CHAPTER I

INTRODUCTION

1.1 Motivation of proposer

The sample preparation process is an essential step before analysis especially for complex samples and trace level analysis. There are two purposes for this process. One is for separating analytes from sample matrices. The other is for preconcentrating trace analytes, which cannot be directly detected by the instruments. The conventional sample preparation is liquid-liquid extraction (LLE). Despite it is popular, it has many drawbacks; i.e., consuming large amount of sample and organic solvent, laborious procedures, time-consuming, high cost and hazardous for environment. Later, liquid phase microextraction (LPME) has been developed in an effort to overcome these problems. Its principle is based on "like dissolves like" being similar to LLE but it uses much less volume of organic solvent (in microliter level) than LLE. LPME provides high preconcentration factor (so called enrichment factor) in the final extract.

There are several LPME techniques have been reported. One is direct immersion single drop microextraction (DI-SDME) [1], where a droplet of solvent is suspended at the tip of a syringe needle directly immersed in the aqueous sample. One is directly-suspended droplet microextraction (DSDME) [2], where solvent droplets float on the aqueous sample surface itself. One is continuous-flow microextraction (CFME) [3], where a solvent droplet suspended at a syringe tip is inserted in a glass extraction chamber continuously exposed to the sample solution stream. The shortcoming of aforementioned techniques is that the solvent droplet is difficult to handle; i.e., unable to control the droplet size, losing the droplet from the needle tip in high-speed convection and difficult to collect the droplet after extraction. Recently, hollow-fiber membrane liquid phase microextraction (HFM-LPME) has been introduced to overcome such problems.

In HFM-LPME, the extracting solvent is supported by the hollow-fiber membrane, in the pores at the wall of the membrane. HFM-LPME can be operated in

two modes; 2-phase mode and 3-phase mode. In 2-phase mode, the organic phase is immobilized in the pores and filled in the lumen of the membrane. Typically, the 2-phase HFM-LPME is applied for extraction of non-polar analytes. In 3-phase mode, the organic phase is impregnated in the pores of the membrane; so called supported liquid membrane (SLM), and the lumen of the membrane is filled with the aqueous acceptor solutions. Typically, the 3-phase HFM-LPME is applied for extraction of dissociated analytes. Since the mass transfer of the analyte in 3-phase mode is based on passive diffusion from the aqueous donor phase across the organic phase into the aqueous acceptor phase, it usually takes a long extraction time in the ranges 15-60 min [4].

To shorten the extraction time, Pedersen-Bjergaard, S. and Rasmussen, K.E. [5] applied an electrical field in the HFM-LPME system; so called electromembrane extraction (EME), in order to enhance the transportation of charged analytes across the SLM. The analytes could be driven across the SLM by electrokinetic migration. If the analyte is cation, the positive electrode is placed in the sample solution and the negative electrode is placed in the acceptor solution and vice versa for the anion.

From the past studies, there were various types of analytes being extracted by EME technique such as hydrophobic basic drug [6], hydrophilic basic drug [7], acidic drug [8], peptides [9], chlorophenol [10], and metal ions (Pb^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}) [11, 12]. Nevertheless, some metals are normally available in anion forms such as As (as arsenate ion) and Cr (as chromate ion). So, the author is interested in developing an EME method for extraction and preconcentration of metals in anion form.

The author choose chromate ion as a model analyte for this study because it is easy to detect by colorimetric method (by complexation to 1,5-diphenylcarbazide) and it is toxic for life.

1.2 Literature review

Many researches in liquid phase microextraction under electrical field so called electromembrane extraction (EME) have been reported. The diversified

analyte models have been studied that are hydrophobic basic drugs, hydrophilic basic drugs, acidic drugs, peptides and cationic metals. Moreover, different set-ups and determination methods have been proposed for improving their extraction efficiency. Reviews of these works are summarized as follow.

