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# Level B and C in vivo/in vitro correlations: statistical considerations

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**Abstract**

An approach to in vivo/in vitro correlation is proposed based on the suggestion that formulations are acceptable, or equivalent to a reference product, if the confidence interval for the in vivo variable lies within a therapeutically acceptable range. An acceptable in vivo/in vitro correlation is defined as a correlation for a range of formulations for which the confidence interval of the predicted in vivo variable lies within the therapeutically acceptable range. Causes of failure of correlations are discussed, with emphasis on the need to understand the mechanism of release from the formulation and the need to control critical variables in experimental studies. © 1997 Elsevier Science B.V.

*Keywords:* In vitro/in vivo correlation; Therapeutic range; Statistics

## 1. Introduction

One of the traditional aims in investigating in vivo/in vitro correlations is to provide a better general understanding of drug absorption and of the dependence of the overall absorption process on the release processes that can be studied in vitro. In such studies the use of $r^2$ as a measure of the proportion of total variance explained, or a test of statistical significance on the slope of the regression, appear to be reasonable measures of the extent to which in vitro behaviour is associated with in vitro release characteristics. However, recent interest in in vivo/in vitro correlations has been directed toward more specific issues such as the use of in vitro studies in quality control procedures and as an alternative to in vivo bioequivalence studies. When the aim of a study is specifically the prediction of an in vivo variable, quite different statistical questions arise. For example, the demonstration of a statistically significant correlation does not imply that the

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correlation is of use in making a prediction of clinical performance which could serve as a substitute for a bioequivalnce study. Similarly, when in vitro tests are used as quality control procedures, it is not sufficient to establish that a statistically significant correlation exists to predict that a minor formulation change will have no significant therapeutic consequence.

This article develops further some preliminary suggestions (Cutler, 1995) on the use of a therapeutic range of the in vivo variable as a basis for establishing in vivo/in vitro correlations when the focus of attention is the therapeutic use of the formulation, rather than general issues concerning absorption mechanisms or efficiency.

## 2. Types of correlation

Three types of in vivo/in vitro correlation have been suggested (Skelly and Shiu, 1993; USP, 1995). Level A correlations involve a comparison between an in vitro dissolution profile and an in vivo input function. Level B correlations involve comparison between the mean in vitro dissolution time (as a single point measure of in vitro dissolution or release rate) with either the mean residence time or the mean in vivo dissolution time (as single point measures of the in vivo drug input rate). Level C correlations involve a comparison between a single point measure of dissolution or release rate (such as mean dissolution time or time for 50% release) and a single point measure of the extent of absorption in vivo (such as AUC). This article will concentrate on Level B and Level C correlations which involve similar statistical considerations.

Level B and C correlations are open to the criticism that they are based on single point measures which may fail to reflect the complexity of the release and absorption mechanisms (this is in part the motivation for Level A correlations which are based on entire time profiles). In particular, two formulations may have different release profiles but the same value of a single point measure such as mean dissolution time. If these differences in release profile are of significance in in vivo performance, they will contribute to failure of an attempted correlation. On the other hand Level B and C correlations have the major advantage of being very simple to perform compared with Level A correlations. We propose that Level B and C correlations, implemented as described below, be regarded as the first step in an overall program of relating in vitro and in vivo performance of dosage forms. When the assumptions on which Level B and C correlations are based are seriously in error the correlations can be expected to fail and the more difficult Level A correlations may be needed (although it needs to be appreciated that some of the reasons for failure of Level B and C correlations will also tend to result in failures in Level A correlations).

## 3. Criteria for an acceptable in vivo/in vitro correlation

The term 'correlation' is applied in general whenever there appears to be a relationship between two (or more) variables. In normal usage the term is usually reserved for the situation in which the correlation is statistically significant. However, as noted above, and in more detail in the following, this is not sufficient for a correlation to be of use in predicting the in vivo performance of a dose form. The present section is concerned with establishing the criteria that distinguish correlations that are 'acceptable' for this purpose.

Although the treatment of data with error is obviously of central importance in considering in vivo/in vitro correlations, it is of value to consider, as a first step, the case in which both in vivo and in vitro parameters are error-free (Fig. 1). In particular, consideration of this special case focuses attention on the purpose in investigating the correlation. The form of Fig. 1 is chosen to represent the case in which an increase in the in vitro variable (say, time for 50% release or mean dissolution time) is expected to be associated with a decrease in the in vivo variable (say, $C_{max}$ or AUC). However, the general considerations apply equally to the case in which the in vivo variable increases with an increase in the in vitro variable.

