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นางสาวอัญชิสา ชินวรกิจ

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HOLLOW FIBER LIQUID PHASE MICROEXTRACTION FOR Cr(III) AND Cr(VI) SPECIATION

Miss Anchisa Chinworakit

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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อัญชิสา ชินวรกิจ: การสกัดแบบวัฏภาคเหลวระดับจุลภาคด้วยเส้นใยกลวงสำหรับการวิเคราะห์รูปแบบทางเคมีของโครเมียม (III) และโครเมียม (VI) (HOLLOW FIBER LIQUID PHASE MICROEXTRACTION FOR Cr(III) AND Cr(VI) SPECIATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.ปกรณ์ วรานุศุภากุล, 55 หน้า.

งานวิจัยนี้ได้พัฒนาการวิเคราะห์รูปแบบทางเคมีของโครเมียม (III) และโครเมียม (VI) ในแหล่งน้ำธรรมชาติ โดยใช้เมมเบรนเหลวที่พยุงด้วยเส้นใยกลวงและวิธีการตรวจวัดทางสเปกโทรโฟโตเมตรีด้วยการเกิดสารเชิงซ้อนกับไดฟีนิลคาร์บาไซด์ อะลิควอท 336 เป็นตัวสกัดวัฏภาคของเหลวที่พยุงด้วยเส้นใยกลวงชนิดพอลิโพรพิลีนขนาด 2 เซนติเมตรจุ่มลงในน้ำตัวอย่าง โครเมียม (VI) ที่อยู่ในรูปประจุลบจะถูกสกัดออกมาจากน้ำตัวอย่างเข้าสู่อะลิควอท 336 ด้วยกลไกการแลกเปลี่ยนประจุลบ หลังจากสกัดนำเส้นใยกลวงออกมาและชะด้วยสารละลาย 1, 5 ไดฟีนิลคาร์บาไซด์ (ในเอทานอล) เกิดสารประกอบเชิงซ้อนสีม่วงซึ่งสามารถตรวจวัดด้วยเครื่องยูวี-วิสิเบิลสเปกโทรโฟโตมิเตอร์ที่ความยาวคลื่น 548 นาโนเมตร โครเมียม (III) สามารถหาได้โดยการออกซิไดซ์โครเมียม (III) เป็นโครเมียม (VI) ด้วยไฮโดรเจนเปอร์ออกไซด์ในภาวะเบส และทำการหาปริมาณโครเมียม (VI) ทั้งหมด จากนั้นหาผลต่างระหว่างปริมาณโครเมียมทั้งหมดในระบบกับปริมาณโครเมียม (VI) ที่หาได้ ในงานวิจัยนี้ได้หาภาวะที่เหมาะสมของการสกัด ได้แก่ ความเข้มข้นของอะลิควอท 336, เวลาที่ใช้ในการสกัด, ปริมาตรสารตัวอย่าง, อัตราเร็วในการคนสารละลาย และผลของเมทริกซ์ ค่าเอนริชเมนท์แฟคเตอร์สำหรับการใช้สกัดน้ำปราศจากไอออนและแหล่งน้ำธรรมชาติเท่ากับ 15 และ 5 ตามลำดับ การตรวจสอบความใช้ได้ของวิธีการพบว่ามีความแม่นและความเที่ยงในการหาปริมาณโครเมียม (III) และโครเมียม (VI) ในน้ำตัวอย่าง โดยได้เปอร์เซ็นต์การกลับคืนในช่วง 81.2-114.8 % และเปอร์เซ็นต์การเบี่ยงเบนมาตรฐานสัมพัทธ์ในช่วง 4-11% ขีดจำกัดการตรวจวัดในน้ำกลั่นและแหล่งน้ำธรรมชาติเท่ากับ 3 และ 10 ไมโครกรัมต่อลิตรตามลำดับ วิธีการวิเคราะห์นี้มีความง่าย ราคาถูก และใช้ปริมาณตัวทำละลายอินทรีย์น้อย ซึ่งเป็นมิตรกับสิ่งแวดล้อม

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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ANCHISA CHINWORAKIT: HOLLOW FIBER LIQUID PHASE MICROEXTRACTION FOR Cr(III) AND Cr(VI) SPECIATION ADVISOR: ASST.PROF.PAKORN VARANUSUPAKUL, Ph.D., 55 pp.

A technique of hollow fiber liquid phase microextraction (HF-LPME) followed by diphenylcarbazide-based UV-Vis spectrophotometric method was developed for speciation of chromium in surface water samples. Aliquat 336 was used as the extraction solvent. A 2 cm-polypropylene hollow fiber membrane impregnated with Aliquat 336 was attached to a medical syringe needle tip and immersed into the sample solution. The method was based on the extraction of Cr(VI) anion into Aliquat 336 via anion-exchange mechanism. After extraction, the membrane was removed from the needle and Aliquat 336 enriched with Cr(VI) was eluted with a small volume of 1,5 diphenylcarbazide in ethanol solution. The violet complex was formed and determined by UV-Vis spectrometer at 548 nm. For the determination of Cr(III), Cr(III) was first oxidized to Cr(VI) by hydrogen peroxide in basic medium, extracted and determined as total chromium, then the amount of Cr(III) was obtained by subtracting of Cr(VI) from the total amount of chromium. Aliquat 336 concentration, extraction time, sample volume, stirring rate and matrix effect were studied and optimized. The enrichment factor about 15 and 5 were obtained for extraction of deionized water and surface water, respectively. The method evaluation showed good accuracy and precision for the determination of Cr(III) and Cr(VI) in surface water samples with recoveries ranging of 81.2-114.8 % and relative standard deviation of 4-11 %. The limits of detection were 3 and 10 μg L^{-1} for deionized water and surface water, respectively. The method is simple, cheap, and uses less organic solvent, which is environmental friendly.

Department Chemistry Student's Signature .....
Field of Study Chemistry Advisor's Signature .....
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LIST OF ABBREVIATIONS

cm	centimeter
mg L^{-1}	milligram per liter
µg L^{-1}	microgram per liter
mL	milliliter
v/v	volume per volume
min	minute
rpm	round per minute
R^2	correlation coefficient
RSD	Relative standard deviation
LOD	Limit of detection
LPME	Liquid phase microextraction
LLE	Liquid-liquid extraction
SPE	Solid Phase microextraction
SDME	Single drop microextraction
HF-LPME	Hollow fiber liquid phase microextraction
ICP-AES	Inductively coupled plasma-atomic emission spectrometry

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Chromium and its compounds are widely used in various industries, such as plating, tanning, paint and pigment production, and metallurgy, which possibly contaminate the environment. The two common oxidation states of chromium present in the environment are Cr(III) and Cr(VI) [1]. Cr(III) is known to be an essential trace nutrients in human bodies, and plays an important role in the metabolism of glucose and certain lipids, whereas Cr(VI) compounds are extremely irritating, toxic and carcinogenic to human body tissue owing to its oxidizing potential [2-3]. Due to the different toxicities of Cr(III) and Cr(VI), it is necessary to determine them separately. The United State Environmental Protection Agency (USEPA) has regulated the permissible limit of 100 μg L^{-1} of total chromium in drinking water. [4] In addition, the World Health Organization (WHO) states the guideline values of 50 μg L^{-1} of Cr(VI) in drinking water [5]. Hence, development of an accurate and highly sensitive speciation method of chromium in environment is absolutely required.

The speciation of two species of chromium, Cr(III) and Cr(VI) can be classified in two types depending on the detection; non-specific and specific detection. The non-specific detections, such as atomic absorption spectrometry (AAS) [6] and inductively coupled plasma-atomic emission spectrometry (ICP-AES) [7] are usually used for the determination of chromium in a total form. However, it seems to be possible to determine each species of chromium by separation of chromium species prior to instrument analysis. The specific detections, such as stripping voltammetry (SV) [8] and spectrophotometry [9] are used for determination of one species of chromium. The spectrophotometric method of Cr(VI) with 1,5 diphenylcarbazide (DPC) has been used worldwide and is highly sensitive and selective to Cr(VI) species [10]. The speciation of the other chromium species can be done by turning the other species into the former one by either reduction of oxidation process, determining both of them as a total and subtracting the former species from the total.

Spectrophotometry is widely used because of its simplicity and low-cost instrumentation.

Since chromium in environmental water systems is in trace amount, the preconcentration step is important. The preconcentration processes that are commonly used are coprecipitation [11], liquid-liquid extraction (LLE) [12], and solid phase extraction (SPE) [13]. Coprecipitation technique is complicated and time consuming and can cause loss of analytes. Conventional liquid-liquid extraction (LLE) is less attractive because it is time consuming and requires large amount of toxic solvent [14]. Solid phase extraction uses less solvent consumption and provides high enrichment factor but low recovery and poor repeatability may be experienced. Recently, liquid-phase microextraction (LPME) [15-17], involving the miniaturization of the LLE has been developed. Single-drop microextraction (SDME), where the liquid extracting phase was hung as a microdrop at the tip of the syringe was developed [18]. Despite the use of very small volume of the solvent, drop stability and low sensitivity were its disadvantages. Subsequently, liquid-phase microextraction based on hollow fiber membrane called hollow fiber liquid phase microextraction (HF-LPME) has been introduced in order to improve solvent stableness [19-21]. In HF-LPME, the organic solvent is impregnated and contained within the lumen of the porous hollow fiber as an interface between the sample solution and the extracting phase. This technique is simple, less solvent consumption, inexpensive and offers high enrichment factor.

In this work, the new-designed HF-LPME method was developed for speciation of chromium. Since Cr(VI) is present as a chromate anion (CrO_4^{2-}) or dichromate anion ($Cr_2O_7^{2-}$), a quaternary ammonium chloride, Aliquat 336 was used as an extracting solvent according to its anion exchanger property [22]. Aliquat 336 was filled in the pores of the membrane and eluted after extraction. The determination method of Cr(VI) was based on DPC method. Cr(III) was oxidized to Cr(VI) by hydrogen peroxide and then determined by the method as total Cr. Speciation of Cr(III) was calculated by subtraction of the concentration of total Cr by the concentration of Cr(VI). The method could improve enrichment factor and sensitivity for speciation of chromium and used less solvent consumption. Parameters influencing extraction performance and enrichment factors such as Aliquat 336

concentration, extraction time, sample volume, and stirring rate were studied and optimized.

1.2 Objective

The objective of this research is to develop a preconcentration of Cr(VI) and speciation for chromium using hollow fiber liquid phase microextraction (HF-LPME) method.

1.3 Scope of this research

1.3.1 To optimize the parameters affecting the performance of HF-LPME method including Aliquat 336 concentration, extraction time, sample volume, and stirring rate for preconcentration and speciation of chromium.

1.3.2 To evaluate the method and apply for speciation and determination of Cr(III) and Cr(VI) in environmental water.

1.4 Benefit of this research

A method for preconcentration and speciation of chromium is obtained, that is simple, inexpensive and reliable providing high sensitivity, which is suitable for determination of Cr(III) and Cr(VI) in environmental water.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Chromium and its contamination into the environmental water system

Chromium and its compound are widely used in various industries, such as metallurgical (stainless steel), electroplating, pigments, tanning and battery. In industrial procedures, the large amount of chromium is discarded in solid, liquid, or gas form into environmental atmosphere affecting the biological and ecological systems. Chromium is commonly present in the environment in two forms, which are Cr(III) and Cr(VI) [1]. Cr(III) and Cr(VI) are different in charge, oxidation state, and chemical properties. Cr(III) is a trace element that is essential for human. It plays a role in controlling glucose and lipid metabolism. While Cr(VI) is toxic and can affect ecology system. Inhalation and skin contact of Cr(VI) can cause perforation and irritation. Moreover Cr(VI) compounds are extremely carcinogenic to human body tissue due to its oxidizing potential [2-3]. The United State Environmental Protection Agency (USEPA) has regulated the permissible limit of 100 $\mu g\ L^{-1}$ of total chromium in drinking water. [4] In addition, the World Health Organization (WHO) states the guideline values of 50 $\mu g\ L^{-1}$ of Cr(VI) in drinking water [5].

The chromium species, Cr(III) and Cr(VI) in environmental water originate from natural sources, including rock constituents, wet precipitation. The concentration of chromium in surface water (rivers and lakes) and sea water were usually limited to 0.1-6.0 $\mu g\ L^{-1}$ and 0.3-0.5 $\mu g\ L^{-1}$, respectively [23]. The concentration of chromium can be much higher in polluted areas. The wastewater from metallurgical, electroplating, tanning, and other chemical industries can cause the chromium contamination in water system (mainly in rivers) [24].

2.2 Chromium speciation

Due to the different toxicities of Cr(III) and Cr(VI), it is necessary to determine them separately which called speciation analysis. Generally, speciation is an analytical process consisting of identification and quantification of various forms of a given element present in analyzed samples. It usually requires a multistep approach, including sampling, sample storage, sample preparation and instrumental analysis. The sampling and storage should be such that trace element species remain unaffected by the procedures [24].

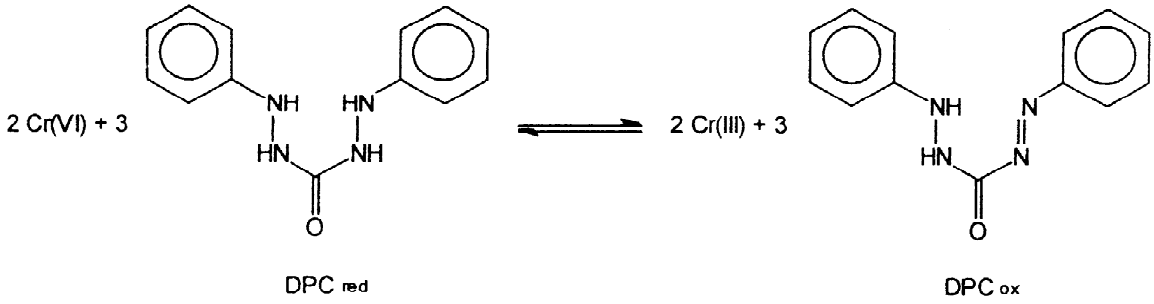
The preparation procedures that commonly used for determination of chromium in environmental samples are filtration, acidification, and extraction. The type of data required (i.e., dissolved or total recoverable) is considered. For the total metal content determination, liquid samples are usually acidified to dissolve large particles. For the determination of the dissolved elements, the samples must be filtered through a 0.45 μm pore diameter membrane filter at the time of collection [25].

