

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Apparatus

A Shimadzu Model AA-670 atomic absorption/flame emission spectrophotometer equipped with a graphic printer Model PR-4 integrator.

A Hewlett Packard HP 8452 A a diode array UV/VIS spectrophotometer.

An Orion Research Digital Ionalyzer 701A pH-meter, USA.

A RC-2 magnetic bar and a magnetic stirrer, Japan.

A Baker-10 Extraction System, J.T. Baker, USA.

Reservoir 75 mL, J.T. Baker, USA.

Disposable column 4 mL, J.T. Baker, USA.

Glassfibre prefilter dimension 10 mm, Sartorius, W. Germany.

Membrane type HA 0.45  $\mu$ m. Millipore, USA.

Hollow Cathode Lamps of

Ag-lamp, Varian techtron, Australia

multi-lamp of Co, Cr, Cu, Fe, Mn, Ni, Varian techtron, Australia

Cd-lamp, S & J Juniper & Co, England

Pb-lamp, S & J Juniper & Co, England

Graduated pipettes 1.00, 2.00, 5.00, and 10.00 mL

Volumetric pipettes 1.0, 5.0, 10.0, and 25.0 mL

Volumetric flasks 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, and 1,000.0 mL

Beakers 25, 50, 100, 250, 500, and 1,000 mL

Measuring cylinder 50, 100, and 250 mL

A Gast Model 05222 V4B, G21Dx general electric A-C motor to use as a vacuum pump , USA

### 3.2 Chemicals

3.2.1 Octadecyl ( $C_{18}$ ) bulk packing for flash chromatography (40  $\mu$ m average particle diameter) was purchased from J.T. Baker Inc., Phillipsburg, USA.

3.2.2 4-(2-Pyridylazo) resorcinol (PAR) was purchased as the monosodium monohydrate salt from E. Merck, Darmstadt, Germany.

3.2.3 The standard metal solutions (1,000 ppm) of cadmium (II) nitrate, nickel (II) nitrate, and silver nitrate were purchased from BDH Chemical Ltd., Poole, England and the standard metal solutions (1,000 ppm) of copper (II) nitrate and lead (II) nitrate were purchased from E. Merck, Darmstadt, Germany. All of standard metal solutions were standard solutions for atomic absorption spectroscopy (Spectro Pure Grade).

3.2.4 Cadmium (II) sulfate octahydrate ( $3CdSO_4 \cdot 8H_2O$ , MW 769.56), anhydrous copper (II) sulfate (Min 99%, MW 159.60), lead (II) nitrate ( $Pb(NO_3)_2$ , MW 331.21), Nickel (II) sulfate hexahydrate ( $NiSO_4 \cdot 6H_2O$ , MW 262.80), and silver nitrate ( $AgNO_3$ , MW 169.88). All of these compounds were analytical reagent grade.

3.2.5 Nitric acid (AR Grade) was purchased from E. Merck, Darmstadt, Germany.

3.2.6 Hydrochloric acid (AR Grade) was purchased from E. Merck, Darmstadt, Germany.

3.2.7 ammonia solution (AR Grade) was purchased from BDH Limited, poole, England.

3.2.8 Sodium hydroxide pellet was purchased from Vidhyasom Co. Ltd., Thailand.

3.2.9 Absolute methanol was purchased from J.T. Baker Chemical Company, Deventer, Holland.

### 3.3 Preparation of the Standard Solutions

3.3.1 The Standard Solutions for Visible Spectra of Ag-PAR, Cd-PAR, Cu-PAR, Ni-PAR, and Pb-PAR Complexes at Various pH.

3.3.1.1 The Stock Solution of PAR.

The solution of PAR ( $1.0 \times 10^{-2}$  M) was prepared by dissolving 0.2572 g in 100 mL of deionized water.  $1.0 \times 10^{-3}$  M and  $1.0 \times 10^{-4}$  M of PAR were prepared by further dilution of this stock solution.

3.3.1.2 The Standard Metal Solutions.

The  $1.0 \times 10^{-2}$  M standard solutions of Ag (I), Cd (II), Cu (II), Ni (II), and Pb (II) were prepared by dissolving 0.1780 g, 0.2680 g, 0.1594 g, 0.2652 g, and 0.3356 g of silver nitrate, tri (cadmium sulfate) octahydrate, anhydrous copper sulfate, nickel nitrate hexahydrate, and lead nitrate, respectively in deionized water, 1.0 mL of concentrated hydrochloric acid was also

added and then each solution was made up to five volumetric flasks of 100.0 mL.  $1.0 \times 10^{-3}$  M of these metal solutions were prepared by further dilution of the  $1.0 \times 10^{-2}$  M solutions.

