# Copper(II) Complexation by D-Glucosamine. Spectroscopic and Potentiometric Studies

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Received November 14, 1984

## Abstract

The Cu(II) complex formation equilibria of D-glucosamine were studied in aqueous solution by potentiometric and spectroscopic (ESR, CD, absorption spectra) techniques. All data agree that two major species are formed in the pH region 6–9 involving two D-glucosamine ligand molecules bound to the cupric ion via $NH_2(CuL_2)$ or $NH_2$ and $O^-$ ($CuH_{-2}L_2$). In the latter case deprotonated hydroxyls were found to be very effective coordination sites for Cu(II) giving rise to chelate complexes. On the contrary, no complex formation was observed for the Cu(II) N-acetyl-D-glucosamine system.

## Introduction

Amino sugars, which are among the most abundant natural organic compounds, are known to bind metal ions. In this connection, the chitin derivatives, and particularly chitosan [poly(β-1,4-D-glucosamine)], its deacetylation product, are employed for the selective removal of transition metal ions from brines and acidic solutions [1].

Being found in soil, largely associated with other organic constituents such as humic matter [2], amino sugars may have an important role to play in the environmental behaviour of trace metals.

The complexing behaviour of amino sugars is still not well understood. In fact, although it is generally accepted that the amino nitrogen is involved in coordination, conflicting conclusions have been drawn as to the structure of the complex species formed even by simple amino sugars such as D-glucosamine. Earlier investigations excluded the participation of hydroxyl groups of amino sugars in the metal coordination [3, 4]. The formation of 1:2 metal to ligand molar ratio species with involvement of hydroxyl group binding was suggested recently [5], but no precise description of the complexes is really available.

In order to obtain a more complete insight into the interaction of metal ions with these biomolecules, we have undertaken a spectroscopic and potentiometric study of the copper(II)–D-glucosamine system in aqueous solution. For the purpose of comparison, the interaction with N-acetyl-D-glucosamine has also been considered.

## Experimental

Potentiometric studies were performed using analytical potentiometry (F.I.C.S.) [6]. The solutions were prepared in deionized and bidistilled water under argon atmosphere. Carbonate free 0.1013 M NaOH was prepared in 0.15 M NaCl and standardized against phthalate (National Bureau of Standards). 0.1 M HCl was standardized with 0.1013 M NaOH. Puratronic cupric chloride was used to prepare a 0.2 M (in $3 \times 10^{-2}$ M HCl) solution checked by titration with EDTA. D-Glucosamine and N-acetyl-D-glucosamine hydrochlorides were used as obtained from Sigma.

The potentiometric titrations were carried out on a Tacussel ISIS 20000 pH-meter at 25 ± 0.03 °C

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TABLE I. Quantity in Micromol of Copper and D-Glucosamine Used in Titrations.

| Cu | D-glucosamine |
|---|---|
| 0, 2, 3, 4 | 28.75 |
| 3 | 15.8 |
| | 28.75 |
| | 40.7 |

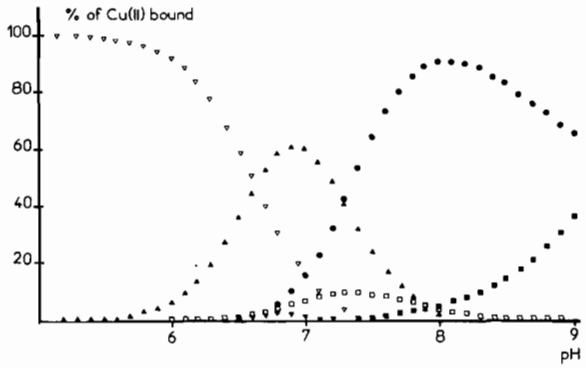


Fig. 1. Species distribution in the Cu(II)–D-glucosamine system as a function of pH. Curve (▽▽▽) unbound Cu(II), curve (▼▼▼) ML, curve (▲▲▲) $ML_2$, curve (□□□) $MH_{-1}L_2$, curve (●●●) $MH_{-2}L_2$, curve (■■■) $MH_{-3}L_2$.

with a TB 10/HA glass electrode and a KCl saturated reference electrode.

