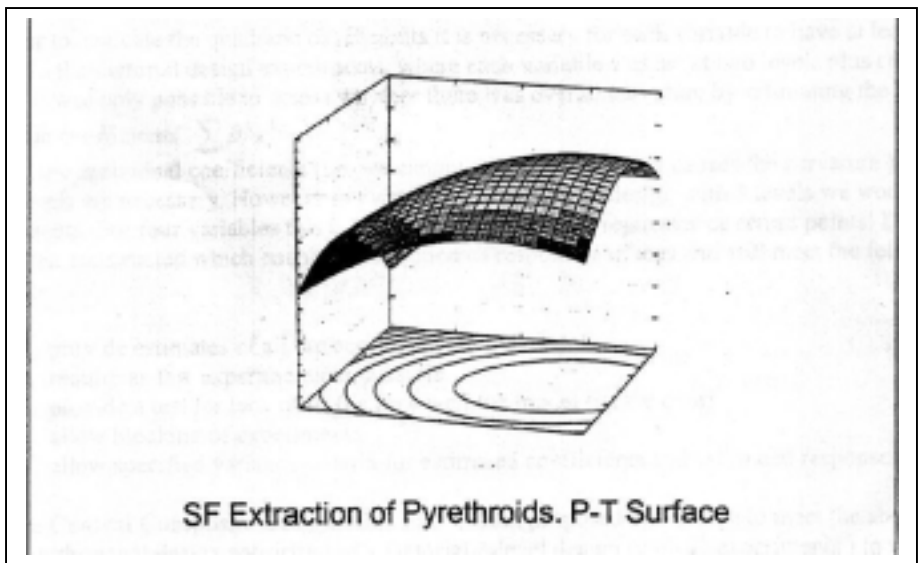


Chemometrics

Unit 4

Response Surface Methodology



Chemometrics

Unit 4. Response Surface Methodology

In Unit 3 the first two phases of experimental design - definition and screening - were discussed. In this unit we will deal with optimisation and validation/ruggedness.

Response surface methodology has a dual aim - to find the optimum settings for the variables and to see how the variables perform over the whole experimental domain, including any interactions.

It is assumed that screening has been done (if needed) to reduce the number of variables to four or less. A maximum of four variables to consider is desirable for two reasons. The first reason is that it keeps the number of experiments to a manageable number (24 + centre points for the CCD design for 4 variables). The second reason involves the nature of a response surface. To understand this we must first define what we mean by a response surface.

4.1 Response Surfaces. A response surface is a k-dimensional surface in k+1 dimensional space, where k is the number of variables. It is a graph of a general second order equation:-

$$y = \beta_0 + \beta_i x_i + \beta_i^2 x_i^2 + \beta_{ij} x_i x_j$$

This is the same model as was used for factorial designs with the addition of the quadratic term in x^2 to allow for curvature in the model. Thus we can test for the case where a variable might have a maximum (or minimum) effect on response in the experimental domain. The **response surface** is then a graph of the response function, y, as a function of the variables x_i . For two variables we thus have a 3-D surface (2-D surface in 3-D space) where the axes are x_1 , x_2 and y (see example on front cover). Since graphs are usually shown on computer screens or flat paper we usually see a 2-D projection of this 3-D plot. However if we have three variables the response surface is a 3-D surface in 4-D space - impossible to depict! The usual method to examine this surface is to hold one of the variables constant (usually at its centre point) and examine y as a function of the other two (see appendix for example). This procedure generates three graphs - y vs x_1, x_2 (x_3 held constant), y vs x_1, x_3 (x_2 constant) and y vs x_2, x_3 (x_1 held constant). For five variables it is necessary to hold two of them constant at a time when drawing graphs. There are now six possible pairings and the possibility that we are getting a more distorted view greatly increases. This is why preliminary screening experiments are desirable if the number of variables is greater than four.

