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Optimization of pharmaceutical formulations based on response-surface experimental designs

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Summary

Optimization techniques for pharmaceutical formulations based on second order orthogonal or rotatable designs as well as simplex lattice designs have been revisited. A digest of the procedures for optimizing pharmaceutical formulations is given.

Introduction

In addition to the art of formulation, techniques are available that can aid in the pharmacist's choice of formulation components which optimize one or more product attributes (Bolton, 1984; Bergman and Gittins, 1985) and recently these chemometric procedures have been applied successfully to pharmaceutical formulations. The experimental design based on surface-response model building is a well-known topic and, consequently, its foundations cannot be introduced here (Box et al., 1978; Box and Draper, 1987; Morgan et al., 1989; Morgan, 1991). However, a brief practical outline is given in the following (Akhnazarova and Kafarov, 1982).

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It is well known that traditional experimentation involves a good deal of effort and time, especially where complex processes are evaluated. A very efficient way to enhance the value of research and to minimize the process development time is through designed experiments.

Today experimental design and optimization are based on mathematical modelling – deterministic or black-box models. Although the first seems to be philosophically the most attractive, unfortunately it often remains inapplicable as there is not always a known law to describe the studied phenomenon. This is the general case of pharmaceutical formulations. A typical mathematical model is:

$$y = y(z_1, z_2, \ldots, z_k) \quad (1)$$

where y is the dependent variable (response) and z_is are the independent variables (factors). They occupy what is called factor space and a plot of y

on this space is called the response surface. By expanding the response function into a truncated Taylor's series, the mathematical model is frequently a polynomial:

$$y = \beta_0 + \sum \beta_i z_i + \sum \beta_{ij} z_i z_j + \sum \beta_{ii} z_i^2 + \ldots \tag{2}$$

Therefore, we will suppose that the values of y vary when a certain numbers of experimentally controlled factors take on different fixed values called levels.

In real processes there always are uncontrollable factors and the output produced varies at random. All these uncontrolled factors form the background noise or experimental error which is assumed to be normally distributed. This hypothesis seems acceptable remembering the central limit theorem taking into account that the background noise is the outcome of summing the uncontrolled factors depending on various laws considered to be mutually independent. The use of multiple linear regression techniques yields sample regression coefficients, b_0, b_i, b_{ij} and b_{ii} which are the estimates of the true coefficients (βs). So, the estimated regression equation take the form:

$$\hat{y} = b_0 + \sum b_i z_i + \sum b_{ij} z_i z_j + \sum b_{ii} z_i^2 + \ldots \tag{3}$$

This regressive procedure is more straightforward than Yates' algorithm for calculations of both main effects and interaction effects (Morgan et al., 1989).

A full factorial design is one where all possible combinations of the factors at all levels involved in the experiment are used. Without replication and taking the same number n of levels for each factor, the number N of possible combinations (treatments or runs) to be performed during the experiment is given by $N = n^f$, f being the number of selected factors.

Consider that our factor space has dimension f. For each factor z_j ($j = 1$–f) we have n levels, the lowest of which is $z_j^{\min}$ and the highest of which is $z_j^{\max}$. So each level of the factor z_j belongs to the interval $(z_j^{\max}, z_j^{\min})$. Then we can define:

the center of the interval, $z_j^0 = \left(z_j^{\max} + z_j^{\min}\right)/2$ (4)

the length of the interval,

$$\Delta z = \left(z_j^{\max} - z_j^{\min}\right)/2 \tag{5}$$

The point whose coordinates are $(z_1^0, z_2^0, \ldots z_f^0)$ is called the center of design.

In order to obtain a dimensionless factor space coordinate system, it is advisable to transform the z_j coordinates into new dimensionless x_j coordinates according to the coding equation:

$$x_j = \left(z_j - z_j^0\right)/\Delta z \tag{6}$$

In the dimensionless coordinate system, the upper and lower levels are $+1$ and -1, respectively, and the center of design is now the point $(0, 0, \ldots 0)$.

After coding, Eqns 2 and 3 may be rewritten as:

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2 + \ldots \tag{7}$$

$$\hat{y} = b_0 + \sum b_i x_i + \sum b_{ij} x_i x_j + \sum b_{ii} x_i^2 + \ldots \tag{8}$$

Now, y, $\hat{y}$, βs and bs are referred to the new coordinate system. However, we continue with the same notation for avoiding an extra amount of new symbols, perhaps superfluous after this indication. So, in the remainder, any parameter will be referred to the coding factors x unless otherwise explicitly indicated.

