Spectrophotometric determination of complex formation equilibria of copper (II), metformin and halides in methanol.

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Abstract- Metformin is prescribed against Diabetes Mellitus II, nevertheless its mechanisms of action are not fully understood. Several studies suggest that mitochondrial copper is chelated by metformin and the properties of the copper-metformin complex could generate the therapeutic activity.

In this work, a spectrophotometric study was performed in order to evaluate in methanol, the stability of the species copper-metformin and copper-metformin-halides. The formation constants and the calculated electronic spectrum of each species at 298 K, are reported. According with our results and considering the mitochondrial chloride concentration reported elsewhere, we hope that these data can be correlated with possible implications in vivo.

Index Terms- Copper(II) complexes; equilibria; metal-based drugs; Metformin.

I. Introduction

Diabetes Mellitus II is considered a group of syndromes affecting the carbohydrate metabolism, in which the common symptom is chronic hyperglycemia. The etiology of Diabetes Mellitus type II, which is not curable but treatable, is not well understood yet. The main symptom is usually a high glucose level; this promotes the beginning of several disorders related to the deficiency of the available insulin, manifested through the secretion, absorption and muscle resistance to insulin [1, 2], later several long-term metabolic consequences, are generated.[3] The prevalence of Diabetes around the world is alarmingly growing. The world health organization estimated that by 2030 the diabetic individuals would rise to 336 million[4] on the other hand the International Diabetes Federation (IDF), shows a higher number, they estimate that by 2035 the diabetics individuals would be about 592 millions.[5] Some risk factors, such as sedentary lifestyle and obesity, may promote Diabetes [6], on the opposite, Diabetes sometimes can be controlled by exercise.[7]

Usually, an oral hypoglycemic agent is required for an adequate blood glucose control.[1] Dimethylbiguanide or metformin(metf) is usually the first-line drug most widely prescribed as glucose-lowering agent in the treatment against Diabetes type II. [8, 9] This prescription has been effectively used as a single drug or combined commonly with glibenclamide.[10] Globally, over 100 million patients are prescribed with this drug annually.[8] Metformin promotes several effects, usually decreases the hepatic glucose production, increases glucose disposal by insulin mediated, decreases fatty-acid oxidation and the glucose intestinal absorption.[11] Also population studies suggest that metformin exposure is associated with reduced cancer risk or an improved prognosis, possibly by their antineoplastic activity[12] Recently, is being evaluated to be incorporated in the treatment or prevention of several diseases, such as colon cancer[13], papillary thyroid cancer[14] and Alzheimer's disease.[15]

Metformin has been known since 1957, however despite the simplicity of their chemical structure and multiple detailed investigations, its cellular mechanism remains unknown. Several theories have been elaborated to explain metformin action[8]. Yet, its molecular mechanism of action remains an important area of Diabetes research.[9] Recent studies propose a relationship between mitochondrial copper and metformin, in which metformin could be related to mitochondrial respiration. These studies suggest that mitochondrial copper is targeted by metformin and cellular effects generated by metformin, are probably related to the properties of the copper-metformin species.[16, 17] A mechanism suggested of action for the metformin, is through inhibition of complex I, affecting the mitochondrial respiratory chain. However, the precise mechanism of this inhibition has not yet been established.[16, 17] This allows theorize that metformin could act in cells at least in part as a copper-binding prodrug.[17]

We are interested to contribute to the understanding of the binding properties of the metformin with copper(II), and establish a possible softness or hardness character of the complexes. In this work, we report the formation constants in methanol of the copper complexes with metformin, and their respective halogen ternary complexes.

II. Methods and Materials

2.1 Materials.

$Cu(NO_3)_2 \cdot 2.5H_2O$, sodium chloride and sodium bromide (Fermont, México) were analytical grade and used without further purification. Metformin was obtained from tablets Predial (Silanes, México). Methanol HPLC grade (Fermont, México) was used as purchased as solvent for the determination of formation constant and for the extraction of metformin from tablets. Due to solubility problems no ionic strength was used.

