	**6**
	1	**6**	**6**

Type 2: 180



Q2b: Fraction?

2. If there are $m = 4$–7 components, say: a reasonable way to select a fraction of all $m!$ runs?

- How to *select* a good fraction? Consider $t = 2$ only
  - When $N = 0 \ mod \ 12$?
  - In other cases?...

Selecting good fractions

- Very limited closed-form methods—for $m \geq 4$, only for larger N (Peng, Mukerjee, Lin, 2018)
  - $m = 4, 5, 6, 7 \ \rightarrow N = 12, 60, 120, 840$
- In general
  - D-criterion (done here)—model-based
  - χ^2 criterion—balance-based.

From χ^2 to D

- Theorem: An OofA OA of $t = 2$ exists ($\chi^2 = 0$ for its PWOF design matrix $\mathbf{P}$) in N runs if and only if its associated $\mathbf{X} = [\mathbf{1}|\mathbf{P}]$ has D-efficiency = 1. (Voelkel, 2017; Peng, et al., 2018)
  - D-efficiency measured wrt the full OofA design

Some $m = 4{,}5$ results

- $m = 4{,}5, N = 12$
  - D-eff'y 1, so OofA OA
  - $m = 4$: two non-isomorphic designs found (not equal)
  - There are good, and there are better, OofA OA's!
  - $m = 5$: only one design found.

Some $m = 5, N = 24$ results

- $m = 5, N =$24
  - D-eff'y = 1 for top 37 of 100 (SAS Optex) and for top 1 (at least) of 20 (R AlgDesign)
  - Only two of these 38 were isomorphic! 37 were not
  - How to see if some designs are better than others?
    - A technical measure (Minimum-moment aberration (Xu, 2003)) works—we will show this idea
    - We will mostly use more intuitive measures here.

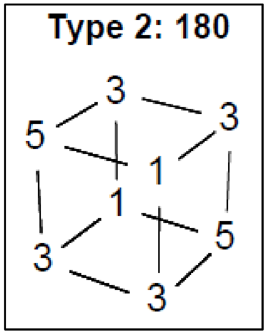
Some $m = 5, N = 24$ results

- $m = 5, N = 24$
  - 37 designs, non-isomorphic, had D-eff'y = 1
  - How to see if some designs are better than others?
- Minimum-moment aberration (Xu, 2003)
  - Idea: better designs have rows that are less similar

$$\begin{pmatrix} 1 & 0 & 1 & 1 \\ 1 & 0 & 0 & 1 \end{pmatrix} \qquad \begin{pmatrix} 0 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \end{pmatrix}$$

  - Similarity = 3 ☹ Similarity = 1 ☺

Some $m = 5, N = 24$ results



- Some more intuitive statistical measures (recall: all 37 are OofA OA's with strength $t = 2$)
- How do designs compare wrt strength $t = 3$?
  - Important! Interactions likely in OofA experiments
- For each design, what fraction of the $\binom{10}{3} = 120$ sets of $t = 3$ columns are OofA orthogonal?
  - Worst: 0.58. Best 4: 0.85-0.77
- For each design, average χ_3^2 over 120 sets of 3 columns?
  - Worst: 1.71. Best 4: 0.51-0.81
- Extremely similar to results using Xu's measures
- Projective properties: two of the five 4 components.

Some $m = 6, 7, N = 24$ results

- $m = 6, N =$24
  - D-eff'y = 1 for top 3 of 1000 (SAS Optex) and for top 1 (at least) of 100 (R AlgDesign)
  - None of these 4 were isomorphic
  - Xu's rankings: Design #'s 3, 4, 2, 1
  - Fraction of the $\binom{15}{3} = 455$ sets of $t = 3$ columns OofA orthogonal? Rankings: # 3, 4, 1, 2
  - Average χ_3^2? # 3, 4, 2, 1
  - Projective properties: four 4-component sets
- $m = 7, N =$24. Best D-eff'y = 0.990.

