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Intrinsic dissolution simulation of highly and poorly soluble drugs for BCS solubility classification

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ABSTRACT

Intrinsic dissolution testing allows characterizing drug substances through its dissolution rate when exposed to a specified surface area in a specific dissolution media. This can be used to determine if a drug substance is highly or poorly soluble. In this work, DDDPlus version 4.0 (Simulations Plus, Inc.) was used to simulate intrinsic dissolution experiments for pyrimethamine and metronidazole. These drugs have low and high solubility properties. Predicted intrinsic dissolution rates (IDR) were compared to observed in vitro IDR. Physicochemical parameters from literature and the experimental conditions of the intrinsic dissolution tests for each drug were used as input data into the software. The program was able to predict the IDR of pyrimethamine and metronidazole within a pH range of 1.0 to 7.2. Observed and predicted IDR values for both drugs showed high correlations ($R^2 > 0.9424$). The IDR values from simulations showed the pH-dependent solubility of pyrimethamine and metronidazole, allowing us to classify the solubility according to the Biopharmaceutics Classification System (BCS). Intrinsic dissolution test simulations using DDDPlus can be used to obtain a BCS solubility classification of a drug substance, helping to reduce the number of laboratory experiments.

KEYWORDS: pyrimethamine; metronidazole; intrinsic dissolution rate; BCS; DDDPlus; dissolution

INTRODUCTION

Intrinsic dissolution is a characterization test for active pharmaceutical ingredients (APIs) where the dissolution rate of a drug is determined from a specific surface area exposed to a dissolution medium at certain rotation speed. (*1, 2*) The intrinsic dissolution rate (IDR) can be used to estimate the solubility class of a substance according to the Biopharmaceutics Classification System (BCS) guidelines. (*3, 4*) It can be also valuable to evaluate differences between polymorphs and solvates. (*2*)

According to the BCS, drugs are classified as high or low solubility. (*5*) Equilibrium solubility testing (shake flask method) is recommended by the US Food and Drug Administration to determine the solubility of drugs, allowing to obtain their BCS class. (*6–10*) However, this method requires the saturation of aqueous solutions, which can be a challenge depending on the drug characteristics, mainly in the early stages of development. For this purpose, intrinsic dissolution is an important characterization test that can be used to obtain the solubility of drugs according to BCS guidelines. (*3, 4*)

The dissolution of an API in a formulation is affected by different factors such as the test conditions (temperature, rotation speed, pH, nature of dissolution medium) and formulation factors, such as compaction pressure and excipient interactions. (*1*) Using design of experiments (DOE) is one way to evaluate the most appropriate test conditions. (*11, 12*)

Although only a few milligrams of API is used in intrinsic dissolution testing, developing a method requires a reasonable number of experiments to evaluate the impact of the test conditions on the IDR. One of the strategies to reduce the number of experiments is using fractional factorial design to develop appropriate intrinsic dissolution methods. (*11, 12*) However, depending on the quantity of the available sample, this step can be unfeasible, in early stages of the development process, where only limited material is available. Approaches, which can further reduce experimental testing, are highly desirable.

DDDPlus™ (Dose Disintegration and Dissolution) version 4.0, designed by Simulations Plus Inc., is a computer program used to simulate in vitro dissolution tests

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employing USP apparatuses 1 (basket), 2 (paddle), and 4 (flow-through cell) and intrinsic dissolution using the rotating disk method. (*13*) The use of DDDPlus to simulate in vitro dissolution from tablets containing low soluble drugs, montelukast sodium and glyburide, was previously demonstrated. (*14*) This software can simulate intrinsic dissolution tests, saving time, reducing the number of experiments to investigate suitable IDR conditions.

The objective of this work was to demonstrate the use of the computer program DDDPlus as a tool for BCS solubility classification by simulating intrinsic dissolution tests for the poorly soluble drug, pyrimethamine, and the highly soluble drug, metronidazole.

MATERIALS AND METHODS

Materials

Pyrimethamine and metronidazole were kindly donated by Farmanguinhos Laboratory/Fiocruz (Rio de Janeiro, Brazil) and Micro Service Micronização e Processos (Diadema, Brazil), respectively. Both APIs were of pharmaceutical grade and were used as received. Hydrochloric acid (HCl) P.A. 37%, glacial acetic acid, sodium acetate, potassium phosphate monobasic monohydrate, potassium chloride, and sodium hydroxide were purchased from Labsynth Products Laboratories (Diadema, Brazil) and used to prepare the buffer solutions as described below.

