In Vitro/In Vivo Correlation of Dissolution Using Moments of Dissolution and Transit Times

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Summary

It is stated that extended release formulations can serve to trace the absorption profile of the gastro-intestinal tract with respect to a drug considered. The methodology combines the technique of deconvolution and correlation of the in vitro and hypothetical in vivo dissolution profile and is based on statistical moment analysis. Some basic approaches to the evaluation of the mean and variance of in vitro dissolution times and in vivo residence times are therefore reviewed. Furthermore, the differences and messages originating from discrete and continuous in vitro/in vivo correlation need to be examplified.

Rigorous in vitro testing of a new extended release formulation under different dissolution conditions — including non-official apparatuses — is an important source of information to support the above mentioned analysis of the intestinal absorption. This demand is based and discussed on results from physiological and clinical pharmacological experiments.

Combining all — physiological information and the results of in vitro and biopharmaceutical testing — and the continuous in vitro/in vivo correlation by statistical moment analysis shows that the absorption features of the gastrointestinal tract can be traced quantitatively in order to separate the variability in bioavailability due to pharmaceutical and physiological reasons.

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1 Introduction

Drugs are no longer panaceae of an ingenious witch doctor but industrial products. As such they have to fulfil the quality criteria which are expected of industrial products of our time. Even more, since they exert an influence directly on the health of man the quality criteria must exceed those usually applied to industrial products.

Individualization of drug therapy must consider a wide range of factors such as age, state of health, body weight and size, sex, genotype, nutrition, and life-style among others. A physician should therefore be sure that the drug product he intends to use is of high quality and not a source of variability and/or insecurity itself.

In this sense in vitro/in vivo correlation of dissolution must be considered as the only meaningful extension to in vitro quality control, since the ultimate standard in drug dissolution is man. In vitro/in vivo correlation can not claim to enable the prediction of blood level profiles for any possible situation (1). It can, however, trace the variability of drug products under realistic conditions and separate the product variability from that caused by biological factors.

The main object of the present paper is to compile the mathematical tools neccessary to correlate quantitatively the results of in vitro and the in vivo experiments using statistical moment analysis. The theoretical essay is explained with data from a clinical trial and an in vitro experiment with molsidomine and examples taken from the literature.

2 Mathematical tools

2.1 Introductory remarks

One of the most useful properties of the statistical analysis of concentration time data using moments is the additivity of mean times (2, 3). For instance, considering an oral administration of a readily available dosage form, the distribution and elimination of drug within and from the body must be preceded by absorption, which is trivial. However, as a consequence, the total mean time of the drug molecules available is the sum of the mean absorption time MT_{abs} and the mean time in the steady state volume of distribution MT_{vss}.

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It is obvious that we can estimate the two components of the total mean time, i. e. MT_{abs} and MT_{vss}, by an appropriate experimental setting. Because of this very useful property of the statistical analysis of concentration time data by moments, this approach has been entitled component analysis (4). However, before further aspects of component analysis will be discussed, some techniques for evaluating mean times are described.

2.2 Computation of mean times

In vitro release

In case of a theoretical curve describing cumulatively the in vitro release from a solid dosage form M(t), the mean in vitro dissolution time is defined as follows (5):

$$MT_{diss.vitro} = \frac{{}_0\int^{\infty} t\ dM(t)}{{}_0\int^{\infty} dM(t)} = \frac{{}_0\int^{\infty} t\ dM(t)}{M_0} \qquad (1)$$

where M_0 ist the amount of drug finally released from the dosage form. Yamaoka and his colleagues have used the differential form of the above definitions (6, 7) and by this introduced a redundant computational step in the practical evaluation of mean times (8).

In reality, however, we handle with measurements ΔM_i of the amounts released from the dosage form during certain time periods (t_i, t_{i+1}) and may be unable or do not wish to formulate a theoretical dissolution profile M(t). Nevertheless, we want to estimate the mean in vitro dissolution time using the actual readings ΔM_i. The integration described by Eq. 1 is simply substituted by summation (5):

$$MT_{diss.vitro} = \frac{{}_i\Sigma\, \bar{t}_i \cdot \Delta M_i}{{}_i\Sigma\, \Delta M_i} \qquad (2)$$

where $\bar{t}_i$ is the midpoint of the time period during which the fraction ΔM_i of the drug has been released from the dosage form in vitro.

An analogous formula holds for the moments of dissolution times of order k (5):

$$m_k = \frac{{}_i\Sigma\, \bar{t}_i^{\,k}\, \Delta M_i}{{}_i\Sigma\, \Delta M_i} \qquad (3)$$

The moments of dissolution times around the mean ($MT_{diss.vitro}$) are denoted as central moments, e. g. the variance of dissolution times is estimated as follows (9):

$$VT_{diss.vitro} = \frac{{}_i\Sigma\, (\bar{t}_i - MT_{diss.vitro})^2\, \Delta M_i}{{}_i\Sigma\, \Delta M_i} \qquad (4)$$

The term "variance of the *mean* residence time" is erroneous; this term was unfortunately introduced by Riegelman and Collier (10) together with an ambiguous definition of the variance of residence times (for details see (11)).

In Table 1, the single computational steps are examplified for a controlled oral release system for molsidomine (M-CoRS, (12)); the dissolution of this formulation in cumulative terms is depicted in Fig. 1. In the in vitro dissolution test the Sartorius dissolution model was used with a change in pH after 1 h (details are given in (12) and (13)). More samples than listed were taken during the actual test. The mean in vitro dissolution time was 2.275 h and the variance of dissolution times 4.129 h^2. If we consider the fractional amount ΔM_i in terms of number of molecules rather than weights by multiplying the molar weight with Avogadro's constant, i. e. $6 \cdot 10^{23}\ mol^{-1}$, Eqs. 2 and 4 become identical to that used in mathematical statistics to evaluate the mean and variance for grouped samples (14).

Table 1 Computation of mean and variance of in vitro dissolution times. In vitro release of molsidomine from controlled oral release system was studied using a Sartorius dissolution model. Computation of moments follows Eq. 2 in the text. Release data were taken from Ostrowski et al. (13)

Time (h)	Cumulative amount dissolved (%)	$\bar{t}_i$ (h)	ΔM_i (%)	$\bar{t}_i \cdot \Delta M_i$ (h %)	$\bar{t}_i^2 \cdot \Delta M_i$ (h^2 %)
0.50	25.2	0.25	25.2	6.30	1.5750
1.00	42.0	0.75	16.8	12.60	9.4500
1.50	47.7	1.25	5.7	7.125	8.9063
2.00	54.5	1.75	6.8	11.90	20.8250
2.50	61.9	2.25	7.4	16.65	37.4625
3.00	67.8	2.75	5.9	16.225	44.6188
4.00	77.5	3.50	9.7	33.95	118.8250
5.00	86,4	4.50	8.9	40.05	180.2250
6.00	93.3	5.50	6.9	37.95	208.7250
7.00	98.8	6.50	5.5	35.75	232.3750
8.00	100.0	7.50	1.2	9.00	67.5000
			100.00	227.50	930.4876

$MT_{diss.vitro}$ = 2.275 h m_2 = 9.305 h^2 $VT_{diss.vitro}$ = 4.1293 h^2

In vivo excretion from the body

In pharmacokinetics, the amount eliminated from the body can be calculated using Dost's law of areas (15) provided that the body system response is linear with respect to the dose administered and elimination takes place from the compartment monitored:

$$M_{elimin}(t) = M(t) = Cl_{tot} \cdot {}_0\int^{t} C(t') \cdot dt' \qquad (5)$$

If we substitute the integration by **A**rea **U**nder the **D**ata values $AUD(0,t_i) = AUD_i$, usually computed using the trapezoidal rule, we obtain an estimate of the amount eliminated from the body system in cumulative terms M(t). The analogy to amounts dissolved in vitro is obvious and Eqs. 2 and 4 can be applied.