In order to evaluate the quadratic coefficients it is necessary for each variable to have at least three levels. In the factorial design experiments, where each variable was set at two levels plus centre points, it was only possible to assess whether there was overall curvature by estimating the **sum** of the quadratic coefficients

$$\beta_i^2 x_i^2$$

and not the individual coefficients (i.e. we cannot tell **which** variable causes the curvature). To do this three levels are necessary. However to carry out a full factorial design with 3 levels we would need 3^k experiments. For four variables this is 81 experiments without replicates or centre points! Designs have been constructed which enable construction of response surfaces and still meet the following criteria:-

- (i) provide estimates of all the coefficients in the model
- (ii) require as few experiments as possible
- (iii) provide a test for lack of fit (i.e how well the model fits the data)
- (iv) allow blocking of experiments
- (v) allow specified variance criteria for estimated coefficients and estimated responses to be met

4.2 The Central Composite Design. Box and Wilson proposed this design to meet the above criteria. It is an orthogonal design consisting of a factorial 2-level design (with 2^k experiments) to which is added a 'star' design with 2k points plus centre points. These star points project out from each face. The designs for 2 and 3 variables are shown in figs 1 and 2. The levels for each variable are shown in **coded** form. When the regression

model is determined with coded variables the size of each coefficient then gives a direct measurement of the importance of each effect. With uncoded variables scaling effects come into the equation and the coefficients are no longer directly comparable (see the Minitab analysis in the case study).

To see how the number of experiments is reduced consider the following table:-

| Number of factors k | 3-level factorials 3^k | CCD $2^k + 2k$ |
|------------------------|-----------------------------|-------------------|
| 2 | 9 | 8 |
| 3 | 27 | 14 |
| 4 | 81 | 24 |
| 5 | 243 | 42 |
| 5 | 81 (1/3 fraction) | 26 (1/2 fraction) |
| 6 | 729 | 76 |
| 6 | 243 (1/3 fraction) | 44 (1/2 fraction) |

(Note that centre points have not been included in either design)

The Centre Composite Design (CCD) has two important features - it is **orthogonal** and **rotatable** providing the correct spacing for the axial parameter (α) is chosen. It can be shown that the design is **rotatable** if

$$\alpha = \sqrt[4]{2^k} \quad \text{where } 2^k \text{ is the number of factorial points.}$$

A design is said to be **rotatable** if the variance, or error, associated with design points, is determined by their distance from the centre and not the direction. Thus in the CCD with α at the optimum value there is equal weighting for all the design points (except the centre points) so each is equally important in determining the response surface. It thus does not matter which variable we call x_1 , which is x_2 etc. To achieve orthogonality the axial spacing must satisfy

$$\alpha^2 = \frac{\sqrt{(N_c + N_a + N_o)} * N_c - N_c}{2} \quad \text{where } N_c \text{ is the number of cubic or factorial points, } N_a \text{ the}$$

axial points and N_o the number of centre points.

Since there are two criteria to fill (and for a given number of factors k only the number of centre points can be adjusted) it is not always possible for a design to be both orthogonal and rotatable but it can generally be approximated fairly well.

Rotatable and orthogonal designs for k factors:-

| K | $N_c (2^k)$ | $N_a (2k)$ | N_o | alpha |
|---|-------------|------------|-------|-------|
| 2 | 4 | 4 | 8 | 1.414 |
| 3 | 8 | 6 | 9.3 | 1.682 |
| 4 | 16 | 8 | 12 | 2 |
| 5 | 32 | 10 | 16.6 | 2.378 |
| 6 | 64 | 12 | 24 | 2.828 |

In particular the number of centre points for achieving both criteria is rather large. Practical considerations often dictate that this number is reduced. It means the design is no longer strictly orthogonal but providing we use the value of α listed then it will still be rotatable.

Case Study no. 1 The Acetylation of Ferrocene

This example illustrates the use of the CCD design to optimize the synthesis of acetylferrocene from ferrocene (ref. *J. Chem. Ed.*, **68**, 879, Oct 1991) The variables studied were reflux time, reflux temperature and mole ratio of ferrocene: acetic anhydride. the response variable was the fractional yield of acetylferrocene. The experimental domain was as follows:-

| | |
|-------------|------------|
| time | 30 - 210 s |
| temperature | 85 - 115°C |
| mole ratio | 3 - 17 |

In the CCD the values of +/- alpha are the low and high values of the variables. For 3 variables the optimum value of alpha is 1.682. The design in coded and uncoded form is shown:-

