การพัฒนาระบบวิเคราะห์แบบออนไลน์อัตโนมัติร่วมกับหน่วยการแยกด้วยเมมเบรนสำหรับตัวอย่างที่มีเมทริกซ์ซับซ้อน

ว่าที่ร้อยตรีศิระ นิธิยานนทกิจ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

DEVELOPMENT OF AUTOMATED ONLINE-ANALYSIS SYSTEM COUPLED WITH MEMBRANE SEPARATION UNIT FOR COMPLEX MATRIX SAMPLES

ActingSub.Lt. Sira Nitiyanontakit

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2013

Thesis Title	DEVELOPMENT OF AUTOMATED ONLINE-ANALYSIS SYSTEM COUPLED WITH MEMBRANE SEPARATION UNIT FOR COMPLEX MATRIX SAMPLES
By	ActingSub.Lt. Sira Nitiyanontakit
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Pakorn Varanusupakul, Ph.D.
Thesis Co-Advisor	Dr. Passapol Ngamukot, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

.....Chairman

(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....Thesis Advisor

(Assistant Professor Pakorn Varanusupakul, Ph.D.)

.....Thesis Co-Advisor

(Dr. Passapol Ngamukot, Ph.D.)

.....Examiner

(Assistant Professor Narong Praphairaksit, Ph.D.)

.....External Examiner

(Dr. Phansak Iamraksa, Ph.D.)

ศิระ นิธิยานนทกิจ : การพัฒนาระบบวิเคราะห์แบบออนไลน์อัตโนมัติร่วมกับหน่วยการแยกด้วยเมมเบรนสำหรับตัวอย่างที่มีเมทริกซ์ซับซ้อน. (DEVELOPMENT OF AUTOMATED ONLINE-ANALYSIS SYSTEM COUPLED WITH MEMBRANE SEPARATION UNIT FOR COMPLEX MATRIX SAMPLES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ปกรณ์ วรานุศุภากุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.ภัสสร์พล งามอุโฆษ, 54 หน้า.

ในงานนี้เป็นการพัฒนาระบบการวิเคราะห์อัตโนมัติโดยร่วมกับหน่วยการแยกด้วยเมมเบรน โดยทั้งนี้ผู้วิจัยได้ทำการออกแบบหน่วยการแยกด้วยเมมเบรนเพื่อใช้ในระบบที่ได้ออกแบบขึ้น อีกด้วย โดยผู้วิจัยทำการออกแบบระบบวิเคราะห์อัตโนมัติสองระบบซึ่งระบบทั้งสองอาศัยวิธีตรวจวัดด้วยความเข้มสีในสารละลาย ในระบบแรกเป็นระบบวิเคราะห์หาไอออนเหล็กในตัวอย่างน้ำผลไม้ และระบบที่สองสำหรับการหาไอออนโครเมี่ยมในตัวอย่างสิ่งแวดล้อม ในระบบแรกอาศัยหลักการเตรียมตัวอย่างด้วยเมมเบรนไดอะไลซิส สามารถกำจัดสิ่งรบกวนต่อการวิเคราะห์ตัวอย่างได้อย่างมีประสิทธิภาพโดยสามารถวิเคราะห์หาปริมาณไอออนเหล็กที่เติมลงไปในตัวอย่างน้ำผลไม้ได้ 24 ตัวอย่างต่อชั่วโมงโดยไม่ต้องอาศัยวิธีการเตรียมตัวอย่างอื่นเพิ่มเติม โดยสามารถวิเคราะห์หาปริมาณไอออนเหล็กที่เติมลงไปในตัวอย่างที่ความเข้มข้นตั้งแต่ 3 ถึง 30 มิลลิกรัมต่อลิตร ส่วนในระบบที่สองเป็นระบบวิเคราะห์สำหรับการหาปริมาณไอออนโครเมียมในตัวอย่างทางสิ่งแวดล้อม โดยใช้เทคนิคการสกัดด้วยเมมเบรนแบบสามวัฏภาค โดยระบบสามารถทำการล้างทำความสะอาดแล้วสร้างวัฏภาคเมมเบรนเหลวใหม่ได้ด้วยตัวเองเพื่อให้ระบบสามารถทำงานได้เองอย่างอัตโนมัติสมบูรณ์แบบ ระบบสามารถวิเคราะห์หาปริมาณโครเมี่ยมในแหล่งน้ำรวมถึงน้ำเสียที่ระดับความเข้มข้นตั้งแต่ 30 ถึง 500 ไมโครกรัมต่อลิตร ใช้เวลา 20 นาทีต่อตัวอย่าง ในระบบนี้สามารถวิเคราะห์ได้มากกว่า 100 ครั้งโดยใช้เมมเบรนเส้นใยกลวงเพียงเส้นเดียว โดยจากระบบทั้งสองได้ใช้หน่วยการแยกด้วยเมมเบรนที่ทางผู้วิจัยได้ออกแบบขึ้นซึ่งหน่วยการแยกด้วยเมมเบรนดังกล่าวใช้กับเมมเบรนชนิดเส้นใยกลวงเพียงหนึ่งเส้นซึ่งสามารถถอดประกอบกอบได้ง่ายและมีต้นทุนการผลิตที่ไม่สูงสามารถต่อเข้ากับระบบวิเคราะห์ได้อย่างมีประสิทธิภาพ

ภาควิชา เคมี	ลายมือชื่อนิสิต .....
สาขาวิชา เคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....
ปีการศึกษา 2556	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5072485023 : MAJOR CHEMISTRY
KEYWORDS: HOLLOW FIBER MEMBRANE / ONLINE-ANALYSIS SYSTEM / LIQUID PHASE MICROEXTRACTION

SIRA NITIYANONTAKIT: DEVELOPMENT OF AUTOMATED ONLINE-ANALYSIS SYSTEM COUPLED WITH MEMBRANE SEPARATION UNIT FOR COMPLEX MATRIX SAMPLES. ADVISOR: ASST. PROF. PAKORN VARANUSUPAKUL, Ph.D.,DR. PASSAPOL NGAMUKOT, Ph.D., 54 pp.

The automated on-line analysis systems coupled with membrane separation unit have been developed. The membrane separation units were designed for incorporation with automated on-line analysis systems. Two systems were proposed; (1) on-line hollow fiber membrane dialysis for determination of Fe(II) ion in fruit juices (2) on-line hollow fiber membrane liquid phase microextraction (HF-LPME) for determination of chromium ion in environmental samples. In the first system, the membrane separation unit was used as an on-line dialysis for screening off interferences in the juice samples before colorimetric determination of iron ion. The working range was at 3 to 30 mg/L. The system provided high sample throughput of 24 samples per hour without any sample pretreatment processes. The second system was proposed for determination of chromium ion by on-line HF-LPME. The system was developed for fully automation, where the hollow fiber membrane was conditioned for extraction, cleaned and regenerated for the next extraction, automatically. The working range was at 30 to 500 µ/L. It could be used for more than 100 times of analyses. Both membrane separation units were easy assembly, durable and cost effective.

Department:	Chemistry	Student's Signature	.....
Field of Study:	Chemistry	Advisor's Signature	.....
Academic Year:	2013	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

I would like to thank my advisor, Assistant Professor Dr. Pakorn Varanusupakul and my co-advissor Dr. Passapol Ngamukot, for all advices, kindness, assistance and support. I would like to thanks Assistant Professor Dr. Warinthorn Chavasiri, Assistant Professor Dr. Narong Praphairaksit and Dr. Phansak Iamraksa for valuable suggestions for my thesis.

This project was financially supported from Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program (PHD/0068/2551). I would like to express my special gratitude to Professor Manuel Miró Lladó for his supervision, supports, encouragement, as well as his friendship during my short-term research at Flow Injection and Trace analysis group (FI-TRACE), the department of chemistry, university of the Balearic Islands, Palma de Mallorca, Spain. In addition, this research was partially supported by the Center of Excellence on Petrochemical and Materials Technology Department of Chemistry, Chulalongkorn University.

This thesis cannot be completed without supports and helps from many people. I would like to thank Dr. Mahitti Puanngam, Mr. Taweesak Janduang and Miss Suprawee Wongsuchoto for their suggestions, supports and helps. In addition, I would like to thank all people in Chromatography and Separation Research Unit group (CHSRU) from Chulalongkorn University for their helps, good friendship and supports.

Finally, I would like to thank my beloved family for their loves, supports and knowledge.

