Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 295–302
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Study of the interaction between hydroxymethylnitrofurazone and 2-hydroxypropyl-β-cyclodextrin

Renato Grillo [a], Nathalie F.S. de Melo [a], Carolina Morales Moraes [b], Renata de Lima [b], Carla M.S. Menezes [c], Elizabeth Igne Ferreira [c], André Henrique Rosa [a], Leonardo Fernandes Fraceto [a,b,*]

[a] *Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Sorocaba, Av. Três de Março 511, CEP 18087-180, Sorocaba, São Paulo, Brazil*
[b] *Departamento de Bioquímica, Instituto de Biologia, Unicamp, Cidade Universitária Zeferino Vaz s/n, Campinas, São Paulo, Brazil*
[c] *LAPEN, Faculdade de Ciências Farmacêuticas, USP, São Paulo, Brazil*

Received 18 June 2007; received in revised form 8 January 2008; accepted 8 January 2008
Available online 16 January 2008

Abstract

Chagas disease is a serious health problem in Latin America. Hidroxymethylnitrofurazone (NFOH) is a nitrofurazone prodrug more active than nitrofurazone against *Trypanosoma cruzi*. However, NFOH presents low aqueous solubility, high photodecomposition and high toxicity. The present work is focused on the characterization of an inclusion complex of NFOH in 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). The complexation with HP-β-CD was investigated using reversed-phase liquid chromatography, solubility isotherms and nuclear magnetic resonance. The retention behavior was analyzed on a reversed-phase C_{18} column, using acetonitrile–water (20/80, v/v) as the mobile phase, in which HP-β-CD was incorporated as a mobile phase additive. The decrease in the retention times with increasing concentrations of HP-β-CD enables the determination of the apparent stability constant of the complex ($K = 6.2 \pm 0.3\ M^{-1}$) by HPLC. The solubility isotherm was studied and the value for the apparent stability constant ($K = 7.9 \pm 0.2\ M^{-1}$) was calculated. The application of continuous variation method indicated the presence of a complex with 1:1 NFOH:HP-β-CD stoichiometry. The photostability study showed that the formation of an inclusion complex had a destabilizing effect on the photodecomposition of NFOH when compared to that of the "free" molecule in solution. The mobility investigation (by NMR longitudinal relaxation time) gives information about the complexation of NFOH with HP-β-CD. In preliminary toxicity studies, cell viability tests revealed that inclusion complexes were able to decrease the toxic effect ($p < 0.01$) caused by NFOH.

Keywords: Hydroxymethylnitrofurazone; 2-Hydroxypropyl-β-cyclodextrin; Solubility isotherm; Nuclear magnetic resonance; Toxicity

1. Introduction

Chagas disease affects about one quarter of the Latin American population. According to the World Health Organization, there are about 120 million people living in risk of contracting parasitosis and 16–18 million people infected with the parasite [1]. The main problem with the treatment is the resistance of *Trypanosoma cruzi* to drugs [2]. Hydroxymethylnitrofurazone (NFOH, Fig. 1) is one of the new candidate drugs for the Chagas disease chemotherapy. It showed to be, *in vitro*, very potent against *T. cruzi* [3]. However, NFOH presents low aqueous solubility, high photodecomposition and high toxicity.

Complex formation with cyclodextrin (CD) provides a way to increase the solubility, stability and bioavailability of drugs [4,5]. CD is able to form inclusion complexes with different classes of molecules, modifying their physical, chemical and biological properties [6]. These cyclic polymers are formed by glucose molecules bound through 1–4 bonds and can be composed by 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucose units. β-Cyclodextrin (Fig. 1, R = H) has been an extensively polymer studied despite its very low aqueous solubility. Moreover, its alkylated derivatives, e.g. 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), have attracted growing interest due to their improved complexing ability, great water solubility and low toxicity. The

* Corresponding author at: Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Sorocaba, Av. Três de Março 511, CEP 18087-180, Sorocaba, São Paulo, Brazil. Tel.: +55 15 3238 3414; fax: +55 15 3228 2842.
E-mail address: leonardo@sorocaba.unesp.br (L.F. Fraceto).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.01.010

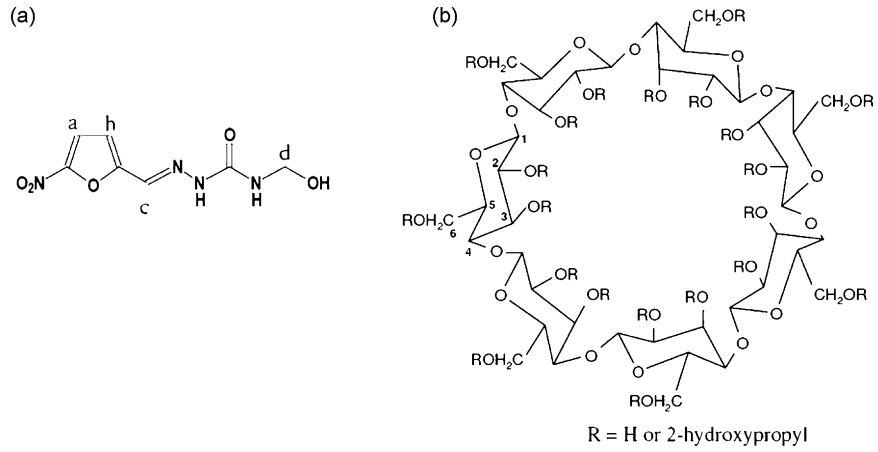

Fig. 1. Chemical structure of (a) NFOH and (b)HP-β-CD (R = H or 2-hydroxypropyl group)—schematic representation.

non-polar cavity of CDs can form inclusion compounds with a variety of guest molecules, being the binding governed by the molecular polarity and ability to closely fit within the cavity [7]. Furthermore, CD allows the accommodation of the apolar part of some molecules such as hydroxymethylnitrofurazone. No reports about the complex formation between antiChagasic compounds and cyclodextrin were found in the literature.

