J. Braz. Chem. Soc., Vol. 10, No. 4, 281-286, 1999.
Printed in Brazil.

0103 – 5053 $6.00 + 0.00

Article

Determination of Correlation Times from Selective and Non-Selective Spin-Lattice Relaxation Rates and their Use in Drug-Drug and Drug-Albumin Interaction Studies

Luzineide Wanderley Tinoco, and José Daniel Figueroa-Villar*

Departamento de Química do Instituto Militar de Engenharia,

Praça General Tibúrcio, 80 - Urca, Rio de Janeiro - RJ, Brazil

Os efeitos da variação da concentração da amostra nos valores de deslocamento químico e nas velocidades de relaxação spin-rede seletiva (R_1^S) e não seletiva (R_1^{NS}) foram medidos em solução para os três isômeros das guanil hidrazonas derivadas do nitrobenzaldeído (NBGH) puros e com a albumina do soro bovino (BSA). Os resultados foram usados para determinar o tempo de correlação (τ_c), mostrando que o grau de interação intermolecular droga-droga varia com a posição do grupo nitro no anel aromático e que este grau de associação interfere na interação destas drogas com a BSA. Os resultados sugerem que o grau de interação droga-droga e droga-BSA estão relacionados com a atividade *in vitro* destas drogas contra o *Trypanosoma cruzi*.

The effects of the changes in sample concentration on the NMR chemical shifts and on the selective and non-selective spin-lattice relaxation rates (R_1^S and R_1^{NS}) of the three isomers of nitrobenzaldeyde guanyl hydrazone (NBGH) pure and with bovine serum albumin (BSA) were measured in solution. The results were used to determine the correlation times (τ_c), showing that the degree of intermolecular drug-drug association varies with the nitro group position on the ring and that this degree of association interferes with the interaction of these drugs with BSA. The results suggest that the degree of drug-drug and drug-BSA association are related to the *in vitro* anti-*Trypanosoma cruzi* activity of these compounds.

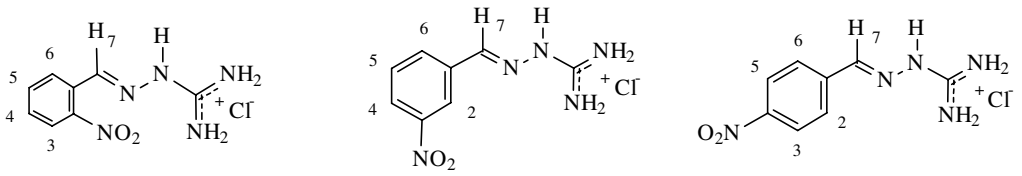
Keywords: *intermolecular interactions, relaxation rates, correlation times, guanyl hydrazones*

Introduction

Aromatic guanyl hydrazones are a new family of cationic compounds which we have shown to be potential candidates for the chemotherapy of Chagas disease[1]. This disease is third in the ranking of importance for the World Health Organization, and is responsible for about 45.000 deaths per year in South America. The transfusion of blood contaminated with *T. cruzi* is the second most important way of infection of this disease in endemic areas, and the only way of contagion in countries outside the endemic zone[2,3]. The probability of receiving a blood transfusion with blood contaminated with this protozoon can be as high as 2.19% in certain regions of South America[4]. Some guanyl hydrazones are able to eliminate the trypomastigote forms of *Trypanosoma cruzi* from contaminated blood in concentrations as low as 17 μM, making them good candidates for the prophylaxis of blood in blood banks in endemic areas[1]. The mechanism of action of these cationic antibiotics is still unknown, but it is believed that it may be related to their capacity to interact with either the cellular membrane or the DNA of the parasite[5]. In any case, it is clear that this interaction occurs through the cationic side chain. In order to determine if the guanyl hydrazones can be safely used in blood prophylaxis it necessary, among other studies, to determine the type and degree of their interaction with plasmatic proteins.