However, several algorithms have been suggested to estimate the total mean time: Benet and Galeazzi (16) describe the

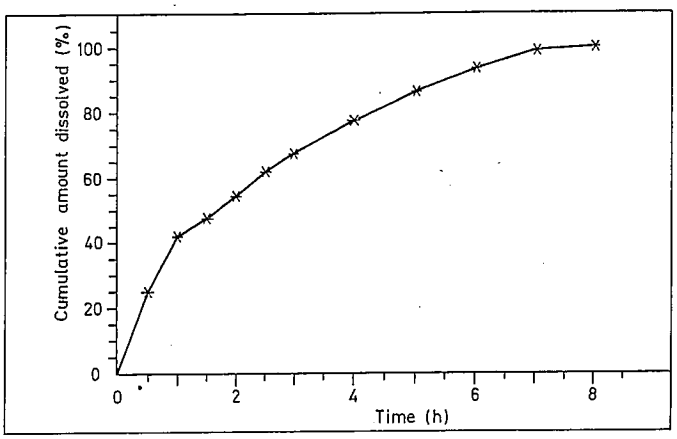

Fig. 1 In vitro release of molsidomine from a controlled oral release system. The amount dissolved is plotted versus time and scaled in percent of the labelled amount (8 mg). Dissolution was tested with the Sartorius dissolution model at 37° and 100 ml dissolution medium (for further details refer to (13)).

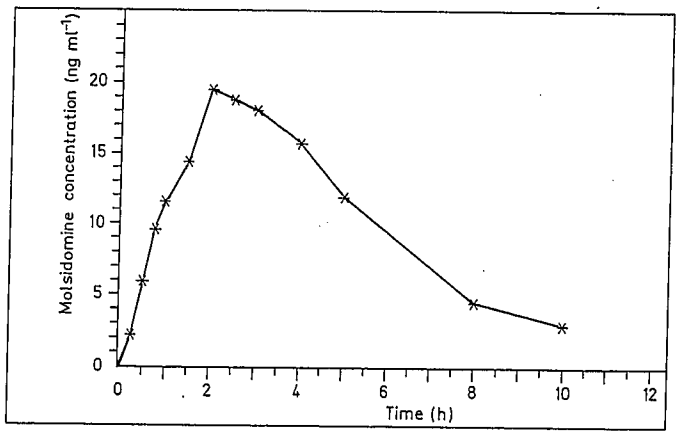

Fig. 2 Plasma concentrations of molsidomine after administration of a controlled oral release system. The formulation was administered to 10 male patients with coronary heart disease (for further details refer to (13)). Mean values are depicted.

computation of the **A**rea **U**nder the "first **M**oment **C**urve" (AUMC) from which they derive the mean time as:

$$MT_{total} = AUMC/AUC \quad (6)$$

Von Hattingberg and Brockmeier (2) have deduced a different algorithm for total mean time by partial integration of Eq. 1: Integration of the concentration/time curve yields a curve AUC(t) proportional to the amounts eliminated which asymptotically approaches AUC(∞).

Using the **A**rea **B**etween the **C**urve AUC(t) and its asymptote AUC(∞) — this area is named ABC — gives an estimate of the total mean time:

$$MT_{total} = ABC/AUC \quad (7)$$

A recent publication (8) has generalized the latter idea for moments of residence (or linger) times of any order, and by inverting the integration, the problem of estimating the asymptote was omitted.

The algorithm is exemplified in Table 2 using molsidomine readings in plasma after administration of a controlled oral release system (for details of the study refer to (13)). The blood level profile is depicted in Fig. 2.

The sequence of integration is inverted yielding **P**rospective **A**reas **U**nder the **C**urve (PAUC) and starts with the extrapolation to infinity, i. e. $PAUC_{k.extrap} = C_z/(\lambda_z)^k = 2.9/(0.542)^k$, where the rate constant λ_z was taken from a study in which a readily available formulation was administered (see below). These extrapolations are listed in the first line of Table 2. From these values downward to the first sample, the area under the data, i. e. PAUD, is calculated using the linear trapezoidal rule: e. g. $PAUD_0(8—10) = (2.9 + 4.5)\,(10—8)/2 = 7.4$ which is added to 5.35 and so on. Note that the time runs from high values to low. Columns 3 and 4 are evaluated in the same way using the values of the preceding column (mathematical details of the algorithm are found in (8)). The values in the bottom line yield an estimate of the moments of residence (or linger) times according to the following formula:

$$MT_{total} = PAUC_1/PAUC_0 \quad (8)$$

and in general

$$m_k = k!\ PAUC_k/PAUC_0 \quad (9)$$

For the formulation considered the total mean time is 4.507 h while the variance of residence (or linger) times is 8.733 h^2.

Alternatively, in pharmacokinetics the blood level profile C(t) is frequently described by an adjusted function consisting of several exponential terms (17):

$$C(t) = {}_j\Sigma\, C_j\, e^{-\lambda_j t} \quad (10)$$

In this case the total mean time can be calculated easily using the characteristics of the function, i. e. C_j and λ_j (2, 18, 19):

$$MT_{total} = \frac{{}_j\Sigma\ C_j/(\lambda_j)^2}{{}_j\Sigma\ C_j/(\lambda_j)} \quad (11)$$

The second moment around zero of residence (linger) times for drug molecules in the body is:

$$m_2 = \frac{2\,{}_j\Sigma\ C_j/(\lambda_j)^3}{\Sigma\ C_j/(\lambda_j)} \quad (12)$$

and the second moment around the mean, i. e. the variance, can be calculated by a well known identity as follows:

$$VT_{total} = m_2 - (m_1)^2 \quad (13)$$

Fig. 3 shows the blood level profile of molsidomine after oral administration of a fast desintegrating tablet. The mean in vitro dissolution time is 3.2 min. Adjusted by the Bateman function, the

Table 2 Computation of mean and variance of in vivo residence (transit) times by Prospective Areas Under the Curve. Concentration-time data for molsidomine after administration of a controlled oral release system: Prospective Areas Under the Curve substituted by Prospective Areas Under the Data Completed by extrapolation. The single computational steps are described in the text. Concentration-time data taken from Ostrowski et al. (13).

