

## On the Complex Formation between Copper(II) and Glycine-Formaldehyde Reaction Products

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The formation of a copper(II) complex with glycine and formaldehyde in aqueous solutions has been confirmed by spectrophotometry, potentiometric titration, and polarography. Addition of formaldehyde to solutions of copper(II) and glycine resulted in an increase of the molar extinction coefficient from 38 to 84, and the system glycine-formaldehyde was found to coordinate with copper(II) in 1:1 and 1:2 chelates depending on the metal ligand ratio. Glycine and formaldehyde were found to combine in equal proportions to give a ligand, the formation of which required an excess of formaldehyde in the solutions. Isolation of the complexes was not possible due to polymerization. Copper(II) solutions with excess of both glycine and formaldehyde changed upon standing to copper-containing amorphous polymers the structure of which (Schiff's base or methylol complexation as the initial reaction) is discussed on the basis of qualitative IR spectral and analytical data.

Schiff's base formation has been confirmed by polarographic studies of formaldehyde solutions in the presence and in the absence of glycine. The copper(II) complex with excess of Schiff's base ligand was found to be reduced at  $-0.52$  V *vs.* S.C.E. in acetate buffer solution.

The reaction between the lower amino acids and formaldehyde has been dealt with by several authors (*e.g.* Tomiyama,<sup>1</sup> Walker<sup>2</sup>). The complex nature of the reaction depends highly on the preparative conditions. The Sørensen formol titration<sup>3</sup> of amino acids in 10 % formaldehyde solutions is based upon the assumption of a condensation reaction as originally postulated by Schiff<sup>4</sup> to give azomethine groups  $=C=N-$ , *i.e.* Schiff's bases, while addition reactions yield mono- or di-methylol derivatives.<sup>5,6</sup>

In aqueous solutions the direct demonstration of Schiff's base formation between glycine and formaldehyde is complicated by the unstable nature of the compound together with the strong polymerizing tendency,<sup>7</sup> and it is difficult to decide whether a Schiff's base or a methylol derivative is formed. According to Eichhorn,<sup>8</sup> some Schiff's bases are stabilized by metal-complexa-

tion as found for copper(II)-salicylaldehyde-glycine. During the last years several Schiff's bases, as model systems for intermediates formed in some biologically important reactions, have been studied as chelating agents, *e.g.*<sup>9-13</sup> but the system metal-glycine-formaldehyde does not seem to have been studied systematically since the early works of Krause.<sup>14</sup> The present work was carried out in order to study the nature of the copper(II)-glycine-formaldehyde complex.

## EXPERIMENTAL

*Materials.* Glycine obtained from Merck was dried at 110°C before preparation of stock solutions by weighing out the appropriate quantity and neutralizing with an equivalent amount of sodium hydroxide. The formaldehyde, Merck reagent grade, 35 weight %, was used without further purification. A standard copper(II) solution was prepared by dissolving an accurately weighed amount of electrolytic copper in nitric acid. Part of the excess of acid was evaporated, and the residue diluted to appropriate volume. Standard sodium hydroxide was prepared by dissolving reagent grade sodium hydroxide in carbon dioxide-free water, and standardizing by potentiometric titration against amidosulphonic acid. Ethanol solutions were of technical grade quality, and the remaining chemicals of reagent grade quality, and were used without further purification.

*Apparatus.* Absorption curves were recorded on Beckman DB Spectrophotometer using 1.000 cm glass cells. Potentiometric titrations and pH-measurements of all solutions were carried out with a Beckman Zeromatic pH-meter using glass and calomel electrodes. Polarograms were recorded with a Tast-Polarograph Selector D (Atlas Werken, Bremen, Germany). The conventional type of dropping mercury electrode and of electrolysis cell was used. All experiments were performed at  $25 \pm 0.1^\circ\text{C}$ .