The aim of optimization is to determine those values $(x_1^*, x_2^*, \ldots, x_f^*)$ that maximize the objective function $y = y(x_1, x_2, \ldots, x_f)$. Two different methodologies (Bergman and Gittins, 1985) may be applied for finding these optimum values. First, one may determine the general configuration of the response surface by evaluating y over a grid of different values according to an experimental

planning. Thereafter, a quadratic function is fitted over the relevant data points. Second, one may proceed to find the maximum of y without determining the general form of the response surface. Direct search methods such as simplex algorithm or steepest ascent hill-climbing algorithms are useful. An advantage of these later algorithms is that they find a maximum with relatively few observations; the drawback is that this maximum may only be a local, rather than a true maximum.

So, chemometrically speaking two well separated generic designs may be performed: simultaneous and sequential designs (Brerenton, 1990). The former involves a given set of experiments according to the established conditions. All these experiments need to be carried out. Then, the output response is fitted to the mathematical model. From the adjusted response surface, optimal conditions can be obtained. Conversely, in sequential designs the experiments are successively performed until the desired optimum is reached by using direct search algorithms. The present survey deals only with simultaneous factorial designs.

The most widely simultaneous designs of interest for pharmaceutical formulation development (Bolton, 1984; Bohidar and Peace, 1988) are the so-called composite designs and simplex-lattice designs. Both kinds of planning will be outlined in the following.

Second-order composite designs

As was indicated previously, considering that the surface region in the factor space close to the optimum is substantially non-linear, one can search for the optimum by adjusting the response surface by second-order non-linear polynomials. For this purpose the factors in the experimental designs should take at least three different values, i.e., a three-level full factorial design is needed. The number ℓ of coefficients of Eqn 8 can be given by (Akhnazarova and Kafarov, 1982):

$$\ell = (f+1)(f+2)/2 \qquad (9)$$

A 3^f full factorial design involves a very great number of observations that exceeds considerably the number of coefficients to be estimated as indicated in Table 1. To alleviate such situations, Box and Wilson (1951) developed new designs, called second order composite designs, for fitting second-order polynomial response surfaces. The kernel of this design is a 2^f design for $f < 5$ or a half replicate thereof at $f > 5$ (Akhnazarova and Kafarov, 1982). Let $N_k = 2^f$. Now we add $2f$ star points positioned on the coordinate axes of factorial space: $(\pm\alpha, 0, \ldots, 0)$, $(0, \pm\alpha, 0, \ldots, 0), \ldots$ $(0, 0, \ldots, \pm\alpha)$, where α is the distance from the center point of the design to a star point. Let $N_\alpha = 2f$. Finally, we add N_0 extra points (generally one) at the center of design $(0, 0, \ldots, 0)$. Therefore, composites of complete factorials require at least $N_k + N_\alpha + 1 = 2^f + 2f + 1$ runs. Composite designs can readily be transformed into orthogonal ones by an appropriate selection of the star arm or axial spacing α (Morgan et al., 1989):

$$\alpha = \left[\left(\left[(N_k + N_\alpha + N_0)N_k\right]^{1/2} - N_k\right)/2\right]^{1/2} \qquad (10)$$

Values for the axial spacing depending on the number of factors involved and the central runs are collected in Table 2.

Orthogonality eliminates covariances between estimated pure second-order coefficients (b_{ii}). Rather than using a criterion for individual estimated coefficients, it is possible to use criteria based on the joint effect of all coefficients. Box and Hunter (1957) have suggested that a second-order rotatable design should be considered an optimum. A design is said to be rotatable if its

TABLE 1

Number of experimental runs required for 3^f factorial and central composite designs

Number of factors	Number of coefficients	Three-level factorial 3^f	Composite $2^f + 2f + 1$
2	6	9	9
3	10	27	15
4	15	81	25
5	21	243 (81) [a]	43 (27) [b]
6	28	729 (243) [a]	77 (45) [b]

[a] Third fractional design.
[b] Half fractional design.

