Differential Pulse Polarographic Study of Simple and Mixed Complexes of Copper Ions with the Antidepressant Drug Imipramine and Glutamic Acid or Histidine

M. KHODARI

Department of Chemistry, Faculty of Science,
South Valley University, Qena, Egypt

The formation of binary and ternary complexes of glutamic acid or histidine and imipramine with Cu(II) has been examined using differential pulse polarographic technique. The reduction of both simple and mixed systems is reversible and controlled by diffusion. The obtained results reveal the formation of mixed complexes. For the system Cu—Glu—Imip, one mixed complex was formed: Cu(Glu)(Imip)$_2$ with stability constant log$\{\beta_{12}\}$ = 10.51, while the system Cu—His—Imip forms two complexes Cu(His)(Imip) and Cu(His)(Imip)$_2$ with stabilities log$\{\beta_{11}\}$ = 6.25 and log$\{\beta_{12}\}$ = 8.77, respectively. These experimental values were compared with the statistical ones.

Mixed complexes are usually formed when the metal ion is present in a mixture of two or more complexing species in solution [1—3]. Different electrochemical techniques were applied to study such systems [4—6]. The method of *DeFord* and *Hume* [7] as extended by *Schaap* and *McMasters* [1] was used to calculate the formation constants of the mixed system using direct current polarographic technique. Differential pulse polarography has been demonstrated to be useful in determination of the formation constants of simple [8] and mixed ligand complexes [9, 10].

Formation of low-molecular mass (l.m.m.) complexes in biofluid may be described as multimetal multiligand equilibrium involving essential exchangeable metal ions and amino acids or other biomolecules. Because potential ligands greatly exceed biological transition metal ions in number and quantity, mixed ligands complexes must be more common than binary and multinuclear ones.

Biological systems have developed a number of ways of keeping the concentration of free (aquated) metal ions to very low levels. Too high concentration of these free metal ions, even the essential elements, is clinically damaging and even fatal. Proven examples are the effects of excess Cu ions in Wilson's diseases [11].

Amino acids are by far the most effective and important biological ligands considering the available coordination sites of a metal ion and the multidentate nature of amino acids and other potential bioligands. Mixed ligand complexes with two different ligands, ternary complexes, are most important in biological systems [12].

Histidine and other amino acids are involved in copper(II) transport in blood and exchange interactions with serum albumin [13]. Imipramine is one of the most widely used drugs for the treatment of depression [14]. Hence their binary and ternary complexes are of great interest. So the present work is aimed to study the expected formed complexes between Cu ions and imipramine and histidine or glutamic acid using differential pulse polarography.

EXPERIMENTAL

The polarograms were recorded using a potentiostat model 263 (EG & G PARC) coupled with 303A static mercury drop electrode (SMDE). The used parameters are: pulse amplitude 25 mV, scan rate 10 mV s^{-1}, drop time 0.5 s. An IBM computer was used to control the potentiostat and the cell.

The pH-metric measurements were carried out using an Orion-Research model 601A.

Imipramine (Sigma), glutamic acid (Merck), and histidine (Sigma) were used without further purification and dissolved in double-distilled water. The total concentration of imipramine, glutamic acid or histidine was recalculated taking into consideration their pK's values, these being 9.50, 9.59, and 9.09 for imipramine [15], glutamic acid [16], and histidine [16], respectively. A stock solution of 0.001 mol dm^{-3} copper was prepared by dissolving a suitable amount of copper nitrate in 100 cm^3 of bidistilled water. All other reagents were of anal. grade. Sodium perchlorate (0.1 mol dm^{-3}) was used as a supporting electrolyte. The dissolved oxygen of the analyzed solution was removed

by passing a stream of nitrogen during 8 min. All the experiments were performed at room temperature and using unbuffered media (sodium perchlorate, pH 7.0).

RESULTS AND DISCUSSION

The expected simple complexes between Cu(II) and each previously named ligand have been investigated in order to determine their stability constants under the same conditions used for the study of the mixed complexes. The stability constants of the formed binary complexes were calculated using the *DeFord* and *Hume* [7] method as modified by *Heath* and *Hefter* [8], which may be expressed as follows

$$F_0(X) = \beta j [X]^j =$$
$$= \text{antilog}\,(0.434\,nF/RT\,\Delta E_p) + \log I_s/I_c \qquad (1)$$

where [X] is the analytical concentration of the ligand, ΔE_p is the peak potential shift, and I_s and I_c are diffusion currents of the free and complexed metal ions, respectively. The other symbols have their usual meaning.

