EXPERIMENTAL DESIGNS FOR BIOAVAILABILITY / BIOEQUIVALENCE TRIALS

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Abstract: The bioavailability of a drug is the rate and extent to which the active drug ingredient is absorbed and becomes available at the site of drug action. The designs that are often considered for bioavailability and bioequivalence studies include some families of parallel group designs, crossover designs and incomplete block designs. Selecting a design for bioavailability study is determined by several factors and are discussed here. Methods of constructing appropriate designs are also described.

Key words: Bioavailability, Bioequivalence, Parallel Designs, Crossover Designs, Balanced and Partially Balanced Incomplete Block Designs, Balanced and Partially Balanced Incomplete Sequence Crossover Designs.

1. Introduction
Bioavailability is an important area of pharmaceutical research. In broad terms, bioavailability includes the study of the factors that influence and determine the amount of active drug that gets from the administered dose to the site of pharmacological action as well as the rate at which it gets there. The evaluation of bioavailability in an intact biological system is termed as biopharmaceutics and should be included in the development programme of every drug product (Metzler, 1974).

In particular, bioavailability denotes the relative absorption efficiency of the formulation, i.e., the amount of drug that is made available to the circulatory system whereas bioequivalence refers to the degree to which clinically important outcomes after receiving a new preparation resemble those of a previously established preparation.

1.1 In Veterinary Medicinal Products
A veterinary medicinal product is a finished dosage form that contains the active ingredient with or without inactive ingredients. Bioequivalence techniques are used for the comparison of the bioavailability of different veterinary products containing the same active ingredients, different batches of the same veterinary medicinal products, as well as different routes of administration. Bioequivalence exists between veterinary medicinal products or between routes of administration if, under identical and appropriate experimental conditions, the bioavailability of the active ingredient only differs within acceptable limits. Limits must be qualified a priori, according to the aim of the tests.

Need and Aim
(i) When changing the specification of the dosage form, its composition or manufacturing process, the new product must be demonstrated to be bioequivalent to the product with which the clinical trials were made.
(ii) If reference is made to an approved product in terms of efficacy and / or safety, bioequivalence to this product must be demonstrated. In this case, the aim of bioequivalence testing is to assure that the reference and test products can be used interchangeably.

(iii) When changing the route of administration, the bioequivalence based upon plasma (or blood) concentration profiles or tissue concentration profiles, between the reference route with which the clinical trials were made, and to which reference would be given in terms of efficacy and safety, must be demonstrated.

1.2 Importance

There are several reasons why the topic of bioequivalence currently attracts so much attention but the overriding one concerns the equivalence of so-called generic drugs to pharmaceutical equivalents. When an innovator (brand-name) drug product is going off patent, the innovator drug company may develop a new dosage form of the innovator drug product with the same active ingredient to extend its market exclusively of this product. At the same time, generic drug companies may file an abbreviated new drug application to obtain generic approval of the innovator drug product under its chemical or generic name—usually at a considerably lower price. For approval of a new dosage form or a generic copy of an innovator drug product, most regulatory agencies require only that evidence of equivalence in average bioavailabilities be provided through a bioequivalence trial. As a result, the design, conduct, analysis, report and presentation of bioequivalence trials are extremely important to ensure the validity of bioequivalence between a test drug product and the reference drug product under the current regulations of bioequivalence.

2. The Design

Unlike clinical trials, bioavailability studies are often conducted with healthy volunteers. Thus, the choice of the design and the statistical methods for the analysis of the data becomes two important aspects in planning a bioavailability study.

Basic Design Considerations

A basic design for an in vivo bioavailability study is determined by the following:

1. The scientific questions to be answered
2. The nature of the reference material and the dosage form to be tested.
   (For example, a suspension may not be an appropriate reference material because of high variability in bioavailability of the suspension dosage form)
3. The availability of analytical methods

In addition to these basic design considerations, some specific considerations when planning a design for a bioavailability study are given as:

(i) The number of formulations to be compared
(ii) The characteristics of the drug and its disposition
(iii) The study objective
(iv) The availability of subjects
(v) The inter and intra subject variabilities
(vi) The duration of the study or the number of periods allowed
(vii) The cost of adding a subject relative to that of adding one period
(viii) Dropout rates
3. Commonly Used Experimental Designs
The designs that are often considered for bioavailability and bioequivalence studies include the complete randomized designs (parallel designs), randomized block designs, crossover designs, Latin square designs and incomplete block designs (Chow and Liu, 2000).

