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Discriminatory Dissolution Test for Tablets Containing α- and β-Thalidomide Polymorphs

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ABSTRACT

Over the years, thalidomide has been prescribed for an increasing number of diseases, including multiple myeloma and erythema nodosum leprosum. In Brazil and other countries, thalidomide is available in tablets, and there is no official dissolution testing available for this dosage form. Considering this, a dissolution method was developed and validated for tablets containing 100 mg of each polymorph using 1-L vessels to verify that it can differentiate between polymorphs, since drug product is supposed to be formulated with the α form. In addition, the possibility of using smaller volumes of dissolution medium was also explored.

This method was compared with the *USP* dissolution method for thalidomide capsules (4-L vessel) with independent models using difference and similarity factors as well as dissolution efficiency. Dissolution kinetics was evaluated using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The kinetic parameters and the suitability of the models for experimental data were evaluated. The developed dissolution method was fully validated. It allowed a better discrimination of thalidomide polymorphs than the *USP* method for the formulations tested. Considering that it uses a conventional dissolution apparatus with 1-L vessels and there is no method described for tablets, it can be used for quality control of thalidomide in this dosage form.

INTRODUCTION

Many solid drugs exist in different physical forms. Polymorphism is often characterized as a drug's ability to exist as two or more crystalline phases that have different arrays, molecular conformations, or both (*1*, *2*). Thalidomide (Figure 1) has two known polymorphic forms, α and β, each one isolated by crystallization using different conditions (*3*, *4*).

Their characteristics in the solid state exert a significant influence on the drug dissolution rate. Drug polymorphs may have different aqueous solubilities and rates of dissolution. When these differences are sufficiently large, bioavailability may change, and could lead to deviations in product quality (*1*, *5*, *6*). For these reasons, it is essential to pay extra attention to drugs presenting polymorphism during the development of generic medications (*1*, *2*).

The low solubility of thalidomide in water, which is around 50 µg/mL for the racemic mixture (*7*, *8*), led to the development of thalidomide exclusively for oral use. The polymorphic form of drugs with poor aqueous solubility, such as thalidomide, must be controlled to ensure product quality.

It is therefore important for the dissolution method to be capable of detecting changes in the analyzed product, and it is mainly important to monitor low solubility drugs for critical parameters of the formulation (*9*). Thus, this work aimed at the development of a discriminative dissolution method able to detect differences in dissolution profiles between tablet formulations obtained from α- and β-thalidomide polymorphs. The method will be compared to that proposed by *USP* (*10*).

MATERIAL AND METHODS

Materials

Thalidomide reference standard was acquired from USP (Lot FOC 107, Rockville, MD, USA). Drug substances of α- and β-thalidomide were kindly donated by Microbiologica (lots TH.T.004 and SEE–052, respectively). Sodium lauryl sulfate (SLS) was purchased from Synth (São Paulo, Brazil). Polyoxyethylene lauryl ether (Brij 35) and hydrochloric acid were acquired from Vetec (Rio de Janeiro, Brazil). Acetonitrile (HPLC grade) was purchased from Honeywell

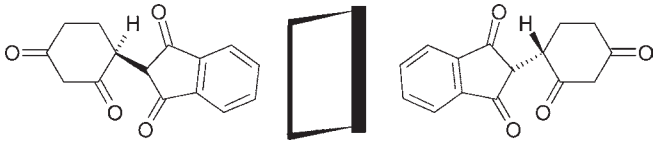

Figure 1. Chemical structures of R-(+)-thalidomide and S-(-)-thalidomide.

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(Muskegon, MI, USA). Orthophosphoric acid was acquired from Merck (Rio de Janeiro, Brazil).Dimethicone 350 was acquired from Dow Corning (Porto Alegre, Brazil). Ultrapure water (Milli-Q Plus, Millipore Corp., Billerica, MA, USA) was used throughout all analyses. All other reagents were of analytical grade.

Physical Characterization of α- and β-Thalidomide Tablets

For this study, 100-mg tablets of α- or β-thalidomide were used. Both formulations were made in Brazil by FUNED (Belo Horizonte, Minas Gerais), a public laboratory that produces medicines for the Brazilian Department of Health on the same equipment used for commercial batches.

