Research Article

A Novel Beads-Based Dissolution Method for the *In Vitro* Evaluation of Extended Release HPMC Matrix Tablets and the Correlation with the *In Vivo* Data

Uroš Klančar,¹ Boštjan Markun,¹ Saša Baumgartner,^{2,3} and Igor Legen¹

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Abstract. The aim of this work was to establish alternative *in vitro* dissolution method with good discrimination and *in vivo* predictability for the evaluation of HPMC extended release matrix tablets. For this purpose, two different HPMC matrix tablet formulations were first evaluated by a range of conventional dissolution testing methods using apparatus 1, apparatus 2, and apparatus 3 according to US Pharmacopoeia. Obtained results showed low discrimination between the tested samples. Afterward, a novel dissolution testing method which combines plastic beads and apparatus 3 was developed with the aim to better mimic the mechanical forces that occur *in vivo*. Results showed that sufficiently large mechanical stress with high dips per minute program setting (apparatus 3) was needed to obtain *in vitro* discriminative results, which are in accordance with the *in vivo* data. The *in vivo* relevance of the method was confirmed with the establishment of the level A *in vitro*–*in vivo* correlation.

KEY WORDS: dissolution method; extended release; HPMC; IVIVC; matrix tablets; quality by design.

INTRODUCTION

Matrix tablets are one of the most common extended-release solid dosage forms. The mechanism and the modeling of a drug's release from hydrophilic matrices have been extensively studied and reviewed in the literature (1–5). The dissolution of drugs from various matrix tablets is mostly tested by *in vitro* dissolution testing. Moreover, the dissolution methods with good *in vivo* predictability contribute significantly to the development of a bio-equivalent product and the establishment of an *in vitro*–*in vivo* correlation (IVIVC) or *in vitro*–*in vivo* relationship (IVIVR). This is especially emphasized in the quality-by-design concept development strategy initiated by the US Food and Drug Administration (6–8).

For the development of *in vivo* predictive dissolution methods, various physiological conditions along the gastrointestinal (GI) tract must be considered. The influence of pH, bile acids, surfactants, ionic strength, and other constituents in the dissolution medium can affect the release profile from matrix tablets (9–12). Additionally, the influence of different hydrodynamic stress conditions should be considered, since the release of the drug from the matrix tablets is usually the combination of erosion of a polymeric matrix and diffusion of the drug out of the matrix (2,4).

The erosion of the nascent polymer layer in the matrix tablet is affected by the surrounding medium and the different hydrodynamic, shear-stress, and frictional forces in the GI tract, all of which are difficult to simulate with *in vitro* testing. In the fasted state, the stress exerted on the tablet during a GI transit depends mostly on the migrating myoelectric complex (MMC), which cycles every 90–120 min and normally starts in the stomach. The movement of the tablets during first and second MMC phase is relatively slow; however, during the short, gastric emptying phase (phase III), the tablets are prone to a greater mechanical stress, since stomach contractions are intense (13–16). The gastrointestinal transit times, hydrodynamics, gastric emptying rates, and forces have been studied *in vivo* using different techniques (14,15,17). Pressures during the gastric emptying phase can be about 60 Pa in the fasted state and up to 96 Pa in the fed state (18). Furthermore, the gastric motility after food intake is elevated which exerts an even greater mechanical stress on the matrix tablets (18–20). There is also evidence that food intake stimulates the transport of the GI content from the terminal small intestine into the colon, a mechanism that is known as the gastro-ileal or gastro-ileocecal reflex, which can again exert elevated pressures on the matrix tablet. This phenomenon can be important when administering food some hours after the intake of the matrix tablet (14,15).

Considering the above, one would suspect that method parameters that affect the hydrodynamic conditions around the matrix tablets are the most relevant for *in vivo* performance. Different hydrodynamic conditions, however, are difficult to simulate with conventional *in vitro* dissolution methods. So far, some modifications to conventional

¹ Lek Pharmaceuticals d.d., Verovškova 57, 1526 Ljubljana, Slovenia.

² Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia.

³ To whom correspondence should be addressed. (e-mail: sasa.baumgartner@ffa.uni-lj.si)

