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Development of a Dissolution Test for Extended-Release Bromopride Pellets with In Vivo–In Vitro Correlation

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ABSTRACT

The aim of this study was the development of a dissolution test with IVIVC for extended-release bromopride (BPD) pellets using bioavailability data. BPD is a Biopharmaceutics Classification System Class 2 drug, and its absorption is primarily limited by its dissolution rate. Despite this, there are no reports describing a dissolution test for BPD dosage forms. The dissolution medium was selected based upon the sink condition and the gastrointestinal pH. Furthermore, USP Apparatus 1 and 3 were tested, and the dissolution method was compared with that outlined by the manufacturer of the reference drug. Deconvolution by the Wagner–Nelson method was used to obtain the fraction of drug absorbed. The dissolution test that yielded IVIVC for both the fasted and fed states ($R^2 > 0.97$) and also the discriminative power used Apparatus 1 at 75 rpm in 900 mL of HCl. The dissolution medium (900 mL PBS pH 7.2) was changed after 1 h. Additionally, quantitative analysis was successfully validated by UV absorption (273 nm). Drug release is controlled by the pellet coating and by BPD solubility. The Higuchi release kinetics model best describes the dissolution of commercial BPD pellets batches.

KEYWORDS: Bromopride; dissolution; extended-release pellets; IVIVC; USP Apparatus 3.

INTRODUCTION

Bromopride (BPD) is an antiemetic drug that blocks the effect of dopamine D_2 receptors in the gastrointestinal tract (GIT) and in the central nervous system; it is used as a therapy for gastrointestinal disorders. BPD stimulates GIT motility and enhances the gastric emptying rate (*1*, *2*). BPD is generally indicated for use in gastroesophageal reflux, irritable bowel syndrome, functional dyspepsia, diabetic gastroparesis, radiological examination of the GIT, and chemotherapy-induced vomiting (*3–5*). This compound is a bromo-analogue of metoclopramide with two ionizable groups ($pK_a = 9.35$) and is categorized as a Class 2 drug according to the BCS with low solubility and high permeability (*6–9*).

An extended-release (ER) dosage form containing coated BPD pellets that can optimize the therapeutic effects of this drug is commercially available. BPD has a half-life of around 4–5 h (*9*, *10*), and the ER extends its pharmacological effects and allows delivery of the drug at specific sites in the GIT. This improves its bioavailability and reduces side effects that result from fluctuations in plasma concentration due to frequent administration (*11*, *12*). Thus, the evaluation of the in vitro ER dissolution profile should include the various conditions to which the drug will be exposed while passing through the GIT (*13–15*).

Dissolution tests that can simulate the in vivo performance of solid oral dosage forms are used to establish the in vitro–in vivo correlation (IVIVC), a regulatory requirement for bioequivalence studies. These tests can effectively evaluate in vivo behavior and reduce the number of clinical trials needed to investigate efficacy of a new drug (*16–18*). The correlations between the in vitro dissolution data and the in vivo absorption data are categorized as levels A, B, and C according to the FDA (*19*). The level A correlation consists of a linear point-to-point relationship between the in vitro dissolution profile and the fraction of the drug absorbed in vivo (*19–23*). This correlation is a predictive mathematical model that describes the relationship between in vitro dissolution and drug absorption over time (*20*). Several dissolution apparatus were used during the development of the dissolution method using IVIVC; however, the reciprocating cylinder or BioDis (Apparatus 3) was designed especially for evaluating ER (*24–26*).

BPD is a Class 2 drug, and its absorption is primarily limited by its dissolution rate. Despite this, there are no other reports in the literature that describe the development of dissolution tests for ER BPD dosage forms. The aim of this study was to develop and evaluate different dissolution methods for extended-release pellets that contained BPD using USP Apparatus 1 and 3 and investigate an IVIVC using bioavailability data. The dissolution kinetics was calculated

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by the model-dependent method, and its discriminatory power was also challenged.

EXPERIMENTAL

Materials

BPD reference standard (purity 99.95%) was purchased from the Genix Pharmaceutical Industry (São Paulo, Brazil). The reagents hydrochloric acid, monobasic potassium phosphate, sodium chloride, and sodium hydroxide were obtained from Tedia (Rio de Janeiro, Brazil). The biobatch Digesan Retard extended-release capsules containing 20 mg BPD microgranules, the placebo (shellac, talc, sucrose, starch, corn starch, Eudragit, and ethyl alcohol), and the test batch were kindly donated by Sanofi–Synthelabo (Rio de Janeiro, Brazil). The Digesan Retard batches A and B were purchased at the local market. For all filtration procedures, 10-µm polyethylene filters (Hanson Research, Chatsworth, CA, USA) and 0.45-µm polyvinylidene fluoride filters (Millex Millipore, São Paulo, Brazil) were used. Water was obtained using the Milli-Q water purification system (Millipore, Bedford, MA, USA).

In Vivo Study

The oral bioavailability studies of the reference drug product Digesan Retard 20 mg were performed at the Integrated Unit of Pharmacology and Gastroenterology (UNIFAG, São Paulo, Brazil). Sanofi–Synthelabo provided the bioavailability data as a courtesy to the Laboratory of Quality Control of Drugs and Medicines (LabCQ, Rio de Janeiro, Brazil). An open-label, randomized, crossover, single-dose study was performed using 24 healthy volunteers (12 men and 12 women) between 18 and 50 years of age, who had previously undergone clinical electrocardiogram assessments and additional laboratory studies. The volunteers were admitted a day before the study began; one group fasted overnight while the other group received a specific diet 30 min before oral administration of the BPD capsules with 200 mL of water. The washout period was considered to be 7 days. Therefore, blood samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 18.0, 20.0, 24.0, and 48.0 h and centrifuged at 3,000 rpm for 10 min. The plasma was separated and stored at -20 °C until analysis.

