In Vitro-In Vivo Relationship of Oral Extended-Release Dosage Forms

Fang-Yu Liu,[1] Nancy C. Sambol,[2] Robert P. Giannini,[3] and Chui Yu Liu[2,4]

Received January 19, 1996; accepted July 17, 1996

Purpose. A method to establish the in vitro-in vivo relationship of oral extended-release products is proposed.

Methods. The approach utilizes incremental amounts of drug released and absorbed within defined time intervals, to construct a χ^2 distributed variable for testing in vitro-in vivo similarity.

Results. A case study is used to demonstrate that the similarities between incremental values of in vivo absorbed and in vitro dissolved fractions are distinguishable for different dissolution profiles despite naturally significant linear correlations between cumulative in vivo absorbed and in vitro dissolved fractions (with different dissolution tests) of an oral extended-release product.

Conclusions. The method enables investigators to compare different in vitro dissolution profiles of an oral extended-release product to find an optimized dissolution profile to be the surrogate of the in vivo release process of the product.

KEY WORDS: extended-release; in vitro-in vivo correlation; in vitro dissolution; in vivo release; absorption; in vitro-in vivo similarity.

INTRODUCTION

Since the 1940s (1), in vitro characteristics of a dosage form have long been sought as a mirror of in vivo behavior. In vitro-in vivo correlation can be described as "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 property of the dosage form" (2). The pharmacokinetic system of an extended-release dosage form can decompose as release (R), absorption (A), distribution (D), metabolism (M) and elimination (E), or RADME (3). In vivo release kinetics is the most important physicochemical property for an extended-release product, but this process is generally unobservable. The in vitro dissolution process can be sought as a substitution for the in vivo release process of a dosage form. The in vivo absorption process, a biological property of the dosage form, can be estimated by drug concentrations observed in the body. Thus, a special definition of "in vitro-in vivo correlation" for extended-release products can be addressed as the establishment of a relationship between in vitro dissolution behavior and in vivo absorption behavior. If the correlation is of the time courses of these two processes [e.g., percent dose dissolved relative to the total dose (dissolved fraction) *vs.* percent dose absorbed relative to the total dose absorbed (absorbed fraction) over a defined time period (4–9)], it is a level A correlation (2,5). In this paper, we address the level A correlation.

Delay in absorption, relative to in vivo release, is a characteristic of extended-release products (3), because the absorption of a drug is usually controlled by solubility (dissolution) and permeability. The curve for the absorbed fraction *vs.* time is expected to be below the curve for the released fraction *vs.* time. These two curves start at the same origin (0 fraction at time 0), separate later, begin to merge after release is complete (after which released fraction is a constant, 100%), and merge into one after absorption is complete (Fig. 1). Therefore, the relationship between absorbed fractions and released fractions is nonlinear, and the unity line, as conventionally used in the literature for demonstrating in vitro-in vivo correlation, is generally not the expected relationship. In addition, the absorbed and dissolved fractions are traditionally expressed in cumulative percentages. Theoretically, a positive relationship is inherent with the use of cumulative fractions, and the linear correlation of any two sets of cumulative fractions is naturally high (see Discussion).

We propose a method to analyze the in vitro-in vivo relationship of extended-release drug products. This method uses incremental values of dissolved and absorbed fractions, instead of cumulative values, to construct a χ^2 for demonstrating the in vitro-in vivo similarity of an extended-release product. These χ^2s enable comparison of different in vitro dissolution profiles of a product to find an appropriate dissolution profile that represents the in vivo release process of the product. The details of this method are demonstrated in a case study.

METHODOLOGY

The in vitro-in vivo relationship of an extended-release product is meaningful only in the time period during which both in vivo release and absorption are occurring (i.e., before the in vivo release is complete). Within this time period, for an extended-release product the absorbed amount should be highly associated with the released amount. After the duration of in vivo release, T, the released fractions are always equal to 100% without variation. Therefore, the absorbed fractions should be normalized by the absorbed fraction at T, $A(T)$, to ensure the profile of normalized absorption fractions presenting similar patterns to the profile of released fractions, and the two profiles have the same scales for both time (from 0 to T) and percentage (from 0 to 100%). If an in vitro dissolution profile approximates to the in vivo release profile, a similarity should exist between the in vitro dissolution profile and the normalized absorption profile.