dissolution apparatus, as well as novel dissolution methods for elucidating the mechanical stress, have been proposed (20–24). Abrahamsson *et al.* designed a rotation-beaker dissolution apparatus that enabled an *in vivo* predictable shear stress on the surface of the hydrophilic polymer matrix tablet (20). Sako *et al.* applied a mechanical stress to swollen matrices using glass beads, and they achieved a good correlation with the *in vivo* data (21). Garbacz *et al.* developed a novel dissolution test apparatus that mimics various GI pressure forces which influences the oral formulation during the gastrointestinal transit. They observed a good correlation between the *in vitro* sensitivity and the *in vivo* release from the matrix tablets (22). Bogataj, together with co-workers, developed a peristaltic-movement stirring device and showed a correlation with the *in vivo* results for diclofenac extended release tablets (23). Aoki *et al.* tested matrix tablets using apparatus 2 with the addition of polystyrene beads to better reflect *in vivo* conditions in gastrointestinal tract of beagle dogs. By using different bead amounts, media volumes and revolutions per minute values, they found the optimal conditions that were able to predict *in vivo* dissolution (24). The use of the USP dissolution apparatus 3 (reciprocating cylinder, Bio-dis) has also been suggested for simulating the mechanical conditions within the GIT, but it was not extensively studied for evaluating matrix tablets (25–27). Apparatus 3 and novel dissolution methods are therefore still of interest when searching for *in vivo* predictive dissolution methods, which enables the establishment of an IVIVC or IVIVR. The FDA defines three correlation levels: A, B, C and multiple level C. Level A is the highest level of correlation. The level A IVIVC model should be able to predict the entire *in vivo* time course from the *in vitro* data. Establishment of level A type of correlation is an important aspect of pharmaceutical science since it may be used to minimize the number of human studies required during product development, assist in setting meaningful *in vitro* dissolution specification, and justify biowaivers for scale-up and post-approval changes (28). However, a level A correlation is sometimes difficult to achieve due to the complexity and interplay of the various GI conditions affecting the matrix tablet (29). Additionally, poor dissolution method development (only few methods tested), the lack of physiological knowledge, and challenging drug properties may also attribute to poor level A correlation establishment.

The present study describes the development of a novel dissolution testing method for hydrophilic hydroxypropylmethylcellulose (HPMC) matrix tablets containing sulfonamide model drug with aim to discriminate samples used in bio-equivalence (BE) study. First, the dissolution testing of two challenging formulations containing different HPMC types was performed using different dissolution media and dissolution apparatus: apparatus 1 (basket method), apparatus 2 (paddle method), and apparatus 3 (Bio-dis) according to US Pharmacopoeia. All tested methods showed very low discrimination between the formulations, although a significant difference was observed in the BE study. Considering *in vivo* BE study results, a new dissolution testing system that combines apparatus 3 and plastic beads was developed with water as dissolution media. The “bead” system enables the simulation of a mechanical stress on swollen matrices with respect to the physiological GI conditions. The *in vivo*

predictability of the novel dissolution method was confirmed by predicting *in vivo* plasma profiles from the *in vitro* dissolution profiles.

MATERIALS AND METHODS

Materials

The tablets containing 1.5 mg of a sulfonamide model drug (thiazide diuretic) were prepared. The drug shows weakly acidic properties (pK_a 8.8) and solubility about 0.1 mg/ml in aqueous buffer solutions ranging from pH 1 to 8 at 37°C. Calculated dose/solubility volume is about 15 ml; therefore, the compound has good solubility according to BCS classification. The permeability is not a limiting factor for absorption (own data); thus, the drug may be classified in BCS class I. The drug's solubility rises above pH 8 when more soluble ionized species are formed; however, these pH values are physiologically not relevant; therefore, it can be stated that dissolution of this compound is pH independent within physiological pH ranges.

The excipients used in the formulations were Hypromellose USP Type 2208, grades K15M and K4M (DOW Chemical Company, Midland, MI, USA), lactose monohydrate 200 mesh (Friesland Campina, the Netherlands), polyvinylpyrrolidone PVP K30 (BASF, Germany), microcrystalline cellulose Avicel PH 102 (FMC BioPolymer, Norway), colloidal silica dioxide Aerosil 200 (Degussa, Germany), and magnesium stearate (Mallinckrodt Chemical Inc., USA).

Reagents for the dissolution testing were of analytical grade, purchased from Merck, Germany. This includes the NaOH, HCl, NaCl, and $NaH_2PO_4 \times H_2O$. Sodium taurocholate was purchased from Fluka (Sigma-Aldrich GmbH, USA) and Soluthin MD (hydrophilic lecithin) from Phospholipid GmbH, Germany. The reagents for the HPLC assay, acetonitrile, methanol, EDTA disodium salt, and glacial acetic acid were also obtained from Merck, Germany.

Tablet Preparation

The model drugs, lactose monohydrate and polyvinylpyrrolidone, were blended and granulated with purified water in a Collette Gral—PRO 25 (Machines Collette, Belgium) high-shear granulator. The granulate, with an incorporated drug, was dried under vacuum and sieved with a Frewitt MG 636 (Key International, USA) oscillating sieve, mesh size 0.5 mm. The granulate loss on drying was 2.6% tested 30 min at 80°C using loss on drying balance (Mettler-Toledo International Inc.). For each formulation, a prescribed amount of granulate (Table I), selected HPMC (Hypromellose USP Type 2208 K15M and K4M), colloidal anhydrous silica, and microcrystalline cellulose (present in formulation A) or lactose monohydrate (present in formulation B) were blended in a bin blender (Erweka, Germany). Magnesium stearate was then added and the whole composition was blended again to obtain the final mixture (Table I). The final mixture was compressed into tablet cores of diameter 8 mm using a tablet press (Ima Kilian LX 18, Germany) with a compression force of 7–10 kN to obtain tablets with a hardness of 100–120 N and a target mass of 200 mg (Kraemer automatic tablet tester, Germany). The compositions A and B

