

# COMPLEX FORMATION EQUILIBRIA OF COPPER(II), METFORMIN AND D-(+)-MANNOSE OR D-(+)-GLUCOSE IN METHANOL

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**Abstract-** Diabetes Mellitus is a metabolic disorder affecting carbohydrate metabolism, metformin is a drug widely used in the treatment of this disease. However, its mechanism of action has not been known yet, although several theories have been proposed to explain its hypoglycemic activity. A recent theory suggests a relationship between metformin and mitochondrial copper. On the other hand, glucose is the most abundant carbohydrate working as energy source; this carbohydrate has an important role in Diabetes Mellitus. In the present research work is reported a UV-VIS study with the purpose of analyzing the in vitro interaction between the ionic copper, metformin and glucose or mannose. The formation constant of ternary complexes of copper with metformin and glucose or mannose and its calculated electronic spectrum of each species in methanol solution, are reported. Hopefully, this information can be used in order to predict some of the possible interactions in vivo.

**Index Terms-** Diabetes mellitus; D-(+)-mannose; D-(+)-glucose; metformin

## I. INTRODUCTION

Diabetes mellitus II is the fifth leading cause of death globally [1-3]. This disease interferes with the carbohydrate metabolism with which generate a chronic hyperglycemia [4-6]. The main effect in diabetes consists of excessive hepatic glucose production, peripheral insulin resistance and a defective secretion of insulin in beta-cells [7]. Type 2 diabetes mellitus is a treatable but not curable [8], currently the most commonly used medications for treatment of Type 2 diabetes are the metformin and sulfonylureas [9]. Metformin or dimethylbiguanide is the first-line drug [10-13], which lowers the blood glucose level by possibly suppressing hepatic glucose output and enhancing peripheral glucose uptake [8, 14]. The mechanism of action has not been defined [11, 15, 16], yet several theories have been elaborated to explain metformin action, its activity is probably related to AMPK (AMP-activated protein kinase) which promotes the use of blood glucose in the muscle[17, 18]. On the opposite, other studies propose the possibility that metformin might interact with mitochondrial copper and the therapeutic effect by metformin is linked to the properties of a copper-metformin species and its possible interaction with glucose [19, 20]. Glucose is an insulin dependant carbohydrate [21, 22], mannose, in contrast to glucose, fails to stimulate a measurable pancreatic release of insulin[23-25]. In this work, with the purpose to analyze an in-vitro direct interaction between the copper-metformin complex and the sugars: D-glucose, D-mannose and also chlorides, a spectrophotometric study was carried out. The calculated electronic spectrum and the formation constants of the complex copper (II)-metformin with each sugar and chlorides in methanol, is reported. Hopefully this information allows theorizing an in vivo interaction between metformin and these sugars.

## II. MATERIALS AND METHODS

Cu(NO<sub>3</sub>)<sub>2</sub>·2.5H<sub>2</sub>O (Fermont, Mexico), D-(+)-mannose and D-(+)-glucose (Sigma Aldrich) were of analytical grade and used without further purification. Metformin was obtained from Ficonax tablets (PiSA, Mexico). Methanol HPLC grade (Fermont, Mexico) was used as the solvent for the extraction of metformin from tablets and the determination of the formation constant for the complexes.

### A. Physical measurements

The spectral measurements were performed using a Shimadzu 1800 UV-Vis Spectroscopy system at 298 K (RT), using quartz cuvettes with 1 cm path length and 3mL of volume. The spectral observed region observed was from 210 to 350 nm and was used for all experiments. The formation constants were determined by the spectrophotometric data refinement using the HypSpec software[26] and the distribution diagrams of species were calculated using software Hyperquad simulation and speciation (HySS) [26]. The use of this spectrophotometric method has been reported before [15, 27].

### B. Extraction and purification of metformin

For the extraction and purification of metformin, tablets were pulverized and homogenized with methanol HPLC. The solution was filtered under vacuum and stored at 277 K until the formation of crystals of metformin; later were separated by vacuum filtration.