The speciation of two species of chromium, Cr(III) and Cr(VI) can be classified in two types depending on the detection; non-specific and specific detection. The non-specific detections, such as atomic absorption spectrometry (AAS) [6] and inductively coupled plasma-atomic emission spectrometry (ICP-AES) [7] are usually used for the determination of chromium in total forms. The separation techniques are used prior to instrument analysis for the determination of each species of chromium. The specific detections, such as stripping voltammetry (SV) [8] and spectrophotometry [9] are used for determination of one species of chromium. The speciation of chromium can be done by determining one species of chromium which is specific for the detection and then determining both of them as a total form after turning the other species into the former one by either reduction of oxidation process. The speciation of the other species can be done by subtraction the former species from the total.

2.3 Determination of Cr(VI) by Diphenylcarbazide-based UV-Vis spectrophotometric method

Diphenylcarbazide-based UV-Vis spectrophotomethic method is usually used for determination of Cr(VI) due to the sensitivity, selectivity, and simplicity. It is the most common spectrophotometric method for Cr(VI) [26]. The selective reaction of Cr(VI) with diphenylcarbazide (DPC) produces a strongly violet complex, which is highly sensitive. The reaction can be described by the following steps (Figure 2.5) [27]:

(1) Cr(VI) reduction step:



(2) Complexation step: Cr^{3+} + DPC_{ox} ⇌ $CrDPC_{ox}$ (violet complex)

Figure 2.1 The reaction of Cr(VI) and diphenylcarbazide [27].

2.4 Methods for chromium preconcentration

Since chromium in environmental water systems is in trace amount, the preconcentration are important. Cr (III) is usually present as cation form (Cr^{3+}) and Cr(VI) is present as chromate anion (CrO_4^{2-}) or dichromate anion ($Cr_2O_7^{2-}$). For preconcentration of these species, ion exchange separation mode is the technique that is wildly used.

2.4.1 Precipitation

Precipitation is a separation method when the analyte is precipitated with a precipitant and then filtered from the sample solution. For example, Cr(III) forms precipitates with $Fe(OH)_3$, $Al(OH)_3$ or $Ti(OH)_3$. Precipitation method is a

sample preparation based on the difference in solubility of each component. The significant characteristic of the precipitant is that it should selectively precipitate with the target analyte. The precipitate should be easily isolated from the solution and have high stability. However, precipitation technique can cause loss of analytes from uncompleted precipitation [28].

2.4.2 Liquid liquid extraction

Liquid liquid extraction (LLE) is a conventional extractive method based on distribution of elements between two immiscible liquid mediums: aqueous and organic solvent. The extraction performance can be enhanced by choosing additives such as ion pair of chelating agents, and salts for improving salting out effect and compromising pH of the sample. It is simple but it has many proprietary limitations including tediousness, large amount of toxic organic solvent, formation of emulsion between water and organic interface, and un-successive extraction [29].

There are some extraction methods for Cr(VI), using non-toxic, nonflammable, stable and selective organic solvent, such as trioctylphosphine oxide, trioctylphosphine amine, methyltrialkyl-ammonium chloride (Aliquat 336), methylisobutylketone (MIBK), and chloroform. Cr(VI) can be directly determined in the organic solvent or back extracted into an acid solution and then determined.

Aliquat 336 is an anion exchanger, in which Cr(VI) as chromate and dichromate anion can transport into it. Aliquat 336 was used as the extractant for preconcentration and separation of Cr(VI) [22].

Aliquat 336 is a mixture of C_8 (octyl) and C_{10} (decyl) chains, which C_8 is predominating. It is a quaternary ammonium salt used as an extracting reagent. The molecular structure of Aliquat 336 is shown in Figure 2.1.

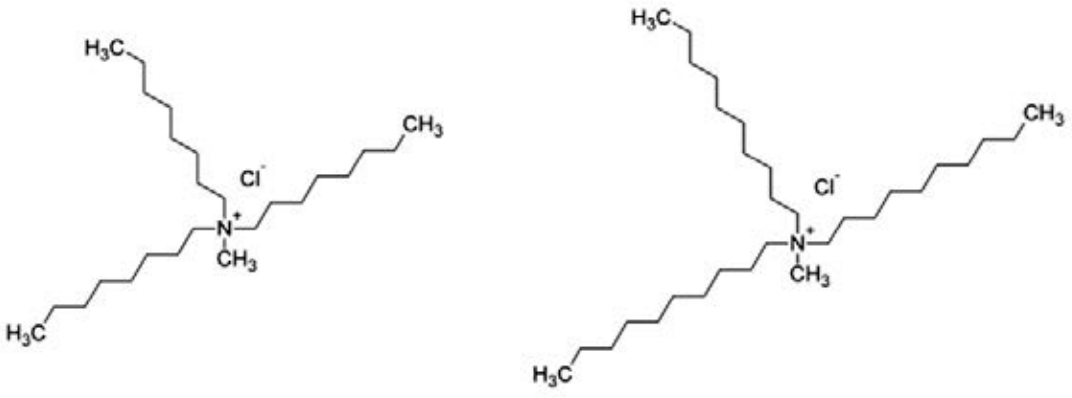


Figure 2.2 The chemical structure of Aliquat 336.

The Cr(VI) separation mechanism of a quaternary ammonium salt is shown in Eq. (1) and Eq. (2). Cr(VI) species in chromate (CrO_4^-) or dichromate ($Cr_2O_7^{2-}$) form are exchanged with chloride ion in Aliquat 336 via anion exchange, whereas the cation form of Cr(III) species are not extracted by Aliquat 336.

$$2R\text{-}Cl + CrO_4^{2-} \rightleftharpoons 2R\text{-}CrO_4 + 2Cl^- \quad (1)$$

$$2R\text{-}Cl + Cr_2O_7^{2-} \rightleftharpoons 2R\text{-}Cr_2O_7 + 2Cl^- \quad (2)$$

2.4.3 Solid phase extraction

Solid phase extraction (SPE) is a simple preparation technique based on the distribution of the analyte in the bulk solution and the solid sorbent. The analyte can be separated from the sample matrices into the sorbent (or solid phase) on account of their greater affinity for the solid sorbent than the sample impurities. It could quantitatively and exhaustively separate the analyte from the matrix solution. The commonly used solid phases for SPE methods are such as Dowex M 4195 chelating rasin [30] and Chromosorb 108 resin [31]. The benefits of SPE method over solvent extraction are high selectivity, ease of removal of the sorbent from the sample solution, and low amount of organic solvent. Although this method uses much less solvent than solvent extraction methods, the SPE column utilizes toxic organic solvents for pretreatment and elution. Moreover, SPE methods give low recovery and poor repeatability.

2.4.4 Liquid phase microextraction

Liquid phase microextraction (LPME) technique is widely used for sample preparation of trace amount of analyte from complex matrices. The principle of LPME is similar to the conventional LLE but LPME uses a few microliters of organic solvent instead of several hundreds of milliliters [32]. LPME is more environmental friendly than other extraction techniques because it used less toxic organic solvent consumption. Single drop microextraction (SDME) was the first LPME technique developed [33]. A single drop of organic solvent is hung in aqueous sample. Then, the analyte is extracted from aqueous phase to the microdrop of organic solvent at the tip of micro syringe. Although SDME is simple and environmental friendly, it gives low stability of the hanging microdrop which can be lost during extraction.

In the LPME system [34], the target analytes are extracted from the aqueous sample into the organic solvent. This process is shown in Figure 2.2 and can be illustrated with Eq. (3)

$$A_{aq} \rightleftharpoons A_{org} \qquad (3)$$

where A_{aq} represents the target analyte in aqueous phase

A_{org} represents the target analyte in organic phase

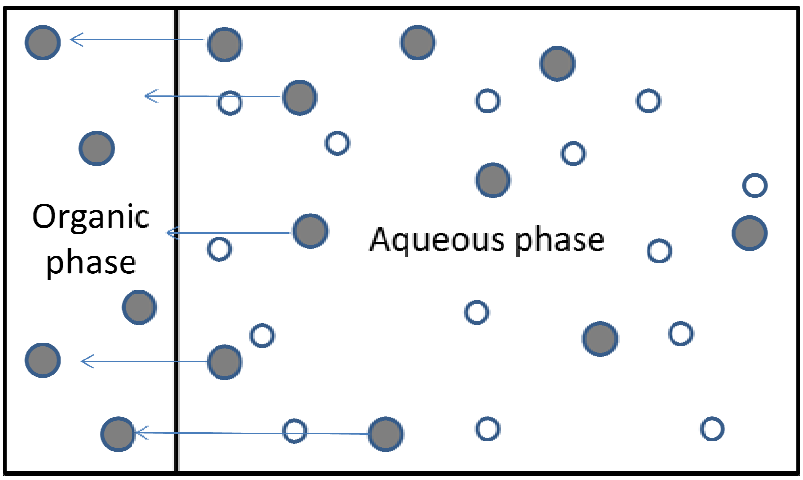


Figure 2.3 Schematic representation of LPME when analyte was extracted to organic phase (black circles: analytes, white circles: other matrices).

The partition coefficient ($K_{org/aq}$) for A is defined as Eq. (4).

$$K_{org/aq} = \frac{C_{eq.org}}{C_{eq.aq}} \quad (4)$$

where $C_{eq.org}$ is the concentration of A in the organic phase at equilibrium
$C_{eq.aq}$ is the concentration of A in the aqueous phase at equilibrium

The enrichment factor (EF) is the ratio of the concentration of A in an enriched organic phase to its concentration in the original aqueous sample as defined in Eq (5). EF can indicate the performance of extraction. It shows how much the analyte concentration is preconcentrated. In LPME, organic phase is in micro-volume

$$EF = \frac{C_{org}}{C_o} \quad (5)$$

where C_{org} is the concentration of A in the organic phase
C_o is the concentration of A originally presented in the aqueous sample

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

2.5.1 Hollow Fiber Membrane

Hollow fiber or tubular membrane is tube-like structure as illustrated in Figure 2.3. Hollow fiber membrane provides high surface area because of its high porosity. The membrane can be used in several separation functions, which depend on the samples (i.e. aqueous, non-aqueous, air) and the properties of analytes. Typical polymer materials used in fabricating hollow fiber membranes are polypropylene (PP), polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), and polysulfone (PS). These materials are stable at all pH ranges, inert to many other chemicals and resistant to high temperature [35-36].

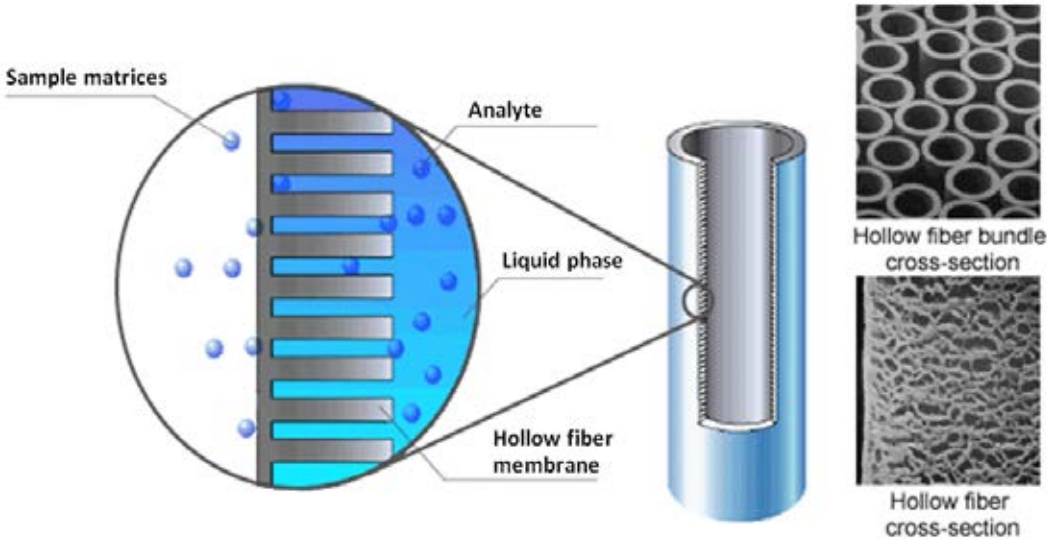


Figure 2.4 Hollow fiber membranes (adapted from [37]).

2.5.2 Hollow fiber liquid phase microextraction (HF-LPME)

Hollow fiber liquid phase microextraction (HF-LPME) is the method that uses the hollow fiber membrane for supporting the extraction solvent inside the porous wall. The advantages of HF-LPME compared to LLE and SPE techniques are using less sample volume, environmental friendly, low cost, and short time of analysis [15]. HF-LPME provides higher enrichment factors. Moreover, it is easy for miniaturization [16-17].

HF-LPME has been developed for solving the problem of instability of microdrop in SDME [17]. In HF-LPME system, the microvolume of the organic solvent is contained within the porous wall of the hollow fiber membrane, so the organic solvent is not directly contact with the sample solution. The major advantage of this technique is the stability of organic solvent, so it is not easily lost into the aqueous solution when stirred vigorously.

HF-LPME can be classified into two modes: three-phase and two-phase HF-LPME. In three-phase HF-LPME, an organic solvent is immobilized in the pores in the wall of the hollow fiber, and an aqueous acceptor solution is held within the lumen. The analytes are extracted into the organic phase and subsequently into the

aqueous phase as shown in Figure 2.4 (a). Three-phase HF-LPME is also called hollow fiber supported liquid membrane (HF-SLM). Another mode of HF-LPME is based on a two-phase system in which the organic solvent is used to fill both the pores in the wall and the lumen of the hollow fiber membrane, as shown in Figure 2.4 (b).

