DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 12(8&9), 1109-1123 (1986)



# STATISTICAL EXPERIMENTAL DESIGN AND ITS APPLICATION TO PHARMACEUTICAL DEVELOPMENT PROBLEMS

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Pharmaceutical scientists are often confronted with the problem of developing formulations and processes for difficult products and must do so in spite of competing objectives. Pressures, placed on the scientist to balance variables and meet these objectives, can be compounded when limited funds, time and resources require rapid and accurate development activities. Statistical experimental designs provide an economical way to efficiently gain the most information while expending the least amount of experimental effort.

Some commonly used techniques called Response Surface Designs are presented. These include the Central Composite, Extreme Vertices, Simplex and Evolutionary Operations designs. Procedures for planning experiments and for analyzing and interpreting data are also discussed. Guidance is provided on the implementation of experimental results and the practical use of these techniques for routine experiments.



 0363-9045/86/1208-1109$3.50/0

## INTRODUCTION

For many years, formulation development scientists have used knowledge based on prior experience to develop pharmaceutical dosage forms. This was, and is, generally sufficient for the development of formulations without critical aspects, such as high dosage, poor solubility, poor compressibility or other physical-chemical properties. Often the scientist develops a formulation or process that is adequate but does not have the time to invest in developing the best possible formulation or process. Selection of appropriate levels for controllable variables can be complicated by the presence of competing objectives, such as minimizing the dissolution time while maximizing the tablet hardness. In order to minimize or maximize particular characteristics of a dosage form, the relationship between controllable (independent) variables and performance or quality (dependent) variables must be understood.

An efficient and economical method of obtaining the necessary information to understand relationships between variables is the application of statistical principles. The application of these principles provides not only efficient use of resources, but also provides a method of obtaining a mathematical model which can be used to characterize and optimize a formulation or process. Without the use of a mathematical model, it is unlikely that the optimum formulation or process conditions will be determined.

## CHARACTERISTICS OF STATISTICAL EXPERIMENTAL DESIGNS

Discussion of the details of the many different types of experimental designs is not within the scope of this text. The reader is directed to the statistical texts (1,2) for these details.

Statistical design techniques were developed to obtain the greatest amount of information, using the least number of experiments, while identifying the presence of interactions between variables and accounting for experimental error. An additional advantage of using these designs is that they require careful planning and adherence to statistical rules. This compels the experimenter to accurately define the goals of the experiments and the steps needed to achieve them. These rules require potential sources of experimental error and their magnitude to be determined. This planning stage can often identify problems which might not have been discovered until after the experiments were completed.

When an entirely new process or formulation is desired, such as the use of a high speed mixer or the development of a controlled release product, this planning may make the scientist realize that a sequential approach is necessary. This approach utilizes small pilot experiments to add information for the decision making process and provides some estimate of experimental error.

Steps to be followed when planning studies using statistical experimental designs are:

1. Carefully describe the problem to be solved.
2. Select the independent variables and the upper and lower limit for each.
3. Identify the dependent variables to be measured.
4. Identify the mathematical model to be used.
5. Provide an estimate of experimental error.
6. Determine how the data will be collected.
7. Determine how the data will be analyzed.
8. Determine how the results will be implemented.

## RESPONSE SURFACE METHODOLOGY

One particular group of statistical experimental designs that can be applied to pharmaceutical development problems is called Response Surface Methodology. A response surface is an area of space defined within the upper and lower limits of the independent variables and is a function of the relationship of these variables to the measured response (dependent variable). The goal of response surface studies is to obtain a regression model that provides a means of mathematically evaluating changes in the response due to changes in the independent variables. When the minimum or the maximum of a surface is sought, this process is called optimization.

This group of designs was introduced in the early 1950's by Box and Wilson (3,4). The use of these techniques was readily accepted and implemented by researchers in many different fields, such as agriculture (5-8), engineering (9,10), chemistry (11,12), veterinary science (13) and other disciplines. Recently, these techniques have been applied to pharmaceutical systems (14-20).

### Selecting The Variables

After carefully describing the problem to be solved, such as optimizing the hardness and dissolution rate of a tablet formulation or controlling the release of a drug from a dosage form, appropriate variables should be selected. The independent variables selected should be quantifiable and easily controlled. Qualitative variables, such as mixer A or mixer B, cannot be utilized in these designs. Examples of suitable quantifiable variables include tabletting machine compression force and speed, mixing time, ingredient concentration or ratios and temperature.

