BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902017000216128

Article

Solubility evaluation of didanosine: a comparison between the equilibrium method and intrinsic dissolution for biopharmaceutics classification purposes

André Bersani Dezani, Thaisa Marinho Dezani, Julie Caroline Ferrari Ferreira, Cristina Helena dos Reis Serra*

Department of Pharmacy, Faculty of Pharmaceutical Sciences of the University of São Paulo, São Paulo, Brazil

BCS (Biopharmaceutics Classification System) and BDDCS (Biopharmaceutics Drug Disposition Classification System) were proposed as tools for classifying drugs into four categories. Both systems consider the solubility as an important characteristic for the classification of compounds in drug development and *in vivo* disposition prediction. Although some results of drug solubility can be found in the literature, the aforementioned characteristic is not entirely clear when considering didanosine (ddI). Based on that, the solubility of ddI was evaluated using equilibrium and intrinsic dissolution methods. For the equilibrium method, excess amount of ddI was added to each media until obtaining a supersaturated solution and the mixture was submitted to agitation at 37 °C. For the intrinsic dissolution method, the drug was compressed into the Wood's apparatus matrix and subjected to dissolution in each media with agitation at 37 °C. The results obtained from the equilibrium method indicated that it was necessary 139.37 mL of pH 1.2 media, 87.72 mL of pH 4.5 media, 12.54 mL of pH 6.8 media, 5.03 mL of pH 7.5 media and 7.65 mL of purified water for drug solubilization. Furthermore, a very fast intrinsic dissolution rate (IDR) was obtained for each media: 0.1 mg/min/cm² (pH 1.2), 0.2 mg/min/cm² (pH 4.5), 0.2 mg/min/cm² (pH 6.8), 0.1 mg/min/cm² (pH 7.5) and 0.1 mg/min/cm² (purified water). Based on these results, ddI can be considered as a highly soluble drug for both equilibrium and intrinsic dissolution methods.

Uniterms: Didanosine/solubility. Biopharmaceutics Classification System (BCS). Biopharmaceutics Drug Disposition Classification System (BDDCS). Intrinsic dissolution. Equilibrium solubility.

INTRODUCTION

The Biopharmaceutics Classification System (BCS) is a scientific tool used for classifying drugs based on their aqueous solubility and intestinal permeability characteristics (Amidon *et al.,* 1995). According to the BCS, drugs are classified as follows: class I (high solubility and high permeability); class II (low solubility and high permeability); class III (high solubility and low permeability) and class IV (low solubility and low permeability). For immediate-release oral dosage forms, rates of dissolution are constantly discussed since the publication of the guidance for industry by the FDA (Food and Drug Administration). Thus, when in combination with the dissolution, the BCS takes into consideration three major factors guiding the rate and extent of drug absorption from immediate release solid oral dosage forms: dissolution rate, solubility and permeability (Amidon *et al.,* 1995; FDA, 2000; Dezani *et al.,* 2013a).

In addition, the Biopharmaceutics Drugs Disposititon Classification System (BDDCS), although it does not currently offer any regulatory support, it has a great importance in drug disposition. In this system, drugs are classified as follows: class I (high solubility and extensive metabolism); class II (low solubility and extensive metabolism); class III (high solubility and poor metabolism) and class IV (low solubility and poor metabolism). Therefore, solubility is used for both BCS and BDDCS systems since this characteristic is mandatory for the upcoming processes of ADME properties (absorption, distribution, metabolism and excretion), which are extremely important in drug development, *in vivo* disposition and classification of drugs (Wu, Benet, 2005).

***Correspondence:** C.H.R. Serra. Departamento de Farmácia, Faculdade de Ciências Farmacêuticas – USP. Av. Professor Lineu Prestes, 580 - Bl. 13, 05508-900, São Paulo, SP, Brasil. E-mail: chserra@usp.br

For biowaiver purposes, the FDA guidance (2000) recommends solubility and permeability assays in order to classify drugs according to the BCS (FDA, 2000)new drug applications (NDAs. For permeability studies, several methods can be used for evaluating intestinal permeation of drugs, such as: *in situ* intestinal perfusion in animals or humans (*in vivo* perfusion), *in vitro* permeation studies using artificial membranes or cell culture monolayers and *ex vivo* method using isolated intestinal segments (FDA, 2000; Dezani *et al.,* 2013b, 2016; Reis *et al.,* 2013).

