

THE EVALUATION OF *IN-VITRO* DISSOLUTION OF MYCOPHENOLATE MOFETIL IN AQUEOUS BUFFER MEDIA USING BASKET METHOD

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ABSTRACT: The paper presents the evaluation of the *in-vitro* dissolution profiles of four immediate release oral dosage forms containing mycophenolate mofetil in three aqueous buffer systems. The basket method was adopted and the results were cross-analyzed with the experimental results generated previously in a similar protocol using the paddle apparatus. The influence of the design of the sinkers was also evaluated. The data was analyzed based on pairwise comparison procedures and on calculation of specific dissolution parameters. The results indicated that the conclusions on similarity are dependent on both pH of media and stirring rate. It was confirmed that the acidic media with low pH values are not adequate for routine quality control purposes, because it induced a very rapid and complete release of the drug. The non-sink testing conditions generated by the phosphate buffer pH=6.8 emphasized a considerable impact of the dose solubility ratio.

Keywords: mycophenolate mofetil, *in-vitro* dissolution, USP apparatus 1, Japanese sinkers.

INTRODUCTION

The *in-vitro* dissolution tests gained a central role in the assessment of quality attributes and performance for a pharmaceutical dosage forms. They are also implemented as the main procedures for reducing the regulatory burden in the development, selection and registration of adequate oral formulations (Shah VP et al., 2014b), especially after official adoption of the Biopharmaceutical Classification System, BCS (Shah VP et al, 2014a). The applicability of the *biowaiver* concept, meaning the possibility to waive the costly, time consuming *in-vivo* demonstration of bioequivalence based on *in-vitro* relevant testing, is continuously extending beyond the initial framework (Tsume Y et al., 2010, 2012). Previously, we have analyzed the main differences between various official guidance documents, and we underlined the fact that several testing apparatus, under the recommended operating conditions may generate different conclusions on the similarity between the same two products (Aqel NN et al., 2014). Our previous paper was focused on the use of USP apparatus 2 at two levels of the stirring rate for the evaluation of dissolution profile for a weak base drug from immediate release, oral solid dosage forms, using the three pH-stages assessment. One of the main observations was that, although generating sink conditions, the hydrochloric acid 0.1N pH=1.2 is probably not an appropriate media for the development of a discriminatory quality control test. The dissolution was rapid and complete, independent on the stirring intensity and on the type of dosage form. Moreover, it was concluded that for the last stage of comparison, simulating the pH conditions in the distal segments of

the small intestine, the profiles are solubility limited and the mean fractions dissolved are determined by the dose to solubility ratio. The current paper adds more information to the initial report, by implementing two other testing equipments and cross-analyzing the *in-vitro* results, using various pairwise comparison procedures and calculation of specific dissolution parameters.

MATERIALS AND METHODS

In-vitro dissolution testing procedure

The *in-vitro* dissolution tests were performed on a group of four immediate release oral dosage forms containing mycophenolate mofetil: three coated tablet formulations containing 500 mg of the active pharmaceutical ingredient (noted C500, M500 and O500), respectively one hard capsule formulation of 250 mg (noted C250). C250 and C500 represented the reference listed drug.

The experimental procedure included the standard USP apparatus 1 (40 mesh baskets) at 100 rpm, installed on a Hanson SR8Plus equipment (Hanson Research Inc., USA). In case of formulation C250, an additional test was conducted, by using USP apparatus 2 at 50 rpm and Japanese sinkers. The dissolution media consisting of hydrochloric acid 0.1 N pH=1.2, acetate buffer pH=4.5 and phosphate buffer pH=6.8 were prepared according to the United States Pharmacopoeia (USP36/NF31, 2013). After degassing by filtration under vacuum, 900 mL of each aqueous media was added to the compendial 1000 mL round bottom vessels and allowed to heat to 37±0.5°C. The tests were performed on six individual dosage units.

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Samples of 5 mL were collected at 5, 10, 15, 20, 30, 45 and 60 minutes and were replaced by an equal volume of fresh media. Details on the products, reagents, analytical standards and quantitative evaluations were provided previously (Aqel NN et al., 2014).

