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. Effects or differences can be calculated as usual and the statistical tests are still correct. 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.

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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

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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

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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 …..

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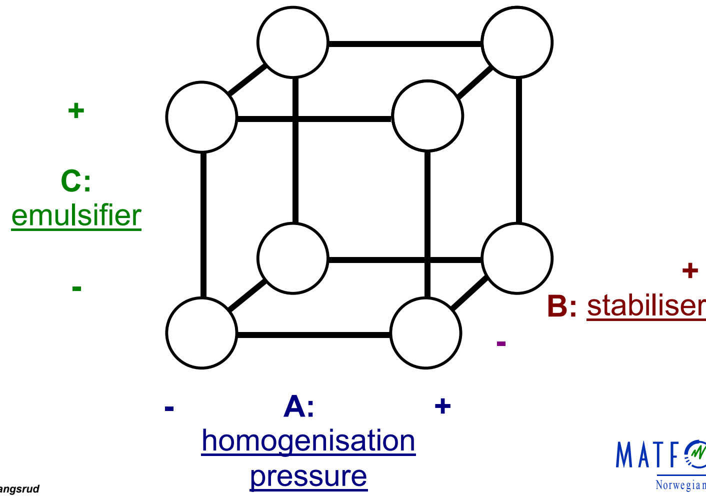
2^3 design with 3 extra center points



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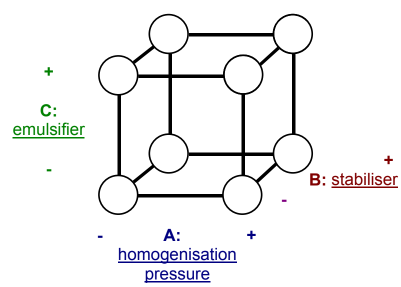
2^3 design with 3 extra center points



Homogenisation pressure	Stabiliser	Emulsifier
-1	-1	-1
1	-1	-1
-1	1	-1
1	1	-1
-1	-1	1
1	-1	1
-1	1	1
1	1	1
0	0	0
0	0	0
0	0	0

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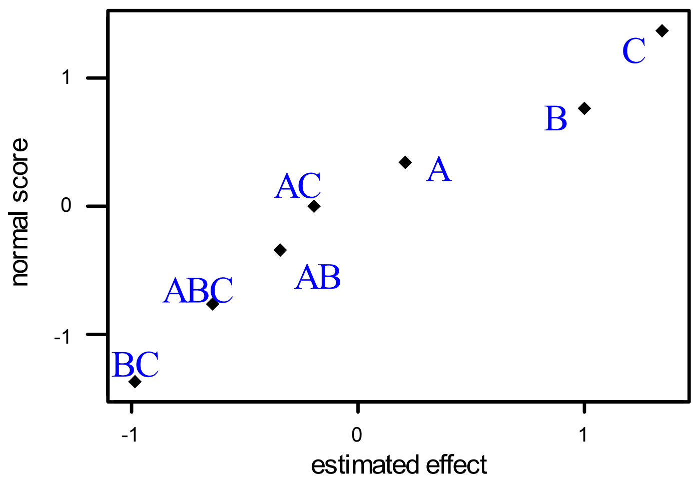
Stability - Normal Probability Plot



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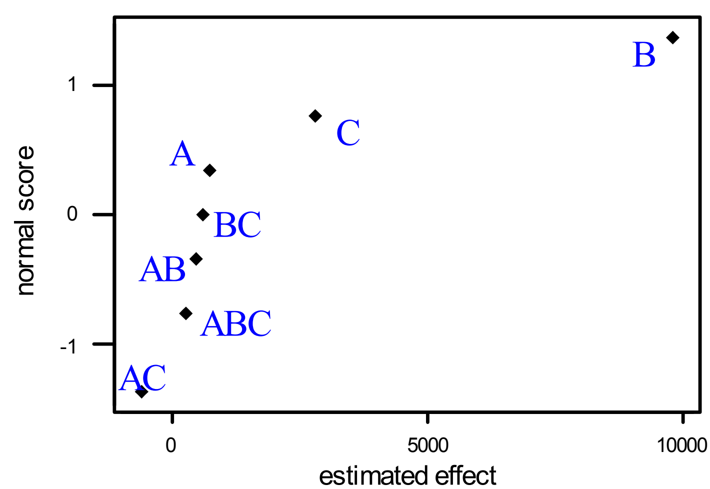
Viscosity - Normal Probability Plot



