CHAPTER V

CONCLUSION AND RECOMMENDATION

The complex formations between the 8 essential amino acids for human with the interesting transition metal ions, Mn(II), Cd(II) and Pb(II) ion, were investigated in this study. The essential amino acids used ~~amino acids used~~ are analar grade of L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan and L-valine. The Mn(II), Cd(II) and Pb(II) ion are the heavy metal ions which are believed to be toxic and these metal ions were representatively sampled from the first, second and third rows of the transition elements, respectively.

The electrochemical techniques, pH-metric titration and polarography, were used for elucidating and determining the complexes at the experimental conditions reported, the constant ionic strength of 0.10 M KCl and the constant temperature of $37.0 \pm 0.5^{o}C$.

The purity of the essential amino acids was checked by pH-metric titration technique. The examination of the entire pH-metric titration curve was not diviated from the calculated shape for every essential amino acids studied. Therefore, the purity of the essential amino acids was satisfied for this study.

The pKa values the essential amino acids at the experimental conditions were evaluated by the pH-metric titration technique and they

were 9.242, 9.261, 8.636, 8.765, 8.857, 8.675, 9.198 and 9.145 for L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan and L-valine, respectively. These pKa-values gave the good agreement to the literature (37) and these values were used throughout in present work.

The complex formations by pH-metric titration technique of the 5.00×10^{-4} M Cd(II) ion with the 1.00×10^{-3} M essential amino acids were shown by the inflection of the pH-metic titration curves of the complexes. The clear inflection curve illustrated that one proton was displaced from the essential amino acid. The stepwise stability constants, K_1 and K_2, were calculated and found to be in the order of seventh and sixth, respectively. The precipitate was taked place in the Cd(II)-L-lysine system by pH-metric titration analysis.

The pH-metric titration study of the complex formations of 5.00×10^{-4} M Mn(II) ion with 1.00×10^{-3} M essential amino acids showed the unclear inflection on pH-metric titration curves, but the calculation gave the stepwise complexes for the systems of Mn(II)-L-isoleucine and Mn(II)-L-tryptophan with the stepwise stability constants, K_1 and K_2, of the order of both second. The complexes of the Mn(II) ion to L-leucine, L-methionine, L-phenylalanine, L-threonine and L-valine were calculated with the stability constants, K_1, of the order of third and the precipitate was taken place during the pH-metric titration of the Mn(II) ion-L-lysine system.

No complex formation was taken place in the systems of 5.00×10^{-4} M Pb(II) ion and 1.00×10^{-3} M essential amino acids showing be the occurrence of precipitate during the pH-methric titration.

All of the essential amino acids studied showed no reduction waves in the polarographic mode in the voltage range of (0.000) - (-3.000) volts. Their solutions were studied at the concentration range of 1.00×10^{-1} - 5.00×10^{-6} M in the pH range of 3.01 - 10.97 by means of the Michaelis borate buffer and the 0.10 M KCl supporting electrolyte solution.

The successive complex formations by the polarographic technique of the 1.00×10^{-4} M Cd(II) ion in the various excess concentrations of the essential amino acids (in the range of 4.00×10^{-4} - 1.00×10^{-1} M) were shown by the shifting in $E_{\frac{1}{2}}$ of Cd(II) ion to more negative potentials and by the decreasing in i_d. All systems studied at pH 7.4 were shown reversibly and diffusion-controlled. The coordination number (j) were to be 1 and 2 corresponding to the 1:1 and 1:2 Cd(II) ion to essential amino acid complexes with the stability constants, log β_1 and log β_2, in the range of, 8 and 15, respectively. In case of Cd(II) ion-L-lysine system, the 1:1 Cd(II) ion to L-lysine complex was carried out only by j nearly 1 with the stability constants, log β_1 in the range of 7.

The polarographic study of the complex formations of 5.00×10^{-4} M Mn(II) ion in the various excess concentrations of the essential amino acids (in the range of 2.00×10^{-4} - 8.00×10^{-2} M) was shown by the decreasing in i_d and by the shifting in $E_{\frac{1}{2}}$ of Mn(II) ion to more negative potentials. The reversible and diffusion-controlled systems were indicated at the pH studied, pH 8.50. The 1:1 Mn(II) ion to essential amino acid complex was carried out by j nearly 1 with the stability constants log β_1 in the range of 3. For Mn(II)-L-lysine

system, the ill-defined wave was shown and the complex formations can nto be carried out by the polarographic technique.

The complex formations by polarographic technique of the 1.00 x 10^{-4} M Pb(II) ion in the various excess concentrations of the essential amino acids (in the range of 2.00 x 10^{-4} - 8.00 x 10^{-2} M) were shown by the shifting in $E_{\frac{1}{2}}$ of Pb(II) ion to more negative potentials and by the decreasing in i_d. The systems studied at pH 6.50, to prevent precipitation, showed reversibly and diffusion-controlled. The 1:1 Pb(II) ion to essential amino acid complexes were indicated by j nearly 1 with the stability constants, log β_1 in the range of 4. In case of Pb(II)-L-lysine system, no evidence of complex formations was shown by no shifting in $E_{\frac{1}{2}}$ of Pb(II) ion as the concentrations of L-lysine increased.

