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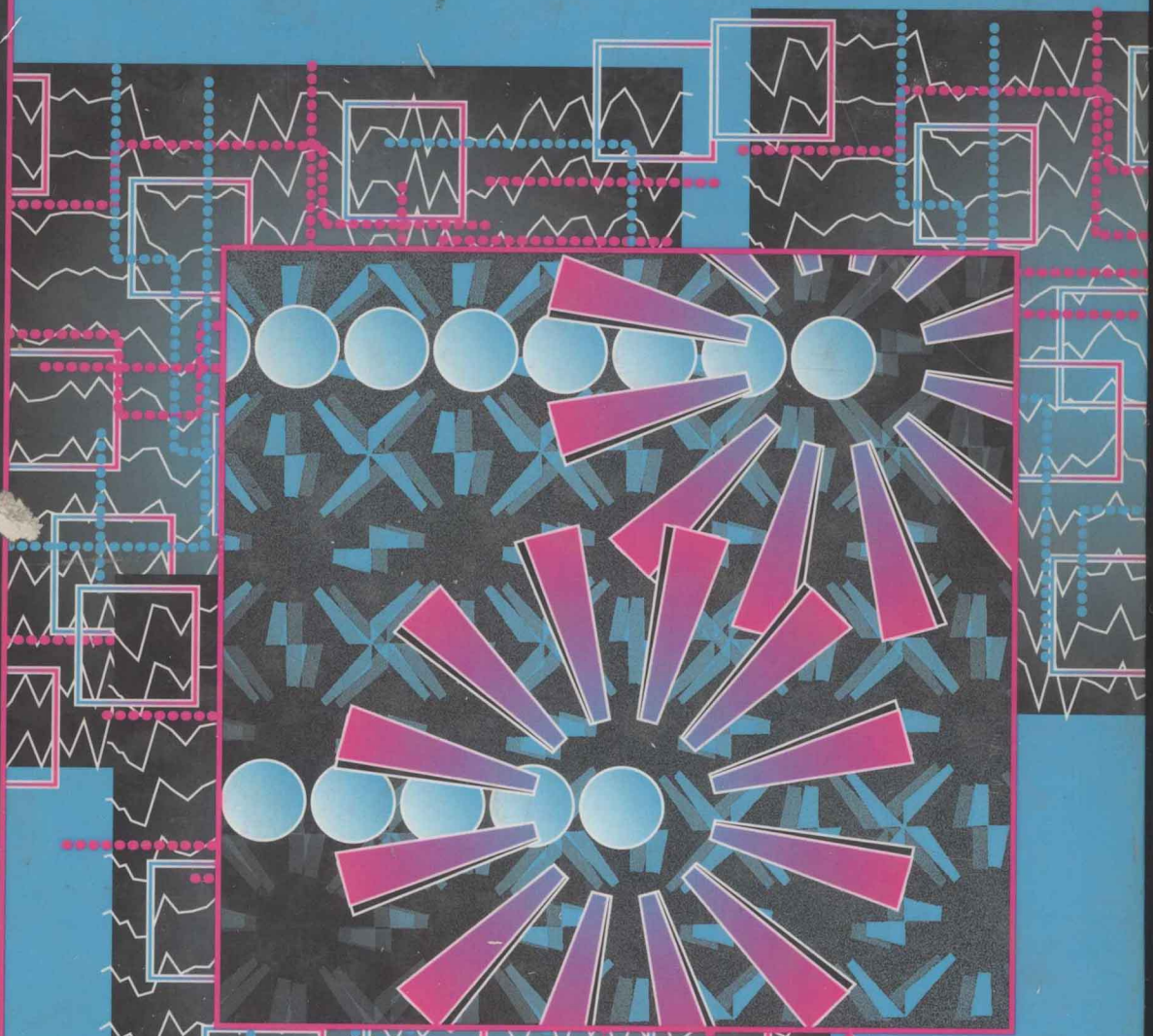

Pharmaceutical Experimental Design and Interpretation

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Introduction to experimental design

1.1 The experimental process

Experimentation is expensive in terms of time, manpower and resources. It is therefore reasonable to ask if experimentation can be made more efficient, thereby reducing expenditure of time and money.

