ORIGINAL ARTICLE

COMPARISON OF THE *IN VITRO* DISSOLUTION PROFILES FOR A HIGH SOLUBILITY DRUG FROM IMMEDIATE RELEASE FORMULATIONS USING USP APPARATUSES 3 AND 4

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Abstract

The current paper represents the continuation of a previous report focusing on the role of *in vitro* dissolution testing in the estimation of the *in vivo* performance for immediate release solid oral dosage forms containing a BCS class 3 drug, metformin hydrochloride. The methodology was extended by application of alternative compendial apparatuses (flow-through cells and reciprocating cylinders, using basket or paddle method as reference) and testing of a higher strength (850 mg). The experimental results suggested the complementarity of information generated by different hydrodynamic parameters and volumes of media.

Rezumat

Prezenta lucrare reprezintă continuarea unui raport anterior asupra rolului testelor de dizolvare *in vitro* în estimarea performanței *in vivo* a formelor farmaceutice solide orale conținând o substanță medicamentoasă din clasa 3 BCS, metformin clorhidrat. Metodologia a fost extinsă prin aplicarea unor aparate compendiale alternative (celulele în flux continuu și cilindri cu mișcări alternative, utilizând metodele cu paletă sau coșuleț ca referință) și testarea unei doze mai mari (850 mg). Rezultatele experimentale au sugerat complementaritatea informației generate de parametri hidrodinamici și volume de mediu diferite.

Keywords: *in vitro* dissolution, BCS class 3, immediate release, reciprocating cylinder, flow-through cell

Introduction

The utility of the *in vitro dissolution* methodologies for the evaluation of the quality and potential *in vivo* performance of oral solid dosage forms containing a high solubility, low permeability drug has been screened previously [1]. The methodologies were direct applications of the specific monographs or adaption based on non-compendial approaches, with notable hydrodynamic differences and variable impact on the disintegration of dosage units and dissolution of metformin hydrochloride as a model drug with limited absorption through the intestinal barrier. Paddle and basket methods are preferred for the development of routine quality control procedures of conventional formulations, based on the considerable experience gained since their official adoption. Both are mentioned as part of the waiver procedures based on the Biopharmaceutics Classification System [2-4], with differences between the guidance documents issued by several regulatory authorities. However, there are known limitations of the dissolution apparatuses, related not only to the hydrodynamics. The conventional round bottom vessels are described as closed systems, with volumes of aqueous fluids of at least 500 mL. The volume used for BCS classification is 250 mL, therefore the utility of small

vessels has been considered for development of more bio-relevant approaches [5-7].

The standardization of the testing parameters, in order to assess the outcome of the dissolution test as a property of the dosage form combining the drug with excipients in a controlled manufacturing process [8], makes it difficult to simulate in an *in vitro* setup the complexity of the *in vivo* phenomena. Particularly the mechanical stress acting on the pharmaceutical dosage unit is a critical factor, as well as the limited and variable volume available for dissolution. The reciprocating cylinder and flow-through cells are mostly used for the assessment of modified release formulations. The availability of several rows of small volume, flat bottom vessels gives the opportunity to simulate the pH change specific to the gastro-intestinal tract [9, 10]. At the same time, setting the frequency of reciprocating movements allows the control of the shearing forces acting on the solid dosage forms. For the flow-through cells, the versatility and the theoretically unlimited volume of media with more or less hydrodynamic standardization are two of the most frequently mentioned advantages [11, 12]. In the current version of the general chapter 1092 of the United States Pharmacopoeia [13] it is suggested that its potential application in case of immediate release formulation can provide insights for the very first moments of the dissolution processes. This is of particular importance for instances where very rapid dissolution occurs (more than 85% of the labelled claimed amount in 15 minutes or less) and it is highly relevant for the elucidation of sources of variability associated with early sampling time in conventional approaches. In this context, it is to be mentioned that recently US Food and Drug Administration issued the final version of the BCS-based biowaiver guidance [4], which extends the application limits to class 3. Additional requirements of qualitative and quantitative similarity (Q1 and Q2) of the compared immediate release solid dosage forms are included in this attempt of international harmonization [14].