2.2 Physical Measurements.

All spectral measurements were made in a Cary 50 UV–Vis Spectroscopy System, at 298 K (RT) using a quartz cell with 1 cm of path length. For the determination of formation constants, the spectrophotometric data were refined with the program HypSpec[18-20]. The observed spectral region was from 250 to 350 nm for all the experiments, excluding determination of the molar absorptivity coefficient for metformin, where the observed spectral region was from 200 to 800 nm. Distribution diagrams of species were calculated using the software Hyperquad simulation and speciation (HySS).[21]

2.3 Metformin extraction and purification

Tablets of metformin were finely powdered and homogenized with methanol. The solution was filtered in vacuum and stored at 277 K until formation of metformin crystals; these were separated with vacuum filtration.

2.4. Determination of the molar extinction coefficient of Metformin

A stock solution 0.00009M of metformin was prepared. Concentrations of metformin were varied from 0.00001 to 0.00009 M. A total of 10 points were used for the molar absorptive coefficient.

2.5. Copper(II)-metformin equilibrium studies

Experiments were performed using two different stock solutions of metformin (0.00371M and 0.00620M). $Cu(NO_3)_2 \cdot 2.5H_2O$ was used to prepare copper stock solutions (0.00210M and 0.00348M). The metformin concentrations were varied from 0.00004M to 0.00074M and 0.00006M to 0.00124M, respectively, in each experiment. The final copper concentration was set constant at 0.00021M and 0.00035M, respectively. A total of 40 spectra were used for the refinement.

2.6. Copper(II)-metformin chloride and bromide equilibrium studies.

For the copper(II)-metformin-halide system, two stock solutions of metformin (0.00223M and 0.00372M), $Cu(NO_3)_2 \cdot 2.5H_2O$ (0.00206M and 0.00344M) and sodium chloride or sodium bromide (0.00125M and 0.00202M) were prepared. For both experiments, the final copper concentration was set constant at 0.00021M and 0.00034M and metformin at 0.00022M and 0.00037M, respectively. The chloride or bromide concentrations were varied from 0.00001M to 0.00025M and 0.00002M to 0.00044M, respectively. A total of 39 spectra were used for the refining process.

III. RESULTS AND DISCUSSION

In all the equilibrium determinations, it is required to avoid the use of ionic strength, because it promotes the precipitation of the copper complexes. Also, has been reported that copper-metformin complexes are sensitive to pH, possess a strongly hydrophilic character with a pKa within the physiological pH range.[17] Because of this, in order to obtain a clear association between copper(II) and metformin, is necessary to avoid the generation of hydroxyl- or protonated species, methanol was used instead of water. This solvent has a donor number of 19 and water of 18, yet methanol is slightly a better nucleophile than water.[22, 23]

3.1 Determination of the molar absorptivity of metformin

The determination of molar extinction coefficient for metformin has been reported before under several conditions, there is a few reports about the determination of the extinction coefficient of metformin in pure methanol (Table 1).

Table 1. Molar absorbance of metformin under several conditions.

$\varepsilon(L \cdot mol^{-1} \cdot cm^{-1})$, λ_{max}(nm)	Solvent, Reference	Ionic strength (M), salt , pH
12400 ± 400, 232	Water,[31]	0.01, sodium phosphate, 7.1
13340 , 216	Water,[32]	0.1, NaOH.
12360, 237	Methanol, [33]	
13785.87, 237.5	Methanol, [34]	

Electronic spectra of metformin at different concentrations in methanol solution are presented in Fig. 1a.

A maximum absorption value for metformin solutions was detected at 236 nm, the extinction coefficient was calculated using the Lambert and Beer Law and the value obtained is $11302.60 \pm 275.25\ L \cdot mol^{-1} \cdot cm^{-1}$ with a R^2 of 0.9959 (Fig. 1b). In which the obtained value in this work is within the error under the conditions reported before.

3.2. Formation constants of the copper(II)-metformin.

Copper(II)-metformin complexes have been studied before, crystal structures of the *bis*-complexes are reported in alkaline aqueous solution as monohydrate[24], octahydrate[25] or using several counter ions such as ClO_4^-, CO_3^{2-}, or Cl^-.[17, 26, 27]. Nevertheless, as far as we know there are no reports about equilibrium determinations of this system in pure methanol.