3.1 The Parallel Design
A parallel design is a complete randomized design in which each subject receives one and only one formulation of a drug in a random fashion.

The simplest parallel design is the two-group parallel design, which compares two formulations of a drug. Each group usually contains the same number of subjects.

The parallel design is not widely used for bioavailability studies owing to the incapability of identifying and removing the intersubject variability from the comparison between formulations. There are some rare occasions in which a parallel design may be more appropriate than a crossover design.

*When it is preferred*
(a) the intersubject variability is relatively small compared with the intrasubject variability
(b) the drug is potentially toxic or has a very long elimination half-life
(c) the population of interest consists of very ill patients
(d) the cost for increasing the number of subjects is much less than that of adding an additional treatment period

In practice, subjects account for a large source of variability in plasma or blood drug concentrations. Thus, an appropriate design should allow estimation and removal of the intersubject variability from drug comparison.

3.2 The Crossover Design
A crossover design is a modified, randomized block design in which each block receives more than one formulation of a drug at different time periods. A block may be a subject or a group of subjects. Subjects in each block receive a different sequence of formulations.

There are several situations, where it is essential to go for the crossover designs like:

(i) the formulations do not have a serious damaging effect on the subjects
(ii) subjects require special training over a long period of time
(iii) objective of the experiment is to find out the effect of different subjects
(iv) homogeneous subjects are scarce
(v) budget constraint

A crossover design is called a *complete crossover design*, if each sequence contains each of the formulations. We shall refer to a crossover design as g x p crossover design if there are g sequences of formulations administered at p different time periods.

For bioavailability and bioequivalence studies, the crossover design is viewed favorably because of the following advantages:
1. Each subject serves as his or her own control. It allows a within subject comparison between formulations.
2. It removes the intersubject variability from the comparison between formulations.
3. With a proper randomization of subject to the sequence of formulation administrations, it provides the best unbiased estimates for the differences (or ratios) between formulations.

*Washout and Carryover Effects*

It is helpful to introduce the concepts of washout and carryover effects (or residual effects) in a crossover design because the presence of carryover effects usually has an influence on statistical inference of bioavailability between formulations.

The washout period is defined as the rest period between two treatment periods for which the effect of the formulation administered at one treatment period should be long enough for the formulation effects to wear off so that there is no carryover effect from one treatment period to the next. The washout period depends on the nature of the drug. A suitable washout period should be long enough to return any relevant changes that influence bioavailability to baseline (usually, at three times the blood-plasma elimination half-life of the active ingredient, therapeutic moiety or its metabolite, or the decay of the immediate pharmacological effect).

If a drug has a long half-life or if the washout period between treatment periods is too short, the effect of the drug might persist after the end of dosing period. In this case, it is necessary to distinguish the difference between the direct drug effect and the carryover effects. The direct drug effect is the effect that a drug product has during the period in which the drug is administered, whereas the carryover effect is the drug effect that persists after the end of the dosing period. Carryover effects that last only one treatment period are called first order carryover effects. A drug is said to have \(c\)-order carryover effects if the carryover effects last up to \(c\) treatment periods. In bioavailability and bioequivalence studies, however, it is unlikely that a drug effect will carry over more than one treatment period because a sufficient length of washout is usually considered.