Time (h)	Plasma concentration (ng ml^{-1})	$PAUDC_0$[a] (ng h ml^{-1})	$PAUDC_1$[b] (ng h^2 ml^{-1})	$PAUDC_2$[c] (ng h^3 ml^{-1})
10.00	2.9	5.35[d]	9.89[e]	18.26[f]
8.00	4.5	12.75	27.99	56.14
5.00	12.0	37.50	103.39	253.22
4.00	15.7	51.35	147.82	378.82
3.00	18.0	68.20	207.60	556.52
2.50	18.8	77.40	244.00	669.42
2.00	19.5	86.98	285.10	801.70
1.50	14.4	95.45	330.70	955.65
1.00	11.6	101.96	380.06	1133.34
0.75	9.6	104.61	405.88	1231.58
0.50	5.9	106.54	432.27	1336.34
0.25	2.2	107.56	459.03	1447.76
0.00	0.0	107.83	485.95	1565.88
$MT_{total.vivo}$ = 4.507 h		m_2 = 29.044 h^2		$VT_{total.vivo}$ = 8.733 h^2

[a] PAUD means the **P**rospective **A**rea **U**nder the **D**ata, i. e. the area under the data from a certain time t up to the last data pair.

[b] PAUC means the **P**rospective **A**rea **U**nder the **C**urve, i. e. the area under the curve from a certain time t up to infinity.

[c] PAUDC is used when PAUD values are extrapolated in general by a single exponential to estimate the area under the curve beyond the last data pair.

[d] $5.35 = C_z/\lambda_z = 2.9/0.5426$

[e] $9.89 = PAUDC_{0.z}/\lambda_z = 5.35/0.5426$

[f] $18.26 = PAUDC_{1.z}/\lambda_z = 9.89/0.5426$

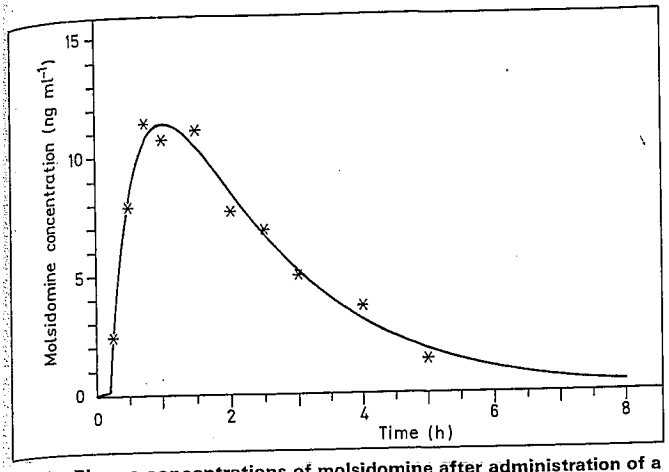

Fig. 3 Plasma concentrations of molsidomine after administration of a fast dissolving tablet. The tablet was administered to 10 patients with coronary heart disease (13). Mean values are depicted.

total mean time emerges as $1/k_a + 1/k_e = 2.32$ h, the variance being $1/(k_a)^2 + 1/(k_e)^2 = 3.7374\ h^2$.

2.3 Additivity of mean times

Calculating the mean time from cumulative elimination curves or concentration time data yields an estimate of the total mean time in the system, which depends on the mode of administration: This is the reason why the term "total mean time" has been used up to now. When specifying the mean time as in the following it is not only necessary that the mode of administering the drug is stated but also that a model for the pathway of the drug molecules is specified (20).

Upon bolus injection the mean time (MT_{vss}) includes distribution within and elimination from the body (19, 21).

$$MT_{vss} = MT_{vc} + MT_{tissues} \quad (14)$$

where MT_{vc} is the mean residence (linger) time in the central volume of distribution and $MT_{tissues}$ is the respective mean time in the peripheral tissues.

Upon administration of an oral solution the mean time encompasses the transport (MT_{LT}) to the site of absorption and the absorption (MT_{abs}), distribution and elimination (MT_{vss}) (2, 6, 10, 22).

$$MT_{total.sol} = MT_{LT} + MT_{abs} + MT_{vss} \quad (15a)$$

$$VT_{total.sol} = VT_{LT} + VT_{abs} + VT_{vss} \quad (15b)$$

If the transport of drug to the site of absorption is considered as a simple lag-time, the mean MT_{LT} is only the lag-time itself and the variance VT_{LT} is zero. One must consider carefully whether the mean transport time should be included in the further calculation, e. g. correlation.

We are interested in the in vivo dissolution process of a solid oral dosage form. After administration of this formulation the mean in vivo dissolution time $MT_{diss.vivo}$ is included in the total mean time (2, 3, 23):

$$MT_{total.vivo} = MT_{diss.vivo} + MT_{LT} + MT_{abs} + MT_{vss} \quad (16a)$$

$$VT_{total.vivo} = VT_{diss.vivo} + VT_{LT} + VT_{abs} + VT_{vss} \quad (16b)$$

The principles of component analysis are obvious from the above examples: Based on the physicochemical properties of a drug, the concept of galenic formulation and the handling of the drug by the body, the sum of components in terms of mean and variance are stated and are investigated in an appropriate experimental setting.

3 In vitro/in vivo correlation

3.1 Introductory remarks

Several attempts have been undertaken to correlate in some way in vitro results with clinical pharmacological findings in vivo.

Purich (24) has stated that the FDA is mainly interested in the relative merits of various in vitro/in vivo correlations in screening drug products, "e. g. the most highly correlated in vitro parameter being the best predictor of in vivo results". He, therefore, did not see any need to justify theoretically the in vitro or in vivo characteristics on which the correlation is based. However, this approach is unsatisfactory from a scientific point of view, although it must be accepted that the regulatory agencies may look at this topic more pragmatically.

In vitro/in vivo correlations cover a wide range of methods ranging from intuitive comparison of in vitro test results with the outcome of bioavailability trials (25) on one end of the scale and the continuous correlation of in vitro and in vivo dissolution profiles (e. g. (26)) on the other. However, it should be emphasized that intuitive comparison of the in vitro properties of a formulation with pharmacokinetic findings ultimately led to the regular study of bioavailability in drug development, the necessity of which is no longer controversial.

In the following sections we will discuss the discrete and continuous quantitative correlation of in vitro and in vivo results based on moment analysis of dissolution and residence times.

3.2 Correlation of discrete characteristics

A first step in comparing the in vitro and in vivo characteristics of a solid oral dosage form can be read from Eqs. 15 and 16. The difference between the total mean times after administering a solution and another formulation under consideration yields an estimate of the mean in vivo dissolution time:

$$MT_{diss.vivo} = MT_{total.vivo} - MT_{total.sol} \quad (17)$$

The sustained release from the solid dosage form under in vivo conditions must make a sufficiently contribution large enough to the total mean time in the body to be extractable by conventional evaluation routines. Needless to say one may test $MT_{diss.vivo}$ for significant differences from zero by conventional inference statistics. If the mean in vitro dissolution time is negligible when compared to the total mean time $MT_{total.vivo}$ it will be impossible to make a reasonable estimate of the mean in vivo dissolution time. This situation is found with drugs with large mean times in their steady state volume of distribution and with formulations with very short in vivo dissolution times.