For the ternary systems, the *Schaap* and *McMasters* model [1] as modified by *Killa et al.* [5] was used to calculate the formation constants. The method may be expressed as follows

$$F_{00}(XY) = \text{antilog}\,[(0.434\,nF/RT)\Delta E_P + \log I_s/I_c]\,(2)$$

where

$$F_{00} = A + B[X] + C[X]^2 + D[X]^3 \qquad (2a)$$

and

$$A = \beta_{01}[Y] + \beta_{02}[Y]^2 + \beta_{03}[Y]^3 \qquad (2b)$$

$$B = \beta_{10} + \beta_{11}[X] + \beta_{12}[Y]^2 \qquad (2c)$$

$$C = \beta_{20} \qquad (2d)$$

The values of A, B, and C were obtained from the plots of F_{i0} *vs.* [X] by the extrapolation of F_{10} and F_{20} to [X] equal to zero. The values of F_{i0} were calculated from the following relations

$$F_{10} = [F_{00} - A]/[X] \qquad (3)$$

$$F_{20} = [F_{10} - B]/[X] \qquad (4)$$

$$F_{30} = [F_{20} - C]/[X] \qquad (5)$$

The simple complexes occurring between copper(II) and each previously named ligand were studied under the same conditions used to study the mixed systems. For both simple and mixed complexes, the reversibility of the reduction process was demonstrated by the
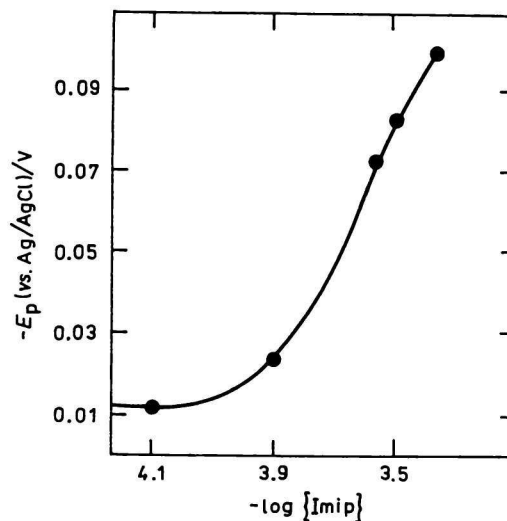

Fig. 1. Peak potential of Cu(II) as a function of log {Imipramine}, $c(NaClO_4) = 0.1$ mol dm^{-3}.

half-width values which are in good agreement with those calculated by *Dillard* and *Haneck* [17]. The reversibility was also confirmed by cyclic voltammetric measurements at the scan rate of 100 m V s^{-1}, the difference between E_{pa} and E_{pc} being very close to the theoretical value of $0.059/n$ V [18, 19]. On the other hand, the direct proportionality of d.c. polarographic current to the square root of the effective mercury height indicates that the reduction process of both simple and mixed systems is controlled by diffusion. The obtained results show that the peak potential of copper is shifting (E_p) to more negative values with increasing Imipramine concentration. Fig. 1 shows the plots of E_p *vs.* log Imipramine concentration. The obtained graph throws the light on the method that should be used to calculate the formation constant of the expected formed complexes between Cu ions and Imipramine. As the resulting graph is a smooth curve, the method of *DeFord* and *Hume* [7] should be used to treat the polarographic data.

The plots of $F_i(X)$ *vs.* [Imip] are indicated in Fig. 2. The curvature is decreased as the function increases, $F_1(X)$ is a straight line with a definite slope and $F_2(X)$ is a parallel to [X] axis, *i.e.* the maximum stoichiometric ratio is two. The formation constants are determined from the interception of the curves with $F_i(X)$ axis at zero concentration of imipramine. The estimated values are indicated in Table 1. These values agree with the reported ones [4, 16].