CONTENTS

Page

Page

Page

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

LIST OF ABBREVIATIONS AND SYMBOLS

FIA	=	flow injection analysis
SIA	=	sequential injection analysis
kg	=	kilogram
g	=	gram
mg	=	milligram
µg	=	kilogram
mL	=	milliliter
L	=	liter
M	=	mole per lite
RSD	=	relative standard deviation
SD	=	standard deviation
LPME	=	liquid phase micro extraction
R^2	=	correlation coefficient
sec	=	second
min	=	minute
cm	=	centimeter
mm	=	millimeter
µm	=	micrometer
hr	=	hour
ID	=	inner diameter
mmol	=	millimole
DPC	=	diphenylcarbazide

CHAPTER I
INTRODUCTION

1.1. Introduction

The on-line analysis systems such as flow injection analysis (FIA) [1-4], sequential injection analysis (SIA) [5-7] and hybrid flow injection analysis [8] are the systems to which sample can be treated, loaded and analyzed automatically in manifold. The on-line analysis system has become more attractive in many chemical analysis fields (food, environmental, petroleum) because there are many advantages over typical wet chemical analysis: for example, automation, speed, low labor, and high precision.

One of the challenges in development of on-line analysis system is integrating sample preparation technique into the system so called on-line sample preparation technique, especially for extraction, isolation, preconcentration or clean-up of such complicated matrix samples for example food, soil and biological fluid [9-12]. Generally, the samples are prepared or treated in batches (off-line) before being loaded into the analysis system. There are attempts to incorporate sample preparation techniques into the on-line analysis system such as on-line solid phase extraction [9], on-line liquid-liquid extraction [12] and on-line membrane separation [13, 14].

On-line membrane separation techniques selectively separate or extract analytes from sample matrices using semi-permeable membrane. The selectivity of the membrane separation technique depends on membrane materials and characteristics of the membrane such as hydrophobicity, functional group and porosity, which lead to variety of separation mechanisms. This technique has been successfully used for sample preparation in many complex matrix samples such as milk, juice, tap water and sediments. Early reports for on-line membrane separations were in the formats of flat sheet membrane. The membrane separation unit was mainly made from acrylic blocks that attached both sides to a flat sheet membrane like a sandwich. In the present, membrane formats are available in many types such as spiral, hollow fiber, and rod, which offer flexibility in design and usage. Recently, the hollow fiber membrane format has been wildly used for several modes of sample preparation now a day.

Hollow fiber membrane liquid phase microextraction (HF-LPME) [15-17] has become an attractive sample preparation technique because only small volumes of

extracting phase are employed so that it can be promptly analyzed by analytical instruments such as gas chromatography [18, 19], high performance liquid chromatography [20, 21] and graphite furnace atomic absorption [22, 23] resulting in high enrichment factor. In addition, due to the tubular shape of the hollow fiber membrane, it is more suitable for connecting with the tubing system for on-line analysis than the flat sheet membrane. From our recent literature reviews, there are applications using single strand hollow fiber membrane as on-line gas phase separation for a flow through gas sensor [24, 25]. However, there are a few reports using on-line single strand hollow fiber separation unit for liquid samples. There are some attempts of using on-line single strand hollow fiber membrane separation units for liquid samples such as liquid phase microextraction (LPME) microfluidic device [26] or on-line membrane dialysis sampling [27], which offer low cost and solvent consumption. Nevertheless, applications of single strand hollow fiber membrane for on-line sample preparation such as dialysis or liquid phase microextraction are still challenging.

In this work, we have designed and introduced the on-line membrane separation units using single strand hollow fiber membrane for incorporation with automated online-analysis system for handling complex matrix samples in routine analysis.

1.2. Objective of the thesis

The objectives of the thesis are, (i) design and construct the single strand hollow fiber membrane separation unit for incorporation with the automated flow-based analysis system; (ii) develop the automated analytical method that incorporates the designed single strand hollow fiber membrane separation unit for on-line sample preparation and determination of analytes in complex matrix samples.

1.3. Scope of the thesis

The scope of this work included design the membrane separation units using single strand hollow fiber membrane, incorporate the units with automated on-line flow based analysis systems and apply for determination of selected analytes in complex matrix samples. The designed membrane units are developed based on three different modes such as dialysis, two-phase extraction and three-phase extraction, which were described in chapter individually. Factors influencing method efficiency were studied and optimized for each application. Results were shown and discussed in each chapter.

1.4. Theory

1.4.1. The semi-permeable membrane in on-line separation

According to the IUPAC recommendation, membrane is ‘‘a structure, having lateral dimensions much greater than its thickness, through which mass transfer may occur under a variety of driving forces’’. Membrane is used to separate solutions into two sides; one is called donor solution, where the sample is input and the other side is called acceptor solution, into which the target species are permeated. Hylton et al. [28] have categorized membranes based on their function, morphology, geometry, chemistry and structure as summarized in Figure 1.1

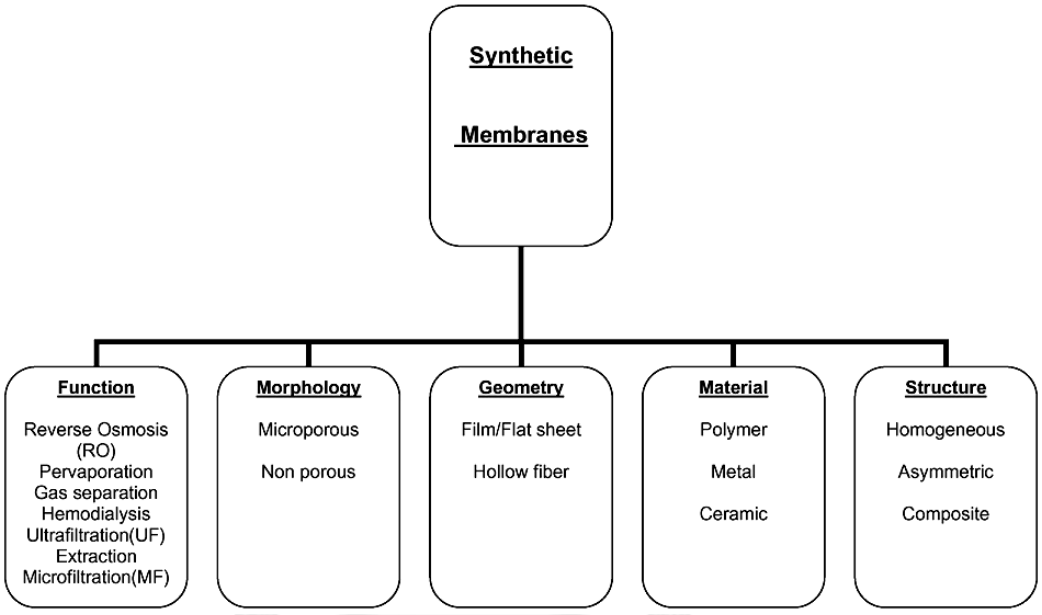

Figure 1.1 Schematic diagram classification of membrane [28]

In addition, Miró et al. [25] have classified membranes based on structure and chemical composition (Table 1.1). Membrane structures are divided into two groups: porous and non-porous membranes. In the case of porous membrane, the selectivity of the membrane is based on size of the pores compared to the size of the species. The membrane materials are usually inert so that there is no interaction between the analyte and the membrane surface that may cause fouling problems. In the case of nonporous membrane such as dense polymeric membranes as well as supported liquid porous membranes, analytes are permeated not only by diffusion but also by partitioning into the membrane phase. Therefore, the selectivity of the membrane is influenced by the interaction between membrane materials and analytes.

Table 1.1 Classification of membranes according to their role in mass transport (modified from [25])

Membrane characteristics	Common materials
Inert membranes	
Microporous(0.1–1.0μm)/ hydrophobic	Polytetrafluoroethylene, polypropylene
Nanoporous (3 – 10 nm)/hydrophilic	Cellulose acetate, polysulphone, polyamide, regenerated cellulose
Homogeneous/hydrophobic	Polydimethylsiloxane, Latex
Reactive membrane	
Ionomeric membrane	Perfluorosulphonate (Nafion), copolymers with pendant sulphonic or quaternary ammonium groups
Ion-carrier membranes with entrapped reagents	Polytetrafluoroethylene, polyvinyl chloride, polypropylene

1.4.2. **Transport mechanisms in membrane separation processes**

The separation processes are achieved by selective permeation by using membrane as a barrier. The chemical species are driven from one phase (donor solution) across the membrane barrier into another phase (acceptor solution) when driving forces are applied.