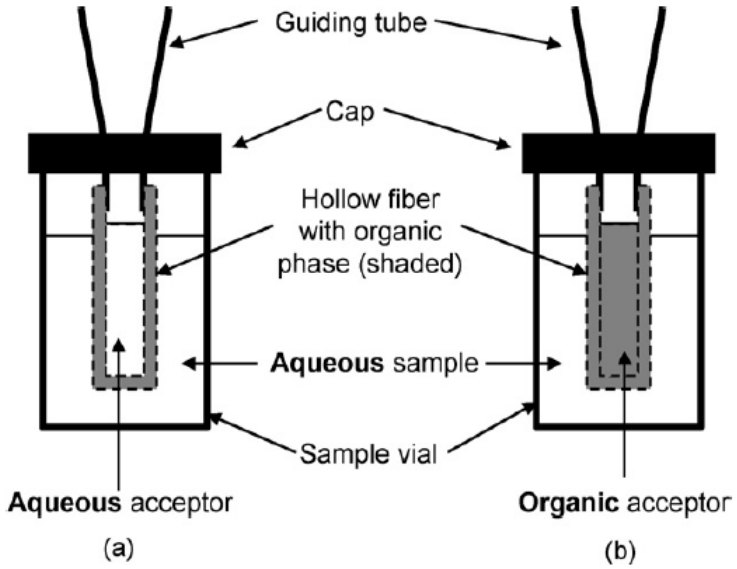


Figure 2.5 Schemes of (a) three- and (b) two-phase LPME [13].

2.6 Literature reviews

There are many studies on chromium determination using liquid phase microextraction techniques. The various types of liquid organic phase have been used such as Cyanex-923 [38], Aliquat 336 [22], triphenyl phosphine [39]. Aliquat 336 are widely used for preconcentration of Cr(VI) because of its anion-exchange property for separation mode. For this reason, many studies on speciation of chromium were reported as follows,

In 1999, Djane et al. [22] developed a supported liquid membrane (SLM) method for the speciation of chromium. The experimental set-up using two serially connected SLM units as shown in Figure 2.6. The extractants impregnated in flat sheet membranes were di-(2-ethylhexyl) phosphoric acid (DEHPA) and methyltrialkyl-ammonium chloride (Aliquat 336), respectively. The principle of

method based on an extraction and enrichment of cationic Cr(III) and anionic Cr(VI) species. The sample solution was pumped to membrane device 1 (M1) containing DEHPA in kerosene where Cr(III) was extracted. The solution carried further through membrane device 2 (M2) containing Aliquat 336 in di-n-hexyl ether where anionic Cr(VI) was extracted. After the analytes were enriched, the acceptor solution was pumped through the two membranes devices and collected in two different vials. The method was applied for the determination of Cr(III) and Cr(VI) in surface water flowing from tannery site. The limit of detection was 0.01 μg L^{-1}.

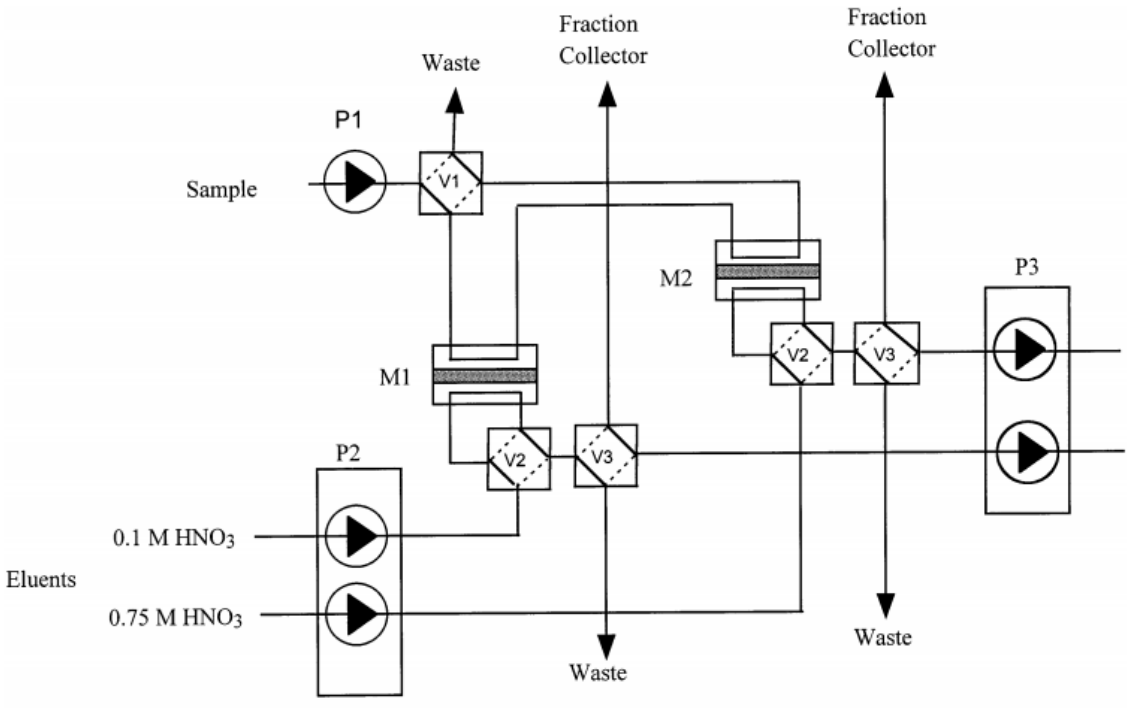


Figure 2.6 Schematic diagram of the serially connected flat sheet SLM system for the speciation of chromium [22].

In 2002, Castillo et al. [27] developed an optical sensor for monitoring of Cr(VI) using flat sheet liquid-supported membranes (FSLSM). Aliquat 336 dissolved in hexane, kerosene, toluene and mixtures with n-decanol was used as an extractant filled in the flat sheet membrane. The extraction procedure was three-phase system. The feed solution, Cr(VI) solution was contacted with the membrane, so Cr(VI) was

extracted into Aliquat 336. The stripping solution, diphenylcarbazide (DPC) in H_2SO_4, then reacted with Cr(VI) producing a strongly coloured complex.

In 2008, Gűell et al. [40] developed a hollow fiber liquid phase microextraction (HF-LPME) system for the removal and preconcentration of Cr(VI) using Aliquat 336 as extractant. In the schematic diagram of the set-up as shown in Figure 2.7, a hollow fiber module contained one coiled polypropylene hollow fiber membrane. The extraction procedure was three-phase system, where the Cr(VI) aqueous solution was a feed solution and $NaNO_3$, HNO_3 and hydrazine sulphate were stripping solutions. All of the solutions were continuously recirculated. The membrane based separation system was effectively applied to removal of Cr(VI) from different aqueous samples, such as spiked natural waters and industrial waters at $\mu g\ L^{-1}$ levels.

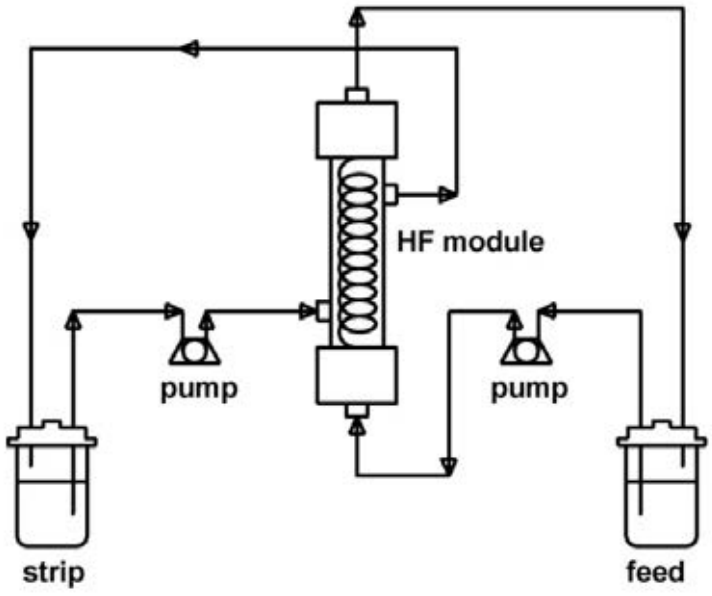


Figure 2.7 Schematic diagram of the continuous HF-LPME process for the removal and preconcentration of Cr(VI) [40].

In 2011, Bey et al. [41] prepared a hydrophilic hollow fiber membranes based on a modified polyether ether ketone (PEEK-WC) and used them as membrane contactors for removal of Cr(VI) from aqueous solutions. Aliquat-336 dissolved in kerosene was used as an extractant. In the system as shown in Figure 2.8, the

membrane contactor modules contained three hollow fiber membranes. The aqueous and organic phases contacted in parallel flow were recirculated. The aqueous phase flowed through the lumen of the fibers while the organic phase circulated through the shell side of the membranes. The working ranges of Cr(VI) were 15-100 mg L^{-1}.

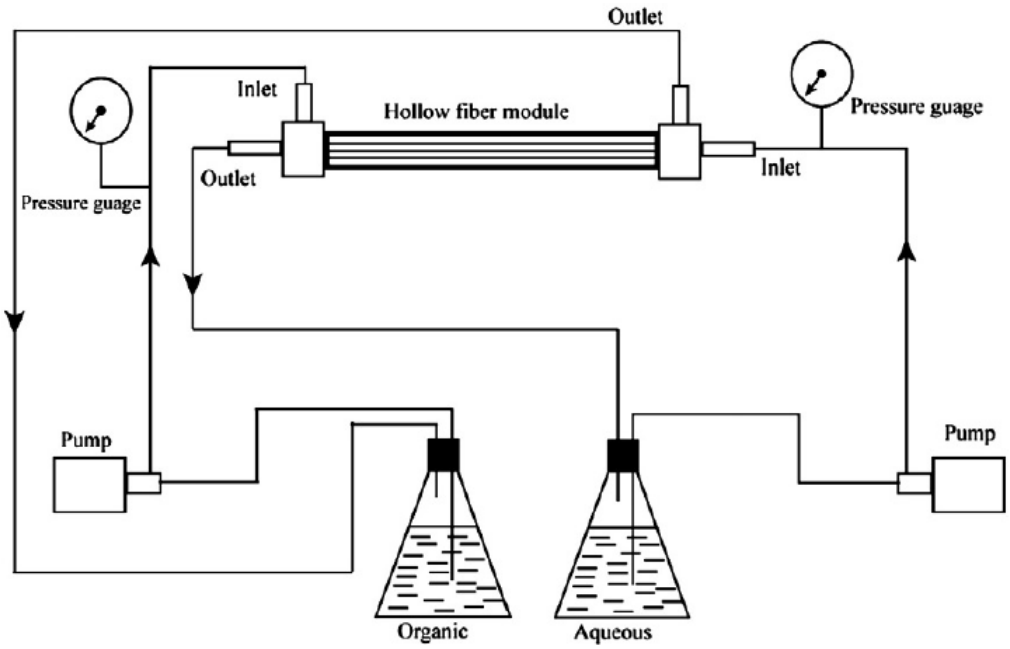


Figure 2.8 Schematic diagram of the hollow fibers membrane contactors for the removal of Cr(VI) [42].

Recently, hollow fiber membrane has been widely used for microextraction techniques due to its stability and inexpensiveness. Hollow fiber membrane could be operated in batch, modules, and automation mode. Many set-up experiments of hollow fiber membrane for microextraction have been reported as follow,

In 2002, Zhao and Lee [43] developed a static and dynamic hollow fiber-liquid phase microextraction (HF-LPME) as a sample preparation technique prior to gas chromatography/mass spectrometry. The schematic representation of the static HF-LPME was shown in Figure 2.9. In extraction process, GC microsyringe was used to introduce organic solvent into the 1.5-cm hollow fiber membrane, and also to serve as a sample introduction device for the GC/MS. The membrane pores were first

impregnated by the organic solvent and filled with 3 μL of the same solvent. Then, the assembly was placed in the aqueous sample. In the static mode, the microsyringe was held still, while the dynamic mode, the microsyringe was withdrawn and depressed alternately.

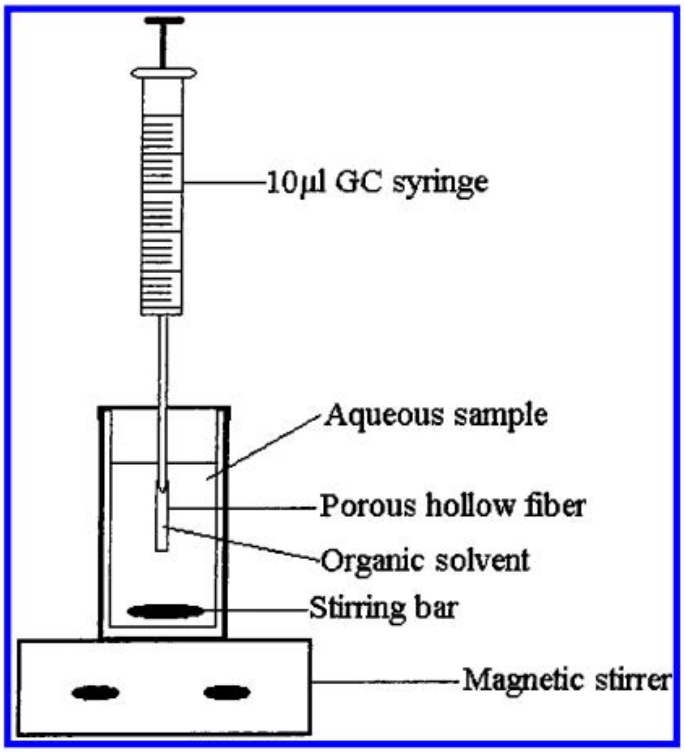


Figure 2.9 Schematic diagram of the static HF-LPME [43].

In 2004, Jiang and Lee [44] developed a new and simple liquid phase microextraction method called solvent bar microextraction (SBME). In the experimental setup as shown in Figure 2.10, the organic solvent was filled within a 2-cm length of a hollow fiber membrane where both ends were sealed, called solvent bar. The solvent bar was placed in a stirred aqueous sample solution for extraction. After extraction, the solvent bar was taken out. One end of the hollow fiber was cut off, and an analyte-enriched organic solvent was withdrawn into the syringe for further instrumental analysis.