In our previous studies we showed, using non-selective T_1 studies, that the guanyl hydrazones derived from nitrobenzaldeyde (NBGH - Fig. 1) interact with bovine serum albumin (BSA) with different affinities[6]. These compounds showed different activity depending on the isomer, for 2-nitrobenzaldehyde (2NBGH, ID_{50} 87.5 μM), 3-nitrobenzaldehyde (3NBGH, ID_{50} 182.6 μM) and 4-nitrobenzaldehyde (4NBGH, ID_{50} 55.9 μM)[1]. Our initial hypothesis to explain this difference in activity was based on the possible

e-mail d5figuer@epq.ime.eb.br



2NBGH 3NBGH 4NBGH

Figure 1. Nitrobenzaldeyde Guanyl Hydrazones (NBGH).

different degrees of drug-drug association, which could affect the availability of the cationic side chain of the drug for interaction with the parasite. This hypothesis was also supported by molecular modeling studies[6]. In this work, selective and non selective spin-lattice relaxation rates measurements were used to study the intermolecular interactions between the NBGH and BSA and the importance of the self-association of the nitroguanyl hydrazones on their interaction with BSA and their *in vitro* activity.

The measurement of selective and non selective spin-lattice relaxation rates was suggested by Freeman *et al* to ascertain whether the relaxation mechanism is dipolar or other, such as chemical shift anisotropy or spin rotation[7]. The use of mono-, bi- and non selective spin-lattice relaxation rates have been applied to conformational studies of amino acids and peptides and in studies of interactions between small molecules with macromolecules[8-14].

To study systems were there is fast conversion between the bound and free states, as is usually the case for the drug-macromolecule complexes, it have been demonstrated that selective spin-lattice relaxation rates (R_1^S) are more sensitive to the process than the non selective spin-lattice relaxation rates (R_1^{NS})[8-14].

In a multi-spin system the relaxation rate can be approximated by a sum of pairwise ^1H-^1H dipolar interactions, yielding an initial rate constant for the recovery of the I_z magnetization given by Eq. 1[12-15].

$$R_1^{NS} = \sum_{i \neq j} \rho_{ij} + \sum_{i \neq j} \sigma_{ij} \quad (1)$$

In this equation ρ_{ij} is the direct relaxation rate and σ_{ij} is the cross relaxation term for a *ij* proton pair.

Selective excitation inverts only the magnetization of spin *i* while not perturbing all other spins ($j \neq i$). Consequently, the cross-relaxation rates do not contribute to the initial recovery constant, which is now given by Eq. 2[12-15]:

$$R_1^S = \sum_{i \neq j} \rho_{ij} \quad (2)$$

Some researchers have shown that R_1 can be measured in the initial rate approximation with the 180° - τ - 90° sequence provided that the 180° pulse is selective and that, in the extreme narrowing limit ($\omega\tau_c << 1$ - were ω is the Larmor frequency and τ_c is the correlation time) the R_1^{NS} / R_1^S ratio is 1.5[12-15].

Experimental

The three nitroguanyl hydrazones used in this work were prepared by treatment of the respective nitrobenzaldehydes with aminoguanidine hydrochloride in refluxing ethanol containing a catalytic amount of HCl[1]. The solutions of the pure nitroguanyl hydrazones (13 mM, 25 mM, 50 mM and 75 mM) were prepared in acetate buffer 0.1 M in D_2O with pD 4.75 at 20 °C. The solutions for the interaction studies were prepared by dissolving the respective NBGH until the desired concentration (13 mM, 25 mM, 50 mM and 75 mM) in a 7.25 x 10^{-5} M stock solution of BSA (98% - *Aldrich Chemical Company*). NMR measurements were carried out on a Varian Unity -300 (300 MHz) spectrometer at 37.0 ± 0.1 °C. Spin-lattice relaxation rates were measured with the inversion-recovery pulse sequence with the first relaxation delay varying from 12.0 s to 32.0 s, depending on the sample concentration, and the second delay from 12.5 ms to 32.0 s. Nine points were used for each measurement. For non selective measurements the 90° ^1H pulse length was 16 μs with 58 dB transmitter power (attenuation of 5dB). For the selective experiments, the selective inversion pulse was achieved by replacement of the hard 180° pulse of the inversion-recovery sequence by a DANTE[16] train consisting of 300 identical hard pulses of the same nutation angle (0.6°) separated by 0.0001 ms delays with the transmitter power adjusted to 30 dB (28 dB attenuation in relation to the non-selective pulse) to give a selective 180° ^1H pulse length of 702.4 μs. All the reported relaxation rate results are the average of at the least three measurements. In all cases, the relative uncertainty was less than 20%, with most results having uncertainties lower than 10%. The absolute uncertainty of the R_1^{NS} / R_1^S ratios was calculated according to standard procedures[17], and was always equal to or less than ±0.1.