The driving forces for the membrane separation processes such as concentration gradient, pressure force and electric forces depend on separation techniques, characteristics of the membranes, target analytes and sample matrices (Figure 1.2). The membrane separation processes are categorized into four important sample preparation purposes as shown in Table 1.2

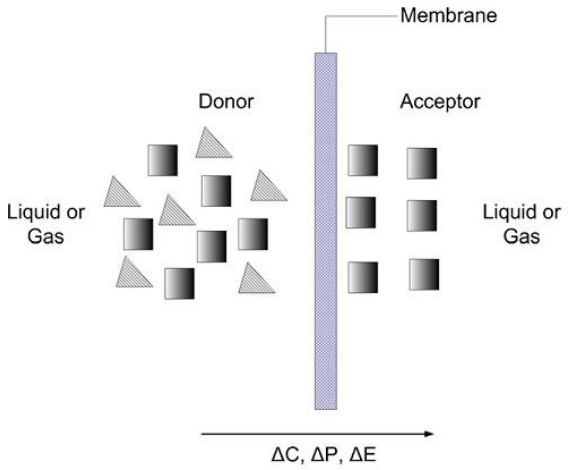

Figure 1.2 Membrane separation process models, which ΔC, ΔP, ΔE are the concentration, pressure and electrochemical gradients, respectively [28]

Table 1.2 Categorization of membrane separation techniques for sample preparation [25]

Technique	Membrane type	Principle	Driving force
Dialysis	Porous	Size-exclusion	Concentration difference
Electrodialysis	Porous	Size-exclusion and selective ion transport	Potential difference
Filtration	Porous	Size-exclusion	Pressure difference
Memebrane extraction	Nonporous	Difference in partition coefficient	Concentration difference

In membrane dialysis, the separation process is based on size, where the species that are smaller size than the pore size of the membrane is transferred across the membrane driven by the concentration gradient of species between two sides of the membrane. In membrane filtration, the separation process is based on size, where the smaller sized species are pushed or pulled through the pores of the membrane by an applied pressure. In electrodialysis, the separation is based on size and interaction between charged species and the ion-exchanged membrane driven by an applied potential across the membrane. In membrane extraction, the separation is based on partition or diffusion of the species into the membrane or into the

supported liquid membrane driven by the concentration gradient of analyte from the bulk solution into the membrane and across the membrane.

1.4.3. **The Hollow fiber membrane**

Hollow fiber membrane was first developed in 1960s by DuPont. The hollow fiber membrane is semi-permeable capillary membrane with inner diameter more than 25 μm and outside diameter less than 1 mm, which the lumen of hollow fiber is porous polymer (Figure 1.3). The hollow fiber membrane can be used singly or in a bundle that contains hundreds of fibers and up to several million fibers.

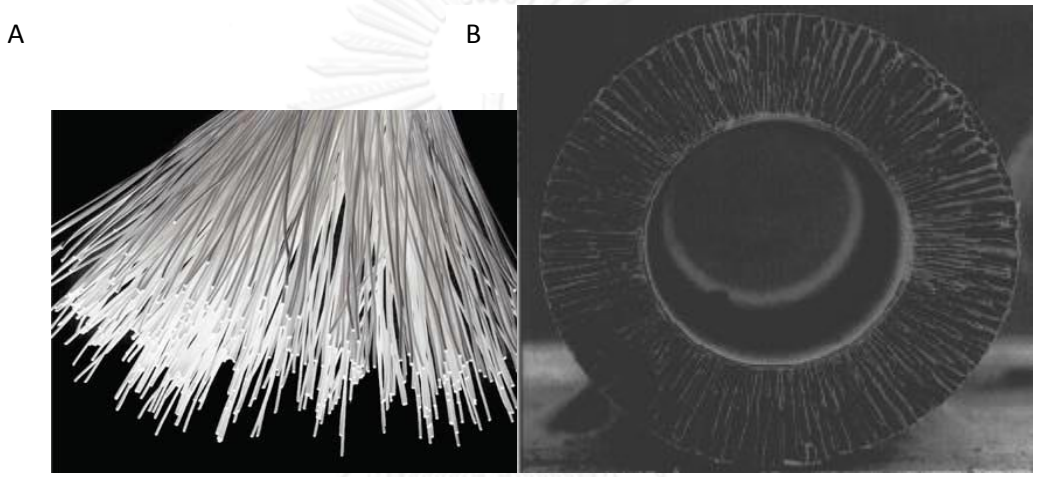

Figure 1.3 The hollow fiber membrane A) hollow fiber membrane B) Scanning electron microscope (SEM) picture of hollow fiber (cross section) [29]

The hollow fiber membrane is used in many separation processes such as ultra-filtration, reverse-osmosis and membrane extraction. It provides many advantages such as large surface area, easy replacement and cost effective.

1.4.4. **Automated on-line analysis system**

The first generation of the on-line analysis system is Flow Injection Analysis (FIA), which was first described by Ruzicka and Hansen in 1975 [1]. In FIA, the sample solution is injected into a carrier stream and mixed with reagents. The analyte is reacted with the reagents and detected by the detector. The typical schematic diagram of (FIA) is illustrated in Figure 1.4.

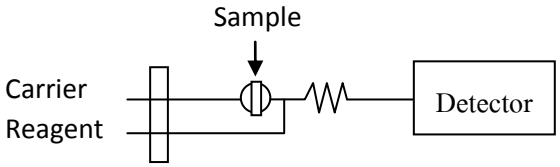

Figure 1.4 Schematic diagram of typical FIA (modified from [1])

FIA systems can be operated automatically or semi-automatically and are frequently employed in routine analyses. Typically, the FIA system consists of four parts: solvent delivery (i.e., peristaltic pump or syringe pump), sample introduction (i.e., injection value or autosampler), sample treatment (i.e., holding coil, on-line heater or on-line dialysis) and detector (Spectrometer or electrochemical device). The sample solution is loaded into the system via the sample introduction part, and carried with the carrier stream by the carrier delivery part to the sample treatment part for proper treatment and finally to the detector part for detection and determination.

The second generation of the on-line analysis system is Sequential Injection Analysis (SIA) system[7], SIA, employs a bi-directional syringe pump to control flow direction of the solution via a multipositional selection valve. The SIA system is more precise and robust than the FIA system because using of peristaltic pump for a long time frequently damages the tubing system resulting in irreproducibility. However, the analytical method based on SIA system usually takes longer analysis time than that based on FIA system because the SIA system takes time to collect all the solutions (i.e., sample and reagent) into the holding coil before pushing forward into the detector. Recently, the next generation of the on-line analysis system has been developed by combining the advantages of the two previous techniques (FIA and SIA) so called hybrid flow analysis (HFA) system[8], where the single syringe pump in the conventional SIA system was replaced with a multisyringe pump and a flow manifold as used in the FIA system. The HFA system is more robust and precise than the conventional FIA because the peristaltic pump is replaced with the multisyringe pump. The HFA provides shorter analysis time than the conventional SIA because the analysis time is reduced by the flow manifold that the sample solution and the reagent solution are mixed and developed at the same time before passing to the detector. The advantages of the on-line analysis system are automatic, precise, cost effective, fast, and environmental friendly. Recently, on-line analysis systems have been developed for many fields of analyses that serve for routine analysis.

The most important part in an automatic on-line analysis system is the computer control device. In the system, the equipment such as pump, multipositional selection valve and solenoid valve controller have a special microchip called "Microcontroller"[30] that is embedded in the electronic circuit. The microcontroller has a function to communicate with the computer and control parts of the instrument. The microcontroller is the microcomputer that configures in microchip size, which can be programmed for user's desires. The microcontroller has many specifications depending on the purposes of the user. The microcontrollers are commercially available from many companies such as PIC® Microcontrollers from Microchip Company, MSC-51 from Intel and AVR from Atmel Corporation. In this thesis, PIC® Microcontrollers from Microchip Company was implemented in our custom-made circuit for controlling the flow-based analysis instruments such as peristaltic pump, auto sampler and valves (Details about circuit and program were described in each chapter and appendix).

1.4.5. **Flow-through membrane-based configurations**

There are some reports reviewing the use of flow-through membranes for sample preparation of several types of samples i.e., air, liquid, sediment, and biological fluid [25, 27, 28]. Most of them have been coupled with flow-based analysis systems such as HPLC, GC and CE.

Miró et al. has classified the flow-through membrane based configurations into two modes according to the way that the sample is exposed to the membrane, that are stagnant flow mode and continuous flow mode as shown in Figure 1.5

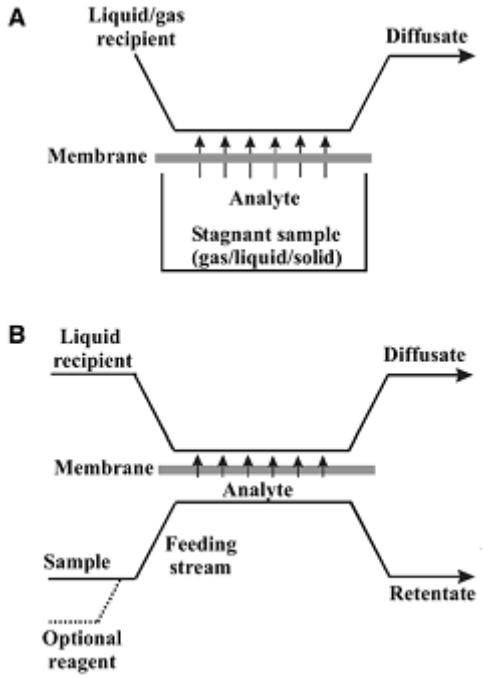

Figure 1.5 Schematic diagrams of flow-through membrane-based configurations: A) Stagnant flow mode; B) continuous flow mode (modified from [25])

In the static or stagnant flow mode, the target analyte is isolated from the stagnant sample solution into the flow stream as shown in Figure 1.5. Examples of stagnant flow mode membrane-based separation are such as probe-type dialysis for continuous trapping of low molecular weight species from the sample and membrane-based dynamic headspace/pervaporation for stripping volatile compounds from samples. In the continuous flow mode, the sample solution is continuously fed while the target analyte is isolated into the flow stream. Example applications of this mode are liquid and gas extraction units before introduction into gas chromatography (GC) or high performance liquid chromatography (HPLC) instrument.

