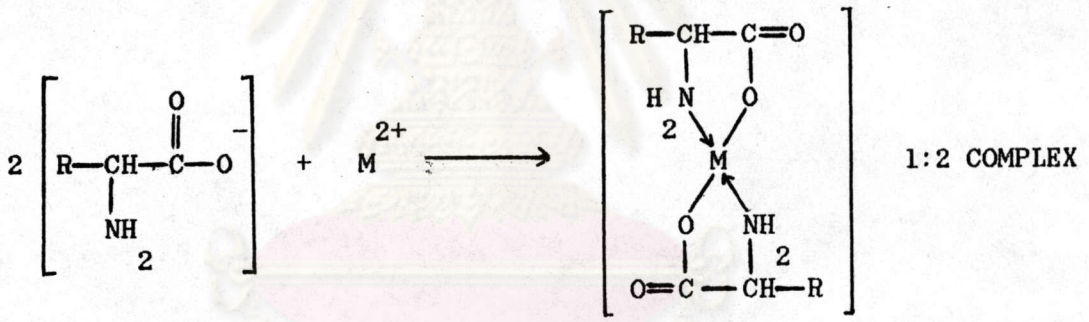
CHAPTER 4

CONCLUSION

Carboxyl group and amino group are the functional groups that contained in all amino acids. The oxygen atom of carbonyl group and the lone pair electrons of nitrogen atom of amino group are able to take part in binding with the metal ion. The preferable five-membered chelate rings of amino acid and metal ion have been reported (21,97) and, here ,the possible formations of the amino acids with metal ion can be generalized by the following reaction:



where $R-CH(NH_2)-C(=O)-O^-$ is the amino acid anion (free ligand) and M^{2+} is the metal(II) ion. Generally, this complexation may be regarded as an instantaneous, irreversible reaction (20).

Amperometric detection of amino acids in various buffer system is possible by means of the enhanced copper(II) ion dissolution from an anodized copper electrode to form complex with amino acids. This work involved a comprehensive study in the characteristics of the anodized copper electrode in both static solution (batch system) and

flowing solution (flow injection system), and an investigation into the possibility of using this electrode as an amperometric detection in HPLC.

From the study in batch system, the most suitable supporting electrolyte was phosphate buffer pH 7.0, and the fixed operating potential for amperometry was +150 mV VS SCE. The increase of the anodic current, when amino acid is added to the solution, is proportional to the amino acid concentration and is dependent on the rate of complexation reaction between the amino acids and copper(II) ions. Electrode characteristics in batch analysis with amperometric detection from Table 3.9 show that the linear range of three amino acids such as cysteine, glycine and threonine were approximately the same (10^{-5} - 10^{-4} M) because of the limiting of the electrode surface. The sensitivity of this detection method for amino acid analysis is determined by the stability constants of the complexation reaction which are 1.23×10^{15}, 2.09×10^{15} and 1.50×10^{19} for glycine, threonine and cysteine (98), respectively. The greater the value of stability constant is, the higher the sensitivity is. Detection limit are in the range of 10^{-7} - 10^{-8} M and therefore this detection method is a sensitive one. The reproducibility was obtained in the range of 0.9-1.2%.

In FIA, the effect on analyte signal was studied as a function of flow rate, residence time, injection volume and sample concentration. The peak current increases according to injection volume and sample concentration, and the peak current decreases due to flow rate and residence time. The analysis of 10 µL amino acids samples were used to study at sampling rate of 103 samples h^{-1} for glycine and threonine, and 80 samples h^{-1} for cysteine. Detection

limits are in the order of 10^{-5} M of 10 µL injected volume. A vary wide linear working range of 3 orders of magnitude (10^{-4} -10^{-1} M) was found for glycine and threonine, and in the range of 10^{-5} -10^{-3} M for cysteine. The reproducibility was obtained in the range of 1.1-1.5% and the carryover was 0-2.7% while the sampling rate varied in the range of 80-144 samples h^{-1}.

