# Bioavailability Assessment under Quasi- and Nonsteady-State Conditions II: Study Designs

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**Abstract** □ The flexibility of bioavailability assessment at quasi- and nonsteady state is demonstrated by systematically removing experimental constraints from the study design. Mathematical expressions are derived to describe each design variation. From the resultant solutions, it is evident that the proposed method can accommodate nonuniformities in dose, dosage interval, dosage regimen, dosing cycle, sampling interval, plasma half-life, washout period, and protocol adherence. Nominal requirements for the method are linear kinetics and mean plasma concentrations estimated over time intervals beginning and ending in the log–linear region.

**Keyphrases** □ Bioavailability—assessment under quasi- and nonsteady-state conditions, mathematical expressions, practical alternatives in study design □ Drug disposition—bioavailability assessment under quasi- and nonsteady-state conditions, mathematical expressions, practical alternatives in study design

In a previous report (1), the rationale for bioavailability estimations at quasi- and nonsteady state was presented. The advantage of the method over conventional approaches is that it needs neither steady-state plasma levels nor extended washout periods. Equations were derived to permit rigorous examination of the basic premise and underlying assumptions. In practice, however, some modification of these equations usually will be required to accommodate variations in experimental design.

The purpose of this article is to demonstrate the flexibility of the proposed method by considering some practical alternatives in study design. Accordingly, various experimental complexities will be introduced in a systematic manner. The corresponding mathematical expressions will be presented in closed form. The discussion will evolve from a relatively simple situation and will favor solutions in general form so that further extensions can be facilitated.

## THEORETICAL: DESIGN VARIATIONS

The following equations were derived (1) on the assumptions that drug disposition obeys linear kinetics and that successive doses are administered during the log–linear region of the plasma concentration curve:

$$\bar{C}p^{(l)} = \bar{C}p^{(ss)}\,(1 - e^{-l\omega\tau}) \qquad \text{(Eq. 1)}$$

$$\bar{C}p^{(ss)} = \frac{F_x D}{\tau\omega V_0} \qquad \text{(Eq. 2)}$$

and:

$$\frac{F_y}{F_x} = \left[\frac{\bar{C}p^{(m+l)}}{\bar{C}p^{(l)}} - e^{-m\omega\tau}\right]\frac{(1 - e^{-l\omega\tau})}{(1 - e^{-m\omega\tau})} \qquad \text{(Eq. 3)}$$

where $\bar{C}p^{(l)}$ is the observed mean plasma concentration during the $l$th interval following the administration of $l$ doses of formulation $x$; $\bar{C}p^{(ss)}$ is the mean plasma concentration at steady state, projected from $\bar{C}p^{(l)}$; $\bar{C}p^{(m+l)}$ is the observed mean plasma concentration during the $(m + l)$th dosage interval when $l$ doses of formulation $x$ are followed immediately by $m$ doses of formulation $y$; $\tau$ is the dosage interval; $\omega$ is the terminal slope, which is assumed to be constant throughout; $V_0$ is an operational constant such that the product $\omega V_0$ is equal to body drug clearance; and $F_x$ and $F_y$ are, respectively, the fraction, $F$, of the dose, $D$, absorbed from formulations $x$ and $y$.

Equations 1–3 are descriptive of a simple and perhaps idealized situation, where the dosage interval, $\tau$, is uniformly spaced around the clock, *e.g.*, when $\tau$ is equal to 8, 12, or 24 hr. However, when the drug is given more frequently than twice a day, a uniform dosing interval may not always be clinically relevant or compatible with the subject's normal sleeping habits. Thus, whenever feasible, dosing schedules should be modified so as to minimize unnecessary sources of inconvenience and noncompliance in bioavailability studies. Accordingly, alternative expressions to Eqs. 1–3 are needed to accommodate these and other variations in experimental design.

**Dosing Intervals**—Suppose a drug is given $r$ doses daily with recurring dosage intervals $\tau_1, \tau_2, \ldots, \tau_r$ such that $\tau_1 + \tau_2 + \ldots + \tau_r = 24$ hr. The mean plasma concentrations during the first dosage interval on the $L$th day and at steady state can be obtained by summing all administered doses, including the first dose on Day $L$; *i.e.*:

$$\bar{C}p^{(L,\tau_1)} = \bar{C}p^{(ss,\tau_1)}\left\{1 - \frac{\left[1 + \sum_{j=1}^{r-1}\left(\prod_{i=1}^{j} e^{\omega\tau_i}\right)\right]}{(e^{-24\omega})\left[\sum_{j=1}^{r}\left(\prod_{i=1}^{j} e^{\omega\tau_i}\right)\right]}(e^{-24L\omega})\right\} \qquad \text{(Eq. 4)}$$

and:

$$\bar{C}p^{(ss,\tau_1)} = \frac{FD}{\tau_1\omega V_0}\frac{(1 - e^{-\omega\tau_1})}{(1 - e^{-24\omega})}(e^{-24\omega})\left[\sum_{j=1}^{r}\left(\prod_{i=1}^{j} e^{\omega\tau_i}\right)\right] \qquad \text{(Eq. 5)}$$

where, in general, $\tau_i$'s need not be equal to one another[1]. It can be easily verified that Eq. 5 reduces to Eq. 2 when all $\tau_i$'s are equal. As an example of Eq. 5, consider a qid regimen in which doses are administered at 7 am, 11 am, 5 pm, and 10 pm. With $\tau_1 = 4$ hr, $\tau_2 = 6$ hr, $\tau_3 = 5$ hr, and $\tau_4 = 9$ hr:

$$\bar{C}p^{(ss,\tau_1)} = \frac{FD}{4\omega V_0}\frac{(1 - e^{-4\omega})}{(1 - e^{-24\omega})}[1 + e^{-20\omega} + e^{-14\omega} + e^{-9\omega}] \qquad \text{(Eq. 6)}$$

Obviously, the relative bioavailability between two formulations may be studied at nonsteady state in many ways. One of the simpler designs calls for estimations of both the mean plasma concentration and the terminal slope at the end of each treatment period. A typical dosing sequence in such a study is schematically depicted in Fig. 1*a*. During Period I, which lasts for $L$ days, the standard formulation, $s$, is given $r$ doses daily according to a prescribed regimen for $(L - 1)$ days, but only the first dose is given on Day $L$. Appropriate plasma samples are taken on Day $L$ to permit estimations of $\bar{C}p^{(\mathrm{I},\tau_1)}$, the average plasma level during interval $\tau_1$, and of $\omega_\mathrm{I}$, the terminal slope.