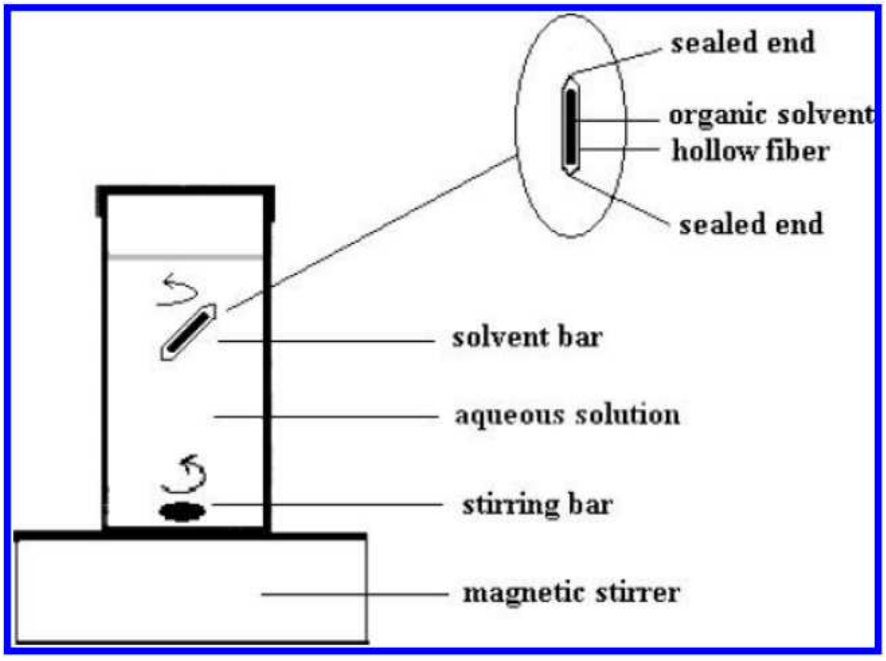


Figure 2.10 Schematic diagram of the solvent bar microextraction [44].

In 2006, Zhang et al. [45] developed a liquid-gas-liquid microextraction (LFLME) technique which was a new organic solvent-free microextraction technique. In the experimental set-up as shown in Figure 2.11, a 2.65-cm polypropylene hollow fiber membrane was used as barrier between the aqueous sample solution and the aqueous acceptor phase (0.5 M NaOH) for extraction of phenols in water. The sample solution is separated from the acceptor phase in the channel of the hollow fiber by the hydrophobic microporous hollow fiber wall with air inside its pores. In extraction procedures, the needle of the microsyringe was pierced throught the septum of the sample vial. Six microliters of 0.5 M NaOH solution in a microsyringe was then filled into the lumen of the hollow fiber as the acceptor solution. Then, the assembly was placed in the sample solution. Phenols in the solution diffused through the microporous hollow fiber membrane and were trapped by the acceptor solution. After an appropriate time, the acceptor solution was withdrawn back into the microsyringe for further instrumental analysis.

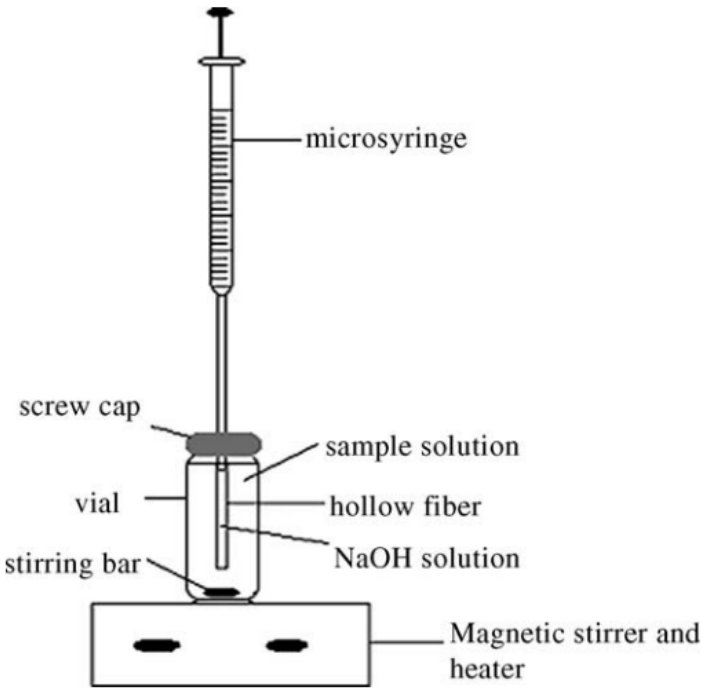


Figure 2.11 Schematic diagram of the liquid-gas-liquid microextraction (LGLME) [45].

In order to form the violet complex with diphenylcarbazide (DPC), the Cr(III) species must be oxidized to Cr(VI). The oxidation may be conveniently performed in acid solution with permanganate, MnO_4^- [46]. In basic medium, the oxidation may be performed with bromine, Br_2 [47] or with hydrogen peroxide, H_2O_2 [48]. For spectrophotometric purposes, H_2O_2 was preferred, since it does not absorb at 548 nm. The oxidation of Cr(III) to Cr(VI) in basic solution may be formulated as Eq. (6).

$$2Cr^{3+} + 8OH^- + 3H_2O_2(aq) \rightleftharpoons 2HCrO_4^-(aq) + 6H_2O \qquad (6)$$

In acidic medium, the reaction presented by Eq. (6) is reversed and Cr(VI) is reduced to Cr(III). So the reaction should go forward in basic medium.

From the literature reviews mentioned above, hollow fiber membrane-based Aliquat 336 is the attractive operation mode for separation of chromium due to anion-exchange property of Aliquat 336 and stability of supported liquid in hollow fiber

membrane. This research aimed to separate and preconcentrate two species of chromium, Cr(III) and Cr(VI) in water using hollow fiber supported liquid membrane extraction and determine by diphenylcarbazide-based spectrophotometric method.

The new design method based on anion exchange HF-LPME was studied for preconcentration and speciation of chromium. A short hollow fiber membrane impregnated with an anion exchanger; Aliquat 336 was proposed. Only the pores of the hollow fiber membrane were filled with the aliquat 336 and immersed into the water sample for extraction of Cr(VI) ion. The extracted Cr(VI) in the membrane was then eluted and formed complex with DPC for direct determination of Cr(VI). Cr(III) could be proceeded and determined in the same way after oxidation to Cr(VI) and subtracted by the Cr(VI). The approach reduced the use of volume of the organic extraction solvent, which was environmental friendly and improved the simplicity and inexpensiveness.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and reagents

1. Potassium chromate ($K_2Cr_2O_7$) (BDH Chemicals, UK)
2. Chromium nitrate nonahydrate ($Cr(NO_3)_3.9H_2O$) (Fisher Scientific, UK)
3. 1,5 diphenylcarbazide (DPC) (Sigma-Aldrich, USA)
4. Methyltrialkyl-ammonium chloride (Aliquat 336) (Merck, Germany)
5. Kerosene (Carco Chemical CO., LTD., Thailand)
6. Sodium hydroxide (NaOH) (Merck, Germany)
7. Hydrogen peroxide 30% (H_2O_2) (Merck, Germany)
8. Ethanol (Merck, Germany)
9. Sulfuric acid (H_2SO_4) (J.T. Baker, Thailand)
10. Nitric acid 65% (HNO_3) (Merck, Germany)

3.2 Instruments and equipments

1. Fiber optic UV-Vis spectrophotometer with Z-flow cell (Avantes BV, the Netherlands)
2. Inductive coupled plasma atomic emission spectrometer (ICP-AES) iCAP 6000 series (Thermo-scientific, USA)
3. Polypropylene hollow fiber membrane Accurel® Q3/2, 600 μm ID 200 μm thickness, 0.2 μm pore size (Membrana, Wuppertal, Germany)
4. pH meter model 744 (Metrohm, Switzerland)
5. Magnetic stirrer (Fisher Stirrer®, USA)
6. Hot plate stirrer (Stuart, UK)
7. Magnetic stirring bar (Spinbar, USA)
8. Medical syringe 3 mL (Becton Dickinson, Singapore)
9. Medical syringe needle with O.D. x length: 0.55 x 25 (mm) (Nipro, Japan)
10. Micro syringe 100 μL (SGE, Australia)
11. Micro filter holder (Advantec, Japan)

12. Filtration membranes (Nylon membrane filter 47 mm 0.45 μm) (Munktell filter, Germany)
13. Autopipettes and tips 100 μL, 1000 μL, and 10 mL (Eppendorf, USA)
14. EPA Vial Kit 20 mL (Vertical chromatography, Thailand)
15. Micro insert vial 300 μL (Vertical chromatography, Thailand)

3.3 Experiment

3.3.1 Preparation of chemical solutions

The stock of 100 mg L^{-1} standard Cr(III) solution was prepared from $Cr(NO_3)_3.9H_2O$ in deionized water. The stock of 100 mg L^{-1} standard Cr(VI) solution was prepared from $K_2Cr_2O_7$ in deionized water. The extracting organic solvent, 10% (v/v) Aliquat 336, was prepared by diluting Aliquat 336 in kerosene. A 6.5 mM 1,5 diphenylcarbazide (DPC) solution was prepared by dissolving 16 mg of DPC in 10 mL ethanol and acidified by 60 μL conc. H_2SO_4.

The oxidant was prepared by dilution of 30% H_2O_2 to 3% with deionized water. A 0.013 M sodium hydroxide solution was freshly prepared by dissolution NaOH in deionized water.

3.3.2 Hollow fiber supported liquid membrane method

The hollow fiber membrane was manually cut into 2.0 cm pieces, and sonicated for 5 min in acetone to remove any contaminants, and allowed to evaporate completely prior to use. The experimental setup was illustrated in Figure 3.1. The hollow fiber membrane was immersed into the extracting organic solvent (10% (v/v) Aliquat 336 in kerosene) for a few minutes in order to fully impregnate the extraction solvent into the porous wall of the hollow fiber. Then, a medical syringe with a stainless steel needle tip was inserted into one end of the hollow fiber. The excess solvent in the lumen was removed using mild air blow. The sample solution of 20 mL containing Cr(VI) was placed in a 20 mL sample vial. The syringe was clamped to set its position. The membrane (together with the syringe needle) was put into the sample solution that was stirred at 600 rpm. After an appropriate extraction time, the

syringe was removed and the membrane was transferred into a 0.3 mL micro insert vial for elution of Cr(VI) as described in next section.

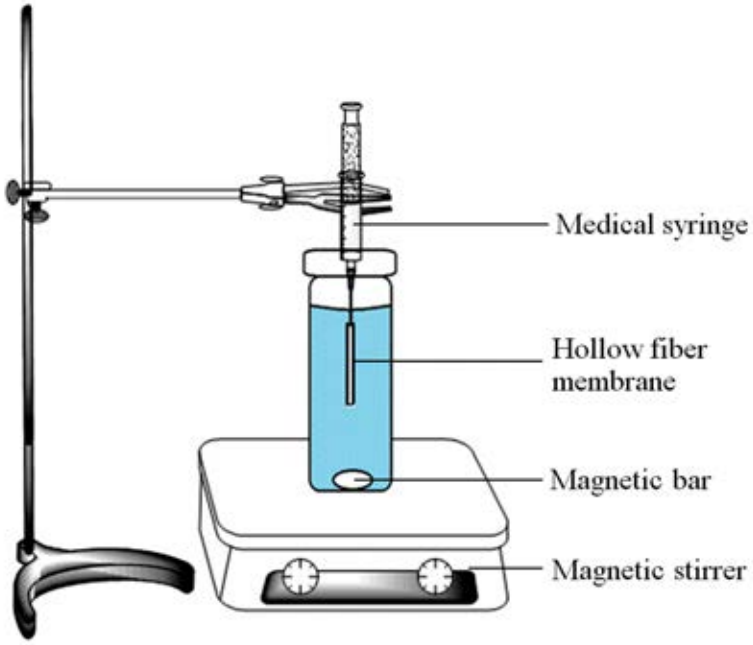


Figure 3.1 Schematic diagram of the hollow fiber liquid phase microextraction (HF-LPME) system.

3.3.3 Determination of chromium

After extraction, the hollow fiber membrane was soaked with a 200 µL of 6.5 mM DPC in ethanol solution for 15 min to ensure that the violet complex of Cr(VI)-DPC was completely formed, as shown in Figure 3.2. In this procedures, ethanol could eluted Aliquat 336 from the hollow fiber membrane due to its solubility enabling DPC to react with Cr(VI) enriched in Aliquat 336. The volume of 200 µL was the minimum volume that completely soaked the whole length of membrane in insert vial. A portion of the complex solution was withdrawn by micro syringe and injected into the micro Z-flow cell connected to the fiber optic UV-Vis spectrometer at 548 nm.

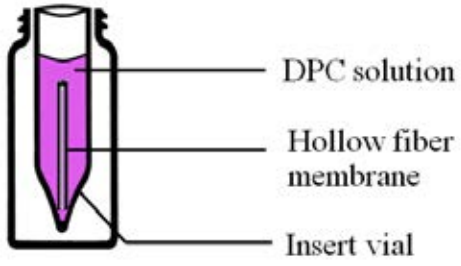


Figure 3.2 Scheme of complex forming of Cr-DPC procedure.