TABLE 2
Values of α for orthogonal second-order composite designs

N_0 central points	f, number of factors			
	2	3	4	5 [a]
1	1.00	1.21	1.41	1.54
2	1.08	1.28	1.47	1.61
3	1.15	1.35	1.54	1.66
4	1.21	1.41	1.61	1.72
5	1.27	1.47	1.66	1.77
6	1.32	1.52	1.72	1.82
7	1.37	1.58	1.77	1.87
8	1.41	1.62	1.82	1.91
9	1.45	1.67	1.87	1.96
10	1.50	1.71	1.91	2.00

[a] Half fractional design.

variance-covariance matrix is invariant to the orthogonal rotation of the coordinates. This means that the variance of the predicted responses depends only on the distance from the design center and not on the direction. The axial spacing in a rotatable design depends only on the number of kernel runs (N_k):

$$\alpha = (N_k)^{1/4} \tag{11}$$

Table 3 lists the values of α and N_0 for different values of f in uniform precision rotatable designs (Box and Hunter, 1957).

The problem of fitting Eqn 8 becomes quite straightforward because in matrix form we have:

$$y = X\beta \tag{12}$$

Thus, the column vector for the estimated coefficients is:

$$B = (X^T X)^{-1} X^T y \tag{13}$$

the estimation of the residual variance is:

$$s^2_{res} = \sum (\hat{y}_i - y_i)^2 / (N - \ell) \tag{14}$$

and the coefficient variance-covariance matrix is:

$$\mathrm{COV} = (X^T X)^{-1} s^2_{res} \tag{15}$$

It is assumed that $X^T X$ is nonsingular and thus has an inverse.

Consider, for instance, that a three-factor orthogonal composite design is applied for optimizing a dosage form and N total experiments $2^3 + 2 \times 3 + 1 = 15$ have been performed. The vectors y and B (written in transpose form) will be:

$$y^T = [y_1 y_2 \ldots y_{15}] \tag{16}$$

$$B^T = [b_0 b_1 b_2 b_3 b_{11} b_{22} b_{33} b_{12} b_{13} b_{23}] \tag{17}$$

and the data matrix X is:

$$X = \begin{vmatrix}
1 & -1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 \\
1 & 1 & -1 & -1 & 1 & 1 & 1 & -1 & -1 & 1 \\
1 & -1 & 1 & -1 & 1 & 1 & 1 & -1 & 1 & -1 \\
1 & 1 & 1 & -1 & 1 & 1 & 1 & 1 & -1 & -1 \\
1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & -1 & -1 \\
1 & 1 & -1 & 1 & 1 & 1 & 1 & -1 & 1 & -1 \\
1 & -1 & 1 & 1 & 1 & 1 & 1 & -1 & -1 & 1 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
1 & -\alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 & 0 \\
1 & \alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & -\alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 \\
1 & 0 & \alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & -\alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 \\
1 & 0 & 0 & \alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{vmatrix} \tag{18}$$

Each column represents the values of the factors in the following order: 1, x_1, x_2, x_3, x_1^2, x_2^2, x_3^2,

TABLE 3
Values of α and N_0 for uniform precision rotatable second-order composite designs

	f, number of factors								
	2	3	4	5	5 [a]	6	6 [a]	7	7 [a]
Kernel	2^2	2^3	2^4	2^5	2^4	2^6	2^5	2^7	2^6
α	1.41	1.68	2.00	2.38	2.00	2.83	2.38	3.36	2.83
N_0	5	6	7	10	6	15	9	21	14

[a] Half fractional design.

x_1x_2, x_1x_3 and x_2x_3. The design matrix $\boldsymbol{D}$ is within $\boldsymbol{X}$ (columns 2–4). The first eight rows of $\boldsymbol{D}$ correspond to all possible values for the coded variables in the 2^3 full factorial design. The following six rows are the star augmentations and the last row is the central run. The columns to the right of these three are derived by multiplying the levels corresponding to the two factors involved.

Once the multiple regression calculations have been made, the optimum in y occurs for those x^* values for which the derivatives $\partial y/\partial x_i$ (i = 1–3) are simultaneously zero. If such a maximum exists x^*, it can be found from:

$$x^* = -S^{-1}s/2 \tag{19}$$

where:

$$x^{*T} = [x_1^* x_2^* x_3^*] \tag{20}$$

$$s^T = [b_1 b_2 b_3] \tag{21}$$

$$S = \begin{vmatrix} b_{11} & b_{12}/2 & b_{13}/2 \\ b_{12}/2 & b_{22} & b_{23}/2 \\ b_{13}/2 & b_{23}/2 & b_{33} \end{vmatrix} \tag{22}$$

This formulation may easily extended to f factors, although if f is equal to or greater than 5 it is more advisable to use fractional factorial designs (Philippe, 1967; Box et al., 1978; Morgan, 1991).