| Experiment | time | temp | moleratio | time | temp | moleratio | %yield |
|------------|---------|---------|-----------|--------|--------|-----------|--------|
| 1 | 66.486 | 91.081 | 5.8378 | -1 | -1 | -1 | 38 |
| 2 | 173.514 | 91.081 | 5.8378 | 1 | -1 | -1 | 62 |
| 3 | 66.486 | 108.919 | 5.8378 | -1 | 1 | -1 | 40 |
| 4 | 173.514 | 108.919 | 5.8378 | 1 | 1 | -1 | 69 |
| 5 | 66.486 | 91.081 | 14.1622 | -1 | -1 | 1 | 39 |
| 6 | 173.514 | 91.081 | 14.1622 | 1 | -1 | 1 | 51 |
| 7 | 66.486 | 108.919 | 14.1622 | -1 | 1 | 1 | 40 |
| 8 | 173.514 | 108.919 | 14.1622 | 1 | 1 | 1 | 55 |
| 9 | 30 | 100 | 10 | -1.682 | 0 | 0 | 30 |
| 10 | 210 | 100 | 10 | 1.682 | 0 | 0 | 65 |
| 11 | 120 | 85 | 10 | 0 | -1.682 | 0 | 52 |
| 12 | 120 | 115 | 10 | 0 | 1.682 | 0 | 57 |
| 13 | 120 | 100 | 3 | 0 | 0 | -1.682 | 55 |
| 14 | 120 | 100 | 17 | 0 | 0 | 1.682 | 47 |
| 15 | 120 | 100 | 10 | 0 | 0 | 0 | 66 |
| 16 | 120 | 100 | 10 | 0 | 0 | 0 | 67 |
| 17 | 120 | 100 | 10 | 0 | 0 | 0 | 68 |
| 18 | 120 | 100 | 10 | 0 | 0 | 0 | 66 |
| 19 | 120 | 100 | 10 | 0 | 0 | 0 | 65 |
| 20 | 120 | 100 | 10 | 0 | 0 | 0 | 66 |

Each row represents one experiment. The yields for the experiments are also shown . The runs have not been randomized so the pattern of the design can be seen more easily but normally the order of doing the experiments would be randomised. You will notice that some of the variable settings look strange e.g 68.486 for time in run 1. This is a consequence of setting +alpha(1.682 in coded form) to be 210s. In practice you would round this off to the nearest practical value (e.g 66s). The design can then be altered for analysis to include the actual time used. This should not have any significant effect on the outcome.

The Minitab results output is shown below. Two forms are possible - using coded and uncoded data. As discussed previously we should examine the model using coded data The points to note are:-

- (i) The coefficient of regression (R^2), which gives the percentage variation in the response explained by our regression model. In this case we can explain 94% of the variation. As a rough rule of thumb R^2 values of over 80% are desirable to have confidence that the response model is useful in giving information on your experiment. If R^2 is less than 80% the data should be examined for outliers (by checking the residuals) or perhaps the experiment overall is very 'noisy'. This can be checked by examining the reproducibility of the centre points.

- (ii) The model coefficients are listed together with their standard deviations, t values and associated probabilities. The t values are for a null hypothesis that the coefficients are zero. A p value < 0.05 suggests there is less than 5% chance this is true (i.e. reject the null hypothesis). In this case study the coefficients for time, mole ratio, time², temperature², molaratio² and time*molaratio are all significant. The fact that all the quadratic terms are significant suggests considerable curvature in the model.
- (iii) An analysis of variance table is also included. The table in this case backs up that the second order model is appropriate - linear, quadratic and interaction effects are all significant and there is no evidence of 'lack-of-fit' (p = 0.479 >> 0.05). Consult unit 2 for further explanation of 'lack-of-fit'
- (iv) An 'unusual' observation is reported, which is a possible outlier, but since the overall fit is very good there is no need to exclude points to get an improved fit.
- (v) For comparison the analysis using the uncoded (actual) values of the variables is shown. Notice now that the coefficient for temperature is now larger than that for time. This is a scaling effect as the range for temperature (85-115) was smaller than for time (30-210). The scaling effect has masked the 'importance' effect. When coded variables are used the time coefficient is larger, reflecting that time also a larger linear effect.

