

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Chemicals and reagents

- 1) 1,5-diphenylcarbazide (Sigma-Aldrich, USA)
- 2) Sodium hydroxide (Merck, Germany)
- 3) Sodium chloride (Carlo erba, Italy)
- 4) Sulfuric acid (J.T. Baker, Thailand)
- 5) Potassium dichromate (BDH Chemicals, UK)
- 6) 2-nitrophenyloctyl ether (NPOE) (Sigma-Aldrich, USA)
- 7) 1-octanol (Sigma-Aldrich, USA)
- 8) 1-heptanol (Sigma-Aldrich, USA)
- 9) Kerosene (local shop)
- 10) methyltrioctylammonium chloride (Aliquat 336) (Merck, Germany)
- 11) Nitric acid 65% (Merck, Germany)
- 12) Ethanol (Merck, Germany)

#### 3.2 Instrument and equipment

- 1) DC power supply 375W, 0-60V, 0-3A (Electronics Source Co., Ltd., Thailand)
- 2) Polypropylene hollow fiber membrane Accurel® Q3/2, 600 µm ID 200 µm thickness, 0.2 µm pore size (Membrana, Wuppertal, Germany)
- 3) Pt wire 0.3 mm diameter (local supplier)
- 4) Fiber optic UV-Vis spectrometer with Z-flow cell (Avantes BV, the Netherlands)
- 5) pH meter model (METTLER TOLEDO, Switzerland and USA)

- 6) Magnetic stirring bar (Spinbar, USA)
- 7) Magnetic stirrer (IKA, Germany)
- 8) Microsyringe 100  $\mu\text{L}$  (Hamilton Company, USA)
- 9) Medical syringe 3 mL (Becton Dickinson and Company, USA)
- 10) Filtration membranes (Nylon membrane filter 47 mm 0.45  $\mu\text{m}$ )  
(Munktell filter, Germany)
- 11) EPA Vial Kit 20  $\mu\text{L}$  (vertical chromatography, Thailand)
- 12) Autopipettes and tips 10  $\mu\text{L}$ , 100  $\mu\text{L}$ , 1000  $\mu\text{L}$  and 10 mL (Eppendorf, USA)

### 3.3 Experiment

#### 3.3.1 Preparation of chemical solutions

##### 3.3.1.1 Stock solution potassium dichromate solution; 1000 ppm

The stock 1000  $\text{mg L}^{-1}$  standard potassium dichromate solution was prepared by dissolving 0.1414 g of potassium dichromate with milli Q water in 50 mL volumetric flask.

##### 3.3.1.2 1,5-diphenylcarbazide; 6.5 mM

The 6.5 mM 1,5-diphenylcarbazide solution was prepared daily by dissolving 0.008 g 1,5-diphenylcarbazide in 5 mL volumetric flask with ethanol.

##### 3.3.1.3 Sulfuric acid; 4 M

The 4 M sulfuric acid was prepared by diluting 11.11 mL of concentrated sulfuric acid in 50 mL volumetric flask and made to the volume with milli Q water.

#### 3.3.1.4 Sodium hydroxide; 1 M

The 1 M sodium hydroxide solution was prepared by dissolving 1 g of sodium hydroxide in 25 mL volumetric flask with milli Q water.

#### 3.3.2 Electrical field assisted liquid phase microextraction procedure

An 8-cm hollow-fiber membrane was immersed in the organic solvent for 1 hr to ensure complete impregnation. The excess organic solvent in the lumen was pushed off and replaced by the acceptor solution. After that, the hollow-fiber membrane was sealed at the end by a sealer. Two 4.5 cm of 0.3 mm i.d. platinum wires were used for electrodes. One was placed in the lumen of the membrane as an anode (+) and the other was placed in the sample solution as a cathode (-). The distance between two electrodes was 1 cm. These electrodes were connected to the power supply. The setup of electrical field assisted liquid phase microextraction is shown in Figure 3.1.

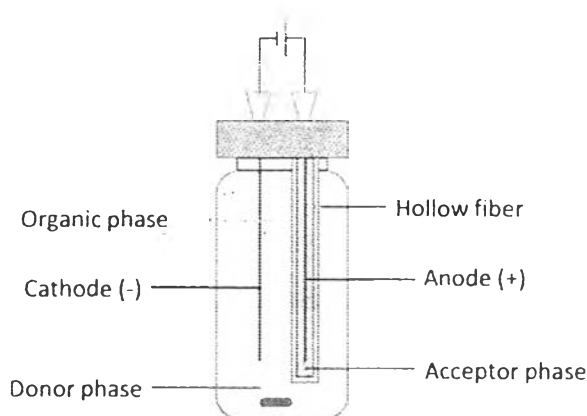


Figure 3.1 Electrical field assisted liquid phase microextraction

#### 3.3.3 Chromium determination method

Chromium(VI) was determined by colorimetric method of Cr-diphenylcarbazide (DPC) complex. The acceptor solution was mixed with 20  $\mu$ L of 6.5

mM DPC and 10  $\mu$ L of 4 M  $H_2SO_4$  solution. The solutions were mixed for 15 min. The violet solution was detected by fiber optic UV-Vis spectrophotometer at 544 nm.