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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)

* Langsrud, Ø. and Næs, T. (1998)
"A Unified Framework for Significance Testing in Fractional Factorials",
Computational Statistics and Data Analysis, **28**, 413-431

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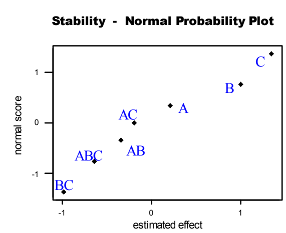
Analysis of *Stability*

by forward selection (Langsrud and Næs, 1998)



	p-values	psi	q
C	0.466105	4.91	4
B	0.540302	3.75	3
BC	0.504727	3.39	2
AB	0.935020	0.34	1
A	0.948044	0.09	0
AC	0.788238	0.09	0

- 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)

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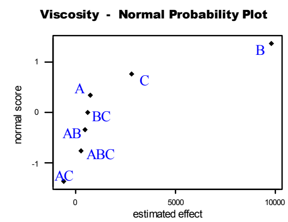
Analysis of *Viscosity*



	p-values	psi	q
B	0.000004	358.47	4
C	0.013365	33.99	3
A	0.576899	2.81	2
AC	0.442565	2.99	1
BC	0.381862	2.18	0
AB	0.314082	1.46	0

- B is clearly significant
- At 5% level C is also significant

- The method controls the experimentwise error rate

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Illustrating the effects on *Viscosity*

- The significant main effects of B and C

B	mean
-1	10625
1	20495

C	mean
-1	14160
1	16960

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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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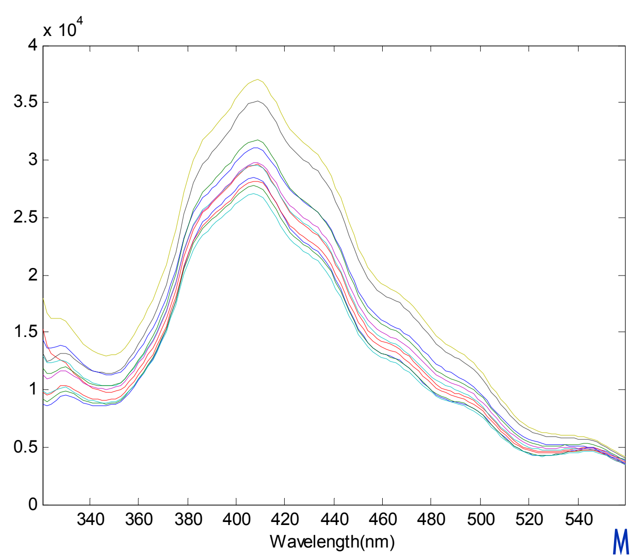
Fluorescence spectra as response



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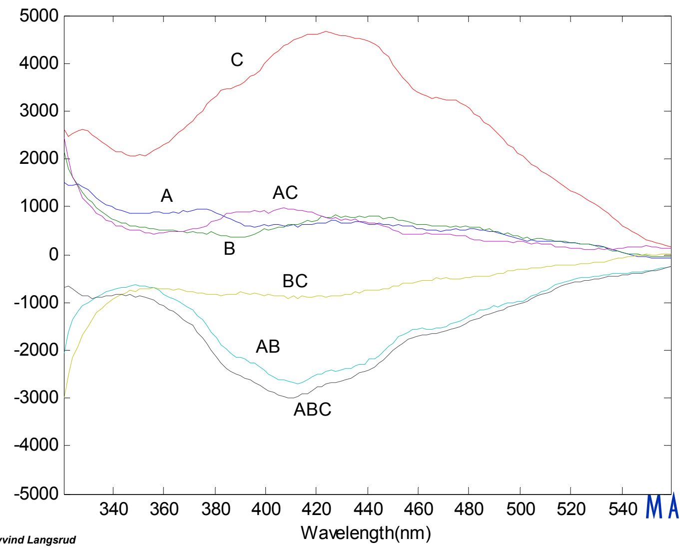
The estimated fluorescence effects



