Zinc(II) and Cadmium(II) Binary Complexes with Creatinine and Their Mixed-Ligand Complexes with L-Asparagine or L-Glutamic Acid: Potentiometric Studies

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ABSTRACT

Creatinine is a physiological component of blood, brain and muscles and an important bioligand, which is the last product of the nitrogen metabolism in the vertebrates. In this study , the stability constants of binary complexes formed by creatinine with Zn(II) and Cd(II), and of the mixed-ligand complexes Zn(II)-creatinine-l-asparagine and Zn(II) creatinine-l-glutamic acid were determined by the Calvin-Bjerrum and Irving-Rossotti methods.

The conditional formation constants and the formation pH ranges of complexes were determined. Relative abundance was plotted using conditional formation constants of the complexes and the steps of the complexes are shown in graphs. The composition of metal-creatinine complexes was determined as metal/ligand = 1: 1.

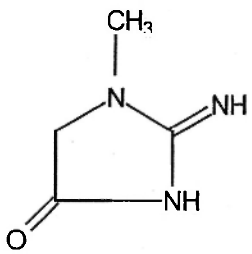
Keywords: stability constant, creatinine, l-asparagine, l-glutamic acid, zinc, cadmium, complex, ligand

1. INTRODUCTION

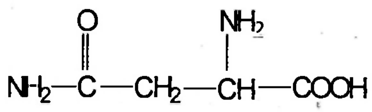
Creatinine (2-amino-1, 5 dihydro-1-methyl-4-H-imidazol-4-one; abbr. creat), being a natural metabolite of creatin, is a very important bioligand. The presence of several donor groups in its main tautomeric form determines its strong coordination capacity. The importance of creatinine in clinical chemistry is well recognized ; its level in serum and urine is indicative of the renal function. The latter participates in energy flow in muscle tissues and is present in blood, muscles and the brain /1, 2/. The complexation ability of creatinine is well recognised and studies on the metal ion interactions with creatinine may be helpful in deciphering creatinine metabolic pathways /2, 3, 4, 5, 6, 7/. The study of binary and ternary complexes of this ligand should be of interest, due to the fact that creatinine metabolism might be connected with its complexation to different metal ions /1, 8/. Five co-ordination modes have been established by X ray crystallography: bidentate bridging through N(1)(ring) and deprotonated exocyclic NH site /9/, bidentate binding via N(1) (ring) and the exocyclic O(C=O) /10/; monodentate binding through the N(1)(ring)site /11/;

monodentate binding through the exocyclic O(C=O)(12); and monodentate fashion through the deprotonated exocyclic NH group /10/. Creatinine is soluble in water and in aqueous solution it shows acidic properties(pKa =4, 89).

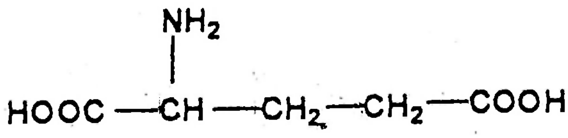
L-asparagine and l-glutamic acid are amino acids which largely exist in several plants.



Creatinine



L-asparagine



L-Glutamic acid

In the present study, the stability constants of creatinine, l-glutamic acid and l-asparagine complexes with Zn(II) and Cd(II) were determined using the potentiometric method by the Calvin-Bjerrum and Irving-Rossotti procedure /13/.

The conditional formation constants depending on pH values were calculated The mole fractions of different species from mixed compound were investigated using calculated conditional formation constants /14/.

The protonation constants and the acid constants of l-asparagine and l-glutamic acid which were used as ligands were taken from the literature /15,16/. The formation constants of Zn(II)-creat and Cd(II)-creat complexes were found, as well as those for Zn(II) and Cd(II) with L-asparagine and l-glutamic acid. In addition, the formation of metal-L-asparagine-creatinine and l-glutamic acid-creatinine mixed-ligand complexes was proven.

