การพัฒนาวิธีการสกัดระดับจุลภาคด้วยเฟสของเหลวโดยเมมเบรนเส้นใยกลวงแบบหน้าสัมผัสเดี่ยว
สำหรับการตรวจวัดไอออนโลหะและสารอินทรีย์ที่แตกตัวเป็นไอออนได้

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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METHOD DEVELOPMENT OF SINGLE-INTERFACE HOLLOW FIBER

MEMBRANE-LIQUID PHASE MICROEXTRACTION FOR DETERMINATION OF

METAL IONS AND IONIZABLE ORGANIC COMPOUNDS

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รุ่งอรุณ พิมพ์ปรุ : การพัฒนาวิธีการสกัดระดับจุลภาคด้วยเฟสของเหลวโดยเมมเบรนเส้นใยกลวง
แบบหน้าสัมผัสเดี่ยวสำหรับการตรวจวัดไอออนโลหะและสารอินทรีย์ที่แตกตัวเป็นไอออนได้
(METHOD DEVELOPMENT OF SINGLE-INTERFACE HOLLOW FIBER MEMBRANE-LIQUID
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ORGANIC COMPOUNDS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ปกรณ์ วรานุศุภากุล, 61 หน้า.

ในงานวิจัยนี้ได้ทำการศึกษาวิธีการสกัดระดับจุลภาคด้วยเฟสของเหลวโดยเมมเบรนเส้นใยกลวง
แบบหน้าสัมผัสเดี่ยวโดยการสกัดและการชะสารที่ต้องการวิเคราะห์ภายในท่อของเมมเบรนเส้นใยกลวงด้าน
เดียวกัน ซึ่งงานนี้ได้ใช้โครเมียม (VI) และกรดซาลิไซลิกเป็นต้นแบบในการศึกษา วิธีการสกัดระดับจุลภาค
ด้วยเฟสของเหลวโดยเมมเบรนเส้นใยกลวงแบบหน้าสัมผัสเดี่ยวได้มีการออกแบบให้สารละลายตัวอย่างไหล
ผ่านเข้าไปในท่อที่แคบของเมมเบรนมีผลทำให้ความดันภายในท่อเมมเบรนเพิ่มขึ้น ดังนั้นจึงพบเห็นการรั่วซึม
ของตัวทำละลายอินทรีย์ออกมาด้านนอกของผนังเมมเบรนซึ่งปัญหานี้สามารถทำให้ลดลงได้ด้วยการปรับ
สมดุลความดันของการไหลของสารละลายภายในท่อ นอกจากนี้ยังได้ทำการศึกษาพฤติกรรมของโครเมียม
(VI) และกรดซาลิไซลิกที่เกิดขึ้นในระบบการสกัดระดับจุลภาคด้วยเฟสของเหลวโดยเมมเบรนเส้นใยกลวง
แบบหน้าสัมผัสเดี่ยว และศึกษาหาสภาวะที่เหมาะสมที่ส่งผลต่อประสิทธิภาพการสกัดซึ่งแสดงด้วยค่าเอนริช
เมนท์แฟคเตอร์ เช่น อัตราการไหลเข้า อัตราการชะ ตัวทำละลายอินทรีย์และเวลาที่ใช้ในการสกัด สำหรับ
การตรวจสอบประสิทธิภาพของวิธีการสกัดภายใต้สภาวะที่เหมาะสมของการทดลองพบว่า ช่วงความเป็น
เส้นตรงของการสกัดโครเมียม (VI) ในช่วงความเข้มข้น 5 ถึง 30 ไมโครกรัมต่อลิตรให้ค่าสัมประสิทธิ์ความ
เป็นเส้นตรง (R^2) เท่ากับ 0.9994 และค่าความเข้มข้นต่ำสุดที่ตรวจวัดได้ของโครเมียม (VI) คือ 1.2
ไมโครกรัมต่อลิตร สำหรับช่วงความเป็นเส้นตรงของการสกัดกรดซาลิไซลิกในช่วงความเข้มข้น 0.25 ถึง 2.00
มิลลิกรัมต่อลิตรให้ค่าสัมประสิทธิ์ความเป็นเส้นตรง (R^2) เท่ากับ 0.9990 และค่าความเข้มข้นต่ำสุดที่
ตรวจวัดได้ของกรดซาลิไซลิก คือ 0.2 มิลลิกรัมต่อลิตร วิธีการสกัดนี้สามารถนำไปประยุกต์ใช้ในการสกัด
โครเมียม (VI) ในตัวอย่างน้ำได้ค่าเปอร์เซ็นต์การกลับคืนของโครเมียม (VI) อยู่ในช่วง 92% ถึง 111% และ
ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 10% และนำไปใช้ในการสกัดกรดซาลิไซลิกในตัวอย่างยาได้ค่า
เปอร์เซ็นต์การกลับคืนของกรดซาลิไซลิกอยู่ในช่วง 92% ถึง 103% และค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อย
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RUNGAROON PIMPARU: METHOD DEVELOPMENT OF SINGLE-INTERFACE HOLLOW FIBER MEMBRANE-LIQUID PHASE MICROEXTRACTION FOR DETERMINATION OF METAL IONS AND IONIZABLE ORGANIC COMPOUNDS. ADVISOR: ASST. PROF. PAKORN VARANUSUPAKUL, Ph.D., 61 pp.

A concept of single-interface hollow fiber liquid phase microextraction (HF-LPME), where the target analytes was extracted and eluted inside the lumen of the HF membrane was explored. Cr(VI) and salicylic acid (SA) were used as the model for this study. The single-interface HF-LPME was designed. The sample solution was fed into the narrow lumen of the membrane resulting in the increased positive pressure inside the lumen, thus the leakage of organic solvent to the outer of the membrane wall was observed. This problem was overcome by flow balancing pressure. The single-interface HF-LPME behaviors of Cr(VI) and salicylic acid were discussed. The parameters influenced the extraction efficiency on the enrichment factor such as inlet flow rate, elution flow rate, organic solvent and extraction time were optimized. The performance of the method was evaluated under the optimal conditions. For Cr(VI), the working range of 5 to 30 µg L^{-1} (R^2 = 0.9994) and the limits of detection of 1.2 µg L^{-1} were obtained. For salicylic acid, the working range of 0.25 to 2.00 mg L^{-1} (R^2 = 0.9990) and the limit of detection of 0.2 mg L^{-1} were obtained. The proposed methods were successfully applied to a various water samples and drug samples. The recoveries of Cr(VI) spiked water samples were in the range 92 to 111% with RSD less than 10%. The recoveries of salicylic acid spiked drug samples were in the range 92 to 103% with RSD less than 3%. The method is simple to operate, cheap and utilizing of less organic solvent, which is environmental friendly.

Department:	Chemistry	Student's Signature ....
Field of Study:	Chemistry	Advisor's Signature ....
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CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATION

LLE	Liquid-liquid extraction
LPME	Liquid-phase microextraction
SDME	Single-drop microextraction
HF-LPME	Hollow-fiber membrane liquid phase microextraction
SLM	Supported liquid membrane
SBME	Solvent bar microextraction
PP	Polypropylene
Aliquat336	methyltrioctylammonium chloride
DPC	1,5-diphenylcarbazide
EAAS	Electrothermal atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
HPLC	High performance liquid chromatography
MS	Mass spectrophotometry
ICP	Inductively coupled plasma spectrometry
EPA	United States Environmental Protection Agency
EF	enrichment factor
RSD	Relative standard deviation
mg L^{-1}	milligram per liter
μg L^{-1}	micro per liter
μm	micrometer
mm	millimeter
cm	centimeter
mL	milliliter
μL	microliter
g	gram
min	minute

CHAPTER I

INTRODUCTION

1.1 Motivation proposer

Liquid phase microextraction (LPME) is the extraction of target analyte from the aqueous sample into a microvolume of a water-immiscible organic solvent that has been developed to overcome some weaknesses of traditional liquid-liquid extraction (LLE). LPME has been reported in various configurations such as single drop microextraction (SDME), dispersive liquid liquid microextraction (DLLME) and hollow fiber membrane liquid phase microextraction (HF-LPME). SDME has been suffered from the stability and reproducibility of the drop. DLLME is difficult to collect the extract from the top or the bottom of the sample tube with a syringe. HF-LPME is one attractive technique that the organic solvent is protected inside the pores of HF membrane. The advantages of HF-LPME are low cost, simple to operate, utilizing less organic solvent, providing high enrichment factor and easy to be automated.

HF-LPME can be performed in two modes; two-phase and three-phase modes. In two-phase mode, the organic solvent is impregnated in the pores of the HF membrane, so-called supported liquid membrane (SLM) and filled inside the lumen as the acceptor solution. The analyte from the aqueous sample is extracted into the SLM and transferred across the membrane into the acceptor solution (the same organic solvent). Two-phase HF-LPME is usually exploited for extraction of non-ionized or non-polar organic compounds. For three-phase mode, it is similar to two phase mode but the lumen of the HF membrane is filled with an acceptor solution. Three-phase HF-LPME has been widely used for extraction of the ionizable organic compounds or the ionic analytes via extraction-back extraction process or ion exchange process. The former process, the ionizable organic compounds are usually adjusted to the non-ionized form and partitioned into the organic SLM and turned into the ionizable form in the acceptor solution. For the latter process, the addition of ionic carrier in the organic SLM, the ionic analyte exchange with counter ion of ionic carrier at the interface between the SLM and the sample solution to form ion-

paired complex, which diffuses into the SLM and further releases the ionic analyte at the interface between the acceptor solution and the SLM via exchanging with counter ion containing the acceptor solution [1-5].

Recently, HF-LPME has been developed for incorporation with a flow-based system [6-8]. Various on-line/automation configurations involving the feeding system have been designed. Generally, before the extraction, the organic solvent is pumped to fill in the pores of the membrane and then the excess organic solvent in the lumen is flushed out. The sample solution flows outside the membrane and the acceptor solution is held stagnantly in the lumen of the membrane during the extraction. After each extraction, the inside and outside of the membrane is cleaned up with the organic solvent and water, respectively to prepare new organic extracting phase for subsequent experiment. Although these systems are developed to fully automate, the configurations are complicated and the membrane clean-up step may be taken a long time.

In this work, the single-interface HF-LPME where the analyte extracted and then eluted inside the lumen of the HF membrane similar in-tube SPME was explored as an alternative setup for simple on-line HF-LPME. Cr(VI) and salicylic acid (SA) were used as the model of inorganic ion and ionizable organic compound. The analytes would be easily detected by a colorimetric method.

1.2 Literature review

Many publications in HF-LPME have been reported with respect to the application for the determination of ionic analytes or metal ions, non-polar organic compounds and ionizable organic compounds in various samples. Reviews of these publications are summarized as follow:

1.2.1 Literature reviews for HF-LPME of ionic analytes or metal ions

In 2007, Peng et al. [9] reported three-phase HF-LPME coupled with graphite furnace atomic absorption spectrometry (GFAAS) for the determination of Cd(II) in sea water. The Cd(II) in the sample solution was extracted into 1-octanol containing a

mixture of dithizone as carrier and oleic acid which was immobilized in the pores of the membrane and further back-extracted into microvolume of 0.05 M HNO$_3$ as acceptor solution filled in the lumen of the membrane. The detection limit of 0.8 ng L^{-1} and a relative standard deviation of 2.5% at 50 ng L^{-1} level were achieved.

In the same year, Xia et al. [10] employed two-phase HF-LPME prior to low-temperature vaporization ICP-MS for the trace element determination of Cu, Zn, Pd, Cd, Hg, Pb and Bi in environmental and biological samples. The anlytes were complexed with diethyldithiocarbmate (DDTC) and extracted into carbon tetrachloride and determined with low-temperature ETV-ICP-MS. DDTC was used as both complexing agent and chemical modifier; moreover, it was promoted to the enhancement of analytical signal. The enrichment factors in the range of 20–305 for the target analytes were obtained with the extraction time of 15 min.

In 2009, Jiang et al. [11] proposed two-phase HF-LPME for speciation of As(III) and As(V) in fresh water and human nail extracts. As(III) could form complex with ammonium pyrroldinedithiocarbamate (APDC) to As(III)-APDC in pH range of 3.0-4.0 and then was extracted into the toluene as the extracting solvent and the acceptor solution. After that, the organic solvent was withdrawn into a microsyringe and directly injected to electrothermal atomic absorption spectrometry (ETAAS) for the determination. For As(V) remaining in the sample solution was reduced to As(III) by l-cysteine to As(III) before formimg complex with APDC. The enrichment factor of 78 was achieved at the extraction time of 10 min.

In the same year, Saleh et al. [12] developed HF-LPME combined with HPLC for extraction and determination of ultra-trace amount of Se(IV) after derivatization in natural water and biological samples (urine and plasma). Se(IV) was reacted with o-phenylenediamine to form piazselenol and extracted into 20 µL of 1-octanol located in the lumen of a hollow fiber. Finally, this solution was injected into HPLC-UV for determination. Under the optimal conditions, preconcentration factor of 130 was accomplished and the relative standard deviation (%RSD) of the method was < 3.7%.

In 2010, Jafar, A. et al. [13] presented HF-LPME coupled with ETAAS for the determination of lead (Pb) and nickel (Ni) in environmental and biological samples.

The ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate, [C6MIM][PF6] as the extracting solvent was employed due to environmentally friendly solvents. In this work, APDC was chosen as chelating agent for Pb and Ni. The optimized extraction time was 12 and 15 min for Ni and Pb, respectively.

In 2012, López-García et al. [14] reported three-phase HF-LPME combined with ETAAS for the determination of Hg(II) in water samples. The sample solution adjusted at pH 7 by a phosphate buffer. Hg(II) was reacted with 1-(2-pyridylazo)-2-naphthol (PAN) as the chelating agent to form the Hg–PAN complex and transferred to toluene as the SLM and then back-extracted in a ammonium iodide solution contained in the lumen of the membrane. The detection limit of 0.06 µg L^{-1} and the enrichment factor of 270 were obtained.

1.2.2 Literature reviews for HF-LPME of organic compounds

In 2010, Saraji et al. [15] proposed three phase HF-LPME combined with electrospray ionizationion mobility spectrometry (HF-LPME-ESI-IMS) was used for the determination of pentazocine in urine and plasma samples. The sample solution was adjusted at pH=9 so that the analyte was deionized, while the acceptor solution would be acidified to be sufficient for ionizing the analytes to enhance the transport of analyte across the SLM into the acceptor solution. The 0.5 M acetic acid was chosen as acceptor solution and extraction time was 30 min. The limit of detection and relative standard deviation of the method were 2 ng mL^{-1} and 5.3%, respectively.

In 2011, Sun et al. [16] proposed two phase HF-LPME as a clean-up procedure for the determination of eight organophosphorus pesticides (OPPs) in fish tissue. The OPPs in fish samples were first extracted with acetone and evaporated. After that, the extract was redissolved with water–methanol (95:5, v/v) solution, followed by polyvinylidene difluoride (PVDF) HF-LPME. O-xylene was used as the SLM and the acceptor solvent and the extraction time was 30 min. The limits of detections were in the range of 2.1–4.5 ng g^{-1}.

In 2012, Wang et al. [17] studied two phase HF-LPME combined with UHPLC–MS/MS for determine seven pesticides in cucumber samples. In this work, porous polyvinylidene fluoride (PVDF) hollow fiber was used. Chloroform as extracting phase was filled in the pores and the lumen of the membrane. Pesticides were extracted from the sample solution to chloroform and then desorbed in a mixture of methanol:water (1:1 v/v) prior to chromatographic analysis. The extraction time was 20 min and the enrichment factor in the ranges of 100 to 147.

In 2013, González-Curbelo et al. [18] developed HF-LPME for the determination of a group of organophosphorus pesticides in two commercial cereal-based baby foods and one wheat flour prior to gas chromatography-nitrogen phosphorus detection. Before HF-LPME procedure, sample were prepared by ultrasound-assisted extraction with acetonitrile (ACN) containing 1.25% (v/v) of formic acid and then evaporation and reconstitution in Milli-Q water. After that, the organophosphorus pesticides in sample solution at pH 7.0 was extracted to 1-octanol as extraction solvent at extraction time of 45 min and was applied followed by a desorption step in ACN. The limits of detection were between 0.29 and 3.20 µg kg^{-1}.