The range for each variable should be established from the experimenter's prior experience or from the results of small pilot experiments. Each variable should be easily controlled to provide appropriate increments within the range selected. Establishing proper ranges is important because the product produced by each experiment in the design should have enough integrity to undergo testing (i.e. measure a response). For instance, setting the lubricant range for a tablet formulation at 0-10% will probably prevent the formation of suitable tablets within this range. A more suitable range might be 0.25-1.0%. This of course, would depend upon the properties of the formulation and the prior experience of the experimenter. The ranges selected should not be too narrow or too wide. If the range is too narrow, the ability to optimize a formulation or process is limited. If the range is too wide, the model selected may not be sufficient to estimate the response surface, and some experiments may produce products not suitable for testing.



## Selecting A Model

As mentioned earlier, a model to be used to describe the relationship between independent and dependent variables should be selected during the planning stage, before the experiments are conducted. This is necessary because the choice of an appropriate experimental design depends, in part, upon the type and number of regression coefficients to be estimated. Statistical theory is used to adequately distribute the experimental points within the experimental space, so that each of the coefficients will be estimated with the same degree of certainty. In this way, the model that is developed will estimate all regions of the surface with similar reliability. Examples of some common regression models are shown below.

$$Y = B_0 + B_1X \qquad \text{(Equation 1)}$$

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 \qquad \text{(Equation 2)}$$

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2 \qquad \text{(Equation 3)}$$

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 \qquad \text{(Equation 4)}$$

where Y = measured response (physical characteristic)

$X_i$ = values of an independent variable

$B_0$ = constant

$B_i$ = coefficient of the linear terms

$B_{ii}$ = coefficient of the quadratic terms

$B_{ij}$ = coefficient of the cross product or interaction terms

where i = (1 to n) and j = (2 to n)
n = number of variables

Equations 1-3 can be used to describe linear relationships or planes (flat surface). Equation 1 is a simple linear model with $B_0$ representing the y-intercept and $B_1$ representing the slope. Estimates of $B_0$ and $B_1$ can be calculated for various values of X and the corresponding response, Y. This is performed by a statistical software package using a computer. The same procedure can be used to estimate the coefficients in equations 2-4. Equation 2 is another linear model but has three independent variables, $X_1$, $X_2$ and $X_3$. Equation 3 represents another linear model that includes cross-product or interaction terms requiring the estimation of cross-product coefficients $B_{12}$, $B_{13}$, and $B_{23}$. Equation 4 is used in many response surface designs because it utilizes quadratic terms ($X_i^2$) to account for curvature in the response surface. This model is called a second order polynomial and is useful because many response surfaces encountered have some degree of curvature. The selection of a model should be based on an estimate of the type of response expected. Often such information is not available, so an equation of the type represented by Equation 4 is used. The experimenter can also use his own model based on theory or prior empirical data.

## Experimental Error and Lack of Fit

Another important part of the planning stage is providing an estimate of experimental error. This error is a measure of the variability inherent in the system under study. A good estimate is necessary because if a system has a large amount of variability, it may be difficult to obtain a suitable mathematical model.

To obtain an estimate of this error, complete experiments need to be replicated. This is in contrast to repeated measurements which simply check the precision of the measurement operations. A replicate provides a means of comparing the results of two identical experiments, which includes all errors present during the entire experimental process.

During the planning stage a sufficient number of replicates should be determined. It is not necessary to replicate all of the experiments in a design.

Lack of fit, on the other hand, is a measure of how well a regression model fits the data. This is obtained by subtracting the experimental error from the total error listed after a computerized regression analysis is performed. The statistical significance of the lack of fit can be tested using an F-Test. Details on this test are described by Ostle (20).

## SOME COMMON RESPONSE SURFACE DESIGNS

As mentioned earlier, there are many examples in the literature of the application of these designs to industrial and research problems. Since details of all these designs would be too lengthy for this discussion, the reader will be referred to appropriate literature sources in the following sections. Two basic categories of designs are fixed and sequential. Fixed designs determine all the experiments needed before any experiments are performed. Sequential designs use the result of one experiment to determine conditions for the next experiment and are suitable primarily for optimization studies.

