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EXPERIMENTAL DESIGN IN DISSOLUTION TESTING

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ABSTRACT

Comparison of dissolution profiles may be facilitated by blocking the individual units of a given batch, thus greatly reducing the possibility of error from variation between experimental runs. Experimental designs are described which allow valid comparisons to be made between batches, as well as allowing the between run variation to be assessed and identifying any systematic errors resulting from differences between vessels. The number of tests required may frequently be reduced, and the need for replicate testing eliminated. The limitation of 6 vessels per run imposes certain restrictions in the experimental designs possible.
Applications of these experimental designs in characterisation of dosage forms by their pH-dissolution topography and their use in factorial formulation experiments are described.

INTRODUCTION

Dissolution testing, using the techniques described in the various pharmacopoeas, has become a routine method for testing solid dosage forms, both for quality control and during drug development. Different batches of the same formulation of the drug are frequently compared as well as different formulations. It is usual and advisable during pharmaceutical development to measure the dissolution rate in a variety of different media.

This is however a technique whose results are sensitive to internal and external factors some of which are difficult to control, such as vibration and non-centralisation of paddles and baskets (1, 2, 3). For this reason the geometry of the apparatus must be verified at frequent and regular intervals, and the

dissolution rate of calibrator tablets, disintegrating and non-disintegrating, is used as an additional verification (3, 4). "Internal" calibrators, formulations frequently tested by the laboratory concerned, and known to give consistent results within and between batches, are also useful. There remains nevertheless the possibility that a residual variation between experimental runs may remain, as well as systematic differences between dissolution vessels. There is thus an advantage in estimating and, where possible, reducing these errors.

Differences between experimental runs are clearly the more important. There should be no significant differences between the vessels of a properly calibrated apparatus. However, comparative data provide a useful verification without needing extra experimental runs. In our own laboratories we frequently wish to compare the profiles of two or more batches, when developing formulations, reformulating, comparing original and reprocessed material used in clinical trials, defining the effect of process variables as part of the optimisation or validation of a manufacturing procedure, or characterisation of the dissolution of a dosage form in various media. It is clearly an advantage if as well as detecting variation and error one can minimise its effect by an appropriate experimental design.

It is achieved by distributing the different samples tested between the experimental runs and the vessls, and regrouping the results for statistical analysis. For manual systems this would be long and tedious.However, most dissolution measurements by UV spectrophotometry use automatic data collection and processing by microcomputer. Spectrophotometers coupled to Commodore 4000 and 8000 series microcomputers are used in our laboratories. These are simple to programme, using Basic. The calculated parameters from dissolution testing are stored on disk for off-line analysis. More powerful microcomputers, IBM PC etc, may be programmed to analyse the absorbances directly.

In this paper we describe experimental designs appropriate to dissolution testing and the restraints imposed by the nature of the test.

COMPLETE BLOCKED DESIGNS

The dosage units are distributed in a regular manner, that is "blocked" between experimental runs and the vessels of the dissolution apparatus. The simplest case is one in which six formulations A-F are compared. Six tablets of each are tested, blocked between experimental runs and vessels, giving a latin square :

Schema (1)

Vessel / Experiment	1	2	3	4	5	6
1	A	B	C	D	E	F
2	F	A	B	C	D	E
3	E	F	A	B	C	D
4	D	E	F	A	B	C
5	C	D	E	F	A	B
6	B	C	D	E	F	A

The latin square should ideally be selected at random. To avoid error and simplify programming we have preferred to use the square given above. However the experimental runs are carried out in random order.

A suitable parameter for comparison is then selected. This may be the dissolution time for a certain percentage of the drug susbstance, or a parameter determined by fitting a model to the data. A useful model-independent parameter is the mean dissolution time determined by moment analysis (5). Methods for defining level and shape of dissolution profiles have been proposed by Mauger et al. (6). The parameters are then compared by three way analysis of variance. If an F-test shows that significant differences exist between formulations they may be identified using Duncan's test or other suitable methods.

Four variances are thus estimated, representing the differences between batches ($\nu = 5$ degrees of freedom), between experimental runs ($\nu = 5$), between vessels ($\nu = 5$) and the residual variance or error ($\nu = 20$) which represents the random variation between tablets in the same batch, as well as imprecision of the analytical method within an experimental run. If the variation between vessels or runs is statistically significant this represents an uncontrolled factor which should be corrected. It may otherwise be suspected but not significant. In either case confidence in the measured differences between the formulations is improved. In addition to this, the repeatability of the experiment is estimated without a large number of repeated analyses.

A similar design is described by Cox et al. (3), who used two way ANOVA to analyse the effect of lack of alignment of paddle shafts and differences in vessel shapes on prednisone tablet dissolution. Each vessel was tested at each of the six positions.

This type of analysis is limited by the fact that six vessels for each apparatus and six dosage units of each batch are normally examined. Comparison of two or three batches is obviously simple; for three batches the design may be blocked as:

Schema (2)

A	A	B	B	C	C
C	C	A	A	B	B
B	B	C	C	A	A

As for the 6 factor design we assume that batches, vessels and runs are independent and non-interacting.