In 2014, Wang et al. [19] reported three phase HF-LPME combined with electrochemiluminescence detection (ECL) for kanamycin sulfate (KAM) in milk and water sample Ru(bpy)$_3^{2+}$ was used ECL luminophore due to its unique photochemical propertie and solubility in a variety of solvent media. The phosphate buffer solution (PBS) containing kanamycin sulfate (pH 8) as donor phase, ionic liquid of 1-octyl-methylmidazolium hexafluorophosphate ([OMIM]PF6) as extracting solvent and aqueous solution (pH 10) as acceptor phase were used for the extraction. The optimal experimental conditions were ECL detection solution pH of 8, donor phase pH of 9 and extraction time of 50 min. The limit of detection (LOD) was 0.67 µg L^{-1}.

In the same year, Yong et al. [20] presented the determination of total thyroxine in human serum. The aqueous sample was acidified and alkyl sulfonic acid salt was added as ion-pairing agent to promote the transport of thyroxine across the 1-octanol SLM into 1 M ammonia solution as acceptor solution at the extraction time of 30 min. The limit of detection of 0.3 ng g^{-1} with a relative standard deviation of o1.3%.were obtained.

In 2016, Fransco et al. [21] reported HF-LPME combined with gas chromatography mass spectrometry for of amphetamines and methamphetamine in urine samples. The aqueous sample was basicified to pH > 12 with 1.0 mol L^{-1} NaOH. The pores of membrane was immobilized with dihexyl ether as extracting solvent and 30 μl of 0.01 mol L^{-1} HCl was filled in the lumen of the membrane. After extraction, the acceptor solution was dried under N_2 stream and the residue was derivatized with trifluoroacetic anhydride prior the injection to GC-MS. The limits of detection were 10 and 20 μg L^{-1} for amphetamine and methamphetamine, respectively.

1.2.3 Literature reviews for flow-based HF-LPME

In 2012, Yamini et al. [22] presented the first automated instrument based on on-line HF-LPME coupled with high performance liquid chromatography (HPLC) for the preconcentration and determination of pyridine and pyridine derivatives. The system consisted of an automated syringe pump for loading the supported liquid membrane and acceptor solvents, a platform lift for moving the sample vial, a sampling loop for on-line injection of the extract to HPLC, along with an electronic board with an AVR microcontroller for storage of data and instrument programs as shown in Figure 1.1. A sample preparation-HPLC method was developed that allowed sample extraction and extract injection to be carried out completely automatically. The extraction time was 40 min and the preconcentration factors were between 40 and 220.
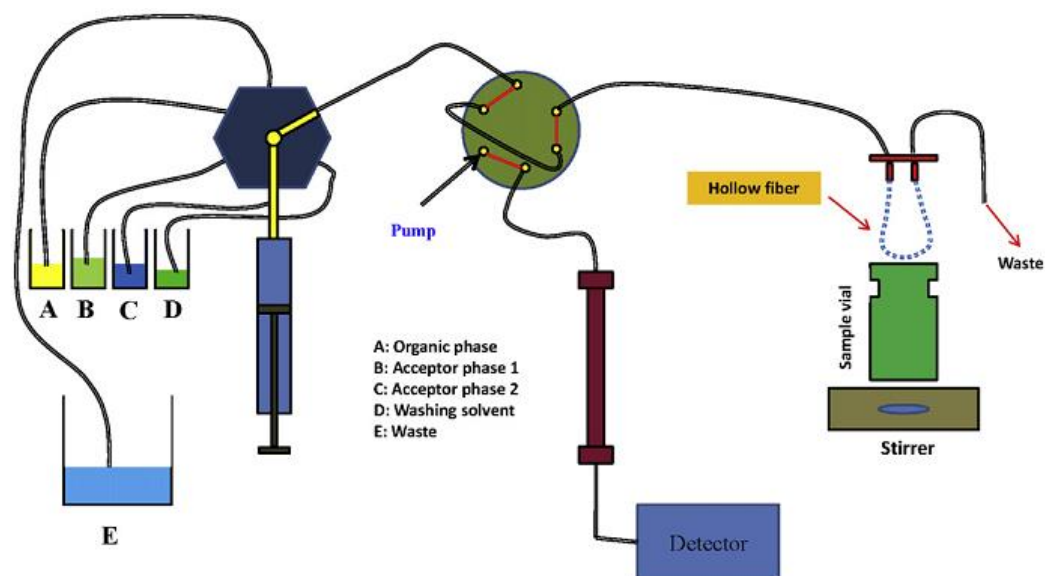
Figure 1.1 Schematic diagram of the on-line automated HF-LPME device [22]

The TT-tube extraction device consisting of two stainless steel tee (T)-connectors attached a stainless steel tube, inside which the HF membrane mounted was presented for an automated dynamic 2-phase HF-LPME [23, 24]. The n-octanol as organic solvent was pumped into the lumen to impregnate in the pores of the HF membrane at the flow rate of 3 μL s^{-1} and then the acceptor solution was filled into the lumen using the syringe pump. The sample solution was pumped along the outside of the HF membrane at the flow rate of 50 mL min^{-1} using the peristaltic pump for 30 min. After each extraction, the inside and outside of the HF membrane were washed with organic solvent and water, respectively for subsequent experiments as shown in Figure1.2.
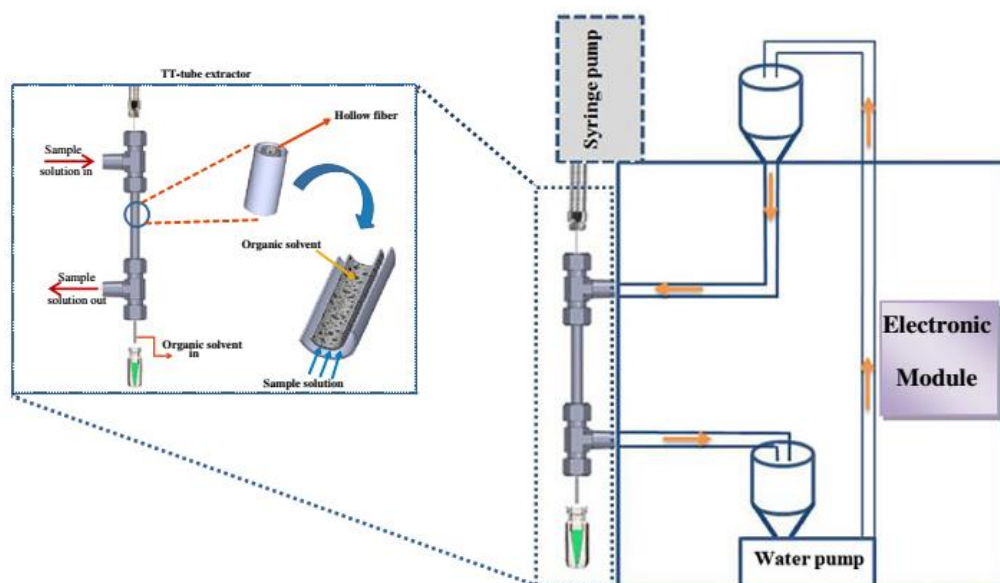
Figure1.2 Schematic setup of dynamic HF-LPME apparatus [23].

In 2013, Sira et al. [8] developed the hybrid flow analyzer based on the sequential injection analysis (SIA) for automation of the overall membrane extraction procedure including in-line immobilization of the organic solvent in pores of the polypropylene HF via programmable flow as shown in Figure 1.3. This method was explored by using Cr(VI) as the model, 10 % (v/v) methyltrioctyl ammonium chloride in kerosene as the SLM and 1,5-diphenylcarbazide as the acceptor solution. The sample volume of 2.8 mL and the extraction time of 4.5 min were achieved with an enrichment factor of ca. 11 and the limit of detection of 4.6 µg L^{-1}. The HF membrane could be used and regenerated the SLM approximately 100 analysis cycles without the decrease of the extraction efficiency.
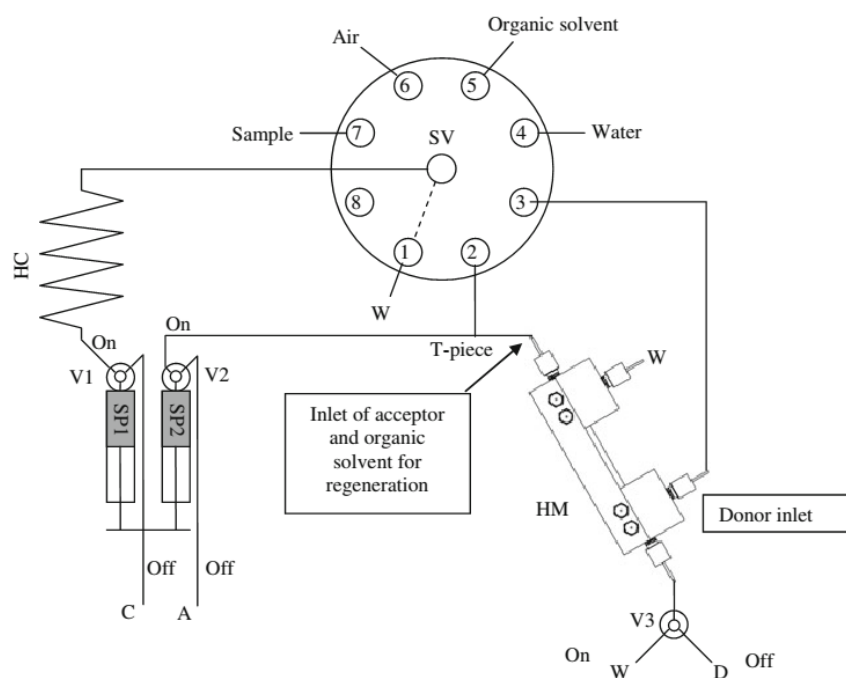
Figure 1.3 Schematic diagram of the hybrid flow analyzer combined HF-LPME. Holding coil (HC),three-way solenoid valve (V), syringe pump (SP), carrier solution (C), acceptor solution (A), selection valve (SV), waste (W), HF-LPME module (HM), and miniaturized CCD spectrophotometer (D) [8].

From the literature review mentioned above, HF-LPME can be applied for the determination of ionic analytes or metal ions, non-polar organic compounds and ionizable organic compounds in various samples. This method has been developed for on-line/automated HF-LPME based on flow system. However, flow-based configuration of the previous publication [8], the sample volume was limited at 2.8 mL and the increase of the extraction efficiency via convection was limited by forward-backward flow. Moreover, after each extraction, the clean-up step of the inside and outside of membrane may be taken a long time. Thus, this work proposed the new concept of single-interface HF-LPME where the analyte extracted and then eluted inside the lumen of the HF membrane.

1.2 Objective and scope of this reach

The objective of this research is to explore the concept of single-interface HF-LPME. The design of the single-interface HF-LPME system was examined. Cr(VI) was chosen as the model of inorganic ions and salicylic acid (SA) as the model of ionizable organic compound. The extraction characteristics were studied. Parameters affecting extraction efficiency were optimized such as inlet flow rate, outlet flow rate, elution flow rate, organic solvent as SLM, pH of acceptor solution and extraction time. The performance of methods were evaluated and applied to water samples and drug samples.

1.3 The benefit of this research

To understand the concept of single-interface HF-LPME for determination of ionic compounds and the option to adapt the concept to design the system for incorporation of HF-LPME with a flow-based system.
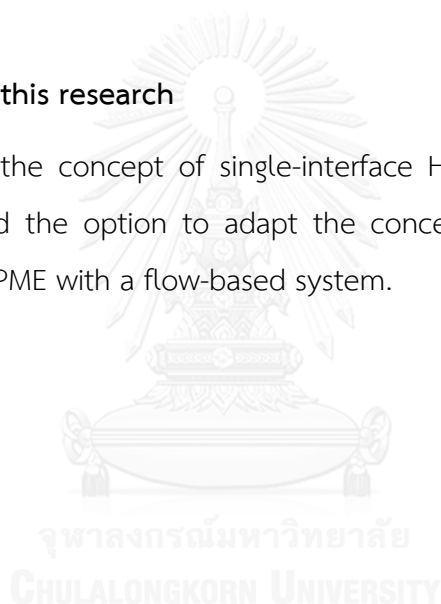
CHAPTER II

THEORY

2.1 Hollow fiber-liquid phase microextraction (HF-LPME)

Hollow-fiber membrane liquid phase microextraction (HF-LPME) has been developed to improve the stability of the extracting solvent which is protected inside the pores of the membrane. The porous membrane is not only utilized to support and protect the extracting solvent but also worked as a filter to prevent large molecule to enter the solvent. Generally, the used hydrophobic membranes are polypropylene (PP), polyvinylidene difluoride (PVDF) and polytetrafluoroethylene (PTFE). Polypropylene membranes are usually chosen owing to their hydrophobicity and compatibility with a wide range of organic solvents [25]. Typical dimensions of the membranes are internal diameter of 600 µm, wall thickness of 200 µm, and pore size of 0.2 µm as illustrated in Figure 2.1.
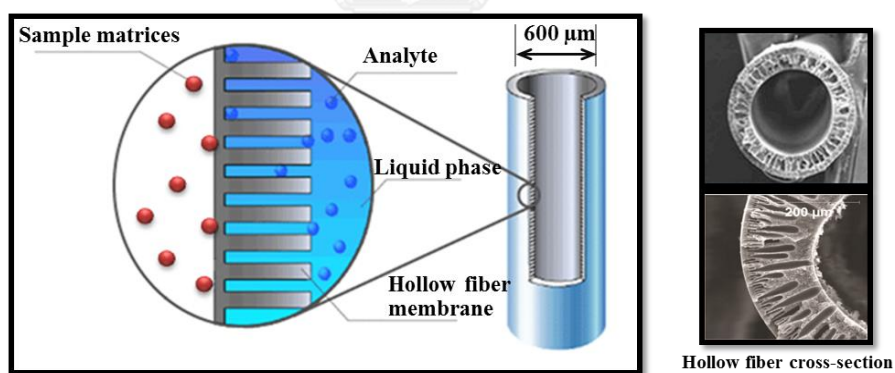
Figure 2.1 Hollow fiber membranes (adapted from [26]).

In HF-LPME, the organic solvent is immobilized in the pores of the membrane by capillary forces, so-called supported liquid membrane (SLM) and the acceptor solution is filled in the lumen of the membrane. The organic solvent considered for extracting solvent must be immiscible with water, strongly immobilized within the fiber pores, low volatility to void losses and compatibility with the selected analytical technique. The analytes are extracted from the aqueous sample across the

SLM and further into the acceptor solution based on diffusion. After the extraction, the acceptor solution is removed for analysis by the analytical instrument. There are several configurations set up as shown in Figure 2.2.
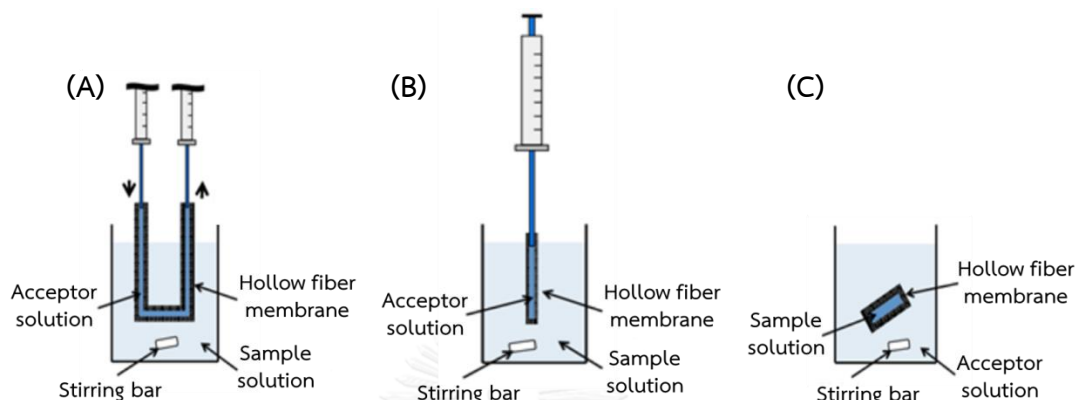
Figure 2.2 Different configurations of HF-LPME: (A) U-shaped; (B) rod-like; and (C) SBME [27].

