

Development and *in vitro* evaluation of tablets based on the antichagasic benznidazole

José Lamartine Soares Sobrinho¹, Luciana Neiva Antunes Lima¹, Diego Cavalcanti Perrelli¹,
Jackson Luiz da Silva¹, Flávia Patrícia Morais de Medeiros^{1,2},
Mônica Felts de La Roca Soares¹, Pedro José Rolim Neto^{1*}

¹Laboratory of Medicine Technology (LTM), Pharmaceutical Sciences Department,
Federal University of Pernambuco, ²State of Pernambuco Pharmaceutical Laboratory (LAFEPE)

This work aimed to verify the interferences caused by the use of excipients for immediate release tablets based on benznidazole obtained by direct compression and the accomplishment of a comparative study between the tablets developed and the reference medicine Rochagan™, obtained by wet granulation. Seven small-scale batches (SSB) were developed and aspects such as compressibility, powder flow, mean weight, friability, disintegration, hardness, assay, content uniformity, kinetic of release in vitro (dissolution) and drug/excipients physical-chemical compatibility were evaluated. Based on the obtained results it can be verified that the analyzed powders presented adequated characteristics for the direct compression process, beyond the inexistent evidence of a physical-chemical interaction between the drug and the tested excipients. The tablets obtained from SSB I and III were chosen for the comparative study with the reference medicine, demonstrating similarity with the statistically treated obtained results, becoming an alternative option of a medicine product for the treatment of Chagas' disease with reduced cost and satisfactory quality.

Uniterms

- Benznidazole
- Excipients
- Direct compression
- Tablet
- Chagas' Disease

*Correspondence:

P. J. Rolim Neto
Laboratório de Tecnologia dos
Medicamentos - LTM
Departamento de Ciências
Farmacêuticas
Universidade Federal de Pernambuco
Av. Prof. Arthur de Sá, S/N - Cidade
Universitária
50740-521 - Recife - PE, Brasil
E-mail: pedro.rolim@pq.cnpq.br

INTRODUCTION

In Latin America the morbidity and mortality rates associated with Chagas' disease possess a greater magnitude than that reached with malaria, schistosomiasis and leishmaniasis, constituting one of the biggest public health problems of developing nations (Lamas *et al.*, 2006). Less than 1% of 1300 new medicines developed in the last 25 years have been developed for the neglected diseases (MSF, 2001). Benznidazole is not only the drug of choice

for Chagas' disease treatment, but nowadays it is also the only one available on the world market (Coura, Castro, 2002; Schofield, Jannin, Salvatella, 2006).

Benznidazole (BNZ) was introduced in Chagas' disease therapy in 1972 by the Pharmaceutical Company Roche™ with the commercial name Rochagan™. On march 3rd, 2003, this company donates your patent rights of the product explore to the Brazilian Government, that passes the privilege to produce this medicine for the State of Pernambuco Pharmaceutical Laboratory (LAFEPE™)

distribution, mean weight, friability, disintegration, hardness, assay, content uniformity, kinetic of release *in vitro* (dissolution) and the drug/excipient physical-chemical compatibility.

Determination of angle of repose and draining speed

The evaluation of the angle of repose (\pm) and speed flow was determined in accordance with the United States Pharmacopeia (USP, 2006; Viana *et al.*, 2006).

Hausner factor and Carr index

The evaluation of the Hausner factor and Carr index was determined in accordance with Rolim Neto (1992). The Hausner factor was determined through the relation between the apparent and compact densities, determined by a Tap Density Tester (Vankel, 230).

The Carr index or Compressibility index was determined by the quotient between the apparent and compact densities in percentage. The compressibility percentage indirectly gives an idea of the cohesion, size uniformity and surface area of powders or their mixtures.

Grain size distribution

The determination of the grain size distribution was carried out in accordance with the method described in the Brazilian Pharmacopeia (1988). A sample of 30 g was placed on the upper sieve and it was submitted to sift for 30 minutes.