Table I. Compositions of the Studied Tablet Formulations in Milligrams

Formulation	A	B
Granulate with model drug	118.1	118.1
Hypromellose USP Type 2208, K15M (DOW)	40.0	
Hypromellose USP Type 2208, K4M (DOW)		70.0
Microcrystalline cellulose	40.0	–
Lactose monohydrate	–	10
Colloidal anhydrous silica	0.4	0.4
Magnesium stearate	1.5	1.5
Target mass	200.0	200.0

differ in terms of the HPMC amount and the viscosity grade. Formulation A contains 20% of HPMC with a viscosity of 15,000 Pas, while formulation B contains 35% of HPMC with a viscosity of 4,000 Pas.

In Vitro Testing

Dissolution Testing Media

The employed dissolution media were deionized water, a 0.05-M phosphate buffer pH 6.8 (USP), simulated gastric fluid (SGF), and a fasted-state simulated intestinal fluid (FaSSIF; Table II). The SGF media was prepared by adding NaCl and 1 M HCl into the water, adjusting pH value to 1.2 and degassing. The FaSSIF medium was prepared as follows: NaCl, NaH₂PO₄, sodium taurocholate, and Soluthin MD were dissolved and mixed in water to obtain a buffer with pH 6.5 (corrected with 1 M NaOH). The composition of the FaSSIF was similar as described previously in the literature (30,31). Sink conditions were achieved for all the tested media.

Dissolution Testing Using Apparatus 1 or Apparatus 2

First, the tablets were tested using dissolution apparatus 1 (basket method) and apparatus 2 (paddle method) in accordance with US Pharmacopoeia (Table III). The dissolution tests were performed using a dissolution tester (Erweka DT6, Germany) coupled with an automatic sampler (Vankel VK8000, USA). Standard vessels with

baskets or paddles were utilized at stirring rates 75, 100, and 150 rpm with 900 ml of dissolution media, however; only 100 rpm tests and results were further explored since there was no difference in discrimination ability between tested rpm values. The dissolution media used in experiments were already described and are shown in Table II. The media temperature was set to 37±0.5°C. For the paddle method, the tablets were put in a 40-mesh basket sinker to prevent floating of the swollen matrix tablets several hours after commencing the test. For each time point, 1.7 ml of sample was automatically collected and filtered through 4.0 µm tip filters (Erweka, Germany) to 2.0 ml vials. The dissolution medium was not replaced. For pH change simulation test, the tablets were tested using apparatus 1 commencing the test in SGF media for first 2 h, followed by FaSSIF media replacement. Sampling times and other dissolution test conditions are shown in Table III.

Apparatus 3 and Bead Dissolution Testing Method

A novel dissolution-testing method was developed using apparatus 3—the reciprocating cylinder (Varian Vankel Bio-dis III, USA) dissolution testing station and plastic beads (Fig. 1). The beads were round, made of a synthetic polymeric material with a density of 1.1 g/cm³, and had a diameter of 8 or 1 mm. The density of the beads was similar to the density of the testing media, which allows the appropriate movement of the beads in the cylindrical vessel under various dipping speeds and enables frequent interactions with the tablet. The density of the beads is a crucial parameter in this method. Prior to the use of plastic beads, also glass beads with density of about 2.5 g/cm³ were tested. It was observed that due to high density, glass beads sunk to the bottom of the cylinders and did not exert any mechanical forces on the tablets even when high dipping speeds were used (40 DPM).

The dissolution test procedure using plastic beads was performed as follows: A total of 250 ml of water medium was poured into each dissolution vessel and placed in a water bath to maintain a temperature of 37 ±0.5°C. Next, beads to fill approximately one fourth of the vessels (8 g) were weighed and placed into reciprocating cylinders. The tablets were weighed and placed on the top of the beads, and the cylinders were attached to the Bio-dis. The polypropylene mesh size on the top and

Table II. Composition (Millimoles per Liter), pH, and Ionic Strength of Different Dissolution Testing Media

Media	Water	Phosphate buffer (USP)	SGF	FaSSIF
Composition	Deionized water	KH ₂ PO ₄ 50 mmol NaOH 22 mmol	NaCl 34 mmol HCl 80 mmol	Sodium taurocholate 3 mmol Soluthin MD 0.75 mmol NaH ₂ PO ₄ ×H ₂ O 28.7 mmol NaOH 13.8 mmol NaCl 106 mmol
pH	7.0	6.8	1.2	6.5
Ionic strength	–	0.072	0.074	~0.152

