Journal de Ciencia e Ingeniería

Journal de Ciencia e Ingeniería, vol. 10, no. 1, pp. 32-38, agosto de 2018
Investigación - Ingeniería Química
ISSN 2145-2628, e-ISSN 2539-066X

Purification of the antichagasic benznidazole from the commercial preparation Rochegan®: characterization of inclusion complexes with β-cyclodextrin

Purificación del antichagásico benznidazol a partir del preparado comercial Rochagan®: caracterización de los complejos de inclusión con β-ciclodextrina

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Recibido: 29/05/2017; revisado: 25/10/2017; aceptado: 08/01/2018

Y. R. Espinosa, F. Galvis-Ovallos, A. Maldonado-Rozo : Purification of the antichagasic benznidazole from the commercial preparation Rochegan®:characterization of inclusion complexes with β-cyclodextrin. *Jou.Cie.Ing*. **10** (1): 32-38, 2018. ISSN 2145-2628, e-ISSN 2539-066X.

Abstract

The Chagas is a endemic disease from the American continent whose etiological agent is the Trypanosoma cruzi. Currently, there is neither a prophylactic vaccine for this disease or a totally effective treatment for the chronic cases. The Nifurtimox and the benznidazole are nitroheterocyclic complexes that have been used since the end of the 60's and the beginning of the 70's, mainly for the treatment of congenital and acute infections. However, these medications can cause a wide variety of adverse effects. Especially, in the benznidazole is necessary to develop new therapeutic alternatives which improve its low solubility in water, the limited gastrointestinal absorption, and the high toxicity. Because of the difficulties that often occur in the achievement of pre-synthesis Benznidazole, high costs in the synthesis process and buy the active compound in countries with limited research economics commodities. We have proposed a method of purification in order to facilitate the experimental procedures from the commercial preparation Rochegan®, obtaining BNZ viable for the formation of the controlled release complex with β-cyclodextrin (βCD) and the characterization of the BNZ-βCD inclusion complex.

***Keywords*:** Chagas disease, Benznidazole, β-cyclodextrin, inclusion complex

Resumen

El Chagas es una enfermedad endémica del continente americano cuyo agente etiológico es el Trypanosoma cruzi. Actualmente, no hay una vacuna profiláctica para esta enfermedad o un tratamiento totalmente eficaz para los casos crónicos. El Nifurtimox y el Benznidazol (BNZ) son complejos nitroheterocíclicos que se han utilizado desde finales de los años 60 y principios de los años 70, principalmente para el tratamiento de infecciones congénitas y agudas. Sin embargo, estos medicamentos pueden causar una amplia variedad de efectos adversos. Especialmente, en el benznidazol es necesario desarrollar nuevas alternativas terapéuticas que mejoren su baja solubilidad en agua, la absorción gastrointestinal limitada, y su alta toxicidad.

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Así mismo, debido a las dificultades en la pre-síntesis del benznidazol, los altos costos en el proceso de síntesis y compra del compuesto activo en países con inversión científica limitada, hemos propuesto un método de purificación a partir del preparado comercial Rochegan®, obteniendo BNZ viable para la formación del complejo de liberación controlada con β-ciclodextrina (βCD) y la caracterización del complejo de inclusión BNZ-βCD.

***Palabras Claves*:** Enfermedad de Chagas, Benznidazol, β-cyclodextrina, Complejo de inclusión.

1. Introduction

The Chagas' disease is a chronic parasitic infection whose etiological agent is the Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae) [1]. This disease is endemic from the American continent, although in the last years its expansion has been associated with the migration of infected people [2]. Nowadays, there is neither a prophylactic vaccine for this disease or a totally effective treatment for the chronic cases [3].

The Nifurtimox [N-(3-Methyl-1,1-dioxido-4-thiomorpholinyl)-1-(5-nitro-2-furyl)methanimine] and the benznidazole [N-benzyl- 2-(2-nitro-1H-imidazol-1-yl) acetamide] [4–6] are nitroheterocyclic complexes that have been used since the end of the 60's and the beginning of the 70's, mainly for the treatment of congenital and acute infections [3,4,7,8]. These complexes are characterized by possessing nitric groups joined to an aromatic nucleus, presenting a wide spectrum of action, like antibiotics and anti-parasites. However, these medications can cause adverse effects like allergies, dermatitis, edemas, nausea, lymphadenopathy, intestinal colic, diarrhea, vomiting, neuritis, loss of weight, loss of calcium, muscle and joint pain, sleep disorders, and cardiac and renal toxicity [9, 10].