#### 3.3.1.3 The pH Adjustment Solutions.

A  $1.0 \times 10^{-2}$  M hydrochloric acid, and (or) a  $1.0 \times 10^{-2}$  M ammonia solution and (or) a  $1.0 \times 10^{-2}$  M sodium hydroxide solution were employed for pH adjustment of the solutions.

### 3.3.2 The Standard Solution for the Study of Sensitivity of Ag, Cd, Cu, Ni, and Pb on Shimadzu AA-670 Atomic Absorption Spectrometer.

All standard solutions of metals (Ag, Cd, Cu, Ni, Pb) were prepared from 1,000.0 ppm of the solutions of standard metal solutions for atomic absorption spectroscopy (spectro pure grade).

#### 3.3.2.1 The Standard Cadmium Solution.

The 100.0 ppm standard solution of cadmium was prepared by pipetting 5.00 mL of 1,000 ppm standard solution of cadmium into a 50.0 mL of volumetric flask, 1.0 mL of concentrated hydrochloric acid was also added and the content was diluted to the mark with deionized water. A series of cadmium solution at the concentration of 0.05, 0.10, 0.20, 0.40, 0.80 and 1.00 ppm was prepared by further dilution of the 100.0 ppm solution.

#### 3.3.2.2 The Standard Lead Solution.

The 100.0 ppm standard solution of lead was prepared by pipetting 5.00 mL of 1,000.0 ppm standard solution of lead into a 50.0 mL of volumetric flask, 1.0 mL of concentrated

hydrochloric acid was also added and the content was diluted to the mark with deionized water. A series of lead solution at the concentration of 0.50, 1.00, 2.00, 4.00, 8.00 and 10.00 ppm was prepared by further dilution of the 100.0 ppm solution.

3.3.2.3 The Standard Solution of Copper, Nickel, and Silver.

The 100.0 ppm standard solution of copper was prepared by pipetting 5.00 mL of 1,000.0 ppm standard solution of copper into a 50.0 mL of volumetric flask, 1.0 mL of concentrated hydrochloric acid was also added and the content was diluted to the mark with deionized water. A series of copper at the concentration of 0.10, 0.50, 1.00, 1.50, 2.00 and 4.00 ppm was prepared by further dilution of the 100.0 ppm solution.

Nickel and silver standard solutions were prepared as same as copper standard solution.

3.3.3 The Standard Solution for Solid-Phase Extraction Study.

3.3.3.1 The Standard Metal Solutions.

All standard solutions of metals ( Ag, Cd, Cu, Ni, Pb ) for standard calibration curves and preparation of complexes were prepared from 1,000 ppm of the standard metal stock solutions (Spectro Pure Grade). The desired concentrations were prepared by further dilution of these 1,000 ppm standard solutions. The appropriate amounts of hydrochloric acid was added in each concentrations.

### 3.3.3.2 The Stock Solution of PAR.

The solution of PAR ( $1.0 \times 10^{-2}$  M) was prepared by dissolving 0.1286 g in 50 mL deionized water. The desired concentrations were freshly prepared by further dilution of this stock solution.

### 3.3.3.3 The pH Adjustment Solutions.

The pH adjustment solutions were prepared as same as section 3.3.1.3.

### 3.3.3.4 The Nitric Acid Solution.

A 6.0 M nitric acid solution was prepared by dissolving 13.5 mL concentrated nitric acid solution (density  $1.40 \text{ g/cm}^3$ ) in 50 mL of deionized water. 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 M were prepared by further dilution of the 6.0 M solution.

## 3.4 Atomic Absorption Spectrometer Conditions.

A Shimadzu AA-670 atomic absorption spectrometer was used for the study. Hollow cathode lamps were employed as radiation sources. The elements were determined according to the manufacture's recommended settings, which are summarized in Table 3.1

Table 3.1 The operational conditions for the analysis of Ag, Cd, Cu, Ni, and Pb by the Shimadzu AA-670 atomic absorption spectrometer.

Metal	Light - source	Lamp Current mA	Wavelength nm	Slit width nm
Ag	Ag - lamp	4	328.1	0.6
Cd	Cd - lamp	4	228.8	0.3
Cu	Co, Cr, Cu, Fe Mn, Ni lamp	6	324.8	0.5
Ni	Co, Cr, Cu, Fe Mn, Ni lamp	8	232.0	0.15
Pb	Pb - Lamp	7	217.0	0.3

### 3.5 The Study of Visible Spectra of the Ag-PAR, Cd-PAR, Cu-PAR, Ni-PAR, and Pb-PAR complexes at various pH.

#### 3.5.1 The Study of Visible Spectra of PAR alone at Various pH

The procedure of the study of visible spectra of PAR alone at various pH was described as follows:

1. Eleven 5.0 mL of  $1.0 \times 10^{-4}$  M PAR solution were pipetted into a series of 50 mL beakers. The pH of the solutions were adjusted to the desired pH (2.0 to 12.0 with the interval of 1.0) by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measure by a pH-meter. The pH probe was washed throughly with deionized water into the solution which was then made the volume to 50.0 mL of volumetric flask.
2. A series of these solutions were used for the study of visible spectra at the wavelength of 360-800 nm by using deionized water as a reference.
3. The visible spectra were recorded with a Hewlett Packard HP 8452 A Photodiode array UV /Visible spectrophotometer with 10 mm quartz cell.