The first step of the proposed method is to fit an appropriate pharmacokinetic model (3) to individual drug concentration-time data separately via nonlinear regression to estimate the pharmacokinetic parameters (Table I). Based on the sampling

[1] Impax Pharmaceuticals, Fremont, California 94539.
[2] Department of Pharmacy, University of California, San Francisco, California 94143-0446.
[3] Watson Laboratories, Corona, California 91720.
[4] To whom correspondence should be addressed.

ABBREVIATIONS: t_0: absorption lag time; T: duration of in vivo release; $A(t)$: absorbed fraction at time t; I_r: incremental value fraction of in vivo release; I_d: incremental value fraction of in vitro dissolution; I_a: normalized incremental fraction of in vivo absorption; s: standard deviation of I_a; $C(t)$: plasma drug concentration at time t; $X_p(t)$: amount of drug in the peripheral compartment at time t; k_a: apparent first-order absorption rate constant; k_{10}: exit rate constant from the central compartment to the elimination site; k_{12}: exit rate constant from the central compartment to the peripheral compartment; k_{21}: exit rate constant from the peripheral compartment to the central compartment.

0724-8741/96/1000-1501$09.50/0

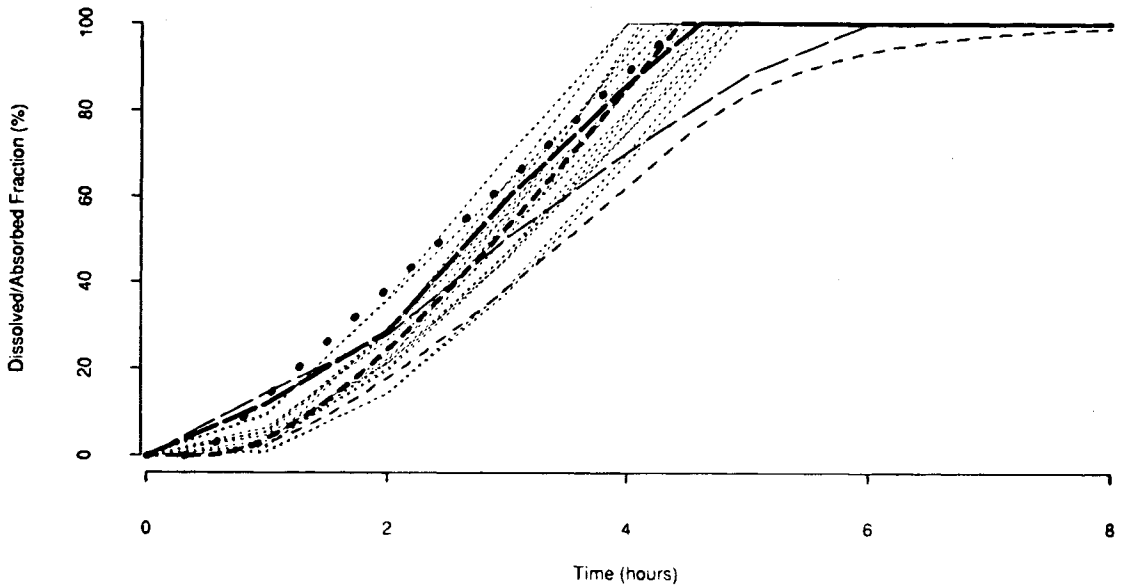

Fig. 1. In vitro and in vivo characteristics of an investigational product. The thin-long dashed line (- - -) represents the dissolution profile of Test 1; the thick-long dashed line (— — —) the dissolution profile of Test 2; the thick dotted line (•••) the population in vivo release profile; the thin-short dashed line (-----) the population in vivo absorption profile; the thick-short dashed line (------) the population normalized in vivo absorption profile; and the thin dotted lines (·····) individual's normalized in vivo absorption profiles.

schedule of both the in vitro dissolution test (its duration is expected to be approximately T) and the drug concentration-time data, a series of time points can be determined for both dissolved and absorbed fractions. Individual absorbed fractions are then calculated, using the observed concentrations and pharmacokinetic parameter estimates, from the first sampling time until the sampling time around T.