In 2006, Pedersen-Bjergaard, S. and Rasmussen, K. E. [5] proposed a rapid sample preparation technique based on electrokinetic migration across an artificial liquid membrane for extraction of hydrophobic basic drugs; i.e., pethidine, nortriptyline, methadone, haloperidol and loperamide in biological fluids (pK$_a$ = 8.3-9.7 and logP = 1.7-5.2). The EME conditions were: 2-nitrophenyloctylether (NPOE) as an extracting solvent; 300 volts; and 5 min extraction time. After extraction, the acceptor solution in microliter level was analyzed by Capillary Electrophoresis (CE). The first setup was shown in Figure 1.1.
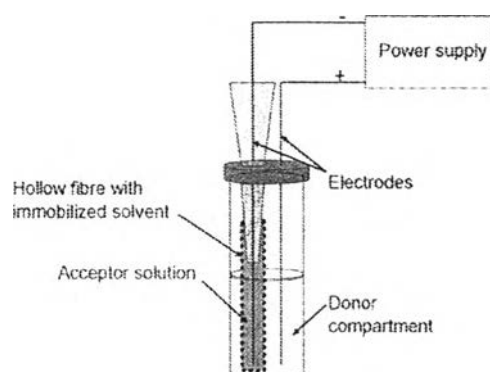

Figure 1.1 Schematic setup of typical electro membrane extraction (EME) [6]

In the same year, Gjelstad, A. et al. [7] studied EME of hydrophilic basic drugs compared to that of hydrophobic basic drugs. In this study, 2-nitrophenyloctylether (NPOE) was used as an extracting solvent for extraction of hydrophobic compounds. For extraction of hydrophilic compounds, an ionic-carrier; i.e., bis-(2-ethylhexyl) phosphate (DEHP) was added to the organic solvent for taking the hydrophilic basic compounds into the acceptor phase through the ion-exchange mechanism as illustrated in Figure 1.2.
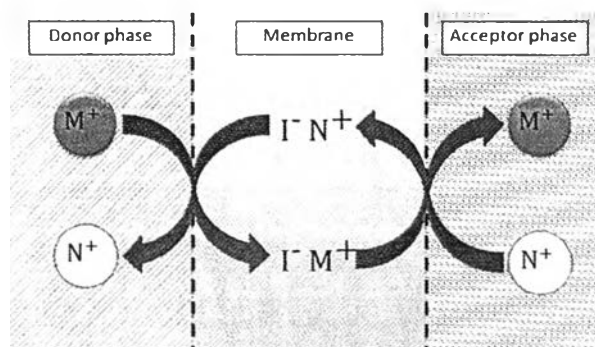
Figure 1.2 Schematic diagram of ion-exchange mechanism in 3-phase HFM-LPME;
I^- = ionic carrier , M^+ = cationic analyte, N^+ = cationic counter ion

In 2007, Balchen, M. et al. studied EME of acidic drugs [8]. The results showed that alcohols enabled extractions of the acidic drug model and 1-heptanol gave the highest efficiency under 50 volts in 5 min. One of the parameters affecting the extraction efficiency is pH adjustment in donor solution and acceptor solution. In the case of basic compounds, the donor and acceptor solution should be acidity for protonation of basic compounds to be cationic species shown in Equation 1.1.

$$B+H^+ \rightleftharpoons BH^+ \qquad\qquad \text{Equation 1.1}$$

On the other hand, for acidic compounds, the donor and acceptor solution should be alkali solution for deprotonation of acidic compounds to be anionic species shown in Equation 1.2.

$$AH \rightleftharpoons A^- + H^+ \qquad\qquad \text{Equation 1.2}$$

From aforementioned passage, these organic model analytes had been studied in both standard solution and biological real samples; i.e., human blood, human plasma, human urine and human breast milk [5, 13, 14].

Later, Gjelstad, A. et al. [15] proposed that the mass transfer of analyte during electromembrane extraction was based on the Nernst-Planck flux equation. as seen in Equation 2.13-2.14.