For solubility characterization, the equilibrium method involves the saturation of the media in which excess amounts of the drug are added. Alternatively, intrinsic dissolution is widely discussed and it consists on submitting the drug to different compression forces until establishment of the optimal condition of compression. The compression force should be sufficient to form the drug disk (die), which remains stable until its complete dissolution in the media throughout the experiment time. The intrinsic dissolution method is not influenced by the compression force, dissolution volume, distance of the drug disk from the bottom of the dissolution vessel or the drug disk rotation speed (Dezani *et al.,* 2013a; Wood, Syarto, Letterman, 1965; Yu *et al.,* 2004; Zakeri-Milani *et al.,* 2009).

The intrinsic dissolution rate (IDR) has been used for solid drug characterization. This property has been studied in order to elucidate the relationship between the dissolution rate and the crystalline form as well as to study the effects of surfactants and pH on the solubilization of poorly soluble drugs (Jinno *et al.,* 2000; Zakeri-Milani *et al.,* 2009)an ionizable water-insoluble drug in physiological pH. The intrinsic dissolution rate (J(total. IDR is generally defined as the dissolution rate of a drug under constant surface area, stirring speed, pH and ionic strength of the dissolution media. The effective IDR can be described as the rate of mass transfered from the solid surface to the liquid phase. The apparatus for intrinsic dissolution testing was originally developed by John Wood, which enables the calculation of the dissolution rate per centimeter square (Wood, Syarto, Letterman, 1965).

The use of IDR has been suggested as an alternative method for the establishment of solubility characterization of a drug. Thus, IDR may correlate better with *in vivo* dissolution rates than solubility, even for those drugs that have a wide range of doses available in the market. In this case, discrepancies may be observed in a comparison between solubility and intrinsic dissolution methods. Thus, the intrinsic dissolution does not take into consideration the dose effect (Dezani *et al.,* 2013a; Yu *et al.,* 2004).

The equilibrium method depends on the dose strength to be calculated. Therefore, solubility characterization using the equilibrium method requires that the dose strength be defined and this method would be used for those drugs that have been approved on clinical trials. On the other hand, the intrinsic dissolution method is very useful in early drug development when the dose strength is not established yet. Based on that, new drugs can have characteristics defined for solubility using the intrinsic dissolution. Besides, as dose strength may vary from country to coumtry due regulatory matters, the intrinsic dissolution method might be used as a universal tool for solubility characterization (Dezani *et al.,* 2013a; Yu *et al.,* 2004; Zakeri-Milani *et al.,* 2009).

Didanosine is an antiretroviral drug used for HIV (Human Immunodeficiency Virus) treatment (Seremeta *et al.,* 2014) and its solubility data are scarce in the literature, especially considering physiologic conditions, which may hamper the biopharmaceutic classification.

Although the permeability mechanism of didanosine is not fully clear, bioavailability problems can be related to both parameters: solubility and/or permeability. Thus, solubility characterization of this antiretroviral drug allows researchers to understand if these parameters can disturb the drug's bioavailability. Despite its low metabolism, didanosine presents low fraction absorbed (25-43%) (Aungst, 1999; Li, Chan, 1999; Moyer *et al.,* 1999; Tavelin *et al.,* 2003).

It is widely known that insufficient drug solubility can lead to a poor oral absorption (Zhao *et al.,* 2002). Since the solubility is mandatory for the next steps (permeability and absorption), there are not solubility studies reported in the literature about didanosine, especially considering physiological parameters as temperature, pH and dose strength. Based on that, this study aims to evaluate the solubility of didanosine using two methods (equilibrium and intrinsic dissolution) in order to characterize the solubility of the drug and figure out if solubility can be a limiting factor for its bioavailability in order to contribute for improvement of the biopharmaceutic-related properties.

MATERIAL AND METHODS

Buffer solutions

Buffer solutions were prepared from different mixture compositions of hydrochloric acid (HCl), potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH, 1 M), as follows: pH 1.2 (HCl), pH 4.5 (KH_2PO_4), pH 6.8 (KH_2PO_4/NaOH), pH 7.5 (KH_2PO_4/NaOH) and purified water obtained from Milli-Q purification system (Millipore, MA, USA). The pH values were adjusted using HCl 0.1 M and NaOH 0.2 M solutions.