The plots of E_p of copper ion against log {Histidine} or log {Glutamic acid} gave a smooth curve and consequently the method of *DeFord* and *Hume* [7] was used to determine the formation constants of the formed complexes. The obtained results indicate that all the studied ligands form 1:1 and 1:2 (metal:ligand) complexes as shown in Table 1.
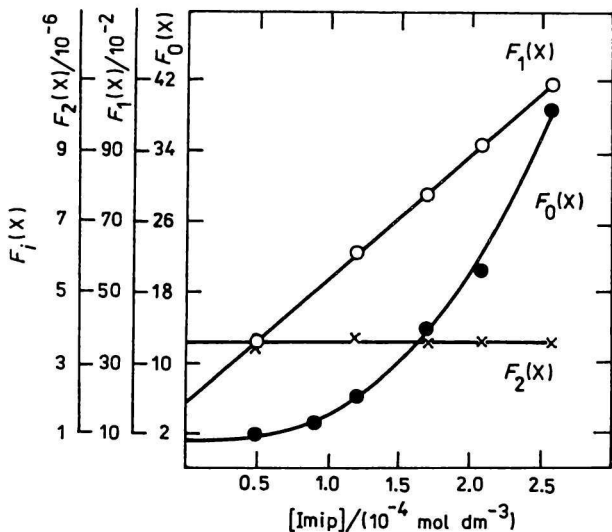
Fig. 2. $F_i(X)$ as a function of imipramine concentration.

Table 1. Stability Constants of the Formed Binary Complexes, Unbuffered Medium, $c(NaClO_4) = 0.1$ mol dm^{-3}, $c(Cu(II)) = 1 \times 10^{-5}$ mol dm^{-3}

System	$\log\{\beta_1\}$	$\log\{\beta_2\}$
Cu—Imip	3.31	5.62
Cu—Glu	5.73	9.66
Cu—His	3.40	6.29

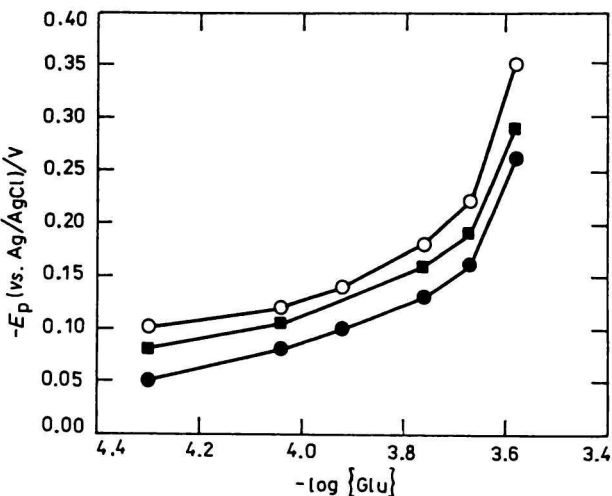**Fig. 3.** Peak potential of $c(Cu(II)) = 1 \times 10^{-5}$ mol dm^{-3} as a function of glutamic acid concentration in the presence of $c(Imip) = 0.00$ mol dm^{-3} (●), $c(Imip) = 9.0 \times 10^{-5}$ mol dm^{-3} (■), $c(Imip) = 2.1 \times 10^{-4}$ mol dm^{-3} (○).

Ternary Systems

The Cu(Glu—Imip), Cu(His—Imip) mixed systems were studied by keeping the concentration of

Table 2. Stability Constants of the Mixed Complexes Formed between Copper, Imipramine, and Glutamic Acid or Histidine. [Imip] = a) 9×10^{-5} mol dm^{-3}, b) 2.1×10^{-4} mol dm^{-3}; Unbuffered Medium, $c(NaClO_4) = 0.1$ mol dm^{-3}, pH 7.0

System		$\log\{A\}$	$\log\{B\}$	$\log\{C\}$	$\log\{\beta_{11}\}$	$\log\{\beta_{12}\}$
Cu—Glu—Imip	a	1.4	4.4	7.90		
						10.51
	b	1.8	5.0	8.20		
Cu—His—Imip	a	0.3	3.6	6.27		
					6.25	8.77
	b	0.5	3.9	6.18		

