Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 351 (2008) 127–132
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Examination of metformin hydrochloride in a continuous dissolution/HDM system

Kyle A. Fliszar[a,*], Natalie Foster[b]

[a] *Merck and Co., Inc. Merck Manufacturing Division, West Point, PA 19486, United States*
[b] *Department of Chemistry, Lehigh University, Bethlehem, PA 18015, United States*

Received 25 April 2007; received in revised form 14 September 2007; accepted 19 September 2007
Available online 25 September 2007

Abstract

A continuous dissolution/absorption system using a hexadecane membrane (HDM) as the permeation measurement has been examined for three distinct formulations of metformin hydrochloride. This system was used to correlate the absorption rate of metformin through the membrane after release from the dosage form to rate of appearance of metformin in the plasma from the same formulations. These correlations were then used to make predictions of the *in vivo* plasma profile for each formulation. Successful predictions of AUC were accomplished for both immediate release and extended release formulations of metformin hydrochloride.

Keywords: Dissolution; Absorption; Metformin hydrochloride

1. Introduction

A simple and rapid *in vitro* method to predict the *in vivo* performance of a drug is a valuable tool for the development of pharmaceutical formulations. Historically, researches have examined either a drug's permeability or its dissolution as a way to predict the *in vivo* performance. Permeability has been examined by a number of methods, including biologically isolated tissues, cultured cells and organ perfusion techniques (Pagliara et al., 1999) as well as artificial membranes including hexadecane and other filter based membranes (Kansay et al., 1998; Wohnsland and Faller, 2001). A number of successful correlations have been established between a drug's permeability across the membrane and its % fraction absorbed (%FA) (Tavelin et al., 2003; Artursson and Karlsson, 1991; Matsson et al., 2005). An extensive body of data also exists correlating a drug's dissolution with its bioavailability, as *in vivo–in vitro* correlations (IVIVCs) have been established for many products (Upoor, 2001; Veng-Pedersen et al., 2000; Li et al., 2005). These correlations are typically made by examining formulations with varying dissolution release rates and determining how the release rate influences *in vivo* measurements such as AUC (Area Under the Curve), C_{max} or T_{max}.

Researchers have previously examined the combined rates of dissolution and permeation using one apparatus. Ginski and co-workers developed a dissolution/Caco-2 system to evaluate the dissolution–absorption relationship of piroxicam, metoprolol and ranitidine (Ginski and Polli, 1999). In these experiments, the researchers demonstrated that their dissolution/absorption system was able to predict whether the absorption of a drug from a specific formulation is dissolution rate limited or permeation rate limited. Another set of researchers examined the absorption of drug through a Caco-2 membrane taking into account the physiological pH shift that a drug encounters as it moves though the GI tract (Kobayashi et al., 2001). In these experiments, small amounts of drug (or drug product) were dissolved at low pH and the solution was then adjusted to pH 6.0 before being exposed to the Caco-2 cells. The permeation rates through the Caco-2 cells were then determined and used to examine potential crystallization of a drug in the GI tract as a function of rising pH.

During the development of drug products, predictability is a desirable attribute for the assurance of drug product quality (Uchiyama, 1996). In the development of pharmaceutical products, many different formulation types or dosage forms are examined prior to settling on the final market image of the

* Corresponding author. Tel.: +1 215 652 2827.
E-mail address: Kyle_Fliszar@Merck.com (K.A. Fliszar).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.09.023

product. Traditionally, researchers had very simple tools such as f_2 comparisons (CDER, 1997; EMEA, 1999) or very complex and costly techniques such as an *in vivo* bio comparison studies to compare different formulations. These formulations can be drastically different (liquid filled capsule versus roller compacted tablet) and the similarity of their performance *in vivo* will not be apparent using *in vitro* techniques.

