Bias in the Wagner–Nelson Estimate of the Fraction of Drug Absorbed

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Purpose. To examine and quantify bias in the Wagner–Nelson estimate of the fraction of drug absorbed resulting from the estimation error of the elimination rate constant (k), measurement error of the drug concentration, and the truncation error in the area under the curve.

Methods. Bias in the Wagner–Nelson estimate was derived as a function of post-dosing time (t), k, ratio of absorption rate constant to k (r), and the coefficient of variation for estimates of k (CV_k), or CV_c for the observed concentration, by assuming a one-compartment model and using an independent estimate of k. The derived functions were used for evaluating the bias with $r = 0.5$, 3, or 6; $k = 0.1$ or 0.2; $CV_c = 0.2$ or 0.4; and $CV_k = 0.2$ or 0.4; for $t = 0$ to 30 or 60.

Results. Estimation error of k resulted in an upward bias in the Wagner–Nelson estimate that could lead to the estimate of the fraction absorbed being greater than unity. The bias resulting from the estimation error of k inflates the fraction of absorption vs. time profiles mainly in the early post-dosing period. The magnitude of the bias in the Wagner–Nelson estimate resulting from estimation error of k was mainly determined by CV_k. The bias in the Wagner–Nelson estimate resulting from to estimation error in k can be dramatically reduced by use of the mean of several independent estimates of k, as in studies for development of an in *vivo-in vitro* correlation. The truncation error in the area under the curve can introduce a negative bias in the Wagner–Nelson estimate. This can partially offset the bias resulting from estimation error of k in the early post-dosing period. Measurement error of concentration does not introduce bias in the Wagner–Nelson estimate.

Conclusions. Estimation error of k results in an upward bias in the Wagner-Nelson estimate, mainly in the early drug absorption phase. The truncation error in AUC can result in a downward bias, which may partially offset the upward bias due to estimation error of k in the early absorption phase. Measurement error of concentration does not introduce bias. The joint effect of estimation error of k and truncation error in AUC can result in a non-monotonic fraction-of-drug-absorbed-vs-time profile. However, only estimation error of k can lead to the Wagner-Nelson estimate of fraction of drug absorbed greater than unity.

KEY WORDS: Wagner–Nelson; fraction absorbed; absorption; estimation error; bias.

INTRODUCTION

The Wagner–Nelson method estimates the fraction of drug absorbed relative to that ultimately absorbed at time infinity (1). The method is frequently applied when the drug's absorption vs. time profile is needed (e.g., 2–20) because it requires no intravenous administration, requires no prior estimate of the volume of distribution, and has no limitations on the order or nature of the absorption process (1). In practice, it is not uncommon that the Wagner–Nelson estimate of the fraction of drug absorbed is greater than unity, and/or the fraction of drug absorbed vs. time profile is a non-monotonic curve. Such observations usually are ignored by presuming that the deviation from the true value of the fraction absorbed is random noise. If the deviation from the true value of the fraction absorbed has a systematic trend, the Wagner–Nelson estimate will be biased and can lead to a wrong conclusion about the absorption characteristics of a drug. It is therefore important to investigate the possible causes that result in biased Wagner-Nelson estimates.

The use of the Wagner–Nelson method to obtain an estimate of the fraction of drug absorbed requires that a value of the elimination rate constant, k, be input. The true value of k is unknown, and as such, an estimate is used. The estimation of k normally is based on the last 3 to 5 data points at the terminal phase of the drug concentration vs. time curve. The estimates of k are influenced by measurement errors in the data points and by the intrasubject variability of the parameter. For highly variable drug products, estimation error of k can be quite large. The Wagner–Nelson estimate may be biased as a result of errors in the input value of k.

The Wagner–Nelson estimate also depends on the concentration-vs-time curve and the area under that curve (AUC). Concentrations can be observed at only a finite number of sampling time points, and the measurements are subject to error. The measurement error of the drug concentrations and the truncation error in the AUC may also introduce bias.

The degree of the possible bias in the Wagner–Nelson estimate may vary over time post-dose. The time trend of such possible bias is useful information when the whole fraction of drug absorption vs. time profile is needed as in the case of level-A *in vivo-in vitro* correlation (IVIVC) (21).

The objectives of this study were to examine and to quantify the possible bias in Wagner–Nelson estimates resulting from estimation error of k, measurement error of the drug concentration, and truncation error in AUC, and to identify an approach to minimize the possible bias.

METHODS

Assumption

A reference immediate release (IR) product and several test products of the same drug ingredient are used in a crossover trial, as with IVIVC studies. The estimate of k is derived from the reference IR product only. It is obtained as the absolute value of the regression coefficient from the regression of log-concentration on time in the terminal phase.

Wagner–Nelson Estimate of the Fraction of Drug Absorbed

Let C be the true concentration-vs-time function for the test product, with $C(t)$ the concentration at time t. Under a one-compartment model with first order elimination, the Wagner-Nelson model of the fraction of drug absorbed up to time t following drug administration, $F(t;k,C)$, can be expressed as follows:

$$F(t;k,C) = \frac{k\int_0^t C(u)du + C(t)}{k\int_0^\infty C(u)du} \qquad (1)$$

where $0 \le F(t;k,C) \le 1$ (1,22).

