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Methods of Establishing In Vitro–In Vivo Relationships for Modified Release Drug Products

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1. Introduction

Many orally administered drugs are produced in more than one formulation. Modified release formulations, also called extended release or controlled release, are designed so that the active drug is released from the dosage form over a period of time, as opposed to conventional (immediate release) dosage forms which are intended to release the active drug nearly instantaneously. The rate of release depends on how quickly the dosage form dissolves in the gastrointestinal tract. A primary benefit of modified release dosage forms is that they allow a reduction in dosing frequency as compared to conventional dosage forms. This is especially valuable with a drug for which it is important that the level of drug remain moderately high over a long period of time, but for which too high levels of drug for any period of time may be toxic. For some drug products the dissolution rate is the primary determinant of the rate of absorption into the bloodstream and subsequent appearance at the site of therapeutic action, while for other drug products the absorption rate depends on both the dissolution rate and the rate at which the drug permeates the wall of the gastrointestinal tract into the bloodstream. The former is called dissolution-rate-limited absorption and the latter combined dissolution- and permeation-rate-limited absorption. In reality, no drug product has instantaneous permeation, but permeation can be fast enough relative to dissolution that it is sensible to speak of dissolution-rate-limited absorption. Likewise, permeation can be slow enough relative to dissolution that it may be called permeation-rate-limited absorption (e.g., many conventional dosage forms). In general, the term absorption refers to the combined processes of dissolution and permeation.

Scientists, drug manufacturers and regulatory agencies all have a strong interest in methods for quantifying the behavior of drug products in humans. From the point of view of the consumer, the most important measure of a drug product is its therapeutic effect (including both intended effects and side effects). However, for many drugs it is not at all clear how to quantify therapeutic effect. Therefore, from both scientific and regulatory points of view the most useful measure for quantitatively evaluating a drug product is bioavailability, which is commonly

defined as the rate and extent of active drug that reaches the bloodstream (Shargel and Yu, 1993). A more ideal measure would be the extent of active drug that reaches the tissue containing its action site, but for many drugs measuring this would be both impractical and unsafe. In most cases the amount of drug in the bloodstream is directly related to the amount of drug at its action site.

A typical bioavailability study involves administering the dosage form to a subject and then measuring the concentration of active drug in the bloodstream at various time points. A plot of drug concentration versus time since the drug product was administered is called the bioavailability profile of the drug product. Different formulations of a drug product may have different bioavailabilities resulting from differences in their absorption rates. The left panel of Figure 1 shows the bioavailability profiles of four different formulations of a drug product measured on four different occasions in the same person. The bioavailability profile depends not only on the absorption rate, but also on the rate of elimination from the bloodstream. Drugs are eliminated from the bloodstream both through excretion, primarily by the kidneys, and metabolism, primarily by the liver. Elimination of active drug does not, in general, depend on the formulation of the drug product, but rather on the physicochemical properties of the active drug. Therefore, one may expect that formulations with different absorption rates will have different bioavailability profiles.

Pharmaceutical scientists use in vitro models to study biological systems which are too difficult or costly to study in vivo. In vitro dissolution is a method for evaluating the rate at which a dosage form would dissolve in the gastrointestinal tract (i.e., in vivo dissolution). This is typically accomplished by placing the dosage form in simulated gastric fluid, often in combination with mechanical agitation, and measuring the fraction of the dosage form that has dissolved at various time points. A plot of fraction dissolved versus time is termed the in vitro dissolution profile. The right panel of Figure 1 shows the in vitro dissolution profiles corresponding to the four formulations yielding the bioavailability profiles in the left panel.

In vitro dissolution studies are most useful when they can be used to study bioavailability, especially for drug products having dissolution-rate-limiting ab-

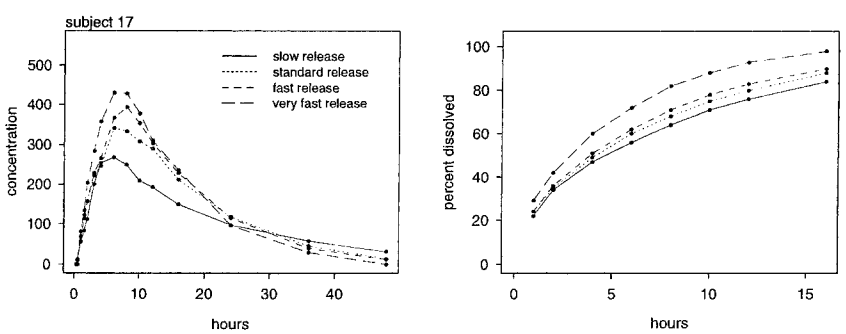

Fig. 1. Example bioavailability and in vitro dissolution profiles for four formulations of a modified release drug product.

sorption. In this case, the bioavailability profiles will be determined primarily by the in vivo dissolution rate; and to the extent in vitro dissolution mimics in vivo dissolution, in vitro dissolution will be useful for predicting bioavailability. On the other hand, when considering drug products having permeation-rate-limiting absorption, in vitro dissolution studies are not likely to be useful for studying bioavailability. In this case, the bioavailability profile will be the same regardless of the in vivo dissolution rate. Since most modified release drug products are not at either end of the dissolution-versus permeation-rate-limiting spectrum, the utility of in vitro dissolution for studying bioavailability must be evaluated on a case-by-case basis. Amidon et al. (1995) and Polli et al. (1996) discuss this issue in detail and propose a theoretical basis for determining whether or not a drug is likely to provide a useful in vitro–in vivo correlation based on its dissolution and permeation properties. They also point out that in many cases, a report of a poor or weak in vitro–in vivo correlation may be reflective of permeation-rate-limiting absorption rather than a failure of the in vitro dissolution test to mimic in vivo dissolution.

In vitro dissolution studies have value apart from their use as models for in vivo dissolution. They can also be used as a quantitative measure of the physicochemical properties of the formulation of a drug product without regard to bioavailability. One important use of in vitro dissolution studies is in monitoring the manufacturing process of a drug product. In vitro dissolution can indicate whether or not drug products from different manufacturing lots, sites, machines, or recipes have the same dissolution profile. In addition, in vitro dissolution can be used to assess the amount of variation in the manufacturing process.

When one intends to apply an in vitro model to make inference about a biological process, it is desirable to be able to demonstrate that the model provides relevant information about the process of interest. This paper is concerned with methods of validating in vitro dissolution as a tool for studying the bioavailability of modified release oral dosage forms. The term "in vitro–in vivo correlation" has been used in the pharmaceutical literature for more than 25 years, but a formal definition did not exist until 1988 when the United States Pharmacopeial (USP) Subcommittee on Biopharmaceutics proposed the following: "the establishment of a relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical characteristic of the same dosage form". There is no doubt that common use of the term prior to and since 1988 is not in conflict with this definition. However, it does appear that many researchers have interpreted this definition rather narrowly.

Most of the in vitro–in vivo correlations reported in the pharmaceutical literature are analyses which quantify some aspect of the relationship in terms of the Pearson correlation coefficient. These kinds of analyses, while providing evidence for the existence of an in vitro–in vivo relationship, may not provide insight into the utility of the relationship. This is not to say that such potential has not been recognized, as Langenbucher (1983) points out: "from the very beginning, the value of in vitro dissolution or release tests has been seen to lie in their ability to

predict the performance of the preparation in vivo". The most recent FDA Guidance for Industry (Malinowski et al., 1997) recognizes the importance of predictive value with a slightly different definition of an in vitro–in vivo correlation: "a predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response (e.g., plasma drug concentration or amount of drug absorbed)".