In this paper we present the results of the *in vitro* dissolution testing of metformin hydrochloride from four marketed immediate release solid oral dosage forms. The methodological approach used comparable parameters, adapted from the available compendial monographs (paddle and basket method) to the particularities of the reciprocating cylinder and flow-through cells. The aim was to evaluate the impact of the assumed differences in volume of media and its pattern of flow on the kinetics and variability of drug release, as well as their discriminatory character for the known differences in composition between the products.

Materials and Methods

Four marketed immediate release, pharmaceutically equivalent solid oral dosage forms containing 850 mg metformin hydrochloride were subjected to the *in vitro* dissolution testing using four distinct methodological approaches, all based on compendial apparatuses. For the paddle and basket method, volumes of 1000 mL of phosphate buffer pH 6.8, prepared according to USP, were used. The media was degassed by filtration under vacuum. All tests were performed on six replicates, at 37 ± 0.5°C. Manual collection of the samples was performed as described previously (7 samples *per* vessel, 60 minutes total duration; [1]), with media replacement. Quantitative analysis of metformin hydrochloride was performed spectrophotometrically at 233 nm, using a validated method [15]. The basket method was applied according to the test 1 of the individual USP monograph (40 mesh conventional basket, at 100 rpm), with Q value of 70% fraction dissolved in 45 minutes as acceptance criteria. The paddle method (75 rpm) corresponded to the test 2, with Q value of 75% in 30 minutes.

For the reciprocating cylinder, the experimental protocol included Erweka Release Rate Tester BioDis RRT10 equipment (Erweka GmbH, Germany), equipped with polypropylene 420 µm screens at both ends of the inner cylinders (lower cap, stainless steel mesh; upper cap, polypropylene mesh). The dosage forms where immersed at a frequency of 20 dips *per* minute on 10 cm in 250 mL aqueous buffer processed as described previously (300 mL flat bottom, conventional vessels). The samples were collected 10 seconds after stopping the movements at indicated times and raising of the inner cylinder.

In a distinct approach, the large tablet flow-through cells (22.6 mm inner diameter, 19 mL volume) were mounted on an Erweka DFZ 720 system (Erweka GmbH, Germany), equipped with a 7 channels HKP 720 piston pump, previously calibrated at 95 - 105 nominal flow rate. The cells were filled with a single 5 mm glass sphere for regulation of unidirectional flow, as well as 7 g of 2 mm glass beads, for hydro-dynamic standardization (columnar flow; [12]). The outflow was passed though glass fibre filters (25 mm diameter, 2.7 µm declared mean pores size, type GD/F) and collected fractionally. The conventional tablets were positioned in holder, above the layer of glass beads. A flow rate of 16 mL/min was used, leading to a total volume of 960 mL of buffer media throughout the test duration.

The mean dissolution profiles were cross analysed for comparison of release kinetics, associated variability and discrimination for the assumed differences between tested multisource products.

Results and Discussion

Considering the specific compendial monograph, all the four generic film coated tablets fulfil the acceptance criteria for both basket and paddle method (Figure 1 and Figure 2). In fact, the dissolution was complete within 30 minutes and independent on the apparatus (more than 95% of the label claimed content of metformin hydrochloride). The tested products had a simple composition, qualitatively similar for A and C (no information on the technical grade of hydroxy-propyl-methylcellulose or povidone was available). The product D, containing a super-disintegrant, represented a particular instance where the dissolution was very rapid independent of the methodology, but highly variable initially.

