

Coordination properties of N,O-carboxymethyl chitosan (NOCC). Synthesis and equilibrium studies of some metal ion complexes. Ternary complexes involving Cu(II) with (NOCC) and some biorelevant ligand

Research Article

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Abstract: In the present study, the acid-base equilibria of N,O- carboxymethyl chitosan abbreviated as (NOCC), is investigated. The complex formation equilibria with the metal ions Cu^(II), Ni^(II), Co^(II), Mn^(II), and Zn^(II) are investigated potentiometrically. The stability constant values of the binary and ternary complexes formed in solution were determined and the binding centers of the ligands were assigned. The relationships between the properties of the studied central metal ions as ionic radius, electronegativity, atomic number, and ionization potential, and the stability constants of the formed complexes were investigated in an effort to give information about the nature of chemical bonding in complexes and make possible the calculation of unknown stability constants. Cu^(II), Ni^(II), and U^(VI) complexes with NOCC are isolated as solid complexes and characterized by conventional chemical and physical methods. The structures of the isolated solid complexes are proposed on the basis of the spectral and magnetic studies. The ternary copper(II) complexes involving NOCC and various biologically relevant ligands containing different functional groups, as amino acids and DNA constituents are investigated. The stability constants of the complexes are determined and the concentration distribution diagrams of the complexes are evaluated.

Keywords: N,O-Carboxymethyl chitosan (NOCC) • Metal complexes • Potentiometry • IR spectroscopy

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1. Introduction

Chitosan, as one of the most abundant natural polymers, is non-toxic, biodegradable and biocompatible. Different from most other natural polymers, chitosan has high reactivity and processability for its specific molecular structure and polycationic nature [1,2]. During recent years, chitosan and its derivatives have received considerable attention due to their potential beneficial biological activities. It has wide application in medicine as antitumor, antiulcer, immunostimulatory, anticoagulant, and antimicrobial [3-10] as well as in cosmetics, textile, paper, food and many other industries. It has several advantages over other types of disinfectants because it possesses a higher antibacterial activity, a broader spectrum of activity, a higher killing rate, and a lower toxicity toward mammalian cells. Chitosan is a powerful

chelating agent, which is easy to form complexes with transition metals and heavy metals [1]. It was reported that chitosan and some of its derivatives are excellent adsorbents for metal ions with much higher selectivity and higher loading capacities than usual commercial chelating agents [11-15]. This is due to its high percentage of nitrogen (6.9%), amino and hydroxyl group which act as chelation sites for metal ions. The free electron doublet of nitrogen on amino group is responsible for the sorption of metal cations. The protonation of amino group in acidic solution is responsible for the electrostatic attraction of anionic complexed cations [16]. However, Chitosan ($pK_a=6.8$) exhibits its biological activity only in an acidic medium because of its poor solubility above pH 6.5 [17]. This obviously limits practical utilization of this material. Thus, water- soluble chitosan derivatives soluble to both acidic and basic physiological circumstances have much

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broader application and physiological importance which has led to great interest in the study of their complexation tendency with metal ions, especially with transition metal ions [1].

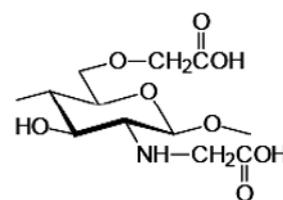
N,O-carboxymethyl chitosan (NOCC), a water soluble chitosan derivative, is the product of the chitosan carboxylation having carboxymethyl substituents on some of both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure [18,19]. NOCC being hydrophilic is an excellent chelating agent since it can be dissolved in a wide pH range from 3 to 11 [17]. Most researches of metal complexes of chitosan and its derivatives focused on their applications in the sequestration or removal of metal ions, dyeing, catalysis, water treatment, and many other industrial processes [1], but few researches pay attention to the study of the stability constant of their metal complexes. Also, the influence of the property of metal ions, on the biological activity of chitosan-metal complexes, which is important for their future application, is still not completely clear.

With the above in mind, and in conjunction with our previous studies in complex formation equilibria of bio-relevant ligands as amino acids, peptides and DNA constituents [20-27], it seems interesting to study the coordination properties of NOCC and its metal complexes. In this investigation we report a quantitative study of the acid base equilibrium of NOCC, as well as the binary complex formation equilibria with $\text{Cu}^{(II)}$, $\text{Ni}^{(II)}$, $\text{Co}^{(II)}$, $\text{Mn}^{(II)}$, and $\text{Zn}^{(II)}$. We have also studied the effect of metal ion properties on the $\log K_1$ values of the binary metal complexes. The solid complexes with the metal ions $\text{Cu}^{(II)}$, $\text{Ni}^{(II)}$, and $\text{UO}_2^{(II)}$ are synthesized and characterized by physicochemical method. Mixed ligand complexes involving Cu-NOCC and some amino acids and DNA constituents are studied and the concentration distributions of the complexes are evaluated.

2. Experimental procedure

2.1. Materials and reagents

NOCC was synthesized and converted into sodium salt according to the method reported in literature [14,15]. The amino acids: glycine, alanine, phenylalanine, threonine, imidazole and methylamine, were provided by the Sigma Chem.Co. The DNA constituents uracil, uridine, thymine, thymidine, inosine, inosine 5'-monophosphat were supplied by BDH-Biochemicals Ltd., UK. The metal salts used are $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$. $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$ obtained from Sigma Chem. Co, UK. NOCC was prepared from two equivalents of HNO_3 acid. Metal salt solutions were prepared and standardized



N,O-carboxymethyl chitosan
(NOCC)

Scheme 1. N,O-carboxymethyl chitosan (NOCC).

as described previously [28]. NaOH solution (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized water.

