**Cu²⁺ Complexes with the Simplest Amino Acid Glycine (Gly)**

### Abstract

Using potentiometric titrations, UV-Vis, IR and speciation diagrams, it appeared that the simplest amino acid Glycine (Gly) is not reacting in a simple manner at all with the copper metal ion (Cu²⁺) in aqueous solutions at 25°C. The potentiometric measurements indicated that Cu²⁺ released a net of two protons (2H⁺’s) into the solution. Free Gly released one proton (H⁺) into the solution from the single ammonium group. On the other hand, when Glycine hydrochloride (Gly.HCl) was used instead of free Gly, both the carboxylate and the ammonium groups released their protons. Upon the reaction of Cu²⁺ with Gly.HCl in any molar ratio, a net of four protons (4H⁺’s) or more were released into the solution; one H⁺ from the carboxylic acid group, the second from the ammonium group and the additional two H⁺’s from the Cu²⁺-aqua ligands. The proposed solution species are in a good agreement with what has been shown in the literature.

**Keywords:** Aqueous solutions; Glycine; Potentiometry; Zwitterion

### Abbreviations:

AA: Amino Acids; Gly: Glycine; Gly.HCl: Glycine Hydrochloride; Cu²⁺: Copper; KHP: Potassium Hydrogen Phthalate; pH: Phenolphthalein; UV-Vis: Ultraviolet-Visible; IR: Infrared; mV: milliVolts; Eq: Equivalents

### Introduction

Most Biology/Chemistry/Physicists and Medicinally related researchers think that the commonly known 20 Amino acids (AA) have been studied to the extent that they know almost everything about them. We believe that studying the simplest amino acid Glycine (Gly) is not that simple when it comes to its reactions with metal ions especially in aqueous solutions under ambient conditions. It is known that Gly is an inhibitory neurotransmitter [1-3]. Typically a 70 kg human body contains about 280 mg copper (Cu²⁺). The copper ion concentration in seawater is in the range of one micro-molar or (1.0x10⁻³ mM), while the human Extracellular Blood Plasma concentration of Cu²⁺ is ~1.5 x10⁻² mM [1].

A very recent chapter by Farkas and Sovago indicated the appearance of 400 papers that discussed metal-complex formation of simple AA and short peptides during the two years span of 2014 & 2015 [4]. The Gly/Cu²⁺ interaction was studied in the following reports [5-9] at which the simple one-to-one [Cu²⁺-Gly] and the bis-[Cu²⁺(Gly)₂] complexes were identified unanimously. Herein, we are showing a very detailed potentiometric and a semi-detailed spectroscopic study (UV-Vis and IR-spectroscopies) that confirm the presence of these complexes observed previously [4-9]. We have seen the appearance of the new ternary [Cu²⁺-Gly-(OH)] complexes. We have recently published two reports that discussed the interactions of copper with an important Phenoxymono-carboxylate (clofibric acid) and the most famous mono-hydroxy tri-carboxylate (citric acid) [10,11]. In these reports we have shown that there is huge body of literature that dealt with the chemistry of copper as one might expect (Figure 1).

### Materials and Methods

**Chemicals/solutions**

All solutions were prepared using 99% purity Sigma reagent grade free Gly formula weight (FW) 75.1 g.mol⁻¹ or Glycine hydrochloride Gly.HCl FW 111.5 g.mole⁻¹. Cu²⁺ solutions were prepared using Copper sulfate penta-hydrate, Cu(SO₄)·5H₂O, formula weight 249.68 g.mol⁻¹. All solutions were prepared by using doubly deionized water: The pH values of all solutions were adjusted using (0.09064 ± 0.00104 mol.L⁻¹) sodium hydroxide (NaOH) solution. The pH values were measured using Orion Membrane pH meter (model 720) with a combination Orion-glass electrode in 0.0 mole.L⁻¹ ionic strength (I).