Hydrochloric acid pH 1.2 was prepared by accurately pipetting 7 mL of HCl P.A. 37% and weighing 3.73 g of potassium chloride per liter. Hydrochloric acid 0.1 M was prepared by pipetting 8.3 mL of HCl P.A. 37% and adding purified water to 1 L.

Acetate buffer pH 4.5 was prepared by dissolving 2.99 g of sodium acetate, 14 mL 2-N acetic acid solution, and purified water. Phosphate buffer pH 7.2 was prepared by dissolving 6.8 g of potassium phosphate monobasic monohydrate and 1.4 g of NaOH, and adding purified water. The amounts of material were used to prepare 1 L of buffer solutions.

Intrinsic dissolution tests

For pyrimethamine, intrinsic dissolution testing was performed according to a fractional factorial design 3^{3-1} using Statistica® 12.0 (StatSoft, Inc., Tulsa, OK, USA) including factors such as compaction pressure (1.75, 3.5, and 7.0 kN), nature of the dissolution medium (HCl pH 1.2; acetate buffer pH 4.5, and phosphate buffer pH 7.2), and rotation speed (50, 100, and 200 rpm), generating the experiments described in Table 1.

Table 1. Pyrimethamine Intrinsic Dissolution Test Conditions

Standard order	Run order	Compaction pressure (kN)	Dissolution media	Rotation speed (rpm)
1	P1	1.75	HCl pH 1.2	50
2	P4	1.75	Acetate buffer pH 4.5	200
3	P7	1.75	Phosphate buffer pH 7.2	100
4	P2	3.5	HCl pH 1.2	200
5	P5	3.5	Acetate buffer pH 4.5	100
6	P8	3.5	Phosphate buffer pH 7.2	50
7	P3	7.0	HCl pH 1.2	100
8	P6	7.0	Acetate buffer pH 4.5	50
9	P9	7.0	Phosphate buffer pH 7.2	200

HCl, hydrochloric acid.

Rotating disk apparatuses from Varian Inc. (Palo Alto, CA, USA) coupled to a D-800 Logan dissolution tester (Logan Instruments Corp., NJ, USA) was used to perform the intrinsic dissolution tests. About 150 mg of the drug was weighed in triplicate and compacted using a hydraulic press (American Lab., São Paulo, Brazil). The temperature used was 37 ± 0.5°C; the volume of the dissolution medium was 900 mL, and aliquots of 5 mL were collected in intervals until a sufficient number of points were obtained.

Each aliquot of dissolution medium was immediately replaced at the same volume and temperature. The amount of drug dissolved was analyzed by a spectrophotometric method in a UV-VIS Cary 50 (Varian Inc.) using quartz cuvettes of 10.0 mm at 273 nm, using each dissolution media as blank.

The visual evaluation of the compacted drug's surface was monitored during the experiments to avoid erroneous data from possible alterations, which can be detected by the presence of curvatures in the plots and low values of linearity. All dissolution media were previously degassed to prevent bubble formation, which could hamper the dissolution of the drug. (*1, 3*)

Intrinsic dissolution rate was calculated according to United States Pharmacopeia (*1*). The amount of drug dissolved (mg) was plotted versus time (s), and through linear regression, R^2 and the corresponding equation was obtained. The slope of this equation is the dissolution rate, and this value was divided by the exposed surface area (0.5 cm^2) to obtain the IDR (mg/s/cm^2).

For metronidazole, the results from an earlier study (*11*) were used for the simulations. The authors conducted the experiments according to a 3^{4-1} fractional factorial design, resulting in 27 experiments, including factors such as compaction pressure, rotation speed, dissolution media, and metronidazole micronization degree. The study confirmed that API micronization did not influence IDR results. (*11*)

Therefore, in this work, we considered only compaction pressure, rotation speed, and dissolution media as parameters, since there is no influence by particle size on IDR. Furthermore, DDDPlus disables the use of particle size distribution of the drug when intrinsic dissolution is selected. It resulted in a 3^{3-1} fractional factorial design (Table 2), from which corresponding in vitro IDR values were used for comparison.