The multiple regression procedure may be easily performed by using suitable software. Good statistical packages like STATGRAPHICS® or CSS (which are available for IBM compatible personal computers) are useful for general purposes in experimental design, optimization, multivariate analysis and a plethora of mathematical and statistical techniques.

Once the regression has been carried out, a number of tests should be applied to check the validity of the regression model which are described in the following.

Adequacy of the model

Generally, the center point is repeated several times (N_0) during the course of the experiments.

TABLE 4

ANOVA of regression model for composite designs

Source	DF
Regression	$\ell - 1$
Residual	$N - \ell$
Pure error	$N_0 - 1$
Lack of fit	$N - \ell - N_0 + 1$
Total	$N - 1$

The total number of experiments is N. The number of regression coefficients is ℓ. The breakdown of the degrees of freedom (DF) for the analysis of variance (ANOVA) is collected in Table 4. The error mean square s_e^2 is calculated from the N_0 central runs. To test the estimated regression equation for the goodness of the fit, use is made of the Fisher F test:

$$F = s_g^2/s_e^2 \tag{23}$$

where s_g^2 is the lack of fit mean square given by:

$$s_g^2 = \left(s_{res}^2(N - \ell) - s_e^2(N_0 - 1)\right) / (N - \ell - N_0 + 1) \tag{24}$$

The regression model is considered adequate if $F < F_{0.95}(N - \ell - N_0 + 1, N_0 - 1)$. An analysis of residuals would also be of interest for testing the adequacy of the model (Draper and Smith, 1981). The statistical packages STATGRAPHICS® and CSS® provide this possibility as well as other diagnostic techniques and the necessary plots in explicit outputs.

Canonical and ridge analysis

The stationary point x^* is not necessarily a maximum (Van Ryswyk and Van Hecke, 1991), it could be a minimum or a saddle point on the fitted response surface. The canonical analysis consists of noting the signs of the eigenvalues λ_i of the matrix S, i.e., the solutions of the f-th order polynomial provided by the determinantal equation:

$$|S - \lambda I| = 0 \tag{25}$$

where I is the identity matrix. The signs of the λ_is reflect the character of the stationary point. If all $\lambda_i s < 0$, the stationary point is a maximum. If all $\lambda_i s > 0$, the stationary point is a minimum. When the λ_is differ in sign, the stationary point is a minimax or saddle point. A contour map or three-dimensional response surface is very illustrative when possible (and may be easily obtained by using any of the statistical packages mentioned). However, it is possible to ascertain some types of response surface without plotting the response surface by examining the pure second-order terms (Morgan, 1991). Consider, for instance, the surface $y(x_1, x_2)$. If b_{11} and b_{22} are positive and of approximately the same order of magnitude, the response surface will be a parabola opening upwards. The stationary point is a minimum. If both coefficients are negative and approximately of the same order of magnitude, the response surface is a parabola opening downwards, and the stationary point is a maximum. When both coefficients are negative with a slight difference in their magnitudes, the response surface becomes flatter. However, if they are both negative but very different a ridge response surface is likely. When one of the parameters is negative and the other positive and both are of about the same size, a saddle is being described. When the number of factors is greater than 2, a pseudo-three-dimensional plot of the response surface may be obtained keeping the remaining other factors at their constant optimal values.

Significance test for regression coefficients

The variance of each regression parameter b_i is easily obtained from the diagonal elements of the variance covariance matrix $\mathrm{COV} = (\boldsymbol{X}^{\mathrm{T}}\boldsymbol{X})\, s^2_{\mathrm{res}}$ (Morgan, 1991). Any statistical package gives this information. Once the variance $s^2\,(b_i)$ is known, the coefficients are tested for significance by Student's t-test:

$$t_i = |b_i| / s(b_i) \tag{26}$$

a coefficient is significant if $t_i > t_{0.95}(\nu)$ where ν is the number of degrees of freedom of the residual variance (Lacroix, 1962; Doerfel, 1987; Tallarida and Murray, 1987). The nonsignificant estimated coefficients are dropped from the regression equation, and the remaining estimated coefficients are recalculated because they are interrelated.