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Analysis of *fluorescence*

	p-values	psi	m	q	expl.var.
C	0.170449	9.93	1	4	0.9679
AB	0.729027	2.37	1	3	0.9441
BC	0.963883	0.45	1	2	0.9285
A	0.950417	0.28	1	1	0.9444
AC	0.879826	0.21	1	0	0.9602
B	0.703025	0.18	1	0	0.9744

- *m* is the number of principal components used for testing
- *expl.var.* is the variance explained by using *m* components.

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Rheology as multivariate respons

y-Shear-stress,Pa	y-Viscosity,Pa-s	s-Shear-stress,Pa	s-Phase,degree	s-G*,Pa
3.610	37.375	1.645	25.355	44.820
3.610	35.515	1.345	26.490	51.010
7.105	160.320	3.910	22.160	74.130
7.105	154.175	4.055	20.960	102.530
5.940	121.835	1.045	24.700	59.585
5.940	57.835	1.040	25.405	64.365
7.105	183.665	4.695	19.845	126.835
7.105	166.020	3.660	20.120	138.610
5.940	98.355	2.700	22.670	78.595
5.940	93.525	2.575	23.550	71.030
5.940	138.325	2.575	23.320	71.665

s-G`,Pa	s-G",Pa	f-Phase,degree	f-G*,Pa	f-G`,Pa	f-G",Pa	f-lutn-G`
40.510	19.180	24.210	48.090	43.870	19.685	0.270
45.650	22.740	25.055	52.375	47.460	22.095	0.285
69.075	26.695	17.145	111.860	106.875	32.975	0.195
95.770	36.585	18.850	114.585	108.435	37.020	0.210
54.155	24.815	24.180	60.925	55.705	24.540	0.260
58.185	27.485	23.905	68.435	62.570	27.715	0.260
119.320	42.990	18.315	142.310	135.090	44.680	0.210
130.315	47.050	18.410	153.410	145.650	47.985	0.215
72.560	30.175	21.005	85.195	79.555	30.465	0.240
65.135	28.310	20.595	87.630	82.025	30.815	0.235
65.825	28.310	21.435	81.745	76.105	29.830	0.245

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Analysis of *Rheology*
with standardisation of the variables

	p-values	psi	m	q	expl.var.
B	0.000001	1013.53	1	4	0.8895
C	0.011701	36.24	1	3	0.5663
BC	0.008968	45.36	1	2	0.6434
A	0.000349	287.58	1	1	0.5592
AC	0.155988	6.02	1	0	0.5410
AB	0.398106	4.55	2	0	0.8017

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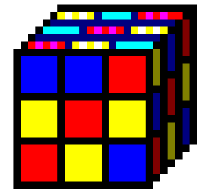
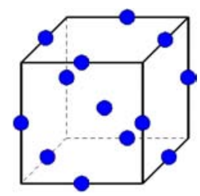
The actual design: 3^3 design with 2 extra center points

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



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



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ANOVA analysis of *Viscosity*

Analysis of Variance for Viscosit, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
A	2	5210297	5320407	2660204	5.40	0.026
B	2	413321269	413287156	206643578	419.10	0.000
C	2	46398397	46410185	23205093	47.06	0.000
A*B	4	1868745	1868476	467119	0.95	0.476
A*C	4	6397751	6407353	1601838	3.25	0.060
B*C	4	7677944	7677944	1919486	3.89	0.037
Error	10	4930680	4930680	493068		
Total	28	485805083				

Least Squares Means for Viscosit

A	Mean	SE Mean
-1	15153	234.1
0	15029	223.0
1	16016	234.1

B	* C	Mean	SE Mean
-1	-1	9220	405.4
-1	0	10500	405.4
-1	1	11893	405.4
0	-1	14560	405.4
0	0	15553	344.7
0	1	16513	405.4
1	-1	17683	405.4
1	0	19983	405.4
1	1	22687	405.4