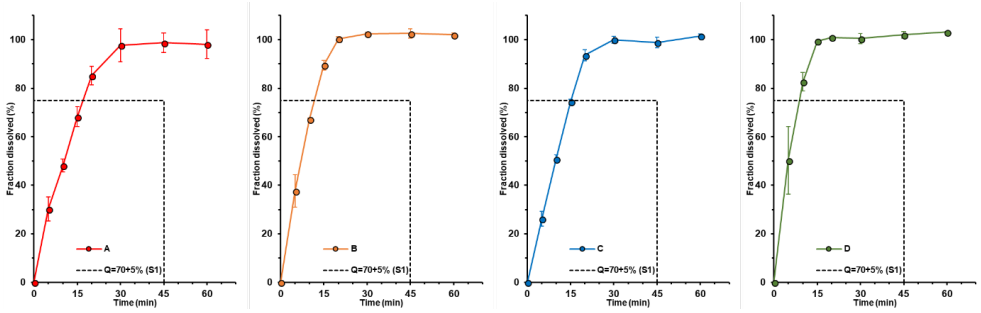

Figure 1.

The *in vitro* dissolution profiles of metformin hydrochloride from conventional immediate release formulations using the basket method (mean ± standard deviation, n = 3)

Note: dotted line represents the acceptance criteria according to test 1 (USP individual monograph)

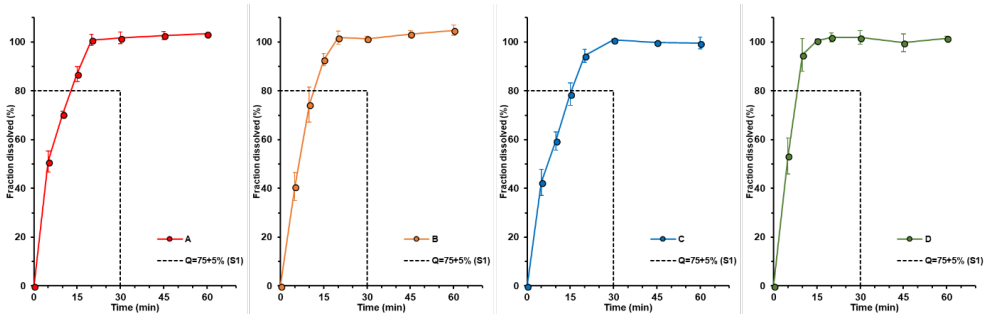

Figure 2.

The *in vitro* dissolution profiles of metformin hydrochloride from conventional immediate release formulations using the paddle method (mean ± standard deviation, n = 6)

Note: dotted line represents the acceptance criteria according to test 2 (USP individual monograph)

The reciprocating cylinders exercised a mechanical stress directly onto the solid dosage form, accelerating the disintegration without significant changes of the dissolution rate. The fraction released after 5 minutes is comparable between the four products, with mean differences lower than 10%. However, this is related to generation of large fragments of both drug and excipients, mainly resident onto the lower sieve. Significant expulsion of solid particles occurred after this first sampling, leading the gradual or almost instantaneous complete release. Even thoughl; deposition on the bottom of the vessel was observed, this didn't increase the variability, nor limited the dissolution rate.

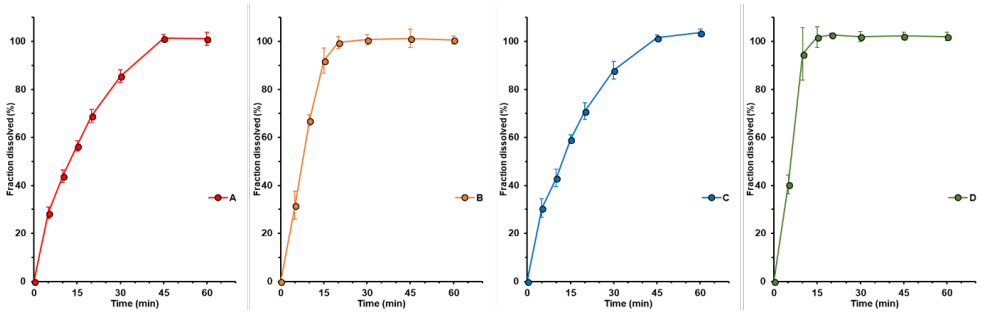

Figure 3.