Figure 2 demonstrates the relatively recent increase in the number of MEDLINE citations containing the keyword phrase in vitro–in vivo correlation. Part of this increase may be a direct result of the formal definition put forth by the USP Subcommittee on Biopharmaceutics, and part of it may be due to a real increase in pharmaceutical research involving in vitro–in vivo correlations. In its recently released Guidance for Industry, the FDA reports that the appearance of in vitro–in vivo correlations in new drug application (NDA) submissions has increased substantially in the past five years. This trend is likely to continue because the FDA is promoting the use of in vitro–in vivo correlations as support for using dissolution studies as surrogates for human bioavailability studies. That is, a strong in vitro–in vivo correlation for a particular drug product could be taken as evidence that the in vitro dissolution model provides sufficient information about bioavailability so that it is unnecessary to conduct further human bioavailability studies. Of course, human bioavailability studies would be required for developing the in vitro–in vivo correlation, but there is still great potential for reducing the overall number of human studies needed for drug applications.

In addition to providing a formal definition of an in vitro–in vivo correlation, the USP Subcommittee on Biopharmaceutics developed four categories of in vitro–in vivo correlations. In brief, the four categories, ranked in order of usefulness from highest to lowest, are as follows:

Level A: A one-to-one relationship between in vitro and in vivo dissolution profiles in which the two curves are superimposable. Since in vivo dissolution is not directly measured in the bioavailability study, it must be

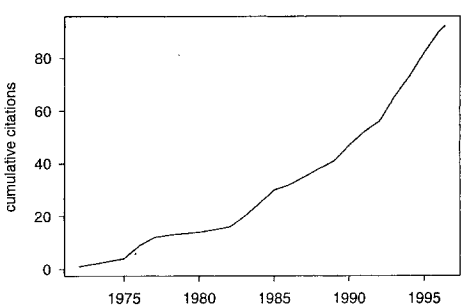

Fig. 2. Cumulative count of MEDLINE citations containing the keyword phrase "in vitro–in vivo correlation" by year.

estimated from the bioavailability profile. They suggest that this be achieved by deconvolution analysis.

Level B: A correlation between the mean in vitro dissolution time and the mean in vivo residence time.

Level C: A correlation between a single in vitro dissolution time (e.g., $t_{50\%}$, the time at which 50% of the drug product has dissolved in vitro) and a single bioavailability parameter such as AUC (area under the bioavailability profile curve), C_{max} (maximum of the bioavailability profile curve), or T_{max} (time to reach C_{max}).

Level D: A qualitative in vitro–in vivo relationship.

The FDA Guidance for Industry extends the definition of the Level A correlation in several ways. First, they allow for a more general point-to-point relationship between in vitro and in vivo dissolution profiles which could be nonlinear. They also propose an equivalent alternative to the Level A correlation which models the relationship between the in vitro dissolution profile and the bioavailability profile instead of the in vivo dissolution profile.

From a statistical point of view, the use of the word "correlation" in the term in vitro–in vivo correlation is somewhat unfortunate because its technical definition is much more restrictive than its common meaning of association or relationship. The problem is compounded because in many published reports the strength of the evidence supporting a valid in vitro–in vivo relationship is quantified by the Pearson correlation coefficient, which seems to be what the USP Subcommittee on Biopharmaceutics had in mind for the Level A correlation. Several authors have been sensitive to this distinction as exemplified by Fairweather (1977): "we employ the term "association" in this context and reserve the use of "correlation" for its technical, statistical meaning". However, this is the exception rather than the rule, as a MEDLINE search on the keyword phrase "in vitro–in vivo relationship" identified only six citations. Most authors of methodological papers use the terms in vitro–in vivo correlation and in vitro–in vivo relationship interchangeably. The statistical literature is seemingly void of applied or methodological work in the area of in vitro–in vivo relationships for modified release drug products, as a search of the Current Index to Statistics failed to find any citations. This is not to say that no statistical methodological work has been done. Rather that it does not appear in statistical journals. Much of the recent methodological work cited in this paper is found in volume 423 of the series *Advances in Experimental Medicine and Biology* edited by Young, Devane and Butler (1997). In the remainder of this paper we shall attempt to provide an overview of some of the methods for quantifying in vitro–in vivo relationships that have been proposed over the past 25 years. In Section 2 we introduce data from an in vitro–in vivo study and discuss some issues related to pharmacokinetic modeling of bioavailability data which impact in vitro–in vivo relationship analyses. In Section 3 we present methods for quantifying in vitro–in vivo relationships and illustrate some of them via the example data of Section 2. We conclude with some general comments and thoughts on areas for future statistical methodological research.

2. In vitro–in vivo studies

In vitro–in vivo studies are a combination of in vitro dissolution studies and bioavailability studies. Typically they involve are least three different formulations of a drug product. The purpose is to evaluate the utility of in vitro dissolution for predicting the bioavailability of the drug product under study. Cardot and Beyssac (1993) give a very nice discussion of the scientific issues which arise with in vitro–in vivo studies for modified release drug products. They discuss the limitations of such studies as well as some of the requirements for initiating in vitro–in vivo studies. Hüttenrauch and Speiser (1985) and Devane (1997) provide insight on the role of in vitro–in vivo studies in drug research and development, and Malinowski (1997) discusses the perspective of regulatory agencies.

In an in vitro dissolution study, the dosage form is dissolved in an apparatus designed to simulate in vivo dissolution conditions and the fraction dissolved is measured at various time points. Such studies typically involve 10–20 replicates with measurements taken at 5–10 time points. Figure 3 shows the in vitro dissolution profiles obtained from a study using 12 tablets each of four different formulations (part of this data appeared in Figure 1). The most notable feature of this dataset is that the inter-tablet variability of the dissolution profiles increases markedly for the more quickly dissolving formulations.

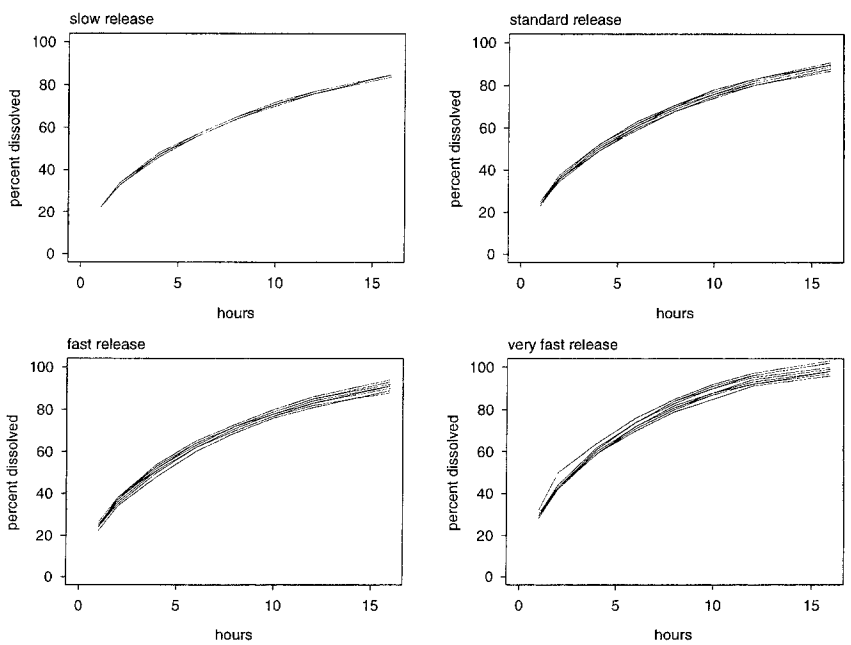

Fig. 3. In vitro dissolution profiles of four formulations of an oral extended release drug product; each panel contains data from twelve tablets.

In a bioavailability study, the dosage form is administered to human subjects and the plasma concentration of the drug is estimated by assay from blood samples collected at various time points. These studies typically involve 10–20 subjects with blood samples taken at 10–20 time points, often over a much longer time period than dissolution studies to account for delayed appearance due to absorption and so that the decrease in drug concentration due to elimination can be observed. Cross-over designs are often used when multiple formulations of the drug are under study. In addition, oral solution or intravenous bolus administration of the drug may be included as an extra study period to facilitate estimation of absorption and elimination kinetics.