Results and Discussion

In order to determine the tendency to self association of the pure drugs, the selective and non-selective relaxation rates were measured in solutions with different concentrations for the three isomers. These data were then used to estimate the correlation times associated using Fig. 2, which can be plotted form Eqs. 1 and 2 assuming a constant distance for the *ij* dipolar interaction[14]. The results are summarized in Tables 1, 2 and 3.

The analysis of selective and non-selective relaxation rates values for the 13 mM and 25 mM solutions of pure 2NBGH (Table 1) show that the R_1^{NS} / R_1^{S} ratio for this compound is around 1.3. This value, for which $\omega\tau_c$ is very close to 1, is expected when the mechanism for relaxation is other than pure dipolar, and the molecule is outside the extreme narrowing region ($\omega\tau_c << 1$), that is, its hydrogens present intermediate τ_c values, which are typical of medium size molecules (see Fig. 2). For the 50 and 75 mM samples, the values of the R_1^{NS} / R_1^{S} ratio are approximately 1.0. In this case, it is possible to say that the molecule is near the $\omega\tau_c \cong 1$ ($\omega\tau_c$ is slightly above 1) and that its hydrogens also present intermediate correlation time values, which are more elevated than those observed for the 13 mM and 25 mM concentrations. Since 2NBGH is a small molecule, these results could only be explained if there is drug-drug intermolecular association, with this interaction being favored at higher concentrations.

On the other hand, the results for 3NBGH, which are summarized in Table 2, show values for the R_1^{NS} / R_1^{S} ratio around 1.0 for the 13 and 25 mM solutions. According with Fig. 2, it is observed that this region corresponds to values of $\omega\tau_c$ of approximately 1, that is, the 3NBGH hydrogens present intermediate τ_c values, similar to the ones observed for the 50 mM and 75 mM solutions of 2NBGH. This fact suggests that, for 3NBGH, it would be possible to consider the existence of drug-drug intermolecular interactions even

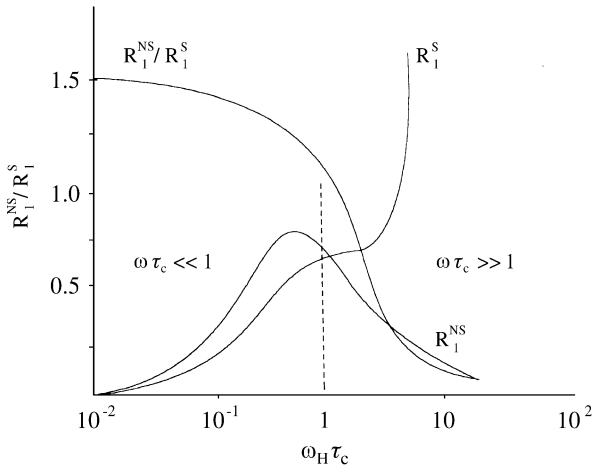
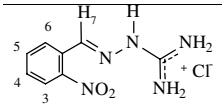


Figure 2. R_1^{NS}, R_1^{S} and the R_1^{NS}/R_1^{S} ratio of a proton pair *vs.* $\omega\tau_c$.

Table 1. R_1^{NS}/R_1^{S} ratios for pure 2NBGH[a].



