Complexation Equilibria and Determination of Stability Constants of Some Divalent Metal Ion Complexes of L-Cysteine and Diphenylamine in Aqueous Media

Abdelatty Mohamed Radalla[1, 2]

[1]Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

[2]Department of Physics, College of Science and Arts in Al-Methnab, Qassim University, Al-Methnab, KSA

Email address:

amradalla@yahoo.com, a.abdelatty@qu.edu.sa

Abstract: *Background.* Metal complexes of biologically active ligands had considerable interests. L-cysteinate residue, L-Cys, is a biologically abundant and important versatile binding site of proteins. Diphenylamine, DPA, is an important aromatic amine containing two phenyl groups. Complexation equilibria of the divalent metal ions, Ca^{2+} and Zn^{2+} with the bio- relevant α-amino acid, L-cysteine and the nitrogen-containing diphenylamine ligand were investigated by means of the potentiometric technique at 25.0 ± 0.1°C and constant ionic strength of 0.200 ± 0.001 mol·dm^{-3} NaNO$_3$. *Objective.* The stability constants and standard free energy changes of the α-amino acid, L-cysteine and diphenylamine complex species were determined at 0.200 ± 0.001 mol·dm^{-3} ionic strength. *Methods.* The formation of the different 1:1 and 1:2 binary complexes and 1:1:1 ternary complexes were inferred from the potentiometric titrations. *Results.* The concentration distribution of L-cysteine species formed in solution was evaluated. The dissociation constants of the α-amino acid and diphenylamine were determined at different ionic strength. The stability constants of these binary and ternary systems were calculated. The values of $\Delta \log_{10} K$, percent relative stabilization, %R.S. and $\log_{10} X$ for the ternary systems were evaluated and discussed. *Concussion.* The ternary complex formation occurred in a stepwise manner with L-cysteine acting as the primary ligand. The obtained values of ΔG^0 indicated that Complex formation reactions are spontaneous. Also, for all systems studied, the ternary complexes formed are more thermodynamically stable than the binary complexes.

Keywords: Potentiometric Studies, Metal (II) Ions, L-Cysteine, Ternary Systems

1. Introduction

Metal complexes of biologically active ligands had considerable interests. L-cysteinate residue, L-Cys, is a biologically abundant and important versatile binding site of proteins. Diphenylamine, DPA, is an important aromatic amine containing two phenyl groups. Ternary complexes formed between metal ions and these two different ligands (L-Cys and DPA) are very mportant interactions in biological fluids and environmental systems [1-10]. Ternary complexes of amino-acids with aromatic nitrogen –containing compounds and metal ions form commonly in biological fluids [11-14].

DPA is a light tan to brown solid with a pleasant odor. It

has been used as a fungicide for the treatment of superficial scald in apples and pears. Diphenylamine has a role as a carotogenesis inhibitor, antioxidant, inhibitor and antifungal agrochemical. It has been converted to 4-hydroxydiphenylamine in rat and man and as an antioxidant. Diphenylamine actually promotes lipid hydroperoxide formation and oxygen consumption while markedly inhibiting generation of thiobarbituric acid reactive substances [15, 16]. Complexes of biologically active ligands with metal ions had considerable interests in the literature [17-24], and in view of this and as a continuation of our published work oriented to study the binary and ternary

complexes of biologically important ligands [25-29], it seems to be of considerable interest to conduct investigations covering complexes of the metal ions, Ca(II) and Zn(II) involving L-cysteine and diphenylamine ligands.

2. Experimental Section

2.1. Materials and Solutions

The diphenylamine is a commercially available chemical (TCI Co., LTD, Tokyo, Japan, MW 169.23 g.mol^{-1}, purity > 98.8% by weight). It is used in this study without further purification. L-Cysteine is an analytical-grade reagent (Aldrich-Merck, MW 121.160 g.mol^{-1}, purity > 99% by weight). The purity of these compounds and the concentrations of the stock solutions were determined [30]. The metal salts were provided by BDH as nitrates or chlorides. Stock solutions of the metal salts were prepared in deionized water, and the metal ion concentrations were determined complexometrically using EDTA and suitable indicators [31]. Carbonate-free sodium hydroxide (0.20 mol·dm^{-3} NaOH (titrant, prepared in 0.20 mol·dm^{-3} NaNO$_3$ solution) was standardized potentiometrically with potassium hydrogen phthalate (Merck AG). A nitric acid solution ($\approx$ 0.05 mol·dm^{-3}) was prepared and used after standardization. Sodium hydroxide, nitric acid, and sodium nitrate were from Merck p.a.

2.2. Apparatus and Procedure

Potentiometric titrations were performed using a Metrohm 702 SM automatic titrator equipped with a 665 dosimat, combined glass electrode and magnetic stirrer (Switzerland). The precision of the instrument was ($\pm$ 0.001) pH unit. The pH titrations were carried out in an 80 cm^3 commercial double-walled glass vessel.

The ionic strength of the solutions is maintained at a constant level by using the desired concentration of NaNO$_3$ solution as supporting electrolyte (Ionic strength, I where, I = 0.200 $\pm$ 0.001 mol·dm^{-3} NaNO$_3$). Generally, the titrated solutions were thermostated at a constant temperature and the temperature was adjusted inside the cell at the desired temperature, by circulating water using a water-thermostat set up (temperature, t, where, t = 25.0 $\pm$ 0.1°C. The calibration of the electrode system was carried out by means of a strong acid (base)/ strong base (acid) titration under the same temperature and ionic strength used in the investigation. A computer program (GLEE, glass electrode evaluation [32] has been used to calibrate the glass electrode. The combination electrode involves the use of a 3 M KCl salt bridge between the internal reference and the external solution. This salt bridge reduces the 24junction potential, but it does not become zero [33].

The investigated solutions were prepared (total volume 50 cm^3) and titrated potentiometrically against standard CO$_2$-free NaOH solution (Figures 2 & 3). A stream of dinitrogen gas was passed throughout the course of the experiment in order to exclude the adverse effect of atmospheric carbon dioxide. Magnetic stirring was used during all titrations.

The ligands concentration was 1x10^{-3} mol·dm^{-3} and the ratios for binary and ternary systems were 1:2 and 1:2:2 (metal ion: primary ligand: or/and secondary ligand). The initial estimates of the ionization constants of the ligands and the stability constants of binary and ternary complexes were calculated by adopting *Irving* and Rossotti technique [34, 35]. A summary of the experimental details, for the potentiometric measurements, is given in Table 1.