*Preparation of solutions.* The solutions used for absorption spectroscopy were made 0.005 M with respect to the total metal concentration and 0.1 M with respect to the buffer concentration. Standard solutions of glycine and reagent grade formaldehyde were added to give the desired concentrations of these components. The ethanol content was 10 % and the concentration of potassium nitrate was 0.2 M. All solutions were either allowed to stand over night or were heated to 50°C for one hour in order to stabilize the optical properties. Adjustments of pH were done through addition of sodium hydroxide, and controlled by means of the pH-meter.

The potentiometric titrations were performed in solutions initially prepared as described above. During the titrations the solutions were allowed to stand some minutes after each addition of titrant, and the resulting curves were controlled by titrating solutions with different starting pH-values.

Solutions with 0.005 M copper(II) and 0.5–1 M of both glycine and formaldehyde were found to change colour from blue to bluegreen and dark green depending on the acidity, during the heating on the water bath. Repeating the procedure with a blank the glycine-formaldehyde mixture turned yellow. The cupric solutions with excess glycine and formaldehyde were allowed to stand, and polymeric masses were formed gradually after some weeks.

## RESULTS AND DISCUSSION

*Spectrophotometric measurements* were performed in order to confirm the existence and to determine the compositions of the complexes in dilute aqueous solutions. Intensification of the blue colour when formaldehyde was added to aqueous mixtures of copper(II) and glycine indicated an enhanced stabilization of the copper(II)-glycine chelate. The absorption curves shown in Fig. 1 demonstrate this increase of extinction value and a shift to lower wavelengths of the absorption maximum in 0.1 M acetate buffer solution, pH 4.6, with constant concentration of copper(II), 0.005 M. The recorded curves A and B

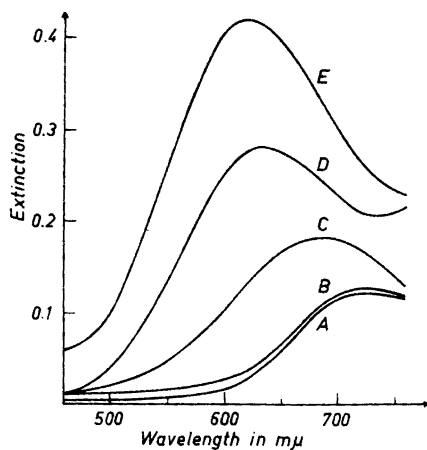


Fig. 1. Light absorption curves of 0.005 M copper(II) in 0.1 M acetate buffer, 0.2 M  $\text{KNO}_3$  and 10 % ethanol (curve A). Curve B = 1 M formaldehyde, curve C = 0.02 M glycine, curve D = 0.5 M glycine, and curve E = 0.02 M glycine and 1 M formaldehyde, added to the solution A.

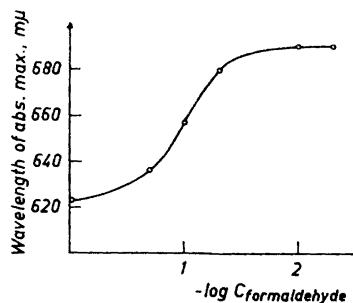


Fig. 2. Variation of the absorption maximum with the concentration of formaldehyde in solutions with 0.005 M copper(II), 0.02 M glycine, 0.2 M  $\text{KNO}_3$ , 10 % ethanol, and 0.1 M acetate buffer pH 4.6.

show the absorption of copper(II) alone and with 1 M formaldehyde present, respectively. The curves C and D were obtained from copper(II)-solutions with 0.02 M (C) and 0.5 M (D) glycine, and curve E with 1 M formaldehyde added to the copper(II)-glycine mixture C. Increase of the pH-values of the solutions gave only small changes in the optical behaviour of the three-component system.

