Journal of Saudi Chemical Society (2014) **18**, 774–782

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Some transition metal ions complexes of tricine (Tn) and amino acids: pH-titration, synthesis and antimicrobial activity

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Received 30 April 2011; accepted 5 August 2011
Available online 12 August 2011

KEYWORDS

pH-titration;
Ternary complexes;
Tricine;
Amino acids;
Biological activity

Abstract Equilibrium studies have been carried out on complex formation of M(II) (M = Co(II), Cu(II) and Zn(II)) with tricine (Tn) and L = amino acids in aqueous solution, at 25 °C and ionic strength of I = 0.1 M ($NaNO_3$). The ternary complexes of amino acids are formed by simultaneous reactions. The concentration distribution of the complexes is evaluated. The solid complexes of [M(II)–Tn–Histidine (Hist)] have been synthesized and characterized by elemental analysis, infrared, magnetic and conductance measurements. The synthesized complexes have been screened for their antibacterial activities and the complexes show a significant antibacterial activity against four bacterial species: *Staphylococcus aureus* (Gram +ve), *Streptococcus pyogenesr* (Gram +ve), *Serratia marcescens* (Gram −ve) and *Escherichia coli* (Gram −ve). The activity increases by increasing the concentration of the complexes.

1. Introduction

N-[tris(hydroxymethyl)methyl]glycine which is also known as (tricine) was first prepared by Good (1962) for use as a useful biological buffer (Bücke et al., 1967) of pH range 7.2–8.5 in animal tissue culture (Virtanen and Bordin, 1999). Metal complexes involving such compounds are of immense biological interest because of their role in the exchange and transport mechanism of trace metal ions in the biological systems (Kapoor et al., 1978). Tricine compound is an interesting chelating agent due to their flexibility to bind with metal ions forming unidentate, bidentate, and tridentate structures (Ramos et al., 2001). This flexibility is established from the fact that the ligand contains various chelating centers; namely, carboxylate oxygen and amido nitrogen as well as alcoholic oxygen. The flexibility is largely dependent on the pH values at which the complex formation is achieved (Kapoor et al., 1978; Ramos et al., 2001). Considerable attention has been paid to the investigation of the metal complex-forming properties involving this ligand using various techniques (Mahmoud, 2007; Iman, 2003; Boraei and Ahmed, 2002). In this study we report the synthesis, characterization and biological activity of

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2011.08.006

the ternary complexes of [M(II)–Tn–Hist]. Solution equilibria of the systems [M(II)–Tn–L] have been studied pH-metrically.

2. Experimental

2.1. Materials and reagent

2.1.1. Chemistry

Tricine (Tn) was obtained from Aldrich. The metal salts [$Cu(NO_3)_2{\cdot}2H_2O$, $Co(NO_3)_2{\cdot}6H_2O$ and $Zn(NO_3)_2$] were provided by BDH-Biochemicals Ltd. Their solutions were prepared and their concentrations were determined by conventional chemical methods (Schwarzenbach and Flaschka, 1957). The amino acids and related compounds, glycine, β-phenylalanine, valine, serine, methylamine, methionine, S-methylcysteine, histidine, histamine, lysine, ornithine and mercaptoethylamine were provided by Sigma. All these chemicals were used as received without any further purification, their purities ranged from 99% to 99.9%. Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized water.

2.1.2. Biological activity

Bacterial species: Gram +ve (*Staphylococcus aureus*, *Streptococcus pyogenesr*) and Gram −ve (*Serratia marcescens*, *Escherichia coli*).

Stock solutions: Stock solutions of the complexes were prepared in DMSO at 1.0 and 2.0 mg/ml.

Media used: Nutrient agar (Biotch).

The paper discs: Whatman filter paper (No. 3) of uniform diameter (6 mm). Ampicillin was used as reference drugs for an in vitro antibacterial test.

2.2. Synthesis of the ternary metal complexes

The ternary complexes of M(II) with Tn and Histidine were prepared in a molar ratio 1:1:1. The reaction mixture was refluxed for 2 h. The isolated solid complexes were obtained by filtration and washed with 50% (v/v) ethanol–water and finally dried in oven at 100 °C for 2 h. KOH was added as a buffering agent during complex formation.