Table 2. Metronidazole Intrinsic Dissolution Test Conditions

Standard order	Run order	Compaction pressure (kN)	Dissolution media	Rotation speed (rpm)
1	M1	3.5	HCl 0.1 M	50
2	M8	3.5	Purified water	100
3	M6	3.5	Phosphate buffer pH 7.2	75
4	M16	7.0	HCl 0.1 M	100
5	M14	7.0	Purified water	75
6	M12	7.0	Phosphate buffer pH 7.2	50
7	M22	10.5	HCl 0.1 M	75
8	M20	10.5	Purified water	50
9	M27	10.5	Phosphate buffer pH 7.2	100

HCl, hydrochloric acid.

Computer simulations using DDDPlus

DDDPlus software was used to simulate the intrinsic dissolution tests. A database for pyrimethamine and one for metronidazole were created in the software.

The ADMET Predictor™ module present in the computer program, GastroPlus™ (Simulations Plus Inc.) can accurately predict absorption, metabolism, elimination, and toxicity characteristics of substances from its molecular structure. This module was used to estimate solubility vs pH data for each drug at 25°C. For this purpose, files containing the molecular structure of each drug were used in this module, and the following solubility data were obtained: pyrimethamine solubility = 0.03 mg/mL at pH 8.37 and metronidazole solubility = 13.42 mg/mL at pH 7.66. Although the estimated solubility was at 25°C, these values were used as input data in DDDPlus because they are in accordance to shake-flask results experiments conducted at 37°C in our lab: pyrimethamine solubility = 0.04 mg/mL at phosphate buffer 0.05 M pH 7.5 and metronidazole solubility = 13.14 mg/mL at phosphate buffer 0.05 M pH 7.2. The pKa values of 7.4 (pyrimethamine) and 2.55 (metronidazole) were obtained from the literature (*15, 16*); particle density (1.2 g/mL), precipitation time (900 s), and diffusion coefficient (0.5 $cm^2/s \times 10^{-5}$) were used as default values from DDDPlus.

Intrinsic dissolution tests conditions for both pyrimethamine (Table 1) and metronidazole (Table 2) were used as input data into the Experimental tab of the software. Single simulations were performed for each experiment. As described in the pyrimethamine intrinsic dissolution tests section, IDR was calculated from the simulated data for both drugs. The values from simulated intrinsic dissolution tests were compared to the in vitro results.

Statistical analysis

Observed IDR values for pyrimethamine and metronidazole were analyzed using Statistica 12.0. Analysis of Variance (ANOVA) was used with $p < 0.05$ to establish statistical significant differences; Pareto charts where generated for each API.

RESULTS

Intrinsic dissolution and computer simulations

For pyrimethamine, the amount of drug dissolved was plotted against time to obtain a slope to calculate the IDR. A simulated amount of pyrimethamine dissolved versus time from DDDPlus was used to predict IDR.

Observed and predicted IDR values for pyrimethamine and the regression coefficients are shown in Table 3.

Similarly, for metronidazole the simulated amounts dissolved versus time were used to predict the IDR, and in vitro IDR data were used to compare to the predicted IDR with the observed IDR (Table 4).

For both drugs, Pareto charts showed that only dissolution media presented a significant influence on IDR (Figures 1 and 2), which was also confirmed by ANOVA of IDR observed results with significant influence for the variable dissolution medium ($p = 0.021$ for pyrimethamine and $p = 0.011$ for metronidazole).

Table 3. In Vitro (Observed) and In Silico (Predicted) Intrinsic Dissolution Rate (IDR) and R^2 Values for Pyrimethamine

Experiment	Dissolution media	IDR (mg/s/cm²)		Observed vs Predicted IDR
		Observed	Predicted	R^2
P1	HCl pH 1.2	0.0028	0.0030	0.9956
P2	HCl pH 1.2	0.0052	0.0050	0.9987
P3	HCl pH 1.2	0.0046	0.0046	0.9919
P4	Acetate buffer pH 4.5	0.0068	0.0072	0.9976
P5	Acetate buffer pH 4.5	0.0072	0.0070	0.9865
P6	Acetate buffer pH 4.5	0.0080	0.0072	0.9860
P7	Phosphate buffer pH 7.2	0.00008	0.00008	0.9762
P8	Phosphate buffer pH 7.2	0.00016	0.00016	0.9881
P9	Phosphate buffer pH 7.2	0.00008	0.00012	0.9424