The quotient of the mean in vivo and mean in vitro dissolution time $MT_{diss.vivo}/MT_{diss.vitro}$ indicates roughly whether the in vitro dissolution runs in front of the in vivo dissolution or lags behind.

$$TS = MT_{diss.vivo}/MT_{diss.vitro} \quad (18)$$

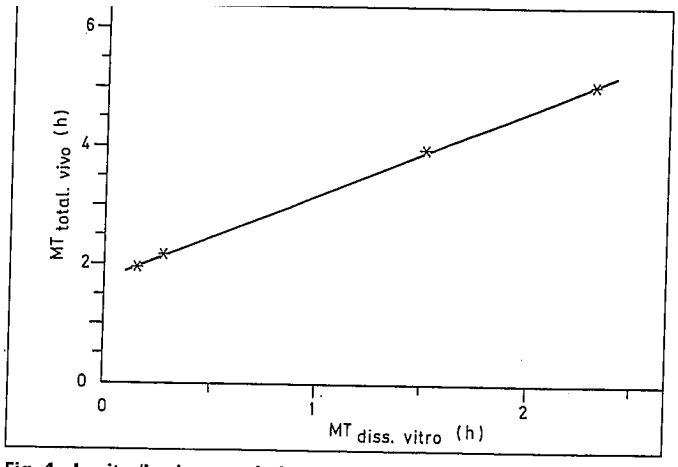

Fig. 4 In vitro/in vivo correlation by mean times. The total mean time in vivo $MT_{total.vivo}$ of four different formulations of carbocromene-HCl administered to volunteers is plotted versus the mean in vitro dissolution times $MT_{diss.vitro}$ of these formulations according to Eq. 19.

Levy et al. (27) have proposed calibrating exactly the in vitro dissolution test procedure on the basis of in vivo measurements. However, this time consuming procedure may be overcome by the mathematical considerations given below.

From Eq. 16 it is obvious that the total mean time may vary if the mean in vivo dissolution time $MT_{diss.vivo}$ is modified by specific changes of the formulation, since we basically assume that the mean absorption time MT_{abs} and mean time in the steady volume of distribution MT_{vss} do not change significantly in an individual. Therefore, when administering different formulations to an individual, we expect linear correlation between the total mean time and the mean in vitro dissolution time. Clearly, since the mean in vitro dissolution time $MT_{diss.vitro}$ does not appear in Eq. 16 we assume a fixed ratio between the mean in vitro and mean in vivo dissolution times, i. e. we assume that the in vitro test equipment is a reasonable substitute for the in vivo "dissolution apparatus":

$$MT_{total.vivo} = TS \cdot MT_{diss.vitro} + MT_{LT} + MT_{abs} + MT_{vss} \qquad (19)$$

where TS is the time scaling factor which mediates between in vitro and in vivo dissolution.

This correlation between $MT_{diss.vitro}$ and $MT_{total.vivo}$ in Eq. 19 was first postulated by Von Hattingberg and Brockmeier (2) and was proved to be valid for carbocromen-HCl (3, 28), which is shown in Fig. 4. The slope of the regression (slope = 1.42) indicates the factor by which the time axis of the in vitro dissolution test result must be regraduated to meet the in vivo situation (29), i. e., the in vitro dissolution test runs in front of the in vivo dissolution process.

Tanigawara et al. (30) found a good correlation between the mean in vitro and mean in vivo dissolution time for four different ampicillin products. Their conversion factor was 1.3 when the four products were tested with the paddle method (50 rpm, 900 ml water, 37° C).

Möller and Langenbucher (31) evaluated the in vivo release profiles of three different theophylline preparations by deconvolution first and then used these hypothetical in vivo dissolution profiles (26) to estimate the mean in vivo dissolution times which were in good correlation to the mean in vitro dissolution times obtained using two different apparatuses. This is particularly surprising, since one formulation showed an extremely wide variation in rate, profile, and extent of in vivo dissolution (31). However, they calculated a time scaling factor of 1.11, 1.01 and 1.02 depending on the in vitro dissolution test equipment.

Nicklasson et al. (32) studied the in vivo performance of three bacampicillin microcapsules and confirmed the dependency stated by Eq. 19 at least for the average $MT_{total.vivo}$ and $MT_{diss.vitro}$ values, but it seems unlikely that the same relation is reflected in each individual. It is clear that mean in vitro dissolution times as short as 6 min and 13 min (formulation M_1 and M_2 respectively) can hardly be extracted under in vivo test conditions (see notes above). However, the time scaling factor when refering to the paddle method (100 rpm, 500 ml water, 37° C) was 0.78.

A rescaling factor of 0.64 was found by Graffner et al. (33); they investigated the in vivo characteristics of four controlled release tablets of the insoluble matrix type for a 5-HT-uptake inhibitor. The paddle method with 50 rpm and 500 ml of water 37° C was used as in vitro reference. Under these conditions a good correlation of mean in vitro dissolution times and total mean time according to Eq. 19 was obtained.

Although Zerbe et al. (34) showed the same dependency between mean in vitro dissolution times and total mean times in vivo they did not give details of the in vitro dissolution conditions or the conversion factor as the slope of the regression line. From their graph, it must be a value clearly below one.

From the studies cited above it is obvious that the theoretically deduced dependency (2) of $MT_{total.vivo}$ and $MT_{diss.vitro}$ is also found under real experimental conditions. The time-rescaling factor, however, is not uniform but clearly depends on the in vitro dissolution conditions used and on the type of galenic formulation.

3.3 Correlation of non-analogous characteristics

The correlation mentioned used analogous characteristics (35), i. e. the average of times the molecules spend in the formulation under in vitro test conditions or the averaged times they spend in the combination, i. e. formulation and body.

Beside the correlation of these reasonable — since analogous — characteristics a variety of euristic correlations of discrete values have been reported. Some of them are simply illogical and thus can be correlated by chance only. These will not be discussed in detail. Those interested might therefore refer to Stricker (35), Lippold (36), and Langenbucher and Möller (37).

Nevertheless, the correlation between mean in vitro dissolution time and the area under the blood level curve for a series of different formulations seems worthy of detailed consideration: At first glance this correlation seems to be illogical since the mean in vitro dissolution time measures an averaged time of release while the area under the blood level curve measures an extent of release and absorption, i. e. an amount absorbed under in vivo conditions (15). (We naturally assume thereby that the labelled amount of the formulation is actually released under in vivo test conditions). However, if we accept the correlation as indisputable after careful and critical re-evaluation, we are left with the duty of interpreting the result:

The most obvious interpretation is that the absorption of drug is limited to a particular segment of the gastrointestinal tract. Therefore, the amount not released from the dosage form during