2. EXPERIMENTAL

2. 1 Materials and Methods

The stock solutions of metal ions were prepared from nitrate salts. Chemicals used were sodium hydroxide(titrisol), sodium perchlorate and perchloric acid.

All chemicals were analytical reagent grade from Merck. Metrohm 654 digital pH-meter with combined glass electrode assembly was used for pH measurements. "Metrohm Multi –Burette E-485" was used as the burette. Computer calculations were performed on the pH-metric data.

Creatinine was dissolved in water . The ionic strength was kept at I=0.1 using $NaClO_4$ during the experiment. Solutions were made up under a N_2 atmosphere in H_2O which was decarbonated and triply distilled. The standard solutions were kept for no longer than 3 weeks. Stock solution of the ligand (0.01M) was prepared prior to use. The exact calibration was done daily by using commercial buffer solutions

(Merck) of pH 4 and 7.

2. 2 Procedure

In order to determine the protonation constants, each of the solutions including 0.1M $HClO_4$ and 0.01M ligand +0.1M $HClO_4$ were titrated potentiometrically with 0.1M NaOH solutions at 25° C. It has been known that, in analytical procedures, the different ligands are effective in the formation of metal complexes. Therefore, the term "conditional formation constants" was introduced into this area. The conditional formation constants can be calculated from the mole fractions of ligand and metals. n_L values were calculated using n_A values , pL values were calculated using n_L values to calculate the stability constants of metal complexes.

The following equation was used to calculate n_L values:

$$n_L = \frac{(V_3 - V_2)\,[N + E^0 + T^0_L\,(y - n_A)]}{(V^0 + V_2).\, n_A\, T^0_M}$$

where

V^o = volume at the beginning	: 50.00ml
N = normality of base	: 0.100N
T^o_L =total ligand concentration	: 0.01M
E^o = concentration of acid	: 0.010M
y = the number of protons given	: 1
T^o_M = total molar metalconcentration	: 20.01M

The following equation was used to calculate pL values:

$$pL = \log \frac{1+\beta_1\,[H^+]}{T^o_L - n_L.\, T^o_M}$$

For creatinine: $\beta_1 = K_1 = 7,94.\,10^4$

pL values were calculated using β-values . n_L =f (pL) graphs were plotted using n_L and p_L values which were calculated for each metal ligand complex. The formation constants of complexes were found from p_L values which corresponded to n_L =0.5.

In order to determine the stability constants of mixed ligand complexes, the solutions including $HClO_4$:(Y+$HClO_4$):(Y+$HClO_4$+L+M) solutions were titrated potentiometrically using NaOH solutions (0.1N)The ionic strength of the reaction media was kept constant at 25°C (I=0.1), using $NaClO_4$ solution. In order to establish the stability constants of mixed ligand complexes the Irving–Rossotti method was used. The stability constants derived from the complexes of all ligands and the metal were evaluated and the ligand which showed a lower stability constant was selected as the second ligand, i.e.:

First ligand : L (L-ASP)

Second ligand : Y(CREAT)

The approach to the mixed system of the Irving-Rossotti method was based on the assumption that the system which has a higher stability constant behaves as the metal in the binary system when binding to the second ligand.