Electronic spectra of methanol solutions of the copper(II)-metformin system are presented in Fig. 2a. For this system when metformin concentration is increased a hyperchromic effect is observed, the spectrum of maximum concentration of metformin shows a maximum at 270 nm.

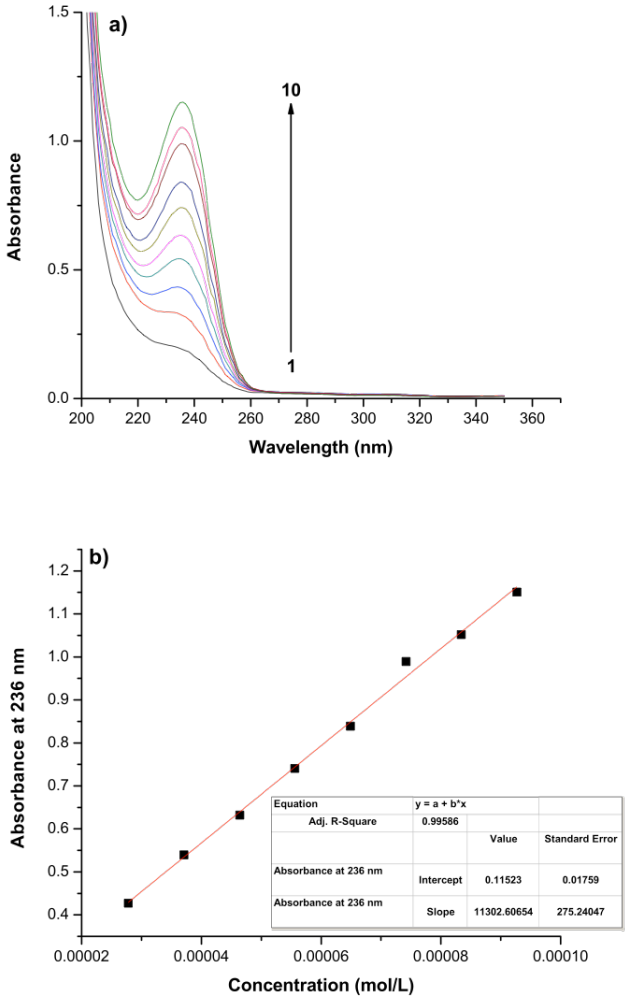

Figure 1. (a) Absorption spectra of the metformin in methanol solution: for spectra 1–10, metformin concentration (M): (1) 0.00000927; (2) 0.0000185;(3) 0.0000278; (4) 0.0000371; (5) 0.0000464; (6) 0.0000556; (7) 0.0000649; (8) 0.0000742; (9) 0.0000834; (10) 0.0000927.

(b) Linear regression using the Lambert-Beer equation for the determination of the molar extinction coefficient for metformin in methanol.

The determination of the formation constant β_{110}, correspond to equilibrium between Cu^{2+} and metformin. This process consist in analyze simultaneously, all the spectra of the two experiments at two different concentrations of stock solutions of copper and use different ranges of concentration of metformin for each experiment. This method involves the correlation between the spectrum obtained, the concentration of metal and ligand used and a proposal of possible colored species. The observed absorbance values at different wavelengths were recorded at 298 K. Considering that only one colored species plus Cu^{2+} and metformin were found, the formation constants determination was achieved using the next model:

$$Cu^{2+} + metf \rightleftharpoons [Cu(metf)]^{2+} \qquad \beta_{110} \qquad (1)$$

The obtained results show the formation only of the *mono* complex $[Cu(metf)]^{2+}$. In Fig.2b is presented the calculated spectrum of the $[Cu(metf)]^{2+}$ which shows a maximum at 270 nm and $\varepsilon = 2627.8\ L \cdot mol^{-1} \cdot cm^{-1}$ with a formation constant of 4.44 ± 0.01. The conditions of the experiment and results are reported in Table 2 in APPENDIX, according to the format suggested by Tuck.[28]