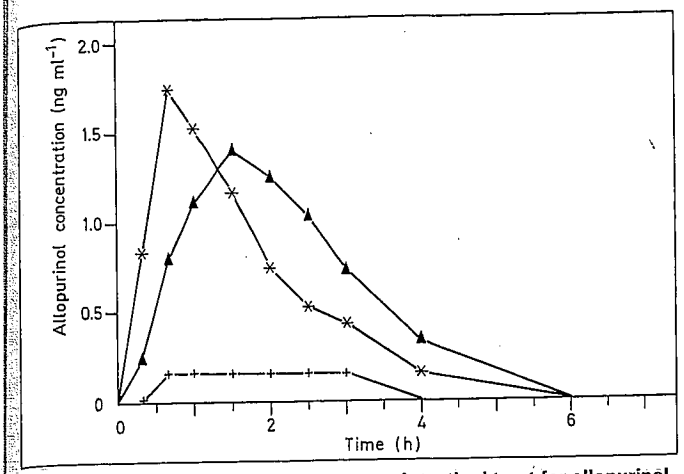

Fig. 5 Absorption spectrum of the gastrointestinal tract for allopurinol. Mean plasma concentration of allopurinol after ingestion of a "high frequency capsule" and release of the drug into the duodenum (*), jejunum (▲) and lower jejunum (+) are shown. Redrawn from data presented by Schuster et al. (40).

a certain period cannot be absorbed. Although this concept was formulated several years ago (38, 39), there are only a few clinical trials showing that it is a real variable in drug absorption, e. g. Schuster et al. has shown such phenomena for allopurinol (40) (see Fig. 5) and site-dependent absorption rate and bioavailability has also been observed for furosemide (41) and piretanide (42), depicted in Fig. 6.

In this situation, an almost linear correlation between the in vitro dissolution time and the area under the concentration time curve might be observed. However, the total mean time theoretically cannot show a linear correlation with the mean in vitro dissolution time: Instead of using the total mean in vitro dissolution time one should use a fractional mean in vitro dissolution time, i. e., the mean time for that fraction which is absorbed under the in vivo test conditions.

Moreover, for digoxin the amount absorbed clearly depends on the rate of release for a large variety of formulations (24, 43, 44), although digoxin is absorbed equally well all along the gastrointestinal tract (45, 46). The explanation is given by Lindenbaum et al. (47), who found that digoxin is degraded within the lumen of the lower intestinal tract by anaerobic organisms. With

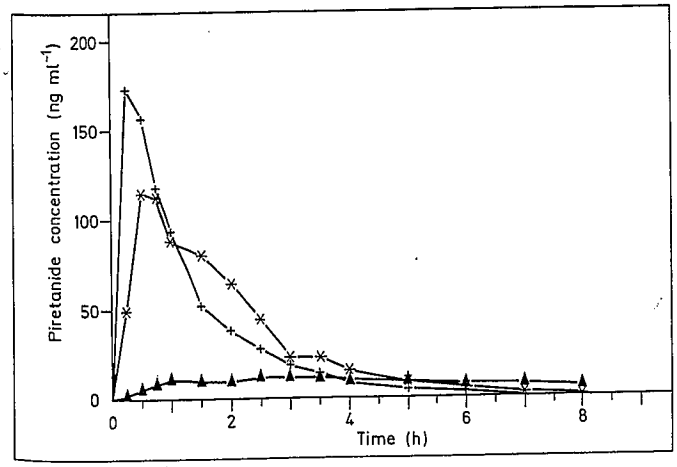

Fig. 6 Absorption spectrum of the gastrointestinal tract for piretanide. Mean plasma concentration of piretanide after endoscopic placement of the drug at three different sites of the gastrointestinal tract under visual control (42) are plotted versus time: stomach (*), duodenum (+), ascending colon (▲). Reproduced from (42) with kind permission of Springer Verlag, Heidelberg.

this interpretation a roughly linear correlation between mean in vitro dissolution time and total amount absorbed is compatible.

A further possible explanation for dependency between amount absorbed and the mean in vitro dissolution time is that saturable first-pass metabolism is present. However, an essential presumption to detect this would be that the rate of release results in hepatic concentrations which are in a relatively small concentration range about two to ten times the concentration which leads to half-maximal metabolisation rate.

These examples clearly show that correlation of even non-analogous characteristics may provoke ideas on the source of variance in bioavailability or at least stimulate additional studies to verify these.

3.4 Continuous in vitro/in vivo correlation

Introductory Remarks

Up to now, we have considered the situation where several different formulations have been tested once with an in vitro dissolution apparatus and once in humans. Testing only one formulation and applying Eq. 18 cannot seriously be regarded as in vitro/in vivo correlation.

A correlation according to Eq. 19 should be carried out with at least three different formulations. It seems, however, somehow redundant to test several formulations if only one is finally selected and to develop different formulations with clearly different mean dissolution times for which there is actually no need — except for the demonstration of a poor mathematical correlation. If the pharmacist has good reasons to assume that one formulation has the in vivo release properties needed, this seems particularly unnecessary. Unless there are no other reasons to develop and test different formulations, discrete in vitro/in vivo correlation alone cannot justify such a strategy.

In this situation, having a single formulation tested in vitro and in vivo, it seems desirable and helpful to analyse quantitatively to what extent or up to which time the in vitro dissolution test has simulated the in vivo dissolution process (26).

Convolution

In order to develop the mathematical tools for in vitro/in vivo correlation for a single formulation, the relation given in Eq. 18 is generalized, which regraduates the in vitro time scale towards the in vivo time base (26):

$$MT_{diss.vivo} = a + b\ MT_{diss.vitro} \qquad (20)$$

The same equation may hold for the total time base of the two systems:

$$t_{vivo} = a + b\ t_{vitro} \qquad (21)$$

Here b is the time scaling factor discussed above. The additive term a has been introduced to account for a possible time lag between in vitro and in vivo dissolution, which, for instance, may be an intentional variable as with an enteric coated formulation. So, Eq. 21 covers the general case, but for special formulations the additive term may be considered as zero (for further interpretation and application see (9, 29)).

Eq. 20 may be valid under several conditions, but Eq. 21 implies that the in vitro and in vivo dissolution profiles are equivalent (9, 29). This concept of equivalence is the essential step in the

continuous in vitro/in vivo correlation: It means that the in vitro and in vivo dissolution profiles do not differ in their general shape or morphology but may only be stretched or shortened with respect to each other; in other words, they can be superimposed by linear regraduation of the time axis. Equivalence can also be interpreted in such a way that the physicochemical principles of drug release from the dosage form are the same for both the in vitro and the in vivo system.

Under these conditions, the variance of dissolution times are related to each other by the following equation (26, 29):

$$VT_{diss.vivo} = b^2\, VT_{diss.vitro} \qquad (22)$$

Since $VT_{diss.vivo}$ is the difference of total variances, once administering the solid dosage form $VT_{total.vivo}$ and once an oral solution $VT_{total.sol}$, we obtain the value b by:

$$b = \sqrt{(VT_{total.vivo} - VT_{total.sol})/VT_{diss.vitro}} \qquad (23)$$

With the value b we can calculate a with the following equation:

$$a = (MT_{total.vivo} - MT_{total.sol}) - b\, MT_{diss.vitro} \qquad (24)$$

Applying this to the formulation of molsidomine mentioned above yields the following values for a and b:

$MT_{total.vivo}$	= 4.507 h	$VT_{total.vivo}$	= 8.733 h^2
$MT_{total.sol}$	= 2.320 h	$VT_{total.sol}$	= 3.737 h^2
$MT_{diss.vivo}$	= 2.187 h	$VT_{diss.vivo}$	= 4.996 h^2
$MT_{diss.vitro}$	= 2.275 h	$VT_{diss.vitro}$	= 4.1293 h^2

$b = \sqrt{4.996/4.129} = 1.10;$

$a = 2.187 - 1.10 \cdot 2.275 = -0.3157$

This result is not acceptable: a subtle interpretation of the value of a would be that under in vivo conditions the release of drug starts 20 min before administration of the formulation to the patients[1]. The negative value of a may partly be the result of error propagation within the individual computational steps. Therefore, the calculation of a time lag, i. e. a, is ommitted. One may either use the value of b for the time regraduation or compute TS according to Eq. 18 (TS = 2.187/2.275 = 0.96). Since both values enclose unity no time transformation of the in vitro dissolution is recommended.