Analysis of the dissolution profile

For comparison of the mean *in-vitro* dissolution profiles, pairwise procedures were adopted using C500 as reference, namely the calculation of official difference and similarity factors, f_1 and f_2 (Polli JE et al., 1997). Supplementary, the Rescigno indexes, ζ_1 and ζ_2 , were calculated according to the following formula:

$$\zeta_i(n) = \frac{\left[\int_0^n |\bar{X}_{Rj} - \bar{X}_{Tj}| \cdot dt \right]^{1/i}}{\left[\int_0^n |\bar{X}_{Rj} + \bar{X}_{Tj}| \cdot dt \right]^{1/i}} = \frac{\left[\sum_{j=1}^{n-1} \left(|\bar{X}_{Rj} - \bar{X}_{Tj}| \right) \left(\frac{t_{j+1} - t_j}{2} \right) \right]^{1/i}}{\left[\sum_{j=1}^{n-1} \left(|\bar{X}_{Rj} + \bar{X}_{Tj}| \right) \left(\frac{t_{j+1} - t_j}{2} \right) \right]^{1/i}},$$

where i is 1 or 2, corresponding to the two Rescigno's indexes, n is the number of experimental points, \bar{X}_{Rj} and \bar{X}_{Tj} are the mean fraction dissolved for the reference and, respectively, tested drug product, in the samples collected at time t_j .

For each mean *in-vitro* dissolution profile, including the previously reported data (Aqel NN et al., 2014), the following parameters were calculated and compared:

- Dissolution efficiency (Khan KA, 1975),

$$DE(\%) = \frac{\sum_{j=1}^{n-1} \left(|\bar{X}_{j+1} + \bar{X}_j| \right) \left(\frac{t_{j+1} - t_j}{2} \right)}{t_n}, \text{ for } 100\%$$

release,

- Mean Dissolution Time (Riegelman S et al.,

$$1980), MDT(\text{min}) = \frac{\sum_{j=1}^{n-1} \left(|\bar{X}_{j+1} - \bar{X}_j| \right) \left(\frac{t_{j+1} + t_j}{2} \right)}{\sum_{j=1}^{n-1} \left(|\bar{X}_{j+1} - \bar{X}_j| \right)},$$

where \bar{X}_j is the mean fraction dissolved in the samples collected at time t_j , whereas t_n is the test duration.

RESULTS AND DISCUSSION

Analysis of the mean *in-vitro* dissolution profiles

The dissolution in the acidic media was very rapid and complete, above the 85% threshold of the label claimed amount of drug, within the initial 5 minutes of the tests (figure 1.a). Except for the capsule formulation C250, a plateau region was noted for all the immediate release formulations beginning with the first sampling point, indicating a faster release rate compared to USP apparatus 2. Within this region, the variability was extremely reduced, the coefficient of variation being lower than 5%. In phosphate buffer pH=6.8 (figure 1.c), the weak base character of mycophenolate mofetil became the dominant of the kinetic profile, limiting the fraction dissolved to 12% for the highest dose strength. For the capsule formulation C250, the amount released was 31%. The difference was higher than the dose ratio, therefore the solubility is not the only determinant. The dissolution kinetics are distinct only in acetate buffer pH=4.5 (figure 1.b), with approximately 11% of dose released within 10 minutes for C250. The pharmaceutical non-equivalence with respect to the reference coated tablet formulation C500 is expressed by a short lag time, corresponding to the opening of the hard capsule. Subsequently, the resulting granulate releases the drug much faster, with mean fraction dissolved of 73% after 60 minutes. In these conditions, the profiles for both dose strengths didn't reach a plateau region, a slow dissolution process being noticed by the end of the testing period. This is probably due to the observed expulsion of drug and excipients particles out of the basket and accumulation of undissolved particles below the stirring element. Noteworthy, this region doesn't have adequate hydrodynamic conditions, despite the high stirring rate.