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In practice, Equation 1 cannot be applied directly because C cannot be observed continuously and without error and k cannot be determined without error. Let us introduce notation that will allow us to examine the effects of measurement error and finite observations:

$t_0 = 0, t_1, \ldots, t_L$:	times at which observations are made.
C_j:	$C(t_j)$, the true concentration at time t_j.
$\hat{C}_j$:	the observed value of C_j.
CV_c:	the coefficient of variation of $\hat{C}_j$, assumed constant.
$\underline{C}$:	the vector of true concentrations (C_0, $C_1, \ldots, C_L$).
$\underline{\hat{C}}$:	the vector of observed concentrations ($\hat{C}_0$, $\hat{C}_1, \ldots, \hat{C}_L$).
AUC_i:	the trapezoidal approximation to $\int_0^{t_i} C(u)du$ using the true concentrations $C_0, C_1, \ldots, C_i$: i.e., $\sum_{j=0}^{i} w_j C_j$, where the w_j are trapezoidal weights, $w_j = (t_{j+1} - t_{j-1})/2$ for $j \in \{1, \ldots, i-1\}$, $w_0 = t_1/2$, and $w_i = (t_i - t_{i-1})/2$.
$A\hat{U}C_i$:	the trapezoidal rule applied to $\hat{C}_0, \hat{C}_1, \ldots, \hat{C}_L$.
$\hat{k}$:	an estimate of k.
$\text{var}[\hat{k}]$, CV_k:	the variance and the coefficient of variation of $\hat{k}$.

$F(t;k,C)$ given by equation (1) is estimated at t_i by

$$F(t_i;\hat{k},\underline{\hat{C}}) = \frac{\hat{k}\,A\hat{U}C_i + \hat{C}_i}{\hat{k}\,A\hat{U}C_L + \hat{C}_L} = \frac{A\hat{U}C_i + \hat{C}_i/\hat{k}}{A\hat{U}C_L + \hat{C}_L/\hat{k}} \tag{2}$$

We can recognize that three sources of errors in equation (2) may result in bias in $F(t_i;\hat{k},\hat{C})$:

(a) Estimation error in $\hat{k}$.

(b) Measurement error in $\hat{C}_j$. Note that we assume that $\hat{k}$ is obtained from the IR product and $\underline{\hat{C}}$ from the test product, so that estimation error in $\hat{k}$ is independent of measurement error in $\underline{\hat{C}}$.

(c) Truncation error in approximating $\int_0^{ti} C(u)du$ by the trapezoidal rule.

Bias in the Wagner–Nelson Estimate of Fraction Absorbed

Bias Due to Estimation Error of k

By assuming that k is estimated from the reference IR product, the expectation of $F(t_i;\hat{k}, \underline{\hat{C}})$ for the test product can be obtained (see Appendix for derivation), as follows:

$$\begin{aligned} E[F(t_i;\hat{k},\underline{\hat{C}})] &= E\{E[F(t_i;\hat{k},\underline{\hat{C}}) \mid \underline{\hat{C}}]\} \\ &\approx E\left\{\frac{k\,A\hat{U}C_i + \hat{C}_i}{k\,A\hat{U}C_L + \hat{C}_L}\right\} \\ &\quad + E\left\{\frac{[\hat{C}_i\,A\hat{U}C_L - \hat{C}_L\,A\hat{U}C_i]A\hat{U}C_L}{[k\,A\hat{U}C_L + \hat{C}_L]^3}\right\}\text{var}[\hat{k}] \end{aligned} \tag{3}$$

Thus the bias in $F(t_i;\hat{k},\underline{\hat{C}})$ can be expressed as,

$$\begin{aligned} Bias[F(t_i;\hat{k},\underline{\hat{C}})] &\approx E[F(t_i;k,\underline{\hat{C}})] - F(t_i;k,C) \\ &\quad + E\left\{\frac{[\hat{C}_i\,A\hat{U}C_L - \hat{C}_L\,A\hat{U}C_i]A\hat{U}C_L}{[k\,A\hat{U}C_L + \hat{C}_L]^3}\right\}\text{var}[\hat{k}] \end{aligned} \tag{4}$$

We can see from equation (4) that estimation error of k introduces bias in $F(t_i;\hat{k},\underline{\hat{C}})$ only by influencing

$$E\left\{\frac{[\hat{C}_i\,A\hat{U}C_L - \hat{C}_L A\hat{U}C_i]A\hat{U}C_L}{[k\,A\hat{U}C_L + \hat{C}_L]^3}\right\}\text{var}[\hat{k}]$$

Bias due to measurement error of C and truncation error in AUC

Equation (4) can be rewritten based on $E\{E[F(t_i;\hat{k},\underline{\hat{C}}) \mid \hat{k}]\}$, using the same approach as for deriving $E\{E[F(t_i;\hat{k},\underline{\hat{C}}) \mid \underline{\hat{C}}]\}$. Then we have

$$\begin{aligned} Bias[F(t_i;\hat{k},\underline{\hat{C}})] &\approx E[(\hat{k}\,AUC_i + C_i)/(\hat{k}\,AUC_L + C_L)] - F(t_i;k,C) \\ &\quad + E\left\{\left[(\hat{k}\,AUC_i + C_i)\left(\hat{k}^2\sum_{j=0}^{L} w_j^2 C_j^2 + 2\hat{k}w_L C_L^2 + C_L^2\right) - \hat{k}(\hat{k}\,AUC_L + C_L)\left(\hat{k}\sum_{j=0}^{i} w_j^2 C_j^2 + w_i C_i^2\right)\right]CV_c^2/(\hat{k}\,AUC_L + C_L)^3\right\} \end{aligned} \tag{5}$$

Assessment of Bias in the Wagner–Nelson Estimate

To quantify the bias in the Wagner–Nelson estimate, a one-compartment model with first-order absorption and first-order elimination is assumed. Under the assumed model, $F(t;k,C)$ can be rewritten as a function of k, r, t, and t_L as follows:

$$F(t;k,C) = [1 - \exp(-rkt)]/[1 - \exp(-rkt_L)] \tag{6}$$

where $r = k_a/k$ and k_a is the absorption rate constant.