The *in vitro* dissolution profiles of metformin hydrochloride from conventional immediate release formulations using the reciprocating cylinders (mean ± standard deviation, n = 3)

The variability distribution profile is different from the flow-through setup. At first sampling point, corresponding to 5 minutes after initiation of the flow, the fractions dissolved are reduced, 1.9% to 6.2%, with coefficients of variation above 10% only for the rapidly disintegrating products but below 20% in all cases. Considering the continuous elution and consecutive collection of the media, these values of the fraction dissolved are possibly reflecting the first 2 - 3 minutes of the analysis. Positioning of the resulting fragments on top of the glass beads layer, as well as their different size during this second half of the test could be an explanation for the apparent increased dispersion of the experimental data.

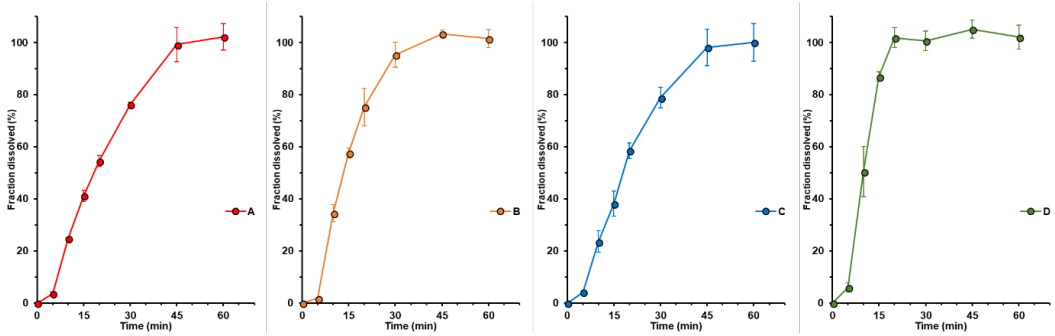

Figure 4.

The *in vitro* dissolution profiles of metformin hydrochloride from conventional immediate release formulations using the large tablet flow-through cells (mean ± standard deviation, n = 3)

To be noted, the kinetic discrimination was not altered, meaning that the fraction released at reference time points (15 and 30 minutes) were well within the regulatory limits for definition of rapid (products B and D) and very rapid dissolution (products A and C). It can be assumed that the short lag-time is not related to the performance of the products, but rather a typical methodological artefact, traced back to the length of the outflow circuit. The dosage forms evolved in a limited momentary volume, although continuously replaced by blank media, therefore the dissolution rates are lower compared to the other dissolution apparatuses.

Even though the experimental approach for BCS-based biowaiver procedures is different from the routine quality control, particularly in this case the volume used for paddle and basket methods, the same limits for model-independent kinetic assessment of the mean *in vitro* dissolution profiles was applied. The data corresponding to the 15 and 30 minutes sampling points are presented in Table I. There are two kinetically-distinct groups, corresponding to the visually observed disintegration pattern. The dissolutions of rapidly disintegrating product coded B and C were not significantly influenced by the hydrodynamic and volume dependent particularities of the experimental setup. The same conclusion is valid for the more slowly dissolving products code A and C. The fraction dissolved for product A at 15 minutes utilizing the paddle method can be considered as a borderline value.

Table I

The assessment of dissolution kinetics based on dissolved fractions at 15 and 30 min

Apparatus	Time point / kinetics	Product			
		A	B	C	D
Basket	15 min	68.26 ± 4.08	89.48 ± 2.06	74.41 ± 1.03	99.43 ± 0.52
	30 min	97.68 ± 6.72	102.47 ± 0.68	100.00 ± 1.38	100.47 ± 2.14
	Dissolution	Rapid	Very rapid	Rapid	Very rapid
Paddle	15 min	86.88 ± 3.02	92.77 ± 2.44	78.65 ± 4.64	100.56 ± 0.63
	30 min	101.67 ± 2.46	101.35 ± 0.97	100.90 ± 0.26	101.80 ± 2.76
	Dissolution	Very rapid	Very rapid	Rapid	Very rapid
Reciprocating cylinder	15 min	56.47 ± 2.13	91.95 ± 5.24	59.19 ± 1.85	101.77 ± 4.41
	30 min	85.54 ± 2.66	100.69 ± 2.18	87.99 ± 3.70	101.99 ± 2.15
	Dissolution	Rapid	Very rapid	Rapid	Very rapid
Flow-through cells	15 min	41.19 ± 2.17	57.63 ± 1.85	38.23 ± 4.81	86.96 ± 1.86
	30 min	76.26 ± 0.74	95.30 ± 4.76	78.81 ± 3.94	100.67 ± 3.74
	Dissolution	Slow	Rapid	Slow	Very rapid