The quantification analyses were performed in a Shimadzu chromatographic system (LC-10 AD VP, Shimadzu Corporation, Kyoto, Japan) coupled with a triple quadrupole tandem mass spectrometer (Micromass Quattro Micro, Waters Corporation, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. The chromatographic analysis was conducted with a Luna C_{18} chromatographic column (100 mm × 4.6 mm, 5 µm) obtained from Phenomenex (Torrance, CA, USA), the flow rate was 0.7 mL/min, the injection volume was 30 µL, and the mobile phase was 80/20 acetonitrile/ammonium acetate buffer (10 mmol/L). Procainamide was used as an internal standard. The ESI was operated as described by Nazare et al. (*27*) in the positive (ES+) and the multiple reaction monitoring mode; analysis was measured at m/z 344.20 > 271.00 for BPD and m/z 236.30 > 163.10 for procainamide.

Drug Solubility

The quantitative evaluation of BPD solubility considered the physiological pH range of the GIT (pH 1.2, 2.0, 4.5, 5.8, 6.9 and 7.2) at 37 ± 0.5 °C. The medium contained 0.01 M HCl, 0.1 M HCl, and phosphate buffer solution (PBS) prepared using potassium phosphate monobasic at differing pH values (4.5–7.2) according to specifications outlined in the *USP* (*28*). The solubility studies were performed in a beaker containing 10 mL of medium, to which approximately 150 mg of BPD was added to ensure an excess of drug in the system. The solutions were constantly mixed by magnetic stirring at 500 rpm for 24 h. Thereafter, the solutions were centrifuged for 15 min (Eppendorf 5430R, Hamburg, Germany) with a rotation speed of 5,000 rpm. The supernatant was filtered through a 0.45-µm Millipore membrane, and the filtrate was assayed according to the UV method for quantifying raw materials as described in the *Brazilian Pharmacopoeia* (*29*). The test was performed in triplicate for each dissolution medium tested.

Dissolution Test Conditions

The dissolution parameters were evaluated considering the physiology of the GIT. The BPD sink conditions in each dissolution medium were verified using solubility tests. The influence of drug release was also investigated, followed by analysis of medium volume and the apparatus.

Dissolution Test Using the Rotating Bottle Apparatus

The rotating bottle apparatus was the dissolution method submitted to the National Agency for Sanitary Vigilance during registration of the Digesan Retard capsules. The rotating bottle apparatus model used in this study was produced, and the conditions are presented in Table 1. This apparatus, also named Diffu Test, is not officially recommended by pharmacopoeias and was used mainly at the beginning of dissolution studies (*30*, *31*). One capsule containing approximately 200 mg of microgranules was placed in an apparatus bottle containing 25 mL of the dissolution medium. Dissolution was performed at 37 ± 0.5 °C using a rotation speed of 30 rpm, according to the specifications described in Table 1. After each dissolution period (1–5), the dissolution medium was filtered, and the microgranules remaining in the tube underwent an additional dissolution period with different dissolution media. During each period, the volume of filtered dissolution medium was transferred to a 100-mL volumetric flask to which 10 mL of 1 M hydrochloric acid was added and brought to volume with purified water. After 8 h of dissolution, the remaining microgranules were

Table 1. Dissolution Test Conditions and Specifications Using the Rotating Bottle Apparatus

Dissolution Medium		Time (h)	Sample	Cumulative % Drug Dissolved
1	0.1 M HCl pH 1.5	1	S1	Maximum 40%
2	PBS pH 4.5	1	S2	Maximum 70%
3	PBS pH 6.9	2		
4	PBS pH 6.9	2	S3	More than 70%
5	PBS pH 7.2	2		

PBS: phosphate buffer solution (potassium phosphate monobasic 0.25 M)
S2: dissolution medium of medium 2 + medium 3
S3: dissolution medium of medium 4 + medium 5

also recovered from the tube (remaining sample - RS). Three samples (S1, S2, and S3) were obtained from each dissolution test. S1was obtained from dissolution medium 1, S2 from dissolution medium 2 plus medium 3, and S3 from dissolution medium 4 plus medium 5. A 5-mL aliquot of each sample (S1, S2, S3, and RS) was placed in separate 50-mL volumetric flasks, 5 mL of 1 M HCl was added to each, then brought up to volume using purified water. The standard sample was also diluted with a 1 M HCl solution. The specifications state that the sum of S1, S2, S3, and RS should equal 100%. In addition, the test was performed in triplicate.