All buffer solutions were prepared based on the British Pharmacopoeia, United States Pharmacopeia and Portuguese Pharmacopoeia in order to mimic the physiological conditions (British Pharmacopoeia, 2016; Farmacopéia Portuguesa, 2008; USP, 2016).

Solubility by equilibrium method

The solubility by equilibrium method was evaluated by adding ddI in each buffer solution (pH 1.2, pH 4.5, pH 6.8 and pH 7.5) and purified water until obtained a supersaturated solution characterized by the presence of precipitate in the bottom of the flasks. For all solutions, an amount of 4000 mg was considered for supersaturation. The experiments were carried out in triplicate. For each plastic flask, 10 mL of buffer solution and excess of ddI powder were added. The amount of drug was sufficient for media supersaturating, which was characterized by an insoluble amount of drug in the bottom of each flask. The samples were kept in an incubator shaker at 37.0±0.5 ºC and orbital agitation of 150 rpm (rotations per minute) for 72h (Dezani *et al.,* 2013a; Okumu, Dimaso, Lobenberg, 2009).

After incubation, each sample was readily filtered (0.45µm) and diluted with the respective solution. Drug measurement proceedings were performed using a UV–Vis spectrophotometer at the maximum absorbance wavelength for each media and the solubility values were calculated using spectrophotometric method previously validated.

For obtaining solubility results, Equation 1 was used for calculation of dose:solubility ratio (*Do*) of ddI in each buffer solution. According to criteria established by the FDA guidance, the D_o value equal or lower than 1 indicates that the compound is highly soluble (FDA, 2000; Lindenberg, Kopp, Dressman, 2004).

$$D_o = \frac{Dose / S}{250\ \text{mL}} \qquad (1)$$

where: D_o is the dose number, *Dose* is the highest prescribed dose (mg) and *S* is the solubility of the drug (mg/mL). The volume of 250 mL was determined in order to represent the standard volume when an oral dosage form is taken, according to the FDA guidance.

Solubility by intrinsic dissolution method

Intrinsic dissolution studies were performed using the Wood's apparatus, which was originally developed by John Wood and used for the calculation of intrinsic dissolution rate per centimeter square (Dezani *et al.,* 2013a; Wood, Syarto, Letterman, 1965; Zakeri-Milani *et al.,* 2009). An amount of ddI (300 mg) was compressed (3000 psi for 3 min) to make a non-disintegrating disk and the exposition area was established in 0.5 cm². In a dissolution system, three vessels were previously filled with 900 mL of buffer solution at a temperature of 37.0 ± 0.5 ºC with apparatus rotation of 50 rpm. The compressed drug in the matrix was submerged into the media and samples were collected at 5, 10, 15, 20, 25, 30, 60, 90, 120 and 150 min with fresh buffer solution replacement.

The establishment of compression force for ddI was made empirically in order to achieve a pattern condition that allowed to get at least six samples throughout the dissolution procedure. Besides, a paper published by Yu and colleagues (2004) describes a range of 600-5000 psi in order to get a die with adequate hardness in the intrinsic dissolution studies. Thus, the die obtained for ddI was hard enough to allow sample collection and not too friable to disintegrate before the sampling time. Furthermore, a compression force around 2000 psi is very common for tablet compression (Yu *et al.,* 2004).

Absorbances were determined in triplicate using a UV-Vis spectrophotometer at the maximum absorbance wavelength for each media. In some cases, a dilution was made in order to allow the absorbance readability by the spectrophotometer considering a range of 10-100 times of dilution factor (Dezani *et al.,* 2013a; Yu *et al.,* 2004). The dissolved drug was mensured for each sample interval according to the volume collected from each vessel. The slope of the linear regression was considered as IDR, which can be calculated by Equation 2.

$$J = \frac{Vdc}{dt}\frac{1}{A} \qquad (2)$$

where: *J* is the intrinsic dissolution rate (IDR), *V* is the volume of the media, and *c* is the drug concentration, *A* is the exposition area in Wood's apparatus and *t* is the time (Yu *et al.,* 2004).

Statistical analysis

Solubility results were obtained for equilibrium and intrinsic dissolution methods, as described previously. Results were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Validation parameters included: linearity (r^2>0.999), precision (>95%), accuracy (>95%) and stability (variation less than 5%), as recommended by ICH (ICH, 2005).