In addition, HF-LPME can be divided in two modes; two-phase and three-phase modes as illustrated in Figure 2.3.
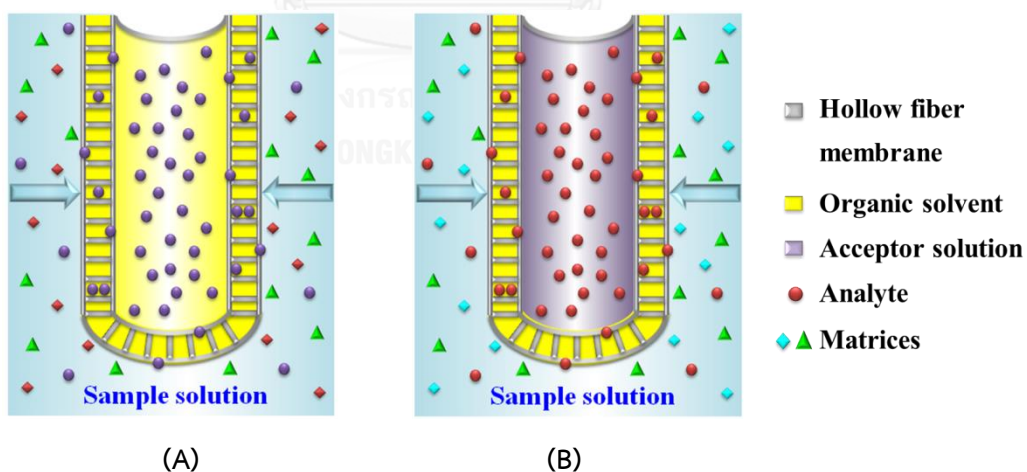
Figure 2.3 Modes of HF-LPME: (A) three-phase HF-LPME and (B) two-phase HF-LPME.

2.1.2 Two-phase HF-LPME

The analyte is extracted from the aqueous sample into the organic solvent filled in the pores and inside the lumen of the HF membrane (acceptor phase). After the extraction, the organic acceptor phase is directly analyzed with GC or normal-phase HPLC. The extraction process is based on passive diffusion, which depends on the partition coefficient of the analyte between the acceptor phase and the donor phase ($K_{a/d}$). This process is shown in equation 2.1 and partition coefficient ($K_{a/d}$) is written as equation 2.2 [28].

$$A_{donor} \rightleftharpoons A_{acceptor} \qquad \text{Equation 2.1}$$
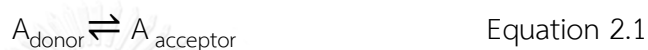
$$K_{a/d} = \frac{C_{eq,\,a}}{C_{eq,\,d}} \qquad \text{Equation 2.2}$$

where A is the analytes; $K_{a/d}$ is partition coefficient of the analyte between acceptor solution and the donor solution; $C_{eq,\,a}$ and $C_{eq,\,d}$ is the concentration of the analyte in the acceptor and donor solution at equilibrium, respectively.

The two-phase system is applied for the extraction of nom-polar organic compounds with high solubility in non-polar organic solvents. For the ionizable organic compounds such as acidic or basic organic compounds, the solubilities are low in non-polar organic solvents. Thus, pH of the aqueous sample is adjusted in order that the organic compounds are in non-ionized form, which are extracted into the organic acceptor phase. The extraction efficiency is increase and can be calculated by the equation below

$$E = \frac{K_{a/d}V_a}{K_{a/d}V_{org} + V_d} \qquad \text{Equation 2.3}$$

where $K_{a/d}$ is partition coefficient of the analyte between acceptor solution and the donor solution; V_a is volume of acceptor solution and V_d is volume of donor solution.

2.1.3 Three-phase HF-LPME

The extraction process is similar to two-phase HF-LPME but the acceptor phase is aqueous solution, which is compatible with CE or HPLC. The partition coefficient of the analyte between the acceptor solution and the donor solution ($K_{a/d}$) and the partition coefficient of the analyte between the acceptor solution and the organic phase ($K_{a/o}$) play important role for the extraction. This process is illustrated in equation 2.4 and partition coefficients are written as the following equation 2.5-2.8 [29].

$$A_{aqueous\ donor} \rightleftharpoons A_{organic\ phase} \rightleftharpoons A_{aqueous\ acceptor} \qquad \text{Equation 2.4}$$

$$K_{o/d} = \frac{C_{eq,\ o}}{C_{eq,\ d}} \qquad \text{Equation 2.5}$$

$$K_{a/o} = \frac{C_{eq,\ a}}{C_{eq,\ o}} \qquad \text{Equation 2.6}$$

$$K_{a/d} = K_{o/d} \times K_{a/o} \qquad \text{Equation 2.7}$$

$$K_{a/d} = \frac{C_{eq,\ a}}{C_{eq,\ d}} \qquad \text{Equation 2.8}$$

where A is the analyte; $K_{o/d}$ is partition coefficient of the analyte between the organic phase and donor solution; $K_{a/o}$ is partition coefficient of the analyte between the acceptor solution and the organic phase; $K_{a/d}$ is partition coefficient of the analyte between the acceptor solution and the donor solution; $C_{eq,\ a}$, $C_{eq,\ o}$ and $C_{eq,\ d}$ is the concentration of the analyte in the acceptor solution, organic phase and the donor solution at equilibrium, respectively.

The three-phase system is usually used for the extraction of the ionizable organic compounds or the ionic analytes via extraction-back extraction process or ion exchange process, respectively. The former process, the ionizable organic compounds are adjusted to the non-ionized form and partitioned into the organic SLM and further back-extracted by turning into the ionizable form in the acceptor solution. For the latter process, the addition of ionic carrier in the organic SLM, the ionic analyte exchange with counter ion of ionic carrier at the interface between the

SLM and the sample solution to form ion-paired complex, which diffuses into the SLM and further releases the ionic analyte at the interface between the acceptor solution and the SLM via exchanging with counter ion containing the acceptor solution as illustrated in Figure 2.4 [30].
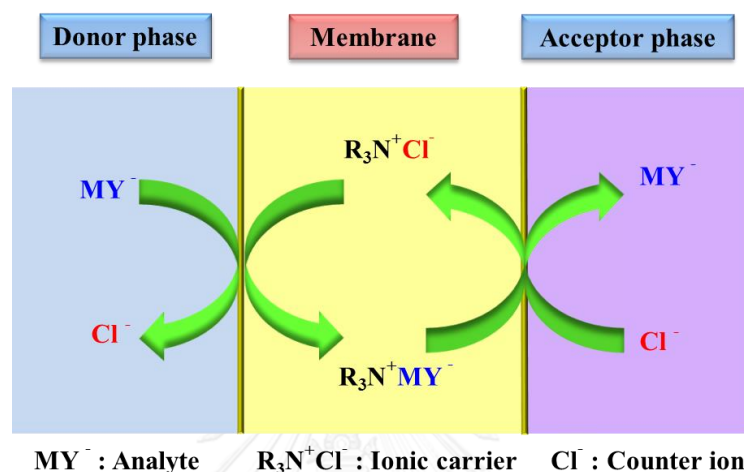
Figure 2.4 Carrier-mediated transport in HF-LPME.

The carriers are employed into three-phase HF-LPME that are neutral carrier, anionic carrier and cationic carrier as shown in Figure 2.5.
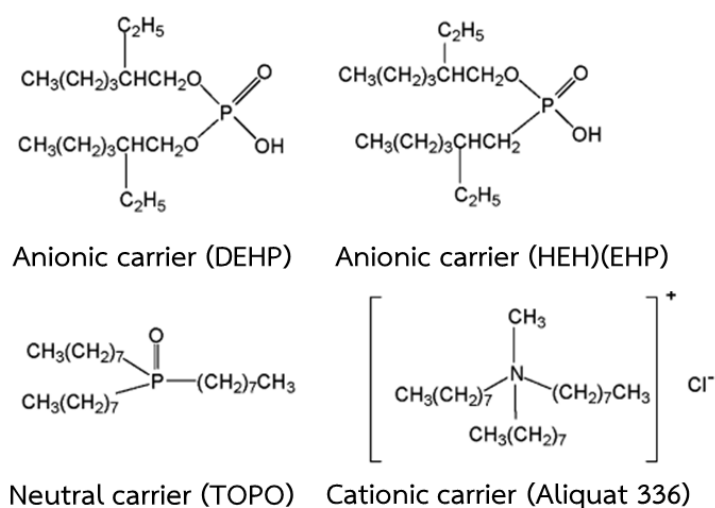
Figure 2.5 Structures of the carriers [31].

The extraction efficiency (E) of three-phase HF-LPME is defined as shown in equation 2.9.

$$E = \frac{K_{a/d}V_a}{K_{a/d}V_a + K_{o/d}V_o + V_d}$$

Equation 2.9

Where $K_{a/d}$ is partition coefficient of the analyte between acceptor solution and donor solution, $K_{o/d}$ is partition coefficient of the analyte between organic solvent and donor solution, V_a, V_o and V_d is volume of acceptor solution, organic solvent and donor solution, respectively.

2.1.4 Colorimetric method for determination Cr(VI)

Cr(VI) can be quantitatively determined by colorimetric method via complexation with 1,5-diphenylcarbazide (DPC) in acidic solution (pH 2) as summarized in Figure 2.6. Cr(VI) is reduced to Cr(III) by 1,5-diphenylcarbazide (DPC in reducing form) and then 1,5-diphenylcarbazone (DPC in oxidized form) reacts with Cr(III) to generate the positively charged Cr(III)-DPC$_{ox}$ complex with violet color. This complex can be detected by UV-Vis spectrophotometer at 544 nm
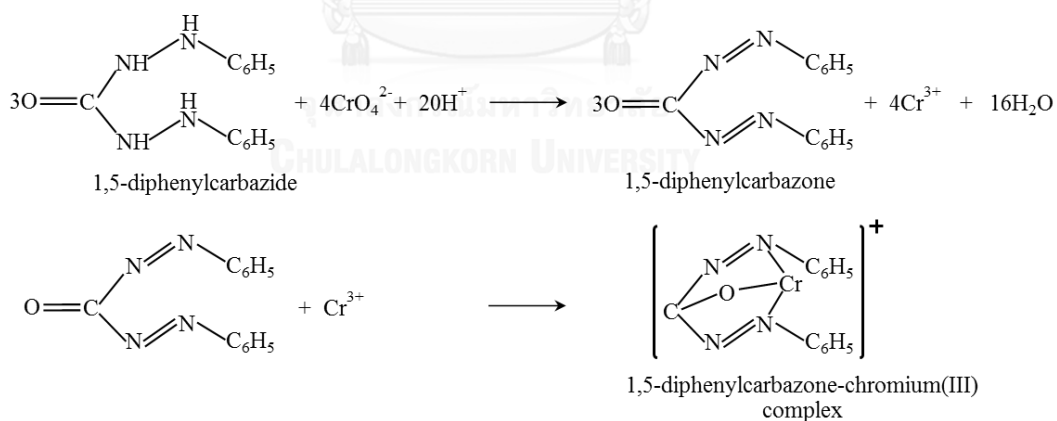
Figure 2.6 Cr-DPC colorimetric method [32].

2.1.5 Colorimetric method for determination salicylic acid

The salicylic acid can be quantitatively determined spectrophotometrically using iron (III) ions. Iron (III) ions react with the phenol (-OH) group on benzene ring's salicylic acid to form a colored violet complex according to the reaction as shown in

Figure 2.7. The Fe(III)-SA complex can be detected by UV-Vis spectrophotometer at 525 nm.
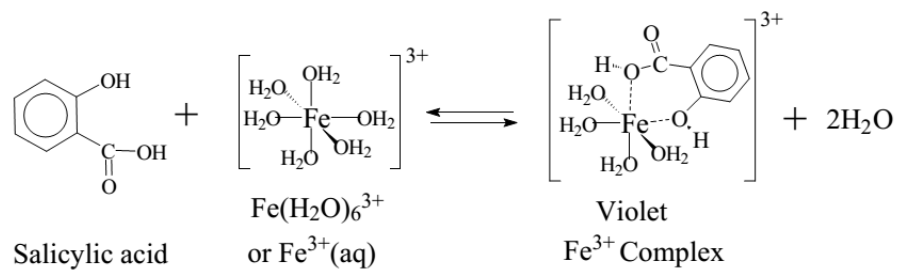
Figure 2.7 Salicylic acid colorimetric method [33].

CHAPTER III

EXPERIMENTAL

3.1 Chemical and reagents

3.1.1 Chemical and reagents for Cr(VI)

1) Potassium dichromate (BDH Chemicals, UK)
2) 1,5-diphenylcarbazide (Sigma-Aldrich, USA)
3) Sulfuric acid (J.T. Baker, Thailand)
4) Kerosene (Carco Chemical CO., LTD., Thailand)
5) 1-octanol (Sigma-Aldrich, USA)
6) 1-heptanol (Sigma-Aldrich, USA)
7) Methyltrioctylammonium chloride (Aliquat 336) (Merck, Germany)
8) Ethanol (Merck, Germany)

3.1.2 Chemical and reagents for Salicylic acid (SA)

1) Salicylic acid (Sigma-Aldrich, USA)
2) Iron(III) chloride (BDH Chemicals, UK)
3) Sodium hydroxide (Merck, Germany)
4) Hydrochloric acid (Merck, Germany)
5) 1-octanol (Sigma-Aldrich, USA)
6) 1-heptanol (Sigma-Aldrich, USA)
7) Dihexyl ether (Sigma-Aldrich, USA)

3.2 Instrument and equipment

1) Polypropylene hollow fiber membrane Accurel® Q3/2, 600 μm ID 200 μm thickness, 0.2 μm pore size (Membrana, Wuppertal, Germany)

2) Syringe pump (Prosense B.V., USA)

3) Peristaltic pump, (Masterflex, USA)

4) Six-port valve (Upchurch Scientific, Oak Harbor, WA, USA)

5) Selection valve (Upchurch Scientific, Oak Harbor, WA, USA)

6) Teflon tubing, 1/16" OD x 0.03" ID (IDEX Health & Science, USA)

7) Tygon tubing, 0.8 mm ID (Tygon, precision tubing, Masterflex)

8) Fiber optic UV-Vis spectrophotometer with sample holder (Avantes BV, the Netherlands)

9) Quartz microcuvette 50 μl (Hellma, Germany)

10) pH meter model (METTLER TOLEDO, Switzerland and USA)

11) Magnetic stirrer (Fisher Stirrer®, USA)

12) Magnetic stirring bar (Spinbar, USA)

13) Micro syringe 100 μL (SGE, Australia)

14) Medical syringe 5 mL (Becton Dickinson, Singapore)

15) Medical syringe needle with O.D. x length: 0.80 x 25 (mm) (Nipro, Japan)

16) Autopipettes and tips 100 μL, 1000 μL, and 10 mL (Eppendorf, USA)

17) EPA Vial Kit 30 mL and micro insert vial 300 μL (Vertical chromatography, Thailand)

18) Filtration membranes (Nylon membrane filter 47 mm 0.45 μm) (Munktell filter, Germany)

3.3 Experiment

3.3.1 Preparation of chemical solutions

3.3.1.1 Preparation of chemical solutions for Cr(VI)

3.3.1.1.1 Stock standard solution of Cr(VI); 1000 mg L^{-1}

The stock 1000 mg L^{-1} standard solution of Cr(VI) was prepared by dissolving 0.1414 g of potassium dichromate with Milli-Q water in 50.00 mL volumetric flask. Then, this stock standard solution was placed in polypropylene bottle and kept at 4 °C.

3.3.1.1.2 1,5-diphenylcarbazide; 10.0 mmol L^{-1}

The 10.0 mmol L^{-1} 1,5-diphenylcarbazide (DPC) solution was prepared daily by dissolving 0.0242 g of 1,5-diphenylcarbazide with ethanol in 10.00 mL volumetric flask.

3.3.1.1.3 Sulfuric acid; 2.0 mol L^{-1}

The 2.0 mol L^{-1} sulfuric acid solution was prepared by pipetting 2.78 mL of concentrated sulfuric acid into 25.00 mL volumetric flask and diluting with Milli-Q water.

3.3.1.1.4 1,5-diphenylcarbazide; 2.0 mmol L^{-1} containing ethanol 80% (v/v) and sulfuric acid 0.05 mol L^{-1} as acceptor solution

The elution solution was prepared by pipetting 4.00 mL of 10.0 mmol L^{-1} 1,5-diphenylcarbazide, 625 µL of 2.0 mol L^{-1} sulfuric acid and 15.00 mL of ethanol into 25.00 mL volumetric flask and then diluting with Milli-Q water.