Dissolution Assay Using the Basket (USP Apparatus 1)

Dissolution studies were performed using USP Apparatus 1 in a Hanson Research SR8 Plus Dissolution Test Station (Hanson Research Corp., Chatsworth, CA, USA). Seven dissolution methods evaluated different dissolution media, test durations, pH values, and volumes, as presented in Table 2. For Methods 1 and 2, only one dissolution medium was used during the entire analysis, while the dissolution media used in Methods 3–5 were initially acidic and subsequently switched to PBS. In Method 6, the dissolution test was carried out using a pH gradient starting at 900 mL of 0.01 M HCl pH 2. After 60 min, the medium was replaced by 500 mL of PBS pH 4.5; after 60 min, 250 mL PBS pH 5.8 was added, which changes the pH of the dissolution medium to 5.1. Finally, after 60 min, an additional 150 mL of PBS pH 7.2 was added, which increased the pH to 5.8, and the assay was allowed to continue another 60 min. Furthermore, Method 7 was similar to Method 6 during the first 120 min; however, 250 mL of 0.012 M NaOH was added to the medium to obtain a pH of 5.8. After an additional 60 min, 150 mL of 0.12 M NaOH was added to the dissolution medium, thus changing the pH to 6.9. All of these experiments were carried out in replicates of six or more.

Dissolution Test Using a Reciprocating Cylinder (USP Apparatus 3)

The dissolution tests using USP Apparatus 3 (BIO-DIS Varian, Varian Inc., CA, USA) were carried out at five pH values over a 270-min period to create a pH gradient and simulate passage through the GIT (pH 2–7.2). The mesh on the top and bottom of the inner tubes was fixed at 405 µm. Each horizontal row of outer tubes was filled with 250 mL of dissolution medium at 37 ± 0.5 °C. Furthermore, the dissolution medium was 0.01 M HCl pH 2 (Method 8), 0.1 M HCl pH 1.2 (Methods 9 and 10), and PBS at pH values of 4.5, 5.8, 6.9, and 7.2, as shown in Table 2. BioDis was programmed so that the inner tubes remained inside each row of outer tubes for the first hour with a rate of 5 dpm (Methods 8 and 9) and 3 dpm (Method 10) with a 5-s holding time between each drop. After this period, the inner

Table 2. Dissolution Test Conditions for Digesan Retard 20-mg Biobatch Performed at 37 ± 5 °C without Medium Replacement

Method	Apparatus	Agitation rate	Time (min) Dissolution Medium Volume				
			60	120	180	240	270
1	1	75 rpm	Water (900 mL)				--
2	1		HCl pH 2 (900 mL)				--
3	1		HCl pH 2 (900 mL)		PBS pH 6.8 (900 mL)		--
4	1		HCl pH 2 (500 mL)		PBS pH 6.8 (500 mL)		--
5	1		HCl pH 2 (900 mL)	PBS pH 7.2 (900 mL)			--
6	1		HCl pH 2 (900 mL)	PBS pH 4.5 (500 mL)	PBS pH 5.1 (750 mL)	PBS pH 5.8 (900 mL)	--
7	1		HCl pH 2 (900 mL)	PBS pH 4.5 (500 mL)	PBS pH 5.8 (750 mL)	PBS pH 6.9 (750 mL)	--
8	3	5 dpm	HCl pH 2 (250 mL)	PBS pH 4.5 (250 mL)	PBS pH 5.8 (250 mL)	PBS pH 6.9 (250 mL)	PBS pH 7.2 (250 mL)
9	3	5 dpm	HCl pH 1.2 (250 mL)	PBS pH 4.5 (250 mL)	PBS pH 5.8 (250 mL)	PBS pH 6.9 (250 mL)	PBS pH 7.2 (250 mL)
10	3	3 dpm	HCl pH 1.2 (250 mL)	PBS pH 4.5 (250 mL)	PBS pH 5.8 (250 mL)	PBS pH 6.9 (250 mL)	PBS pH 7.2 (250 mL)

HCl pH 1.2 (0.1 M)
HCl pH 2 (0.01 M)
PBS: phosphate buffer solution

tubes were automatically elevated and remained above the outer tubes for a few seconds during the dissolution medium run and then moved to the next immediate row containing new dissolution medium. This process was repeated until the inner tubes passed through the other rows of outer tubes containing the dissolution medium. All of these experiments were carried out in a minimum of six replicates (*28*, *32*).

Sampling Parameters

The sampling parameters were the same for all the dissolution apparatus used. Next, 10-mL samples were collected with disposable syringes without medium replacement over a 240-min period for Apparatus 1 and the rotating bottle apparatus, and 300 min for Apparatus 3, while samples were taken at time points of 30, 60, 120, 150, 180, 210, 240, 270, and 300 min. The samples were immediately filtered and then volumetrically diluted 1:1 with 0.1 M HCl. The acidification procedure of the dissolution samples allowed quantitative analysis to be performed using a single standard curve despite the pH differences in the dissolution media. The cumulative percentage of BPD capsule release was calculated based on a calibration curve at 2.5, 5.0, 7.5, 10.0, and 12.5 µg/mL of the BPD standard in 0.1 M HCl (pH 1) obtained at the same day. The samples were analyzed by UV spectrophotometer at 273 nm (UV–2401PC, Shimadzu Corporation, Kyoto, Japan).

Validation of the Quantitative Method

The analytical method parameters of specificity, linearity, precision, accuracy, limit quantification, and solution stability were validated (*28*, *32*). The specificity was determined by comparing the spectral scans from 200 to 400 nm of placebo, diluent (1 M HCl), standard solution, BPD raw material, and a solution of the reference medication Digesan Retard. The linearity of the method was evaluated by linear regression analysis using five different concentrations ranging from 2.5 to 12.5 µg/mL, which are equivalent to 25, 50, 75, 100, and 125% of the working drug concentration. These sample solutions were prepared in duplicate on different days. The accuracy of the method was determined by the recovery test, where a known amount of BPD was added to placebo at low, medium, and high concentrations (5.0, 10, and 12 µg/mL) to determine the working concentration. The recovery and RSD were assessed by comparing the values obtained for the spiked sample solutions with the theoretical concentration. The precision was estimated by calculating the relative standard deviation (RSD) of the assay using six samples at the working concentration (10 µg/mL) prepared similarly as presented in the accuracy method. The limit of quantification (LOQ) was determined by the response and the slope of the regression equation. LOQ was calculated as ten times the noise level of the calibration curve (*33*). The stability of the solution was evaluated at room temperature using a standard and sample solution at 10 µg/mL. Samples were quantified immediately after preparation (time zero) and at each hour thereafter until 12 h, and then at 24 h and 48 h. The RSD was calculated between the samples and time zero.