Let I_r denote the incremental value of in vivo released fraction a specific time interval, I_a the corresponding incremental value of normalized absorption fractions, I_d the corresponding incremental value of dissolved fraction, and s^2 the variance of I_a. The statistical assumption of the proposed method is that I_r approximately follows a normal distribution $N(\mu, \sigma^2)$. With the null hypotheses H_0: $E(I_a) - \mu = E(I_d) - \mu = 0$ and $D(I_a) - \sigma^2 = 0$ (E and D denote the mathematical expectation and variance, respectively), $(I_a - \mu)/s$ and $(I_d - \mu)/s$ follow a standard normal distribution $N(0, 1)$, and $(I_a - I_d)/(\sqrt{2}s)$ then follows $N(0, 1)$ as well. Hence, a χ^2 for the i-th subject can be determined from Eq. (1):

$$\chi^2_{i,df=n} = \sum_{j=1}^{n} \frac{(I_{a,ij} - I_{r,j})^2}{2s_j^2}, \quad (1)$$

in which, $I_{a,ij}$ is the j-th element of the vector of the incremental normalized absorption fractions in the i-th subject, $I_{r,j}$ the j-th element of the vector of the incremental dissolved fractions, n the number of elements in each incremental vector, and s_j^2 the variance of $I_{a,ij}$s (over i) that can be calculated from the following Eq. (2):

$$s_j^2 = \frac{1}{m-1} \sum_{i=1}^{m} (I_{a,ij} - \bar{I}_{a,j})^2, \quad (2)$$

in which m is the number of subjects and $\bar{I}_{a,j} = m \sum_{i=1}^{m} I_{a,ij}$ the mean of $I_{a,ij}$s (over i). These χ^2s are the statistics for demonstrating the in vitro-in vivo similarity of the investigational extended-release product and can be used to compare different in vitro dissolution profiles.

Table I. Individual Estimates of Pharmacokinetic Parameters

Subject ID	Parameter						
	t_0 (h)	T (h)	k_{21} (h^{-1})	k_a (h^{-1})	α (h^{-1})	β (h^{-1})	V_c/F (L)
1	0.464	4.42	0.184	0.634	0.507	0.0747	756
2	0.449	4.40	0.296	1.49	1.34	0.0958	189
3	0.214	3.84	0.319	1.03	0.931	0.0810	760
4	0.587	4.10	0.108	0.522	0.394	0.0595	1180
5	0.230	4.78	0.110	0.470	0.422	0.0650	621
6	0.247	4.72	0.566	1.01	0.611	0.0800	1130
7	0.522	4.60	0.113	0.652	0.460	0.0866	1700
8	0.739	4.87	0.128	0.569	0.463	0.0751	454
9	0.404	4.51	0.265	1.40	0.727	0.0732	581
10	0.768	4.22	0.249	3.02	0.445	0.0795	769
11	0.666	4.14	0.102	0.918	0.436	0.0647	547
12	0.617	4.88	0.144	1.30	0.654	0.0711	526
13	0.379	3.85	0.0934	0.499	0.430	0.0508	2380
14	0.655	4.94	0.0990	0.462	0.271	0.0554	639
15	0.507	4.66	0.353	0.620	0.420	0.0759	1590
16	0.366	4.34	0.161	2.96	0.582	0.0953	484

RESULTS

Dissolved Fractions

An in vitro dissolution profile of an investigational extended-release product is shown in Table II (Test 1) and Fig. 1. The in vitro dissolution test was performed using a USP

Table II. Dissolved and Absorbed Fractions

Dissolution Test	Dissolved Fractions j = 1	2	3	4	5	
1	14.4	27.8	50.5	70.4	88.4	
2	12.0	28.7	59.9	86.0	100.0	
Subject ID (i)	$A(t_{ij})$ (j) 1	2	3	4	5	$A(T_i)$
1	2.17	5.53	26.2	54.0	80.3	63.4
2	4.75	22.9	51.9	70.3	91.6	83.1
3	7.50	17.5	52.5	78.1	97.2	73.9
4	1.17	4.34	19.6	54.1	69.6	54.2
5	2.84	9.29	23.2	44.2	62.1	58.8
6	5.59	15.3	19.9	38.1	100	78.1
7	1.99	4.67	16.9	44.1	87.4	65.0
8	0.52	3.23	15.6	40.8	67.3	61.5
9	5.28	23.4	43.5	71.2	93.2	82.8
10	2.08	26.1	57.1	81.3	96.8	90.4
11	1.37	14.5	36.6	58.9	78.2	69.9
12	2.25	18.9	35.4	58.4	87.6	82.0
13	2.46	7.21	28.9	47.4	62.1	51.4
14	0.65	4.81	10.6	24.0	65.9	56.5
15	1.69	11.4	24.0	52.4	69.7	64.1
16	9.70	32.6	60.0	81.5	97.2	91.5

Apparatus 2, paddle (consisting of 6 tubs) stirrer operating at 100 rpm in 900 ml of simulated gastric fluid (SGF, pH 1.2) for 1 hr and then in 900 ml of simulated intestinal fluid (SIF, pH 7.4) for another 15 hr. The dissolution profile suggests an approximate zero-order release.