#### 3.5.2 The Study of Visible Spectra of the Ag-PAR Complex at Various pH

The procedure of the study of visible spectra of the Ag-PAR complex at various pH was described as follows:

1. Eleven 5.0 mL of  $1.0 \times 10^{-3}$  M standard solution of silver were pipetted into a series of 50 mL beakers containing exact

5.0 mL each of  $1.0 \times 10^{-4}$  M PAR solution. The pH of the solutions were adjusted to the desired pH (2.0 to 12.0 with the interval of 1.0) by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measure by a pH-meter. The pH probe was washed throughly with deionized water into the solution which was then made the volume to 50.0 mL of volumetric flask.

2. A series of these solutions were used for the study of visible spectra at the wavelength of 360-800 nm by using deionized water as a reference.

3. The visible spectra were recorded with a Hewlett Packard HP 8452 A Photodiode array UV /Visible spectrophotometer with 10 mm quartz cell. These spectra are shown in Figure 4.1.

### 3.5.3 The Study of Visible Spectra of the Cd-PAR, Cu-PAR, Ni-PAR, and Pb-PAR Complexes at Various pH

The procedure of the study of visible spectra of the Cd-PAR, Cu-PAR, Ni-PAR, and Pb-PAR complexes at various pH were prepared as same as the study of visible spectra of Ag-PAR complex at various pH. The spectra of Ag-PAR, Cd-PAR, Cu-PAR, Ni-PAR, and Pb-PAR complexes were shown in Figures 4.2 to 4.6, respectively.

### 3.6 The Study of Sensitivity of Cadmium, Copper, Lead, Nickel, and Silver on Shimadzu AA-670 Atomic Absorption Spectrometer.

The procedure of the study of sensitivity of cadmium, copper, lead, nickel, and silver were done as the following steps:

1. The operational conditions for determination each metal by

AAS were set as mentioned in section 3.4.

2. Each series of standard solution of metals which were prepared in section 3.3.2, was aspirated to a flame atomic absorption spectrometer by using deionized water as the blank solution. Deionized water was aspirated to rinse each solution after each measurement and before the changing each solution.

3. The calibration curve of each metal was made ( see Figures 4.6 to 4.10) By using these calibration curves of each metal, the sensitivity of cadmium, copper, lead, nickel, and silver were calculated.

The relationships between absorbance and concentration of Ag, Cd, Cu, Ni, and Pb were shown in Tables 4.1 to 4.5, respectively.

### 3.7 The Study of Detection Limit of Ag, Cd, Cu, Ni, and Pb on Shimadzu AA-670 Atomic Absorption Spectrometer.

The detection limit of each metal was determined by decreasing the concentrations of each metal until the signal to noise ratio was equal to 2. The detection limit of Ag, Cd, Cu, Ni, and Pb results were summarized in Table 4.7.

### 3.8 The Procedure of Packing C<sub>18</sub> into Cartridge

A C<sub>18</sub> SPE cartridge for the study of all variables on percentage of recovery in this study was prepared as follows:

1. One piece of 10 mm glassfibre prefilter was put into 4 mL polypropylene column.

2. 500 mg of C<sub>18</sub> bulk packing were carefully poured into the

cartridge as described in step 1.

3. The another piece of 10 mm glassfibre prefilter was placed over C<sub>18</sub> as mentioned in step 2.

### 3.9 The Procedure of Activated C<sub>18</sub> SPE cartridge on the Conditioning Step

The C<sub>18</sub> SPE cartridge must be activated before usage. The activated cartridge was prepared by passing 3.0 mL of absolute methanol through the C<sub>18</sub> SPE cartridge which was prepared in section 3.8. To push the absolute methanol downward, the vacuum pump must be used at 10 mm Hg pressure at the first time ( about 30 seconds ) and then decreased the pressure to 5 mm Hg. After the absolute methanol nearly dry ( over the top of packing about 2 mm ), 2.0 mL deionized water was passed through the column.

### 3.10 The Study of the Effect of Variables on Percentage of Recovery of C<sub>18</sub> SPE Cartridge by Using Cd-PAR Complex and Cu-PAR Complex as the Models of the Study.