Scanning Electron Microscopy

The morphology of the biobatch coated pellets and the partially coated pellets (test batch) of BPD were evaluated before and after the dissolution test by scanning electron microscopy (SEM) (Jeol, JSM–6460 LV, Tokyo, Japan). The samples were prepared in an aluminum holder, and the surfaces were sputter-coated with gold (20-mm thick) and photographed at 15 kV under a vacuum.

In Vitro–In Vivo Correlation

The percentage of drug absorbed (F_a) versus time was calculated by mathematical deconvolution of the plasma concentration versus the time curve using the modified Wagner–Nelson equation (*34*, *35*)

$$\%\ absorbed = \frac{\frac{C_t}{K_{el}} + AUC_{0-t}}{AUC_{0-\infty}} \times 100$$

where C_t is the plasma concentration at time t, K_{el} is the elimination rate constant, AUC_{0-t} is the area under the curve from time 0 to time t, and $AUC_{0-\infty}$ is the area under the curve from 0 to infinity. The IVIVC was evaluated by level A, obtained by comparison of the fractions of drug absorbed and dissolved in vitro during the same time interval (*20*). Eight different times were used for each dissolution condition to establish the IVIVC. In vitro and in vivo results were taken as independent (x) and dependent (y) variables, respectively, and the data was evaluated by linear regression analysis.

Statistical Analysis

The correlation coefficient (R^2) and the slope were calculated and interpreted using the mean of linear regression analysis (Sigma Plot). The difference between the averages of the two data sets of released BPD was assessed by variance analysis using one-way ANOVA (GraphPad Prism software, Informer Technologies, Inc., La Jolla, CA, USA) and considered significant if $p = 0.05$.

Model-Independent Method

The calculation of difference (f_1) and similarity (f_2) factors is the independent method used by many regulatory agencies to compare dissolution profiles (*28*, *32*). The comparative release profiles were evaluated by the calculation of f_1 and f_2 from the following equations:

$$f_1 = \frac{\sum_{j=1}^{n} R_j - T_j}{\sum_{j=1}^{n} R_j} \times 100$$

$$f_2 = 50 \times \log\left[100 \Big/ \sqrt{1 + \sum (R_t - T_t)^2 / n}\right]$$

where n is the number of sampling times used to calculate f_2, R_t is the percentage of drug dissolved from the reference formulation at time t, and T_t is the percentage of dissolved drug from the test formulation at time t.

Test values of 0–15 for f_1 and 50–100 for f_2 suggest similarity between the drug release profiles of the test formulation and the reference drug product and further suggest an in vivo similarity.

Model-Dependent Method

The kinetics of BPD release was evaluated according to four mathematical models: zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas (*23*, *36*). The most suitable kinetics model (*36*) was selected based on the correlation coefficient values obtained by linear regression.

RESULTS AND DISCUSSION

In Vivo Study

The time profile of the mean plasma BPD concentrations obtained from the pharmacokinetic study was deconvoluted to its fraction absorbed–time profile obtained by the Wagner–Nelson model, as presented in Figure 1A. The fed state indicated that there was a delay at the beginning of absorption; however, absorption of BPD was complete at around 4.5 h in the fasted and fed states. The differences observed in the absorption rate after oral administration of BPD in the fasted and fed states can be justified by known interactions between the formulations and food as well as specific properties of fluids in the GIT, including ionic strength and pH ranges that occur in the GIT during these states. These factors can greatly affect the rate of drug release from a matrix (*37*). Drugs that are poorly water soluble or experience pH-dependent effects on solubility, as in the case of BPD, are greatly affected by changes in the GIT that occur during the fed state. These factors may significantly alter their bioavailability. After meals, the rate of gastric emptying for liquids and solids is much slower than that in fasting conditions, which results in a reduction of the fraction of BPD absorbed and causes a time-dependent increase in the plasma concentration lag time.