**Preparation of the potentiometric titration solutions**

In all free Cu²⁺, or free Gly, or free Gly.HCl, or Cu²⁺ -Gly, or Cu²⁺-Gly.HCl potentiometric titrations in 1:1, 1:2, and 1:3, and 1:4,
and 1:5 ratios, NaOH solution was the titrant. NaOH solutions were prepared from NaOH laboratory grade pellets in carbonate free water. The methods used to prevent the contamination of the titrant with atmospheric CO\textsubscript{2} had been described elsewhere [10-15]. The NaOH solutions were standardized using primary standard potassium hydrogen phthalate (KHP). Both NaOH and KHP were purchased from Fisher Chemical Co. Before any KHP titration, the KHP was dried at 110°C for 24 hours and stored in a desiccator. A stock indicator solution of about 0.2% phenolphthalein in about 90% ethanol was prepared from reagent grade phenolphthalein. KHP was titrated to the phenolphthalein end point. Typically, thirteen-fifteen runs were carried out to standardize the NaOH solution. Standard statistical treatments of the data such as the arithmetic mean, standard deviation, T-test, and Q-test were conducted using Excel software.

**Potentiometric titrations**

The potentiometric titration solutions were contained in a 250 mL beaker equipped with a magnetic stirring bar. The beaker was covered with a custom made Teflon cover. In a typical titration; the Gly or Gly.HCl solutions were added first (in independent experiments) followed by the addition of Cu\textsuperscript{2+} solution. The mixture was allowed to stand for a minimum of five minutes to reach a state of equilibrium. No other solutions were added to adjust the ionic strength of the solution. The total volume of the final titration solution was 100 mL. The final concentration of the Cu\textsuperscript{2+} ion titrated was in the range of 2.0 mmoles.L\textsuperscript{-1}. Before each titration, the titration solution mixtures were allowed to stir for an extra 25 minutes for complete equilibrium.

The NaOH titrant was added in segments of 100 µL increments using an Eppendorf micropipette with continuous stirring. The time intervals between the additions of the NaOH solution were set to 5 minutes, which was sufficient to get each of the pH values stabilized and reach complete equilibrium. The start pH-value was in the range of 3-4 (unless otherwise is specified) and the final pH-value was in the range of 10-11. Each titration took about 5 to 6 hours to complete. All titrations were conducted at room temperature.

**UV-Vis spectroscopy**

We have gathered all UV-Vis spectroscopy measurements on the T60 high-performance spectrophotometer in connection with UWIN software version 5.0, both purchased from Advanced ChemTech (Louisville, KY). UV-Vis Samples were prepared in D.I. water at 25°C. The entire UV-Vis spectrum was scanned from 250 to 1000 nm using quartz cuvettes with optical path length of 1 cm. A reference cuvette filled with D.I. water was used with all measurements. The concentration of Cu\textsuperscript{2+} was = 8.75×10\textsuperscript{-3} mol. L\textsuperscript{-1}. The UV-Vis spectra were collected at the pH values of 3.00 after 60 minutes equilibrium time and the measurements were repeated after 1440 minutes (24 hours equilibrium time) to ensure complete equilibrium.

**IR spectroscopy**

All IR spectroscopy measurements were conducted using Nicolet iS10 spectrophotometer in connection with OMNIC software version 8.1, both purchased from Thermo Fisher Scientific (Madison, WI). Samples were prepared in D.I. water at 25°C. The entire IR spectrum was scanned from 400 to 4000 cm\textsuperscript{-1} using the provided attenuated total reflectance (ATR) accessory cell compartment equipped with a diamond cell that can accommodate solid samples or aqueous solution samples. The following data parameters were used in collecting the IR spectra: number of sample scans and the number of background scans was set at 32 with resolution of 4.000, and Laser frequency of 15798.7 cm\textsuperscript{-1}. Typical IR spectra were generated in which the X-axis was given as Wave numbers in cm\textsuperscript{-1} and Y- axis was recorded as % Transmittance.