$$M + L \longrightarrow ML$$

$$ML + Y \longrightarrow MLY$$

$$MLY + Y \longrightarrow MLY_2$$

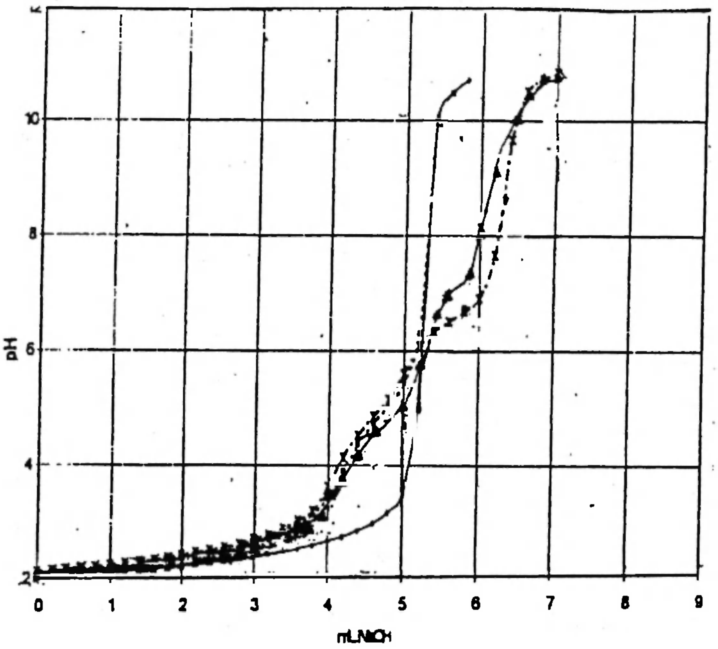
The mixtures consisting of metal and ligands were titrated potentiometricallly.The n=f(pL) graphs were plotted using n and pL values which were calculated from titration curves.The separation between ($HClO_4$) : ($Y+HClO_4$) : and ($Y+HClO_4+L+M$), in all potentiometric titration curves, showed the formation of a mixed compound.

3. RESULT AND DISCUSSION

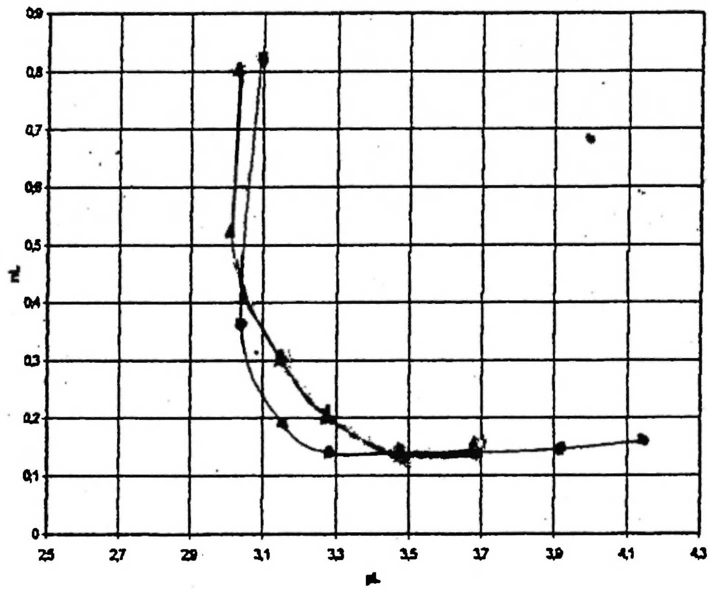
a) Binary complexes with creatinine

In this study the solution, which has a final ligand concentration of 0.002mol/l and an ionic strength of 0.1, was titrated with 0.100mol/l NaOH solution. The protonation constant of the ligand was established in our previous work /15/.