**Table 1.** *Summary of experimental parameters for the potentiometric measurements.*

System Description
Dissociation processes L-cysteine and diphenylamine Binary Complexes: L-cysteine or diphenylamine with Ca^{II} or Zn^{II}. Ternary Complexes L - cysteine and diphenylamine with Ca^{II} or Zn^{II}.
Solution composition [Ligand] = [Metal Ion] = 1x10^{-3} mol•dm^{-3}
Metal Ion: Ligand: = 1:2 for binaries and 1:2:2 for ternaries
Ionic strength From 0.01 mol·dm^{-3} to 0.30 mol·dm^{-3} of NaNO$_3$.
Experimental method pH-metric titration of 50 cm^3 samples in the range 2 to 11.5.
Instrument SM 702 Metrohm Auto- Titrator with a Combined pH Glass Electrode Equipped with a 665 Dosimat and a Magnetic Stirrer.
Calibration By titrating HNO$_3$ solution with NaOH solution at the same Temperature and Ionic Strength as the solution under study.
T (°C) 25 $\pm$ 0.1°C.
n_{tot}[a] About 100 to 120.
n_{tit}[b] About 4 to 6 titrations.
Calculation Method A computer program based on unweighted linear least-squares fit.

[a] number of titration points per titration [b] number of titrations per titration curve.

3. Results and Discussion

3.1. Dissociation Constants of the Free Ligands

The structural formulas of the investigated ligands are given in Figure 1. The proton dissociation constants of the ligands investigated have been determined in aqueous medium (Table 2) at 25°C and I = 0.20 mol·dm^{-3} NaNO$_3$.
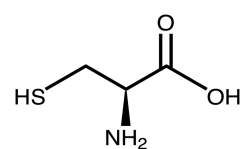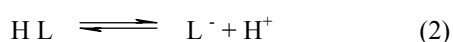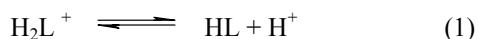

L-Cysteine
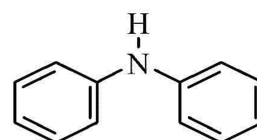
Diphenylamine

Figure 1. *The structural formulas of the investigated ligands.*

The protonated diphenylamine (H$_2$ L $^+$) is expected to have two ionization constants relevant to the following ionization steps (Eqs. (1-2)):

$$H_2L^+ \rightleftharpoons HL + H^+ \tag{1}$$

$$HL \rightleftharpoons L^- + H^+ \tag{2}$$

L - Cysteine (H_3A^+) is expected to have three ionization constants relevant to the following ionization steps [36, 37] (Eqs. (3-5)):

$$H_3A^+ \rightleftharpoons H_2A + H^+ \tag{3}$$

$$H_2A \rightleftharpoons HA^- + H^+ \tag{4}$$

$$HA^- \rightleftharpoons A^{2-} + H^+ \tag{5}$$
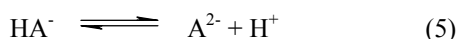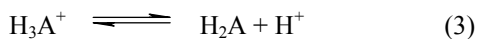
The first dissociation proton of protonated diphenylamine

(H_2L^+) take place at lower buffer regions (1 ≥); therefore, the dissociation constant value (pK_{a1}) could not be determined by the potentiometric method and was not used in the calculations.

The first ionization proton of the L- cysteine molecule is assigned to the *carboxylic* group (-COOH), the second is assigned to the sulfohydryl groups (-SH) and the third is assigned to the amine group ($-NH_3^+$). The second and the third ionization constants of L - cysteine were redetermined potentiometrically in aqueous solutions, under the experimental conditions (t = 25°C, $I = 0.2$ mol·dm^{-3} NaNO$_3$). The values obtained (Table 2) agreed quite well with previously reported ones [36, 37].

Table 2. *Dissociation constants of L-cysteine and diphenylamine investigated in aqueous media at 25.0 ± 0.°C and at different ionic strength.*

| Ionic Strength(mol•dm^{-3} NaNO$_3$)	L-Cysteine		Diphenylamine
	pK_{a2}	pK_{a3}	pK_{a2}
0.01	8.02 ± 0.01	10.22± 0.01	10.14± 0.01
0.02	8.09 ± 0.02	10.29 ± 0.02	10.18± 0.01
0.05	8.14 ± 0.04	10.33 ± 0.04	10.23± 0.02
0.100.20	8.26 ± 0.038.40 ± 0.02	10.37 ± 0.0310.40 ± 0.02	10.28± 0.0110.36± 0.01
0.30	8.44± 0.05	10.45 ± 0.03	10.42± 0.01

The changes in ionic strength affect the dissociation of acids, particularly amino acids [38]. And to explore how this could affect the dissociation of the amino acid investigated, the second and third dissociation constants of l-cysteine have been determined at different ionic strengths (0.01 to 0.30 mol·dm^{-3} NaNO$_3$) at 25.0 ± 0.1°C. The data obtained are given in Table 2. The experimentally measured values of pK_{a2} and pK_{a3} of l-cysteine are plotted against the square root of the ionic

strength of the medium The straight lines in Figure 4 followed the ionic strength equation for a protonated monoprotic amino acid (Eq. (6)), with a charge of z = +1, where A is a constant equal to 0.50.

