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Preparation and Characterization of Poloxamer 407 Solid Dispersions as an Alternative Strategy to Improve Benznidazole Bioperformance

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ABSTRACT

Benznidazole (BZL), the first line drug for Chagas disease treatment, presents a low solubility, limiting the possibilities for its formulation. In this work, solid dispersions' (SDs) technology was exploited to increase BZL kinetic solubility and dissolution rate, seeking for an improvement in its bioperformance. A physical mixture (PM) and an SD using Poloxamer 407 as carrier were prepared and characterized. Dissolution tests were performed, and data were analyzed with the lumped model, which allowed to calculate different parameters of pharmaceutical relevance. A bioactivity assay was also carried out to probe the SD anti-trypanocidal activity. Among the most relevant results, the initial dissolution rate of the BZL SD was near 3, 4 and about 400-fold faster than the PM, a commercial formulation (CF) and an extracted BZL, respectivley. The times needed for an 80% of drug dissolution were 3.6 (SD), 46.4 (PM), and 238.7 min (CF); while the dissolution efficiency values at 30 min were 85.2 (SD), 71.2 (PM), and 65.0% (CF). Survival curves suggested that using Poloxamer 407 as carrier did not alter the anti-trypanocidal activity of BZL. These results allow to conclude that SDs can be an effective platform for immediate release of BZL in an oral administration.

Introduction

Chagas disease represents one of the first causes of cardiac lesions in young, economically productive adults in endemic countries, bringing them out laboral inhability.[1] This is a potentially fatal infection caused by the parasite *Trypanosoma cruzi* and affects around 8 million people worldwide, mainly in Latin America.[2] Although initially Chagas disease occurred almost exclusively in rural areas, socioeconomic phenomena have modified its epidemiological profile, transforming it in a disease with incidence also in urban and periurban areas. Moreover, owing to migration, the disease can now be found in many other parts of the world with important foci in Canada, North America, Europe, and Australia.[3]

Chagas treatment is quite recent, although the disease is an old zoonosis, and when adequate, it improves clinical picture in most cases.[3] The treatment of the human disease with nifurtimox or benznidazole (BZL) dates from the 1970s, and since then is based on an empirical therapy. These drugs are especially valuable in the acute phase of the disease because it is not certain whether the anti-trypanocidal treatment during the indeterminate phase can prevent the development of chronic disease, although favorable results were obtained in a small study in children who received BZL.[4] Nevertheless, both drugs are toxic and their efficacy varies, probably as a consequence of differences among parasite strains. Furthermore, a great variability in the efficacy of nitrofurans and nitroimidazoles was reported across Latin America, apparently associated with biological differences between *T. cruzi* strains, inclusive some of them are naturally resistant to the existing drugs.[5,6] However, BZL is used as the first line of treatment for Chagas disease in most countries because its adverse effects are milder than those of nifurtimox, increasing the compliance of the patients to the treatment.[7]

Chemically, BZL (N-benzyl-2-nitro-1*H*-imidazole-1-acetamide), is a 2-nitroimidazole derivative with low water solubility. Therefore, the design of formulations to enhance its solubility is one of the key drivers for improving the bioavailability of this poorly soluble drug. One of the alternatives to reach this goal is the use of

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solid dispersions (SDs), first described in 1961 by Sekiguchi and Obi,[8] who found that a formulation based on eutectic mixtures could improve the rate of drug release, and consequently, the bioavailability of poorly water-soluble drugs. Later in 1971, Chiou and Riegelman[9] defined SDs as the dispersion of one or more drugs in an inert carrier or matrix in the solid state.

SDs produce a saturated or supersaturated solution, leading to an immediate dissolution of a portion of the drug, which is available for rapid absorption, upon contact with the gastrointestinal fluid. On the other side, the excess drug could precipitate in the gastrointestinal fluid in very finely divided particles. These characteristics often result in remarkably improved drug absorption from an SD as compared to a conventional tablet or capsule formulation. A complete review article discussing the use of SD to improve the dissolution rate and oral bioavailability of poorly water-soluble drugs has been reported by Leuner and Dressman.[10]

Literature reports previous attempts to enhance BZL solubility employing SDs. One study investigated the effect of 2 hydrophilic polymers, polyethylene glycol 6000, and polyvinylpyrrolidone K-30 for BZL SDs, observing an increase in the solubility, attributed to an interaction between the drug and the polymer.[11] Leonardi and Salomon[12] studied later the dissolution behavior of BZL SDs and physical mixtures (PMs) prepared with polyethylene glycol 6000, and unexpectedly concluded that the PMs presented an enhanced dissolution behavior. Fonseca-Berzal et al.[13] evaluated the use of sodium deoxycholate or low substituted hydroxypropylcellulose as carrier to obtain SDs of BZL by a freeze-drying process.

Poloxamers, also known by their trade name Pluronic®, are triblock copolymers based on poly(ethylene oxide)—*b*-poly(propylene oxide)—*b*-poly(ethylene oxide) (PEO—PPO—PEO) and have been researched extensively for their ability to solubilize different compounds.[14] For this reason, they have also been employed to prepare SDs carrying pharmaceutical agents.[15,16] On this regard, Scalise et al.[17] prepared nanoparticles of BZL with poloxamer 188 by a solvent-diffusion method, and tested them in a deadly *T. cruzi* acute infection model.