1.4.6. Flow-through membrane based separation unit

Flow-through membrane-based separation units have been used in variety of types of separations such as dialysis, extraction and phase separation. The simple design of flow-through membrane-based separation unit is sandwich type as illustrated in Figure 1.6. The sandwich type flow-through membrane-based separation unit has been designed for using with a flat sheet membrane. The separation unit is

usually made of acrylic blocks, which are separated to two channels by the flat sheet membrane.

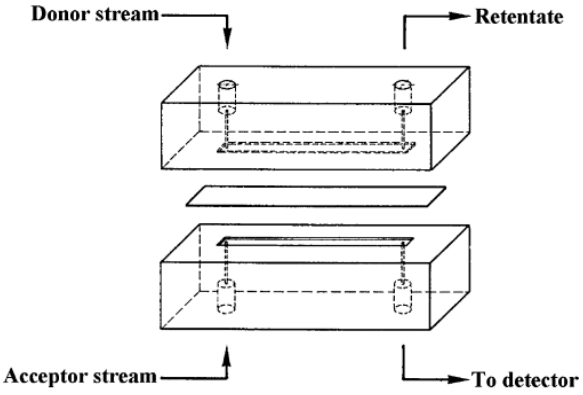

Figure 1.6 The Sandwich type flow-through membrane-based separation unit [25]

The next generation of flow-through membrane-based separation unit is concentric or tubular type, which has been designed for using with a hollow fiber membrane; for example, microdialysis probes, as shown in Figure 1.7

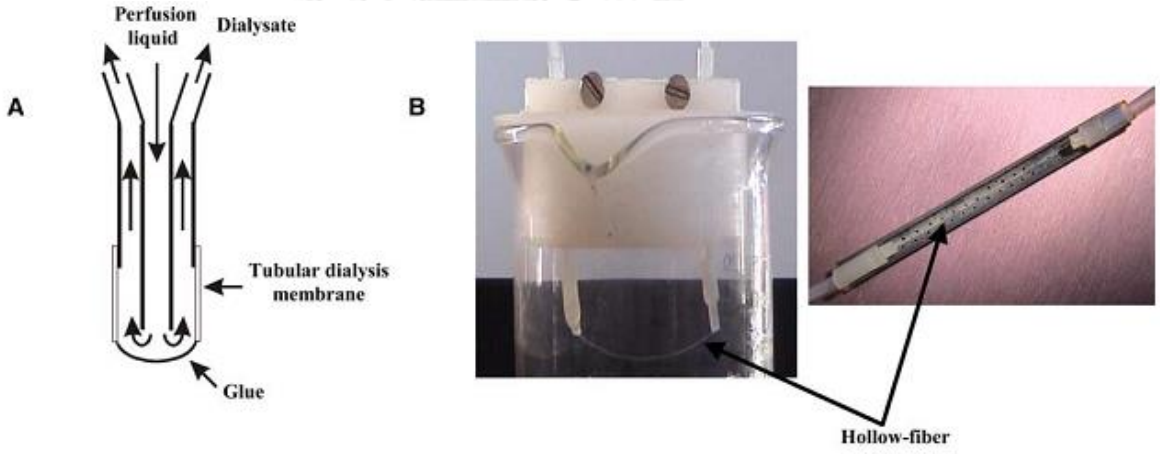

Figure 1.7 Concentric or tubular type flow-through membrane-based separation unit A) Concentric-type probe. B) Single strand hollow fiber membrane unit. [25]

The hollow fiber membrane separation unit has advantage above flat sheet membrane. Hollow fiber membrane is tubing like membrane that can be implemented into the flow system directly. The hollow fiber membrane provides higher active surface area than the flat sheet membrane for on-line membrane extraction.

1.4.7. **The computer-aided design software (CAD)**

The computer-aided design software (CAD) [31] such as SolidWorks® and AutoCAD® is a useful software for design and production of hardware parts, in this case, membrane separation unit. The software helps designing part models in three dimension (3D) and allows real-time editing, which reduces time and cost compared to paper work design. The software generates many types of documents such as 3D model picture, 2D drawing and machine code as shown in Figure 1.8. The simple way of making design part model with CAD program is to construct a 3D model (Figure 1.8 A) by 2 major steps; first, making surface in 2 axes (ex. circle or square); and extruding or cutting it in the rest of the axis. This model is useful for planning and presentation of design before making the real part. The 2D blueprint (Figure 1.8 B) can be generated after complete 3D model. The user can place desired direction and scale information into the picture. This blueprint is used when making the real part.

A B

A

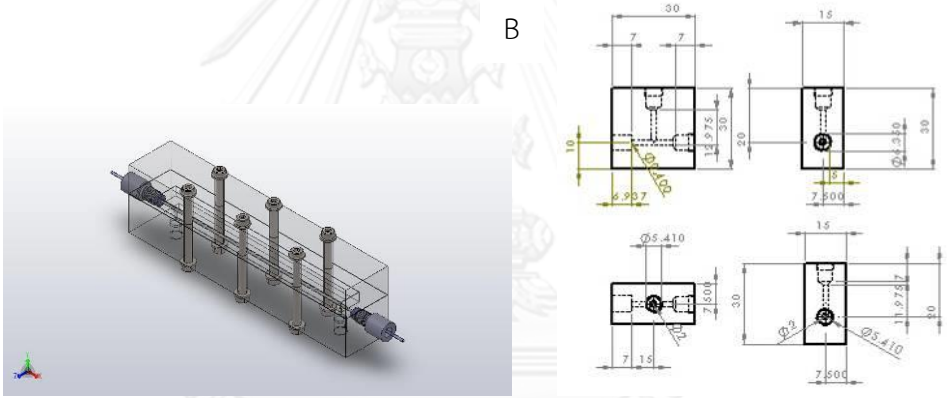

Figure 1.8 Drawing of a membrane unit designed by CAD software: A) 3D model; B) 2D blueprint with actual scale

CHAPTER II
THE DESIGNS OF HOLLOW FIBER MEMBRANE UNIT

2.1. Introduction

Generally, there are two major types of on-line membrane separation unit have been reported that are sandwich and tubular type. The sandwich type (Figure 2.1) consists of two identical blocks, which made from plastic or metal. Each block is engraved into chamber, which allows solution (donor and acceptor) be contacted at both sides of the membrane surfaces.

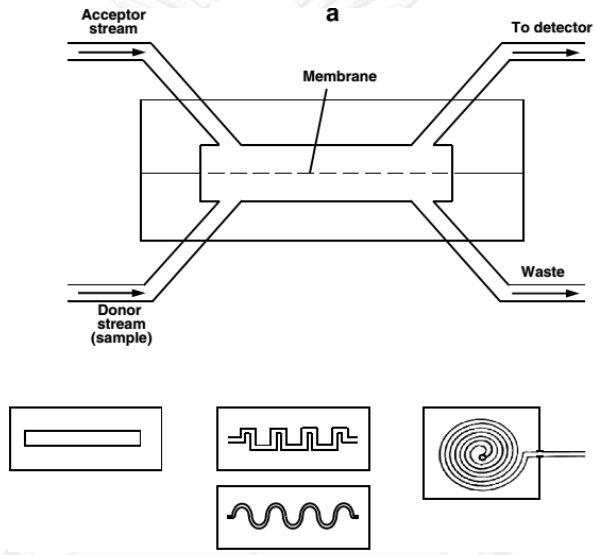

Figure 2.1 Sandwich type membrane separation unit with different designs of solution chamber [27]

Typically, the sandwich type unit has been designed for flat sheet membrane. The unit can be operated by following 4 steps: (1) unit disassembly, (2) cleaning, (3) replacing the membrane and (4) unit reassembly. This sandwich type membrane separation unit has many advantages such as easy to clean, simple to assemble and durable. However, there are some areas of the membrane that are not in contact with the solution.

The further development of membrane separation unit is the tubular type or concentric type unit, which consists of two concentric tubes (Figure 2.2). One is for a donor solution and the other is for an acceptor solution. The concentric type unit has been designed for tubular membrane, especially hollow fiber membrane. The concentric type membrane separation unit provides much higher active surface area than the sandwich type unit. Nevertheless, the concentric type unit is not easy to be

reused or to replace a new membrane because the membrane is usually fixed to the unit with epoxy glue.