$$pKa = pK^0a + 2A (2z - 1) [\sqrt{I} / (1 + \sqrt{I})] \tag{6}$$

Where, at the ionic strength of zero, the value of pK^0a2 and pK^0a3 are equal to 8.00 and 10.20 respectively.
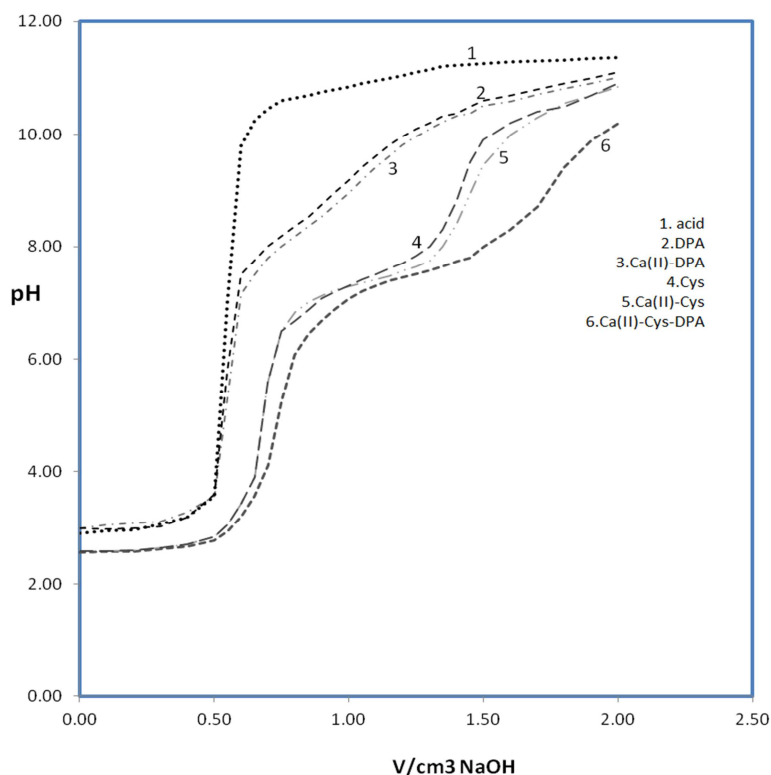
Figure 2. *Potentiometric Titration Curves for Ca(II) – L- Cysteine - Diphenylamine System.*
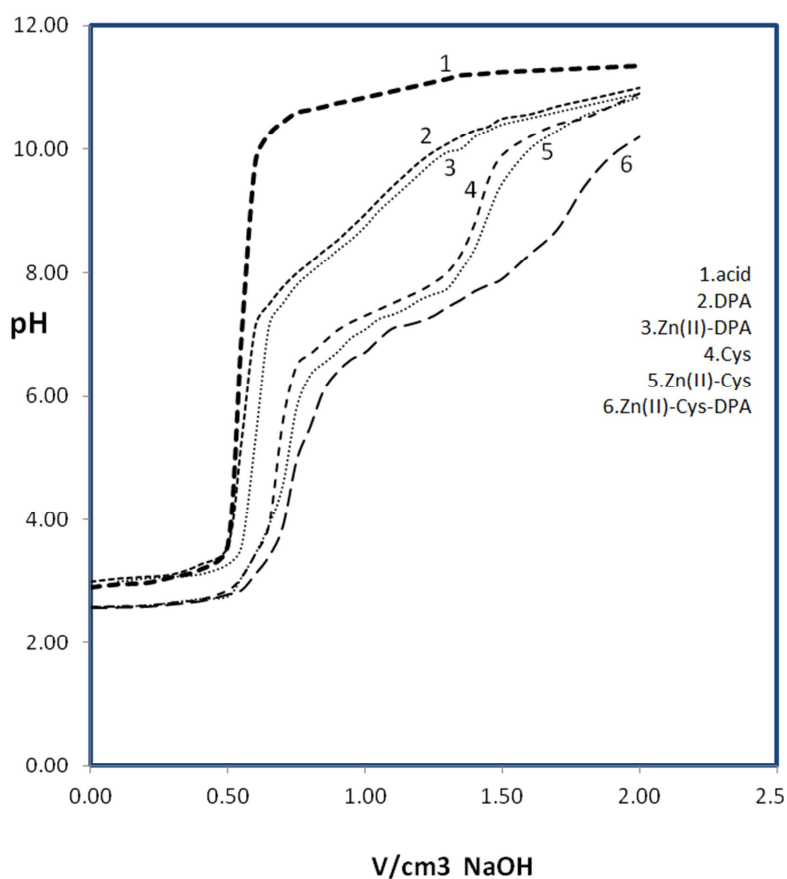
Figure 3. *Potentiometric Titration Curves for Zn(II) - L- Cysteine – Diphenylamine System.*

Figure 4. *Concentration Distribution Diagram of L-Cysteine species at 0.20 mol dm^{-3} NaNO$_3$ ionic strength and 25°C.*