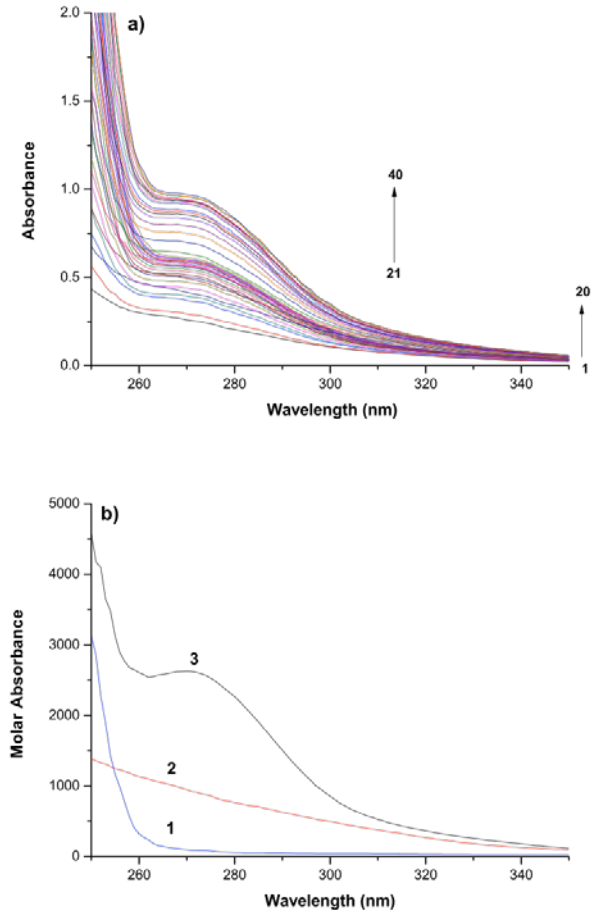

Figure 2: (a) Absorption spectra of the cooper(II)-metformin system in methanol solution: for spectra 1-20, [Cu(II)]= 0.00021M and metformin concentration (M): (1) 0.00004;(2) 0.00007;(3) 0.00011;(4) 0.00015;(5) 0.00019;(6) 0.00022;(7) 0.00026;(8) 0.00030;(9) 0.00033;(10) 0.00037;(11) 0.00041;(12) 0.00044; (13) 0.00048;(14) 0.00052; (15) 0.00056;(16) 0.00059;(17) 0.00063;(18) 0.00067;(19) 0.0007;(20) 0.00074. For spectra 21-40, [Cu(II)]= 0.00035M and metformin concentration (M): (21) 0.00006;(22) 0.00012;(23) 0.00019;(24) 0.00025;(25) 0.00031;(26) 0.00037;(27) 0.00043;(28) 0.0005;(29) 0.00056;(30) 0.00062;(31) 0.00068;(32) 0.00074;(33) 0.00081;(34) 0.00087;(35) 0.00093;(36) 0.00099;(37) 0.00105;(38) 0.00112;(39) 0.00118;(40) 0.00124.

(b) Calculated electronic spectra of the copper(II)-metformin complexes in methanol. (1) Metformin; (2) Cu^{2+}; (3) $[Cu(metf)]^{2+}$.

Nevertheless, as far as we know there are no reports about of equilibrium determinations of this complex in methanol or other solvent. Copper(II)-metformin complexes have been studied before in aqueous systems, in which only the *bis* species was isolated, but not the *mono* species. Also, the crystal structures of the *bis*-complexes has been reported in alkaline aqueous solution, where is confirmed that every metformin molecule is bind through two nitrogen atoms in a square-planar geometry.[25]