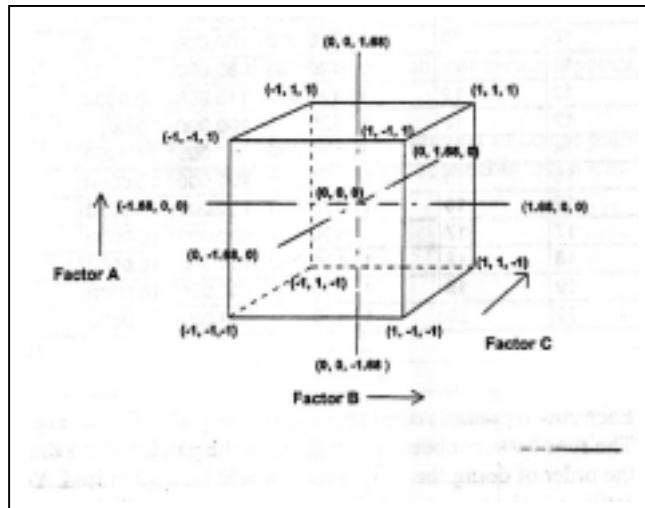
Central Composite Design

Central Composite Design

Factors: 3 Blocks: none Center points: 6
 Runs: 20 Alpha: 1.682

Data Matrix

| Run | A | B | C |
|-----|--------|--------|--------|
| 1 | -1.000 | -1.000 | -1.000 |
| 2 | 1.000 | -1.000 | -1.000 |
| 3 | -1.000 | 1.000 | -1.000 |
| 4 | 1.000 | 1.000 | -1.000 |
| 5 | -1.000 | -1.000 | 1.000 |
| 6 | 1.000 | -1.000 | 1.000 |
| 7 | -1.000 | 1.000 | 1.000 |
| 8 | 1.000 | 1.000 | 1.000 |
| 9 | -1.682 | 0.000 | 0.000 |
| 10 | 1.682 | 0.000 | 0.000 |
| 11 | 0.000 | -1.682 | 0.000 |
| 12 | 0.000 | 1.682 | 0.000 |
| 13 | 0.000 | 0.000 | -1.682 |
| 14 | 0.000 | 0.000 | 1.682 |
| 15 | 0.000 | 0.000 | 0.000 |
| 16 | 0.000 | 0.000 | 0.000 |
| 17 | 0.000 | 0.000 | 0.000 |
| 18 | 0.000 | 0.000 | 0.000 |
| 19 | 0.000 | 0.000 | 0.000 |
| 20 | 0.000 | 0.000 | 0.000 |



Response Surface Regression

The analysis was done using coded units.

Estimated Regression Coefficients for %yield

| Term | Coef | StDev | T | P |
|----------|--------|--------|---------|-------|
| Constant | 66.360 | 0.4086 | 162.418 | 0.000 |

| | | | | |
|---------------|--------|--------|---------|-------|
| time | 10.168 | 0.2711 | 37.509 | 0.000 |
| temp | 1.641 | 0.2711 | 6.053 | 0.000 |
| mratio | -2.743 | 0.2711 | -10.117 | 0.000 |
| time*time | -6.832 | 0.2639 | -25.888 | 0.000 |
| temp*temp | -4.357 | 0.2639 | -16.510 | 0.000 |
| mratio*mratio | -5.594 | 0.2639 | -21.199 | 0.000 |
| time*temp | 1.000 | 0.3542 | 2.823 | 0.018 |
| time*mratio | -3.250 | 0.3542 | -9.176 | 0.000 |
| temp*mratio | -0.500 | 0.3542 | -1.412 | 0.188 |

S = 1.002 R-Sq = 99.6% R-Sq(adj) = 99.3%

Analysis of Variance for %yield

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|----------------|----|---------|---------|---------|--------|-------|
| Regression | 9 | 2823.76 | 2823.76 | 313.752 | 312.64 | 0.000 |
| Linear | 3 | 1551.44 | 1551.44 | 517.148 | 515.31 | 0.000 |
| Square | 3 | 1177.82 | 1177.82 | 392.607 | 391.21 | 0.000 |
| Interaction | 3 | 94.50 | 94.50 | 31.500 | 31.39 | 0.000 |
| Residual Error | 10 | 10.04 | 10.04 | 1.004 | | |
| Lack-of-Fit | 5 | 4.70 | 4.70 | 0.940 | 0.88 | 0.553 |
| Pure Error | 5 | 5.33 | 5.33 | 1.067 | | |
| Total | 19 | 2833.80 | | | | |

Response Surface Regression

The analysis was done using uncoded units.

Estimated Regression Coefficients for %yield

| Term | Coef | StDev | T | P |
|---------------|--------|---------|---------|-------|
| Constant | -588.4 | 35.9485 | -16.368 | 0.000 |
| time | 0.7 | 0.0792 | 8.824 | 0.000 |
| temp | 11.0 | 0.6769 | 16.282 | 0.000 |
| mratio | 8.9 | 1.0216 | 8.709 | 0.000 |
| time*time | -0.0 | 0.0001 | -25.888 | 0.000 |
| temp*temp | -0.1 | 0.0033 | -16.510 | 0.000 |
| mratio*mratio | -0.3 | 0.0152 | -21.199 | 0.000 |
| time*temp | 0.0 | 0.0007 | 2.823 | 0.018 |
| time*mratio | -0.0 | 0.0016 | -9.176 | 0.000 |
| temp*mratio | -0.0 | 0.0095 | -1.412 | 0.188 |