### C. Equilibrium studies

Experiments were performed using two different stock solutions of D-(+)-mannose (man) and D-(+)-glucose (glc) (355.20  $\mu\text{M}$  and 488.40  $\mu\text{M}$ ), metformin (met) (372.00  $\mu\text{M}$  and 496.00  $\mu\text{M}$ ), chloride (Cl) (342.20  $\mu\text{M}$  and 478.80  $\mu\text{M}$ ) and  $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$  was used to prepare stock solutions of ionic copper (344.00  $\mu\text{M}$  and 481.60  $\mu\text{M}$ ). In each experiment the concentration of copper and metformin were varied, metformin from 3.72  $\mu\text{M}$  to 74.40  $\mu\text{M}$  and 4.96  $\mu\text{M}$  to 99.20  $\mu\text{M}$  and  $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$  from 3.44  $\mu\text{M}$  to 68.80  $\mu\text{M}$  and 4.82  $\mu\text{M}$  to 96.32  $\mu\text{M}$ , respectively. In each experiment, the final mannose or glucose concentration was set constant at 35.52  $\mu\text{M}$  and 48.84  $\mu\text{M}$ , respectively. For the systems with chloride, the chloride concentration was varied from 3.42  $\mu\text{M}$  to 68.44  $\mu\text{M}$  and 4.79 to 95.76  $\mu\text{M}$ . A total of 40 spectra were used for the refining process in each system.

### III. RESULTS

In this study, in all the equilibrium determinations, it is required to avoid the use of ionic strength; if the ionic strength is increased a precipitation of the copper complexes occurs. Instead of water, methanol was used as the solvent. Considering that water with a donor number of 18 and methanol of 19, methanol is slightly nucleophilic than water and increase stability the complex[28-30]. Using water as solvent at physiological pH ranges is very important. Nevertheless, using water might generate hydroxylated or protonated species. The generation of several species at the same time might interfere with the formation constant determination. In this first study, water was avoided as a solvent in order to generate a starting point to find an accurate value of a formation constant so that in later studies water can be used as a solvent.

#### A. Formation constants of the copper (II)-metformin with D-(+)-mannose and D-(+)-glucose

The formation of the complexes of copper (II) and metformin with glucose or mannose has not been reported before. Nevertheless we use as a starting point the previously reported values of the formation constants for the species copper(II)-metformin with chloride and bromide[15]. The electronic spectra of the solutions for the systems of copper(II)-metformin-D-(+)-mannose and copper(II)-metformin-D-(+)-glucose are presented in Fig. 1 and 2, respectively. For both systems when the concentration of copper and metformin are increased, a hyperchromic effect at 235 nm is observed, in both systems.

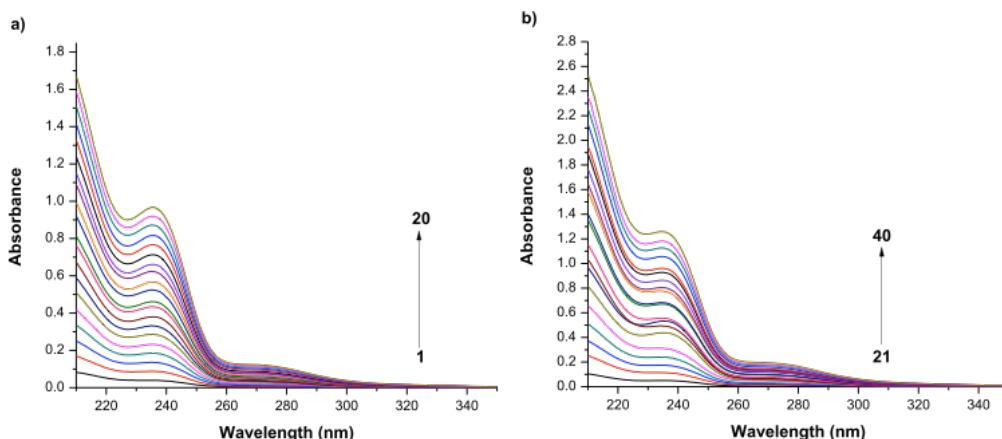
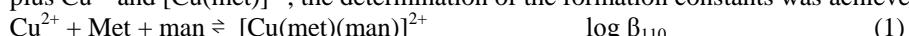