Q3: Adding Process Variables?

- Can be done naturally—and successfully
- Idea: start with a D-optimal OofA design, then add process variables
- Ex: $m = 5$, N =24, with 2^4 (4 main effects)
  - D-eff'y = 1
- Ex: $m = 5$, N =24, with 3×2^2 (3 main effects)
  - D-eff'y = 1 (4000 iterations to find this).

Q4: Analysis?

- Case Study (real problem, real data) used here
- Based on work from Kevin P. Gallagher, PPG Industries, Pennsylvania, USA

Case study: introduction

- Automotive coatings (“paint”). Several layers:
  - Corrosion protection
  - Primer
  - Color
  - Protective clearcoat (the case study)
- Quality influenced by *Viscosity*. For car doors:
  - high enough to prevent excessive flow (sags and drips)
  - low enough to allow paint to flow and level to provide smooth, attractive finish.

Case study: physics of viscosity

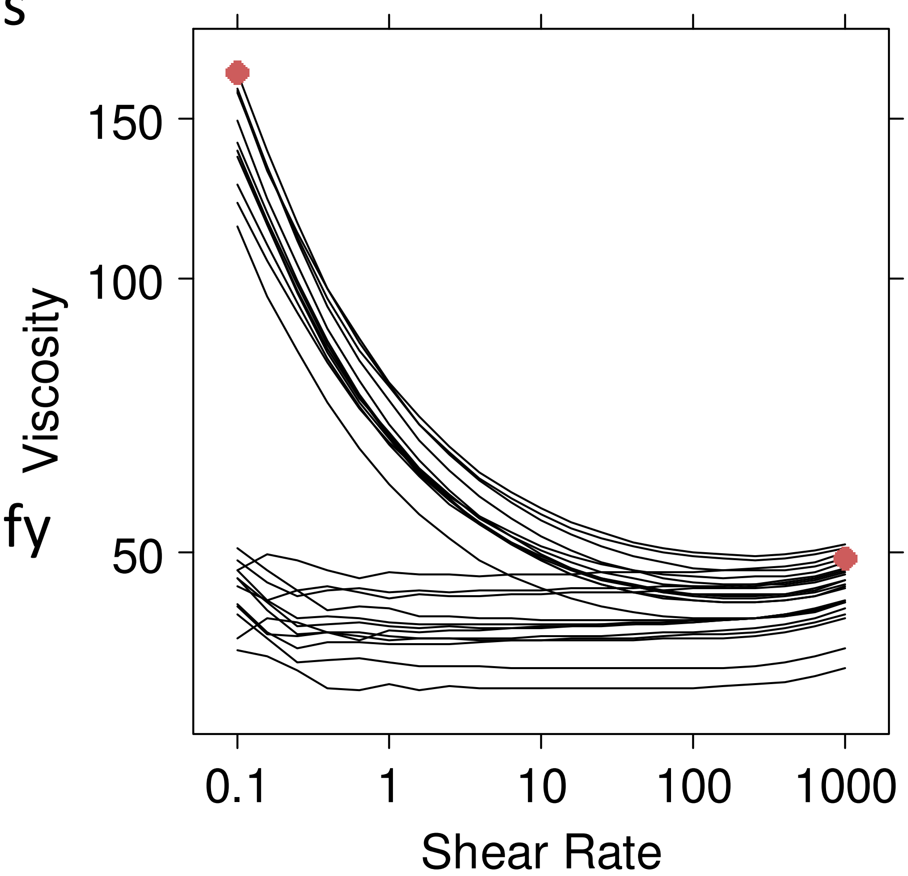
- A coating may:
  - Have a constant viscosity vs. shear rate (Newtonian fluid)
  - Have a changing viscosity vs. shear rate (non-Newtonian fluid)
- Automotive coatings
  - Non-Newtonian behavior desired is *shear thinning*—viscosity decreases at higher shear rates.

Case study: why shear thinning?