2.2. Synthesis of the binary metal complexes

$\text{Cu}^{(II)}$, $\text{Ni}^{(II)}$, and $\text{UO}_2^{(II)}$ complexes of NOCC were prepared in the ratio 1:1 (metal:ligand), by direct mixing of 0.5 mmol of metal salt, 0.5 mmol of ligand and 0.5 mmol of sodium acetate. The metal salt and ligand were dissolved in the smallest possible volume of ethanol and sodium acetate. The mixture was refluxed for 1-3 h. The formed solid complexes were separated by filtration, washed with Ethanol and finally with diethyl ether. The analytical Data are given in Table 1.

2.3. Apparatus and measuring techniques

2.3.1. Instruments, apparatus and measuring techniques

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprocessor and electrode were calibrated with standard buffer solutions prepared according to NBS specifications [28] at $25 \pm 0.1^\circ\text{C}$. and $I = 0.1 \text{ mol dm}^{-3}$, potassium hydrogen phthalate (pH 4.008) and a mixture of KH_2PO_4 and Na_2HPO_4 (pH 6.865). A (0.10 mol dm^{-3}) standard acid solution was titrated with a standard base (0.10 mol dm^{-3}) to convert the pH meter reading into hydrogen ion concentration. The pH values was plotted against $\text{p}[\text{H}]$, where the relation $\text{pH} - \text{p}[\text{H}] = 0.5$ was observed for all the titration data. A pK_w value of 13.997 [29] was used to calculate the $[\text{OH}^-]$. The titrations were performed in a thermostated titration vessel equipped with a magnetic stirring system, under purified N_2 atmosphere using 0.05 M NaOH as titrant. The titrations were performed at a constant ionic strength of 0.1 mol dm^{-3} (NaNO_3). The microchemical analysis of the separated solid complexes for C, H and N was performed in the microanalytical center, Cairo University. The analyses were performed twice to check the accuracy of the analytical data. IR spectra were measured on a 80486-

pc FTIR Shimadzu spectrophotometer using KBr pellets. The magnetic susceptibility measurements for the complexes were determined by the Gouy balance using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as a calibrant at room temperature [30].

The acid dissociation constants of the ligand were determined by titrating 40 ml of ligand solution (1.25×10^{-3} mol dm^{-3}). The formation constants of the complexes were determined by titrating 40 mL of the solution containing metal ion (1.25×10^{-3} mol dm^{-3}) and ligand (1.25×10^{-3} mol dm^{-3}). The formation constant of the mixed ligand complexes were determined by titrating solution mixtures containing equivalent amounts of (1.25×10^{-3} mol dm^{-3}) Cu^{II} , NOCC and other ligands in concentration ratio 1:1:1 for amino acids, and 1:1:2 for the DNA constituents.

The stability constant values were calculated by using the computer program MINQUAD-75 [31]. Various possible composition models were tried to calculate the stoichiometries and stability constants of the system studied. The model selected was that which gave the best statistical fit as described before [31]. The experimental titration data points was compared with the theoretical curve calculated from the acid dissociation constant values of the ligand and the formation constants of their complexes, in order to check the validity of the selected model. Table 3 lists the stability constants together with their standard deviations and the sum of the squares of the residuals derived from the MINQUAD output. The speciation diagrams were obtained using the program SPECIES [32].

2.3.2. Spectrophotometric measurements

A spectrophotometric investigation of the binary and ternary $\text{Cu}(\text{II})$ complexes with NOCC was performed by scanning the visible spectra of solution mixtures (A–C). Under the prevailing experimental conditions and after neutralization of the released hydrogen ions, associated with complex formation, it is assumed that the complexes have been completely formed. The samples utilized for spectrophotometric measurements were prepared as follows:

A- 1 cm^3 (0.01 mol dm^{-3}) $\text{Cu}(\text{II})$ ion

B- 1 cm^3 (0.01 mol dm^{-3}) $\text{Cu}(\text{II})$ ion + 1 cm^3 (0.01 mol dm^{-3}) NOCC + amount of base required to neutralize the H^+ liberated from complex formation.

C- 1 cm^3 (0.01 mol dm^{-3}) $\text{Cu}(\text{II})$ ion + 1 cm^3 (0.01 mol dm^{-3}) NOCC +

1 cm^3 (0.01 mol dm^{-3}) alanine + amount of base required to neutralize the H^+ liberated from complex formation.

In each case the final volume was brought to 10 cm^3 by addition of deionized water, the ionic strength is kept constant at 0.1 mol dm^{-3} NaNO_3 .

3. Results and discussion

3.1. Acid base equilibria of N,O-carboxymethylated chitosan

NOCC is prepared in two equivalents of HNO_3 solution to allow protonation of the two carboxylate groups and formation of H_2L species, which acts as a diprotic acid according to the following equilibria



Generally, the following equilibria could be used to describe the cumulative (overall) formation constant β_{pqr} of the species.



$$\beta_{\text{pqr}} = \frac{[\text{M}_p\text{L}_q\text{H}_r]}{[\text{M}]^p[\text{L}]^q[\text{H}]^r} \quad (4)$$

(charges on individual species are omitted for simplicity).

Here, we have no metal complex formation ($p=0$), then only protonation of NOCC according to Eqs. 1 and 2 will occur.

Fig. 1, shows the potentiometric titration of NOCC with a base. From the Fig, it is clear that there is a first deprotonation stage of the two carboxylate groups with a pK_{a1} value of 5.84 ($\text{pK}_{a1} = \log\beta_{012} - \log\beta_{011}$). The HN group is deprotonated at higher pH in the second deprotonation stage with pK_{a2} value of 8.04 ($\text{pK}_{a2} = \log\beta_{011}$). Fig. 2, shows the concentration distribution diagram of NOCC. At pH 2. the diprotonated species of the ligand, H_2L , appears, and on increasing pH the concentration of H_2L decreases and the mono protonated species HL starts to form and reaches a maximum concentration of 98 % at pH 6. The HL species is predominating in the physiological pH range.