During Period II, which begins on Day $(L + 1)$ and lasts for $M$ days, the same dosage regimen of $r$ daily doses of formulation $x$ is given for $(M - 1)$ days and then only the first dose on Day $M$. Appropriate estimates of $\bar{C}p^{(\mathrm{II},\tau_1)}$ and of $\omega_\mathrm{II}$ are obtained from plasma samples taken on Day $M$. The purpose in giving only one dose on Day $L$ of Period I and on Day $M$ of Period II is to permit more extensive sampling of the terminal slopes. The elapsed time between the last dose of Treatment I and the first dose of Treatment II is defined as the washout period, which in this case is 1 day.

[1] In these and ensuing equations, the summation term is defined to be identically zero and the product term identically unity whenever the upper limit of the running index is smaller than the lower limit; *i.e.*: $\Sigma_a^b \equiv 0$ and $\Pi_a^b \equiv 1$, if $b < a$.

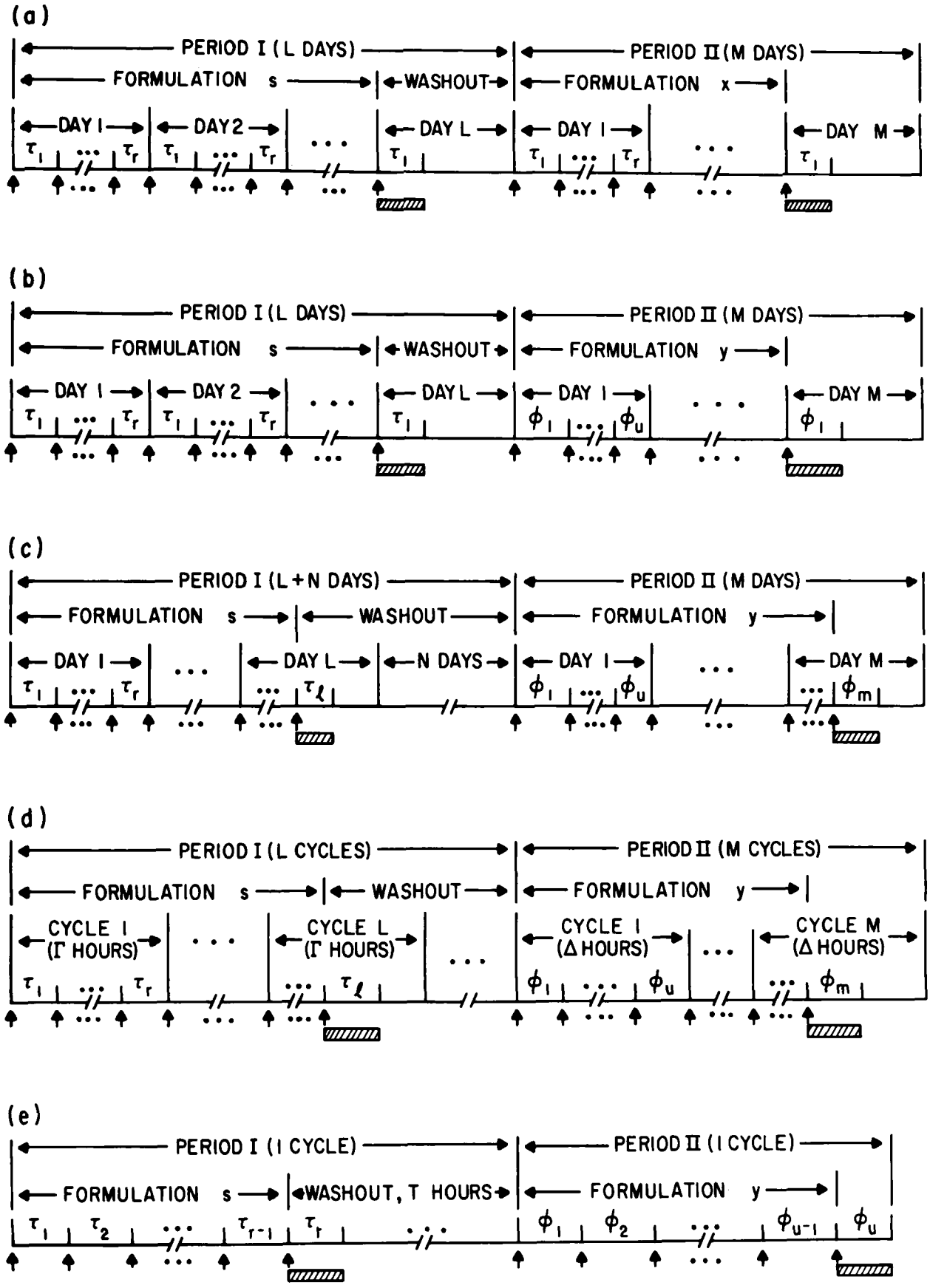


**Figure 1**—*Dosing schemes. Vertical arrows (↑) denote time of administration. Average plasma levels are determined in the last dosing interval of each treatment (shown in shaded area).*