Particle size was determined by laser light diffraction (CILAS 1180, Cilas, France). Dimethicone was used as dispersant. The results were calculated automatically using software. The surface areas of the polymorphs were determined using the BET gas-phase adsorption method (NOVA 1000 Surface Area Analyzer, model Autosorb-1, Quantachrome Instruments, Boynton Beach, FL, USA). Physical characterization of the tablets for average weight, hardness, friability, disintegration, and uniformity of dosage units was performed according to *USP* (*10*).

Dissolution Test Conditions

Method A

The development and validation of the Method A dissolution test were performed using a VANKEL VK 8000 dissolution station comprising a VK two-way peristaltic pump, a VK 750D recirculation/heating controller, and a VK 7010 multibath dissolution station ($n = 8$) with automatic sampler. A paddle device was used (USP Apparatus 2) at a rotation speed of 75 rpm. The volume of the dissolution medium used was 1 L, preheated to 37 ± 0.5 °C. Sample aliquots of 10 mL were collected at 10, 20, 30, 40, 50, and 60 min, and the medium volume was replaced after sampling to maintain constant volume. The samples were filtered through a 0.45-µm filter and analyzed using HPLC.

Method B

The *USP* dissolution test (Method B) was performed using a VANKEL VK 7000 autosampling station with a VK 750D recirculation/heating controller. The conditions of Method B are the same as those described for Method A, but the volume of dissolution medium was 4 L.

Dissolution Medium

Method A

The volume of the dissolution medium was 1 L. The dissolution medium was prepared using a 5 L of a mixture of 1% SLS solution and 1 L of 0.225 M hydrochloric acid. The pH of the dissolution medium was 1.68.

Method B

The volume of the dissolution medium was 4 L (*10*). The dissolution medium was prepared using a mixture of 10 L of 0.075% brij solution and 2 L of 0.225 M hydrochloric acid. The pH of the dissolution medium was 1.70.

Sink Conditions

Tablets containing 100 mg of α- and β-thalidomide were shaken vigorously in 350 mL of medium (Methods A and B) before they were added to the dissolution vessels containing preheated medium at 37 ± 0.5 °C for 2 h. Samples were collected, filtered, and analyzed by HPLC.

HPLC Analysis

The Shimadzu HPLC system (Kyoto, Japan) consisted of an LC–20AT pump, a DGU–20A5 degasser, a SIL–20A automatic injector, a SPD–M20A photodiode array detector, a CBM–20A communication module, and LC Solution software.

Chromatographic Conditions

Thalidomide was analyzed using a Waters XTerra MS C18 (5-µm particle size, 3.9 × 150 mm) reversed phase column. The mobile phase was isocratic and consisted of 0.1% orthophosphoric acid/acetonitrile (80:20 v/v), filtered and degassed. The chromatographic conditions were constant flow rate of 1.0 mL/min, 20-µL sample volume, room temperature, and ultraviolet detection at 237 nm. All quantitative analysis calculations were performed with external standardization based on peak areas.

Validation of the Dissolution Test

The dissolution test was validated for specificity, linearity, accuracy, and precision according to the *USP* (*10*). Stability under test conditions was also evaluated.

Specificity

A placebo was prepared to verify method specificity. A mixture of the excipients (microcrystalline cellulose 102, lactose spray-dried, polyplasdone, magnesium stearate, colloidal silicon dioxide) in an amount proportional to the tablet formulation was transferred to 1 L of the dissolution medium ($n = 6$) at 37 ± 0.5 °C. The test lasted one hour using a paddle apparatus (USP Apparatus 2) with a 100-rpm rotation speed. Samples were collected, filtered, and analyzed by HPLC. The system response was examined for the presence of interference or overlap with the thalidomide peak.

Linearity

For linearity experiments, solutions of five concentrations of thalidomide over the range of 25–125 µg/mL were prepared by diluting a stock solution containing 1 mg/mL in dissolution medium, on three different days. Analyses

were performed in triplicate. The results were represented graphically, which allowed the evaluation of the calibration curve and coefficient of determination. The linearity was estimated by linear regression analysis.