3.3.1.2 Preparation of chemical solutions for salicylic acid

3.3.1.2.1 Stock standard solution of salicylic acid; 1000 mg L^{-1}

The stock 1000 mg L^{-1} standard solution of salicylic acid was prepared by dissolving 0.0500 g of salicylic acid with Milli-Q water in 50.00 mL volumetric flask. Then, this stock standard solution was stored in polypropylene bottle and kept at 4 °C.

3.3.1.2.2 Iron(III) chloride; 0.2% (w/v) in hydrochloric acid 0.10 mol L^{-1} as complexing agent

The complexing agent was prepared by dissolving 0.0500 g of iron(III) chloride and pipetting 0.21 mL of concentrated hydrochloric acid into 25.00 mL volumetric flask and then diluting with Milli-Q water.

3.3.1.2.3 Sodium hydroxide; 1.0 mol L^{-1} as acceptor solution

The 1.0 mol L^{-1} sodium hydroxide solution was prepared by dissolving 1.0000 g of sodium hydroxide into 25.00 mL volumetric flask and diluting with Milli-Q water.

3.3.3 Single-interface HF-LPME procedure

A piece of 12.5 cm hollow fiber membrane was sonicated with acetone for 10 min to remove any contaminants and dried prior to use. The membrane was immersed into the organic solvent for 1 h to ensure that the pores were filled. The excess solvent in the lumen was removed using air blow. Then, the membrane was inserted to a glass tube. Both ends of the membrane were sealed to syringe needles attached to tygon tubings that connect to the sample solution via a peristaltic pump

as shown in Figure 3.1. A 30 mL of sample solution was fed into the lumen of the membrane.
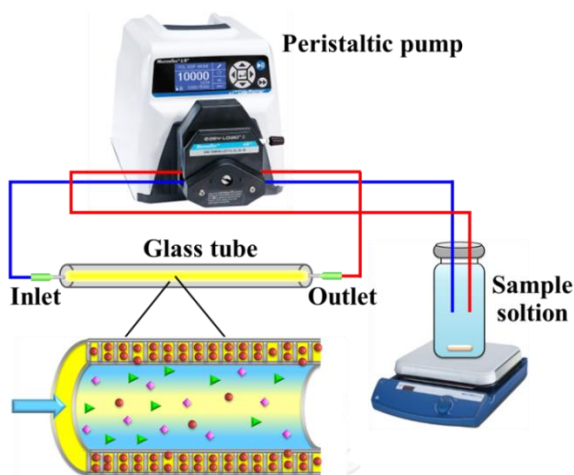
Figure 3.1 Schematic diagram of extraction step in single-interface HF-LPME.

After extraction, the sample solution remaining in the lumen of the HF membrane was flushed out with air blow pushed by a medical syringe. Then, the membrane was removed and reattached one end to a six port injection valve (position 5) and the other end to a multi-position selection valve (position 3) as shown in Figure 3.2. The extract was eluted by an appropriate elution solution, which was filled in a sample loop using a medical syringe at the loading position (position 2 of the six port valve). At the inject position, the elution solution was pulled into the lumen of the membrane by the syringe pump in aspiration mode. The eluent was drawn into a holding coil and then pushed to an insert vial (position 2 of the selection valve) for collection and further analysis. In this study, the hollow fiber membrane was disposed after each use to avoid sample carry over.
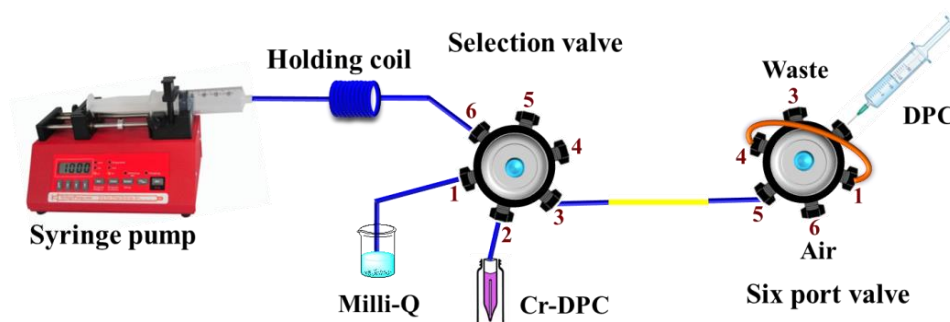
Figure 3.2 Schematic diagram of elution step of single-interface HF-LPME.

3.3.4 Determination of procedure

3.3.4.1 Determination procedure of Cr(VI)

Cr (VI) was determined by Cr-DPC colorimetric method. The violet solution of Cr-DPC complex was formed in the elution step collected in a microcuvette and detected by a fiber optic UV-Vis spectrophotometer at 544 nm.

3.3.4.2 Determination procedure of salicylic acid

Salicylic acid was determined by forming a color complex with ferric chloride solution after the elution step. A violet colored complex was collected in a microcuvette and detected by a fiber optic UV-Vis spectrophotometer at 525 nm.

3.3.5 Method optimization

3.3.5.1 Optimization of single-interface HF-LPME of Cr(VI)

3.3.5.1.1 Effect of flow pressure on stability of SLM

The single-interface HF-LPME was designed that the sample solution was continuously pumped into the lumen of membrane, thus the leakage of the SLM due to the increased pressure inside the lumen was considered. The outlet flow rates in the range of 0.25 to 1.5 mL min^{-1} higher than the inlet flow rate were tested on extraction of Cr(VI). The inlet flow rate was set at 4 mL min^{-1}.

3.3.5.1.2 Effect of elution flow rate

The elution flow rate would affect amount of Cr(VI) eluted with 1,5-diphenylcarbazide (DPC). Normally, the extraction efficiency increased with the decreased flow rate since the contact time between the sample solution and

the SLM was increased, thus the mass transfer of the analyte into the SLM was higher. The elution flow rates in the range of 25 to 250 µL min^{-1} were optimized.

3.3.5.1.3 Effect of inlet flow rate

The extraction efficiency of Cr(VI) in sample solution into the SLM would depend on the inlet flow rate. The inlet flow rates in the range of 2 to 15 mL min^{-1} with the outlet flow rate of 1 mL min^{-1} higher than the inlet flow rate at fixed extraction time of 15 min were explored.

3.3.5.1.4 Effect of organic solvents and concentration of Aliquat 336

The organic solvent impregnated in the pores of the membrane is one of the important parameter that influence the diffusion of Cr(VI) to the SLM. Three types of organic solvents including kerosene, 1-heptanol and 1-octanol were studied. Since Cr(VI) could not extracted into the hydrophobic SLM; therefore, Aliquat 336 was added in the SLM to promote the transfer of Cr(VI). The concentrations of Aliquat 336 in the range of 1 to 20% (v/v) were studied.

3.3.5.1.5 Extraction time profile

The single-interface HF-LPME is a non-exhaustive method that the mass transfer of the analyte is based on time-dependent equilibrium process. The extraction time profile of Cr(VI) in the range of 10 to 30 min was investigated.

3.3.5.2 Optimization of single-interface HF-LPME of salicylic acid

3.3.5.2.1 Effect of organic solvents

Three types of the organic solvent were studied including 1-heptanol, 1-octanol and dihexyl ether based on their hydrophobicity.

3.3.5.2.2 Effect of inlet flow rate

The inlet flow rates in the range of 2 to 15 mL min^{-1} with the outlet flow rate of 1 mL min^{-1} higher than the inlet flow rate at fixed extraction time of 15 min were studied.

3.3.5.2.3 Effect of elution flow rate

The salicylic acid was eluted to the dissociated form by NaOH solution. The contact time between salicylic acid and NaOH solution would affect amount of eluted salicylic acid. The elution flow rates in the range of 5 to 30 μL min^{-1} were examined.

3.3.5.2.4 Effect of acceptor solution pH

The concentrations of NaOH as acceptor solution, which corresponded to pH of the acceptor solution were examined. The pH of NaOH was varied from 10 to 13.

3.3.5.1.5 Extraction time profile

The extraction time profile was established in the range of 10 to 30 min.

3.3.6 Method evaluation

3.3.6.1 Calibration curve and linearity

The linear calibration curve was established in the concentration ranging from 5 to 30 μg L^{-1} for Cr(VI) and 0.25 to 2.00 mg L^{-1} for salicylic acid. The slope, intercept and determination coefficient (R^2) which were obtained from the calibration curve were used to express the linearity of the proposed method.

3.3.6.2 Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) is the lowest concentration of the analyte that can be determined by analytical procedure, whereas limit of quantification (LOQ) is the lowest concentration of analyte that can be quantitatively determined. The LOD and LOQ were calculated from the equation 3.1 and 3.2, respectively.

$$S_{LOD} = S_B + 3SD \qquad \text{Equation 3.1}$$

$$S_{LOQ} = S_B + 10SD \qquad \text{Equation 3.2}$$

where S_{LOD} is the signal at the limit of detection

S_B is the signal of blank soluion

SD   is the standard deviation of blank

Since there was no signal of blank measurement in spectrophotometric method, the standard deviation (SD) was calculated from equation 3.3

$$SD = \sqrt{\frac{\sum (y_i - Y_i)^2}{n-2}} \qquad \text{Equation 3.3}$$

where y_i is the signal of analyte measurement from the experiment

Y_i is the signal of analyte calculated from linear equation

n is the number of concentration of standard solution

3.3.6.3 Precision and accuracy

The precision of the method was carried out in term of intra-day precision and inter-day precision at 10 µg L^{-1} spiked Cr(VI) and 0.5 mg L^{-1} spiked salicylic acid in Milli-Q water. The intra-day precision was determined in one day with seven replicates, while the inter-day precision was determined within five consecutive days in three replicates per day. The results were expressed as %RSD of the determinations.

The accuracy of the method was demonstrated as %recovery at 10 and 30 µg L^{-1} spiked Cr(VI) in water samples and 0.25 mg L^{-1} spiked salicylic acid in drug samples. The %recovery was obtained from equation 3.4.

$$\% \text{ Recovery} = \left(\frac{C_c - C_b}{C_s} \right) \times 100 \qquad \text{Equation 3.4}$$

where C_c is the concentration of analyte found in spiked sample

C_b is concentration of analyte found in unspiked sample

C_s is the concentration of standard that spiked into the sample

3.3.6.4   Enrichment factor

The enrichment factor (EF) was calculated from the equation below.

$$\text{Enrichment factor} = \frac{C_a}{C_d} \qquad \text{Equation 3.5}$$

where C_a is the final concentration of analyte in acceptor aqueous solution

C_d is the initial concentration of analyte in donor aqueous solution

3.3.7   Real samples

Several kinds of water samples were used for determination of Cr(VI). Drinking water samples were purchased from local markets in Bangkok, Thailand. Surface water samples were collected from Chaopraya river, Chulalongkorn University pond and tap water. The water samples were filtered through a membrane filter with 0.45 µm pore size to remove some colloids and matrices.

Drug samples such as keratolytic solution and aspirin tablet were used for determination of salicylic acid. The commercial keratolytic solution and aspirin tablet was obtained from a local drug store. The keratolytic solution (0.2 g mL^{-1} salicylic acid as labeled) was prepared by dissolving 0.50 mL sample solution in small volume of methanol in a 100 mL volumetric flask and diluting with Milli-Q water. Aspirin tablet (81 mg $tablet^{-1}$ acetyl salicylic acid as labeled) was weighed and

homogenized. A 17 mg of aspirin was dissolved in Milli-Q water and heated. The solution was cooled, filtered and made up to a 100 mL with Milli-Q water.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Method optimization

The enrichment factor (EF), which is the ratio of the final concentration of analytes in the acceptor solution after the elution to the initial concentration of analytes in the aqueous solution was utilized to evaluate the extraction performance.

4.1.1 Optimization of single-interface HF-LPME of Cr(VI)

4.1.1.1 Effect of flow pressure on stability of SLM

In the single-interface HF-LPME format, the outer of the membrane was surrounded by air. When the sample solution was fed inside the lumen, small liquid droplets were odserved on the outside surface of the membrane. The leakage of the organic solvent may be due to the increase of positive pressure inside the lumen as a result of the flowing of the solution inside the feeding tube and the membrane, which were different in the diameters. Thus, the flow pressure inside the lumen was the important factor which might be considered [34-36]. The flow pressure was balanced by adjusting the flow rates in and out of the lumen of the membrane using the 2 channel peristaltic pump with a simultaneous individual flowing as shown in Figure 3.1. The leakage was not observed when the outlet flow rate was slightly higher than the inlet flow rate. The outlet flow rates in the range of 0.25 to 1.5 mL min^{-1} higher than the inlet flow rate (4 mL min^{-1}) were optimized. The enrichment factors at the various differences of outlet and inlet flow rates are shown in Figure 4.1. The difference of outlet and inlet flow rate of 1.0 mL min^{-1} was chosen, where the inlet flow rate was 4 mL min^{-1} and the outlet flow rate was 5 mL min^{-1}, although the difference of outlet and inlet flow rate of 1.5 mL min^{-1} gave a slightly higher enrichment factor. The increase of difference of outlet and inlet flow rate probably

caused on air gap inside the lumen of the membrane due to discontinuous flow inside the lumen.
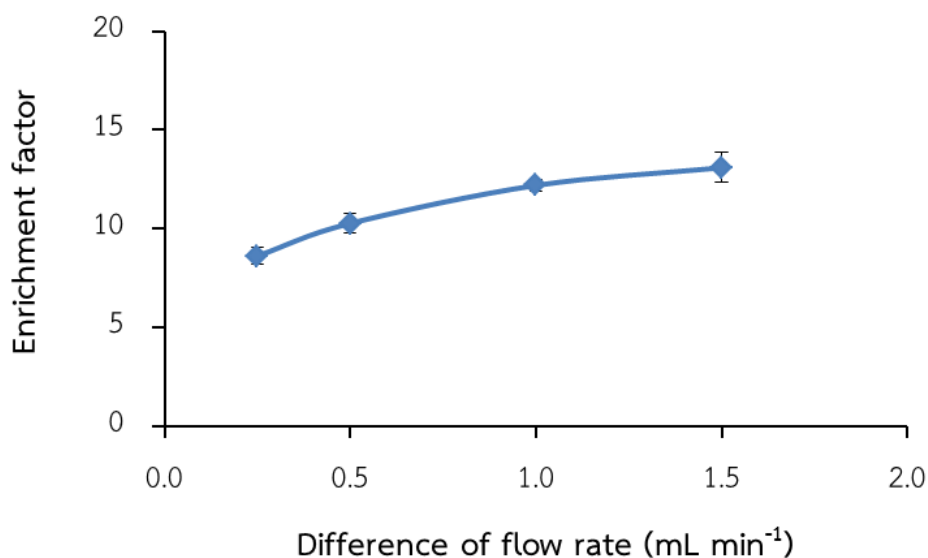
Figure 4.1 Variation of the single-interface HF-LPME performances of Cr(VI) with the differences of outlet and inlet flow rates. The inlet flow rate: 4 mL min^{-1}; (Cr(VI): 20 µg L^{-1}; n=3; elution flow rate 100 µL min^{-1}, SLM: kerosene containing 5 % Aliquat 336; extraction time: 15 min, acceptor solution: DPC)

4.1.1.2   Mass transfer and recirculation configuration

In the single-interface HF-LPME, the mass transfer of the analyte takes place at the contact area between the sample solution and the SLM inside the lumen of the membrane. In this format, there is no driving force to promote the mass transfer of the analyte from the sample solution into the SLM unlike the conventional 3-phase HF-LPME, where the acceptor phase plays an important role in transfering the analyte from the sample solution across the SLM to the acceptor solution. Hence, the approaches enhancing of the mass transfer of the analyte in the single-interface HF-LPME were considered. Normally, the mass transfer of the analyte into the SLM increases with increasing the contact time or the extractive contact area between the sample solution and the SLM. Normally, to increase the contact time between the sample solution and the SLM, the sample solution is fed inside the

lumen at low flow rate. However, this process is quiet time-consuming. Thus, increase the number of contact cycle by recirculating the sample solution at high flow rate was attempted. In this work, a recirculation configuration of the sample solution was performed for increasing the number of contact cycle or total extractive contact area between the sample solution and the SLM in order to increase the mass transfer of the analyte into the SLM.