Figure 1. Absorption spectra of copper (II)-metformin with D-(+)-mannose system in methanol solution, (a) For spectra 1-20: [man] = 35.52  $\mu\text{M}$ , [Cu(II)] = 3.474  $\mu\text{M}$  to 68.80  $\mu\text{M}$  and [Met] = 3.72  $\mu\text{M}$  to 74.40  $\mu\text{M}$ . (b) For spectra 21-40: [man] = 48.84  $\mu\text{M}$ , [Cu(II)] = 4.82  $\mu\text{M}$  to 96.32  $\mu\text{M}$  and [Met] = 4.96  $\mu\text{M}$  to 99.20  $\mu\text{M}$

The determination of the formation constants represents the equilibrium between  $\text{Cu}^{2+}$ , metformin, and mannose or glucose, in each system respectively. This process consisting of the simultaneous analysis of the spectra of the experiments at two different concentrations of the stock solutions of copper and metformin and the use of different concentration range of mannose or glucose. This method is based on the correlation between the spectrum obtained, the concentration of the used metal and ligands and a proposal of possible colored species. Absorbance values were obtained at several wavelengths, considering a single colored species was found plus  $\text{Cu}^{2+}$  and  $[\text{Cu}(\text{met})]^{2+}$ , the determination of the formation constants was achieved using the next model:



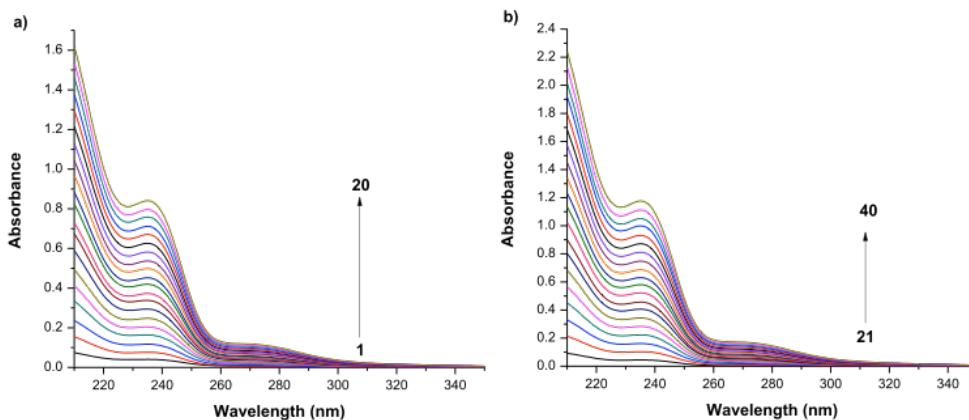


Figure 2. Absorption spectra of copper (II)-metformin with D-(+)-glucose system in methanol solution, (a) For spectra 1-20:  $[glc] = 35.52 \mu M$ ,  $[Cu(II)] = 3.474 \mu M$  to  $68.80 \mu M$  and  $[Met] = 3.72 \mu M$  to  $74.40 \mu M$ . (b) For spectra 21-40,  $[glc] = 48.84 \mu M$ ,  $[Cu(II)] = 4.82 \mu M$  to  $96.32 \mu M$  and  $[Met] = 4.96 \mu M$  to  $99.20 \mu M$ .

The logarithmic value of the formation constants obtained in this study and the summary of the experimental parameters are reported in Table 1. The results obtained show the unique formation of a mono-species for the system with mannose or glucose, with a formation constant of  $\log \beta_{110} = 10.52 \pm 0.02$  and  $\log \beta_{110} = 10.61 \pm 0.01$ , respectively. The calculated electronic spectrum of the species  $[Cu(met)(man)]^{2+}$  and  $[Cu(met)(glc)]^{2+}$  are shown in Fig. 3a), both species show a maximum at 235 nm and  $\epsilon = 15880 \text{ L mol}^{-1} \text{cm}^{-1}$  and  $\epsilon = 13806 \text{ L mol}^{-1} \text{cm}^{-1}$ , respectively.