Absorbed Fractions

The corresponding oral immediate-release product presents a profile that indicates a two-compartment pharmacokinetic model with first-order absorption. The pharmacokinetic model used for an oral extended-release product with zero-order in vivo release, first-order absorption and two-compartment disposition is described by Liu et al. (3).

A study of the investigational product was carried out in 16 healthy volunteers [the first 6 subjects were used in Liu et al. (3) for pharmacokinetics only]. Each subject had up to 16 plasma concentrations measured at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18, 24 and 36 hr after the dose. The pharmacokinetic model, with 7 parameters, t_0, T, V_c/F, k_{21}, k_a, α and β, was fit to each individual's data *separately* via nonlinear regression analysis using the NONMEM software (10). The estimates of the parameters are provided in Table I. The in vitro dissolved fractions and in vivo absorbed fractions based on the geometric means of these parameter estimates are depicted in Fig. 1. The concentrations predicted from the parameter estimates were in good agreement with the observations (Fig. 2).

The in vivo release appears to be complete before 5 hr after the dose. Referring to the sampling schedule of plasma concentrations, 5 time points (1, 2, 3, 4 and 5 hr) were chosen to determine the time course of the in vitro-in vivo relationship (the last time point should be around time T).

With a two-compartment system, the absorbed fraction at time t, $A(t)$, can be calculated using the following equation:

$$A(t) = \frac{C(t) + \frac{1}{V_c} X_p(t) + k_{10} AUC_t}{k_{10} AUC_\infty}, \quad (3)$$

in which,

$$\frac{X_p(t)}{V_c} = k_{12} e^{-k_{21}(t-t_0)} \int_{t_0}^{t} e^{k_{21}u} C(u)\, du.$$

$$\approx k_{12} e^{-k_{21}(t-t_0)} \sum_{j=1}^{n} \frac{e^{k_{21}(t_{(j)}-t_0)} C(t_{(j)}) + e^{k_{21}(t_{(j-1)}-t_0)} C(t_{(j-1)})}{2} \Delta t_{(j)}, \quad (4)$$

$t_0 = t_{(0)} < t_{(1)} < \cdots < t_{(n)} = t$ and $\Delta t_{(j)} = t_{(j)} - t_{(j-1)}$ (3).

With a zero-order release, $A(T)$ can be calculated (3) as follows:

$$A(T) = \frac{1}{k_a(T - t_0)} [e^{-k_a(T-t_0)} + k_a(T - t_0) - 1]. \quad (5)$$

Table II lists the absorbed fractions in all 16 subjects.

In Vitro-In Vivo Similarity

In each subject, $A(t_j)/A(T)$ provides normalized absorption fractions to ensure the relevant comparison with in vivo released fractions. When a normalized absorption fraction exceeds 100%, its value should be taken as 100%. An incremental vector in each subject is then determined by the normalized absorption fractions. These values, along with the incremental vector, for the dissolved fractions, are shown in Table III. Fig. 1 illustrates individual normalized absorption fractions *vs.* time for the 16 subjects. Using Eq. (1), a χ^2 was determined from the absorbed and dissolved incremental values of each subject (Table III). With 5 degrees of freedom, the critical value for a χ^2 at 0.05 nominal level is $\chi^2_{df=5}(0.95) = 11.1$, i.e., $\text{pr}(\chi^2_{df=5} > 11.1) = 0.05$. It appears that the $\chi^2_{df=5}$s of subjects 4, 8 and 14 were greater than the critical value.

According to the additive property of χ^2, one can construct an overall $\chi^2_{df=80}$ by summing up the 16 individual $\chi^2_{df=5}$s together. The overall χ^2 based on this dissolution test was $\chi^2_{df=80} = 144$, and the corresponding critical value at 0.05 nominal level was $\chi^2_{df=80}(0.95) = 102$. Note a greater χ^2 indicates less similarity and vice versa, and an overall χ^2 greater than the critical value indicates an unfavorable in vitro-in vivo similarity.