Simplex-lattice designs

One of the more popular methods of defining response surfaces and optimal regions for formulation characteristics is the application of simplex lattice designs. This term is unfortunate because it creates confusion between the simplex method for direct search optimization and the mixture design considered here, a very different method on all accounts. In order to avoid further confusion, we shall use the term Scheffe's lattice (Akhnazarova and Kafarov, 1982) in connection with the mixture simplex lattice design. This class of design is particularly appropriate in formulation optimization procedures where the total quantity of the different ingredients under consideration must be constant (Bolton, 1984). Consider, for instance, that in a liquid formulation the active ingredient and solvent compose 90% of the product and the remaining 10% consists of preservatives, colouring agents and surfactant. Therefore, for determining optimal proportions we vary the concentrations of these three ingredients with the restriction that their total concentration is 10%. In general, the Scheffe's lattice designs are usually applied to formulation problems in which a mixture of three or more components is to be investigated. The design is conveniently represented by regular-sided figures. For example, a three-component system is represented by an equilateral triangle in two dimensions. In such a case, for the sake of D-optimality (Akhnazarova and Kafarov, 1982), an incomplete third-degree polynomial response surface in terms of the proportions of the components namely, A, B and C is required for fitting the response:

$$y = b_{\mathrm{A}}x_{\mathrm{A}} + b_{\mathrm{B}}x_{\mathrm{B}} + b_{\mathrm{C}}x_{\mathrm{C}} + b_{\mathrm{AB}}x_{\mathrm{A}}x_{\mathrm{B}} + b_{\mathrm{AC}}x_{\mathrm{A}}x_{\mathrm{C}} + b_{\mathrm{BC}}x_{\mathrm{B}}x_{\mathrm{C}} + b_{\mathrm{ABC}}x_{\mathrm{A}}x_{\mathrm{B}}x_{\mathrm{C}} \tag{26}$$

TABLE 5

Coded levels for a three-component Scheffe's lattice design

Run [a]	x_A	x_B	x_C
1	1.0	0.0	0.0
2	0.0	1.0	0.0
3	0.0	0.0	1.0
4	0.5	0.5	0.0
5	0.5	0.0	0.5
6	0.0	0.5	0.5
7	0.33	0.33	0.33

[a] Extra design test point are not included.

where x_A, x_B and x_C are the relative proportions of ingredients A, B and C fulfilling $x_A + x_B + x_C = 1$.

Accordingly, seven runs or formulations must be performed: (i) three formulations, one on each vertex A, B or C which represents the formulations with pure components; (ii) three formulations prepared with 50:50 mixtures of each pair of components AB, AC, BC; (iii) one formulation prepared with one-third of each component at the center of design. This design is depicted in Table 5.

For systems comprising more components a single figure cannot conveniently be constructed, but can be readily treated mathematically as an extension of the three-component system.

In experimentation following Scheffe's lattice designs there are no degrees of freedom to test the equation for the adequacy because the designs are saturated. In the three-component system there are seven runs for determining seven regression parameters (strictly speaking, the problem is reduced to solve a linear system of equations). Thus, to test the adequacy of the fit, it should be advisable to run one or more extra-design points called test points. Rigorous statistical techniques may be applied (Akhnazarova and Kafarov, 1982) but the following simple procedure is sufficient for testing purposes (Bolton, 1984): Once the model equation is fitted to data, the results at the extra-design test points are predicted based on the equation and their agreement with the observed value assessed. If the agreement is close, we have increased faith in the predictive power of the response function.

In the treatment discussed, the components comprise the entire mixture. A more common situation is one in which part of the formulation must remain fixed, such as the drug concentration in a tablet. This is no cause for alarm because we can restrict the treatment to those components which are varied and with adequate transformation, treat the data in the same way as above. For instance, if the components to be changed make up 60% of the total formulation ingredients, we can suitably transform the actual percentages of these components so that the transformed percentage is 100% or unity on a fractional scale. This is easily accomplished by coding the amount of ingredient used (Bolton, 1984) in the following way:

$$\text{actual fraction} = \frac{\text{amount used} - \text{minimum}}{\text{maximum} - \text{minimum}} \quad (27)$$

Formulation parameters for optimization experiments

As Bohidar and Peace (1988) have pointed out, the development of a pharmaceutical formulation and the associated processes usually involve a number of independent and dependent variables. Independent variables (factors) may include the level of a given ingredient or the mixing time for a given process stage. Excipient ingredients are examples of independent variables. Several dependent variables (response values) can be considered. For example, in the case of tablets we have:

disintegration time in min; tablet breaking extent (hardness) in kg; dissolution (%) release in 30 min and in 45 min; friability (%) weight loss; thickness uniformity in % RSD (relative standard deviation); porosity in μm/g; mean pore diameter in μm; weight uniformity in % RSD; tablet breakage as the number of chipped tablets; granulation mean diameter in μm.