4.1.1.3 Effect of elution flow rate

After the extraction, Cr(VI) trapped in the SLM was eluted with 1,5-diphenylcarbazide (DPC) solution as the acceptor solution. The elution flow rate of the DPC would affect the amount of Cr(VI) stripped from the SLM. The elution flow rates in the range of 25 to 250 μL min^{-1} were optimized. As illustrated in Figure 4.2, the enrichment factor decreased significantly after the flow rate of 50 μL min^{-1}. Lower elution flow rate allowed more contact time between DPC and the SLM resulting in more mass transfer of Cr(VI) into DPC. However, the enrichment factors were barely obtained at the elution flow rate of 25 and 50 μL min^{-1}. The elution flow rate of 50 μL min^{-1} was chosen due to decreased total analysis time.

Figure 4.2 Effect of elution flow rate on the extraction performance of single-interface HF-LPME of Cr(VI). (Cr(VI): 20 µg L^{-1}, n=3; inlet flow rate: 4 mL min^{-1}, outlet flow rate: 5 mL min^{-1}, SLM: kerosene containing 5 % Aliquat 336, extraction time: 15 min, acceptor solution: DPC)

4.1.1.4 Effect of inlet flow rate

The sample solution was continuously pumped into the lumen of the membrane. The extraction efficiency of analyte in sample solution into the SLM would depend on the inlet flow rate. The inlet flow rates in the range of 2 to 15 mL min^{-1} with the outlet flow rate of 1 mL min^{-1} higher than the inlet flow rate at fixed extraction time of 15 min were explored. Figure 4.3 shows that the enrichment factor was increased as the inlet flow rate increased and remained constant at the inlet flow rate more than 10 mL min^{-1}. Considerably, the inlet flow rate increased from 2 to 10 mL min^{-1} as total extractive contact area raised approximately from 2000 to 10,000 cm^{2} (inner volume of the membrane: 3.53 x 10^{-2} cm^{3}, the extractive contact area: 2.36 cm^{2} and HF membrane length: 12.5 cm); thus, the analyte was more extracted with increased total extractive contact area. For the inlet flow rate of more than 10 mL min^{-1}, the extraction efficiency was not improved probably because the equilibrium might be reached. Therefore, the inlet flow rate at 10 mL min^{-1} was chosen.
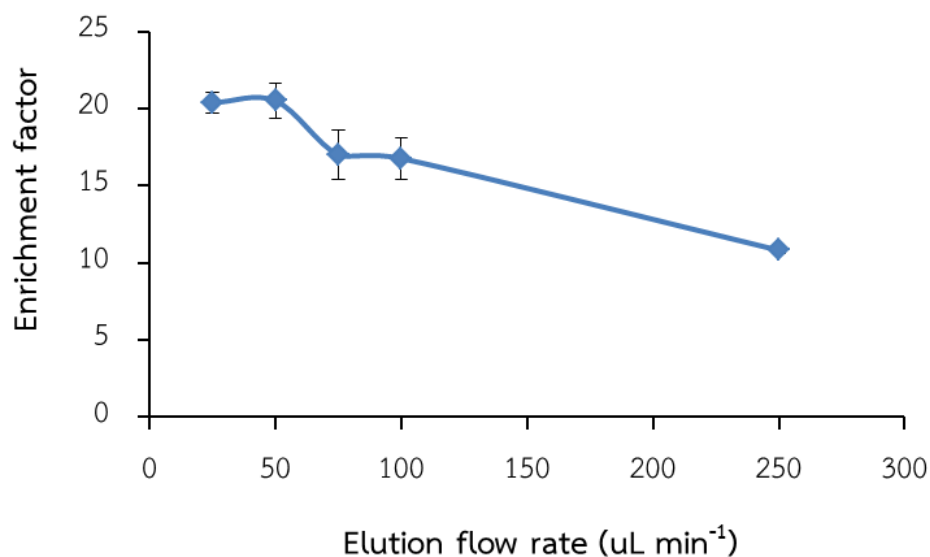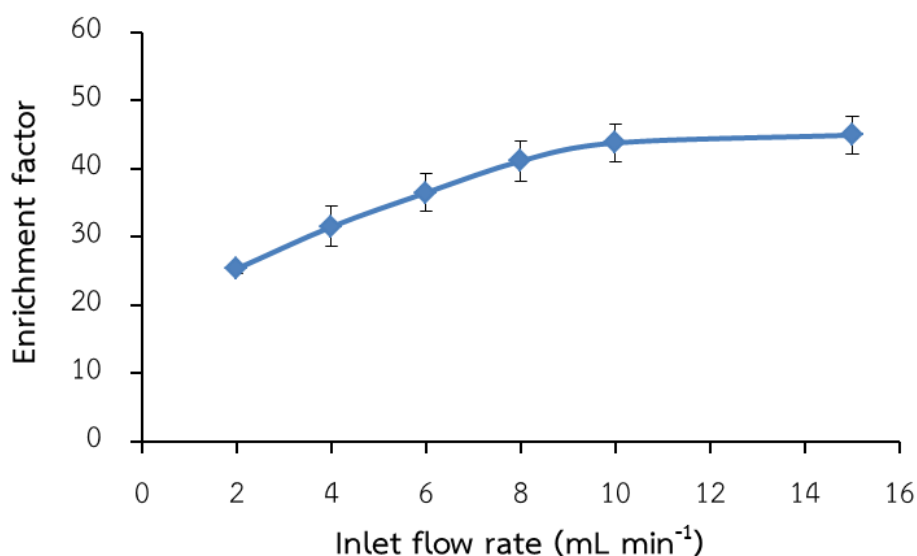
Figure 4.3 Effect of inlet flow rate on the extraction performance of single-interface HF-LPME of Cr(VI). (Cr(VI) 20 µg L^{-1}; n=3; elution flow rate 50 µL min^{-1}, SLM: kerosene containing 5% Aliquat 336; extraction time: 15 min, acceptor solution: DPC)

4.1.1.5 Effect of organic solvents and concentration of Aliquat 336

The organic solvent has influenced the extraction of Cr(VI) from sample solution into the SLM. The appropriate organic solvent should be immiscible with water to prevent dissolving in the sample solution, low volatility to avoid loss during extraction and compatible with the HF membrane. Considering these factors, three types of the SLM including kerosene, 1-heptanol and 1-octanol were studied. To enhance the extraction of Cr(VI), Aliquat 336 as anion exchanger was added in the organic solvent. Since Cr(VI) exists as different ionic forms of oxyanion such as $HCrO_4^-$ and $Cr_2O_7^{2-}$ and CrO_4^{2-} [37]; therefore, the extraction mechanism for Cr(VI) could be the electrostatic interaction with anion exchanger Aliquat 336 as described by the equation below

$$HCrO_{4\,(aq)}^- + R_3CH_3N^+Cl^-_{(SLM)} \longleftrightarrow R_3CH_3N^+HCrO_{4\,(SLM)}^- + Cl^-_{(aq)}$$
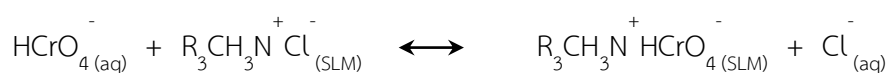
where $R_3CH_3N^+Cl^-$ represents the Aliquat 336

The results in Figure 4.4 shows that the mixture of Aliquat 336 in kerosene yielded the best extraction performance among those three mixtures. The reason may be attributed to that kerosene is the least viscous solvent among those three solvents; therefore, Cr(VI) may diffuse into kerosene easier than into the others according to Table 4.1.
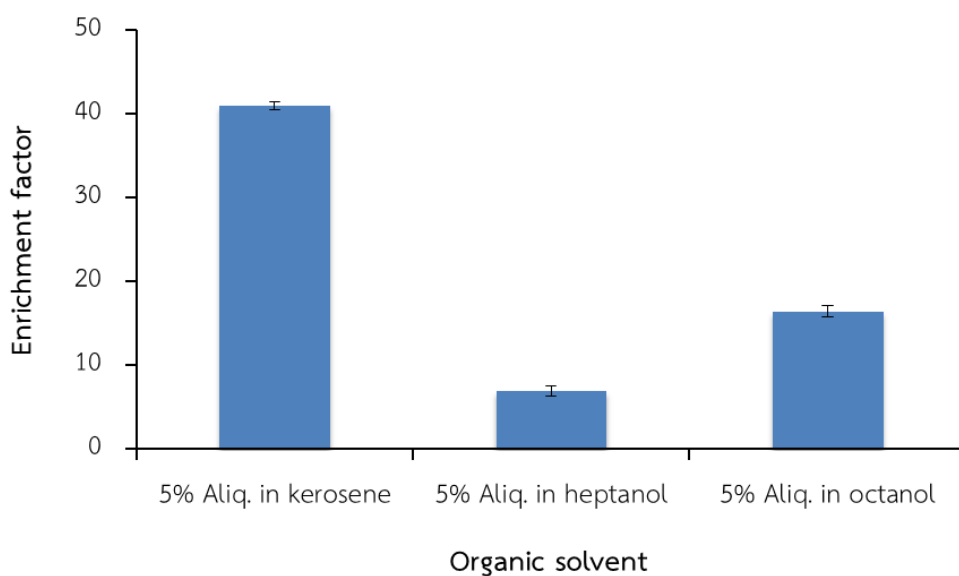
Figure 4.4 Effect of organic solvents on the extraction performance of single-interface HF-LPME of Cr(VI). (Cr(VI): 20 µg L^{-1}; n=3; elution flow rate: 50 µL min^{-1}, inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, extraction time: 15 min, acceptor solution: DPC)

Table 4.1 Properties of organic solvent as SLM

Solvent	Viscosity (mPa·s)	Log P (hydrophobicity)
1-heptanol	5.81[A]	2.62[A]
1-octanol	7.29[A]	3.07[A]
kerosene	1.64[B]	-
Aliquat 336	1450[C]	5.33[D]
dihexyl ether	1.71[A]	5.23[A]

References A: [38], B: [39], C: [40], D: [41]

Aliquat 336 plays an important role to promote the transportation of Cr(VI) into the SLM. The concentrations of Aliquat 336 in the range of 1 to 20% (v/v) in kerosene were examined. The results in Figure 4.5 shows that the extraction performance increased significantly with increased concentration of Aliquat 336 and declined gradually when the concentration of Aliquat 336 was more than 5%. However, the addition of Aliquat 336 may have increased the overall viscosity of the SLM that impeded the diffusion of Cr(VI) into the SLM. Thus, kerosene containing 5% Aliquat 336 was selected as the SLM.
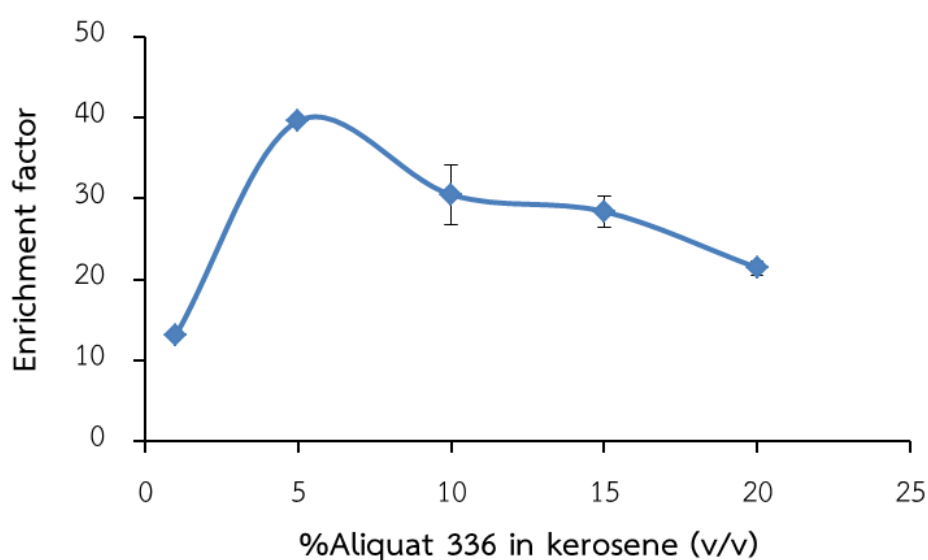
Figure 4.5 Effect of concentration of Aliquat 336 in kerosene on the extraction performance of single-interface HF-LPME of Cr(VI). (Cr(VI): 20 µg L^{-1}, n=3, elution flow rate: 50 µL min^{-1}; inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, extraction time: 15 min, acceptor solution: DPC)

4.1.1.6 Extraction time profile

HF-LPME is a non-exhaustive method based on equilibrium process. The highest distribution coefficient of Cr(VI) between the sample solution and the SLM is obtained at the equilibrium. Therefore, the single-interface HF-LPME time profile in the range of 10 to 30 min was investigated. The results are displayed in Figure 4.6. The enrichment factor increased with the increased extraction time and then

probably reached the equilibrium after 15 min at the inlet flow rate of 10 mL min^{-1}. The extraction time of 15 min was chosen.
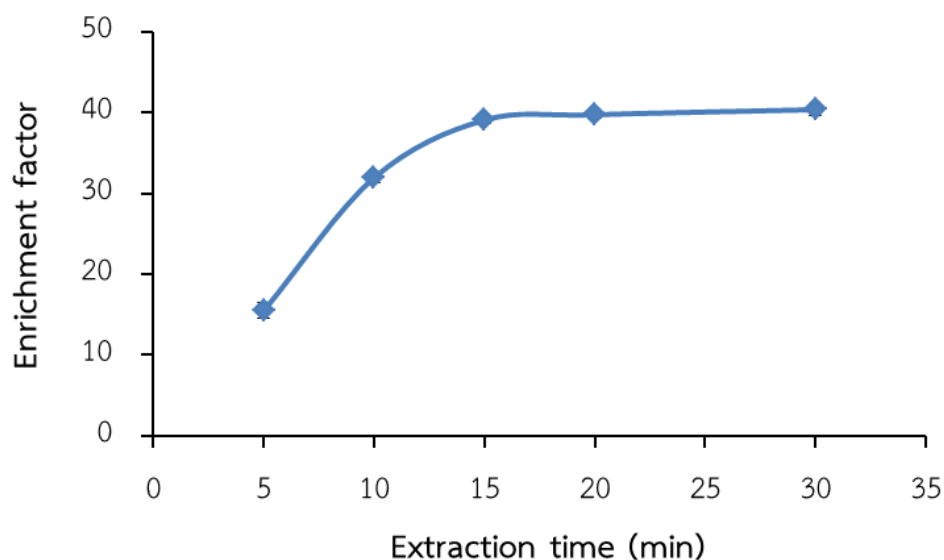
Figure 4.6 Extraction time profile of single-interface HF-LPME of Cr(VI). (Cr(VI): 20 μg L^{-1}; n=3, elution flow rate: 50 μL min^{-1}, inlet flow rate 10 mL min^{-1}, outlet flow rate 11 mL min^{-1}, SLM: kerosene containing 5 % Aliquat 336, extraction time: 15 min, acceptor solution: DPC)

Considerably, the inlet flow rate profile as shown in Figure 4.3, the enrichment factor remains constant at the inlet flow rate more than 10 mL min^{-1} at fixed extraction time of 15 min. It may be attributed to that the increase of inlet flow rate as increased total extractive contact area would not affect the extraction efficiency or the mass transfer of Cr(VI) to the SLM because the equilibrium time of 15 min was reached. Thus, increasing of the inlet flow rate more than 10 mL min^{-1} may be possible for shortening extraction time.

4.1.2 Optimization of single-interface HF-LPME of salicylic acid

The single-interface HF-LPME system was setup and operated in the same way as that for Cr(VI). The sample solution was acidified to pH 1.1 to keep

salicylic acid in non-dissociated form so that it could be effectively extracted by a nonpolar organic solvent.

4.1.2.1 Effect of organic solvents

Three types of the organic solvent were studied including 1-heptanol, 1-octanol and dihexyl ether. The results in Figure 4.7 shows that 1-octanol provided the highest enrichment factor among those three solvents. The extraction efficiency of organic compound typically corresponds to the degree of hydrophobicity of the solvents (log P of SA = 2.26). Moreover, for solvent with high degree of hydrophobicity like dihexyl ether, the analyte might have been strongly retained and hardly eluted in the elution step. Thus, 1-octanol was chosen as the SLM. The hydrophobicity of organic solvents is displayed in Table 4.1.