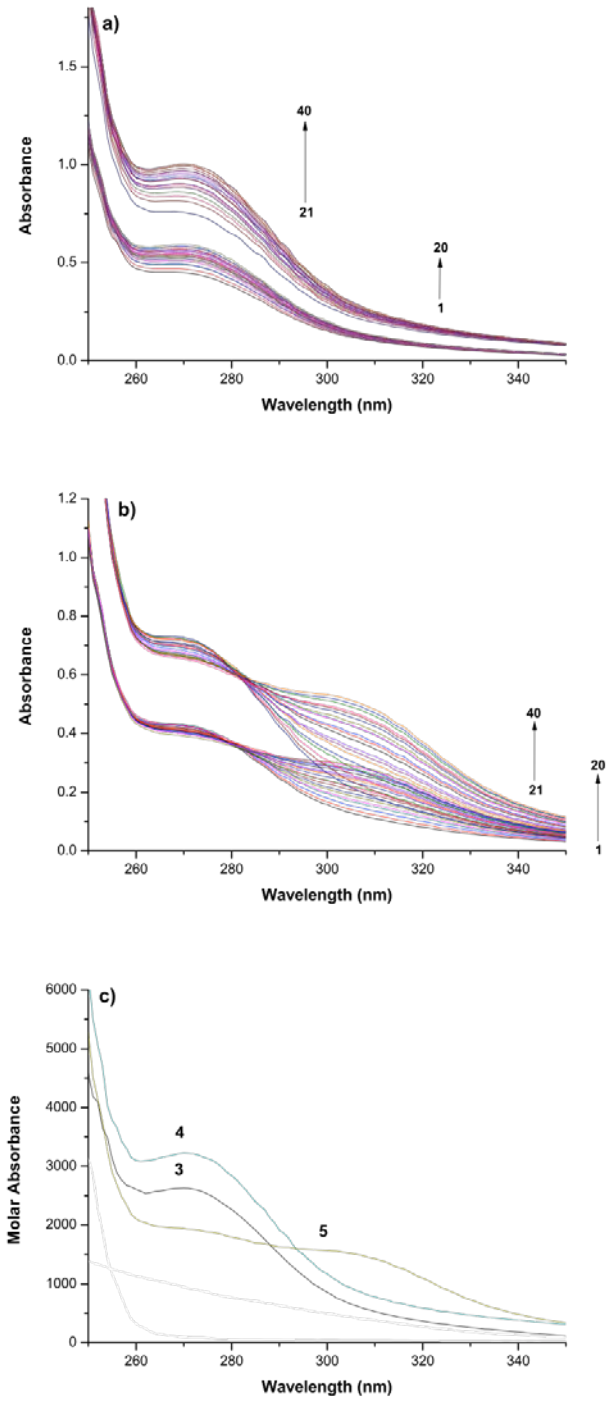

Figure 3 (a): Absorption spectra of the copper(II)-metformin-chloride system in methanol solution: for spectra 1-20, [Cu(II)]= 0.00021M, [metformin]= 0.00022M and sodium chloride concentration (M): (1) 0.00001; (2)0.00003; (3) 0.00004; (4) 0.00005; (5) 0.00006; (6) 0.00008; (7) 0.00009; (8) 0.0001; (9) 0.00011; (10) 0.00013; (11) 0.00014; (12) 0.00015; (13) 0.00016; (14) 0.00018; (15) 0.00019; (16) 0.0002; (17) 0.00021; (18) 0.00023; (19) 0.00024; (20) 0.00025. For spectra 21-40, [Cu(II)]= 0.00034M, [metformin]= 0.00037M and chloride concentration (M): (21) 0.00002; (22) 0.00004; (23) 0.00007; (24) 0.00009; (25) 0.00011; (26) 0.00013; (27) 0.00015; (28) 0.00018; (29) 0.0002; (30) 0.00022; (31) 0.00024; (32) 0.00026; (33) 0.00029; (34) 0.00031; (35) 0.00033; (36) 0.00035; (37) 0.00037; (38) 0.0004; (39) 0.00042; (40) 0.00044.

(b) Absorption spectra of the cooper(II)-metformin-bromide system in methanol solution: for spectra 1-20, [Cu(II)]= 0.00021M, [metformin]= 0.00022M and sodium bromide concentration (M): (1) 0.00001; (2)0.00003; (3) 0.00004; (4) 0.00005; (5) 0.00006; (6) 0.00008; (7) 0.00009; (8) 0.0001; (9) 0.00011; (10) 0.00013; (11) 0.00014; (12) 0.00015; (13) 0.00016; (14) 0.00018; (15) 0.00019; (16) 0.0002; (17) 0.00021; (18) 0.00023; (19) 0.00024; (20) 0.00025. For spectra 21-40, [Cu(II)]= 0.00034M, [metformin]= 0.00037M and bromide concentration (M): (21) 0.00002; (22) 0.00004; (23) 0.00007; (24) 0.00009; (25) 0.00011; (26) 0.00013; (27) 0.00015; (28) 0.00018; (29) 0.0002; (30) 0.00022; (31) 0.00024; (32) 0.00026; (33) 0.00029; (34) 0.00031; (35) 0.00033; (36) 0.00035; (37) 0.00037; (38) 0.0004; (39) 0.00042; (40) 0.00044.