Scientific principles of experimental design have been available for a considerable time. Much of the work originated with Sir Ronald Fisher and Professor Frank Yates. They worked together at the Rothamsted Agricultural Research Station, and there is an undeniably agricultural 'feel' to some of their terminology. The principles that they and others devised have found application in a variety of fields, but it is surprising how little these principles have been used in pharmaceutical systems. The reasons for this neglect are a matter for speculation, but there is no doubt that the principles of experimental design do have a widespread applicability to the solution of pharmaceutical problems.

Experimentation can be defined as the investigation of a defined area with a firm objective, using appropriate tools and drawing conclusions which are justified by the experimental data so obtained. Most experiments consist essentially of measuring the effect that one or more factors have on the outcome of the experiment. The factors are the independent variables and the outcome is the dependent variable.

The overall experimental process can be divided into a number of stages.

1 Statement of the problem. What is the experiment supposed to achieve; what is its objective?
2 The choice of factors to be investigated, and the levels of those factors which are to be used.
3 The selection of a suitable response. This may be defined in Stage 1, the statement of the problem. If so, then we must be sure that the measurement of the chosen response will really contribute to achievement of the objective. The proposed methods of measuring the response and their accuracy must also be considered at this stage.
4 The choice of the experimental design. This is often a balance between cost and statistical validity. The more an experiment is replicated, the greater the reli-

ability of the results. However, replication increases cost and the experimenter must therefore consider what is an acceptable degree of uncertainty. This in turn is governed by the number of replicates which can be afforded. Inextricably linked with this stage is selection of the method to be used to analyse the data.

5 Performance of the experiment: the data collection process. This will follow the experimental design laid down earlier.

6 Data analysis using methods defined earlier.

7 Drawing of conclusions.

The steps in the process can be illustrated using a simple example which is developed further in Chapter 4. As part of a study of diffusion through gels, Gebre-Mariam *et al.* (1991) wished to investigate the relationship between the composition of mixtures of glycerol and water and the viscosity of those mixtures.

Thus the objective (Stage 1) was to establish the dependence of the viscosity of glycerol–water mixtures on their composition. The factor to be investigated (Stage 2) was composition, up to a maximum of about 40% w/w glycerol. The response (Stage 3) was the viscosity of the liquids, measured by an appropriately accurate method, in this case a U-tube viscometer.

At the outset, it was not known if the relationship would be rectilinear or curvilinear. Furthermore it was intended to fit the results to a model equation, and so for both these reasons, an adequate number of data points needed to be obtained. Five concentrations of glycerol were selected, covering the desired range (Stage 4). It was expected that this would be the minimum number which would enable a valid regression analysis to be obtained. A greater number of data points could have been used, thereby improving the reliability of any relationship, but of course this would have involved additional work.

The experiments were then carried out (Stage 5), the data subjected to regression analysis (Stage 6), and the relationship between composition and viscosity established (Stage 7).

Thus the experimental design *and* the method to be used to analyse the data are selected *before* the experiment is carried out. The conclusions that can be drawn from the data depend to a large extent on the manner in which the data were collected. All too often, the objective of the experiment is imperfectly defined, the experiment is then carried out and only at that point are methods of data analysis considered. It is then discovered that the experimental design is deficient and has provided insufficient and/or inappropriate data for the most effective form of analysis to be carried out. Thus the term 'experimental design' must include not only the proposed experimental methodology but also the methods whereby the data from the experiments are to be analysed. The importance of considering both parts of this definition together cannot be overemphasized.

1.2 Computers and experimental design

A point which must be considered at this stage is the availability of computing facilities, whether mainframe or PC or even those of a pocket calculator. The advantages of the computer are obvious. The chore of repetitive calculation has been removed, and so an undeniable disincentive to use statistical methods has been removed at the same time. However, using a computer can give rise to two related

problems. The first is to place absolute reliance on the computer – if the computer says so, it must be so. The second is the assumption that the computer can take unreliable data or data from a badly designed experiment and somehow transform them into a result which can be relied upon. The computer jargon GIGO – garbage in, garbage out – is just as appropriate to problems of experimental design as to other areas in which computers are used.