The aim of this work was to prepare and characterize BZL SDs using poloxamer as carrier with the purpose of improving the solubility of the drug. Phase and saturation solubility studies and dissolution tests were performed. Drug dissolution data were analyzed by a mathematical model developed and validated by our research group.[18-20] Parameters of pharmaceutical relevance such as initial intrinsic dissolution rate, dissolution efficiency (*DE*), sampling time and mean dissolution time (MDT), were calculated, because they can be useful to predict the behavior of the SDs. Furthermore, a bioactivity assay was performed to evaluate the effect of the BZL SD on *T. cruzi* viability.

Materials and Methods

Materials

Commercial tablets of BZL (Abarax® Elea) were kindly provided by the Ministerio de Salud Pública de la Provincia de Salta (Argentina). They were pulverized, sieved, and the 210-μm size fraction used in following assays was named as commercial formulation (CF). Powder BZL (P-BZL) with a purity of 97% was purchased from Sigma-Aldrich (St. Louis, MO). The amphiphilic polymer Poloxamer 407 (P407) was bought from BASF (Buenos Aires, Argentina). All other reagents were of analytical grade.

BZL Extraction Procedure

BZL was extracted from the CF adapting the protocol described by Garcia et al.[21] Briefly, the tablets were mashed and dissolved in ethanol by stirring at 45°C. The obtained suspension was filtered, the solids discarded, and the liquid phase recrystallized by addition of ice-cold distilled water on an ice bath. The solid fraction was recovered by vacuum-filtration, and dried at 45°C until constant weight. This solid was sieved, and 210-μm BZL particles were stored protected from light at room temperature until use. The BZL obtained by this procedure was identified as extracted BZL (E-BZL).

Solid Dispersions

SDs were prepared by the fusion method. Briefly, molten P407 was homogeneously loaded with BZL at 63°C by stirring, rapidly cooled in liquid nitrogen and then pulverized. The drug loading in the carrier was 32 %w/w and was selected according to previous studies (results not shown), which concluded that this proportion was the highest possible to reach by this technique. The 210 μm particle size fraction was obtained by sieving. A PM was prepared mixing the components in the same proportion, using the sifted 210 μm particle size fractions. All preparations were stored in screw-cap vials until use.

Physicochemical Characterization of the Materials

X-Ray Diffraction

To evaluate their crystalline characteristics, E-BZL, P407, SD, and PM were analyzed by X-ray diffraction (XRD) using a DRX Philips PW1800 diffractomer. Assays were performed at 40 KV and 30 mA in a range of 5°-70° 2θ at a rate of 0.02° 2θ/s.

Scanning Electron Microscopy

This technique was used to determine the morphology of P-BZL, E-BZL, P407, SD, PM, and CF. Samples were gold covered using a Denton Vacuum metallizer, LLC, Desk-IV. Observations were carried out with a scanning electron microscope JEOL JSM-6480LV-Japan, in the Laboratory of Scanning Electron Microscopy (LASEM, ANPCyT-UNSa-CONICET).

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis was used to characterize the thermal behavior of E-BZL, P407, SD, and PM. DSC thermograms were obtained using a DSC TA Q200 (TA Instruments). Samples were accurately weighed into hermetic aluminum pans, hermetically sealed with aluminum lids and heated from 25°C to 250°C at a heating rate of 10°C/min under constant purging of dry nitrogen at 20 mL/min. An empty hermetic aluminum pan, sealed in the same way as the sample, was used as reference.

Phase and Saturation Solubility Studies

To perform the phase-solubility studies, an excess of E-BZL was added to 5 mL of a 0.1 N HCl with increasing concentrations of P407 (5%, 10%, 15%, and 20% w/v) in sealed glass vials. They were placed in a bath under stirring at a predetermined temperature for 96 h to reach the solubility equilibrium. After that, suspensions were filtered, and the filtrate was suitably diluted for spectrophotometrical analysis at $\lambda = 324$ nm. BZL concentration was calculated using the corresponding calibration curve made with standard solutions of different concentration of BZL in 0.1 N HCl by triplicate. Phase solubility was determined at 25°C and 37°C.

For saturation solubility studies, an amount of E-BZL, SD, and PM were placed into sealed glass vials with 5 mL of 0.1 N HCl (pH = 1.2), to reach the same BZL quantity in all the samples. They were maintained in a water bath under stirring at 25°C and 37°C for 4 days. After that, the samples were filtered and analyzed spectrophotometrically at 324 nm to determine BZL concentration.

Both assays were performed by triplicate.

Dissolution Tests

Dissolution tests of SD, PM, E-BZL, and the CF were performed using an USPXXIV dissolution apparatus 2 (SOTAX AT 7 smart). Temperature was kept constant at 37 ± 0.5°C, and the rotational paddle speed was set at 50 rpm. The amount of BZL was 100 mg in all experiments. Dissolution medium was 900 mL 0.1 N HCl solution. At predetermined intervals, 4-mm of filtered aliquots were taken, adding the same amount of fresh medium to keep volume constant along the test. Samples were properly diluted, and concentration of dissolved drug was measured at 324 nm by UV-vis spectrophotometry. All measurements were performed by triplicate.