It is also assumed that the parameter tested is normally distributed and the variance is constant. In general the F-test for comparing means is robust to departures from normality, but certain of the parameters determined in dissolution testing - in particular the times for 90 % dissolution - have greatly skewed distributions. Parameters for comparison should be chosen accordingly. After the analysis of variance, residuals may be calculated and tested for departure from constant variance. It is sometimes advantageous to transform the data, for example taking the logarithms of the dissolution times to achieve constant variance.

When comparing two or three formulations there are certain disadvantages to the three way analysis of variance described above. For comparing two formulations there would be only 4 residual degrees of freedom. In such a case it is preferable to assume that the between-vessel variance is not significant, and to analyse the data by two-way analysis of variance, which gives a value for the error variance with 9 degrees of freedom. The design of the experiment remains the same.

For other numbers of batches or formulations, different designs are required if we wish to test six tablets. In the case of five batches we may carry out 6 experimental runs. In the "spare" vessels we may include 6 tablets of an unrelated batch. A similar approach may be used for 4 batches.

INCOMPLETE BLOCK DESIGNS

In spite of the limitations imposed by the number of vessels per run being held normally at six we may in certain cases use blocked designs which are incomplete, in the sense that not all batches may be treated in the same experimental run, but balanced, in the sense that the total number of times a given pair of treatments occurs together in the same run is the same for all pairs.

This type of design enables between block, between treatment and residual variances to be separated as in the complete blocks described above. Where six tablets are to be tested the design may also be blocked by vessel as for the latin square. For 7 treatments A-G we have:

Schema (3)

A	B	C	D	E	F
G	A	B	C	D	E
F	G	A	B	C	D
E	F	G	A	B	C
D	E	F	G	A	B
C	D	E	F	G	A
B	C	D	E	F	G

which is a 7x7 latin square with one column missing, known as a Younden square (7,8). A similar design for 4 treatments consisting of two Younden squares may be used:

Schema (4)

A	A	B	B	C	C
D	D	A	A	B	B
C	C	D	D	A	A
B	B	C	C	D	D

Balanced incomplete designs also exist for 11, 16, 21 and higher numbers of treatments. It is unlikely that one would wish to use these designs for more than 20 batches.

It is also possible that one might wish to test fewer tablets of each batch. For example 16 treatments may be compared in a balanced design of 8 experimental runs with 3 replications of each treatment. It is not possible in this case to construct a Youndeh square. For other numbers of treatments blocked incomplete partially balanced designs may be used, where pairs of treatments occur together different numbers of times. These are discussed in more detail later.

BALANCED DESIGNS IN THE CHARACTERISATION OF DISSOLUTION PROFILES

It is usual during the development of a controlled or modified release product, or where the drug substance has low solubility, to characterise the dissolution under different conditions, for example in media of different pH values characteristic of the gastro-intestinal tract (9). If six tablets are to be tested at each pH the latin square (schema 1) may be used. Controlled release dosage forms are often more homogeneous in their dissolution properties than conventional forms and it may be found that the testing of 3 tablets will give results of sufficient precision. In this case 3 tests only need to be

carried out, with the dissolution media blocked by experiment and randomised between vessels. We thus obtain no information on variation between vessels; this is included in the residual variance.

We may feel that the profile should be established using several batches of the formulation. If we take 5 batches A-E we may test them at 5 pH values between 1 and 8 using a graeco-latin square design. We must assume that the batches behave similarly. If the profiles are different in shape, the formulations being different, the square must be replicated.

Schema (5)

pH 1.0-A	pH 4.0-B	pH 6.0-C	pH 7.0-D	pH 7.5-E
7.5-B	1.0-C	4.0-D	6.0-E	7.0-A
7.0-C	7.5-D	1.0-E	4.0-A	6.0-B
6.0-D	7.0-E	7.5-A	1.0-B	4.0-C
4.0-E	6.0-A	7.0-B	7.5-C	1.0-D

We thus obtain the mean profile for the five batches, the spread among the batches, variation between experiments and between vessels and the residual error is calculated with 8 degrees of freedom. The design is thus effective in spite of the sixth vessel being wasted.

There is no 6x6 graeco-latin square. If six batches are to be tested at six pH values a 6x6 latin square blocked by experiment and by batch is used, with the different media randomised between the vessels.

It does appear however that a balanced design may be obtained for 6 dissolution media and 3 batches of the dosage form (schema 6). Similar plans may be used to investigate the effect of surfactant at different levels over the full pH range.

Schema (6)

A1	B1	C2	D2	E3	F3
F1	A1	B2	C2	D3	E3
E2	F2	A3	B3	C1	D1
D2	E2	F3	A3	B1	C1
C3	D3	E1	F1	A2	B2
B3	C3	D1	E1	F2	A2

We may also wish to characterise the formulation with regard to different levels of agitation. It is well known that a given formulation whose performance <u>in vivo</u> depends on the dissolution

rate may be characterised by an in vitro pH dissolution profile, but in general no single test at a constant or varying pH is sufficient to predict in vivo dissolution. For similar reasons it is advantageous to generate the dissolution profiles at different agitation conditions. Here there is no possibility of blocking by experimental run and by vessel as all vessels in a given run must be stirred at the same speed. Replicate experiments are required. However if other factors are being tested as well as the agitation the number of experimental runs may be significantly decreased.