Metformin hydrochloride is utilized in these experiments to examine a continuous dissolution/absorption system. Metformin is highly soluble in water (Bretnall and Clarke, 1998), but has low permeability as measured by standard Caco-2 techniques (Matsson et al., 2005) and a 500 mg dose is only 50–60% absorbed in humans (Timmins et al., 2005). Based on these characteristics, metformin is characterized as a BCS Class III drug when administered as an immediate release tablet (Amidon et al., 1995). The pharmacokinetics of metformin has been widely studied, and numerous immediate release dosage forms and oral solutions have been shown to be bioequivalent to each other (Cheng et al., 2004). Its absorption into the body is permeation rate limited, and only significant alterations to the dissolution rate, as would be observed from formulating metformin as an extended release tablet, will influence the rate and extent of absorption.

The research presented here builds upon work by Ginski and Polli (1999), and Kobayashi et al. (2001), in the development of a combination dissolution/absorption system to examine pharmaceutical formulations. The system developed couples a dissolution type apparatus with an absorption chamber that employs an artificial hexadecane membrane (HDM) as the permeation measurement. The objective in developing this system is to predict how changes in formulation will influence the *in vivo* performance of a drug while using an *in vitro* system that is simple to prepare.

2. Materials and methods

2.1. Model assumptions

When using a combined dissolution/permeation apparatus, the rate at which drug accumulates in the receiving chamber is a combined measurement of the rate of dissolution (k_d) and the rate of permeation (k_p) and is expressed as the combined rate, $k_{p,d}$. M_x represents the amount of drug administered, M_{pl} represents the amount of drug in the plasma, and k_{el} is the elimination rate of the drug from the plasma. This scheme can be represented as two ordinary differential equations listed below

$$\frac{dM_x}{dt} = -k_{p,d}M_x \quad (1)$$

$$\frac{dM_{pl}}{dt} = k_{p,d}M_x - k_{el}M_{pl} \quad (2)$$

M_x at time zero is defined as:

$$M_{x0} = \frac{DF}{V} \quad (3)$$

In which D is the dose of the drug being administered, F is the fraction of the drug available for transport and V is the volume of distribution of the drug. Solving these equations, one can relate the amount of drug in the plasma and time via the following equation:

$$M_{pl} = \frac{k_{p,d}DF}{V(k_{el} - k_{p,d})}(e^{-k_{p,d}t} - e^{-k_{el}t}) \quad (4)$$

By measuring, the combined dissolution/permeation rate of a drug from a formulation, one can estimate the amount of drug that will appear in the plasma at a given time. This rate is calculated by determining the fraction dose absorbed in the receiving chamber versus time. The fraction dose absorbed is the related to $k_{p,d}$ through the following first-order expression:

$$FDA = (1 - e^{-k_{p,d}t}) \quad (5)$$

In cases where a good linear fit can not be made to calculate the $k_{p,d}$, this rate can be represented as a function (exponential or power) rather than a value. In these cases, a non-linear function is fitted to data for the fraction of the dose absorbed versus time to generate a function that describes the change of $k_{p,d}$ with respect to time.

2.2. Formulations

Three formulations containing metformin hydrochloride were examined. Formulation A contains 1000 mg of metformin hydrochloride at a drug load of 76.9% and standard pharmaceutical excipients. Formulation B contains 1000 mg of metformin hydrochloride at a drug load of 76.9% and is identical in composition to formulation A with the addition of 0.5% sodium lauryl sulfate. Formulation C is glucophage® XR 500 mg obtained from Bristol Myers Squibb (Princeton, NJ).

2.3. Dissolution/absorption system components

The continuous dissolution/absorption system is shown in Fig. 1. This system consists of a Van Kel dissolution vessel (Cary, NC), a laboratory stirrer (Fisher, Pittsburg, PA) and a side-by-side diffusion cell (Permegear, Bethlehem, PA). In this system, dissolution of the full dosage form and permeation across the HDM occurred simultaneously. At the start of the experiment, dissolution medium was filtered through a 10-μm Full Flow® (Van Kel, Cary, NC) filter and transferred via peristaltic pump