Equilibrium solubility and biopharmaceutics classification

Both BCS and BDDCS consider the solubility as an important and mandatory factor for drug development and *in vivo* disposition. Since the FDA guidance publication in 2000, several studies of solubility of drugs were performed in order to classify drugs according to their characteristics. However, it is clear that several studies in the literature do not consider the physiological conditions as pH of the buffer solution and temperature, although it is possible to find manuscripts regarding formulation studies for the ddI pharmaceutical product.

Solubility experiments are of great consideration for early drug development. However, some data found in the literature regarding ddI solubility are not considered adequate since some experiments were performed in different conditions in comparison with physiological conditions.

The solubility determination of ddI in the present study was performed according to the FDA guidance (FDA, 2000) in an incubator with orbital shaking platform. This equipment enables the heating of flasks and adequate control of the temperature and speed. Thus, the conditions adopted in this study remained constant and standardized, considering the physiological conditions of pH and temperature. The stirring time used in this work was 72 hours, which is important period to reach the equilibrium of the media.

The FDA guidance defines a compound as "*highly soluble*" when the highest prescribed dose is soluble in 250 mL or less of aqueous media over the pH range of 1.2-7.5 at 37 °C. However, for European Medicines Agency (EMA) the physiological pH range is 1.2-6.8 (FDA, 2000; EMEA, 2010; Strauch *et al.,* 2011).

Some differences regarding dose strength of the drug can determine variability in the biopharmaceutical classification (Lindenberg, Kopp, Dressman, 2004). Table I shows solubility results, dose:solubility ratio and D_o for ddI. The Figure 1 represents average solubility of ddI.

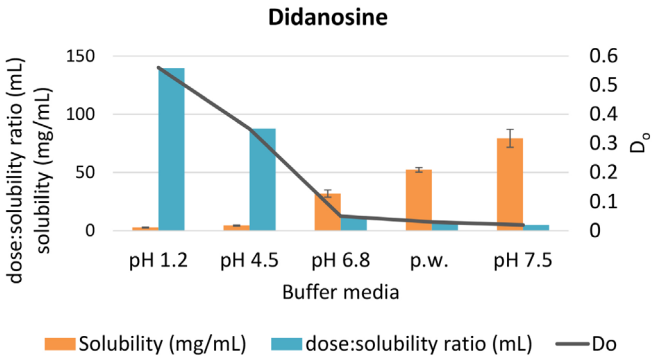

FIGURE 1 - Average solubility, dose:solubility and D_o of ddI in buffer solutions pH 1.2, pH 4.5, pH 6.8, purified water (p.w.) and pH 7.5. Results obtained from equilibrium method for solubility evaluation. Error bars indicate the standard deviation (SD) for each result.

The maximum dose strenght for ddI is 400 mg. By using the equilibrium method, the results showed that the compound presents a high solubility in all buffer solutions and water. Thus, the dose:solubility ratio is lower than 250 mL and $D_o \leq 1$, as demonstrated in Table I (FDA, 2000, 2015; Lindenberg, Kopp, Dressman, 2004).

Based on the solubility results (mg/mL) of ddI, the buffer solution pH 7.5 is considered the optimum media for drug solubilization. That is in accordance to those results presented for dose:solubility and D_o in Table I.

According to the results presented in Table I and Figure I, the ddI solubility increases over the pH and a lower solubility for pH 1.2 can be observed. Studies published in the literature show that the low solubility of ddI in low pH solutions is due to a rapid degradation in acid media, including gastric pH. This acid instability explains the low and highly variable bioavailability of ddI (20-40%) in comparison with others nucleoside reverse transcriptase

TABLE I - Solubility results and their standard deviation (SD) values at 37 °C and dose:solubility ratio of ddI for each buffer media used for the equilibrium method assay

Drug and the highest prescribed dose	pH	solubility (mg/mL) ±SD	dose:solubility ratio (mL)	D_o
ddI (400mg)	1.2	2.87 (±0.42)	139.37	0.56
	4.5	4.56 (±0.48)	87.72	0.35
	6.8	31.91 (±3.07)	12.54	0.05
	p.w.*	52.28 (±1.99)	7.65	0.03
	7.5	79.38 (±7.72)	5.03	0.02

** p.w. = purified water*

inhibitors. Also, the first-pass metabolism of ddI is related to its low bioavailability (Aungst, 1999; Morse, Shelton, O'Donnell, 1993).