It is important to recognise at the outset that a correlation is likely to be of value only when the in vivo parameter is of therapeutic relevance. For this reason, simple in vivo measures such as $C_{max}$ (which can be readily related to a therapeutic concentration range) or AUC (directly related to the extent of absorption) may have an advantage over Level B measures, such as mean absorption time, which are less readily related to therapeutic outcome.

When a therapeutically acceptable range of the in vivo parameter can be identified, this range of in vivo variables can be (in the case error-free data) immediately translated into a range of acceptable values for the in vitro parameter (Fig. 1). In this case any formulation with an in vitro variable within its acceptable range would be therapeutically effective, provided that the in vivo variable is an appropriate indicator of therapeutic performance.

Fig. 2 shows the more realistic case in which the in vivo parameter alone contains significant error. The error in the in vitro parameter will generally be much smaller than the error in the in vivo

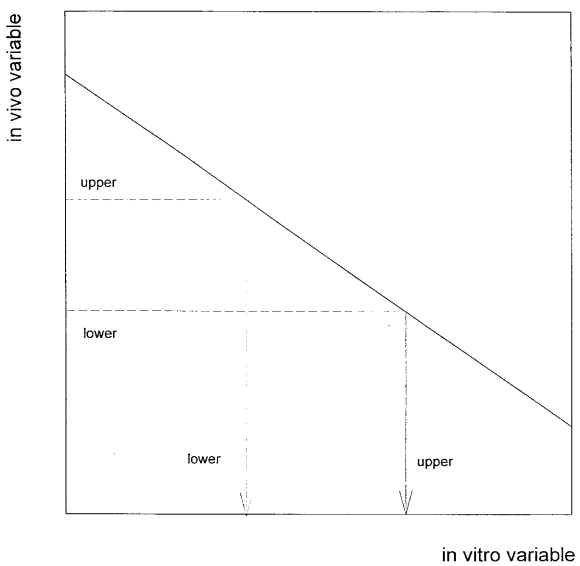


Fig. 1. Error-free correlation between in vivo and in vitro variables. The horizontal lines labelled 'upper' and 'lower' represent the upper and lower limits of the acceptable range of the in vivo variable. The vertical lines indicate the corresponding limits for the in vitro variable.

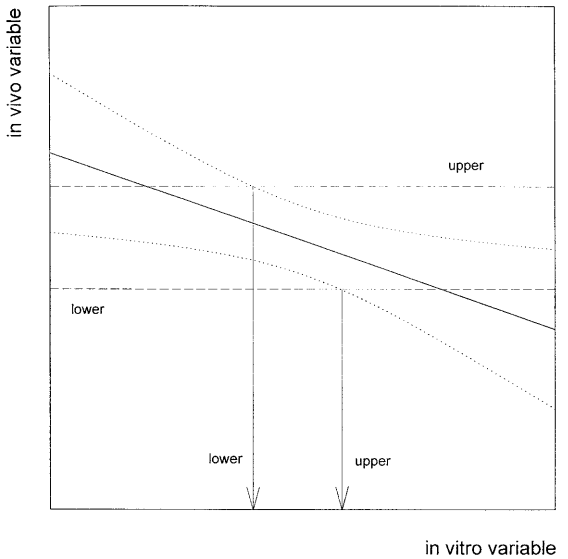


Fig. 2. Limits of the useful range for a Level B or C correlation. The dotted lines show the limits of the confidence interval for the predicted value of the in vivo variable. The vertical lines show the limits of the in vitro variable for which the confidence interval lies within the acceptable range of the in vivo variable.

parameter, so this case is likely to be a reasonable representation of the situation met in practice. In Fig. 2 the solid line indicates the line of best fit for a correlation and the confidence interval for the predicted values of the in vivo variable is shown by the dotted line. For those formulations with an in vitro value above the lower bound and below the upper bound shown in Fig. 2, the confidence interval for the predicted values of the in vivo variable lies within the acceptable therapeutic limits for the in vivo variable. It is proposed that a formulation would be considered acceptable if its confidence interval for the in vivo variable falls within the acceptable range. Applying the same principle to the correlation, it is proposed that the correlation be considered acceptable over the range of values of the in vitro variables for which the confidence interval for the predicted values of the in vivo variable is within the acceptable therapeutic range.