By combining Eqs. 4 and 5, the mean plasma concentration during the first dosage interval, $\tau_1$, on Day $L$ becomes:

$$\bar{C}p^{(I,\tau_1)} = \frac{F_I D(1 - e^{-\omega_I \tau_1})}{\tau_1 \omega_I V_0 (1 - e^{-24\omega_I})} [R_1] \quad \text{(Eq. 7)}$$

where $F_I$ is the fraction of the dose, $D$, absorbed from formulation $s$ in Period I; $\omega_I$ is the observed terminal slope on Day $L$; and:

$$[R_1] = (e^{-24\omega_I}) \left[ \sum_{j=1}^{r} \left( \prod_{i=1}^{j} e^{\omega_I \tau_i} \right) \right] - (e^{-24L\omega_I}) \left[ 1 + \sum_{j=1}^{r-1} \left( \prod_{i=1}^{j} e^{\omega_I \tau_i} \right) \right] \quad \text{(Eq. 8)}$$

The mean plasma level during $\tau_1$ on Day $M$ of Period II, $\bar{C}p^{(II,\tau_1)}$, is obtained by summing the residual contributions from formulation $s$ during Period I, $\bar{C}p^s$, and the cumulative effects of formulation $x$ during Period II, $\bar{C}p^x$:

$$\bar{C}p^s = \frac{F_I D[R_1]}{\tau_1 V_0 (1 - e^{-24\omega_I})} [e^{-24(\omega_I + M\omega_{II} - \omega_{II})}] \int_0^{\tau_1} e^{-\omega_{II} t'} dt' = \frac{F_I D[R_1] (1 - e^{-\omega_{II}\tau_1})}{\tau_1 \omega_{II} V_0 (1 - e^{-24\omega_I})} [e^{-24(\omega_I + M\omega_{II} - \omega_{II})}] \quad \text{(Eq. 9)}$$

and:

$$\bar{C}p^x = \frac{F_{II} D[R_2]}{\tau_1 V_0 (1 - e^{-24\omega_{II}})} \int_0^{\tau_1} e^{-\omega_{II} t'} dt' = \frac{F_{II} D[R_2] (1 - e^{-\omega_{II}\tau_1})}{\tau_1 \omega_{II} V_0 (1 - e^{-24\omega_{II}})} \quad \text{(Eq. 10)}$$

where $t'$ is time from the last dose, *i.e.*, the morning dose on Day $M$ of Period II; $\omega_{II}$ is the observed terminal slope; $F_{II}$ denotes the fraction of dose $D$ absorbed from formulation $x$ during Period II; and:

$$[R_2] = (e^{-24\omega_{II}}) \left[ \sum_{j=1}^{r} \left( \prod_{i=1}^{j} e^{\omega_{II} \tau_i} \right) \right] - (e^{-24M\omega_{II}}) \left[ 1 + \sum_{j=1}^{r-1} \left( \prod_{i=1}^{j} e^{\omega_{II} \tau_i} \right) \right] \quad \text{(Eq. 11)}$$

By combining Eqs. 9 and 10:

$$\bar{C}p^{(II,\tau_1)} = \frac{D(1 - e^{-\omega_{II}\tau_1})}{\tau_1 \omega_{II} V_0} \times \left\{ \frac{F_{II}[R_2]}{(1 - e^{-24\omega_{II}})} + \frac{F_I[R_1] [e^{-24(\omega_I + M\omega_{II} - \omega_{II})}]}{(1 - e^{-24\omega_I})} \right\} \quad \text{(Eq. 12)}$$

The bioavailability of formulation $x$ relative to formulation $s$ is given by Eq. 13, which is obtained by rearranging the ratio of Eq. 12 to Eq. 7:

$$\left(\frac{F_{II}}{F_I}\right)_{s/x} = \frac{[R_1](1 - e^{-24\omega_{II}})}{[R_2](1 - e^{-24\omega_I})} \times \left\{ \frac{\bar{C}p^{(II,\tau_1)} \omega_{II} (1 - e^{-\omega_I \tau_1})}{\bar{C}p^{(I,\tau_1)} \omega_I (1 - e^{-\omega_{II}\tau_1})} - e^{-24(\omega_I + M\omega_{II} - \omega_{II})} \right\} \quad \text{(Eq. 13)}$$

where the subscript $s/x$ denotes the fact that formulation $s$ is given in Period I and formulation $x$ in Period II (Fig. 1*a*). The last term in Eq. 13 may be identified as the residue factor, because it reflects the effect of Treatment I on the observed mean plasma level, $\bar{C}p^{(II,\tau_1)}$. This term approaches zero as $M$ approaches infinity, *i.e.*, at steady state. The ratio $\omega_{II}(1 - e^{-\omega_I\tau_1})/\omega_I(1 - e^{-\omega_{II}\tau_1})$ can be identified as a correction factor for the observed mean plasma levels in the event that the two terminal slopes are not identical.

Implicit in Eq. 13 is the assumption that $V_0$ remains constant but the terminal slope $\omega$ may change between treatments. For the special case of $L = M$ and $\omega_I = \omega_{II}$, Eq. 13 reduces to:

$$\left(\frac{f_{II}}{F_I}\right)_{s/x} = \frac{\bar{C}p^{(II,\tau_1)}}{\bar{C}p^{(I,\tau_1)}} - e^{-24M\omega} \quad \text{(Eq. 14)}$$

In Eqs. 7–13, $\omega_I$ and $\omega_{II}$ are used in the context that they represent the mean terminal slopes of their respective treatment periods. If $\omega_I \neq \omega_{II}$, possible causes of the observed difference are numerous and usually unknown. Among them are systematic changes arising from specific interactions between the drug and the host, random fluctuations in drug disposition, experimental error, and differences in absorption rate between formulations, for in theory at least the terminal slope may be the manifestation of a slow, first-order absorption rate.

In any event, it should be recognized that the use of $\omega_I$ and $\omega_{II}$ represents a simplified and possibly biased correction, which may result in an overestimation or an underestimation of the ratio ($F_{II}/F_I$). This problem of varying terminal slopes is, however, not unique with the proposed method but also pertains to steady-state comparisons. To compensate for these effects, a balanced crossover comparison of the treatments is desirable. Therefore, subsequent discussion assumes that each study design is a crossover comparison.