The calculations were performed on an Apple II computer. The titrated quantities of Cu(II) and D-glucosamine are given in Table I.

ESR measurements were carried out on a Varian E-9 spectrometer at 9.15 GHz. Spectra of either 1:1 or 1:2 metal to ligand ratio solutions ([Cu] = $5 \times 10^{-3}$ M) were recorded at 123 K on ethylene glycol–water (1:3 v/v) glasses.

Absorption spectra were recorded on Beckman Acta M7 and DU7 spectrophotometers.

CD spectra were recorded on a Jobin Yvon Mark III spectropolarimeter.

## Results and Discussion

### *Cu(II)–D-Glucosamine System*

Below pH 11 D-glucosamine undergoes one deprotonation process with $pK_{LH} = 7.70$. The proton binding site is the amino group.

The potentiometric results obtained for the Cu(II)–D-glucosamine solutions indicate the formation of five distinct species in the pH range 5–9.5 (Figs. 1 and 2).

TABLE II. Logarithm of Stability Constants (log $\beta_{pqr}$) of Complex Species $M_pH_qL_r$ (M = Cu(II), L = D-Glucosamine) in 0.15 M NaCl at 25 °C.

| p | q | r | log $\beta_{pqr}$ |
|---|---|---|---|
| 0 | 1 | 1 | 7.70 |
| 1 | 0 | 1 | 3.06 |
| 1 | 0 | 2 ($CuL_2$) | 8.76 |
| 1 | −1 | 2 | 0.83 |
| 1 | −2 | 2 ($CuH_{-2}L_2$) | −5.82 |
| 1 | −3 | 2 | −15.08 |

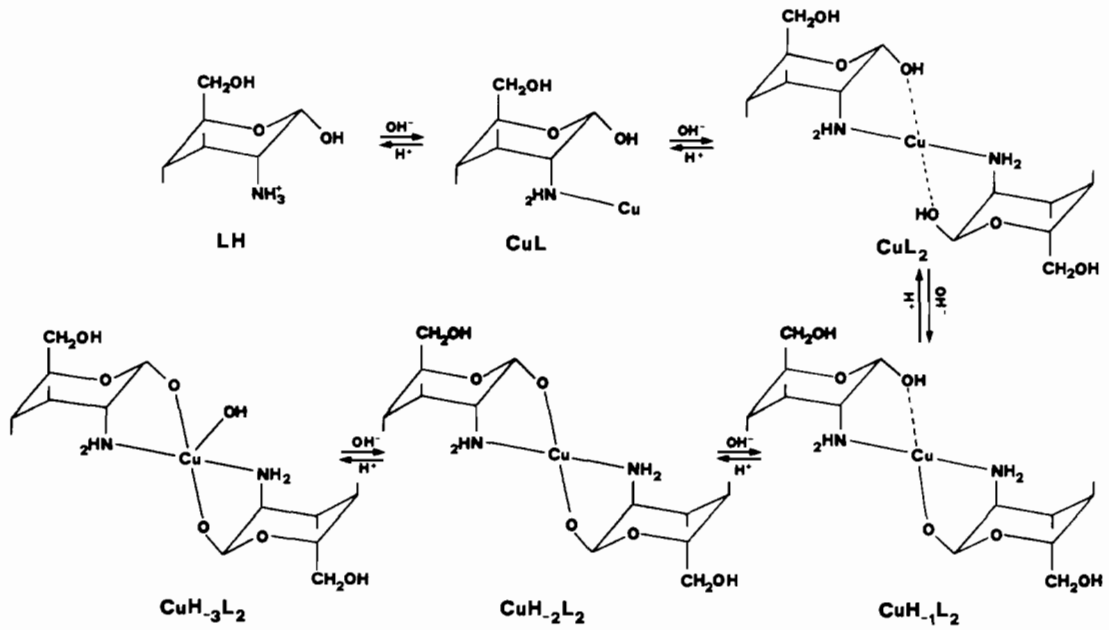


Fig. 2. Schematic presentation of the coordination modes in the Cu(II)–D-glucosamine system.