Note that these considerations apply to establishing the existence of an acceptable correlation between the in vivo and in vitro variables for

formulations for which both variables are known. The use of such a correlation to predict values of the in vivo variable when only the in vitro variable is known involves additional considerations, outlined below. For the moment we note that, in a case such as that depicted in Fig. 2, it is at least possible for an acceptable correlation to exist, that there is some range of formulations that would be judged to be acceptable. The following section shows that it is not necessarily the case that an acceptable correlation can be established.

## 4. Expected problems in establishing acceptable in vivo/in vitro correlations

A failure to establish an acceptable correlation can arise for a number of reasons. For the 'ideal' case (appropriate in vivo and in vitro variables) a failure to establish an acceptable correlation can be expected when the variance in the in vivo variable is large compared with its therapeutic range. This could be due to variability in the pharmacokinetics of the drug, or to a narrow therapeutic range. Fig. 3 shows the case in which the relationship between the in vitro and in vivo variables is the same as in Fig. 2, but with a smaller therapeutic range. In this case, the construction outlined above, to establish upper and lower limits of the in vitro variable, does not produce a range of in vitro values useful for predicting when a therapeutic outcome will be successful. This is because there is no value of the in vitro variable for which the confidence interval for the in vivo variable lies within the therapeutic range.

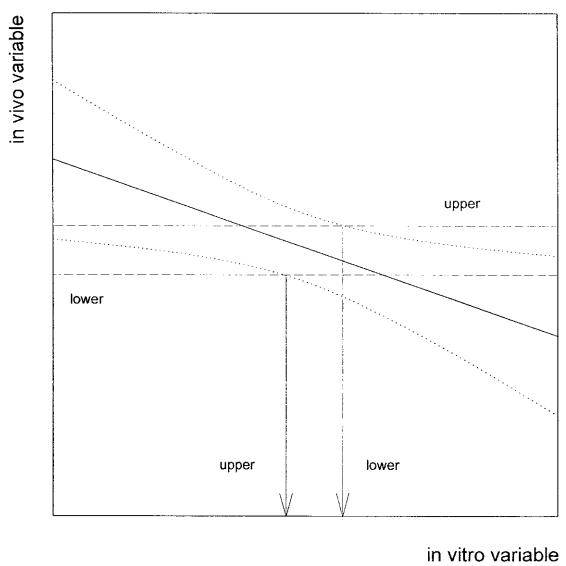


Fig. 3. The same construction as in Fig. 2 but with a narrower range of acceptable values for the in vivo variable. There is no in vitro range for which the confidence interval lies within the acceptable in vivo range; the 'upper' limit of the in vitro variable is below the 'lower' limit.

This illustration also serves to indicate the difference between an acceptable correlation, as defined above, and a statistically significant correlation (a correlation for which the slope is judged to be significantly different from zero). Figs. 2 and 3 differ only in the therapeutic range of the in vivo variable, so if the correlation in Fig. 2 ('acceptable') is statistically significant this is also the case for Fig. 3 (not 'acceptable'). The difference between Figs. 2 and 3 is not in the statistical significance of the slope but in the relative magnitudes of the confidence interval and therapeutic range for the in vivo variable.

The situation shown in Fig. 4 differs from that of Fig. 2 in that a wider confidence interval for the correlation is shown, with the same therapeutic range for the in vivo variable. This is the expected situation for a drug with highly variable pharmacokinetics. As in the case just discussed, attempts to establish acceptable limits for the in vitro variable leads to the result that there is no value of the in vitro variable for which the confidence interval for the in vivo variable is within the therapeutic range.

It can be noted at this point that highly variable pharmacokinetics does not, in itself, preclude an acceptable correlation. If a drug with highly variable pharmacokinetics is nevertheless therapeutically useful, it is likely to have a relatively wide therapeutic range, which will assist in establishing an acceptable correlation.