SGF simulated gastric fluid, USP US Pharmacopoeia, FaSSIF fasted-state simulated intestinal fluid

Table III. Various Dissolution Testing Methods

Test	T1	T2	T3	T4	T5	T6	T7	T8
Test type	Apparatus 1	Apparatus 1	Apparatus 2	Apparatus 1, simulation	Bio-dis, beads 8 mm	Bio-dis, beads 8 mm	Bio-dis	Bio-dis, beads 1 mm
Setting	100 RPM	100 RPM	100 RPM	100 RPM	20 DPM	20:40:25 DPM	20:40:25 DPM	20:40:25 DPM
Sampling times (h)	2, 4, 6, 8, 10, 12, 16	2, 4, 6, 8, 10, 12	2, 4, 6, 8, 10, 12	1, 2, 4, 6, 8, 10, 12, 16, 20	1, 2, 4, 6, 8, 10, 14	1, 2, 4, 6, 8, 10	1, 2, 4, 6, 8, 10	1, 2, 4, 6, 8, 10, 14
Medium	Phosphate buffer (USP)	Water	Water	SGF, FaSSIF	Water	Water	Water	Water

SGF simulated gastric fluid, USP US Pharmacopoeia, FaSSIF fasted-state simulated intestinal fluid

bottom of the cylindrical vessels was 840 μm . At the beginning, the DPM rate program was set simply at 20 DPM but was finally adjusted in three intervals: 1 h at 20 DPM, 15 min at 40 DPM, and the remaining part of the test with 25 DPM. The middle 15-min phase with 40 DPM was set to simulate the forces in gastric emptying phase, which is known for its intense hydrodynamic pressures as seen from literature data (14,15). To evaluate the effect of beads, the test was run also without beads using the same DPM settings. The sampling time points were from 1 up to 14 h depending on the test (Table III; Fig. 2).

HPLC Assay

An HPLC system, Waters separation module 2695D (Waters, USA) with UV detection at a wavelength of 254 nm, was used for the analysis of the collected samples. The

chromatographic column Chromolith Speed ROD RP-18e (Merck, Germany) with dimensions of 50 $\times$ 4.6 mm was thermostated at 36°C during the analysis. The mobile phase had the composition of acetonitrile/methanol/NaEDTA/glacial acetic acid in the ratio 275:175:550:1 (volume ratio). The NaEDTA solution was prepared by dissolving 1 g of EDTA disodium salt in 5,000 ml of purified water. The flow rate of the mobile phase was 2.0 ml/min, and 100 μl of sample solution was injected from the vials maintained at 4°C. The retention time of the model drug was around 1 min; the total run time of the analysis was 1.3 min. The HPLC method was validated.

In Vivo Study Protocol

Both formulations were tested in a human relative bio-availability study under fasting conditions. The study was conducted by an outsourcing agency (Scentryphar, Brasil) on 26 healthy volunteers using an open-label, randomized, crossover-study design. Each subject randomly received both tablets in two treatments and two sequences over two periods. The subjects were confined for 36 h per period; the washout period before the second period was at least 14 days. Over each period, 24 blood samples (6 ml each) were collected over 96 h (see Fig. 3 for exact time points). The active ingredient in the plasma biological matrix was determined by high-performance liquid chromatography connected to mass spectrometry.

In Vitro–In Vivo Correlation

The *in vitro*–*in vivo* correlation was modeled by the direct differential-equation-based IVIVC method described by Buchwald (32). This is a one-stage IVIVC method that directly relates the *in vitro* dissolution profile to the *in vivo* plasma concentration profile. Such a single-step approach to establish the level A *in vitro*–*in vivo* correlation is also recognized in the FDA's Guidance on Extended Release Oral Dosage Forms (28) as an alternative to the two-stage de-convolution-based approach, which aims for a point-to-point relationship between the *in vitro* dissolution and *in vivo* absorption. The principle of the differential equation-based IVIVC is similar to the commonly employed one-stage convolution-based IVIVC methods (33–35). It does, however, offer more modeling

Fig. 1. Beads dissolution testing system: vessel for dissolution medium (1), dissolution medium (2), cylindrical vessel (3), plastic beads of diameter 8 mm (4), tablets (5), perforated bottom and top of cylindrical vessel with polypropylene mesh, size 840 μm (6), sampling probe (7), water bath with temperature of 37°C (8)

Fig. 2. Dissolution profiles for formulation A (straight line) and formulation B (dotted line) with SD error bars ($N=3$) obtained from various dissolution testing methods numbered from T1 to T8. T1 to T8 conditions are presented in Table III. For clarity, only 12 h time scale is shown

flexibility by avoiding the integral transforms used in the de-convolution- or convolution-based IVIVC models and can be also used in cases when no pharmacokinetic data from intravenous or oral solution reference formulation are available.