#### 3.10.1 The Effect of Nitric Acid Concentration

3.10.1.1 The procedure of the study of the effect of nitric acid concentrations at 0.1, 0.5, 1.0, 2.0, and 4.0 M on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. Five 5.0 mL of 1.00 ppm standard cadmium solution were pipetted into a series of 100 mL beakers containing

exact 5.0 mL of  $4.0 \times 10^{-5}$  M PAR solution. The pH of the solutions were adjusted between 7-8 by using the pH adjusting solution. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Five volumetric flasks of Cd-PAR complex solution in No.1 were passed through five C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of each concentrations of nitric acid solution 0.1, 0.5, 1.0, 2.0, and 4.0 M was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers, respectively. The volume of each volumetric flask was made up to 5.0 mL with each concentration of nitric acid solutions.

4. The blank solutions were prepared as same as step 1 to step 3 but the standard cadmium solution was not added in the step 1.

5. Five solutions (from step 3) were aspirated to a flame AAS by using six concentrations of standard cadmium solutions at 0.05, 0.10, 0.20, 0.40, 0.80 and 1.00 ppm as a standard calibration curve. The blank solutions (from step 4) for each cartridge was aspirated to flame AAS before sample solutions (from step 3).

6. The relationships between percentage of recoveries and concentrations of nitric acid solution of Cd-PAR complex were shown in Table 4.10.

3.10.1.2 The procedure of the study of the effect of nitric acid concentrations at 0.1, 0.5, 1.0, 2.0, and 4.0 M on percentage of recovery by using Cu-PAR complex as a model was mentioned as follows:

1. Five 5.0 mL of 0.40 ppm standard copper solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $1.6 \times 10^{-5}$  M PAR solution. The pH of the solutions were adjusted between 7-8 by using the pH adjusting solution. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Five volumetric flasks of Cu-PAR complex solution in No. 1 were passed through five C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of each concentrations of nitric acid solution 0.1, 0.5, 1.0, 2.0, and 4.0 M was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers, respectively. The volume of each volumetric flask was made up to 5.0 mL with each concentration of nitric acid solution.

4. The blank solutions were prepared as same as step 1 to step 3 but the standard copper solution was not added in the step 1.

5. Five solutions ( from step 3 ) were aspirated to a flame AAS by using six concentrations of standard copper solutions at 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm as a

standard calibration curve. The blank solutions ( from step 4 ) for each cartridge were aspirated to flame AAS before sample solutions (from step 3).

6. The relationships between percentage of recoveries and concentrations of nitric acid solution of Cu-PAR complex were shown in Table 4.11.

The effect of concentrations of nitric acid solution on percentage of recoveries by using Cd - PAR complex and Cu - PAR as the models of this study were compared in Figure 4.12.

### 3.10.2 The Effect of Volume of Nitric Acid Solution

3.10.2.1 The procedure of the study of the effect of volume of 1.0 M nitric acid solution at 1.0, 2.0, 3.0, 4.0 and 5.0 mL on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. The preparation of the solution was done as same as step 1 and 2 in section 3.10.1.1.

2. Five volumes of 1.0 M nitric acid solution 1.0, 2.0, 3.0, 4.0 and 5.0 mL were passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers, respectively. The volume of each volumetric flask was made up to the mark with 1.0 M nitric acid solution.

3. The blank solutions were prepared as same as step 1 to step 2 but the standard cadmium solution was not added in step 1.

4. Five solutions (from step 2) were aspirated to a flame AAS by using six concentrations of standard cadmium solutions 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm as a standard calibration curve. The blank solutions (from step 3) for each cartridge was aspirated to flame AAS before sample solutions (from step 2).

5. The relationships between percentage of recoveries and the volumes of nitric acid solution of Cd-PAR complex were shown in Table 4.12.

3.10.2.2 The procedure of the study of the effect of volume of 1.0 M nitric acid solution at 1.0, 2.0, 3.0, 4.0 and 5.0 mL on percentage of recovery by using Cu-PAR complex as a model can be described as follows:

1. The preparation of the solution was done as same as step 1 and 2 in section 3.10.1.2.

2. Five volumes of 1.0 M nitric acid solution 1.0, 2.0, 3.0, 4.0 and 5.0 mL were passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers, respectively. The volume of each volumetric flask was made up to the mark with 1.0 M nitric acid solution.

3. The blank solutions were prepared as same as step 1 to step 2 but the standard copper solution was not added in step 1.

4. Five solutions (from step 2) were aspirated to a flame AAS by using six concentrations of standard copper solutions 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm as a standard calibration curve. The blank solutions (from step 3) for each cartridge was aspirated to flame AAS before sample solutions (from step 2).

5. The relationships between percentage of

recoveries and the volumes of nitric acid solution of Cu-PAR complex were shown in Table 4.13.