Bias Resulting from Estimation Error of k

To isolate the effect of estimation error of k from the effect of measurement error of C_i and truncation error in AUC, we assessed the bias conditional on a known $C(t)$ over continuous time for the test product. Then, Eq. (4) can be simplified as a function of k, r, t, t_L, and CV_k, as follows:

$$\begin{aligned} Bias[F(t;\hat{k},C)] &\approx [\exp(-k(t_L + rt)) - \exp(-k(t + rt_L)) + \exp(-kt) \\ &\quad - \exp(-krt) - \exp(-kt_L) + \exp(-krt_L)] \\ &\quad \times \frac{1 - \exp(-kt_L) - [1 - \exp(-rkt_L)]/r}{(1 - 1/r)^2[1 - \exp(-rkt_L)]^3}\,CV_k^2 \end{aligned} \tag{7}$$

The effects of the influential factors on $Bias[F(t;\hat{k},C)]$ are quantified based on the maximum bias ($Bias_{\max}$) over sampling time $0 - t_L$, and the area under the $Bias[F(t;\hat{k},C)]$-vs-time curve (AUB), where

$$AUB = \int_0^{t_L} Bias[F(t;\hat{k},C)]\,dt.$$

$Bias_{max}$ and AUB are computed with $r = 0.5$, 3 or 6; $k = 0.1$ or 0.2; and $CV_k = 0.2$ or 0.4. These input values are chosen to simulate the situations that are likely encountered in practice. The input of t_L is chosen by assuming that $C(t_L)$ is equal to $C(t = 60)$ for drug products with $r = 3$ and $k = 0.1$.

Bias Resulting from Measurement Error of C and Truncation Error in AUC

To isolate the effect of measurement error of C and truncation error in AUC from the effect of estimation error of k, we assessed the bias conditional on a known k. Thus, we can rewrite Eq. (5) as follows:

$$\begin{aligned} Bias[F(t_i;k,\underline{\hat{C}})] \approx\ & (k\,AUC_i + C_i)/(k\,AUC_L + C_L) - F(t_i;k,C) \\ & + [(k\,AUC_i + C_i)(k^2 \sum_{j=0}^{L} w_j^2 C_j^2 + 2k w_L C_L^2 \\ & + C_L^2) - k(k\,AUC_L + C_L)(k \sum_{j=0}^{i} w_j^2 C_j^2 \\ & + w_i C_i^2)]CV_c^2/(k\,AUC_L + C_L)^3 \end{aligned} \quad (8)$$

To examine the effect of measurement errors in $\underline{\hat{C}}$ with a minimal influence of truncation error in AUC, we use the integral of $C(t)$ over time for AUC in Eq. (8) and a dense sampling time points with an interval of 0.1 for obtaining the inputs in Eq. (8) that contain w_j. Bias attributed to measurement error in $\underline{\hat{C}}$ is quantified using Eq. (8) with $r = 0.5$, 3, or 6; $k = 0.1$ or 0.2; and $CV_c = 0.2$ or 0.4. The input of t_L is chosen in the same way as mentioned above.

The influence of truncation errors in AUC on bias is examined by comparing the results from using the dense sampling points with the results using 16 sampling time points $\underline{t}$ = (0, 0.25, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 30.0), given $r = 3$, $k = 0.2$, and $CV_c = 0.2$.

Joint Effects of Errors in $\hat{k}$ and $\underline{\hat{C}}$ and Truncation Error in AUC

The joint effects of estimation errors in $\hat{k}$ and $\underline{\hat{C}}$ and truncation error in AUC on the bias in the Wagner–Nelson estimate are examined using a stochastic simulation, with $r = 3$, $k = 0.2$, $CV_c = 0.2$, and $CV_k = 0.2$, for $\underline{t}$ = (0, 0.25, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 30.0). By assuming $\hat{C}_i = C_i + \varepsilon_i$, with $\varepsilon_i \sim N(0, C_i^2 CV_c^2)$, we generate 5,000 replicates of $\underline{\hat{C}}$. For each replicate of $\underline{\hat{C}}$, we generate an estimate $\hat{k}$ by assuming $\hat{k} = k + \delta$, with $\delta \sim N(0, k^2 CV_k^2)$. The values of random variables are obtained using the SAS RANNOR function (24). From each replicate, $F(t_i;\hat{k},\underline{\hat{C}})$ is calculated for t_i in $\underline{t}$. The mean of $F(t_i;\hat{k},\underline{\hat{C}})$ over the replicates is used as the estimate of $E[F(t_i;\hat{k},\underline{\hat{C}})]$. The joint effects on $Bias[F(t_i;\hat{k},\underline{\hat{C}})]$ are quantified using the difference between the estimate of $E[F(t_i;\hat{k},\underline{\hat{C}})]$ and $F(t_i;k,C)$.

RESULTS

Bias in the Wagner–Nelson Estimate Attributed to Estimation Error of *k*

The expectation of the Wagner–Nelson estimate based on Eqs. (4), (6), and (7) and the true fraction absorbed based on Eq. (6), as a function of post-dosing time for a drug with $k = 0.2$, r = 3, and CV_k, = 0.4, are presented in Fig. 1. The bias in the Wagner-Nelson estimate as a function of post-dosing time is presented in Fig. 2 showing two levels of k, in Fig. 3 showing three levels of r, and in Fig. 4 showing two levels of CV_k.