3.2. Binary complexes involving NOCC with the metal ions $\text{Cu}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Co}(\text{III})$, $\text{Mn}(\text{III})$ and $\text{Zn}(\text{II})$

Table 2 lists the stoichiometry and stability constants of the binary complexes formed with NOCC together with the proton association constants of NOCC. The metal ions with the ligand are titrated potentiometrically with NaOH . A displacement was observed in the metal-ligand titration curves compared to the ligand titration curve. This indicated the release of protons upon complex formation.

The potentiometric titration data of the binary complex formation equilibria was fitted to various models. The

Table 1. Analytical and physical data for prepared metal salts.

Compound Empirical formula	M.Wt	Colour	M.P., °C	Yield	Found (Calcd.), %		
					C (C)	H (H)	N (N)
$[\text{Cu}(\text{C}_{10}\text{H}_{13}\text{NO}_8)] \cdot \text{H}_2\text{O}$	356.5	Dark blue	185	85%	33.70 (33.60)	3.5 (3.6)	3.75 (3.90)
$[\text{Ni}(\text{C}_{10}\text{H}_{13}\text{NO}_8)] \cdot 3\text{H}_2\text{O}$	387.7	Dark green	>235	90%	30.76 (30.95)	3.4 (3.3)	3.50 (3.60)
$[\text{UO}_2(\text{C}_{10}\text{H}_{13}\text{NO}_8)]$	545.0	Yellow	265	75%	21.96 (22.20)	2.2 (2.3)	2.42 (2.50)

titration data fit satisfactory with the formation of the deprotonated species 110, and the protonated species 111. Comparison of the potentiometric titration data with the theoretically simulated curve of the protonation constant of H_2L and the formation constants of the formed binary complexes in solution was carried out in order to test the validity of the complex formation model selected.

The concentration distribution of the various species formed in solution was estimated in order to provide useful picture of metal ion binding. Fig. 3 represents the speciation diagram of the binary Cu(II) complex with NOCC. In the acidic pH range the protonated complex 111 is formed and its concentration reaches the maximum percentage (40%) at $\text{pH} \approx 3.4$, whereas, the deprotonated complex species 110 starts to form at $\text{pH} \approx 3$ and reaches its maximum percentage (100%) starting from $\text{pH} 7$.

3.3. Relationships between the properties of central metal ions and stability constants of complexes

The relationships between the properties of the studied central metal ions and the stability constants of their complexes were discussed in an effort to give information about the nature of chemical bonding in complexes and make possible the calculation of unknown stability constants.

Fig 4. displays graphically the stability order of the complex forming ability of the transition metal ions. From Fig. 4 it could be seen that $\log K_1$ values are arranged in the order $\text{Mn} < \text{Co} < \text{Ni} < \text{Cu} > \text{Zn}$. This stability order is in accordance with Irving-William's order [33,34] for divalent metals of 3d series. The extra stability of Cu (II) complex could be attributed to Jahn-Teller effect.

It was reported that the stability constants for complexes of metal ions of the same charge is inversely proportional to metal ion radii (39), based on the electrostatic interaction between the metal ion and the ligand. This relationship may be approximately valid for ions of similar electronic configuration. Fig. 4a shows a linear plot of $\log K_1$ of the metal chelates of the ligand

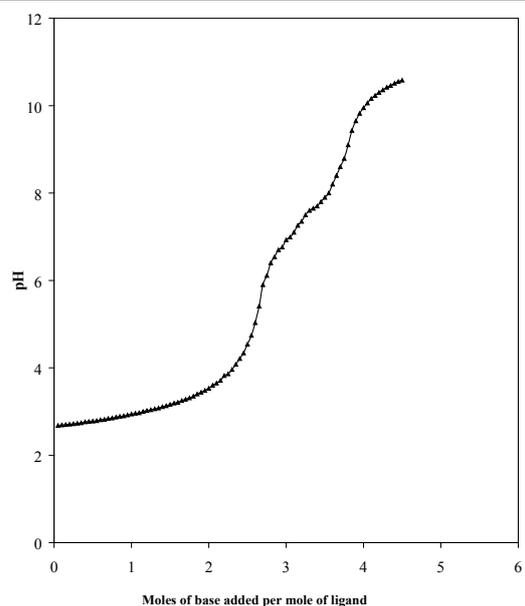


Figure 1. Potentiometric titration curve of N,O- Carboxymethyl chitosan.

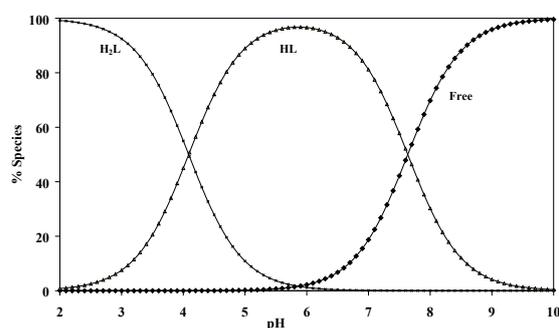


Figure 2. Concentration distribution of various species as a function of pH for N,O-Carboxymethyl Chitosan.

against the reciprocal ionic radii R . The corresponding data are presented in Table 3.

Plotting $\log K$ values against electronegativity of metal atoms give more or less straight line as shown in Fig. 4b. This may be explained on the basis that when the electronegativity difference between a metal atom and a ligand decreases, as a result of increasing the electronegativity of the metals, the covalent character

of the metal-ligand bond would increase which in turn result into higher stability of the metal chelates.

Fig. 4c shows the relationship between $\log K_1$ values and the second ionization potential of the studied bivalent metal ions. A more or less straight line is obtained which is in accordance with the Van Panthaleon- Van Ech Eq. 5 [35].

$$\text{Log } k_1 = P(I-q) \quad (5)$$

Where, I represents the ionization potential for the reaction ($M \rightarrow M^{m+} + me$) in the gaseous phase, P , q are constants independent of the metal ion but depends on the experimental conditions and the nature of ligand. P depends on the number of the donor groups of the ligand and q is the number of electrons involved in complex formation.