Accuracy and Precision

The accuracy of the method was determined by the recovery of thalidomide added to 1 L of dissolution medium at 37 ± 0.5 °C, containing a mixture of excipients in an amount proportional to the tablet formulation. The test was performed over 1 h at 75 rpm. Three different known concentrations of thalidomide were analyzed: 33, 83, and 98 µg/mL for polymorph α; and 27, 63, and 76 µg/mL for polymorph β. After 1 h, samples were collected and analyzed by HPLC. Six samples of each polymorph were analyzed on two days.

Method precision was evaluated by calculating the intraday (repeatability) and interday (intermediate precision) relative standard deviation (RSD) of the results obtained in the accuracy studies.

Standard Solution and Sample Stability

Solutions containing 1 mg/mL of thalidomide ($n = 3$ for each polymorph) were added to 1 L of dissolution medium at 37 ± 0.5 °C, resulting in a final concentration of 100 µg/mL. The test was performed for 2 h using a paddle apparatus (USP Apparatus 2) with a rotation speed of 75 rpm. Aliquots of the samples were filtered and analyzed by HPLC at 0, 8, and 24 h. The responses of the solutions were obtained by comparison with a freshly prepared standard solution.

Comparison of Dissolution Profiles

The dissolution efficiency (%DE) was calculated from the curves of percent drug dissolved versus time (dissolution profile). The dissolution profiles were also compared using difference (f_1) and similarity (f_2) factors. According to the current regulations, two dissolution profiles are considered similar when f_1 is between 0 and 15 and f_2 is between 50 and 100 (*11*).

Evaluation of Release Kinetic

To calculate the kinetic parameters, a model-dependent approach was used. The mathematical model that best represented the dissolution process for each polymorph (α and β) in each method (A and B) was determined. The suitability of models to experimental data was evaluated with the assistance of MicroMath Scientist 3.0 (Micromath, St. Louis, MO, USA), comparing the models based on model selection criteria (MSC) and correlation coefficient (r) values using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models.

The dissolution constant (k) was obtained from the equations defined by the mathematical model that showed the highest MSC and r values. Dissolution half-life ($t_{50\%}$) was obtained from the graphics.

RESULTS AND DISCUSSION

Physical Characterization of the Tablets

The dissolution rate is directly proportional to the surface area of the drug particles. Reduced particle size leads to an increase in the surface area exposed to the dissolution medium, resulting in an increased dissolution rate. Particle size and wettability can be modified by process parameters, but solubility balance is determined by the polymorphic form (*11–13*).

The particle size and surface area of the α- and β-polymorphs did not present a significant difference, according to Table 1. This suggests that the difference in solubility between formulations α and β are related to polymorphism, which may affect drug release from the dosage form as well as drug absorption (*4*, *5*). Regarding the average weight and content uniformity, both formulations were within the recommended limits (*11*). Hardness and friability of both formulations were adequate, which was expected since they were prepared under the same conditions.

Some differences observed in the physicochemical proprieties (particle size, surface area) and physical parameters (hardness, friability) are not relevant

Table 1. Physical Parameters of α- and β-thalidomide

Parameter	α-thalidomide, mean ± SD (RSD)	β-thalidomide, mean ± SD (RSD)
Particle size (µm)[a]	86.88 ± 0.13 (0.15%)	82.29 ± 0.60 (0.74%)
Superficial area (m^2/g)[a]	3.08 ± 0.82	5.17 ± 1.56
Mean weight (mg)[b]	371.55 ± 4.51 (1.21%)	371.00 ± 2.49 (0.67%)
Dose uniformity (mg)[b]	101.63 ± 1.74 (1.72%)	100.33 ± 1.03 (1.03%)
Friability (%)[b]	0.11	0.01
Hardness (N)[b]	8.05 ± 0.70 (8.66%)	9.34 ± 0.49 (5.20%)
Disintegration (min)[b]	0.43	0.56

[a] *Bulk*
[b] *Tablets*

regarding the fast disintegration of the tablets analyzed (Table 1). However, the existence of polymorphism appears to have a much greater effect on the dissolution rate.