Trend of sensitivity of the detector for amino acid in FIA is similar to that in batch system as the sensitivity decreases from cysteine, threonine and glycine, respectively. The reason for this is also the same as that described in batch system that sensitivity depends on the stability constant. However, the sensitivity of the detector for the same amino acid in FIA was lower than that in batch analysis by 3 orders of magnitude due to dilution effect from the dispersion of sample and the non equilibrium complexation reaction between amino acid and copper(II) ions in the carrier stream. From these reasons, high concentration of amino acid can be analysed in FIA system rather than in batch system and the detection limit in FIA is also higher than that in batch analysis.

Disadvantage of the anodized copper electrode used in batch system is the fact that the current measured depends not only on the amino acid concentration, but also on the pH and composition of the matrix of sample solution which are the cause of uncertain electrode response. In flow system, the electrode surface is continuously washed with electrolyte, allowing quantitative determinations of amino acids in pH range evaluated. The injected sample volume was very little comparing to the volume of the carrier stream and therefore the solution around the electrode was not change so much. This brings

about the advantages of maintenance and reproducibility of an active electrode surface ,which was also observed by Hui and Huber (26).

The anodized copper electrode operated in a miniature flow-cell has been shown to be used as a detector in reverse phase HPLC. This detector is the specific detector, showing response only to copper-binding molecules. Five amino acids, threonine, methionine, arginine, phenylalanine and tryptophan, can be separated and detected as shown in Table 3.22. It shows that sensitivity of threonine in HPLC ($93.46\ \mu A\ M^{-1}$ of 20 µL injection or $39.27\ nA\ \mu g^{-1}$ injected) was lower than that in FIA ($108.34\ \mu A\ M^{-1}$ of 10 µL injected or $90.76\ nA\ \mu g^{-1}$ injected from Table 3.18) according to long residence time which caused higher dispersion.

The sensitivity of this detection technique is compared with that of other frequently used methods in Table 4.1. The detection limit is in the same order of magnitude as that of potentiometric detector. Lower detection limits have reported for underivatized amino acids with UV absorbance measurements at 200 nm (99). However, in the analysis of biological fluids, considerable interference from other compounds must be expected with this method. The advantage of amperometry over fluorimetry is its simplicity and the absence of the need for derivatization. Thus it offers short analysis time (compared with pre-column derivatization for fluorimetry) and low cost (compared with pre and post-column derivatization for fluorimetry or UV absorbance). However, a drawback to the use of a copper electrode is the fact that only certain neutral or alkaline buffer solutions which weakly formed complexed with copper(II) ions can be used, while most separations are performed with acidic mobile phases, e.g. , in

ion-exchange and ion-pair chromatoguaphy. This can be overcome by post-column addition of an alkaline solution to an acidic mobile phase.

Table 4.1 Comparison of sensitivity of detection methods for amino acid in HPLC.

Compound separate	Detection method	Typical detection limits (p mole)	Ref
PTH derivatives	UV absorbance	5-50	6,7,8
DNP derivatives	UV absorbance	10-50	9
Dns derivatives	Fluorimetry	0.05-1	11,12
OPA derivatives	Fluorimetry	0.05-0.5	14,15
Amino acids	UV absorbance, 200 nm	1-10	99
Amino acids	Ninhydrin reaction and absorbance	10-100	2
Amino acids	OPA reaction and fluorimetry	0.5-10	4
Amino acids	Potentiometry with copper electrode	100-500	1,16
Amino acids	Amperometry with copper electrode	200-500	This work

While the goal of this research was to investigate the behaviour of the anodized copper electrode as amperometric detector for amino acids, it is probable that the detection method developed will also have direct application to a wide variety of other copper binding species such as the antibiotics tetracyclin, sulpha drugs and

penicillin. Other metal electrode whose metal ions can be dissolved from the electrode and rapidly form metal-ligand complex may enable to use by the similar method. It is suggested that the anodized copper electrode can be used for determination of some metal ions which competitively formed more stable complex with added ligand in electrolyte. These can be extended in the further research work.