Although it is clear from these considerations that pharmacokinetic variability and a narrow therapeutic range will make it more difficult to establish a correlation, in most practical situations it is likely that other factors contribute to a failure

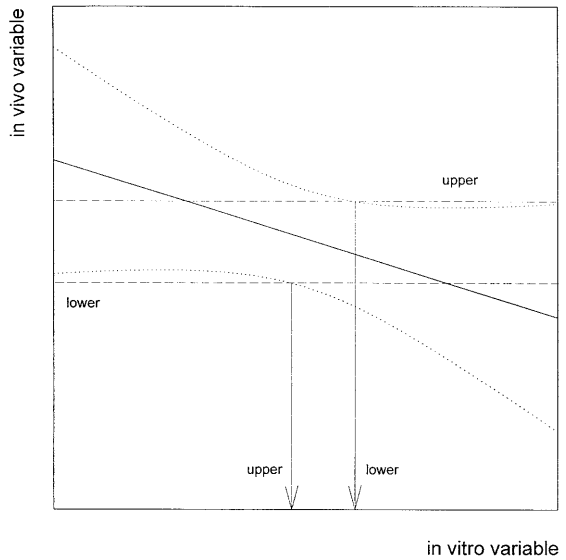


Fig. 4. The same construction as in Fig. 2 but with a wider confidence interval for the correlation (more variable pharmacokinetics). As in Fig. 3 there is no useful in vitro range for a correlation; the 'upper' limit of in vitro variable is below the 'lower' limit.

to obtain a useful correlation. Consider the ideal case in which the in vivo variable is appropriately chosen and that the in vitro measure is indeed a valid measure of in vivo release behaviour. In this case, the failures depicted in Figs. 3 and 4 imply that there is no acceptable formulation at all; that is, not even the reference formulation is acceptable by the criteria set. It is an unlikely situation that an in vivo/in vitro correlation would be attempted before it has been demonstrated that at least one formulation of the active principle is therapeutically effective. It appears then that an outright failure to establish a correlation is probably not due to random variability that can be interpreted within an idealized statistical model, but rather to failure of the statistical model itself.

A central assumption involved in the use of a correlation to predict a value of the dependent variable (here, the in vivo variable) given a value for the independent variable (here the in vitro variable) is that the same statistical model applies to all the data used to establish the correlation. In the present case, it is assumed that there exists a relationship

$$\text{in vivo variable} = f(\text{in vitro variable}) + e$$

where $f$ stands for some function (in the present case a linear function) which describes the underlying relationship between the in vitro and in vivo variables for all the formulations used in the study and $e$ is the random error contribution from an error distribution common to all the data considered. Either of these assumptions of the statistical model can be questioned in an actual study. It is possible that for some formulations in the study the error distribution might be very different from that for other formulations. For example, if some formulations have release characteristics that are pH-dependent, the error distribution for these formulations will depend on in vivo pH variations (within or between subjects) which will not influence the error distribution for formulations with pH-independent release. For the same example, the function $f$, relating the underlying in vitro and in vivo variables, is likely to be quite different for the different types of formulation, as the relationship for one type of formulation depends on both in vitro and in vivo pH characteristics while for the other type the relationship is independent of these pH conditions. Equivalent general comments are likely to apply when formulations differ in other physical or physico-chemical characteristics (Cardot and Beyssac, 1993).

Figs. 5 and 6 illustrate a consequence of failure in the assumptions underlying the statistical model for in vivo/in vitro correlations. Fig. 5 shows the situation for two types of formulations for which the underlying functional relationship (the function $f$) is different. In this illustration, a correlation is possible for both types of formulation, but each with a different range of in vitro values. However, when the two types of formulation are erroneously analyzed together, according to the same statistical model (Fig. 6), there is no range of the in vitro variable for which the predicted confidence interval of the in vivo variable lies within its therapeutic range. That is, the failure to establish a correlation, which appears to be due to large random fluctuations (larger, for example, than would have been seen for a reference formulation alone), is in fact due to differences in the underlying functional relationship between the two variables.

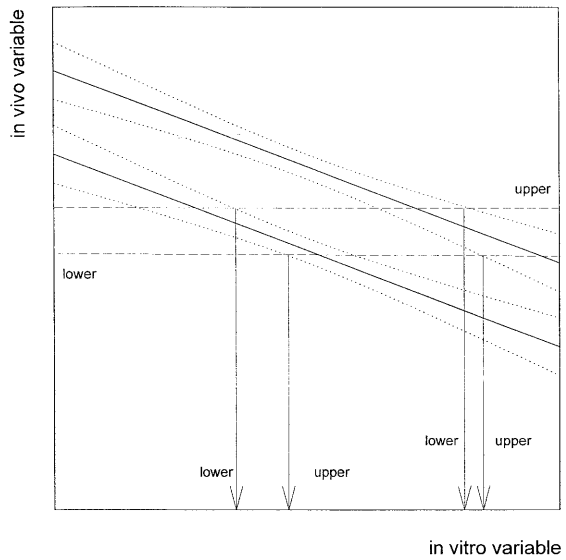


Fig. 5. Acceptable correlations for two types of formulation with distinct statistical models.

