

## A STUDY OF THE REACTION OF RIBOSE bis(THIOSEMICARBAZONATO) COPPER(II) AND NITRIC OXIDE.

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### Abstract

The acid constant of ribose bis(thiosemicarbazone), RibTSC, and the formation constants of ribose bis(thiosemicarbazonato) copper(II), Cu(RibTSC-2H), and Cu(RibTSC-2H)-(NO)<sub>x</sub> were determined (Where x = 1 or 2). The SPEFO version of the LETAGROP was used to determine both constants, and the graphical method was also used for the first constant. The results show that RibTSC has one isosbestic point at 350 nm and a pK<sub>a</sub> value of 11,08, Cu(RibTSC-2H) has a relatively large average formation constant of 6,188\*10<sup>6</sup>, and the logarithm of the formation constants of Cu(RibTSC-2H)-(NO)<sub>1</sub> and Cu(RibTSC-2H)-(NO)<sub>2</sub> were found to be 8,68 ± 0,12 and 11,41 ± 0,09, respectively.

The reaction between aqueous Cu(RibTSC-2H) and saturated aqueous nitric oxide, NO, in a pH 7.4 buffered aqueous solution, at 20 °C, was studied using UV-visible. The absorbance of Cu(RibTSC-2H) at 468 nm decreases as portions of NO were successively added to 2 ml of 2,5\*10<sup>-5</sup> mol/L of this complex. A graph of ε(Cu(RibTSC-2H)) at 468 nm against [NO], at 20 °C, reflects that Cu(RibTSC-2H)-(NO)<sub>2</sub> adduct is formed, which does not decompose when temperature is raised to 60 °C. The concentration of 2.10<sup>-3</sup> molL<sup>-1</sup> for saturated aqueous nitric oxide at 20 °C was used for all calculations.<sup>1</sup>

### 1.0 Introduction

Thiosemicarbazones, TSC, are typical shift bases and possess a range of biological applications: antitumour, antiviral, antibacterial, antimalarial, antifungal, and antileprotic activities. The bioactive properties of some TSCs and their complexes have motivated several researchers to explore their use as pharmacology basis. These studies have demonstrated that the preparation of pharmacology should take into account, besides cell membrane permeability, its physical chemistry properties and a possible mechanism through which it exerts its effects. These properties are: nature of the metal (electronic structure and oxidation state), nature of the ligand (including its bioactive properties), kinetic properties, thermodynamic stability, and structural and electronic characteristics of the complex.<sup>2</sup>

On the other hand, nitric oxide is a strong π-acceptor ligand and a normal endogenous free radical in biological systems, which is synthesised in many cells by the enzymes nitric oxide synthase (NOS) using L-arginine as a substrate, and is now known to possess diverse and indispensable functions in a wide variety of biological phenomena in the cardiovascular, central nervous, and immune systems.<sup>3-5</sup> The chemical biology of NO divides the potential chemical reactions into two distinct categories, direct and indirect. The direct effects are those where NO binds to biological targets, which are primarily heme complexes and reactive radical species. Indirect effects are those that are derived from the chemistry of NO with molecules such as

oxygen, and superoxide to form reactive nitrogen oxide species such as N<sub>2</sub>O<sub>3</sub>, ONOO<sup>-</sup> and ONOOH. These chemical species mediate nitrosative and oxidative stress,<sup>4-6,7</sup> in particular; overproduction of NO is implicated in the catastrophic drop in blood pressure associated with septic shock.<sup>4,8-10</sup> This hypotension does not always respond to intravenous administered adrenaline and dopamine. Therefore, the endogenous equilibrium concentration of nitric oxide should be maintained, since its alteration is potentially highly dangerous. Novel therapeutic approaches now concentrate on the inhibition of the enzyme inducible nitric oxide synthase (iNOS) and/or the removal of NO by use of specific, high affinity, non-toxic scavengers.

Cu(RibTSC-2H) would be a potential nitric oxide scavenger because it has reasonable water solubility and cell membrane permeability due to the remainder of the ribose moiety which is hydrophilic,<sup>20</sup> and its square planar geometry is also most favourable.<sup>5,21-23</sup>

In this research we proposed the following objectives:

- To determine the acid constant of ribose bis(thiosemicarbazone)
- To determine the formation constant of the complex, Cu(RibTSC-2H), and
- To study the spin trapping capacities of Cu(RibTSC-2H) for nitric oxide.