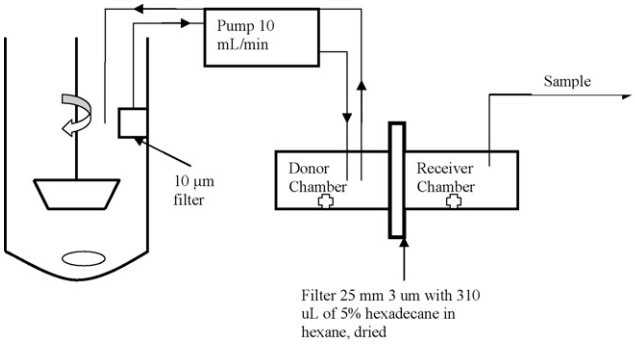

Fig. 1. Schematic of dissolution/absorption system.

(Distek, North Brunswick, NJ) through polyvinylchloride tubing (internal diameter of 1.59 mm) to the donor compartment of the side-by-side diffusion cell. Pressed between the donor and acceptor cell was a HDM prepared on a 25 mm, 3 μm Isopore filter (Millipore, Ireland) as described by Wohnsland and Faller (Wohnsland and Faller, 2001). The filter was placed in a beaker and 310 μL of 5% hexadecane in hexane was added drop wise. The filter was allowed to dry for at least 1 h in a fume hood prior to initiation of the experiment. A stir bar was present in both the donor and acceptor cells to ensure a homogeneous solution was maintained. Medium returned from the donor compartment back into the dissolution vessel via a second polyvinyl chloride tube. The flow rate for both transfers was 10.0 mL/min.

2.4. Dissolution conditions

The dissolution portion of the experiment was carried out in 25 mM NaCl in water maintained at 37 °C using a circulating water bath (Fisher, Pittsburg, PA). The paddle speed of the laboratory mixer was fixed at 100 rpm and the dissolution volume was maintained at 250 mL for all experiments to simulate the chyme volume in the GI tract (Dressman et al., 1985).

For dissolution experiments only, similar conditions were used. The dissolution experiments were conducted in a traditional six vessel compendial apparatus (Van Kel, Cary, NC) with a dissolution medium of 25 mM NaCl (900 mL) and a rotation speed of 75 rpm. Sampling for the immediate release formulations was conducted using an auto sampler with filtration through a 10-μm Full Flow® (Van Kel, Cary, NC) filter at 10, 15, 20, 30, 45 and 60 min. Sampling for the extended release formulation was conducted at 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10 and 12 h via the same auto sampler and filtration system.

2.5. Permeability cell conditions

Both the donor and receiver sides of the cell were 5 mL, and were maintained at 37 °C via a circulating water bath. The donor side of the cell was filled with dissolution medium circulated from the dissolution vessel. The receiver portion of the cell was initially filled with Hank's balanced salt solution (HBSS). The receiver cell was sampled using a 1-mL aliquot at 0.25, 0.50, 1, 2, 3, 4, 5 and 6 h followed by immediate replacement with fresh HBSS.

2.6. HPLC analysis conditions

All samples were analyzed using an Agilent 1100 (Wilmington, DE) HPLC system equipped with a UV detector capable of analyzing 255 nm. The analysis was conducted using a Phenomenex Luna SCX 50 mm × 4.6 mm, 5 μm particle size column and a mobile phase consisting of a 75%/25% (v/v) solution of 50 mM KH_2P0_4 (pH 3.5): acetonitrile at a flow rate of 2.0 mL/min. Standards were prepared using USP reference standard at an appropriate concentration.

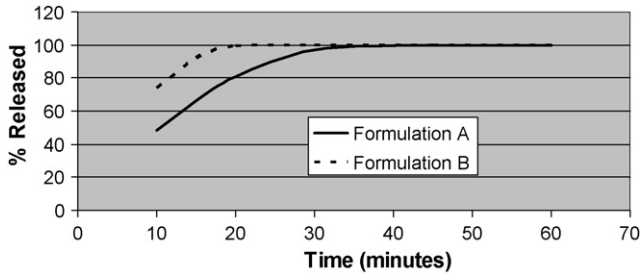

Fig. 2. Dissolution profiles of formulations A and B in 25 mM NaCl at 75 rpm USP II.