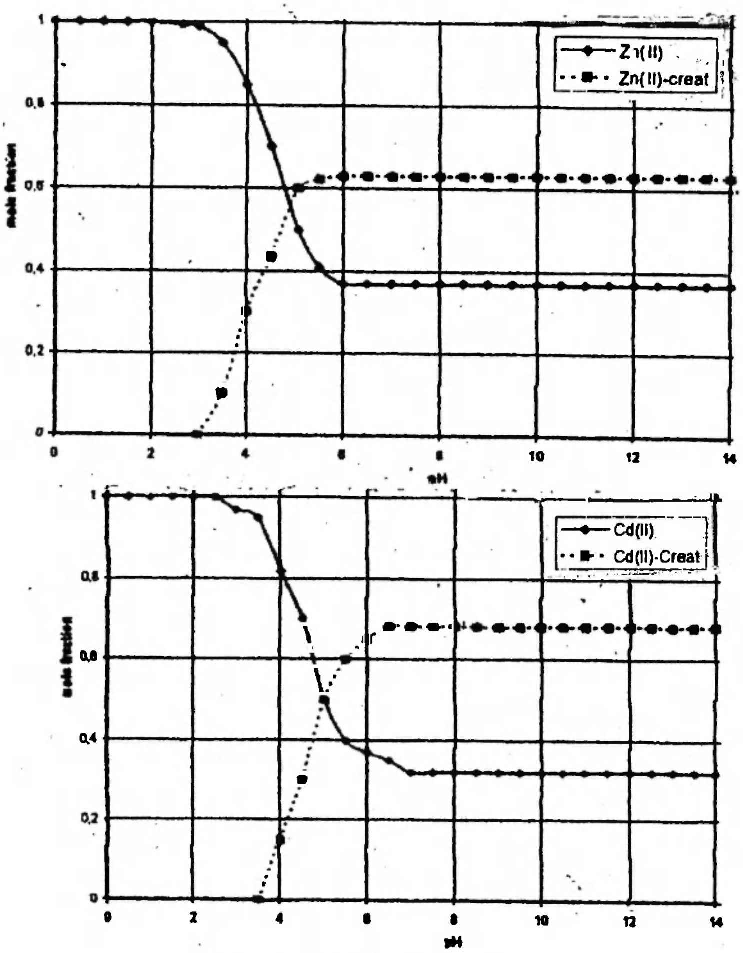
For finding the stability constants of the complexes of metals with ligands, the solutions which contain Cr(III), Mn(II), Co(II), Ni(II), Cu(II), salts in certain concentrations were titrated with NaOH solution potentiometrically at 25°C. Titration curves were obtained by plotting the pH changes versus the 0.100 mol/l NaOH volumes. The resulting titration curves belonging to metals are shown in Graph 1. The n_L =f(pL) curves were plotted by using n_L values, which were calculated by the potentiometric titration curves. The formation constants of the complexes have been read, which correspond to the n=0.5 from n_L = f(pL) curves(Graph 2). In the evaluation of the relative abundance (mole fractions) of the species in the system, these are plotted against the pH (Graph 3). The pH range where the conditional formation constant was at the maximum was overlapped by the pH range where the relative abundance of metal-ligand complexes was at the maximum. The conditional formation constants of the complexes were calculated and they were plotted versus the corresponding pH changes. In calculating the conditional formation constants, it is accepted that the only competitive ligand is the hydronium ion in the reaction medium. The pH ranges in which the complexation occurs , the maximum values of conditional formation constants and the pH values corresponding to these conditional formation constants for 25°C are shown in Table 1 (Graph 4).



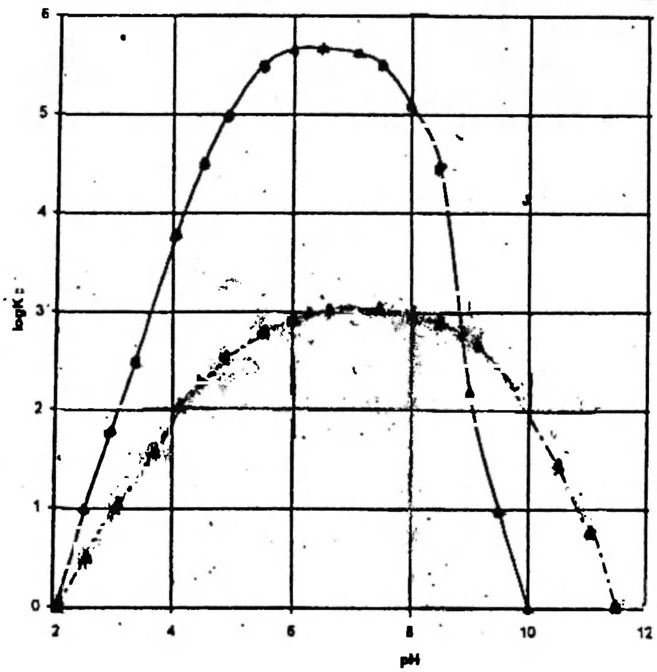
Graph 1: Potentiometric titration curves of metal-ligand:
—•— $HClO_4$; ···•··· Creatinine; --x-- Zn (II); -▲- Cd (II),



