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Original Article

EVALUATION OF *IN VITRO* DISSOLUTION OF BENZNIDAZOLE AND BINARY MIXTURES: SOLID DISPERSIONS WITH HYDROXYPROPYLMETHYLCELLULOSE AND β-CYCLODEXTRIN INCLUSION COMPLEXES

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ABSTRACT

Objective: To increase the solubility/dissolution of benznidazole (BNZ) in water using two systems: solid dispersions (SD) with hydroxypropylmethylcellulose (HPMC) and β-cyclodextrin (β-CD) inclusion complexes (IC).

Methods: The samples were obtained by physical mixtures (PM), kneading (KN), evaporation (EV) and by spray-dryer (SY) atomization The analysis was based on results of *in vitro* dissolution and molecular modeling techniques.

Results: Molecular modeling showed that BNZ can form β-CD complexes in different ways such as in an aqueous solution or a vacuum. *In vitro* dissolution showed significant improvement in BNZ solubility in the PM, SD and IC, and also that the β-CD IC promoted better solubility than SD with HPMC.

Conclusion: Considering the data obtained, it is possible to consider the technique for the formation of β-CD IC as a more effective technique in promoting the improvement of BNZ solubility compared with getting SD with HPMC which, in turn, may increase the bioavailability of the drug and improve their pharmaceutical potential.

Keywords: Benznidazole, Solid dispersion, Inclusion complexes, Dissolution.

INTRODUCTION

Each drug has a different profile and a unique delivery and action in specific targets. This profile refers to its solubility in water. Poorly soluble drugs in aqueous solutions have low bioavailability when administered orally. This fact can be attributed to three factors: the possible degradation of the drug in the digestive tract, the low dissolution rate and low permeability of the drug through the walls of the digestive apparatus [1]. Promoting an increased solubility/dissolution rate of drugs with these characteristics is one of the most important stages in the development of its pharmaceutical form [2].

BNZ, N-benzil-2-nitro-1-imidazolacetamide, is the drug of choice for treatment of Chagas disease [3], which is itself as an endemic disease in many Latin American countries and affects 16-18 million people in the world [4]. BNZ therapy is effective in the onset of the acute and chronic disease, presenting, as main problems, low solubility and high toxicity of this drug. Low water solubility leads, consequently, to the limitations in bioavailability. Therefore, in order to improve poor drug solubility/dissolution in water, several techniques have been used, including solid dispersions (SD) with hydrophilic polymers and cyclodextrins (CD) inclusion complexes (IC) [5].

SD systems can increase the dissolution rate and bioavailability of water-insoluble drugs, once these are exposed to an aqueous medium as the carrier which dissolves and releases the drug as very fine colloidal particles. This greatly reduces particle size and increases the surface area resulting in improved dissolution and oral absorption rates. Furthermore, no energy is required to break up the crystal lattice of a drug during the dissolution process. Drug solubility and wettability may be increased by surrounding hydrophilic carriers [6]. Several highly soluble substances can be use to accelerate the release of poor hydrosoluble drugs [7].

CDs are cyclic oligosaccharides consisting of six, seven and eight glucose units called α, β and γ-cyclodextrins, respectively, and are linked by α-1,4 molecules [8]. These compounds are able to form inclusion complexes with a large variety of organic compounds by replacing the water molecules, which have high enthalpy by appropriate guest molecules. The CD functions as molecular carriers carrying the hydrophobic guest molecules in solution to the lipophilic cell membranes which, with a higher affinity, aid their uptake [9].

β-CD (fig. 1A) is the most used natural cyclodextrin. It offers a low cost and the duration of the patent has expired. Also, its cavity size is suitable to include aromatic and heterocyclic rings [10-12]. Likewise, hydrophilic polymers, such as HPMC (fig. 1B), have been used in solid dispersion systems. Thus, the solubilizing effect of CD may also be enhanced by the use of these polymers [13].

This study aims to assess the increment of BNZ solubility/dissolution in binary systems involving SD with HPMC and β-CD IC. Thus, a physical mixture (PM) and other different techniques of production can be used: kneading (KN), evaporation (EV) and spray-dryer atomization (SY) and detailed study of molecular modeling to evaluate the complexation of BNZ with β-CD.