The formation constant gives us an idea of the relative stability of this complex even in

oxidative environment, such that it may not vastly perturb the biological environment.

## 2.0 Equipment and methods

### 2.1 Reagents

Ribose ( $C_5H_{10}O_5$ ) and dehydrated copper(II) acetate are analytically pure reagents, and the para-aminotoluene and thiosemicarbazide were recrystallised, while ethanol and methanol were distilled before use.

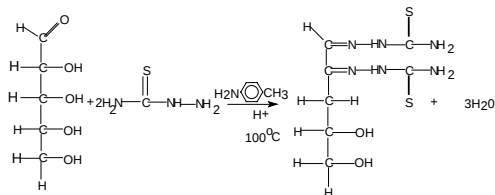
### 2.2 Equipment

The electronic spectra were recorded on an Ultraspec III spectrophotometer (Pharmacia) interfaced with a microcomputer for data acquisition. The temperature of the cell was controlled by a Peltier controller system of Pharmacia (Autofill III) connected to the spectrophotometer and the data were processed by origin version 5 of microcal and SPEFO version of the LETAGROP software.

The NMR spectra were recorded on a Bruker AC 250 F spectrometer that operates at a frequency of 250 MHz ( $^1H$ ) and 62,89 MHz ( $^{13}C$ ).

### 2.3 Synthesis of ribose bis(thiosemicarbazone) and its copper(II) complex.

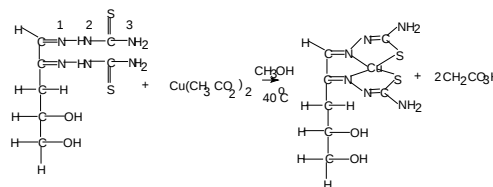
11,2 mmol of ribose and 11,2 mmol of p-aminotoluene were mixed in a 3:1 by volume water-ethanol solution, and 1,2 ml of glacial acetic acid added. The mixture was refluxed at 100 °C for 30 min, after which 22,4 mmol of thiosemicarbazide were added. The reaction mixture was further refluxed for 5 hrs, then cooled to ambient temperature and thereafter kept at 0 °C for 24 hrs before filtration and subsequent washing with ethanol. The dried product was recrystallised in a similar ethanolic solution, and refluxing with vigorous stirring at 80 °C for 2 hrs.<sup>11,13</sup> UV-visible, NMR, and melting point, assessed its purity. A percentage yield of 48.2 was achieved.



### Synthesis of ribose bis(thiosemicarbazone)

The copper(II) acetate and RibTSC ( $C_7H_{14}S_2N_6$ ) were separately dissolved in methanol in a molar ratio of 1:1, and warmed until they completely dissolved before they were mixed and refluxed at

about 40 °C for 3 hrs, followed by cooling, filtration and drying. A percentage yield of 55.4 was achieved.



### Synthesis of ribose bis(thiosemicarbazonato)copper(II)

#### 2.4 (a) Confirmation of the acid-base equilibrium of aqueous RibTSC by NMR

34,6 mg of RibTSC were dissolved in 0,5 ml of  $d_6$ -DMSO and the  $^1H$  spectrum recorded. Two equivalents of NaOD were added and  $^1H$  spectrum recorded, followed by neutralisation by equivalent  $CF_3OOH$  and recording of the  $^1H$  spectrum. The same experiments were repeated and the  $^{13}C$  spectra recorded instead.

#### (b) Determination of the isosbestic point and the acid constant of RibTSC

Two methods were used:

(i) Various 25 ml solutions, each containing 1 ml of RibTSC ( $3,666 \cdot 10^{-4}$  mol/L), 10 ml  $KClO_4$  (0,25 mol/L), 0,5 ml  $HClO_4$  (0,1089 mol/L) and varying volumes of NaOH (0,1001 mol/L) increasing by 25  $\mu$ L from 0 to 1100  $\mu$ L, were prepared and their spectra recorded, then selected data was processed with SPEFO version of LETAGROP software.

(ii) In four separate 25 ml volumetric flasks, 1 ml RibTSC ( $3,666 \cdot 10^{-4}$  mol/L) and 10 ml  $KClO_4$  (0,25 mol/L), were placed and then each flask was filled with a buffer solution of a particular pH value. The selected pH values for the solutions were 5; 9,2; 10 and 13.