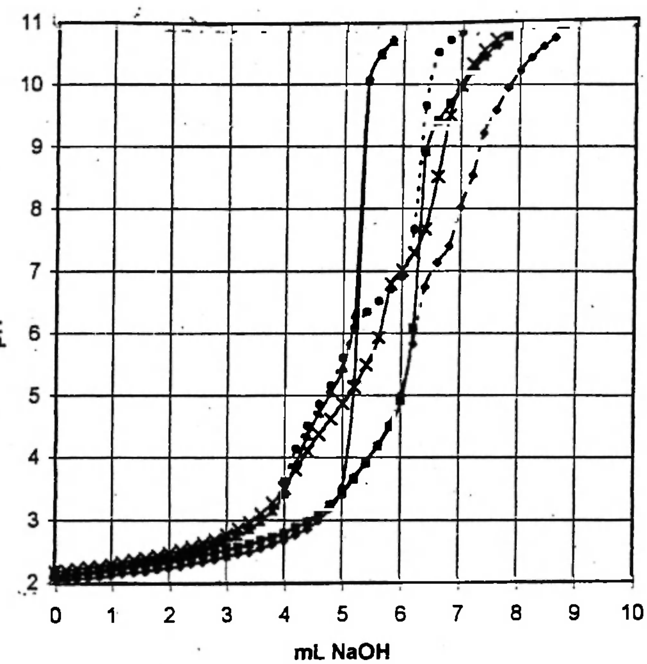
Graph 2: Creatinine-metal pL-nL curves : —•—Zn, -▲- Cd.



Graph 3: Creatinine-metal mole fraction curves.

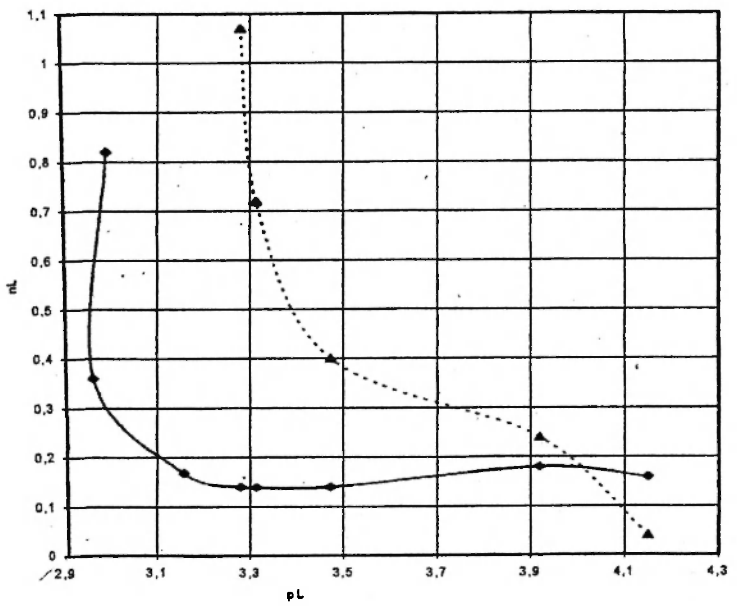


Graph 4: Creatinine-metal conditional formation curves: –•–Zn, –▲– Cd.