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Unbalanced blocks (days) →

A	B	C	Day
-1	-1	-1	4
0	-1	-1	5
1	-1	-1	2
-1	0	-1	3
0	0	-1	5
1	0	-1	3
-1	1	-1	4
0	1	-1	4
1	1	-1	1
-1	-1	0	4
0	-1	0	5
1	-1	0	2
-1	0	0	1
0	0	0	2
1	0	0	5
-1	1	0	4
0	1	0	4
1	1	0	3
-1	-1	1	1
0	-1	1	2
1	-1	1	3
-1	0	1	1
0	0	1	1
1	0	1	4
-1	1	1	4
0	1	1	2
1	1	1	1
0	0	0	2
0	0	0	1

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)

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Stability

Source	DF	exVarSS	p-Value
A	1	0.004334	0.586609
A*A	1	0.001639	0.737202
B	1	0.217732	0.001466
B*B	1	0.001558	0.743540
C	1	0.291266	0.000443
C*C	1	0.004434	0.582350
A*B	1	0.019144	0.261604
A*C	1	0.003016	0.649536
B*C	1	0.026667	0.189030
Block	4	0.037545	0.622876
Error	14	0.195853	

B	adjusted mean
-1	7.1342
1	8.3874

C	adjusted mean
-1	7.0351
1	8.4511

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Viscosity

Source	DF	exVarSS	p-Value
A	1	0.000004	0.945192
A*A	1	0.010226	0.004367
B	1	0.746661	0.000000
B*B	1	0.000065	0.793518
C	1	0.086138	0.000000
C*C	1	0.000417	0.508874
A*B	1	0.003133	0.083323
A*C	1	0.000131	0.709347
B*C	1	0.003740	0.060808
Block	4	0.018681	0.008292
Error	15	0.013652	

C	adj. mean
-1	13811
1	17089

A	adj. mean
-1	15822
0	14692
1	15799

B	adj. mean
-1	10177
1	20580

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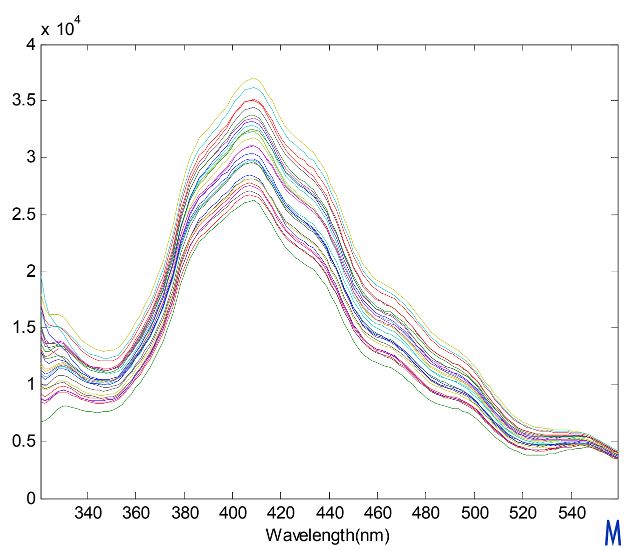
Fluorescence as response



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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.

* Langsrud, Ø. (2000).
"Fifty-Fifty MANOVA: Multivariate Analysis of Variance for Collinear Responses"
Proceedings of Industrial Statistics in Action 2000, University of Newcastle.

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Analysis of *Fluorescence*

```
NOTE: Due to missing values, 1 observation was removed.
  ---  FIFTY-FIFTY MANOVA   VERSION 1.1 ---  128 responses ---
  Source      DF        exVarSS nPC nBu exVarPC exVarBU    p-Value
  A            1       0.017874   1   5  0.949   1.000    0.029414
  A*A          1       0.017322   1   5  0.948   1.000    0.219138
  B            1       0.139253   1   5  0.966   1.000    0.001664
  B*B          1       0.027987   1   5  0.955   1.000    0.237324
  C            1       0.323186   1   5  0.973   1.000    0.000002
  C*C          1       0.004042   1   5  0.947   1.000    0.596164
  A*B          1       0.009311   1   5  0.950   1.000    0.396251
  A*C          1       0.006879   1   5  0.921   1.000    0.670535
  B*C          1       0.011193   1   5  0.937   1.000    0.366550
  Block        4       0.221358   1   5  0.962   1.000    0.009285
  Error       14       0.183920 - STANDARDIZATION OFF ------------
```