(c) Calculated electronic spectra of the copper(II)-metformin-halide complexes in methanol: (3) $[Cu(metf)]^{2+}$, (4) $[Cu(metf)Cl]^{1+}$, (5) $[Cu(metf)Br]^{1+}$.

Differing from the reported studies in aqueous systems, in this work, the copper(II)-metformin *bis* complex cannot be identified. The *mono* species copper-metformin found could be explained considering the nucleophilic character of methanol. Metformin should compete with the solvent molecules bound to the metal ion. Also, has been reported that in general for copper complexes with simple diamine ligands, stability constants are higher in aqueous systems than in methanol.[29] Is conceivable that the nucleophilic environment promoted by methanol could create intramolecular interactions of several nitrogen atoms of the metformin to the copper center, increasing the chelate effect and in consequence, the stability and formation of the *mono* species.

This may decrease the probability of the formation for the *bis* species. In order to evaluate this theory, a crystallographic study should be done to isolate and characterize the *mono* species, using water at different pH values.

3.3. Formation constants of the copper(II)-metformin-halogen complexes.

With the purpose to study the hardness or softness character of the copper-metformin complex, the interaction of halides with the copper center in the complex was studied by generating the ternary complex with metformin and halides. Nevertheless, interaction of the copper(II)-metformin complexes with chloride has been studied before and the structures of *bis*(*N*,*N*-dimethylbiguanide) copper(II) dichloride dihydrate and di-□-chloro*bis*-[chloro-(*N*,*N*-dimethylbiguanide)copper(II)] have been reported.[30] Also, as far as we know, there are no reports about

the equilibrium determinations of the copper– metformin ternary complexes.

For this purpose it was employed an equimolar concentration of copper and metformin and was maintained as constant, in each experiment is varied only the concentration of chloride or bromide. The observed electronic spectra of the copper(II)-metformin-halide solutions are shown in Fig 3. For the chloride system, it can be shown that increasing the chloride concentration generates a hyperchromic effect (Fig. 3a), with a maximum absorption at 270 nm, which indicate the formation on the chloride complex.

On the other hand, increasing the bromide concentrations promotes a hyperchromic and a bathochromic effect to (Fig. 3b); this variation could be related to a slight change in the geometry of the complex.

The determination of the formation constants β_{jkl} corresponds to a successive equilibria between copper(II), metformin and each halide, were made using the same methodology as described above. The observed absorbance values at different wavelengths were recorded at 298 K. Considering that only one colored species plus complex $[Cu(metf)]^{2+}$ were found, the formation constants determination was achieved using the next model:

$$Cu^{2+} + metf + Cl^{-} \rightleftharpoons [Cu(metf)Cl]^{1+} \qquad \beta_{111} \qquad (2)$$

$$Cu^{2+} + metf + Br^{-} \rightleftharpoons [Cu(metf)Br]^{1+} \qquad \beta_{111} \qquad (3)$$

Only the complexes $[Cu(metf)Cl]^{1+}$ or $[Cu(metf)Br]^{1+}$, were found. The formation only of the *mono* halide complexes is probably related to a steric effect generated by the metformin when is bonded to the metal ion; also the nucleophilic character of methanol could contribute. The calculated electronic spectrum of the $[Cu(metf)Cl]^{1+}$ and $[Cu(metf)Br]^{1+}$ complexes are presented in Fig.3c. For the $[Cu(metf)Cl]^{1+}$ shows an absorption maximum at 270 nm and ε=3176.9 $L \cdot mol^{-1} \cdot cm^{-1}$. Meanwhile for the $[Cu(metf)Br]^{1+}$ shows an absorption maximum at 296 nm with ε=1583.6 $L \cdot mol^{-1} \cdot cm^{-1}$. The obtained formation constant for the $[Cu(metf)Cl]^{1+}$ complex is 8.88± 0.01 and for $[Cu(metf)Br]^{1+}$ is 9.62± 0.02 (Table 2 in APPENDIX). This increase of stability and absorption of the halide complexes related to the $[Cu(metf)]^{2+}$, can be explained considering a major charge transfer of the halide to the metal center, this increases the softness of the metal center, being bromide softer than chloride, promotes a higher stability. Also the halide ion could stabilize the geometry of the complex. According to the results presented here, copper-metformin complex might behave as a soft acid with an affinity by soft bases. Considering that mitochondrial chloride concentration is found in a very wide range[25], the results presented in this work, may show new implications. The activity of the copper-metformin-chloride species should be correlated with in vivo studies.