Figure 4 shows the plasma concentration profiles from a bioavailability study with 20 subjects in a four-period cross-over design. The formulations used here are the same as those used in the in vitro dissolution study above. The bioavailability profiles of the four formulations do not appear to be very different, especially relative to the magnitude of the inter-subject variation. A comparison of the panels corresponding to the slow and very fast formulations reveals that the primary difference between the formulations, with respect to bioavailability, is in the height of the plasma concentration profiles rather than the width. It is worth noting that the inter-subject variability is much larger than the inter-tablet variability of the in vitro study (Figure 3).

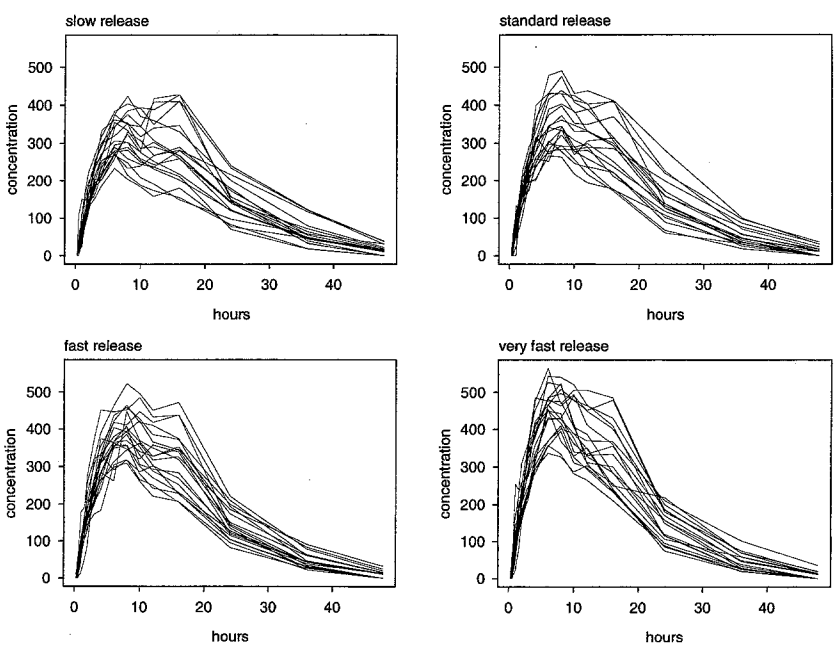

Fig. 4. Plasma concentration profiles of four formulations of an oral extended release drug product; each panel contains data from twenty subjects in a four period cross-over design.

Since bioavailability depends on other processes in addition to in vivo dissolution (i.e., permeation, elimination), there is no hope of relating in vitro dissolution to bioavailability without accounting for these other processes, even if in vitro dissolution mimics in vivo dissolution perfectly. This fact is recognized in all of the proposed methods for determining in vitro–in vivo correlations. The methods differ primarily in the way in which they account for these other processes. Understanding how this can be accomplished requires a knowledge of pharmacokinetics. Lee and Amidon (1996) define the pharmacokinetics of drugs as a combination of physiological compartments and pharmacokinetic processes. The compartments are spaces (stomach, blood, tissue) or states (metabolites) in which a drug may reside. The action of a pharmacokinetic process is to move a drug from one compartment to another. We do not provide an review of pharmacokinetic analysis, but direct the interested reader to one of several texts on the subject: Wagner (1971), Gibaldi and Perrier (1982), Notari (1987), Shargel and Yu (1993), Amidon and Lee (1996).

For the purpose of determining in vitro–in vivo relationships, the most important physiological compartments are the two spaces comprising the gastrointestinal tract and the bloodstream and the two states of the drug product, undissolved and dissolved. The most important pharmacokinetic processes are in vivo dissolution from the undissolved to the dissolved state, permeation from the gastrointestinal tract into the bloodstream, and elimination from the bloodstream. If in vitro dissolution mimics in vivo dissolution and permeation and elimination can be accounted for, then there is hope that a relationship between in vitro dissolution and bioavailability may be found. All of the proposed methods for determining in vitro–in vivo relationships can be classified into one of two approaches.

The first approach can be roughly stated as follows: given the observed bioavailability profile of a drug product, first estimate the unobserved in vivo dissolution profile based on assumptions about the permeation and elimination processes, then develop a model of the relationship between the observed in vitro dissolution profile and the estimated in vivo dissolution profile. If this model is valid, then it may be applied to predict in vivo dissolution, and hence bioavailability, from in vitro dissolution profile. This is also sometimes called the deconvolution approach because the technique used to estimate the unobserved in vivo dissolution profile from the observed bioavailability profile is based on the mathematical process of deconvolution. The second approach is sometimes called the convolution approach and attempts to model bioavailability from in vitro dissolution directly, in one step. This generally requires more formal assumptions about the relationship between in vitro and in vivo dissolutions well as the permeation and elimination processes.

From a theoretical biological point of view, the two approaches are identical in that either can be used to fit the same analytical model. From a statistical point of view, the difference between the two approaches depends on the complexity of the processes being modeled. In the unrealistically simple case where in vitro dissolution mimics in vivo dissolution perfectly, permeation is instantaneous,

elimination is described by first-order kinetics and variability is low, the two approaches are likely to give very similar results in terms of statistical inference. As complexity increase, the differences between the two approaches become more pronounced. From a practical point of view, the utility of the approaches depends on the goal of the study. If the primary goal is to validate the in vitro dissolution model, the deconvolution approach addresses that issue directly. Such a situation could occur if it is desirable to compare several competing in vitro dissolution models (e.g., with different pH levels or amounts of agitation). If the goal is to both validate the in vitro dissolution model and determine how the in vitro dissolution profile can be used to predict bioavailability, the convolution approach addresses that issue more directly.

2.1. Modeling in vitro dissolution data

Some of the methods that have been proposed for determining in vitro–in vivo correlations require the assumption of a particular functional form for the in vitro dissolution profile (Langenbucher, 1983; Drewe and Guitard, 1993; Ishii et al., 1996). Several families of functions are mentioned in the FDA Guidance for Industry, but there are probably other reasonable choices. One family is described by the Hill equation, which states that

$$M(t) = \frac{t^{\beta}}{D_{50}^{\beta} + t^{\beta}} \tag{1}$$

where $M(t)$ is the fraction dissolved at time t, D_{50} is the time at which 50% of the drug is dissolved and β is a shape parameter. When $\beta = 1$, this simplifies to the well known Michaelis–Menten equation. Another family is based on differential equations modeling which leads to functional forms that are sums of exponentials. The most simple of these is based on first-order dissolution for which

$$M(t) = 1 - \mathrm{e}^{-\delta t} \tag{2}$$

where δ, the dissolution rate parameter, is inversely proportional to the dissolution half-life of the drug product. A third family is the well-known Weibull function

$$M(t) = 1 - \mathrm{e}^{-t^{\beta}/\alpha} \tag{3}$$

where α is the dissolution rate parameter and β is a shape parameter. When $\beta = 1$ this is equivalent to (Eq. (2)). $1 - M(t)$ can be viewed as the survival function of the drug product (i.e., the fraction of drug not yet dissolved at time t). In general, any survival function is a candidate for modeling the in vitro dissolution profile. Figure 5 shows the results of fitting the first-order dissolution curve (Eq. (2)) to the in vitro dissolution profiles in Figure 3. Other authors have proposed nonparametric or semiparametric estimation of the in vitro dissolution profile, (Verotta, 1997; Gillespie, 1997). The most simple nonparametric estimation is by linear interpolation. Verotta and Gillespie both also propose semiparametric estimation using smoothing splines.