2.3. Physical measurements

Infrared absorption spectra in the range (200–4000 cm^{-1}) were measured on Mattson 5000 FTIR spectrophotometer. The conductance measurements in solutions were carried out using conductivity bridge TDS model 72. Molar magnetic susceptibility corrected for diamagnetic using Pascal's constant was determined at room temperature (298 K) using Faraday's method. The apparatus was calibrated against $Hg[Co(SCN)_4]$.

2.4. Apparatus and measuring techniques

The pH-measurements were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). The electrode was calibrated with standard buffer solutions prepared according to NBS specifications (Bates, 1975). The pH meter readings were converted in to hydrogen ion concentration by titrating a standard acid solution (0.05 M), the ionic strength of which was adjusted to 0.1 M ($NaNO_3$) with a standard base solution (0.05 M) at 25 °C. The pK_a of water was calculated at ionic strength of 0.1 M to be 13.87 ± 0.05.

2.5. Equilibrium measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 ml) solution (1.25×10^{-3} M) of constant ionic strength 0.1 M, adjusted with $NaNO_3$. The stability constants of the binary complexes were determined by titrating 40 ml of a solution mixture of M(II) (1.25×10^{-3} M), the ligand (2.5×10^{-3} M) and 0.1 M $NaNO_3$. The stability constants of mixed-ligand complexes were determined by titrating 40 ml of solution containing M(II), Tn and amino acids, all of concentration (1.25×10^{-3} M) and 0.1 M $NaNO_3$. All titrations were performed in a purified N_2 atmosphere, using aqueous 0.05 M NaOH as titrant.

The general four component equilibrium can be written as follows (charges are omitted for simplicity):

$$l(\mathrm{M}) + p(\mathrm{Tn}) + q(\mathrm{L}) + r(\mathrm{H}) \rightleftharpoons (\mathrm{M})_L(\mathrm{Tn})_q(\mathrm{L})_q(\mathrm{H})_r \quad (1)$$

$$\beta_{lpqr} = \frac{[\mathrm{M}_l(\mathrm{Tn})_p(\mathrm{L})_q(\mathrm{H})_r]}{[\mathrm{M}^l(\mathrm{Tn})^p(\mathrm{L})^q(\mathrm{H})^r]} \quad (2)$$

Calculations were performed using the computer program SUPERQUAD (Gans et al., 1985).

SUPERQUAD. The stoichiometrics and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and which was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere (Gans et al., 1976). The species distribution diagrams were obtained with the program SPECIES (Pettit, 1984).

2.6. Biological activity

2.6.1. Antibacterial activity

All of the synthesized M(II) complexes were screened in vitro for their biological activity using four bacterial species: Gram +ve (*S. aureus*, *S. pyogenesr*) and Gram −ve (*S. marcescens*, *E. coli*), using the paper disc diffusion technique (Liu and Kwasniewska, 1981). Each of the complexes was dissolved in DMSO at two different concentrations 1 and 2 mg/ml. Paper discs of Whatman filter paper (No. 3) of uniform diameter (6 cm) were sterilized in an autoclave. The paper discs, soaked in the desired concentration of the complex solutions, were placed under aseptic conditions in the petri dishes containing nutrient agar media (Biotch) seeded with all bacterial species separately. The petri dishes were incubated at 37 °C and the inhibition zones were recorded after 24 h of incubation.

The antibacterial activities are calculated as a mean of three replicates. The antibacterial activity of a common standard antibiotic *Ampicillin* was also recorded using the same protocol and at the same concentration and solvent. The antibacterial results of the complexes were compared with the standard and the % activity index for the M(II) complexes were calculated by using the formula:

Table 1 Formation constants of copper(II)–Tn–amino acids complexes at 25 °C and 0.1 M ionic strength.

