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Research Article

A Preformulation Study of a New Medicine for Chagas Disease Treatment: Physicochemical Characterization, Thermal Stability, and Compatibility of Benznidazole

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Abstract. This work aimed the studies of physicochemical characterization, thermal stability, and compatibility of benznidazole (BNZ) drug by spectroscopy (NMR, IR), thermoanalytical (differential thermal analysis, differential scanning calorimetry, and thermogravimetry), and chromatographic (HPLC) techniques, beyond the analytical tools of Van't Hoff equation and Ozawa model. The compatibility study was conducted by binary mixtures (1:1, *w*/*w*) of the drug with microcrystalline cellulose 102 and 250, anhydrous lactose, and sodium starch glycolate. The physicochemical characterization confirmed data reported in scientific literature, guaranteeing authenticity of the analyzed raw material. The drug melts at 191.68°C (ΔH, 119.71 J g^{-1}), characteristic of a non-polymorphic raw material, and a main stage decomposition at 233.76–319.35°C (Δm, 43.32%) occurred, ending the study with almost all mass volatilized. The quantification of drug purity demonstrated a correlation of 99.63% between the data obtained by chromatographic (99.20%) and thermoanalytical technique (99.56%). The Arrhenius equation and Ozawa model showed a zero-order kinetic behavior for the drug decomposition, and a calculated provisional validity time was 2.37 years at 25°C. The compatibility study evidenced two possible chemical incompatibilities between BNZ and the tested excipients, both associated by the authors to the reaction of the BNZ's amine and a polymer carbohydrate's carbonile, being maillard reactions. The BNZ reaction with anhydrous lactose is more pronounced than with the sodium starch glycolate because the lactose has more free hydroxyl groups to undergo reduction by the drug. In this sense, this work guides the development of a new solid pharmaceutical product for Chagas disease treatment, with defined quality control parameters and physicochemical stability.

KEY WORDS: binary mixtures; Chagas disease; drug stability; excipients; preformulation.

INTRODUCTION

It is estimated that the overall prevalence of Chagas disease is 9.8 million infected people (1). This disease results from an infection by *Trypanosoma cruzi*, a parasite that belongs to an ecosystem that is uniquely American. The interruption of vectorial and transfusional transmission has succeeded in endemic countries; however, these infected people still do not have an appropriate drug treatment (2). The current therapy, whose drug of choice is the benznidazole in an immediate release tablet dosage form, is not adequate for the administration in neonates, children, and elderly. Scientists around the world have been working on the development of new therapies for Chagas disease treatment, and the results range from the discovery of new biochemical targets to the develop of new molecules with potent trypanocidal action (3). Based on this situation, the development of differentiated pharmaceutical forms of benznidazole such as modified release, sublingual, or dispersible tablets aims to be available in the near-future medicines that improve patient compliance, efficacy, and safety of therapy.

The development of pharmaceuticals forms requires knowledge about physicochemical properties of drug and excipients, beyond knowledge about its individual and associated compounds stability behaviors. Thermoanalytical techniques make the inquiry of these properties possible, being widely used in the pharmaceutical industries as fast and precise techniques of quality control and development of new products (4–7). These techniques have been used for several purposes, including thermal characterization (8–11), drugs stability studies (12–14), and preformulation studies (15,16).

Thermal decompostion, also called thermolysis, is defined as a chemical reaction in which a compound breaks up into at least two other substances when heated. The

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reaction is usually endothermic as heat is required to break chemical bonds in the compound undergoing decomposition. The nitroimidazole compounds, under high temperatures, tend to split the C-NO_2 bond first, then all the other C-N, C-C, C-H, and N-H bonds of the ring are broken and volatilized as NO_2, CO_2, and CO. The kinetic determination depends of the mathematical model used and method conditions (17).

There are no complete data of the benznidazole's thermal behavior available in the literature; however, information about other nitroimidazoles can be found and correlated with the drug in study. The ornidazole, 1-(2-hydroxy-3-chloropropyl)-2-methyl-5-nitroimidazole, is considered thermally stable up to 166°C. Its main decomposition stage occurs at 166–316°C, with a later mass loss stage of carbonaceous material at high temperatures, having volatilization of 94% of sample. Its decomposition kinetic is determined as zero-order model according to Ozawa's model (18). The metronidazole indicated a high activation energy of thermal decomposition and a good stability. The decomposition occurs at 178–296°C. The Ozawa's model was used in the kinetic studies evidencing a zero-order kinetic behavior (19).