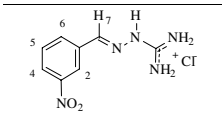
	13 mM	25 mM	50 mM	75 mM
H3	1.4	1.2	1.0	1.2
H4	1.3	1.2	0.8	1.0
H5	1.7	1.8	1.2	1.4
H6	1.3	1.2	1.1	1.1
H7	1.0	1.0	0.7	0.9
average R_1^{NS}/R_1^{S}	1.3	1.3	1.0	1.1

a- The absolute uncertainty for all results is equal to or less than ± 0.1.

at lower 3NBGH concentrations. Interestingly, for concentrations above 25 mM it is observed that the values of the R_1^{NS} / R_1^{S} ratio are around 1.2, thus, according to Fig. 2 the values of τ_c would be smaller than for the 13 and 25 mM solutions. This result suggests that at higher concentrations the molecules of 3NBGH are more mobile than at lower concentrations. This is somehow surprising, as at higher concentrations it would be expected that the increase in solution viscosity and the greater possibility of drug-drug interaction should lead to greater values for the correlation time. This behavior of the 3NBGH is not well understood yet, but one feasible explanation for this phenomenon would be the possibility of shorter half-lives for the molecular aggregates of 3NBGH at higher concentrations. In fact, it seems plausible that at higher concentrations the greater number of drug-drug collisions would decrease the half-life of the molecular aggregates, as compared with the dilute solutions. This might be the case for systems where the drug-drug interaction is much stronger that the solvent-drug interaction, that is, for systems where it would be necessary the collision with a solute molecule to disrupt the aggregate.

For 4NBGH the average value of the R_1^{NS} / R_1^{S} ratio is around 1.3 for all the concentrations (see Table 3). This

Table 2. R_1^{NS}/R_1^{S} ratios for pure 3NBGH[a].



	13 mM	25 mM	50 mM	75 mM
H2	0.6	1.1	1.2	1.0
H4	0.8	1.1	1.3	1.1
H5	0.8	1.2	1.4	1.2
H6	0.9	1.3	1.4	1.1
H7	1.0	1.3	1.2	1.0
average R_1^{NS}/R_1^{S}	0.8	1.2	1.3	1.1

a- The absolute uncertainty for all results is equal to or less than ± 0.1.

value corresponds to the $\omega\tau_c \cong 1$ region (Fig. 2) and suggests the occurrence of intermolecular drug-drug interactions. In any case, the results suggest that the affinity of 4NBGH for drug-drug intermolecular interaction is the lowest of the three isomers, in agreement with the highest overall R_1^{NS} / R_1^{S} ratio. It is also interesting to notice that, for this system, there are not detectable effects of the changes in concentration on the correlation time values.

In general, it can be observed that the three isomers differ greatly on their relaxation behavior at higher concentrations. Since this behavior is somehow anomalous, it seems more appropriate to compare the results of the three compounds at the lowest studied concentration, where the solution behavior is closer to ideality and the differences in the tendency to form molecular aggregates are more likely to be observed. The average R_1^{NS} / R_1^{S} ratio for all the hydrogens of each molecule in the 13 mM solutions (see Tables 1, 2 and 3) are 1.3, 0.8 and 1.3 for 2NBGH, 3NBGH and 4NBGH, respectively. From these results it is clear that 3NBGH displays the highest tendency to self aggregation (τ_c= 1.1 x 10^{-9}), while the other two isomers show a lower potential for drug-drug interaction (τ_c = 3.7 x 10^{-10}), where the τ_c values were calculated by interpolation of the plot shown in Fig. 2.

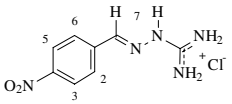
If the values of the R_1^{NS} / R_1^{S} ratio, which are used for the determination of the correlation times for the three isomers, are calculated as an average of the R_1^{NS} / R_1^{S} ratio of all the individual hydrogens at the four concentrations used (2NBGH = 1.2, 3NBGH = 1.1 and 4NBGH = 1.3), the results obtained for τ_c for the NBGH are 4.8 x 10^{-10} s, 5.3 x 10^{-10} s and 3.7 x 10^{-10} s for 2NBGH, 3NBGH and 4NBGH respectively. Since the correlation time is inversely proportional to the degree of molecular mobility, that is the smaller values of τ_c correspond to higher molecular mobility, it is possible to say that the intensity of drug-drug intermolecular interaction for the nitro guanyl hydrazones decrease in the order 3NBGH > 2NBGH > 4NBGH. This result, which shows the lowest molecular mobility or higher tendency for drug-drug interaction for 3NBGH, is in agreement with the results obtained with the relaxation measurements at the lowest concentration (13 mM). However, in this case, it seems that the *ortho* and *para* isomers do not display a similar behavior, with 4NBGH being the least likely to form molecular aggregates.