Figure 4.7 Effect of organic solvents on the extraction performance of single-interface HF-LPME of SA. (SA: 1.00 mg L^{-1}; n=3; inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, elution flow rate: 25 μL min^{-1}, SLM: 1-octanol; extraction time: 15 min, acceptor solution: NaOH)

4.1.2.2 Effect of inlet flow rate

The inlet flow rates in the range of 2 to 15 mL min^{-1} with the outlet flow rate of 1 mL min^{-1} higher than the inlet flow rate at fixed extraction time of 15 min were studied. Figure 4.8 shows that the enrichment factor increases as the inlet flow rate increases gradually and remains stable at the flow rate more than 10 mL min^{-1}. The higher flow rate provides more number of cycles that the analyte could pass through the lumen and allows the analyte having more contact to the organic solvent. The inlet flow rate of 10 mL min^{-1} was chosen on the highest enrichment factor.
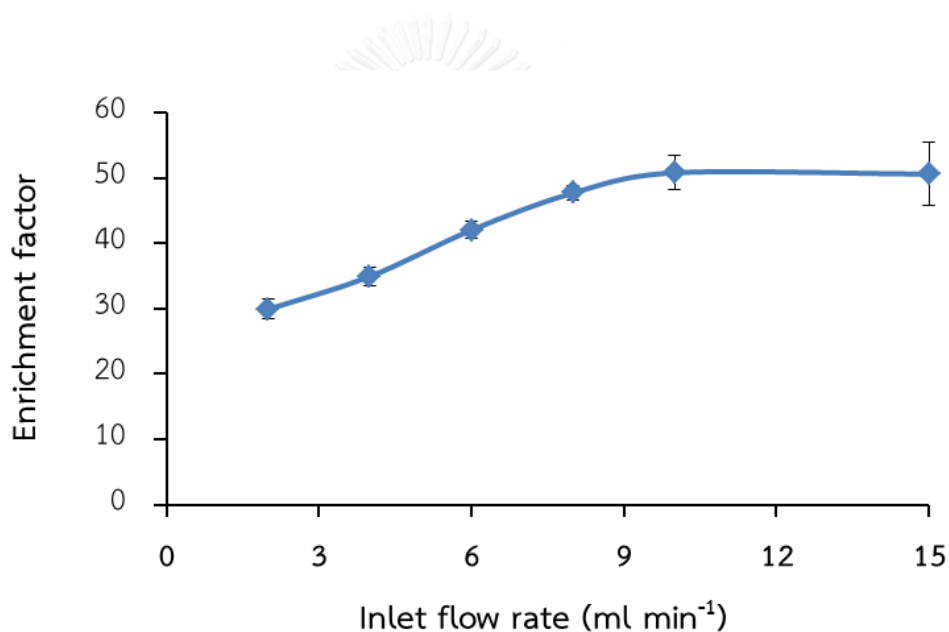
Figure 4.8 Effect of inlet flow rate on the extraction performance of single-interface HF-LPME of SA. (SA: 1.00 mg L^{-1}; n=3; elution flow rate 25 µL min^{-1}, SLM: 1-octanol; extraction time: 15 min, acceptor solution: NaOH)

4.1.2.3 Effect of elution flow rate

In the elution step, the salicylic acid was eluted into the dissociated form by NaOH solution. Typically, the elution flow rate is relatively slow to allow sufficient contact between the eluent and the analyte. The elution flow rates in the range of 5 to 30 µL min^{-1} were examined. As illustrated in Figure 4.9, the lower

elution flow rate exhibited better elution. Despite the elution flow rate of 5 μL min^{-1} showed the highest enrichment factor; however, the flow rate of 10 μL min^{-1} was selected in order to decrease half the time of elution step and further to prevent the loss of SLM during the elution.
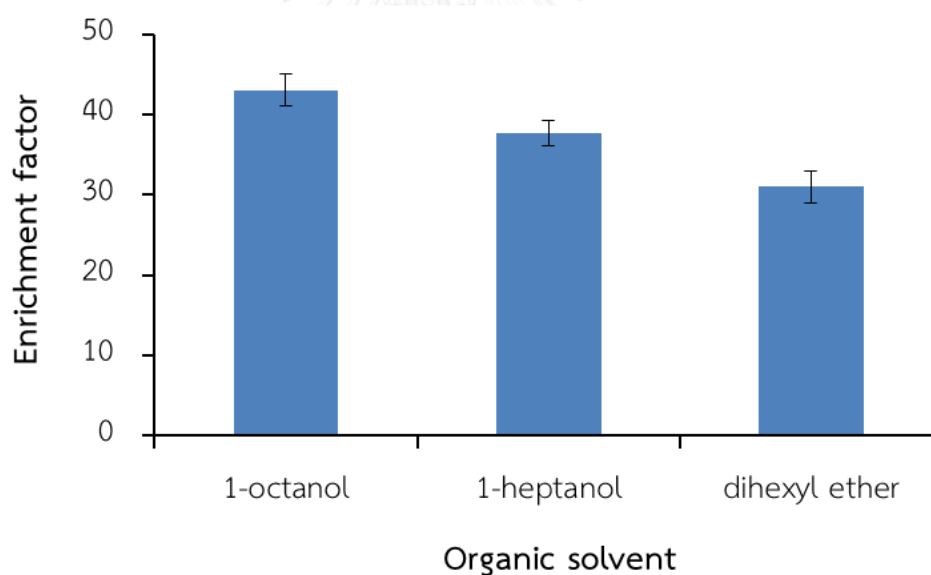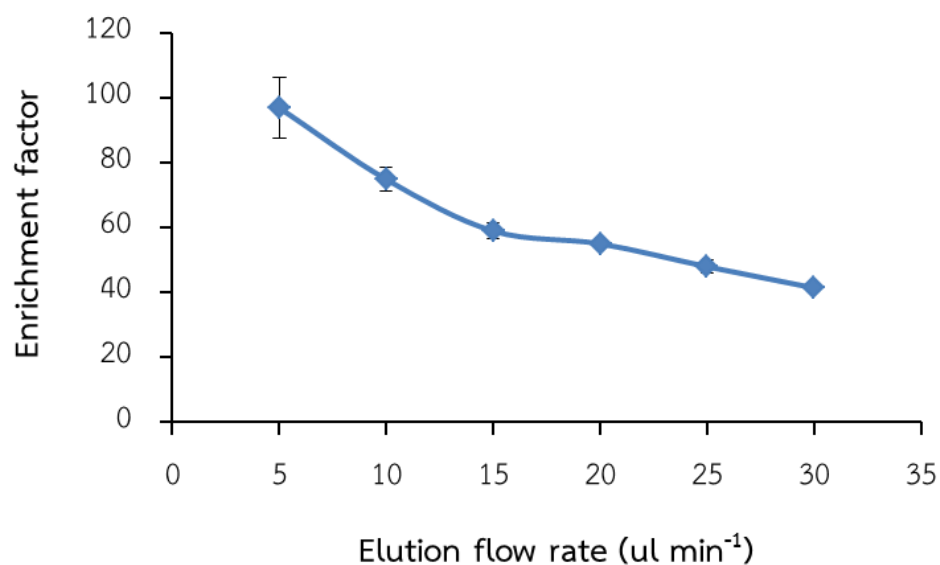

Figure 4.9 Effect of elution flow rate on the extraction performance of single-interface HF-LPME of SA. (SA: 1.00 mg L^{-1}; n=3; inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, SLM: 1-octanol; extraction time: 15 min, acceptor solution: NaOH)

4.1.2.4 Effect of acceptor solution pH

Since in elution step, the salicylic acid would be deprotonated by hydroxide ion and transferred into the acceptor solution, the concentrations of NaOH, which are corresponding to pH of the acceptor solution were examined. The pH of NaOH was varied from 10 to 13. The results in Figure 4.10 shows that, the enrichment factor increased with higher pH and decreased at pH above 12. Increase in hydroxide ion may increase the rate of deprotonation of salicylic acid. However, too high concentration of hydroxide ion could affect the complexation of salicylic

acid and Fe^{3+} in determination step. Therefore, acceptor solution pH of 12 was selected.
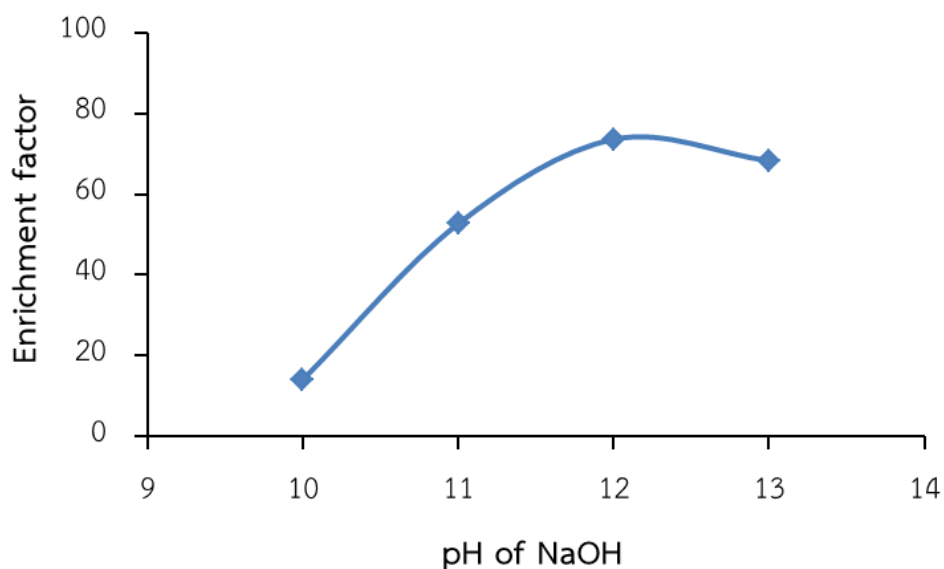
Figure 4.10 Effect of NaOH pH on the enrichment factor of single-interface HF-LPME of SA (SA: 1.00 mg L^{-1}; n=3; inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, elution flow rate: 10 µL min^{-1}, SLM: 1-octanol; extraction time: 15 min, acceptor solution: NaOH)

4.1.2.5 Extraction time profile

The extraction time profile of single-interface HF-LPME of salicylic acid was established from 10 to 30 min. The results are shown in Figure 4.11. The enrichment factor increased with increased extraction time and approached an equilibrium after 25 min. The extraction time of 25 min was chosen giving the enrichment factor of 95.
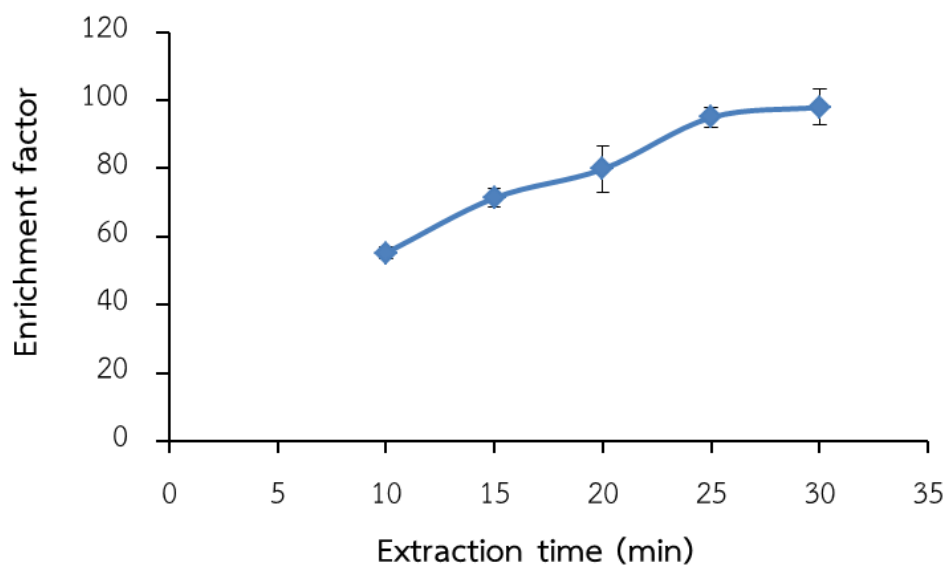
Figure 4.11 Extraction time profile of single-interface HF-LPME of SA (SA: 1.00 mg L^{-1}; n=3; inlet flow rate: 10 mL min^{-1}, outlet flow rate: 11 mL min^{-1}, elution flow rate: 10 μL min^{-1}, SLM: 1-octanol, acceptor solution: NaOH)

The optimal conditions of single-interface HF-LPME for determination of Cr(VI) and salicylic acid were summarized in Table 4.2.

Table 4.2 Optimum conditions of single-interface HF-LPME for determination of Cr(VI) and salicylic acid

Parameters	Optimum conditions	
	Cr(VI)	Salicylic acid
Hollow fiber membrane length	12.5 cm	12.5 cm
Elution flow rate	50 μL min^{-1}	10 μL min^{-1}
Inlet flow rate	10 mL min^{-1}	10 mL min^{-1}
Outlet flow rate	11 mL min^{-1}	11 mL min^{-1}
Organic solvent	kerosene in 5% (v/v) Aliquat 336	1-octanol
Sample solution volume	30 mL	30 mL
Acceptor solution	2.0 mM DPC in 0.05 M H_2SO_4	pH 12 of NaOH
Extraction time	15 min	25 min
Total analysis time	18 min	33 min

4.2   Method evaluation

The single-interface HF-LPME was evaluated the performance of method including linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

4.2.1   Calibration curve and linearity

The calibration curves were established on the extraction of spiked Milli-Q water in the concentration ranging from 5 to 30 μg L^{-1} for Cr(VI) and 0.25 to 2.00

mg L^{-1} for salicylic acid with three replicates (n=3) as shown in Figure 4.12 and Figure 4.13. This method provided a good linearity with determination coefficient (R^2) = 0.9994 and 0.9990 for Cr(VI) and salicylic acid, respectively.
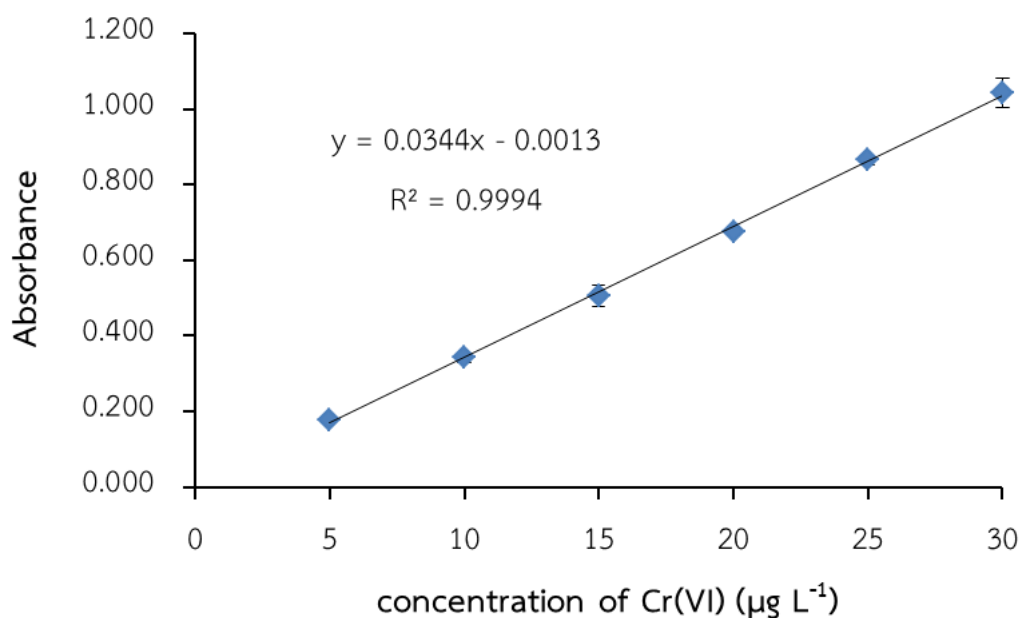
Figure 4.12 Working range of Cr(VI) determined by single-interface HF-LPME in Milli-Q water
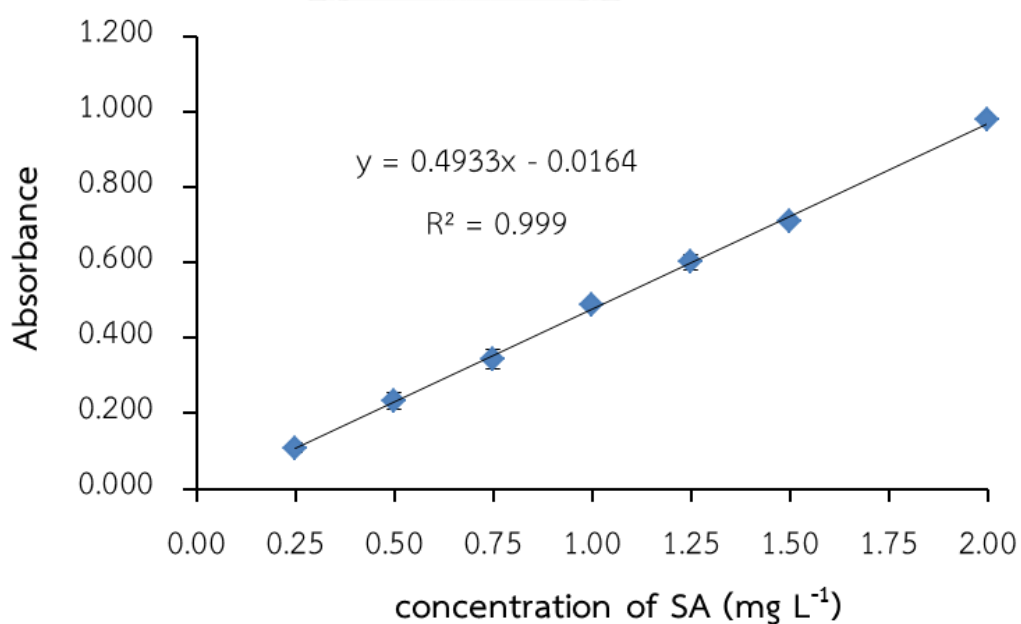
Figure 4.13 Working range of salicylic acid determined by single-interface HF-LPME in Milli-Q water

4.2.2 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ calculated based on three times and ten time of the standard deviation of the regression line were 1.2 µg L^{-1}and 3.5 µg L^{-1}, respectively for Cr(VI) and 0.2 mg L^{-1} and 0.6 mg L^{-1}, respectively for salicylic acid as shown in Table 4.3. The obtained LOD of Cr(VI) was below than the maximum contaminant level of Cr(VI) in drinking water and tap water (50 µg L^{-1}) recommended by the World Health Organization (WHO) thus the proposed method can not only be applied for the determination of low-µg L^{-1} level of Cr(VI) in water samples but also be used a quantitative determination of salicylic acid in drug sample.