Due to the low solubility in water [11], the limited gastrointestinal absorption, and the high toxicity present in the benznidazole is necessary to develop new therapeutic alternatives which improve these aspects [12,13]. Thus, 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 micronization, polymorphs, solid dispersions with hydrophilic polymers, cyclodextrins inclusion complexes, polymeric and lipid nanoparticles, and salt formation [14, 15].

The cyclodextrins are cyclic carbohydrates that possess an hydrophobic cavity and a hydrophilic exterior that allow the formation of inclusion complexes soluble in water, with a great variety of molecules of low solubility [13], due to the weakness of Van der Waals forces, hydrophobics, dipole-dipole, and hydrogen bridges that determine the behavior of the inclusion complex between the cyclodextrin and the host molecules, being widely used in the pharmaceutical industry [16]. This characteristic improves the properties of the drugs under study, improving the solubility, stability, bioavailability, and vectorization (once they are encapsulated in its hydrophobic cavity) which, for the pharmaceutical industry, is faster and cheaper than the development and introduction of new molecules for the treatment [17,18].

Because of the difficulties that often occur in the achievement of pre-synthesis Benznidazole, high costs in the synthesis process and buy the active compound in countries with limited research economics commodities, we have proposed a method of purification in order to facilitate the experimental procedures. Thus, the purpose of this study was the purification of the anti-chagasic benznidazole (BNZ) from the commercial preparation Rochagan®, for the formation of the controlled release complex with β-cyclodextrin (β-CD) and the characterization of the BNZ-β-CD inclusion complex through the Thermogravimetric Analysis (TGA) and Fourier-Transform Infrared spectroscopy (FTIR).

2. Materials and Methods

For the extraction of the [N-benzyl-2-(2-nitro-1H-imidazol-1-yl) acetamide], from the commercial preparation Rochagan® (composition: BNZ, starch, lactose, talc, and magnesium stearate) preliminary washings were made with the solvents: methanol, chloroform, and distilled water [19, 20] according to the solubility of the BNZ in different solvents, reported by Maximiano *et al.* [21]. A TGA control of the pill was made in order to confirm the presence of the active compound, its excipients, and respective fusing temperatures. During the washing with distilled water, the supernatant was taken for evaporation, the obtained solute sample was analyzed by thermogravimetry, where it occurred the solubilization of the lactose and the starch, the latter in lower proportion (See fig. 1), then, the precipitate was treated with methanol, showing a wide spectrum of solubilization, reaching to solubilize the BNZ, lactose, and starch. Finally, those that were washed with chloroform show a very high level of starch extraction in the sam-

ple. The magnesium stearate and the talc are insoluble in these solvents and are present in the final precipitate.

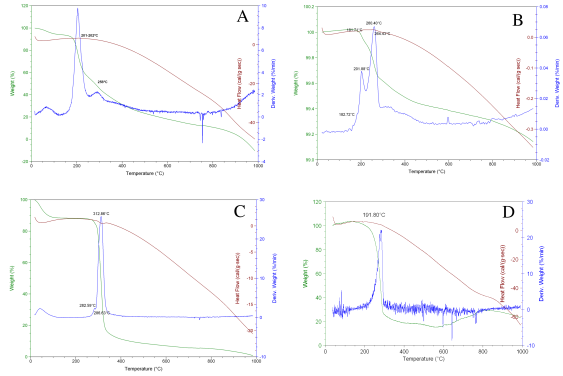

Fig. 1. Thermogravimetric analysis of the solutes obtained by solubility with organic solvents. (A) Solutes present in distilled water. It was registered the presence of lactose (FT: 201-202°C) and starch (FT: 256-258°C) (B) Solutes present in methanol. It was registered the presence of BNZ (FT: 188-190°C), lactose (FT: 201-202°C) and starch (FT: 256-258°C) (C) Solute present in chloroform. It was registered the presence of starch (FT: 256-258°C). (D) TGA where it is evidenced the presence of the N-benzil-2-nitro-imidazolacetamide free from excipients (FT 191.80°C). FT= fusing temperature; in green= Weight; in red= Heat Flow; in blue= Derivate Weight.

2.1. *Extraction method*

30 pills divided in 6 Falcon tubes, previously macerated, were taken. Then, 25 ml of distilled water was additioned to each tube at 40°C, centrifuged at 5000 rpm for 3 minutes, excluding the supernatant and repeating this procedure four times. After this, 5 ml of chloroform was additioned to the precipitate of each tube and centrifuged at 5000 rpm for 3 minutes, excluding the supernatant and repeating this procedure 3 times. 3 ml of methanol was additioned to the precipitate obtained in each of the tubes and centrifuged at 5000 rpm for 3 minutes, repeating the process 10 times. The compound N-benzil-2-nitro-1-imidazolacetamide was obtained by evaporation at 60°C of the supernatant obtained in each washing with methanol (See fig. 1D). The estimation of the purity was made by TGA and FTIR.

