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Stability Constants and Antimicrobial Studies of Metal(II) Complexes with Cinnamaldehyde-(2-hydroxy benzylidene)hydrazide

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Synthesis and structural features of some bidentate Schiff base complexes of the type $[M(NNO)_2]$ [where M = Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and NNO = cinnamaldehyde-(2-hydroxy benzylidene)hydrazide] are reported. The ligand was derived by the condensation of cinnamaldehyde hydrazone with salicyaldehyde. These metal complexes were characterized by elemental analysis, molar conductance, magnetic momentum measurements, IR, ^{1}H NMR spectral data. An octahedral geometry was proposed for all metal complexes. Stability constants of these metal complexes were determined using a potentiometric method at constant ionic strength (0.1 m) at different temperature (25, 30, 35 ºC) in 50 % v/v ethanol-water mixture by calculating the values of η_A, η and P_L, has been found that ligand forms complexes in 1:2 ratio. Antimicrobial activities of ligand and its metal complexes were evaluated against strains- *E. coli*, *Klebsiella*, *Enterococci* and *S. aureus*. It has been found that metal complexes have higher activity than the parent ligand and their bio-potency are discussed.

Key Words: Metal(II) complexes, Schiff base, Cinnamaldehyde-(2-hydroxy benzylidene)hydrazide, Antimicrobial activity, Infrared conductance.

INTRODUCTION

The development of bioinorganic chemistry field has received interest in Schiff base complexes due to their wide application in the field of catalysis and as antimicrobial, antituberculosis and antitumour activity. Schiff bases play an important role in organic chemistry, as they easily form stable complexes with most transition metal ions, since it has been recognized that many of these complexes may serve as models for biologically important species[1-5]. Coordination compounds derived from *bis* aryl hydrazone have been reported to act as enzyme inhibitors and are useful due to their pharmacological application[6-11]. In view of the importance of such hydrazones, in present studies the synthesis, characterization, stability constant and biological activity of Cu(II), Ni(II), Co(II), Mn(II) complexes of cinnamaldehyde-(2-hydroxy benzylidene)hydrazide have been described.

EXPERIMENTAL

All the reagents used were of analytical grade, stock solutions of metal salts were prepared from pure metal salts. The melting point, elemental analysis, IR spectra and ^{1}H NMR spectra have been compared with literature values to check the purity of the ligand.

Synthesis of ligand: The ligand was synthesized in two steps. The first step is the synthesis of cinnamaldehyde monohydrazone (**I**), followed by the cinnamaldehyde-(2-hydroxy benzylidene)hydrazide (**II**) in the second step from monohydrazone.

Cinnamaldehyde (1 mmol 1.33 mL) was dissolved in ethanol (10 mL). To this solution hydrazine dihydrochloride (1 mmol 1.04 g) dissolved in absolute ethanol (10 mL) and a few drops of sodium acetate were added and the mixture was refluxed for 4 h at 70-80 ºC in a water-bath. After 2 h a golden yellow precipitate was formed, which was filtered and washed with $NaHSO_3$ solution to remove any excess of aldehydes. The product was recrystallized from ethanol to give the cinnamaldehyde monohydrazone compound (80 % yield, mp 120 ºC).

Cinnamaldehyde monohydrazone (**I**) (2 g, 1 mmol) dissolved in absolute ethanol (10 mL) was added to solution of salicyaldehyde (1 mmol 1.22 g). The reaction mixture was refluxed for 4 h. After cooling, a yellow precipitate was collected, filtered and finally washed with absolute ethanol (10 mL) and purified by recrystallization from ethanol (77 % yield; m.p. 130 ºC) (Fig. 1).

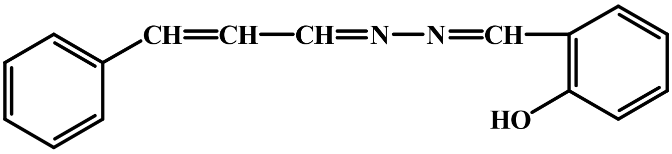

Fig. 1. Structure of cinnamaldehyde-(2-hydroxy benzylidene)hydrazide

Synthesis of metal complexes: Ligand hydrazide was dissolved in absolute ethanol (10 mL) and to this corresponding metal salt (0.1 mmol) such as nickel(II) chloride hexahydrate (2.37 g), cobalt(II) chloride hexahydrate (2.37 g), mangnese(II) chloride hexahydrate (1.97 g) and copper(II) acetate dihydrate (1.99 g) dissolved in bidistilled water were added. The reaction mixture was refluxed for 4 h. After cooling the formed solid was collected, filtered and washed several times with 50 % (v/v) ethanol-water and sodium bisulphate to remove any excess of the unreacted starting materials. The complexes were washed with diethyl ether and dried in vacuum over anhydrous $CaCl_2$. The purity of the compounds was checked by TLC using benzene-water mixture (3:1).