3.3.4 Oxidation of Cr(III) to Cr(VI)

The experiment was adapted from the oxidation method by J.E.T. Andersen [49]. A 60 mL of sample solution was adjusted to pH 10 by addition of 9.0 mL of 0.013 M sodium hydroxide. Then 3.0 mL of 3% H_2O_2 was added into the solution. The solution was heated on a hotplate for 45 minutes at 80 ºC to allow the oxidation reaction to be complete, and then boiled for 15 minutes in order to remove any excess of hydrogen peroxide. The chromium (original Cr(VI) and Cr(VI) after oxidized from Cr(III), were extracted and determined as a total by method described in section 3.3.2 and section 3.3.3, respectively. The concentration of Cr(III) could be calculated from Eq. (7).

$$[\mathrm{Cr(III)}] = [\text{total Cr}] - [\mathrm{Cr(VI)}] \quad (7)$$

3.3.5 Optimization of the hollow fiber liquid phase microextraction (HF-LPME)

3.3.5.1 Aliquat 336 concentration

The Aliquat 336 concentration has a significant effect on the Cr(VI) transportation of Cr(VI) into the membrane. The enrichment factor is the ratio of the concentration of the analyte in the extracting solvent to that in the sample solution, which corresponds to the sensitivity of the method. It is expected that the

enrichment factor of the extraction would increase with the organic concentrations. Thus, Aliquat 336 in various concentration range of 10-50 % (v/v) was studied.

3.3.5.2 Extraction time

Since the liquid phase microextraction is a non-exhaustive extraction, the mass transfer of the analyte into the extracting phase depends on extraction time that relates to the sensitivity of the method. An extraction time in the range of 5-45 min was studied.

3.3.5.3 Sample volume

Since the enrichment factor (EF) of the analyte is related to the sample volume (V_{aq}) as described in the equation below:

$$EF = \frac{C_{org}}{C_o} = \frac{n_{org}/V_e}{C_o} = \frac{\left(\frac{KV_{org}}{1+KV_{org}/V_{aq}}\right)}{V_e}$$

where K is the distribution constant. C_{org} and n_{org} are the concentration and the mole of analyte extracted in the organic solvent, respectively. C_o is the concentration of analyte originally presented in the sample. V_e is the final volume of eluent. As the volume of the organic solvent (V_{org}) is kept constant, the enrichment factor could be influenced by the volume of the sample (V_{aq}). A 10 mL and 20 mL sample volumes were studied.

3.3.5.4 Stirring rate

Stirring rate can enhance the diffusion of the analyte from the bulk to the surface of the membrane, where the mass transfer occurs. Stirring rates in the range of 100-700 rpm was studied.

3.3.5.5 Matrix effect

The presence of other anions may affect the extraction of Cr(VI) ions due to the competition of Cr(VI) ions and other anions on the active sites

of the aliquat 336. Matrix effect was studied using chloride and sulfate as interference ions. Various concentrations of sodium chloride and sodium sulfate were studied.

The Cr(VI) reaction with diphenylcarbazide is usually free from interferences [50]. Although iron(III) ion could produce a yellow colour with DPC, it does not interfere with our method because the anion-exchange extraction mode of Aliquat 336 can extract only Cr(VI) anion from the iron cations prior to DPC reaction. For this reason, the effect of interfering ion on DPC reaction may be negligible.

3.3.6 Optimization of the oxidation of Cr(III) to Cr(VI)

For the determination of Cr(III), Cr(III) must be converted to Cr(VI) which could be determined by diphenylcarbazide-based spectrophotometry method. The parameters influenced the oxidation of Cr(III) to Cr(VI) were studied as follow.

3.3.6.1 Hydrogen peroxide concentration

Hydrogen peroxide was used as oxidizing agent. The amount of hydrogen peroxide should be sufficient for completely oxidizing Cr(III) to Cr(VI) and should disturb the extraction of chromium the least. Hydrogen peroxide concentrations in the range of 0.125-1.125 % (v/v) were studied.

3.3.6.2 Sodium hydroxide concentration

Sodium hydroxide was used for pH adjustment required for oxidation reaction. The amount of sodium hydroxide should be appropriate for oxidizing Cr(III) to Cr(VI) and should disturb the extraction of chromium the least. Sodium hydroxide concentrations in the range of 0.001-0.045 M were studied.

3.3.7 Method evaluation

The performance of the HF-LPME followed by diphenylcarbazide based UV-Vis spectrophotometric method for preconcentration and speciation of chromium in surface waters was evaluated. Linearity, accuracy, precision, limit of detection and enrichment factor were evaluated.

3.3.7.1 Calibration curve and linearity

The linear calibration curve between the absorbance and the concentrations of Cr(VI) was established for the concentration ranging from 40-160 μg L^{-1}. The linear regression method was used to obtain slope, intercept and R^2.

3.3.7.2 Accuracy and precision

Intra-day and inter-day replicate determination of 50 μg L^{-1} of Cr(VI) was tested for method precision. The recoveries of spiked 50 μg L^{-1} of Cr(VI) samples were determined for method accuracy.

3.3.7.3 Limit of detection

Ten blank solutions were analyzed for obtaining the blank signals. The limit of detection was defined as the concentration calculated from the signal at the average blank signal plus 3 times of standard deviation of blank signal. ($\bar{X} + 3SD_B$)

3.3.7.4 Enrichment factor and extraction efficiency

The enrichment factor (EF) of the analyte was calculated from the equation below:

$$\mathrm{EF} = \frac{\mathrm{C_{org}}}{\mathrm{C_o}}$$

where C_{org} is the concentration of analyte extracted in the organic solvent; C_o is the concentration of analyte originally presented in the sample.

3.3.8 Sample collection

Surface water samples were collected from 6 sources; a pond in front of Physics building, Chulalongkorn University; a pond at the main entrance of Chulalongkorn University; a pond at the faculty of Science, Chulalongkorn University; Phasrichareon Canal; Ratburana Canal; and Chaengron Canal. Due to the determination of dissolved chromium in the water samples, they were filtered through

a membrane filter (0.45 μm) to get the clear water, stored in individual polypropylene bottles, and refrigerated.

3.3.9 Determination of chromium by ICP-AES method

Chromium concentrations in the samples were determined ICP-AES method according to the USEPA method 200.7 [25] and compared with those obtained from out method. All samples were collected by the procedures in section 3.3.8 and acidified with 2 drop of conc. HNO_3 prior to analyze by ICP-AES method. The emission line for chromium was 267.716 nm. The torch was in axial view mode. The results were statistically compared using Paired t-Test method at 95% confidence level.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Diphenylcarbazide UV-Vis spectrophotometry method for determination of Cr(VI)

The calibration curve for determination of chromium by diphenylcarbazide UV-Vis spectrophotometry method was established in the concentrations range of 200-1000 µg L^{-1}. The linear regression was obtained in Figure 4.1 with the correlation coefficient (R^2) > 0.99. The method provided the limit of detection about 74 µg L^{-1}.

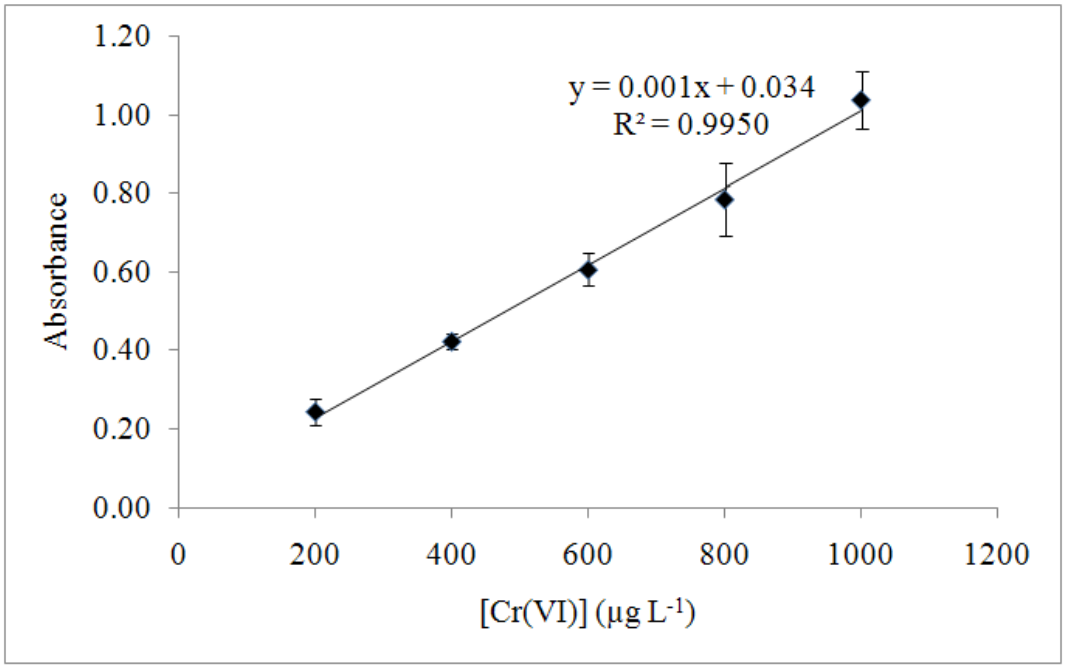


Figure 4.1 Calibration curve obtained when determined Cr(VI) concentration in ranges of 200-1000 µg L^{-1} by diphynylcarbazide-based spectrophotometric method.

4.2 Optimization of the hollow fiber liquid phase microextraction (HF-LPME)

4.2.1 Aliquat 336 concentration

The enrichment factors of the extraction of Cr(VI) obtained from various concentrations of Aliquat 336 in the range of 10-50 % (v/v) on the extraction of Cr(VI) from water are shown in Figure 4.2. It was found that the enrichment factors were not significantly altered in the studied concentration range indicating that the active sites, Cl^- of Aliquat 336 in the concentration of 10 % (v/v) was high enough to exchange with Cr(VI) in the form of $Cr_2O_7^{2-}$. The 10 % (v/v) Aliquat 336 was chosen because it was the minimum concentration of Aliquat 336 that gave high enrichment factor.

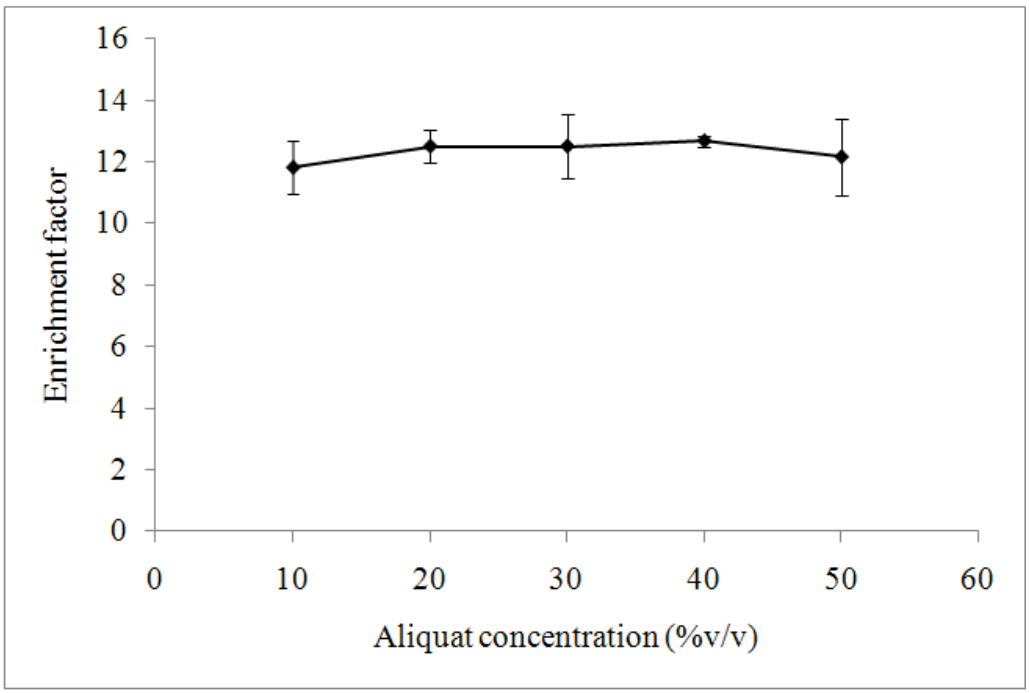


Figure 4.2 Effect of Aliquat 336 concentration on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; extraction time: 15 min; stirring rate: 600 rpm; sample volume: 20 mL)

4.2.2 Extraction time

In this study, the extraction time from 5 to 45 min was optimized. The enrichment factor for each extraction time was determined. As shown in Figure 4.3, the enrichment factors increased as the extraction times increased. The extraction time was optimized between the method sensitivity and the speed of analysis. It was not necessary to choose the extraction time that gives the highest sensitivity because it may be too long. For this reason, the extraction time that gives sufficient sensitivity for determination of chromium below the concentration of 50 µg L^{-1}, required by the WHO regulation, may be considered. In the experiment, the extraction time of 15 min was chosen because it provided limit of detection below 50 µg L^{-1}.

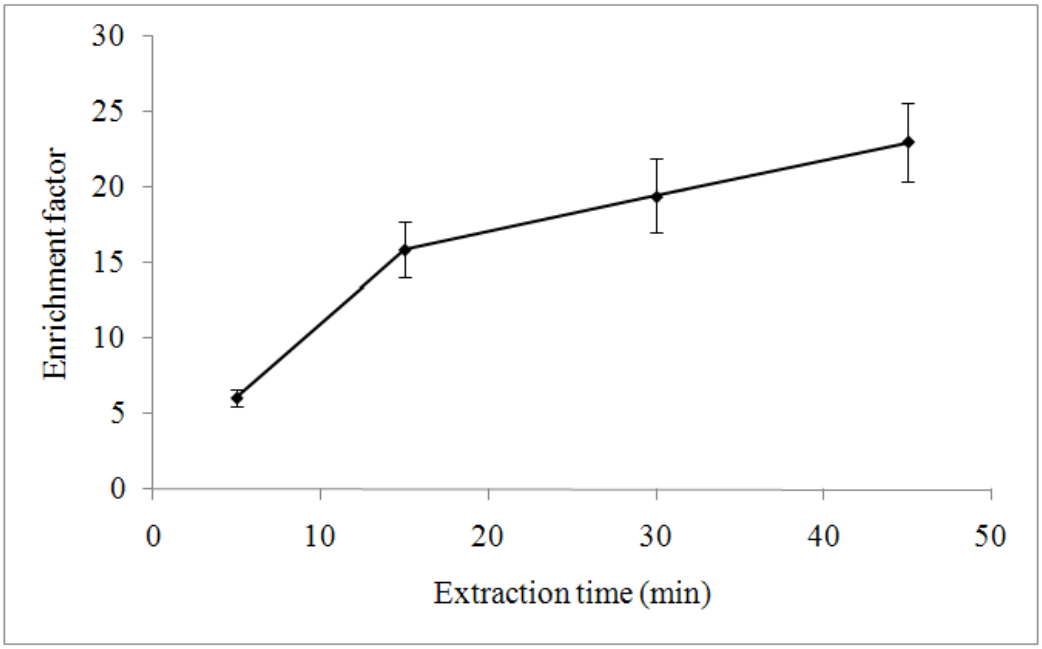


Figure 4.3 Effect of extraction time on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; stirring rate: 600 rpm; sample volume: 20 mL)

4.2.3 Sample volume

In this study, 50 µg L^{-1} sample solutions with volumes varied at 10 and 20 mL were tested. As shown in Figure 4.4, the sample volume of 20 mL gave higher enrichment factor and better sensitivity than that of 10 mL. Theoretically, the more sample volume would provide more analytes being extracted resulting in higher enrichment factor as well as better method sensitivity. The sample volume of 20 mL has provided sufficient sensitivity required by the WHO regulation. Hence, 20 mL was chosen for further studies.