With the differential equation-based method, the *in vivo* plasma concentration profiles are modeled directly by solving the differential equation of underlying pharmacokinetic model. We have used a simple one-compartment open model which was found adequate to model

the observed plasma concentration profiles and is described by the following differential equation (36):

$$dA/dt = r_a(t) - k_{el}A \quad (1)$$

where A is the amount of drug in the central compartment (plasma), $r_a(t)$ the *in vivo* absorption rate, and k_{el} the elimination constant of drug from plasma. The plasma concentration of the drug, C_p , is obtained by dividing the amount A by the volume of distribution V_d . The key step in

Fig. 3. Mean plasma profiles with standard errors (C_p plasma concentration) collected from the test subjects ($n=26$) for formulations A (diamonds) and B (squares): **a** shown for 96 h; **b** first 24 h shown. Arrow indicates the concavity of plasma profile due to change in release rate at approximately 7 h

developing the level A *in vitro*–*in vivo* correlation is to find the relationship between the *in vivo* absorption rate $r_a(t)$ and *in vitro* dissolution rate $r_{dis}(t)$. This can be done by comparing directly $r_a(t)$ with $r_{dis}(t)$ —or more often their corresponding cumulative analogs, the cumulative absorption function $F_a(t)$ and the cumulative dissolution function $F_{dis}(t)$. The *in vivo* absorption function $F_a(t)$ is conveniently estimated from plasma concentration profiles by the Wagner–Nelson method Eq. 2, assuming a one-compartment pharmacokinetic model:

$$F_a(t) = (C_p(t) + k_{el} \text{AUC}_{(0-t)}) / k_{el} \text{AUC}_{inf}, \quad (2)$$

where $C_p(t)$ is the concentration of drug in plasma at time t , k_{el} the elimination constant, $\text{AUC}_{(0-t)}$ the area under plasma concentration curve from time zero to time t , and AUC_{inf} the area from time zero to infinity. Alternatively, the correlation can be established by finding the relationship between the *in vivo* time scale (t) to the *in vitro* time scale (t^*) in such a way that $F_a(t) = F_{dis}(t^*)$ —that is, the total *in vivo* amount of absorbed drug at time t is equal to the total *in vitro* amount of dissolved drug at time t^* . This relationship between t and t^* is usually visualized by the so-called Levy plot (37). In this paper, we follow the time-scaling approach, which has proved very useful in cases when the *in vivo* drug absorption and *in vitro* drug release occur on different time scales (32,34,38,39).

The procedure is as follows: The *in vitro* dissolution profiles of the formulations A and B were first fitted by the Weibull function using the OriginPro 8.0 software (© Origin-Lab Corporation):

$$F_{dis}(t^*) = F_{max} \left[1 - \exp\left(-(\alpha t^*)^\beta\right) \right], \quad (3)$$

where F_{max} is maximum amount of dissolved drug, α and β parameters of the Weibull function, and t^* time *in vitro*. An empirical power law time-scaling relation was applied:

$$t^* = t^a, \quad (4)$$

where a is the model parameter, whose optimal value is yet to be determined. This time-scaling relation was used to transform the *in vitro* dissolution profile $F_{dis}(t^*)$ into the *in vivo* absorption profile $F_a(t)$ by the equation $F_a(t) = F_{dis}(t^*)$. From the cumulative absorption profile $F_a(t)$, the absorption rate $r_a(t)$ is obtained, and the plasma profiles can be simulated by solving the differential equation Eq. 1. The differential equation was solved by the Euler integration method. The time-scaling parameter a was optimized with the Solver Tool in the Excel™ software (Microsoft Office Professional Plus 2010) to obtain the best fit of the simulated plasma profiles to the observed plasma profiles. The plasma profiles for the formulations A and B were fitted simultaneously with the same time-scaling relation, i.e., the same a parameter. This is required as a valid level A correlation should have the same form for both formulations. All other parameters of the pharmacokinetic model, k_{el} and V_d , were kept fixed and the same for both formulations, as the same group of subjects received both formulations in the *in vivo* study. A weighted sum of squares was chosen as the objective function to be minimized by the optimization procedure (40):

$$O_{WLS} = \sum_{i=A,B} W_i (C_i - C'_i)^2, \quad (5)$$

where C_i are the *in vivo* observed plasma concentrations at time points t_i and C'_i the modeled plasma concentrations at time points t_i . The weights W_i were set to $1/\sigma_i^2$, where σ_i is *in vivo* determined standard deviation of C_i . The sum is performed over all time points t_i for both formulations—A and B.

The predictive performance of the model was quantified according to the recommendation of regulatory authorities (28,41) for the evaluation of internal predictability of the IVIVC. To evaluate the internal predictability of the model, the percent prediction error (%PE) for the C_{max} and AUC parameters should be calculated as

$$\%PE = \frac{[(\text{observed value} - \text{predicted value}) / \text{observed value}] \times 100}{(6)}$$

The internal predictability of the model is considered acceptable if the average absolute percent prediction error across all formulations is less than 10% for the C_{max} and the AUC parameters and in addition the absolute %PE for each of the predicted parameters is less than 15%.