RESULTS AND DISCUSSION

The physical properties, analytical data and spectral data of ligand and its metal complexes are given in Tables 1 and 2, respectively. On the basis of these studies, the proposed structure of the metal complexes are given in Fig. 2.

TABLE-1
MICRO-ANALYTICAL DATA'S OF THE LH$_1$ AND METAL COMPLEXES

Compounds		Ligand	L-Cu^{2+} complex	L-Ni^{2+} complex	L-Co^{2+} complex	L-Mn^{2+} complex
Elemental analysis (%): Found (Calcd.)	C	79.67 (79.49)	70.34 (70.24)	70.58 (71.10)	70.83 (70.78)	71.32 (70.63)
	H	5.83 (4.85)	5.51 (5.13)	5.53 (5.19)	5.59 (4.97)	5.59 (5.25)
	N	10.64 (10.51)	9.65 (9.27)	9.68 (9.25)	9.72 (9.43)	9.79 (9.32)
	M	-	10.0 (10.28)	9.78 (8.25)	9.37 (9.12)	8.74 (8.34)
Magnetic moment		-	4.85	3.15	4.86	5.85
Conductivity		-	3.75	3.60	3.23	3.48
Melting point (ºC)		130	150	180	170	200
m.w. Found (calcd.)		254 (250)	580 (575)	578 (573)	574 (576)	572 (569)
Yield (%)		77	75	68	65	69
Colour		Yellow	Brown	Black	Brownish black	Black

TABLE-2
IR, ^{1}H NMR AND UV SPECTRA OF LIGAND LH$_1$ AND ITS METAL COMPLEXES

Compd.	IR spectra (cm^{-1})				^{1}H NMR spectra (ppm)		UV spectra (nm)	
	ν(M-N)	ν(C=N)	ν(Ar-CH)	ν-(C=C-)	δ(Ar-H)	δ(N=H)	(-C=C-)	(-C=N)
LH$_1$	–	1536, 1628	3020	1620	7.00-7.1	9.76	270	300
LH$_1$-Cu^{2+}	578	1506, 1615	3013	1620	6.42-7.7	8.72	271	280
LH$_1$-Ni^{2+}	598	1510, 1609	3009	1620	6.38 -6.6	8.60	271	263
LH$_1$-Co^{2+}	610	1509, 1611	3018	1620	6.18-6.5	8.52	272	258
LH$_1$-Mn^{2+}	620	1511, 1621	3014	1620	6.10-6.8	8.40	272	265

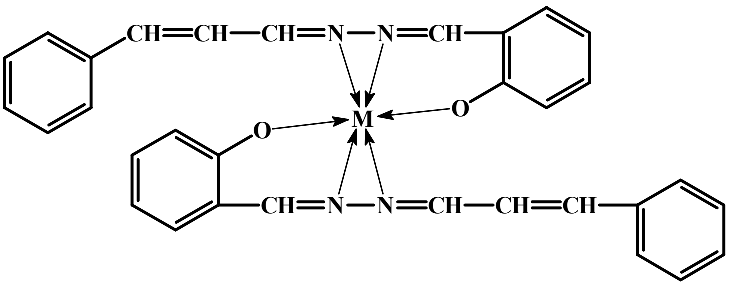

Fig. 2. Proposed structure of metal complexes

Potentiometric studies: Metal ion solutions (C = 0.0002 mol dm^{-3}) were prepared from analar metal chlorides in bidistilled water and were standardized with EDTA.The ligand solution (c = 0.001 mol dm^{-3}) was prepared by dissolving the accurate mass of the solid in ethanol (AnalaR). Solutions of 0.001 M HCl and 1 M NaCl were also prepared in bidistilled water. A carbonate free sodium hydroxide in ethanol-water mixture (30 vol %) was used as titrate and standardized against oxalic acid (AnalaR).