System	*l*	*p*	*q*	*r*[a]	log *β* [b]	Δlog *K*
Tricine (Tn)	0	1	0	1	7.70(0.02)	
	0	1	0	2	10.33(0.03)	
	1	1	0	0	9.45(0.01)	
	1	2	0	0	14.68(0.04)	
	1	1	0	−1	−1.35(0.03)	
Glycine	0	0	1	1	9.62(0.02)	
	0	0	1	2	11.90(0.01)	
	1	0	1	0	8.11(0.02)	
	1	0	2	0	15.07(0.03)	
	1	1	1	0	16.36(0.02)	−1.20
β-phenylalanine	0	0	1	1	9.12(0.01)	
	0	0	1	2	11.01(0.03)	
	1	0	1	0	7.86(0.01)	
	1	0	2	0	14.85(0.01)	
	1	1	1	0	16.54(0.03)	−0.77
Valine	0	0	1	1	9.57(0.02)	
	0	0	1	2	11.70(0.01)	
	1	0	1	0	8.16(0.02)	
	1	0	2	0	14.99(0.02)	
	1	1	1	0	16.28(0.03)	−1.33
Serine	0	0	1	1	9.14(0.05)	
	0	0	1	2	11.40(0.01)	
	1	0	1	0	8.53(0.04)	
	1	0	2	0	14.66(0.03)	
	1	1	1	0	16.89(0.01)	−1.09
	1	1	1	−1	0.98(0.05)	
Methylamine	0	0	1	1	10.55(0.01)	
	1	0	1	0	6.67(0.02)	
	1	0	2	0	11.66(0.05)	
	1	1	1	0	11.76(0.01)	
	1	1	2	0	16.44(0.02)	−4.36
	0	0	1	1	9.12(0.01)	
Methionine	0	0	1	2	11.10(0.04)	
	1	0	1	0	8.86(0.01)	
	1	0	2	0	14.60(0.01)	
	1	1	1	0	16.99(0.02)	−1.32
S-methylcysteine	0	0	1	1	8.25(0.01)	
	1	0	1	0	8.85(0.02)	
	1	1	1	0	16.78(0.03)	−1.52
Histidine	0	0	1	1	9.53(0.01)	
	0	0	1	2	15.81(0.03)	
	0	0	1	3	17.81(0.06)	
	1	0	1	0	10.89(0.01)	
	1	0	2	0	18.23(0.04)	
	1	0	1	1	19.76(0.03)	
	1	1	1	0	20.04(0.02)	−0.30
Histamine	0	0	1	1	9.85(0.01)	
	0	0	1	2	16.05(0.05)	
	1	0	1	0	10.52(0.02)	
	1	0	2	0	17.56(0.01)	
	1	1	1	0	19.20(0.01)	−0.77
Lysine	0	0	1	1	10.44(0.01)	
	0	0	1	2	19.66(0.01)	
	1	0	1	0	10.81(0.05)	
	1	0	2	0	18.89(0.07)	
	1	1	1	0	19.88(0.01)	−0.38
	1	1	1	1	23.76(0.03)	

Table 1 (*continued*)

System	*l*	*p*	*q*	*r*[a]	log *β* [b]	Δlog *K*
Ornithine	0	0	1	1	10.58(0.00)	
	0	0	1	2	19.43(0.02)	
	0	0	1	3	21.39(0.02)	
	1	0	1	0	11.90(0.05)	
	1	0	2	0	16.30(0.02)	
	1	0	1	1	15.13(0.04)	
	1	1	1	0	20.01(0.03)	−1.34
	1	1	1	1	20.75(0.06)	
Mercaptoethylamine	0	0	1	1	10.03(0.04)	
	0	0	1	2	18.64(0.02)	
	1	0	1	0	11.61(0.05)	
	1	0	2	0	18.89(0.07)	
	1	1	1	0	20.34(0.04)	−0.72
	1	1	1	1	26.25(0.05)	

[a] *l*, *p*, *q* and *r* are the stoichiometric coefficient corresponding to Cu(II), tricine, amino acids and H^+, respectively.
[b] Standard deviations are given in parentheses.

$$\%\ \text{Activity index} = \frac{\text{Zone of inhibition by test complexes (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

2.6.2. Determination of minimum inhibitory concentration (MIC) value

In vitro antimicrobial screening concentrations of the complexes were estimated from the minimum inhibitory concentrations (MIC) values. The disc diffusion method was used to determine the MIC values by preparing discs containing 0.1–1.0 mg/ml from each complex against all the bacterial species using the same protocol mentioned above. After incubation, the minimum inhibitory concentrations (MIC) were recorded on the lowest concentrations of the complexes that had visible inhibition. Ampicillin drug was used as reference.

3. Results and discussion

3.1. Formation constants of the various complexes

The acid dissociation constants of the ligands and the formation constants of their binary complexes were previously reported (Perrin, 1979). We have redetermined these constants, Tables 1–3 under the prevailing experimental conditions as those utilized for determining the stability constants of the mixed-ligand complexes. The results obtained are in good agreement with the literature data (Perrin, 1979).