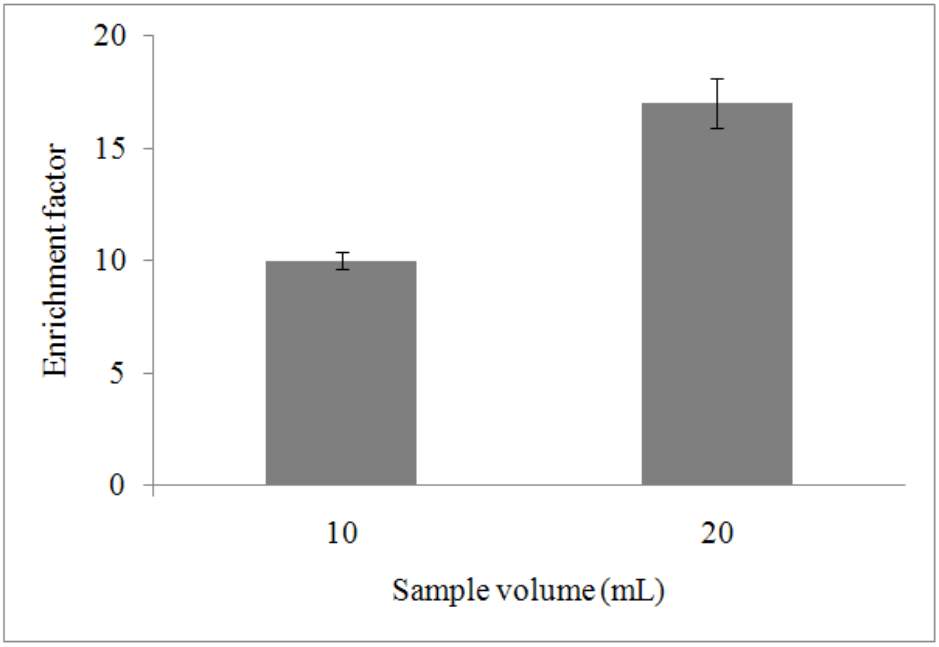


Figure 4.4 Effect of sample volume on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; extraction time: 15 min; stirring rate: 600 rpm)

4.2.4 Stirring rate

The stirring rates ranging from 100-700 rpm was tested. The result shown in Figure 4.5 indicates that stirring the sample greatly improves the enrichment factor. However, the stirring rate at 700 rpm was too fast so that the solution would be vigorously stirred and the magnetic bar would spin unsmoothly. Thus, the stirring rate of 600 rpm was used for subsequent experiments.

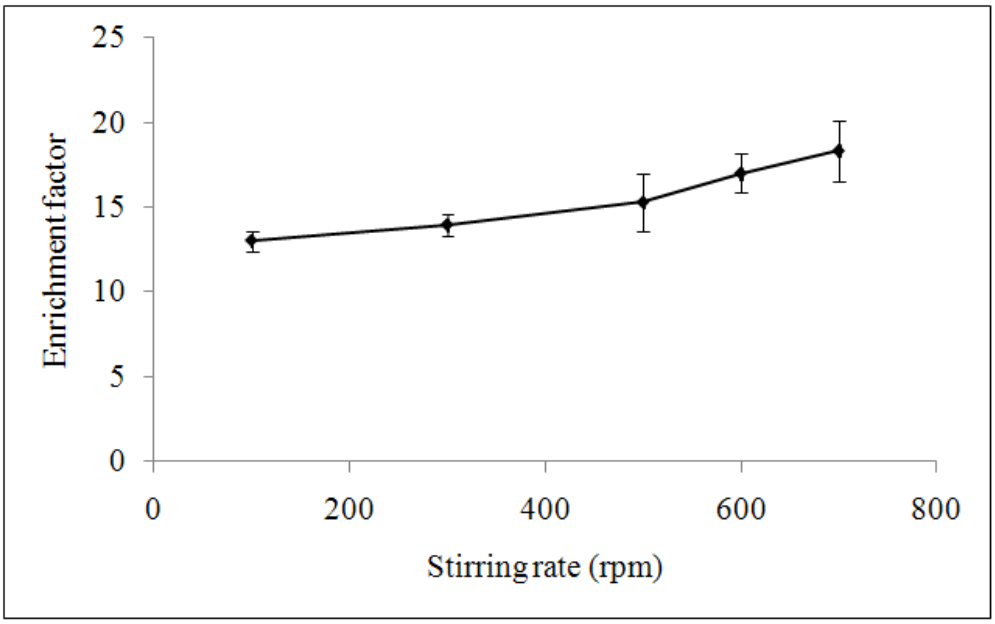


Figure 4.5 Effect of stirring rate on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; extraction time: 15 min; sample volume: 20 mL)

4.2.5 Matrix effect

The presence of other anions may affect the extraction of Cr(VI) ions due to the competition of Cr(VI) ions and other anions on the active sites of the aliquat 336. The common anions in natural water are sulfate (SO_4^{2-}), chloride (Cl^-), and nitrate (NO_3^-). In this study, Cl^- and SO_4^{2-} were investigated

The effect of Cl^- on the enrichment factor for Cr(VI) extraction was investigated using various concentration of sodium chloride. The result is shown in Figure 4.6. It indicates that the enrichment factors decreased when chloride concentration increased. So the presence of Cl^- had an effect on the extraction of Cr(VI) ions. Moreover, the effect was dependent on the concentration of Cl^-. The reason might be explained by the ion exchange equilibrium (Eq. (8)). Compared to the lower concentration of Cl^-, the higher concentration of Cl^- provide more Cl^- in the solution so that it might have caused less $Cr_2O_7^{2-}$ being exchanged with the Aliquat 336, resulting in poor enrichment factor of Cr(VI) extraction.

$$2(\text{R-Cl})_{org} + Cr_2O_7^{2-} \rightleftharpoons 2(\text{R-}Cr_2O_7)_{org} + 2Cl^- \quad (8)$$

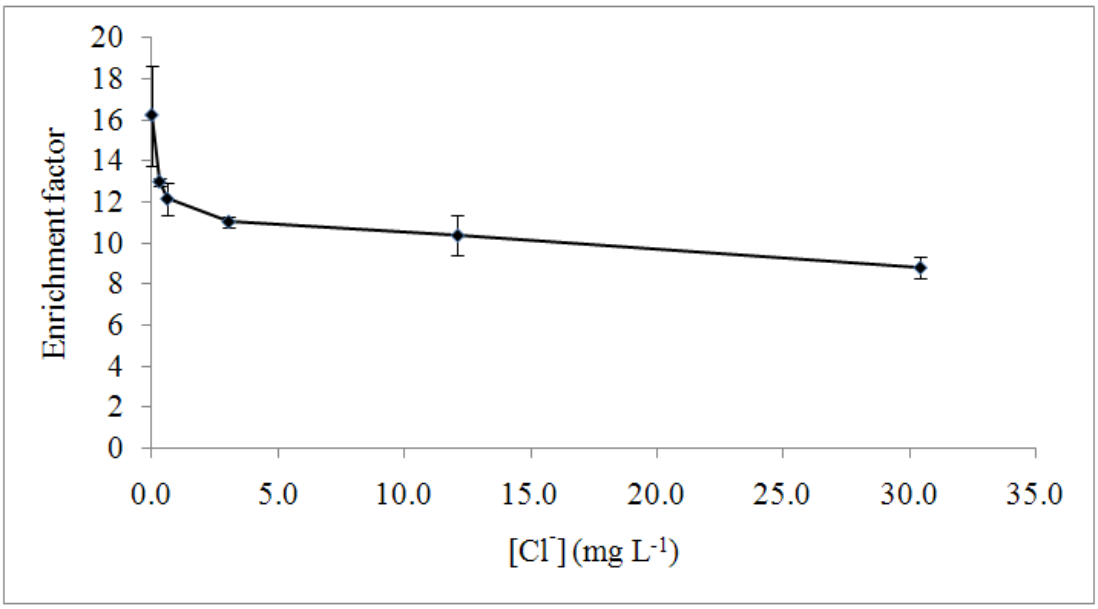


Figure 4.6 Effect of chloride concentration on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; extraction time: 15 min; sample volume: 20 mL)

The effect of SO_4^{2-} on the enrichment factor for Cr(VI) extraction was investigated using various concentration of sodium sulfate. The result is shown in Figure 4.7. It indicates that the enrichment factors decreased when sulfate concentration increased. So the presence of SO_4^{2-} had an effect on the extraction of Cr(VI) ions. Moreover, the effect was dependent on the concentration of SO_4^{2-}. The reason might be explained by the electrostatic attraction between ions and the ion exchanger, which depends on charge and hydrated size of the ions. Since SO_4^{2-} probably is smaller than $Cr_2O_7^{2-}$, the presence of SO_4^{2-} may compete with $Cr_2O_7^{2-}$ for ion exchange on Aliquat 336.

Certainly, this conclusion applies within the concentration range studied and cannot be generalized for all ionic compositions. However, it does indicate that the results obtained with model bi-ionic systems can be extended to multi-ionic systems that are typical for surface water matrices [51].

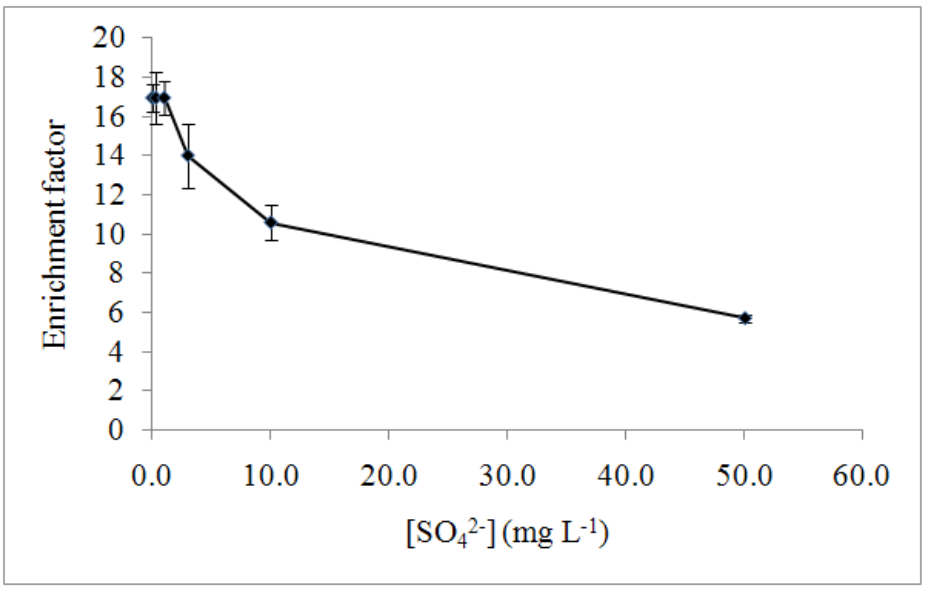


Figure 4.7 Effect of sulfate concentration on enrichment factor for HF-LPME of Cr(VI) in water samples. (50 µg L^{-1}, n=3; extraction time: 15 min; sample volume: 20 mL)

Since the natural water samples contain different ion concentrations depending on the water source, the presence of ions at different concentrations may affect the extraction efficiency of the method. The comparison of the enrichment factors obtained from three different kinds of matrices; i.e. deionized water, drinking water, and surface water is illustrated in Figure 4.7. The enrichment factors of three matrices were different. Deionized water gave the highest enrichment factor because it had less ions. In contrast, the surface water gave the least enrichment factor due to its complicated matrices. This shows that the matrix could affect to the extraction sensitivity. Hence, the matrix matching technique should be considered for compensate background in the calibration procedure. Matrix matching is the technique that can be used to compensate the matrix effects that influence analytical response.