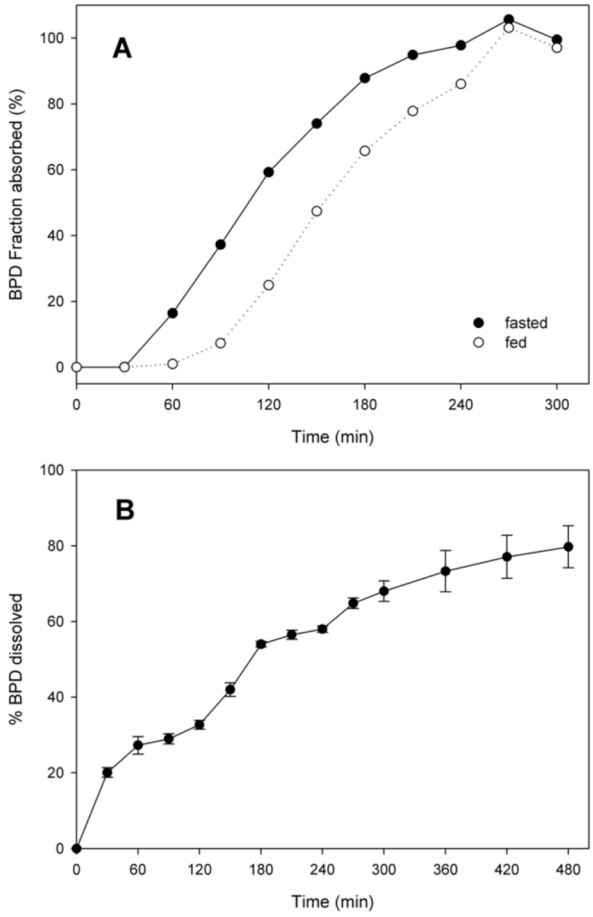

Figure 1. (A) In vivo fraction of BPD absorbed in the fasted and fed states and (B) the BPD biobatch dissolution profile using the method recommended by the manufacturer with the rotating bottles apparatus.

Table 3. Solubility of Bromopride in Various Dissolution Media

Medium	pH	Bromopride (µg/mL)
0.1 M HCl	1.2	29.9
0.01 M HCl	2.0	4.36
	4.5	14.1
PBS	5.8	14.4
	6.9	8.11
	7.2	3.41

PBS: phosphate buffer solution (potassium phosphate monobasic 0.25 M)

BPD Solubility

The solubility of BPD in the different dissolution media within the physiological pH range was presented in Table 3. The solubility decreased with increasing pH since BPD has a pK_a of 9.35, whereas the molecule is ionized in acidic pH (*9*). These results show the importance of acid strength since the 0.1 M HCl medium could solubilize seven times more BPD than could 0.01 M HCl. Also, sink conditions were achieved in all the conditions evaluated.

Development of the Dissolution Test

During the development of a dissolution method, it is necessary to evaluate several of the dissolution test parameters to guarantee that the method is discriminatory. Subsequently, the method should be able to identify changes that occur during the production process and drug formulation. Therefore, the appropriate apparatus, rotation speed, volume and composition of the dissolution medium, pH, ionic strength, and surfactant must be selected (*37*, *39*). The dissolution apparatus commonly used for ER are Apparatus 1 (basket), 2 (paddle), and 3 (reciprocating cylinder). Apparatus 1 was chosen since the baskets were capable of retaining the extended-release BPD pellets even though the capsules had dissolved in the dissolution medium. Apparatus 3 (reciprocating cylinder) is indicated for ER since it is able to simulate GIT pH; therefore

it was tested during this study (*28*). Overall, 10 different dissolution methods were evaluated to determine the most suitable dissolution medium. This requires the investigation of dissolution profiles in the physiological pH range, as presented in Table 2. The definition of the dissolution period for each medium with differing pH accounted for the physiological intestinal transit time and the specific pH in each portion of the GIT (*40*).

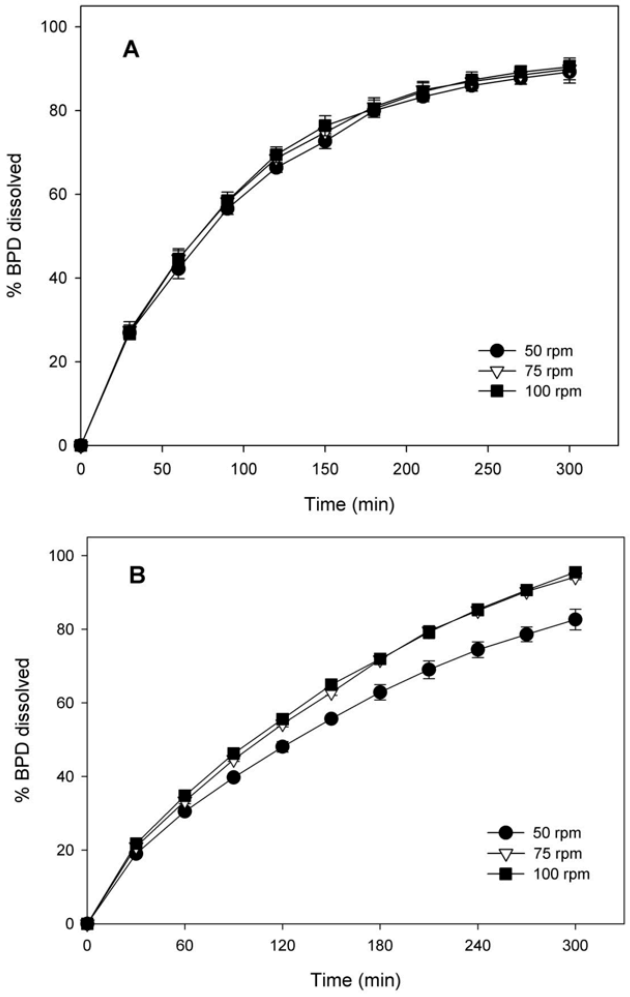

Figure 2. Dissolution profile of the BPD biobatch in (A) 0.1 M HCl and (B) PBS pH 7.2 using Apparatus 1 at rotation speeds of 50, 75, and 100 rpm.