Assuming the in vitro dissolution as a hypothetical in vivo dissolution profile, we can carry out a convolution using the response to the fast disintegrating tablet as a weighting function (26, 48—50). The result, i. e. the prediction of the blood level profile, can be compared with the actual reading after administering the extended oral release system by a correlation coefficient or a correlation plot of actual readings versus prediction, the slope of which should be one and the intercept zero.

The result of convolution for the formulation of molsidomine considered is depicted in Fig. 7a, while Fig. 7b gives the correlation plot. The prediction of the concentration profile is reasonable, particularly in respect to the maximum concentration and

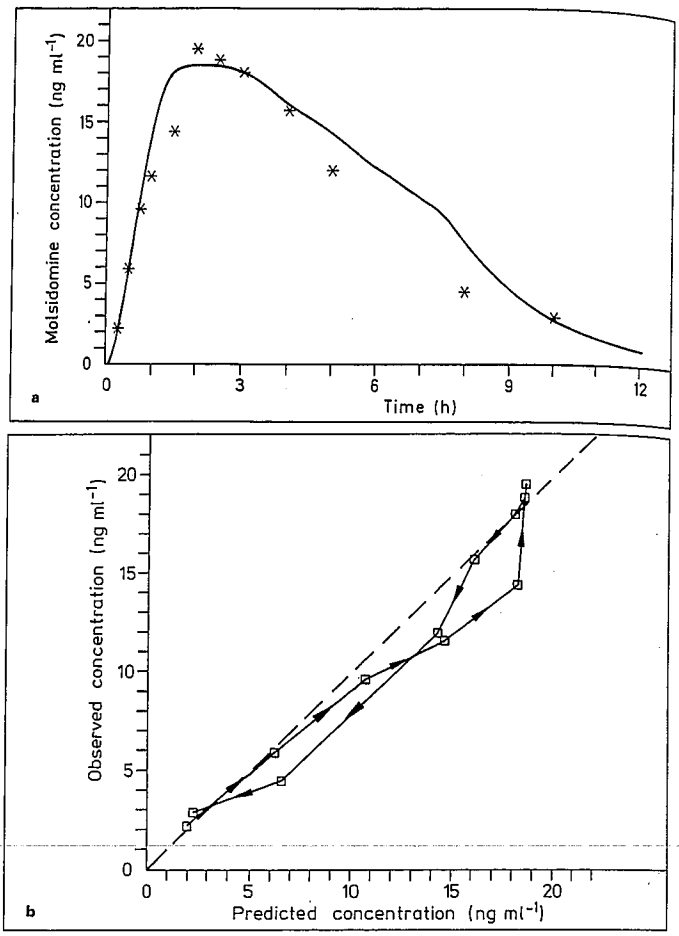

Fig. 7 Continuous in vitro/in vivo correlation by convolution. Fig. 7a: The dots (*) represent the plasma concentrations of molsidomine; mean values of 10 patients are depicted (13). The fine line is the result of convolution. The results of an in vitro dissolution experiment using the Sartorius dissolution model are taken as the input scheme into a body system characterized by a weighting function which is the dose-normalized response to a fast dissolving tablet (Fig. 3). Fig. 7b: Correlation of predicted and observed concentration. The broken line represents the ideal case of identity. For further details refer to the text.

the time of maximum concentration, the correlation coefficient being 0.973. However, a slight but systematic deviation is recognizable when critically reviewed resembling a hysteresis. One may take this result as an indication that in vivo concentration/time profiles cannot be predicted — or may ask for reasons for the discrepancy detected.

But with convolution, conformity or non-conformity between the observed and predicted concentration profile can be perceptedonly; this approach does not indicate any reasons for a possible discrepancy, as in the case discussed.

The procedure described above is schematically outlined in Fig. 8 on the left hand side (26): Time transformation is followed by convolution which results in a predicted concentration time profile which is compared to the actual readings.

Deconvolution

The converse way to test in vitro and in vivo equivalence uses deconvolution sketched on the right of Fig. 8. This approach requires sufficient sampling of the blood level profile during the in vivo dissolution phase. The total result of deconvolution depends very much on the accuracy of the early readings, since

[1] It should be emphasized that a negative value of a alone is not a reason to reject the result. If for instance the in vitro dissolution shows a clear lag-time a negative a may indicate that the lag time is shorter under in vivo conditions, e. g. with an enteric coated formulation.

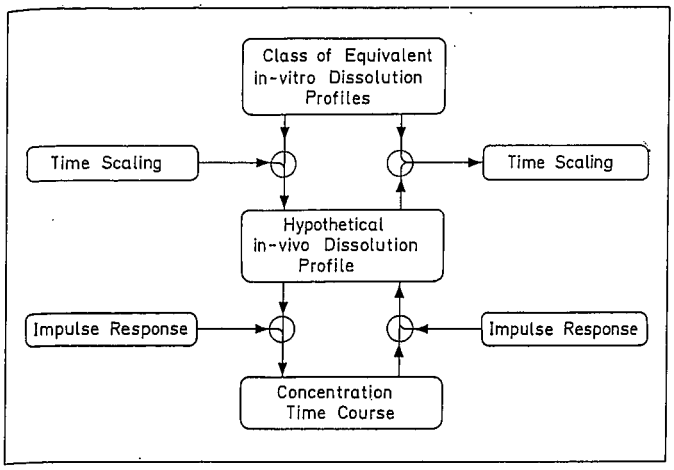

Fig. 8 Strategies for continuous in vitro/in vivo correlation. The scheme sketches the two fundamental approaches to demonstrate the continuous in vivo relevance of an in vitro dissolution profile: On the left the transformation of the in vitro test result towards the in vivo time scale is followed by convolution whilst on the right the hypothetical in vivo dissolution is estimated by deconvolution followed by correlation with the in vitro dissolution profile. (Reproduction from (26) with kind permission of Springer Verlag, Heidelberg.)

they influence the calculation of the further hypothetical dissolution values.

In this context, if the first value calculated for the hypothetical in-vivo release by deconvolution indicates that 80% of the labelled amount has already been dissolved (or even more) one cannot seriously talk of a complete profile. This shows clearly that the study design was not adequate.