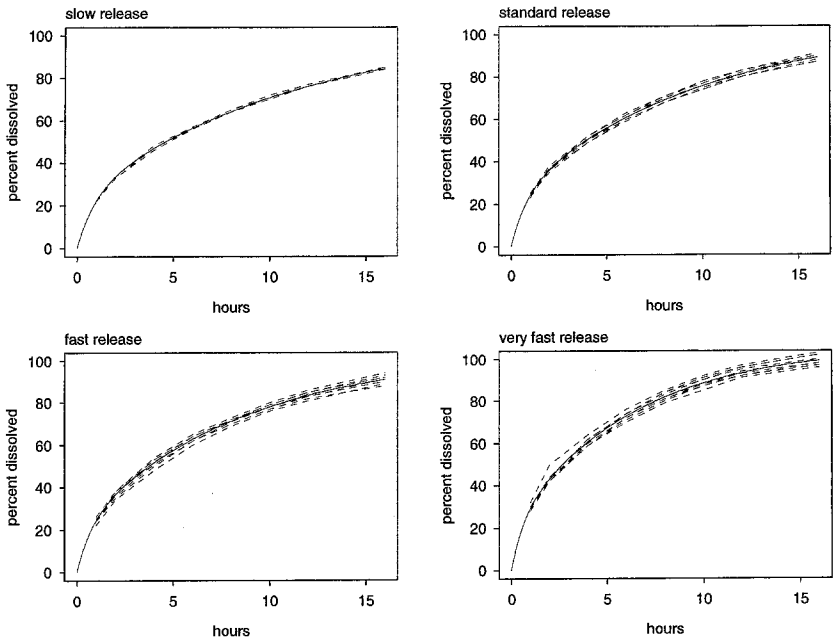

Fig. 5. Results of fitting first-order dissolution curves to in vitro dissolution profiles of four formulations of an oral extended release drug product.

Sometimes (e.g., Level B in vitro–in vivo correlation) it is of interest to calculate the mean in vitro dissolution time. It is reasonable to view $M(t)$ as a cumulative distribution function in the statistical sense. That is, the proportion of drug product dissolved at time t can be thought of as the probability that an individual molecule of the dosage form will dissolve by time t. The mean dissolution time (MDT), the "expected" time for an individual molecule to dissolve, is given by

$$\text{MDT} = \int_0^\infty (1 - M(t))\mathrm{d}t \ . \tag{4}$$

For example, the mean dissolution time of a dosage form with first-order dissolution kinetics (Eq. (2)) is δ^{-1}, the inverse of the dissolution rate. In practice, the mean dissolution time can be estimated either by fitting an assumed functional form or empirically via approximate numerical integration of Eq. (4).

2.2. Modeling bioavailability data

The strategy used for modeling bioavailability data depends on which approach is invoked for finding an in vitro–in vivo relationship. However, all are based on the idea of convolution and its mathematical inverse, deconvolution. Much has been written on the subject of convolution and deconvolution, as well as the pitfalls

associated with algorithms designed to carry out the processes numerically. We shall not spend time reviewing that work here except to give a general overview of the basic ideas. Briefly, in pharmacokinetic modeling the convolution integral is used to describe the combined effects of absorption and elimination on plasma drug concentration. The relationship is generally described as

$$C(t) = \int_0^t W(t-z)I(z)\mathrm{d}z \tag{5}$$

where $C(t)$ is plasma drug concentration at time t, W describes the response of the system to an instantaneous input of drug and I describes the input of drug into the system (Madden et al., 1996). C, W and I are often called the response, weighting and input functions respectively. Various interpretations can be given to the functions W and I. If W describes the theoretical response of the system to an intravenous injection of drug, then I represents the rate of entry of drug into the bloodstream (i.e., dissolution and permeation). On the other hand, if W describes the response of the system to an oral administration of drug in solution, then I represents the rate of entry of dissolved drug into the gastro-intestinal tract (i.e., dissolution). In this case, the process of permeation is included in the response function W. Given W and I, the process of evaluating this integral (Eq. (5)) is called convolution. Given C and either W or I, the unknown function can be calculated by deconvolution. These can be performed either numerically or analytically. An implicit assumption of this model is that of a linear system, i.e., the relevant pharmacokinetics can be mathematically described by a system of linear differential equations.

2.2.1. Deconvolution

Deconvolution analysis is the basis for the first approach to developing in vitro–in vivo relationships. Under this approach the in vivo dissolution (or absorption) profile is estimated using deconvolution (Wagner, 1971; Brockmeier et al., 1985; Polli et al., 1996; Butler, 1997; Rackley, 1997). That is, based on the observed bioavailability profile, C and information or assumptions about W, estimate I and then develop a relationship between this estimate and the observed in vitro dissolution profile. A completely analytic approach based on standard compartmental modeling considerations leads to functional forms for W and I which are linear combinations of exponentials. Nonlinear regression algorithms may be applied to estimate the parameters in these types of models (Langenbucher, 1983).

A somewhat less restrictive approach is to assume a functional form for W only, and estimate I numerically. For some cases of these semi-analytic models, closed-form estimates of I at each observed plasma concentration time point are available. The well-known Wagner–Nelson (1964) and Loo–Riegelman (1968) methods are two such estimators. If W is observed as well C, then there are methods for calculating model-free estimates of I (Langenbucher, 1982, 1983). This occurs if an intravenous bolus or oral solution of drug is given and the corresponding plasma concentration profile is measured as part of the bioavailability study. In this case, a two stage procedure is used in which W is estimated at

the first stage by deconvolution of the observed C (corresponding plasma concentration profile) and known I (intravenous bolus has known input function), this estimate of W is then used in the second stage to calculate the deconvolution estimate of I for the plasma concentration profile corresponding to the experimental drug product formulation.

The right hand panel of Figure 6 shows the results of applying the Wagner–Nelson method to the bioavailability data (left panel) in Figure 1. This is based on a one-compartment model of elimination kinetics, the parameters of which were estimated externally based on data from an intravenous bolus of drug. Therefore, the estimated input function I represents input of drug into the bloodstream and includes both in vivo dissolution and permeation (i.e., absorption). If an oral solution had been used instead of an intravenous bolus, the estimated input function would represent input into the gastrointestinal tract (i.e., in vivo dissolution). When used for calculating an in vitro–in vivo correlation, it is customary to estimate the percent absorbed only over the time frame of the corresponding in vitro dissolution study. However, it is possible to estimate the in vivo absorption profile over the entire plasma concentration profile.

The estimated absorption profiles reflect the percent of the nominal dose absorbed across time. There is no way of knowing what fraction of the drug product actually administered has been absorbed. Therefore, we can only speculate as to why the absorption profile for the slow formulation reaches only 50% after 16 h. One possibility is that the tablet administered during this particular period in fact contained less than the nominal dose, but that it was completely absorbed. Another possibility is that the tablet dissolved so slowly that it was only 50% dissolved after 16 h. A third possibility is that the true elimination kinetics for this particular period were faster than when the intravenous bolus was given. If that were the case, it could appear that less than the nominal dose of drug had been absorbed because it was removed more quickly than expected.

Figure 7 shows the estimated in vivo absorption profiles for all the subjects in the bioavailability study (Figure 4). It is difficult to judge how much of the intersubject variation in the plasma concentration profiles has been removed by this

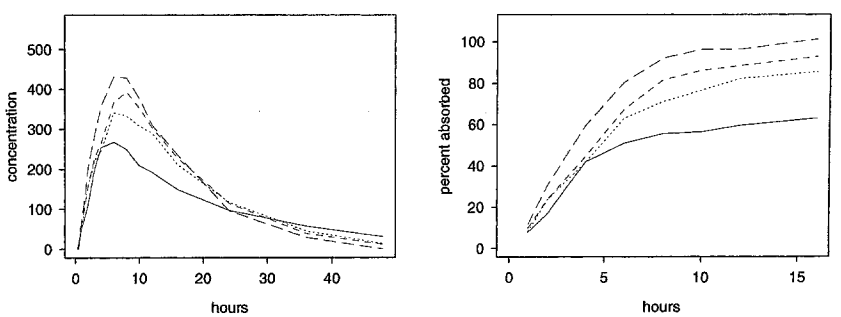

Fig. 6. Example bioavailability and estimated in vivo absorption profiles for four formulations of a modified release drug product.