### 2.5 Determination of the formation constant of $[Cu(RibTSC-2H)]$

Job's method was used to confirm the stoichiometry of the complex. The formation constant of  $Cu(RibTSC-2H)$  was graphically determined using data from Job's method, and from mixtures of equimolar solutions of copper(II) acetate and RibTSC, of equal volumetric ratios.<sup>12</sup>

### Theoretical basis.

The formation of Cu(RibTSC-2H) at 25 °C from copper(II) acetate and RibTSC can be summarised as:



and therefore the stability constant is :

$$K = [\text{Cu}(\text{RibTSC-2H})][\text{CH}_3\text{COOH}]^2 / [\text{Cu}^{2+}][\text{RibTSC}]$$

The collateral formation of a weak electrolyte, acetic acid, would buffer the mixture due to the following equation:



Thus explaining why the pH of the mixture did not vary. The protonic concentration of the system can be considered constant, therefore forms part of  $K'$ :

$$K' = K / [\text{H}^+]^2 = [\text{Cu}(\text{RibTSC-2H})] / [\text{Cu}^{2+}][\text{RibTSC}]$$

To determine the stability constant using data from Job's method, it is necessary to find the concentration of the complex, by means of Beer-Lambert law:

$$a = \log(I_0/I) = \epsilon lc,$$

where  $a$  is absorbance,  $\epsilon$  is the extinction coefficient of the complex,  $l$  the optical path length of the cell, and  $c$  concentration of the complex. Since  $a$  and  $l$  are measurable, the problem is one of finding  $\epsilon$ , or in practice the product  $\epsilon l$ . Various methods can be used.

(a) Let  $x$  be the concentration of  $\text{Cu}^{2+}$  added to the solution and let  $y$  be that of the added ligand, RibTSC. Then for any mixture:

$$\begin{aligned} K' &= [\text{complex}] / [\text{Cu}^{2+}][\text{RibTSC}] \\ &= [\text{complex}] / [x - c(\text{complex})][y - c(\text{complex})] \\ &= [a / \epsilon l] / [(x - a / \epsilon l)(y - a / \epsilon l)]. \end{aligned}$$

Rearranging the above equation:

$$xy / a = 1 / \epsilon l [1/K' + (x + y)] - a / (\epsilon l)^2$$

The total concentration ( $x + y$ ) is constant. It follows that a graph of  $xy / a$  against  $a$  should be linear and that the gradient will yield  $\epsilon l$  and, the intercept and this value of  $\epsilon l$  will then yield  $K'$ .

(b) An alternative method for obtaining  $\epsilon l$  is to measure  $a$  for series of 1:1 by volume mixtures at increasing dilutions. Let the concentration of mixed  $\text{Cu}^{2+}$  and RibTSC in the resulting solution be  $C$ . Then for the series of mixtures the concentration of free  $\text{Cu}^{2+}$  and RibTSC are given by:

$$[\text{Cu}^{2+}] = [\text{RibTSC}] = C - [\text{complex}];$$

therefore:

$$K' = a / \epsilon l (C - a / \epsilon l)^2$$

or

$$C / a^{1/2} = [1 / \epsilon l K']^{1/2} + a^{1/2} / \epsilon l$$

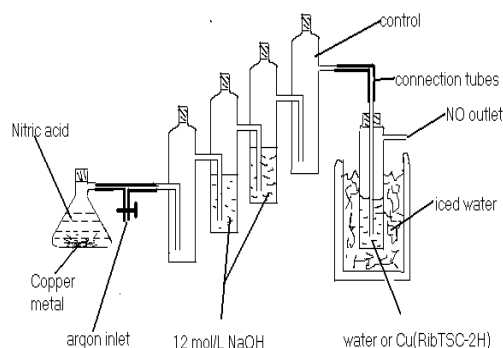
A plot of  $C / a^{1/2}$  against  $a^{1/2}$  should therefore be linear with a gradient  $1/\epsilon l$  and intercept  $(1/\epsilon l K')^{1/2}$ .

The reported log  $\epsilon$  value is approximately 3,71<sup>11,13,18,19</sup>

We preferred the second method (method (b)) to the first one because it had superior lineal correlation factors in all cases. ( $R \approx 0,998$ )

## 2.6 The study of the reaction between Cu(RibTSC-2H) and NO

The saturated aqueous solution of NO was obtained by generating the gas from the reaction of metallic copper with 5 molL<sup>-1</sup> aqueous nitric acid. The gas was purified by bubbling it through two schlenk flasks containing 12 molL<sup>-1</sup> NaOH(aq) before it was collected in 20 ml of water in a container immersed in ice cubes. Passing pure argon for not less than 25 min deoxygenated the whole system, tightly isolated from air, prior to the complex – NO interaction.