Optimization of In Vitro Dissolution Test

The investigational product dissolves faster in pH 3–8, than in pH 1–3, and it is relatively hard and erodible, which makes the dissolution even faster with abrasion. Thus, in the gastrointestinal (GI) tract, the release should be faster after the product reaches the duodenum because of the higher pH and the presence of peristalsis. Therefore, we changed the conditions of the in vitro dissolution test to 900 ml SGF at 50 rpm for 1.5 hr and then 900 ml SIF plus 10 g glass beads at 150 rpm for another 14.5 hr (we increased the stirring rotation to 150 rpm without glass beads, but the dissolution

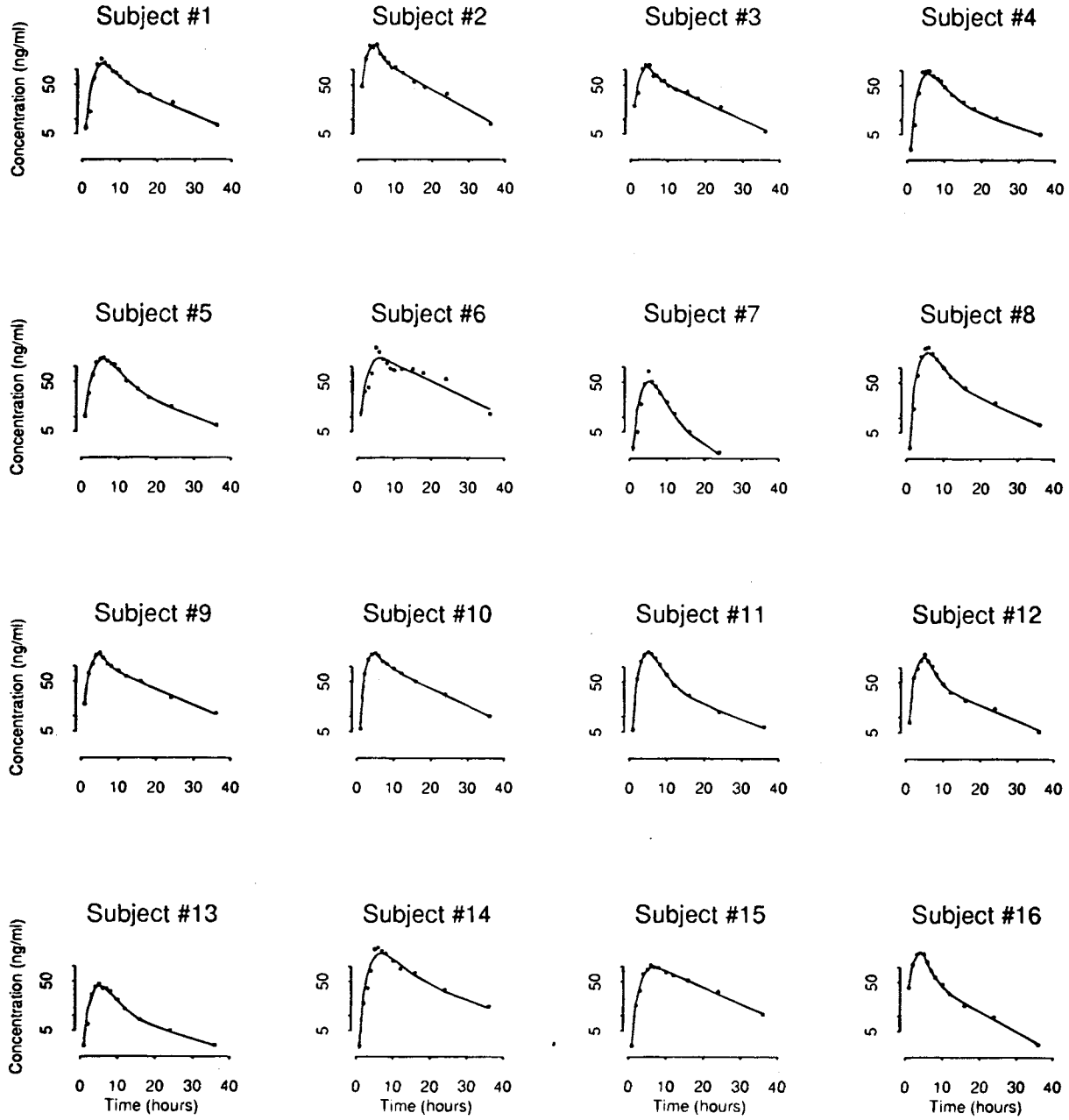

Fig. 2. Predicted concentrations (solid lines) and data (points) for each subject.