Some studies in the literature present formulation strategies to try preserve ddI from acid instability in the stomach. However, even formulations containing excipients used for buffering the gastrointestinal content are not completely capable to avoid ddI degradation, which may limit its bioavailability (Aungst, 1999). Thus, the relationship pH-solubility is extremely important for ddI, which has its permeability and bioavailability hindered by solubility conditions.

In the literature, ddI has a solubility of 20 mg/mL at room temperature (Sanchez-Lafuente *et al.,* 2002a). By using a similar method described in this study, Anderson and colleagues (1988) performed a study of ddI at 25°C and the solubility results ranged from 27.3 to 460.0 mg/mL according to the pH solution (pH range 6.21-10.18). If temperature increases, the solubility increases as well. That is why physiologic conditions are very important for solubility studies in drug development studies as well as the pH of the buffer solution used for these experiments (Anderson *et al.,* 1988).

Once ddI solubility in acid pH is a critical factor, several studies in the literature describe strategies for drug formulation to try protecting the substance against the acid instability and/or try to improve its bioavailability based on pharmacokinetic problems reported in the literature. The use of excipients can aid on buffering the gastrointestinal media, as briefly discussed before, but the development of prodrugs, sustained release matrix tablets and controlled release dosage forms are widely described in the literature for ddI formulation studies. Some of excipients used include anionic copolymer based on acrylic and methacrylic acid with low content in quaternary ammonium functions and ethylcellulose with a high degree of polymerization (Lalanne *et al.,* 2007; Sanchez-Lafuente *et al.,* 2002a, 2002b).

Intrinsic dissolution studies

Additionally to the equilibrium method, several researchers have discussed the possibility of using the intrinsic dissolution method. Although this method is not required for solubility classification of drugs according to biopharmaceutics systems such as BCS and BDDCS, some advantages regarding intrinsic dissolution method have been discussed over the years.

In this study, the intrinsic dissolution method was performed for ddI and considered physiological conditions as pH media (1.2, 4.5, 6.8, 7.5, and purified water) as well as the temperature (37 °C for drugs administered orally).

Figure 2 shows the plot of accumulated concentration *versus* time for ddI at pH 1.2, 4.5, 6.8, 7.5, and purified water. The insignificant discrepancies among the three runs using three disks in three dissolution vessels indicated a good reproducibility. Linearity was also good, which is demonstrated by a correlation coefficient greater than 0.99 for all the buffer solutions, as shown in Table II.

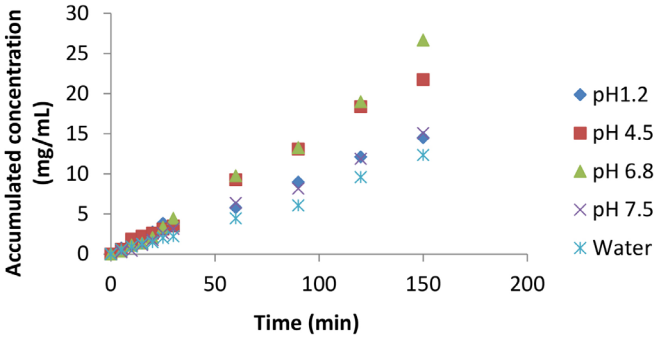

FIGURE 2 - Accumulated concentration (mg/mL) *versus* time (min) profile for ddI in buffer solutions pH 1.2, 4.5, 6.8, 7.5 and purified water.

The intrinsic dissolution study allows obtaining the IDR for each media, i.e., when a drug has a high dissolution rate, the greater is the IDR value. On the other hand, when a drug takes a long time to be solubilized, the IDR value tend to be lower. The Table II shows the results of IDR for ddI in all buffer solutions used.