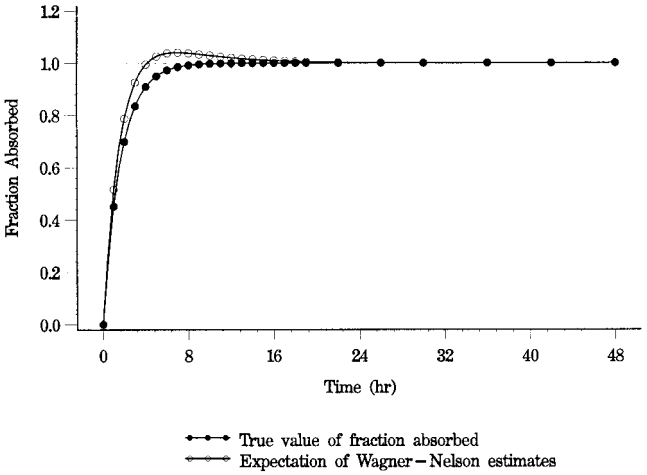

Fig. 1. The true value of fraction absorbed and the expectation of Wagner–Nelson estimates of fraction absorbed as a function of post-dosing time, for drugs with elimination rate constant (k) equal to 0.2, ratio of absorption rate constant vs. k (r) equal to 3, and coefficient of variation for estimate of k (CV_k) equal to 0.4 based on Eq. (3), (6), and (7).

Bias in Wagner–Nelson Estimates of the Fraction of Drug Absorbed

As shown in Figs. 1–4, the bias was greater than 0 over the post-dosing time period before the time of the last quantifiable concentration. Figures 1–4 show that the bias increased with time for the initial time period post-dose and then decreased with time until the time of the last quantifiable concentration, when the bias became 0 (Fig. 1). The $Bias_{max}$ occurs at time $t = \ln[(G + rk)/(G + k]/[k(r - 1)]$, where $G = rk[\exp(-kt_L) - \exp(-rkt_L)]/\{r[1 - \exp(-kt_L)] - 1 + \exp(-rkt_L)\}$.

The degree of bias relative to $F(t;k,C)$ is highest immediately following the dosing, and then decreases at a decreasing rate (Figs. 2–4). The pattern of the bias over time implies that the bias will inflate the absorption rate mainly during the absorption phase of an orally administered drug.

The bias can drive the expectation of the Wagner–Nelson estimate out of its parameter space, i.e., the estimate can be greater than unity (Fig. 1). For example, $E[F(t;\hat{k},C)] > 1$ for $t \in [4.2, 29.9]$, given $k = 0.2$, $r = 3$, $CV_k = 0.4$, and $t_L = 30$ (Eqs. 4, 6, and 7; Fig. 1).

Relationship of the Estimation Bias with k

The $Bias_{\max}$ is not correlated with k (Fig. 2). For example, given $r = 3$ and $CV_k = 0.2$, $Bias_{\max} = 0.023$ or 2.8% of $F(t;k,C)$ for both $k = 0.1$ and $k = 0.2$ (Fig. 2). However, AUB decreases with k (Fig. 2). Given $r = 3$ and $CV_k = 0.2$, $AUB = 0.388$ for $k = 0.1$, whereas $AUB = 0.189$ for $k = 0.2$ (Fig. 2).

A high value of k implies a short half-life. Thus drug products with a short half-life tend to have a lower AUB compared with drugs with a long half-life.

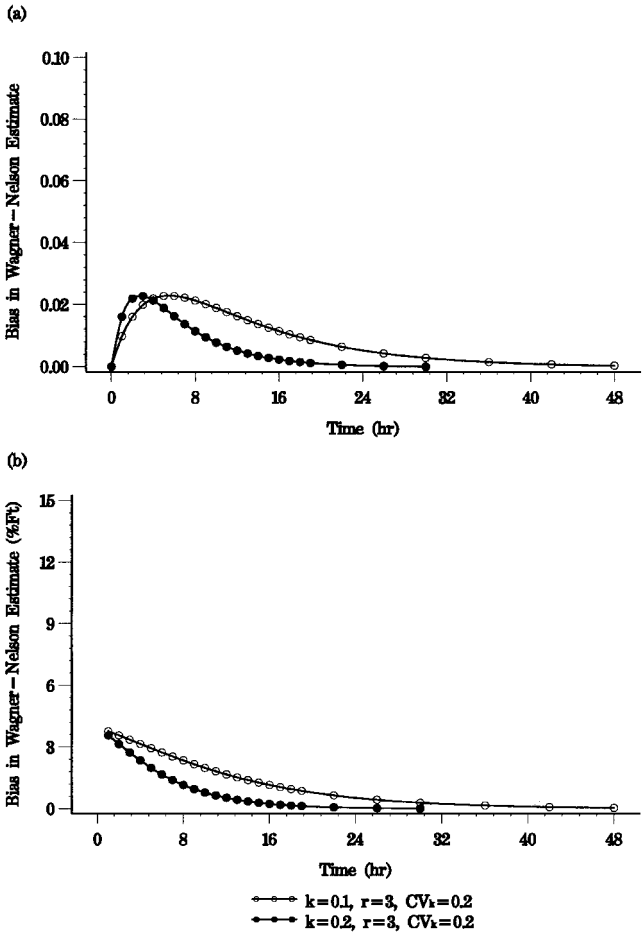

Fig. 2. The bias in Wagner–Nelson estimates of fraction absorbed as function of post-dosing time, for drugs with elimination rate constant (k) equal to 0.1 or 0.2, ratio of absorption rate constant vs. k (r) equal to 3, and coefficient of variation for estimate of k (CV_k) equal to 0.2 based on Eq. (7). (a) Absolute value; (b) in percentage of the true fraction absorbed (F_t).