In a two-way crossover study, Eq. 13 is applicable to half of the subjects who received the depicted (Fig. 1*a*) sequence of formulation $s$ for $L$ days in Period I followed by formulation $x$ for $M$ days in Period II. For subjects in the complementary group who received formulation $x$ for $L$ days in Period I and formulation $s$ for $M$ days in Period II, the resultant bioavailability ratio becomes $(F_{II}/F_I)_{x/s}$. This result does not alter the general form of Eqs. 7–14 but merely means that the identification of formulations $s$ and $x$ with Periods I and II is reversed, *i.e.*:

$$\left(\frac{F_{II}}{F_I}\right)_{s/x} = \frac{F_x}{F_s} \quad \text{(Eq. 15)}$$

and:

$$\left(\frac{F_{II}}{F_I}\right)_{x/s} = \frac{F_s}{F_x} \quad \text{(Eq. 16)}$$

In addition, more estimates of $\omega_I$ and $\omega_{II}$ may be included in the design to observe better their time courses of change throughout the study. This may entail skipping dosages more than once per treatment. Alternatively, since successive doses are to be administered during the log-linear phase, it may be sufficiently reassuring to include a few strategically placed plasma sample points at the end of some selected dosage intervals.

**Dosages and Dosage Regimens**—Sometimes, bioavailability studies are performed to assess new formulations that differ from the standard formulation not only in potency but also in dosage regimen. Often, the new formulation may be one designed to deliver the same daily dosage at less frequent intervals.

Suppose formulation $y$ is to be compared with formulation $s$ in a crossover study. One dosing sequence in such a study is shown in Fig. 1*b*. During Period I, which lasts for $L$ days, $r$ doses of formulation $s$ of potency $D_I$ are given each day for $(L - 1)$ days, but only the first dose is administered on Day $L$. During Period II, which begins on Day $(L + 1)$, $u$ doses of formulation $y$ of potency $D_{II}$ are given daily for $(M - 1)$ days, and again only one dose is given on Day $M$. In general, $u$ may not be equal to $r$. Mean plasma concentrations and terminal slopes are determined on the last day of each treatment; the washout period is again 24 hr.

The expression for $\bar{C}p^{(I,\tau_1)}$ is analogous to Eq. 7, *i.e.*:

$$\bar{C}p^{(I,\tau_1)} = \frac{F_I D_I (1 - e^{-\omega_I \tau_1})[R_1]}{\omega_I \tau_1 V_0 (1 - e^{-24\omega_I})} \quad \text{(Eq. 17)}$$

where the subscript I is identified with formulation $s$. Also by analogy to Eq. 13 and defining the daily dosing intervals for formulation $y$ as $\phi_1, \phi_2, \ldots, \phi_u$ such that $\phi_1 + \phi_2 + \ldots + \phi_u = 24$ hr, it can be shown that the mean plasma level, $\bar{C}p^{(II,\phi_1)}$, during the first dosage interval $\phi_1$ on Day $M$ of Period II is:

$$\bar{C}p^{(II,\phi_1)} = \frac{(1 - e^{-\omega_{II}\phi_1})}{\omega_{II}\phi_1 V_0} \times \left\{ \frac{F_{II} D_{II}[R_3]}{(1 - e^{-24\omega_{II}})} + \frac{F_I D_I[R_1]}{(1 - e^{-24\omega_I})} e^{-24(\omega_I + M\omega_{II} - \omega_{II})} \right\} \quad \text{(Eq. 18)}$$

where the subscript II is now identified with formulation $y$, and:

$$[R_3] = (e^{-24\omega_{II}}) \left[ \sum_{j=1}^{u} \left( \prod_{i=1}^{j} e^{\omega_{II}\phi_i} \right) \right] - (e^{-24M\omega_{II}}) \left[ 1 + \sum_{j=1}^{u-1} \left( \prod_{i=1}^{j} e^{\omega_{II}\phi_i} \right) \right] \quad \text{(Eq. 19)}$$

Combining Eqs. 17 and 18 gives:

$$\left(\frac{F_{II}}{F_I}\right)_{s/y} = \frac{D_I[R_1](1 - e^{-24\omega_{II}})}{D_{II}[R_3](1 - e^{-24\omega_I})} \times \left\{ \frac{\bar{C}p^{(II,\phi_1)}(\phi_1\omega_{II})(1 - e^{-\omega_I\tau_1})}{\bar{C}p^{(I,\tau_1)}(\tau_1\omega_I)(1 - e^{-\omega_{II}\phi_1})} - e^{-24(\omega_I + M\omega_{II} - \omega_{II})} \right\} \quad \text{(Eq. 20)}$$

It is evident that Eq. 13 is a special case of Eq. 20 where $D_I = D_{II}$ and that a common daily dosage regimen is used for formulations $s$ and $y$. The correction factor now includes the ratio $\phi_1/\tau_1$, which compensates for possible differences in the length of sampling intervals between treatments.