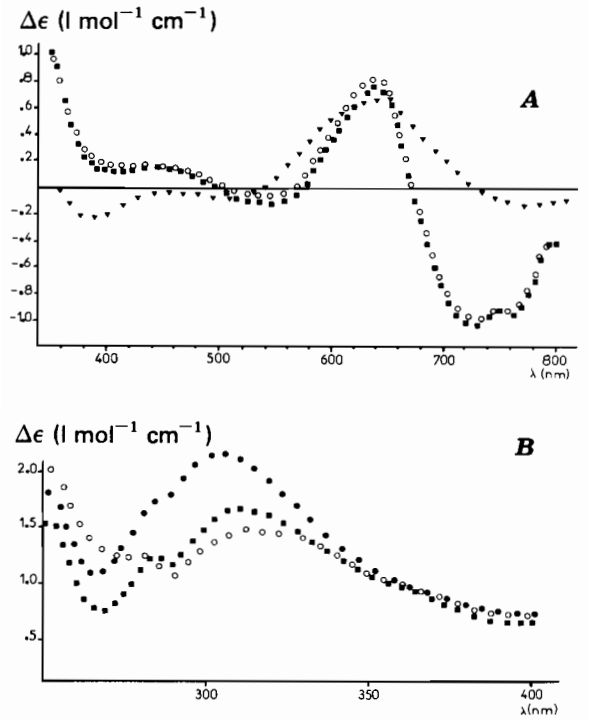


Fig. 3. Circular dichroism of Cu(II)–D-glucosamine solutions for a 1:5 metal/ligand ratio in the d–d region (A) at pH 6.46 (▼▼▼), 8.57 (○○○), 9.42 (■■■) and in the charge transfer region (B) at pH 6.42 (○○○), 7.28 (■■■), 8.57 (●●●).

The $CuL_2$ complex (Table II and Fig. 1), the major species around pH 7 (~55% at pH 6.9), is also easily distinguished by the absorption, CD and ESR spectra. The d–d transition energy of 660 nm ($\epsilon$ = 44) strongly suggests the involvement of two nitrogen atoms in the metal coordination [7–10]. The CD measurements, Fig. 3, which show a main Cotton effect at 640 nm ($\Delta\epsilon$ = +0.06) and the ESR spectra ($g_\parallel$ = 2.317 and $A_\parallel$ = 175 × $10^{-4}$ $cm^{-1}$), Fig. 4, also support the formation of a complex with two nitrogen atoms bound to the copper ion [7–11].

The log $\beta$ value of 8.76 for the $CuL_2$ complex (Table II) is about one order higher than that found for the corresponding monodentate-bonded species in the Cu(II)–$NH_3$ system (log $\beta_{102}$ = 7.6) [12, 13] and considerably lower than that found for the (N,O) chelate coordination in $CuL_2$ complexes, *e.g.*, with amino acids [8, 9]. This suggests a slight, if any, involvement of the protonated hydroxyl groups in the metal coordination of the $CuL_2$ species.

Above pH 7 two other complexes are formed resulting from one- and two-proton dissociation from $CuL_2$, namely $CuH_{-1}L_2$ (~10% at pH 7.4) and $CuH_{-2}L_2$ (~90% at pH 8.1).