2.2. *Synthesis of the complex*

The preparation of the complex was made by the co-precipitation method reported by Szejtli [22] in 1998 and was standardized by Demicheli in 2004 [23], in a 1:1 and 1:1.5 molar ratio, BNZ and βCD, respectively. The mixture was kept in magnetic agitation at 60°C for 48 hours, until an homogeneous paste was obtained.

2.3. *Physico-chemical characterization*

2.3.1. *Thermogravimetric analysis*

The TGA analysis was made by using the DSC-TGA SDT Q600 V7.0 Build 84 in nitrogen atmosphere with a flow rate of 100 ml/min and with a temperature increase of 10°C/min in a range of 50-1000°C.

2.3.2. *Fourier-Transform Infrared spectroscopy*

The infrared spectra were obtained in the 4000–400 nm region by using IR Prestige - 21 Fourier Transform Infrared Spectrophotometer Shimadzu. An hydraulic press was used for the fabrication of the compressed pills of potassium bromide (KBr), in 1:10 ratio (Sample:KBr, respectively)

3. Results and Discussion

3.1. *Identification for Fourier-Transform Infrared spectroscopy*

[N-benzyl- 2-(2-nitro-1H-imidazol-1-yl) acetamide] ($C_{12}H_{12}N_4O_3$) was described in terms relative to its orientation in three planar fragments: (1) imidazol group; (2) benzyl group and (3) acetamide fragment [20].

In the analysis by FTIR it was observed an intense band near 3281 cm^{-1}, characteristic of the tense absorptions of the N-H bond of the secondary amines present in the acetamide fragment (See fig. 2).

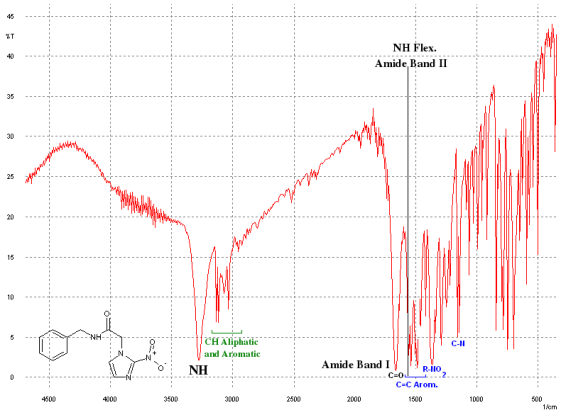

Fig. 2. Infrared spectrum of the N-benzyl-2-(2-nitroimidazol-1-yl) acetamide.

The amide band I was observed at 1665 cm^{-1}, characteristic of the C=O bond, while the amide band II shows the deformations of the N-H flexion at 1585 cm^{-1} and near 1400 cm^{-1}; where it is masked by the peaks of the double bond C=C of the aromatic nucleus, which is consistent with the one reported by Soares-Sobrinho *et al.* (2012) [13].

The region of the C-H bond showed the tension of these for the aliphatic and aromatic forms over 3000 cm^{-1} and reaching 3131 cm^{-1}. As well, deformation bands of flexion of the C-H bond were observed appearing in the level of the region which goes from 1100 cm^{-1} to 900 cm^{-1}. The C=C tense bands of the benzyl group were visible at 1540 cm^{-1} and 1486 cm^{-1} [24].

The intensity of the band in the tension of the C-N bond present in the imidazol group was observed at 1157 cm^{-1}, the absorption of the double bond carbon-nitrogen was produced in the region within 1700 cm^{-1} and 1615 cm^{-1} and was attached to effects of contour of form analogous to the absorption bands of the C=O and C=C bond (See fig. 2) [24].

The functional group R-NO2 showed symmetric vibration and a maximum absorbance at 1372 cm^{-1}.

3.2. *Synthesis and characterization of the BNZ-βCD inclusion complex*

The synthesis of the inclusion complex was carried out by the co-precipitation method. To determine the fusing temperature of the BNZ-βCD inclusion complex, first, it was determined the fusing temperature of the βCD within 293-295°C (See fig. 3A).