Dissolution data were analyzed by a lumped second-order kinetic model developed by our research group[18,20] and validated.[19] To compare the different dissolution profiles, initial intrinsic dissolution rate, *DE*, dissolution time, sampling time, and MDT were calculated, according to the independent statistical analysis methods.[22]

Bioactivity

To evaluate the effect of the SDs on the parasite viability, epimastigotes of *T. cruzi* stock 1, Tulahuén strain, lineage VI were used. Parasites were grown in standard conditions at 28°C in axenic medium liver infusion tryptose supplemented with 10% fetal bovine serum, and 2% penicilin-streptomicyn. Epimastigote viability was determined by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT is a yellow tetrazolium salt, which is reduced by active cells yielding purple formazan crystals. The final purple color can be detected spectrophotometrically at 570 nm.[23,24] We followed a protocol modified for *T. cruzi* epimastigotes, adding phenazine methosulphate (PMS, 0.22 mg per mL of MTT) to the MTT solution. PMS is an intermediate electron carrier that diminishes the incubation time to obtain the purple formazan crystals.[25] Briefly, 1:2 serial dilutions of E-BZL or SD were carried out in 96-well plates. E-BZL was dissolved in dimethylsulfoxide (DMSO) at 1 M; whereas, SD was dissolved in sterile H_2O at 15.4 mM. Both E-BZL and SD were diluted in liver infusion tryptose to 4 mM, starting the assay at 2 mM. Parasites were seeded at 4×10^7 cells/mL to a final volume of 100 μL per well. After 48 h, 20 μL of MTT with PMS were added to each well, followed by 30 min incubation at 28°C. The MTT reaction was stopped by adding 100 μL of solubilizer (10% sodium dodecyl sulphate, 45% dimethylformamide, pH 4.5 in acetic acid).[26] Plates were read in a Tecan plate reader at 570 nm. Assays were repeated 4 times, and each concentration per duplicate.

Data Analysis

Data are presented as the mean ± standard deviation of triplicate repeat experiments ($n = 3$) in solubility and dissolution studies. Biological assays are presented as the mean ± standard error of 4 experiments ($n = 4$).

Dissolution profiles were statistically analyzed using Polymath 6.0 software. A p-value less than 0.05 ($p < 0.05$) was considered significant.

In the bioactivity assay, survival curves were obtained with GraphPad Prism 7.0 using a nonlinear regression with log (inhibitor) versus response (3 parameters). IC50 values were compared using the nonparametric Mann-Whitney test (GraphPad Prism 7.0).