**Results and Discussion**

**Potentiometric titrations of free Gly, Gly.HCl, and free Cu\textsuperscript{2+} ion**

Figure 2 is the potentiometric titration experiments of free Gly. HCl which shows plots of three independent titrations at which the acidity constants of both the carboxylic acid functional group and the ammonium groups are separated by a well-defined sharp inflection point. Figure 3 is the speciation diagram of free Gly.HCl generated in aqueous solutions using Hyperquad simulation and speciation (Hyss) software program [16], pKa values were used from Martell & Smith [17], pKw value of 13.78 was taken from the literature [18]. Gly.HCl releases a net of two protons due to the fact that Gly.HCl has two titratable functional groups; the carboxylic acid (−COOH) group and the ammonium (NH\textsubscript{4}\textsuperscript{+}) group as shown in Figure 2. Data of this ligand has been reported in the NIST standard reference database of critically selected stability constants of metal complexes [17]. Data about the reaction of Cu\textsuperscript{2+} and Gly.HCl are catalogued in Table 1.

![Figure 2. Potentiometric titration graph of free Gly.HCl](image)

Figure 4 is the potentiometric titration graph of free Gly. Three titration plots were overlapped to show data consistency. The initial pH of the solution was about 8.50 which are totally different compared to that shown in Figure 2. This is due to the

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fact that free Gly shown in Figure 4 has lost its carboxylic acid proton before the addition of the first increment of NaOH titrant. In another word, free Gly exists in its Zwitterion form. So that the degree of protonation or de-protonation of the reacting ligand is a governing factor for the identity of the metal complexes, or nanometal species, or medicinal, or chemical species formed.

We have shown in the supplementary material the detailed potentiometric titrations of free phosphoric acid (H₃PO₄) and that of free Cu²⁺ solutions (Supplementary Figures 1-6) in which the total number of protons released by each species is shown. For example, titrating free Cu²⁺ releases a net of two protons (2H⁺) or two equivalents into the aqueous solutions. This is due to metal ion hydrolysis. This term is defined in equations 1-2 [18-20] and it is valid for any metal ion in aqueous solutions. The number of equivalents is defined as the number of milli-moles of added titrant (NaOH in this case) per number of milli-moles of metal ion present in solution (Cu²⁺ ion in this case).

$$\text{[Cu(H}_2\text{O)}_6\text{]}^{2+} \rightarrow \text{[Cu(H}_2\text{O)}_5\text{(OH)}]}^{+} + \text{H}^+ \quad (1)$$

$$\text{[Cu(H}_2\text{O)}_5\text{OH]}^{+} \rightarrow \text{[Cu(OH)}_2\text{]}\text{ppt} + \text{H}^+ \quad (2)$$

Supplementary Figures 7-14 are the detailed potentiometric titration graphs of the Cu²⁺:Gly.HCl in various molar ratios (1:1, 1:2, 1:3, 1:4, and 1:5 ratios)
It will suffice to discuss the 1:1 titrations (Cu\(^{2+}\): Gly•HCl) as an example, in which three replicas overlapped at 4.00 equivalents. The important point here is that four equivalents of protons have been released from the reaction of Cu\(^{2+}\) with Gly•HCl and went into the solution. Two protons were clearly released from the Gly•HCl. The source of the other two protons must be accounted for. These two protons came from the aqua ligand attached to the Cu\(^{2+}\) ion. It is established in the literature that such hydroxo-complexes with Cu\(^{2+}\) have been observed previously [8,10,17-20]. The proposed and the most plausible species to be formed in solution will be the ternary copper hydroxo-glycinate complex [Cu\(^{2+}\)(Glycinate\(^{-}\))(OH\(^{-}\))]\(^{2-}\). Any complex we have observed in the current study is shown in Table 1 to be compared to the literature values. Table 2 is the summary of all potentiometric titrations carried out in the current study.