The aim of the present study was to characterize the inclusion complex formed between NFOH and HP-β-CD through the study of the HPLC retention behavior of hydroxymethylnitrofurazone in presence of HP-β-CD, through solubility isotherm, nuclear magnetic resonance, photostability and cytotoxic assays. This is an elementary study for the characterization of a potential formulation to be used as a therapeutic option for the Chagas disease.

2. Experimental

2.1. Reagents and chemicals

Hydroxymethylnitrofurazone was synthesized as previously described [3]; HP-β-CD was purchased from Roquette and characterized by a substitution degree of 4.2. HPLC-grade acetonitrile (ACN) was obtained from J.T. Baker and deionized water at 18 mΩ from a Waters ultra pure water system.

2.2. Effect of cyclodextrin on NFOH retention time by HPLC

The chromatographic experiments were performed using a Shimadzu SCL-10VP controller pump, a Shimadzu SIL-10AD VP auto injector, a UV–vis SPD-10A VP detector (detection: 260 nm for NFOH) and Class-VP 6.12 as software. A reversed-phase Phenomonex Gemini C_{18}, 5 μm, 10 cm × 0.46 cm was employed. The mobile phase used for these studies was acetonitrile–water (20/80, v/v), in which HP-β-CD was dissolved (0, 5, 10, 15, 20, 30 mM). The whole solution was filtered through a 0.2 μm pore size nylon membrane filter. The mobile phase was pumped at a flow rate of 1.0 mL/min. The chromatographic experiments were carried out at 25 °C. The NFOH concentration in the injected solution was 60 μM and the injection volume was 0.2 mL in all experiments.

The retention behavior of NFOH was governed by the drug partition coefficients between the mobile and stationary phases. In presence of cyclodextrins, there is an additional contribution in the drug retention behavior due to the complexation process.

The capacity factors for NFOH were monitored in the presence of increasing concentration of HP-β-CD. The apparent stability constant of the complex, K, was determined in triplicate, using equation (1) [8]:

$$\frac{1}{k'} = \frac{1}{k'_s} + \frac{K[\mathrm{CD}]^x}{k'_s} \quad (1)$$

where k' is the capacity factor at each cyclodextrin concentration [CD], and k_s' is the solute capacity factor in absence of cyclodextrin, x is a stoichiometry coefficient. For a 1:1 stoichiometry complex, a plot of $1/k'$ versus [CD] yields a straight line and K is obtained from the slope-to-intercept ratio.

2.3. Determination of the apparent stability constants

Excess amounts of NFOH were added to 10 mL glass tubes containing different concentrations of HP-β-CD. The tubes were shaken until equilibrium was reached (32 h) at 25 °C. Then, the solutions were centrifuged and the concentration of NFOH was spectrophotometrically determined at 260 nm using a Femto spectrophotometer. The presence of HP-β-CD did not interfere in the spectrophotometric assay of NFOH.

When a linear relationship was obtained between the NFOH solubility and the concentration of HP-β-CD, the diagram was classified as A_L, according to Higuchi and Connors [9] and the experimental data fit equation (2):

$$K = \frac{\text{slope}}{S_o(1 - \text{slope})} \quad (2)$$

where S_o is the molar solubility of NFOH. The apparent stability constant of the complex formed, K, is obtained from the slope of the straight line.

2.4. Photostability assays

The photostability of NFOH (60 μM) was assessed in water solution in the presence (60 μM) and absence of HP-β-CD. The suspensions were filtered through 0.22 μm membrane filters in order to obtain a clear solution. These solutions were positioned 30 cm away from a 1.8 kW xenon lamp as a light source, transmitting light corresponding to exposure behind window glass (cut-off approximately 310 nm). The solutions were inserted in an incubator at 30 °C, sampled at specified time intervals (0, 1, 2, 3, 4, 5 and 6 h) and the concentration of NFOH was determined by HPLC assay, using an analytical curve (concentration range: 10–100 μM, area = 1.6×10^7 [NFOH] − 2.7×10^4, $r = 0.999$). The results were expressed as percentages of the remaining NFOH. Each test was carried out in triplicate. The HPLC conditions were described in Section 2.2.

2.5. Study of the complex by nuclear magnetic resonance

One-dimensional ^{1}H NMR spectra were recorded on a Varian Inova 500 MHz spectrometer, in unbuffered deuterated water (D_2O) for NFOH, HP-β-CD and the complex. NFOH (0.2 mM) and HP-β-CD (0.2 mM) stock solutions were mixed in 5 mm NMR tubes, in a total sample volume of 600 μL, and left 32 h for equilibration before the NMR analysis. The probe temperature was regulated to 25 °C.

The ^{1}H NMR spectra were recorded using a simple pulse-acquire sequence with solvent presaturation. Typical acquisition parameters consisted of 32 K points covering a sweep width of 6000 Hz, a pulse width (pw 90) of 10 μs and digital zero filling to 128 K; a 0.5 Hz exponential function was applied to FID before Fourier transformation. Resonance at 4.81 ppm, which presents a residual solvent peak, was used as internal reference. Data were collected without an external reference to avoid possible interactions with HP-β-CD.