Sink Conditions

Sink conditions are often recommended, considering that the dissolution test, when used as a batch-to-batch quality control tool, is planned so that most of the drug is released (80–85%) (*14*). Tablets of α- and β-thalidomide tablets did not present sink conditions for Method A, according to Table 2. A medium that does have sink conditions can be justified if it shows higher discriminatory power or if it proves to be more reliable than one that does (*9*, *10*). It is also known that during in vivo dissolution, sink conditions may not exist, especially for drugs that present low solubility in water (*15*).

Dissolution Method Validation

Specificity

The specificity test shows that the excipients do not interfere with the thalidomide peak (Figure 2). The chromatogram obtained by injecting the placebo solution did not present any other peak at the same retention time (5 min). The chromatographic peak purity tool available in the LC Solution software was used to verify the purity. This tool analyzes the peak providing values between 0 and 1. The value obtained (>0.9999) shows that the peak was pure, without interference.

Linearity

Three standard curves in the 25.0–125.0 µg/mL range were evaluated to assess linearity. The resulting linear equation is

$y = 48475\ (\pm 96.0)\ x + 37736\ (\pm 64.1)$

where y is the peak area and x is the concentration of the analyte. The average correlation coefficient (r) is 0.9999, indicating that the method is linear in the evaluated range.

Accuracy and Precision

Accuracy of the method was evaluated by the recovery test. As indicated in Table 3, the average recoveries obtained for three concentration levels ranged from 100.60% to 101.05% for α-thalidomide tablets and 99.66% to 100.55% for β-thalidomide, showing that the method is accurate. Recoveries from 95.0% to 105.0 % are acceptable (*10*).

Likewise, method precision was assessed by determination of repeatability (intraday analysis) and intermediate precision (interday analysis). The results, shown in Table 4, are expressed as relative standard deviation (RSD). The method is considered precise, with RSD values less than 1%.

Table 2. Dissolution of Thalidomide in Different Media[a]

	Method A mean ± SD (RSD)	Method B mean ± SD (RSD)
α-thalidomide	57.26 ± 1.80 (3.15%)	80.78 ± 0.35 (0.43%)
β-thalidomide	54.45 ± 0.27 (0.49%)	72.71 ± 0.34 (0.46%)

[a] *(n = 3)*

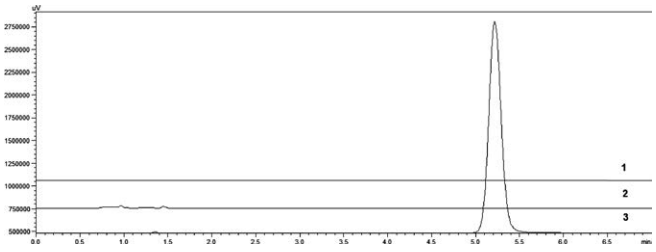

Figure 2. Specificity test for thalidomide with (1) mobile phase, (2) placebo, and (3) USP reference standard.

Table 3. Accuracy Results for Thalidomide[a]

Polymorph	Concentration added to the matrix (µg/mL)	Recovered Concentration (µg/mL ± DPR)	Recovery (%)
α	33	33.34 ± 0.16	100.89–101.20
	82	82.60 ± 0.71	100.21–101.73
	98	98.59 ± 0.56	100.52–100.68
β	27	27.01 ± 0.24	99.26–100.85
	63	63.75 ± 0.22	100.28–100.82
	76	75.74 ± 0.30	99.53–99.80

[a] *(% recovery)*

Table 4. Precision Results for Thalidomide[a]

Polymorph	Day	Concentration added to the matrix (µg/mL)	Intraday RSD	Interday RSD
α	1	33	0.11	0.47
	2	33	0.68	
	1	82	0.66	0.85
	2	82	0.77	
	1	98	0.70	0.56
	2	98	0.53	
β	1	27	0.07	0.87
	2	27	0.11	
	1	63	0.28	0.35
	2	63	0.10	
	1	76	0.16	0.40
	2	76	0.57	

[a] *(n = 3)*

Stability of Sample Solutions

Both α- and β–thalidomide were stable under dissolution test conditions. The results demonstrate that sample and standard solutions remained at 100.0 ± 2.0% over 24 h at room temperature.