4.2.3 Precision

The precision of the method was expressed as intra-day and inter-day precision with the relative standard deviations (RSDs) by the extraction and determination of 10 µg L^{-1} spiked Cr(VI) in Milli-Q water and 0.5 mg L^{-1} spiked SA in Milli-Q water. The intra-day precision was obtained in one day with seven replicates, while the inter-day precision was evaluated from the result within five consecutive days in three replicates per day. Both intra-day and inter-day precision were less than 10% RSD as reported in Table 4.3.
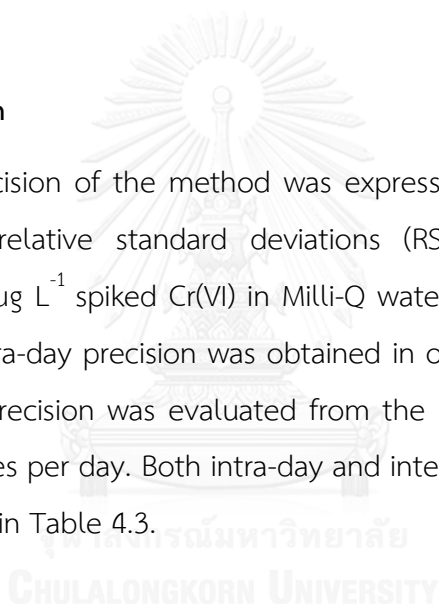
Table 4.3 Method evaluation of single-interface HF-LPME of spiked Cr(VI) and salicylic acid in Milli-Q water

Analytical parameter	Cr(VI)	Salicylic acid
Linear range	5-30 µg L^{-1}	0.25-2.00 mg L^{-1}
Linear equation	y = 0.0344x+0.00134	y = 0.4933x+0.0164
Determination coefficient (R^2)	0.9994	0.9990
LOD	1.2 µg L^{-1}	0.2 mg L^{-1}
LOQ	3.5 µg L^{-1}	0.6 mg L^{-1}
Enrichment factor (EF)	41	95
Intra-day precision (%RSD, n=7)	5.1	8.5
Inter-day precision (%RSD, n=5)	7.3	9.0

4.2.4 Application of single-interface HF-LPME for the determination of Cr(VI) in water samples and the determination of salicylic acid in drug samples

The proposed method was applied for determination of Cr(VI) in various water samples; i.e., drinking waters and mineral water (local markets), surface waters (Chaopraya river water and Chulalongkorn University pond water) and tap water along with the determination of salicylic acid in drug samples. Due to the matrices in samples could affect the extraction efficiency, the matrix-matched calibration curve and the standard addition method were used.

The matrix-matched calibration curves were established on the extraction of Cr(VI) spiked water samples in the concentration range of 10 to 30 µg L^{-1} Cr(VI). The recoveries of spiked Cr(VI) at 10 and 30 µg L^{-1} in water samples were in the range of 92 to 111% with %RSD less than 10%.

The standard addition calibration curves were established on the extraction of salicylic acid spiked drug samples in the concentration range of 0.25 to 2.00 mg L^{-1} salicylic acid. The recoveries of spiked salicylic acid at 0.25 mg L^{-1} in drug samples were in the range of 92 to 103% with %RSD less than 3%. Moreover, the single-interface HF-LPME could be determined the amount of salicylic acid in the keratolytic solution (labeled 2 mg mL^{-1}) and aspirin tablet (labeled 81 mg) were 2.1 mg mL^{-1} with %RSD 1.8 and 73 mg with %RSD 2.3, respectively.

The results of single-interface HF-LPME of Cr(VI) in water samples and salicylic acid in drug samples were summarized in Table 4.4 and Table 4.5 respectively. The method has been successfully applied to real samples providing good accuracy and precision.

Table 4.4 Single-interface HF-LPME of spiked Cr(VI) in water samples

Sample	Linear equation	Concentration of Cr(VI) ($\mu g\ L^{-1}$)			%Recovery	%RSD
		Original	Spiked	Found		
Drinking water 1	y = 0.0213x - 0.0102 (R^2=0.9997)	< 1.2	10	10.3	103	0.5
			30	31.6	105	6.4
Drinking water 2	y = 0.0296x - 0.0581 (R^2=0.9954)	< 1.2	10	10.4	104	6.2
			30	29.5	98	7.6
Drinking water 3	y = 0.0151x - 0.0162 (R^2=0.9922)	< 1.2	10	11.1	111	10.8
			30	31.1	104	7.7
Mineral water	y = 0.0188x - 0.0297 (R^2=0.9948)	< 1.2	10	10.8	108	5.0
			30	30.5	102	1.4
Chaopraya river water	y = 0.0228x - 0.0275 (R^2=0.9974)	< 1.2	10	10.4	104	0.8
			30	30.2	101	3.8

Table 4.4 Single-interface HF-LPME of spiked Cr(VI) in water samples (to be continued)

Sample	Linear equation	Concentration of Cr(VI) (μg L^{-1})			%Recovery	%RSD
		Original	Spiked	Found		
CU pond water	y = 0.0125x - 0.0014 (R^2=0.9978)	< 1.2	10	10.5	105	1.5
			30	28.9	96	4.2
Tap water	y = 0.0257x - 0.0001 (R^2=0.9966)	< 1.2	10	9.2	92	4.6
			30	29.6	99	3.0

Table 4.5 Single-interface HF-LPME of spiked salicylic acid in drug samples

Sample	Linear equation	Concentration of Salicylic acid (mg L^{-1})			Recovery (%)	RSD (%)
		Original	Spiked	Found		
Keratolytic solution (diluted)	y = 0.495x + 0.2504 (R^2 = 0.9973)	0.27	0.25	0.52	103	2.0
Salicylic acid (diluted from aspirin tablet)	y = 0.4672x + 0.2106 (R^2 = 0.9980)	0.23	0.25	0.46	92	2.7

CHAPTER V

CONCLUSION

5.1 Conclusion

The concept of single-interface HF-LPME similar to in-tube SPME has been explored. The analyte was extracted and then eluted inside the lumen of the HF membrane. Cr(VI) and salicylic acid (SA) were used as the model for inorganic ion and ionizable organic compound, respectively The single-interface HF-LPME format was designed. Because the sample solution was fed into the lumen of the membrane, thus the extractive contact area between the sample solution and the SLM was limited. The mass transfer of the analyte extracted into the SLM could be increased with increasing total extractive contact area. Therefore, the recirculation was attempted to enhance the extraction efficiency. However, the sample solution was fed into the narrow lumen, the leakage of organic solvent immobilized in the pore of membrane due to the increased pressure inside the lumen was considered. The leakage was overcome by flow balancing pressure. Parameters that influenced the extraction efficiency on the enrichment factor were investigated. The optimal conditions for extraction of Cr(VI) were inlet flow rate of 10 mL min^{-1}, outlet flow rate of 11 mL min^{-1}, elution flow rate of 50 µL min^{-1}, kerosene in 5% (v/v) Aliquat 336 and extraction time of 15 min. The optimal conditions for extraction of salicylic acid were inlet flow rate of 10 mL min^{-1}, outlet flow rate of 11 mL min^{-1}, elution flow rate of 10 µL min^{-1}, 1-octanol, NaOH pH of 12 and extraction time of 25 min.

The performance of method shows good linearity with working range of 5 to30 µg L^{-1} Cr(VI) and 0.25 to 2.00 mg L^{-1} salicylic acid with determination coefficient (R^2) more than 0.99. The accuracy and precision are in the acceptable range. Moreover, the single-interface HF-LPME methods can be applied to the determination of Cr(VI) in water sample and the determination of salicylic acid in drug sample.

The single-interface HF-LPME for ionic analyte with low distribution coefficient may yield limited mass transfer into the SLM; nonetheless, the equilibrium could be

rapidly achieved and the extraction time could be shortened with increasing the inlet flow rate.

5.2 Suggestion of future work

The single-interface HF-LPME time profile with various inlet flow rates may be studied to ensure that the increase of inlet flow rate will shorten extraction time for the determination of inorganic ions. Besides, assembling the single-interface HF-LPME with the flow-based analysis system for on-line or automation system would also be interesting.

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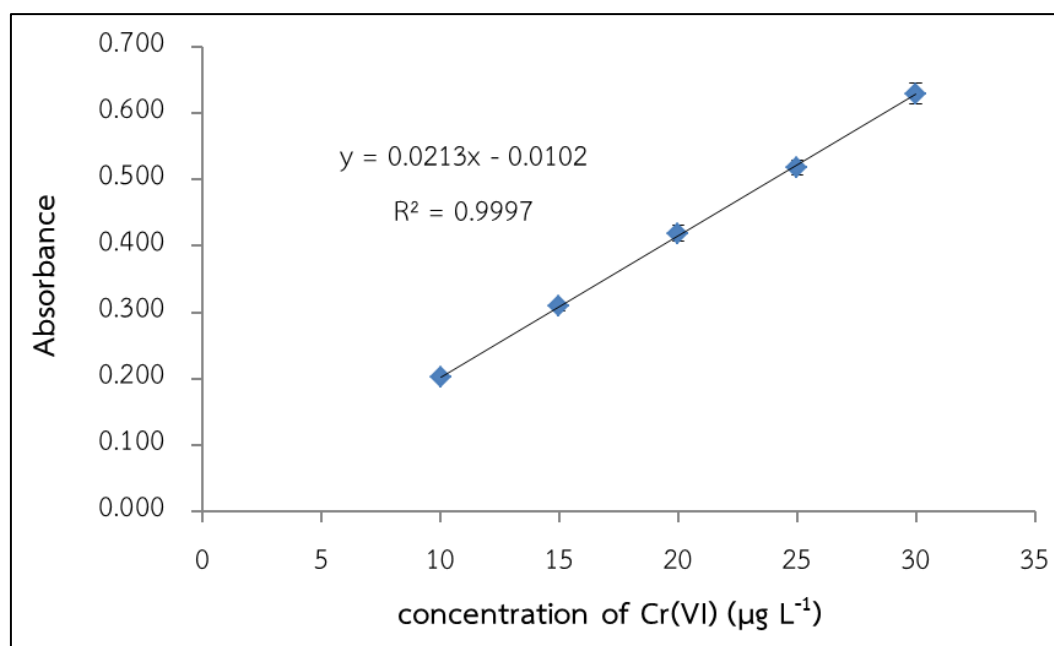
APPENDIX

Figure A.1 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in drinking water 1.
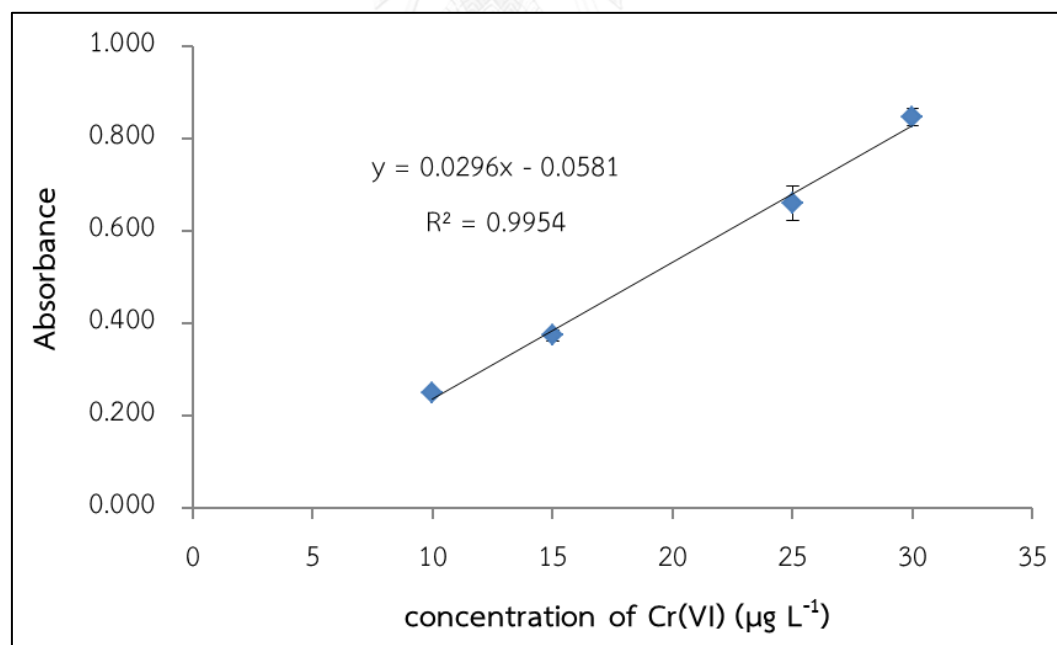