However it is undeniable that access to a computer is invaluable. Many readers will have access to a mainframe computer equipped with comprehensive statistical packages such as MINITAB (Minitab Inc., USA), SPSS (McGraw-Hill, USA) or SAS (SAS Institute, USA). Bohidar (1991) has described the application of SAS to problems of pharmaceutical formulation.

Appendix 2 contains references to the use of MINITAB in some of the techniques described in the book. However a desktop computer will suffice for many of the calculations described in this text, as many statistical packages for PCs are now on the market. Alternatively, useful programs can be written in BASIC, and some are given in Appendix 2. Neither should the possibilities of PC spreadsheets be overlooked. Spreadsheet packages such as Lotus 1.2.3 (Lotus Development Corporation) and Excel (Microsoft Corporation) can be of great value.

Several software packages specifically intended for experimental design and optimization purposes are now available. One example is the RS/Discover suite of programs from BBN Software Products Corporation (Cambridge, USA). The menu-driven program in this package invites the user to specify the independent variables, together with their units, the ranges of values for the variables, the required degree of precision and to indicate if the value of a given variable can be easily altered. The program then produces a worksheet which gives the design of the experiment (full factorial, central composite etc.) and the values of the independent variables for each experiment. The experiments are usually given in random order except in those cases where a particular experimental variable cannot be easily altered in value. In such cases, the experiments are grouped so that the time taken to alter that variable is minimized. After the experiments have been carried out, the responses are added to the worksheet. Data can then be analysed, fitted to models and contour plots and response surfaces produced. Applications of this package have been given by McGurk *et al.* (1989) and Jones *et al.* (1989).

The Design-Ease and Design-Expert packages offered by Stat-Ease Inc. (Minneapolis, USA) provide facilities for the design and analysis of factorial experiments. The programs generate worksheets of experiments in random order or in blocks for experiments involving process variables or mixtures, and from the results can produce a statistical analysis and three-dimensional and contour graphs.

Similar programs include ECHIP (Expert on a Chip, Hockessin, USA), which has been reviewed by Dobberstein *et al.* (1994), CHEOPS (Chemical Operations by Simplex, Elsevier Scientific Software, Amsterdam, The Netherlands) and CODEX (Chemometrical Optimization and Design for Experimenters, AP Scientific Services, Stockholm, Sweden).

Release 8 of MINITAB, designed to run on personal computers, contains many features which are relevant to experimental design. In addition to useful statistical techniques, it includes programs for determinant analysis (Chapter 5) and principal components analysis (Chapter 6). The commands FFDESIGN and PBDESIGN generate fractional factorial designs and Plackett–Burman designs for a specified number of experimental factors (Chapter 9). Randomization of the order in which

the experiments are to be performed can also be carried out. The command FFACTORIAL analyses data from experiments based on these designs, and facilities for drawing contour plots from the data are also available (Chapter 10). Details are given in Ryan and Joiner (1994).

1.3 Overview of experimental design and interpretation

This is not a textbook on statistics. However, some statistical knowledge is essential if the full power of techniques in experimental design are to be appreciated. Neither does this book set out to be a compendium of methods of experimental design. Rather it sets out to discuss methods which are of value in the design of experiments and the interpretation of results obtained from them.

The literature in this area is considerable, and should readers wish to develop their knowledge of a particular technique, references to further reading are given at the end of each chapter. Statistical textbooks and some general texts on experimental design, which the authors have found to be of value, are given at the end of this chapter.

Many experiments consist of acquiring groups of data points, each group having been subjected to a different treatment, and methods for evaluating data from such experiments are included in Chapter 2. Essentially these methods are based on establishing if the mean values of the various groups differ significantly. When there are only two groups of data, the Student's *t*-test is usually applied, but for three or more groups, analysis of variance is the method of choice. The latter also forms the basis of many of the methods of experimental design described in later chapters.