Dissolution Profile Using the Rotating Bottle Apparatus

The dissolution profile obtained using the rotating bottle apparatus is presented in Figure 1B, which indicates that the BPD pellets reached a plateau of around 78.9% of constant drug release after a 4 h dissolution period. A certain amount of the drug remained in the tube after 8 h of dissolution. This method showed a moderate IVIVC, yielding correlation coefficients of 0.925 and 0.943 in the fasted and fed states, respectively. However, the rotating bottle apparatus is an unofficial apparatus, which made it necessary to develop a method using a USP apparatus.

Dissolution Profile Using a Basket (USP Apparatus 1)

Rotation speed is an important parameter in the development of a dissolution test since it directly influences drug release. Conditions such as mild agitation are commonly used to provide discriminating power of the dissolution method (*28*, *39*). In the case of Apparatus 1, rotation speeds between 50 and 100 rpm are usually employed (*28*, *32*), thus the rotation speeds of 50, 75, and 100 rpm were evaluated in this work. The influence of agitation on the release of BPD from the Digesan Retard 20-mg biobatch was investigated in two dissolution media that simulated the gastric (0.1 M HCl, pH 1.2) and enteric conditions (PBS, pH 7.2), as presented in Figure 2. Under gastric conditions, variation of the rotation speed from 50 to 100 rpm did not affect the dissolution profile or the BPD release rate (Figure 2A). However, under enteric conditions, there was a reduced release rate relative to simulated gastric fluid conditions, mainly at a rotation speed of 50 rpm (Figure 2B), due to the lower solubility of BPD in PBS pH 7.2, as presented in Table 3. No significant differences were observed between the BPD release profiles at 75 and 100 rpm in the simulated enteric fluid ($p > 0.05$), thus an intermediate rotation speed of 75 rpm was adopted in order to use the mildest discriminative dissolution conditions.

Figure 3A presents the BPD dissolution profile using a rotation speed of 75 rpm and dissolution Methods 1–7 described in Table 2. All dissolution methods reached a plateau of constant drug release within 4 h of analysis, except when using Method 1. The dissolution of the drug in water using Method 1 was unsatisfactory and presented an incomplete (<20%) release of the drug, probably due the pellet coating insolubility in this medium. Furthermore, BPD is a drug administered just before or after meals, when the pH of the stomach is closer to 2, which is similar to the conditions utilized in Methods 2–7. Therefore, Method 2 (pH 2) provided a milder dissolution profile compared with the same rotation speed presented in Figure 2A (pH 1.2), which indicates that acid strength greatly influenced the BPD release. Dissolution was accelerated when BPD was introduced to a low pH (Figure 2A); this can be explained by the high BPD solubility at pH 1.2 (Table 3). Methods 3 and 4 differed only in the volume of PBS used and presented similar dissolution profile results. Although the dissolution Methods 5–7 are quite different, the dissolution profiles were similar among the methods, and dissolution was accelerated after the first hour of analysis. This occurs when simulated gastric fluid (pH 2) is replaced by simulated enteric fluid, probably due the high BPD solubility.

Dissolution Profile Using the Reciprocating Cylinder (USP Apparatus 3)

The dissolution test performed using Apparatus 3 at five pH values for a 300-min period is shown in Figure 3B. Also, Method 8 showed slower release of BPD during the first hour compared with the other methods that utilized the same apparatus. This can be attributed to the use of

higher pH during the gastric stage (0.01 M HCl), which reduced drug solubility. Despite the different agitation rates used in Methods 9 and 10, BPD release was similar.

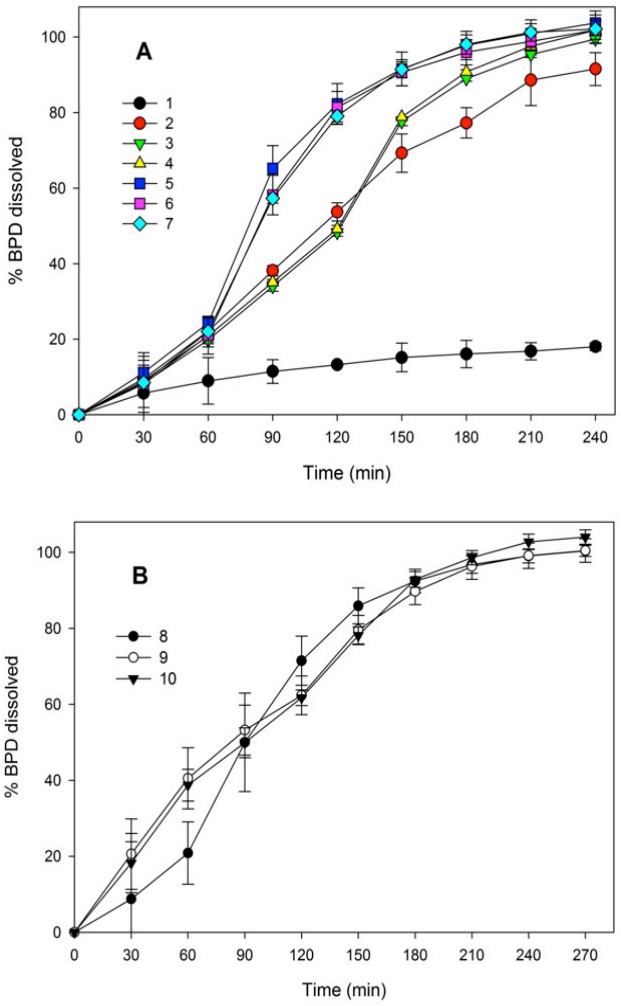

Figure 3. Dissolution profiles of the BPD biobatch using different dissolution media in (A) Apparatus 1 and (B) Apparatus 3.