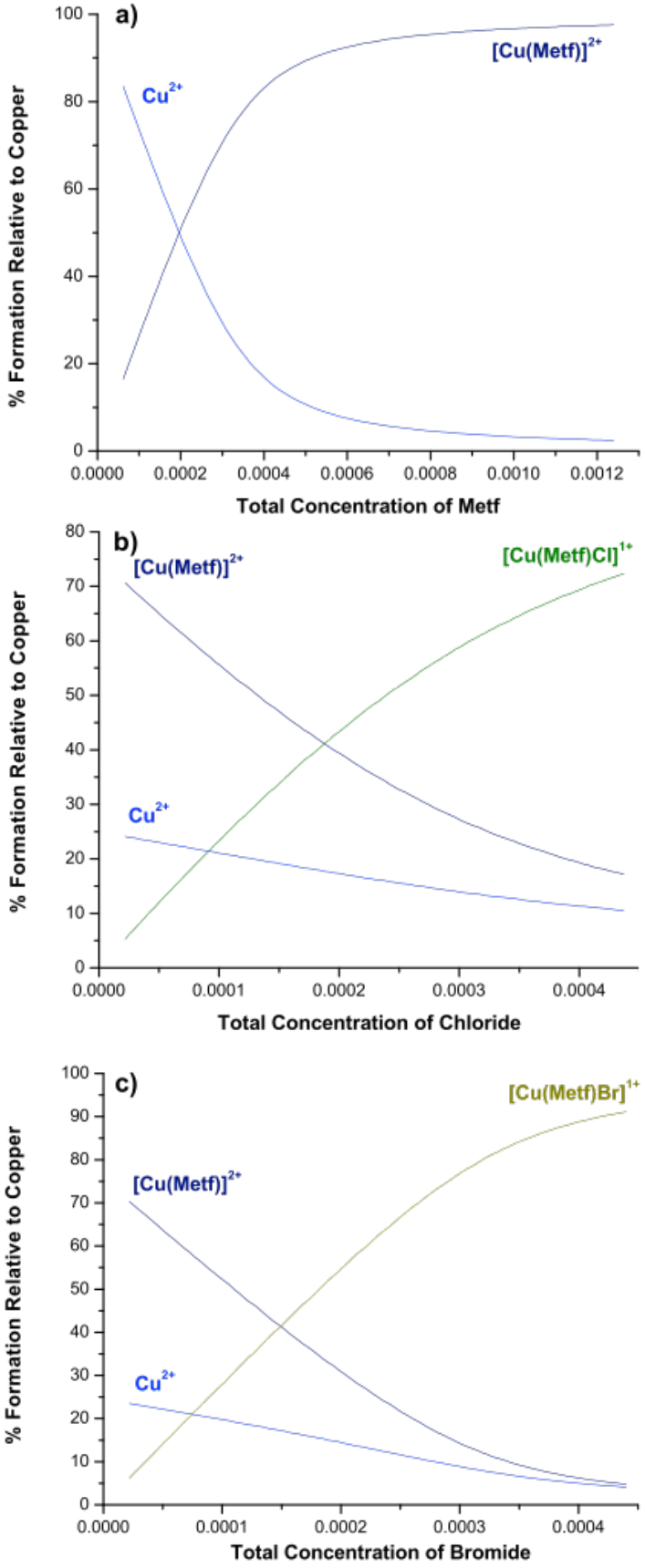

Figure. 4. (a) Formation curves of the copper(II)-metformin complex in methanol. $[Cu^{2+}]$= 0. 00035M and metformin range from 0.00006M to 0.00124M.