S = 1.002 R-Sq = 99.6% R-Sq(adj) = 99.3%

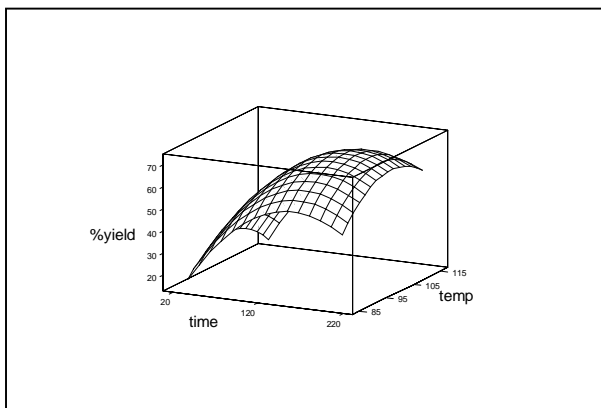
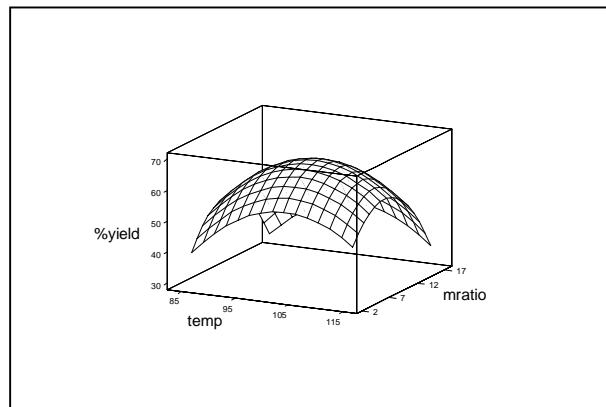
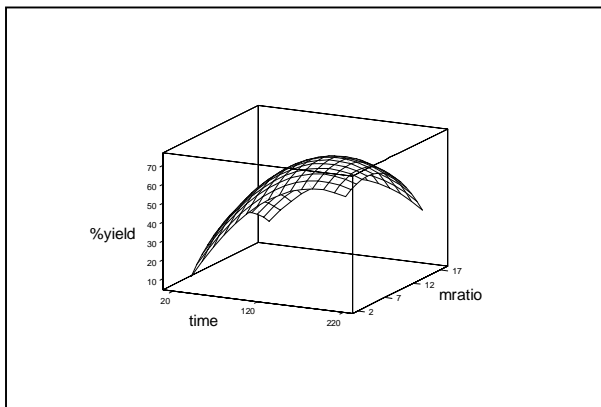
Analysis of Variance for %yield

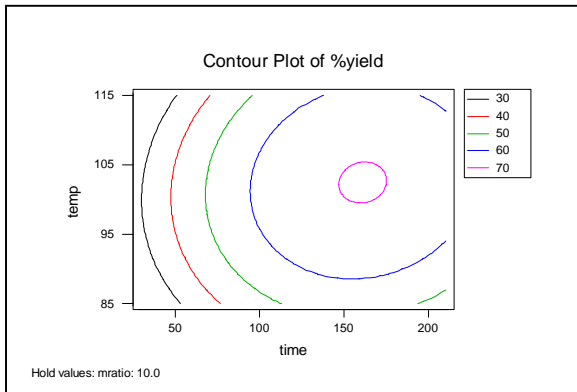
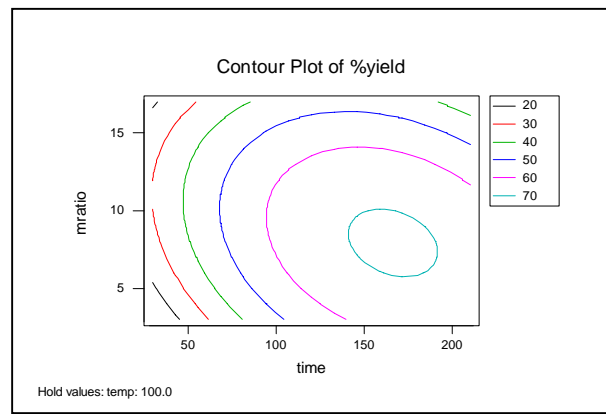
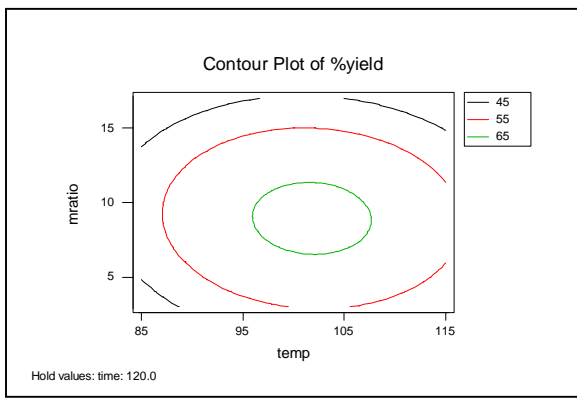
| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|----------------|----|---------|---------|---------|--------|-------|
| Regression | 9 | 2823.76 | 2823.76 | 313.752 | 312.64 | 0.000 |
| Linear | 3 | 1551.44 | 344.29 | 114.765 | 114.36 | 0.000 |
| Square | 3 | 1177.82 | 1177.82 | 392.607 | 391.21 | 0.000 |
| Interaction | 3 | 94.50 | 94.50 | 31.500 | 31.39 | 0.000 |
| Residual Error | 10 | 10.04 | 10.04 | 1.004 | | |
| Lack-of-Fit | 5 | 4.70 | 4.70 | 0.940 | 0.88 | 0.553 |
| Pure Error | 5 | 5.33 | 5.33 | 1.067 | | |
| Total | 19 | 2833.80 | | | | |