3.1.1. Acid–base equilibria of tricine (Tn)

Tricine (Tn) exists in solution as zwitterionic amino acids. In acid medium both the carboxylate and imino groups are protonated. The calculated acid dissociation constants, expressed as pK_a values, are in good agreement with reported values (Kapoor et al., 1978; Ramos et al., 2001) amounting to 7.70 and 2.63. The highest pK_a value was due to the protonated imino group while the other value was due to the carboxylic proton. When the proton dissociation constant value of tricine ($10^{-7.70}$) is compared with that of glycine ($10^{-9.45}$), the higher value of the former may be due to the inductive electron attraction by the hydroxyl oxygen.

3.1.2. Binary M(II) complex formation equilibria with tricine

The formation constants of binary complexes of M(II) with tricine were determined by fitting potentiometric data on the basis of possible composition models. The selected model with the best statistical fit was found to consist of 110, 120 and 11-1 complexes. The stability constants of their complexes are given in Tables 2–4. It was reported (Boraei and Ahmed, 2002) that the chelating ligand in the 110 complex has a tendency to undergo deprotonation from one of the (–CH_2OH) groups, affording the 11-1 complexes and tricine acts as an OON tridentate ligand, where it chelates to the metal ion through the carboxylic oxygen and imino nitrogen in addition to one of the (–CH_2OH) alcoholic oxygen atoms after deprotonation.

The concentration distribution diagram of Cu(II)–(Tn) system is shown in Fig. 1, taken as a representative example. The 110 complex starts to form at pH value 2, reaching a maximum concentration (87.61%). On the other hand, 120 complex concentration was found to increase with increasing the pH and becomes predominant (66.31%) at pH = 8. The ionization of the OH group starts after pH ~6 and the 11-1 predominate (98%) at pH = 10.8.

3.1.3. Ternary M(II) complex formation equilibria involving tricine and some amino acids

The ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potentiality of tricine and other ligands. The formation constants of 1:1 M(II) complexes with tricine or amino acids are of the same order of magnitude, Tables 1–3. Consequently the ligation of tricine and amino acid will proceed simultaneously. The titration data of the ternary complexes with amino acids (HL) and tricine (HA) fit satisfactory with formation of the species M(Tn), M(L), M $(L)_2$ and M(Tn)(L).

The formation of mixed-ligand complex by simultaneous mechanisms may be confirmed by comparing the theoretical curve, conducted based on the calculated formation constants and the experimental titration data points, Fig. 2, for the Co(II)–Tn–glycine system, taken as a representative. The good fit obtained is indicative of the validity of the complex formation model. Thus, the formation of ternary complexes can be

Table 2 Formation constants of cobalt (II)–Tn–amino acids complexes at 25 °C and 0.1 M ionic strength.

System	*l*	*p*	*q*	*r*[a]	$\log\beta$[b]	$\Delta\log K$
Tricine (Tn)	1	1	0	0	5.45(0.03)	
	1	2	0	0	9.54(0.05)	
	1	1	0	−1	−2.56(0.03)	
Glycine	1	0	1	0	5.78(0.04)	
	1	0	2	0	10.23(0.03)	
	1	1	1	0	9.97(0.04)	−1.26
β-phenylalanine	0	0	1	0	4.75(0.01)	
	1	0	2	0	8.66(0.02)	
	1	1	1	0	9.87(0.03)	−0.33
Valine	1	0	1	0	5.41(0.03)	
	1	0	2	0	9.72(0.04)	−0.92
	1	1	1	0	9.94(0.01)	
Serine	1	0	1	0	5.51(0.03)	
	1	0	2	0	10.11(0.04)	
	1	1	1	0	9.57(0.01)	−1.39
	1	1	1	−1	0.75(0.03)	
Methylamine	1	0	1	0	4.44(0.01)	
	1	0	2	0	9.87(0.03)	
	1	1	1	0	6.45(0.02)	−3.44
	1	1	2	0	13.11(0.06)	
Methionine	1	0	1	0	5.23(0.02)	
	1	0	2	0	9.78(0.04)	
	1	1	1	0	9.46 (0.04)	−1.22
S-methylcysteine	1	0	1	0	5.39(0.02)	
	1	0	2	0	10.11(0.03)	
	1	1	1	0	9.61(0.02)	−1.23
Histidine	1	0	1	0	7.87(0.04)	
	1	0	2	0	14.01(0.06)	
	1	0	1	1	11.31(0.03)	
	1	1	1	0	12.23(0.04)	−1.09
Histamine	1	0	1	0	6.81(0.02)	
	1	0	2	0	11.33(0.03)	
	1	1	1	0	11.45(0.01)	−0.81
	1	1	1	1	17.00(0.07)	
Lysine	1	0	1	0	7.56(0.02)	
	1	0	2	0	11.24(0.03)	
	1	1	1	0	10.44(0.02)	−2.61
	1	1	1	1	19.17(0.04)	
Ornithine	1	0	1	0	7.31(0.02)	
	1	0	2	0	11.02(0.02)	
	1	1	1	0	11.20(0.03)	
	1	1	1	1	21.23(0.05)	−1.56
Mercaptoethylamine	1	0	1	0	10.78(0.04)	
	1	0	2	0	18.23(0.02)	
	1	1	1	0	15.57(0.03)	−0.66
	1	1	1	1	25.11(0.06)	

