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Host–guest interactions between benznidazole and beta-cyclodextrin in multicomponent complex systems involving hydrophilic polymers and triethanolamine in aqueous solution

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ABSTRACT

Association of hydrophilic compounds with cyclodextrins to increase drug solubility has been extensively studied in aqueous solution. However, the mechanism of interaction among these components remains unclear. In this study, the mechanism of interaction of seven different hydrophilic polymers (HPs) and triethanolamine (TEA) in aqueous solution with beta-cyclodextrin (β-CD) to modify the aqueous solubility of benznidazole (BNZ) was well investigated using solubility diagrams, thermodynamic experiments, molecular modeling and NMR studies. Solubility diagrams in different pH values confirmed linear soluble BNZ-β-CD inclusion complexes, with 1:1 stoichiometry (AL type). A synergistic effect in the association of TEA with BCD did not occur, due to competition between TEA and BNZ β-CD cavity, which led to obtain inclusion complexes with limited solubility (B type). The increment of BNZ solubility occurred only at higher TEA concentrations by cosolvency mechanism, which was evidenced by solubility diagrams, molecular modeling and NMR studies. The association of different hydrophilic polymers with β-CD contributes thermodynamically to stabilize the formed complexes, in which POL 407 and PVA increased considerably the observed $K_{1:1}$ value. An enthalpic contribution of hydrophilic polymers led to enhance the spontaneity of BNZ-β-CD interaction and a slight increasing in entropy change (ΔS) did possible to stabilize the interaction between BNZ and β-CD.

1. Introduction

Chagas disease is an endemic infection caused mainly by the parasite *Trypanosoma cruzi*. According to the World Health Organization (WHO), more than 10 million people are infected, mainly in Latin America. Furthermore, more than 25 million people are at risk of infection. Benznidazole, N-benzyl-2-(2-nitroimidazol-1-yl) acetamide (BNZ — Fig. 1), is the mainly available drug for its treatment [1], which is classified as a poor soluble drug with high permeability through biological barriers. Due to these characteristics, alternatives that lead to an increment in drug solubility can considerably increase its efficacy. Among several techniques that have been studied for enhancing equilibrium solubility of non-polar drugs in aqueous vehicles, cosolvency and complexation with cyclodextrins are well established for this purpose [2,3].

Cyclodextrins (CD) are cyclic oligosaccharides with hydroxyl groups on the outer surface and a cavity in the center. The three most common naturally occurring CD are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) with six, seven and eight (α-1,4)-linked D-glucopyranose units, respectively. Due to their cone-shaped structure with an outer hydrophilic surface and a cavity with a lipophilic character, these molecules can host hydrophobic or water insoluble compounds by "host-guest" mechanism, with a favorable change in enthalpy by reducing the free energy of the aqueous environment [4–6]. Thus, the cyclodextrin inclusion complexes may alter some physicochemical properties of drugs, such as stability, solubility and consequently bioavailability [4–6].

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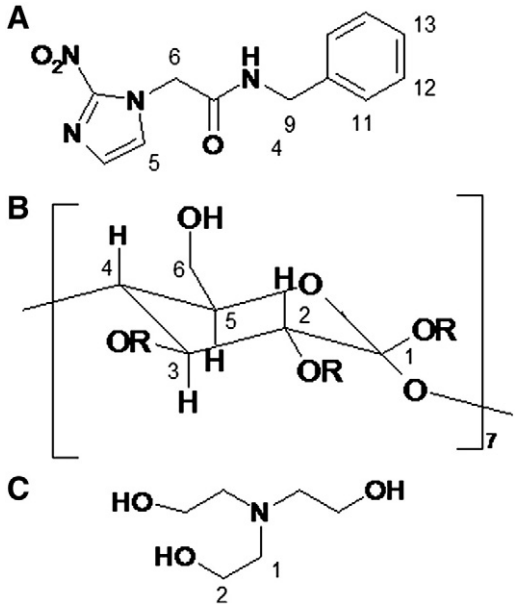

Fig. 1. Schematic representation of chemical structures of (A) benznidazole, (B) β-CD and (C) TEA.

The phase-solubility diagram described by Higuchi and Connors is the most used approach in inclusion complex characterization. This is a well-established method to estimate not only the value of the stability constant but also to give insight into the stoichiometry involved with components in equilibrium. Some types of diagram can be identified in solubility studies, which may be classified into two main categories; A- and B-types. A-type diagram is observed when the apparent solubility of the substrate increases as a function of CD concentration. B-type diagrams are indicative of the formation of complexes with limited water solubility [6–8].