RESULTS AND DISCUSSION

It is well-known that the release from HPMC matrix tablets is a combination of the erosion of the gel layer and the diffusion of the dissolved drug from the swollen gel layer on

the tablet. Swollen matrices with a predominately erosion-controlled release mechanism are susceptible to the surrounding mechanical forces, depending on various factors, especially the amount and the viscosity grade of the dissolution-rate-controlling polymer. In our previous work, we showed that there appears to be a minimum threshold value of polymer viscosity and/or polymer amount that assures a controlled, non-accelerated, drug-dissolution profile even in the presence of increased stress conditions in the gastrointestinal tract (42). Additionally, it was shown that in matrices with lower polymer amounts or lower polymer viscosity grades the release is predominately erosion controlled and the tablets have high mechanical susceptibility. This is also the case for formulations in the present study; thus, the introduction of mechanical stress *in vitro* may better discriminate the samples.

Development of Dissolution Method Using Apparatus 1 and Apparatus 2

The testing of tablet formulations A and B started by using conventional dissolution methods. The results from different dissolution tests are presented in Fig. 2.

Test T1 in the phosphate buffer pH 6.8 medium produced the smallest difference between the studied tablet formulations. Additionally, only about 60% of the drug was released in 12 h from both studied formulations (T1; Fig. 2).

When water was used as a dissolution medium (T2; Fig. 2), the dissolution profiles for both formulations were faster than in the case of the phosphate buffer pH 6.8. Moreover, the test was more discriminatory.

In test T3 using apparatus 2 and water as a dissolution medium, comparable results to test T2 were observed (T2, T3; Fig. 2). Comparing the results from tests T2 and T3 with T1 showed that the dissolution tests performed in water were more discriminative compared to the phosphate buffer pH 6.8. This may be attributed to the higher ionic strength of the phosphate buffer medium, which normally decreases the drug-release rate from the HPMC matrix tablets, which can diminish the discriminatory ability of the method. The mechanism of slowing the drug release from the HPMC matrix tablets in the presence of different salts was studied previously (43–45). One of the possible explanations for the slower release is the competition of the polymer chains with the ionic constituents for the water molecules; therefore, there is less water available for the polymer hydration and consequently the drug release from matrix tablet is slower.

To better reflect *in vivo* conditions, the formulations were further tested in pH change simulation test using apparatus 1 in SGF changed by FaSSIF media after 2 h. The discriminatory ability of this method was low as evident from dissolution profiles for test T4 in Fig. 2.

On the basis of results from tests T1 to T4 and *in vivo* BE study results (see below), it was concluded that apparatus 1 and apparatus 2 did not provide *in vivo* discriminative dissolution results. Considering this, we continued experiments with apparatus 3, where the beads were incorporated. Water was selected as a suitable and most discriminatory medium since the dissolution results from tests T1–T4 were most discriminative in water.

Development of Dissolution Method Using Apparatus 3 with and Without Beads

All results obtained by testing the tablets using apparatus 3 (reciprocating cylinder, Bio-dis) with or without beads (T5–T8; Fig. 2) showed a faster drug release in comparison to the tests T1–T4. The hydrodynamic conditions in Bio-dis are different from those in apparatus 1 or 2 since the tablet is trapped in a reciprocating cylindrical vessel, which moves vertically throughout the medium. This causes faster drug release rates compared to basket or paddle methods. In comparison to previously published paddle-beads method (24), we found the use of apparatus 3—beads method beneficial since the matrix tablets in our particular case floated on the top of the media while tested with the paddle method. This is a common phenomenon in hydrophilic matrix tablets that swells and the density is reduced. Therefore, it was assumed that beads in the vessels of apparatus 2 could not impact the matrix tablets in the same manner as in apparatus 3 since in the apparatus 3, the tablets and the beads float constantly up and down throughout the cylindrical vessels enabling frequent collisions.

The results from test T5 where 8 mm beads were added and DPM value set to 20 showed better discriminatory level compared to apparatus 1 and 2 tests (Fig. 2). However, to find out if the discriminatory ability can be increased, the DPM settings at apparatus 3 were further adjusted and divided into three phases. The first phase was 1 h at 20 DPM, the second 15 min at 40 DPM, and the third phase at 25 DPM. Second phase was introduced to mimic gastric emptying phase where mechanical forces exerted on the tablet are elevated (14,15). Using this DPM program together with beads, the discriminatory power was the highest among all the methods tested (T6; Fig. 2). Evidently, the presence of the beads causes additional friction forces that enable a faster erosion of the matrix tablet's gel layer, leading to faster drug release rates and better discrimination between erosion susceptible matrices. The importance of the beads was confirmed further with results obtained by the method using the same DPM program settings, but without the presence of beads (T7; Fig. 2) where the discriminatory power was lower compared to the test with the beads (T6; Fig. 2).