The minor species, $CuH_{-1}L_2$, dominated by $CuL_2$ and $CuH_{-2}L_2$, is not really shown by any of the

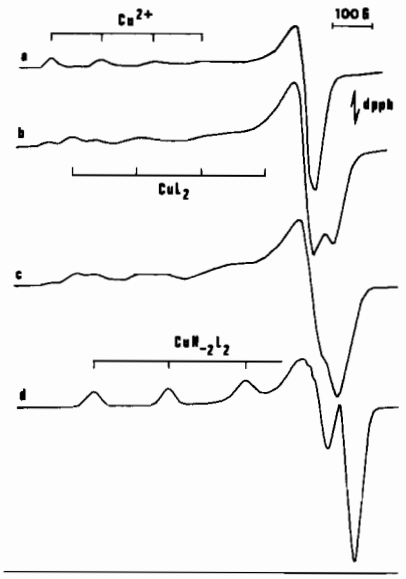


Fig. 4. ESR spectra of Cu(II)–D-glucosamine solutions (1:2 metal to ligand ratio) at (a) pH 4.51, (b) pH 6.82, (c) pH 7.15 and (d) 8.48.

spectroscopic techniques used, but it is reasonable that the deprotonation process involves one of the hydroxyl groups of glucosamine and that a chelate (N,O) ring is formed (Fig. 2).

Thus, the $CuH_{-2}L_2$ species arising from the deprotonation of $CuH_{-1}L_2$ should be a complex in which two (N,O) chelate rings are formed by amino groups and deprotonated hydroxyls.

The potentiometric as well as spectroscopic data support this assumption quite well. The d–d transition energy increases from 660 nm ($CuL_2$) to 620 nm ($\epsilon$ = 40). The $A_\parallel$ and $g_\parallel$ values also change to $g_\parallel$ = 2.255 and $A_\parallel$ = 196 × $10^{-4}$ $cm^{-1}$ indicating stronger metal–ligand interaction [7, 10, 11]. The involvement of the second binding site of D-glucosamine in metal coordination is clearly indicated by the remarkable changes of the CD spectra between pH ~ 6.8 ($CuL_2$) and pH ~ 8 ($CuH_{-2}L_2$). The formation of $CuH_{-2}L_2$ is followed by the appearance of strong negative effects centered at 730 nm ($\Delta\epsilon$ = −0.15). The positive effect at 640 nm ($\Delta\epsilon$ +0.04 at pH 7.8) remains throughout the whole pH range up to 10, although it changes considerably in shape and increases the $\Delta\epsilon$ to +0.1 (Fig. 3).

In the charge transfer region complex formation is followed by positive Cotton effects centered at 300 nm (Fig. 3). These effects may be assigned to $NH_2$ → Cu(II) charge transfer transitions. The $\Delta\epsilon$ of the transition centered at 315 nm (broad band) characteristic for the $CuL_2$ complex reaches a value of +0.6, while for $CuH_{-2}L_2$ at pH ~ 8 a $\Delta\epsilon$ value of +2.2 (300 nm) is observed.

The major variations of the Cotton effects are probably accounted for by distinct rearrangements

occurring in the ligand due to the deprotonation process $CuL_2 \rightarrow CuH_{-2}L_2$ and formation of (N,O) chelate rings involving bonds between copper and deprotonated hydroxyls.

The increase of pH to the region above 10 where $CuH_{-3}L_2$ predominates, according to the potentiometric results, does not result in any distinguishable variation of absorption or ESR spectra.

*Cu(II)–N-Acetyl-D-glucosamine System*

In both 1:1 and 1:2 metal to ligand ratio solutions the copper(II) solvated ion is detected by ESR spectroscopy also after addition of base. However, at pH above 5 precipitation of copper hydroxide is observed. Accordingly, the electronic absorption spectra do not display any shift of the d–d absorption maximum from the value corresponding to the free ion.

## Conclusions

The spectroscopic as well as potentiometric results have shown that D-glucosamine is a quite effective chelating agent for copper(II) ions. The formation constant values indicate that the amino sugar molecule may be a competitive ligand in soil even as a monomeric unit especially in a slightly basic medium.

On the other hand, the protected amino group of N-acetyl-D-glucosamine has negligible tendency to coordinate copper(II). Consequently, copper(II) binding is not allowed at pH values below those required for the ionisation of the sugar hydroxyl groups.

## Acknowledgement

The authors wish to thank Dr. M. H. Loucheux-Lefebvre for helpful discussions concerning this work.

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