Study of drug/excipient physical-chemical compatibility

Analyses were carried out to verify the possible physical-chemical interactions between the drug and excipients of the two best developed formulations. The DTA thermogram of benznidazole, excipients and respective binary mixture was obtained by a DTA-50 cell Shimadzu using alumina crucibles with about 8.0 mg of samples, under a flow dynamic nitrogen atmosphere at 50 mL \cdot min⁻¹ and heating rate of 10 °C \cdot min⁻¹ until 500 °C and 30 °C \cdot min⁻¹ until 900 °C. The DTA cell was calibrated initially with indium (m.p. 156.6 °C; ΔH_{fus} = 28.54 J \cdot g⁻¹) and then zinc (m.p. 419.6 °C) (Bazzo, Silva, 2005).

Evaluation of developed tablets and reference medicine

Mean Weight: the method used is described in the Brazilian Pharmacopeia (1988), with tolerance range of

5% for tablets with mean weight above 350 mg. *Hardness:* the determination of tablet hardness was carried out using a Vankel® K 200 hardness tester. The minimum hardness established for tablets is 4 Kgf in accordance with the Brazilian Pharmacopeia (1988). *Friability:* the friability of the tablets was determined in accordance with Brazilian Pharmacopeia (1988) and the acceptable value must be inferior of 1.5% of the established weight. *Disintegration:* the disintegration test was carried out by the method described in the Brazilian Pharmacopeia (1988), by a Nova Ética® disintegrator, with the maximum time specification of 15 minutes. *Assay:* a spectrophotometer method described by Soares Sobrinho *et al.* (2006) was developed and validated. The method uses a wavelength of 324 nm for BNZ, water as blank and a final solute concentration of 20 μ g/mL. *Dissolution:* according to a method supplied by the pharmaceutical company LAFEPE™, that uses artificial non enzymatic gastric juice as dissolution medium (HCl 0.1 M), medium volume of 1000 mL at 37°C \pm 0,5°C; apparatus 1 (basket) and rotation of 100 rpm, carried out by a Varian™ Dissolutor, Model VK-7000, 7010 and 750D with automatic collector, model VK-8000. The quantification of the BNZ dissolution percent was carried out in Micronal B582 Spectrophotometer in a 324 nm wavelength and artificial non enzymatic gastric juice as the blank. The results may present according specification method: Qe”60% of drug dissolved in 60 minutes and Qe”80% of drug dissolved in 120 minutes.

Kinetic of *in vitro* release

The tablets kinetics of release from SSBI, SSBIII and reference medicine, Rochagan™, follows the method used for dissolution tests, being the times of collection 10, 30, 45, 60 and 120 minutes. The results were used to calculate the dissolution efficacy (DE) and later compared with the variance analysis (ANOVA) (Aguiar *et al.*, 2005; Anderson *et al.*, 1998; Khan, 1975; Khan, Rhodes, 1972).

RESULTS AND DISCUSSION

Quality Control of the SSB developed

The grain size distribution (Figure 1) showed a high percentage of fine powder in the drug analysis, a factor that characterizes a powder with poor rheological properties of flow. It can also be observed that the mixture of these excipients with the drug improves significantly the grain size distribution of the mixture giving a flow to the powders.

Generally the small-scale batches presented very similar rheological advantages in comparison with the

isolated drug material, a fact that can be correlated with the angle of repose (with the exception of SSB V) and flow speed (Table II). SSB I, III, IV and VII resulted in the best grain size distribution from the technological point of view, presenting themselves as more homogeneous.