When we use a full second order model we get main, interaction and quadratic effects so it becomes harder to discern which variables have the most influence on yield. We can get more information by looking at the **response surfaces**. The response surface is an n-dimensional surface in n+1 dimensional space. In this case it will be a graph of yield as a function of time, temperature and molar ratio. This would be a 3-D surface in 4-D space - impossible to draw! In order to get a graph we can draw in 3-D (or, in fact, a 2-D representation of a 3-D graph) we hold one of the variables constant (at the centre point) and look at yield as a function of the other two variables. The graph can be represented as a surface (wire-frame) or contour graph. In this case we have three pairs of graphs with one of the variables held constant each time.

The following can be observed by examining the graphs:

- (i) Each of the three variables have optimal settings as evidenced by each of the surfaces showing maxima (the 'peaks' of the hills - shown most clearly in the contour diagrams). This supports the fact that all three quadratic coefficients are significant (when using coded values).
- (ii) Time shows a strong positive effect - there is a definite slope to higher time, indicating longer times are better, reflected in the significant positive time main effect. Similarly mole ratio has a smaller negative effect - lower mole ratios are better.
- (iii) Temperature only has a significant quadratic coefficient. Low and high temperatures are about the same but there is a better intermediate temperature. Also the contours are more widely spaced along the temperature axes - indicating change in temperature is less important than the other two variables i.e. the variable is more 'rugged'





Determining Optimal Settings

The next phase of the analysis is to determine the optimal settings of the variables - in the ferrocene case study we want to know the best temperature, time and mole ratio to get the best yield. There are three methods we can use:-

- (i) **Inspection of the response surfaces.** For example we could examine the temperature-time surface to get estimates of the optimal settings. From the contour diagram the 'peak' in the surface appears to be at about 104°C and 165s. **Caution!** This is NOT the true response surface, which is a 3-D surface in 4-D space. To get a 2-D surface in 3-D space (and hence one we could actually visualise) it was necessary to set the other variable, mole ratio, to a constant value (in this case its mid point value of 10). We can only get true optima from the graphs in the 2-variable case where the true surface can be viewed in 3-D space
- (ii) **Canonical Analysis.** This is a mathematical treatment which finds stationary points of polynomials. The defining equation is :

$$\mathbf{x}_s = -\mathbf{B}^{-1}\mathbf{b}/2$$

where \mathbf{x}_s is a vector containing the values of the variables at the stationary point. B is a matrix where the diagonal elements are the quadratic coefficients from the second order model equation; the off-diagonal elements are half the interaction coefficients i.e $B(i,i) = b_{ii}$ and $B(i,j) = B(j,i) = b_{ij}/2$. The B matrix gives additional information in that the eigenvalues of the matrix determine what kind of stationary point it is. If the values are all negative it is a maximum; if all positive it is a minimum while if the signs are mixed it is a saddle point. **Problem!** While this process gives a global maximum or minimum (if one exists) this point may not be within the experimental domain i.e they might be unattainable or undesirable settings (even negative!) so the information may not be of use. What we want are the optimum settings **within** the experimental domain.