The comparison of the mean *in vitro* dissolution profiles was performed using the EMA (2010) conditions, considering a single time point after the 85% limit of the dissolved fraction for each product. To be noted, *in vitro* similarity was assessed within the same product, the goal being to underline the impact of

each methodology. The results presented in Table II address only to products coded A and C, where the calculation of the similarity factors was possible (at least three pairs on mean data available).

Table II

Within product evaluation of *in vitro* dissolution similarity based on the values of the difference (f_1) and similarity factors (f_2)

Comparison	Product: **A**		Product: **C**	
	f_1	f_2	f_1	f_2
Basket *vs*. Paddle	-	-	**12.15***	**51.16***
Basket *vs*. Reciprocating cylinder	**14.67***	49.26	20.41	42.11
Basket *vs*. Flow-through cells	46.30	28.45	49.14	25.66
Paddle *vs*. Reciprocating cylinder	-	-	25.84	36.90
Paddle *vs*. Flow-through cells	-	-	54.65	21.24
Reciprocating cylinder *vs*. Flow-through cells	29.15	38.06	30.27	36.38

* – *in vitro* similarity concluded

Due to the high solubility profile of the active pharmaceutical ingredient and intense stirring pattern, the paddle and basket method [15] were not able to discriminate between the different compositions. In all the other cases, the main source of the *in vitro* dissolution non-similarity is to be traced to the initial 15 minutes of the testing procedure. Moreover, the flow-through cells seem to provide the slowest release rates, with distinct dissolution patterns for the same product, when compared to the other three approaches. Presumably, there is an increased bio-relevance of the 15 minutes sampling point, because it corresponds roughly to 250 mL cumulative volume passing through the equipment. When compared to the reciprocating cylinder, the kinetic differences reflect mainly the critical role of the shearing forces induced by the amplitude and frequency of the alternative movements.

It is the authors' personal opinion that the current dissolution criteria for immediate release solid oral dosage forms containing BCS class 3 drugs are too restrictive. Based on *in silico* simulations, previous reports suggested that, within large limits, dissolution kinetics have not a significant impact on the *in vivo* exposure parameters [16]. The main limitations in absorption come from the physico-chemical properties of hydrophilic molecules. Theoretically, the location of intestinal absorption windows and the associated transit times necessary for reaching it should be considered in setting the time interval of complete release. This could trigger a case-by-case approach, not feasible for regulatory purposes. The application of various experimental setups, able to emphasize on the *in vitro* performance of the dosage forms when subject to controlled variables such as mechanical stress or limited volume of aqueous fluid, may be adequate for the bio-inequivalence risk assessment. Recent reports [17] suggest that the requirement of qualitative and quantitative similarity of composition may be too restrictive. The methodologies based on flow-through cells or reciprocating cylinder present several advantages in the evaluation of immediate release dosage forms and should be used as part of an aggregate *in vitro* similarity assessment.

Conclusions

The current report illustrates the complementarity of information generated by various methodological approaches based on compendial dissolution apparatuses in case of immediate release solid oral dosage forms containing metformin hydrochloride, a BCS class 3 drug. The flow-through cells and reciprocating cylinders provided alternative insights on the role of mechanical stress and limited momentary volume of aqueous media in dissolution, as the first step for the *in vivo* absorption. The *in vitro* test displayed different discriminatory character in relation to the composition of the products, therefore it is suggested that the combined results of several dissolution methods will provide an adequate risk assessment for bio-waiver procedures.

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