### 3.3.4 Method optimization

#### 3.3.4.1 Types of organic extracting solvent

Organic solvent is one of the crucial parameters that affect extraction efficiency. The organic solvent should have suitable properties such as high electrical conductivity, low vapor pressure and low viscosity. Four types of organic solvents; 2-nitrophenyl octyl ether (NPOE), 1-octanol, 1-heptanol and mixed 1-octanol/NPOE were studied in the electrical field assisted LPME procedure.

#### 3.3.4.2 Aliquat 336 (ionic carrier) concentration

According to literature reviews, metal ions were not easily extracted by using only organic solvent that immobilized in the wall of the membrane. Ionic carrier was sometimes added to the organic solvent for improving the extraction efficiency. Therefore, Aliquat 336 was chosen as ionic carrier and the concentrations of Aliquat 336 were studied in the range of 0-20 % (v/v).

#### 3.3.4.3 Types and concentration of acceptor solution

In ion-exchange mechanism, analytes formed ion-paired complexes with the carrier at the interface between the donor solution and the SLM, then exchanged with the counter ions in the acceptor solution. Accordingly, types and concentration of the counter ions in the acceptor solution could affect the transportation of the analytes. Two types of acceptor solutions; sodium chloride (NaCl) and sodium hydroxide (NaOH) in the range of 0.1-1.5 M were studied in the electrical field assisted LPME procedure.

#### 3.3.4.4 Extraction time and applied voltage

The transportation of analytes through the SLM depends on applied voltage which was described in Nernst-Planck flux equation in section 2.1.4. Thereby, the voltages in the range of 0-50 volts were studied.

For reaching the equilibrium distribution process of the extraction, the extraction time is varied. The extraction times in the range of 1-30 min were studied.

#### 3.3.4.5 Stirring speed

Stirring speed plays an important role in improving the kinetics and extraction efficiency by reducing the thickness of boundary layer around the SLM. Consequently, the stirring speeds in the range of 0-1000 rpm were studied.

### 3.3.5 Method evaluation for extraction

#### 3.3.5.1 Calibration curve, linearity and limit of detection

The linear calibration curve between the absorbance and the concentrations of chromium(VI) was established for the concentration ranging from 3-15  $\mu\text{g L}^{-1}$ . The linear regression method was used to obtain slope, intercept and  $R^2$ .

The limit of detection (LOD) for the method was determined by extraction of blanks and calculated from the Equation 3.1 [51].

$$S_{\text{LOD}} = S_{\text{B}} + 3\text{SD} \quad \text{Equation 3.1}$$

Where  $S_{\text{LOD}}$  is the signal at the limit of detection;  $S_{\text{B}}$  is the signal of blank solution; SD is the standard deviation of blank.

The standard deviation (SD) was calculated from Equation 3.2 [51] because there was no signal of blank in UV-Vis spectrophotometric method.

$$\text{SD} = \sqrt{\frac{\sum (y_i - \bar{y})^2}{n-2}} \quad \text{Equation 3.2}$$

Where  $y_i$  and  $Y_i$  are the signal of analyte measurement from the experiment and from linear equation, respectively as well as  $n$  is the number of concentration of standard solution.

### 3.3.5.2 Precision and accuracy

Replicate extractions of spiked  $10 \mu\text{g L}^{-1}$  of Cr(VI) water samples were analyzed in the same day and for several consecutive days. The standard deviations of concentrations of Cr(VI) obtained were attributed to intra-day precision and inter-day precision, respectively. The accuracy of the method was expressed as %recovery of spiked Cr(VI), which is shown in the equation below.

$$\% \text{ Recovery} = \left( \frac{C_c - C_b}{C_s} \right) \times 100 \quad \text{Equation 3.3}$$

Where  $C_c$  is the concentration of Cr(VI) found in spiked sample, which calculated from the calibration curve;  $C_b$  is the concentration of Cr(VI) found in unspiked sample, which calculated from the calibration curve; and  $C_s$  is the concentration of Cr(VI) standard that spiked into the sample.

### 3.3.5.3 Enrichment factor

The enrichment factor of the method was calculated by the equation below.

$$\text{Enrichment factor} = \frac{C_a}{C_d} \quad \text{Equation 3.4}$$

Where  $C_a$  is the final concentration in acceptor aqueous solution and  $C_d$  is the initial concentration in donor aqueous solution.

### 3.3.5.4 Matrix effect

For application to the real samples, there are various ions in the solution that can interfere the extraction. Thus, three types of common ions; chloride ( $\text{Cl}^-$ ), sulfate ( $\text{SO}_4^{2-}$ ) and hydrogen phosphate ( $\text{HPO}_4^{2-}$ ) were studied.

### 3.3.6 Real samples

Drinking water samples were purchased from local markets in Bangkok. CU pond water was filtered through a membrane filter (0.45  $\mu\text{m}$ ) to remove some matrices. After that, the samples were extracted by the proposed method.