## Central Composite Design

This fixed design was first described by Box and Wilson (3). Their paper and two texts (1,2) are excellent sources of fundamental information on how these designs are constructed. This design is based on factorial designs, with additional points added to estimate curvature of the response surface. The model

TABLE 1

| Number of variables k | Number of Experiments: Three-leveled factorial, $3^k$ | Number of Experiments: Composite, $2^k + 2k + 1$ |
|---|---|---|
| 2 | 9 | 9 |
| 3 | 27 | 15 |
| 4 | 81 | 25 |
| 5 | 243 | 43 |
| 5 (1/3 fractional) | 81 | (1/2 fractional) 27 |
| 6 | 729 | 77 |
| 6 (1/3 fractional | 243 | (1/2 fractional) 45 |

Comparison of the Number of Experiments for Factorial and Composite Designs

used is typically a second order polynomial. Table 1 shows how this design reduces the number of experiments compared to a standard three leveled factorial.

Additional reductions can be made if a fractional factorial is used for the center of the design. The distance of the additional points from the center of the design is dependent upon the number of variables. This distance can be found in a statistical text (1) or calculated (2). The Central Composite Design has been successfully applied to pharmaceutical systems (14,19).

## Extreme Vertices Design

This fixed design was first described by Anderson and McLean (22). The design was developed for experiments with mixtures, where the only independent variables are the amount of each of the ingredients in the mixture. This design does not incorporate any process variables, such as temperature, pressure, time, etc. The response variable is usually some direct measure of each mixture prepared, such as viscosity, dispersibility and solubilization or lubrication capacity. Concentration ranges for each ingredient in the mixture are set by the experimenter and an algorithm is used to determine the experimental points. The algorithm developed by Anderson and McLean has been improved by Snee and Marquardt (23,24). Recently, the Extreme Vertices design was applied to a pharmaceutical solubility problem (25).

## Simplex Design

One type of sequential design is the Simplex Design, which is used primarily for optimization purposes. The experimenter is responsible for selecting the experimental points for the first set of n + 1 experiments (where n equals the number of variables). These points should be selected based on prior experience,so they are close to where the optimum is expected. After the initial responses are measured for each of these points, design rules are used to determine points for the next experiments. Basically, these rules force movement over a response surface in a direction opposite to the lowest previous response. In this way, the experimenter can eventually reach a point of maximum response or optimum.

The strength of this design is that the optimum can be determined without the need to assume a mathematical model to fit to the response surface. The drawbacks of this design is that the experimenter does not know in advance how many experiments are needed to reach the optimum, and a regression model may not be available if all regions of the surface have not been adequately covered. This may limit the ability to characterize areas of the surface in greater detail. This design was recently applied to a capsule formulation project (17).

## Evolutionary Operations (EVOP)

Another sequential design that is useful in production settings is called Evolutionary Operations (EVOP). EVOP allows the experimenter to slightly alter process variables during actual production operations and measure responses of interest. Some common response variables include cost, time, yield, purity and product quality aspects. The slight variations in the process variables are selected so that an acceptable product is still produced. This procedure allows the process to be optimized without economic losses.

One restriction on this procedure is that many batches of product must be manufactured in order to move toward an optimal response. This is due to the small variations utilized. Another limitation is the constraint placed upon parts of the manufacturing process filed in an NDA. The use of this design in pharmaceutical production was described by Rubenstein (18). This is another design technique that does not provide a mathematical model to describe the response surface.

## DATA ANALYSIS

After appropriate experiments for a fixed design have been performed, values for each response are paired with corresponding values for each independent variable. Data is entered in a statistical software package on a computer, and the model selected is used in a regression analysis for each set of independent variable/response variable data. Coefficients of the regression model (B terms) are estimated for each data set so that the effects of the independent variables on each response can be estimated. The completed model must be checked to see if it adequately describes the response surface based on statistical tests. This can be done using the following techniques.

1. Compare the absolute value of the regression coefficient with its standard error. The standard error should be less than 50% of the value of the coefficient.

2. Examine the residuals which show the difference between the actual responses measured and those predicted by the regression model. The residuals include variation due to experimental error and variations of the model from actual values.

3. Measure lack of fit for the model by subtracting pure experimental error from the total error. The result of the calculation is the amount of error due to lack of fit, which can be tested using an F-Test. Lack of fit determines how well a model fits the data, independent of experimental error. This procedure is most useful for models based on theory that have been developed by the experimenter and for which there is no data demonstrating the performance of such a model.

4. Examine the correlation coefficient to see if it is close to 1.0. Usually 0.90-1.0 is acceptable for most development work, but a coefficient greater than 0.95 is very good.

5. The ultimate test of a model, if possible, is to predict a response by setting the levels of the independent variable to some level not used in the experiments. The prediction is then tested by performing an experiment at the selected conditions and comparing the measured response to the prediction.

Once the model has been judged suitable, it can be used to optimize the system under study or assist in the characterization of the system.