Pellets SEM

The surface of the coated pellets of the BPD biobatch was characterized by SEM before and after the dissolution test. The images reveal that the Eudragit and shellac coating was uniform (Figure 4A,B) and that the spherical shape of the pellets was maintained after exposure to the gastric and enteric media (Figure 4C,D), although considerable cracking could be observed on the surface of the pellets after dissolution, which indicates that the coating did not form a continuous barrier. No swelling was observed after the pellets contacted the dissolution medium, characterizing the inflow of liquid that occurs due to pressure differences. Considering the dissolution and the SEM data, the dissolution process of the BPD pellets was controlled by the formulation, especially the coating, and also by the BPD solubility.

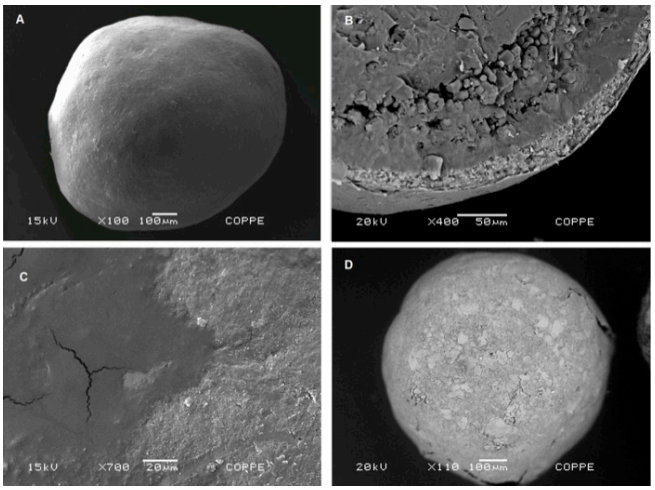

Figure 4. (A) Scanning electron micrographs of the biobatch pellet surface using 100× magnification. (B) Cross section using 400× magnification, which increases visualization of the surface details of the pellet layers. (C) Image of the pellet after exposure to gastric media for 1 h in the gastric media at 700× magnification. (D) Image at 110× magnification after 4 h of dissolution in the rotating bottles apparatus.

Validation of the Quantitative Method

No peaks were observed in the placebo or the dissolution medium at the wavelengths maximally absorbed by BPD (300, 273, and 214 nm), which demonstrates the specificity of the method. Good linearity was observed between 2.5 and 25 µg/mL BPD diluted in 0.1 M HCl. The linear equation obtained by the least-squares method was $y = 33.84x + 0.009$ ($n = 3$) and showed an adequate correlation coefficient, >0.9996. The precision of the method was determined by the RSD (1.78%) among the six samples tested at the BPD working concentration. The accuracy of the method was satisfactory, and recoveries were obtained between 98.8% and 101.7% at the three concentration levels evaluated. The LOQ was 0.8999 ng/mL. The BPD solution remained stable after storage for 48 h at room temperature, and the RSD was 0.77% among the evaluated samples.

Each dissolution test was carried out until at least 80% of the drug was released into the dissolution medium (*28*).

Investigation of IVIVC

The correlation coefficient (R^2) and the slope was obtained by plotting the in vivo absorption (F_a) against the in vitro dissolution data assuming that $t_{vitro} = t_{vivo}$ without any time-scaling. These data are presented in Table 4. Establishment of the IVIVC was performed by plotting the data obtained during the 30–240 min intervals in Methods 1–7 and 30–270 min for Methods 8–10. These interval times were chosen to reflect the time at which the BPD plasma concentration begins to decrease during clearance (Figure 1B). The IVIVC was considered achieved when the in vitro methods showed a correlation coefficient >0.95 and a slope close to 1.0 for both the fed and fasted states. This is important since drug administration should occur just before and after meals. As shown in Table 4, all of the methods tested (1–10) had correlation coefficients

>0.96 in the fasted state. However, in the fed state, only Method 3 presented values >0.97. These data indicate that this condition may have a high degree of IVIVC and demonstrate a high degree of proportionality between the in vivo and in vitro data as well as a more linear relationship between the point-to-point evaluations. In Figure 5, the point-to-point relationship is shown in the fasted and fed states obtained using Method 3. Also, Method 3 is the safest for use in quality control since it showed an IVIVC for the fasted and fed states ($R^2 > 0.97$), which is considered an IVIV point-to-point correlation (*20*, *39*).

Table 4. Correlation Coefficients (R^2) and Slopes Obtained for the Absorbed Fraction of BPD in the Fasted and Fed States, and the Percentage of BPD Released as a Function of Time in the Dissolution Medium of Each Method

Dissolution Method	Fasted		Fed	
	slope	R^2	slope	R^2
Diffu test	2.27	0.9247	2.13	0.9429
1	0.868	0.9831	0.772	0.8610
2	1.20	0.9944	1.11	0.9372
3	1.02	0.9767	0.959	0.9705
4	1.00	0.9759	0.946	0.9623
5	1.01	0.9503	0.856	0.7629
6	0.988	0.9580	0.845	0.7771
7	0.986	0.9691	0.851	0.7997
8	1.08	0.9793	1.02	0.8232
9	1.32	0.9880	1.30	0.9005
10	1.22	0.9875	1.21	0.9177

Values were calculated by plotting in vivo and in vitro data from 30 to 240 min for Methods 1–7 and from 30 to 270 min for Methods 8–10 and Diffu test.