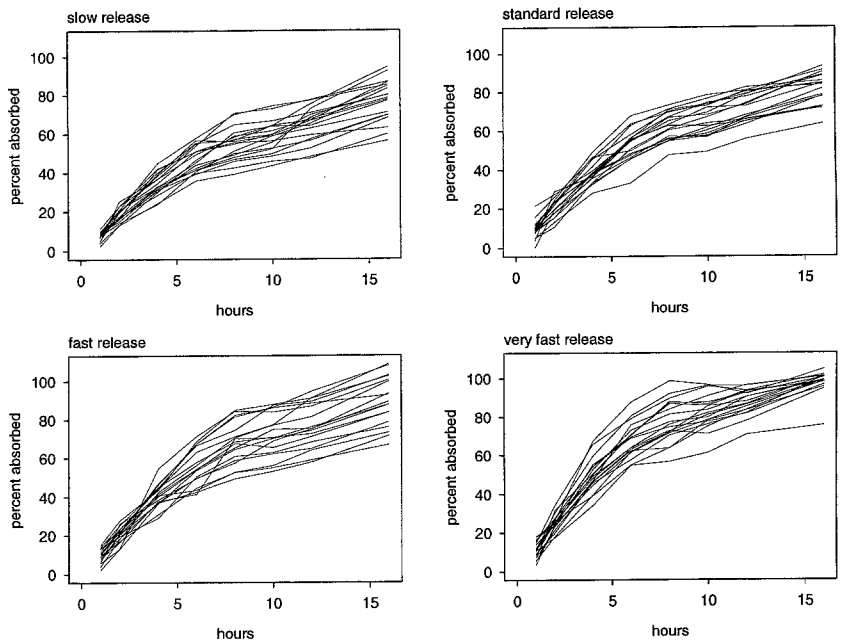

Fig. 7. Estimated in vivo absorption profiles of four formulations of an oral extended release drug product; each panel contains data from twenty subjects in a four period cross-over design.

standardization, but there is still noticeable inter-subject variation among the estimated in vivo absorption profiles. We can conclude from this that at least part of the inter-subject variation in plasma concentration is due to variation in the in vivo dissolution and/or permeation processes, and that not all of it is due to variation in elimination kinetics.

2.2.2. Convolution

Convolution is the basis for the second approach to developing in vitro–in vivo relationships. Under this approach the observed bioavailability profile is modeled from the observed in vivo dissolution profile using convolution (Vaughn and Leach, 1976; Langenbucher, 1982, 1983; Gillespie, 1997; Mauger and Chinchilli, 1997; Verotta, 1997). Such a model requires information or assumptions about the functional forms of W and I, and the relationship between in vitro dissolution and I. As with deconvolution, one can take a completely parametric approach (Langenbucher, 1983; Gillespie, 1997; Mauger and Chinchilli, 1997), a semiparametric approach (Langenbucher, 1982, 1983; Verotta, 1997) or a model-free approach (Vaughn and Leach, 1976; Langenbucher, 1983). An advantage of the completely analytic approach is that it does not require external information about W.

Figure 8 shows the results of fitting a convolution model to the bioavailability data in Figure 1. In this case, a one-compartment model with first-order per-

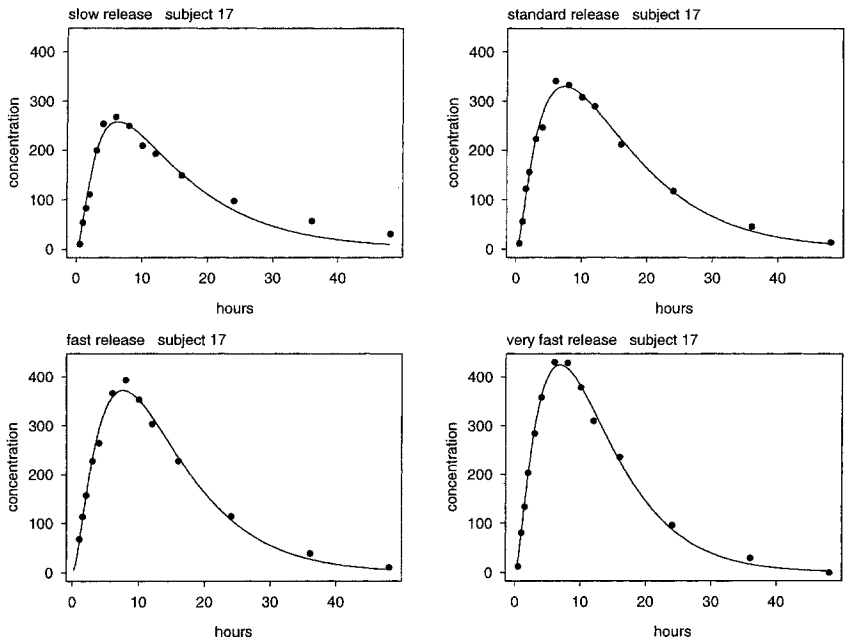

Fig. 8. Example of convolution model fitted to in vivo bioavailability profiles for four formulations of a modified release drug product.

meation was assumed. It was also assumed that the in vitro dissolution profile was equivalent to the unobserved in vivo dissolution profile. Since the in vitro dissolution profile of the particular tablet used in the in vivo study cannot be observed, the convolution model was based on the in vitro dissolution profile of an average tablet as depicted in Figure 5. Given the relative magnitudes of the inter-subject and inter-tablet variation in these studies, it is likely that inter-tablet variation is a negligible component of inter-subject variation.

This model fits the observed bioavailability profiles very well. However, it would be misleading to use this model to validate the relationship because it is not sensible to validate a model with the same data that were used to estimate the model in the first place. A potential solution would be to use cross-validation to obtain a less biased estimate of the true prediction error of the model. Figure 9 shows the results of fitting the same model in a leave-one-out fashion. That is, the parameters of the W function used for calculating the predicted bioavailability profile for the slow formulation were estimated using only the bioavailability profiles of the other three formulations, not the slow formulation. This model does not fit as well as the previous model, indicating that Figure 8 gave a biased view of the true prediction error. Interestingly, the slow formulation is noticeably overpredicted. This is consistent with our observation that the deconvolution based estimate of the in vivo absorption profile was lower than the in vitro dissolution profile and illustrates the point that a thorough analysis of an

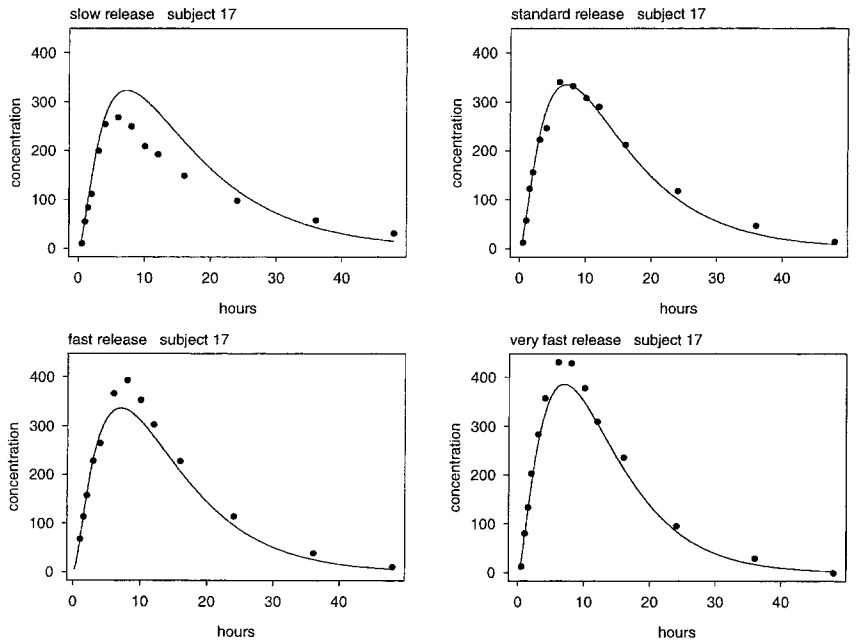

Fig. 9. Example of cross-validation convolution model fitted to in vivo bioavailability profiles for four formulations of a modified release drug product.

in vitro–in vivo study may include both the convolution and deconvolution approaches.