3. Results

3.1. Dissolution comparison

The dissolution profiles of formulations A, B, and C are shown in Figs. 2 and 3. The similarity factor (f_2) calculated between formulations A and B is 31.2 using the following equation, where n represents the number of dissolution measurements, R_t represents the amount of drug release from the reference formulation at time t, and T_t represents the amount of drug released from the test formulation at time t:

$$f_2 = 50 \log \left\{ \left[1 - \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t) \right]^{-0.5} \times 100 \right\} \quad (6)$$

A value less than 50 indicates that these two release profiles are dissimilar. Given the significant difference in release rate of formulation C, one can assume that this formulation would be considered dissimilar from both formulations A and B. An f_2 comparison was not done between formulations A and C or formulation B and C due to the different number and time of sampling points. The dissimilar nature of these two formulations indicates that a bioequivalence study should be run to demonstrate that the two formulations behave identically *in vivo*.

3.2. In vivo comparison

The plasma pharmacokinetic profiles for each of the three formulations were examined. In all cases the *in vivo* data comes from $N = 12$ patients dosed under fasting conditions. The *in vivo* studies were conducted as open-label, randomized, three parts studies using healthy male and female subjects ages 18–50. As

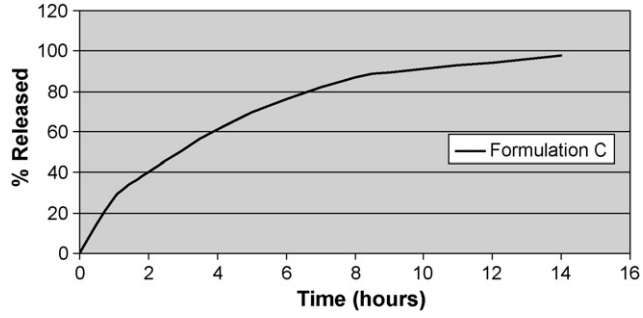

Fig. 3. Dissolution profile of formulation C in 900 mL 25 mM NaCl at 75 rpm USP II.

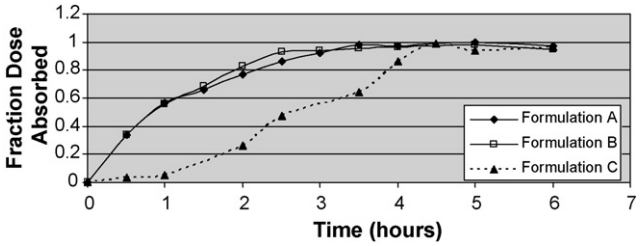

Fig. 4. The fraction dose absorbed for the three formulations evaluated.

shown in Fig. 4, the fraction of the dose absorbed in the plasma was plotted for each formulation. The rates of absorption for formulations A and B were nearly identical, while the absorption rate for formulation C was significantly slower.

3.3. Dissolution/Absorption system data

The absorption rate of metformin hydrochloride, as measured by appearance of metformin hydrochloride in the receiving chamber, was determined for each of the formulations. The absorption profiles for all three formulations are shown in Fig. 5. These profiles were generated by measuring the concentration of the drug in the receiving chamber at a given time. This concentration was then converted to a total amount of drug absorbed through the membrane by taking into account the volume of chamber and subsequent samplings of the chamber. The absorption rates for formulations A and B were also similar in this experiment (1.61 and 1.56 mg/h for formulations A and B, respectively) relative to one another as seen *in vivo*. In addition, formulation C also shows a significantly slower absorption rate (1.06 mg/h) as was observed in the *in vivo* data.