These problems indicate that (as for any other correlation) attempts need to be made to control sources of variation that are likely to confound the correlation. For example, if the aim of the study is to establish a basis for in vitro measures as quality control procedures, the underlying statistical model is more likely to be valid for the range of formulations tested if these are similar products, with the same release mechanism and structure, reflecting the range and type of variation that is expected in practice.

Note that inconsistency of the statistical model will also lead to the same kind of problems when Level A correlations are used (although the statistical model will be more complicated than that for Level B and C correlations). If Level B and C correlations fail because of a variety of release mechanisms across the formulations used for the correlation, it is not likely that good correlations can be established by any procedure which does not incorporate mechanistic details as part of the model.

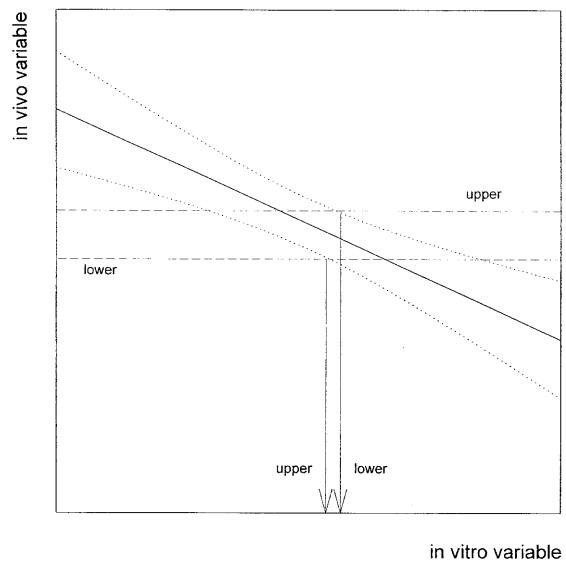


Fig. 6. Failure to establish an acceptable correlation when the two data sets of Fig. 5 are analyzed assuming a single statistical model.

## 5. Prediction using an acceptable in vivo/in vitro correlation

For the ideal case (relevant in vivo and in vitro variables and the same statistical model for all formulations) the procedure for predicting the value of the in vivo variable based on a measurement of the in vitro variable for a 'test' formulation is straightforward. It is simply a matter of measuring the value of the in vitro variable of the test formulation to determine whether it is within its acceptable range.

This use of a correlation relies on the assumption that the same statistical model applies not only to the formulations used to establish the correlations but also to the 'unknown' test formulation whose in vivo performance is to be predicted. When this assumption is false, the consequences are quite different from when the aim is to establish, rather than use, a correlation. As shown in Figs. 7 and 8, when an incorrect statistical model is used the result can either be a prediction that the formulation is satisfactory when it is not (Fig. 7) or the prediction that the formulation is unsatisfactory when it is in fact satisfactory (Fig. 8).

Although it is easy to see the prediction errors in Figs. 7 and 8, in an actual study the true value