Imipramine constant at 9×10^{-5} mol dm^{-3} and 2.1×10^{-4} mol dm^{-3} and varying concentration of Glu and His. The stability constants were calculated using eqn (2). Since Cu(II) forms individually four-coordinated complexes with amino acids of bidentate nature, there can be the possibility of the existence of only two mixed species 1:1 or 1:2. In each of the mixed systems, a shift in the peak potential to more negative side was observed with increasing glutamic acid or histidine concentration. This shift is in the presence of Imipramine greater than in its absence (Fig. 3) indicating the formation of mixed complexes. The functions F_{00}, F_{10}, and F_{20} were obtained as described above for each of the systems under investigation. The values of A, B, and C were abstracted from the intercept of F_{00}, F_{10}, and F_{20} with the [X] axis at [X] equal to zero, respectively. Using eqn (2c) and the two values of B, the stability constants β_{11} and β_{12} for the ternary systems MAL and MAL$_2$, respectively, were determined. The obtained results are given in Table 2.

For the Cu—Glu—Imip the value of β_{11} is negative, *i.e.* the Cu(Glu)(Imip) does not exist in the solution under our experimental conditions and the value of $\log\{\beta_{12}\}$ was calculated to be 10.51.

For the Cu—His—Imip, the stability constants $\log\{\beta_{11}\}$ and $\log\{\beta_{12}\}$ were calculated from the two values of B and were found to be 6.25 and 8.77, respectively.

The binary and ternary complexes have been compared. The mixing constant, K_m [20] was used to compare the stabilities of the binary and ternary complexes. For the following reaction

$$1/2[Cu(A)_2] + 1/2[Cu(L)_2] = [Cu(A)(L)]$$

the mixing constants ($\log\{K_m\}$) could be calculated from the relation

$$\log\{K_m\} = \log\{\beta_{11}\} - 1/2\log\{\beta_{20}\} - \log\{\beta_{02}\}$$

For the Cu(His)(Imip) system, a value of 0.35 was determined for $\log\{K_m\}$. This value shows that the

Table 3. Equilibria Involved in the Formation of Ternary Complexes for the Cu—Glu—Imip System

	Equilibria	$\log\{K\}$
1	$Cu + Glu + 2Imip \rightleftharpoons Cu(Glu)(Imip)_2$	10.51
2	$Cu(Glu) + 2Imip \rightleftharpoons Cu(Glu)(Imip)_2$	4.78
3	$Cu(Imip)_2 + Glu \rightleftharpoons Cu(Glu)(Imip)_2$	4.89
4	$Cu(Glu)_2 + 2Imip \rightleftharpoons Cu(Glu)(Imip)_2 + Glu$	0.85

Table 4. Equilibria Involved in the Formation of Ternary Complexes for the Cu—His—Imip System

	Equilibria	$\log\{K\}$
1	$Cu + His + Imip \rightleftharpoons Cu(His)(Imip)$	6.25
2	$Cu(His) + Imip \rightleftharpoons Cu(His)(Imip)$	2.85
3	$Cu(Imip) + His \rightleftharpoons Cu(His)(Imip)$	2.94
4	$Cu + His + 2Imip \rightleftharpoons Cu(His)(Imip)_2$	8.77
5	$Cu(Imip)_2 + His \rightleftharpoons Cu(His)(Imip)_2$	3.15
6	$Cu(His)_2 + 2Imip \rightleftharpoons Cu(His)(Imip)_2 + His$	2.48
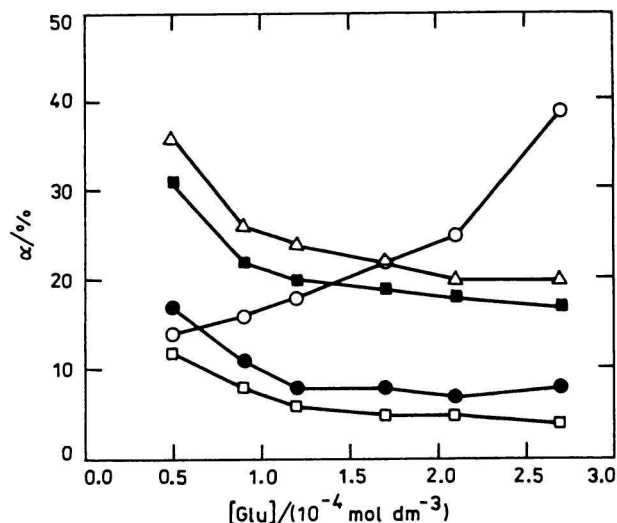
Fig. 4. Distribution curves of the different complexes formed in the Cu(II)—His—Imip system, $c(\text{Imip}) = 9 \times 10^{-5}$ mol dm^{-3}. ● Cu(His)(Imip)$_2$, ■ Cu(His)(Imip), ○ Cu(His)$_2$, △ Cu(His), □ Cu(II).

mixed complexes are more stable than the simple ones.