The second and third dissociation constant values of l-cysteine determined at 25°C at the ionic strength of 0.20 mol·dm^{-3} NaNO$_3$ ionic str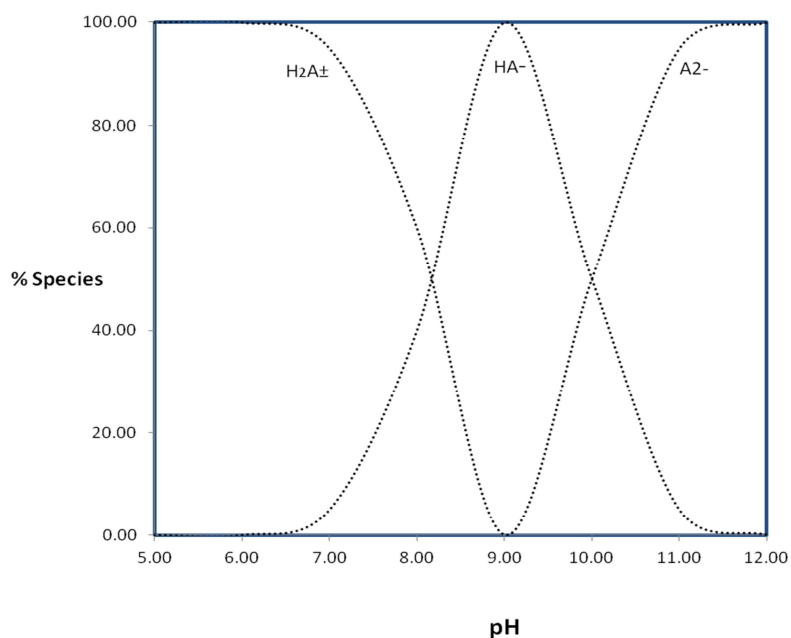ength are used to plot the species concentration distribution diagram (Figure 4). The plot shows that the H$_2$A$^{\pm}$ species predominates in solution up to pH 7.0. Above pH 8.0, the concentration of this species decreases, and HA^{-} species, starts to form and reaches its maximum concentration of 99% at pH > 10.0. Above pH 10.50, species A^{2-} starts to form. It is concluded that HA^{-}species of l-cysteine predominates mainly in the physiological pH range of biological fluids.
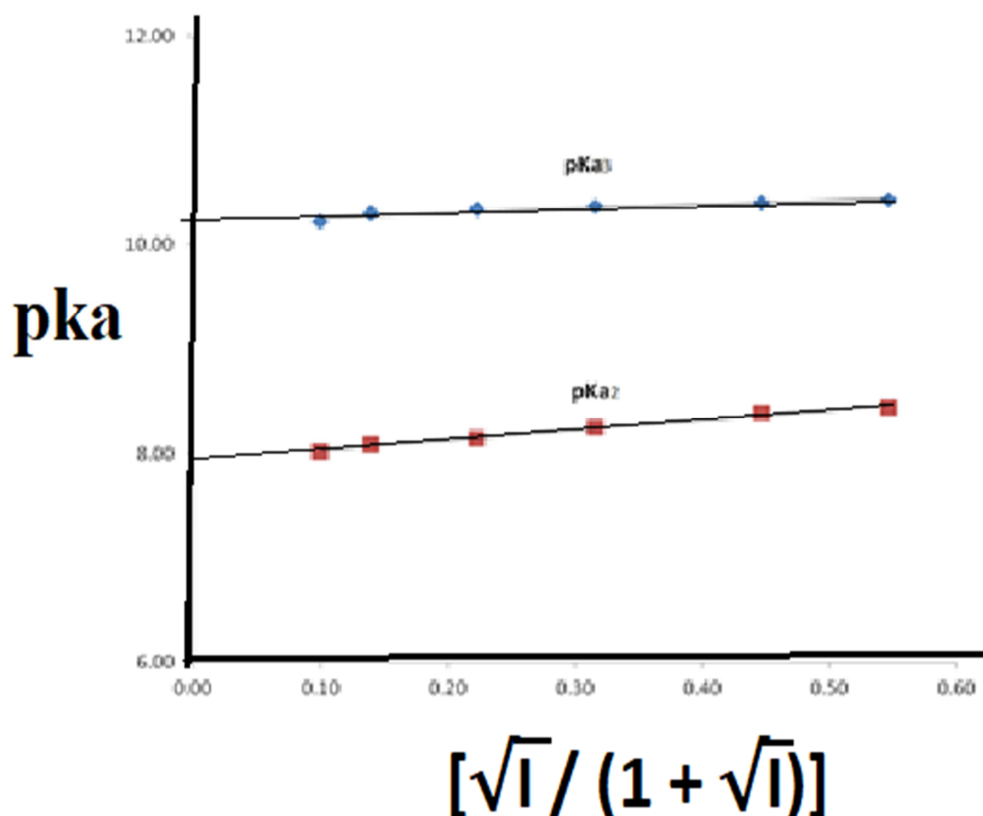
Figure 5. Plot of the effect of the ionic strength of the medium on the dissociation constants of L-Cysteine at 25°C.

The second and third ionization constants of protonated L-cysteine were determined potentiometrically in aqueous solutions at 25°C, and $I = 0.20$ mol·dm^{-3} NaNO$_3$. The values obtained (pKa$_2$ = 8.40 and pKa$_3$ = 10.40) agreed well with literature data [39, 40].

3.2. Binary Metal Complex Systems

Analyses of the complexed ligands curves of diphenylamine and L-cysteine systems (Figures 2 & 3) indicate that the addition of metal ion to the free ligand solutions shifts the buffer regions of ligands to lower pH values (curves c and e). This shows that complex formation reactions proceed by releasing protons from such ligands as shown (Eqs. (7–10)):

$$M^{2+} + HL \rightleftharpoons ML^+ + H^+ \qquad (7)$$

$$ML^+ + HL \rightleftharpoons ML_2 + H^+ \qquad (8)$$

$$M^{2+} + H_2A \rightleftharpoons MA + 2H^+ \qquad (9)$$

$$MA + H_2A \rightleftharpoons MA_2^{2-} + 2H^+ \qquad (10)$$

The binary complexes observed for L-cysteine and diphenylamine systems investigated begin to form in the pH range of 2.8 - 5.2 for Zn^{2+} and Ca^{2+} metal ions, respectively. These values were obtained from the appearance of a divergence of the binary complexes titration curves from that of the free ligands curves. The stability constants of 1:1 and 1:2 binary complexes have been determined in an aqueous medium at 25°C and $I = 0.20$ mol dm^{-3} NaNO$_3$. The values obtained (Table 3) agreed well with the reported literature values [36, 37].

It is observed that the stability constants of the different 1:2 metal-ligand complexes are lower than the corresponding 1:1 systems (Tables 3), as expected from the statistical considerations [41]. This is the normal trend in neutral ligands where the enthalpy is more favorable for a 1:1 species (exothermic) as compared to a 1:2 species. Thus, for the stepwise coordination in binary complexes of investigated ligands, the entropy contribution to the free energy change becomes less favorable from one coordination step to another.

Examination of the stability constants of the binary complexes (Table 3), reveals the following:

Table 3. *Stability constants of 1:1 (log$_{10}$ K$_1$) and 1:2 (log$_{10}$ K$_2$) binary complexes of L-cysteine and diphenylamine investigated in aqueous media at 25.0°C and $I = 0.20$ mol·dm^{-3} NaNO$_3$.*

Metal Ion	Ca(II)		Zn(II)	
	log$_{10}$ K$_1$	log$_{10}$ K$_2$	log$_{10}$ K$_1$	log$_{10}$ K$_2$
L-Cysteine	5.98 ± 0.01	4.79 ± 0.02	7.82 ± 0.01	6.04 ± 0.03
Diphenylamine	5.39±0.01	4.58 ± 0.01	7.06 ± 0.01	5.14 ± 0.02

Stabilities of the 1:1 binary complexes for investigated ligands are higher than the corresponding 1:2 binary ones for the same metal ion.
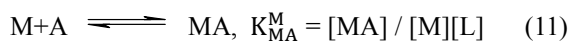
Normal 1:1 and 1:2 binary complexes of investigated ligands are formed with all metal ions studied.

Stabilities of the 1:1 binary complexes for Zn^{2+} metal ion are higher than the corresponding 1:1 binary ones of Ca^{2+} metal ion.