2.5.1. Study of the complex stoichiometry

The continuous variation method was adopted to determine the stoichiometry of the complex (Djedaïne et al.). ^{1}H NMR spectra were obtained for a series of NFOH:HP-β-CD mixtures, in which the total initial concentration of both species was kept constant (0.2 mM) but the mol fraction of each component varied from 0 to 1 [10].

2.5.2. Relaxation times measurement (T_1)

The relaxation time measurements (T_1) experiments were recorded at 25 °C on a Varian Inova 500 MHz NMR spectrometer. Samples were degassed, bubbling nitrogen slowly for 5 min through the solution using a thin hard plastic tube (PTFE 1/32 in. diameter), to avoid the interference of dissolved O_2. For ^{1}H NMR, the 90° pulse was typically of 10–15 μs and the recycling time was set to three times the largest value of T_1, i.e., 15 s [11]. Longitudinal relaxation times were obtained by the conventional inversion-recovery method.

2.6. Cell culture and cytotoxic assays

Balb/c mice fibroblasts (3T3 cells) were cultured in DMEN supplemented with 15% fetal bovine serum, 100 UI/mL penicillin and 100 μg/mL streptomycin sulfate (pH 7.2–7.4) under a humidified atmosphere, at 37 °C and 5% CO_2. Cells were seeded (1×10^4 cells/well) in 98 wells tissue culture plates and cultured for 48 h. The cells were incubated for 3 and 24 h with three different concentrations, 0.050, 0.075 and 0.100 mM of the vehicle (NFOH, HP-β-CD or complex). Cell viability was assessed by the tetrazolium reduction [12] (MTT test). 0.5 mg/mL of MTT was incubated for 3 h with treated 3T3 cells, at 37 °C. The number of viable cells was determined by measuring the amount of MTT converted to formazan by mitochondrial dehydrogenases [12]. The formazan crystals formed were dissolved in ethanol and shaken for 20 min at room temperature. Cytotoxic assays data were analyzed by one-way analysis of variance (one-way ANOVA) with Tukey–Kramer as a post hoc test. Statistic significance was defined as $p < 0.05$.

3. Results and discussion

3.1. Chromatographic determination of the apparent stability constant

When cyclodextrins are added to the mobile phase, solute retention is driven by the drug partition between the mobile and stationary phases and the solute complexation with the cyclodextrins. According to the solute retention time and the void time, capacity factors were calculated for each solute in the presence of increasing concentrations of HP-β-CD. As expected, the retention times decrease as the concentration of HP-β-CD in the mobile phase increases due to the formation of the analyte–cyclodextrin complex, which enhances the guest solubility in the mobile phase and reduces its residence time in the column [13,14] (Fig. 2). The variation in the retention times obtained by replication was always lower than 1%.

The above mentioned formation constant for the NFOH:HP-β-CD complex was calculated according to Fig. 3 and Eq. (1). The linear relationship between $1/k'$ and HP-β-CD concentration (Fig. 3) with correlation coefficient higher than 0.99 indicates that the NFOH behavior is well described by the model, assuming a 1:1 stoichiometry between the guest and HP-β-CD [15,16].

The apparent stability constant of NFOH with HP-β-CD was $6.2 \pm 0.3\ M^{-1}$ ($1/k' = 5.72$ [HP-β-CD] + 0.92), indicating a weak interaction in this complex formation at these chromatographic conditions.

3.2. Determination of the apparent stability constant by solubility isotherm

The solubility enhancements obtained with cyclodextrins have been widely employed in the improvement of drugs bioavailability [17,18]. Despite the fact that the solubility

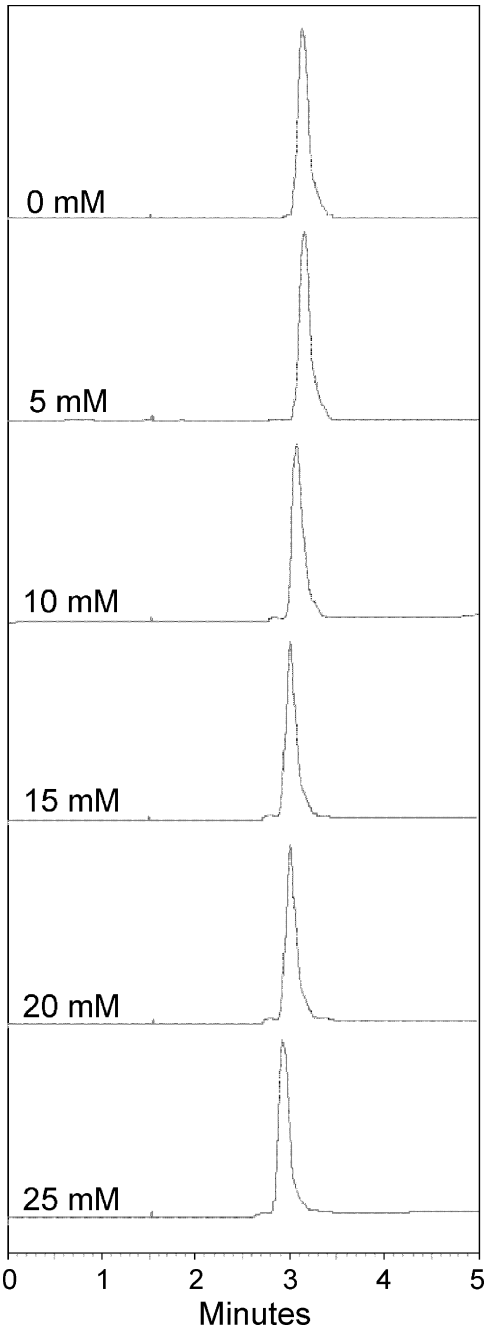

Fig. 2. Decrease in NFOH retention time in the presence of increasing concentrations of HP-β-CD (0, 5, 10, 15, 20, 30 mM) at 25 °C. Chromatographic conditions—column: Phenomenex C18, 5 μm, 10 cm × 0.46 cm; mobile phase: acetonitrile–water (20/80, v/v).