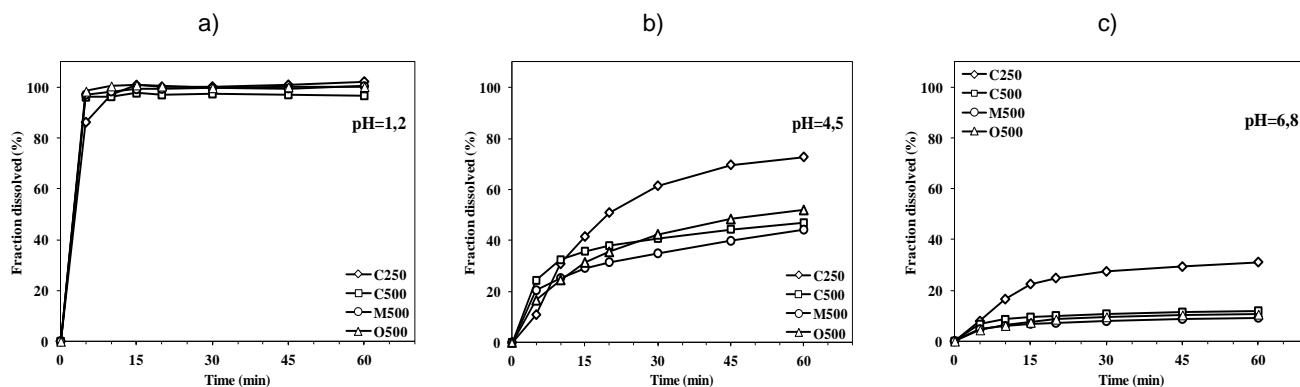


Fig. 1 The mean *in-vitro* dissolution profiles of mycophenolate mofetil in three media ($n=6$; standard deviation was not represented, for the clarity of the graphs): a) hydrochloric acid 0.1N pH=1.2; b) acetate buffer pH=4.5; c) phosphate buffer pH=6.8.

The influence of the sinker design

It concerns the use of Japanese sinkers, presented as an alternative device in chapter 711 of the USP36-NF31 (2013), the mean dissolution profiles of C250 are highly different, when compared to the previous setup based on the reference, USP compliant sinkers (Aqel NN et al., 2014). Globally, the mean fractions dissolved are lower and less variable (figure 2). For the acetate buffer pH=4.5 stage, the initial release phase seems to occur at a higher rate, correlated with the design of the Japanese sinker, i.e. higher volume, allowing an unrestricted opening of the capsule's shell (Soltero RA et al, 1989). After this phase, the external wire screen of the sinker probably altered the flow pattern of the media under the paddle and retarded the release from the undissolved particles of active pharmaceutical ingredient. It should be noted that the highest values of the coefficient of variation was observed in the acidic conditions. This observation could not be linked to a conning effect, because the hydrochloric acid solution 0.1N pH=1.2 provided adequate sink conditions. Therefore, it is probably a methodological artifact, generated by the small differences in positioning of the capsule formulation within the sinker, before opening of the shell.

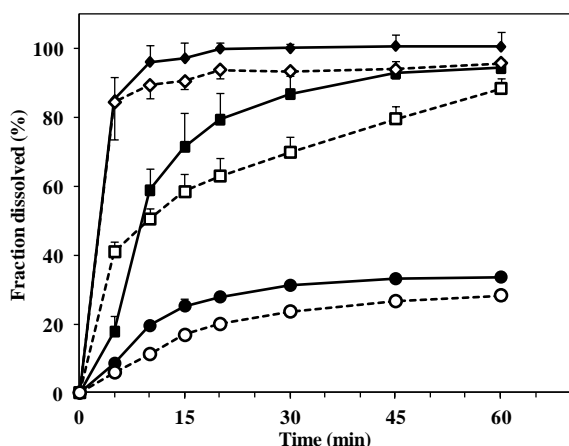


Fig. 2 The influence of the sinker on the *in-vitro* dissolution profiles of mycophenolate mofetil from C250 capsule formulation (n=6; mean +/- standard deviation; \diamond and \blacklozenge , pH=1.2; \blacksquare and \square , pH=4.5; \circ and \bullet , pH=6.8; open symbols with dotted lines for Japanese sinker; black symbols with straight lines for USP sinkers, data from Aqel NN et al., 2014)

Evaluation of the dissolution parameters

For the assessment of the interplay between the hydrodynamic conditions and experimental setup, solubility of the drug in compendial aqueous media with physiological pH, dose strength and type of dosage form, dissolution efficacy and mean dissolution time for each mean *in-vitro* release profiles were calculated (table 1). It becomes apparent that, based on the weak base characteristics of mycophenolate mofetil (Scheubel E et al., 2012), the solubility decreases as the pH is increased. This was transposed in a consistent drop in dissolution efficiency from more than 88% at pH=1.2 to less than 27% at pH=6.8. In the same testing

conditions, the mean dissolution time increased in a non-proportional manner from 2.60 - 4.46 min to, respectively, 8.83 - 17.06 min, with slightly higher values for the acetate buffer system with pH=4.5. Except for acidic stage of evaluation, the values of dissolution efficiency were almost double for the lower dose strength and presented a clear dependence the sinker design. For the phosphate buffer pH=6.8, the results are obviously generated by the dose solubility ratio. For the paddle method, the increase of the stirring rate from 50 to 75% generated a reduction of mean dissolution time up to 38.71% and doubled the dissolution efficiency at pH=4.5.