## UTILIZATION OF MODELS

With optimization, the goal is to find the levels of each independent variable that will produce the best response. The range of values used to search for the optimum must be less than or equal to the range used in the experimental design. The method used to find the optimum point is call a grid search. This is a brute force method which forces the calculation of all responses within the ranges of the independent variables.

The output of the search could be all calculated responses (Y values), or a listing of independent variable values that yield Y values within selected constraints . For example, a tablet is desired that has a hardness (kp) within a range of $Y_{H1}$ to $Y_{H2}$ and a dissolution time ($T_{80}$) within a range of $Y_{T1}$ to $Y_{T2}$. Assume a mathematical model has been developed for each response variable, yielding model H and model T. Calculations can be performed, using a computer program, and the only independent variable values, ($X_i$), output from the program yield responses that meet the following constraints.

$$\text{Lower} < Y_H < \text{Upper}$$

AND

$$\text{Lower} < Y_T < \text{Upper}$$

Another data analysis option, if the response surface shape needs to be examined, is to produce graphics. These graphics can take the form of contour plot or three-dimensional plots. Caution should be used, however, if more than two variables are studied, because some of the variables must be held constant and all effects may not be clear. Examples of contour plots are shown in Figures 1 and 2. Contours lines represent points of equal response and serve as a two-dimensional representation of a three-dimensional surface. Graphical analysis allows the experimenter to visualize how changes an independent variable can alter the shape of the response surface. This analysis can often be used to determine critical points in a process or formulation. Ideally, it is desirable to have a process or formulation that lies on a plateau, (Figure 1), instead of on the

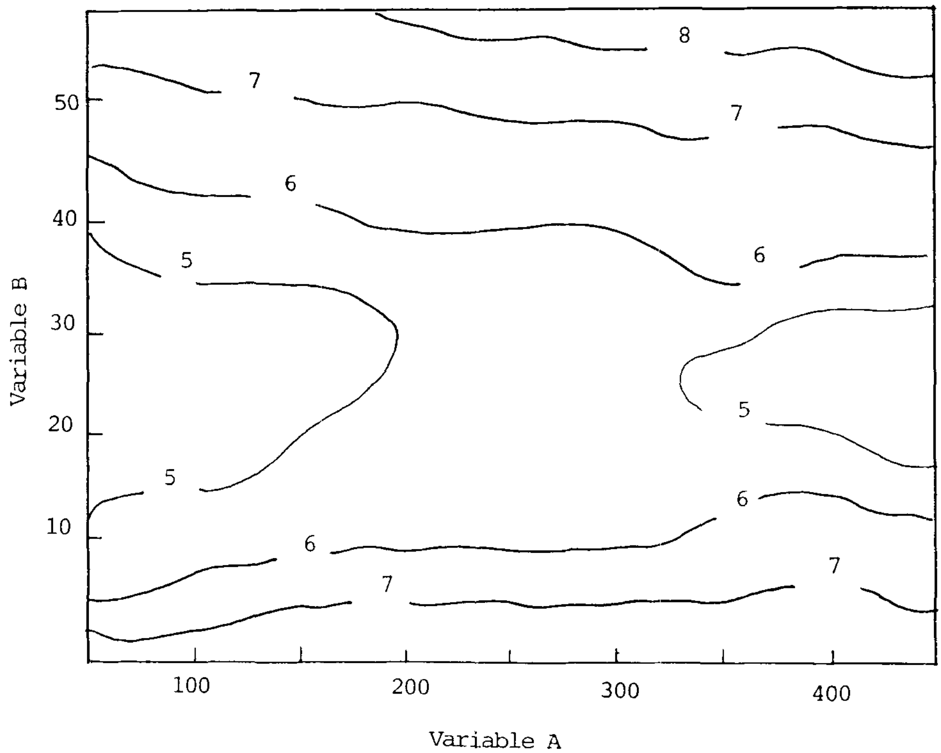


Figure 1. An example of a contour plot showing a view of a plateau at the center of the response surface. (Contour lines connect responses of equal value.)

edge of a ridge (Figure 2). Placement on a plateau indicates that variations in the independent variable will not appreciably affect the output of the process or formulation. A more complex subjective view of the surface can be obtained by generating a three-dimensional surface.