The synthesis of the inclusion complex was initiated with a 1:1 BNZ-βCD molar ratio. The TGA showed at 190°C a weight decrease of 18.39% that continued up to 230°C, indicating the presence of free BNZ that was not part of the inclusion complex. The second loss of weight was of 6.91% and was registered between 240°C and 280°C, representative of the BNZ-βCD inclusion complex, with a fusing temperature between 248°C and 250°C. Finally, in the temperature range which goes from 290°C to 350°C, we found βCD that was not part of the inclusion complex, with a loss of weight of 67.80% (See fig. 3B).

In order to obtain more inclusion efficiency of the BNZ in the βCD, it was taken a bigger molar ratio between these, 1:1.5 BNZ- βCD, respectively. In the TGA the period between 190°C and 230°C, a loss of weight of 6.18% was observed, which indicated a decrease in the free BNZ (See fig. 3C).

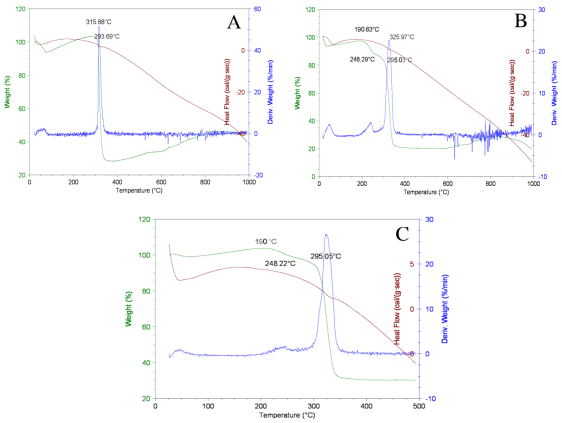

Fig. 3. (A) fusing temperature of the β-cyclodextrin, 293.69°C. (B) TGA of the BNZ-βCD inclusion complex in 1:1 molar ratio. (C) TGA of the BNZ-βCD inclusion complex in 1:1.5 molar ratio, respectively. In green= Weight; in red= Heat Flow; in blue= Derivate Weight.

Between the temperature range that goes from 240°C to 280°C, characteristic of the inclusion complex, it was observed a loss of weight of 7.28%. In the last temperature range, 290-350°C, it is remarkable the large percentage of βCD, 73.08%, that was not part of the inclusion complex, although the quantity of free BNZ-βCD had decreased and the concentration of the BNZ-βCD had increased.

The low solubility in water that shows the benznidazole is determining in the formation of the inclusion complex because of the repulsive forces between the involved molecules. However, the raise of the βCD concentration increases the solubility in water of the BNZ [12, 25], but does not greatly improve the formation ratio of the BNZ-βCD complex.

For the FTIR analysis of the BNZ-βCD complex, first, it was characterized the βCD, showing an intense wide band between 3800-3000 cm^{-1} characteristic of the tension of the O-H bond [24], may be because of the formation of intramolecular hydrogen bridges between the C-2-OH groups of a glucopyranose with the C-3-OH group of the adjacent glucopyranose causing a decrease in the frequency and a widening of the band, which is known as effect of the hydrogen bridge [22, 24]. The band of the C-H bond appears at 2928 cm^{-1} as a little peak followed to the O-H bond zone. For its part, the flexion deformation of the C-H bond appeared in the region that goes from 1200 cm^{-1} to 900 cm^{-1}, masked by the tension of the C-O-C bond in which is characteristic an unfolded intense band in the interval 1310-1000 cm^{-1} and in this spectrum it was registered in the region that goes from 1160 cm^{-1} to 1024 cm^{-1} [24]

(See fig. 4A).