3.4. Infrared, electronic spectra and magnetic measurements

The study of the spectral and magnetic properties of the solid complexes provides information that can throw considerable light on their geometry. The analytical data show that the ligand forms 1:1 complex with $\text{Cu}^{(II)}$, $\text{Ni}^{(II)}$, and $\text{UO}_2^{(II)}$. Generally, it is difficult to use infrared spectroscopy to fully elucidate the structure of complexes. However, it can provide some useful information on the functional groups of the ligand chelated with metal ions, especially with metal complexes of carboxylic acids [36,37]. This is usually performed by examination of the carbon-oxygen stretching frequencies and their related values to the nature of carboxylate coordination. The IR spectrum of the ligand display a broad characteristic band in the range $3475\text{--}3400\text{ cm}^{-1}$ attributed to the amine ($-\text{NH}_2$) and the antisymmetric and symmetric OH vibrations of the $-\text{OH}$ groups present in the ligand. The presence of this band with little change in shape and frequency in the metal complexes indicates that neither the $-\text{OH}$ nor the $-\text{NH}_2$ group are involved in complex formation

In general, the un-ionized and un-coordinated COO^- stretching band occurs at $1750\text{--}1700\text{ cm}^{-1}$, whereas, the ionized and coordinated COO^- occurs at $1650\text{--}1590\text{ cm}^{-1}$ [38]. The spectra of NOCC displays strong band at 1600 and 1423 cm^{-1} corresponding to the asymmetric and symmetrical stretching in the carboxylate anion, respectively, indicating that the carboxyl groups in NOCC have a high degree of an ionic characteristic. These bands change in shape and frequency in the metal complexes, which indicates the participation of the carboxylate groups in complex formation. This is also supported by the appearance of new bands at $520\text{--}530\text{ cm}^{-1}$ in the spectra of complexes,

Table 2. Stability constants of NOCC complexes.

System	p	q	r ^a	Log β^b	S ^c
NOCC	0	1	1	8.04 (0.01)	5.10×10^{-7}
	0	1	2	13.72 (0.03)	
Cu- NOCC	1	1	0	10.54 (0.02)	5.70×10^{-8}
	1	1	1	14.85 (0.01)	
Cd- NOCC	1	1	0	5.90 (0.01)	8.80×10^{-7}
	1	1	1	14.38 (0.05)	
Mn- NOCC	1	1	0	5.30 (0.04)	9.70×10^{-7}
	1	1	1	14.02 (0.06)	
Ni- NOCC	1	1	0	8.32 (0.01)	4.82×10^{-7}
	1	1	1	14.60 (0.04)	
Co- NOCC	1	1	0	6.34 (0.02)	8.80×10^{-7}
	1	1	1	14.44 (0.05)	
Zn- NOCC	1	1	0	7.24 (0.03)	6.40×10^{-7}
	1	1	1	14.20 (0.04)	

^a p , q and r are the stoichiometric coefficients corresponding to M^{II} , NOCC, and H^+ respectively; ^b Standard deviation are given in parentheses; ^c Sum of square of residuals

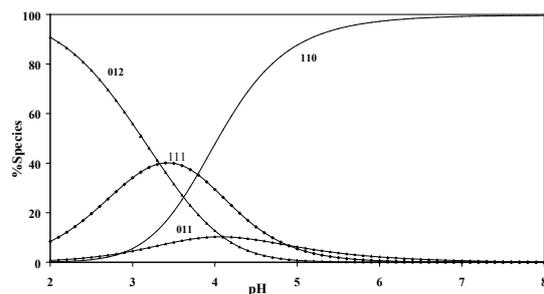


Figure 3. Concentration distribution of various species as a function of pH in the [Cu-NOCC] system.

which were not found in the spectrum of the free ligand, possibly assignable to ν M-O stretching vibration. The IR spectra of the ligand also display bands appearing in the $2958\text{--}2600\text{ cm}^{-1}$ region (medium to weak) assigned to C-H stretching vibration.

The structures of the isolated solid complexes are proposed on the basis of the spectral and magnetic studies. The electronic spectrum of the $\text{Cu}(\text{II})$ complex gives a broad band with a maximum at 14211 cm^{-1} , in consistence with that reported for square planner geometry [24,39]. The square planner geometry for the $\text{Cu}(\text{II})$ complex is also supported by calculating the room temperature magnetic moment. Copper(II) complexes have magnetic moments ranging from 1.7-2.2 b.m. Moments in the higher region between 1.9-2.2 b.m. are, however, characteristic of four-coordinate complexes containing ionic or weak covalent bonds. The $\text{Cu}(\text{NOCC})$ complex has magnetic moment value of 2.21 indicating the presence of Cu^{2+} .

The electronic spectrum of the $\text{Ni}(\text{NOOC})$ complex shows a maximum at 18552 cm^{-1} , in consistence with the square planner geometry [40]. This is further

Table 3. Atomic number, ionic radius, electronegativity and ionization potential of the investigated bivalent metal ion^a.

Metal ion	Mn ²⁺	Co ²⁺	Ni ²⁺	Cu ²⁺	Zn ²⁺
Atomic Number	25	27	28	29	30
Ionic Radius (R.Å ^o)	0.81	0.79	0.72	0.71	0.74
Electronegativity(E.N)	1.55	1.88	1.91	2.00	1.65
Second Ionization Potential (K J mol ⁻¹)	1509	1648	1753	1958	1733.3

^aValues from [48]

supported by the diamagnetic behaviour of the [Ni(L)]⁺ complex.