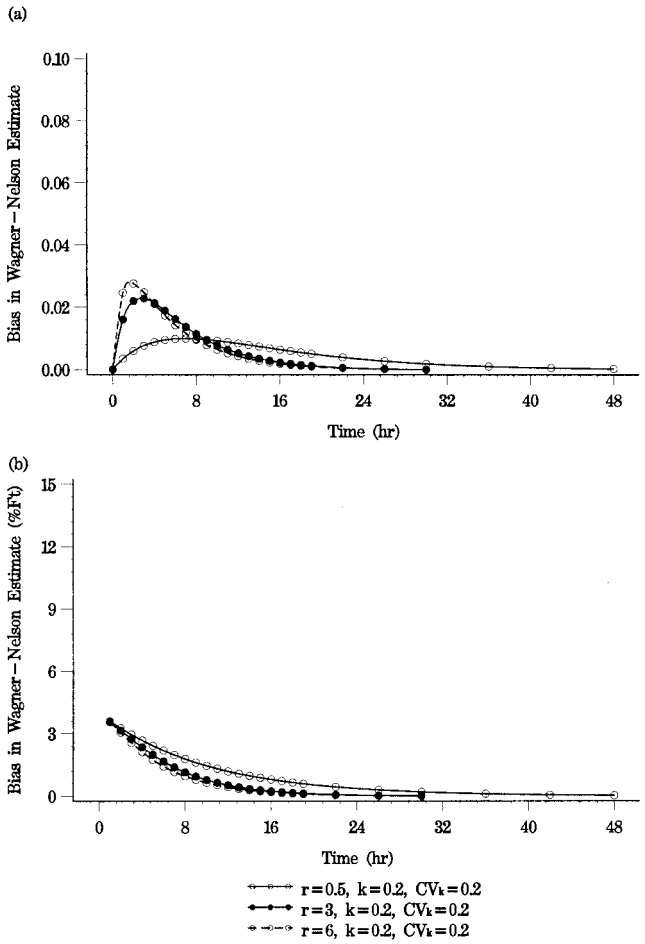

Fig. 3. The bias in Wagner–Nelson estimates of fraction absorbed as function of post-dosing time, for drugs with elimination rate constant (k) equal to 0.2, ratio of absorption rate constant vs. k (r) equal to 0.5, 3 or 6, and coefficient of variation for estimate of k (CV_k) equal to 0.2 based on Eq. (7). (a) Absolute value; (b) in percentage of the true fraction absorbed (F_t).

Relationship of the Estimation Bias with r

The $Bias_{\max}$ is positively correlated with r (Fig. 3). For example, given $k = 0.2$ and $CV_k = 0.2$, $Bias_{\max} = 0.023$ [2.8% of $F(t;k,C)$] for $r = 3$, whereas $Bias_{\max} = 0.028$ [3.1% of $F(t;k,C)$] for $r = 6$ (Fig. 3), representing a 21% increase in $Bias_{\max}$. However, AUB is insensitive to changes in r (Fig. 3). Given $k = 0.2$ and $CV_k = 0.2$, $AUB = 0.194$ for $r = 0.5$ and $AUB = 0.189$ for either $r = 3$ or $r = 6$ (Fig. 3). This implies that r influences mainly $Bias_{\max}$.

Relationship of the Estimation Bias with $\mathrm{CV_k}$

$Bias_{\max}$ and AUB are positively correlated with CV_k (Fig. 4). For example, given $k = 0.2$ and $r = 3$, $Bias_{\max} = 0.023$ [2.8% of $F(t;k,C)$] and $AUB = 0.189$ for $CV_k = 0.2$ whereas $Bias_{\max} = 0.092$ [11.3% of $F(t;k,C)$] and $AUB = 0.758$ for $CV_k = 0.4$.

The sensitivity of $Bias_{\max}$ and AUB to change in CV_k is much higher compared to the sensitivity to the change in k or r. Given $k = 0.2$ and $r = 3$, a change in CV_k from $CV_k = 0.2$ to $CV_k = 0.4$ results in a 300% increase in $Bias_{\max}$and a 301% increase in AUB.

When a mean of two independent estimates of k is used as $\hat{k}$ in the Wagner–Nelson estimator, $Bias_{\max}$ and AUB will be reduced by 50% from the value when a single estimate was used because CV_k^2 will reduced by 50% according to Eq. (7).

Bias Attributed to Measurement Error of *Ci*

The bias attributed to measurement error in $\hat{\underline{C}}$ based on Eq. (8) using dense sampling time points is close to 0 ($Bias_{\max} < 0.002\%$ of $F(t;k,C)$] with all the parameter combinations: $k = 0.1$ or 0.2; $r = 0.5$, 3, or 6; and $CV_c = 0.2$ or 0.4. The results suggest that the measurement errors in $\hat{\underline{C}}$ alone do not introduce bias in the Wagner-Nelson estimate of the fraction of drug absorbed.

Bias Attributed to Truncation Error in AUC

The truncation error in AUC resulted in a negative bias. Given $k = 0.2$, $r = 3$, and $CV_c = 0.2$, the bias vs. time curve goes down first and then turns up towards 0 at $t_L = 30$ (see Fig. 5). The maximum bias is equal to –0.015, that is, approximately –2.1% of $F(t;k,C)$.