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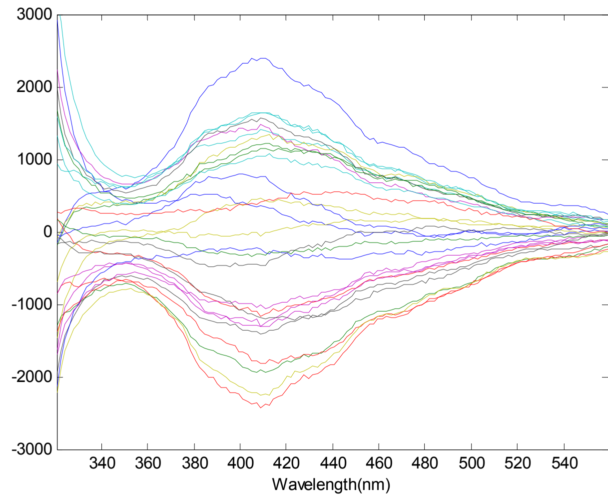
The residuals



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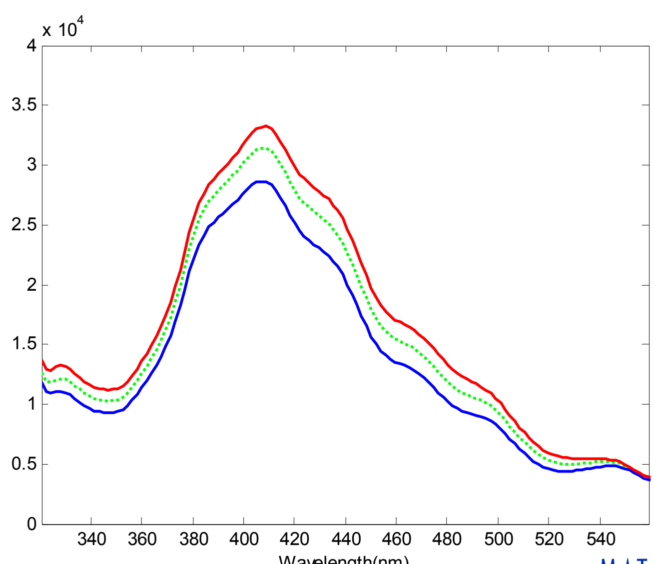
Illustrating the effect of C (emulsifier): -1, 0, 1



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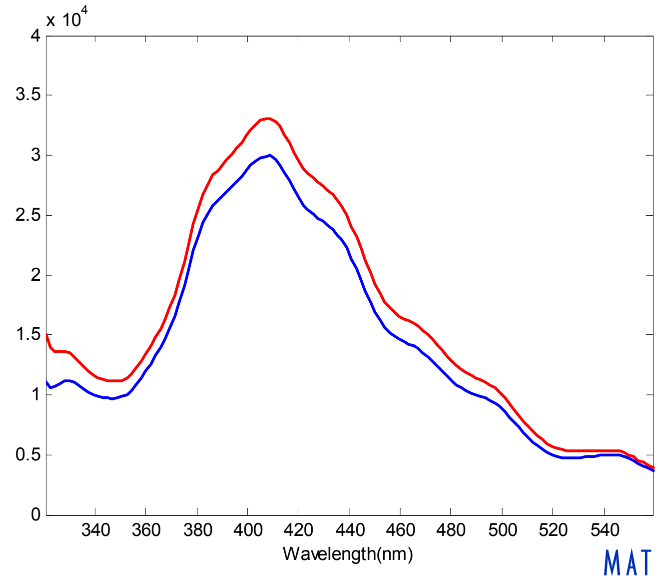
Illustrating the effect of B (stabiliser): -1, 1