For subjects in the complementary group who received formula-

tion $y$ ($u$ daily doses) during Period I and formulation $s$ ($r$ daily doses) during Period II, the corresponding solution is:

$$\left(\frac{F_{II}}{F_I}\right)_{y/s} = \frac{D_I[R_4](1-e^{-24\omega_{II}})}{D_{II}[R_5](1-e^{-24\omega_I})} \times \left\{\frac{\bar{C}p^{(II,\tau_1)}(\tau_1\omega_{II})(1-e^{-\omega_I\phi_1})}{\bar{C}p^{(I,\phi_1)}(\phi_1\omega_I)(1-e^{-\omega_{II}\tau_1})} - e^{-24(\omega_I+M\omega_{II}-\omega_{II})}\right\} \quad \text{(Eq. 21)}$$

where:

$$[R_4] = (e^{-24\omega_I})\left[\sum_{j=1}^{u}\left(\prod_{i=1}^{j} e^{\omega_I\phi_i}\right)\right] - (e^{-24L\omega_I})\left[1+\sum_{j=1}^{u-1}\left(\prod_{i=1}^{j} e^{\omega_I\phi_i}\right)\right] \quad \text{(Eq. 22)}$$

and:

$$[R_5] = (e^{-24\omega_{II}})\left[\sum_{j=1}^{r}\left(\prod_{i=1}^{j} e^{\omega_{II}\tau_i}\right)\right] - (e^{-24M\omega_{II}})\left[1+\sum_{j=1}^{r-1}\left(\prod_{i=1}^{j} e^{\omega_{II}\tau_i}\right)\right] \quad \text{(Eq. 23)}$$

The quantity $[R_i]$ can be defined as the regimen factor, which serves to maintain proper accounting of the dosing sequence. More specifically, the regimen factor tracks the following: the total number of doses per treatment, the number of doses per day, the order among dosing intervals within the day, the number of days per treatment, and the order in which the treatments are administered. The systematic changes in $[R_i]$ among Eqs. 8, 11, 19, 22, and 23 should be noted in relation to the corresponding variations in dosing sequence. In subsequent discussion, these regimen factors will assume added significance as the complexity in experimental design increases.

**Sampling Interval and Washout Period**—Equations 4–23 were derived to accommodate experimental designs where estimates of mean plasma concentrations are confined to the first dosage interval on the last day of each treatment period and where the elapsed time between the last dose of the first treatment and the first dose of the second treatment is 24 hr. Expressions with greater general utility will be needed when neither condition prevails.

The general solution for the mean plasma concentration during interval $\tau_k$ on Day $L$ following the repeated administration of $r$ daily doses with recurring intervals $\tau_1, \tau_2, \ldots, \tau_k, \ldots, \tau_r$ may be represented by:

$$\bar{C}p^{(L,\tau_k)} = \bar{C}p^{(ss,\tau_k)}\left[1-\frac{[G]}{[H]}\right] \quad \text{(Eq. 24)}$$

where:

$$\bar{C}p^{(ss,\tau_k)} = \frac{FD[H](1-e^{-\omega\tau_k})}{\omega\tau_k V_0(1-e^{-24\omega})} \quad \text{(Eq. 25)}$$

$$[G] = (e^{-24L\omega})\left(\prod_{i=1}^{k-1} e^{-\omega\tau_i}\right)\left[1+\sum_{j=1}^{r-1}\left(\prod_{i=1}^{j} e^{\omega\tau_i}\right)\right] \quad \text{(Eq. 26)}$$

and:

$$[H] = (e^{\omega\tau_k})\left[\sum_{j=1}^{k}\left(\prod_{i=j}^{k} e^{-\omega\tau_i}\right)\right] + (e^{-24\omega})\left[\sum_{j=k}^{r-1}\left(\prod_{i=k}^{j} e^{\omega\tau_i}\right)\right] \quad \text{(Eq. 27)}$$

The working forms of Eqs. 26 and 27 are usually much simpler than their general forms. For example, the expressions for the case of $r = 3$ and $k = 2$ are:

$$[G] = (e^{-24L\omega})(1+e^{-\omega\tau_1}+e^{\omega\tau_2}) \quad \text{(Eq. 28)}$$

and:

$$[H] = 1+e^{-\omega\tau_1}+e^{-\omega(\tau_1+\tau_3)} \quad \text{(Eq. 29)}$$

It can be verified that Eqs. 24 and 25 reduce to Eqs. 4 and 5 when $k = 1$.

Suppose the experimental design includes a dosing scheme like that shown in Fig. 1c. As in Fig. 1b, formulation $s$ is given in Period I and formulation $y$ in Period II. However, instead of terminating Treatment I with the first dose on Day $L$, $l$ doses of formulation $s$ are administered on Day $L$. The mean plasma level $\bar{C}p^{(I,\tau_l)}$ is determined during the interval $\tau_l$. Similarly, $m$ doses of formulation $y$ are given on Day $M$ of Period II, and the mean plasma level $\bar{C}p^{(II,\phi_m)}$ is determined over $\phi_m$. Terminal slopes $\omega_I$ and $\omega_{II}$ are estimated as before. Thus, if both treatment periods begin at the same clock hour and $l > 1$, the washout period is less than 24 hr when Treatment II begins on Day $(L+1)$.

On the other hand, the initiation of Treatment II may be postponed for $N$ days (Fig. 1c) and the washout period is extended to $(24N + \Sigma_{i=l}^{r}\tau_i)$ hr. Under these circumstances, the bioavailability ratio is given by:

$$\left(\frac{F_{II}}{F_I}\right)_{s/y} = \frac{D_I[R_6](1-e^{-24\omega_{II}})}{D_{II}[R_7](1-e^{-24\omega_I})} \times \left\{\frac{\bar{C}p^{(II,\phi_m)}}{\bar{C}p^{(I,\tau_l)}}\frac{\omega_{II}\phi_m(1-e^{-\omega_I\tau_l})}{\omega_I\tau_l(1-e^{-\omega_{II}\phi_m})} - W\right\} \quad \text{(Eq. 30)}$$

where:

$$W = e^{-24(N\omega_I+M\omega_{II}-\omega_{II})}\left[\prod_{i=l}^{r} e^{-\omega_I\tau_i}\right]\left[\prod_{i=1}^{m-1} e^{-\omega_{II}\phi_i}\right] \quad \text{(Eq. 31)}$$