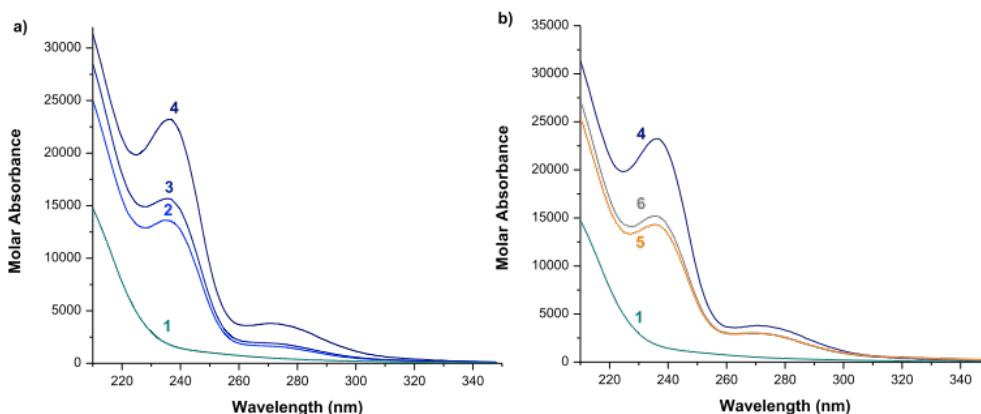


Figure 3. a) Calculated electronic spectra of the copper (II)-metformin with D-(+)-mannose and D-(+)-glucose complexes in methanol. (1)  $Cu^{2+}$ ; (2)  $[Cu(met)(glc)]^{2+}$ ; (3)  $[Cu(met)(man)]^{2+}$ ; (4)  $[Cu(met)]^{2+}$ . b) Calculated electronic spectra of the copper (II)-metformin with D-(+)-mannose and D-(+)-glucose with chloride complexes in methanol. (1)  $Cu^{2+}$ ; (5)  $[[Cu(met)(glc)]Cl]^{1+}$ ; (6)  $[[Cu(met)(man)]Cl]^{1+}$ ; (4)  $[Cu(met)]^{2+}$ .

In this work, the mono species of copper-metformin with mannose and glucose were found, but the *bis*-complex was not identified. This could be explained considering the nucleophilic character of methanol, which is higher than water [28, 30], also is possibly that the oxygen atoms of the sugar molecules increases the stability of the complexes and might generate a steric hindrance.

#### B. Formation constants of the copper (II)-metformin with D-(+)-mannose or D-(+)-glucose with chloride

The electronic spectra of the solutions for the systems of copper(II)-metformin-D-(+)-mannose and copper(II)-metformin-D-(+)-glucose both with chloride are presented in Fig. 4 and 5. For both systems when the concentration of the solution with copper, metformin and chloride is increased, a hyperchromic effect at 235 nm is observed, in both systems. The determination of the formation constants represents the equilibrium between  $Cu^{2+}$ , metformin, mannose or glucose and chloride. This process consists also in the simultaneous analysis of the spectra of the experiments at two different concentrations of the stock solutions of copper, metformin, mannose or glucose and chloride, and the use of different concentration ranges of mannose or glucose in each experiment. This method is based on the correlation between the spectrum obtained, the metal and the ligands concentrations used, as well as a suggestion of possible colored species.