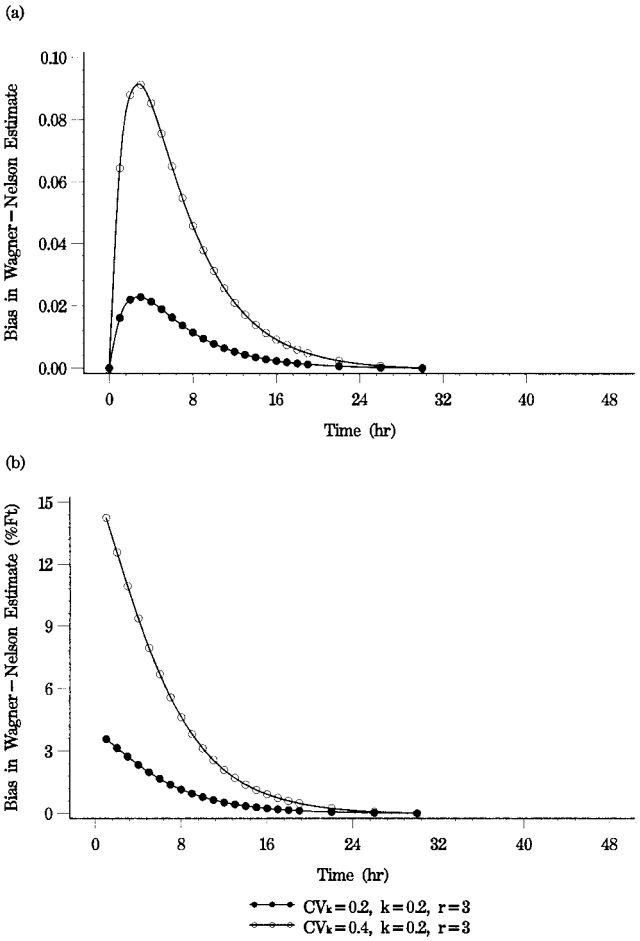

Fig. 4. The bias in Wagner–Nelson estimates of fraction absorbed as function of post-dosing time, for drugs with elimination rate constant (k) equal to 0.2, ratio of absorption rate constant vs. k (r) equal to 3, and coefficient of variation for estimate of k (CV_k) equal to 0.2 or 0.4, based on Equation 7. (a) Absolute value; (b) in percentage of the true fraction absorbed (F_t).

Joint Effects of Estimation Errors in $\hat{k}$ and $\hat{\underline{C}}$ and Truncation Error in AUC

Results of the stochastic simulation with $r = 3$, $k = 0.2$, $CV_k = 0.2$, and $CV_c = 0.2$ using the selected time points are presented in Fig. 5. As shown in Fig. 5, the bias resulting from the joint effects increased with time over the initial post-dosing period and then decreased. After decreasing to –0.006, the bias showed a slow increasing trend towards 0 at $t_L = 30$. Figure 5 shows that $Bias_{\max}$ due to the joint effect is equal to 0.019 at $t = 3$.

The bias resulting from the joint effects is not equal to the sum of the biases as a result to the separate effects of estimation error in $\hat{k}$ and truncation error in AUC. For example, at $t = 3$, the bias due to estimation error in $\hat{k}$ is 0.023; the bias due to the truncation error in AUC is –0.012; their sum is 0.011; but the actual bias is 0.019. This implies that the effects of estimation error in $\hat{k}$ and of truncation error in AUC are not additive.

As shown in Fig. 5, the bias due to the joint effects during the initial period post-dose is mainly attributed to the influence from estimation error in $\hat{k}$, whereas the bias in the terminal phase post-dose is mainly attributed to the influence from the truncation error in AUC.

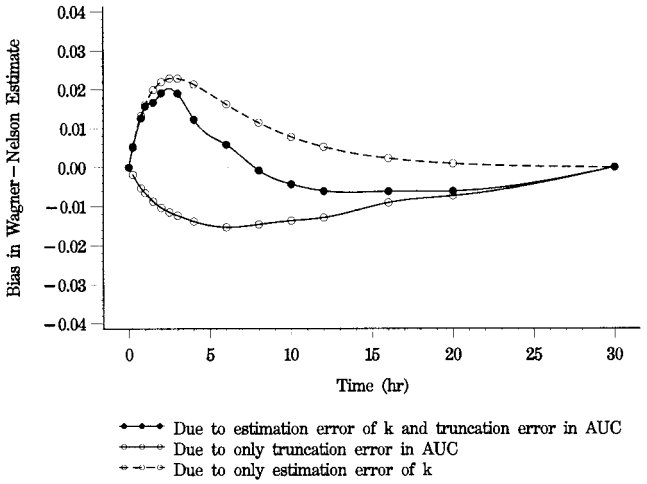

Fig. 5. The bias in Wagner–Nelson estimates of fraction absorbed as function of post-dosing time, for drugs with elimination rate constant (k) equal to 0.2, ratio of absorption rate constant vs. equal to 3, coefficient of variation for estimate of k equal to 0.2, and coefficient of variation for drug concentration equal to 0.2 based on one-compartment model, where the bias due to only estimation error of k is from Eq. (7), the bias due to only the truncation error is from Eq. (8) based on the sampling time points defined in the text, and the bias due to their joint effect is from the simulation.

DISCUSSION

In this study, we investigated the influence of estimation errors of the elimination rate constant, measurement error of the drug concentration, and the truncation error in AUC on the Wagner–Nelson estimate of the fraction of drug absorbed. Estimation error of the elimination rate constant results in an upward bias in the Wagner–Nelson estimate, mainly in the early drug absorption phase. The truncation error in AUC can result in a downward bias in the Wagner–Nelson estimate, which may partially offset the upward bias due to estimation error of the elimination rate constant in the early absorption phase. The bias due to the truncation error tends to be dominant in the terminal phase. Measurement error of concentration does not introduce bias in the Wagner–Nelson estimate. The joint effect of estimation error of the elimination rate constant and truncation error in AUC can result in a non-monotonic fraction of drug absorbed vs. time profile. However, only estimation error of the elimination rate constant can lead to the Wagner–Nelson estimate of fraction of drug absorbed greater than unity.

The magnitude of the bias due to estimation error of the elimination rate constant is determined by the elimination rate constant, the ratio of the absorption rate to the elimination rate, and the variability of the estimated elimination rate constant. The variability of the estimated elimination rate constant shows the biggest effect. The bias inflates the fraction of drug absorbed vs. time profiles mainly in the early post-dosing period, which would erroneously lead to a conclusion of a quicker absorption rate than the true rate for the drug product.