- Higher viscosity levels *before* use, in can (longer times without settling)
- Lower viscosity levels *during* spray application (smoother coating finish)
- Higher viscosity on vertical surfaces *after* being applied (less sagging)

Case study: graphs

- *Rheology* : branch of physics on deformation and flow of matter, including non-Newtonian flow of liquids.
- *Rheological-flow curves* (viscosity vs. shear rate) characterize viscosity shear dependence
- log(LSV/HSV) used to quantify shear thinning
  - LSV = low shear visc'y (shear rate = 0.1 s^{-1})
  - HSV = high shear visc'y (shear rate = 1000 s^{-1}).



Case study: three experiments

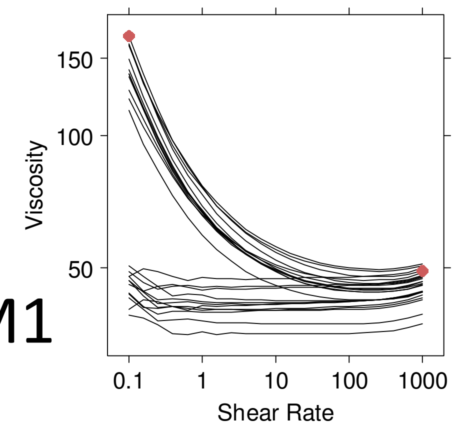
- Experiments on components of new premium automotive clearcoat
- Solvent: always added first (so, not part of DOE)
- Three experiments were run (shown on next slide)
- *Resin* (Wikipedia)
  - Polymer chemistry: solid or highly viscous substance that is typically convertible into polymers.
- *Crosslinking*
  - Chemistry: a bond that links one polymer chain to another.

Case study: three experiments

Component	Abb	In (4,24)	In (5, 15)	In (6,24)
primary binder resin	R1	✓	✓	✓
secondary binder resin	R2	✓	✓	✓
flow and leveling additive	A1	✓	✓	✓
rheology modifier #1	M1	✓	✓	✓
crosslinking resin	X		✓	✓
rheology modifier #2	M2			✓

- We will only examine (m=4,N=24) and (6,24) here.