The following mixtures (i-iii) were prepared and titrated potentiometricallly at 298 K against standard 0.004 M NaOH in ethanol-water mixture (50 vol %): (i) 5 mL 0.001 M HCl +5 mL 1 M NaCl + 20 mL ethanol, (ii) 5 mL 0.001 M HCl + 5 mL 1 M NaCl + 5 mL 0.001 M ligand + 15 mL ethanol, (iii) 5 mL 0.001 M HCl + 5 mL 1 M NaCl + 15 mL ethanol + 5 mL 0.001 M ligand + 5 mL 0.0002 M metal salt.

For each mixture, the volume was made up to 50 mL with bidistilled water before the titration. The pH measurements were carried out using Equiptronics-610 pH meter accurate to ± 0.01 units. The pH meter readings in ethanol-water (50 v/v) are corrected according to Van-Uitert and Hass relation[12].

Antibacterial studies: Muller Hinton agar was used for the growth of specific bacterial species. The ligand and its metal complexes were dissolved at a concentration of 10 mg/mL in DMF. In all the 3 concentrations of the drug were prepared (1.00, 0.10 and 0.01 mg/mL) for the microbial testing. Then they were applied to a paper disc with the help of micropipette. The discs were left in an incubator for 48 h at 37 °C and then applied to the bacteria grown on agar plates. They were incubated at 37 °C for 24 h. The zones of inhibition (diameter in mm) were then measured around the disc and are given in Table-3.

TABLE-3
ANTIMICROBIAL ACTIVITY OF *BIS* HYDRAZIDE AND THEIR METAL COMPLEXES

Compd.	Microorganisms											
	Staphylococus aureus (mm)			*Klebsiella* (mm)			*Enterococci* (mm)			*E. coli* (mm)		
	A	B	C	A	B	C	A	B	C	A	B	C
LH_1	15	16	17	13	14	15	14	16	18	15	17	19
LH_1-Cu^{2+}	19	23	24	20	22	23	18	20	22	20	23	25
LH_1-Ni^{2+}	18	19	21	17	18	19	17	19	22	17	19	22
LH_1-Mn^{2+}	19	21	26	16	18	19	16	19	23	18	20	23
LH_1-Co^{2+}	20	23	26	15	17	19	18	20	22	16	18	20

A = 0.01 mg/mL, B = 0.10 mg/mL, C = 1.00 mg/mL

Antimicrobial activity: Hydrazide and its metal complexes were evaluated for antimicrobial activity against strain gram(+)ve and gram(-)ve bacteria (*Escherichia*) and *Cocci* (*Staphylococcus aureus*) *bis*hydrazone metal complexes were found to be biologically active. It is known that chelation tends to make ligands act as more powerful and potent bactericidal agent and this was confirmed by the fact that the metal complexes showed enhanced antimicrobial activity against one or more strains. It may be suggested that the chelated complexes deactivate various cellular enzymes, which play vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more proteins of the cell, which as a result, impairs normal cellular process[13-15].

Stability constant: The average number of the protons associated with the ligand molecule L, η_A, was determined at different pH values applying the following equation.

$$\eta_A = y + \frac{(V_1 - V_2)(N + E^0)}{(V^0 + V_1)\, C_L^0} \tag{1}$$

where Y = number of available protons in L (Y = 1) and V_1 and V_2 are the volumes of alkali required to reach the same pH on the titration curve of hydrochloric acid and organic ligand, respectively. V^0 = initial volume (50 cm^3) of the mixture; N^0 = concentration of sodium hydroxide solution and C^0_L = initial concentration of free acid. The titration curves (η_A *vs.* pH) for the proton-ligand systems were constructed and found to extend between 0 and 1 on the η_A scale. This means that L has one ionizable proton. It can be seen that for same volume of NaOH added the ligand titration curves had a lower pH value than the acid titration curve. The displacement of a ligand titration curve along the volume axis with respect to the acid titration curve is an indication of proton dissociation. The proton-ligand stability constants were calculated using the method of Irving and Rossoti[16]. The data obtained are listed in Table-4.