For the Student's *t*-test and analysis of variance to be applicable, the data should, strictly speaking, be normally distributed about the mean, and must have true numerical values. Such tests cannot be applied to adjectival information, or when data has been assigned to numbered but arbitrarily designated categories. In such cases, non-parametric methods come into their own. These do not depend for their validity on a normal or Gaussian distribution, and 'adjectival' data can be assessed using them. However, such methods depend on there being an adequate number of data points to facilitate comparison, and hence the degree of replication in the experiment must be appropriate if such methods are to be used. Non-parametric methods can involve paired data, where each subject acts as its own control, or unpaired data. Both are discussed in Chapter 3.

Having obtained raw data from the experiment, the next decision is how to use it to its best advantage. The decision may be simple, for example if all that is required is a mean value and standard deviation, or the plot of one value against another, which gives a perfect straight line. Usually more is required, in which case the statistical method which is most applicable to the problem must be chosen.

An obvious example involves a series of pairs of results for which it is required to know if they are related, and if so how. A simple example would be the variation of the weights of a collection of laboratory animals with their heights. A plot of height (h) against weight (w), drawn on graph paper, may not give a definite answer, as the points could be such that it is not certain whether or not the results are scattered around a straight line. The probability that the results are so related is given by regression analysis, together with the value of the line in predicting unknown results. Alternatively, the relationship may be curved, but fits a quadratic equation. The

relationship between shampoo viscosity and salt concentration, given in Chapter 4, is a good example, and describes methods used to determine the predictive properties of the equation.

If the results are not related, a third property, for example age (A), may make an important contribution. It is not possible to plot a graph in this situation, although one could construct a three-dimensional model.

It will not be possible to visually express equations with more than three variables, but such higher relationships can be expressed in terms of an equation. Thus for example, if the variation of the animals' weights (w) with height (h), age (A) and waist circumference (c) is examined, a relationship of the form shown in (1.1) can be devised, i.e.

$$w = b_0 + b_1 h + b_2 A + b_3 c, \tag{1.1}$$

in which b_0, b_1, b_2 and b_3 are constants and can be derived by regression analysis. A minimum of four sets of data (because there are four variables) would be required to derive such an equation, and a perfect relationship would result. For a reliable relationship, a minimum of five sets of data for each unknown, giving a minimum total of 20 sets of results, are necessary.

Other relationships can be detected, either by trial and error, by suspected relationships, derived theoretically or found for similar systems in the literature, for example logarithmic (1.2), ternary (1.3) or square root (1.4). Some examples are given in the text, and methods for calculating them and evaluating their reliability described.

$$y = b_0 + b_1 \log x \tag{1.2}$$

$$y = b_0 + b_1 x + b_2 x^2 + b_3 x^3 \tag{1.3}$$

$$y = b_0 + \sqrt{b_1 x} \tag{1.4}$$

Sometimes one is presented with a collection of data in which some properties may be related and others are not. This is given in the form of a matrix, an example of which is:

$$\begin{bmatrix} a_1 & a_2 & a_3 & a_4 \\ b_1 & b_2 & b_3 & b_4 \\ c_1 & c_2 & c_3 & c_4 \\ d_1 & d_2 & d_3 & d_4 \\ e_1 & e_2 & e_3 & e_4 \end{bmatrix} \tag{1.5}$$

Each column represents a property of the materials under examination, and each row a combination of the properties representing one example. Thus each row could represent the properties of a different tablet formulation, for example 1 could represent tablet weight, 2 disintegration time, 3 crushing strength and 4 moisture content. To work with these data one must have a knowledge of matrices and their manipulation, which differs from basic algebraic methods. The basic matrix algebra necessary to understand this section is given in Appendix 4, followed by examples of its use.

Alternative ways in which the results can be expressed are also described in Chapter 5, together with ways in which relationships can be detected or eliminated.

When a series of results is presented, individual results can frequently be arranged into unrelated groups within which the results are related. This is called cluster analysis. Alternatively the validity of preconceived classifications can be examined by discrimination analysis. These techniques are described in Chapter 6.

Relationships within sets of results can often be detected and used to simplify data. Thus the number of rows shown in (1.5) could possibly be reduced to four or even less by principal components analysis, and the columns reduced in a similar manner by factor analysis. The procedure can often be improved by rotating the data. These procedures are covered in Chapter 7.