Table 1: Summary of experimental parameters for the complex system of copper (II)-metformin-D-(+)-mannose or D-(+)-glucose and systems with halides in methanol

Solution composition		[T <sub>L</sub> ] range from 3.72 to 74.40 $\mu\text{Mol L}^{-1}$ and 4.96 to 99.20 $\mu\text{Mol L}^{-1}$ [T <sub>M</sub> ] range from 3.44 to 68.80 $\mu\text{Mol L}^{-1}$ and 4.82 to 96.32 $\mu\text{Mol L}^{-1}$ [T <sub>X</sub> ] constant at 35.52 and 48.84 $\mu\text{Mol L}^{-1}$	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric titration	
Temperature		25°C	
Total number of data points		Cu complexation: 40 solution spectra	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
[Cu(met)(man)] <sup>2+</sup>	[Cu(met)] <sup>2+</sup> + man ⇌ [Cu (met)(man)] <sup>2+</sup>	log β <sub>110</sub> = 10.52 ± 0.02	0.0147
Solution composition		[T <sub>L1</sub> ] range from 3.42 to 68.44 $\mu\text{Mol L}^{-1}$ and 4.79 to 95.76 $\mu\text{Mol L}^{-1}$ [T <sub>L</sub> ] range from 3.72 to 74.40 $\mu\text{Mol L}^{-1}$ and 4.96 to 99.20 $\mu\text{Mol L}^{-1}$ [T <sub>M</sub> ] range from 3.44 to 68.80 $\mu\text{Mol L}^{-1}$ and 4.82 to 96.32 $\mu\text{Mol L}^{-1}$ [T <sub>X</sub> ] constant at 35.52 and 48.84 $\mu\text{Mol L}^{-1}$	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric titration	
Temperature		25°C	
Total number of data points		Cu-ligand-chloride to mannose complexation: 40 solution spectra	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
[Cu(met)(man)Cl] <sup>+</sup>	[Cu(met)Cl] <sup>+</sup> + man ⇌ [Cu(met)(man)Cl] <sup>1+</sup>	log β <sub>111</sub> = 15.64 ± 0.01	0.0059
Solution composition		[T <sub>L</sub> ] range from 3.72 to 74.40 $\mu\text{Mol L}^{-1}$ and 4.96 to 99.20 $\mu\text{Mol L}^{-1}$ [T <sub>M</sub> ] range from 3.44 to 68.80 $\mu\text{Mol L}^{-1}$ and 4.82 to 96.32 $\mu\text{Mol L}^{-1}$ [T <sub>X</sub> ] constant at 35.52 and 48.84 $\mu\text{Mol L}^{-1}$	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric titration	
Temperature		25°C	
Total number of data points		Cu complexation: 40 solution spectra	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
[Cu(met)(glc)] <sup>2+</sup>	[Cu(met)] <sup>2+</sup> + glc ⇌ [Cu (met) (glc)] <sup>2+</sup>	log β <sub>110</sub> = 10.61 ± 0.01	0.0052
Solution composition		[T <sub>L1</sub> ] range from 3.42 to 68.44 $\mu\text{Mol L}^{-1}$ and 4.79 to 95.76 $\mu\text{Mol L}^{-1}$ [T <sub>L</sub> ] range from 3.72 to 74.40 $\mu\text{Mol L}^{-1}$ and 4.96 to 99.20 $\mu\text{Mol L}^{-1}$ [T <sub>M</sub> ] range from 3.44 to 68.80 $\mu\text{Mol L}^{-1}$ and 4.82 to 96.32 $\mu\text{Mol L}^{-1}$ [T <sub>X</sub> ] constant at 35.52 and 48.84 $\mu\text{Mol L}^{-1}$	
		Ionic strength, electrolyte	Not used
		pH range	Not used
Experimental method		Spectrophotometric titration	
Temperature		25°C	
Total number of data points		Cu-ligand-chloride to glucose complexation: 40 solution spectra	
Method of calculation		HypSpec	
Species	Equilibrium	Log β	σ
[Cu(met)(glc)Cl] <sup>+</sup>	[Cu(met)Cl] <sup>+</sup> + glc ⇌ [Cu(met)(glc)Cl] <sup>1+</sup>	log β <sub>111</sub> = 16.18 ± 0.01	0.0066

Absorbance values were obtained at several wavelengths, considering a single colored species were found plus  $\text{Cu}^{2+}$ ,  $[\text{Cu}(\text{met})]^{2+}$  and  $[\text{Cu}(\text{met})(\text{man})]^{2+}$  or  $[\text{Cu}(\text{met})(\text{glc})]^{2+}$ , the determination of the formation constants was achieved using the next model:

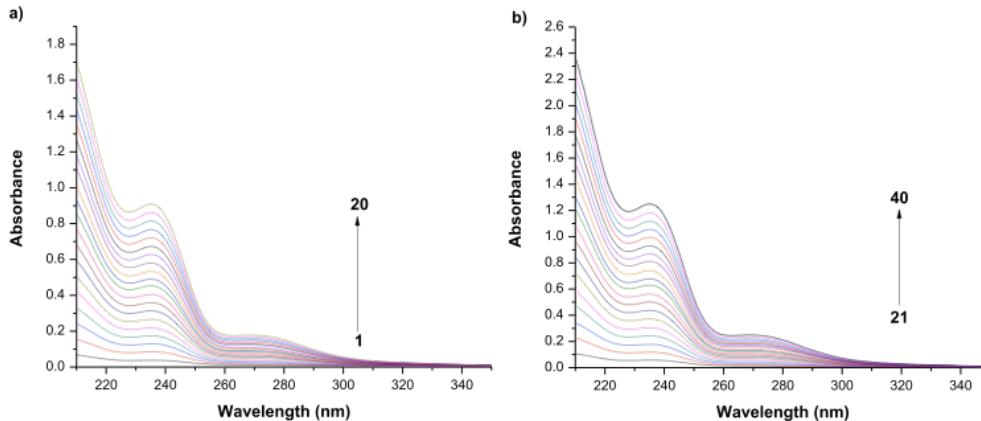
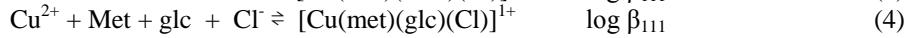
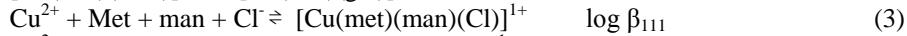


Figure 4. Absorption spectra of copper (II)-metformin-D-(+)-mannose with chloride system in methanol solution: (a) For spectra 1-20,  $[\text{man}] = 35.52 \mu\text{M}$ ,  $[\text{Cu}(\text{II})] = 3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ ,  $[\text{Met}] = 3.72 \mu\text{M}$  to  $74.40 \mu\text{M}$  and  $[\text{Cl}] = 3.42 \mu\text{M}$  to  $68.44 \mu\text{M}$ . (b) For spectra 21-40,  $[\text{man}] = 48.84 \mu\text{M}$ ,  $[\text{Cu}(\text{II})] = 4.82 \mu\text{M}$  to  $96.32 \mu\text{M}$ ,  $[\text{Met}] = 4.96 \mu\text{M}$  to  $99.20 \mu\text{M}$  and  $[\text{Cl}] = 4.79 \mu\text{M}$  to  $95.76 \mu\text{M}$ .