**Fig 1** Nitric oxide generation in argon atmosphere.

The reaction between Cu(RibTSC-2H) and NO gas was carried out in a pH 7,4 phosphate buffer aqueous solution by:

(i). Directly bubbling NO gas into 20 ml of aqueous Cu(RibTSC-2H), ( $9,49 \cdot 10^{-5}$  mol/L), in a glass tube immersed in ice cubes. The

spectrum of the resultant mixture was recorded at 20 °C.

(ii). Successively adding 10  $\mu$ L aliquots of saturated aqueous solution of NO to 2 ml Cu(RibTSC-2H) (aq) ( $9,49 \cdot 10^{-5}$  mol/L) at 1,5 min intervals, using the wavescan repeat scan facility. The temperature was later raised to 60 °C then repeat scan four times at 10 min intervals before reducing the temperature back to 20 °C.

Both reactions were followed by UV-visible spectrophotometry.

### 3.0 RESULTS AND DISCUSSIONS

#### 3.1 Determination of the isosbestic point and the acid constant of RibTSC

The hydrolysis of TSC, in general, proceeds by an initial protonation of the azomethine nitrogen atom followed by water or hydroxyl ions' bonding to the azomethine carbon atom. Subsequently, in our first experimental procedures, we used RibTSC in an excess of NaOH. The results were inconsistent, in general. It was noticed that in alkaline media RibTSC produces species that weakly absorb at 280 nm. We therefore had to use the above-described methods.

Computer structure modelling and spectroscopic data, particularly  $^1\text{H}$ -RMN,  $^1\text{H}$ - $^1\text{H}$  COSY, and NOE-DEF, indicate that the TSC moieties of free RibTSC ligand are both in the E-configuration,<sup>13</sup> therefore the C2 arm is in a more open environment. The chemical shifts of 10,83 and 11,68 for the N2-H protons of RibTSC were observed and steric crowding, as well as electronic effects, may account for the lack of equally strong hydrogen bonding by DMSO to the hydrazine protons. (Fig 2(a))

These different chemical shifts suggested a stepwise deprotonation of this compound, and therefore two equilibria and subsequently two pKa values and probably two isosbestic points. Contrary to these expectations, RibTSC has one isosbestic point at 350 nm and a pKa value of  $11,82 \pm 0,169$  when titrated with NaOH. However, the small electronic environment difference about these protons, reflected by  $\Delta\delta < 1$ , maybe of smaller significance in the presence of a much stronger base like OH<sup>-</sup>. The equal chemical shifts of corresponding protons of all the centrosymmetric bis(TSC) investigated by West et al,<sup>13</sup> in which these protons are in equivalent chemical environments support the above suggestion.

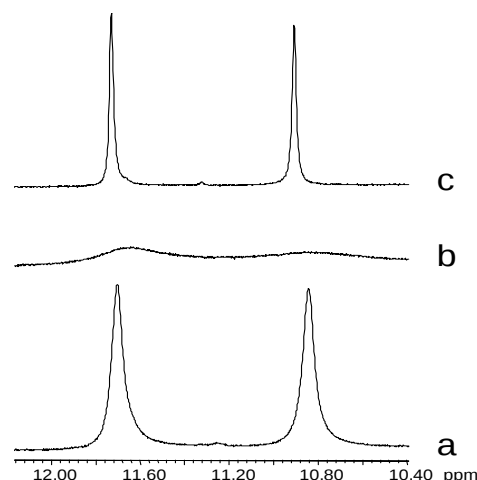
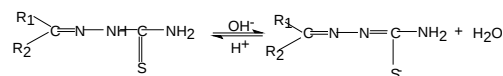


Fig 2 (a) RibTSC in  $d_6$ -DMSO, (b) Two equivalents of NaOD were added to (a), (c) Two equivalents of  $\text{CF}_3\text{COOH}$  were added to (b).