The sink condition in the dissolution media during the experiment is kept by the comparison of the final concentration of ddI and its solubility in the dissolution media. According to Zakeri-Milani and colleagues (2009), compounds with high solubility could be successfully demonstrated by an IDR greater than 3 mg/min/cm^2, while

TABLE II - Intrinsic dissolution rate (IDR) (mg/min/cm^2) for ddI

	Didanosine				
	pH 1.2	pH 4.5	pH 6.8	pH 7.5	Water
R^2	0.9913	0.9963	0.9912	0.9954	0.9908
IDR	0.1	0.2	0.2	0.1	0.1

compounds with low solubility show an IDR less than 1 mg/min/cm^2. However, the method used by Zakeri-Milani *et al* is different from that one described in this manuscript (Dezani *et al.,* 2013a; Zakeri-Milani *et al.,* 2009). Another study carried out by Yu and colleagues (2004) discuss a different boundary value for drug classification, where drugs with IDR greater than 0.1 mg/min/cm^2 could be classified as highly soluble compound (Dezani *et al.,* 2013a; Yu *et al.,* 2004). In this study, the parameter used for drug classification was 0.1 mg/min/cm^2 due similarities in the experiment conditions.

According to the results presented in the Table II, good qualitative correlation between the solubility classification and IDR values was observed. In other words, ddI presented a high velocity of dissolution for all buffer solutions according to Yu and colleagues (2004).

IDR and permeability are a rate phenomenon instead of an equilibrium phenomenon. Moreover, it might correlate more closely with *in vivo* drug dissolution than solubility. For intrinsic dissolution studies, the dose is not taken into consideration for classification. Actually, by using equilibrium method, the dose is considered for drug classification, which can lead to wrong classification, since the dose may vary from country to country. Thus, when the dose is either extremely high or extremely low (for exemple, a drug with solubility of 1.0 g/mL and its dose is 0.25 mg and another drug 4.0 mg/mL and its dose is 1000 mg) a discrepancy between the current solubility classification and the IDR occurs. Furthermore, when the dose is extremely high, the *in vivo* absorption process may be limited by the solubility (Dezani *et al.,* 2013a; Issa, Ferraz, 2011; Singh *et al.,* 2010; Yu *et al.,* 2004).

The comparison between the equilibrium method and intrinsic dissolution shows that the IDR is a good tool for drug classification according to solubility. In intrinsic dissolution, the dose is not taken into consideration as mentioned before. In addition, the duration of the experiment is very short and no media saturation is reached. Furthermore, Yu and colleagues discuss about variables regarding the method and no significant difference could be observed for those drugs tested. All these conditions seem to be closest to the *in vivo* conditions, which make the IDR suitable for drug classification according to the biopharmaceutics classification systems and this method can be used for pH-sensitive substances (Dezani *et al.,* 2013a; Yu *et al.,* 2004).

CONCLUSION

A high solubility is mandatory for drug absorption and its bioavailability. Both BCS and BDDCS systems consider the solubility as an important characteristic for drug development and disposition. With the publication of the FDA guidance based on BCS, several solubility studies have been performed in order to classify drugs and establish their classification into one of the four classes considered for BCS and BDDCS.

The equilibrium method is widely used and it is based on media saturation. Futhermore, the dose must be considered and, then, the solubility classification of the drug can be divergent, since each country has its own prescribed doses available in the market. Based on that, the intrinsic dissolution method seems to be suitable for drug classification, since this technique does not consider the prescribed dose and the experiments were conducted considering the physiological conditions as pH and temperature of the buffer solutions.

In this study, the data of solubility and IDR of ddI are aligned with each other. Based on the results, the IDR could be used as a tool for classifying drugs in early drug development, which seems to be adequate since this method does not require a large amount of drug to be performed.

Yu and colleagues consider 0.1 mg/min/cm^2 as a boundary value, which was applied to our study for the discussion on the IDR of ddI since their method is considered very similar to that one described in this manuscript. Then, based on the classification defined by Yu and colleagues, the ddI may be considered as highly soluble drug. Thus, the data obtained from the intrinsic dissolution test confirm the results obtained in the equilibrium solubility procedure (Dezani *et al.,* 2013a; Yu *et al.,* 2004).

ACKNOWLEDGMENTS

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for financial support and fellowship. Thanks to Fundação para o Remédio Popular (FURP, Brazil) and Cristália Produtos Químicos Farmacêuticos for donation of the chemical substances.

REFERENCES

AMIDON, G.L.; LENNERNAS, H.; SHAH, V.P.; CRISON, J.R. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res*, v.12, n.3, p.413-420, 1995.