For example if we wish to characterise a product at 6 pH values and 3 agitation levels - for example with paddles rotating at 50, 75 and 100 rpm this may be done in six experimental runs, two at each speed. All six media, randomly distributed between the vessels, are tested in each run. The following sources of variation are tested and the variances estimated:

Between dissolution media	5	degrees of freedom
Between runs	3	" "
Between paddle speeds	2	" "
Interaction media/speed	10	" "
Residual	15	" "

The model may be extended for comparison of two formulae, or batches.

FACTORIAL STUDIES IN FORMULATION

Statistical experimental design is being used more extensively in pharmaceutical research and development (10). Where dissolution is a critical parameter there is often a clear advantage in using the above experimental designs, particularly in improving confidence and removing need for verification of unexpected results. However the type of blocking discussed above is not always necessary. In the case of the characterisation of a manufacturing process all factors likely to influence the operation are varied. As example we may consider a design requiring 22 experiments where five main effects of process variables are measured along with 6 second order effects. These latter terms are either interactions between process variables or quadratic terms in certain of the main effects. For each of the parameters measured the reproducibility between batches may be estimated with 11 degrees of freedom. Thus in the case of dissolution testing there is no need to block or randomise the tablets between experimental (dissolution) runs as data from all experiments are used to calculate an effect. Testing of batches should be, as far as possible, in random order. If we still wish to run the dissolution tests using a blocked design it will be

necessary to carry out 31 experimental runs (with 9 "dummies") if a balanced design is required.

A common exploratory study is carried out using a simple factorial design where several factors are studied at two levels. For 3 factors 8 experiments are required, and for 4 factors, 16 and for 5 factors 32 experiments, and so on. The number of experiments may be reduced if some of the interactions are to be neglected. Thus 5 factors may be studied in a 2^{5-2} design in 8 experiments, or a 2^{5-1} design in 16 experiments. These designs have been used to study the effect of process variables on extrusion spheronisation (11). Since relatively few experiments are carried out there is in this case a clear advantage in blocking by experimental run, in order to save duplication. As described above a balanced incomplete block design exists for 16 treatments. It does not exist for eight treatments. Since 8 is a very common number of treatments the problem should be examined in some detail. There are two possibilities:

(a) Randomise the treatments between dissolution runs and vessels. Any differences between dissolution runs (and vessels) is contained in the residual error term.

(b) Use an incomplete partially balanced design. The following design

Schema (7)

A	B	C	D	E	F
H	A	B	C	D	E
G	H	A	B	C	D
F	G	H	A	B	C
E	F	G	H	A	B
D	E	F	G	H	A
C	D	E	F	G	H
B	C	D	E	F	G

is partially balanced by experimental runs and is incomplete by vessel. The pairs of treatments AB, BC, CD..., HA occur in the same block 5 times whereas all others occur four times. The experiments must be calculated out in random order. The statistical analysis is described by Montgomery (8).

DISSOLUTION METHOD VALIDATION AND RUGGEDNESS TESTING

Certain methods discussed above provide important elements in the analytical validation of a dissolution method, namely the reproducibility and the dosage form characterisation. An important part of validation of a dissolution method, as with

other analytical methods, is testing for ruggedness. Factors thought to influence the dissolution are varied somewhat beyond the levels they may be allowed to assume in the test specification. Two levels are normally used, above and below the nominal level. We have studied the effect of pH, surfactant concentration, temperature, paddle speed, height of sampling probe, degassing, etc, using an incomplete factorial design of the type proposed by Plackett and Burman (12). The designs require 4, 8, 12, 16... experiments of different combinations of factors, allowing up to 3, 7, 11, 15 factors to be studied. Normally only main effects are calculated though it is sometimes possible to identify interactions (13).

Clearly it is not possible to block by experimental run as the paddles must turn at the same speed in all vessels at one time. As all factors are varied together and the main effect of a given factor is calculated using all the data this is not a serious drawback. Nevertheless it is necessary to estimate the reproducibility of the method, by repeating the experiments with the factors at their normal levels or by using a design with more experiments, for example 12 experiments to test 7 factors, with the extra degrees of freedom used for calculating the reproducibility.

We may broaden the ruggedness test by introducing other discrete factors. The series of experiments may be done with two technicians, two different apparatus and two batches of the dosage form.

CONCLUSIONS

We have shown how well-established methods in experimental design may be applied to dissolution testing, giving improved accuracy, and at the same time allowing the performance of method and apparatus to be evaluated. In certain cases the numbers of tests required may be considerably reduced. Routine use of this approach is greatly facilitated by the use of microcomputers for automatic data colection. The fact that only six units may be tested imposes restrictions on the possible designs but the use of partial balancing allows most series of experiments to be so tested.

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