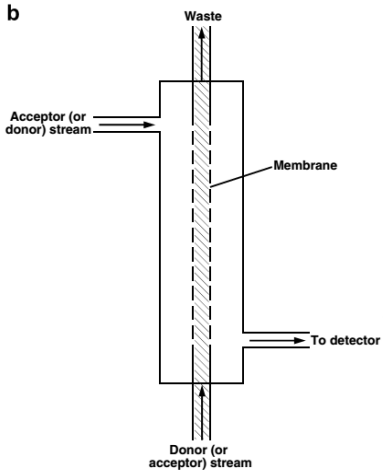

Figure 2.2 Concentric type membrane separation unit [27]

Nordmeyer, F. R. et al. [32], introduced the single strand hollow fiber membrane unit for on-line dialysis sampling of Ca(II) ion in human blood serum as shown in Figure 2.3. Cuprophane hollow fiber membrane was used for dialysis. The membrane was inserted inside the TFE tubing and connected with Lexan fittings. The membrane was fixed with silicone rubber glue at the end of the compartment. This system was useful for the analysis of ions and small molecules in many sample types such as blood, soil extracted and pharmaceuticals samples. However, this unit took 24 hours to setup due to the glue-curing step.

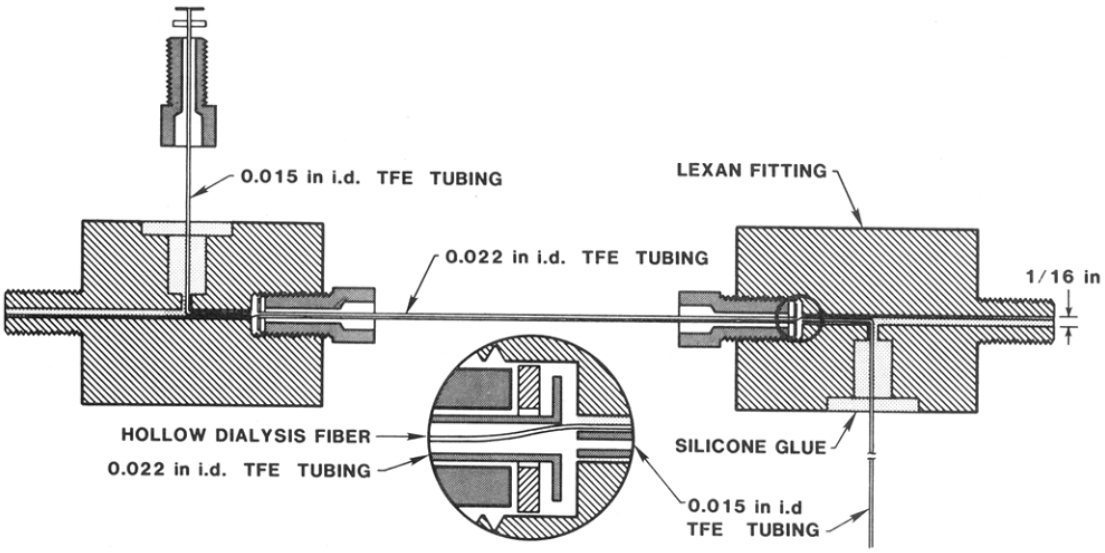

Figure 2.3 The single strand hollow fiber membrane unit designed by Nordmeyer, F. R. et al. [32]

To solve the drawback mentioned above, the new membrane separation unit should be setup instantly within the day of experiment.

In this thesis, membrane separation units have been designed and developed for hollow fiber membrane to allow the unit to be assembled for replacement of a new membrane like in sandwich type unit.

2.2. The Easy assemble hollow fiber membrane unit

The first design of the membrane unit was made of two acrylic blocks. One was drilled into 3 mm width×10 mm length×5 mm depth (approximately 1.5 mL) for a donor chamber and drilled at the end of the chamber for the donor inlet and for the membrane insertion as shown in Figure 2.4 (see more detail of 2D with actual scale in appendix A.1). The unit was sealed with a polypropylene plastic sheet and closed with a tightly screwed flat acrylic block lid. The hollow fiber membrane was inserted from the side of the acrylic block and sealed with glue. The syringe needle was attached at the end of both sides of the hollow fiber for acceptor inlet and outlet. This design was easily built with not many steps and low cost. The unit can be manually disassembled and reassembled for deep cleaning and replacing with a new membrane.

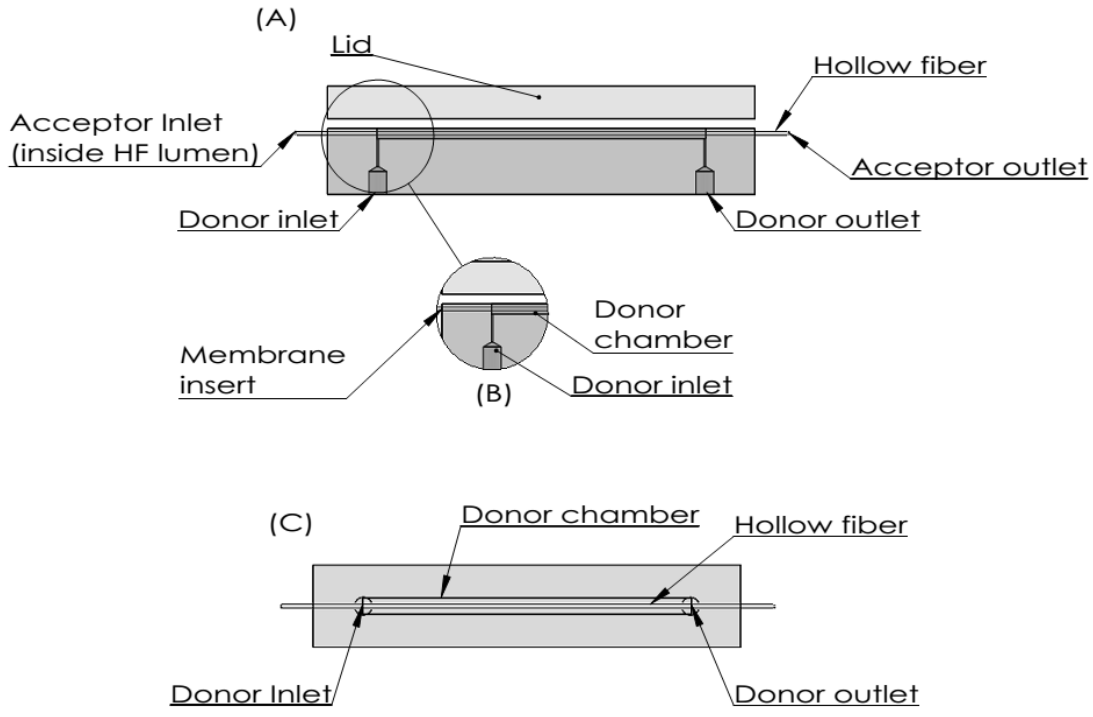

Figure 2.4 The first design of easy assemble hollow fiber membrane unit A) side view B) zoom at connector C) top view

The design was improved by changing the membrane insertion port into fitting tight compatible port (Figure 2.5 and more detail of 2D with actual scale in appendix A.2) for more convenient assembly. The cover area was reduced for more tight seal cover.

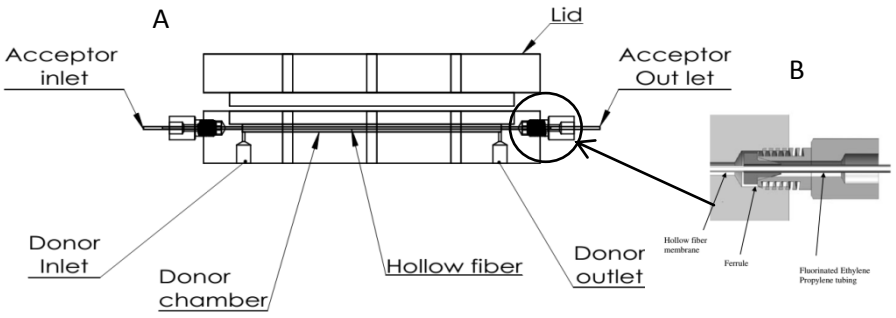

Figure 2.5 The improved design of easy assemble hollow fiber membrane unit A) side view schematic B) zoom fitting type port for hollow fiber membrane insertion

The design was used for on-line membrane dialysis of Fe(II) [33] as described in Chapter III. The donor solution was pumped into the donor chamber at the donor inlet. The donor solution was allowed to contact with the outside surface of the hollow fiber membrane. This design was easy to build and cost effective because only two pieces of acrylic were used for the unit. However, the limit of this unit was the chemical resistance of the acrylic plastic that could be used normally for aqueous solution due to the chemical resistance limit of the acrylic plastic. In addition, the Teflon plastic could be replaced to improve chemical resistance properties of the unit but the cost of the unit would be extremely high.