isotherm for NFOH and HP-β-CD (Fig. 4) has presented a low increase in solubility, it has occurred as a linear function of HP-β-CD concentration, corresponding to the A_L-type profile defined by Higuchi and Connors [9]. This relationship suggests the formation of a 1:1 NFOH:HP-β-CD complex. The apparent stability constant (K) determined from the slope and the intercept ([NFOH] = 0.010 [HP-β-CD] + 1.28, correlation coefficient, $r = 0.995$) of this plot was $7.9 \pm 0.2\ M^{-1}$, indicating the formation of a weak complex [19].

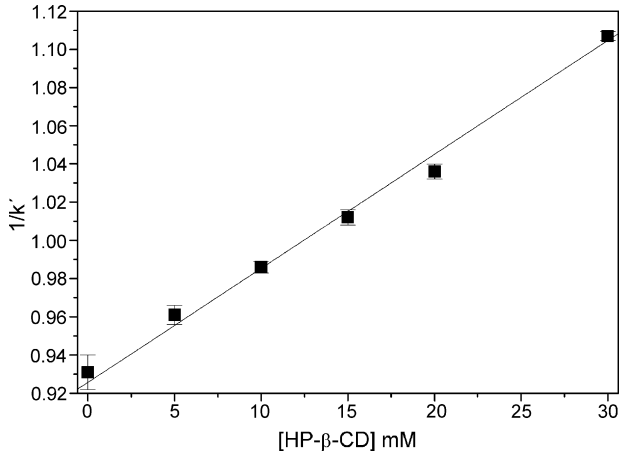

Fig. 3. Plot of 1/k vs. HP-β-CD concentration for NFOH at 25 °C. Chromatographic conditions—the same used in Fig. 2.

The increase in solubility caused by the complexation of NFOH with HP-β-CD was lower than those reported for others drugs in literature [20,21]. The apparent stability constant obtained is similar to the one measured by chromatography experiments.

3.3. Photostability assays

In this study, the photostability of NFOH and NFOH:HP-β-CD inclusion complex were examined in aqueous solution. A control was carried out using a solution of NFOH in water, in absence of cyclodextrin. The data produced indicated that HP-β-CD retarded the photodegradation of NFOH, making it more stable in the presence of light (Fig. 5). The same effect is described in the literature for other drugs [22,23].

The data revealed that the addition of HP-β-CD was not able to completely inhibit the photochemical decomposition of NFOH. HP-β-CD decreases the observed apparent-first-order rate of photochemical decomposition of NFOH in a non-linear relationship. This result was consistent with a kinetic system in which a free drug is degraded at higher rates than the drug in the

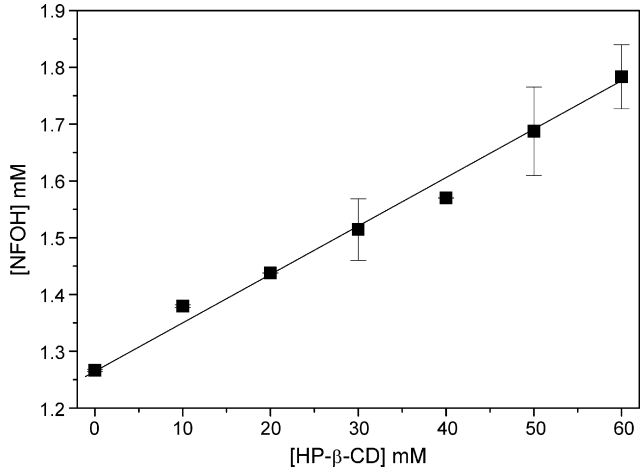

Fig. 4. Solubility diagram of NFOH (mM) with HP-β-CD (mM) at 25 °C.

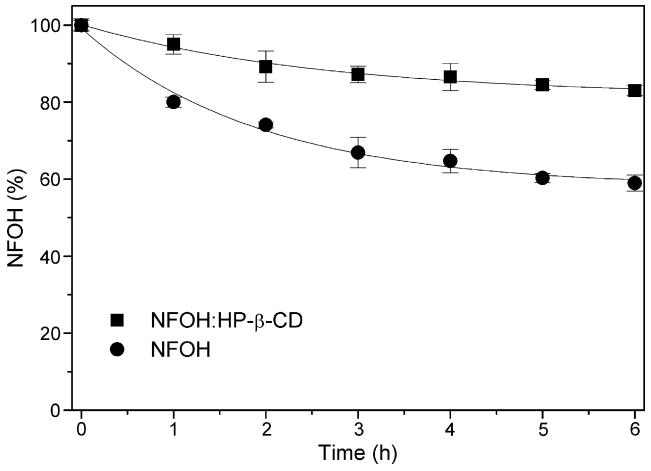

Fig. 5. Photodegradation profiles of NFOH under photoexposure radiation of an aqueous solution containing NFOH free and complexed with HP-β-CD.

complex form [23]. Photochemical degradation constants (k_{obs}) for NFOH in the absence and presence of HP-β-CD were determined by the data from Fig. 5, using the first-order kinetics. The values of k_{obs} for NFOH in absence and presence of HP-β-CD are $5.19 \times 10^{-1} \pm 0.03\,min^{-1}$ and $3.83 \times 10^{-1} \pm 0.07\,min^{-1}$, respectively. The formation of an inclusion complex had a stabilizing effect on NFOH regarding the photodecomposition compared to that of the "free" molecule in solution [24]. Based on the binding constant between HP-β-CD and NFOH and on the concentrations of HP-β-CD and NFOH under the experimental conditions, about 4% of the NFOH can form an inclusion complex with HP-β-CD. However, the observed k_{obs} values are far beyond the expected maximum effect, indicating that not only the inclusion complex formation but also other mechanisms must be considered [25].