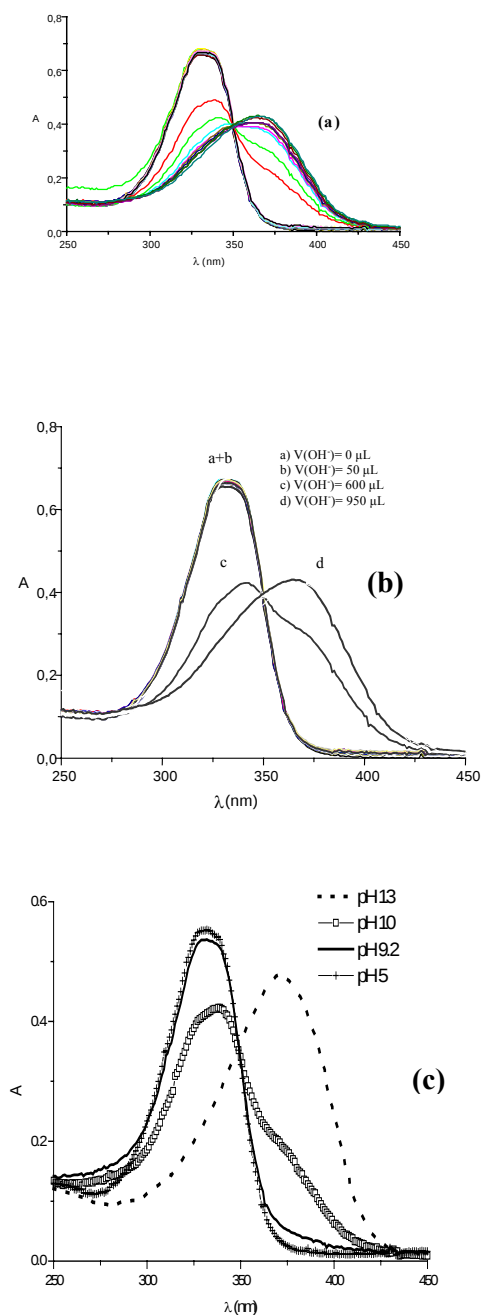
The acid-base equilibrium of RibTSC was confirmed by NMR method. (Fig 2(a-c)) This method had to be used in order to confirm that RibTSC does not decompose in alkaline media, at least within the experimental time ranges. The hydrazine protons disappeared on addition of two equivalents of NaOD to RibTSC in  $d_6$ -DMSO (Fig 2(b)) and the peaks were re-established on addition of two equivalents of trifluoroacetic acid,  $\text{CF}_3\text{OOH}$ . (Fig 2(c)) When a similar experiment was repeated and the  $^{13}\text{C}$  spectra recorded instead, the spectra coincided in the three cases, demonstrating that there was no cleavage of the carbon chain of RibTSC.

From Fig 3(a) and (b), it is observed that bis(RibTSC) has maximums at 331 nm in aqueous solutions of  $9,2 \geq \text{pH}$  and 368 nm in aqueous solutions of  $\text{pH} \geq 11$ , and an isosbestic point at 350 nm. It is worthy noting that the presence of the other species is detectable as from pH 10. In spite of the different chemical shifts assigned to the hydrazine protons, the presence of a single isosbestic point within the experimental pH range, clearly demonstrates the existence of only two species in equilibrium.



The formation of a second N=C in each TSC moiety allows greater  $\pi$ -orbital delocalisation, thus accounting for the bathochromic shift in

alkaline media and also supporting the  $\pi \rightarrow \pi^*$  assignation of these transitions.



**Fig. 3** Determination of Isosbestic point at 25 °C.

**a)** Spectra of RibTSC at different pH media

**b)** Titration of RibTSC in excess  $\text{HClO}_4$  with NaOH

**Fig 3c** Comparison of theoretical (—) and experimental (...) curves of RibTSC acid-base titration

The observation that acidification of an aqueous solution of RibTSC is not accompanied by any significant variation in neither intensity nor on the position of maximum absorption, allows the inference that this equilibrium is well displaced to thione tautomer and this may also explain why cationic complexes are very common.<sup>11,13,14</sup> Nevertheless, no plausible explanation could be forwarded for the observed decrease in the extinction coefficient of the conjugate base of RibTSC, which might be due to planar deviation of the RibTSC backbone. In addition, wavelength increase due to additional double bonds is about 30 nm, almost half the predictions by Fieser's rules, probably indicating minimal  $\pi$ -orbital delocalisation enhancement.