2.3. The adjustable concentric membrane unit

This design was consisted of two t-pieces of Teflon blocks, glass tube, sleeves, PEEK nuts, and metal holder as shown in Figure 2.6. The two T-pieces were attached at both ends of the glass tube and placed on the metal holder to fix all components together. A hollow fiber membrane was inserted into the unit at the end of T-piece and held with sleeves, ferrules and nuts. The sample solution would be pumped through at the sample inlet flowing outside of the membrane surface. The flexible tubing was attached at the end of the sleeve. The acceptor would be pumped into the lumen of the hollow fiber membrane.

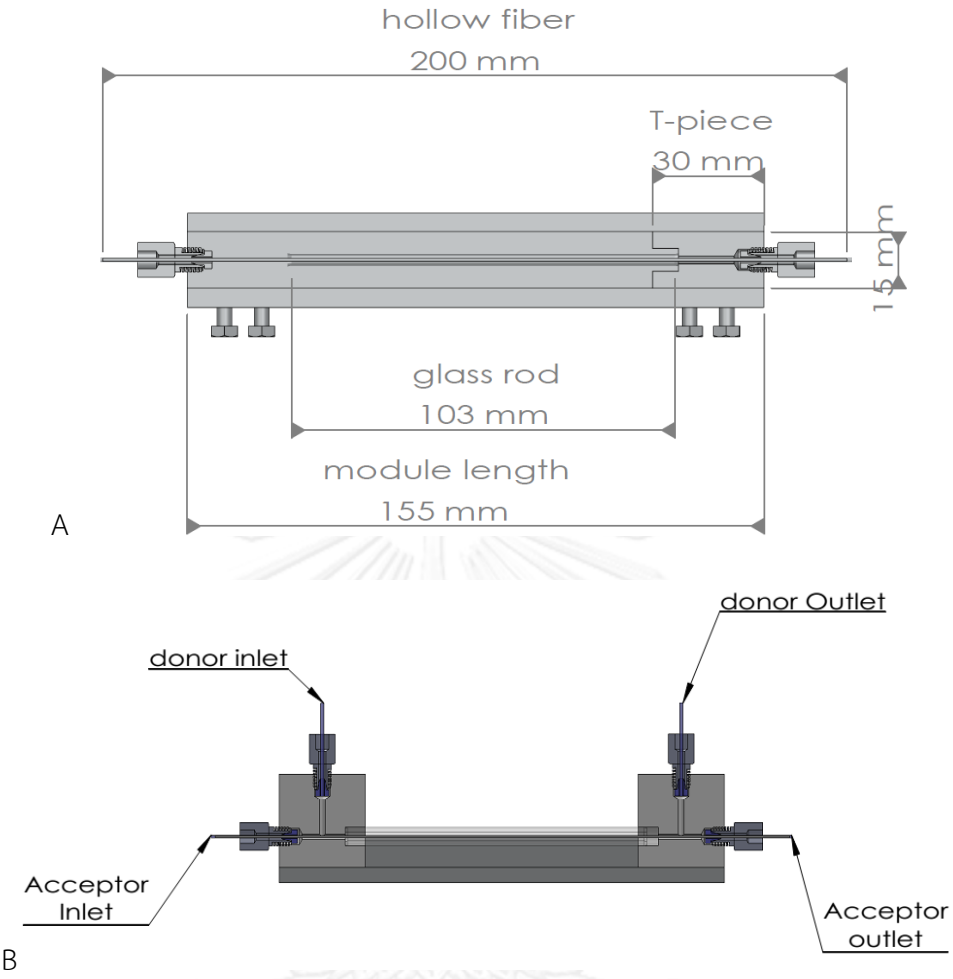

Figure 2.6 Adjustable concentric membrane unit for extraction A) top view cross section B) side view cross section

This adjustable concentric membrane unit provided more chemical resistance. The glass tube was used as donor chamber for two reasons; first, the glass tube is easy to adjust the length and the internal volume by changing the dimension of the glass tube; and second, the glass material is less expensive than the Teflon that is used in the previous easy assembly hollow fiber membrane unit. This design was applied as a phase separation for extraction of selenium sulfide in cosmeceutical product [34] and as a support liquid membrane for extraction chromium in environment sample [35]. In addition, the unit can be modified to be used in electromembrane mode by inserting Pt wires in the donor chamber and in the acceptor inlet as shown in Figure 2.7.

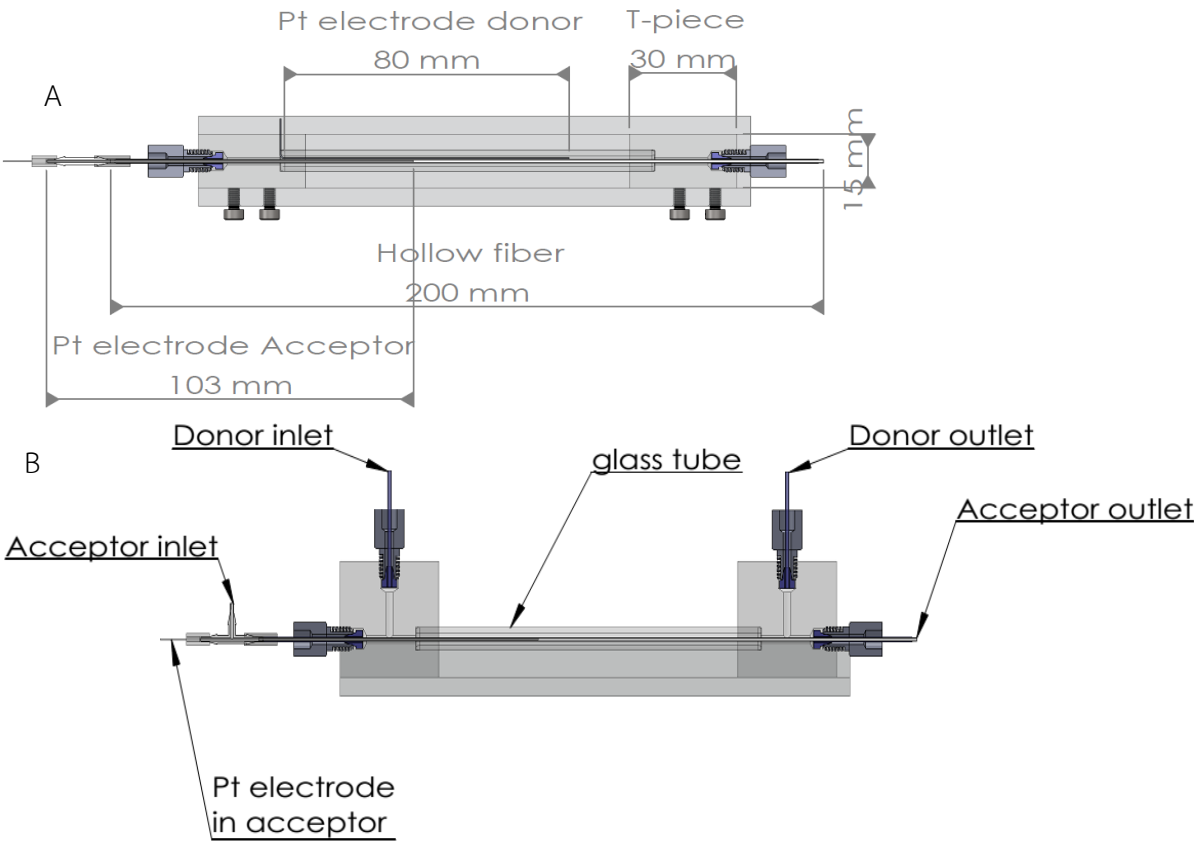

Figure 2.7 Adjustable concentric membrane unit for electro membrane mode A) top view cross section B) side view cross section

CHAPTER III
EASY ASSEMBLED HOLLOW FIBER MEMBRANE UNIT FOR ON-LINE DIALYSIS OF Fe(II) IN FRUIT JUICES SAMPLE

3.1. Introduction and literature reviews

The metal ion analysis is an interest topic in chemical analysis. The metal ion level relates to the quality or toxicity of the sample. Now a day, there are many analytical methods for metal ion analysis such as ion-selective electrode, ion chromatography, titration, gravimetric analysis and colorimetric spectrometry, which the metal ions are analyzed as total or individual oxidation number (speciation). One of the common metal ion analysis methods is colorimetric method, where the metal ions are complexed with specific chromic reagents and forming color species before detection with spectrophotometer. This method has been used in many applications of metal ion analysis. The method offers many advantages above other techniques such as simplicity, high selectivity and cost effectiveness. In addition, it can be operated off-line and on-line procedures [20, 27]. However, the limitations of this method are optical interfering species such as suspended particles or solid matters and colored interfering species such as pigment that cause high background or interfering signal in the spectrophotometric measurement. Thus, sample preparation steps are required to eliminate the interferences before using colorimetric method. In the case of complex matrix sample; for example, fruit juice sample, high molecular weight interferences such as peel, pigment and polysaccharide should be eliminated before using the colorimetric analysis. Thus the sample preparation method based on size exclusion mechanism is suitable for separation of high molecular weight interferences before analysis. One of the membrane separation methods that base on size exclusion mechanism is called the membrane dialysis.