In sequential analysis, described in Chapter 8, results are examined continuously as they become available. The procedure has particular advantage in trials involving serious diseases, where it is important that if there is a significant improvement, it is obtained with as few patients as possible, so that the controls can be stopped and all subsequent patients given the new treatment.

Other techniques discussed are Free–Wilson analysis (Chapter 4), a technique in which biological activity is related directly to chemical structure, and Andrews' plots (Chapter 6) in which more than two variables are examined in two dimensions.

Experimental programmes can, if not efficiently designed, consume large amounts of time, materials and labour, and hence it is essential that programmes are designed in the most cost-effective manner. In Chapter 9, the principles of factorial design are fully discussed. Factorial design, when allied to statistical techniques such as analysis of variance, is a powerful tool in gaining the maximum amount of information from a limited number of experiments.

Factorial design involves the variation of two or more experimental variables or factors in a planned manner, the factors being investigated at two or more levels. The technique establishes the relative order of importance of the factors, and can also indicate if factors interact and if such interactions are significant. Even so, full factorial designs involving several factors at three or even more levels can demand considerable resources. Therefore methods by which the number of experiments can be reduced in factorial designs are also explored. The potential hazards in using such limited designs are also discussed.

Many pharmaceutical formulations and processes lend themselves to optimization procedures, whereby the best possible result is sought, given a series of limits or constraints. Thus the best possible solution is not necessarily a maximum (or minimum) value, but is rather a compromise, taking a number of factors into account. There are two principal methods of optimization. One is model-dependent optimization, in which a group of experiments is carried out and the results then fitted to an equation (the model). Such techniques are discussed in Chapter 10. Adequate experimental design, usually factorial in nature, is a prerequisite. The construction of the model and establishing its validity draws heavily on the correlation and regression techniques described in Chapter 4.

Once the model has been established, it can be used to construct contour plots. These are diagrams of the value of the response in terms of the values of the experimental variables. Such plots are invaluable in visualizing relationships between independent and dependent variables, and also in assessing the robustness of the response.

Model-dependent methods require that a series of experiments be carried out and the results assessed only when the whole series has been completed. Methods by which the results of only a few experiments govern the conditions of further experi-

ments are model independent. No attempt is made to express results in a model equation. Such methods are described in Chapter 11, which also includes a comparison between model-dependent and model-independent techniques.

Many pharmaceutical formulations involve a number of ingredients in mixtures, the total mass or volume of which is fixed. The contents of a hard shell capsule or the composition of a fixed volume injection are good examples. In such circumstances, it follows that if the proportion of one ingredient is changed, then the proportion of at least one of the others must also change. Such mixtures are amenable to the principles of experimental design, the applications of which are described in Chapter 12.

Each chapter is illustrated by a number of worked examples. Their selection has sometimes caused problems. Inevitably the authors have tended to select examples which they have found of value, and which are therefore in fields in which they are personally interested. However they accept that there are many other areas of pharmaceutical science which could have been explored. Therefore many of the chapters end with a bibliography which indicates those areas where a particular technique has been used, and the reader is referred to the original articles.

The appendices of the book contain material to which reference may be required, but which would be intrusive if contained in the main body of the text.

Tabulated statistical data (e.g. values of Student's t, F, correlation coefficients at given significance levels) have been reduced to a minimum, and only include material which is needed in the worked examples used in the text. Complete tables are readily available elsewhere.

As stated earlier, the ready availability of computing power has removed much of the drudgery associated with repetitive statistical calculations. The authors have found the MINITAB suite of statistical programs particularly useful, and in Appendix 2 give the MINITAB instructions for many of the statistical procedures used in the text. Release 8 of MINITAB, which is suitable for personal computers, also contains some programs of specific relevance to experimental design, and makes the package even more valuable. For readers without access to MINITAB or similar programs, several computer programs written in BASIC are included in Appendix 2. These are specifically linked to worked examples in the text.

Much of the material described in Chapters 5 and 6 demands a knowledge of matrix algebra. It may be that some readers do not possess such knowledge and so a short introduction to matrix algebra forms Appendix 4. This can be referred to as necessary.

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