$$[R_6] = (e^{\omega_I\tau_l})\left[\sum_{j=1}^{l}\left(\prod_{i=j}^{l} e^{-\omega_I\tau_i}\right)\right] + (e^{-24\omega_I})\left[\sum_{j=l}^{r-1}\left(\prod_{i=l}^{j} e^{\omega_I\tau_i}\right)\right] - (e^{-24L\omega_I})\left(\prod_{i=1}^{l-1} e^{-\omega_I\tau_i}\right)\left[1+\sum_{j=1}^{r-1}\left(\prod_{i=1}^{j} e^{\omega_I\tau_i}\right)\right] \quad \text{(Eq. 32)}$$

and:

$$[R_7] = (e^{\omega_{II}\phi_m})\left[\sum_{j=1}^{m}\left(\prod_{i=j}^{m} e^{-\omega_{II}\phi_i}\right)\right] + (e^{-24\omega_{II}})\left[\sum_{j=m}^{u-1}\left(\prod_{i=m}^{j} e^{\omega_{II}\phi_i}\right)\right] - (e^{-24M\omega_{II}})\left(\prod_{i=1}^{m-1} e^{-\omega_{II}\phi_i}\right)\left[1+\sum_{j=1}^{u-1}\left(\prod_{i=1}^{j} e^{\omega_{II}\phi_i}\right)\right] \quad \text{(Eq. 33)}$$

In this case, the regimen factor $[R_i]$ has the added responsibility of tracking dosing intervals over which the mean plasma concentrations are determined. In general, $[R_i]$ represents the difference between $[G]$ and $[H]$ after appropriate substitutions therein.

The reason for skipping doses on Day $L$ is to facilitate estimations of the terminal slope. Ideally, this elapsed time, or washout period, should only be long enough to provide a satisfactory estimate of $\omega$. In the situations depicted in Figs. 1b and 1c, the difference in the length of the washout period is manifested in the terms $e^{-24\omega_I}$ and $(e^{-24N\omega_I})$ $[\Pi_{i=l}^{r} e^{-\omega_I\tau_i}]$ in Eqs. 20 and 31, respectively. This can be easily verified by noting that when $l = 1$ and $N = 0$, the two terms are identical:

$$\prod_{i=1}^{r} e^{-\omega_I\tau_i} = e^{-24\omega_I} \quad \text{(Eq. 34)}$$

Equation 30 is the most general form for the cases discussed so far. Experimental parameters and observed variables required for its application are summarized in Table I. The choice of design parameters is large, even in a simple two-way crossover study. For example, if $D_I = 2$ mg, $D_{II} = 5$ mg, $L = M = 2$ days, $N = 1$ day, $r = 2$, $u = l = m = 1$, $\tau_1 = 10$ hr, $\omega_I = 0.05$ hr$^{-1}$, $\omega_{II} = 0.06$ hr$^{-1}$, $\bar{C}p^{(I,\tau_1)} = 4$ ng/ml, and $\bar{C}p^{(II,\phi_1)} = 5$ ng/ml, it can be verified that the ratio $F_{II}/F_I$ is equal to 1.067.

**Dosing Cycles**—Up to now, arguments have been developed on the basis that each dosing sequence repeats itself every 24 hr. This assumption is not unreasonable, since most current chronic therapy calls for medication at least once daily. Not coincidentally, in terms of study expedition, more frequent dosing also means that useful plasma concentrations can be attained sooner and study duration can be minimized. With increasing knowledge in chronopharmacology and controlled drug delivery systems, dosing cycles longer or shorter than 24 hr may become more meaningful for some drugs and dosage forms.

Suppose the dosing cycle for formulation $s$ is $\Gamma$ hr instead of 24 hr and that for formulation $y$ is $\Delta$ hr. By reference to Fig. 1d, the mean plasma level of formulation $s$ is estimated over the interval $\tau_l$ during the $L$th cycle in Period I; the mean plasma level of formulation $y$ is estimated over $\phi_m$ during the $M$th cycle in Period II. The elapsed time between the last dose of treatment $s$ and the first dose of treatment $y$ is $T$ hr. The solution for this design can be obtained by appropriate extensions of Eq. 30:

$$\left(\frac{F_{II}}{F_I}\right)_{s/y} = \frac{D_I[R_8](1-e^{-\Delta\omega_{II}})}{D_{II}[R_9](1-e^{-\Gamma\omega_I})} \times \left\{\frac{\bar{C}p^{(II,\phi_m)}\phi_m\omega_{II}(1-e^{-\omega_I\tau_l})}{\bar{C}p^{(I,\tau_l)}\tau_l\omega_I(1-e^{-\omega_{II}\phi_m})} - W\right\} \quad \text{(Eq. 35)}$$

Table I—Experimental Parameters and Observed Variables Applicable to Fig. 1c and Eq. 31

| Parameters and Variables | Period I | Period II |
|---|---|---|
| Dosage potency | $D_I$ | $D_{II}$ |
| Length of period, days[a] | $L + N$ | $M$ |
| Length of cycle, hr | 24 | 24 |
| Number of doses per cycle | $r$ | $u$ |
| Dosage intervals, hr[b] | $\tau_1, \ldots, \tau_l, \ldots, \tau_r$ | $\phi_1, \ldots, \phi_m, \ldots, \phi_u$ |
| Last dose given[c] | $l$th dose on Day $L$ | $m$th dose on Day $M$ |
| Sampling interval, hr | $\tau_l$ | $\phi_m$ |
| Washout period, hr | $T = 24N + \sum_{i=l}^{r} \tau_i$ | — |
| Terminal slope, $hr^{-1}$ | $\omega_I$ | $\omega_{II}$ |
| Average plasma level | $\bar{C}p(I, \tau_l)$ | $\bar{C}p(II, \phi_m)$ |

[a] $N$ is optional; $N \geq 0$. [b] By definition, $\tau_1 + \tau_2 + \ldots + \tau_r = \phi_1 + \phi_2 + \ldots + \phi_u = 24$ hr, and all daily doses are assumed to begin at the same clock hour (*e.g.*, 8 am). [c] $r \geq l \geq 1$; $u \geq m \geq 1$.

