Analysing Designed Experiments with Multiple Responses

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Multiple responses are common in industrial and scientific experimentation. Often these multiple response variables are related in some way. Digitizations of continuous curves and several related measures of the same physical phenomena are examples of such data. The analysis is still founded on the classical experimental design methodology, but additional tools are needed.

Principal component analysis is a very powerful technique for this type of data. One possibility is to perform univariate analysis of some individual principal components.

It is, however, possible to improve this type of tests. There are also multivariate alternatives and a newly developed framework is called Fifty-Fifty MANOVA. A related method is presented in a forthcoming article that considers screening experiments with few or zero error degrees of freedom.

When significant effects are found, one should illustrate these effects for easier interpretation. The sums of squares summed of over all responses illustrate the relative importance of each factor. Different (adjusted) mean values can be illustrated in a principal component score plot or directly as mean curves. In this talk, the discussion will be based on examples from food research with multiple responses from fluorescence spectra, rheology and particle size distributions.

INTRODUCTION

Multi-response Experiments:

- Analysis of individual responses by ANOVA.
  - There may be “too many” responses.
- Analysis of all responses by classical MANOVA
  - Can not be used when: #responses > #observations
- Analysis of individual principal components by ANOVA
  - This method works fairly well (but can be improved)
- Generalised MANOVA (Fifty-Fifty MANOVA)
  - Combines MANOVA and principal components
- Related methods for fractional factorial designs
Analysing Designed Experiments with Multiple Responses

- Significance tests for fractional factorial designs
  - Univariate
  - Multivariate
- Analysis of variance
  - Univariate (by using type II sums of squares)
  - Multivariate (Fifty-Fifty MANOVA)
- Illustrating the effects
  - (adjusted) mean values
  - Mean curves
  - Principal component score plot

A Dressing Experiment
One of several experiments in the dressing project

- Three design variables were varied
  - Homogenisation pressure
  - Amount of stabiliser
  - Amount of emulsifier

- Several responses were measured
  - Stability
  - Viscosity
  - Fluorescence (curves)
  - Rheology (several parameters)
  - Particle size distributions (curves)
  - and many others ….
2³ design with 3 extra center points

<table>
<thead>
<tr>
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<th>Stabiliser</th>
<th>Emulsifier</th>
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</table>

C: Emulsifier  
B: Stabiliser  
A: Homogenisation pressure
Significance tests for unreplicated designs

Several methods exist
- The methods are based on order statistics
- Important difference
  - Control of individual error rates
  - Control of experimentwise error rates
- Another difference
  - Forward selection / Step-down
  - Backward elimination / Step-up
- Langsrud and Næs (1998)* incorporates independent sources of variation (from center points)

  "A Unified Framework for Significance Testing in Fractional Factorials",
  *Computational Statistics and Data Analysis, 28, 413-431*

Analysis of Stability
by forward selection (Langsrud and Næs, 1998)

<table>
<thead>
<tr>
<th></th>
<th>p-values</th>
<th>psi</th>
<th>q</th>
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<tbody>
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- The largest (absolute value) effect is first tested
- An “F-statistic” where the denominator contains
  - q smallest effects
  - independent sources of variation
    - Here: ABC (1 df) + center points (2 df)
Analysis of Viscosity

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- B is clearly significant
- At 5% level C is also significant
- The method controls the experimentwise error rate

Illustrating the effects on Viscosity

- The significant main effects of B and C

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<th>C mean</th>
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<td>16960</td>
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</table>
A multivariate significance testing procedure for fractional designs by Langsrud (2001)*

- Generalisation of the univariate procedure
- Forward selection
- Control of experimentwise error rates
- Elements in the computation of test statistic
  - Sequential principal component decomposition
    - Number of components chosen according to an explained variance criteria (50%)
  - Hotelling $T^2$ test statistic
  - Single response statistic

* Langsrud, Ø. (2001)
"Identifying Significant Effects in Fractional Factorial Multiresponse Experiments", Technometrics, November 2001, Vol. 43, No. 4