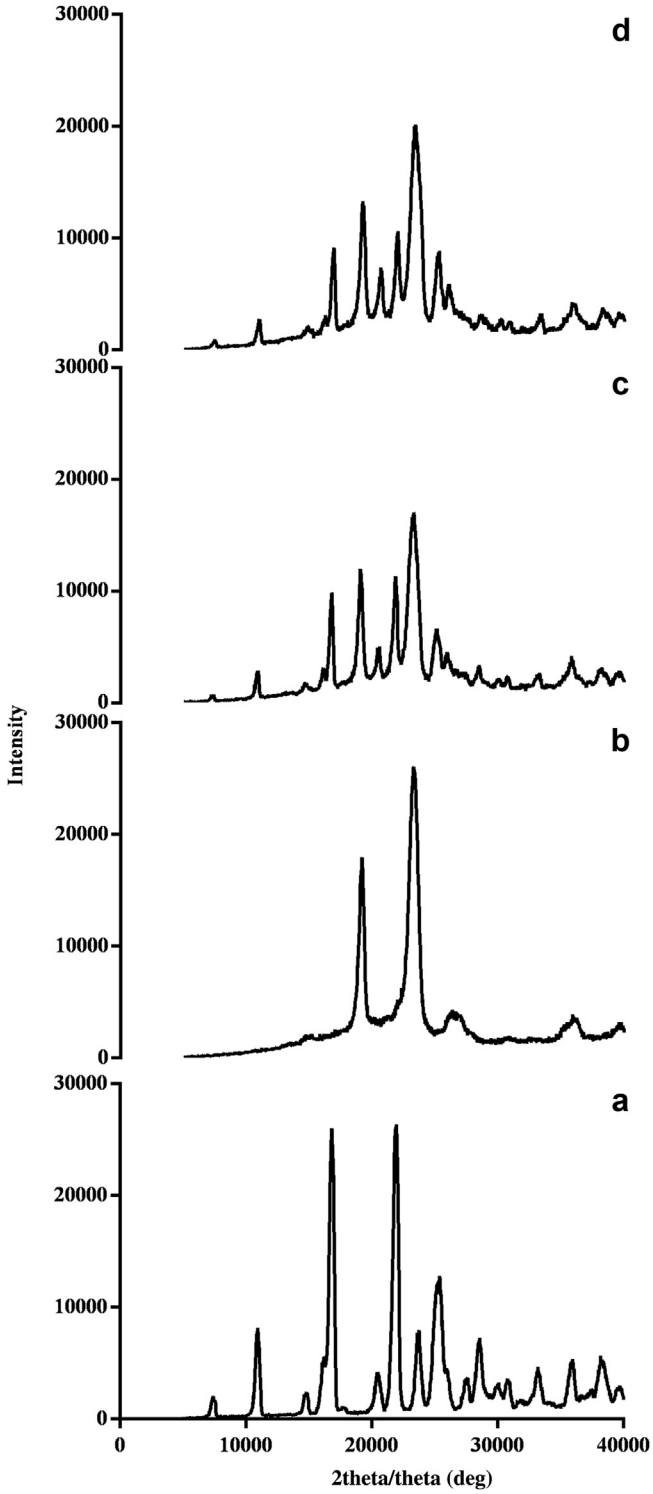

Figure 1. XRD diffractograms of E-BZL (a), P407 (b), PM (c), and SD (d).

Results and Discussion

Physicochemical Characterization

X-Ray Diffraction

Samples of E-BZL, P407, SD, and PM, were analyzed by XRD to determine the crystalline state of drugs in the materials.

As shown in Figure 1, the diffraction profile of E-BZL demonstrated its crystalline nature, showing diffraction peaks at 7.41°,

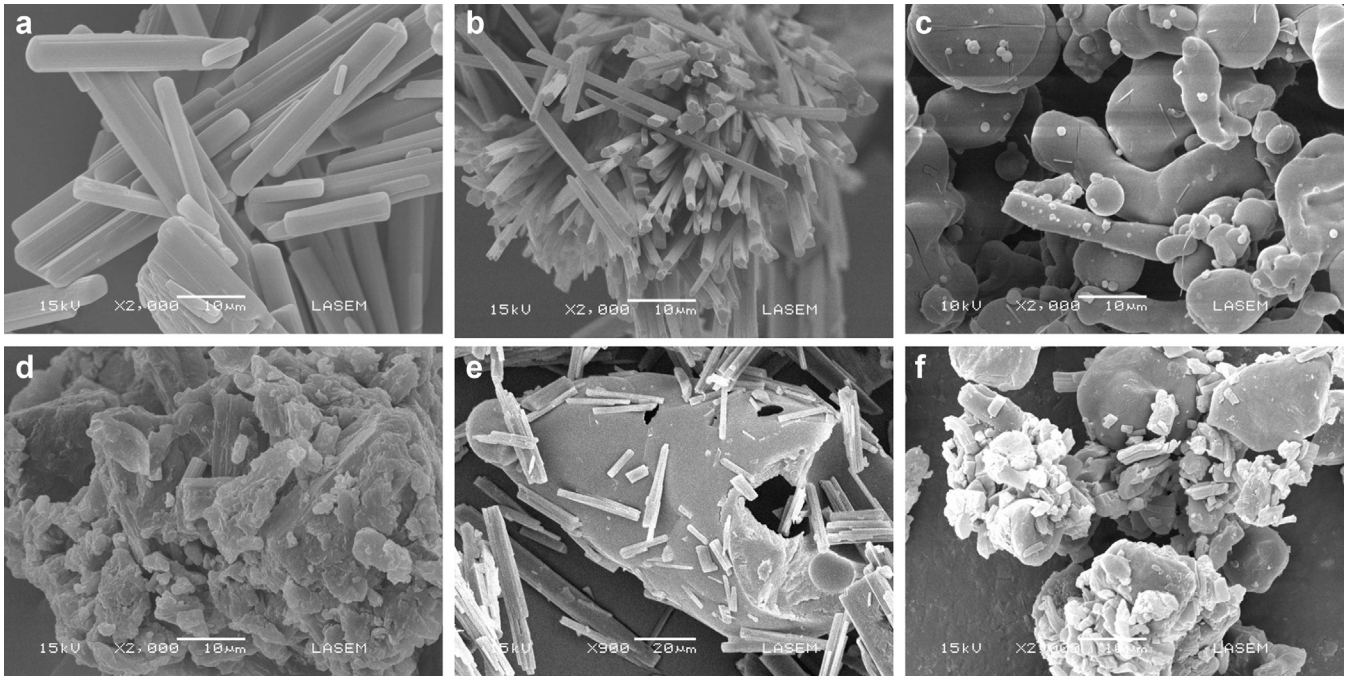

Figure 2. Scanning electron micrographs: P-BZL (a), E-BZL (b), P407 (c), SD (d), PM (e), and CF (f).