In the study of drug-BSA intermolecular interaction the analysis of selective and non-selective relaxation rates was carried out as for the pure drugs. The values of the R_1^{NS} / R_1^{S} ratio were evaluated for each NBGH isomer in 13 mM, 25 mM, 50 mM and 75 mM solutions in the presence of BSA (7.25 x 10^{-5} M). The results are shown in Tables 4, 5 and 6.

Initially, the analysis was carried out using only the average R_1^{NS} / R_1^{S} ratio for all the isomers at the 13 mM solutions. Then, the same procedure was carried out using the overall average values for all the hydrogens at all the different concentrations, as it was done for the pure drugs. For each case, the correlation times were determined from Fig. 2, and the results are summarized in Table 7.

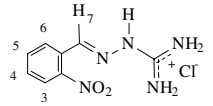
For the 13 mM 2NBGH solution, the average value of the relaxation rate ratio in the presence of BSA is 0.6, which indicates that τ_c corresponds to 1.7 x 10^{-9} s. This τ_c value is significantly longer than the τ_c value for the pure drug (3.7 x 10^{-10} s). In fact it is possible to say that the molecule now presents long correlation times values, and that it is on the $\omega\tau_c >> 1$ region. This result strongly suggests that 2NBGH is forming a complex with BSA. It is well known that BSA presents a correlation time of 3,8 x 10^{-8} s and that the small molecules present values of τ_c in the range of 10^{-10} s to 10^{-12} s.[18] Clearly, because of the association with BSA, 2NBGH is showing a τ_c value proximal to the τ_c value of BSA. An identical conclusion is reached if the analysis is carried out using the overall average values of the R_1^{NS} / R_1^{S} ratio for 2NBGH. In this case, the data from Tables 4 and 7 show that the average of the values of R_1^{NS} / R_1^{S} ratio is 0.6 for the 13 mM and 25 mM 2NBGH solutions, and 0.1 for the 50 mM and 75 mM 2NBGH solutions. Both results, when evaluated on the plot of Fig. 2, show that the 2NBGH molecules in the presence of BSA are on the $\omega\tau_c >> 1$ region, which is indicative of complex formation with BSA. These results also show that the increase in 2NBGH

Table 3. R_1^{NS}/R_1^{S} ratios for pure 4NBGH[a].

	13 mM	25 mM	50 mM	75 mM
H2,6	1.3	1.5	1.2	1.3
H3,5	1.3	1.2	1.2	1.2
H7	1.4	1.2	1.2	1.7
Average R_1^{NS}/R_1^{S}	1.3	1.3	1.2	1.4

a- The absolute uncertainty for all results is equal to or less than ± 0.1.

Table 4. R_1^{NS}/R_1^{S} ratios for 2NBGH with BSA[a].

	13 mM	25 mM	50 mM	75 mM
H3	0.6	0.6	0.1	0.1
H4	0.7	0.4	0.1	0.1
H5	0.7	0.6	0.1	0.1
H6	0.5	0.6	0.1	0.1
H7	0.5	0.7	0.1	0.1
average R_1^{NS}/R_1^{S}	0.6	0.6	0.1	0.1

a- The absolute uncertainty for all results is equal to or less than ± 0.04.

concentration facilitates the drug-BSA intermolecular interaction.

The results for 3NBGH in the presence of BSA, are summarized in Table 5. The analysis of the data obtained with the 13 mM solution affords an average value for the R_1^{NS} / R_1^{S} ratio of 0.7, which corresponds to $\tau_c = 1.4 \times 10^{-9}$ s, which is slightly greater than τ_c for the pure drug (1.1×10^{-9} s). This value is indicative of a weaker drug-BSA interaction for 3NBGH. When the analysis is carried out for all the data, it is observed that the average R_1^{NS} / R_1^{S} ratio is 0.7 for all the concentrations. The value of the average overall R_1^{NS}/R_1^{S} ratio for the pure 3NBGH solutions is 1.1 ($\tau_c = 5.3 \times 10^{-10}$), indicating that the increase in *tc* due to the presence of BSA for 3NBGH (0.9×10^{-9} s) is only about half of what it was observed for 2NBGH (2.0×10^{-9} s). With these results it is possible to conclude that 3NBGH interacts with BSA, but with lower affinity than 2NBGH.