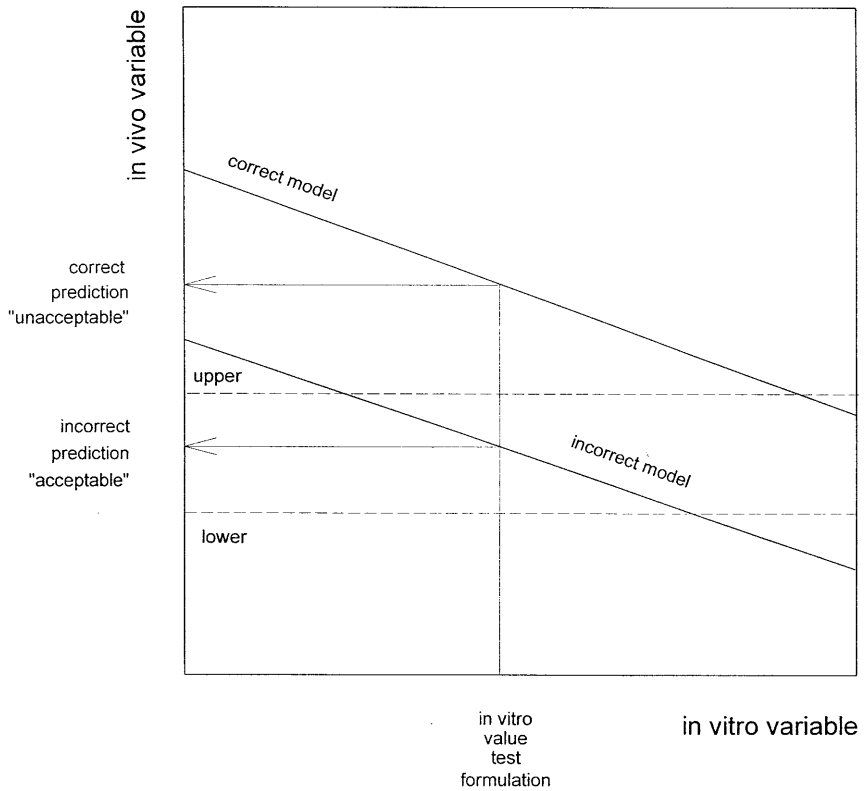


Fig. 7. Incorrect prediction that a formulation is acceptable arising from an incorrect statistical model.

of the in vivo variable is not known. Instead, all that is available is the value of the in vitro variable and the 'successful' or 'unsuccessful' prediction. There is no statistical information available to indicate whether the test formulation can be described by the same statistical model as the correlation. In this case a judgement on the reliability of the prediction has to be made based on general considerations of the circumstances of the studies involved. Thus, for a formulation with a release mechanism different from the formulations used to establish the correlation, any prediction should be regarded with scepticism, except perhaps when previous studies have clearly demonstrated that the correlation applies to a wide range of types of formulations. On the other hand, a prediction could be regarded with confidence when the release mechanism of all the formulations is well-understood and when the variables known to be significant for the release process have been adequately controlled.

As noted above, the same problems exist when Level A correlations are used to predict in vivo behaviour if there is uncertainty about the consistency of the statistical model. No procedure that fails to incorporate mechanistic details is likely to provide reliable predictions when there are significant differences in mechanism of release across formulations.

## 6. Illustration based on experimental data

Fig. 9 shows data derived from a study of four related aspirin formulations (Aiache et al., 1988), for which release profiles in vitro (used to calculate mean dissolution time) and plasma concentration profiles in 12 volunteers ($C_{max}$ obtained by inspection) were obtained. The line of best fit is shown as a solid line and the 95% confidence

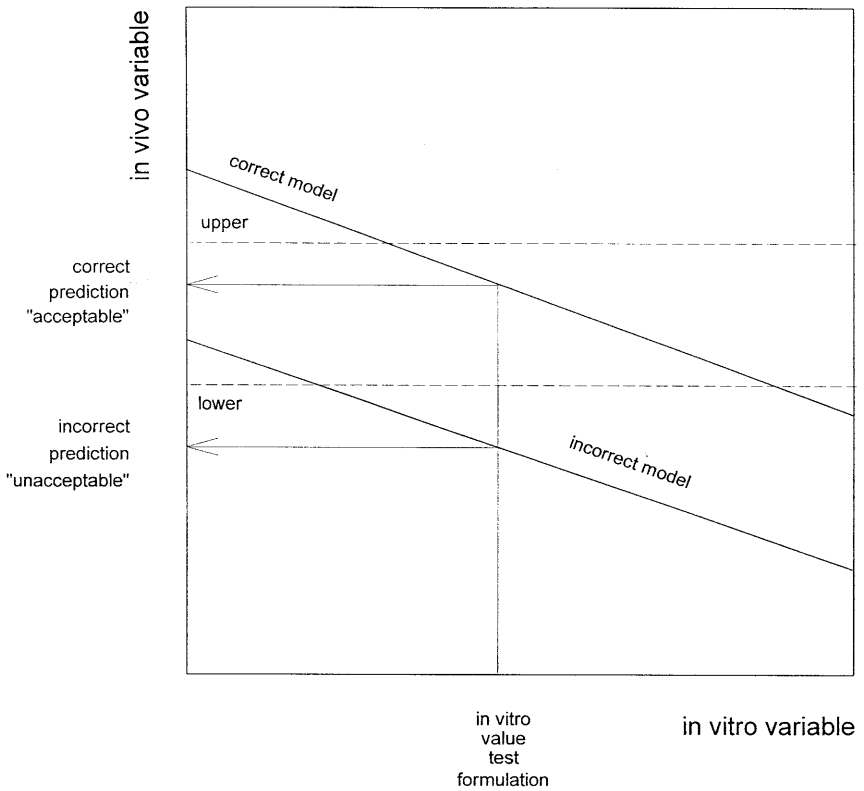


Fig. 8. Incorrect prediction that a formulation is unacceptable arising from an incorrect statistical model.

