CHAPTER III

EXPERIMENTS

3.1 Chemicals

The 8 essential amino acids employed, namely L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan and L-valine were FLUKA reagents of puriss. grade. The purity of these essential amino acids was checked by pH-metric titration and examination of the entire curve for deviation from the calculated shape (1). The metal ions used were BDH reagents of AnalaR grade in the forms of following salts : cadmium (II) nitrate, lead (II) nitrate and manganese (II) chloride. Sodium hydroxide and potassium hydrogen phthalate were BDH reagents of AnalaR grade. The other chemicals used for preparations of buffer systems and electrolyte solutions were of analar grade. These chemicals were used without further purification unless otherwise mentioned. Hygroscopic compounds were kept in a desiccator over the anhydrous silica gel.

Mercury (BDH, AnalaR grade) was filtered through a small hole in the apex of filter paper (Whatman number 42) for many times before using.

Purified nitrogen gas used for dearating the test solutions was prepared by passing the oxygen-free nitrogen gas (obtained from Thai Industrial Gas Co.) through three bubbling towers in the gas

line. The first tower contained amalgamated zine metals in a vanadium (II) chloride solution for reduction of any contaminated oxygen gas. The second tower contained an alkaline solution for neutralization of the nitrogen gas (27). The last one was trapped tower for preventing of any solution from the second tower to flow into the test solution.

All solutions were prepared with the double deionized water.

3.2 Apparatus

The pH-values of the test solutions were measured with the pH meter (ORION RESEARCH Digital Ionalyzer Model 501) equipped with the glass electrode.

Polarograms were obtained with the Princeton Applied Research (PAR) Model 174A Polarographic Analyzer equipped with the Hewlett-Packard 7040A X-Y Recorder.

The cell employed in all polarographic analyses is a conventional two compartments cell (H-shaped cell), one compartment served for the reference electrode, saturated calomel electrode (SCE), and the other compartment served for the test solution. Dropping mercury electrode (DME) was used as a working electrode.

All potentials reported herein were measured against SCE.

All calculations were made by the scientific calculator (CASIO Scientific Calculator Model 3600P) that the linear regression by least square treatments and programmed calculations can be made.

The temperature of the water bath that used to equilibrate the temperature of the test solution was controlled by the temperature controller board (33). Dependence of time and temperature of the water bath was measured as shown in Table 3.1

Table 3.1 Dependence of time and temperature of the water bath

time(hrs.)	water bath temperature (^{o}C)
0.5	36.5
1.0	37.0
2.0	37.2
4.0	37.5
8.0	37.2
10.0	37.2

As seen in Table 3.1, the water bath temperature was fluctuated in the range of $\pm$ 0.5^{o}C . Therefore, all measurements were reported at a constant temperature of 37.0 $\pm$ 0.5^{o}C.

3.3 Procedure

3.3.1 Double Deionized Water

The double deionized water used throughout this study was prepared by passing the distilled water through a set of three columns. Each column had an inside diameter of 3.5 cm and a length of 55.0 cm. The first column was packed with anion-exchange resins, Amberlite IR-45 (OH). In the second column cation-exchange resins,

Amberlite Ir-120 (H), was packed. The last column was half-filled with the anion exchangers and the other half with the cation exchangers.

3.3.2 Salt Bridge and Reference Electrode

3.3.2.1 Preparation of Salt Bridge (34)

A 0.4 g of agar-agar was molten in ca. 10 cm^3 of saturated KCl solution by gently warming the mixture on a hot plate. The cross member of the clean and dry cell was clamped vertically and the molten agar gel was transferred into the cross member by means of a dropper. The cell was allowed to stand undisturbed until the gel had solidified. No air bubble should be present in the KCl-agar salt bridge. When the H-shaped cell is not used, the test solution compartment should be kept filled with the double deionized water to prevent the agar plug from drying out.

3.3.2.2 Preparation of the Reference Electrode (34)

The SCE was prepared in one compartment of the H-shaped cell. Electrical connection with the SCE was made by means of a platinum wire, sealed through a glass tube, the glass tube contained a little mercury into which an amalgamated copper wire was dipped. To set up the electrode, a saturated solution of KCl and Hg_2Cl_2 was first prepared. Pure mercury was placed in the bottom of the dry electrode compartment of the H-shaped cell for a depth of 0.5 cm. The mercury was then covered with the calomel paste. The latter was prepared by rubbing pure Hg_2Cl_2, mercury and saturated

solution of KCl in a clean beaker with a clean glass rod. The electrode compartment was filled with a saturated solution of KCl and Hg_2Cl_2 and the rubber bang with the glass tube associated with electrical connection was then inserted.