The aim of this work was a preformulation study of benznidazole with devoted pharmaceutical excipients for the development of a new tablet formulation. The study encompassed the studies of physicochemical characterization, thermal stability, and compatibility of benznidazole (BNZ) drug.

MATERIALS AND METHODS

The benznidazole raw material was supplied by F. Hoffmann-La Roche Ltd. (Basiléia, Switzerland), batch 13871 (Fig. 1). The excipients used were anhydrous lactose, microcrystalline cellulose 102, microcrystalline cellulose 250, and sodium starch glycolate, all supplied by Blanver Farmoquímica (Tabuão da Serra, Brazil). The preference of work with classic excipients was based upon the low cost required to this new product, since Chagas disease is considered a negligence disease.

The binary mixtures were obtained by physical mixture of the compounds in glass vial by a vortex machine for 15 min (Barnstead Thermolyne Maxi Mix II, 37600, Lote 39296EJ). The 1:1 (*w*/*w*) ratio was chosen to maximize the interactions between the compounds, since the excipients will be used in smaller proportions in the formulation.

^{1}H-NMR and IR Spectroscopic Analyses

The drug IR spectrum was obtained by Raman technique in a Perkin-Elmer system model 720, and the ^{1}H-NMR spectrum was obtained in deuteron chloroform by a spectrometer Unity Plus 300 RMN operated at 300 MHz.

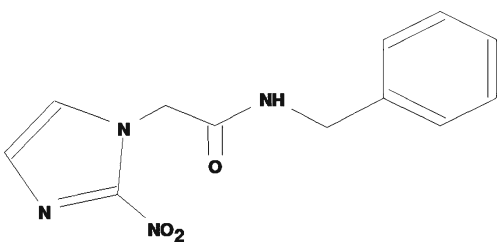

Fig. 1. Molecular structure of benznidazole

Determination of the Purity

The benznidazole purity determination was carried by a Shimadzu® liquid chromatograph with PDA detector and software Class-VP (Kyoto, Japan). The HPLC method used is reported in scientific literature (19). The data obtained by HPLC technique was confronted to the data obtained by differential scanning calorimetry (DSC) and treated by Van't Hoff equation (20).

Thermoanalytical Techniques

The DSC was used to drug characterization, purity quantification, and thermal decomposition kinetic determination by a Shimadzu DSC-50 cell (Kyoto, Japan), under a 50 mL min^{-1} of nitrogen flow, sample of 5 mg, aluminum pan, and heating rates of 10°C min^{-1}, 15°C min^{-1}, and 20°C min^{-1}. The differential thermal analysis (DTA) was used to study the drug compatibility by a Shimadzu DTA-50 cell (Kyoto, Japan), under a 50 mL min^{-1} of nitrogen flow, sample of 8 mg, alumina pan, and heating rate of 10°C min^{-1}. The DTA and DSC have different sensitivities and capabilities, but their uses were determined by the need for these differing purposes, as well as their analytical reliability and routine laboratory practicality. The DTA and DSC cells were calibrated with indium (mp, 156.6°C; ΔH_{fus}, 28.54 J g^{-1}) and zinc (mp, 419.6°C; ΔH_{fus}, 108 J.g^{-1}).

The thermogravimetry (TG) and first derivative of TG (DTG) were obtained to drug characterization, decomposition kinetic determination, and compatibility study by a Shimadzu thermobalance TGA-50H (Kyoto, Japan), under a 50 mL min^{-1} of nitrogen flow, samples of 8 mg (±0.5), alumina pan, and heating rates of 10°C min^{-1}, 15°C min^{-1}, and 20°C min^{-1}. The isothermal curves were obtained of 180°C, 200°C, 220°C, 230°C, 240°C, and 250°C during 120 min, corresponding to the onset of the drug decomposition process, seen in the dynamic method, in order to establish the provisional validity time of benznidazole, and the obtained data was used to calculate the degradation constant by the Arrhenius model. The thermal analytical data had been analyzed by Shimadzu TASYS software using the tangent peak delta values.