Absorption curves were also recorded of solutions, at constant pH, containing an increasing amount of formaldehyde. The concentration of copper(II) and glycine were 0.005 M and 0.02 M, respectively, and pH 4.6 in 0.1 M acetate buffer. This amount of glycine relative to copper should facilitate the formation of possible higher complexes. In Fig. 2 the wavelengths of the absorption maximum has been plotted against the negative logarithm of the formaldehyde concentration. The figure shows that the wavelength of the absorption maximum does not change when the formaldehyde concentration is less than 0.02 M, but is shifted to lower wavelengths with increasing concentration of formaldehyde. Only minor shifts of the maximum are observed with more than 1 M formaldehyde present. These results indicate that in the concentration range 0.02 M to 1 M formaldehyde the solutions, with constant pH and constant glycine concentration, contain mixtures of copper(II)-glycine and copper(II)-glycine-formaldehyde complexes, and that one mole of glycine combines with one mole of formaldehyde. The simultaneous change in the molar extinction coefficient from 38 to 84, and the fact that 0.02 M seems to be the concentration of formaldehyde when the first observed changes occur, confirm these assumptions. Optimal conditions for the copper(II)-glycine-formaldehyde

complex are obtained in solutions with 1 M formaldehyde or more, which is also the most convenient concentration of formaldehyde in the Sørensen formol titration.

In order to determine the composition of the complex, continuous variation studies were performed at different pH-values. Copper(II) and glycine solutions were mixed in various ratios keeping a constant total concentration of 0.002 M, in 0.1 M acetate and phosphate buffers with 1 M formaldehyde, 10 % ethanol and 0.2 M potassium nitrate present. The extinctions were measured against distilled water. The curves obtained at various wavelengths in acetate buffer, pH 4.6, showed maxima corresponding to 1:1 and 1:2 complexes, as should be expected from what is known of copper(II)-glycine chelation.<sup>15-18</sup> In phosphate buffer solutions, pH 6.0, maxima corresponding only to the 1:2 complex were obtained, as precipitation of copper(II) hydroxide occurred in solutions with lower ligand content.

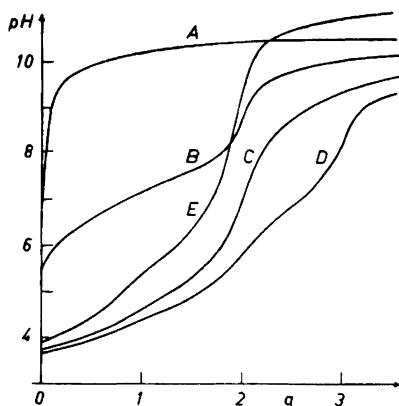
The above results indicate that the copper(II)-glycine-formaldehyde complex is of the same nature as the copper(II)-chelates reported by Klotz *et al.*<sup>19</sup> and Graddon and Munday.<sup>20</sup> In solutions with high concentrations of glycine, or with pH values above 8, they observed the same shift of the wavelength of the absorption maximum. The extinction values observed in the present investigation are twice as high as those for the copper(II)-glycine complex, but are in order of magnitude equal to the extinction values reported for the copper(II)-polypeptide complexes.<sup>19</sup> A further increase of the molar extinction coefficient to about 150 was observed in the solutions with great excess, more than 1 M, of both glycine and formaldehyde, while no further shift in the wavelength of the absorption maximum was found.

*Potentiometric titrations.* In order to obtain additional information on the copper(II)-glycine-formaldehyde reaction mixture, in dilute aqueous solutions, potentiometric titrations were carried out. The acid content in the 1 M formaldehyde used in all the solutions was determined by titration with sodium hydroxide in absence of the reagents. The titration curve obtained is plotted in Fig. 3 (A).