An example of a MathCad worksheet to evaluate canonical analysis for the Ferrocene case study is shown:

Ferrocene Optimisation Canonical Analysis

$$b := \begin{bmatrix} .6989 \\ 11.0208 \\ 8.8971 \end{bmatrix}$$

$$B := \begin{bmatrix} -0.00239 & 0.001048 & -0.0073 \\ 0.001408 & -0.0548 & -0.00673 \\ -0.0073 & -0.00673 & -0.32291 \end{bmatrix}$$

$$x := -B^{-1} \cdot \frac{b}{2}$$

$$x = \begin{bmatrix} 167.907 \\ 103.909 \\ 7.815 \end{bmatrix}$$

$$\text{eigenvals}(B) = \begin{bmatrix} -2.188 \cdot 10^{-3} \\ -0.323 \\ -0.055 \end{bmatrix}$$

(iii) Numerical methods - the Excel 'Solver'

This method uses iterative numerical methods to approximate an optimal solution, starting from initial guesses and making 'improved' guesses till an optimal solution fitting the constraints is found. The 'Solver' utility in Excel is a convenient way to do this. To set it up we need to enter the coefficients for the uncoded model. We also need initial guesses (values estimated from the response surfaces are good starting points). A value of the yield is then calculated, using the regression equation. We then set up the solver to maximise this value of the yield by varying the three variables subject to the constraints ($30 \leq \text{time} \leq 210$, $85 \leq \text{temperature} \leq 115$, $3 \leq \text{mole ratio} \leq 17$). The spreadsheet is shown below. Notice that, in this case, we get almost identical result to canonical analysis because the maximum point is within the experimental domain. **Caution.** The Solver does not always get to the optimum value. This can occur if the surface is irregular and depends on the initial guesses. For example, one of the surfaces illustrated is a ridge with a saddle point. If we are trying to **minimise** the response quite different results will be obtained depending on what side of the ridge we start.

Using Excel Solver to find optimal conditions

Uncoded coefficients Initial Guesses

| | | | |
|-----|----------|------------------|----------|
| b0 | -588.403 | time | 100 |
| b1 | 0.699893 | temp | 90 |
| b2 | 11.02075 | moleratio | 5 |
| b3 | 8.897133 | | |
| b11 | -0.00239 | | |
| b22 | -0.05477 | Optimised values | |
| b33 | -0.32291 | | |
| b12 | 0.002095 | time | 167.5493 |
| b13 | -0.01459 | temp | 102.8489 |
| b23 | -0.01347 | moleratio | 7.846165 |
| y | 71.87101 | | |

4.3 Blocking

It may be necessary, as described in unit three, to divide the experiments due to constraints of time, reagents, personnel etc. The CCD design allows an easy method of blocking into orthogonal blocks. The factorial or cube points go in one block and the star points go in the other. centre points are divided evenly between the blocks. If more than two blocks are needed the cube points can be further blocked as described in unit three.

4.4 Ruggedness/Validation

The last phase of the design is to check or validate the predicted optimal settings and to perform a 'ruggedness' check. The aim here is to first check that the predicted conditions do in fact give optimal results and to check how 'stable' or 'rugged' the method is if the variables are changed slightly from these settings. A rugged method is one where the surface is fairly flat around the optimum i.e. the variables have some flexibility in their settings and still get close to optimal response.

There is no accepted single design used for ruggedness/validation work. Full or fractional factorial designs plus modified factorial designs such as Plackett-Burmann and Taguchi designs have been used.

For example, consider the ferrocene case study. The predicted optimal settings become the centre points for the validation/ruggedness design i.e 167s, 103°C, 7.8 MR. The design used here is a full factorial plus centre points. Using a criterion of 10% of the original range for each variable the new design is:-

| t(coded) | T(coded) | MR(coded) | t(uncoded) | T(uncoded) | MR(uncoded) |
|----------|----------|-----------|------------|------------|-------------|
| -1 | -1 | -1 | 157 | 101 | 7.1 |
| 1 | -1 | -1 | 177 | 101 | 7.1 |
| -1 | 1 | -1 | 157 | 105 | 7.1 |
| 1 | 1 | -1 | 177 | 105 | 7.1 |
| -1 | -1 | 1 | 157 | 101 | 8.5 |
| 1 | -1 | 1 | 177 | 101 | 8.5 |
| -1 | 1 | 1 | 157 | 105 | 8.5 |
| 1 | 1 | 1 | 177 | 105 | 8.5 |
| 0 | 0 | 0 | 167 | 103 | 7.8 |

The centre point would be replicated (say) 4 times and the order randomised.