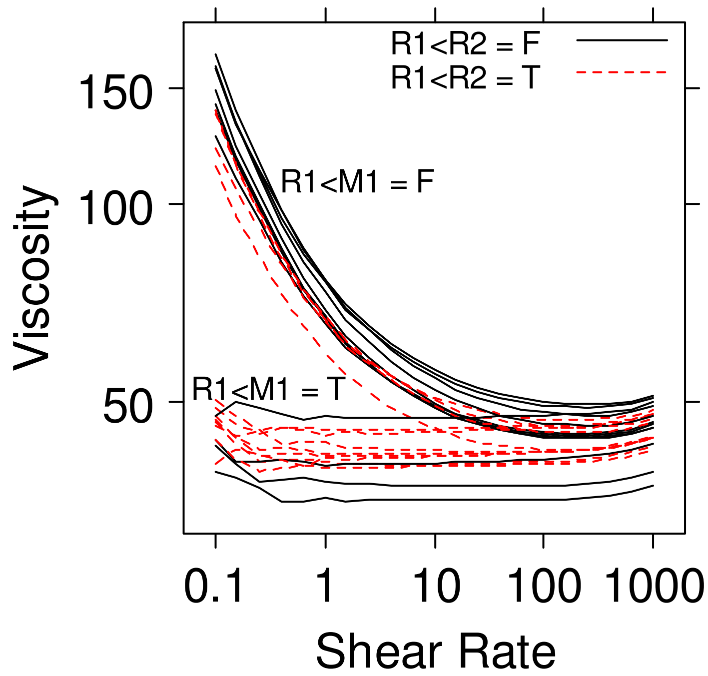
Case study: analysis method



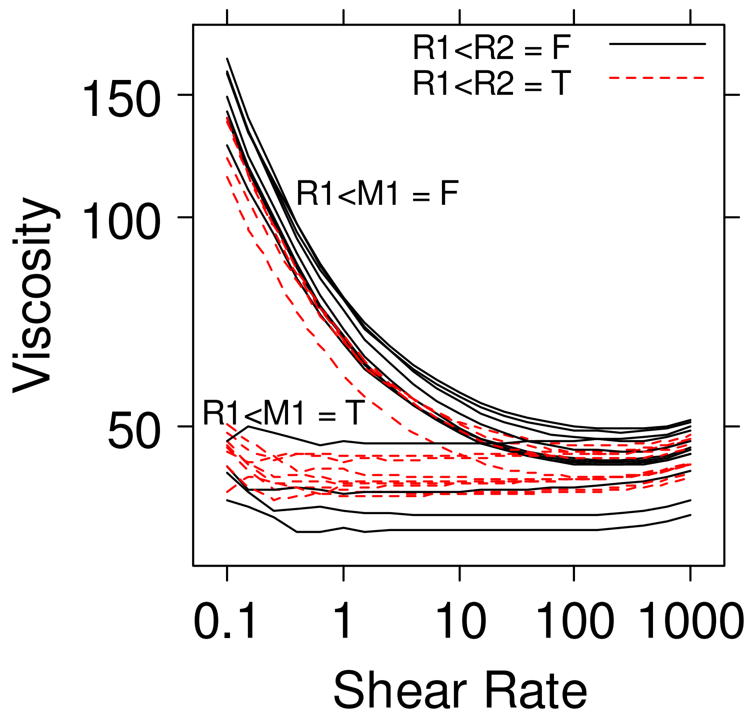
- Response: log(LSV/HSV)
- For (4,24), 4 components: R1, R2, A, M1
- So, 6 PWO factors:
  R1<R2, R1<A, R1<M1, R2<A, R2<M1, A<M1
- Analysis method:
  - Fit full main-effect model (6 factors)
  - Use a stepwise regression method to reduce the model (AIC, small-sample adjusted)
  - From this reduced model, add two-factor interactions (2fi's) of largest-effect terms. Run this "reduced + 2fi's model" using stepwise regression again.

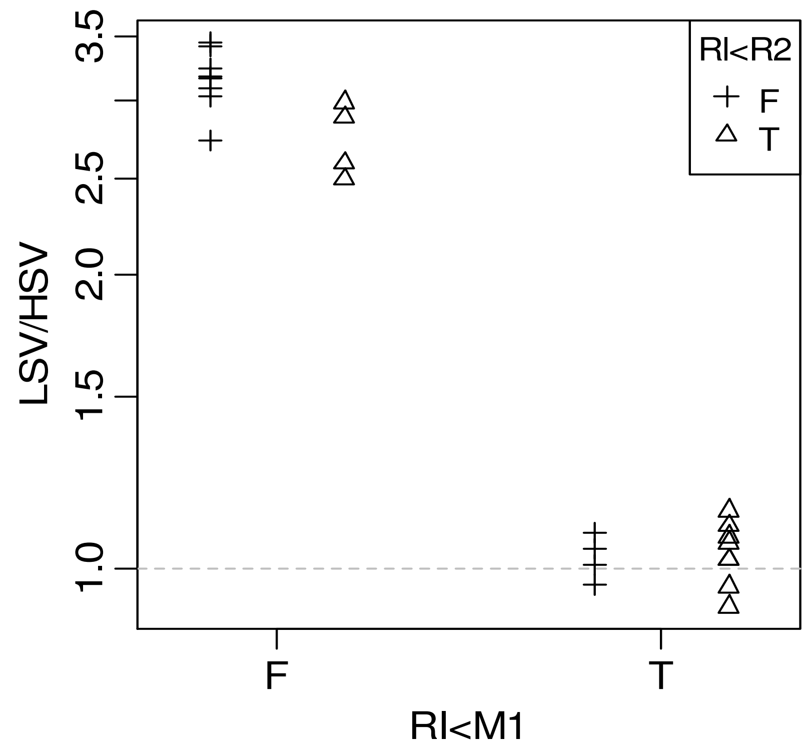
Case study: (4,24) results

- Largest effect: R1<M1
- Smaller effects:
  – R1<R2 effect existed
  – Interaction with R1<M1 also detected.
- Next: summaries using actual response—log(LSV/HSV)