The bias due to estimation error of the elimination rate constant is most sensitive to variability of the estimates. For

drug products that show high intrasubject variability in clearance, the Wagner–Nelson estimate of the fraction absorbed can be significantly inflated by the estimation error of the elimination rate constant. The bias can be reduced dramatically by use of mean estimates of the elimination rate constant. This is very helpful information when absorption of highly variable drugs is estimated using the Wagner–Nelson method and several independent estimates of the elimination rate constant of the same drug are available, as in studies for development of IVIVC, where there are several test products and a reference of the same drug ingredient.

The influence of the truncation error in AUC was evaluated under one set of time points for the cases selected. The selection of sampling time points follows the common practice in pharmacokinetic studies, in which dense sampling points are selected in the early post-dosing period and around the time for the presumed peak concentration. The observed bias in the Wagner–Nelson estimate of the fraction absorbed due to the truncation error in AUC may vary when a different set of time points is selected. However, the trend and the magnitude of the bias relative to the bias resultin from estimation error of the elimination rate constant are similar when using different sets of the sampling time points (results not shown).

In development of a level-A IVIVC, a common approach is to find a linear relationship between the absorption-time profile with the *in vitro* dissolution-time profile. The Wagner–Nelson estimate of the fraction of drug absorbed is frequently explored to transform the drug concentration-time profile into a fraction of drug absorbed vs. time profile. The bias in the Wagner–Nelson estimate can change the absorption-time profile, and as such can change the true relationship between the fraction of drug absorbed vs. time profile and the *in vitro* dissolution-time profile. Therefore, an unnecessary adjustment for time discrepancy, i.e., time scaling (21), may be encountered, particularly for highly variable drug products. For the purpose of IVIVC development, one may bypass the Wagner–Nelson transformation to avoid influence from the bias by predicting $C(t)$ or AUC_i/AUC_L directly from the *in vitro* dissolution-time profile.

APPENDIX: DERIVATION OF $E[F(t_i;\hat{k},\underline{\hat{C}})]$

Let $\underline{\hat{C}}$ be the vector of observed concentrations ($\hat{C}_0$, $\hat{C}_1, \ldots, \hat{C}_L$), where $\hat{C}_i$ is the observed concentration at time t_i ($i = 0,1, \ldots, L$). Let $X = \hat{k}\, A\hat{U}C_i + \underline{\hat{C}}_i$ and $Y = \hat{k}\, A\hat{U}C_L + \hat{C}_L$, where $\hat{k}$ is the estimate of elimination rate constant k and $A\hat{U}C_i$ is the area under the observed plasma concentration-time curve from time 0 to t_i by the trapezoidal rule. Thus, we have $F(t_i;\hat{k},\underline{\hat{C}}) = X/Y$, and $E[F(t_i;\hat{k},\underline{\hat{C}})|\underline{\hat{C}}] = E[X/Y|\underline{\hat{C}}]$. Because there is no closed form for $E[X/Y|\underline{\hat{C}}]$, we may explore the second-order Taylor expansion to obtain an accurate approximation to $E[F(t_i;\hat{k},\underline{\hat{C}})|\underline{\hat{C}}]$. Following Mood *et al.* (23), we have:

$$E[X/Y|\underline{\hat{C}}] \approx \frac{E[X|\underline{\hat{C}}]}{E[Y|\underline{\hat{C}}]} - \frac{1}{E[Y|\underline{\hat{C}}]^2}\operatorname{cov}[X,Y|\underline{\hat{C}}] + \frac{E[X|\underline{\hat{C}}]}{E[Y|\underline{\hat{C}}]^3}\operatorname{var}[Y|\underline{\hat{C}}] \tag{1}$$

For given data on a test product, by assuming $\hat{k}$ is estimated from a reference IR product of the same drug ingredient, we have:

$$E[X|\underline{\hat{C}}] = kA\hat{U}C_i + \hat{C}_i,$$

$$E[Y|\underline{\hat{C}}] = k\,A\hat{U}C_L + \hat{C}_L,$$

$$\operatorname{cov}[X, Y|\underline{\hat{C}}] = A\hat{U}C_i\, A\hat{U}C_L \operatorname{var}[\hat{k}], \text{ and}$$

$$\operatorname{var}[Y|\underline{\hat{C}}] = A\hat{U}C_L^2 \operatorname{var}[\hat{k}]$$

assuming $\hat{k}$ is an unbiased estimate of k derived from the linear regression of log-concentration on time in the terminal phase.

From substituting $E[X|\underline{\hat{C}}]$, $E[Y|\underline{\hat{C}}]$, $\operatorname{cov}[X,Y|\underline{\hat{C}}]$, and $\operatorname{var}[Y|\underline{\hat{C}}]$ in Eq. (1) by the above corresponding expressions, we can obtain the following:

$$E[F(t_i;\hat{k},\underline{\hat{C}})|\underline{\hat{C}}] \approx \frac{k\,A\hat{U}C_i + \hat{C}_i}{k\,A\hat{U}C_L + \hat{C}_L} + \frac{[\hat{C}_i\, A\hat{U}C_L - \hat{C}_L\, A\hat{U}C_i]A\hat{U}C_L}{[k\,A\hat{U}C_L + \hat{C}_L]^3}\operatorname{var}[\hat{k}].$$

Thus, we have,

$$E[F(t_i;\hat{k},\underline{\hat{C}})] = E\{E[F(t_i;k\hat{C}|\hat{C}]\} \approx E\left\{\frac{k\,A\hat{U}C_i + \hat{C}_i}{k\,A\hat{U}C_L + \hat{C}_L}\right\} + E\left\{\frac{[\hat{C}_i\, A\hat{U}C_L - \hat{C}_L\, A\hat{U}C_i]A\hat{U}C_L}{[k\,A\hat{U}C_L + \hat{C}_L]^3}\right\}\operatorname{var}[\hat{k}].$$