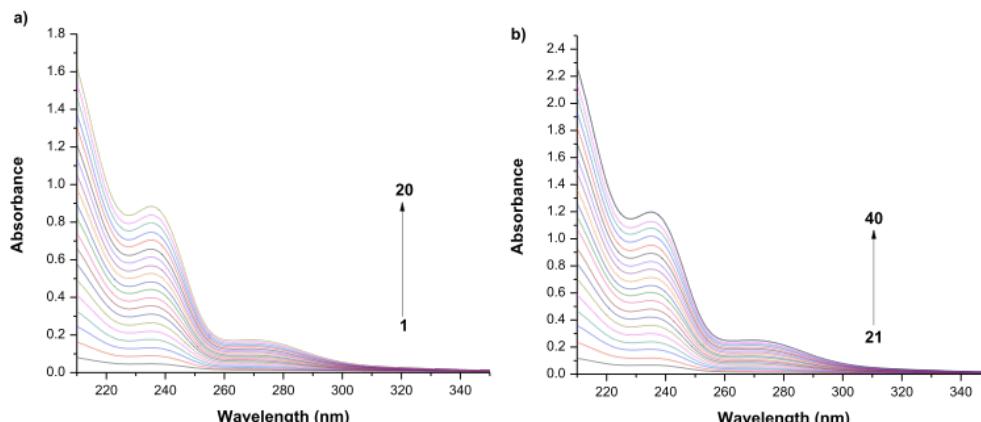


Figure 5. Absorption spectra of copper (II)-metformin-D-(+)-glucose with chloride system in methanol solution: (a) For spectra 1-20,  $[\text{glc}] = 35.52 \mu\text{M}$ ,  $[\text{Cu}(\text{II})] = 3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ ,  $[\text{Met}] = 3.72 \mu\text{M}$  to  $74.40 \mu\text{M}$  and  $[\text{Cl}] = 3.42 \mu\text{M}$  to  $68.44 \mu\text{M}$ . (b) For spectra 21-40,  $[\text{glc}] = 48.84 \mu\text{M}$ ,  $[\text{Cu}(\text{II})] = 4.82 \mu\text{M}$  to  $96.32 \mu\text{M}$ ,  $[\text{Met}] = 4.96 \mu\text{M}$  to  $99.20 \mu\text{M}$  and  $[\text{Cl}] = 4.79 \mu\text{M}$  to  $95.76 \mu\text{M}$ .

The logarithmic value of the formation constants obtained in this study and the summary of the experimental parameters are reported in Table 1. The results obtained show the unique formation of a mono-species for the system with mannose or glucose and chloride, with a formation constant of  $\log \beta_{111} = 15.64 \pm 0.01$  and  $\log \beta_{111} = 16.21 \pm 0.01$ , respectively. The calculated electronic spectrum of the species  $[\text{Cu}(\text{met})(\text{man})(\text{Cl})]^{1+}$  and  $[\text{Cu}(\text{met})(\text{glc})(\text{Cl})]^{1+}$  are shown in Fig. 3b, this spectrum have a maximum at 235 nm and  $\epsilon = 15183 \text{ L mol}^{-1} \text{ cm}^{-1}$  and  $\epsilon = 14293 \text{ L mol}^{-1} \text{ cm}^{-1}$ , respectively.

#### C. Distribution curves of the systems of copper(II)-metformin-D-(+)-mannose and copper(II)-metformin-D-(+)-mannose-chloride

The Fig. 6a and b shows the distribution diagrams of the system copper(II)-metformin-D-(+)-mannose and copper(II)-metformin-D-(+)-mannose-chloride. A solution with an equimolar concentration of copper (II), metformin and mannose has a formation of 94.6% for  $[\text{Cu}(\text{met})(\text{man})]^{2+}$  and about of 5.40% of ionic copper. For the chloride experiments, a solution with an equimolar concentration of copper(II), metformin, mannose and chloride, yields approximately 98.65% of  $[[\text{Cu}(\text{met})(\text{man})]\text{Cl}]^{1+}$  and 1.35% of ionic copper.