10.9°, 16,9°, 22°, 23.7°, and 25.4°, in concordance with the reported for BZL by other authors.[11,27] According to the literature, P407 produces 2 characteristic peaks at 19.2° and 24.0°,[28] which were observed in the present work at 19.2° and 23.2°. PM and SD spectra showed that some BZL peaks were attenuated, indicating a partial loss of BZL crystalline state in the SD, probably due to the solubilization of part of the drug into the polymer matrix, as reported by other authors.[11,27] This attenuation in the strong BZL peaks may contribute to solubility improvement since amorphous forms are more easily soluble than the crystalline forms.[11]

Scanning Electron Microscopy

To assess the physical state of BZL within the polymeric matrix, samples of P-BZL, E-BZL, P407, SD, PM, and CF were studied by scanning electron microscopy. Both P-BZL and E-BZL appeared as irregular needle-shaped crystals of different sizes (Figs. 2a and 2b), in agreement with the data from XRD. P407 was observed as spheres of smooth surface and irregular shape of different sizes (Fig. 2c). Figure 2d shows that the SD is composed by particles of different sizes with irregular surface, in which the components, P407 and BZL, could not be morphologically distinguished. This result could indicate the formation of a new structure in the SD due to drug solubility in the polymer. On the contrary, in the PM, BZL was distributed on the surface of P407, each compound maintaining its own structure (Fig. 2e). Figure 2f corresponds to the microscopic analysis of the CF and shows that BZL crystals were in a low proportion, since they represent around 20% w/w of the tablet, and adsorbed on the excipients of the formulation, probably due to the manufacturing process of the CF.

Differential Scanning Calorimetry

To determine the thermal behavior, samples of E-BZL, P407, SD, and PM thermograms were obtained by DSC (Fig. 3). E-BZL and P407 presented an endothermic peak at 191.8°C (126 J/g) and at 53.31°C (127 J/g), respectively, corresponding to their melting points.[11,29,30] It could also be detected that the endothermic peak of BZL was attenuated in the PM (60 J/g), whereas it almost disappeared in the SD, probably because the BZL got dissolved in the molten carrier throughout the test. The peak corresponding to the melting temperature of the carrier did not undergo a major change neither in the PM (45 J/g) nor in the SD (77 J/g), revealing an ordered state of P407 in both preparations. Nevertheless, the peak of P407 was slightly displaced in both SD and PM, which would indicate the formation of a eutectic mixture between the 2 components (drug and polymer) during the assay. The lack of a BZL-melting peak in the SD thermogram could evidence the formation of a single phase mixture of the drug because of a total solubility of the BZL during the test, preventing the display of the endothermic peak of fusion. In the case of the PM, the melting point of the drug was decreased and broadened due to a partial solubilization of the BZL. These results are in agreement with the observed in another study.[11]

Phase and Saturation Solubility Studies

The effect of P407 on E-BZL solubility was studied by dissolution assays in 0.1 N HCl (pH = 1.2) at 25°C and 37°C (Fig. 4). BZL solubility increased linearly along with polymer content. For both temperatures, a nearly 2-fold increase was observed when P407 content was 20 %w/w in comparison with 5%w/w. This behavior was expected since P 407 concentrations in this assay were above its critical micelle concentration, reported at 2.8×10^{-6} M.[14] As the concentration of P 407 increases reaching the critical micelle concentration, the polymer chains start to associate to form micelles with a hydrophobic core that can incorporate water insoluble molecules, like BZL, promoting its faster and greater solubility. The increase in BZL solubility could be also explained by the decrease in the interfacial tension between the drug and the dissolution medium provoked by the P 407.[31]

Extrapolating the lines to 0%w/w P407, values of BZL solubility were estimated to be 0.266 ± 0.013 mg/mL at 25°C and 0.486 ± 0.027 mg/mL at 37°C. These values are in close agreement with the ones experimentally obtained for BZL in 0.1 N HCl (0.248 mg/mL