TABLE-4
THERMODYNAMIC FUNCTION FOR THE DISSOCIATION OF LIGAND IN ETHANOL-WATER (50 v/v) MIXTURES AND 0.1 M NaCl AT DIFFERENT TEMPERATURE

Temp. (K)	Formation constant (log K_1)	(Gibbs energy change)i (KJ mol^{-1})	(Enthalpy change)i (KJ mol^{-1})	(-Entropy change)i (J mol^{-1} K^{-1})
298	7.62	43.40	–	36.44
308	7.42	43.75	32.54	36.39
318	7.28	44.32	–	37.04

The formation curves for the metal complexes were obtained by plotting the average number of ligands attached per metal ions (η) *vs.* the free ligand exponent (pL), according to Irving and Rosotti[17]. The average number of the reagent molecules attached per metal ion, η and free ligand exponent, P_L, can be calculated using the equations:

$$\eta = \frac{(V_3 - V_2)\,(N + E^0)}{(V^0 + V_2)\,\eta_A\, C_M^0} \tag{2}$$

and

$$P_L = \log 10 \frac{\sum \beta_n^H (1/\text{anti}\log pH)^n}{C_L^0 - \eta C_M^0} \times \frac{V^0 + V_3}{V^0} \quad (3)$$

where C^0_M = total concentration of the metal ions present in the solution, β_n^H = overall proton-reagent stability constant. V_1, V_2, V_3 are the volumes of alkali required to reach the same pH on the titration curves of hydrochloric acid, organic ligand and complex, respectively. These curves were analyzed and the successive stability constants were determined using different computational methods[18]. The values of the stability constants log K_1 and log K_2 are given in Table-5.

TABLE-5
STEPWISE STABILITY CONSTANTS FOR METAL COMPLEXES OF HYDRAZIDE IN ETHANOL-WATER (50 vol %) MIXTURES AND 0.1 M NaCl AT DIFFERENT TEMPERATURE

M^{n+}	298 K		308 K		318 K	
	log K_1	log K_2	log K_1	log K_2	log K_1	log K_2
Mn^{2+}	7.30	4.07	7.42	4.15	7.50	4.25
Co^{2+}	7.15	4.02	7.32	4.00	7.42	4.27
Ni^{2+}	6.00	3.50	6.10	3.62	6.29	3.71
Cu^{2+}	6.50	3.52	6.60	3.65	6.72	3.70

The following general remarks can be made: (i) The maximum value of η was 2 indicating the formation of 1:2 (n [metal]:n[ligand]) complexes only (Fig. 3). (ii) The metal ion solution used in the present study was very dilute (2×10^{-5} mol dm^{-3}), hence there was no possibility of formation of polynuclear complexes[19]. (iii) The titration curve of the metal ion complexes were displaced to the right hand side of the ligand titration curves along the volume axis, indicating proton release upon complex formation of the metal ion and ligand. The large decrease in pH for the metal ion complexes titration curves relative to ligand titration curves points to the formation of strong metal ion complexes[20]. (iv) In most cases, the colour of the solution after complex formation was found to be different from the colour of the ligand at the same pH. (v) For the same ligand at constant temperature, the stability of the chelates increases[21] in the order Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}. This can be attributed to the decrease of charge on the central metal ion which decreases the electronic attraction between the cation and the ligand.

The formation constant (log K_1) for L, as well as the stability constants of its complexes with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} have been evaluated at 298, 308 and 318 K. The enthalpy change (ΔH) for the dissociation and complexation process was calculated from the slope of the plot pk_a or log k *vs.* 1/(T) using the graphical representation of von't Hoff equations:

$$2.303\ RT \log K = \Delta H - T\Delta S \quad (4)$$

or

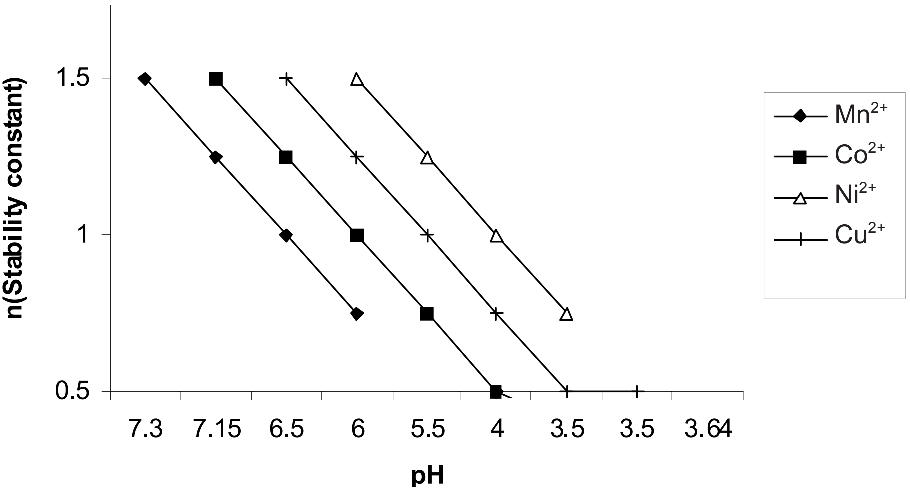

Fig. 3. Plot of stability constant of metal complexes of bis hydrazones *vs.* pH

$$\log K = (-\Delta H/2.303R)\,(1/T) + (\Delta S + 2.303R) \tag{5}$$

From the Gibbs energy change (ΔG) and (ΔH) values one can deduce the entropy changes (ΔS) using the well known relationships.

$$\Delta G = -2.303\ RT \log K \tag{6}$$

$$\Delta S = (\Delta H - \Delta G) / T \tag{7}$$

where the gas constant R= 8.314 JK^{-1} mol^{-1}, K is the dissociation constant for the ligand or the stability constant of the complexes and T absolute temperature.

All thermodynamic parameter of the formation process of ML are recorded in Table-6.

TABLE-6
THERMODYNAMIC FUNCTIONS FOR METAL COMPLEXES OF LIGAND IN ETHANOL-WATER (50 v/v) MIXTURES AND 0.1 M NaCl AT DIFFERENT TEMPERATURE

M^{n+}	Temp. (K)	(Gibbs energy Change)i (KJ mol^{-1})		(Enthalpy change)i (KJ mol^{-1})		(-Entropy change)i (J mol^{-1} K^{-1})	
		1	2	1	2	1	2
Mn^{2+}	298	37.02	20.05	21.06	23.25	194.890	145.30
	308	38.85	21.48			194.510	145.22
	318	40.84	22.49			194.650	143.83
Co^{2+}	298	34.17	19.93	27.76	20.104	207.810	134.32
	308	35.91	21.31			206.720	134.44
	318	38.23	22.55			207.510	134.11
Ni^{2+}	298	40.79	22.89	25.84	23.93	223.570	157.11
	308	43.16	23.29			224.020	153.31
	318	45.17	25.95			223.300	156.85
Cu^{2+}	298	41.65	22.93	19.147	17.23	203.990	134.78
	308	43.75	24.47			204.188	135.38
	318	45.66	25.87			203.810	135.55

From these result the following conclusions can be made: (i) The pk_a values decreases with increasing temperature revealing that their acidity increases with increasing temperature[22]. (ii) A positive value of ΔH indicates that the process is endothermic. (iii) A large positive value of ΔG indicates that the dissociation process is not spontaneous[23]. (iv) The dissociation process for ligand has negative values of ΔS due to the increased order as a result of salvation processes[24].

All the thermodynamic parameters of the stepwise stability constants of complexes are recorded in Table-6. It is known that the metal ions exist in solution as octahedral hydrated species[25]. Examination of these values showed that: (i) The stepwise stability constants (log K_1 and log K_2) (Figs. 4 and 5) for ligand complexes decrease with increasing temperature. (ii) The negative value of ΔG for the complexation process suggests that spontaneous nature of such process[26]. (iii) The ΔH values are negative, meaning the these processes are exothermic and favourable at lower temperature. (iv) The ΔS values for the ligand complexes are positive confirming that the complex formation is entropically favourable[27].

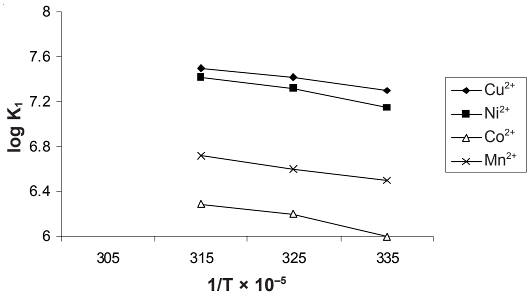

Fig. 4. Van't off plot of log K_1 of M^{n+} complexes *vs.* 1/T

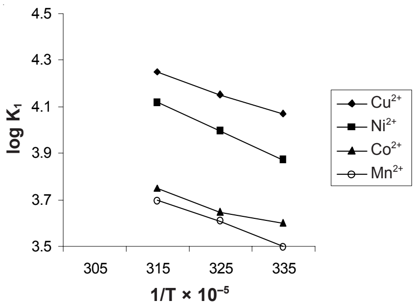

Fig. 5. Van't Hoff plot of log K_2 of M^{n+} complexes *vs.* 1/T

Conclusion

The dissociation process is non-spontaneous, exothermic and entropically unfavourable while the complexation process is spontaneous, exothermic and entropically favourable. Cinnamaldehyde-(2-hydroxy benzylidene)hydrazide was found to be biologically active and its metal complexes displays enhanced antimicrobial activity against one or more strains. Chelation tends to make the ligand acts as more powerful and potent bactericidal agent.