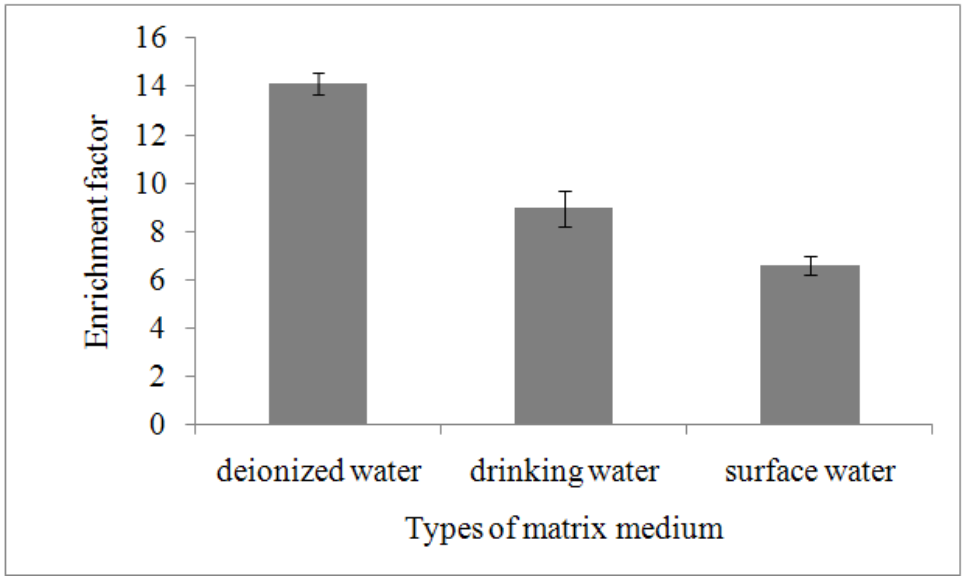


Figure 4.8 Effect of different matrix mediums on enrichment factor for HF-LPME of Cr(VI). (50 µg L^{-1}, n=3; extraction time: 15 min; sample volume: 20 mL)

4.3 Optimization of the oxidation of Cr(III) to Cr(VI)

In oxidation reaction as shown in Eq. (9), Cr(III) is oxidized by hydrogen peroxide and giving anion Cr(VI) in $HCrO_4^-$ form. Then $HCrO_4^-$ can change into $Cr_2O_7^{2-}$ by the equilibrium in Eq. (10). No matter what species of anion are (i.e. $HCrO_4^-$, $Cr_2O_7^{2-}$), they can form complex with DPC, similarly. So the Cr(VI) anion in any forms can be determined by the DPC method.

$$2Cr^{3+} + 8OH^- + 3H_2O_2(aq) \rightleftharpoons 2HCrO_4^-(aq) + 6H_2O \quad (9)$$

$$2HCrO_4^-(aq) \rightleftharpoons Cr_2O_7^{2-}(aq) + H_2O \quad (10)$$

4.3.1 Hydrogen peroxide concentration

The hydrogen peroxide concentrations in the range of 0.125-1.125 % (v/v) were studied as shown in Figure 4.8. The results showed that the enrichment factors were not significantly altered in the studied concentration range. The reason for this can be explained by stoichiometrically by Eq. (8) that the hydrogen peroxide

of 0.125 % (v/v) had been excessive to oxidize Cr(III) to Cr(VI). Hence, 0.125 % (v/v) hydrogen peroxide was chosen.

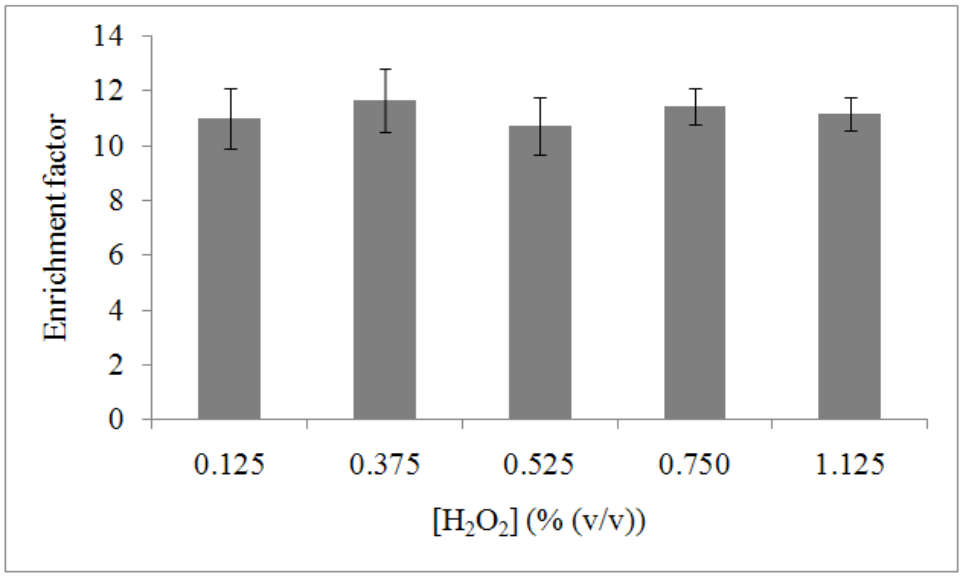


Figure 4.9 Effect of hydrogen peroxide concentrations on enrichment factor for the oxidation of Cr(III) to Cr(VI) prior to HF-LPME. (50 µg L^{-1} Cr(III), 0.02 M NaOH, n=3; extraction time: 15 min; sample volume: 20 mL)

4.3.2 Sodium hydroxide concentration

Sodium hydroxide plays a role as pH-adjustment solution. The oxidation of Cr(III) to Cr(VI) is occurred at pH 10. Considering that additional of ions may affect the extraction of chromium ion in the solution based on matrix effect as described in section 4.2.5, the amount of sodium hydroxide should be at least as possible. The concentrations of sodium hydroxide in the range of 0.001-0.045 M were studied as shown in Figure 4.9. The results showed that the sodium hydroxide concentration of 0.001 M gave the highest enrichment factor because it interfered the system the least. Hence, the 0.001 M of sodium hydroxide was used in further studies.

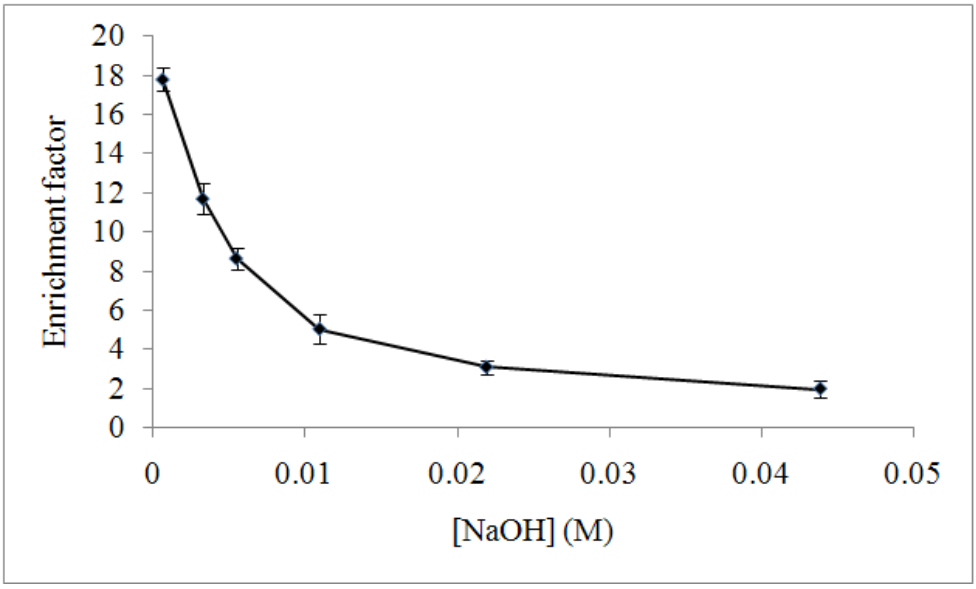


Figure 4.10 Effect of sodium hydroxide concentrations on enrichment factor for the oxidation of Cr(III) to Cr(VI) prior to HF-LPME. (30 µg L^{-1} Cr(III), n=3; extraction time: 15 min; sample volume: 20 mL)

Since there was an addition of NaOH in the oxidation of Cr(III) to Cr(VI) and determined as a total as well as there was an influence of other ions in the extraction of Cr(VI) ion (section 3.3.2), the sensitivities of extraction of Cr(VI) with and without addition of NaOH were shown in Figure 4.10.

The sensitivities of the determination of Cr(VI) with and without addition of NaOH were indicated by the slopes which were 0.0086 and 0.0274, respectively. It should be noticed that the sensitivities of the determination of Cr(VI) with- and without- addition of NaOH were not comparable. In the determination of Cr(III) by oxidizing to Cr(VI) using hydrogen peroxide and sodium hydroxide as Eq. (8), the sensitivity of method was 0.0079 which was close to the sensitivity of the determination of Cr(VI) with addition of NaOH. For these reasons, in the speciation of Cr(VI), the same amount of NaOH was added in order to keep the sensitivity comparable.

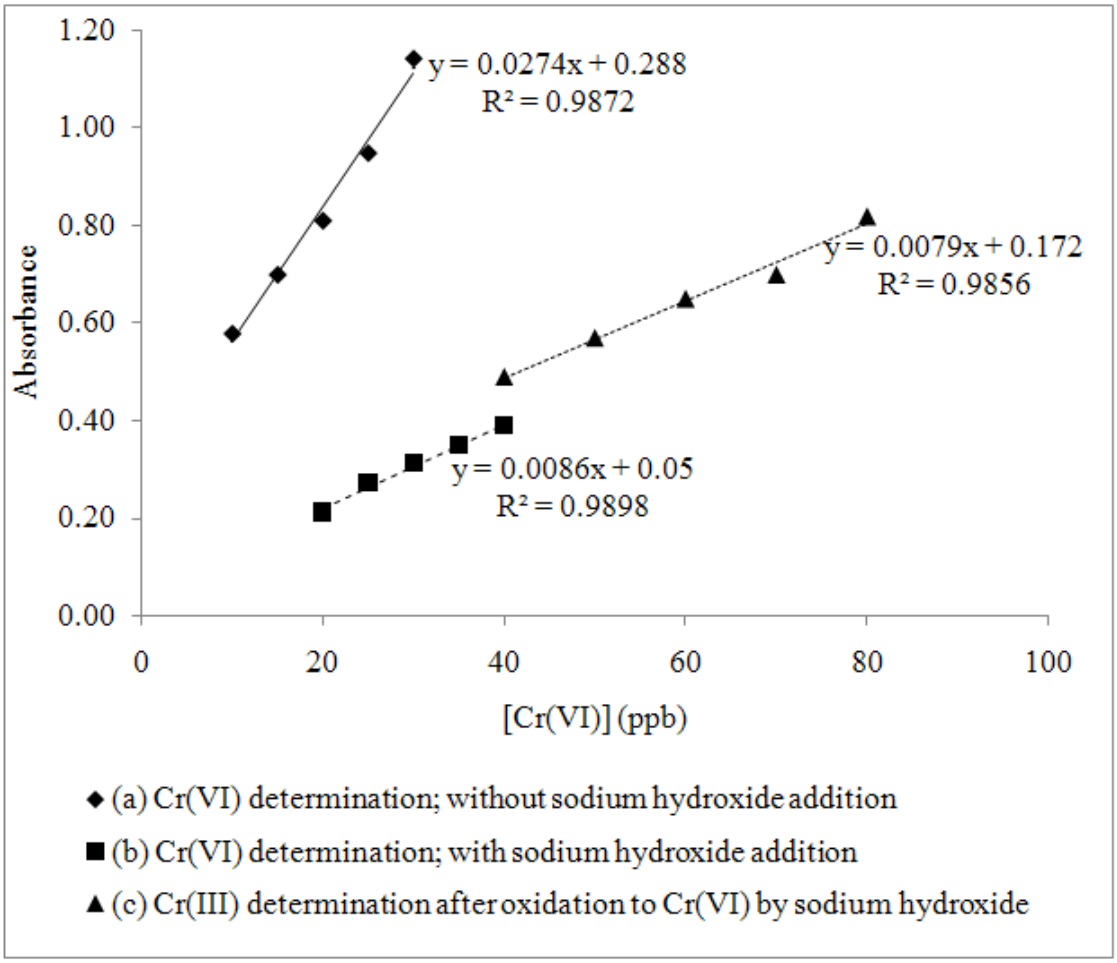


Figure 4.11 Calibration curves obtained when determined: (a) Cr(VI) without sodium hydroxide addition; (b) Cr(VI) with sodium hydroxide addition; (c) Cr(III) after oxidation to Cr(VI) by sodium hydroxide.

4.3.3 Performance of oxidation of Cr(III) to Cr(VI)

The oxidation efficiency of Cr(III) to Cr(VI) was studied by calculation of yield that Cr(III) could be oxidized to Cr(VI). It was calculated based on Eq. (11).

$$\text{yield } (\%) = \frac{A_{\text{tot. Cr}} - A_{\text{Cr(VI)}}}{A_{\text{Cr(VI)}}} \times 100 \qquad (11)$$

where $A_{tot.\,Cr}$ is the absorbance of total chromium in Cr(VI) form

$A_{Cr(VI)}$ is the absorbance of Cr(VI) at the equivalent concentration of Cr(III)

Figure 4.11 shows the spectrum of Cr(VI) and Table 4.1 shows the absorbances obtained from a mixture of the same concentrations of Cr(III) and Cr(VI) (25 μg L^{-1}) before and after oxidation. The oxidation efficiency of Cr(III) to Cr(VI) was about 95 % with relative standard deviation less than 7 %. So the oxidation method was high performance and good precision for oxidizing Cr(III).