The IR spectrum of the UO₂(II) complex is used to estimate the force constant of the U-O bond in Å. The spectrum shows three characteristic bands assigned to ν_3 , ν_1 and ν_4 vibrations, respectively, of the dioxouranium ion [41]. The force constant (F) of $\nu(U=O)$ could be calculated by the method of McGlynn *et al.* [42] using the value of ν_3 given by:

$$(\nu_3)_2 = (1307)_2 (F_{u-o})/14.103$$

By substituting the value of the force constant of the uranyl complex into the relation given by Jones [41],

$$R_{u-o} = 1.08 (F_{u-o})^{-1/3} + 1.17$$

The bond length of the U-O bond could be calculated in Å. The values calculated for F_{u-o} and R_{u-o} are 6.9 mdynes Å⁻¹ and 1.53 Å, respectively. These values fall in the usual range of the uranyl complexes [39,42].

3.5. Ternary complexes involving Cu(II), NOCC and some biorelevant ligands

The proton association constants of the ligands and the formation constant of the binary complexes of amino acids and DNA units were determined, and was found to be in good agreement with literature data [24,25,44]. The ternary complexes of Cu^{II}-NOCC with the ligands are determined under the same experimental conditions of temperature and ionic strength. The results are presented in Tables 4 and 5.

The mechanism of ternary complex formation depends on the chelating potential of NOCC and other ligands. The results obtained show that the formation constants of the 1:1 copper (II) complexes formed with either NOCC or amino acids are nearly of the same order. This indicates that the ligation of amino acids and NOCC

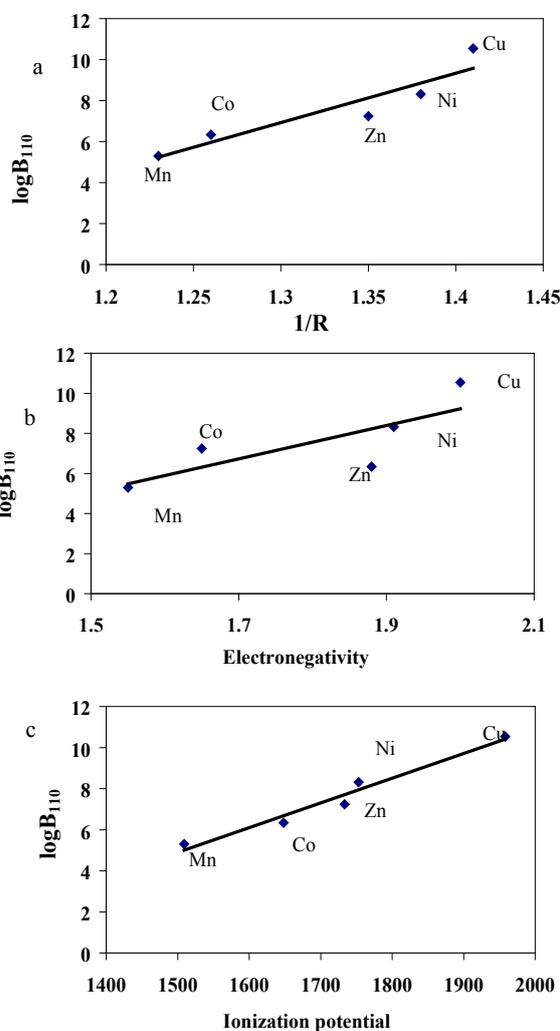


Figure 4. Effect of metal ion properties on the stability constant of NOCC-complexes.

would proceed in a simultaneous mechanism. This mechanism was verified by comparing the experimental titration data with the theoretically calculated curve. The two curves were completely coinciding indicating the validity of model selected.

Table 4. Formation constants of the binary and ternary complexes in the Cu^{II}-NOCC-amino acids at 25°C and 0.1M ionic strength.

System	l	p	q	r ^a	Log ₁₀ β ^b	S ^c	ΔlogK
[Cu(H₂O)₄]²⁺	1	0	0	-1	-6.44(0.07)		
	1	0	0	-2	12.99(0.02)	9.6×10 ⁻⁷	
Cu- NOCC	1	1	0	0	10.54(0.01)	1.7×10 ⁻⁸	
	1	1	0	1	14.85(0.03)		
Glycine	0	0	1	1	9.60(0.01)	1.6×10 ⁻⁷	
	0	0	1	2	11.93(0.02)		
	1	0	1	0	8.26(0.07)	3.0×10 ⁻⁷	
	1	0	2	0	13.57(0.09)		
	1	0	1	-1	0.34(0.08)		
	1	0	1	-2	10.07(0.06)		
	1	1	1	0	20.93(0.01)	4.0×10 ⁻⁸	2.13
	1	1	1	1	25.26(0.01)		
Alanine	0	0	1	1	9.69(0.01)	9.3×10 ⁻⁸	
	0	0	1	2	11.89(0.02)		
	1	0	1	0	7.99(0.03)	3.1×10 ⁻⁸	
	1	0	2	0	14.18(0.02)		
	1	0	1	-1	-0.03(0.05)		
	1	0	1	-2	-9.30(0.01)		
	1	1	1	0	19.86(0.02)	1.4×10 ⁻⁷	1.33
	1	1	1	1	25.24(0.03)		
β-phenyl-alanine	0	0	1	1	9.12(0.01)	2.0×10 ⁻⁸	
	0	0	1	2	11.01(0.03)		
	1	0	1	0	7.53(0.04)	4.3×10 ⁻⁸	
	1	0	2	0	13.73(0.03)		
	1	0	1	-1	0.001(0.05)		
	1	0	1	-2	-10.08(0.03)		
	1	1	1	0	19.23(0.02)	1.6×10 ⁻⁷	1.16
	1	1	1	1	24.70(0.03)		
Methylamine	0	0	1	1	10.03(0.04)	1.6×10 ⁻⁷	
	1	0	1	0	6.82(0.04)		
	1	0	2	0	11.56(0.09)	1.6×10 ⁻⁷	
	1	0	1	-1	13.54(0.06)		
	1	0	1	-2	20.33(0.06)		
	1	1	1	0	16.62(0.03)	1.6×10 ⁻⁷	-0.74
	1	1	2	0	28.60(0.06)		
imidazole	0	0	1	1	7.04(0.01)	1.6×10 ⁻⁷	
	1	0	1	0	4.20(0.01)		
	1	0	2	0	7.62(0.04)	1.6×10 ⁻⁷	
	1	0	3	0	10.35(0.05)		
	1	1	1	0	16.98(0.08)	1.6×10 ⁻⁷	
	1	1	1	1	23.35(0.03)		2.24
Threonine	0	0	1	1	9.11(0.01)	7.0×10 ⁻⁸	
	0	0	1	2	11.32(0.02)		
	1	0	1	0	7.97(0.03)	3.1×10 ⁻⁸	
	1	0	2	0	14.13(0.04)		
	1	0	1	-1	0.90(0.05)		
	1	0	1	-2	-8.39(0.02)		
	1	1	1	0	15.81(0.02)	1.6×10 ⁻⁷	
	1	1	1	1	20.65(0.07)		2.14
	1	1	1	-1	5.31(0.06)		
Histidine	0	0	1	1	9.53(0.01)	1.8×10 ⁻⁷	
	0	0	1	2	15.81(0.02)		
	1	0	1	0	11.48(0.01)	1.6×10 ⁻⁷	
	1	0	2	0	19.70(0.06)		
	1	0	1	1	15.75(0.04)		
	1	0	1	-1	6.82(0.03)		
	1	0	1	-2	-3.77(0.04)		
	1	1	1	0	21.75(0.05)	1.6×10 ⁻⁷	-0.27
	1	1	1	1	29.93(0.05)		3.64