### Table 1: Critically Selected stability constants in the form of Log values for Cu\(^{2+}\)/Gly.

| Cu\(^{2+}\)/Gly Compounds | Log β ± SD\(^a\) | Log β ± SD\(^b\) | Net Charge | Remarks |
|--------------------------|------------------|------------------|------------|---------|
| H2Gly                    | 2.32 ± 0.01      | 2.33 ± 0.01      | 1          | c       |
| HGly                     | 9.62 ± 0.01      | 9.57 ± 0.01      | 0          | c       |
| Cu-Gly                   | 8.28 ± 0.01      | 8.19 ± 0.04      | 1          | c       |
| Cu-(Gly)\(_2\)           | 15.38 ± 0.01     | 15.1 ± 0.01      | 0          |        |
| Cu-(Gly)\(_2\)           | 19.55 ± 0.03     | -                | -1         |        |
| Cu-(Gly)\(_2\)H\(^{-}\)   | 1.28 ± 0.01      | -                | 0          |        |
| Cu-(Gly)\(_2\)H\(^{-}\)   | -9.25 ± 0.02     | -                | -1         | c       |
| Cu-(Gly)\(_2\)H\(^{-}\)   | 4.91 ± 0.05      | -                | -1         |        |
| Cu-(Gly)\(_2\)H\(^{-}\)   | -4.96 ± 0.04     | -                | -2         |        |

\(^{a}\)Reference [8]  
\(^{b}\)Reference [17]  
\(^{c}\)Observed in the current study

### Table 2: Summary of all potentiometric titrations of Cu\(^{2+}\): Gly•HCl in 0:1, 1:0, 1:1, 1:2, 1:3, 1:4, and 1:5 molar ratios (ionic strength I = 0.0).

| Cu\(^{2+}\):Gly ratios | Volume of Added Titrant (mL) | No. of Equivalents of Titrant (Eq.) | Remarks |
|------------------------|------------------------------|----------------------------------|---------|
| 0:01                   | 1.65 ± 0.07                   | 1.05 ± 0.07                      | 1 Proton (H\(^{+}\)) released |
| 1:00                   | 2.17 ± 0.06                   | 1.97 ± 0.06                      | 2 Protons (H\(^{+}\)) released |
| 1:01                   | 4.70 ± 0.17                   | 4.18 ± 0.16                      | 4 Protons (H\(^{+}\)) released |
| 1:02                   | 6.40 ± 0.14                   | 6.15 ± 0.20                      | 6 Protons (H\(^{+}\)) released |
| 1:03                   | 7.70 ± 0.01                   | 7.38 ± 0.06                      | 7.4 Protons (H\(^{+}\)) released |
| 1:04                   | 9.03 ± 0.29                   | 8.74 ± 0.27                      | 8.75 Protons (H\(^{+}\)) released |
| 1:05                   | 10.95 ± 0.07                  | 10.55 ± 0.13                     | 10.55 Protons (H\(^{+}\)) released |

### High equilibrium UV-Vis spectroscopy of Cu\(^{2+}\) with free Gly

We have conducted novel UV-Vis absorption spectroscopy experiments. In these experiments, Cu\(^{2+}\) was reacted with the free Gly that was potentiometrically titrated in Figure 4. The Cu\(^{2+}\) solution was mixed with Gly solution in 1:1 molar ratio. Figure 5 shows the UV-Vis absorption spectra for the control (DI H\(_2\)O), free copper sulfate solution (Cu\(^{2+}\)) and Cu\(^{2+}\):Gly solution in 1:1 ratio after 60 minutes equilibrium time. The experiment was repeated after 24 hours on the same set of cuvettes to observe if there were any changes in the absorption pattern of the Cu\(^{2+}\):Gly reaction system after a very long equilibrium time i.e. 1440 minutes. Figure 6 shows the UV-Vis absorption spectra for the control (DI H\(_2\)O), free Cu\(^{2+}\) solution and Cu\(^{2+}\):Gly solution in 1:1 ratio after (24 hours) or 1,440 minutes equilibrium time. It is noteworthy that researchers in [6,7] showed some UV-Vis absorption spectra for copper Glycine systems however none were similar to the spectra presented in the current study.

The absorption peaks that shown had a maximum absorption peak at \(\lambda_{\text{max}} = 810\) nm (Absorbance value of 0.521) which is the typical region for the d\(^{9}\) metal ion such as Cu\(^{2+}\) [19]. With a simple Beer's-Lambert equation calculation one can calculate the molar absorptivity (c) as shown in equation (3).
eq.1:1

Citation:

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