was not significantly faster than that with 100 rpm). The incremental values of dissolved fractions under these conditions are listed in Table III (Test 2). The $\chi^2_{df=5}$s for the 16 subjects are shown in Table III, and the overall $\chi^2_{df=80}$ with this dissolution test was 98.8 [it is less than the critical value $\chi^2_{df=80}(0.95) = 102$; although the $\chi^2_{df=5}$s of Subjects 4 and 14 were still greater than $\chi^2_{df=5}(0.95) = 11.1$]. Therefore, as a surrogate of the in vivo release process of the product, the second dissolution profile is better than the first one, and the in vitro-in vivo similarity based on the second dissolution profile is generally acceptable.

Since the release of the product might vary at different GI sites, we tried to fit a model incorporating a two-piece zero-order in vivo release kinetics to the data. Corresponding parameter estimates were in a good agreement with those listed in Table I, but neither the goodness-of-fit nor the in vitro-in vivo similarity improved significantly. The real in vivo release rate of the product may not be a constant, but the process can be approximated to a one zero-order reasonably.

Note that a normalized in vivo absorption fraction is not expected to be the same as the in vivo released fraction at the same time $t < T$ (e.g., Fig. 1, in which both populational fractions are based on the geometric means of parameter estimates listed in Table I). Therefore, the minimization of χ^2 is not applicable to the optimization of the in vitro dissolution test. An appropriate dissolution profile of an extended-release product should be the one that incorporates physicochemical and physiological considerations and demonstrates a reason-

Table III. In Vitro-in Vivo Similarity in the 16 Subjects

Dissolution Test	Increment of Dissolved Fractions, $l_{r,j}$ j = 1	2	3	4	5
1	14.4	13.4	22.7	19.9	18.0
2	12.0	16.7	31.2	26.1	14.0

Subject ID (i)	Increment of Normalized Absorbed Fractions, $l_{a,ij}$ j = 1	2	3	4	5	$\chi^2_{i,df=5}$ Based on Test 1	Test 2
1	3.42	5.30	32.6	43.9	14.8	10.3	6.63
2	5.72	21.8	34.9	22.1	15.4	5.53	2.59
3	10.2	13.5	47.4	29.0	0.00	4.52	1.78
4	2.16	5.85	28.2	63.7	0.18	17.8	12.8
5	4.83	11.0	23.7	35.7	24.8	6.38	4.03
6	7.16	12.4	5.89	23.3	51.2	6.27	6.79
7	3.06	4.12	18.8	41.9	32.2	10.5	8.20
8	0.85	4.41	20.1	41.0	33.7	13.5	10.5
9	6.38	21.9	24.3	33.5	14.0	5.08	2.45
10	2.30	26.6	34.3	26.8	10.1	10.7	6.24
11	1.96	18.8	31.6	31.9	15.8	9.97	5.90
12	2.74	20.3	20.1	28.1	28.8	8.64	5.91
13	4.79	9.24	42.2	36.0	7.78	8.27	4.42
14	1.15	7.36	10.3	23.7	57.5	13.9	12.8
15	2.64	15.2	19.7	44.3	18.3	10.4	6.97
16	10.6	25.0	30.0	23.5	10.9	2.34	0.75
					$\chi^2_{df=80}$	144	98.8

able-fit according to the defined absorption profile. Evidently, the second dissolution test (with a set of testing conditions reflecting the release conditions in the gastrointestinal tract, a reasonable dissolution completion closer to the estimated T, and a reasonable overall χ^2 less than the critical value) approached optimization more than the first test. It is apparent that the in vitro-in vivo relationship depends on the in vitro experimental method, and calls attention to the need to optimize in vitro dissolution testing. Note also that when two overall χ^2s are both less than the critical value, an F-test is then possibly needed, which can be conducted using the following formula:

$$F = \frac{\chi^2_{df=n\cdot m,larger}}{\chi^2_{df=n\cdot m,smaller}}. \quad (6)$$

DISCUSSION

Natural Correlation of Cumulative Fractions

Based on Table II, the mean absorbed fractions for the investigational product at 1, 2, 3, 4 and 5 hr were estimated to be 3.25, 13.9, 32.6, 56.2 and 81.6, respectively. The correlation coefficient between these absorbed fractions and the dissolved fractions of Test 1 was estimated to be 0.993, and that of Test 2 was estimated to be 0.980. The linear regression coefficient of absorbed fractions *vs.* dissolved fractions was estimated to be 1.045 with a 95% confidence interval (0.809, 1.281) for Test 1, and to be 0.839 (0.525, 1.153) for Test 2. Both correlations are statistically significant, but one does not differ significantly from the other.