RESULTS AND DISCUSSION

NMR and IR Spectroscopic Analyses

The IV spectrogram of BNZ (Fig. 2) shows characteristic bands, especially regarding the amides typical bands (stretching vibration N-H), carbonyl stretch (amide I band), and NH deformation (amide II band), besides the vibrations due to the benzyl and imidazole group, and in particular, to the nitro group. The band due to stretching vibrations of N-H is at 3,330 cm^{-1}.

The carbonyl stretching band is at 1,685 cm^{-1}, and the N-H deformation (amide II) is at 1,565 cm^{-1}, characteristic of a secondary amide. Besides these, the band at 1,318 cm^{-1} is assigned to C-N stretching. The set of bands at 3,180; 3,160; 3,120; 3,090; and 3,000 cm^{-1} arise from stretching vibrations of symmetrical and asymmetrical methylene of benzyl group and the aromatic C-H stretch, as shown in Fig. 2.

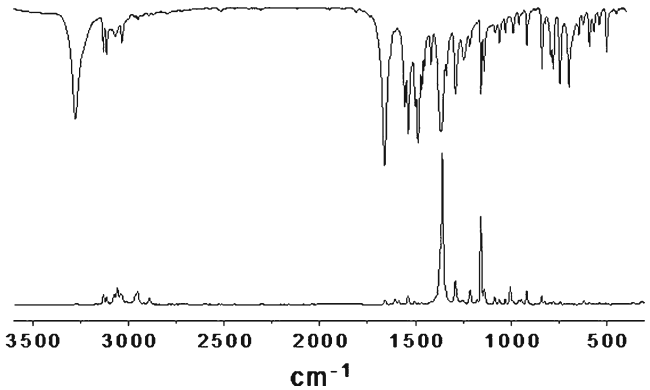

Fig. 2. IR and spectrum Raman of benznidazole

According to Fig. 3, the BNZ has a characteristic singlet at 7.58 ppm corresponding to the hydrogen of the nitroimidazole ring's carbon 5, and its corresponding vicinal proton singlet at 7.28 ppm. The protons attached to the amide group are identified in the singlets at 5.19 and 4.32 ppm found separately due to difference in deprotonation. Finally, between these, two assignments are attributed to the triplet phenyl protons at 4.73 ppm and the integration explained by the greater number of protons in the molecule of the BNZ.

The spectroscopic analyses of benznidazole (NMR and IR) results confirmed the reported data in scientific literature (21), demonstrating similarity in the resonance bands characteristics of the presence of assignments and similar functional groups, guaranteeing authenticity for the analyzed raw material, as demonstrated in Figs. 2 and 3.

Determination of Drug Purity

The Van't Hoff equation in chemical thermodynamics relates the change in temperature (T) to the change in the equilibrium constant (K) given the standard enthalpy change (ΔH^{θ}) for the process. The equation was first derived by Jacobus Henricus van 't Hoff (Eq. 1) and can also be written by Eq. 2. If the enthalpy change of reaction is assumed to be constant with temperature, the definite integral of this differential equation between temperatures T_1 and T_2 is given by Eq. 3. In this equation, K_1 is the equilibrium constant at absolute temperature T_1, and K_2 is the equilibrium constant at absolute temperature T_2. ΔH^{θ} is the standard enthalpy change, and R is the gas constant. Counting with Eqs. 4 and 5, it follows the Eq. 6. Therefore, a plot of the natural logarithm of the equilibrium constant *versus* the reciprocal temperature gives a straight line. The slope of the line is equal to the negative standard enthalpy change divided by the gas constant, $-\Delta H^{\theta}/R$, and the intercept is equal to the standard entropy change divided by the gas constant, $\Delta S^{\theta}/R$. Differentiation of this expression yields the Van 't Hoff equation (22).