Fig. 6 illustrates the fraction of the dose absorbed in dissolution/absorption system for each formulation over time factoring in the *in vivo* fraction dose absorbed data to determine an endpoint. The data from Fig. 4 is used to set a time at which the fraction of the dose absorbed *in vivo* approaches a plateau. This time point is then used as a reference time to end the *in vitro* experiment. In the dissolution/absorption system, there is no elimination occurring and fresh media is added to the receiving chamber creating constant sink conditions. This makes calculating a fraction dose absorbed versus time very difficult, as the amount of drug absorbed will continue increasing with time indefinitely. By examining the *in vivo* fraction dose absorbed curves, the time at which the amount of drug absorbed levels off (F_a approaches 1.0) was used as the end point for the *in vitro*

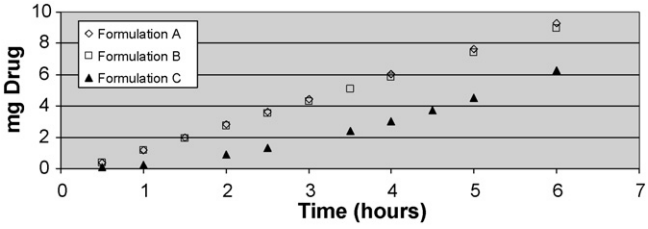

Fig. 5. Metformin absorbed through the artificial membrane as measured by drug in the receiving compartment.

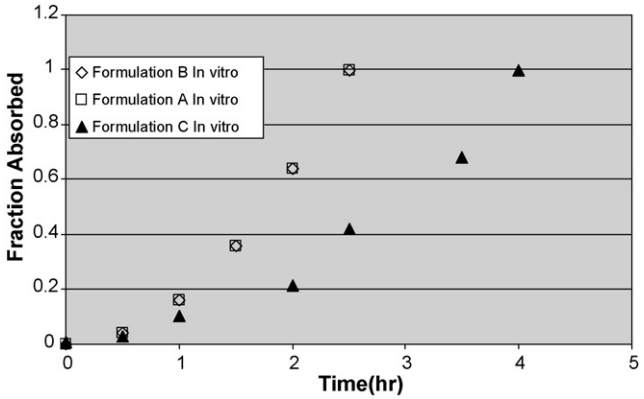

Fig. 6. Fraction dose absorbed of metformin as measured via the dissolution/absorption system.

experiments. For formulations A and B this was 2.5 h, and for formulation C this was 4.5 h. The amount of drug absorbed in the *in vitro* experiment at this ending time was used to determine the fraction of the drug absorbed at the times sampled up to that point. This plot again illustrates the near-identical absorption between formulations A and B, and the significantly slower absorption for formulation C.

Fig. 7 illustrates the ability of the dissolution/absorption system to predict differences or similarities between formulations observed *in vivo*. When the same data plotted in Fig. 6 are combined with fraction dose absorbed data generated *in vivo*, one can see a distinct difference between the immediate release formulations and the extended release formulation. In addition, the data from the model illustrates that no difference will be observed between the two immediate release formulations. The deconvoluted *in vivo* data was generated using the AUC for each formulation through 2.5 or 4.5 h without factoring in k_{el}.

3.3.1. Pharmacokinetic profile prediction

Using the combined rate of dissolution/absorption measured in the in vitro system, a prediction of pharmacokinetic profile was attempted. Fig. 8 illustrates the predicted and observed pharmacokinetic profiles for each of the three formulations

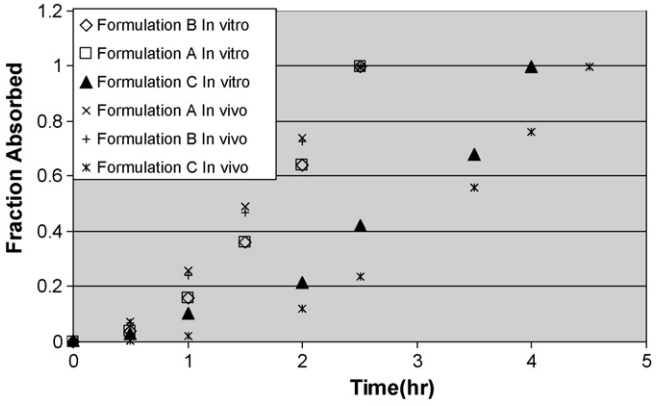

Fig. 7. Fraction dose absorbed as measured in the dissolution/absorption system compared with data generated from the *in vivo* analysis.