The effect of volumes of nitric acid solution on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as the models of this study were compared in Figure 4.13.

### 3.10.3 The Effect of pH

3.10.3.1 The procedure of the study of the effect of pH at 5.02, 6.07, 7.03, 8.05, 9.07, and 10.01 on percentage by using Cd-PAR complex as a model of recovery can be described as follows:

1. Six 5.0 mL of 1.00 ppm standard cadmium solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $4.0 \times 10^{-5}$  M PAR solution. The pH of the solutions were adjusted to the desired pH ( 5.0 to 10.0 with the interval of 1.0 ) by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measured by pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Six volumetric flasks of Cd-PAR complex solution which were prepared in No. 1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Six 5.0 mL of volumetric flasks were

employed as the receivers for each cartridge.

4. The blank solution for each pH was prepared as same as step 1 to step 3 but the standard cadmium solution was not added in step 1.

5. Six solutions ( from step 3 ) were aspirated to a flame AAS by using six concentrations of standard cadmium solutions 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm as a standard calibration curve. The blank solutions for each cartridge was aspirated to flame AAS before sample solutions ( from step 3 ).

6. The relationships between percentage of recoveries and pH value of Cd-PAR complex were shown in Table 4.14.

3.10.3.2 The procedure of the study of the effect of pH at 5.02, 6.07, 7.03, 8.05, 9.07, and 10.01 on percentage by using Cd-PAR complex as a model of recovery can be described as follows:

1. Six 5.0 mL of 0.40 ppm standard copper solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $1.6 \times 10^{-5}$  M PAR solution. The pH of the solutions were adjusted to the desired pH ( 5.0 to 10.0 with the interval of 1.0 ) by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measured by pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Six volumetric flasks of Cu-PAR complex solution which were prepared in No. 1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Six 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for each pH was prepared as same as step 1 to step 3 but the standard copper solution was not added in step 1.

5. Six solutions ( from step 3 ) were aspirated to a flame AAS by using six concentrations of standard copper solutions 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm as a standard calibration curve. The blank solutions for each cartridge was aspirated to flame AAS before sample solutions ( from step 3 ).

6. The relationships between percentage of recoveries and pH value of Cu-PAR complex were shown in Table 4.12.

The effect of pH values on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as the models of this study were compared in Figure 4.14.

#### 3.10.4 The Effect of Flow rate in the Retention Step

3.10.4.1 The procedure of the study of the effect of flow rate in the retention step at 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mL/min on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. Six solutions of Cd-PAR complex were done as same as step 1 in section 3.10.1.1.

2. Six volumetric flasks of Cd-PAR complex solution in No. 1 were passed through six C<sub>18</sub> SPE cartridges which

were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward. The pressure of vacuum pump was adjusted to control the flow rate at 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mL/min

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Six 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for each flow rate was prepared as same as step 1 to step 3 but the standard cadmium solution was not added in step 1.

5. Six solutions (from step 3) were aspirated to a flame AAS by using six concentrations of standard cadmium solutions 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm as a standard calibration curve. The blank solutions (from step 4) for each cartridge was aspirated to flame AAS before sample solutions (from step 3).

6. The relationships between percentage of recoveries and flow rate in the retention step of Cd-PAR complex were shown in Table 4.16.

3.10.4.2 The procedure of the study of the effect of flow rate in the retention step at 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mL/min on percentage of recovery by using Cu-PAR complex as a model was mentioned as follows:

1. Six solutions of Cu-PAR complex were done as same as step 1 in section 3.10.1.2.

2. Six volumetric flasks of Cd-PAR complex solution in No. 1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward. The pressure of vacuum pump was adjusted to control the flow rate at 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mL/min.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Six 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for each flow rate was prepared as same as step 1 to step 3 but the standard copper solution was not added in step 1.

5. Six solutions (from step 3) were aspirated to a flame AAS by using six concentrations of standard copper solutions 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm as a standard calibration curve. The blank solutions (from step 4) for each cartridge was aspirated to flame AAS before sample solutions (from step 3).

6. The relationships between percentage of recoveries and flow rate in the retention step of Cu-PAR complex were shown in Table 4.17.

The effect of flow rate in the retention step on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as the models of this study were compared in Figure 4.15.

### 3.10.5 The Effect of Flow rate in Elution Step

3.10.5.1 The procedure of the study of the effect of flow rate in the elution step at 0.1, 0.5, 1.5, 3.0, 6.0 and 9.0 mL/min on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. Six solutions of Cd-PAR complex were done as same as step 1 in section 3.10.1.1.