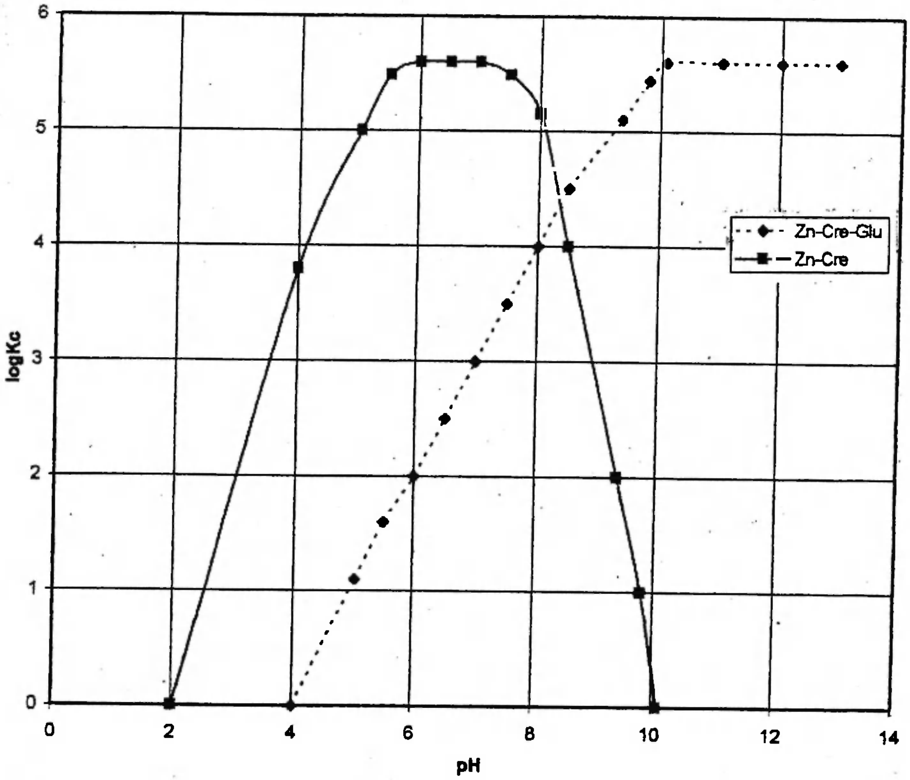


Graph 5: Potentiometric titration curves

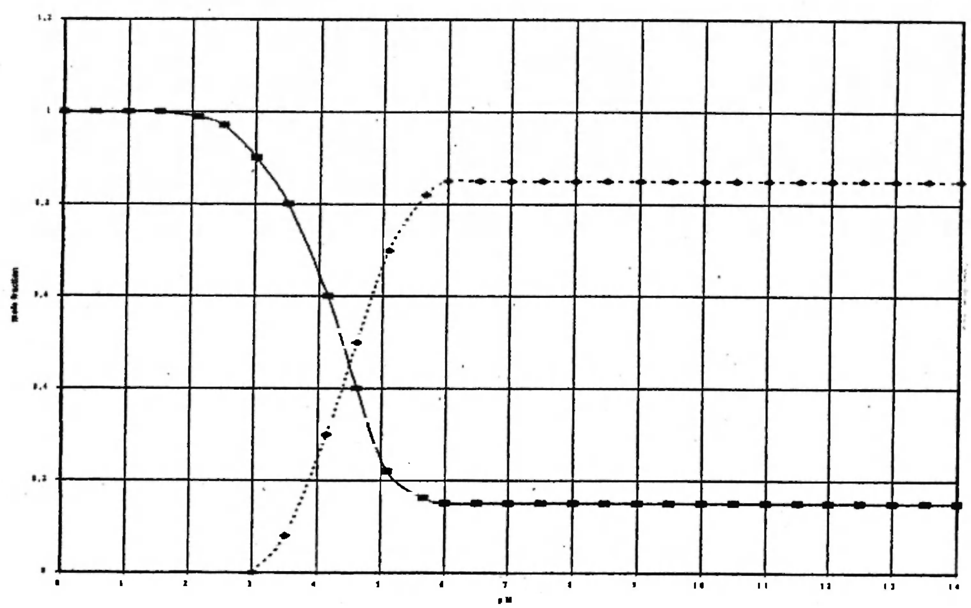
- $HClO_4 + NaClO_4$
- $HClO_4 + NaClO_4$ + L-Glutamic acid
- $HClO_4 + NaClO_4$ + Creatinine
- $HClO_4 + NaClO_4$ + Zn(II) + Creatinine + L-Glutamic acid
- $HClO_4 + NaClO_4$ + Zn(II) + Creatinine