3.4. Study of the complex by nuclear magnetic resonance

NMR spectroscopy is the most powerful tool for the study of inclusion complex formation between CDs and a variety of guest molecules, which has been successfully used to confirm the conformations of inclusion complexes [26].

The interaction of NFOH and HP-β-CD was investigated by NMR, which revealed the stoichiometry of the complex and the dynamic properties of NFOH in the presence of cyclodextrin.

Fig. 6 and Table 1 show the ^{1}H NMR spectra of NFOH, HP-β-CD and NFOH in the presence of HP-β-CD as well as the NMR chemical shifts and assignments, respectively. The assignments of NFOH [3] and HP-β-CD [27–30] are in agreement with the literature.

Chemical shifts variations of specific host or guest nucleus could provide evidence for the formation of inclusion complexes in solution, since significant changes in microenvironment are known to occur between the free and bound states. Information about the interaction of NFOH and HP-β-CD is primarily inferred from the changes in chemical shifts. From Fig. 6 and Table 1, it can be seen that the H_3 hydrogens, located inside the cavity close to the end of the HP-β-CD, shift up-field. The other HP-β-CD hydrogens present changes in the chemical shift

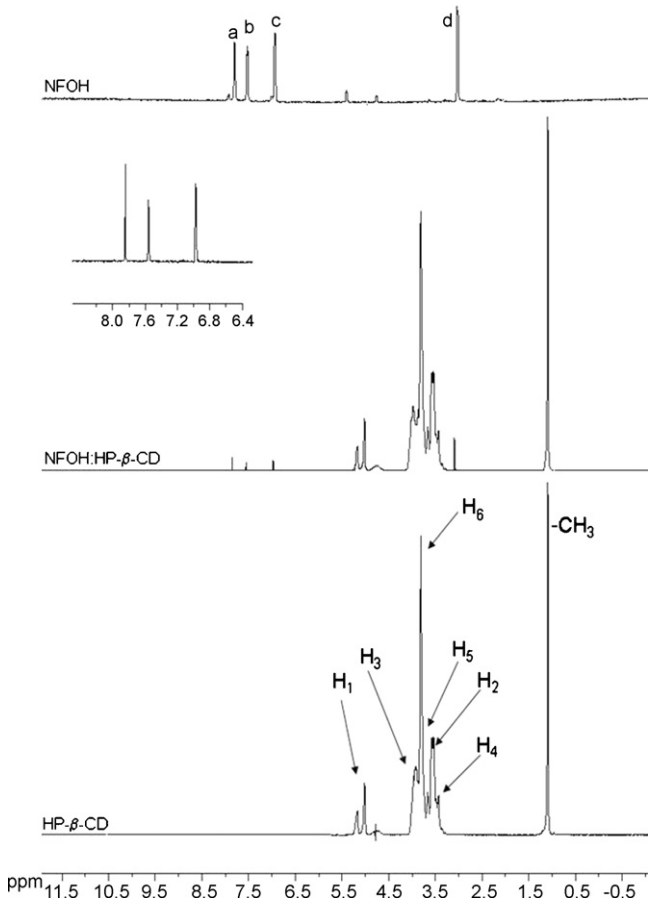

Fig. 6. ^{1}H NMR spectra (500 MHz) of NFOH (0.2 mM), HP-β-CD (0.2 mM) and NFOH:HP-β-CD (1:1 molar ratio). Samples in D_2O at 25 °C.

<0.010 ppm. The effect on H_3 hydrogens in comparison to H_5 (and H_6) was higher, thus it can be assumed that NFOH was preferentially inserted into the HP-β-CD close to secondary hydroxyls [27]. For NFOH, all the hydrogens presented up-field shift.

Table 1
^{1}H chemical shifts (ppm) data and assignment of NFOH hydrogens (in the presence and absence of HP-β-CD) and HP-β-CD (in presence and absence of NFOH), at 25 °C in D_2O

Hydrogen	$\delta_{absence}$	$\delta_{presence}$	$\Delta\delta$
NFOH			
H_a	7.610	7.685	0.075
H_b	7.512	7.563	0.051
H_c	6.803	6.833	0.030
H_d	2.821	2.849	0.028
HP-β-CD			
H_1	5.114	5.115	0.001
H_2	3.674	3.677	0.003
H_3	4.030	4.092	0.062
H_4	3.540	3.541	0.001
H_5	3.758	3.767	0.009
H_6	3.918	3.923	0.005

[NFOH] and [HP-β-CD] = 0.2 mM. The chemical shifts are referenced by the residual solvent peak (H_2O and HDO) at 4.81 ppm.