Where D_i is diffusion coefficient for the charged analyte in the organic membrane, h is the thickness of the SLM, χ is ratio of the total ionic concentration on the donor side to that on the acceptor side (ion balance), C_{ih} and C_{i0} are

concentration of analyte close to the membrane in sample solution and acceptor solution, respectively. v is a dimensionless driving force. z_i is charge of the ith cationic substance, e is the elementary charge, $\Delta\phi$ is the electrical potential across the SLM, k is a constant and T is the temperature. From these equations, both the diffustion coefficient (D_i) and the SLM thickness (h) are constant, the flux of analyte can be improved by increasing the potential difference $\Delta\phi$ or by lowering the ion balance (χ) over the SLM.

In 2008, Kjelsen, I.J.Ã.s. et al. [13] proposed a low voltage EME using a common battery for extraction of basic drugs from biological samples, illustrated in Figure 1.3. The range of potential was 1-10 volt. The low voltage makes the EME technique more simple.
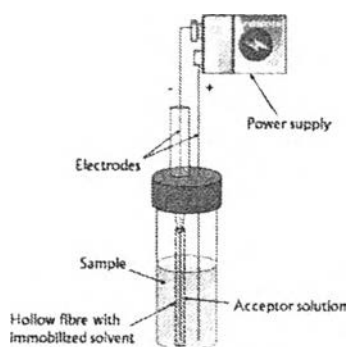

Figure 1.3 Schematic setup of electromembrane extraction (EME) with common battery [16]

Balchen, M. et al studied EME of peptides [9, 17]. Different structures of peptides were studied; i.e., the number of amino peptides, number of ionized groups, amino acid sequence and their polarity. 1-octanol mixed with bis(2-ethylhexyl) phosphate. (DEHP) was chosen as anionic carrier for supported liquid membrane. After extraction, the aqueous acceptor solution was injected to High Performance Liquid Chromatography (HPLC). Wide range of recoveries (0-56%) was found because of their differences in physio-chemical properties of the peptides. It indicated that the polarity and the number of ionized groups were significant parameters for the extraction.

In 2009, Basheer, C. et al. first introduced EME of lead ion (Pb^{2+}) [11]. Toluene was used as the SLM. Phosphoric acid adjusted by disodium tetraborate buffer was used as the acceptor. Lead ion complexed with ethylenediamminetetracetic acid (EDTA) solution was extracted under 300 volts for 15 min. Limit of detection was 0.019 mg L^{-1}. Then, the optimized method was applied to analysis of lead ions in urine, lipstick, blood serum and amniotic fluid samples.

In the same year, Lee, J. et al. studied EME of chlorophenols from seawater [10]. Chlorophenols were extracted by 1-octanol under only 10 volt for 10 min. As chlorophenols are weak acid, the donor and acceptor solution were adjusted to pH 12. Limit of detection and quantification was 0.1 and 0.4 ng mL^{-1}, respectively.

In 2010, Eibak, L.E.E. et al [18] invented the specialized configuration of EME as shown in Figure 1.4. Three parallel extractions were simultaneously performed with a 9 volt battery under stagnant conditions. Amitriptyline, citalopram, fluoxetine, and fluvoxamine were isolated by 1-ethyl-2-nitrobenzene (ENB) as the organic liquid membrane for 1 min. The results showed that it was no significant difference compared to the ordinary single extraction.
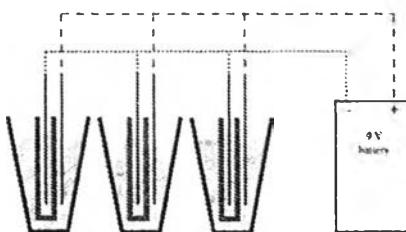

Figure 1.4 Schematic setup of electromembrane extraction (EME) with three parallel extraction [18]