Dissolution Profile of α- and β-Thalidomide

The rotation speed was set to 75 rpm. If the rotation speed is set too high, differences in drug formulations or batches of in vivo relevance may not be observed. On the other hand, at low rotation speeds the method may become too sensitive, detecting differences that will not influence in vivo absorption (*11*). The dissolution volume of the method was chosen based on drug solubility. The maximum recommended volume for regular vessels (1 L) was used. The choice of surfactant is an important parameter for the dissolution study. When the solubilizing capacity of the surfactant is very high, the dissolution medium may not discriminate changes between formulations (e.g., polymorphic form), as suggested by ICH Q6A (*14*).This was observed for the poorly soluble drug mebendazole (*16*, *17*).

Considering that the discriminatory power of the dissolution method can be used to detect changes in batches of the same formulation, it is important to demonstrate this capacity, especially for monitoring drugs or critical parameters in the formulation, to achieve the desired performance of a low solubility drug product. Determining whether a dissolution method can discriminate these changes presents a challenge. One of the best ways to analyze the discriminatory power of the method is to test the formulations with drugs that have different characteristics (particle size, crystalline form, density). If the result presents a significant difference in the variables, then the method can be considered discriminatory for the critical production variables (*9*, *18*).

The in vitro dissolution profiles of the tablets are shown in Figure 3. Each data point represents a mean of twelve measurements for each product. The RSDs follow the recommendations of the guidance: ≤20.0% until 10 min and ≤10.0% after that. Tablets of α- and β-thalidomide presented different dissolution profiles for Methods A and B.

Typical acceptance criteria for percent drug dissolved at the end of the test are in the range of 75–80% of the nominal content. Acceptance criteria including time of dissolution are usually established based on the dissolution profile data (*11*). In the dissolution profiles shown in Figure 3, β-thalidomide tablets did not meet this criterion for Method A, which is not observed in the dissolution profiles of the α and β tablets analyzed using Method B.

The Biopharmaceutics Classification System (BCS) supplies a regulatory scientific structure taking into account polymorphic drugs. For drugs where the rate and extent of absorption are limited by dissolution, differences in the solubility of the polymorphic forms may affect bioavailability considerably (*14*). For Class 2 drugs, it is expected that the dissolution rate will be a limiting step for drug absorption, and it may be possible to establish an in vivo–in vitro correlation.

Thalidomide presents low solubility (*7*, *8*) and high permeability (bioavailability of 67–93%), and it is a Class 2 drug according to the BCS (*19*, *20*). In this case, due to the high risk that a change of polymorph will have on bioavailability, it is important to have appropriate control of the polymorphic forms.

Dissolution Profile Comparison

The dissolution profiles for Methods A and B of α- and β-thalidomide tablets were compared. The results of the dissolution efficiencies, difference (f_1) and similarity (f_2) factors between methods A and B are shown in Table 5.

The results of f_1 and f_2 for both α- and β-thalidomide tablets show that the profiles are similar. However, β-thalidomide tablets present f_1 and f_2 values very close to the acceptance limits, proving the low similarity of

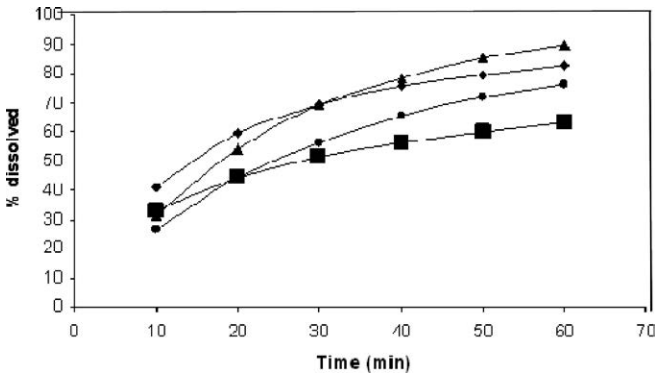

Figure 3. Mean dissolution profiles of α-thalidomide tablets in (♦) 1 L and (▲) 4 L of dissolution media, and β-thalidomide tablets in (■) 1 L and (●) 4 L of dissolution media.