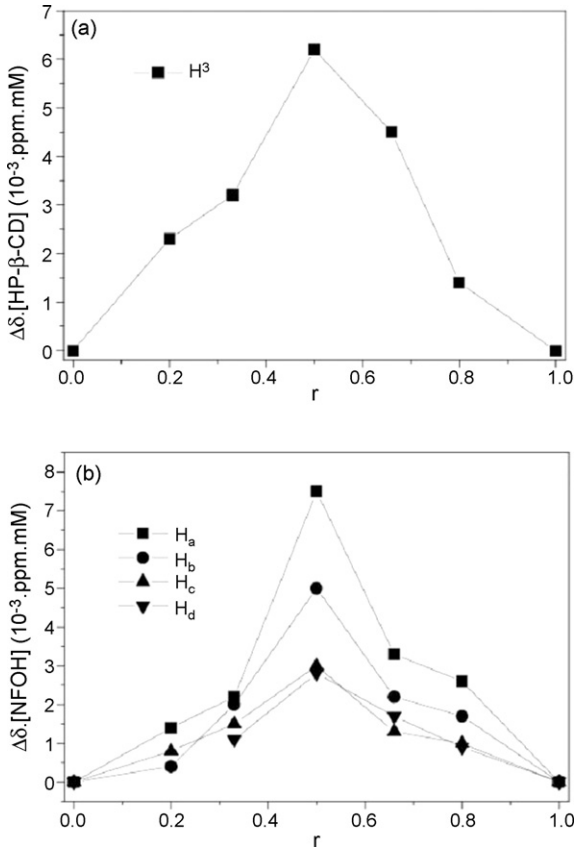

Fig. 7. Job's plots corresponding to the chemical shift displacement of (a) HP-β-CD (H_3) and (b) NFOH hydrogens.

3.4.1. Stoichiometry

The continuous variation method was employed to establish the stoichiometry of the complex using NMR. If a physical parameter directly related to the concentration of the complex is plotted as a function of the mol fraction (r) of NFOH or HP-β-CD, its maximal value occurs at $r_{NFOH} = m/(m+n)$ or $r_{HP\text{-}\beta\text{-}CD} = n/(m+n)$, where m and n are the molar ratios of NFOH and HP-β-CD in the complex, respectively. In the NMR spectra and under fast conditions, for a signal belonging to NFOH, for example, the calculated quantity $\Delta\delta$. [NFOH] will be proportional to the complex concentration, being able to be plotted against r [10].

The continuous variation method was applied for all hydrogens of the molecules (host and guest) and yielded identical results. The NFOH and HP-β-CD that experienced the largest shifts are reported in Fig. 7.

In all cases, Job's plots show a maximum value at $r = 0.5$ and high symmetrical shape, indicating the existence of a complex with a 1:1 stoichiometry, within the range of the investigated concentrations. These results were in agreement with the chromatography and phase-solubility studies between NFOH and HP-β-CD.

In general, inclusion complex formation is a diffusion-controlled process; so, most of the inclusion complex formation can instantly reach the equilibrium. Thus, for the HP-β-CD:NFOH inclusion complex, the complexation-induced shift in ^{1}H NMR were utilized for estimating the complexation molar ratio and these comportaments were observed for a fast equilibrium process

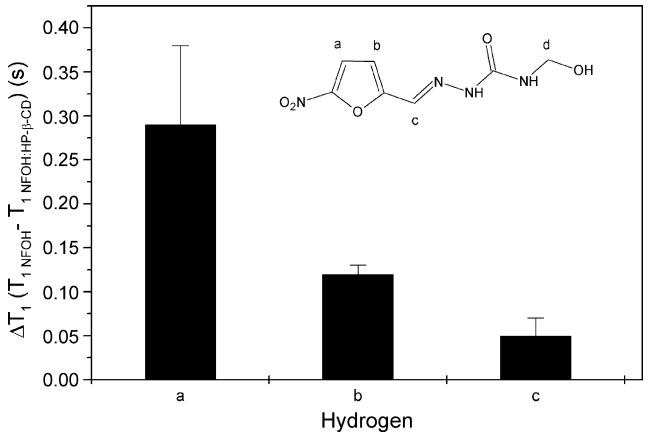

Fig. 8. Changes in the longitudinal relaxation times (ΔT_1, s) of NFOH at 25 °C, 500 MHz.

3.4.2. Dynamic properties

The longitudinal relaxation times (T_1) give information about the nucleus mobility in the solution. To study the interaction between host and guest molecules, the measurement of T_1 can give information about which region of the molecule presents mobility changes after the interaction with another molecule [11]. Fig. 8 shows the ΔT_1 variation of the NMR signals of the hydrogen from NFOH in the presence and absence of HP-β-CD.

Spin-lattice relaxation times (T_1) were measured for NFOH hydrogens before and after the addition of HP-β-CD. A reduction in T_1 values of the hydrogens from the nitroheterocyclic group of the NFOH molecule is an indicative that the movement of the whole molecule is changed in the presence of HP-β-CD [31]. Owens et al. [31] have showed that the reduction in T_1 values for hydrogens of molecule on addition of CD molecules was reported to be due to increased correlation time, τ_c, confirming the restricted rotation of hydrogens atoms as a result of a probably existence of a complex in an aqueous solution.

The effect on the T_1 of the hydrogens from NFOH in the presence of cyclodextrin is due to the interaction between these molecules and not due to possible changes in the solution viscosity, since the CD concentration was very small (0.2 mM)

3.5. Cell culture and cytotoxic assays

Measurement of NFOH, complex and HP-β-CD effects on 3T3 cell viability was a way to evaluate the cytotoxicity of these chemical substances. The treatment of 3T3 cells was realized in three different concentrations (0.05, 0.075 and 0.100 mM) of the compounds. Fig. 9 shows the concentration effect on the cell viability of 3T3 (%) after 3 and 24 h of incubation.