$$\frac{d\ln K}{dT} = \frac{\Delta H^{\ominus}}{RT^2} \tag{1}$$

$$\frac{d\ln K}{d\frac{1}{T}} = -\frac{\Delta H^{\ominus}}{RT^2} \tag{2}$$

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H^{\theta}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{3}$$

$$\Delta G^{\theta} = \Delta H^{\theta} - T\Delta S^{\theta} \tag{4}$$

$$\Delta G^{\theta} = -RT\ln K \tag{5}$$

$$\ln K = -\frac{\Delta H^{\theta}}{RT} + \frac{\Delta S^{\theta}}{R} \tag{6}$$

The DSC data were treated by Van't Hoff equation to determine the drug purity. The DSC curves were obtained by heating rates (β) of 10°C, 15°C, and 20°C, showing drug

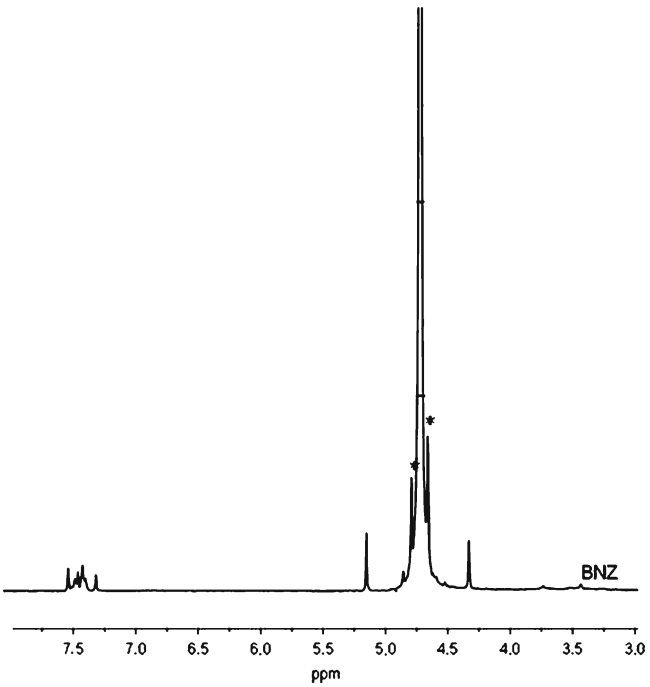

Fig. 3. NMR of benznidazole

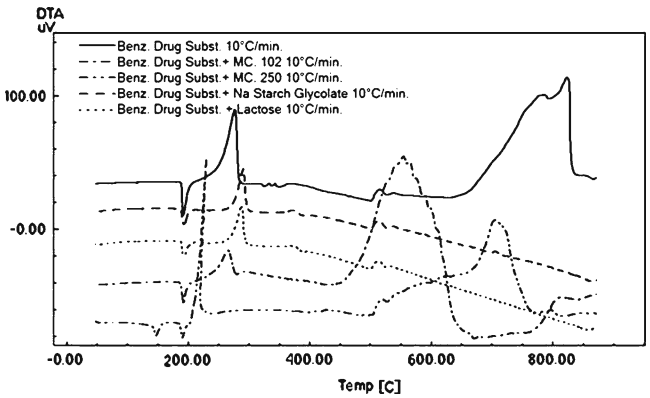

Fig. 4. DTA curves of benznidazole drug substance and binary mixture

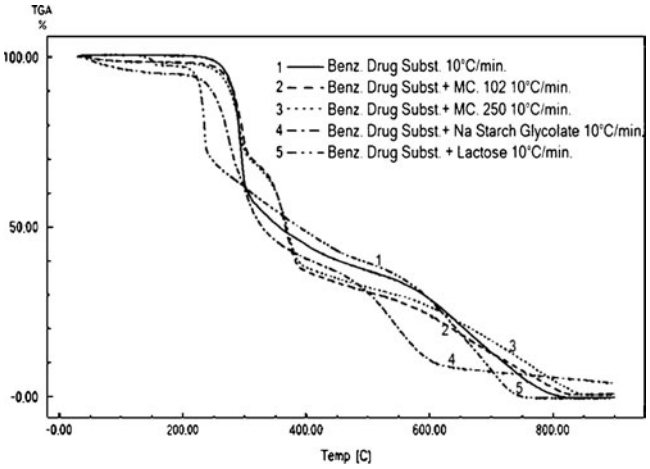

Fig. 5. TG curves of benznidazole drug substance and binary mixture

purity values of 99.20%, 98.78%, and 98.02%, respectively to each β used. The β of 10°C min^{-1} is the most sensitive and was demonstrated to be more adjusted when compared with the purity value obtained by the chromatography method, of 99.56%. The obtained correlation between techniques was 99.63%, demonstrating that the thermoanalytical technique can be used as powerful tool in the evaluation of the purity of the drug substance.