Continued **Table 4.** Formation constants of the binary and ternary complexes in the Cu^{II}-NOCC-amino acids at 25°C and 0.1M ionic strength.

System	l	p	q	r ^a	Log ₁₀ β ^b	S ^c	ΔlogK
Histamine	0	0	1	1	9.88(0.01)	1.6×10 ⁻⁷	
	0	0	1	2	15.97(0.01)		
	1	0	1	0	10.20(0.01)	1.6×10 ⁻⁷	
	1	0	2	0	17.53(0.03)		
	1	0	1	1	14.89(0.01)		
	1	0	1	-1	3.48(0.04)		
	1	0	1	-2	-5.86(0.02)	1.6×10 ⁻⁷	
	1	1	1	0	21.50(0.02)		0.76
	1	1	1	1	29.70(0.02)		4.27

^a l, p, q and r are the stoichiometric coefficients corresponding to Cu^{II}, NOCC, other ligands and H⁺, respectively; The coefficient -1 refers to a proton loss.
^bStandard deviation are given in parentheses; ^cSum of the squares of residuals.

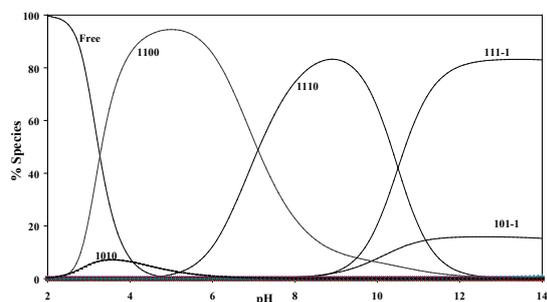


Figure 5. Concentration distribution of various species as a function of pH in the [Cu-NOCC-Threonine] system (at a concentration of 1.25m mol L⁻¹ for each).

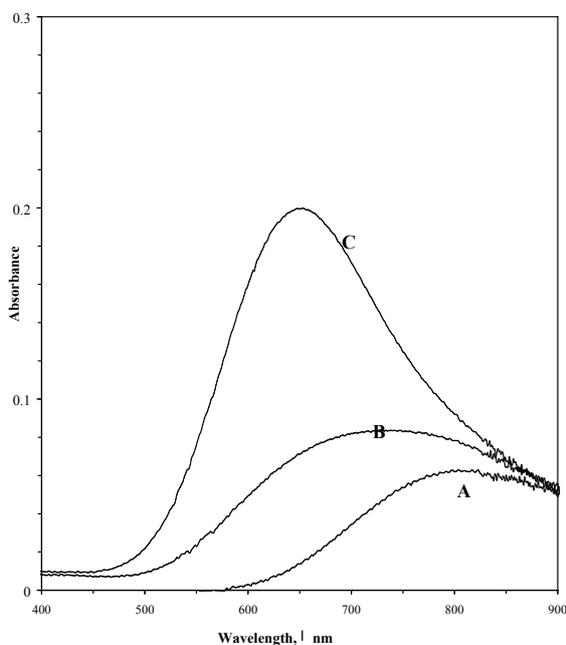


Figure 6. Visible spectra of [Cu-NOCC-Alanine] system. Curves: A: Cu(II); B: [Cu-NOCC]²⁺; C: [Cu-NOCC-Alanine].

3.5.1. Complexes involving amino acids

Fitting of the titration data of the ternary complexes with amino acids and NOCC show the formation of the species: Cu(NOCC), Cu(L), Cu(L)₂, Cu(NOCC)(L) and Cu(NOCC).

The results show that glycine is most likely coordinating to Cu(NOCC)²⁺ as a bidentate rather than as a monodentate ligand. This is because the calculated stability constant value of the monodentate methylamine complex is lower than that of glycine.

The steric effect exerted between the phenyl group of phenylalanine, and the lower basicity of its amino group would result into the formation of a less stable complex compared to that of alanine.