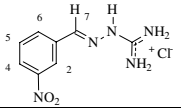
The values of the R_1^{NS}/R_1^{S} ratio for 4NBGH with BSA are summarized in Table 6. It is possible to observe that the average ratio is 0.5 for the 13 to 50 mM solutions and 0.3 for the 75 mM solution. This values correspond to the $\omega\tau_c >> 1$ region in Fig. 2. The average R_1^{NS} / R_1^{S} ratio values for the 13 mM solution, as well as for all other solutions is 0.5, which is significantly smaller than the value for the pure drug (1.3). The difference between the correlation time for pure 4NBGH (3.7×10^{-10} s) and the correlation time for the drug with BSA (2.3×10^{-9} s) is 1.9 x 10^{-9} s, showing that 4NBGH interacts with BSA with a similar affinity as 2NBGH.

Since the correlation time presents an inverse correlation with the degree of molecular mobility, it is possible to say that the intensity of drug-BSA intermolecular interaction for the nitro guanyl hydrazones decrease in the order 2NBGH > 4NBGH >> 3NBGH, suggesting that the 2NBGH have, for a short margin over 4NBGH, the greater affinity for BSA. However, if we calculate the $\tau_{c\,(PURE)}/\tau_{c\,(BSA)}$ ratio for each drug (Table 7), thus considering the effect of drug-drug intermolecular interaction, it can be observed that the lower $\tau_{c\,(PURE)}/\tau_{c\,(BSA)}$ ratio corresponds to 4NBGH, indicating that this drug has the lowest propensity for drug-drug interaction and the highest tendency for drug-BSA interaction. In this case, it is possible to say that the degree of drug-BSA intermolecular interactions decrease in the order 4NBGH > 2NBGH >> 3NBGH. Interestingly, this last result suggests that there is a correlation between the $\tau_{c\,PURE}/\tau_{c\,BSA}$ ratio and the *in vitro* ID_{50} values, which follow the order 4NBGH > 2NBGH > 3NBGH.

Conclusion

Selective T_1 measurements are very sensitive and convenient for the investigation of the binding of small molecules to macromolecules, allowing the calculation of the correlation times. Our results indicate that all the NBGH are able to establish intermolecular interactions with BSA, and that of the three isomers 4NBGH and 2NBGH present the highest affinity for this protein, with 4NBGH being the most affine. The *meta* isomer, 3NBGH, on the other hand

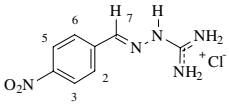
Table 5. R_1^{NS}/R_1^{S} ratios for 3NBGH with BSA[a].



	13 mM	25 mM	50 mM	75 mM
H2	0.7	0.8	0.7	0.6
H4	0.7	0.7	0.7	0.6
H5	0.6	0.7	0.8	0.8
H6	0.6	0.9	0.7	0.7
H7	1.0	0.6	0.7	1.0
average R_1^{NS}/R_1^{S}	0.7	0.7	0.7	0.7

a- The absolute uncertainty for all results is equal to or less than ± 0.05.

Table 6. R_1^{NS}/R_1^{S} ratios for 4NBGH with BSA[a].



	13 mM	25 mM	50 mM	75 mM
H2,6	0.5	0.5	0.5	0.3
H3,5	0.5	0.6	0.5	0.4
H7	0.6	0.6	0.5	0.2
average R_1^{NS}/R_1^{S}	0.5	0.6	0.5	0.3

a- The absolute uncertainty for all results is equal to or less than ± 0.05.

Table 7. R_1^{NS}/R_1^{S} ratio and correlation times for the NBGH.