(b) Formation curves of the copper(II)-metformin-chloride system in methanol. $[Cu^{2+}]$= 0.00034M, metformin 0.00037M and sodium chloride range from 0.00002M to 0.00044M.

(c) Formation curves of the copper(II)-metformin-bromide complexes in methanol. $[Cu^{2+}]$= 0.00034M, metformin 0.00037M and sodium bromide range from 0.00002M to 0.00044M.

3.4 Distribution curves for the complexes Copper(II)-metformin and Copper(II)-metformin-chloride and bromide.

Speciation diagrams of copper(II)-metformin, copper(II)-metformin-chloride and copper(II)-metformin-bromide are shown in Fig. 4a, b and c, respectively.

A solution with an equimolar concentration of copper(II) and metformin yields 95.65% of the complex $[Cu(metf)]^{2+}$ and less of 5% of ionic copper. In the case of ternary systems, a solution with an equimolar concentration of copper(II), metformin and chloride generate 72% of the $[Cu(metf)Cl]^{1+}$, about 11% of ionic copper and 17% of $[Cu(metf)]^{2+}$. In the case of the experiments with bromide, a solution with equimolar concentration of copper(II), metformin and bromide yields, about 91% of the complex $[Cu(metf)Br]^{1+}$, 4% of ionic copper and about 5% of $[Cu(metf)]^{2+}$.

IV. CONCLUSION

Because the solubility of the complexes, no ionic strength was used, the formation constants reported here should be only used in a comparative way with other systems measured in similar conditions and should not be considered as true thermodynamic equilibrium constants. For the copper(II)-metformin system was observed only the formation of the *mono* species, this differ from previously reported studies in aqueous systems in which the *bis* species was isolated, but not the *mono* species.

Further studies should be done to isolate *mono* species, also crystallographic studies could help establish analyze the structure of the species. Also, in order to evaluate the nucleophillic character of these complexes, studies in several solvents with different donor numbers, including water at different pH values, should be done. The formation constant for $[Cu(metf)Cl]^{1+}$ and $[Cu(metf)Br]^{1+}$ are higher than the constant for $[Cu(metf)]^{2+}$. Is possible that metformin increases the softness of the copper center, this promotes an affinity for a softer base. The formation constant for the bromo-species is higher than for the chloro species, considering that bromide is a softer base than chloride, copper center should work as a softer acid. This work shows that chloride possibly could be coordinated to a copper(II)-metformin species. This could generate a far more stable species: $[Cu(metf)Cl]^{1+}$. Considering the chloride concentration in mitochondria, this opens the possibility to theorize a new active and stable species. Hopefully, these data can be used to correlate with the in vivo studies and analyze the possible implications of these findings.

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APPENDIX

Table 2. Summary of experimental parameters for the system: copper(II)–metformin and copper(II)–metformin–halogen complexes in methanol.

Solution composition		$[T_L]$ range from 0.00004 to 0.00074M and 0.00006 a 0.00124M $[T_M]$ constant at 0.00021M and 0.00035M.	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric	
Temperature		25ºC	
Total number of data points		Cu complexation: 39 solution spectra	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
Cu(metf)	$Cu^{2+}+metf \rightleftharpoons [Cu(metf)]^{2+}$	$\log \beta_{110} = 4.44 \pm 0.01$	0.0131
Solution composition		$[T_X]$ range from 0.00001 to 0.00025M and 0.00002 to 0.00044M $[T_{L1}]$ constant at 0.00022M and 0.00037M $[T_M]$ constant at 0.00021M to 0.00034M.	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric	
Temperature		25ºC	

Total number of data points		Cu-Ligand to halide complexation: 39 solution spectra for chloride ion and 39 solution spectra for bromide ion.	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
Cu(metf)Cl	$Cu^{2+}+metf+Cl^{-} \rightleftharpoons [Cu(metf)Cl]^{1+}$	$\log \beta_{111} = 8.88 \pm 0.01$	0.0025
Cu(metf)Br	$Cu^{2+}+metf+Br^{-} \rightleftharpoons [Cu(metf)Br]^{1+}$	$\log \beta_{111} = 9.62 \pm 0.02$	0.0076