[a] *l*, *p*, *q* and *r* are the stoichiometric coefficients corresponding to Co(II), tricine, amino acids and H^+, respectively.
[b] Standard deviations are given in parentheses.

described by the following equilibria (charges are omitted for simplicity):

$$H(Tn) \rightleftharpoons H + Tn$$

$$HL \rightleftharpoons H + L$$

$$M + L \rightleftharpoons ML$$

$$M + 2L \rightleftharpoons ML_2$$

$$M + Tn \rightleftharpoons M(Tn)$$

Table 3 Formation constants of zinc (II)–Tn–amino acids complexes at 25 °C and 0.1 M ionic strength.

System	*l*	*p*	*q*	*r*[a]	$\log\beta$[b]	$\Delta\log K$
Tricine (Tn)	1	1	0	0	6.77(0.05)	
	1	2	0	0	8.80(0.07)	
	1	1	0	−1	−1.78(0.05)	
Glycine	1	0	1	0	6.43(0.03)	
	1	0	2	0	11.89(0.01)	
	1	1	1	0	12.67(0.04)	−0.53
β-phenylalanine	1	0	1	0	6.65(0.05)	
	1	0	2	0	11.76(0.01)	
	1	1	1	0	12.43(0.04)	−0.99
Valine	1	0	1	0	6.12(0.01)	
	1	0	2	0	11.06(0.03)	
	1	1	1	0	12.21(0.02)	−0.68
Serine	1	0	1	0	6.33(0.01)	
	1	0	2	0	11.55(0.01)	
	1	1	1	0	12.62(0.01)	−0.48
	1	1	1	−1	−1.33(0.02)	
Methylamine	1	0	1	0	4.98(0.02)	
	1	0	2	0	9.04(0.06)	
	1	1	1	0	8.34(0.01)	
	1	1	2	0	15.64(0.03)	−3.41
Methionine	1	0	1	0	6.20(0.02)	
	1	0	2	0	10.13(0.01)	
	1	1	1	0	12.92(0.02)	−0.05
S-Methylcysteine	1	0	1	0	6.13(0.01)	
	1	0	2	0	10.23(0.03)	
	1	1	1	0	12.68(0.01)	−0.22
Histidine	1	0	1	0	8.21(0.03)	
	1	0	2	0	15.32(0.02)	
	1	1	1	0	13.48(0.04)	−1.50
Histamine	1	0	1	0	7.96(0.02)	
	1	0	2	0	13.95(0.03)	
	1	1	1	0	13.36(0.05)	−1.37
Lysine	1	0	1	0	8.32(0.04)	
	1	0	2	0	14.22(0.02)	
	1	1	1	0	14.02(0.02)	−0.98
	1	1	1	1	21.33(0.02)	
Ornithine	1	0	1	0	8.47(0.03)	
	1	0	2	0	14.56(0.03)	
	1	1	1	0	14.78(0.03)	−0.46
	1	1	1	1	21.55(0.04)	
Mercaptoethylamine	1	0	1	0	10.34(0.03)	
	1	0	2	0	17.33(0.05)	
	1	1	1	0	16.23(0.02)	−0.88
	1	1	1	1	24.29 (0.02)	

[a] *l*, *p*, *q* and *r* are the stoichiometric coefficient corresponding to Zn(II), tricine, amino acids and H^+, respectively.
[b] Standard deviations are given in parentheses.

$$M + Tn + L \rightleftharpoons M(Tn)(L)$$

The results (Tables 2–4) show that the amino acids form 1110 complexes but methylamine forms 1110 and 1120 complexes. The methylamine complex (1110) is less stable than those of the amino acids. This indicates that amino acids function as bidentate ligands coordinating through the amino and carboxylate groups.