3.3.3 Stock Solutions

3.3.3.1 Stock Solutions of Metal Ions

The stock solution of 5.00×10^{-2} M of each metal ion desired, Cd (II), Pb (II) and Mn (II) ion, was prepared by dissolving the appropriate amounts of its salt with the double deionized water

3.3.3.2 Stock Solutions of Essential Amino Acids

The essential amino acids were dried for 3 hours at 120°C before weighing (1,6,35). The stock solution of 1.00×10^{-2} M of each essential amino acid desired was prepared by dissolving its appropriate amounts with the deionized water.

3.3.3.3 Electrolyte Solution

Potassium chloride was dried at 120°C for 3 hours before weighing (6). The stock solution of 1.00 M KCl that used as electrolyte solution for controlling the ionic strength of the test solution was prepared by dissolving the appropriate amounts with the deionized water.

3.3.4 pH-metric Titration Technique

3.3.4.1 Standardization of Standard NaOH Solution

Potassium hydrogen phthalate was dried for 3 hours at 120°C before weighing (6). The standard NaOH solution used as titrant in pH-metric titration technique was standardized by titrating with the primary standard solution, potassium hydrogen phthalate solution, for three times. Then, it was immediately used after standardization to prevent the CO_2 absorption.

3.3.4.2 pH-metric Titration of the Test Solutions

The complex formation of Cd (II), Mn (II) and Pb (II) ions with essential amino acids was first studied by pH-metric titration technique. The ionic strength of all test solutions was kept constant by maintaining a concentration of 0.10 M KCl and by keeping a low concentration of essential amino acid and metal ion (35). The concentration of essential amino acid in the titration cell was 1.00×10^{-3} M and the metal ion was 5.00×10^{-4} M. The total volume of the test solutions was 50.0 cm^3, whether metal ions were present or not, by preparing in the volumetric flask and transferred quantitatively into the titration cell.

All titrations were performed in a 100 cm^3 titration cell that immersed in a constant temperature water bath of 37.0 ± 0.5°C. The titration cell was fitted with a magnetic stirrer and the purified nitrogen gas inlet and outlet tubes, small buret tube and glass electrode were inserted through a tightly fitting rubber stopper. Presaturated purified nitrogen gas was kept over

the surface of the test solution to maintain an inert atmosphere and to prevent the CO_2 absorption.

The glass electrode was standardized before and after each titration with the standard buffer solution (ORION RESEARCH INC.) of a pH 6.97 at 37°C. Each essential amino acid was titrated with standard NaOH solution, first in the absence of metal ions and then in the presence of metal ions. The pH values of the resulting solution were recorded after each addition of standard NaOH solution and the hydrogen-ion concentrations were obtained directly from the pH values measured. The acid-dissociation constants (Ka) of the essential amino acids were obtained from similar titration under the same experimental conditions that the metal ions had been omitted. The titrations were performed at least three times for each of the test solution.

3.3.5 Polarographic Technique

3.3.5.1 Buffer Solution

Michaelis borate buffer solution was prepared by mixing the appropriate volumes of 0.10 M NaOH or 0.10 M HCl solution with borax solution (containing boric acid in NaOH solution) for the pH range of 3.01-10.97.

3.3.5.2 Polarograms of the Test Solutions

The complex formations of Cd (II) , Pb (II) and Mn (II) ions with the essential amino acids were also studied by polarographic technique. All test solutions were prepared in 50.0 cm^3 volumetric flasks by mixing required amounts of the solution of

metal ion and essential amino acid, then adding the required amounts of KCl solution to maintain the constant ionic strength of 0.10 M and finally diluting with buffer solution for a desired pH to give the final volume of 50.0 cm^3.

The H-shaped polarographic cell was immersed in a water bath at a constant temperature of 37.0 $\pm$ 0.5^{o}C. Before the test solution was placed in the cell, the test compartment was washed two or three times with the double deionized water and lastly rinsed with the test solution. The pH of the test solution was checked in the test compartment before the deareation started.

Purified nitrogen gas was used to remove dissolved oxygen from the test solution for ten minutes by means of a disposable capillary. During the polarographic performance, a stream of purified nitrogen gas was maintained over the solution surface to prevent the redissolution of oxygen into the test solution. The desired potential range, current sensitivity, the height of mercury reservior, scan rate and polarity were set on the instrument and the polarogram was recorded.