Other strategies have been investigated to enhance both the apparent aqueous solubility and dissolution rate of poor water-soluble drugs. Among them, solid dispersion using hydrophilic polymers (HP) is well established for this purpose [9]. Some studies have also found that the use of hydrophilic compounds such as triethanolamine (TEA) isolated or associated with cyclodextrins has demonstrated interesting results [10,11]. It has also been described in literature papers aiming to increase BNZ solubility by applying several types of CD [12,13] and studies using ternary complexes with CD and hydroxyethylmethylcellulose to increase the BNZ aqueous solubility [14]. However, the effect of TEA and the involved mechanism of its interaction in inclusion complex formation have not been considered. These liquid hydrophilic compounds may interact with insoluble drugs by cosolvency or complexant mechanisms. Some models have been proposed for the prediction of this cosolvency, in which the log-linear model still remains to be the most useful.

Hence, the purpose of this study was to investigate the interaction of BNZ with β-cyclodexdrin (β-CD) in aqueous solution containing hydrophilic compounds, such as TEA and hydrophilic polymers. The polymers studied included polyethyleneglycols with different molecular weights (PEGs 1500, 4000 and 10000); hydroxypropylmethylcellulose (HPMC); Polyvinyl alcohol (PVA); polyvinylpyrrolidone (PVP) and poly (oxyethylene) block copolymer (poloxamer 407). The mechanisms of interaction among these components were followed and clarified using, solubility phase diagrams, thermodynamic experiments, molecular modeling and NMR studies. The analytical results were checked using a validated methodology to access analytical concentration of drug in the samples.

2. Experimental

2.1. Materials

BNZ, N-benzyl-2-(2-nitroimidazol-1-yl) acetamide was obtained from Roche (Brazil); βCD was a gift from Roquette® (Labonathus, Brazil); polyethyleneglycol (PEG) 1500, 4000 and10000, polyvinilpirrolidone PVP-k 30 and triethanolamine were purchased from Synth (Brazil); polaxamer (POL 407) and hydroxypropylmethylcellulose (HPMC) were purchased from Sigma-Aldrich (USA) and polyvinyl alcohol from Vetec (Brazil). All other reagents were analytical grade. The purified water (1.3 μS) was prepared from reverse osmosis purification equipment, model OS50 LX, Gehaka (Brazil).

2.2. Method validation

2.2.1. Instrumental conditions

An UV spectrophotometric method for quantitative analysis of BNZ was validated according to the guidelines established by the International Conference on Harmonisation (ICH) [15] and Brazilian regulatory National Agency of Sanitary Monitoring (ANVISA) [16]. The equipment used consisted of a UV–Vis spectrophotometer Thermo Fisher Scientific, Evolution 60S, USA. All absorbance readings were taken in a 1-cm path-length cuvette at room temperature, at wavelengths between 200 and 400 nm.

2.2.2. Analytical parameters

The standard curve was obtained from different aliquots taken from a BNZ stock solution of 500 μg ml^{-1} prepared in ethanol, which were diluted with water to prepare solutions with different concentrations (2.5 to 40 μg ml^{-1}). A 20 μg ml^{-1} solution was scanned from 200 to 400 nm, to find the best wavelength (324 nm) for BNZ quantifications. Furthermore, the data used to build the standard curve were subjected to analysis of variance (one-way ANOVA) to assure the linearity of method. Specificity of method was determined by comparing the analytical plots of absorbance, of a matrix solution containing different compounds (TEA + β-CD + HP/TEA).

Intra and inter-day precision tests were performed by calculating the relative standard deviation (RSD) of analyses of BNZ solutions at five different concentrations (5, 10, 20, 30 and 35 μg ml^{-1}) (n = 5). Analyses were carried out on the same day (intra-day test) and on five different days (inter-day test), at intervals of at least two days, with the same spectrophotometer equipment. Accuracy was accessed using the standard addition method, in which solutions containing the components of matrix (β-CD + HP/TEA) were mixed with distinct amounts of standard BNZ solution to obtain five different drug concentrations (5, 10, 20, 30 and 35 μg ml^{-1}). Accuracy level was calculated as the mean of five tests at each level (n = 5), from the relationship:

$$\text{Accuracy} = \left[\frac{\text{mean experimental concentration}}{\text{theoretical concentration}}\right] \cdot 100. \quad (1)$$

In the apparent robustness, the influence on precision of several analytical parameters, such as different days was described in previous sections. Additionally, the effect of pH of the analytical solution on the accuracy and precision of the method was investigated. To this end, the pH of the analytical solutions was about pH 6.5, which was adjusted to lower and higher levels (pH 4.5 and 8.5) with 0.05 mol l^{-1} phosphate ($KHPO_4$) buffered solution.

2.3. Solubility studies

2.3.1. Phase solubility studies

The solubility diagrams were obtained according to the method established by Higuchi and Connors [17], in which an excess of BNZ was added to flasks containing different β-CD concentrations (0 to