Histidine is a tridentate ligand and may coordinate in a glycine-like or histamine-like way. The stability constant value of the histidine complex is higher than that of α-amino acids and close to that of histamine. This indicates that histidine is coordinating in the histamine-like way.

The stability constant values of methionine and S-methylcysteine complexes are in fair agreement with those of α-amino acids and lower than that of mercaptoethylamine (N, S-donor

Table 4 Analytical and some physical data for M(II) complexes.

Complexes	M.Wt.	Color	M.P. (°C)	Found (Calcd.) (%)			Λ_m in DMSO (Ω^{-1} cm^2 mol^{-1})	U_{eff} (B.M.)
				C	H	N		
$[Co(Tn)(Hist)\cdot(H_2O)_2]C_{12}H_{22}$ Co N_4O_9	393.23	Brownish red	> 300	36.7(36.0)	5.0(4.9)	14.3(14.6)	6.0	2.80
$[Zn$ $(Tn)(Hist)\cdot H_2O)_2]C_{12}H_{22}Zn$ N_4O_9	399.67	White	> 300	36.1(36.5)	5.4(5.1)	14.0(13.6)	7.0	–
$[Cu(Tn)(Hist)\cdot(H_2O)_2]C_{12}H_{22}CuN_4O_9$	397.84	Blue	> 300	36.2(36.4)	5.6(5.4)	14.1(14.5)	4.0	2.76

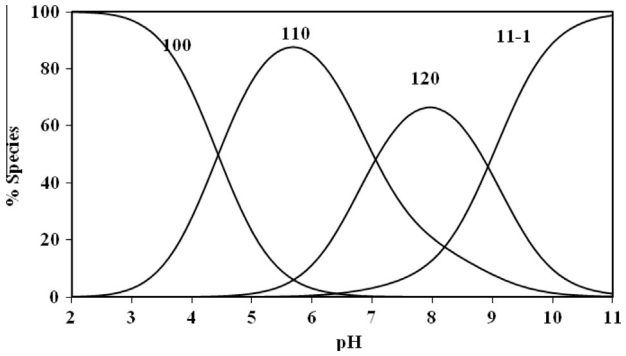

Figure 1 Concentration distribution of various species as a function of pH in the Cu(II)–Tn system.

set). This may be taken to indicate that methionine and S-methylcysteine chelate through the α-amino and carboxylate groups.

Ornithine was found to form a more stable complex than α-amino acids. The stability constant of its complex is higher than those of α-amino acids, by about four logarithmic units. This may be taken to indicate that ornithine most likely chelates through the two amino groups.

Unlike ornithine, the stability constant of the complex, M(Tn)–lysine, is in fair agreement with those of α-amino acids. This may be taken to indicate that lysine most likely chelates through the α-amino and carboxylate groups, because chelates formed through binding with the two amino groups will form strained eight-membered rings.

Estimation of the concentration distribution of various complex species in solution provides a useful elucidation of

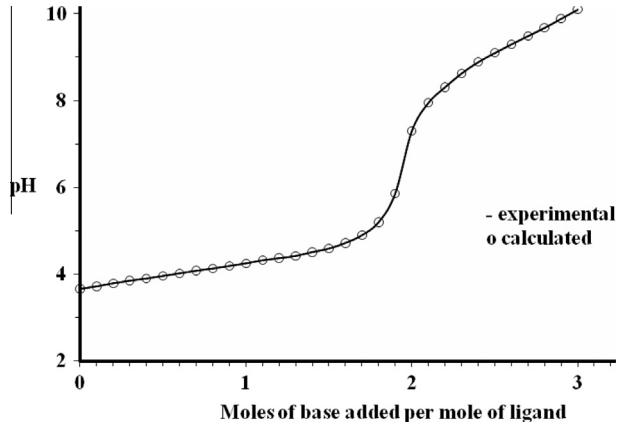

Figure 2 Potentiometric titration curve of the Co(II)–Tn–glycine.