The comparison of the results from those two techniques was first attempted here. The comparative values of the complex stability constants were shown in Table 5.1. It can see that the comparison was generally in good agreement of stability constants determined under comparable condition by pH-metric titration and polarographic measurements. The comparison of the stability constants of Cd(II), Mn(II) and Pb(II) complexes of each essential amino acid showed that their stability constants decreased in the following order; Cd > Pb > Mn. This work showed that the polarographic method was capable of giving reasonably accurate values for both the overall and stepwise stability constants, log β and log K, of metal complexes. It is the author's view that the polarographic method should be used in conjumetion with, and complementary to, the pH-metric titration technique.

Table 5.1 Comparison of the complex stability constants from the pH-metric titration technique and the poparographic technique

Essential Amino Acids	Cd(II) ion						Mn(II) ion						Pb(II) ion					
	log K_1		log K_2		log β_2		log K_1		log K_2		log β_2		log K_1		log K_2		log β_2	
	T	P[a]	T	P[b]	T[b]	P	T	P[a]	T	P[b]	T[b]	P	T	P[a]	T	P[b]	T[b]	P
L-isoleucine	7.71	7.90	6.72	7.15	14.43	15.05	2.88	3.38	2.58	-	5.46	-	-	4.33	-	-	-	-
L-leucine	7.90	7.97	6.73	7.02	14.63	14.99	3.05	3.61	-	-	-	-	-	4.59	-	-	-	-
L-lysine	-	7.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-methionine	7.54	7.90	6.61	6.80	14.15	14.70	2.75	3.22	-	-	-	-	-	4.10	-	-	-	-
L-phenylalanine	7.67	8.03	6.72	6.83	14.39	14.86	3.15	3.25	-	-	-	-	-	4.35	-	-	-	-
L-threonine	7.70	8.26	6.62	6.53	14.32	14.79	3.01	3.02	-	-	-	-	-	4.36	-	-	-	-
L-tryptophan	7.84	8.23	7.32	7.22	15.16	15.45	2.91	3.45	1.93	-	4.84	-	-	5.07	-	-	-	-
L-valine	7.65	7.97	6.73	6.99	14.38	14.96	2.72	3.19	-	-	-	-	-	4.42	-	-	-	-

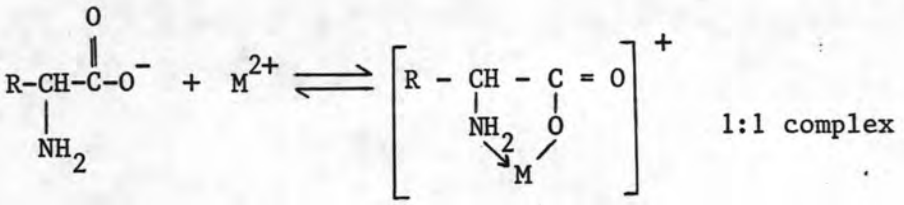
T = determined by pH-metric titration technique

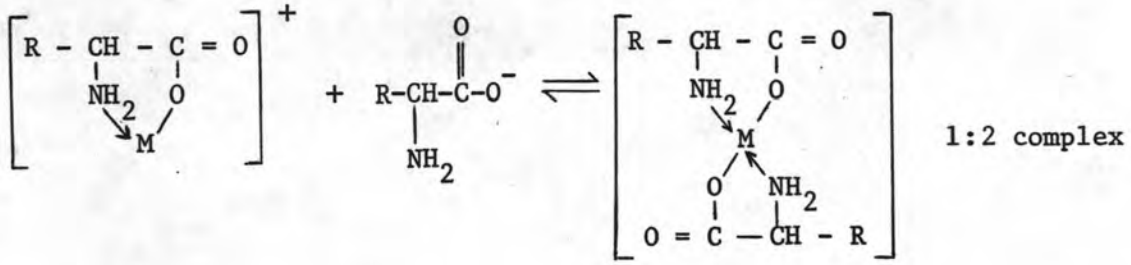
P = determined by polarographic technique

[a] log K_1 = log β_1

[b] determined from $\beta_2 = K_1K_2$ (by equation 2.2)

In addition, all essential amino acids contained one carboxyl group and one amino group. The oxygen atom of carboxyl group and the lone pair electrons of nitrogen atom of amino group are able to take part in bonding with the metal ion. The preferable five-membered chelate rings of amino acid and metal ion have reported (1,5) and, here, the possible formations in stepwise manner of the essential amino acid with metal ion can be generalized by the following reactions :





where $R\text{-}CH(NH_2)\text{-}C(=O)\text{-}O^-$ is the essential amino acid anion (free ligand) and M^{2+} is the metal (II) ion.

Finally, because of the biological importance of the essential amino acids, further study of the complex stability contants of the other metal ions with the essential amino acids should be made in the variation of methods, the variation of temperatures and the variation of ionic strengths. Especially, the metabolism of the essential amino acids in the presence of heavy metal ions under the physiological systems would be suggested for study. In addition, the mixed-ligand

complexes of the essential amino acids with the metal ions should be very interested in the future study for analytical chemist.