NBGH	R_1^{ns}/R_1^{s} pure		τ_c pure (s, x 10^{10})		R_1^{ns}/R_1^{s} BSA		τ_c BSA (s, x 10^{9})		τ_c pure/ τ_c BSA		ID_{50} (μM)
	a	b	a	b	a	b	a	b	a	b	
2NBGH	1.3	1.2	3.7	4.8	0.6	0.4	1.7	2.5	0.22	0.19	87.5
3NBGH	0.8	1.1	11.0	5.3	0.7	0.7	1.4	1.4	0.79	0.38	182.6
4NBGH	1.3	1.3	3.7	3.7	0.5	0.5	2.3	2.3	0.16	0.16	55.9

a- Values calculated for the 13 mM solutions only.
b- Values calculated using the average of all the hydrogens at all the studied concentrations.

is the drug with the highest tendency to drug-drug association, and the less affine to BSA. The analysis of the values of $\tau_{c\ PURE}/\tau_{c\ BSA}$ ratio shows that as the self association tendency of the drugs increases, the tendency for drug-BSA association and the *in vitro* activity decreases. These results strongly favor the hypothesis that the mechanism of interaction of these compounds with *T. cruzi* is of electrostatic nature, and that it occurs through the guanidinium moiety. In this way, the different tendencies for self association in solution, which disfavors the interaction of the drugs with the parasite, would explain the *in vitro* activity differences between the three isomers.

Acknowledgment

We gratefully acknowledge the financial support given by PADCT/CNPq, the scholarship awarded by CNPq (J.D. Figueroa-Villar) and studentship awarded by CAPES (L.W. Tinoco).

References

1. Messeder, J.C.; Tinoco, L.W.; Figueroa-Villar, J.D.; Souza, E.M.; Santa Rita, R.; De Castro, S.L. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 24, 3079.
2. Chmunis, G. A. *Transfusion* **1991**, *31*, 547.
3. Hamerschlak, N.; Pasternak, J.; Amato Neto, V.; Carvalho, M.B.; Guerra, C.S.; Coscina, A.L. *Rev. Soc. Bras. Med. Trop.* **1997**, *30*, 205.
4. Center for Disease Control (CDC). *Emerging Infectious Diseases* **1998**, *4*, 1, 5.
5. Santos Filho, O.A.; Figueroa-Villar, J.D. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 13, 1797.
6. Tinoco, L.W.; Ph.D. Thesis 1998, Instituto Militar de Engenharia, RJ, Brazil.
7. Freeman, R. ; Hill, H.D.W.; Tomlinson, B.L.; Hall, L.D. *J. Chem. Phys*. **1974**, *61*, 466.
8. Niccolai, N.; Miles, M.P.L.; Hehir, S.P.; Gibbons, W.A. *J. Am. Chem. Soc.* **1978**, *100*, 20, 6528.
9. Gaggelli, E.; Gaggelli, N.; Maccotta, A.; Valensin, G. *J. Magn. Reson.* **1994**, *B 104*, 9.
10. Gaggelli, E.; Gaggelli, N.; Maccotta, A.; Valensin, G. *Arch. Biochem. Biophys.* **1994**, *308*, 1, 48.
11. Gaggelli, E.; Valensin, G.; Kushinir, T.; Navon, G. *Magn. Reson. Chem*. **1992**, *30*, 61.
12. Camparini, B.; Gaggelli, E.; Marchettini, N.; Valensin, G. *Biophys. J.* **1985**, *48*, 247.
13. Valensin, G.; Kushinir, T.; Navon, G. *J. Magn. Reson.* **1982**, 23.
14. Bonechi, C.; Donati, A.; Picchi, M.P.; Rossi, C.; Tiezzi, E. *Colloids and Surfaces*: A **1996**, *115*, 89.
15. Hall, L.D.; Hill, H.D.W. *J. Am. Chem. Soc.* **1976**, *98*, 5, 1269.
16. Bodenhausen, G.; Freeman, R.; Morris, G.A. *J. Magn. Reson*. **1976**, *23*, 171.
17. Harris, C.D. *Quantitative Chemical Analysis*; W. H. Freeman and Company; New York, 1996.
18. Wallach, D. *J. Chem. Phys.* **1967**, *47*, 5258.

Received: March 29, 1999