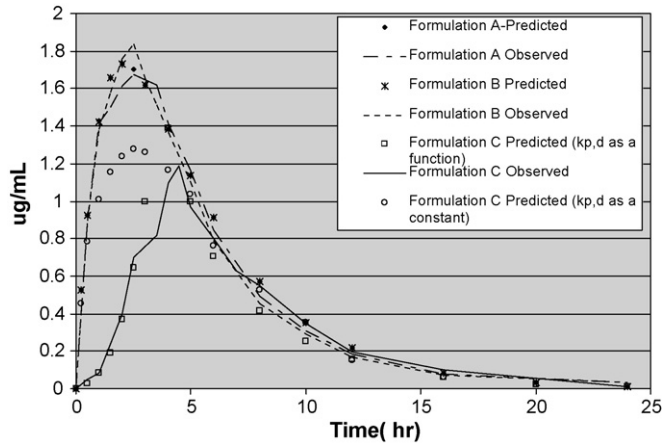

Fig. 8. Mean observed (from $N = 12$ healthy volunteers) and predicted plasma concentration values for metformin.

Table 1

Value	Formulation	Observed	Predicted	%PE
C_{max} (ng/mL)	A	1,870	1,730	−7.4
	B	1,920	1,730	−9.8
	C ($k_{p,d}$ constant)	1,150	1,240	+7.6
	C ($k_{p,d}$ change over time)	1,150	1,390	+20.8
$AUC_{(0\rightarrow\infty)}$ (ng h/mL)	A	11,700	11,900	+1.6
	B	11,800	11,900	+0.72
	C ($k_{p,d}$ constant)	7,620	11,900	+56.0
	C ($k_{p,d}$ change over time)	7,620	7,050	−7.5

examined. For the extended release formulation, the prediction was performed using $k_{p,d}$ as a constant and as a function related to time. Table 1 illustrates the predicted and observed AUC and C_{max} values, as well as % Prediction Errors (PE) obtained for each formulation. For all formulations a V (Volume of distribution) of 35,000 mL and an F of 1 were used. The value was derived from a prediction of the effective volume of distribution for most drugs in highly perfused organs (Usansky and Sinko, 2005).

4. Discussion

The biopharmaceutical classification system (BCS) indicates that class I drugs are considered high solubility/high permeability, and class III drugs are high solubility/low permeability (Amidon et al., 1995). Furthermore, based on the BCS, metformin hydrochloride is defined as BCS Class III drug (Lindenberg et al., 2004). In 2000, the FDA issued a guidance on requesting biowaivers for drugs falling into the BCS Class I category and maintaining rapid dissolution (>85% in 30 min) (FDA, 2000). In addition, researchers have proposed that BCS Class III drugs are also strong candidates for consideration of a biowaiver given that similar formulations should all approximate the performance of an oral solution given a rapid dissolution (Cheng et al., 2004; Blume and Schug, 1999). The rapid dissolution of an immediate release BCS III drug for consideration of a biowaiver has been defined as >90% in 30 min (Yu and Amidon, 1999), or if comparing two immediate release formulations an f_2 of >50 should exist between the two profiles (Cheng et al., 2004).

The immediate release metformin hydrochloride formulations examined in this work do not have dissolution profiles that are considered similar, but they do exhibit nearly identical absorption profiles when monitored using the dissolution/absorption system and *in vivo* plasma analysis. Although both immediate release formulations showed >90% drug released in 30 min, the differences noted in the formulations at the early time points make bioequivalence between the two formulations difficult for the FDA to accept. The third formulation examined has a significantly slower release rate (only 22% released in 30 min) leading to a decreased absorption rate both *in vitro* and *in vivo*. This observation of *in vivo* performance of the two immediate release dosage forms relative to the extended release dosage form agrees well with behavior indicated by the BCS. For a BCS III drug, absorption is permeation-rate limited unless the dissolution rate is depressed to a point that it becomes rate limiting as in the case of the extended release formulation. The dissolution/absorption system examined in this work serves as an excellent direct measure of the influence of the interplay between dissolution and permeation on absorption. The ratio of absorption rates *in vitro* between the immediate release formulations and the extended release formulation are nearly identical to the behavior observed *in vivo*. This conclusion would not be reached by comparing only the *in vitro* dissolution curves of the individual formulations, as evidenced by the f_2 values obtained when comparing the two immediate release formulations.