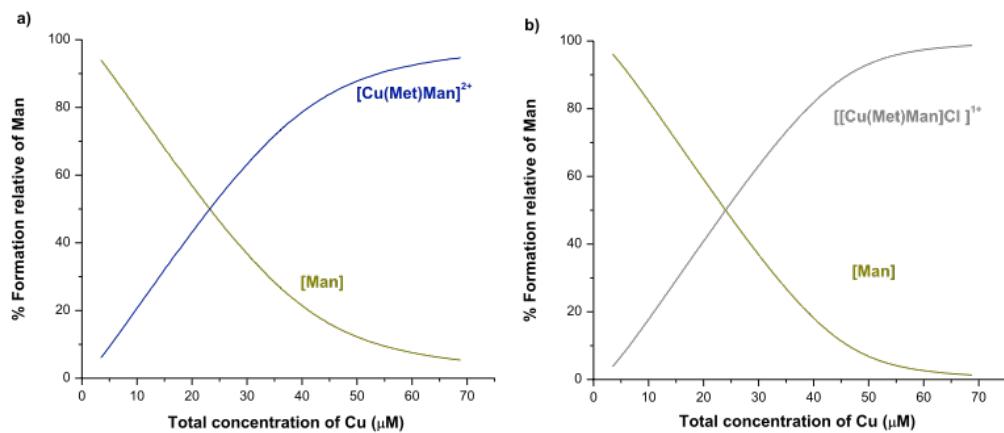


Figure 6. Formation curves of the copper (II)-metformin-D-(+)-mannose complexes in methanol a)  $[\text{man}] = 35.52 \mu\text{M}$  and copper (II) range from  $3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ . b) For system with chloride.  $[\text{man}] = 35.52 \mu\text{M}$  and copper (II) range from  $3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ .

#### D. Distribution curves of the systems of copper(II)-metformin-D-(+)-glucose and copper(II)-metformin-D-(+)-glucose-chloride

The Fig. 7a and b shows the distribution diagrams of the system copper(II)-metformin-D-(+)-glucose and copper(II)-metformin-D-(+)-glucose-chloride. A solution with an equimolar concentration of copper (II), metformin and glucose has a formation of 95.41% for  $[\text{Cu}(\text{met})(\text{glc})]^{2+}$  and less of 4.59% of ionic copper. For the chloride experiments, a solution with an equimolar concentration of copper(II), metformin, glucose and chloride, yields approximately 99.73% of  $[[\text{Cu}(\text{met})(\text{glc})]\text{Cl}]^{1+}$  and 0.27% of ionic copper.

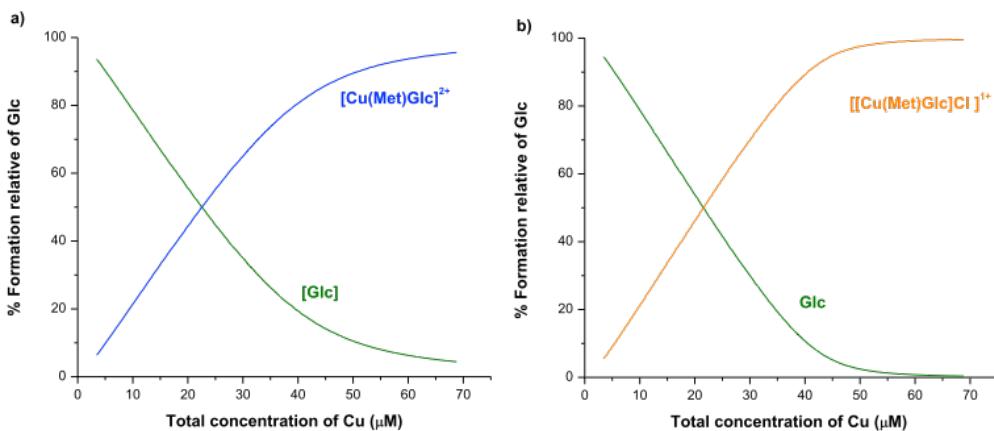


Figure 7. Formation curves of the copper (II)-metformin-D-(+)-glucose complexes in methanol a)  $[\text{glc}] = 35.52 \mu\text{M}$  and copper (II) range from  $3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ . b) For system with chloride  $[\text{glc}] = 35.52 \mu\text{M}$  and copper (II) range from  $3.474 \mu\text{M}$  to  $68.80 \mu\text{M}$ .

## IV. CONCLUSION

The study of the stability of metal complexes is a great research area to theorize possible interactions in biological systems. According to the results obtained in this study, the complex copper-metformin-glucose have a slightly higher formation constant compared with the other system with mannose, nevertheless the glucose and mannose systems have a similar stability. On the other hand, the use of chloride increases the affinity for interacting with the sugar molecule. Also the formation constants of the systems with chloride are quite similar. Nevertheless, considering that the formation constants values of the copper-metformin species with each sugar molecule are non-statistically distinguished. If this interaction occurs in vivo systems, the copper-metformin complex is not capable to distinguish the interacting sugar and possibly an additionally mechanism should be present.

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