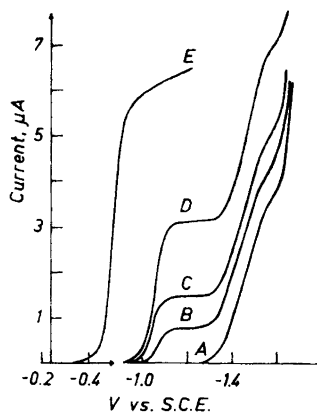
When glycine is titrated with standard base in presence of 1 M formaldehyde, a single steep inflexion is observed on the titration curve B. This inflexion occurs when the amount of base added is equivalent to the amount of amino acid present, and is in accordance with curves obtained by the Sørensen formol titration.<sup>3</sup> The abscissa in Fig. 3 gives the number,  $a$ , of moles of alkali added per mole of copper(II) ion, corrected for the acid content of the formaldehyde. Thus curve B corresponds to 0.002 M glycine, since the copper(II)-containing solutions, curves C, D, and E, were 0.001 M with respect to copper(II).

The titration curves C and D, of solutions containing two and three moles of glycine per mole of copper, respectively, exhibit a slight inflexion at  $a = 1$  and a pronounced inflexion at  $a = 2$ , indicating a reaction taking place in two steps. Curve D also shows an inflexion at  $a = 3$  corresponding to the titration of the third mole of ligand, not bonded to copper. No inflexion occurred at  $a = 3$  on the curves obtained from solutions with higher glycine content.

Titration curves were also performed in solutions without formaldehyde, and curve E represents the titration of the 1:2 mixture of copper(II) and glycine.



*Fig. 3.* Potentiometric titration curve of 1 M formaldehyde solution, 0.2 M  $\text{KNO}_3$  and 10 % ethanol (curve A). Curve B = 0.002 M glycine added, curve C = copper(II) and glycine in ratio 1:2, and curve D = copper(II) and glycine in ratio 1:3, added to 1 M formaldehyde. Curve E = copper(II) and glycine in ratio 1:2 without formaldehyde. The copper concentration was 0.001 M.



*Fig. 4.* Polarograms of solutions with 0.1 M formaldehyde, 0.2 M  $\text{KNO}_3$  and 10 % ethanol in 0.1 M acetate buffer with various amounts of glycine. Curve A = 0, B = 0.01 M glycine, C = 0.02 M glycine, and D = 0.04 M glycine. Curve E = 0.001 M copper(II) and 0.5 M glycine in the same supporting electrolyte but with 1 M formaldehyde.

It is evident from the titration curves (Fig. 3) that solutions with glycine and 1 M formaldehyde (curve C) show stronger chelating tendencies toward copper(II) than solutions without formaldehyde (curve E). The formation of 1:1 and 1:2 chelates is demonstrated by the inflexion points. It is also evident that the 1:3 chelate is not formed in the solutions studied.

The experimental data given above indicate that the copper(II)-glycine complex retains the chelate structure in aqueous solutions even at high concentration of formaldehyde. It is likely that the change in the ligand structure may account for the observed high extinction values at low glycine concentrations. A greater degree of complex formation thus seems to occur, as a result of a reaction between glycine and formaldehyde, as is indicated also by the increased acidity of the solutions. Whether the reaction proper between glycine and formaldehyde is an addition or condensation reaction, is not possible to decide from these experiments, since both reactions will give rise to an increase of the acidity.

*Solid compounds.* The necessity of an excess of formaldehyde for the copper complexes to form is demonstrated above. Isolation experiments, with copper and glycine in stoichiometric ratios, gave only amorphous masses, as is also reported by Galeotti<sup>21</sup> to have been the result of isolation experiments on glycine-formaldehyde compounds.

Copper(II) solutions with an excess of both glycine and formaldehyde were observed to deposit polymers when standing for some weeks. The dried amor-

phous compounds were readily soluble in water but insoluble in acetone, and decomposed at 176°C. IR spectra were obtained and elementary analysis performed on samples prepared at various degrees of acidity. However, the interpretation of the data from the non-stoichiometric mixtures is only qualitative.