Stabilities of the 1:1 binary complexes of L-cysteine (tri-dentate ligand) are higher than the corresponding 1:1 binary ones of diphenylamine (mono-dentate ligand).

3.3. Formation of Ternary Complexes

For the formation of ternary complexes of the selected bivalent metal ions in presence of diphenylamine (secondary ligand = L) and L- cysteine (primary ligand = A), the following equilibria may be considered (Eqs. (11 & 12)):

$$M + A \rightleftharpoons MA, \quad K_{MA}^M = [MA] / [M][L] \quad (11)$$

$$MA + L \rightleftharpoons MAL, \quad K_{MAL}^{MA} = [MAL] / [MA][L] \quad (12)$$

Here, complex formation is considered to take place in a stepwise manner. Therefore, it is assumed that, in the presence of both ligands, the L- cysteine (A) interacts first with the metal ion forming a 1:1 MA binary complex, which is then followed by ligation of the diphenylamine (L), i.e., stepwise manner. The overall stability constant β_{MAG}^M may be represented in Eq. (13):

$$M + A + L \rightleftharpoons MAL, \quad \beta_{MAL}^M = [MAL] / [M][A][L]$$

$$= K_{MAL}^{MA} \times K_{MA}^M \quad (13)$$
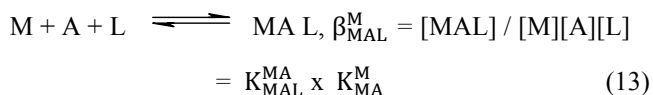
The β_{MAL}^M constant expresses the stability of the mixed-ligand species; it does not represent the binding strength between L and M^{2+} ions directly in the presence of A.

This effect is much better reflected by the equilibrium constant, K_{MAL}^{MA}, calculated according to Eq. (14):

$$\log_{10} K_{MAL}^{MA} = \log_{10} \beta_{MAL}^M - \log_{10} K_{MA}^M \quad (14)$$

This latter reaction constant (the reaction is shown as in Eq. (18), and indicates how tightly L is bound to the simple MA binary complex.

Based on the stability constant values of ternary complexes formed (Table 4), the following conclusions could be drawn:

Stabilities of the 1:1:1 ternary complexes of investigated ligands are higher than the corresponding 1:1 binary ones for the same metal ion.

The stability of the ternary complex of Zn^{2+} is higher than that of ternary complex of the Ca^{2+} system.

3.4. Comparison of Stabilities of Ternary Complexes with Binary Complexes

Different methods are used to estimate the formation and the relative stability of the different ternary complexes

formed in solution [42]. The Comparison of stabilities of the ternary complexes investigated with the corresponding binary ones can be evaluated in many ways.

3.4.1. The Δ log₁₀ K parameter

In most cases, the relative stability of the mixed-ligand species [MAL] is expressed in terms of the $\Delta \log_{10} K$ parameter, the constant due to the equilibrium (Eq. (15)):

$$MA + ML = MAL + M \quad (15)$$

$$\Delta \log_{10} K = \log_{10} K_{MAL}^{MA} - \log_{10} K_{ML}^{MA}$$

which is a way to characterize the tendency toward formation of mixed-ligand complexes. The $\Delta \log_{10} K$ expresses the effect of the bounded primary ligand (A) towards an incoming secondary ligand (L). The positive $\Delta \log_{10} K$ values obtained (Table 4) for ternary systems indicate favored formation of ternary complexes over the corresponding binary ones. This can be ascribed to inter-ligand interactions or some cooperation between the primary and secondary ligands such as H-bond formation.

### 3.4.2. Percent Relative Stabilization (% R. S.)

Another parameter, percent relative stabilization (% R.S.) to quantify stability of a ternary complex relative to binary complex of the secondary ligand [43], may be defined as in Eq. (16):

$$\% R. S = [\log_{10} K_{MAL}^{MA} - \log_{10} K_{ML}^M / \log_{10} K_{ML}^M] \times 100 \quad (16)$$

This parameter shows how much a ternary complex is stable relative to the binary complex of secondary ligand. The values obtained for our ternary complexes are given in Table 4. The ternary complexes are relatively stable than binary complexes of the secondary ligand. Also, the percent relative stabilization (% R.S.) data are in good agreement with the $\Delta \log_{10} K$ data.

3.4.3. The Disproportionation Constant (log₁₀ X)

A third parameter known as $\log_{10} X$, is frequently used to characterize the stability of ternary or mixed-ligand complexes. It measures the tendency of one mole each of the binary complexes MA_2 and ML_2 to disproportionate forming two moles of MAL as follows (Eq. (17)):

$$MA_2 + ML_2 \rightleftharpoons 2MAL, \quad X = [MAL]^2 / [MA_2][ML_2] \quad (17)$$
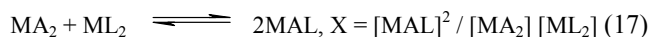
It is therefore calculated according to Eq. (18):

$$\log_{10} X = 2 \log_{10} \beta_{MAL} - (\log_{10} \beta_{MA2} + \log_{10} \beta_{ML2}) \quad (18)$$

The value of the constant X expected on statistical ground is 4. Whenever it deviates from this value, it must be the result of inter-ligand electronic and / or sterric interactions [44]. The $\log_{10} X$ values were calculated and the results are showed Table 4. The values obtained are always greater than the statistically expected ones, which means formation of more stable ternary complexes.

Table 4. *Stability constants and Parameters of 1:1:1 ternary complexes of L-cysteine (A) and diphenylamine (L) with Ca(II) and Zn(II) metal ions investigated in aqueous media at 25.0°C and I = 0.20 mol·dm⁻³NaNO₃.*

Stability Constantsor Parameters	Ternary Complex Systems	
	Ca(II) - L-Cysteine-Diphenylamine	Zn(II) - L-Cysteine-Diphenylamine
$\log_{10} K_{MAL}^{MA}$	6.39 ± 0.01	9.35 ± 0.03
$\log_{10}\beta_{MAL}^{M}$	12.37 ± 0.01	17.17 ± 0.02
$\Delta \log_{10} K$	1.00 ± 0.01	2.29 ± 0.02
X	4.00 ± 0.01	8.28 ± 0.03
$\log_{10} X$	0.60 ± 0.01	0.92 ± 0.01
% R.S.	18.55 ± 0.10	32.44 ± 0.20