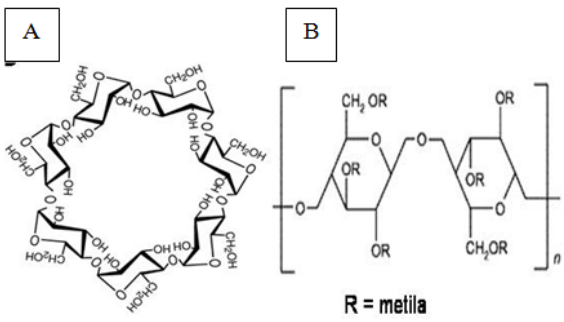


Fig. 1: Chemical structure of β-cyclodextrin (A) and Hydroxypropylmetylcellulose (B)

MATERIALS AND METHODS

Material

Benznidazole (BNZ), reference substance (99.9%), manufactured by Roche® was supplied by the Pharmaceutical Laboratory of the State of Pernambuco (LAFEPE®); Hydroxypropylmetylcellulose (HPMC) was supplied by Dow®; β-cyclodextrin (β-CD) was supplied by Apsen Phamraceutics®; etanol, was supplied by Vetec®, and pure water was obtained by a reverse osmosis system (Gehaka® 20) with an attached Gehaka®. deionizer

BNZ assay

The assay was performed according to a methodology developed and validated by Soares-Sobrinho *et al.* (2006) [14], using a UV-visible spectrophotometer at a wavelengh of 324 nm.

Preparation of samples

Preparation of physical mixtures

Initially, the BNZ PM and HPMC were precisely weighed at a ratio of 1:1 (w/w) and the BNZ PM and β-CD were mixed at a ratio of 1:1 (molar ratio). Each of these were pulverized in a porcelain mortar with the aid of a pestle until a homogeneous mixture of powders was obtained. The mixtures were then shifted into 250 µm meshes, transferred to suitable containers and kept in a desiccator. The PMs obtained were called a HPMC PM and a β-CD PM.

Preparation of solid dispersion and inclusion complexes

Evaporated

A SD of BNZ with HPMC (1:1, w/w) and a BNZ IC with β-CD (1:1, molar ratio) were prepared by the solvent evaporation method. Each compound was separately dissolved using ethanol and water (2:1) as solvents at a minimum ratio necessary to solubilize them. The solvents were removed by a MA 120 Marconi® rotary evaporator at a fixed temperature (60 ºC), reduced pressure (-800 mbar) and a rotation of 130 rpms. After spending 12 h in a desiccator, samples were pulverized in a mortar with a pestle and sieved with the aid of a 250 µm mesh. The SD and the resulting IC were transferred to appropriate containers, protected from light and kept in a desiccator for further characterization. The samples were named HPMC EV and β-CD EV.

Kneaded

Samples were precisely weighed in the same proportions and sprayed earlier in a porcelain mortar with the aid of a pestle, with simultaneous wetting of the powder with a ethanol/water solution at a ratio of 2:1 (V/V) until a homogeneous mixture was obtained. Samples were dried at 60 °C for 14 h for the removal of the ethanol/water solution. The resulting samples were sieved in a mesh of 250 µm and transferred to suitable containers for future characterizations. The samples obtained by kneading method were called HPMC KN and β-CD KN.

Spray dryer systems

The SD of BNZ with HPMC (1:1, w/w) and IC involving BNZ with β-CD (1:1, molar ratio) were prepared using a mini Büchi B-191 Spray Dryer. BNZ, β-CD and HPMC were separately dissolved in a solution containing ethanol and water (2:1) at a minimum ratio necessary to solubilize them. The samples were obtained under the following conditions: inlet temperature at 140 °C, air flow at 5 ml/min and outlet temperature at about 80-110 °C. The samples were named HPMC SY and β-CD SY.

Molecular modeling of BNZ-β-CD inclusion complex

The geometry optimization of the BNZ-βCD and BNZ-βCD inclusion complexes were performed in vacuum and in the presence of water molecules using an MM2 force field as an implement in Hyper Chem (release 6.03 for windows). The presence of water was simulated by placing the solute molecules into a 25x25x25 Å box with approximately 200 water molecules and a minimum distance of 2.3 Å between the solute and water molecules. The most stable complex conformation was considered through the lowest interaction energy [15, 16].