A pharmaceutical dosage form or drug delivery system consist of the active ingredient as well as the excipient ingredients. The effects of these excipient ingredients on the response variable are of primary interest in formulation development (Bohidar, 1984).

Worked examples

In order to demonstrate the practicability and value of the concepts explained we have selected some practical examples. The data for the first and third examples were taken from Bolton (1984). The second example was inspired by the paper of Van Ryswyk and Van Hecke (1991).

The data treatments have been performed according to the techniques explained here. The computations were carried out on an 80386 IBM compatible personal computer by using the CSS: STATISTICA statistical package from StatSoft.

Example 1

A combination drug product is tested for obtaining the dose of each drug which would result in an optimal response. The product contains two drugs, A and B. The experiment consists of formulating combinations containing each drug at two dose levels. The doses for A were 5 and 10 mg, those for B being 50 and 100 mg. These levels were carefully selected to cover a range of doses which would include the appropriate dose to be chosen as the prime candidate for the final marketed product, which is a local anesthesic. The response (y) is the average time to anesthesia for 12 patients per group.

First we will attempt to fit the response to the following equation:

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2$$

where x_1 and x_2 are the coded levels for A and B. We need to calculate six regression parameters. An orthogonal second-order composite design may be useful to solve it. Thus, a 2^2 full factorial kernel plus four star points and (at least) an additional central point are needed. The set of nine experiments is presented in the table shown below (the α value is taken from Table 2):

Coded levels		Response
x_1	x_2	y
−1	−1	9.7
−1	1	8.4
1	−1	8.2
1	1	4.1
1	0	7.5
−1	0	9.0
0	1	3.8
0	−1	5.3
0	0	4.8

After multiple regression analysis, the parameters were:

$b_0 = 4.98 \pm 0.47$

$b_1 = -1.22 \pm 0.26$

$b_2 = -1.15 \pm 0.26$

$b_{11} = 3.18 \pm 0.45$

$b_{22} = -0.52 \pm 0.45$

$b_{12} = -0.70 \pm 0.32$

Owing to the lack of replication ($N_0 = 1$), we will obtain the experimental F value as the ratio between the variance of the model and the residual variance. The variance due to the model is calculated as follows. First, compute the sum of squares due to the model as the difference between the total sum of squares (with respect to the mean) and the residual sum of squares. Then the calculated sum of squares is divided by the number of degrees of freedom ($\ell - 1$). Thus, the experimental value of F is 19.71, higher than the tabulated value $F(5,3,95\%) = 9$. This indicates a fair adequacy of the fitted model.

The coded levels where the response is optimal are calculated from Eqn 19 which yields the values $x_1 = 0.065$ and $x_2 = -1.15$. However, a canonical analysis of the corresponding matrix S gives two eigenvalues which differ in sign, i.e., at

the chosen working levels, a saddle point is obtained.

If a maximum response is desired, say 5 min, various combinations of x_1 and x_2 may satisfy the requirement. The ultimate choice will probably depend on other factors, such as cost, toxicity, etc.

On the other hand, by considering the significance of the regression coefficients according to the t-test, b_{22} and b_{12} would be disregarded, yielding a more straightforward polynomial expression.

Example 2

In order to illustrate how to develop the response surface and use it to maximize the yield of a synthetic procedure, we will now consider the production of a pharmacologically active compound by industrial chemical synthesis. The most important experimental variables are temperature T, reactant mole ratio R and reaction time t. In a previous work, two level experiments were performed:

	t	R	T
High level	210 min	17	115°C
Low level	85 min	3	85°C

Thus, the zero level corresponds to 120 min, 10 and 100°C.