Table 4. In Vitro (Observed) and In Silico (Predicted) Intrinsic Dissolution Rate (IDR) and R^2 Values for Metronidazole

Experiment	Dissolution media	IDR (mg/s/cm²)		Observed vs Predicted IDR
		Observed	Predicted	R^2
M1	HCl 0.1 M	0.0752	0.0752	0.9994
M8	Purified water	0.0294	0.0298	0.9997
M6	Phosphate buffer pH 7.2	0.0231	0.0236	0.9996
M16	HCl 0.1 M	0.1072	0.1082	0.9996
M14	Purified water	0.0256	0.0254	0.9992
M12	Phosphate buffer pH 7.2	0.0195	0.0198	0.9974
M22	HCl 0.1 M	0.0874	0.0878	0.9993
M20	Purified water	0.0204	0.0214	0.9970
M27	Phosphate buffer pH 7.2	0.0254	0.0262	0.9998

DISCUSSION

Solubility is one of the most relevant API physicochemical characteristics evaluated in preformulation studies because it is related to drug dissolution and presumably to in vivo performance of the drug product. (*17*) In early stages of drug discovery, solubility can be evaluated using small amounts of API via intrinsic dissolution testing. (*3*)

Pyrimethamine is a weak base with pKa 7.4 (*15*) expected to be ionized at acid pH. This drug had higher observed IDR values for the experiments conducted at pH 4.5 (P4, P5 and P6), followed by pH 1.2 (P1, P2 and P3) and lower values for the experiments P7, P8 and P9, conducted at pH 7.2 (Table 3). Metronidazole is also a weak basic compound and is more soluble at pH values below 2 (*18*), which was confirmed by the higher observed IDR values when intrinsic dissolution was performed using HCl 0.1 M as dissolution medium (M1, M16, and M22). Lower observed IDR values for this drug were observed for the experiments at higher pH test conditions (Table 4).

The determination of IDR from APIs can be used to evaluate and classify the API's solubility according to BCS guidelines when the dose is not too high or very low. (*19*) Intrinsic dissolution test results show that IDR represents the pH-dependent solubility of pyrimethamine and metronidazole, confirming IDR's suitability to determine the solubility classification of both low and highly soluble drugs. The use of intrinsic dissolution tests to determine the solubility of drugs according to BCS has been reported in the literature. (*3, 4*)

For intrinsic dissolution testing, the conditions of surface area, temperature, rotation speed, pH, and ionic strength must be kept constant, as described in Equation 1. (*13, 20*)

$$\frac{dM_u}{dt} = -0.62\gamma A D^{\frac{2}{3}} \omega^{\frac{1}{2}} \mu^{\frac{-1}{6}} C_s \qquad \text{(Equation 1),}$$

where

M_u = amount of undissolved drug

γ = calibration constant (unitless)

A= surface area of the tablet (cm^2)

D= diffusion coefficient (cm^2/min)

ω = rotational rate of the disk apparatus (radians/sec)

μ = dynamic viscosity ($g\ cm^{-1}s^{-1}$)

C_s= saturation solubility (mg/mL)

According to Equation 1, IDR increases as the rotation speed is improved, but the statistical analysis for experimental conditions used (independent factors and respective levels) showed that, for pyrimethamine (low soluble drug), the influence of rotation speed was only observed in the experiments conducted using HCl pH 1.2 (Table 3). For metronidazole, this influence could be noted within the results of the same medium (Table 4), but when comparing to the others, different rotation speeds can lead to similar IDR values. Thus, dissolution medium was the variable that showed significant influence on IDR for pyrimethamine (Figure 1) and metronidazole (Figure 2), with $p < 0.05$.