Dissolution *in vitro*

The dissolution of BNZ was performed with the isolated drug, the PM and samples obtained by EV, KN and SY. The dissolution test was performed using the paddle dissolution apparatus, at 37±5 ºC and 75 rpms. For this analysis, the samples were weighed exactly equivalent to 50 mg of BNZ and transferred to vats containing 900 ml of dissolution medium containing HCl 0.1 N with NaCl. A 4 ml sample was collected at predetermined intervals and immediately filtered (0.45 µm mesh).

The volume removed from inside the vessel was immediately restored by the addition of a medium at 37±5 °C. To do a reading, 2 ml of a dilution sample were collected from 10 ml of water. The BNZ concentration at each time interval was determined by UV spectrophotometry. Each sample was determined fourfold.

The results were assessed based on the dissolution efficiency and were subsequently compared by analysis of variance (ANOVA) One Way, considering $P<0.05$.

RESULTS AND DISCUSSION

Molecular modeling of inclusion complexes

For 1:1 stoichiometry in a vacuum, can appreciate important differences in the energies, and complexation by side large of the CD more favorable energetically however, the simulation in an aqueous solution had inverted values, and the complexes formed by the narrow portion of β-CD were the most favorable (table 1).

Unlike the data obtained by the solubility diagram, the simulation of the formation of IC between BNZ and β-CD exhibits a more favorable stoichiometric complexation form 1:2 (drug: CD) in a vacuum. The values obtained in aqueous solution are equivalent to those obtained with 1:1 stoichiometry, in exactly the same way, so it is more likely that there simultaneously exists, in equilibrium, two stoichiometries in solution (table 2).

Table 1: Enthalpy calculated for the formation of 1:1 stoichiometric complexes BNZ-βCD

Included group	ΔH* (Kcal·mol^{-1})	
	vacuum	Aqueous medium
imidazol-narrow	27.0	-2448.0
benzyl-narrow	26.2	-2613.3
benzyl-broad	10.9	-476.4
imidazol-broad	10.9	-434.4

* ΔH-enthalpy change

Table 2: Enthalpy calculated for the formation of 1:2 stoichiometric BNZ-βCD complexes.

Included group	ΔH* (Kcal·mol^{-1})	
	vacuum	Aqueous medium
broad-benzyl/imidazol-narrow	-22.1	-2898.6
narrow-benzyl/imidazol-broad	-32.1	-2415.2
broad-benzyl/imidazol-broad	-16.2	-2813.9
narrow-benzyl/imidazol-narrow	-41.6	-2834.1

* ΔH-enthalpy change

Experimental studies are needed to confirm these theoretical indications of complexity, among them spectra of nuclear magnetic resonance (NMR) are able to explain clearly the nature of inclusion complexes obtained in practice.

fig. 2 show an illustrative example of the formation of IC in an aqueous state and fig. 3 and 4 show the spatial representation of all possible valued complexes.

Dissolution *in vitro*

The dissolution tests were performed in a total time of 60 min and, the dissolution curves shown, represent the profiles of the BNZ release rate percentage dissolved obtained for binary mixtures and pure BNZ *versus* time.

In the dissolution curves of the PM, the SD and the IC, there was an increase in the BNZ dissolution rate when compared to the behavior of the drug alone. The dissolution rate of pure BNZ was slow: less than 10% of BNZ were dissolved in 1 hour, as fig. 5 shows.

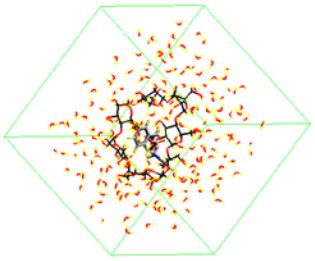
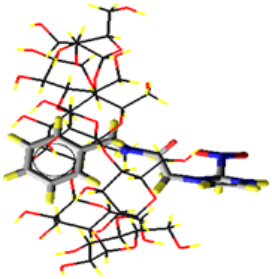
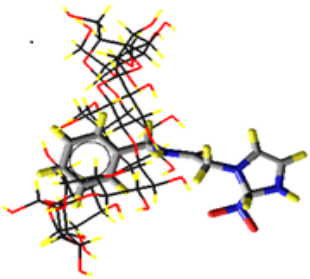