Figure A.2 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in drinking water 2.
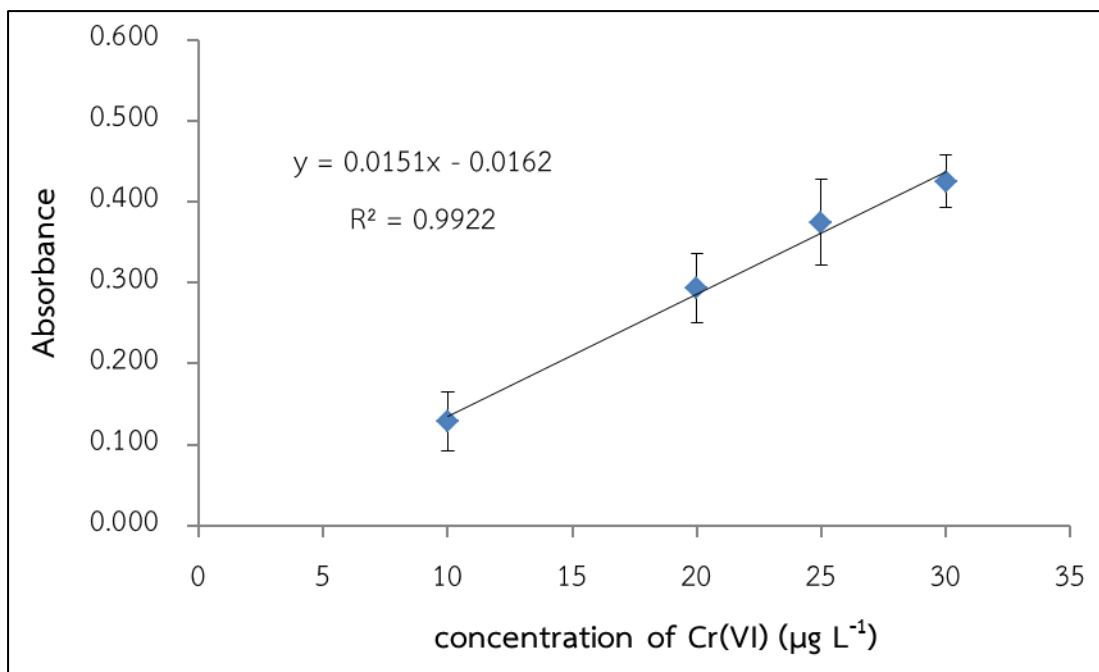
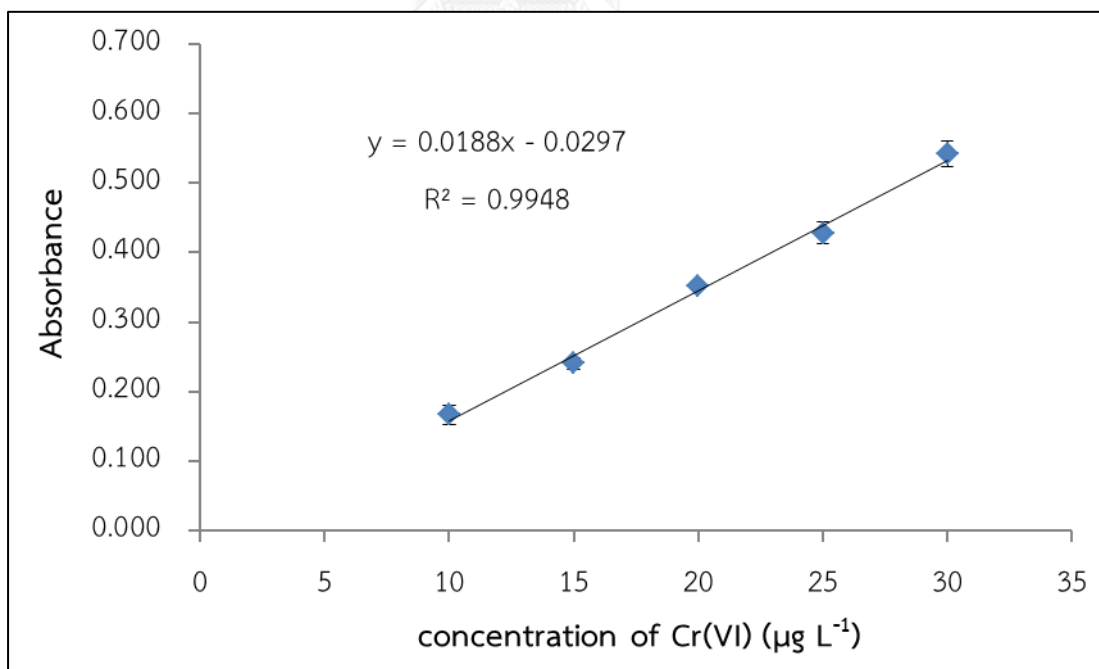Figure A.3 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in drinking water 3.

Figure A.4 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in mineral water.
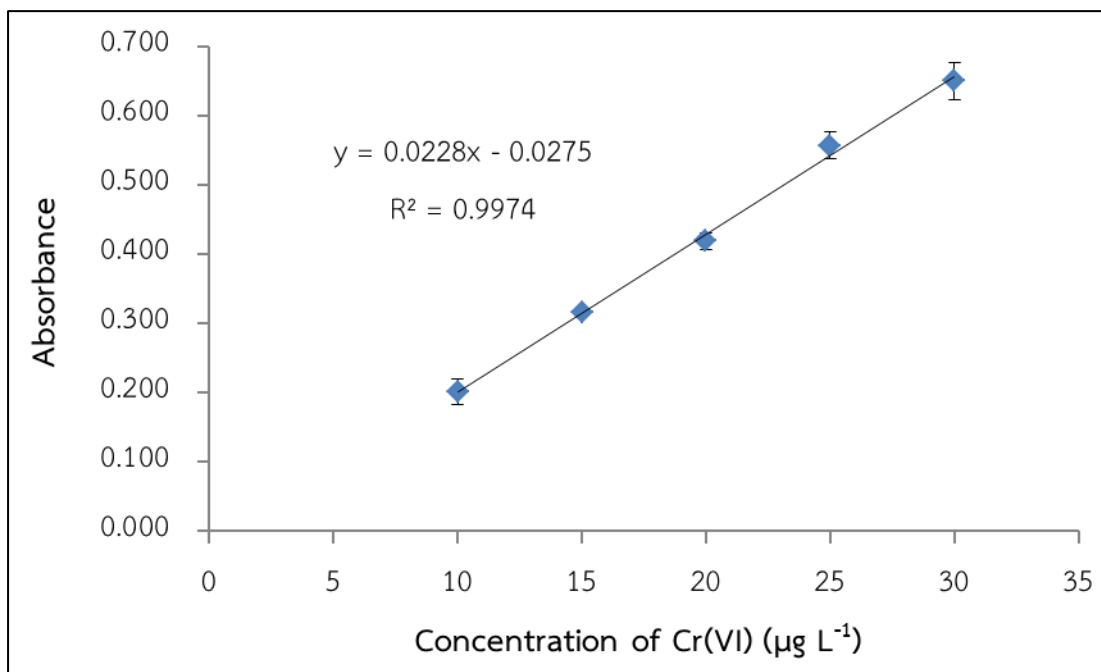
Figure A.5 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in Chaopraya river water.
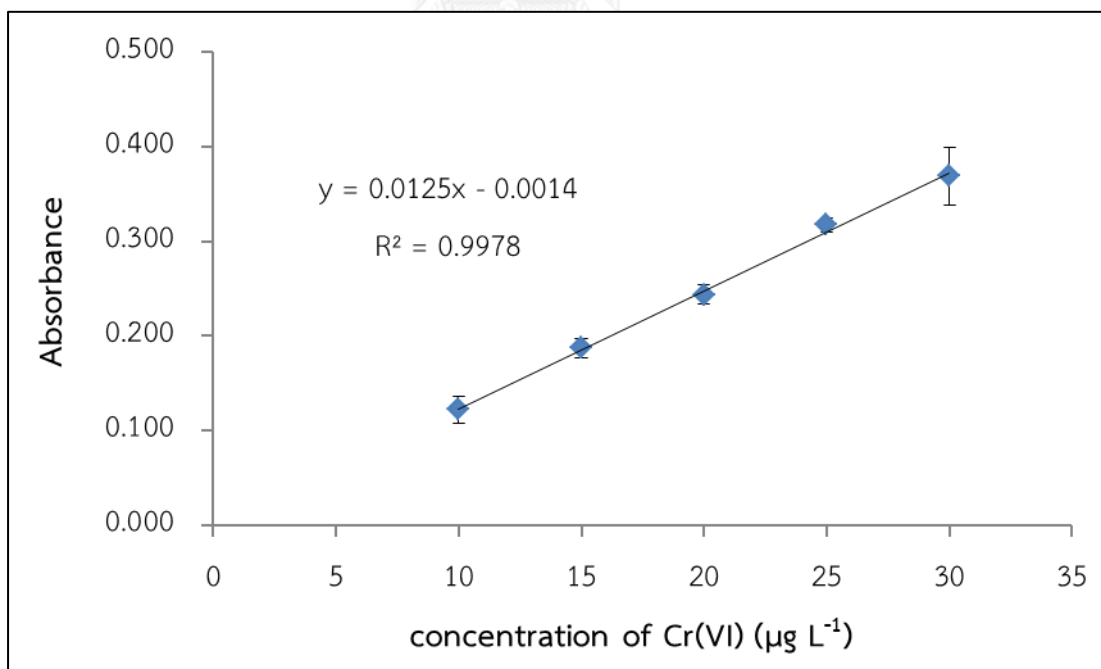

Figure A.6 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in CU pond water.
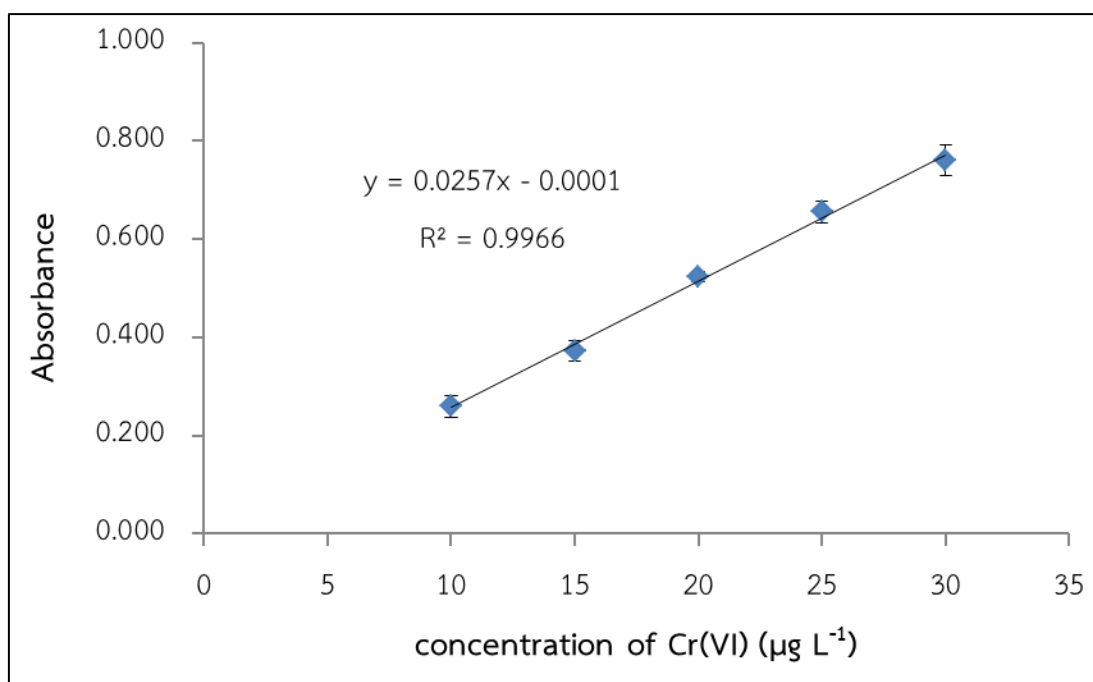
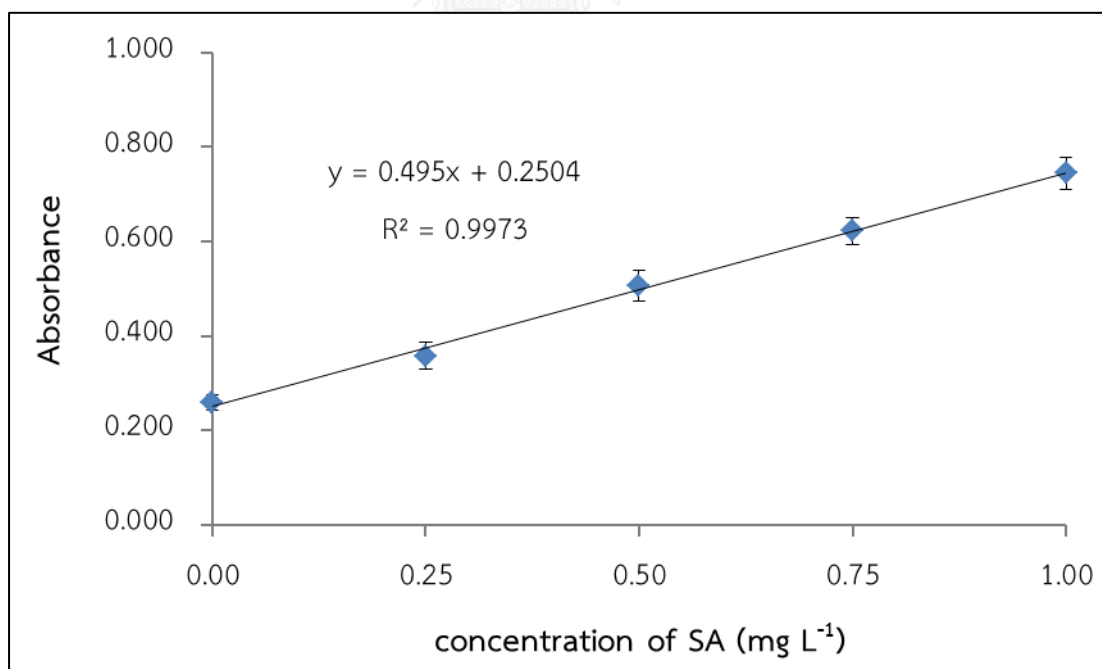Figure A.7 Matrix-match calibration curve of Cr(VI) determined by single interface HF-LPME in tap water.

Figure A.8 Standard addition calibration curve of salicylic acid determined by single interface HF-LPME in keratolytic solution.
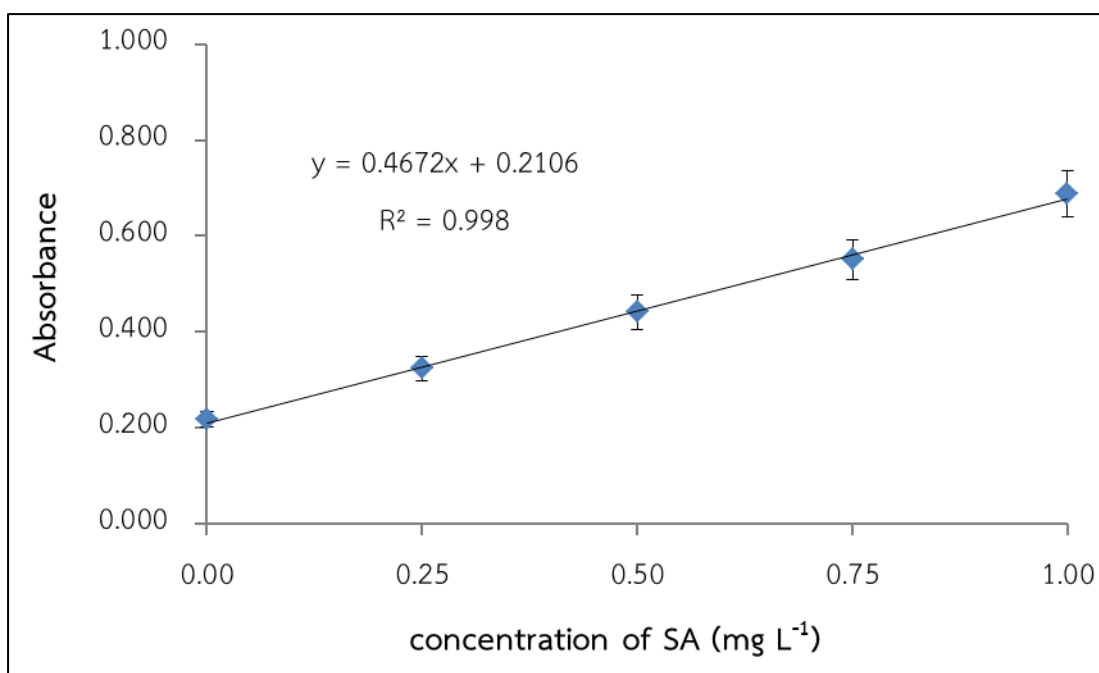
Figure A.9 Standard addition calibration curve of salicylic acid determined by single interface HF-LPME in aspirin tablet.

VITA

Miss Rungaroon Pimparu was born on the 24th of August 1980 in Nakhonratchasima, Thailand. She received her Bachelor's degree of Science from Khon Kaen University in 2001 After that, she becomes a Master's degree of Science from Chiang Mai University, Chiang Mai in 2008. Then, she entered her academic for Doctor of Philosophy degree at Department of Chemistry, Faculty of science, Chulalongkorn University in 2010 and has become a member of Chromatography and Separation Research Unit (ChSRU).

Poster presentation and proceeding

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