To evaluate the significance of the beads size, 1-mm beads were additionally tested. It was observed that 1-mm beads stick to the gel layer surface; consequently, the

Fig. 4. Dissolution profiles of formulation A and B in the dissolution test T6. Measured dissolution points (A—diamonds, B—squares) and corresponding fitted Weibull functions (A—straight line, B—dotted line) are shown

Table IV. The Estimated Parameters of the Weibull Function

$F_{\text{dis}}(t^*) = F_{\text{max}} (1 - \exp(-(\alpha t^*)^\beta))$		
	Formulation A	Formulation B
$F_{\text{max}} (\%)$	101.8	101.5
$\alpha (1/\text{h})$	0.2996	0.2309
$\beta (/)$	1.469	1.635

release was slower and the discriminatory ability was low (T8; Fig. 2).

Interestingly, in our experiments, relatively high dipping speeds were used to discriminate between the samples in comparison to studies presented elsewhere in literature where the authors described dipping speeds of about 10 DPM (27,46,47). The highest discriminatory ability was observed in method using high DPM settings in combination with 8 mm size beads. This finding indicates that sufficient hydrodynamic stress is needed to discriminate between the studied tablets. Furthermore, the importance of short 15-min phase with 40 DPM (gastric emptying phase) was emphasized since results incorporating this phase showed better discriminatory power and *in vivo* relevance compared to test T5 where all the testing was performed at 20 DPM.

Above findings indicate that the novel dissolution method with beads of proper size and density that apply the additional mechanical stress is advantageous for evaluating matrix tablets where erosion is involved in the release mechanism. Additionally, the mechanism of release can be explained. Evidently, formulations A and B exhibit different release profiles due to the different susceptibilities to mechanical stress. It seems that formulation A forms a less robust gel layer, even if the viscosity of the HPMC K15M is higher than in formulation B (HPMC K4M). However, in formulation A, polymer represents only 20% of the whole tablet, whereas HPMC K4M in formulation B represents 35% of the tablet. Probably the amount of polymer in formulation A is not sufficient to form homogenous matrix even if the polymer viscosity grade is higher compared to formulation B. The higher sensitivity of the gel layer on the formulation A leads to faster erosion and dissolution of the drug from the matrix tablet, especially in the presence of beads.

It is important to note that on the basis of conventional dissolution testing, one could come to the conclusion that 20% of the HPMC K15M (formulation A) is enough to form a robust gel layer and that these tablets will be bio-equivalent to the tablets with 35% of HPMC K4M (formulation B). The results obtained from analyzing the tablets with USP apparatus 1 or 2 did not provide sufficient discrimination between the formulations. In contrast, the results obtained with the newly developed Bio-dis beads method showed good discrimination between the samples which could prevent such simplified conclusions that lead to the bio-non-equivalence of the products.

In Vivo Results

The mean plasma profiles from the *in vivo* studies are presented in Fig. 3. The release and, consequently, the absorption of the drug from formulation A was faster than from formulation B, which is evident from the shorter t_{max} and the higher C_{max} (Fig. 3).

From the average plasma profiles, it is clear that the release from formulation B is more constant, since the C_{max} peak of the curve is relatively flatter than in the case of formulation A (Fig. 3b). The concavity seen on the curves at approximately 7 h (Fig. 3b), the point at which the release gets faster (especially for formulation A), could be attributed to the elevated hydrodynamic stress in the GIT after the consumption of a meal given to the test subjects (the meal was given 5 h after the dosing). As explained in the "INTRODUCTION," after a meal the GIT's mechanical activity is elevated, which can affect the release from the matrix tablets, especially if the release is more erosion controlled (14,15).

Furthermore, the plasma profiles show that the t_{max} is long which means that the rate of absorption of the drug into systemic circulation is slow. The elimination half-life of the drug is also long, approximately 15 h. The drug accumulates in the body slowly; therefore, rapid peaks in the plasma profiles are not to be expected, especially if the drug release from the matrix tablet is consistent and robust. However, the average plasma profile from formulation A exhibits a narrower C_{max} peak than that of formulation B (Fig. 3), which leads to the conclusion that the consistency of the gel layer of the matrix tablet A can be disrupted more easily than that of tablet B.