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REFERENCES

1. W.H. Leung, E.Y.Y. Chan, E.K.F. Chow, I.D. Williams and S.M. Peng, *J. Chem. Soc, Dalton Trans*, 1229 (1996).
2. D.A. Atwood and M.J. Harvey, *Chem. Rev.*, **101**, 37 (2001).
3. G.A. Moris, H. Zhou, C.L. Stern and S.T. Nguyen, *Inorg. Chem.*, **40**, 3222 (2001).
4. M. Bandini, P.G. Cozzi and A. Umani-Ronichi, *Chem. Commun.*, 919 (2002).
5. L. Canali and D.C. Sherrington, *Chem. Soc. Rev.*, **28**, 85 (1999).
6. N.Y. Ito and T. Kasturi, *Bull. Chem. Soc. (Japan)*, **72**, 603 (1999).
7. T. Katsuki, *Curr. Org. Chem.*, **5**, 663 (2001).
8. Z.H. Chohan and S.K.A. Sheazi, *Synth. React. Inorg. Met.-Org. Chem.*, **29**, 105 (1999).
9. C. Jayabalakrishnan and K. Natarajan, *Synth. React. Inorg. Met.-Org. Chem.*, **30**, 1023 (2001).
10. T. Jeeworth, H.L.K. Wah, M.G. Bhoeon, D. Ghoorhoo and K. Babooram, *Synth. React. Inorg. Met.-Org. Chem.*, **30**, 1023 (2000).
11. E.-I. Ochiai, Bioinorganic Chemistry-An Introduction, Allyn and Bacon, Boston (1977).
12. L.G. Van Uitert and C.G. Hass, *J. Am. Chem. Soc.*, **75**, 451 (1953).
13. M. Tofazzal, H. Tarafdar, A. Manaf, D. Ali, W. Juan and K. Azahri, *Transition Met. Chem.*, **10**, 456 (2004).
14. D.-H. Shi, Z.-L. You, C. Xu, Q. Zhang and H.-L. Zhu, *Inorg. Chem.*, **10**, 404 (2007).
15. V.D. Athawale and S.S. Nerkar, *Monatsh Chem.*, **131**, 267 (2000).
16. H.M. Irving and H.S. Rosotti, *J. Chem. Soc.*, 2904 (1954).
17. H.M. Irving and H.S. Rosotti, *J. Chem. Soc.*, 3397 (1953).
18. F.J.C. Rosotti and H.S. Rosotti, *Acta Chem. Scand.*, **9**, 1166 (1955).
19. M.T. Beck and L. Nagybal, Chem. of Complex Equillibria, Wiley New York (1990).
20. A.A. Al-Sarawy, A.A. El-Bindary and A.Z. El-Sombati, *Bull. Electrochem.*, **20**, 453 (2004).
21. F.R. Harlly, R.M. Burgess and R.M. Alcock, Solution Equillibria, Ellis Horwood Chichester, p. 257 (1980).
22. M.P. El-Sherbiny, A.A. Al-Sarawy and A.A. El-Bindary, *Boll. Chim. Farm.*, **141**, 274 (2004).
23. A.Z. El-Sonbati, A.A. El-Bindary and R.M. Ahmed, *J. Solution Chem.*, **32**, 617 (2003).
24. C.S.G. Phillips and R.J.P. Williams, Inorganic Chemistry, Oxford University, New York, Vol. 2, p. 268 (1968).
25. G.H. Jeffery, J. Basett, J. Mendham and R.C. Deney, Vogel's Text book of Quantitative Chemical Analysis, Longman, London, edn. 5 (1989).
26. A.A. Al-Sarawy, *Chem. Papers*, **58**, 109 (2004).
27. A.T. Mubarak, *J. Solution Chem.*, **33**, 149 (2004).

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