Table 1

The values of dissolution efficiency (%) and mean dissolution time (min) for mycophenolate mofetil in different media

Apparatus, conditions	Parameter	Dissolution Efficiency (%), DE			Mean Dissolution Time (min), MDT		
		pH=1.2	pH=4.5	pH=6.8	pH=1.2	pH=4.5	pH=6.8
USP1, 100 rpm	C250	95,16	52,64	23,98	4,19	16,61	13,83
	C500	92,96	37,93	9,92	2,32	11,54	10,28
	M500	95,23	32,96	7,33	3,13	15,30	12,18
	O500	95,97	37,92	8,49	2,60	16,22	11,99
USP2, 50 rpm ³	C250 ¹	95,35	37,00	23,30	3,24	19,72	17,06
	C250 ²	88,75	65,93	20,44	4,47	15,30	16,71
	C500	97,39	35,09	11,44	4,24	14,50	12,08
	M500	88,73	30,27	9,75	4,72	17,11	14,87
USP2, 75 rpm ³	O500	93,23	25,62	9,58	4,39	18,61	15,12
	C250 ¹	94,39	75,39	26,91	3,72	12,09	11,98
	C500	97,75	71,18	12,33	3,92	9,98	8,83
	M500	97,96	65,35	11,49	3,70	13,39	10,17
O500	97,92	61,60	11,65	3,01	13,90	10,10	

¹USP sinker; ²Japanese sinker; ³Experimental data from Aqel NN et al., 2014.

Interestingly, the conclusions on the *in-vitro* similarity seem to be dependent on the experimental design, particularly on the type of USP apparatus and on the pH of the media. In the previous report (Aqel NN et al., 2014), the mean dissolution profiles for the formulation O500 were considered non-similar to the reference listed drug (C500), based on the values of both difference and similarity factors. As presented in table 2, the hydrodynamic conditions induced by the adoption of USP apparatus 1 at 100 rpm were discriminatory only for dose strength, but not for the difference in composition or manufacturing process. The values of both Rescigno's indexes support this conclusion. The high stirring rate probably generates a rapid disintegration of the pharmaceutical formulations, thereafter the dissolution being dependent mainly on the solubility of the drug. This is probably a confirmation of the assumed limitations of

the basket method. Despite the simplicity, robustness, advanced standardization and adequate experience as one of the first testing devices that were officially adopted, the USP apparatus 1 has several disadvantages, such as the currently observed disintegration-dissolution interactions and the deficient stirring conditions under the basket (Fotaki N, 2014).

Table 2

The pairwise comparison of mean *in-vitro* release profiles obtained using USP apparatus 1 at 100 rpm (reference product: C500)

Media	Metric s	Test product		
		C250	M500	O500
Acetate buffer pH=4.5	f_1	40,19 ¹	14,14	12,62
	f_2	39,38 ²	63,95	65,06
	ζ_1	0,19	0,07	0,05
	ζ_2	0,20	0,07	0,06
Phosphate buffer pH=6.8	f_1	131,6 9 ¹	26,78 ¹	17,07 ¹
	f_2	43,69 ²	78,61	85,84
	ζ_1	0,41	0,15	0,08
	ζ_2	0,43	0,15	0,08

¹Values of the difference factor outside the regulatory acceptance interval for similarity, 0-15.

²Values of the similarity factor outside the regulatory acceptance interval for similarity, 50-100.