Case study: (4,24) results



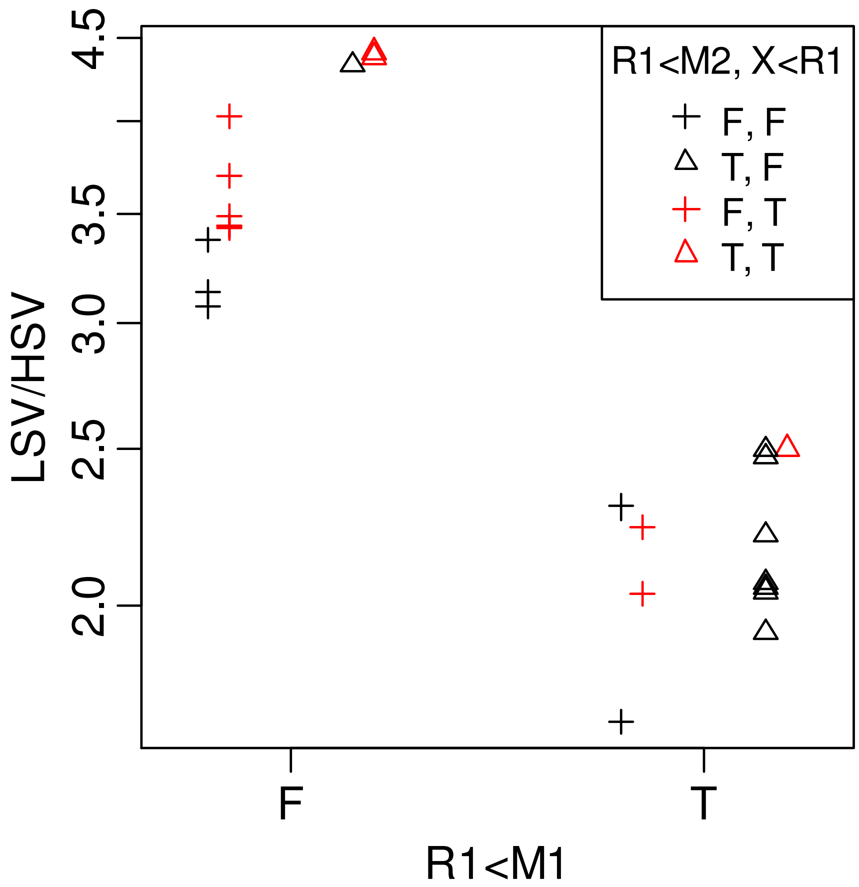


Case study: (6,24)

- 6 components: X, R1, R2, A1, M2, M1
- 15 PWO factors:
  - X<R1, X<R2, X<A1, X<M2, X<M1
  - R1<R2, R1<A1, R1<M2, R1<M1
  - R2<A1, R2<M2, R2<M1
  - A1<M2, A1<M1
  - M2<M1
- Modeling: fit 15 main effects, stepwise, ...

Case study: (6,24) results

- Largest effect: R1<M1
  - Same as (4,24)
- Smaller effects:
  - R1<R2, same as (4,24)
  - X<R1
  - R1<M2
- All non-Newtonian, shear-thinning



What have we found?

- Order-of-Addition Experiments—not unusual
- Little information available to construct good designs
- The factors in an m-component OofA exp't: PWO's
- Optimal ways to select a fraction of all $m!$ runs
- Addition of process variables to an OofA design
- The analysis of such experiments.

Thank you!
Questions?