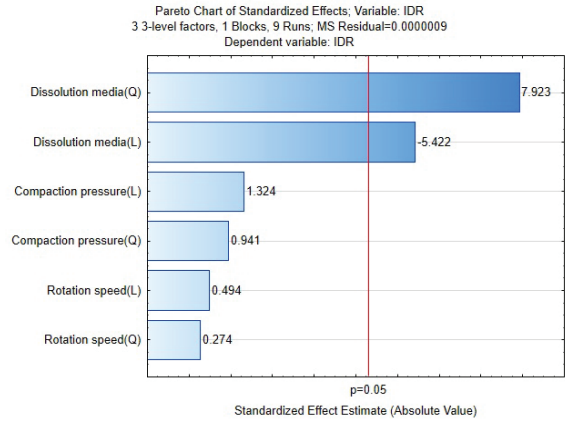

Figure 1. Pareto chart for the effects of variables: dissolution media, compaction pressure, and rotation speed on intrinsic dissolution rate (IDR) for pyrimethamine.

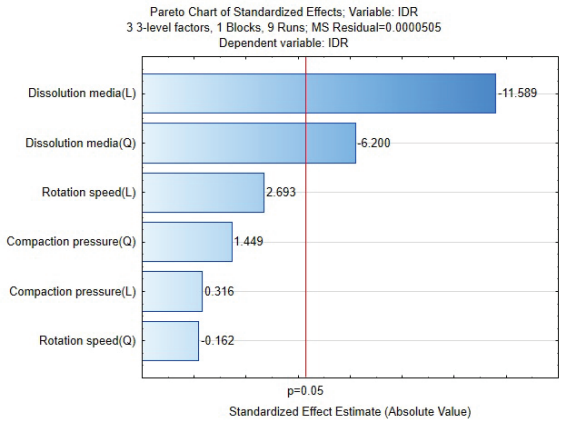

Figure 2. Pareto chart for the effects of variables: dissolution media, compaction pressure, and rotation speed on intrinsic dissolution rate (IDR) for metronidazole.

Due to its higher solubility at pH 4.5, acetate buffer could be chosen as a suitable dissolution medium for intrinsic dissolution tests for pyrimethamine. For metronidazole, although the dissolution medium influenced IDR, all studied media are suitable, due to the high solubility. Any of the tested compaction pressures and rotation speeds for both drugs can be used for the intrinsic dissolution tests, because they did not have a significant effect on IDR; however, it is recommended to fix one set of conditions to compare samples of these drugs from different suppliers.

The simulations obtained in this work showed a high correlation between observed and predicted IDR values for pyrimethamine (Table 3) and metronidazole (Table 4), showing that DDDPlus can be used to estimate the intrinsic dissolution of both, low and highly soluble drugs. The IDR values found for both drugs showed a pH-dependency on their solubility.

DDDPlus was able to predict the intrinsic dissolution values that were used to calculate IDR for each test condition, showing its accuracy in estimating differences in solubility as the pH changed. IDR values above 0.0017 mg/s/cm^2 indicate a highly soluble drug. (*3, 19*) Pyrimethamine did not show IDR results higher than this value for all pH tested; therefore, it can be considered a low soluble drug. Metronidazole presented IDR values above 0.0017 mg/s/cm^2, confirming its high solubility. These findings correlate with the BCS classification of the drugs: pyrimethamine – class II or IV and metronidazole – class I. (*21*)

Metronidazole's degree of micronization was used as additional factor by a previous study (*11*), which confirmed that particle size does not impact IDR. DDDPlus does not use particle size distribution when intrinsic dissolution is selected for simulation, thus micronization differences between metronidazole samples were not considered in simulations. The software was able to simulate IDR values for metronidazole with a high correlation $R^2 \geq 0.9970$ (Table 4) with the in vitro data for different intrinsic dissolution test conditions.

DDDPlus can accurately predict IDR for the studied drugs, showing that this software can be used to estimate drug's solubility according to BCS classification and the best conditions for intrinsic dissolution tests, reducing the number of laboratory experiments, and helping pharmaceutical companies to save time and costs.

CONCLUSION

Computer simulations using DDDPlus can help to gain biopharmaceutical understanding of APIs in the drug development process. Software simulations can be used to predict the intrinsic dissolution of API's in the physiologically relevant pH range of 1 to 7.2. This can help streamline and minimize experimental lab work. Key experiments can be identified by the simulations and be confirmed by lab results to characterize important biopharmaceutical API properties.

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CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

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