## IMPLEMENTATION

Successful implementation of information gained through response surface experiments depends upon how careful an experimenter was during the planning stage. The problem and important variables should have been defined in such a way that the results of the experiments can be applied to actual development projects. For instance, assessing the performance of a tablet granulation using a single punch press cannot predict

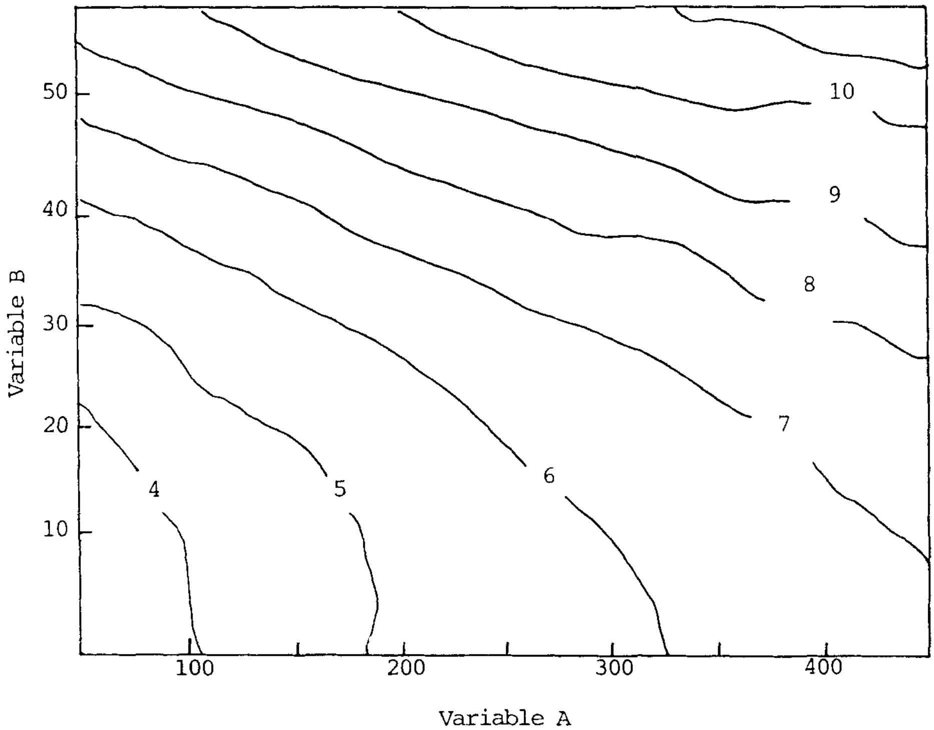


Figure 2. An example of a contour plot showing a view of a rising slope of a response surface. (Contour lines connect responses of equal value.)

what will happen on a high speed rotary production machine. In such a case, appropriate experiments should be used to simulate actual process conditions, such as using a high speed press with fewer punches and an adjustable speed force-fed feed frame.

If an optimum formulation or process is desired, and an acceptable mathematical model has been generated, the model can be used to provide the appropriate levels for each of the variables. If a characterization is desired, the model can be used to identify the range of each variable that produces an acceptable product. As described above, this can be done by setting the range for each of the critical response variables and calculating, with a computer, all the combinations of the independent variables that meet these constraints. A surface can then be mapped which will show how much variability a system can tolerate. This is a way to reduce errors when a scientist is asked to approve a change in the original formulation or process. Of course, if a proposed change involves an independent variable that was not studied, an answer may not be clear.

## PRACTICAL ASPECTS

Although many scientists may realize the usefulness of generating a mathematical model, it may not be clear as to how modelling techniques can be utilized in practical terms in development projects. It is true that these techniques may not be practical for every development project, since many products may not present any great difficulty for the scientist.

Some examples of situations where the use of response surface designs may be questionable include:

1. There is uncertainly about whether all experiments are feasible(i.e., all experiments must yield a usable product).
2. The requirements for supply of a costly ingredient are clearly greater than for a conventional experimental approach.
3. The presence of critical qualitative variables.
4. The presence of critical variables to be encountered during a production scale process that cannot be reproduced for study on a smaller scale.
5. The tasks facing the scientist are considered unproblematic.

However, in the case of products with troublesome physical-chemical properties, or a need for controlled release, these designs can be very efficient. Another point to be considered is that these designs can be applied to a system on a smaller scale. An example would be using only two or three independent variables. This is a reasonable approach as long as these variables have a significant influence on the system. Reducing the number of variables may not give a detailed view of a formulation or process, but it can provide some useful insight. The data generated can also serve as a data base to add more knowledge to an experimenter's experience, so that better decisions can be made during planning stages for future projects.

Although it seems that using only conventional development procedures and saying, "We've done it this way for so many years. . ." may be sufficient to solve development problems, one needs only to use these statistical techniques to solve a complex problem to be convinced of their usefulness.

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