The equilibria between various species existing in solution for Cu—Glu—Imip and Cu—His—Imip systems with the equilibrium constants $(\log\{K_m\})$ values are given in Tables 3 and 4, respectively.

From Table 3 one can observe that the equilibria 2, 3, and 4 favour the formation of mixed complexes over the simple ones. The same table shows also that the addition of Glu to Cu(Imip)$_2$ is more easier than the addition of Imip to Cu(Glu)$_2$, (equilibria 3 and 4).

From the values of $\log\{K\}$ in Table 4 one can observe that the equilibria 2, 3, 5, and 6 favour the formation of mixed systems over the simple ones. It was also found that the addition of His to Cu(Imip) is more easier than the addition of Imip to Cu(His) (equilibria 3 and 2) and Imip can replace His readily (equilibrium 6).

The distribution curves of the different complexes formed in each system have been calculated as mentioned elsewhere [4—21]. Fig. 4 is a representative diagram showing the distribution of the Cu(His)$_i$(Imip)$_j$ complexes $(\alpha/\%)$ as a function of the histidine concentration in the presence of 9×10^{-5} mol dm^{-3} imipramine.

REFERENCES

1. Schaap, W. B. and McMasters, D. L., *J. Am. Chem. Soc. 83*, 4699 (1961).
2. Khurana, S. C. and Newman, L., *Inorg. Chem. 11*, 2855 (1972).
3. Couoto, K. C. and Kour, T., *Electrochim. Acta 24*, 1133 (1979).
4. Khodari, M., Vire, M. J. C., Patriarche, G. J. and Ghandour, M. A., *Anal. Lett. 22*, 445 (1988).
5. Killa, M. H., Mabrouk, E. M., and Ghoneium, M. M., *Transition Met. Chem.* (Weinheim, Germany) *17*, 59 (1992).
6. Khodari, M., Ghandour, M. A., Mansour H., and El-Din, H. S., *Chem. Papers 49*, 21 (1995).
7. DeFord, D. D. and Hume, D. N. *J. Am. Chem. Soc. 73*, 5321 (1953).
8. Heath, G. A. and Hefter, G., *J. Electroanal. Chem. 84*, 295 (1977).
9. Jaber, A. M., *Polyhedron 5*, 1701 (1986).
10. Killa, H. M. and Zaky, M., *Anal. Lett. 18*, 167 (1985).
11. Berthon, G., *Handbook of Metal-Ligand Interactions in Biological Fluids*, p. 583. Dekker, New York, 1995.
12. Kiss, T., *Biocoordination Chemistry.* (Burger, K., Editor.) P. 56. Ellis Horwood, London, 1990.
13. Sarkar, B., *Metal Ions in Biological Systems.* Vol. *12.* (Sigel, H., Editor.) P 233. Dekker, New York, 1981.
14. Wang, J., Golden, T., Ozsoz, M., and Lu, Z., *Bioelectrochem. Bioenerg. 23*, 217 (1989).
15. Green, A., *J. Pharm. Pharmacol. 19*, 10 (1967).
16. Martel, A. E. and Smith, R. M., *Critical Stability Constants.* Vol. *5.* Plenum Press, New York, 1982.
17. Dillard, M. and Haneck, K. W. *Anal. Chem. 48*, 216 (1976).
18. Delhay, P., *New Instrumental Methods in Electrochemistry.* Interscience, New York, 1954.
19. Killa, H. M., Mercer, E. E., and Philip, R. H., *Anal. Chem. 56*, 2401 (1984).
20. Jain, S. L., Kishan, J., and Kapoor, R. C., *Indian J. Chem., A 18*, 133 (1979).
21. Abo El-Maali, N., *M.Sc. Thesis.* Faculty of Science, Assiut University, Assiut, Egypt, 1986.