Convolution and deconvolution based methods have drawbacks, beginning with the fact that numerical instabilities can occur with either of them. Particularly when fitting models based on sums of exponentials. For the model-based approaches, overfitting can be a serious problem. The semiparametric and model-free methods require external estimates of the elimination kinetics and their accuracy depends on the validity of these estimates for all periods in the cross-over study. The difficulty is that it is not possible to assess whether or not they are in fact valid. Several papers have appeared in the pharmacology literature related to these issues in the context of pharmacokinetic analysis and in vitro–in vivo relationships (Cutler, 1981; Chan et al., 1986; Süverkrüp et al., 1988; Liu et al., 1995; Purves, 1996; Madden et al., 1996). In the next section we will investigate how these methodologies can be used to develop in vitro–in vivo relationships.

3. Methods of quantifying in vitro–in vivo relationships

There is still much work needed in the area of statistical inference for in vitro–in vivo relationships. The majority of the methodological work reported in the pharmacology literature is limited to looking at relationships graphically and

quantitative evaluation using the Pearson correlation coefficient. There are limitations to this approach, not least of which is that it does not provide a basis for making a decision as to whether or not a particular in vitro–in vivo relationship is sufficiently strong to warrant use of the in vitro dissolution test as a surrogate for bioavailability. The different levels of in vitro–in vivo correlations described by the USP Subcommittee on Biopharmaceutics (1988) provide a reasonable starting point for discussion of the methods for quantifying in vitro–in vivo relationships. Methods for quantifying in vitro–in vivo relationships based on relating the entire in vitro dissolution profile with the entire bioavailability profile provide the strongest evidence of an in vitro–in vivo relationship (Malinowski et al., 1997). We distinguish between methods which relate (1) in vitro dissolution with in vivo dissolution as estimated by deconvolution of the bioavailability profile and (2) in vitro dissolution with bioavailability. They require distinctly different methodologies, although they are based on the same underlying theoretical model. They each have drawbacks and advantages; however, the former appears to be much more widely used in the pharmacology literature.

3.1. Relationships between dissolution profiles

Level A correlations, as described by the USP Subcommittee on Biopharmaceutics, typically follow the first approach outlined in the previous section and relate observed in vitro dissolution with estimated in vivo dissolution across time. Some authors appear to use the terms in vivo dissolution and in vivo absorption interchangeably, although the general use of the term absorption refers to the combined processes of dissolution and permeation, the two processes which determine the rate of appearance in the bloodstream. These two are equivalent only when permeation is instantaneous. In the previous section we discussed methods for estimating both in vivo dissolution and absorption from the observed bioavailability profile. Figure 10 shows the estimated in vivo absorption profiles (solid lines) from Figure 6 overlaid with mean in vitro dissolution profiles (dashed lines) from Figure 5. In vivo absorption lags behind in vitro dissolution for the first few hours with all four formulations, but eventually catches up with the exception of the slow release formulation.

If, as it appears in our example, the estimated in vivo absorption profile is similar to the in vitro dissolution profile, it may be reasonable to look for direct relationships between the two. Wagner (1971) was one of the earliest authors to propose calculating the correlation between the profiles. If the time points of the in vitro and in vivo studies are coincident, then the profiles can be paired by time and plotted against each other. Figure 11 shows this plot for the example data. One notable feature of this plot is that the lines corresponding to the three faster releasing formulations are nearly coincident, which may be evidence that the relationship is fairly consistent across these three formulations. If the time points are not coincident, the plot can be constructed by interpolating one of the curves. Wagner suggested summarizing the strength of the in vitro–in vivo relationship with the Pearson correlation coefficient. The Pearson correlation coefficient for

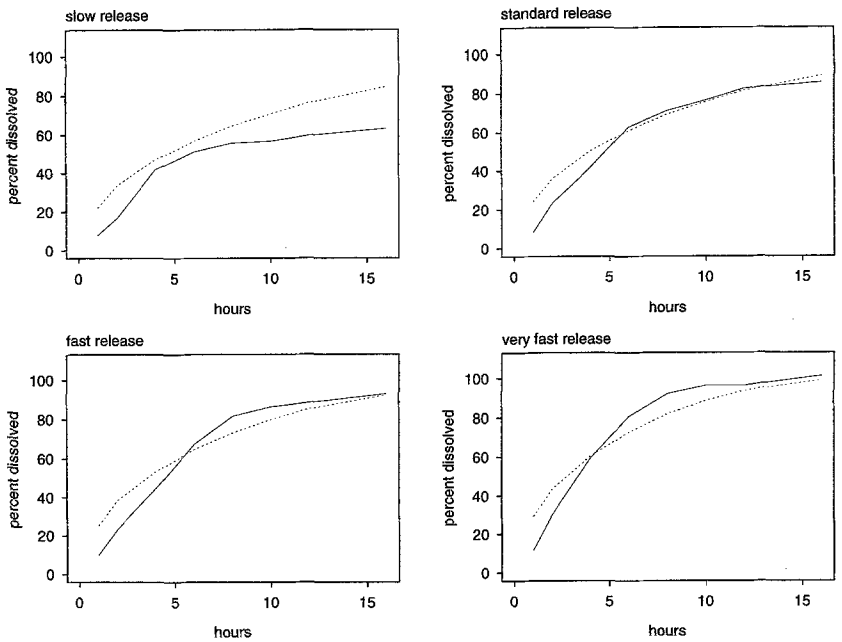

Fig. 10. In vitro dissolution and estimated in vivo absorption profiles for four formulations of a modified release drug product.

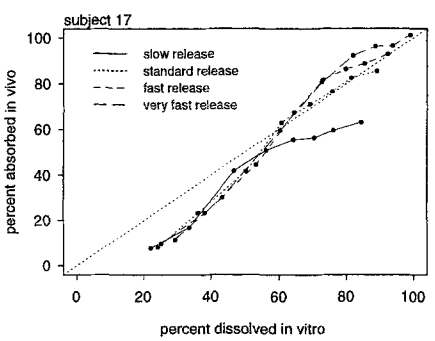

Fig. 11. Estimated in vivo absorption versus mean in vitro dissolution for four formulations of a modified release drug product.

each formulation in this example is greater than 0.96. A review of recent reports of in vitro–in vivo relationships (randomly selected from a MEDLINE search) reveals that this is a popular approach (e.g., Mojaverian et al., 1997).

Is the correlation coefficient a reasonable summary statistic? It does seem like a reasonable measure by which to compare two or more competing dissolution tests, but does it have any absolute meaning? Certainly it is not prudent to evaluate the strength of the relationship based on the common benchmarks for

correlations in every day use, since lack of in vitro–in vivo relationship is not equivalent to no correlation. In fact, the correlation coefficient can be reasonably large even when the in vitro–in vivo relationship is poor. This is because the in vitro dissolution and in vivo absorption (or dissolution) profiles are both zero at time zero and greater than zero at some time later (unless, of course, the dosage form does not dissolve or is not absorbed, but in this case it is unlikely that one would be trying to develop an in vitro–in vivo relationship). Consequently, the correlation-associated p-values typically reported in these studies are of little value. Fairweather (1977) and Liu et al. (1996) recognized this problem, and Dunne et al. (1997) performed a simulation study to investigate potential problems with this methodology. This remains an open question, and a number of papers discussing scientific and regulatory issues of these types of in vitro–in vivo relationships have appeared over the past ten years (USP Subcommitte on Biopharmaceutics, 1988; Skelly et al., 1990; Siewert, 1993; Siewert et al., 1993; Cardot and Beyssac, 1993). In addition to Dunne et al. (1997) Mendell-Harary et al. (1997) and Bigora et al. (1997) have investigated nonlinear in vitro–in vivo correlations relating the observed in vivo dissolution profile to the estimated in vivo dissolution profile.