Fig. 2: BNZ-β-CD inclusion complex, 1:1 stoichiometrically optimized by MM2 with a benzyl group introduced by a cyclodextrin narrow cavity

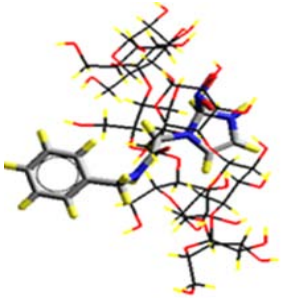


benzyl-narrow

imidazol-broad

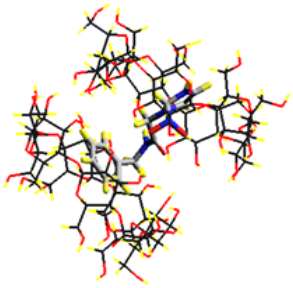


benzyl-broad

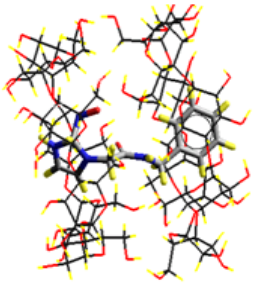


imidazol-narrow

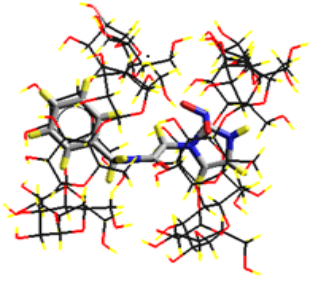
Fig. 3: Possible stoichiometric structures of BNZ-βCD 1:1 by MM2



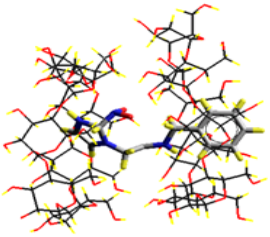
broad-benzyl/imidazol-broad



narrow-benzyl/imidazol-narrow



broad-benzyl/imidazol-broad



narrow-benzyl/imidazol-narrow

Fig. 4: Possible stoichiometric structures of BNZ-βCD 1:2 by MM2

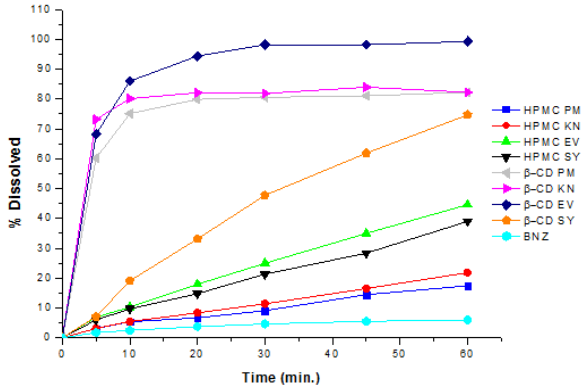


Fig. 5: BNZ dissolution profile and the various binary systems (PM, SD and IC)

According to this graph, comparing with pure BNZ, the speed and the dissolution rate of the drug in a PM with HPMC (HPMC PM) was higher, showing that, even without chemical interaction, just a mixture of poorly soluble drug with HPMC was able to improve the dissolution characteristics of BNZ.

From the dissolution curves comparing the PMs with the SDs, all dispersions provided a major effect on the dissolution rate than the corresponding PM. Compared with pure BNZ, there was more speed in the release of drug in the evaporated dispersions (HPMC EV) than in those that were atomized (HPMC SY) and kneaded (HPMC KN). Thus, the rate of BNZ dissolution presents in the PMs and all the SDs with HPMC was significantly higher than BNZ alone. The improvement in dissolution rate relative to BNZ can be explained by the wetting effect of HPMC to BNZ.

Regarding the β-CD IC, it becomes evident that all the complexes increased the BNZ dissolution rate and the complex obtained by evaporation (β-CD EV) had better results when compared to the kneaded (β-CD KN) and atomized (β-CD SD) complexes. The fact that the KN complexes achieved better results than the SY release is due to the processing of atomized samples, and these should be further optimized for observation of better results.