This work also demonstrates the viability of using an artificial membrane as the permeation barrier rather than Caco-2 cells, as had been shown in previous work. An artificial membrane can be ready for use in hours rather than weeks, it requires simple techniques to prepare, and it offers significantly simplified storage and handling conditions compared with live cell lines. Although this experiment used 25 mM sodium chloride as the dissolution medium, the artificial membranes allow for a wider range of media, such as low pH or surfactant based media, which may destroy live cell lines, rendering them useless for measuring permeability. The absorption characteristics of the drug being examined should be strongly considered before using an artificial membrane in such studies. The artificial membrane enables the measurement of the passive diffusion of a molecule and does not take into account active transport. If a molecule is primarily or even significantly absorbed via an active transport mechanism, the artificial membrane may not serve as an appropriate tool for measuring the permeability of the drug.

The predicted pharmacokinetic curves for both of the immediate release metformin formulations agree well (<10% PE) with the *in vivo* curves. The extended release formulation has good agreement for C_{max}, but the prediction is poor for AUC when a constant rate is used for $k_{p,d}$. The poor prediction of AUC has been reported for drugs that exhibit site-dependent absorption or have incomplete absorption (Gillespie, 1997). Given the simplistic nature of the system used to describe the pharmacokinetics, it is not unreasonable to expect a poorer prediction for more complex systems such as extended release tablets. However, the prediction did show that a significant discrepancy between the

shapes of the pharmacokinetic profiles exists between immediate release and extended release. The shape of the curve and the accuracy of prediction for AUC were significantly better when a function was used to describe $k_{p,d}$ instead of a constant. The equation used to fit the fraction dose absorbed data was an exponential expression that had a significantly better correlation ($r = 0.98$ for exponential fit versus $r = 0.88$ for linear fit). In the case of the extended release formulation, using a model with a time dependent $k_{p,d}$ is a better representation of the *in vivo* process. The immediate release formulations essentially provide the entire drug in a bolus dose, and $k_{p,d}$ is really a measure of the permeation rate because the dissolution rate is so rapid and $k_{p,d}$ as a constant value is a good representation. In the case of the extended release formulation, $k_{p,d}$ is not a direct measure of k_p as k_d still influences the process by determining how much drug is available at a given time to permeate the membrane. In this case, a time dependent $k_{p,d}$ is a better representation of the system.

A dissolution/absorption system gives researchers an added tool to gain confidence in the performance of a formulation *in vivo* prior to running a costly and time-consuming bioequivalence study. This tool will allow researchers to quickly screen a number of formulations that may have different release rates, compositions or manufacturing processes to determine how those changes may affect the absorption rate of the drug. This system can be further evaluated to determine its performance using other BCS class drugs, different media and different dissolution conditions.

References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Artursson, P., Karlsson, J., 1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochim. Biophys. Acta 175, 880–885.

Blume, H., Schug, B., 1999. The biopharmaceutic classification system (BCS): Class III drugs—better candidates for BA/BE waiver? Eur. J. Pharm. Sci. 9, 117–121.

Bretnall, A., Clarke, G., 1998. Metformin hydrochloride. In: Brittain, H.G. (Ed.), Analytical Profiles of Drug Substances and Excipients, 25. Academic Press, San Diego (CA), pp. 243–293.

Cheng, C., Yu, L., Lee, H., Yang, C., Lue, C., Chou, C., 2004. Biowaiver extension potential to BCS Class III high solubility-low permeability drugs: bridging evidence for metformin immediate-release tablet. Eur. J. Pharm. Sci. 22, 297–304.