The effect of increasing concentrations of the compounds in the cell viability seems to be an increase in the cytotoxic effect. NFOH reduces cell viability up to values of 69 and 40% at 0.100 mM of NFOH for 3 and 24 h, respectively, indicating that the increase in concentration causes more cytotoxic effect than the observed for NFOH:HP-β-CD and HP-β-CD.

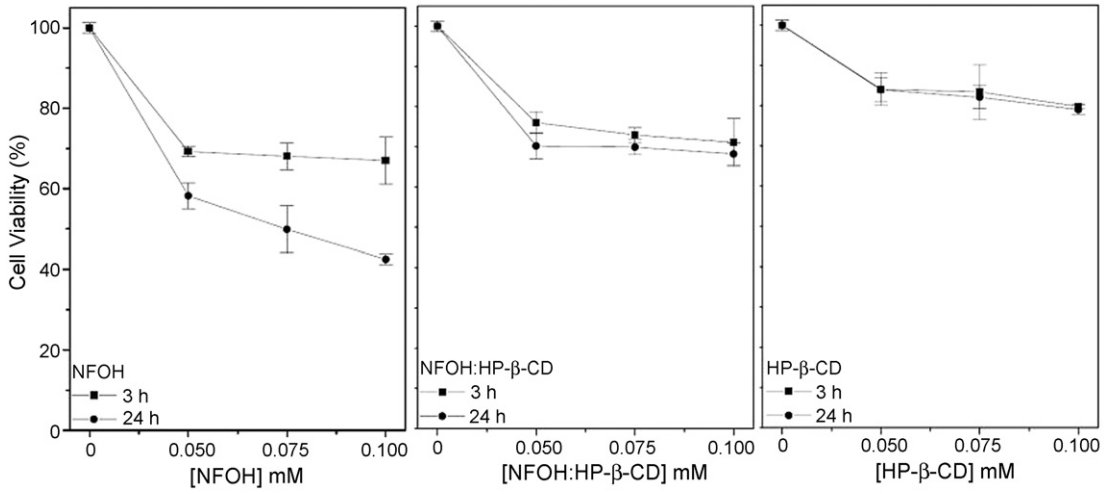

Fig. 9. Cytotoxic effects of NFOH, NFOH:HP-β-CD, HP-β-CD at 0.05, 0.075 and 0.100 mM on Balb/c 3T3 cells incubated for 3 h at 37 °C and 5% CO_2 as evaluated by MTT reduction test. Data expressed as % cell viability (mean ± S.D., $n = 8$ experiments). Cell viability without NFOH, CD and complex is 100% (data not shown).

The NFOH:HP-β-CD induced maximum effects at 0.100 mM, values comparable to the ones obtained by HP-β-CD, i.e., not affecting cell viability up to 3 and 24 h ($p < 0.001$), as compared to NFOH.

One of the most important considerations about this *in vitro* toxicity model is that NFOH induced cytotoxic effects in a dose-dependent manner and the cellular protective effects observed after the treatment with NFOH:HP-β-CD could be explained by the sustained release of NFOH from HP-β-CD cavity. These results were in agreement to related study in literature [32,33].

Therefore, since free HP-β-CD showed low activity over the concentration range used in this study, the low cytotoxicity of the NFOH:HP-β-CD complex compared to free NFOH was attributed to the formation of a host–guest complex between NFOH and HP-β-CD, thereby avoiding the NFOH side effects.

Cyclodextrins accelerate or decelerate various types of reactions, with kinetic features similar to those of enzyme reactions, i.e., catalyst–substrate complex formation, competitive inhibition, saturation and stereospecific catalysis [34]. The most important primary consequence of the interaction between a poorly soluble guest and a cyclodextrin in aqueous solution that could explain the reduction in the cytotoxicity of NFOH when complexed with HP-β-CD is a modification in the reactivity of the included molecule. In most cases, the reactivity decreases due to guest stabilization, but in other cases, the cyclodextrin behaves as an artificial enzyme that can accelerate and modify the reaction pathway [34] . The rate of reaction is changed by the inclusion because the guest is transferred from the polar environment of water to a less polar one of the cyclodextrin cavity, i.e., there is a microsolvent effect. The reaction rate increases when flexible guest molecules are forced to adjust to a reactive conformation and vice versa [35].

4. Conclusion

This study showed the physical–chemical characterization and *in vitro* evaluation of an inclusion complex of NFOH and HP-β-CD. Based on the results reported here, it is possible to see that the complexation of NFOH with HP-β-CD modified the physicochemical characteristics of the guest molecule and diminished its cytotoxicity. Then, we believe that the inclusion complex between NFOH and cyclodextrin might be a potential formulation as a therapeutic option for the treatment of the Chagas disease.

Acknowledgments

This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (05/03045-9). R.G. (06/00787-7) and C.M.M. (04/02091-4) are the recipients of fellowships from FAPESP; C.M.S.M. is the recipient of fellowship from Prodoc-CAPES (00019-08-03); E.I.F. is the recipient of a fellowship from CNPq. We would like to thank the reviewer's comments, which helped to improve the quality of the manuscript. L.F. Fraceto dedicate this work to Profa. Dra. Eneida de Paula (Depto of Biochemistry/IB/Unicamp by contribution in his academic formation, scientific collaboration and friendship.