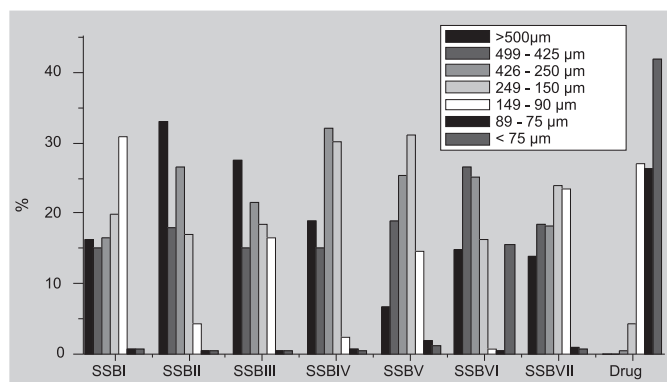


FIGURE 1 - Grain size distribution of benznidazole drug and respective mixtures of powders composing the small-scale batches obtained.

The flow characteristics (angle of repose and flow speed) and the compressibility aptitude (Hausner factor and Carr index) demonstrated that some of the seven tested formulations had not obtained results in the range of the specifications in accordance with the United States Pharmacopeia (2006), specifically the SSB II, IV and VII.

However, due to the small differences in the obtained results the compression of all the previously formulated SSB was undertaken. The first evaluated aspect after the compression process was the mean weight of the tablets, also taking into consideration the standard deviation of each obtained batch. In this stage of choice an ideal formulation, it was noted that the majority of the

SSB presented results inside the specifications ($\pm 5\%$), however batches II, IV, V, VI and VII presented a high standard deviation.

For the rest of physical-chemical tests there were not differences in results between the tested SSB. In the friability analyses, disintegration, hardness, assay, content uniformity and dissolution test the SSB gave results in the range of the specifications with the exception of LB IV in the hardness test (Table II). Based on the obtained results two formulations (LB I and III) were chosen for carrying out the drug/excipient interaction study and posterior kinetic of release, to compare the results with the reference medicine RochaganTM from Roche Pharmaceutical.

Drug/excipients physical-chemical compatibility study

DTA curves of benznidazole show an endothermic process characteristic of melting in the range of 187.68 to 198.88 °C, with melting peak at 191.1 °C and enthalpy value of -144.96 J.g^{-1} , which corresponds to the melting range the literature (Farmacopéia Brasileira, 1988) and exothermic process characteristic of decomposition at the range of 259.94 to 285.16 °C, with decomposition peak in 278.62 °C and enthalpy value of 975.77 J.g^{-1} (Figure 2).

The results obtained with the DTA technique showed that the association of benznidazole with the tested excipients does not show evidence of any significant physical interaction, due to the values of reaction and fusion enthalpy, when the value of the melting peak of benznidazole remains uncharged for all the binary mixtures around 191 °C (Table III e Figure 2). Also they do not show evidence of any significant chemical interaction, the discrete displacement evidenced retarding the decomposition peak of the drug in the binary mixtures thermogram and the reduction of decomposition enthalpy

TABLE II – Quality control of the SSB obtained

Parameters	Specifications	SSB I	SSB II	SSB III	SSB IV	SSB V	SSB VI	SSB VII
Mean weight	350 mg \pm 5%	339.4 \pm 3.5	333.9 \pm 15.1	339 \pm 5.5	334 \pm 12.8	352 \pm 13.2	341 \pm 11.9	334 \pm 16.7
Friability	< 1.5%	0.11	0.22	0.14	0.5	0.91	0.18	0.25
Disintegration	< 15 min	0.4	0.25	0.26	0.46	0.3	0.2	0.35
Hardness	> 4.0 kgf	4.65	5.28	6.16	3.25	4.85	5.28	4.76
Assay	95 - 105%	99.15	98.57	100.07	—	—	101.07	99.02
Content Uniformity	85 – 115%	98.57	—	99.82	—	—	100.51	100.31
Dissolution	> 80%	92.70	—	81.54	—	—	84.46	85.61
Angle of repose	< 35°	34.03	32.73	29.91	37.09	34.05	32.60	31.20
Flow speed	< 10 s	5	9	6	5	5	5	5
Hausner factor	< 1.25	1.15	1.48	1.1	1.13	1.2	1.22	1.35
Carr index	<25%	18	22	9	11	16	19	26

value can be explained at the moment benznidazole melts and the microcrystalline cellulose continues to be solid and incorporates it. Therefore, the cellulosis does not show a process of fusing and its decomposition occurs at approximately 370 °C. Generally chemical interactions reactivity, characterized for the displacement of the decomposition peak, potentially occur when two compounds are in liquid form.