The experimental results confirm several previous findings. The acidic media with low pH values are not adequate for the evaluation of dissolution profiles for mycophenolate mofetil from immediate release oral solid dosage forms, at least for routine quality control purposes. The selection of these testing conditions induced a very rapid and complete release of the drug (within 5 to 10 minute), independent on the apparatus or stirring rate. Secondly, the decrease in solubility of the drug from pH=1.2 to 4.5 is not as drastic as for other standard weak basic compounds, e.g. ketoconazole or dipyridamol. It is possible that the supersaturation phenomenon will affect the absorption profile to lower extent, since the fraction dissolved in the proximal segments of the intestine may still be high. The current approach for the BCS class II drugs is that extension of the applicability of the *biowaiver* principles could be considered for drugs that are highly soluble at the site of gastro-intestinal absorption, mainly weak acids such as non-steroidal anti-inflammatory agents. The high solubility of the weak bases has been discussed only in relation to the nucleation process, possibly occurring when the solution generated in acidic, gastric environment is emptied into the duodenal compartment. The weak bases are not chemical entities with homogenous physico-chemical characteristics. In fact, they present highly different molecular structures, with consequently distinct values for several biorelevant parameters, for example dissociation constants and pH-dependent solubility. Therefore, it rather

unsubstantiated to exclude the feasibility of BCS-based biowaiver approaches for all immediate release oral formulations containing weak bases drugs.

Perhaps one of the main conclusions is that selection of USP apparatus is critical in the development of a dissolution methodology, able to signal potential alteration of the *in-vivo* performance of the dosage form. The experimental results indicated that the conclusion on the *in-vitro* similarity is strongly dependent upon the hydrodynamic conditions. Moreover, the dose-solubility ratio had a considerable influence in case of non-sink conditions. Especially for regulatory purposes, evidence on the comparative performance of the tested formulations using both compendial apparatus at various stirring rate, with possible evaluation of two dosage unit for dose-proportionality analysis, must be submitted.

CONCLUSIONS

The *in-vitro* dissolution profiles of four immediate release oral dosage forms containing mycophenolate mofetil were evaluated in three aqueous buffer systems, using the basket method. The Japanese sinkers and paddle apparatus were adopted for the lower strength, capsule formulation, and the experimental data was cross-analyzed with previously reported data. The results indicated that the conclusions on similarity are dependent on both pH of media and stirring rate. The non-sink testing conditions emphasized a considerable impact of the dose solubility ratio.

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REFERENCES

- Aqel NN, Lupuleasa D, Mitu MA, Stanescu AA, Donea C, Miron DS, Radulescu FS, The impact of differences between regulatory guidance for *in-vitro* testing on the dissolution profiles of mycophenolate mofetil. *Farmacia* (in press). 2014.
- Fotaki N. Dissolution testing: principles and compendial testing. In: Mircioiu C, Shah VP (eds.), *School of Advanced Studies. Biowaivers, development of in vitro and quality generic drugs*. SoAS 2014. Carol Davila Publishing House, 60-69, 2014.
- Khan KA, The concept of dissolution efficiency. *J Pharm Pharmacol*, 27, 48-49, 1975.
- Polli JE, Rekhi GS, Augsburg LL, Shah VP, Methods to compare dissolution profiles and a rationale for wide dissolution specifications for

- metoprolol tartrate tablets. *J Pharm Sci*, 86, 690-700, 1997.
- Riegelman S, Collier P, The application of statistical moment theory to the evaluation of in vivo dissolution time and absorption time. *J Pharmacokinet Biopharm*, 8, 509-534, 1980.
- Scheubel E, Adamy L, Cardot JM. Mycophenolate mofetil: use of a simple dissolution technique to assess generic formulation differences. *Diss Tech*, 2, 52-58, 2012.
- Shah VP, Amidon GL. G.L. Amidon, H. Lennernas, V.P. Shah, and J.R. Crison, 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, 1995-Backstory of BCS. *AAPS J*, 2014a.
- Shah VP, Cecil TL, Srinivasan SV, Williams RL, Progressively reducing regulatory burden. *AAPS J*, 16, 621-624, 2014b.
- Soltero RA, Hoover JM, Jones TF, Standish M, Effects of sinker shapes on dissolution profiles. *J Pharm Sci*, 78, 35-39, 1989.
- Tsume Y, Amidon GL, The biowaiver extension for BCS class III drugs: the effect of dissolution rate on the bioequivalence of BCS class III immediate-release drugs predicted by computer simulation, *Mol Pharm*, 7, 1235-1243, 2010.
- Tsume Y, Langguth P, Garcia-Arieta A, Amidon GL, In silico prediction of drug dissolution and absorption with variation in intestinal pH for BCS class II weak acid drugs: ibuprofen and ketoprofen. *Biopharm Drug Dispos*, 33, 366-377, 2012.
- United States Pharmacopoeia 36 - National Formulary 31 (USP36-NF31), First supplement. Chapter <711> - Dissolution, 301-313, 2013.