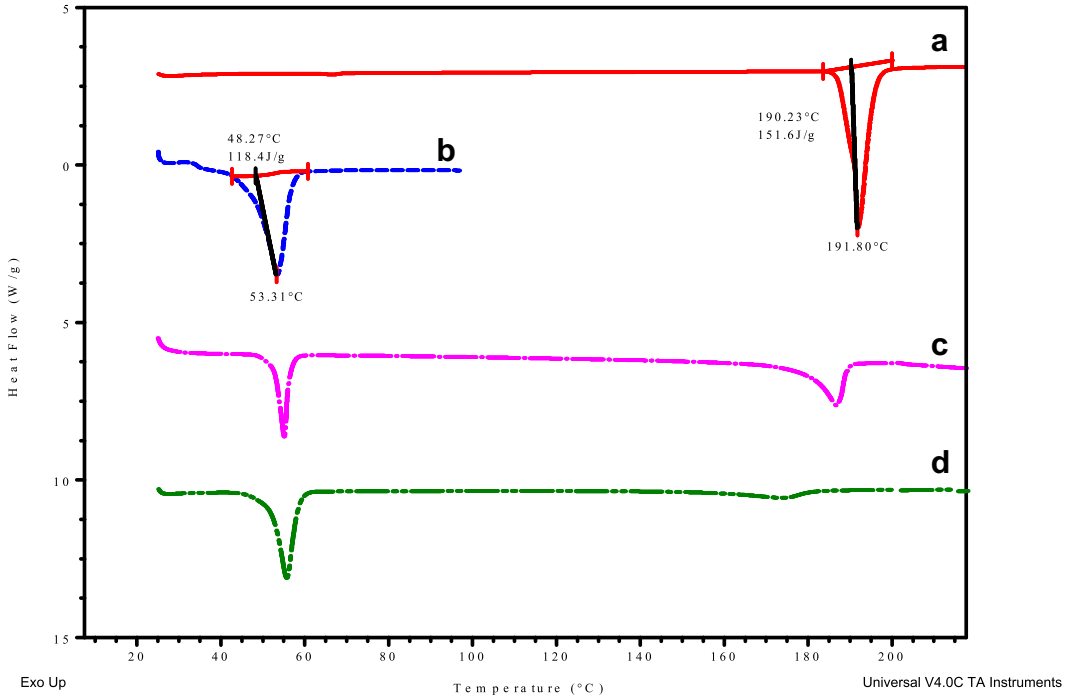

Figure 3. DSC profiles of E-BZL (a), P407 (b), PM (c), and SD (d).

and 0.410 mg/mL, at 25°C and 37°C, respectively). From these results, it was also concluded that BZL solubility is strongly influenced by temperature.

The saturation solubility of BZL also increased significantly with temperature, both for SD and PM, being 0.260 ± 0.001 and 0.274 ± 0.010 mg/mL at 25°C, and 0.420 ± 0.012 and 0.428 ± 0.007 mg/mL at 37°C, respectively.

Biopharmaceutical Characterization

Since bioavailability is often directly related to the extent and maintenance of the supersaturation state generated by a SD,[32] dissolution performance can be used to predict the *in vivo* behavior. Hence, dissolution testing at all stages of development, from screening studies to evaluation of the final dosage form, can provide information of how formulation and process parameters can impact the *in vivo* performance.

Figure 5 shows the results of *in vitro* dissolution studies of the CF, the E-BZL, and its binary mixtures (SD and PM) in 0.1 N HCl

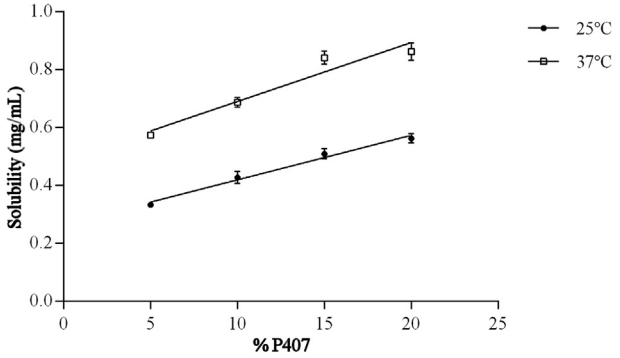

Figure 4. Influence of P407 content on BZL solubility in 0.1 N HCl.

solution. The experimental data were fitted using our drug dissolution profile model, deduced by a theoretical analysis considering a second order kinetic expression for the process:

$$M\% = \frac{a \times t}{1 + b \times t} \quad (1)$$

where M% is the percentage of drug released at time t, and parameters a and b are given in %*min^{-1} and min^{-1}, respectively. Table 1 shows the values of the model parameters, the correlation coefficient (R^2), and the standard deviation (s) for each case, which is defined as:

$$s = \sqrt{\frac{(SSD)}{(n-1)}} \quad (2)$$

where *SSD* is the sum of the squares of the differences between the experimental value and the one calculated by the model, and n is the number of samples taken in an experimental run.

The correlation coefficient values (from 0.9964 to 0.9991) and standard deviations (from 0.27 to 1.63) suggested a good fit of the experimental data and supported the accuracy of the model.

On the other hand, the model also allowed to calculate the initial dissolution rate because the rate of drug release at any time is given by:

$$\frac{dM\%}{dt} = \frac{a}{(1 + b \times t)^2} \quad (3)$$

And therefore, when t = 0, the initial dissolution rate is:

$$\left.\frac{dM\%}{dt}\right|_{t=0} = a \quad (4)$$

Analysis of the parameter a values indicated that the SD initial dissolution rate was near 3, 4, and about 400-fold faster than the