Dressman, J.B., Amidon, G.L., Fleisher, D., 1985. Absorption potential: estimating the fraction absorbed for orally administered compounds. J. Pharm. Sci. 74, 588–589.

FDA Guidance for Industry, 2000. Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based ona biopharmaceutics classification system. CDER/FDA.

Gillespie, W.R., 1997. Convolution based approaches for in vivo in vitro correlation modeling. Adv. Exp. Med. Biol. 423, 53–65.

Ginski, M.J., Polli, J.E., 1999. Prediction of dissolution–absorption relationships from a dissolution/Caco-2 system. Int. J. Pharm. 177, 117–125.

Guidance for industry immediate release solid oral dosage forms scale-up and post approval changes: Chemistry, manufacturing and controls, 1997. In vitro dissolution testing and in vivo bioequivalence documentation. Center for drug evaluation and research (CDER), CMC 5.

Human medicines evaluation unit, 1999. Note for guidance on quality of modified release products: A. oral dosage forms; B. Transdermal dosage forms; Section 1 (Quality). The European agency for the Evaluation of Medicinal Products (EMEA), CPMP/QWP/604/96.

Kansay, M., Senner, F., Gubernator, K., 1998. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. J. Med. Chem. 41, 1007–1010.

Kobayashi, M., Sada, N., Sugawara, M., Iseki, K., Miyazaki, K., 2001. Development of a new system for prediction of drug absorption that takes into account drug dissolution and pH change in the gastro-intestinal tract. Int. J. Pharm. 221, 87–94.

Li, S., He, H., Parthiban, L., Yin, H., Serajuddin, A., 2005. IV-IVC considerations in the development of immediate-release oral dosage form. J. Pharm. Sci. 94, 1396–1414.

Lindenberg, M., Kopp, S., Dressman, J., 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines. Eur. J. Pharm. Biopharm. 58, 265–278.

Matsson, P., Bergstrom, C., Nagahara, N., Tavelin, S., Norinder, U., Artursson, P., 2005. Exploring the role of different drug transport routes in permeability screening. J. Med. Chem. 48, 604–613.

Pagliara, A., Reist, M., Geinoz, S., Carrupt, P.A., Testa, B., 1999. Evaluation and prediction of drug permeation. J. Pharm. Pharmacol. 51, 1339–1357.

Tavelin, S., Taipalensuu, J., Soderberg, L., Morrison, R., Chong, S., Artursson, P., 2003. Prediction of the oral absorption of low-permeability drugs using small intestine-like 2/4/A1 cell monolayers. Pharm. Res. 20, 397–405.

Timmins, P., Donahue, S., Meeker, J., Marathe, P., 2005. Steady-state pharmacokinetics of a novel extended-release metformin formulation. Clin. Pharm. 44, 721–729.

Uchiyama, M., 1996. Prospect of drug product quality-regulatory considerations. Proc. Int. Symp. Control. Release Bioact. Mater. 23, 63.

Upoor, V., 2001. Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations. J. Control. Release 72, 127–132.

Usansky, Helen H., Sinko, Patrick J., 2005. Estimating Human Drug oral absorption kinetics from Caco-2 permeability using an absorption-disposition model: model development and evaluation and derivation of analytical solutions for ka and Fa. J. Pharmacol. Exp. Ther. 314, 391–399.

Veng-Pedersen, P., Gobburu, J., Meyer, M., Straughn, A., 2000. Carbamazepine level-A in vivo in vitro correlation (IVIVC): a scaled convolution based predictive approach. Biophar. Drug Dispos. 21, 1–6.

Wohnsland, F., Faller, B., 2001. High-throughput permeability pH profile and high-throughput alkane/water logP with artificial membranes. J. Med. Chem. 44, 923–930.

Yu, L.X., Amidon, G.L., 1999. A compartmental absorption and transit model for estimating oral drug absorption. Int. J. Pharm. 186, 119–125.