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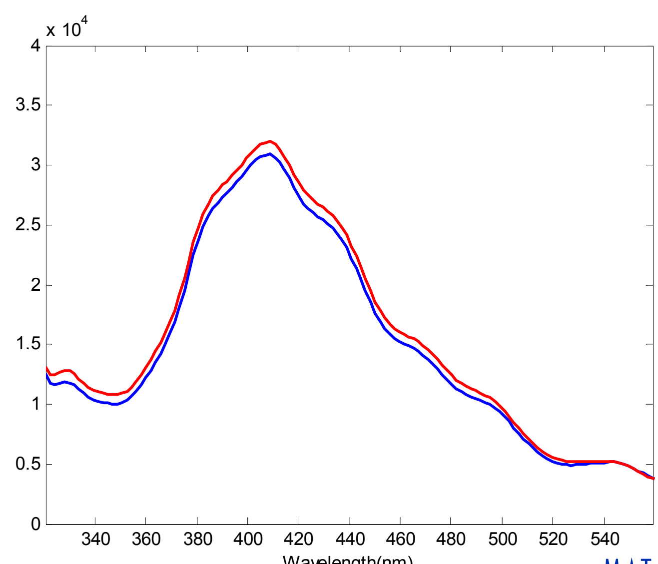
Illustrating the effect of A (pressure): -1,1



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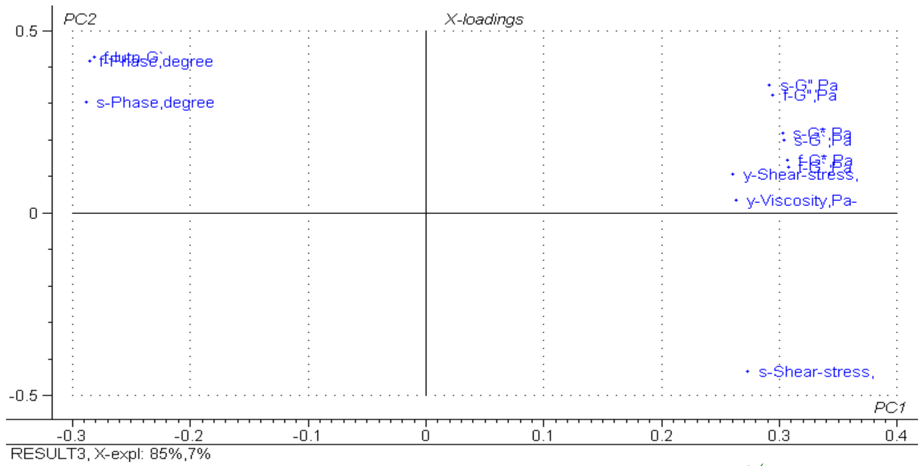
Rheology as multivariate respons
– principal component loadings



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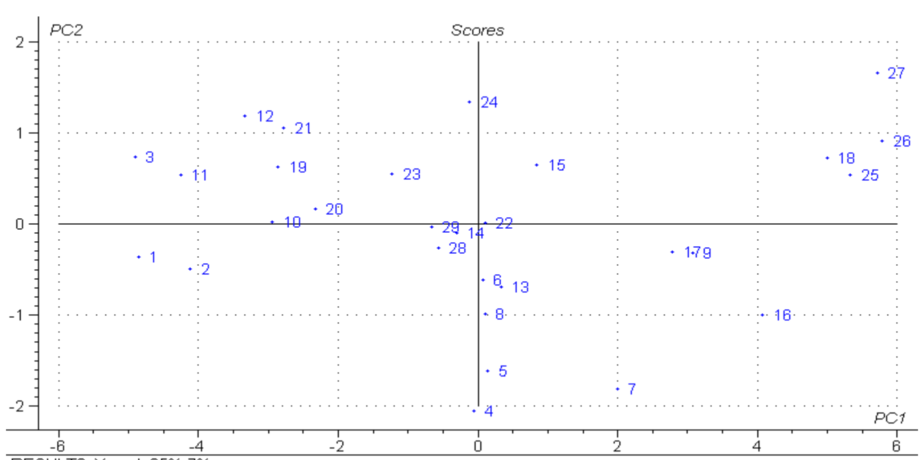
principal component scores