3.4.4. The Standard Gibbs Free Energy Change Parameter (ΔG^0)

The change in the standard state Gibbs free energy is a key thermodynamic parameter for systems under equilibrium. Under a particular set of equilibrium conditions, the ΔG^0 value dictates the direction of the equilibrium. The Gibbs energy is a balance between enthalpy and entropy of an equilibrium [45-51]. The change in the Gibbs free energy for the dissociation equilibria of the ligands (- $\log_{10} K_a$ = pK_a) investigated can be calculated by Eq. (19)

$$\Delta G = - RT \ln K_a \text{ or}$$

$$\Delta G = - 2.303 RT \log_{10} K_a \text{ or}$$

$$\Delta G = 2.303 RT pK_a \qquad (19)$$

And at 298.15 K, the change in the standard state Gibbs free energy can be:

Calculate for pKa with R equal to 8.314 J K⁻¹ mol⁻¹ (Eq. (20)):

$$\Delta G^0 = 5.708 pK_a \qquad (20)$$

In the same way, the change in the standard state Gibbs free energy for the formation equilibria ($\log_{10}$ K) of the complexes investigated can be calculated by Eq. (21):

$$\Delta G^0 = - 5.708 \log_{10} K \qquad (21)$$

Regarding Table 5, ΔG^0 values are positive for dissociation reactions of the ligands (non-spontaneous) and negative for the complex formation reactions (spontaneous).

Table 5. *The ΔG^o values for the dissociation equilibria of L-cysteine (A) and diphenylamine (L) and their binary and ternary complexes with Ca(II) and Zn(II) metal ions in aqueous media at I = 0.20 mol•dm⁻³ NaNO₃.*

Equilibrium	Equilibrium constantor Parameter	ΔG^o / kJ.mol⁻¹
L-Cysteine		
$H_2A^\pm = H^+ + HA^-$	$8.40 \pm (0.01)$	$+89.21 \pm (0.10)$
$HA^- = H^+ + A^{2-}$	$10.40 \pm (0.03)$	$+110.24 \pm (0.10)$
Diphenylamine		
$HL = H^+ + L^-$	$10.36 \pm (0.02)$	$+110.03 \pm (0.10)$
Ca(II) - L-Cysteine		
$Ca^{2+} + A^{2-} = CaA$	$5.98 \pm (0.01)$	$-63.51 \pm (0.10)$
$CaA + A^{2-} = CaA_2^{2-}$	$4.79 \pm (0.02)$	$-50.83 \pm (0.20)$
$Ca^{2+} + 2A^{2-} = CaA_2^{2-}$	$10.77 \pm (0.02)$	$-114.37 \pm (0.10)$
Ca(II) – Diphenylamine		
$Ca^{2+} + L^- = CaL^+$	$5.39 \pm (0.01)$	$-57.24 \pm (0.10)$
$CaL^+ + L^- = CaL_2$	$4.58 \pm (0.02)$	$-48.64 \pm (0.20)$
$Ca^{2+} + 2L^- = Ca L_2$	$9.97 \pm (0.02)$	$-105.88 \pm (0.20)$
Ca(II) - L-Cysteine – Diphenylamine		
$CaA + L^- = CaAL^-$	$6.39 \pm (0.01)$	$-67.86 \pm (0.10)$
$Ca^{2+} + A^{2-} + L^- = CaAL^-$	$12.37 \pm (0.02)$	$-131.36 \pm (0.20)$
$CaA_2^{2-} + CaL_2 = 2CaAL^-$	$0.60 \pm (0.01)$	$-6.42 \pm (0.10)$
$CaA + CaL^+ = CaAL^- + Ca^{2+}$	$1.00 \pm (0.02)$	$-10.62 \pm (0.20)$
Zn(II) - L-Cysteine		
$Zn^{2+} + A^{2-} = ZnA$	$7.82 \pm (0.01)$	$-83.05 \pm (0.10)$
$ZnA + A^{2-} = ZnA_2^{2-}$	$6.04 \pm (0.03)$	$-64.14 \pm (0.30)$
$Zn^{2+} + 2A^{2-} = ZnA_2^{2-}$	$13.86 \pm (0.02)$	$-147.19 \pm (0.20)$
Zn(II) – Diphenylamine		
$Zn^{2+} + L^- = ZnL^+$	$7.06 \pm (0.01)$	$-74.97 \pm (0.10)$
$ZnL^+ + L^- = ZnL_2$	$5.14 \pm (0.03)$	$-54.58 \pm (0.30)$
$Zn^{2+} + 2L^- = ZnL_2$	$12.20 \pm (0.02)$	$-129.56 \pm (0.20)$
Zn(II) - L-Cysteine – Diphenylamine		
$ZnA + L^- = ZnAL^-$	$9.35 \pm (0.01)$	$-99.93 \pm (0.10)$
$Zn^{2+} + A^{2-} + L^- = ZnAL^-$	$17.17 \pm (0.01)$	$-182.34 \pm (0.10)$
$ZnA_2^{2-} + ZnL_2 = 2 ZnA(HL)$	$0.92 \pm (0.01)$	$-9.93 \pm (0.10)$
$ZnA + ZnL^+ = ZnAL^- + Zn^{2+}$	$2.29 \pm (0.01)$	$-24.32 \pm (0.10)$

For all systems studied, the ternary complexes formed are more thermodynamically stable than the binary cones.

4. Conclusion

Complex formation reactions of Zn^{2+} and Ca^{2+} metal ions with L-cysteine and diphenylamine are studied. The complex formation reactions are investigated to ascertain complex compositions and to calculate complex stabilities. Normal 1:1 and 1:2 binary complexes and 1:1:1 ternary complexes are formed with all investigated metal ions. The stabilities of the ternary complexes are evaluated and quantitatively compared with their binary complexes. The $\Delta \log_{10} K$, $\log_{10} X$, and % R.S values indicated marked stabilities of the ternary complexes over the binary ones. The obtained values of ΔG^0 indicated that Complex formation reactions are spontaneous. Also, for all systems studied, the ternary complexes formed are more thermodynamically stable than the binary complexes.

References

[1] Frausto da Silva, J. J. R., Williams, R. J. P.: The Biological Chemistry of the Elements; The Inorganic Chemistry of Life. Clarendon Press, Oxford (1991).