where $\Gamma = \tau_1 + \tau_2 + \ldots + \tau_r$, $\Delta = \phi_1 + \phi_2 + \ldots + \phi_u$, and:

$$W = (e^{-\omega_I T}) \left( \prod_{i=1}^{m-1} e^{-\omega_{II}\phi_i} \right) [e^{-\omega_{II}\Delta(M-1)}] \quad \text{(Eq. 36)}$$

$$[R_8] = (e^{\omega_I \tau_l}) \left[ \sum_{j=1}^{l} \left( \prod_{i=j}^{l} e^{-\omega_I \tau_i} \right) \right] + (e^{-\Gamma\omega_I}) \left[ \sum_{j=l}^{r-1} \left( \prod_{i=l}^{j} e^{\omega_I \tau_i} \right) \right] - (e^{-\Gamma L \omega_I}) \left( \prod_{i=1}^{l-1} e^{-\omega_I \tau_i} \right) \left[ 1 + \sum_{j=1}^{r-1} \left( \prod_{i=1}^{j} e^{\omega_I \tau_i} \right) \right] \quad \text{(Eq. 37)}$$

$$[R_9] = (e^{\omega_{II}\phi_m}) \left[ \sum_{j=1}^{m} \left( \prod_{i=j}^{m} e^{-\omega_{II}\phi_i} \right) \right] + (e^{-\Delta\omega_{II}}) \left[ \prod_{j=m}^{u-1} \left( \prod_{i=m}^{j} e^{\omega_{II}\phi_i} \right) \right] - (e^{-\Delta M \omega_{II}}) \left( \prod_{i=1}^{m-1} e^{-\omega_{II}\phi_i} \right) \left[ 1 + \sum_{j=1}^{u-1} \left( \prod_{i=1}^{j} e^{\omega_{II}\phi_i} \right) \right] \quad \text{(Eq. 38)}$$

Thus, the lengths of dosing cycles also influence the regimen factors, $[R_i]$.

The very idea of a dosing cycle (of whatever length) demands unerring adherence to the prescribed regimen without which steady state can never be achieved and the equations so far developed cannot be applied. Therefore, the requirements for assessing bioavailability at quasi- and nonsteady state apparently are less demanding only with respect to study duration, not in exactitude. Accordingly, on purely pragmatic grounds, it is important that the proposed method accommodate those occasional deviations from perfect adherence to protocol.

Suppose a bioavailability study were to be performed such that Eq. 35 would apply. Suppose further that, in spite of best intentions, the prescribed dosage schedule could not be followed exactly but that the precise time for each dose was duly recorded. Under these circumstances, it is inconceivable that the integrity of the dosing cycles would have been preserved. Nevertheless, estimates of relative bioavailability are still possible by treating the entire Period I as a single dosing cycle for formulation $s$ and treating the entire Period II as a single dosing cycle for formulation $y$. Mean plasma levels, $\bar{C}p^{(I,\tau_r)}$ and $\bar{C}p^{(II,\phi_u)}$, are determined during the last dosing interval of each treatment. This situation is schematically represented in Fig. 1e. The expression for this scheme represents a special case of Eq. 35, in which $M = L = 1$ with sampling intervals $\tau_r$ and $\phi_u$. Substituting into Eq. 35 and simplifying give:

$$\left(\frac{F_{II}}{F_I}\right)_{s/y} = \frac{D_I}{D_{II}} [R] \times \left\{ \frac{\bar{C}p^{(II,\phi_u)}(1 - e^{-\omega_I \tau_r})\omega_{II}\phi_u}{\bar{C}p^{(I,\tau_r)}(1 - e^{-\omega_{II}\phi_u})\omega_I \tau_r} - (e^{-\omega_I T}) \left( \prod_{i=1}^{u-1} e^{-\omega_{II}\phi_i} \right) \right\} \quad \text{(Eq. 39)}$$

where:

$$[R] = \frac{\sum_{j=1}^{r} \left( \prod_{i=j}^{r-1} e^{-\omega_I \tau_i} \right)}{\sum_{j=1}^{u} \left( \prod_{i=j}^{u-1} e^{-\omega_{II}\phi_i} \right)} \quad \text{(Eq. 40)}$$

When recalling their intent, the relative simplicity of Eqs. 39 and 40 is ironic. They were derived to accommodate deviations from the prescribed regimen. Nevertheless, they suggest that mean plasma level determinations should always be confined to the last dosing interval of each treatment period and that each treatment period should be considered as a single dosing cycle even though protocol adherence may have been perfect. In practice, this approach should be preferred since the number of doses per treatment period need not be large under quasi- and nonsteady-state conditions.

In some instances, the doses ingested are not uniform throughout a given treatment period. Loading or booster doses may be intentionally given to raise the plasma levels initially or during the sampling interval, or the test subject may try to compensate for doses he or she accidentally missed. In either case, Eqs. 39 and 40 remain useful; their general formats need not be changed so long as all ingested doses are exact multiples of a common unit dose.

The number of terms in the denominator of the regimen factor, $[R]$, is always equal to the number of unit doses ingested within the corresponding treatment period. For example, if a unit dose $D_I$ were to be given at 0, 8, 24, 32, 36, and 48 hr, the numerator of Eq. 40 would contain six terms: $e^{-48\omega_I} + e^{-40\omega_I} + e^{-24\omega_I} + e^{-16\omega_I} + e^{-12\omega_I} + 1$. However, if the doses were taken as one unit, each at 0 and 32 hr and as two units each at 24 and 48 hr, and nothing was taken at 8 and 36 hr, the six terms would become:

$$e^{-48\omega_I} + e^{-24\omega_I} + e^{-24\omega_I} + e^{-16\omega_I} + 1 + 1 = e^{-48\omega_I} + 2e^{-24\omega_I} + e^{-16\omega_I} + 2 \quad \text{(Eq. 41)}$$

Under these conditions, the booster, the loading, or the compensatory doses are merely multiples of unit doses given with zero dosage intervals.