REFERENCES

1. J. G. Wagner. *Fundamentals of Clinical Pharmacokinetics*. Drug Intelligence Publications, Inc. Hamilton, Illinois, 1975 pp. 174–177.
2. P. Mojaverian, E. Radwanski, C. C. Lin, P. Cho, W. A.Vadino, and J. M. Josen. Correlation of in vitro release rate and in vivo absorption characteristics of four chlorpheniramine maleate extended-release formulations. *Pharm. Res.* **9**:450–456 (1992).
3. C. Caramella, F. Ferrari, M. C. Bonferoni, M. E. Sangalli, M. De Bernardi di Valserra, F. Feletti, and M. R. Galmozzi. In vitro/in vivo correlation of prolonged release dosage forms containing diltiazem HCI. *Biopharm. Drug Dispos.* **14**:143–160 (1993).
4. R. A. Fassihi and W. A. Ritschel. Multiple-layer, direct-compression, controlled-release system: in vitro and in vivo evaluation. *J. Pharm. Sci.* **82**:750–754 (1993).
5. T. Hayashi, T. Ogura, and Y. Takagishi. New evaluation method for in vitro/in vivo correlation of enteric-coated multiple unit dosage forms. *Pharm. Res.* **12**:1333–1337 (1995).
6. M. Durisova and L. Dedik. Modeling in frequency domain used for assessment of in vivo dissolution profile. *Pharm. Res.* **14**:860–864 (1997).
7. P. Mojaverian, J. Rosen, W. A. Vadino, S. Liebowitz, and E. Radwanski. In-vivo/in-vitro correlation of four extended release formulations of pseudoephedrine sulfate. *J. Pharm. Biomed. Anal.* **15**:439–445 (1997).
8. J. E. Polli. In vitro-in vivo relationships of several "immediate" release tablets containing a low permeability drug. *Adv. Exp. Med. Biol.* **423**:191–198 (1997).
9. I. Mahmood. Simple method for the estimation of absorption rate constant (ka) after oral administration. *Am. J. Ther.* **5**:377–381 (1998).
10. K. Yu, M. Gebert, S. A. Altaf, D. Wong, and D. R. Friend. Optimization of sustained-release diltiazem formulations in man by use of an in-vitro/in-vivo correlation. *J. Pharm. Pharmacol.* **50**:845–850 (1998).
11. M. C. Meyer, A. B. Straughn, R. M. Mhatre, V. P. Shah, R. L. Williams, and L. J. Lesko. The relative bioavailability and in

vivo-in vitro correlations for four marketed carbamazepine tablets. *Pharm. Res.* **15**:1787–1791 (1998).
12. L. H. Emara, B. S. el-Menshawi, and M. Y. Estefan. In vitro-in vivo correlation and comparative bioavailability of vincamine in prolonged-release preparations. *Drug. Dev. Ind. Pharm.* **26**:243–251 (2000).
13. L. H. Emara, B. S. el-Menshawi, and M. Y. Estefan. In vitro-in vivo correlation and comparative bioavailablity of vincamine in prolonged-release preparations. *Drug Dev. Ind. Pharm.* **26**:243–251 (2000).
14. J. G. Wagner, D. A. Ganes, K. K. Midha, I. Gonzalez-Younes, J. C. Sackellares, L. D. Olson, M. B. Affrime, and J. E. Patrick. Stepwise determination of multicompartment disposition and absorption parameters from extravascular concentration-time data. Application to mesoridazine, flurbiprofen, flunarizine, labetalol, and diazepam. *J. Pharmacokinet. Biopharm.* **19**:413–455 (1991).
15. M. F. Williams, G. E. Dukes, W. Heizer, Y. H. Han, D. J. Hermann, T. Lampkin, and L. J. Hak. Influence of gastrointestinal site of drug delivery on the absorption characteristics of ranitidine. *Pharm. Res.* **9**:1190–1194 (1992).
16. K. Murata and K. Noda. Pharmacokinetics of multiparticulate sustained-release diltiazem preparations in dogs. *J. Pharm. Sci.* **83**:38–41 (1994).
17. A. A. Nomeir, P. Mojaverian, T. Kosoglou, M. B. Affrime, J. Nezamis, E. Rodwanski, C. C. Lin, and M. N. Cayen. Influence of food on the oral bioavailability of loratadine and pseudoephedrine from extended-release tablets in healthy volunteers. *J. Clin. Pharmacol.* **36**:923–930 (1996).
18. M. Sanaka, Y. Koike, T. Yamamoto, S. Mineshita, S. Yamaoka, S. Hirama, H. Tanaka, and Y. Kuyama. Reliable and convenient parameter of the rate of paracetamol absorption to measure gastric emptying rate of liquids. *Int. J. Clin. Pharmacol. Ther.* **35**: 509–513 (1997).
19. Q. F. Xing. S. Lin, and Y. W. Chien. Transdermal testosterone delivery in castrated Yucatan minipigs: pharmacokinetics and metabolism. *J. Control. Release* **52**:89–98 (1998).
20. S. Mallick, B. K. Gupta, and S. K. Ghosal. Assessment of bioavailability of experimental controlled release microcapsules of nifedipine. *Acta Pol. Pharm.* **57**:175–180 (2000).
21. FDA. Guidance for industry. *Extended Release Oral dosage Forms: Development, Evaluation, and Application in in vitro/in vivo Correlations*. Center for Drug Evaluation and Research, Rockville, Maryland, 1997.
22. M. Gibaldi and D. Perrier. *Pharmacokinetics*. Marcel Dekker, Inc. New York, 1975 pp 149–155.
23. A. M. Mood, F. A. Graybill, and D. C. Boes. *Introduction to the theory of statistics.* McGraw-Hill Publishing Company, New York, 1974 pp 180–181.
24. SAS. *SAS Language, Version 6*. SAS Institute Inc., Cary, North Carolina, 1990 pp 589.