Fig. 5. **a** Drug absorption profiles for the formulations A and B obtained by the Wagner–Nelson method; **b** Levy plot demonstrating the relationship between the *in vivo* (t) and *in vitro* (t^*) time scales for the dissolution test T6. The dotted line shows the function $t^* = t^{0.61}$, obtained by optimal fitting of the plasma profiles

Fig. 6. Observed mean plasma profiles (A—diamonds, B—squares) and simulated plasma profiles (A—straight line, B—dotted line) for the formulations A and B

In Vitro–In Vivo Correlation

The dissolution of formulations A and B in the novel dissolution test T6 and the corresponding fitted Weibull functions are shown in Fig. 4. In Table IV, the estimated parameters of the Weibull function are shown. The mean plasma profiles of the formulations A and B in the BE study are shown in Fig. 3.

The elimination constant k_{el} was estimated from the terminal phase of the plasma profiles (Fig. 3), and the values 0.048/h and 0.045/h were obtained for the formulations A and B, respectively. In addition, the apparent volume of distribution was calculated by the equation $V_d/F = \text{Dose}/(k_{el} \text{AUC}_{inf})$, where F stands for the fraction of dose absorbed. We obtained V_d/F 44 and 49 L for the formulations A and B, respectively. A higher value of the apparent volume of distribution for formulation B, in addition to lower AUC, is an indicator that less drug was absorbed from this formulation. The difference between the elimination constants is not considered significant as the drug does not show the “flip-flop” effect. In the *in vivo* study, the same subjects received both formulations; therefore, also the same pharmacokinetic parameters were used when simulating plasma profiles for both formulations, A and B ($k_{el}=0.048/\text{h}$; $V_d=44$ L). It was assumed that the absorption from the formulation A was complete in the reported *in vivo* study ($F=1$).

The absorption kinetics was calculated using the Wagner–Nelson method (Fig. 5a), and the Levy plot was constructed (Fig. 5b). As can be seen from the Levy plot, the relationship between the *in vitro* (t^*) and *in vivo* (t) time scales is not linear. This is probably due to changing physiological conditions as the dosage form proceeds along the gastrointestinal tract, which precludes the establishment

of a unique linear time scaling between the *in vitro* dissolution and *in vivo* absorption (37,39,48). The time scaling relation was approximated by a one-parametric power law function: $t^*=t^a$ Eq. 4. The plasma profiles were then simulated with optimization of the time scaling parameter a . The following optimal time scaling relation was obtained, $t^*=t^{0.61}$, giving the best fit of the simulated plasma concentration profiles to the observed profiles. The simulated and observed plasma profiles are shown in Fig. 6.

The simulated plasma profiles mimic the *in vivo* plasma profiles quite well. The observed and predicted values of the C_{max} and AUC parameters and the corresponding prediction errors are shown in Table V.

It can be concluded that the presented IVIVC model predicts the C_{max} and AUC parameters with good accuracy and satisfies the conditions for the internal predictability of the level A correlation, set by the regulatory agencies. It should be, however, noted that because only two formulations were used to develop the correlation, the evaluation of external predictability is recommended in this case for additional confirmation and subsequent full application of the *in vitro*–*in vivo* correlation.

With the presented modeling approach, it was also possible to develop the level A correlation for the dissolution tests T3, T5, T7, and T8—according to the criteria set by the regulatory guidance. However, as these tests are less discriminatory, the prediction errors were higher compared to the model based on the dissolution test T6. In addition, the fit of the simulated plasma profiles to the observed plasma profiles was poorer for these dissolution tests as compared to the fit based on the T6 test. Therefore, tests T3, T5, T7, and T8 are considered less useful for supporting the development of a bioequivalent formulation in comparison to the T6 dissolution test, even though they can satisfy the criteria for the level A correlation from the regulatory point of view. We were not able to develop the level A correlation based on the dissolution tests T1, T2, and T4, as these test were not discriminatory enough.

CONCLUSION

A new, simple dissolution method utilizing Bio-dis apparatus with beads for testing hydrophilic matrix tablets was developed. The method was able to predict *in vivo* performance and enabled the development of level A IVIVC for the tested formulations. It was noted that high mechanical forces *in vitro* were necessary to provide a satisfactory correlation with the *in vivo* data. Established IVIVC was used to simulate plasma profiles and to predict C_{max} and

Table V. Internal Predictability of the IVIVC Model

Values	C_{max} (ng/ml)			AUC_t (ng h/ml)			AUC_{inf} (ng h/ml)		
	Obs.	Pred.	% PE	Obs.	Pred.	% PE	Obs.	Pred.	% PE
Formulation A	20.7	19.1	7.7	697	714	−2.4	707	726	−2.7
Formulation B	15.6	16.8	−7.7	665	707	−6.3	679	722	−6.3

Obs. and Pred. values of the C_{max} and AUC parameters and the corresponding prediction errors are shown

Obs. observed, Pred. predicted

AUC values. The use of beads-based dissolution method may be useful in the future since robust matrix formulations that are bio-equivalent to the reference product could be planned during early stages of the development. In spite of good correlation established in our case, it is important to stress that the method needs to be tested on a “case by case” principle since different formulations and compounds respond differently to the surrounding environment *in vivo* and *in vitro*.

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