The prediction equation for optimizing the procedure is:

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2b_{12}x_1x_3 + b_{23}x_2x_3$$

where x_1, x_2 and x_3 are the coded factor levels:

$$x_1 = (t - 120)/90$$

$$x_2 = (R - 10)/7$$

$$x_3 = (T - 100)/15$$

In this three-variable experiment there are 3^3 total combinations to explore. In order to alleviate this, we will use a rotatable second-order composite design. According to Table 3, a kernel full factorial design of 2^3 experiments plus $2 \times 3 = 6$ star points (with $\alpha = 1.68$) and a 5-fold replication of the central design point are needed. The yields obtained are compiled in the table shown below:

Coded levels			yield in g
x_1	x_2	x_3	y
−1	−1	−1	negligible
−1	−1	1	0.03
−1	1	−1	0.02
−1	1	1	0.03
1	−1	−1	0.41
1	−1	1	0.21
1	1	−1	0.53
1	1	1	0.28
1.68	0	0	0.41
−1.68	0	0	negligible
0	1.68	0	0.33
0	−1.68	0	0.23
0	0	1.68	0.16
0	0	−1.68	0.31
0	0	0	0.66
0	0	0	0.65
0	0	0	0.66
0	0	0	0.67
0	0	0	0.66

The regression parameters obtained were:

$$b_0 = 0.661 \pm 0.014$$

$$b_1 = 0.149 \pm 0.009$$

$$b_2 = 0.028 \pm 0.009$$

$$b_3 = -0.048 \pm 0.009$$

$$b_{11} = -0.166 \pm 0.009$$

$$b_{22} = -0.140 \pm 0.009$$

$$b_{33} = -0.156 \pm 0.009$$

$$b_{12} = 0.021 \pm 0.012$$

$$b_{13} = -0.061 \pm 0.012$$

$$b_{23} = -0.009 \pm 0.012$$

According to the value of the tabulated t-test ($t(9,95\%) = 2.26$) the parameters b_{12} and b_{23} would be neglected.

From the replication of the central point ($N_0 = 5$), the pure error variance $s_e^2 = 5 \times 10$ was obtained. From this value and the residual variance s_r^2es = 0.001076, the lack of fit variance was computed according to Eqn 24, giving $s_g^2 = 0.001897$. Thus, the experimental F value is 37.94, much greater than that tabulated, $F(6,4,95\%) = 6.16$, which indicates that the data provide a satisfactory fit to the model equation.

The optimum point calculated from Eqn 19 gives $x_1 = 0.505$, $x_2 = 0.146$ and $x_3 = -0.257$. The canonical analysis yields three negative eigenvalues which indicates that the optimal point computed is a true maximum.

The uncoded values gives the optimal variables to be applied to the synthesis: a reaction time of 165.5 min, a reaction temperature of 102.2°C and a reagent ratio of 8.2.

Example 3

The example presented below is an experiment in which a simplex design is used to obtain a formulation with optimal properties. The experiment was prompted by problems with tablet hardness for a large volume marketed product. The cause could be traced to three components of the tablets which we shall denote as ingredients A, B and C. Together, these components consist of 25% of the original formulation, or 75 mg of the total tablet weight of 300 mg. A careful evaluation of the products ingredients indicates that the three components had to be present in an amount equal to at least 10 mg of each in order for the tablet to be satisfactorily compressed. Thus, the recommended simplex design to obtain a satisfactory tablet hardness consists of varying the three components with the constraint that the sum of the components must be 75 mg, and that each component be present in an amount equal to at least 10 mg. Accordingly, the minimum amount is 10 mg and the maximum 55 mg. In order to apply the simplex polynomial in a suitable manner, the actual amounts used should be transformed into fractions such that the minimum corresponds to 0 and the maximum to 1. This is accomplished by using Eqn 27. The fractions x_1, x_2 and x_3 refer to ingredients A, B and C, respectively.

The three-component simplex design is given in the following table. The response to be optimized is the hardness average of 20 tablets drawn at random from experimental batches.

fractions			average hardness
x	x	x	y
1	0	0	6.1
0	1	0	7.5
0	0	1	5.3
0.5	0.5	0	6.6
0.5	0	0.5	6.4
0	0.5	0.5	6.9
0.333	0.333	0.333	7.3
0.5	0.25	0.25	7.2
	(check point)		

The fitted equation was:

$$\hat{y} = 6.1x_1 + 7.5x_2 + 5.3x_3 - 0.8x_1x_2 + 2.8x_1x_3 + 2.0x_2x_3 + 15.24x_1x_2x_3$$

The predicted value for the check point was $\hat{y} = 7.1$, in good agreement with the experimental value of 7.2. Therefore, the equation satisfactorily predicts the tablet hardness.

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