The results prove that the pharmaceutical form tablets of immediate release based on benznidazole is possible, using the tested excipients, with stability, a requirement that could be confirmed and correlated in the stability test following the international guidelines for pharmaceutical forms. This makes the continuity of the pharmaceuticals development in a rational form possible.

The magnesium stearate, even though it was part of the two selected formulations, was not tested because of the possibility of physical-chemistry interactions due to the small percentage of the excipient in the formulation (1%).

After the confirmation that there was no physical or chemical interaction between the drug and the two tested excipients, selected by the best two small-scale batch (SSB I and III), the dissolution profile tests of the elected SSB were carried out together with the reference medicine, making it possible to observe in accordance with the dissolution efficacy (DE) of SSB I (58.02), SSB III (56.11) and the reference medicine (54.89). The similarity between the studied profiles (Figure 2), the variance analysis (ANOVA) demonstrated with level of 5% of probability an insignificant value of F, and a value of p bigger than the probability level. Therefore, the averages showed to be statistically equal (calculated F = 3.9255 and priced F = 4.2564).

The BNZ drug is characterized by the manufacturer (Roche™) as poorly soluble in water and very permeable, however the data available in the scientific papers published are confuse and dubious related to the drug solubility, because some of them classified the drug as soluble in water (Lindenberg, Kopp, Dressman, 2004;

TABLE III - Calorimetric parameters of benznidazole and binary mixtures

Sample	DTA fusion peak (°C)	DTA ΔH fusion (J/g)	DTA decomposition peak (°C)	DTA ΔH Decomposition (J/g)
BNZ	191.10	-144.96	278.62	975.77
BNZ:MC 102	192.90	-65.21	291.19	324.47
BNZ:MC 250	192.73	-65.45	287.95	379.63

Kinetic of release in vitro (dissolution)

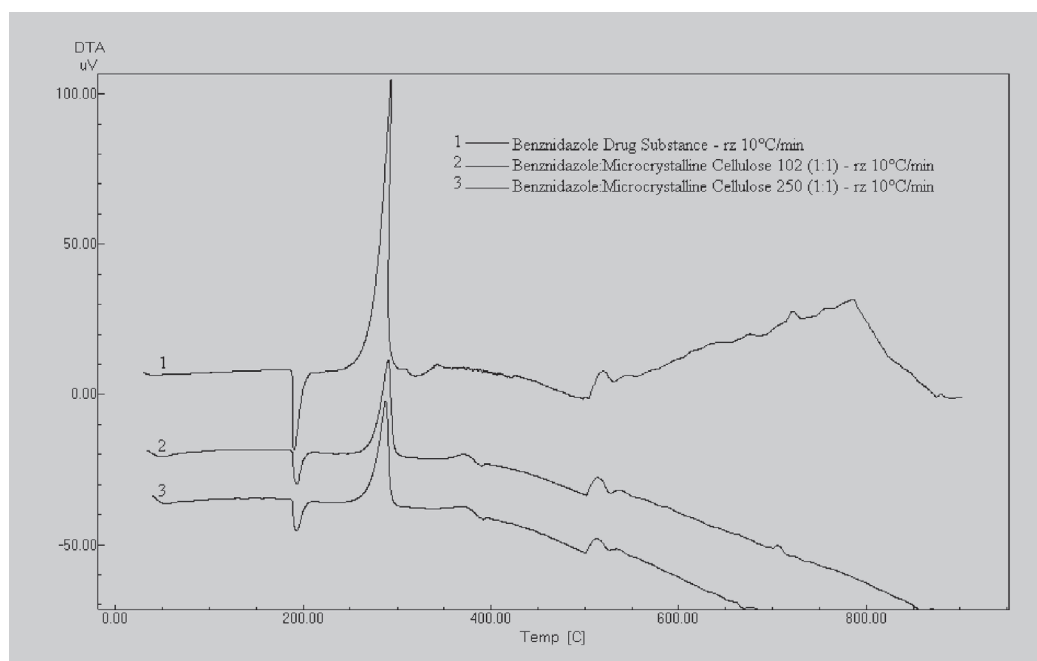


FIGURE 2 - DTA curves of Benznidazole drug substance and excipients.