The dose-normalized response following the administration of an oral solution or a fast dissolving tablet is regarded as weighting function w(t). The actual readings of the concentration are considered as response $r(t_i) = C(t_i)$. The hypothetical input scheme of drug D_k may be calculated by the following general deconvolution formula (26):

$$D_k = \frac{C_{obs}(t_{k+1}) - C_{predict}(t_{k+1})}{w(t_{k+1} - t_k)} \qquad (25)$$

where $C_{obs}(t_{k+1})$ means the (k+1)th reading when the solid dosage form is administered, and $C_{predict}(t_{k+1})$ is the predicted concentration after k individual doses D_i considered as already accounted for as inputs into the system up to the time t_k. The nominator, $w(t_{k+1} - t_k)$, represents the value of the dose-normalized weighting function at time $(t_{k+1} - t_k)$ which can be any sum of exponential functions.

If w(t) is composed of a sum of pure exponentials, i. e. $w(t) = {}_j\Sigma h_j e^{-\lambda_j t}$, the input D_k is considered as a bolus input. If w(t) consists of a sum of infusion functions, i. e. $w(t) = {}_j\Sigma h_j/((t_{k+1}-t_k)\lambda_j)(1 - e^{-\lambda_j t})$, then D_k is considered as the dose administered by a constant infusion during the period t_k to t_{k+1}. According to this interpretation of administration of the drug, the value of $C_{predict}(t_{k+1})$ must be calculated.

The hypothetical in vivo dissolution profile is obtained in cumulative terms by summing up the individual doses:

$$M_k = M(t_k) = \sum_{i=1}^{k} D_i \qquad (26)$$

The deconvolution formula (Eq. 25) has been applied to the formulation of molsidomine. The in-vivo dissolution profile is depicted cumulatively in Fig. 9a together with the in vitro dissolution test result. It is immediately seen that in vitro dissolution runs slightly ahead of the hypothetical in vivo rate for the first three hours and clearly ahead thereafter. A test for equivalence is applied in order to analyse in more detail the extent to which both profiles are isomorphic (9): One can read equivalence or non-equivalence from a graph where the time to dissolve certain amounts under test condition A are plotted versus the corresponding times needed to dissolve the same amount under condition B. This graphic method has been titled the Levy-plot (9).

Times related to equal amounts dissolved are taken from the graph or determined numerically (9). These times are depicted in Fig. 9b where the x-axis gives those times related to the in vitro dissolution whilst the y-axis is related to the hypothetical in vivo dissolution.

The relation is clearly curvilinear, but the relationship can be approximated by two linear phases; the first one, phase I, being valid from zero to approximately 3.4 h indicating a small lag time of 0.11 h (intercept) and a time scaling factor of 1.26 (slope). The second phase lasts from 3.4 h onwards with a conversion factor of 3.0. During the first phase, 5.83 mg (73% of the dose) was hypothetically dissolved in vivo and absorbed.

The possible interpretations for a curvilinear relation between those times related to equal amounts dissolved have been

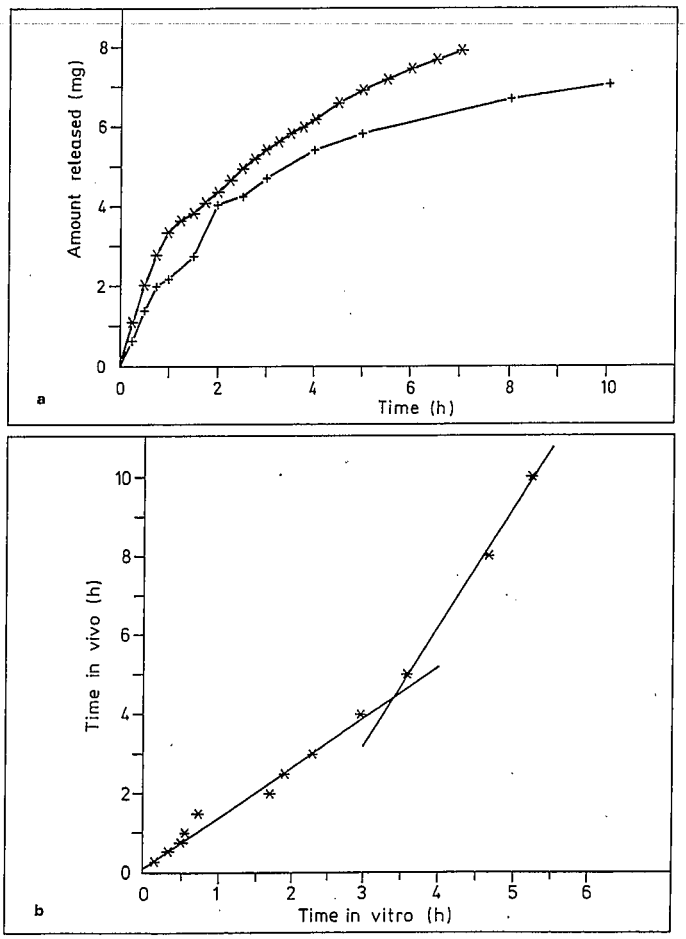

Fig. 9 Continuous in vitro/in vivo correlation by deconvolution. Fig. 9a: The hypothetical in vivo dissolution profile (+) estimated by deconvolution and the in vitro dissolution profiles using the Sartorius dissolution apparatus (*) are depicted. Fig. 9b: The correlation of times — $t_{diss.vivo}$ versus $t_{diss.vitro}$ — related to identical amounts dissolved under both conditions is shown. For further details refer to the text.

discussed in detail for theophylline (26). The most likely interpretation for a curvilinear Levy-plot is that the rate of absorption changes while the drug is moving down the gastrointestinal tract. This purely theoretical conclusion has been confirmed by another group of scientists using an alternative experimental approach (51). The essential requirement for this conclusion is, however, that the dissolution profile is reasonably independent of changes in pH and modes of agitation, i. e. that equivalent dissolution profiles result from different modes of dissolution. Specific trials have revealed differences in the rate of drug absorption for several drugs (40, 42, 45, 46, 52, 53).

If this interpretation is valid for molsidomine, the in vivo dissolution profile obtained from deconvolution is actually only a hypothetical one and the true in vivo release profile may be completely equivalent to the in vitro one. The latter statement is confirmed by the fact that the release from the M-CoRS-formulation is hardly affected by different dissolution test equipments (54) and can be considered as independent from environment to a certain extent.

It must be emphasized that the preceding conclusions require intensive in vitro testing of the formulation considered. It is not sufficient to test the formulation under only one condition — although it may be an officially recommended one — but to challenge the isomorphism (or equivalence) for several apparatuses and modes.

4 Testing equivalence or in vitro/in vitro correlation

Essential trials to prove the in vivo relevance of the in vitro dissolution properties of a drug formulation can already be carried out in the in vitro situation alone: This means that we can partly challenge the hypothesis that the release profile will be reflected in the concentration time profile after administration (of the formulation in question) without having to perform a clinical trial. Although this should be the first step in the development of extended release formulations, it is discussed last.

Since isomorphism (or equivalence) will play an essential role in the following considerations, as it does for continuous in vitro/in vivo correlation in general, the reader may refer to the definition given above or to the original publications for further details (9, 29).