If the bands were due to  $n \rightarrow \pi^*$  transitions, in a highly polar media such as the one used in our experiments, the position of the maximum in the spectrum of the conjugate base would shift hypsochromically given the expected selective stabilisation of the lone pair electrons by ion-permanent dipole interactions.<sup>15</sup> In fact, it may be inferred that the slight increase in absorbance on the few first  $\text{OH}^-$  additions are due to the deprotonation of the azomethine nitrogen thereby re-establishing a more planar  $\text{sp}^2$  nitrogen centre, thus somewhat enhancing delocalisation.

The  $\text{pK}_a$  of 11,82  $\pm$  0.169, calculated from the spectral data of Fig 3(a), using the SPEFO version of the LETAGROP software compares very well with that obtained by Beraldo and Tosi<sup>16</sup> and Knight et al.,<sup>17</sup> that is 11,50  $\pm$  0,2 and 10,97 respectively for 2-formylpyridine in aqueous solutions in the presence of 0,5% DMSO. Once deprotonation of RibTSC starts, it is highly energetically favoured due to the exothermic neutralisation process, coupled with stabilisation by enhanced  $\pi$ -orbital delocalisation that is reflected by the shortening of the N2-C-N3 bonds and extensive hydration of the resultant anion.

The second method produced very similar results to the first as fig 3(b) illustrates. The major disadvantage of this method could be the different chemical media as a result of different buffer systems used.

In Fig 3c there is a notable deviation when the LETAGROP predicted curves were compared to the experimental ones. This could suggest that there is probably a second equilibrium that builds up at a much slower rate than acid-base equilibrium. This second equilibrium is

responsible for the formation of the species that absorbs at 280 nm, and we eventually agreed that this could be a result of trans-cis isomerism as was observed by Beraldo and Tosi<sup>16</sup>

### 3.2 Determination of the formation constant of [Cu(RibTSC-2H)]

Job's method confirmed the stoichiometry of the complex Cu(RibTSC-2H) and as reflected by Fig 4(a) the complex has a 1:1 Cu<sup>2+</sup>:RibTSC molar ratio.

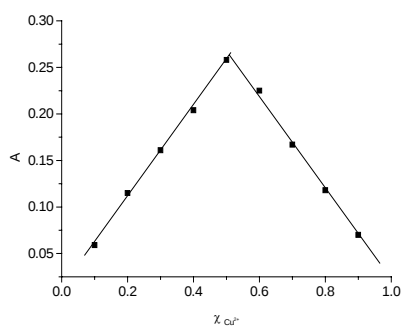


Fig 4 (a) Confirmation of Cu<sup>2+</sup>-RibTSC stoichiometry

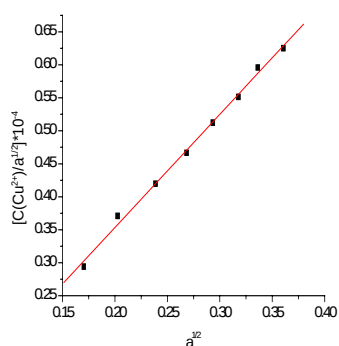


Fig. 4 (b) Determination of Formation constant of Cu(RibTSC-2H)

The gradient of the graph in Fig 4(b) is  $1,714.10^{-4}$  and the intercept  $1,066.10^{-6}$ , and using the equation:  $C / a^{1/2} = [1 / \epsilon l K']^{1/2} + a^{1/2} / \epsilon l$ , therefore,

$$\epsilon = 5833 \text{ (} l = 1 \text{),}$$

therefore:

$$\begin{aligned} [1 / \epsilon l K']^{1/2} &= 1,066.10^{-6} \\ K &= 1,51.10^8 \end{aligned}$$

The validity of our assumptions was confirmed by:

(a) The log  $\epsilon$  value obtained is 3,77, which is quite agreeable to those reported in the literature.<sup>11,13,18,19</sup>

(b) The high lineal correlation factors, of R > 0,99 in all cases for both methods.

The use of the LETAGROP software permitted us to verify the validity of the above-mentioned method and the results derived thereafter. The spectral data used to determine the formation constant of Cu(RibTSC-2H) above were processed by this software and gave us a very similar result of  $6,18.10^6$ . Also this programme calculates theoretical absorbance values basing on the provided experimental data and high correlation between these data, as reflected in Fig 4(c), further confirms the obtained result.