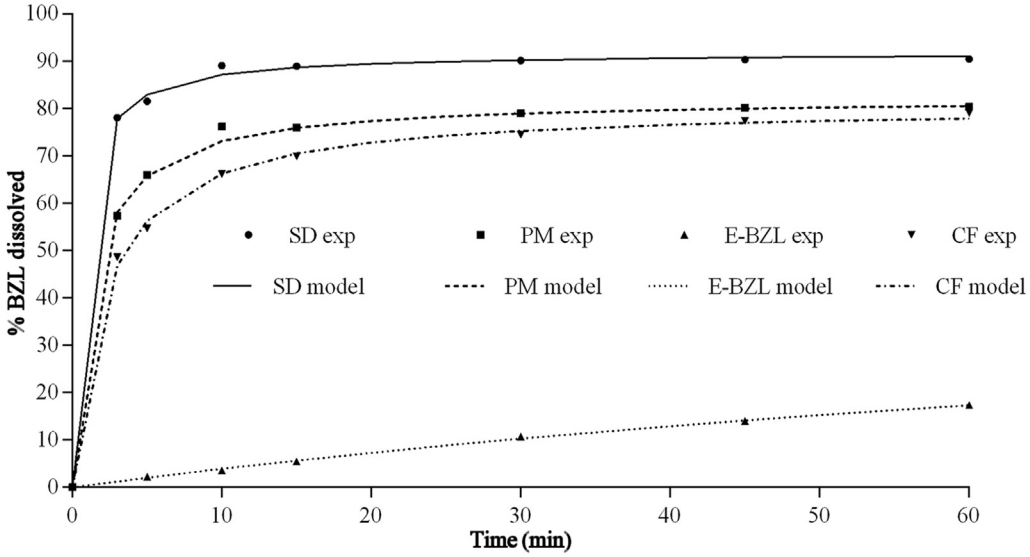

Figure 5. BZL dissolution profiles in 0.1 N HCl at 37°C.

PM, CF, and E-BZL, respectively. This is important since although the polymer would increase BZL solubility both in SD and PM, the enhancement in the SD initial dissolution rate would also play a fundamental role in the BZL bioavailability, taking into account that an oral administration is desired. Similarly, from parameter *a* values, and considering that the volume of the assay was 900 mL and that 100 mg is 100 %w/w of BZL, an absolute parameter, the "initial specific dissolution rate" (*ISDR*) (μg/mL min), could be calculated (Table 1). As it was expected, this value showed the same behavior as the initial dissolution rate among the different preparations tested. These results allowed to conclude that P407 used as a carrier for the preparation of the SD produced a significant increase in the initial release rate of BZL in comparison with the other preparations.

Other interesting parameters, such as sampling time (t_{tmin}), *DE*, and dissolution time ($t_{X\%}$), were used to evaluate the behavior of the different preparations. The t_{tmin} is the amount of drug dissolved in a given time, whereas $t_{X\%}$ is the time necessary to reach the dissolution of a given amount of drug. On the other hand, *DE* is defined as the area under the dissolution profile up to a certain final time (t_F), expressed as the percentage of the rectangular area described by 100% dissolution at the same t_F. The *DE* value can be calculated from the following equation:

$$DE = \frac{\int_0^{t_F} M\%dt}{100 \times t_F} \times 100 \quad (5)$$

Therefore, $DE = 100\%$ indicates that a drug was instantaneously dissolved.

Table 1
Parameters of the Kinetic Model

Sample	Lumped Model Parameters				*ISDR* (μg/mL min)
	a (%*min^{-1})	*b* (min^{-1})	R^2	*s* (%)	
SD	170.2272	1.85281	0.9991	0.95	195.6804
PM	66.0302	0.80385	0.9964	1.63	73.1025
E-BZL	0.4225	0.00764	0.9983	0.27	0.4694
CF	37.2440	0.46137	0.9983	1.08	48.6703

According to the model equation (Eq. 1), the *DE* and $t_{X\%}$ can be simply determined as:

$$DE = \left(\frac{a}{b^2}\right) \frac{[b \times t_F - \ln(1 + b \times t_F)]}{100 \times t_F} \times 100 \quad (6)$$

And

$$t_{X\%} = \frac{X\%}{(a - b \times X\%)} \quad (7)$$

It is worth noting that *DE* considers the effects of the amount of drug dissolved, as well as the dissolution rate on the dissolution process. Moreover, since *DE* is mathematically related to the integral of the drug's dissolution profile, the effect of the experimental error and the correlation deviations are minimized. For these reasons, *DE* seems to be the best parameter to compare different drug formulations.

Table 2 shows t_{tmin} at 5, 10, 15, and 30 min; *DE* at 30 min and $t_{80\%}$. At all sampling times, the SD showed a higher percentage of BZL dissolved in comparison with the other preparations. The time needed for an 80% dissolution of BZL for the SD was almost 13 times lower than the one for the PM and around 66 times lower than the needed for the CF. Pharmacopeias use this parameter as an acceptance limit of the dissolution test,[22] for example, a $t_{45min} \geq 80\%$, which is the same as a $t_{80\%} \geq 45$ min. It was observed that this acceptance limit is fully reached only by the SD. Although PM $t_{80\%}$ is almost acceptable, CF value is far from the desired one, and it was not mathematically reached for E-BZL.

Among the different methods available to compare drug release profiles,[22] the independent statistical analysis ones were chosen. These methods include ratio tests that are based on the relation between characteristic parameters estimated from the dissolution assay. One of the test commonly used is the MDT, which is defined as:

$$MDT_{X\%} = \frac{\sum_{j=1}^{n} t_{jm} \times \Delta M\%}{\sum_{j=1}^{n} \Delta M\%} \quad (8)$$

where $t_{jm} = (t_j + t_{j-1})/2$ is the midpoint time between 2 samples and $\Delta M\%$ is the additional amount of drug release between t_j and

Table 2
Parameters for the Independent Statistical Analysis Method

Sample	Sampling Time (%)				DE_{30} (%)	$t_{80\%}$ (min)	$MDT_{65\%}$ (min)	$MDT_{75\%}$ (min)
	t_5	t_{10}	t_{15}	t_{30}				
SD	81.6	89.1	90.2	90.3	85.2	3.6	0.40	0.58
PM	65.9	76.2	75.9	79.9	71.2	46.4	1.22	2.08
E-BZL	2.3	3.6	5.5	10.7	5.5	NR	NR	NR
CF	54.7	66.2	69.90	74.5	65.0	238.7	2.24	4.01