One essential requirement for in vivo relevance of an in vitro dissolution profile is that the in vitro dissolution profile is reasonably independent from the pH gradient used in the in vitro experiment. This is because the interindividual differences between the pH in the resting stomach are extreme: Kuna (55) has shown that the gastric pH in a representative population (312 subjects, 1556 measurements) may range from pH = 1.0 to pH = 9.0. These values have been confirmed by other authors using different analytical tools (56, 57). When the drug is given with a meal with different constituents, the pH in the stomach and the upper part of the intestine will cover the same range. Therefore, a formulation which shows clear dependency of the release profile on pH and/or pH gradient will show a good in vitro/in vivo correlation (if any) only by chance. Nevertheless, if dissolution with different pH gradients result in different but equivalent dissolution profiles, there may still be a good continuous correlation of in vitro and in vivo results, though interpretation becomes more difficult.

The situation becomes even more complex if the drug is r leased pH-dependent in the stomach but not absorbed from th stomach; dose-dumping in some individuals is then unavoidab with such formulations.

Even in the case of an enteric coating, the time the formul tion stays in the stomach may have an influence on the releas profile when the pH which triggers release is reached (4).

All these features, especially the equivalence or nor equivalence under different pH conditions and with or witho pre-incubation in an acidic medium can be tested in vitro; it is nc advisable to test these properties in man only, since then the cannot be separated clearly from other effects.

Another demand is that the formulation should show at leas equivalent profiles (9, 29) when tested in different dissolutio equipments or with different modes, i. e. the shape of the curve should be independent of the mode of agitation by the dissolvin medium; the profile may be stretched or shortened in total but the morphology should be the same.

If this demand is not fulfilled there is no chance of linking the in vitro dissolution profile with the in-vivo concentration time response. Clearly, if different agitation modes or dissolution apparatuses result in non-equivalent (non-isomorphic) dissolution profiles one does not know which of the different profiles should be considered as the input to the body system model (Fig. 8).

In the case of equivalent in vitro dissolution profiles, however, any representative of this class can be used to link in vitro and in vivo dissolution by at least sectionally rescaling the time base as described above. Each of the class of equivalent dissolution profiles will produce the same type of Levy-plots (9) although the characteristic transformation parameters may differ in their values.

In contrast, if the dissolution profiles resulting from different test conditions show non-equivalence it can be concluded that they will show the same instability in morphology under in vivo conditions due to the intra- and interindividual variability in the frequency, direction and intensity of the migrating motility complexes along the gastro-intestinal tract (58).

Therefore, if a formulation is highly sensitive to agitation with respect to the general profile this will cause high intraindividual and interindividual variability of in vivo release and thus no isomorphism (or equivalence) of in vitro and in vivo dissolution profiles can be expected.

Since equivalence is the basic assumption for discrete und continuous in vitro/in vivo correlation — and also for those correlations not based on the mean time concept — non-equivalence which is already obvious under broadly comparable in vitro test conditions withdraws the rationale for in vitro/in vivo correlation[2].

In the ideal case the profile is independent in absolute terms of any pH gradient and mode and extent of agitation by the dissolving medium, i. e. showing an environment independent release. This is really the challenge in the development of extended release formulations. In such a case, we can assume hypothetically with a low risk that the dissolution profile in vivo will be the same as in the in vitro experiments.

[2] Recently reported results with slow release formulations of theophylline (59—63) have clearly underlined the need for in vitro dissolution models which can simulate the influence of food on the release profile.

The latter independency has been partly demonstrated for the formulation of molsidomine discussed above (54). It is therefore logical that the prediction of concentration profiles by convolution without any transformation of the time base is in good correlation to what is actually oberserved in patients (64). Clearly, the variability due to the individual clearance of the patients and individual absorption profiles of the GI-tract cannot be reduced by pharmaceutical properties of the drug formulation.

5 Conclusions

Two different methods have been outlined to correlate the in vitro results of dissolution with the pharmacokinetic response in vivo: the first — i. e. the discrete parameter correlation — requires several formulations tested in parallel under in vitro and in vivo conditions and is a correlation on averages; the second — i. e. the continuous correlation — can be carried out with a single formulation tested in vitro and in vivo.

In both cases the principles are based on the fact that single events forming a sequence, e. g. dissolution, absorption, elimination, are additive with respect to the time elapsed for these events and the mean of these times, respectively. It is the basis of component analysis in pharmacokinetics and can be revealed by an adequate experimental setting. This concept, although simple is compelling; it is applicable to both in vitro and in vivo dissolution test results and enables a complex process to be broken down into its basic components.

In addition, the concept presumes that there is a fixed ratio between the times elapsed for in vitro dissolution events and the respective times for the in vivo events. Nevertheless, it must be emphasized that any in vitro/in vivo correlation presumes that some in vitro dissolution characteristics are reflected in the in vivo concentration time profile. Therefore, each of these methods presumes a fixed ratio, at least for average characteristics, although the theoretical background to alternative correlations has not been elaborated as clearly as the mean time concept.

Moreover, with continuous in vitro/in vivo correlation, this presumption implies that the total in vitro dissolution profile can be projected isomorphically to yield the in vivo dissolution profile: it means that the in vitro and in vivo dissolution profiles do not differ in their morphology but only may be stretched or shortened with respect to each other; this property has been termed equivalence.

Even with a difference in time base the in vitro and in vivo dissolution profiles must not differ in their general morphology. Otherwise neither the prediction of the concentration by convolution (including time base rescaling) nor the correlation of the hypothetical in vivo dissolution obtained by deconvolution with the in vitro test will produce satisfying results. However, whether the assumption of equivalent in vitro/in vivo profiles is rational can be tested under in vitro conditions. For example, equivalence with respect to pH and agitation can be extensively tested under in-vitro conditions alone, and additional in vitro dissolution models for testing the influence of food, for example, should be considered. Therefore, this testing for in vitro/in vitro equivalence as a first step is a duty and the essential rationale for going even further in correlation, i. e. correlating any in vitro with any in vivo characteristic. One should therefore not stick to officially recommended in vitro test equipment when developing a new extended release formulation.

In contrast to continuous correlation the presumption for the discrete in vitro/in vivo correlation are less restrictive, equivalence "on average" may be sufficient.

From our experience, the technique of plotting according to times needed to dissolve the same amount under different conditions (Levy-plot) — in vitro and/or in vivo — is the most efficient way of revealing equivalence, i. e. conservation of the morphology of the dissolution profiles.

Moreover, with an extended release formulation showing equivalent dissolution profiles under clearly different in vitro test conditions it is possible to study the absorption features of the gastrointestinal tract with respect to the drug considered by combining the technique of deconvolution and Levy-plot. This has been confirmed for theophylline, and similar experiments for molsidomine are under way.

So, why not to use the highly developed pharmaceutical technologies in drug formulation to trace this important physiological variable for drug absorption and by this separate the variabilities in bioavailability due to physiological reasons from those due to the formulation?

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Acknowledgements

The author is indebted to Dr. D. Voegele for his helpful suggestions concerning the manuscript. The support of Mr. A. Reeves in revising the manuscript is gratefully acknowledged. Mrs. K. Herrnkind rendered expert secretarial assistance.