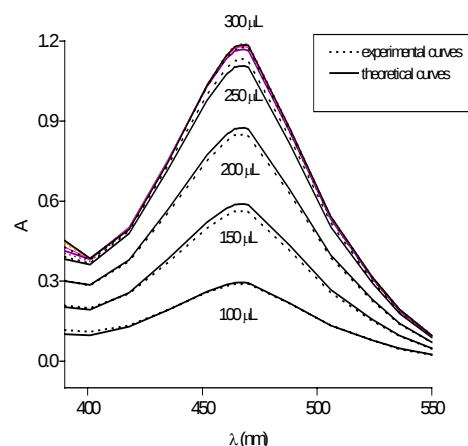


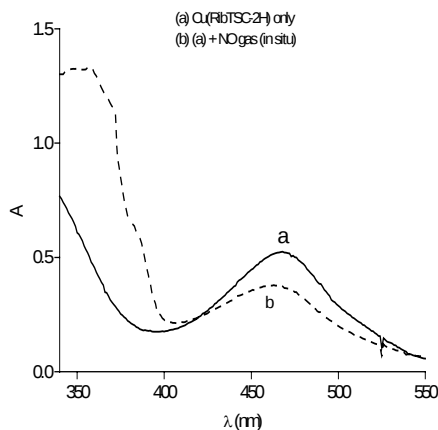
Fig. 4 (c) Comparison of theoretical and experimental curves

### 3.3 The study of the reaction between Cu(RibTSC-2H) and NO

Nitric oxide, NO, and ribose bis (thiosemicarbazonato)copper(II), have the complementary relationship for structural recognition, because the copper(II) ion has an unpaired electron, the substrate's ligands and the complex's geometry would not present significant steric effects, in addition to the mainly  $\sigma$ -donating effects of the N-atoms. The guest (entering) ligand is a relatively small free radical and the sulphur atoms would tend to promote greater back donation by the copper atoms into the  $\pi^*_{(NO)}$  orbitals. Consequently, the entrance of a second nitric oxide molecule could be electronically hindered because of the strong trans effect of the already bonded NO molecule and the Jahn-Teller effect which tend to inhibit the attainment of the maximum co-ordination number by copper(II) ions. The above stated considerations are likely to greatly reduce the stability of the Cu-(NO)<sub>2</sub> adduct with respect to Cu-NO, if both NO moieties are "linearly" coordinated. This may effectively widen the possibility of Cu-(NO)<sub>2</sub> complex formation, in

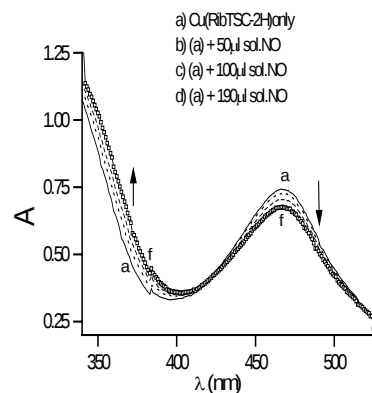
which the “linear” and “bent” bonding modes co-exist.

When NO gas was directly bubbled through buffered Cu(RibTSC-2H) aqueous solution, there was an appreciable decrease of the maximum absorbance at 468 nm, a consistent small hypsochromic shift to 465 nm, and a new band at about 358 nm.(Fig 5(a)) The tendency to shift to lower wavelengths could be a result of the greater degree of axial coordination by NO, thereby creating a larger positive charge on the copper ion due to the marked  $\pi$ -acceptor of NO. The greater polarisation of the highly polarisable thiolate group (CS<sup>-</sup>) would increase absorbance of the charge transfer band that usually became deformed as the experiments progressed. (Fig 5(a)) This analysis apparently lends more support to its classification as a sulphur to copper transfer band.<sup>13,16,20</sup>

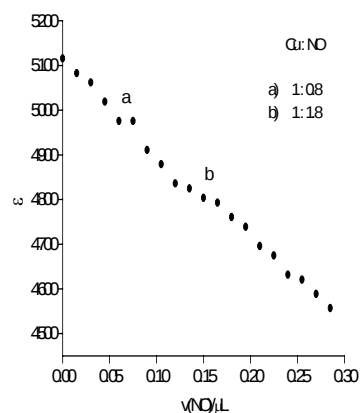


**Fig 5(a)** Direct bubble of NO in Cu(RibTSC-2H)

Fig 5(b), like Fig 5(a), reflects the decrease in the absorbance of Cu(RibTSC-2H) at 468 nm as aliquots of saturated aqueous NO solution were added. The new band at 358 nm is more pronounced in Fig 5(a) than in Fig 5(b) due to the presence of an excess of NO. Plots of  $\epsilon$  (Cu(RibTSC-2H)) against volume of NO added at 468 nm show two deflection points, (a) and (b), at NO: Cu(RibTSC-2H) ratios close to 1:1 and 2:1, where the  $\epsilon$  values tend to be constant.(Fig 5(c))