The potentiometric data of threonine are much better fitted assuming the formation of the Cu(NOCC) (LH₋₁) species. This complex is formed through induced ionization of the β-alcohol group of threonine as mentioned in the literature [45]. The pK_a value of the β-alcoholato-group incorporated in the Cu (II) complex (logβ₁₁₁₀ - logβ₁₁₁₋₁) is 10.5. This is in good agreement with that reported in literature for Cu-threonine complex [24]. Fig. 5. shows the Species distribution of threonine, taken as a representative amino acid. From the figure it is clear that the deprotonated species (1110) starts to form at pH~4 and attains a maximum concentration of 83% at pH 8.9. Upon increase of pH, the concentration of the (1110) complex species decreases together with an increase in (111-1) complex concentration.

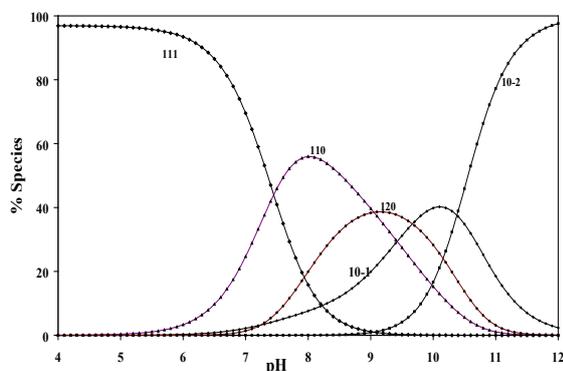
Histidine is known to coordinate in either glycine-like or histamine-like mode depending on the available coordination sites of the primary ligand. It is a trientate ligand having, amino, imidazole and carboxylate group as binding sites. Cu(NOCC)²⁺, has only two binding sites available for complex formation. The Results show that stability constant values of the ternary complexes formed with histidine or histamine and Cu(NOCC)²⁺ are of the same order, and lower than those of amino acids. This indicates that both histidine and histamine would preferably coordinate through amino and imidazole group.

Fig. 6 presents the visible spectra scanned for the binary and ternary Cu(II) complexes with NOCC and alanine. The aquated aquated copper(II) ion (mixture A) shows a broad, weak band with a maximum wavelength at 817 nm being attributed to the ²T_{2g} ← ²E_g transition

Table 5. Formation constants of [Cu-NOCC] - DNA complexes, at 25°C and 0.1 M ionic strength.

System	p	q	r ^a	log β ^b	S ^c	logK _{CuD} ^{Cu}	ΔlogK
Cu(NOCC)-(H₂O)	1	0	-1	-7.02(0.02)	1.6 × 10 ⁻⁷		
	1	0	-2	-17.40(0.06)			
Uracil	0	1	1	9.28(0.006)	2.4 × 10 ⁻⁸	5.49	-0.31
	1	1	0	5.18(0.04)	1.3 × 10 ⁻⁸		
	1	2	0	11.23(0.01)			
Uridine	0	1	1	9.01(0.01)	1.1 × 10 ⁻⁷	4.6	0.54
	1	1	0	5.14(0.03)	2.5 × 10 ⁻⁸		
	1	2	0	10.66(0.01)			
Thymine	0	1	1	9.58(0.00)	8.7 × 10 ⁻⁸	5.77	0.71
	1	1	0	6.48(0.03)	1.7 × 10 ⁻⁸		
	1	2	0	12.56(0.06)			
Thymidine	0	1	1	9.50(0.00)	8.1 × 10 ⁻⁸	4.7	0.82
	1	1	0	5.52(0.02)	1.1 × 10 ⁻⁸		
	1	2	0	10.89(0.08)			
Inosine	0	1	1	8.43(0.01)	4.1 × 10 ⁻⁸	4.5	0.87
	1	1	0	5.37(0.01)	8.7 × 10 ⁻⁸		
	1	2	0	8.44(0.03)			
	1	1	1	12.82(0.02)			
Inosine 5'-monophosphate	0	1	1	9.21(0.01)	4.3 × 10 ⁻⁸	3.5	3.75
	0	1	2	15.21(0.01)			
	1	1	0	7.25(0.01)	7.9 × 10 ⁻⁹		
	1	1	1	13.48(0.01)			
	1	1	2	17.41(0.02)			

p, q and r^a are the stoichiometric coefficients corresponding to [Cu-NOCC], DNA and H⁺ respectively; ^b Standard deviation are given in parentheses; ^c Sum of square of residuals.

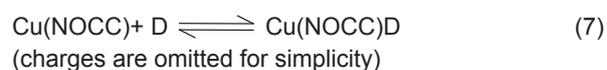
**Figure 7.** Concentration distribution of various species as a function of pH in the [Cu-NOCC]-inosine system.

[25]. The binary [Cu(NOCC)]²⁺ complex (mixture B) shows an absorption maximum at ≈ 715 nm. On the other hand, the spectra obtained from the ternary complex of copper(II) with NOCC and alanine, taken as a representative of amino acids, (mixture C) exhibits a band at 648 nm. This shift in the absorption spectrum towards shorter wave length (blue shift) is an evidence of the ternary complex formation together with the potentiometric measurements.

3.5.2. Complexes involving DNA constituents

Comparison of the stability constant value of Cu^{II}-NOCC with that at of Cu^{II}-DNA complexes shows that the Cu^{II}-NOCC complex is considerably higher than that of Cu^{II}-DNA complexes. This implies a stepwise mechanism for the formation of the ternary complexes of Cu^{II}-

NOCC with DNA units, whereby Cu^{II}-NOCC complex is first formed due to its greater stability, followed by chelation of DNA constituent. The results are presented in Table 4. The stepwise mechanism of the ternary complex formation could be described by the equilibria presented in Eqs. 6 and 7.