ANDERSON, B.D.; WYGANT, M.B.; XIANG, T.-X.; WAUGH, W.A.; STELLA, V.J. Preformulation solubility and kinetic studies of 2',3'-dideoxypurine nucleosides: potential anti-AIDS agents. *Int. J. Pharm.*, v.45, n.1-2, p.27-37, 1988.

AUNGST, B.J. P-glycoprotein, secretory transport, and other barriers to the oral delivery of anti-HIV drugs. *Adv. Drug Deliv. Rev.*, v.39, n.1-3, p.105-116, 1999.

BRITISH PHARMACOPOEIA. *British Pharmacopoeia*. London, 2016.

DEZANI, A.B.; PEREIRA, T.M.; CAFFARO, A.M.; REIS, J.M.; SERRA, C.H.R. Equilibrium solubility *versus* intrinsic dissolution: characterization of lamivudine, stavudine and zidovudine for BCS classification. *Braz. J. Pharm. Sci.,* v.49, n.4, p.853-863, 2013a.

DEZANI, A.B.; PEREIRA, T.M.; CAFFARO, A.M.; REIS, J.M.; SERRA, C.H.R. Determination of lamivudine and zidovudine permeability using a different *ex vivo* method in Franz cells. *J. Pharmacol. Toxicol.*, v.67, n.3, p.194-202, 2013b.

DEZANI, T.M.; DEZANI, A.B.; JUNIOR, J.B.S.; SERRA, C.H.R. Single-Pass Intestinal Perfusion (SPIP) and prediction of fraction absorbed and permeability in humans: a study with antiretroviral drugs. *Eur. J. Pharm. Biopharm.*, v.104, p.131-139, 2016.

EUROPEAN MEDICINES AGENCY. CPMP/EMEA. *Guideline on the investigation of Bioequivalence*. London, 2010. 27p. Available at: <http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:GUIDELINE+ON+THE+INVESTIGATION+OF+BIOEQUIVALENCE#0>. Accessed in: Mar 28, 2016.

FARMACOPÉIA PORTUGUESA IX. *Farmacopéia Portuguesa IX.* Lisboa: Infarmed, 2008.

FOOD AND DRUG ADMINISTRATION. FDA. Guidance for Industry. Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Rockville: Center for Drug Evaluation and Research, August, 2000. 13p.

FOOD AND DRUG ADMINISTRATION. FDA. *FDA Approved Drug Products.* Available at: <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Overview&DrugName=DIDANOSINE>. Accessed in: 1 jan. 2015.

INTERNATIONAL CONFERENCE ON HARMONISATION. ICH. *Validation of a analytical procedures: text and methodology* Q2(R1). International Conference on Harmonisation of Technical Requirements for registration of pharmaceuticals for human use. Geneva, ICH, 2005. p.17

ISSA, M.G.; FERRAZ, H.G. Intrinsic dissolution as a tool for evaluating drug solubility in accordance with the biopharmaceutics classification system. *Dissolut. Technol.*, v.18, n.3, p.6-13, 2011.

JINNO, J.; OH, D.-M.; CRISON, J.R.; AMIDON, G.L. Dissolution of ionizable water-insoluble drugs: The combined effect of pH and surfactant. *J. Pharm. Sci.*, v.89, n.2, p.268-274, 2000.

LALANNE, M.; ANDRIEUX, K.; PACI, A.; BESNARD, M.; RÉ, M.; BOURGAUX, C.; OLLIVON, M.; DESMAELE, D.; COUVREUR, P. Liposomal formulation of a glycerolipidic prodrug for lymphatic delivery of didanosine via oral route. *Int. J. Pharm.*, v.344, n.1-2, p.62-70, 2007.

LI, X.; CHAN, W.K. Transport, metabolism and elimination mechanisms of anti-HIV agents. *Adv. Drug Deliv. Rev.*, v.39, n.1-3, p.81-103, 1999.

LINDENBERG, M.; KOPP, S.; DRESSMAN, J.B. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *Eur. J. Pharm. Biopharm.*, v.58, n.2, p.265-278, 2004.

MORSE, G.D.; SHELTON, M.J.; O'DONNELL, A.M. Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin. Pharmacokinet.,* v.24, n.2, p.101-123, 1993.

MOYER, T.P.; TEMESGEN, Z.; ENGER, R.; ESTES, L.; CHARLSON, J.; OLIVER, L.; WRIGHT, A. Drug monitoring of antiretroviral therapy for HIV-1 infection: method validation andresults of a pilot study. *Drug Monitor. Toxicol.*, v.1476, p.1465-1476, 1999.