The recorded IR spectra indicated a system obviously more complex than those from the copper(II)-glycine chelate.<sup>20</sup> In the  $6\ \mu$  region, two strong peaks near  $1600\ \text{cm}^{-1}$  and  $1680\ \text{cm}^{-1}$  may be attributed to ionic carboxyl-copper linkage and non-conjugated C=N stretching vibration. Hydrogen bondings together with coordinated as well as free water may explain the strong and broad band in the  $3\ \mu$  region.

The elemental analyses showed that the number of groups linked to each copper ion increased with increasing pH-values. The lowest number calculated was four glycine and two or three formaldehyde molecules per copper(II) ion, and a low oxygen content indicated that the polymer might be formed through cross-linking methylene groups, by condensation reactions. In alkaline solutions each copper ion seems to be surrounded by a large number of glycine-formaldehyde condensation products, before the cross-linking with the neighbouring copper ion occurs. The transformation of initially simple copper chelates into higher polymer structures with cross-linking methylene bridges is indicated by Gimblett.<sup>22</sup>

*Polarography.* Polarograms of 0.001 M copper(II) in the presence of 0.5 M glycine and 1 M formaldehyde in solutions prepared as described above were recorded at pH 4.6. The halfwave potential of the reduction wave was  $-0.52\ \text{V vs. S.C.E.}$  (Fig. 4, curve E), and the measured value of  $E_{3/4} - E_{1/4}$  was 0.040 V indicating an irreversible electrode reaction. An increase in the pH of the supporting electrolyte resulted in a splitting of the wave in two parts; the height of the second one increased with increasing pH of the electrolyte and the half-wave potential shifted to more negative values. A double wave was also obtained when polarograms were recorded from solutions with copper and glycine present in stoichiometric ratios, and excess of formaldehyde.

All polarograms exhibited two waves at more negative potentials. One of these is attributed to the reduction of formaldehyde at  $-1.5\ \text{V vs. S.C.E.}$ , and according to Zuman<sup>23</sup> the wave near  $-1.0\ \text{V}$  may correspond to the reduction of an azomethine group  $=\text{C}=\text{N}$  (or a Schiff's base). No reduction of the glycine itself was observed. Polarograms recorded from solutions without copper exhibited the same two waves, and a few polarograms from solutions with increasing amount of glycine and constant concentration, 0.1 M, of formaldehyde, and 10 % of ethanol, are shown in Fig. 4, curves A, B, and D. The height of the waves near  $-1.0\ \text{V vs. S.C.E.}$  is almost proportional to the concentration of glycine in the concentration range 0.005 M to 0.1 M provided buffered solutions. In 0.1 M acetate buffer, pH 5.0, the observed value of the limiting current was  $0.75\ \mu\text{A}$  with 0.01 M glycine present. Some preliminary studies showed that the limiting current increased with increasing pH values, and that the height of the wave was independent of the height of the mercury column. This indicates that the electrode reaction is controlled by the rate of a chemical reaction and that further kinetic studies on this system may be of interest.

In contrast to the pyruvic acid-glycine system studied by Zuman,<sup>24</sup> the aldehyde is present in excess in the present study, but the mechanism of the reduction is probably the same and the appearance of the wave at  $-1.0$  V indicates the formation of the Schiff's base suggested above. The supposed stabilization of the C=N structure by metal complexation<sup>9,12</sup> seems to be of minor importance in the polarographic reduction of the double bond in the solution studied. However, the magnitude of the reduction potential of the formed copper(II) chelate indicates an enhanced stability of the chelate compared to that of copper-glycine, as confirmed above by spectrophotometric and potentiometric methods. The observed splitting of the reduction wave of the copper-complex, depending on the pH of the supporting electrolyte, may be due to an adsorption reaction at the dropping mercury electrode in the solutions used, and further adsorption studies on this system, together with polarographic investigations on the kinetic nature of the glycine-formaldehyde mixtures, are in progress in this laboratory.

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