This high purity value, 99.20%, can be correlated to the benznidazole melting process, which presented a small enthalpy value (119.7 J g^{-1}), a sharp peak at 192°C and a narrow range of melting, characteristic of non-polymorphous raw materials.

Thermal Characterization of Benznidazole and Excipients

The benznidazole's DTA curves showed an endothermic process characteristic of melt in the range of 188–199°C, with peak at 192°C and enthalpy value of 935 J g^{-1}, in agreement to the literature (23,24) and to the DSC result. The exothermic process characteristic of decomposition occurred in the range of 260–285°C, with peak at 279°C. TG curves show that benznidazole is thermally stable until 234°C, showing a main decomposition stage between 234–320°C, with 43% of weight loss.

Regarding the excipients under study, the anhydrous lactose's DTA curves showed one endothermic phase transition between 145–156°C, with peak at 151°C correlated with crystalline transition of amorphous material, an endothermic process characteristic of melting in the range of 215–226°C, with peak at 221°C, and a decomposition process following the melting. TG and DTG curves showed a first loss mass stage between 66–92°C (Δm, 0.11%) associated to the superficial water loss—even being the material anhydrous, small mass of water can be absorbed by the powder—and four stages of thermal decomposition process. The decomposition start at 148°C and showed stages between 148–190°C (Δm, 3.2%), 237–299°C (Δm, 32.35%), 315–365°C (Δm, 17.15%), and 465–620°C (Δm, 24%).

The sodium starch glycolate's DTA curves showed a first endothermic event characteristic of melt in the range of 29–127°C and two exothermic events characteristics of decomposition between 231–284°C and 301–316°C. TG and DTG curves showed a first loss mass stage between 40–125°C (Δm, 5.6%) associated to the superficial water loss and two decomposition ranges between 250–380°C (Δm, 45%) and 622–717°C (Δm, 20%).

The microcrystalline cellulose 102 and 250's DTA curves showed a similar profile, with first event in the range of 32–107°C representing the residual water loss, confirmed by TG curves, and a second endothermic event in 370°C which is a characteristic of the cellulose decomposition. The absence of melting is characteristic of amorphous material. TG and DTG curves showed two stages of decomposition in the range of 260–407°C (Δm, 74.4%) and 537–582°C (Δm, 7.15%).

Compatibility Study

The DTA and TG obtained results showed the association of benznidazole and the studied excipients to the two possible significant chemical interactions (Figs. 4, 5; Table I).

By DTA technique, it was possible to observe an interaction between benznidazole and anhydrous lactose by the decomposition reaction change behavior (T onset, T peak, and ΔH) (Table I). The TG and DTG showed three stages of decomposition, being 124–165°C (Δm, 2.9%), 195–262°C (Δm, 30%), and 543–759°C (Δm, 37%) (Fig. 5). In this case, the interaction resulted in a reduction of thermal stability, once the drug is stable until 234°C, and this binary mixture is stable until 124°C.

The possible chemical interaction observed between the compounds is through the classical reaction between lactose's carbonile and benznidazole's amine, a maillard reaction. The temperature of fusion of these two compounds is very close, so the interaction is intensified because on the liquid state, their reactivity has potentially increased.

Anhydrous lactose is very unstable because it absorbs one water molecule for every lactose molecule forming the α-lactose monohydrate, whereas the anhydrous β-lactose is more stable under conditions of relatively low humidity.