*Crossover Designs for Two Formulations*

The most commonly used statistical design for comparing average bioavailability between two formulations of a drug probably is a two sequence, two period, crossover design, known as a *standard* \(2 \times 2\) crossover design. For this design each subject is randomly assigned to either sequence RT or sequence TR at two dosing periods. In other words, subject within RT (TR) receive formulation R (T) at the first dosing period and the formulation T (R) at the second dosing period. The dosing periods are separated by a washout period of sufficient length for the drug received in the first period to be completely metabolized or excreted from the body. Although the crossover design is a variant of the Latin square design, the number of formulations in a crossover design does not necessarily have to be equal to number of periods. One example is a \(2 \times 3\) crossover design for comparing two formulations but in three periods. Subjects in each sequence receive one of the formulations, twice at two different dosing periods. The design of this kind is known as a *higher order crossover design*.

*Crossover Designs for Three or More Formulations*

The crossover designs for comparing three or more formulations is more complicated than those for comparing two formulations. For comparing three formulations of a drug, there are a total of three possible pair wise comparisons between formulations. It is desirable to
estimate these pair wise differences in average bioavailability between formulations with the same degree of precision. In other words, it is desirable to have equal variances for each pair wise differences in average bioavailability between formulations \([i.e., V (F_i - F_j) = \nu \sigma_e^2, i, j = 1, 2, 3]\), where \(\nu\) is a constant and \(\sigma_e^2\) is the intrasubject variability. A design with this property is known as variance balanced design. It should be noted that, in practice, \(\nu\) may vary from design to design. Thus, an ideal design is one with the smallest \(\nu\), such that all pair wise differences between formulations can be estimated with same best precision. However, to achieve this goal, the design must be balanced. A design is said to be balanced if it satisfies the following conditions (Jones and Kenward, 1989):

1. Each formulation occurs only once with each subject.
2. Each formulation occurs the same number of times in each period.
3. The number of subjects who receives formulation \(i\) in some period followed by formulation \(j\) in the next period is the same for all \(i \neq j\).

Under the constraint the number of periods (\(p\)) being equal to the number of formulations (\(t\)), balance can be achieved by using a complete set of “orthogonal Latin squares” (John 1971; Jones and Kenward, 1989). A complete set of orthogonal Latin squares consist of \(t\) \((t-1)\) sequences except for \(t = 6\).

**Example 3.1**: Orthogonal Latin squares for three formulations \((t = 3)\)

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
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<tr>
<td>2</td>
<td>T₁</td>
</tr>
<tr>
<td>3</td>
<td>T₂</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>T₁</td>
</tr>
<tr>
<td>6</td>
<td>T₂</td>
</tr>
</tbody>
</table>

As a result, when the number of formulations to be compared is large, more sequences and consequently more subjects are required. This however, may not be of practical use. A more practical design has been proposed by Williams (1949), known as Williams design (WD). A WD possesses balance property and requires fewer sequences and periods.

**Method I**
The algorithm for constructing a Williams’s design with \(t\) periods and \(t\) formulations is summarized in the following steps:

1. Number of formulations from 1,2…\(t\).
2. Start with a \(t \times t\) Standard Latin Square (SLS). In this square, the formulations in the \(i^{th}\) row are given by \(i, i+1…t, 1, 2…i-1\).
3. Obtain a mirror image of the SLS.
4. Interlace each row of the SLS with the corresponding mirror image to obtain a \(t \times 2t\) arrangement.
5. Slice the $2 \times t$ arrangement down in the middle to yield two $t \times t$ squares. The columns of each $t \times t$ square correspond to the periods and the rows are the sequences. The numbers within the square denote the formulations.

6. If $t$ is even choose any one of the two $t \times t$ squares. If $t$ is odd, use both squares.

**Example 3.2:** Construction of WD with $t = 4$ (one reference and three test formulations):

1. Denote the reference formulation by 1, and test formulations 1,2, and 3 by 2,3,and 4 respectively.
2. A $4 \times 4$ SLS is given as

   \[
   \begin{array}{cccc}
   1 & 2 & 3 & 4 \\
   2 & 3 & 4 & 1 \\
   3 & 4 & 1 & 2 \\
   4 & 1 & 2 & 3 \\
   \end{array}
   \]