DE_{30}, dissolution efficiency at 30 min; $t_{80\%}$, time needed to reach an 80% of drug dissolved; NR, not reached.

t_{j-1}. However, as we pointed out previously, since our proposed model (Eq. 1) presents a good fit for the experimental data, the $MDT_{X\%}$ can be calculated as:

$$MDT_{X\%} = \frac{\int_0^{M\%_j} t \times dM\%_j}{\int_0^{M\%_j} dM\%_j} \tag{9}$$

Considering Equation 3:

$$MDT_{X\%} = \frac{\int_0^{t_{X\%}} \frac{a \times t}{(1+b \times t)^2} dt}{M\%(t_{X\%})} \tag{10}$$

Finally:

$$MDT_{X\%} = \frac{a}{b^2} \frac{\left[ln(1+b \times t_{X\%}) - \frac{b \times t_{X\%}}{(1+b \times t_{X\%})}\right]}{M\%(t_{X\%})} \tag{11}$$

where $M\%(t_{X\%})$ is the percent of drug accumulated at $t = t_X$ and t_X is obtained from Equation 7. To compare the dissolution profile of SD with those of PM and CF, $MDT_{65\%}$ and $MDT_{75\%}$ were calculated and are presented in Table 2. In both cases, SD presented the lowest MDT, followed by PM, and CF, while E-BZL stayed out of the analysis due to its low water solubility. A low MDT is desired when a new formulation is designed for immediate release by oral administration.

Following the independent analysis method and considering the relation between all determined parameters from dissolution profiles, SD stood out as a promising alternative for a fast dissolution of BZL in gastric fluid. Significant differences (95% confidence level) were observed in every parameter estimated for the different BZL formulations. These results showed that SD had the best performance according to sampling time, *DE*, dissolution time, and MDT.

The lumped model developed by our research group allowed the analysis of the dissolution data from *in vitro* experiments to obtain the information needed to determine the feasibility of BZL delivery using the technological strategy of SDs.

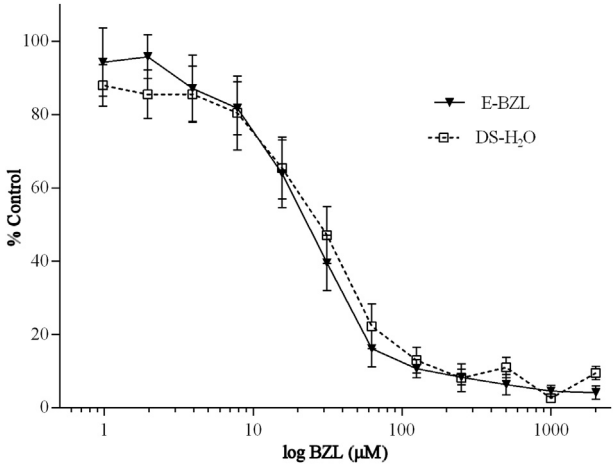

Figure 6. Survival curves of epimastigotes treated with E-BZL (solid line), and SD (dotted line) for 48 h ($n = 4$ determinations). Values were referred as % of control (untreated) parasites.

Effect on T. cruzi *Viability: Bioactivity*

Since SD showed the best performance in the *in vitro* determinations, it was further tested in a bioactivity assay to have an indication of its behavior on *T. cruzi* parasites. IC50 values were determined for SD and E-BZL, being 21.68 ± 1.6 and 32.47 ± 4.9 μM, respectively. Figure 6 shows the survival curve for each formulation using data obtained from all assays. Differences found in IC50 values and survival curves were not statistically significant. These results indicate that using P407 as carrier did not alter the antitrypanocidal activity of BZL. It is worth mentioning that *in vitro* tests usually employ DMSO to dissolve compounds with low solubility in water, such as BZL. When using BZL in SD, the use of the toxic DMSO was avoided because the formulation was easily resuspended directly in the culture medium.

Conclusions

Chagas disease is a “neglected disease” and currently represents one of the greatest therapeutic challenges in tropical medicine. In this context, improving the biopharmaceutical properties of BZL will lead to a better clinical management for patients.

BZL SD using P407 as carrier can be an effective platform for immediate release by oral administration because it allows a higher kinetic solubility in water and a faster dissolution rate. This could result in a better absorption and increased bioavailability, thus enhancing the biopharmaceutical behavior of BZL. Furthermore, the SD maintained the effectiveness of BZL in regard to its antitrypanocidal activity.

Although SDs are of particular interest due to the potential for oral bioavailability enhancement of poorly soluble drugs, these are challenging systems considering their inherent thermodynamic instability due to their high energy state as compared to their crystalline counterparts. For this reason, further studies are required to evaluate SDs chemical and physical stability, which are the key for successful development and commercialization. Undoubtedly, understanding both thermodynamic and kinetic processes is critical for a rational formulation design.

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