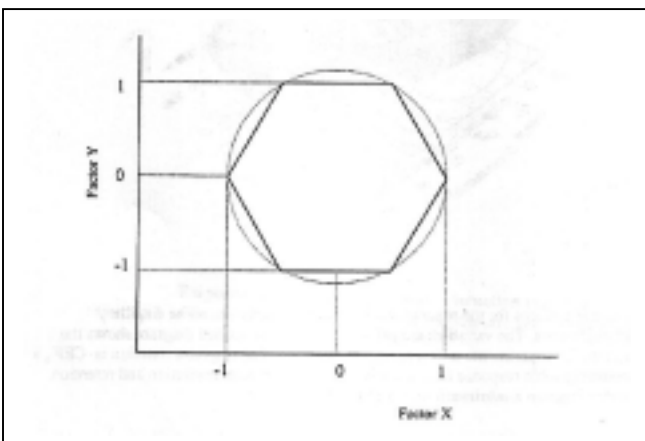
4.5 Alternative Designs

The CCD is not the only design that can be used for response surface modelling. It is the most widely used and has many advantages. However one disadvantage to this design is that each variable has to be set at five levels. This may be experimentally inconvenient (for example, if five different buffer solutions need to be prepared). Also for any 'high' or 'low' setting of the variable it is only combined with the others at the mid-point setting (i.e. points like $\pm\alpha, 0, 0$). We never carry out experiments with two or more variables at the high settings. Both these disadvantages can be overcome if α is set to 1 (i.e. a face-centred cube design). The design is no longer rotatable but this may be outweighed by the extra experimental convenience.

Another design which uses only three settings for each variable is the **Box-Behnken** design. These designs are rotatable second-order designs that are incomplete 3^k factorial designs. For three factors, for example, the design can be constructed as three blocks of 4 experiments consisting of a full factorial design for two factors with the third set at zero. To this design centre points are added:

| A | B | C |
|----|----|----|
| -1 | -1 | 0 |
| -1 | 1 | 0 |
| 1 | -1 | 0 |
| 1 | 1 | 0 |
| -1 | 0 | -1 |
| -1 | 0 | 1 |
| 1 | 0 | 1 |
| 1 | 0 | -1 |
| 0 | -1 | -1 |
| 0 | 1 | 1 |
| 0 | -1 | 1 |
| 0 | 1 | -1 |
| 0 | 0 | 0 |
| 0 | 0 | 0 |
| 0 | 0 | 0 |

Another possibility is the Doehlert, or uniform shell, design. For two variables the experiments form a hexagon centred on the centre points, giving six experiments + centre points. One variable is studied at three levels and one at five (see diagram).



We can summarise then that multi-level experimental designs provide a way of picturing how the method is performing overall. It not only predicts the optimal conditions for the experiment but gives a 'picture' of how each variable is affecting the result.

References

The following textbooks provide a good general coverage of this topic:

1. A First Course in Design and Analysis of Experiments, G. W. Oehlert (Freeman, New York, 2000)
2. Chemometrics: Experimental Design, E. Morgan (Wiley, London, 1991)

For a more theoretical treatment the following references are useful:

1. Response Surfaces: Design and Analysis, A.I. Khuri and J.A. Cornell (Dekker, New York, 1996)
2. Empirical Model-Building and Response Surfaces, G.E.P. Box and N.R. Draper (Wiley, New York, 1987)

The following review articles also provide good introductory summaries:

1. Attaining Optimal Conditions, H.V. Ryswyck and G.R. van Hecke, J. Chem. Ed., **68**, 878 (1991)
2. Introduction to Experimental Design for Chemists, R.S. Strange, J. Chem. Ed., **67**, 113 (1990)
3. Central Composite Design Experiments, J.A. Palasota, S.N. Deming, J. Chem. Ed., **69**, 560 (1991)
4. Experimental Design and Optimization, T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, A. Nystrom, J. Petterson and R. Bergman, CHemoetrics and Intelligent Laboratory Systems, **42**, 3 (1998)
5. Experimental Design 1. Screening and 2. Optimization, P. W. Araujo and R. G. Brereton, Trends in Analytical Chemistry, **15**, 26-31 and 63 - 70 (1996)