2. Six volumetric flasks of Cd-PAR complex

solution in No.1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. The vacuum pump was adjusted to control the flow rate in this step at 0.1, 0.5, 1.5, 3.0, 6.0, and 9.0 mL/min. Six 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for each flow rate was prepared as same as step 1 to step 3 but the standard cadmium solution was not added in step 1.

5. Six solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm of standard cadmium solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and flow rate in the elution step of Cd-PAR complex were shown in Table 4.18.

3.10.5.2 The procedure of the study of the effect of flow rate in the elution step at 0.1, 0.5, 1.5, 3.0, 6.0 and 9.0 mL/min on percentage of recovery by using Cu-PAR complex as a model was mentioned as follows:

1. Six solutions of Cu-PAR complex were done as same as step 1 in section 3.10.1.2.

2. Six volumetric flasks of Cu-PAR complex solution in No.1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. The vacuum pump was adjusted to control the flow rate in this step at 0.1, 0.5, 1.5, 3.0, 6.0, and 9.0 mL/min. Six 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for each flow rate was prepared as same as step 1 to step 3 but the standard copper solution was not added in step 1.

5. Six solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.10, 0.50, 1.00, 1.50, 2.00 and 4.00 ppm of standard copper solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and flow rate in the elution step of Cu-PAR complex were shown in Table 4.19.

The effect of flow rate in the elution step on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as the models of this study were compared in Figure 4.16.

### 3.10.6 The Effect of PAR Concentration

3.10.6.1 The procedure of the study of the effect of PAR concentration at  $2.0 \times 10^{-5}$ ,  $4.0 \times 10^{-5}$ ,  $8.0 \times 10^{-5}$ ,  $2.0 \times 10^{-4}$ , and  $4.0 \times 10^{-4}$  M on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. Five 5.0 mL of 1.00 ppm standard cadmium solution were pipetted into a series of 100 mL beakers containing exact

5.0 mL of  $2.0 \times 10^{-5}$ ,  $4.0 \times 10^{-5}$ ,  $8.0 \times 10^{-5}$ ,  $2.0 \times 10^{-4}$ , and  $4.0 \times 10^{-4}$  M PAR solutions, respectively. The pH of each solution was adjusted between 7-8 by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Five volumetric flasks of Cd-PAR complex solution which were prepared in No.1 were passed through five C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solutions were prepared as same as step 1 to step 3 but the standard cadmium solutions were not added in step 1.

5. Five solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm of standard cadmium solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and PAR concentrations of Cd-PAR complex were shown in Table 4.20.

3.10.6.2 The procedure of the study of the effect of PAR concentration at  $8.0 \times 10^{-6}$ ,  $1.6 \times 10^{-5}$ ,  $3.2 \times 10^{-5}$ ,  $8.0 \times 10^{-5}$ , and  $1.6 \times 10^{-4}$  M on percentage of recovery by using Cu-PAR complex as a model was mentioned as follows:

1. Five 5.0 mL of 0.40 ppm standard copper solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $8.0 \times 10^{-6}$ ,  $1.6 \times 10^{-5}$ ,  $3.2 \times 10^{-5}$ ,  $8.0 \times 10^{-5}$ , and  $1.6 \times 10^{-4}$  M PAR solutions, respectively. The pH of each solution was adjusted between 7-8 by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Five volumetric flasks of Cu-PAR complex solution which were prepared in No.1 were passed through five C<sub>18</sub> SPE cartridges which were activated before usage (see section 3.9). The vacuum pump was used to suck the solution downward at 15 mm Hg pressure.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as the receivers for each cartridge.

4. The blank solutions were prepared as same as step 1 to step 3 but the standard copper solutions were not added in step 1.

5. Five solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm of standard copper solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and PAR concentrations of Cu-PAR complex were shown in Table 4.21.

The effect of PAR concentrations on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as the models of this study were compared in Figure 4.17.

### 3.10.7 The Effect of Sorbent Mass

3.10.7.1 The procedure of the study of the effect of sorbent mass at 100, 200, 300, 400, and 500 mg on percentage of recovery by using Cd-PAR complex as a model can be described as follows:

1. The C<sub>18</sub> SPE cartridges were prepared at various mass (100, 200, 300, 400, 500 mg). The procedure of packing C<sub>18</sub> into the cartridge was done as same as section 3.8 and the procedure of activated C<sub>18</sub> SPE cartridge was prepared as mentioned in section 3.9.

2. The preparation of the solution for the study of this effect was done as same as step 1 and step 2 in section 3.10.1.1.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as a receivers for each cartridge.

4. The blank solution for each sorbent mass was prepared as same as step 2 and step 3 but the standard cadmium solution was not added in step 2.

5. Five solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm of standard cadmium solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and sorbent mass of Cd-PAR complex were shown in Table 4.22.