**Fig. 5 (b)** 10  $\mu$ L NO additions to Cu(RibTSC-2H) ( $9.49.10^{-5} \text{ molL}^{-1}$ )



**Fig. 5(c)** Variation of  $\epsilon(\text{Cu(RibTSC-2H)})(9.305. 10^{-5} \text{ molL}^{-1})$  at 468 nm as 15 ml aliquots of saturated aqueous NO were added at 20 °C

The Selected absorbance data from the NO addition method, processed by the SPEFO version of LETAGROP software, deduced the logarithms of the stability constants  $\beta_1$  and  $\beta_2$ , corresponding to the formation of Cu(RibTSC-2H)-NO and Cu(RibTSC-2H)-(NO)<sub>2</sub> adducts respectively.

**Table 1** Formation constants of Cu(RibTSC-2H)-(NO)<sub>x</sub> adducts

| Log       | Value            |
|-----------|------------------|
| $\beta_1$ | $8.68 \pm 0.12$  |
| $\beta_2$ | $11.41 \pm 0.09$ |

Fig 5(d) below reflects very high correlation between the theoretical and experimental curves.

On raising the temperature to 60 °C the absorption is slightly decreased and reducing the temperature back to 20 °C shows that the solution regains its previous absorption at this

temperature, as reflected in Fig 6. This behaviour could, at a priori, imply that the Cu(RibTSC-2H)-NO<sub>x</sub> adducts do not decompose within this temperature range and that the formation reactions of the adducts are exothermic and reversible.

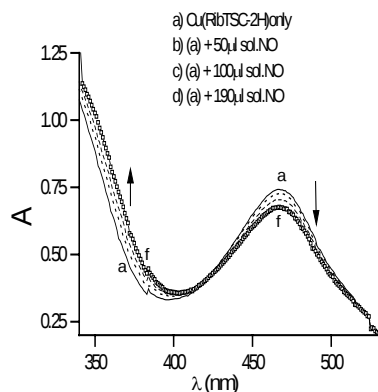


Fig 5(d) Comparison of theoretical and experimental curves

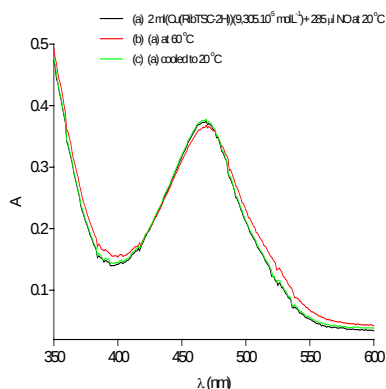


Fig 6 Temperature effect on 467 nm band

NO forms adducts with nucleophiles such as amines, sulphites, and thiols (nitrosation).<sup>6</sup> We, therefore, carried out the NO addition method with the ligand instead of the complex. The effect of NO on RibTSC was also followed by UV spectrophotometry at 330 nm. The results clearly demonstrated opposite effects of NO on the free ligand and the complex. Though we do not have much information as to what really happens, but from the information from these experiments, it is more than safe to infer that the spectral changes on Cu(RibTSC-2H) are not due to thiol-NO interactions.

#### 4.0 CONCLUSIONS

The following conclusions were drawn from this project:

1. (a) It was established that RibTSC has only one isosbestic point at 350 nm when titrated with NaOH.

(b) The pK<sub>a</sub> of RibTSC was found to be 11,82 ± 0,169.

2. The average formation constant of Cu(RibTSC-2H) was determined as 6.188 × 10<sup>6</sup>.

3. (a) There are interactions between Cu(RibTSC-2H) and NO, forming Cu(RibTSC-2H)-NO and Cu(RibTSC-2H)-(NO)<sub>2</sub> adducts.

(b) The logarithms of the formation constants of these adducts were determined by LETAGROP software as 8,68 ± 0,12 and 11,41 ± 0,09, respectively.

5. Therefore, it can be inferred to that the axial positions of Cu(RibTSC-2H) are relatively active in the presence of stronger nucleophiles.

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