In general, bioavailability studies involve a dozen or more subjects. It is common therefore, to correlate the mean estimated in vivo absorption (or dissolution) profile with the mean in vitro dissolution profile. Figure 12 shows the plot for our entire example data set. The notable features of this plot are that the lines are all fairly straight and coincident, but they all lie slightly below the unity line and have slopes greater than one, indicating either that in vitro dissolution is faster that in vivo dissolution, or that permeation is not instantaneous. The apparent difference with the slow release formulation noted in Figure 11 does not appear here, indicating a probable subject-specific phenomenon. This plot seems to indicate even more strongly that the in vitro–in vivo relationship is consistent across these four formulations. The Pearson correlation coefficient for each formulation in this figure is greater than 0.997, it is clear why it is tempting to report this statistic. The in vitro–in vivo relationship looks extremely good by this

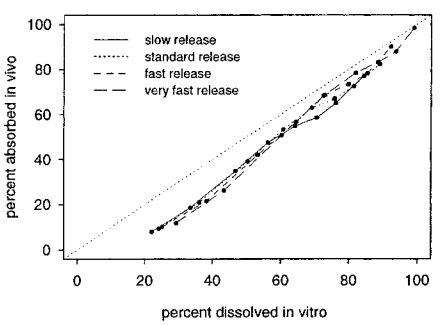

Fig. 12. Mean estimated in vivo absorption versus mean in vitro dissolution of four formulations of an oral extended release drug product.

analysis, even without the correlation coefficient. On the other hand, Figure 13 shows the same relationship for each individual separately. We see that there is a large amount of variation in the in vitro–in vivo relationship.

From a statistical modeling point of view, there are problems with this sort of analysis. Perhaps most troublesome is that there is no straightforward way to assess the effects of misspecification of the deconvolution model. That is, it is not possible to evaluate whether or not the estimated in vivo absorption profile is in any way reflective of the true in vivo absorption profile. One could attempt a sensitivity analysis in which the results of different deconvolution techniques are compared, but is there any reason to believe that the correct deconvolution model is the one that leads to superimposability of the in vitro dissolution and in vivo absorption? Polli et al. (1996) provide a theoretical argument that, in general, one should not expect the in vitro dissolution and in vivo absorption profiles to be superimposable because the permeation process is, in general, not instantaneous. One objective of their paper was to explain the apparent lack of in vitro–in vivo relationships for drugs which have permeation-rate-limited absorption. Ishii et al. (1995) and Hayashi et al. (1995) discuss methods to account for other processes affecting gastrointestinal transit and their impact on in vitro–in vivo relationships.

A slightly different approach to finding relationships between dissolution profiles is to plot in vivo dissolution (or absorption) times versus in vitro dissolution times. The resulting profile would represent the time required for in vivo

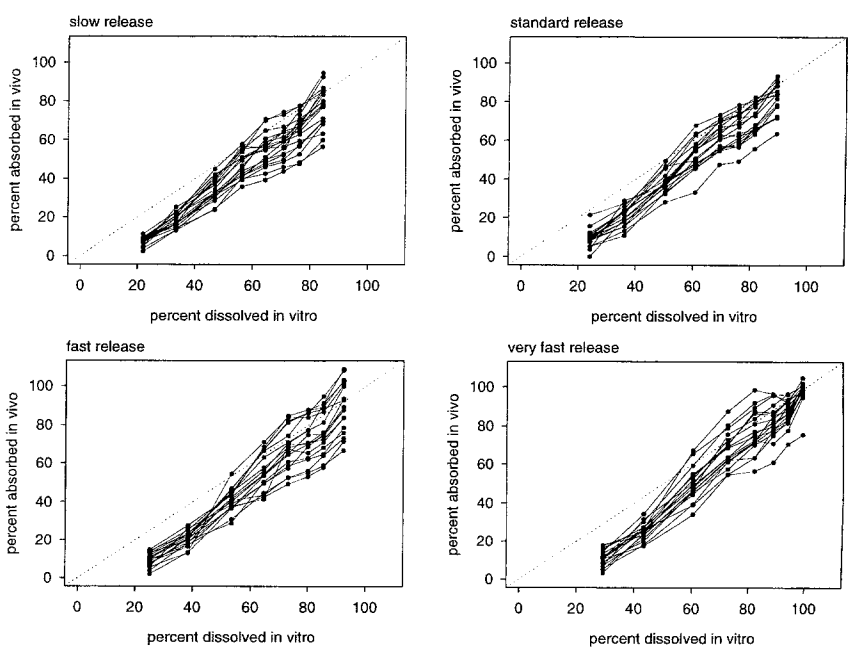

Fig. 13. Estimated in vivo absorption versus mean in vitro dissolution of four formulations of an oral extended release drug product.

dissolution relative to in vivo dissolution. This plot is typically more complicated to generate, because it involves interpolation of either the in vitro dissolution or in vivo absorption profile. It does allow a visual assessment of the relative dissolution times, which may be interesting in itself. Brockmeier et al. (1982, 1983, 1984) wrote a series of papers on time scaling of dissolution profiles in relation to in vitro–in vivo correlations.

3.2. Comparing observed and predicted bioavailability profiles

The other approach to developing profile based (Level A) in vitro–in vivo relationships is to relate in vitro dissolution directly with bioavailability (Vaugh and Leach, 1976; Langenbucher, 1982; Brockmeier et al., 1985; Gillespie, 1997; Mauger and Chinchilli, 1997; Verotta, 1997). As discussed in the previous section, much work has been developed on methods for selecting and fitting convolution models, but relatively little has been developed on methods for evaluating relationships. Young et al. (1997) provide a nice discussion of the important issues that must be addressed when validating an in vitro–in vivo relationship, and make some informal suggestions about possible statistical criteria for evaluating validity. It seems clear that an appropriate evaluation of convolution based models will compare the observed and predicted bioavailability profiles. The FDA Guidance for Industry (Malinowski et al., 1997) suggests using estimates of prediction error. They recommend prediction error as a "method for evaluation of predictability" and distinguish between internal predictability, the ability of the model to predict bioavailability for the data from which the model was estimated, and external predictability, the ability of the model to predict bioavailability from a different bioavailability study. However, they stop short of giving any specific guidelines as to what level of prediction error constitutes an acceptable in vitro–in vivo relationship. "Methodology for the evaluation of IVIVC predictability is an active area of investigation and a variety of methods are possible and potentially acceptable. Therefore, definitive recommendations regarding methods and criteria cannot be made at this time. Ideally, it is wished to determine that a correlation is accurately and consistently predictive of in vivo performance. Once this goal has been achieved, in vitro dissolution could be used confidently as a surrogate for in vivo bioavailability of extended release drug products."

Choice of prediction error metric will be critical. To illustrate this point, we calculated two potential measures of prediction error: relative error (RE)

$$\mathrm{RE} = \frac{\text{observed value} - \text{predicted value}}{\text{predicted value}} \tag{6}$$

and absolute relative error (ARE)

$$\mathrm{ARE} = \left| \frac{\text{observed value} - \text{predicted value}}{\text{predicted value}} \right| . \tag{7}$$

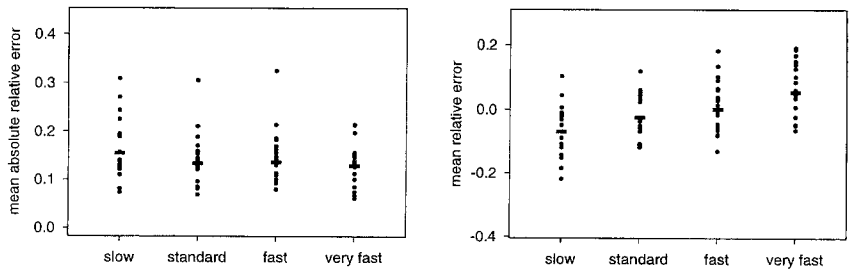

Fig. 14. Prediction error of convolution-based model for four formulations of a modified release drug product.