Graph 6: nL=f(pL) curves for Zn(II)-Creatinine and Zn(II)-Creatinine-L-Glutamic acid



Graph 7: Conditional formation curves for Zn(II)-Creatinine and Zn(II)-Creatinine-L-Glutamic acid.



Graph 8: The relative abundance curve for Zn(II)-Creatinine-L-Glutamic acid (mole fraction curve)

------- M . . . . ML

(compare to Graph 4)

b) Mixed complexes with creatinine and l-asparagine or l-glutamic acid

In this study, the conditional formation constants were calculated and these constants were found to be in accordance with the mixed complex formation constants.

Graphs 5-8 show, for Zn(II) as example, the comparison between curves obtained with metal + creatinine alone and with metal + creatinine + l-glutamic acid.

This result allows us to find the stability constants of mixed complexes. In this calculation, the pK values of ligands and the formation constants of complexes which they formed with metals are used as given in Tables 1 and 2.

Table 1

Binary ligand complex	Max pH range of the metal complexes	PH of the max. in metal complexes	$\log K'_e$	K'_e
Zn(II) Creat	2.00 – 10.00	6.50	2.93	$8.50.10^2$
Cd(II) Creat	2.00 – 11.50	7.00	3.00	$1.00.10^3$

Table 2

Formation Constants of mixed – ligand complexes(I=0,11,t=25°C)

Ligand-metal	$\log K_1$	$\log K_2$	$\log \beta_3$
L-Aspargine-Zn(II)	4.00	3.47	7.47
L-Aspargine-Cd(II)	3.93	3.46	7.39
L-Glutamic Acid-Zn(II)	5.66	4.90	10.56
L-Glutamic Acid-Cd(II)	4.63	4.03	8.66

The conditional formation constant, namely the stability constant of mixed complexes, can also be calculated. The difference between the formation constants of mixed and binary systems is a parameter which characterises the formation behaviour of mixed ligand complexes /19/.

$$\Delta \log K = \log K_{MLY} - \log K_{MY2}$$

This difference is the equilibrium constant of the following equation:

$$ML + MY_2 \longrightarrow MLY_2 + M \quad (1)$$

If logK is negative and the equilibrium of reaction (1) is more to its left hand side.

$$M + L \longrightarrow ML \quad (2); \quad ML + Y \longrightarrow MLY \quad (3)$$

$$MLY + Y \longrightarrow MLY_2 \quad (4); \quad M + Y \longrightarrow MY \quad (5)$$

Equation (1) can be calculated from the difference of equations (4) and (5).The conditional formation constant equals the β values of mixed complex and the constants found in this work are in accordance with the calculated conditional formation constants of $\beta_2=K_1.K_2$ mixed complex. The $logK_1$ = 4,04 value observed for n=0,5 from the n = f(pL) graph for Cu-Creat-L-Asparagine system is in line with the conditional formation constant. However, there is another value observed for n=1,5 value (logK=3,74).

The dissociation constants of the ligands used were

Creatinine	$logK_1 = 4,90$
L-Asparagine	$logK_1 = 8,85$; $logK_2 = 2,05$
L-Glutamic Acid	$logK = 9.75$; $logK_2 = 4.20$; $logK_3 = 2.35$.