3.10.7.2 The procedure of the study of the effect of sorbent mass at 100, 200, 300, 400, and 500 mg on percentage of recovery by using Cu-PAR complex as a model was described as follows:

1. The C<sub>18</sub> SPE cartridges were prepared at various mass (100, 200, 300, 400, 500 mg). The procedure of packing C<sub>18</sub> into the cartridge was done as same as section 3.8 and the procedure of activated C<sub>18</sub> SPE cartridge was prepared as described in section 3.9.

2. The preparation of the solution for the study of this effect was done as same as step 1 and step 2 in section 3.10.1.1.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge. Five 5.0 mL of volumetric flasks were employed as a receivers for each cartridge.

4. The blank solution for each sorbent mass was prepared as same as step 2 and step 3 but the standard copper solution was not added in step 2.

5. Five solutions (from step 3) and the blank solutions (from step 4) were aspirated to flame AAS. 0.10, 0.50, 1.00, 1.50, 2.00, and 4.00 ppm of standard copper solutions were employed as a calibration curve.

6. The relationships between percentage of recoveries and sorbent mass of Cu-PAR complex were shown in Table 4.23.

The effect of sorbent mass on percentage of recoveries by using Cd-PAR complex and Cu-PAR complex as models of this study were compared in Figure 4.18.

### 3.11 The Study of Precision on C<sub>18</sub> SPE cartridges for Determination Ag, Cd, Cu, Ni, and Pb as a Single Component and as the Mixture Components.

The suitable conditions for determination Cd and Cu by using Cd-PAR complex and Cu-PAR complex respectively were concluded in Table 4.24. These conditions have been employed for each metal ( Ag, Cd, Cu, Ni, Pb ) as a single component and as the mixture components in water.

#### 3.11.1 The Study of Precision of Each Metal as a Single Component

3.11.1.1 The procedure for the study of precision of Cd in water as a single component can be described as follows:

1. Six 5.0 mL of 1.00 ppm standard cadmium solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $4.0 \times 10^{-5}$  M PAR solution. The pH of the solutions were added. The pH of the solution was measured by a pH-meter. The pH probe was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Six volumetric flasks of Cd-PAR complex solution in No.1 were passed through six C<sub>18</sub> SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 12 mm Hg pressure. The flow rate at this pressure was about 6.0 mL/min.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge at the same flow rate in the retention step. Six volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for the study of precision of Cd was prepared as same as step 1 to step 3 but the standard cadmium solution was not added in step 1.

5. Six solutions ( from step 3 ) were aspirated to a flame AAS by using six concentrations of standard cadmium solutions 0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 ppm as a standard calibration curve. The blank solution ( from step 4 ) was aspirate to a flame AAS before sample solutions ( from step 3).

6. The precision result of C<sub>18</sub> SPE cartridges for determination of Cd in this study was shown in Table 4.25.

3.11.1.2 The procedure for the study of precision of Cu, Ag, Ni, and Pb in water as a single component can be done as same as the procedure for the study of precision of Cd in water as a single component which was described above. Table 3.2 has been summarized the desired concentration of each metal in 100.0 mL volumetric flask (before passage through the C<sub>18</sub> SPE cartridge), the PAR concentration for each metal, and the concentration of each metal for each standard calibration curve.

The precision results of C<sub>18</sub> SPE cartridges for determination of Cu, Ag, Ni, and Pb in water as a single component were shown in Tables 4.26 to 4.29, respectively. The results of the study of Ag, Cd, Cu, Ni, and Pb on C<sub>18</sub> SPE cartridge as a single component in water were summarized in Table 4.30.

Table 3.2 The summarization of each metal concentration, PAR concentration, and concentration of each metal for the standard calibration curve for the study of precision on C<sub>18</sub> SPE cartridges

Metal	Metal conc. (ppm)	PAR (M)	Concentration for Standard Calibration Curve (ppm)
Ag	0.10	$4.0 \times 10^{-6}$	0.10, 0.50, 1.00, 1.50, 2.00, 4.00
Cd	0.05	$2.0 \times 10^{-6}$	0.05, 0.10, 0.20, 0.40, 0.80, 1.00
Cu	0.20	$8.0 \times 10^{-6}$	0.10, 0.50, 1.00, 1.50, 2.00, 4.00
Ni	0.20	$8.0 \times 10^{-6}$	0.10, 0.50, 1.00, 1.50, 2.00, 4.00
Pb	0.50	$2.0 \times 10^{-5}$	0.50, 1.00, 2.00, 4.00, 8.00, 10.00

### 3.11.2 The Study of Precision of Ag, Cd, Cu, Ni, and Pb as the Mixture Components

The procedure for the study of precision of Ag, Cd, Cu, Ni, and Pb as the mixture components can be described as follows:

1. Ten 5.0 mL of each metal ( Ag 2.00 ppm, Cd 1.00 ppm, Cu 4.00 ppm, Ni 4.00 ppm, and Pb 10.00 ppm ) was pipetted into into a series of 100 mL beakers containing exact 5.0 mL of  $8.0 \times 10^{-4}$  M PAR solution (at the desired concentrations of each metal which was shown in Table 3.2). The pH of the solutions were adjusted between 7-8 by using the pH adjustment solution. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in volumetric flask. The pH value of the solution was checked again.