Membrane dialysis is most wildly used for cleanup sample in routine clinical and environmental analysis. In the case of passive dialysis, the driving force is concentration gradient. Normally, the large molecules are restricted by the pore size of the membrane so that they cannot cross into the other side of the membrane. This technique is effective for elimination of the large molecules such as particles, pigment, colloids and fibers from biological or environmental samples. The membrane dialysis can be operated in on-line mode, where donor and acceptor solutions flow between two sides of the membrane. Ganeshjeevan et al. [21] used on-line membrane dialysis coupled with ion chromatography (IC) for spectrophotometric determination of Cr(VI) and Cr(III) in high colorant matrices. The

on-line membrane dialysis offered high efficiency in the background signal suppression that causes colored interferences as shown in Figure 3.1. The system provided LOD at 5 µg/L. The analysis time was only 20 min per sample.

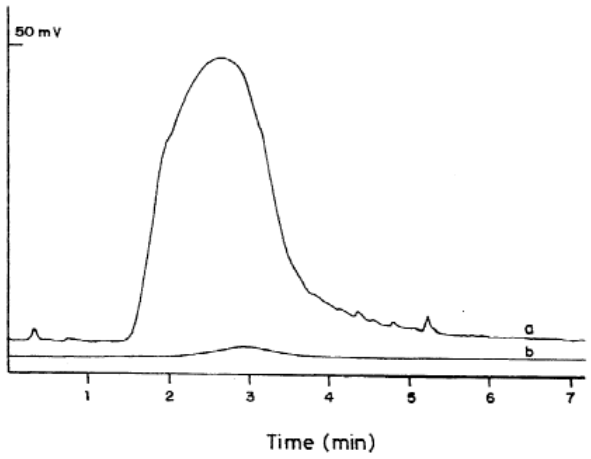

Figure 3.1 Peak of dye (red) in colored wastewater sample detected at 520 nm a) directed injection (original signal of dye) b) with on-line dialysis (background suppression) [21]

In this work, we developed membrane separation unit, incorporated to on-line analysis system and tested for on-line dialysis screening for colorimetric determination Fe(II) ion in fruit juice samples, which were rich in color and particle species that might interfere to the spectrophotometric detection. The Fe(II) was determined by 1,10-phenanthrolien colorimetric method.

3.2. Experiment

3.2.1. Chemical

All chemicals and reagents used were analytical grade and summarized in Table 3.1

Table 3.1 Chemicals

Chemical	Supplier
Ferrous (II) sulfate ($FeSO_4$)	Ajax chemical
1,10 Phenanthroline	Merck
Hydroxylamine HCl ($HONH_2{\cdot}HCl$)	Ajax chemical
Nitric acid (HNO_3)	Merck
Sulfuric acid (H_2SO_4)	Merck
Ferric ammonium sulfate ($NH_4Fe(SO_4)_2$)	CarloErba
Calcium sulfate ($CaSO_4$)	Baker Analyzed, USA
Magnesium sulfate ($MgSO_4$)	BDH lab supplies
Zinc sulfate ($ZnSO_4$)	Carlo Erba Reagents
Potassium sulfate (K_2SO_4)	Merck
Sodium sulfate (Na_2SO_4)	Ajaxchemical
Red grape juice samples	Local market

3.2.2. **Preparation of solution**

3.2.2.1. ***Stock standard Fe(II) solution; 1000mg/L***

A 0.2714 g of ferrous (II) sulfate was dissolved in 100 mL of deionized water.

3.2.2.2. ***1,10-phenanthroline; 2mmol/L***

A 0.36 g of 1,10-phenanthroline was dissolved by 1000 mL of deionized water.

3.2.2.3. ***hydroxylamine HCL; 10% (w/v)***

A 10 g of hydroxylamine HCL was dissolved in 100 mL of deionized water.

3.2.3. **Easy assembled hollow fiber membrane unit incorporated with flow-based analysis system for automated on-line dialysis sampling and colorimetric determination of Fe(II) ion in fruit juice samples**

The on-line membrane dialysis system was semi-automated at the beginning of this work. The system was setup according to the basic concept of flow injection analysis as shown in Figure 3.2.

The system consisted of two peristaltic pumps (masterflex), Easy assembly hollow fiber membrane unit (describe in section 2.2) with a single strand of polyethersulfone hollow fiber membrane (0.7 mm ID, 0.3 mm thickness, Membrana, GmbH, Germany), injection valve, Teflon tubing, cuvette based configuration flow cell, spectrophotometer with analog voltage output (LW scientific model V325XS), data acquisition card (DAQ) (USB-6009 National Instrument and use Labview® for recorder program). This system was operated only continuous flow mode. The Fe(II) ion was formed complex with 1,10 Phenanthroline at the end of the unit where the pink color was formed and flowed to the spectrophotometer. The signal from spectrophotometer at wavelength 505 nm was corrected continuously by DAQ card. Further development was to make the system fully automated with addition of the custom made autosampler. The program (SIrA V1.0) for controlling the whole system was written by Labview®. This system could be operated in continuous and stopped flow mode.

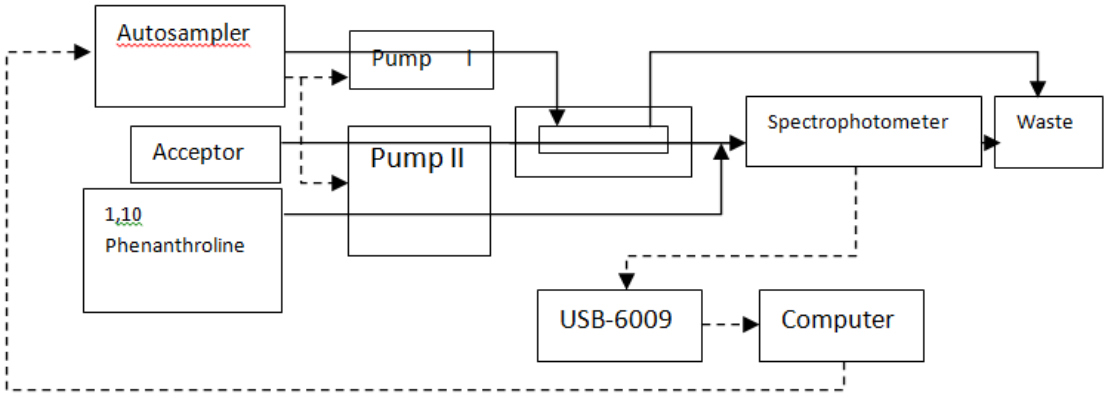

Figure 3.2 Schematic diagram of the automated easy assembly hollow fiber on-line dialysis system using Easy Assembly Hollow Fiber Membrane Unit for colorimetric determination of Fe(II) ion.

3.2.4. Mode of on-line membrane dialysis

The system can be operated in two modes; continuous mode and stopped flow mode.

3.2.4.1. *Continuous flow mode*

Two peristaltic pumps were pumped continuously. The flow rate of each pump can be varied. The peristaltic pump I was used for sample loading (donor stream). The peristaltic pump II was used for acceptor solution and reagent. The sample was loaded into the donor stream via an injection valve. While the sample was carried

into the donor chamber, the analyte (Fe(II) ion) was diffused through the membrane into the acceptor stream.

3.2.4.2. ***Stopped flow mode***

When the method started, the autosampler arm was lifted and moved into sample vial. The sample was pumped into the donor chamber and stopped for a certain of time. The sample volume was controlled by the predetermined pumping time. Then, both peristaltic pumps were turned on to carry the donor solution into the waste and the acceptor solution into the detector simultaneously.

3.2.5. **The conventional method for determination iron in fruit juice sample**

A 2 mL sample was digested by addition of 2 mL of a 1:1 (v/v) concentrated HNO_3: concentrated H_2SO_4. The aliquot was heated by a hot plate about 30 min until the solution turned into clear solution and left the solution until room temperature. A 2 mL of 10% (w/v) hydroxylamine HCL and 2 mL of 1,10-phenanthroline were added. The solution was adjusted to pH 6–8 by ammonium hydroxide. The absorbance of the pink or red color solution was measured at the wavelength of 505 nm.

3.2.6. **Optimization of parameters affecting the performance of the easy assembled hollow fiber membrane unit for on-line dialysis sampling of Fe(II) ion**

3.2.6.1. ***Continuous flow mode***

In the preliminary study, the continuous flow mode was used because it was simpler simply system than the stopped flow mode. Two parameters; i.e., sample volume and donor flow rate were studied due to their effect on sensitivity.