[2] Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J. S.: Bioinorganic Chemistry. University Science Books, Mill Valley (1994).

[3] Goodman Gilman, A., Goodman, L. S.: Goodman and Gilman's he Pharmacological Basis of Therapeutics, 10th edn. Mc-Graw Hill, New Jersey (2002).

[4] Kimura, E., Kurogi, Y., Shionoya, M., Shira, M.: Synthesis, properties, and complexation of a new imidazole-pendant macrocyclic 12-membered triamine ligand. Inorg. Chem. 30, 4524–4529 (1991).

[5] Schayer, R. W.: The metabolism of ring labeled histamine. J. Biol. Chem. 196, 469–475 (1952).

[6] Baldridge, R. C., Tourtellotte, C. D.: The metabolism of histidine. III. Urinary metabolites. J. Biol. Chem. 233, 125–127 (1958).

[7] Khandelwal, J. K., Prell, G. D., Morrishow, A. M., Green, J. P.: Presence and measurement of imidazoleacetic acid, a c-aminobutyric acid agonist, in rat brain and human cerebrospinal fluid. J. Neurochem. 52, 1107–1113 (1989).

[8] Garcı´a-Raso, A., Fiol, J. J., Adrover, B., Tauler, P., Pons, A., Mata, I., Espinosa, E., Molins, E.: Reactivity of copper(II) peptide complexes with bioligands (benzimidazole and creatinine). Polyhedron. 22, 3255–3264 (2003).

[9] Ranford, J. D., Sadler, P. J.: Cytotoxicity and antiviral activity of transition-metal salicylato complexes and crystal structure of bis(diisopropylsalicylato)(1,10-phenanthroline)copper(II). Dalton Trans. 22, 3393–3399 (1993).

[10] Majella, G., Vivienne, S., Malachy, M., Michael, D., Vickie, M.: Synthesis and anti-candida activity of copper(II) and manganese(II) carboxylate complexes: X-ray crystal structures of [Cu(sal)(bipy)] _C2H5OH_H2O and [Cu(norb)(phen)2]_6.5H2O (salH2 = salicylic acid; norbH2 = cis-5-norbornene- endo-2,3-dicarboxylic acid; bipy = 2,20-bipyridine; phen = 1,10-phenanthroline). Polyhedron 18, 2931–2939 (1999).

[11] Saha, D. K., Sandbhor, U., Shirisha, K., Padhye, S., Deobagkar, D., Ansond, C. E., Powell, A. K.: A novel mixed-ligand antimycobacterial dimeric copper complex of ciprofloxacin and phenanthroline. Bioorg. Med. Chem. Lett. 14, 3027–3032 (2004).

[12] Zoroddu, M. A., Zanetti, S., Pogni, R., Basosi, R.: An electron spin resonance study and antimicrobial activity of copper(II)–phenanthroline complexes. J. Inorg. Biochem. 63, 291–300 (1996).

[13] Sigel, H.: Metal Ions in Biological Systems, vol. 3. Marcel Dekker, New York (1974).

[14] Lau, S., Sarkar, B.: Kinetic studies of copper(II)-exchange from L-histidine to human serum albumin and diglycyl-L-histidine, a peptide mimicking the copper(II)-transport site of albumin. Can. J. Chem. 53, 710–715 (1975).

[15] Bensichem, M. V., Crystal and molecular structure of trans-dichlorobis (thiazole) platinum(II). Inorg. Chem. 31, 634–639 (1992).

[16] Acta Cryst. Diphenyamine (2003). E59, o1123-o1125.

[17] Khalil M. M.; Attia, A. E.; Potentiometric Studies on the Binary and Ternary Complexes of Copper(II) Containing Dipicolinic Acid and Amino Acids. J. Chem. Eng. Data, 1999, 44, 180-184.

[18] Khalil, M. M.; Complexation Equilibria & Determination of Stability Constants of Binary and Ternary Complexes with Ribonucleotides (AMP, ADP, and ATRP) and Salicylhydroxamic Acid as Ligands. J. Chem. Eng. Data, 2000, 45, 70-74.

[19] Khalil, M. M.; Solution Equilibria & Stabilities of Binary and Ternary Complexes with N-(2-Acetamido) iminodiacetic Acid and Ribonucleotides (AMP, ADP, and ATP). J. Chem. Eng. Data, 2000, 45, 837-840.

[20] Khalil, M. M.; Attia, A. E.; Potentiometric Studies on the Formation Equilibria of Binary and Ternary Complexes of some Metal Ions with Dipicolinic Acid and Amino Acids. J. Chem. Eng. Data, 2000, 45, 1108- 1110.

[21] Khalil, M. M.; Taha, M.; Equilibrium Studies of Binary and Ternary Complexes involving Tricine and some Selected Amino Acids. Monatschefte fur Chemie, 2004, 135, 385-395.

[22] Khalil, M. M.; Fazary, A. E.; Potentiometric Studies on Binary and Ternary Complexes of Di- and Trivalent Metal Ions involving some Monats Chem., 2004, 135, 1455- 1474.

[23] Khalil, M. M.; El-Deeb, M. M.; Mahmoud, R. K.; Equilibrium Studies of Binary Systems involving Lanthanide and Actinide Metal Ions and some selected Aliphatic and Aromatic Monohydroxamic Acids. J. Chem. Eng. Data, 2007, 52, 1571-11579.

[24] Khalil, M. M.; Mahmoud, R. K.; New Insights into M(II)-Hydroxamate Interactions: The Electro-Analytical Behavior of Metal(II) Complexes Involving Monohydroxamic Acids and Diamines in an Aqueous medium, J. Chem. Eng. Data, 2008, 53, 2318-2327.

[25] Khalil, M. M.; Radalla, A. M.; Mohamed, A. G.; Potentiometric Investigation on Complexation of Divalent Transition Metal Ions with Some Zwitterionic Buffers and Triazoles. *J. Chem. Eng. Data*, 2009 (in press).

[26] Khalil, M. M.; Radalla, A. M; Mohamed, N. A.; Solution Equilibria and Thermodynamic studies on Complexation of Divalent Transition Metal Ions with Some Triazoles and Biologically Important Aliphatic Dicarboxylic Acids in Aqueous Media. *The First Scientific Conference for Postgraduate Students*. Beni-Suef University, Beni-Suef, Egypt, 2009, Jun. 26-28. P-200.