References

[1] WHO. Chagas disease: strategic direction for research. Disease burden and epidemiological trends, http://www.who.int/tdr/diseases/chagas/direction.htm, accessed in January 2007.
[2] J.R. Coura, S.L. de Castro, Mem. Inst. Oswaldo Cruz 97 (2002) 3–24.
[3] M.C. Chung, R.V.C. Guido, T.F. Martinelli, M.F. Gonçalves, M.C. Polli, K.C.A. Botelho, E.A. Varanda, W. Colli, M.T.M. Miranda, E.I.F. Ferreira, Bioorg. Med. Chem. 11 (2003) 4779–4783.
[4] A. Karim, S. Ahmed, R. Siddiqui, Am. J. Med. 111 (2001) 150–153.
[5] G. Dollo, D.O. Thompson, P. Le Corre, F. Chevanne, R. Le Verge, Int. J. Pharm. 164 (1998) 11–19.
[6] R.A. Rajewski, V.J. Stella, J. Pharm. Sci. 85 (1996) 1142–1169.
[7] K.A. Connors, Chem. Rev. 97 (1997) 1325–1357.
[8] J.L. Atwood, J.E.D. Davies, D.D. Macnicol, F. Vögtle, in: J. Szejtli, T. Osa (Eds.), Comprehensive Supremolecular Chemistry, Elsevier Science Ltd., Oxford, 1996.
[9] T. Higuchi, K.A. Connors, Adv. Anal. Chem. Inst. 4 (1965) 117–212.
[10] F. Djedaïne, S.Z. Lin, B. Perly, D. Wouessidjewe, J. Pharm. Sci. 79 (1990) 643–646.

[11] L.F. Fraceto, A. Spisni, S. Schreier, E. de Paula, Biophys. Chem. 115 (2005) 11–18.
[12] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[13] K. Uekama, F. Hirayama, T. Irie, Chem. Lett. 661 (1978).
[14] K. Uekama, F. Hirayama, S. Nasu, N. Matsuo, T. Irie, Chem. Pharm. Bull. 26 (1978) 3477–3484.
[15] C. Ravelet, A. Geze, A. Villet, C. Grosset, A. Ravel, D. Wouessid-jewe, E. Peyrin, J. Pharm. Biomed. Anal. 29 (2002) 425–430.
[16] C.M. Moraes, P. Abrami, E. de Paula, A.F. Braga, L.F. Fraceto, Int. J. Pharm. 331 (2007) 99–106.
[17] F. Hirayama, K. Uekama, Adv. Drug Deliv. Rev. 36 (1999) 125–141.
[18] S. Tommasini, D. Raneri, R. Ficarra, M.L. Calabró, R. Stancanelli, P. Ficarra, J. Pharm. Biomed. Anal. 35 (2004) 379–387.
[19] Y.L. Loukas, V. Vraka, G. Gregoriadis, J. Pharm. Biomed. Anal. 16 (1997) 263–268.
[20] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017–1025.
[21] C. Gazpio, M. Sánchez, I.X. García-Zubiri, I. Vélaz, C. Martinez-Ohárriz, C. Martín, A. Zornoza, J. Pharm. Biomed. Anal. 9 (2004) 487–492.
[22] K.L. Yap, X. Liu, J.C. Thenmozhiyal, P.C. Ho, Eur. J. Pharm. Sci. 25 (2005) 49–56.
[23] C.Y. Chen, F.A. Chen, A.B. Wu, H.C. Hsu, J.J. Kang, H.C. Cheng, Int. J. Pharm. 141 (1996) 171–178.Wednesday, February 13, 2008 at 4:55 am
[24] H.H. Tonnesen, M. Másson, T. Loftsson, Int. J. Pharm. 244 (2002) 127–135.
[25] S. Tommasini, M.L. Calabro, P. Donato, D. Raneri, G. Guglielmo, P. Ficarra, R. Ficarra, J. Pharm. Biomed. Anal. 35 (2004) 389–397.
[26] H.J. Schneider, F. Hacket, V. Rudiger, Chem. Rev. 98 (1998) 1755–1785.
[27] H.L. Ma, J.J. Wu, W.J. Liang, J.B. Chao, J. Incl. Phenom. Macrocyclic Chem. 58 (2007) 221–226.
[28] C. Jullian, S. Miranda, G. Zapata-Torres, F. Mendizabal, C. Olea-Azar, Bioorg. Med. Chem. 15 (2007) 3217–3224.
[29] N.E. Polyakov, T.V. Leshina, E.O. Hand, A. Petrenko, L.D. Kispert, J. Photochem. Photobiol. A: Chem. 161 (2004) 261–267.
[30] G. Xiliang, S. Shaomin, D. Chuan, F. Feng, M.S. Wong, Spectrochim. Acta Part A 61 (2005) 413–418.
[31] P.K. Owens, A.F. Fell, M.W. Coleman, M. Kinns, J.C. Berridge, J. Pharm. Biomed. Anal. 15 (1997) 1603–1619.
[32] C.M. Moraes, D.R. Araújo, M.G. Issa, H.G. Ferraz, F. Yokaichiya, M.K.K.D. Franco, I. Mazzaro, P.S. Lopes, M.M. Gonçalves, E. de Paula, L.F. Fraceto, Rev. Ciênc. Farm. Basica Apl. 27 (2006) 207–212.
[33] D.R. Araújo, S.S. Tsuneda, C.M.S. Cereda, F.D.G.F. Carvalho, P.S.C. Preté, S.A. Fernandes, F. Yokaichiya, M.K.K.D. Franco, I. Mazzaro, L.F. Fraceto, A.F.A. Braga, E. de Paula, Eur. J. Pharm Sci. 33 (2008) 60–71.
[34] J. Szejtli, Chem. Rev. 98 (1998) 1743–1754.
[35] D.W. Griffiths, M.L. Bender, J. Am. Chem. Soc. 95 (1973) 1679–1680.