2. Ten volumetric flasks of mixture metal-PAR complex solutions in No. 1 were passed through ten C<sub>18</sub> SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 12 mm Hg pressure. The flow rate at this pressure was about 6.0 mL/min.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge at the same flow rate in the retention step. Ten volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for checking the study of precision was prepared as same as step No.1 to No. 3 but the standard solution of each metal was not added in step No.1.

5. Ten solutions ( from No.3 ) were aspirated to a flame AAS by using six concentration solution of each metal for standard calibration curve which were shown in Table 3.2. The blank solution was aspirated before sample solutions.

6. The precision results of these metals were shown in Table 4.31.

### 3.12 The Procedure for the Study of the Accuracy for Solid-Phase Extraction Technique.

The synthetic unknown mixture solutions were prepared to evaluate the accuracy of solid-phase extraction technique. The concentration of each metal ( Ag, Cd, Cu, Ni, Pb, ) was determined by means of the calibration curve method. The procedure was described as follows :

1. Three 75.0 mL of the synthetic unknown solution were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $8.0 \times 10^{-4}$  M PAR solution. The pH of the solutions were adjusted between 7-8 by using the pH adjustment solutions. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in a volumetric flask. The pH value of the solution was checked again.

2. Three volumetric flasks of the solution from No.1 were passed through three C<sub>18</sub> SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 12 mm Hg pressure. The flow rate at this pressure was about 6.0 mL/min.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge at the same flow rate in the retention step. Three volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for checking the accuracy was prepared as same as the step No.1 to No.3 but the synthetic unknown was not added in step No.1.

5. Three solutions (from No.3) were aspirated to a flame AAS. The blank solution was aspirated before sample solutions.

6. The concentration of each metal in aqueous solution was determined from calibration curve.

### 3.13 The Determination of Heavy Metals in Real Samples

Six collected water samples from several places can be described as follows:

A. Purist-drinking water collected from Chem. Bldg. 3 in December 23<sup>rd</sup>, 1992 time 11.55 p.m.

B. British Portacel-drinking water collected from a store in December 23<sup>rd</sup>, 1992 time 8.30 a.m.

C. Tap water collected from Chem. Bldg. 3 in December 24<sup>th</sup>, 1992 time 7.00 a.m.

D. Rain water collected from a collected tank at Chem. Bldg. 1 in December 24<sup>th</sup>, 1992 time 6.30 a.m.

E. River water collected from Chao Phya River at Sathupradit Port in November 9<sup>th</sup>, 1992 time 10.30 p.m.

F. Pool water collected from Chulalongkorn University Pool in December 8<sup>th</sup>, 1992 time 8.30 a.m.

Each collected water samples were filtered by using 0.45  $\mu\text{m}$  membrane filter prior to use the SPE technique. The collected water samples were analyzed by solid-phase extraction technique that can be described as follows:

1. Three 75.0 mL of water sample were pipetted into a series of 100 mL beakers containing exact 5.0 mL of  $1.0 \times 10^{-3}$  M PAR solution. The pH of the solutions were adjusted between 7-8 by using the pH adjustment solutions. Suitable amount of water was added. The pH of the solution was measured by a pH-meter. The pH probe was washed thoroughly with deionized water into the solution which was then made the volume to 100.0 mL in a volumetric flask. The pH value of the solution was checked again.

2. Three volumetric flasks of solutions in No. 1 were passed through three  $\text{C}_{18}$  SPE cartridges which were activated before usage ( see section 3.9 ). The vacuum pump was used to suck the solution downward at 12 mm Hg pressure. The flow rate at this pressure was about 6.0 mL/min.

3. 5.0 mL of 1.0 M nitric acid solution was passed through each cartridge at the same flow rate in the retention step. Three 5.0 mL volumetric flasks were employed as the receivers for each cartridge.

4. The blank solution for the study of real water sample was prepared as same as step No. 1 to No. 3 but the water sample was replaced by deionized water.

5. Three solutions of each water sample and blank solution were aspirated to a flame AAS. By using the calibration curve, the

concentration of each metal in sample water which was treated with C<sub>18</sub> SPE cartridge has been read and recorded by a flame atomic absorption. The concentrations of each metal in water samples can be calculated (see Appendix B).