In the same year, Nojavan, S. et al. proposed EME coupled with Capillary Electrophoresis (CE) for extraction and determination of amlodipine enantiomers [19]. In this work, the longer cathode and hollow fiber membrane was used to increase the contact area and mass transfer as shown in Figure 1.5. The SLM was 2-

nitrophenyl octylether (NPOE); the voltage was 200 volts; and the extraction time was 15 min. The limits of detection for both enantiomers were 3 ng mL^{-1}.
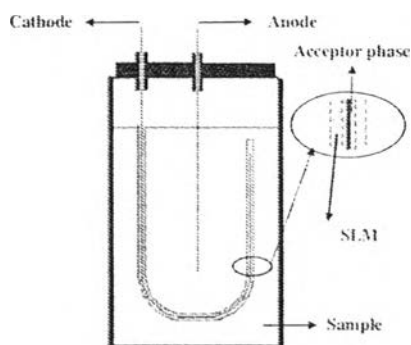

Figure 1.5 Schematic setup of electromembrane extraction (EME) with long cathode [19]

In 2011, Kubán, P. et al. studied EME of several heavy metal ions; i.e., Mn^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} in tap water and milk powder [12]. The extraction solvent was 1-octanol mixed with 0.5% v/v bis(2-ethylhexyl)phosphonic acid; the applied voltage was 75 volts; and the extraction time was 5 min. The acceptor was analyzed for metal ions by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C4D). The detection limit was 25-200 nM.

Payán, M.R. et al. introduced EME coupled with HPLC-diode array detection and fluorescence detection for extraction and determination of non-steroidal anti-inflammatory drugs (NSAIDs) in urban wastewater samples [20]. 1-octanol was used as the SLM; the applied voltage was only 10 volts; and the extraction time was 10 min. High selectivity and no interfering signals were detected.

In the same year, Seidi, S. et al. studied EME of thebaine in water sample, biological fluids, poppy capsule and narcotic drugs [21]. The SLM was 2-nitrophenyl octylether (NPOE); the applied voltage was under 300 volts; and the extraction time was 15 min. Moreover, the ion balance effect also was studied. Increasing ions in both donor and acceptor solutions led to increase in migration of ions through the

SLM producing higher current level and Joule heating that might affect instability of the SLM.

Alhooshani, K. et al. developed EME of haloacetic acids (HAAs) and aromatic acetic acids, which are disinfection by-products (DBPs) from water treatment. Toluene was used as the SLM; applied voltage was 200 volts; and extraction time was 30 min. The recoveries were in the range of 87-106%. The limit of detection was between 0.072 and 40.3 ng L^{-1} [22].

Besides, Petersen, N.J. et al. proposed the new EME configuration that was applicable to lab-on-chip with online ultraviolet and mass spectrometric detection [23]. A 25 μm thick porous polypropylene flat sheet membrane was bonded between two poly(methyl methacrylate) (PMMA) substrates as shown in Figure 1.6. Basic drugs pethidine, nortriptyline, methadone, haloperidol, loperamide, and amitriptyline were extracted by 2-nitrophenyl octylether (NPOE) as the SLM at 15 volts.
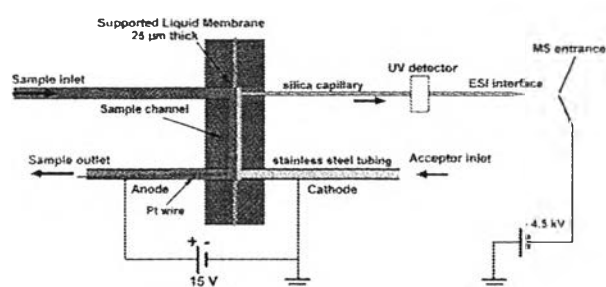

Figure 1.6 Schematic setup of on-chip EME [23]

In 2012, Jamt, R.E.G. et al introduced longer hollow fiber membrane for large volume of acceptor solution as depicted in Figure 1.7 [24]. Six basic drugs from undiluted whole blood were extracted by 1-ethyl-2-nitrobenzene (ENB) as the SLM at 15 volts for 5 min in a totally stagnant system.
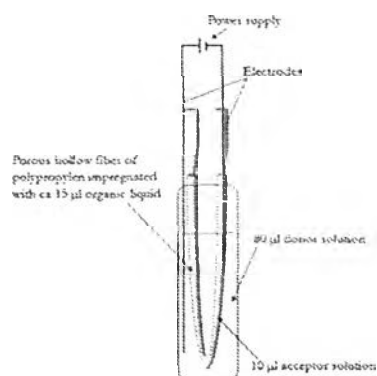
Figure 1.7 Schematic setup of electromembrane extraction (EME) with long hollow fiber [24]