3. The minor image of the $4 \times 4$ SLS is given by

   \[
   \begin{array}{cccc}
   4 & 3 & 2 & 1 \\
   1 & 4 & 3 & 2 \\
   2 & 1 & 4 & 3 \\
   3 & 2 & 1 & 4 \\
   \end{array}
   \]

4. The $4 \times 8$ arrangements after interlacing the $4 \times 4$ SLS with its mirror image is

   \[
   \begin{array}{cccc|cccc}
   1 & 4 & 2 & 3 & 3 & 2 & 4 & 1 \\
   2 & 1 & 3 & 4 & 4 & 3 & 1 & 2 \\
   3 & 2 & 4 & 1 & 1 & 4 & 2 & 3 \\
   4 & 3 & 1 & 2 & 2 & 1 & 3 & 4 \\
   \end{array}
   \]

5. The two $4 \times 4$ squares obtained by slicing the above $4 \times 8$ arrangement are

<table>
<thead>
<tr>
<th>Periods</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
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<td>3</td>
<td>2</td>
<td>4</td>
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<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td>(2)</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
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<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
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<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Therefore, the final WS design for 4 formulations is:
### Experimental Designs for Bioavailability / Bioequivalence Trials

#### Periods

<table>
<thead>
<tr>
<th>Sequence</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>T₃</td>
<td>T₁</td>
<td>T₂</td>
</tr>
<tr>
<td>2</td>
<td>T₁</td>
<td>R</td>
<td>T₂</td>
<td>T₃</td>
</tr>
<tr>
<td>3</td>
<td>T₂</td>
<td>T₁</td>
<td>T₃</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>T₃</td>
<td>T₂</td>
<td>R</td>
<td>T₁</td>
</tr>
</tbody>
</table>

#### Method II

(i) For even $v$

Develop the initial sequence cyclically mod $(t)$ to get $t$ sequences.

\[
1 \ t \ 2 \ t-1 \ \ldots \ \frac{t}{2} \ \frac{t}{2}+1
\]

**Example 3.3:** WS design for $t=6$

```
1 6 2 5 3 4
2 1 3 6 4 5
3 2 4 1 5 6
4 3 5 2 6 1
5 4 6 3 1 2
6 5 1 4 2 3
```

The final WS design for 4 formulations is:

<table>
<thead>
<tr>
<th>Sequences</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>T₅</td>
<td>T₁</td>
<td>T₄</td>
<td>T₂</td>
<td>T₃</td>
</tr>
<tr>
<td>2</td>
<td>T₁</td>
<td>R</td>
<td>T₂</td>
<td>T₅</td>
<td>T₃</td>
<td>T₄</td>
</tr>
<tr>
<td>3</td>
<td>T₂</td>
<td>T₁</td>
<td>T₃</td>
<td>R</td>
<td>T₄</td>
<td>T₅</td>
</tr>
<tr>
<td>4</td>
<td>T₃</td>
<td>T₂</td>
<td>T₄</td>
<td>T₁</td>
<td>T₅</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>T₄</td>
<td>T₃</td>
<td>T₅</td>
<td>T₂</td>
<td>R</td>
<td>T₁</td>
</tr>
<tr>
<td>6</td>
<td>T₅</td>
<td>T₄</td>
<td>R</td>
<td>T₄</td>
<td>T₁</td>
<td>T₂</td>
</tr>
</tbody>
</table>
```

(ii) For odd $v$

Developing the initial sequences

\[
1 \ t \ 2 \ t-1 \ \ldots \ \frac{(t+3)}{2} \ \frac{(t+1)}{2} \text{ and}
\]

\[
t \ 1 \ t-1 \ 2 \ \ldots \ \frac{(t-1)}{2} \ \frac{(t+1)}{2} \text{ cyclically mod (t) to get } 2t \text{ sequences.}
\]
Example 3.4: WS design for Five formulations (t=5):

\[
\begin{array}{cccccc}
1 & 5 & 2 & 4 & 3 \\
2 & 1 & 3 & 5 & 4 \\
3 & 2 & 4 & 1 & 5 \\
4 & 3 & 5 & 2 & 1 \\
5 & 4 & 1 & 3 & 2 \\
5 & 1 & 4 & 2 & 3 \\
1 & 2 & 5 & 3 & 4 \\
2 & 3 & 1 & 4 & 5 \\
3 & 4 & 2 & 5 & 1 \\
4 & 5 & 3 & 1 & 2 \\
\end{array}
\]

The final WS design for 4 formulations is:

\[
\begin{array}{cccccc}
\text{Periods} \\
\hline
\text{Sequences} & I & II & III & IV & V \\
\hline
1 & R & T_4 & T_1 & T_3 & T_2 \\
2 & T_1 & R & T_2 & T_4 & T_3 \\
3 & T_2 & T_1 & T_3 & R & T_4 \\
4 & T_3 & T_2 & T_4 & T_1 & R \\
5 & T_4 & T_3 & R & T_2 & T_1 \\
6 & T_4 & R & T_3 & T_1 & T_2 \\
7 & R & T_1 & T_4 & T_2 & T_3 \\
8 & T_1 & T_2 & R & T_3 & T_4 \\
9 & T_2 & T_3 & T_1 & T_4 & R \\
10 & T_3 & T_4 & T_2 & R & T_1 \\
\end{array}
\]

3.3 Balanced Incomplete Block Designs

When comparing three or more formulations of a drug product, a complete cross over design may not be of practical interest for the following reasons (Westlake, 1973):

1. If the number of formulations to be compared is large the study may be too time-consuming since t formulations require t -1 washout periods.
2. It may not be desirable to draw many blood samples for each subject owing to medical concerns.
3. Moreover a subject is more likely to drop out when he or she is required to return frequently for tests.

These considerations suggest that one should keep the number of formulations that a subject receives as small as possible when planning a bioavailability study. For this, a randomized incomplete block design may be useful. However, if we compare several test formulations with a reference formulation, the within-subject comparison is not reliable, as the subject in some sequences may not receive the reference formulation.
An *IBD* is a randomized block design in which not all formulations are present in every block. A block is called incomplete if the number of formulations in the block is less than the number of formulations to be compared. For an incomplete block design, the blocks and formulations are not orthogonal to each other; *i.e.*, the block effect and formulation effects may not be estimated separately.

When IBD is used, it is recommended the formulation in each block be randomly assigned in a balanced way so that the design will possess some optimal statistical properties. We shall refer to such a design as a BIBD. A BIBD is an IBD in which any two formulations appear together an equal number of times. The advantages of using a BIBD, rather than an IBD, are given below:

1. The difference in average bioavailability between the effects of any two formulations can always be estimated with the same degree of precision.
2. The analysis is simple in spite of the non-orthogonality provided that the balance is preserved.
3. Unbiased estimates of formulation effect are available.

Suppose there is *t* formulations to be compared and each subject can only receive exactly *p* formulations (*t* > *p*). A BIBD may be constructed by taking \( \binom{t}{p} \), the combination of *p* out of *t* formulations, and assigning a different combination of formulations to each subject. However to minimize the period effect, it is preferable to assign the combinations in such a way that the design is balanced over period (*i.e.*, each formulation appears the same number of times in each period). In general, if the number of formulations is even (*i.e.*, *t*=2\(n\)) and *p*=2, the number of blocks (sequences) required is *g*=2\(n(2n-1)\). On the other hand, if the number of formulations is odd (*i.e.*, *t*=2\(n+1)\) and *p*=2, then *g*=(2\(n+1)\)\(n\).

**Example 3.5:** BIBD for \(t=4\) with \(p=2\)

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
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<tr>
<td>2</td>
<td>(T_1)</td>
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<tr>
<td>3</td>
<td>(T_2)</td>
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<tr>
<td>4</td>
<td>(T_3)</td>
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<td>R</td>
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<tr>
<td>6</td>
<td>(T_1)</td>
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<tr>
<td>7</td>
<td>(T_3)</td>
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<td>8</td>
<td>(T_2)</td>
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<td>9</td>
<td>R</td>
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<tr>
<td>10</td>
<td>(T_3)</td>
</tr>
<tr>
<td>11</td>
<td>(T_2)</td>
</tr>
<tr>
<td>12</td>
<td>(T_1)</td>
</tr>
</tbody>
</table>

For *p*=2, the first six blocks are required for balanced incomplete block design. However, to ensure balance over periods an additional six blocks (7 through 12) are needed.
3.4 Partially Balanced Incomplete Block Designs

BIBD is, in fact, a special case of variance-balanced design. For an IBD balanced may be achieved with fewer than C(t, p) blocks. Such designs are known as *partially balanced incomplete block designs*.

Suitable designs can be selected directly from Clatworthy Tables (1973) or can be obtained by rearranging these designs in such a way that in the resulting design, each period is uniform, *i.e.*, every treatment appear in each period an equal number of times.

**Example 3.6**: PBIB Design for t=5 (Design C1 in Clatworthy Tables, 1973) and p=2:

<table>
<thead>
<tr>
<th>Periods</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>ii</td>
<td>2</td>
<td>4</td>
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<tr>
<td>iii</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>iv</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>v</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

3.5 Balanced Incomplete Sequence Crossover designs (BISCOD)

The crossover designs considered in Section 3.2 require number of periods equal to number of formulations to be tested, *i.e.*, the sequences in the design are complete. Crossover designs for number of periods less than number of formulations, are available in literature which may also be used for bioequivalence trials.

**Method I**

Patterson and Lucas (1962) obtained a method of construction of balanced incomplete sequence crossover designs by constructing a balanced crossover design for each set of k treatments that form a block of a balanced incomplete block design. Let the parameters of the considered BIB design be v′, b′, r′, k′ and λ′ with b′ blocks are arranged in b′ rows. Now, construct a crossover design with v* = k′, p* and n* using the block contents of each block of the above BIB design, *i.e.*, from each row, we get n* sequences which are again arranged in rows. The resultant design is a BISCOD with parameters v = v′, p = p* and n = b′n*, if rows are treated as sequences and columns as periods.

**Example 3.7**: Consider the following BIB design with parameters v′=4, b′=6, r′=3, k′=2 and λ′=1:

```
1  2
1  3
1  4
2  3
2  4
3  4
```
Construct a crossover design with number of treatments $v^* = 2$ in $p^* = 2$ periods and $n^* = 4$ units using each block of the BIB design. Let the crossover design considered be as follows:

\[
\begin{array}{cc}
1 & 2 \\
2 & 1 \\
1 & 1 \\
2 & 2 \\
\end{array}
\]

The resultant BISCOD with $v = 4$, $p = 2$ and $n=24$ is as follows:

<table>
<thead>
<tr>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period I</td>
</tr>
<tr>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>Period II</td>
</tr>
<tr>
<td>2 1 1 2 3 1 1 3 4 1 1 4</td>
</tr>
</tbody>
</table>

| Period I  |
| 13 14 15 16 17 18 19 20 21 22 23 24 |
| Period II |
| 2 3 2 3 2 4 2 4 3 4 3 4 |

Method II

Using the series of BIB designs with $v=mk+1$ ($v$ is prime or prime power), $b=mv$, $r=mk$, $k$, $\lambda=k-1$ constructed by developing mod $v$, each of the $m$ initial blocks: $(x^i, x^{i+m}, x^{i+2m}, \ldots, x^{i+(k-1)m})$, $i = 0, 1, 2, \ldots, m-1$; $x$ being a primitive element of GF ($v$), Dey and Balachandran (1976) obtained balanced crossover designs. If the blocks of the above BIB design are written as columns, a crossover design in $v$ treatments, $mv$ sequences and $k$ periods, where the columns are treated as sequences and rows as periods can be obtained. In order to make this crossover design balanced, the last treatment in each sequence is also placed in the same sequence in a period preceding the first period. Thus, from the BIB design, an arrangement of $(k+1)$ rows (periods) and $mv$ columns (sequences) are obtained. The first row of this arrangement is called initial period or pre-period. The treatments are applied from the initial period to which the experiment really starts, though the observations in this period are not to be used for analysis. Actually, only the residual effects of the treatments applied in the initial period enter into the data collected from the second period.

**Example 3.8**: Let $m=2$, $k=3$, so that $v=7$. The balanced crossover design in 14 sequences and 3 periods is shown below:

<table>
<thead>
<tr>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periods I II III IV V VI VII VIII IX X XI XII XIII XIV</td>
</tr>
<tr>
<td>O 4 5 6 7 1 2 3 5 6 7 1 2 3 4</td>
</tr>
<tr>
<td>i 1 2 3 4 5 6 7 3 4 5 6 7 1 2</td>
</tr>
<tr>
<td>ii 2 3 4 5 6 7 1 6 7 1 2 3 4 5</td>
</tr>
<tr>
<td>iii 4 5 6 7 1 2 3 5 6 7 1 2 3 4</td>
</tr>
</tbody>
</table>

The period number O is the initial period.
3.6 Partially Balanced Incomplete Sequence Crossover designs (PBISCOD)

To further reduce the experimental resources, partially balanced incomplete sequence crossover designs were obtained by Patterson and Lucas (1962). Let the parameters of the considered m-associate class PBIB design be $v^{'}, b^{'}, r^{'}, k^{'}, \lambda^{'i}$ (i = 1, 2, …m) with $b^{'}$ blocks are arranged in $b^{'}$ rows. Now, construct a crossover design with $v^* = k^{'}, p^*$ and $n^*$ using the block contents of each block of the PBIB design considered, i.e., from each row, we get $n^*$ sequences which are again arranged in rows. The resultant design is a PBISCOD with parameters $v = v^{'}, p = p^*$ and $n = b^{'n^*}$, if rows are treated as sequences and columns as periods.

**Example 3.9:** Consider the following PBIB(2) design with $v^{' = 5}, r^{' = 2}, k^{' = 2}, \lambda^{'1} = 1$ and $\lambda^{'2} = 0$:

```
1  3
2  4
3  5
4  1
5  2
```

Constructing a COD with $v^* = k^{' = 2}, p^* = 2$ and $n^* = 4$ from each block of the PBIB(2) design, the following PBISCOD with $v = 5, p = 2, n = 20$ can be obtained:

```
Sequences
1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20
Period I
1  3  1  3  2  4  2  4  3  5  3  5  4  1  4  1  5  2  2  5
Period II
3  1  1  3  4  2  2  4  5  3  3  5  1  4  4  1  2  5  2  5
```

4. Conclusions

If the intrasubject variability is the same as or larger than the intersubject variability, the reference on the difference in average bioavailability would be the same regardless of which design is used. Actually a crossover design in this situation would be a poor choice, because blocking results in the loss of some degrees of freedom and will actually lead to a wider confidence interval on the difference between formulations.

If the drug has very long half life or it possesses a potential toxicity, or bioequivalence must be established by clinical endpoint because some drugs do not work through systemic absorption, then a parallel design may be a possible choice. With this design the study avoids a possible cumulative toxicity from the carryover effect from one treatment period to the next. In addition the study can be completed quickly. However, the drawback is that the comparison of average bioavailability is made based on the intersubject variability. If the intersubject variability is large relative to the intrasubject variability, the statistical inference on the difference in average bioavailability between formulations is unreliable. Even if the intersubject variability is relatively small, a parallel design may still require more subjects to reach the same degree of precision by a crossover design.
In practice, a crossover design which can remove the intersubject variability from the comparison of average bioavailability between formulations is often considered to be the design of choice, if the number of formulations to be compared is small, say, not more than three. When the number of formulations is large, a balanced or partially balanced incomplete block design may be preferred over a crossover design. Balanced / partially balanced incomplete sequence crossover designs may be used more advantageously when it is difficult to conduct the experiment for more number of periods but, the formulations are known to have carryover effects.

The selected design may affect the data analysis, the interpretation of the results, and the determination of bioequivalence between formulations. Thus all factors and listed in the above should be carefully evaluated before an appropriate design is chosen for the experiment.

References

Additional References