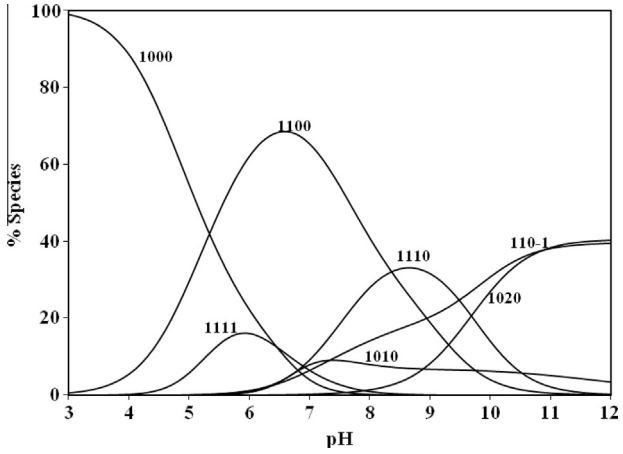

Figure 3 Concentration distribution of various species as a function of pH in the Zn(II)–Tn–Lysine system.

the concentration of the complexes as a function of pH. In all the investigated systems, the concentration of the ternary complex increases with increasing pH. The concentration of [M–Tn–L] ranges from about 23% to 48% in the pH range 8.4–9 with different amino acids investigated. Protonated ternary complex species has been found to be most favoured at lower pH values. The species distribution for Zn–Tn–Lysine complex, taken as a representative, is given in Fig. 3.

The parameter $\Delta \log K$ values are generally used to indicate the relative stability of the ternary complexes as compared to the binary ones as in the equations:

$$M(Tn) + M(L) \rightleftharpoons M(Tn)(L) + M$$

$$\Delta \log K = \log K^{M(Tn)}_{M(Tn)L} - \left(Log\, K^{M}_{M(Tn)} + Log\, K^{M}_{M(L)}\right)$$

Since more coordination sites are available for bonding of the first ligands to a metal ion than for the second ligands, $\Delta \log K$ values for the most ternary complexes are of negative order.

The stability constants were found to increase with increasing ionic radii, electronegativity and ionization potentials of the metal ions. The order of stability of the different binary or ternary complexes in the systems under investigations, in terms of the nature of metal ion, follows the usual sequence: Cu > Zn > Co (Mellor and Maley, 1947).

3.2. Characterization of the isolated solid complexes

The analytical data for the prepared ternary complexes (Table 4) revealed the coordination of the di-anion of the primary ligand tricine (Tn^{2-}) and the di-anion of the secondary

ligand L^{-2} to the central divalent metal ion. The general molecular formula of the ternary complexes were found to be $[M(Tn)(Hist)\cdot(H_2O)_2]$. Their solubilities varied in different common organic solvents. The complexes are air-stable having high melting points and are insoluble in H_2O, but soluble in coordinating solvents such as DMF and DMSO. The formulation of these complexes is based on the elemental analysis, IR, magnetic and conductance measurements.

The IR spectrum of the prepared ternary complexes shows the broad band occurring in the vicinity of 3320cm^{-1} characterizing the stretching vibration of the alcoholic OH group of tricine that appeared in the IR spectra of the complexes without a significant change, denoting that this group plays no role in the coordination. New broad bands appeared in the 3483–3324 cm^{-1} range in the IR spectra of all complexes. These bands can be likely attributed to the stretching vibration of H_2O molecules. Moreover, the band characteristics of NH stretching vibration of the primary ligand (tricine) have completely disappeared. The band observed in the 1590–1625 cm^{-1} range in the IR spectra of the complexes is assigned to the asymmetric stretching vibration of the coordinated carboxylate group. This assignment is based on the fact that the unionized and uncoordinated COO^- asymmetric stretching vibration band appears at 1750–1700 cm^{-1} whereas the ionized and coordinated COO^- stretching vibration band occurs in the 1650–1590 cm^{-1} range (Nakamoto, 1978).

Further, the band responsible for the symmetric stretching vibration of the coordinated carboxylate group is observed in the 1420–1380 cm^{-1} region in the IR spectra of the complexes. This behavior indicates that the carboxylate groups of the primary (tricine) and secondary (Hist) ligands are involved in the coordination of such ligands to the central metal ion. Moreover, the absence of any band in the 1750–1700 cm^{-1} region in the IR spectra of the complexes is considered as further evidence for the participation of the carboxylate group in the complex formation. The negative shift of both the NH_2 imidazole nitrogen (1594 cm^{-1}) and (1509 cm^{-1}) groups in the IR spectrum of complexes suggest the participation of those groups in bonding. Those two bands are observed at 1620 and 1550 cm^{-1}, respectively, in the IR of the histidine. The bands observed at 410, 360 and 303 cm^{-1} are assigned to ν(M–O) (Mehrotra and Bohra, 1983), ν(M–N) (NH_2) and ν(M–N) (imidazole) vibrations, respectively.