interval is shown as a dotted line. The horizontal dashed lines show the therapeutic range for the analgesic effect of aspirin (Ritschel, 1986). The predicted confidence interval for the correlation lies well within the therapeutic range for the plasma concentration, indicating an acceptable correlation, as defined above, over the entire range of measured in vitro values. Further studies would be needed to specify the maximum range of an acceptable correlation (as shown, for example, in Fig. 2) but these would not be needed for quality control purposes if the range of in vitro values expected in practice is spanned by the range used to establish the correlation. For all the aspirin formulations in this study it is expected that the rate controlling mechanism of release is aqueous diffusion. It appears that this common mechanism of release allows for a consistent statistical model for the range of formulations. For related formulations (say, the result of minor formulation changes to the products used to establish the correlation), with release also rate limited by aqueous diffusion, the correlation appears to be useful for quality control purposes involving prediction of the in vivo performance of the test products.

## 7. Summary

Level A and B in vivo/in vitro correlations are expected in general to be characterized by a considerable degree of scatter. In this article we propose that the question of the acceptable level of scatter needs to be considered in terms of a therapeutically acceptable range of the in vivo variable. According to this proposal a correlation is considered to be acceptable over a range of variable values for which the 95% confidence interval of the correlation lies within the therapeutically acceptable range of the in vivo variable.

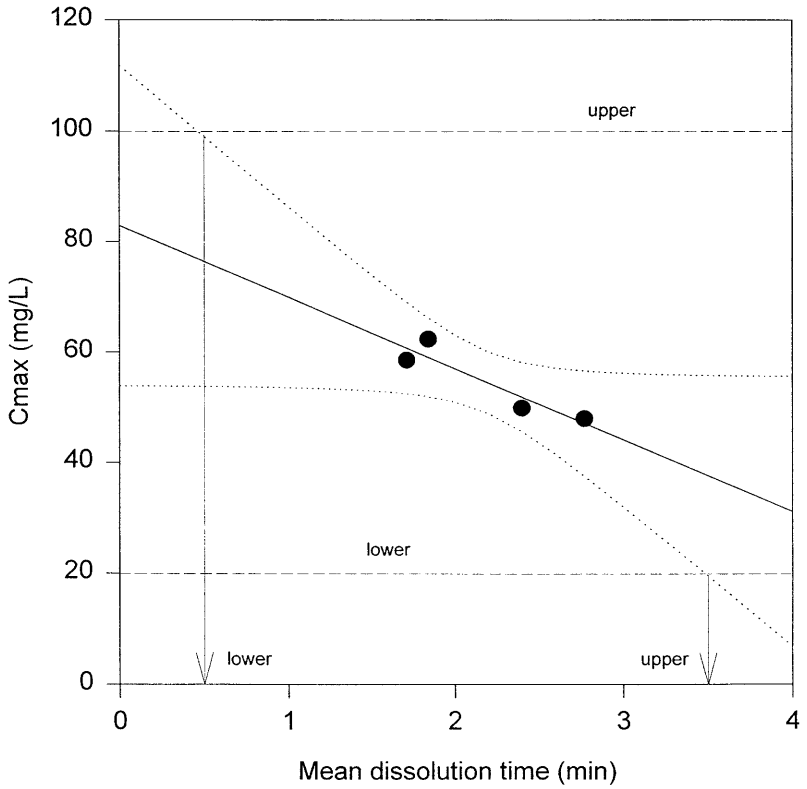


Fig. 9. Correlation between $C_{max}$ and mean dissolution time for four aspirin formulations.

The feasibility of the approach has been demonstrated using experimental data and a conventional therapeutic range of plasma concentrations. However, this is not the only approach consistent with the general method outlined here. All that is required is that some range of values of the in vivo variable be specified which is consistent with an adequate therapeutic outcome. If it is not possible to make such an assignment for a particular in vivo variable there would seem to be little value in measuring that variable, and little value in attempting to develop correlations using it.

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