An opposite result was obtained by Patil *et al.* (2014) [17], which described the highest drug release rate for lornoxicam from the spray dried ciclodextrin compared to those prepared by kneading and ultra sonification methods. Other recent works also highlight the increase in solubility of the drugs using β-CD [18-20].

The increase in the dissolution rate seen for the IC with different drugs can be explained based on the higher solubility of amorphous state energy/reduction of crystallinity, the lower interfacial tension between the drug and water insoluble in CD-induced dissolution and higher solubility complex in water [21].

The rapid dissolution of the drug in the first 5 min and the detection of more than 80% of BNZ dissolved in about 10 min, shows there was a BNZ complexation with β-CD. This behavior must be attributed to the high energy of the amorphous state and the formation of IC [22]. The PM also shows the corresponding improvement in solubility.

Thus, according to the obtained data, rapid and excellent behavior in the formation of IC dissolution of the drug with β-CD is clearly demonstrated. Although SD with HPMC increased the rate of BNZ dissolution, it showed lower results than with the IC.

Based on the dissolution profile dissolution efficiency at times 30 min (DE 30) and 60 min (DE 60) of each of the binary mixtures (table 3) were calculated. According to these figures, the dissolution of the drug dispersion with HPMC proved to be highly favored and, especially, in systems based on β-CD complexation relative to BNZ alone.

Table 3: BNZ Dissolution Efficiency (DE) and binary mixtures at 30 and 60 min

BNZ and binary mixtures	% DE 30 min	% DE 60 min
β-CD EV	179,21%	89,60%
β-CD KN	156,31%	78,15%
β-CD PM	150,14%	70,07%
β-CD SY	86,52%	43,26%
HPMC EV	48,73%	24,36%
HPMC SY	41,17%	20,58%
HPMC KN	23,10%	11,55%
HPMC PM	19,48%	9,74%
BNZ	8,31%	4,15%

The Analysis of Variance (ANOVA) revealed that with the level of significance of 5% when comparing the techniques of preparation of the samples (PM, KN, EV and SY) given, there is a greater statistical difference between these techniques when they form IC ($F_{calculated}$: 8.96; $F_{tabulated}$: 2.4) than in SD ($F_{calculated}$: 4.7; $F_{tabulated}$: 2.4).

This data enables us to say that to accommodate BNZ with β-CD, the choice between either preparation techniques will be of great relevance to ensure better solubility of the drug. Then, the statistical comparison was made between the two excipients (HPMC and β-CD), using as constant preparation technique.

Among all the treatments used in the KN mixtures, a higher difference ($F_{calculated}$: 82.3; $F_{tabulated}$: 4.08) between the formation of SD with HPMC and β-CD IC confirms a better drug complex with CD solubility profile instead of mixing it with a polymer was shown.

Similar results were observed for PM ($F_{calculated}$: 82.21; $F_{tabulated}$: 4.08), showing an advantage for complexation. Whereas with the EV technique the differences were less dramatic but still significant ($F_{calculated}$: 49.2; $F_{tabulated}$: 4.08). The lowest result was detected for SY ($F_{calculated}$: 8.0; $F_{tabulated}$: 4.08), which denotes the smallest interference of this technique compared to the excipient of choice for BNZ.

CONCLUSION

In this study it was evident that the contribution of IC and SD systems, using three different techniques, increased the rate/speed of BNZ dissolution. The theoretical study of molecular modeling showed that BNZ can complex with β-CD in different ways, in an aqueous solution and in the presence of a vacuum so that, an excellent stoichiometric complexation can be brought about to improve BNZ solubility.

The system of complexation of the drug with β-CD, represented by IC, proved to be more efficient when compared with their PM and the SD with HPMC, which guarantees a greater capacity for solubility/dissolution of the drug. The calculations regarding the DE at 30 and 60 min corroborate these findings. Considering the data obtained, it is possible to interpret the technique for the formation of β-CD IC as more effective in promoting the improvement of BNZ solubility when compared with obtaining a SD with HPMC which, in turn, may increase the bioavailability of the drug and improve its pharmaceutical potential.

CONFLICT OF INTERESTS

Declared None

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