It must be remembered that the proposed method is predicated on the assumption that successive doses are administered during the log-linear phase of plasma decay (1). On closer examination, however, this requirement can be relaxed to the extent that it applies only to the doses over which intervals the mean plasma levels are to be determined. It is not a constraint for all other doses. In other words, given an accurate record of the dosing sequence and its precise timing, it is sufficient that the mean plasma concentrations be determined over dosing intervals beginning and ending in the log-linear region. The justification for this conclusion is a consequence of the principles of superposition wherein the manifestation of log-linear plasma decay represents total accountability of the cumulative effects of all preceding dosages. The more restrictive requirement that successive doses must be administered during the log-linear region (1) is necessary only when dosage intervals are uniform.

Similarly, the constraint relative to continuing absorption during the log-linear phase (1) was necessary only because the relevant equations ignored the possibility that the terminal half-lives might differ between treatments. This constraint does not apply when estimates of $\omega$ are made in each treatment period.

## SUMMARY AND CONCLUSIONS

An attempt has been made to examine the effect of potential constraints on the design of bioavailability studies under quasi- and nonsteady-state conditions. Given linear kinetics and mean plasma levels estimated over time intervals beginning and ending in the log-linear region, there appears to be no other constraints,

provided there is an accurate accounting of the doses ingested and their precise timing.

In general, the relative bioavailability between two formulations in a crossover study is a function of the ratio of their respective mean plasma concentrations at quasi- and nonsteady state. Appropriate correction factors may be introduced to compensate for the effects of dose, dosing sequence, half-life, sampling interval, and residuals. Each of these elements can be readily identified in the equations developed for each design variation.

Mathematical solutions in closed form have been derived for crossover studies in which: (*a*) the potency of the two treatments may be different, (*b*) time intervals between doses need not be uniform, (*c*) the daily dosage regimen for the two treatments may be different, (*d*) adjustments may be attempted for possible changes in half-life between treatments, (*e*) mean plasma concentrations may be sampled over any convenient dosing interval and need not be the same interval between treatment, (*f*) the elapsed time between the last dose of the first treatment and the first dose of the second treatment may be as long or as short as needed, (*g*) the dosage cycle of recurring sequences is not restricted to 24 hr and may differ between treatments, (*h*) treatment periods may vary in length, and (*i*) dosing sequences may be completely random, *i.e.*, acyclic.

Most of these design alternatives are equally applicable to comparisons at steady state. There is, however, one important difference. With the proposed method, steady-state plasma levels are inferred and, therefore, need not be experimentally attained. In this way, even though the dosing sequence may be acyclical, the corresponding steady state can be inferred by depicting the entire sequence as a single cycle repeated indefinitely. That is to say, steady state is more useful as a concept than an experimental reality.

The number of imaginable permutations in the design of bioavailability studies is limitless. Undoubtedly, many more will surface with time. No attempt has been made to consider any but the simplest of situations in the present discussion. Hopefully, sufficient details have been provided to permit useful extensions to more complex experimental designs.

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# Correlation of Phase Inversion Temperature with Kinetics of Globule Coalescence for Emulsions Stabilized by a Polyoxyethylene Alkyl Ether

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**Abstract** □ The phase inversion temperatures, globule coalescence rates, and long-term stability of oil-in-water emulsions stabilized by polyoxyethylene 4 cetyl ether were measured. Addition of sodium chloride to the aqueous phase depressed the phase inversion temperatures of the emulsions and the cloud point of the surfactant. Linear correlations were obtained between phase inversion temperature and cloud point and also between phase inversion temperature and the logarithm of the globule coalescence rate at constant temperature. This latter finding is consistent with a theory of emulsion type based upon the kinetics of coalescence. The programmed viscometric technique of determining inversion revealed the presence of a liquid crystalline phase below 35°, which contributes significantly to emulsion stability.

**Keyphrases** □ Emulsions—phase inversion temperature correlated with kinetics of globule coalescence, long-term stability measured, polyoxyethylene alkyl ether as stabilizer □ Polyoxyethylene alkyl ether—as stabilizer in emulsions □ Phase inversion temperature—emulsions, correlated with kinetics of globule coalescence □ Stability, long term—emulsions, polyoxyethylene alkyl ether as stabilizer

When oil-in-water emulsions stabilized by nonionic surfactants are heated, they may invert because of the decreased water solubility of the emulsifiers. This phenomenon has been examined extensively (1–3), particularly the relation between phase inversion temperature and formulation variables such as the hydrophilic–lipophilic balance (HLB) of the emulsifier, the nature and volume of the oil phase, and the presence of additives. The correlations obtained between emulsion stability and phase inversion temperature generally have been of a qualitative nature.

The work described here was concerned with the quantitative relationship between the phase inversion temperature and the rates of globule coalescence of a series of emulsions stabilized by a single nonionic surfactant. The relation of these parameters to long-term storage also was investigated. Emulsions with differing inversion temperatures and stabilities were produced by adding an electrolyte to their aqueous phases. The data were interpreted in terms of a quantitative kinetic theory for predicting emulsion type (4).

## EXPERIMENTAL

**Materials**—The water was twice distilled from a quartz glass still. Liquid paraffin[1] was of BP quality. The surfactant[2], a commercial sample of polyoxyethylene cetyl ether containing an average of four ethylene oxide units (polyoxyethylene 4 cetyl ether), was used without purification (HLB 8.6). Sodium chloride[3] and glycerol[3] were analytical grade reagents.

**Preparation of Emulsions**—Oil-in-water emulsions were pre-

[1] Vestan grade A350, Fina S.A. Brussels, Belgium.
[2] Texofor A4, batch G964, Glovers Chemicals Ltd., Leeds, England.
[3] B.D.H. Chemicals Ltd., Poole, United Kingdom.