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Fluorescence spectra as response

![Fluorescence spectra graph](image-url)
The estimated fluorescence effects

Analysis of fluorescence

<table>
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- \( m \) is the number of principal components used for testing
- \( \text{expl.var.} \) is the variance explained by using \( m \) components.
**Rheology as multivariate response**

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<th>y-Viscosity, Pa-s</th>
<th>s-shear stress, Pa</th>
<th>s-phase, degree</th>
<th>s-G´, Pa</th>
<th>s-G”, Pa</th>
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<table>
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<th>s-G”, Pa</th>
<th>f-phase, degree</th>
<th>f-G´, Pa</th>
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**Analysis of Rheology**

with standardisation of the variables

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<th>m</th>
<th>q</th>
<th>expl.var.</th>
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<td>C</td>
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</table>
The actual design: $3^3$ design with 2 extra center points

The previous results was based on a subset of the whole data set

Alternative designs:
- Central Composite Design
- Box-Behnken Design

ANOVA analysis of Viscosity

Analysis of Variance for Viscosity, using Adjusted SS for Tests

<table>
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<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
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<td>C</td>
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<td>23205093</td>
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<td>A*B</td>
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Least Squares Means for Viscosity

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### Modified analysis

- **Continuos instead of categorical variables (second order model)**
- **Unbalanced analysis**
  - Use Type II Instead of Type III
- **Sums of Squares**
  - Report sums of squares as the fraction of the total sums of squares (explained variance)

### Stability

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<tr>
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</table>

C adjusted mean: -1 7.0351  
B adjusted mean: -1 7.1342  
1 8.3874
### Viscosity

<table>
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</table>

**A** adj. mean

-1 15822
0 14692
1 15799

**B** adj. mean

-1 10177
1 20580

**C** adj. mean

-1 13811
1 17089

---

### Fluorescence as response

![Fluorescence plot](chart)
Fifty-Fifty MANOVA
Langsrud (2000)*

- The testing of significance is based on the same statistical principles as classical MANOVA: Classical test statistics and classical statistical distributions.
- HOWEVER: Three types of dimensionality reduction.
  - Adjust the response data for other factors in the model (according to type II sums of squares).
  - Perform PCA on adjusted (see above) response data. The testing is based on a few (=nPC) important components.
  - In addition some components (=nBu) are used as buffer components.


Analysis of Fluorescence

NOTE: Due to missing values, 1 observation was removed.
--- FIFTY-FIFTY MANOVA VERSION 1.1 --- 128 responses ---

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>exVarSS</th>
<th>nPC</th>
<th>nBu</th>
<th>exVarPC</th>
<th>exVarBU</th>
<th>p-Value</th>
</tr>
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<td>0.001664</td>
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<td>5</td>
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<td>1.000</td>
<td>0.237324</td>
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<td>1</td>
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</table>
Illustrating the effect of C (emulsifier): -1, 0, 1
Illustrating the effect of B (stabiliser): -1, 1

Illustrating the effect of A (pressure): -1, 1
Rheology as multivariate response – principal component loadings

principal component scores
## Analysis of Rheology

--- FIFTY-FIFTY MANOVA VERSION 1.1 --- 12 responses ---

<table>
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<tr>
<th>Source</th>
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<th>nBu</th>
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</tbody>
</table>

--- STANDARDIZATION ON

The effect of A (and A*A)

- Scores for the first two components
- Adjusted mean values
- Coordinates in the score plot

![Score Plot](image.png)
The effect of B, C and B*C:  \((B,C)\)

Concluding remarks

- Fifty-Fifty MANOVA is a new framework for testing significance in multivariate linear models
  - A generalisation of classical MANOVA
  - Handles several collinear responses
  - A MATLAB program is available at http://www.matforsk.no/ola/ffmanova.htm
- A related method for fractional factorial designs
- Illustrating the effects
  - (adjusted) mean values
  - Mean curves
  - Principal component score plot