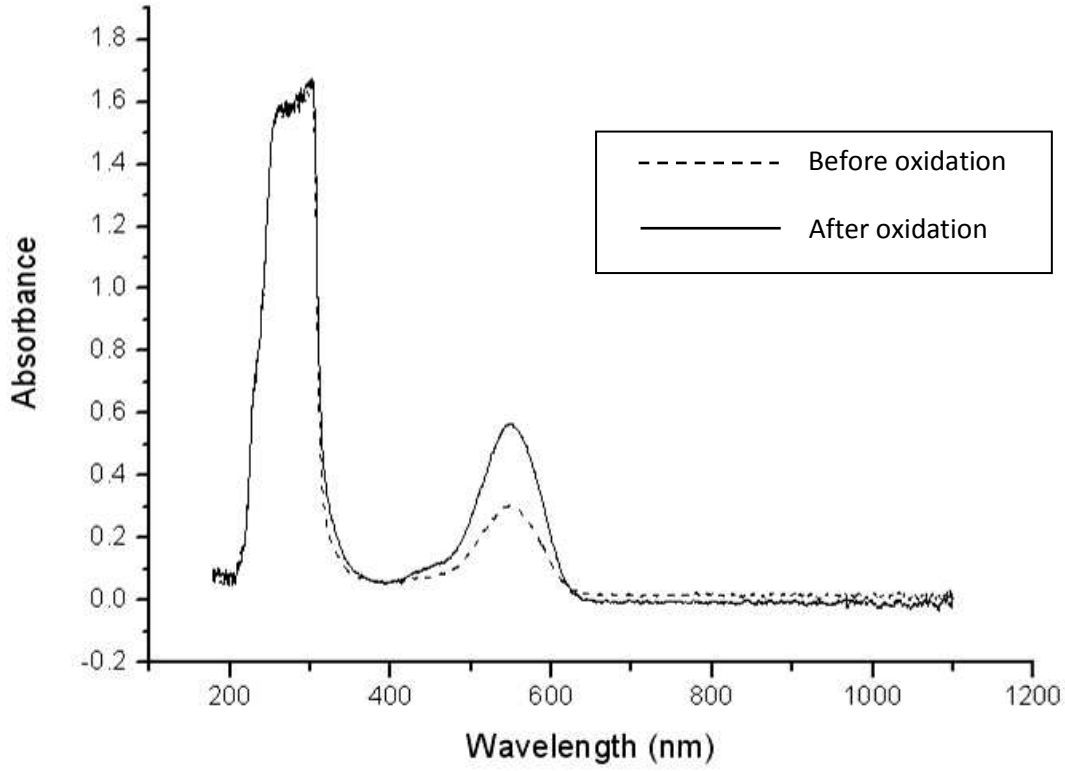


Figure 4.12 Spectrum of Cr(VI) obtained from a mixture of Cr(III) and Cr(VI) before and after oxidation process. (25 μg L^{-1} Cr(III); 25 μg L^{-1} Cr(VI))

Table 4.1 Absorbances of Cr(VI) obtained from a mixture of Cr(III) and Cr(VI) before and after oxidation process (25 μg L^{-1} Cr(III); 25 μg L^{-1} Cr(VI))

Batch	Absorbance of Cr(VI)		%Yield
	Before oxidation (as Cr(VI))	After oxidation (as total Cr(VI))	
1	0.28	0.56	100
2	0.24	0.45	88
3	0.29	0.57	97
		Mean	95
		SD	6
		%RSD	7

The optimal parameters of hollow fiber liquid phase microextraction followed by diphenylcarbazide based spectrophothmetric method for determination of Cr(VI) were summarized in table 4.2. The total analysis time was about 20 minutes.

Table 4.2 Optimum condition of HF-LPME extraction for speciation of chromium

Parameters	Optimum
Length of hollow fiber membrane	2 cm
Extracting solvent (acceptor solution)	10 % (v/v) Aliquat 336 in kerosene
Extraction time	15 min
Sample volume	20 mL
Stirring rate	600 rpm
Total analysis time	20 min
Hydrogen peroxide concentration	0 % (v/v) for non-oxidized sample; Cr(VI)
	0.125 % (v/v) for oxidized sample; total Cr
Sodium hydroxide concentration	0.001 M

4.4 Method evaluation

To study the performance of the method for speciation of chromium in water sample, surface water samples obtained from the pond in front of Physics Building, Chulalongkorn University were used as a model aqueous sample. Since the method is matrix dependent, the performance of method between real sample and deionized water were compared. Enrichment factor, linearity, accuracy, precision, and limits of detection were evaluated.

4.4.1 Linearity, accuracy, precision, limit of detection, and enrichment factor

Matrix matching technique was used for making calibration curve for determination of chromium. For analytical method using external standard, the standard solution was prepared by using sample-like medium. For determination of Cr(III) and Cr(VI) in surface water samples, the matrix matched calibration was obtained.

The calibration curves for determination of Cr(VI) were established for the concentrations ranging from 20-160 μg L^{-1}. The calibration is shown in Figure 4.12. In deionized water, a working range of 20-100 μg L^{-1} was observed giving the linear regression equation: $y = 0.0101x + 0.0090$ with correlation coefficient (R^2) = 0.9920. In surface water, a working range of 40-160 μg L^{-1} was observed giving the linear regression equation: $y = 0.0058x + 0.0752$ with correlation coefficient (R^2) = 0.9976. The linearity of the method was very good because it provided correlation coefficient greater than 0.99.

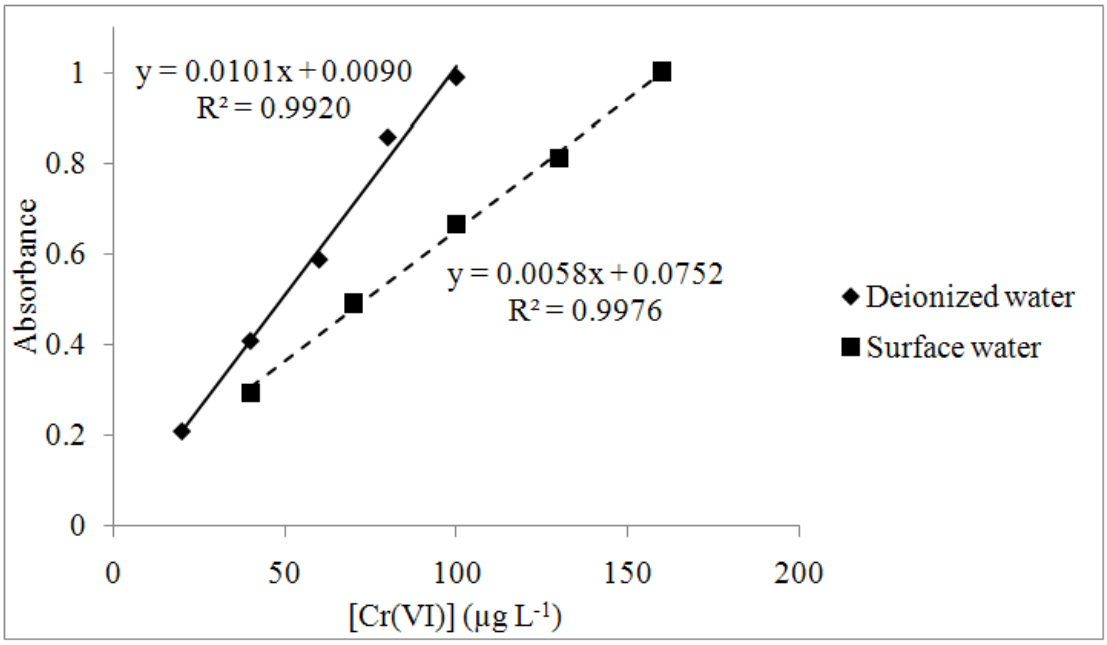


Figure 4.13 Calibration curves of Cr(VI) determination in deionized water and surface water.

The limit of detection (LOD) and limit of quantification were calculated based on these equations.

$$S_{LOD} = S_B + 3SD \quad (12)$$

$$S_{LOQ} = S_B + 10SD \quad (13)$$

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 Eq. (14)

$$SD = \sqrt{\frac{\Sigma(y_i - Y_i)^2}{n-2}} \quad (14)$$

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

The proposed method provides low limit of detection for both of deionized water and surface water. The limit of detection and enrichment factor were showed Table 4.3.

Table 4.3 Method evaluation of HF-LPME of Cr(VI) in water samples determined by diphenylcarbazide-based UV-Vis spectrophotometric method

Method evaluation	Deionized water	Surface water
Linear equation	y = 0.0101x + 0.0090	y = 0.0058x + 0.0752
R^2	0.9920	0.9976
Enrichment factor	15	5
RSD (%)	< 12	< 12
LOD (μg L^{-1})	3	10
LOQ (μg L^{-1})	12	29

Three non-spiked and spiked surface water samples; (1) a pond at the main entrance of Chulalongkorn University; (2) Phasrichareon Canal; and (3) Ratburana Canal were tested for accuracy of the speciation method. The accuracy of the method was expressed in term of recovery of spiked samples and summarized in Table 4.4. The recoveries in the range of 81.2-114.8 % were obtained for both species, Cr(III) and Cr(VI). The recovery of spiked samples was in the satisfactory range [52].

Table 4.4 Analytical results (mean ± standard deviation, n=3) for Cr(III) and Cr(VI) determination in surface water samples

Sample solution	Spiked (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Great pond, CU	-	-	20.0 ± 2.4	BDL*	-	-
	50	50	60.0 ± 1.0	40.6 ± 0.9	85.8	81.2
Phasrichareon Canal	-	-	BDL	BDL	-	-
	50	50	47.5 ± 2.1	51.7 ± 4.7	95.1	103.3
Ratburana Canal	-	-	BDL	BDL	-	-
	50	50	57.4 ± 1.9	57.2 ± 4.8	114.8	114.4

*BDL: below the detection limit

The 50 μg L^{-1} of Cr(VI) spiked surface water samples obtained from the pond in front of Physics Building, Chulalongkorn University was tested for precision of the speciation method. The precision of the method was determined by intra-day precision and inter-day precision. For intra-day precision, three samples (n=3) were analyzed during the same day, under the same experimental conditions. For inter-day precision, the samples were analyzed on six different days (n=6). The results as shown in Table 4.5, the relative standard deviation (RSD) was 4 and 11 % for intra-day precision and inter-day precision, respectively which were in the satisfactory range [52]. The method showed good precision with RSD not greater than 11 %.

Table 4.5 Precision of the proposed method evaluated using 50 μg L^{-1} of Cr(VI)

Precision	RSD (%)
Intra-day (n=3)	4
Inter-day (n=6)	11

4.4.2 Comparison between the proposed method and the standard method using ICP-AES

The method was compared with the standard method (USEPA method 200.7) using ICP-AES for determination of total Cr in surface water samples. Five surface water samples: (1) a pond at the main entrance of Chulalongkorn University; (2) a pond at the faculty of Science, Chulalongkorn University; (3) Phasrichareon Canal; (4) Ratburana Canal; and (5) Chaengron Canal were tested. A mixture of the same concentrations of Cr(III) and Cr(VI) (50 μg L^{-1}) were spiked into the surface water samples. The validation results were expressed in terms of concentration for Both UV-Vis and ICP-AES determination as shown in Table 4.5. The paired t-test was used for comparison of the results obtained from two different methods applied to different samples. The results for both method were not statistically different (Paired t-test, $P>0.05$)

Table 4.6 Comparison between the proposed method and the ICP-AES method for determination of Cr(VI) in surface water samples (spiked 100 μg L^{-1} of total chromium)

Sample	[total Cr] (μg L^{-1})	
	Proposed method	ICP-AES
Great pond, CU	106.0 ± 4.9	107.9 ± 4.6
Science pond, CU	110.9 ± 2.1	112.5 ± 3.6
Phasrichareon Canal	99.2 ± 2.8	123.0 ± 3.5
Ratburana Canal	114.6 ± 2.8	113.1 ± 0.8
Chaengron Canal	108.5 ± 11.2	116.1 ± 7.1

The concentrations were reported as mean ± standard deviation.

CHAPTER V

CONCLUSION

5.1 Conclusion

A hollow fiber supported liquid phase microextraction (HF-LPME) followed by 1,5 diphenylcarbazide-based UV-Vis spectrophotometric method has been developed for speciation of chromium in water sample. The speciation method based on the extraction of Cr(VI) in the form of dichromate ion ($Cr_2O_7^{2-}$) via anion exchange mechanism through Aliquat 336 impregnated in hollow fiber membrane. After the extraction, Cr(VI) in hollow fiber membrane was eluted with 200 µL of 1,5 diphenylcarbazide in ethanol solution to form violet complex and sequentially determined with UV-Vis spectrophotometer at 548 nm. Cr(III) that existed as cation was not extracted by the HF-LPME but determined after oxidation to Cr(VI) by hydrogen hydroxide and calculated by subtracting the amount of Cr(VI) from the amount of total Cr.

Parameters that influenced the method sensitivity were investigated. The optimal conditions for extraction were 10% (v/v) Aliquat in kerosene, 15 min extraction time, 20 mL sample volume, and 700 rpm stirring rate. The interfering ion was also studied. The presence of other ions in the sample matrices could affect the sensitivity of the method, so the matrix matching technique was used for speciation of chromium in real surface water samples.

The method showed good linearity in the concentration range of 20-160 µg L^{-1} with correlation coefficient (R^2) greater than 0.99. The enrichment factors were 15 and 5 for deionized water and surface water, respectively. The limits of detection were 3 and 10 µg L^{-1} for deionized water and surface water, respectively, which are below the guideline value of WHO regulation. The method validation showed good accuracy (81.2-114.8 % recovery for determination of Cr(III) and Cr(VI)) and good precision (3.85 and 10.64 % RSD for intra-day and inter-day determination, respectively). Comparison between the purposed method and ICP-AES method was

also investigated. The results for both methods were not statistically different (Paired t-test, $P>0.05$).

The proposed method can be applied for speciation of chromium in water samples. The proposed method provides low limit of detection, good linearity good precision and accuracy. Even though the extraction time is about 15 min, the setup is so simple that it can handle several samples at the same time. The method is inexpensive and uses less solvent consumption, which could be also considered as a green chemistry.

5.2 Suggestion of future work

The idea of hollow fiber supported liquid membrane microextraction technique may be apply to other new design of set-up experiment, such as the number of membrane, type of analyte, type of organic solvent, for improving sensitivity and widely used in various analyte. Furthermore, this method should be applied for other water samples.

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APPENDIX

Appendix

Table A.1 Paired t-test data analysis (n=3, confidential level 95%)

	Proposed method	ICP-AES
Mean	107.8333333	114.504
Variance	33.53147386	31.13608
Observations	5	5
Pearson Correlation	-0.587451229	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.47236669	
P(T<=t) one-tail	0.107452406	
t Critical one-tail	2.131846782	
P(T<=t) two-tail	0.214904812	
t Critical two-tail	2.776445105	

VITA

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Poster presentation and proceeding

"Hollow fiber membrane based liquid-phase microextraction (HF-LPME) for the preconcentration of chromium(VI) determined by diphenylcarbazide-based UV-Vis spectrophotometric method" Anchisa Chinworakit, Pakorn Varanusupakul. Poster presentation and proceeding, *Pure and Applied Chemistry International Conference (PACCON 2012)*, The Empress Hotels, Chiangmai, Thailand, 11-13 January, 2012.