[27] Khalil, M. M.; Radalla, A. M.; Qassim, F.; Equilibrium and Thermodynamic Studies on Binary and Ternary Complex Systems Including Some Triazoles and Aromatic Carboxylic Acids. *The First Scientific Conference for Postgraduate Students*. Beni-Suef University, Beni-Suef, Egypt 2009, Jun. 26-28. P-400.

[28] Khalil, M. M.; Mohamed, S. A.; Radalla, A. M.; Potentiometric and Conductometric Studies on the Binary and Mixd-ligand Complexes in Solution: M^{II}-Dipicolinic Acid-Glycine Systems. *Talanta*, 1997, 44, 1365-1369.

[29] Khalil M. M.; Radalla, A. M.; Binary and Ternary Complexes of Inosine *Talanta*, 1998, 46, 53-61.

[30] Gran, G. Determination of the Equivalence Point in Potentiometric Titration. *Analyst* 1952, 77, 6, 61-671.

[31] Welcher, F. J. *The Analytical Uses of Ethylenediaminetetraacetic acid*; Von Nostrand: Princeton, NJ, 1965.

[32] Gans, P.; O'Sullivan, B. Glee, a New Computer Program for Glass pH-Electrode Calibration. *Talanta* 2000, *51*, 33-37.

[33] Longhi, P.; D'Andrea, F.; Mussini, T.; Rondinini, S.; Verification of the approximate equitransference of the aqueous potassium chloride salt bridge at high concentrations. *Anal. Chem.*, 1990, 62, 1019-1021.

[34] Irving, H. M.; Rossotti, H. S. Methods for Computing Successive Stability Constants from Experimental Formation Curves. *J. Chem. Soc.*, 1953, 3397-3405.

[35] Irving, H. M.; Rossotti, H. S. The Calculation of Formation Curves of Metal Complexes from *pH* Ttitration Curves in Mixed Solvent. *J. Chem. Soc.* 1954, 2904.

[36] Martell, A. E.; Smith, R. M.; *Critical Stability Constants.* Plenum Press, New York, 1976.

[37] Inezedy, J.; *Determination of Equilibrium Constants, In: Analytical Applications of Complex Equilibria*. Ellis Horwood, Chechester, 1976.

[38] C. D. Kennedy; Ionic Strength and the Dissociation of Acids. Biochemical Education, 1990, 18 35-40.

[39] James, P. D., Parameswaran, K. N., Acidic Dissociation Constants of Thiols. J Chem. Eng. Data, 1955, 6, 386-389.

[40] Elliott, D. C. Dawson, R. M.; James, K. M.; Data for Biochemical Research, Oxford Science Publications, 3rd ed, 1986, pp 1-31.

[41] Martin, R. B.; Prados, R. J.; Some Factors Influencing Mixed Complex Formation. *J. Inorg. Nucl. Chem.*, 1974, 36, 1665-1674.

[42] H. Sigel: Metal Ions In Biological Sytems, Vol. 2. Marcel Dekker, New, York, 1973.

[43] Martell, A. E.; Smith, R. M.; *Critical Stability Constants.* Plenum Press, New York, 1974.

[44] Laurie, S. H.; James, C.; Binary and Ternary Complexes of hydroxmic Acids. *Inorg. Chim. Acta,* 1983, 78, 225-229.

[45] J. B. Chaires; Calorimetry and thermodynamics in drug design. Annu. Rev. Biophys., 2008, 37, 135-151.

[46] Shahbazi, Mina, Farzaneh Mehrzad, Masoud Mirzaei, Hossein Eshtiagh-Hosseini, Joel T. Mague, Mehdi Ardalani, and Mojtaba Shamsipur. "Synthesis, single crystal X-ray characterization, and solution studies of Zn (II)-, Cu (II)-, Ag (I)-and Ni (II)-pyridine-2, 6-dipicolinate N-oxide complexes with different topologies and coordination modes." Inorganica Chimica Acta 458 (2017): 84-96.

[47] Mirzaei, Masoud, Hossein Eshtiagh-Hosseini, Zahra Karrabi, Behrouz Notash, Antonio Bauzá, Antonio Frontera, Morteza Habibi, Mehdi Ardalani, and Mojtaba Shamsipur. "Synthesis, structure, solution and DFT studies of a pyrazine-bridged binuclear Cu (II) complex: On the importance of noncovalent interactions in the formation of crystalline network." Journal of Molecular Structure 1079 (2015): 78-86.

[48] Mirzaei, Masoud, Hossein Eshtiagh-Hosseini, Mojtaba Shamsipur, Mahdi Saeedi, Mehdi Ardalani, Antonio Bauzá, Joel T. Mague, Antonio Frontera, and Morteza Habibi. "Importance of polarization assisted/resonance assisted hydrogen bonding interactions and unconventional interactions in crystal formations of five new complexes bearing chelidamic acid through a proton transfer mechanism." RSC Advances 5, no. 89 (2015): 72923-72936.

[49] Hosseini-Hashemi, Zahra, Masoud Mirzaei, Ameneh Jafari, Peyman Hosseinpour, Mohammad Yousefi, Antonio Frontera, Mahmoud Lari Dashtbayaz, Mojtaba Shamsipur, and Mehdi Ardalani. "Effects of N-oxidation on the molecular and crystal structures and properties of isocinchomeronic acid, its metal complexes and their supramolecular architectures: experimental, CSD survey, solution and theoretical approaches." RSC advances 9, no. 44 (2019): 25382-25404.

[50] Hosseini Hashemi, Zahra, Masoud Mirzaei, Hossein Eshtiagh-Hosseini, Fereshteh Sadeghi, Mojtaba Shamsipur, Mehdi Ardalani, and Alexander J. Blake. "Solid and solution states studies of two Mn (II) complexes based on N-oxidized pyridine-2, 5-dicarboxylic acid." Journal of Coordination Chemistry 71, no. 24 (2018): 4058-4071.

[51] Soleimannejad, J., F. Moghzi, Sh Hooshmand, Z. Dankoob, M. Ardalani, and M. Shamsipur. "A comparison of ligand behaviors and interactions during supramolecular assembly using molecular dynamics simulation: Synthesis, solid state and solution studies of two Ni (II) compounds." Polyhedron 133 (2017): 24-32.