Table 5. Comparison of the Dissolution Profiles through Model-Independent Methods: Difference (f_1) and Similarity (f_2) Factors and Analysis of Variance

Product	f_1	f_2	ANOVA
Biobatch × Batch A	3.71	77.94	$p > 0.05$
Biobatch × Batch B	6.19	67.07	$p > 0.05$
Biobatch × Test Batch	19.77	38.41	$p < 0.001$
Batch A × Batch B	7.50	62.08	$p > 0.05$
Batch A × Test Batch	19.37	38.69	$p < 0.001$
Batch B × Test Batch	23.66	34.37	$p < 0.001$

f_1: difference factor (0–15)
f_2: similarity factor (50–100)
One-way ANOVA–Tukey´s test ($\alpha = 0.05$)

Application of the Dissolution Method

To evaluate the discriminative power of the developed dissolution test, a BPD pellet batch, named as test batch with a partial coating that compromises the sustained release, was produced. Figure 6 shows the photomicrograph of the test batch before and after dissolution. Compared with the commercially obtained batch, these pellets exhibited decreased coating and greater surface roughness, which caused an insufficient control of the drug release (Figure 4). The test pellet coating was easily eroded during dissolution, and the drug was released following the Korsmeyer–Peppas kinetic model (Figure 7), which presents different behavior compared with the commercial pellets, which followed the Higuchi model (Figure 4). Two other commercial batches of Digesan Retard 20 mg, named batches A and B, were tested as shown in Figure 7. The dissolution profiles of the commercial batches, when compared with the biobatch, showed no statistically significant differences as calculated by ANOVA and by f_1 and f_2 (Table 5). In addition, the test batch was statistically different from the biobatch and the two commercial batches. The BPD release kinetics for batches A and B followed the Higuchi-type model.

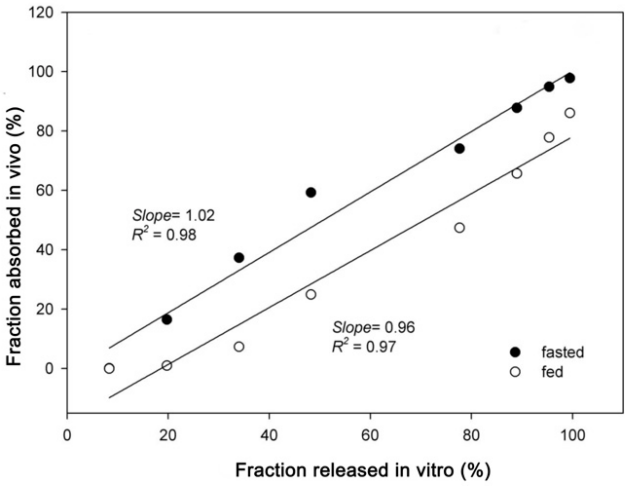

Figure 5. In vitro–in vivo correlation established for the in vitro dissolution data of biobatch using dissolution Method 3 and the in vivo absorption data in the fasting and in the fed state.

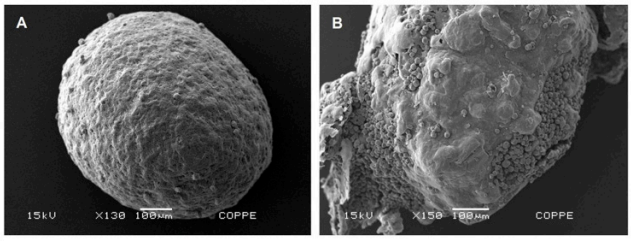

Figure 6. (A) Image by scanning electron microscopy of the pellet surface of the test batch using 130× magnification. (B) Image of the pellet after exposure during 1 h in the gastric media at 150× magnification.

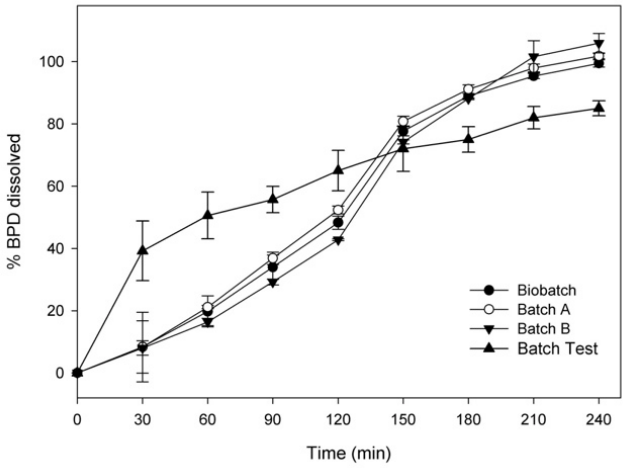

Figure 7. Dissolution profiles of the biobatch, two commercial batches (Batches A and B), and the test batch using Method 3.

CONCLUSIONS

A dissolution test with point-to-point IVIVC and discriminative power was established to evaluate extended-release BPD pellets. The dissolution test that was developed employs USP Apparatus 1 at 75 rpm, with 900 mL of HCl pH 2 for 1 h after which the medium is changed to 900 mL of PBS pH 7.2, at 37 ± 0.5 °C. Quantitative analysis was successfully validated using UV absorption spectrophotometry at 273 nm. The drug release from the pellet is controlled by the coating and by BPD solubility. The dissolution test has adequate discriminative power, and the Higuchi-type release kinetics model best describes BPD dissolution of the commercial batches and the Digesan Retard biobatch.

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