The stability constants of mixed complexes are given in Table 3.

Table 3

Formation Constants of mixed-ligand complexes (I = 0,11, t = 25°C)

Mixed-ligand complex	$LogK_1$	$Log\ \beta_1$
Zn(II)-L-Asparagine-Creat	3.28	3.28
Cd(II)-L-Asparagine-Creat	2.96	2.96
Zn(II)-L-Glutamic Acid Creat	3.49	3.49
Cd(II)-L-Glutamic Acid-Creat	4.02	4.02

In this condition,the free metal equation and the second ligand(Y) according to equation(3) can form this complex. It is observed that the pH area in which the conditional formation constants is maximum lies within the pH area of the mixed complex.

Table 4

PH range of the mixed ligand complexes

Mixed ligand complex	pH range of the metal complexes	Max pH range of the metal complexes
Zn(II)-L-Asparagine-Creat	4.00-14.00	11.00-14.00
Cd(II)-L-Asparagine-Creat	4.00-14.00	11.0-14.00
Zn(II)-L-Glutamic Acid-Creat	4.00-14.00	11.00-14.00
Cd(II)-L-Glutamic Acid-Creat	4.00-14.00	11.50-14.00

4. CONCLUSIONS

The purpose of the present work was to determine stability and formation constants for creatinine-L-asparagine metal complexes by potentiometric method. This method is simple and sensitive for the metal-ligand complex.

REFERENCES

1. S. Muralidharan, K.S. Nagaraja and M.R. Udupa, *Polyhedron*, **3**, 619 (1984).
2. C.N.H. Long, *Biochemists Handbook*, Van Nostrand, Princeton, 1961.
3. S. du Pre and H. Mendel. *Acta Cryst.* **8**, 311 (1955).
4. M.R. Udupa and B. Krebs. *Inorg. Chim. Acta* **33**, 241, 1979.
5. M.R. Udupa and B. Krebs. *Inorg. Chim. Acta* **55** , 153 (1981).
6. S. Muralidharan, K.S. Nagaraja and M.R. Udupa. *Trans. Met. Chem.* **9**, 218 (1984).
7. J.L. Hughes, R.C. Liu, T. Enkoji, C.M. Smith, J.W. Bastian and P.D. Luna. *J. Med. Chem.* **18**, 1077 (1975).
8. M. Mitewa. *Coord. Chem. Rev.* **140** (1), (1995).
9. A.J. Conty, M. Fyfe and B.M. Gatehouse. *Inorg. Chem.*, **17**, 1467 (1978).
10. J.M. O'Connor, K. Hübner, A. L. Rheingold and LM Liable-Sands. *Polyhedron* **16**, 2029 (1997).
11. B. S. Parojon Costa, E. J. Baran and O. E. Piro, *Polyhedron* **16**, 3379 (1997).
12. A. Panfil, J. J. Fiol and M. Sabat. *J. Inorg. Biochem.* **60**, 109 (1995).
13. H.M. Irving and H.S. Rossotti, *J. Chem. Soc.*, **1953**, 3397-3405; **1954**, 2904-2910.
14. H.A. Laitinen and W.E. Harris, *Chemical Analysis* 2^{nd} ed. Mc. Graw Hill, Tokyo, 1975
15. S. Karaderi and D. Bilgic, *Revs. Inorg. Chem.*, **25**, 387 (2005).
16. Y. Lurie, *Handbook of Analytical Chemistry*, Khimiya, Moscow, 1971.
17. K.Burger, *Biocoordination Chemistry*: *Coordination Equilibra in Biologically Active Systems*, Ellis Horwood, New York, 1990.
18. H. Demircan, M. Pekin, C. Kurt and E. Dölen, *Toxicological and Environ. Chem*, **71**, 357-367 (1999).
19. E.Still, *Anal.Chim.Acta*, **107**,105-112 (1979).