Table I. Calorimetric Parameters of Benznidazole

Sample	DTA (°C) fusion peak	DTA ΔH (J/g) fusion	DTA (°C) decomposition peak	DTA ΔH (J/g) decomposition
BNZ drug substance	191.16	−145.45	278.62	975.77
BNZ+Na starch glycolate	191.84	−88.86	262.52	328.76
BNZ+anhydrous lactose	190.88	−58.05	229.35	450.88
BNZ+CM 102	193.54	−65.21	291.19	324.47
BNZ+CM 250	192.76	−71.86	287.95	379.63

CM microcrystalline cellulose

These different forms of lactose interfere with tablet properties. The anhydrous α-lactose presents a better thermal behavior in relation to the α-lactose monohydrate.

The preformulation study obtained results justify the substitution of lactose for microcrystalline cellulose, as a diluent excipient; although microcrystalline cellulose is also a carbohydrate, it presents a bigger polymer structure causing a steric hindrance of the carbohydrate's carbonile that possibly blocks the reaction with benznidazole's amine.

The DTA results showed also an interaction by the association of benznidazole drug with sodium starch glycolate due the displacement of the drug substance thermal decomposition peak (Table I). The TG and DTG curves of the binary mixture of benznidazole and sodium starch glycolate (1:1) showed three decomposition stages, being 92–253°C (Δm, 8.8%), 253–283°C (Δm, 18%), and 283–602°C (Δm, 60%).

This observed interaction was more discrete than the interaction observed with lactose, and the explanation involves the fact that the sodium starch glycolate is also a polymer carbohydrate; however, the reaction with anhydrous lactose is more pronounced because the lactose has more free hydroxyl groups to undergo reduction by the drug. Beyond the reaction occurring in a lesser extent, the ratio used of the sodium starch glycolate in a solid formulation, a disintegrant excipient, in accordance with the Handbook of Pharmaceutical Excipients (25), is between 2–8%, being a smaller ratio than the one used for this study.

The binary mixtures of benznidazole and microcrystalline cellulose 102 (1:1) and benznidazole and microcrystalline cellulose 250 (1:1) presented three decomposition process. The decomposition stages of the mixture with cellulose 102 were 274–305°C (Δm, 21.2%), 305–383°C (Δm, 31.8%), and 383–817°C (Δm, 38.9%). The decomposition stages of the mixture with cellulose 250 were 260–299°C (Δm, 22.3%), 299–388°C (Δm, 33.1%), and 388–843°C (Δm, 38.9%) (Fig. 5).

Thermal Decomposition Kinetic Investigation

The Arrhenius equation and Ozawa model was used to determine the kinetic parameters such as apparent activation energy (E_a), frequency factor (A), and reaction order (n), using the data obtained with the thermogravimetry (TG) study. The calculated data showed one kinetic behavior of zero order for benznidazole with E_a (95.91 KJ mol^{-1}) and frequency factor (1.25×10^8 min^{-1}). The confirmation of this kinetic should be done by applying more than one kinetic model; however, the result obtained by Ozawa's model is in accordance with the results obtained for other nitroimidazole compounds' kinetic published in the literature.

The extrapolated constant in 25°C was determined using previous calculated data in the studied temperatures and benznidazole presented a thermal degradation kinetic with constant of $K=1.4086\times10^{-9}$ and validity provisional time of 2.37 years.

CONCLUSION

The physicochemical characterization confirmed data reported in scientific literature, guaranteeing authenticity of the analyzed raw material. The quantification of drug purity demonstrated a good correlation between the data obtained by chromatographic and thermoanalytical techniques, demonstrating that the thermoanalytical technique can be used as powerful tool in the evaluation of the purity of the drug substance. The Arrhenius equation and Ozawa model showed a zero-order kinetic behavior for the drug decomposition, and the calculated provisional validity time was 2.37 years at 25°C. The compatibility study evidenced by DTA and TG two possible chemical incompatibilities between BNZ and the tested excipients, both associated by the authors to the reaction of the BNZ's amine and a polymer carbohydrate's carbonile, being maillard reactions. The BNZ reaction with anhydrous lactose is more pronounced than with the sodium starch glycolate because the lactose has more free hydroxyl groups to undergo reduction by the drug. In this sense, this work guides the development of a new solid pharmaceutical product for Chagas disease treatment, with defined quality control parameters and physicochemical stability.

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