Davarani, S.S.H. et al. invented a home-made glass vial, at the bottom of which, there was a hole for insertion of a syringe to inject and remove the sample solution as shown in Figure 1.8 [25]. The sample solution was displaced before cutting off the voltage to avoid back extraction of analytes from the acceptor solution to the donor solution.
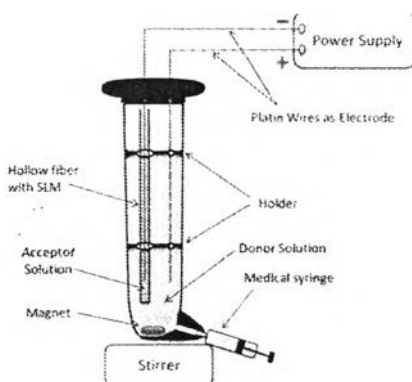

Figure 1.8 Schematic setup of EME with home-made glass vial [25]

In the same year, Šlampová, A. et al. proposed EME system using stabilized constant direct current for extraction of basic drugs and amino acids from human urine and serum samples [26]. 1-ethyl-2-nitrobenzene (ENB) and 2-nitrophenyl octylether (NPOE) were used as the SLM. The extraction performances were compared at 5 min extraction between using constant electric current at 4.5 μA and

using constant voltage at 4 volts. The constant electric current provided significant improvement in repeatability of the extraction process.

Tan, T.Y. et al. presented EME of biological anions including nitrite, adipate, oxalate, iodide, fumarate, thiocyanate and perchlorate [27]. They were extracted using methanol as the SLM at 12 volts for 5 min. EME provided more efficient transport mechanism leading to shorter extraction time. Besides, limits of detection were in the range of 0.01-0.04 μg mL^{-1}.

Moreover, Rezazadeh, M. et al. introduced pulsed electromembrane extraction (PEME) that is a new concept of EME that creates pulsed voltage in combination with common DC constant power supplies [28]. From Figure 1.9, PEME provided increasing extractability by eliminating mass transfer barrier and improved the stability by reducing the thickness of double layer.
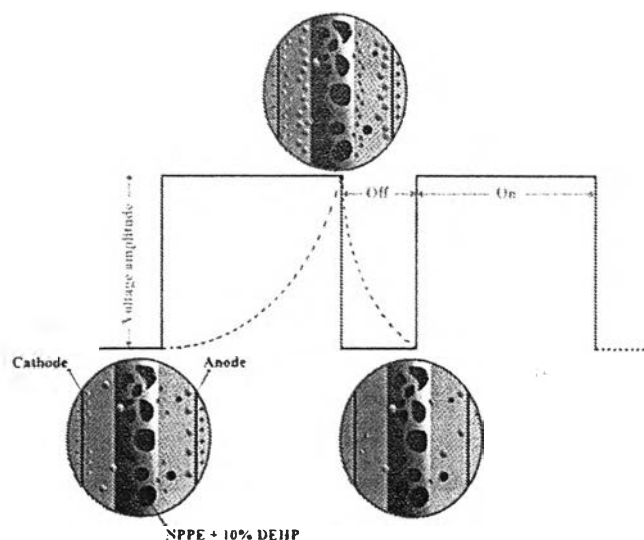

Figure 1.9 Schematic diagram of PEME setup [28]

In 2013, Rezazadeh, M. et al. proposed solid phase microextraction in an association to electromembrane extraction, namely "electromembrane surrounded solid phase microextraction (EM-SPME)" as shown in Figure 1.10 [29]. The ionic analytes migrated from aqueous sample solution through the SLM into the acceptor phase, where the pencil lead was immersed serving as the cathode in EME and as