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Analysis of *Rheology*

```
---  FIFTY-FIFTY MANOVA   VERSION 1.1 ---  12 responses ---
Source      DF        exVarSS nPC nBu exVarPC exVarBU    p-Value
A            1       0.024601  2   5  0.685   0.997    0.000002
A*A          1       0.019789  2   5  0.790   0.996    0.005452
B            1       0.671111  1   5  0.947   0.999    0.000000
B*B          1       0.011262  2   5  0.718   0.993    0.235250
C            1       0.082027  2   5  0.763   0.998    0.000000
C*C          1       0.014066  2   5  0.754   0.996    0.197659
A*B          1       0.007798  2   5  0.753   0.996    0.037500
A*C          1       0.003366  2   5  0.771   0.995    0.154337
B*C          1       0.014732  2   5  0.722   0.996    0.000064
Block        4       0.046025  2   5  0.836   0.995    0.060237
Error       15       0.064372 - STANDARDIZATION ON  ------------
```

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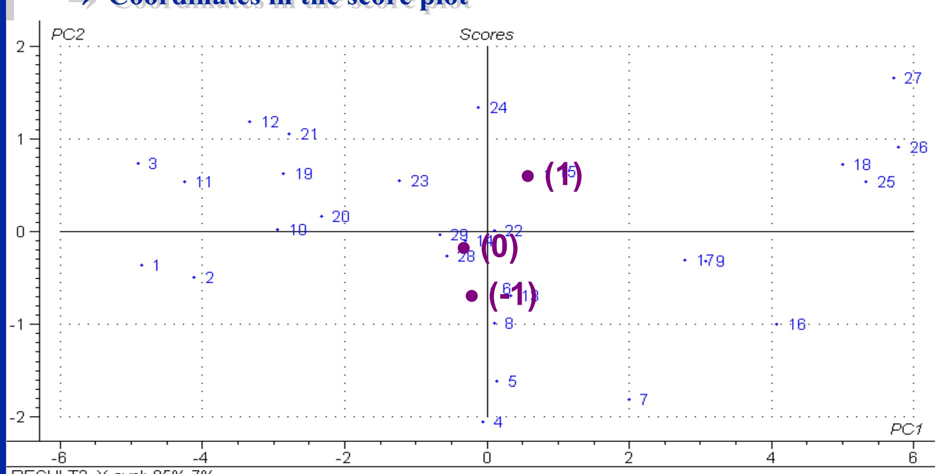
The effect of A (and A*A)

Scores for the first two components
→ Adjusted mean values

→ Coordinates in the score plot



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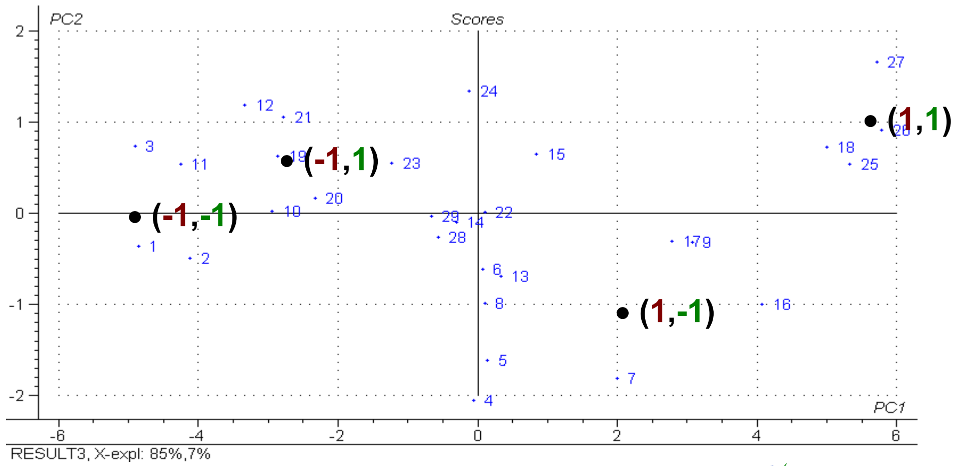
The effect of B, C and B*C: (B,C)



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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

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