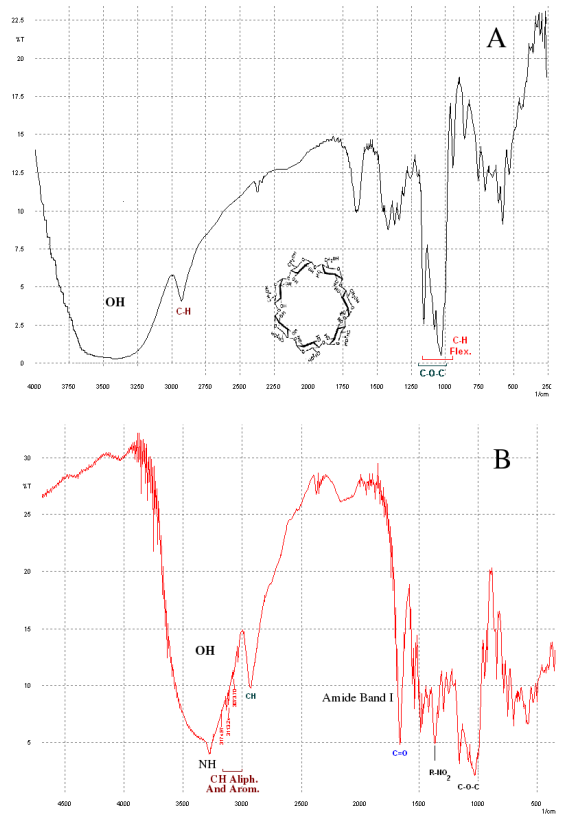

Fig. 4. FT-IR analysis. (A) Infrared spectrum of the β-cyclodextrin. (B) Infrared spectrum of the BNZ-βCD inclusion complex.

The infrared spectrum of the BNZ-βCD inclusion complex of the figure 4B showed an intense band between 3640-3000 cm^{-1} in which it persisted the effect of hydrogen bridge with a decrease in the bandwidth, which could be due to the hydrogen intramolecular bridges which became intermolecular in union with the BNZ and, so, the length of the O-H bonds became shorter, leading to the formation of a narrower band [13, 17], (See fig. 4B).

Immersed in the band of the O-H bond, it was evidenced like a little peak at 3275 cm^{-1}, corresponding to the tension band of the N-H bond of the secondary amide belonging to the acetamide fragment of the BNZ, where it was observed the decrease of its frequency indicating, may be, the formation of an hydrogen bridge with the hydrogen from the hydroxyl group of the βCD. The amide band I of the C=O bond was observed at 1665 cm^{-1} as an intense peak and, when confronting with the region of the C-H bond for the aliphatic and aromatic forms, the tension of these bonds were over 3000 cm^{-1}, reaching 3131 cm^{-1}. For its part, the vibration of the C-H skeleton of the βCD remained as a little peak at 2928 cm^{-1} followed to the zone of O-H bond and to the C-H region for the aliphatic and aromatic forms of the BNZ.

The double bands of C=C tension of the benzyl group showed a modification in the spectrum and are not shown in the graphic. Following that, the symmetric vibration of the R-NO_2 group it is not registered at 1372 cm^{-1}. Finally, it was observed the tension of the C-O-C bond of the βCD represented by an unfolded band in the region that goes from 1160 cm^{-1} to 1024 cm^{-1} and that it masks flexion of the C-H bond that goes from 1200 cm^{-1} to 900 cm^{-1}.

By comforting the analyzed spectrums for each bond and functional group characteristic in the BNZ, the βCD and the BNZ-βCD complex (See fig. 2, 4A and 4B, respectively), it shows that, may be, the formation of the inclusion complex was carried out by the interaction of the benzyl group with the cavity of the βCD and the acetamide fragment of the BNZ with the hydroxyl groups of the βCD in 1:1 stoichiometric ratio [25] (See fig. 5).

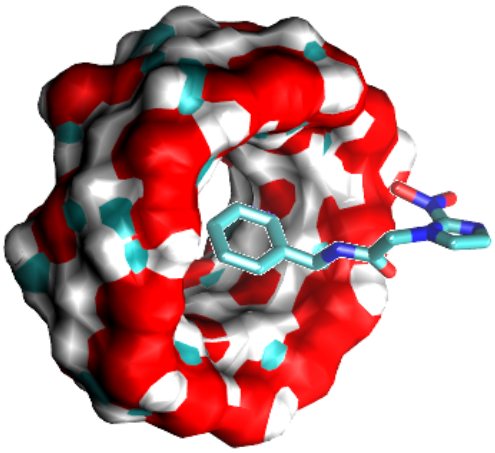

Fig. 5. Representation of the BNZ-βCD inclusion complex. In white, red, cyan and blue; hydrogen, oxygen, carbon and nitrogen atoms, respectively. Graphical representation using VMD package [26].

4. Conclusions

In this work, we propose a extraction protocol of the BNZ by organic solvents and its validation by TGA and FTIR allowed to obtain viable BNZ for the study of the

BNZ-βCD inclusion complex. Unfortunately, the complexation efficiency of βCDs is rather low and, therefore, a significant amount of cyclodextrins is needed to solubilize a small amount of water-insoluble compounds. So it is recommendable the study of chemical derivatives of the CD like alkyl, sulphobutyl and hydroxyl groups that increase its solubility in water, as well as changes in the CD-drug molar ratio and the use of a third component, like water-soluble polymers that interact with the inclusion complex (multicomponent complexes [15]), improving drug's physical, chemical and biological properties.

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