We calculated both the mean relative error and the mean absolute relative error (across time) between the predicted and observed bioavailability profiles (9) for each formulation, within each subject separately. This can be viewed as a cross-validation type estimate of prediction error since the models were fit in a leave-one-out fashion. Figure 14 shows the results of this analysis for the example data. The average (across subjects) mean relative error is less than 10% for all four formulations. However, the average mean absolute relative error is greater than 10% for all four formulations. There are many other metrics that could potentially be used (e.g., root mean square prediction error) and methods other than cross-validation for estimating them (e.g., bootstrap). The FDA has indicated that definitive recommendations cannot be made at this time, but it would seem reasonable to expect that the criteria eventually adopted will be consistent with the current criteria for demonstrating bioequivalence between two different drug formulations. In the case of validating an in vitro–in vivo relationship, one potential goal would be to demonstrate bioequivalence between the observed bioavailability profile and the predicted bioavailability profile based on the in vitro–in vivo relationship.

4. Discussion

Although there are many potential methods for developing in vitro–in vivo relationships, there seems to be general consensus that something equivalent to the USP Level A correlation is the gold standard and that Level B/C correlations should be considered as fall-back positions (USP Subcommittee on Biopharmaceutics, 1988; Siewert et al., 1993; Cardot and Beyssac, 1993; Malinowski et al., 1997). However, it is not clear how to interpret an in vitro–in vivo study which produces a strong Level B/C correlation, but no Level A correlation. It depends on what is meant by "produces no Level A correlation". If it means that no Level A correlation is found because the data are of insufficient quality (e.g., too noisy or too sparse), then it may be reasonable to make inference from a Level B/C correlation. On the other hand, if the data indicate that there is no valid Level A correlation (i.e., in vitro dissolution does not mimic in vivo dissolution),

then it seems likely that a strong Level B/C correlation would be a spurious result. That is, evidence of a mathematical relationship between single parameters of in vitro dissolution and bioavailability cannot be taken as evidence of a mathematical relationship between the in vitro dissolution profile and (some transformation of) the bioavailability profile. This is not to say that lower level correlations are of no value, because they may provide evidence for a relationship between some aspects of in vitro dissolution and bioavailability, but they must be critically evaluated. Consideration must also be given as to the precise goal of the in vitro–in vivo relationship.

A general statement of purpose for in vitro–in vivo studies is to define a direct relationship between in vitro and in vivo data such that measurement of in vitro dissolution alone is sufficient to determine the biopharmaceutical fate of the dosage form (Cardot and Beyssac (1993)). The term "biopharmaceutical fate" could be taken to mean bioavailability profile, C_{max}, mean residence time, area under the bioavailability profile curve (AUC), etc. If the outcome of interest is AUC, then either a Level A correlation or a Level B/C correlation framed in terms of AUC would be reasonable. On the other hand, if the outcome of interest is the bioavailability profile, then only a Level A correlation is sufficient. If the outcome of interest is to be chosen based on exploratory data analysis, then an external validation study is mandated. An alternative is to evaluate biopharmaceutical fate in terms of more than one parameter, e.g., C_{max} and AUC. This is analogous to the approach commonly taken in bioequivalence trials where the goal is to demonstrate that two different drug product formulations have equivalent biopharmaceutical fates.

The purpose statement is also unclear as to whether the goal is to "determine the biopharmaceutic fate of the dosage form" in an individual, or on average across a population of individuals. This issue is an ongoing debate in bioequivalence testing. At this point the FDA relies on average bioequivalence (Patnaik et al., 1997). There are practical reasons for considering average rather than individual data. It allows for incorporating data from an in vitro–in vivo study of a new formulation of the drug product which was not available at the time of the original study. In addition, external validation of the in vitro–in vivo relationship, by definition, can only be done in terms of the relationship between averages. The primary advantage of in vitro–in vivo relationships based on individual data is that it permits direct assessment of inter-subject variability. It seems that inter-subject variation should play some role in the assessment of in vitro–in vivo relationships. If two separate in vitro–in vivo studies of the same drug product yield the same in vitro–in vivo relationship based on average data, but have different amounts of inter-subject variability, it would seem reasonable to give more weight to the one with less variability.

There are advantages to both of the approaches for developing profile based in vitro–in vivo relationships that we have discussed. The primary advantages of a deconvolution based method which relates in vitro dissolution with in vivo dissolution are that it allows for a direct visual assessment of the degree to which in vitro dissolution mimics in vivo dissolution, and that it is easier to carry out

when no assumptions are made about the functional forms of the dissolution, permeation, and elimination processes. The primary advantages of the convolution based method for predicting bioavailability directly are that the bioavailability profile is generally the relevant biopharmaceutical fate, the statistical issues involved in developing an in vitro–in vivo relationship based on individual data are more easily addressed, and that the validity of the model can be evaluated directly with respect to the observed data rather than a complicated nonlinear transformation thereof (deconvolution). The convolution based method requires development of the pharmacokinetic model that is used. In contrast, there are a number software packages available for performing deconvolution with relatively little input from the user required. A careful analysis using either approach requires thoughtful use of diagnostic procedures for assessing the effects of model misspecification and both inter- and intra-subject variation on the model fitting process. The importance of this can be easily obscured by the relative ease with which these "black box" deconvolution algorithms provide results. By focusing on the bioavailability profile as the outcome of interest, the complexity of the assumptions of model selection are made more explicit.

There is no general family of functions to select from when specifying functional forms for W and I (Eq. (5)), although mixtures of exponentials are likely candidates if one assumes a compartmental model. Ideally, choices for these functional forms should be based on results from previous pharmacokinetic studies of the drug. Often times, such analyses have been performed if one is at the stage of attempting to establish an in vitro–in vivo relationship. The pitfalls and numerical hazards associated with fitting models which are sums of exponentials to highly variable data are many. An acceptable strategy is to start with the most simple reasonable model, assess model misspecification, and reformulate as necessary. Starting with over-parameterized models is likely to lead to very good data fitting, but fails when validated externally or internally (cross-validation).

Two very recent papers (Hussain, 1997; Dowell et al., 1997) propose the use of neural networks to predict in vivo dissolution profiles. A main advantage of this type of approach is that directly relating of in vitro dissolution data to in vivo bioavailability data via a generic network avoids the choice of a particular model, and thus model misspecification. Semiparametric models also have this advantage. These methods may prove valuable for developing in vitro–in vivo relationships. However, from a regulatory point of view it seems important that the nature of the in vitro–in vivo relationship be clearly understood if a biowaiver for human bioavailability studies is to be granted. The FDA Guidance for Industry does not give any indication that model-free methods alone can provide an acceptable and convincing interpretation of an in vitro–in vivo study.

The statistical properties of estimators under the convolution and deconvolution approaches have not been investigated, but it is reasonable to expect that trade-offs will exist. Other areas ripe for statistical development include inference for evaluating the strength of the in vitro–in vivo relationship. The methods discussed here are ad hoc and have not been objectively evaluated. This is a

critical issue from a regulatory perspective since it has been proposed that waivers of bioavailability studies can be granted based on the strength of an in vitro–in vivo relationship. The sample sizes commonly in use for in vitro–in vivo studies are those of independent in vitro dissolution and bioavailability studies. Research is needed in the area of designing in vitro–in vivo studies with the purpose of developing an in vitro–in vivo relationship.

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