Correlation of any two sets of cumulative fractions is naturally high. To demonstrate this point, we randomly sampled 10 numbers twice from the interval (0, 100) and obtained the following two ascending vectors:

Cumulative vector 1:	2.5	5.1	10.7	13.6	23.9	33.3	68.1	76.0	79.5	99.2
Cumulative vector 2:	8.4	16.8	38.9	41.5	49.9	59.2	62.7	83.9	88.5	97.0

The correlation coefficient between these two random generated vectors was 0.943, with a p-value much smaller than 0.001 (the critical value for correlation coefficients with sample size 10 is 0.632 at 0.05 nominal level, 0.765 at 0.01 level, and 0.872 at 0.001 level). We repeated this random sampling 1,000 times, and the mean correlation coefficient was 0.935. Thus, the correlation between cumulative vectors is naturally high even if no meaningful relationship exists.

Instead, the incremental values (each element of the cumulative vector minus the previous element, or zero for the first element) of the two cumulative vectors can be determined as follows:

Incremental vector 1:	2.5	2.6	5.6	2.9	10.3	9.4	34.8	7.9	3.5	19.7
Incremental vector 2:	8.4	8.4	22.1	2.6	8.4	9.3	3.5	21.2	4.6	8.5

The correlation coefficient of these two vectors was −0.229. Thus linear correlation is generally not an appropriate tool for demonstrating an in vitro-in vivo relationship of oral extended-release products.

Specifying an Oral Dissolution Controlled-Release Product

Over the past two decades, "in vitro-in vivo correlation" has been the topic of countless discussions. The overall pharmacokinetic profile of a drug product is probably too complex to be accurately described by in vitro data. Nevertheless, the in vitro-in vivo relationship is still an extremely useful tool for comparing different formulations of a given compound, and dissolution specifications remain a core issue. Although in vivo absorption varies and is largely uncontrollable, it is well-recognized that an absorption matrix may be estimated by analyzing concentration-time observations. Generally, the in vitro-in vivo relationship is a unique characteristic reflecting the link between biological properties and physicochemical properties of an extended-release product. For specifying an extended-release product, a measurement is needed that is sensitive enough to distinguish an appropriate in vitro dissolution profile that is consistent with a normalized in vivo absorption profile. A reasonable in vitro-in vivo relationship is the presupposition of setting dissolution specifications. Therefore, a reasonable in vitro-in vivo similarity gives investigators a firm basis for setting appropriate dissolution specifications for an extended-release product.

In addition, if investigators can find a set of appropriate dissolution conditions and the in vivo absorption profiles of a

series of modified formulations respond according to the modified in vitro dissolution profiles (i.e., absorption profiles will be sensitive to the changes in dissolution process); they might be able to optimize the in vivo behavior of a product via modifying in vitro dissolution profiles.

REFERENCES

1. USP, XIV, 1950.
2. USP Subcommittee on Biopharmaceutics. *Pharm. Forum* 4160–4161 (1988).
3. F. Y. Liu, N. C. Sambol, R. P. Giannini and C. Y. Liu. *Pharm. Res.* 1995, **12**:720–728.
4. J. Drewe and P. Guitard. *J. Pharm. Sci.* 1993, **82**:132–137.
5. J. P. Skelly, G. L. Amidon, et al.. *Pharm. Res.* 1990, **7**:975–982.
6. Z. Hussein and M. Friedman. *Pharm. Res.* 1990, **7**:1167–1171.
7. K-H. Yuen, A. A. Arvind, A. Desmukh and J. M. Newton. *Pharm. Res.* 1993, **10**:588–592.
8. H. Humbert, M-D. Cabiac and H. Bosshard. *J. Pharm. Sci.* 1994, **83**:131–136.
9. P. Mojaverian, E. Radwanski, et al. *Pharm. Res.* 1992, *9*:450–456.
10. S. L. Beal and L. B. Sheiner. *NONMEM User's Guides*, UCSF: NONMEM Project Group, San Francisco, 1992.