OKUMU, A.; DIMASO, M.; LÖBENBERG, R. Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib solid oral drug products. *Eur. J. Pharm. Biopharm.*, v.72, n.1, p.91-98, 2009.

REIS, J.M.; DEZANI, A.B.; PEREIRA, T.M.; AVDEEF, A.; SERRA, C.H.R. Lamivudine permeability study: A comparison between PAMPA, *ex vivo* and *in situ* Single-Pass Intestinal Perfusion (SPIP) in rat jejunum. *Eur. J. Pharm. Biopharm.*, v.48, n.4-5, p.781-789, 2013.

SANCHEZ-LAFUENTE, C.; FAUCCI, M.T.; FARNÁNDEZ-ARÉVALO, M.; ÁLVAREZ-FUENTES, J.; RABASCO, A.M.; MURA, P. Development of sustained release matrix tablets of didanosine containing methacrylic and ethylcellulose polymers. *Int. J. Pharm.*, v.234, n.1-2, p.213-221, 2002a.

SANCHEZ-LAFUENTE, C.; RABASCO, A.M.; ÁLVAREZ-FUENTES, J.; FERNÁNDEZ-ARÉVALO, M. Eudragit RS-PM and Ethocel 100 Premium: Influence over the behavior of didanosine inert matrix system. *Farmaco*, v.57, n.8, p.649-656, 2002b.

SEREMETA, K.P.; TUR, M.I.; PÉREZ, S.M.; HOCHT, C.; TAIRA, C.; LÓPEZ-HERNANDEZ, O.D.; SOSNIK, A. Spray-dried didanosine-loaded polymeric particles for enhanced oral bioavailability. *Colloids Surf. B Biointerf.*, v.123, p.515-23, 2014.

SINGH, S.; DOBHAL, A.K.; JAIN, A.; PANDIT, J.K.; CHAKRABORTY, S. Formulation and evaluation of solid lipid nanoparticles of a water soluble drug: Zidovudine. *Chem. Pharm. Bull*, v.58, n.5, p.650-655, 2010.

STRAUCH, S.; JANTRATID, E.; DRESSMAN, J.B.; JUNGINGER, H.E.; KOPP, S.; MIDHA, K.K.; SHAH, V.P.; STAVCHANSKY, S.; BARENDS, D.M. Biowaiver monographs for immediate release solid oral dosage forms: Lamivudine. *J. Pharm. Sci.*, v.100, n.6, p.2054-2063, 2011.

TAVELIN, S.; TAIPALENSUU, J.; SODERBERG, L.; MORRISON, R.; CHONG, S.; ARTURSSON, P. Prediction of the oral absorption of low-permeability drugs using small intestine-like 2/4/A1 cell monolayers. *Pharm. Res.*, v.20, n.3, p.397-405, 2003.

UNITED STATES PHARMACOPEIA. *United States Pharmacopeia 39 /National Formulary 34*. Rockville: USP, 2016.

WOOD, J.; SYARTO, J.; LETTERMAN, H. Improved holder for intrinsic dissolution rate studies. *J. Pharm. Sci.*, v.54, n.7, p.1068, 1965.

WU, C.Y.; BENET, L.Z. Predicting drug disposition via application of BCS: Transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.*, v.22, n.1, p.11-23, 2005.

YU, L.X.; CARLIN, A.S.; AMIDON, G.L.; HUSSAIN, A.S. Feasibility studies of utilizing disk intrinsic dissolution rate to classify drugs. *Int. J. Pharm.*, v.270, n.1-2, p.221–227, 2004.

ZAKERI-MILANI, P.; BARZEGAR-JALALI, M.; AZIMI, M.; VALIZADEH, H. Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability. *Eur. J. Pharm. Biopharm.*, v.73, n.1, p.102-106, 2009.

ZHAO, Y.H.; ABRAHAM, M.H.; LE, J.; HERSEY, A.; LUSCOMBE, C.N.; BECK, G.; SHERBORNE, B.; COOPER, I. Rate-limited steps of human oral absorption and QSAR studies. *Pharm. Res.*, v.19, n.10, p.1446-57, 2002.

Received for publication on 29th June 2016
Accepted for publication on 11th April 2017