Uracil, uridine, thymine and thymidine have only basic nitrogen donor atom (N₃-C₄O group) in the measurable pH range. Fitting the potentiometric data of these pyrimidinic species was found to be consistent with the formation of the 1:1 and 1:2 complexes with Cu(NOCC)²⁺ ion. The inductive effect of the extra electron-donating methyl group of thymidine increases the basicity of its N₃ group, consequently the thymidine complex is more stable than that of uridine. As a result of the high pK_a values of the pyrimidines (pK_a > 9) and the fact that they are monodentates, their complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of the pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges. The stability constant values of the nucleosides are given in Table 5. The calculated values show that the mixed ligand complexes of nucleosides are less stable than the corresponding bases. This is

due to the steric hindrance caused by the sugar residue in the nucleotides, which may reduce the overall basicity of the metal complexes and reduce their complexation tendency with the metal ions. Inosine and inosine 5'-monophosphate (IMP), as examples of purines, are reported to have two metal ion binding centres, N₁ and N₇ nitrogen's. Inosine forms the complexes 110 and 111, while inosine 5'-monophosphate forms 110, 111 and 112 complexes. The extra stabilization of inosine-5'-monophosphate (5'-IMP) complex with Cu(NOCC)²⁺ compared to that of inosine is due to the triply negatively charged 5'-IMP³⁻ ion. Fig. 7 shows the speciation of inosine complex, where the species distribution of the complexes is plotted against pH. From Fig. 7 it is clear that the species 111 is formed in the acidic pH region and it corresponds to the N₇ coordinated complex, while N₁ nitrogen is in protonated form. The pK_a of the protonated form (logβ₁₁₁ - logβ₁₁₀) amounts to 7.45. The protonated species formed in case of 5'-IMP corresponds to N₇ coordinated complex, where the N₁ nitrogen and the phosphate group are protonated. The pK_a values of the protonated species of the IMP complex which corresponds to N₁H group is 3.93 (logβ₁₁₂ - logβ₁₁₁). Whereas, the second protonated species which corresponds to the -PO₂(OH) has a pK_a value of 6.23 (logβ₁₁₁ - logβ₁₁₀). This means that acidification by 5.25 pK_a units for the N₁H groups will occur upon complex formation, which is in consistent with that reported previously for IMP and GMP complexes [46]. The different coulombic forces operating between the ions resulting from the negatively charged phosphate group in IMP would result in extra stabilization of the complexes species formed with IMP compared to that of pyrimidines.

3.6. Comparison of the stability constant of the ternary complexes with those of the binary complexes

The stability of the [Cu(NOCC)L] mixed-ligand species expressed by the constant β_{ipqr} does not represent directly the binding strength between (L) and Cu-NOCC complex. Different methods are known to estimate the formation of ternary complexes. ΔlogK has been used in comparing the stabilities of ternary and binary complexes [24,25,47]. It expresses the effect of the bounded primary ligand (NOCC in this case) towards an incoming secondary ligand.

The ΔlogK value for the protonated ternary complexes are calculated using Eq. 8 and the corresponding value of the deprotonated ternary complexes are calculated using Eq. 9.

$$\Delta\log K = \log\beta_{1111} - \log\beta_{1100} - \log\beta_{1011} \quad (8)$$

$$\Delta\log K = \log\beta_{1110} - \log\beta_{1100} - \log\beta_{1010} \quad (9)$$

It is well known that the Lewis-acidity of the copper(II) ion is decreased upon complex formation, therefore, the amino acid would coordinate more easily with the free metal ion Cu²⁺ than with the complexed metal ion [Cu(NOCC)]²⁺. This in turn would result in a negative values of Δlog K. Table 1 lists the Δlog K values calculated in this work. Previous studies on similar systems have reported that the theoretical Δlog K value for a square planar copper(II) complex is -0.9 [24,25]. The formation of ternary complexes would be favoured if the calculated Δlog K values are higher than -0.9. The results obtained in this work show that all the calculated Δlog K values for α- amino acids are higher than the theoretical value (-0.9), indicating that that ternary complexes are favoured.

The stability of the ternary complexes formed with [Cu-NOCC]²⁺ and the DNA-units, through the stepwise mechanism, are calculated using the following Eq. 10.

$$\Delta\log K = \log K_{\text{Cu(NOCCD)}}^{\text{CuNOCC}} - \log K_{\text{CuD}}^{\text{Cu}} \quad (10)$$

The values are compared to the corresponding binary complexes. The calculated Δlog K values (Table 4) are all invariably positive. This indicates that the ternary complexes formed with [Cu(NOCC)]²⁺ and DNA-units have higher stability than the corresponding binary ones.

4. Conclusion

The present work describes the acid-base equilibria of N,O- carboxymethylated chitosan abbreviated as (NOCC), and the complex formation equilibria with the metal ions Cu^(II), Ni^(II), Co^(II), Mn^(II) and Zn^(II), to ascertain the composition and stability constants of the complexes. The results showed that the stability of the complexes can be ordered as Mn(NOCC) < Co(NOCC) < Ni(NOCC) < Cu(NOCC) > Zn(NOCC). The effect of metal ion properties on the stability of the complexes was investigated. Some solid complexes were synthesized and characterized. Their structures and formation are determined using microanalysis, magnetic, and different spectral tools. The ternary copper(II) complexes involving NOCC and various biologically relevant ligands containing different functional groups as amino acids and DNA constituents are investigated. The results showed that the ternary complexes involving amino acids are formed through a simultaneous reaction mechanism, whereas the ternary complexes formed with DNA constituents follows a stepwise mechanism. The Stoichiometry and stability

constants of the complexes formed are reported at 25°C and 0.1 M ionic strength. The results show the formation of 1:1 complexes with amino acids. DNA constituents form 1:1 and 1:2 complexes. The concentration distribution diagrams of the complexes were evaluated.

In combination of stability constants data of such $[\text{Cu}(\text{NOCC})]^{2+}$ complexes with amino acids, and DNA constituents, it would be possible to calculate

the equilibrium distribution of the metal species in biological fluids where all types of ligands are present simultaneously.

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