The magnetic moment values (2.80 and 2.68 B.M.) suggest the presence of an octahedral geometry around the M(II) ion (Schlapp and Penney, 1932). The molar conductance values of the complexes are given in Table 1. The molar conductivity values (Λ_m) of the M(II) complexes were found to be between 4.0 and 7.0 Ohm^{-1} cm^2 mol^{-1}. The low values indicate that all M(II) complexes are nonelectrolytes.

3.3. Antibacterial activity

A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or to kill them. Commercial products, which incorporate these compounds, are available for controlling microbial infections in many different circumstances. In the present investigation the antibacterial activity of the complexes was studied. The biological activity of the isolated mixed-ligand complexes and *Ampicillin* (as a standard) were tested against *S. aureus*,

Table 5 Antibacterial activity of the isolated mixed-ligand complexes.

Bacterial species	Complexes														Ampicillin (standard) (mg ml^{-1})			
	Cu(Tn)(Hist)·$(H_2O)_2$]				Co(Tn)(Hist)·$(H_2O)_2$]				Zn(Tn)(Hist)·$(H_2O)_2$]									
	MIC[a]	Diameter of inhibition zone (mm)		% Activity index		MIC[a]	Diameter of inhibition zone (mm)		% Activity index		MIC[a]	Diameter of inhibition zone (mm)		% Activity index		MIC[a]	Diameter of inhibition zone (mm)		% Activity index
		1.0	2.0	1.0	2.0		1.0	2.0	1.0	2.0		1.0	2.0	1.0	2.0		1.0	2.0	1.0	2.0
Staphylococcus aureus Gram + ve	0.4	9	11	82	85	0.5	4	6	36	46	0.3	6	9	55	69	0.2	11	13	100	100
Streptococcus pyogenesr Gram + ve	0.5	10	12	83	86	0.5	5	6	42	43	0.4	6	8	50	57	0.3	12	14	100	100
Serratia marcescens Gram –ve	0.7	6	8	67	73	0.6	3	5	33	45	0.5	5	7	56	64	0.3	9	11	100	100
E. coli Gram –ve	0.6	5	7	63	70	0.6	4	6	50	60	0.6	5	7	63	70	0.4	8	10	100	100

[a] MTC unit is mg/ml.

S. pyogenesr (Gram +ve) and *S. marcescens*, *E. coli* (Gram −ve). The results of the antibacterial activity of the synthesized complexes are recorded in Table 5. The activity of the complexes has been compared with the activity of a common standard antibiotic *Ampicillin* and % activity index has been calculated.

The % activity index data indicate that the [Cu(Tn)(Hist)·$(H_2O)_2$] complex shows the highest activity (86%) against *S. aureus* and 85% against *S. pyogenesr* at a concentration of 2.0 mg/ml. [Co(Tn)(Hist)·$(H_2O)_2$] complex has lowest activity against *S. marcescens* while [Zn(Tn)(Hist)·$(H_2O)_2$] has moderate activity against *E. coli* and *S. aureus*.

The activity of the isolated complexes increases as the concentration increases, as expected, since it is well known that concentration plays a vital role in increasing the degree of inhibition (Chaudhary and Singh, 2003). The order of antibacterial activity of these complexes is as follows:

[Cu(Tn)(Hist)·$(H_2O)_2$] > [Zn(Tn)(Hist)·$(H_2O)_2$] > [Co(Tn)(Hist)·$(H_2O)_2$].

The tested complexes were more active against Gram +ve than Gram −ve bacteria; it may be concluded that the antibacterial activity of the compounds is related to cell wall structure of the bacteria.

The differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram +ve bacteria and are ineffective against Gram −ve pathogens (Koch, 2003).

The cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting the synthesis of peptidoglycan (a component of bacterial cell wall). Gram +ve bacteria possess a cell wall containing a thick layer of peptidoglycan and teichoic acid, while Gram −ve bacteria have a relatively thin cell wall consisting of one layer of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These may explain why the complexes have a high antibacterial activity against Gram +ve bacteria than Gram −ve bacteria.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the Research Group Project No. RGP-VPP-062.

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