SPME sorbent. The analytes were absorbed on the carbonaceous cathode (pencil lead). After that, the pencil lead was inserted into the GC injection port for thermal desorption at 280°C. This technique was suitable for analyzing nonvolatile or ionizable compounds. The model analytes were amitriptyline and doxepin. They were extracted using 2-nitrophenyl octylether (NPOE) as the SLM at 120 volts for 20 min.
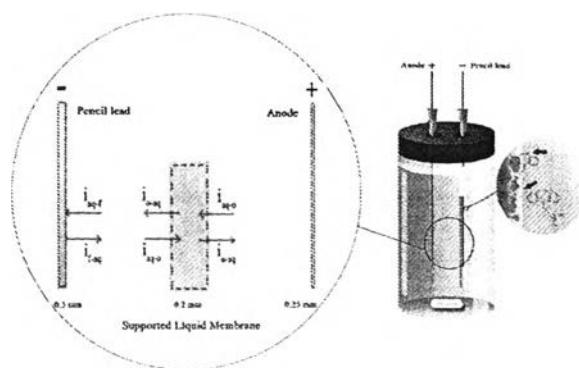

Figure 1.10 Scheme of electromembrane surrounded solid phase microextraction (EM-SPME) [29]

Davarani, S.S.H. et al. studied EME of uranium(VI) using NPOE mixed with 1% DEHP at 80 volts for 14 min with fluorometric detection [30]. The study showed that EME could reduce the interfering effect of other ions in real water samples.

Safari, M. et al proposed dual EME system for speciation of chromium [31]. Both cathode and anode were inserted in two different acceptor solutions in two different hollow fiber membranes as seen in Figure 1.11. Chromium(III) migrated to the cathode while Chromium(VI) moved to the anode. The SLM was 1-octanol; the voltage was 30 volts; and the extraction time was 9 min.
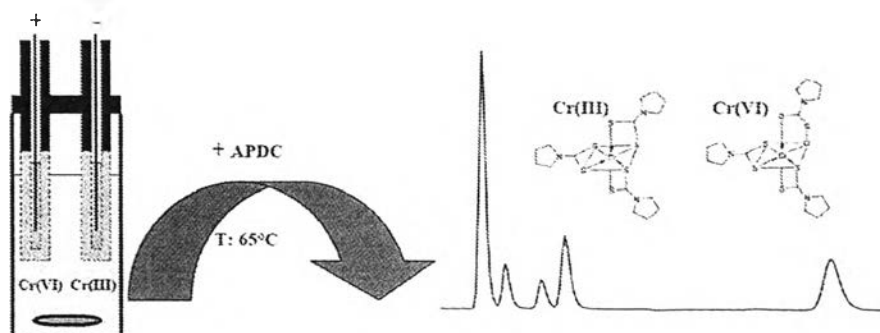
Figure 1.11 Scheme of dual electromembrane extraction [31]

According to the literature reviews, various analyte models were studied including acids, bases, hydrophobic and hydrophilic compounds as well as metal ions such as Pb^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}, which are in cation form. Although, there was one work that extracted metal in anion form by electromembrane extraction, the efficiency was limited and the preconcentration factor was not high enough because pure organic solvent was used as SLM. Consequently, this work was attempted to improve EME efficiency of metal in anion form; i.e., chromate ion as a model, by studying the types of organic solvents and the use of ionic carrier.

1.3 Objective and scope of this research

The objective of this research is to develop a liquid phase microextraction method assisted by an electrical field so called electromembrane extraction for determination and preconcentration of metal in anion form. Chromate ion is selected as a model ion for this study because it is relatively easy to detect by colorimetric method. Besides, Cr(VI) is a toxic species in the environment. Parameters affecting extraction efficiency are investigated such as organic extracting solvent, concentration of ionic carrier, applied voltage and extraction time are investigated and optimized. The method is evaluated and applied to real samples.

1.4 The benefit of this research

      The extraction method for the metal in anion form based on liquid phase microextraction assisted by an electrical field is obtained that can reduce time, and provide high preconcentration.