Kasim *et al.*, 2004). For this reason it is not possible to propose a correlation *in vivo-in vitro* using the published drug data, but it could be proposed for a drug with this characteristics that the dissolution step will be the critical factor in the drug biodisponibility. Therefore, the pharmacokinetics profile of the developed medicine could be similar to the commercialized one, once the *in vitro* profile of dissolution is considered statistically equal.

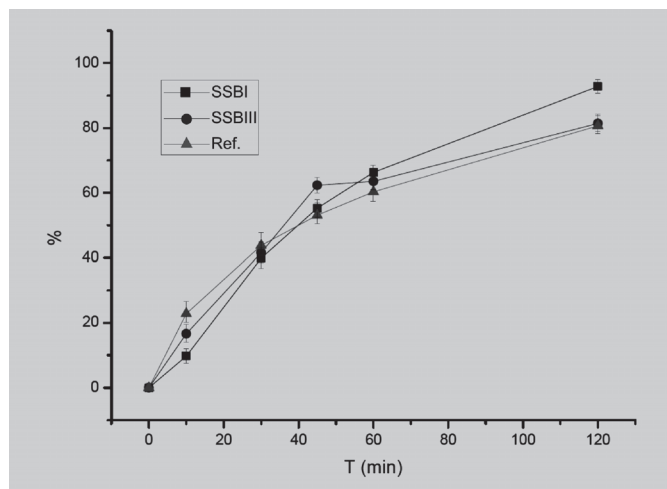


FIGURE 3 – Kinetics of *in vitro* release.

CONCLUSION

The presented results comprised the characterization of the flow and compressibility properties of seven small-scale batches mixtures and drug/excipient physical-chemical compatibility, as well as the confirmation of quality of the two batches chosen from the *in vitro* similarity with the reference medicine kinetics release.

Based on the the results the developed formulations are considered alternatives for the production of immediate release tablet based on benznidazole, using the technology of direct compression. It is a fast process, of practical attainment and production reduced cost, and the developed product has the quality demanded for oral solid pharmaceutical form.

RESUMO

Desenvolvimento e avaliação *in vitro* de comprimidos a base do antichagásico benznidazol

O trabalho teve como objetivo a verificação das possíveis interferências dos excipientes utilizados na obtenção do comprimido de liberação imediata à base de

benznidazol por meio do processo de compressão direta e realização de estudo comparativo entre os comprimidos obtidos e o medicamento de referência Rochagan®, obtido por meio da granulação por via úmida. Sete lotes de bancada (LB) foram produzidos e aspectos, tais como compressibilidade, fluxo do pós, peso médio, friabilidade, desintegração, dureza, teor, dissolução, uniformidade de conteúdo, cinética de liberação *in vitro* (dissolução) e compatibilidade físico-química fármaco/excipientes foram avaliados. Diante dos resultados obtidos pode-se verificar que os pós analisados apresentaram características adequadas para o processo da compressão direta, além da comprovação da não existência de interação de natureza físico-química entre o fármaco e os excipientes testados. Os comprimidos obtidos a partir do LB I e III foram escolhidos para o estudo comparativo frente ao medicamento de referência, demonstrando semelhança com os resultados observados, tratados estatisticamente. Torna-se, assim, uma alternativa para a produção de medicamento destinado ao tratamento da doença de Chagas com um custo reduzido e qualidade satisfatória.

UNITERMOS: Benznidazol. Excipientes. Compressão Direta. Comprimido. Doença de Chagas.

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