Table 5. Comparison of Tablet Dissolution Profiles through the Dissolution Efficiency (%DE), Difference Factor (f_1), and Similarity Factor (f_2)

Parameter	Method A	Method B	Method A	Method B
%DE α	74.1	67.6		
%DE β	73.0	66.1		
$f_1\alpha^a$		7.5		
$f_2\alpha^a$		59.0		
$f_1\beta^a$		13.5		
$f_2\beta^a$		50.6		
$f_1{}^b$			24.4	16.5
$f_2{}^b$			36.4	44.7

[a] *Method B used as reference*
[b] *Intramethod for both polymorphs*

Methods A and B for this polymorph. When comparing each method for both polymorphs, it was observed that Method A was more discriminatory than Method B for α and β-thalidomide tablets.

Another approach used to compare drug dissolution is dissolution efficiency (%DE). This parameter can be defined as the area under the dissolution curve at a specific time, compared with the area of the rectangle described by 100% of dissolution at this time. This parameter is related to the actual amount of drug that is dissolved in the medium, and thus a better prognosis of in vivo results may be achieved (*21*). According to Table 5, Method A showed higher values for %DE.

Kinetics of Drug Release

Zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetics models were applied to describe the dissolution profiles. Based on these values obtained by mathematic modeling (Scientist 3.0, Micromath, St. Louis, MI, USA), the model that describes the profiles best is Korsmeyer–Peppas for α and β tablets using Method A. For Method B, the dissolution profile of polymorph α was best described by the first-order equation and polymorph β by Higuchi (Table 6).

The Korsmeyer–Peppas model relates drug release according to time. This model is generically used to analyze the release from polymeric matrices when the mechanism is not well known or when more than one releasing process is involved (*21*). On the other hand, the first-order model is based on the relationship of the *ln* function of the percent of undissolved drug versus time, and it is mainly related to immediate-release formulations, where the amount of drug released is proportional to the remaining amount in the dosage form, which decreases over time. The Higuchi model is used to describe drug release as a diffusion process based on Fick's law (*21*, *22*).

According to the results of the Table 6, polymorph α was more soluble than polymorph β by both methods.

CONCLUSIONS

A dissolution method was developed for tablets containing α- and β-thalidomide polymorphs, and it was successfully validated according to the *USP*. The method, which uses regular 1-L dissolution vessels, was compared with the *USP* method (4-L vessels).

Considering the overall dissolution profiles, the Higuchi model best described them, suggesting that for the tested formulations, drug release behaved as a diffusion process based on Fick's law. The results of f_1 and f_2 for both α- and β-thalidomide tablets showed that the profiles are similar for Methods A and B. On the other hand, the use of f_1 and f_2 factors to verify the discriminatory power of the methods for both polymorphs showed that Method A was able to better differentiate the profiles.

The developed method is easier to perform and presented similar results when compared with the *USP* method (capsules) for both thalidomide polymorphs, and it is more discriminatory for the dosage forms evaluated. It is thus an important alternative for quality control of thalidomide.

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Table 6. Correlation Coefficients (r) and Model Selection Criteria (MSC) Obtained from Mathematical Treatment of Dissolution Data and Kinetic Parameters for Methods A and B

	α-thalidomide				β-thalidomide			
	Method A		Method B		Method A		Method B	
Release Model	***r***	**MSC**	***r***	**MSC**	***r***	**MSC**	***r***	**MSC**
Zero-order	0.8960	0.64	0.9488	1.43	0.8984	0.62	0.9576	1.58
First-order	0.9867	2.86	**0.9999**	**8.32**	0.9573	1.64	0.9975	4.53
Higuchi	0.9864	3.09	0.9942	4.14	0.9880	3.14	**0.9965**	**4.58**
Korsmeyer–Peppas	**0.9965**	**4.38**	0.9942	3.88	**0.9993**	**5.99**	0.9970	4.54

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