3.2.6.1.1. Sample volume

The sample loading volume is corresponding to the mass of the analyte that inputs to the system and affects the signal. Nevertheless, at the same loading flow rate, the higher sample loading volume takes more time to be loaded into the system. The sample volume were studies at 0.5 and 1.5 mL of sample (20 mg/L Fe(II)) by changing the sample loop of a injector port. The flow rate of the system was fixed at 0.6 mL/min for the acceptor solution and 2.7 mL/min for the donor solution. The system was operated in continuous flow mode.

3.2.6.1.2. Donor flow rate

The donor flow rate is corresponding to the time that the sample passes into the donor chamber where the analyte transfers through the membrane into the acceptor phase. In this experiment, the donor flow rate has been studied within the range of 1.7 to 4.6 mL/min. A 1.5 mL of 20 mg/L standard Fe(II) solution was used. The accepter flow rate was fixed at 0.6 mL/min in the order to keep the longest contact time.

3.2.6.2. ***Stopped flow mode***

The stopped flow mode is the mode the donor solution and the acceptor solution are stagnant in the chamber at a certain of time providing higher mass transfer than the continuous flow mode [36]. However, the system is more complicated than the continuous flow mode because the system requires an additional controlling part to stop the pump at the certain of time.

3.2.6.2.1. Stopped time

To improve the contact time, the stopped flow mode was studied to allow the donor and the acceptor phase be contacted longer than the continuous flow mode.

3.2.6.2.2. Acceptor flow rate

The acceptor flow rate influences the diffusion of the analyte into the acceptor stream that might affect the peak shape of the signal. The acceptor flow rate was varied from 0.6 to 2.2 mL/min. The donor flow rate was fixed at 3.1 mL/min. Loading volume was 1.5 mL. Standard Fe(II) solution of 20 mg/L was used for this study.

3.2.6.3. ***Interference study***

The foreign ions in the sample may interfere two important processes; i.e., complexation of 1,10 phenanthroline [37] and ion transfer of Fe(II) across the membrane [27]. The interference was studied by spiking interfering ions that might be contained in fruit juice samples such as Na(I) K(I) Ca(II) Mg(II) Zn(II) at various concentration levels in the sample with 10 mg/L Fe(II) ion. The results were reported as the concentration of Fe(II) found in the sample at different spiked levels of each interfering ion.

3.2.7. **Method evaluation**

The method performance was evaluated at the optimized condition using standard Fe(II) solution and spiked fruit juice samples. The method limit of detection

was reported. The linear working range was determined. The precision of the method was expressed as relative standard deviation (%RSD) of replicate analyses of spiked fruit juices at 10 mg/L of standard Fe(II) ion. The accuracy of the method was evaluated by recovery study of spiked fruit juice samples at 3 and 5 mg/L. In addition, the results were compared to those obtained by the conventional method (see 3.2.5.).

3.3. Result and discussion

3.3.1. The performance of the easy assembled single strand hollow fiber membrane unit for on-line dialysis sampling of Fe(II) in fruit juice samples

The performance of the easy assembled hollow fiber membrane unit on background suppression in spectrophotometric detection of Fe(II) in fruit juice samples was shown in Figure 3.3. In the case of fruit juice, the colorimetric determination of Fe(II) could not be measured directly in the color background fruit juice sample. The signal would be interfered by pigments and particulate matters suspended in the sample giving high background signal as shown in Figure 3.3a). When the easy assembled hollow fiber membrane unit was attached to the system, the membrane could effectively screen off pigments and macromolecule interference species (Figure 3.3b) and allow only small ions and molecules pass through the membrane resulting in suppression of the background signal. Figure 3.3c) showed the signals of Fe-1,10 phenanthroline complex obtained in the acceptor solution ensuring that Fe(II) had been transferred across the membrane.

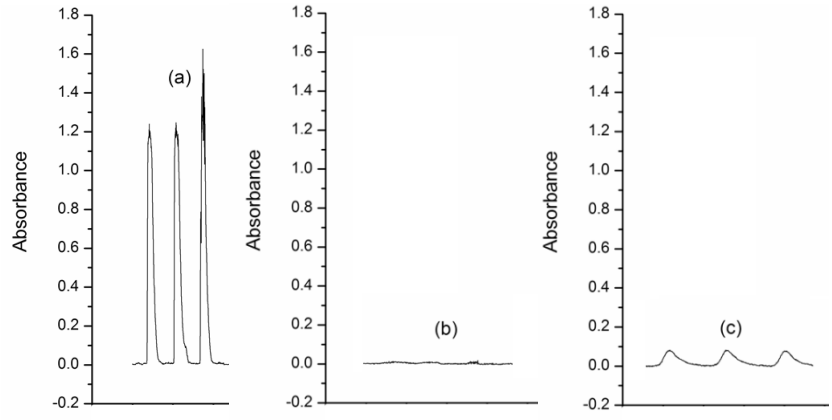

Figure 3.3 The screening ability of the on-line dialysis flow based system. The signal of a) the red grape juice sample directly injected to the detector; b) the acceptor solution after screening the grape juice by the on-line dialysis and c) the Fe(II) 1,10-phenanthroline complex in the acceptor solution after screening the grape juice by the on-line dialysis. Data shown are representative of 3 replicate Injections.

3.3.2. Method optimization

3.3.2.1. *Sample volume*

The sample volume is corresponding to the mass of analyte loaded into the system. However, when the sample volume is increased the analysis time also increases. Figure 3.4 compared the absorbances of Fe(II)-1,10-phenanthroline complexes in the acceptor solutions obtained at sample volumes of 0.5 and 1.5 mL. The signal of 1.5 mL of loading volume was 4 times higher than that of 0.5 mL, while the analysis time of 1.5 mL sample loading volume (4 min) was 2 times longer than that of 0.5 mL (6 min). Thus the sample loading volume of 1.5 mL was chosen for the rest of experiment

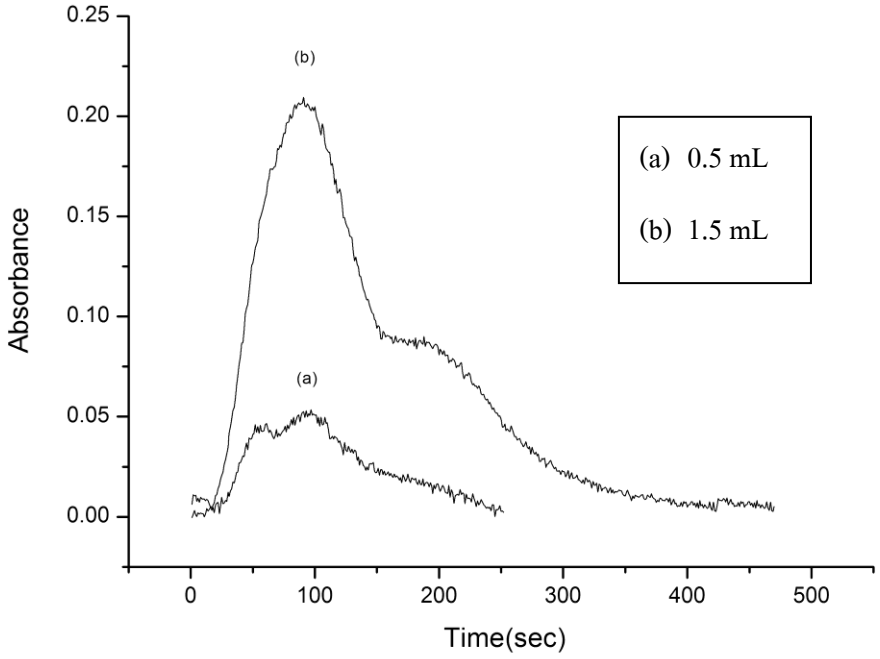

Figure 3.4 The signal of the Fe(II)-1,10-phenanthroline complex in the acceptor solution after on-line dialysis screening of a 20 mg/L standard Fe(II) solution at loading volumes of 0.5 and 1.5 mL. (Donor flow rate = 2.7 mL/min; acceptor flow rate = 0.6 mL/min)

3.3.2.2. ***Donor flow rate***

The absorbances of Fe(II)-1,10-phenanthroline complexes in the acceptor solutions at various donor flow rates were shown in Figure 3.5. At the higher donor flow rate, the peak shape was sharper and higher than those at the lower flow rate. Since the driving force of dialysis is influenced by the concentration gradient, when using the continuous flow mode, the higher flow rate seems to maintain the concentration gradient than the lower flow rate while the dialysis was taking place. However, too high donor flow rate might gain the higher back pressure of the donor chamber cause to leaking of the donor chamber, thus the donor flow rate of 3.1 mL/min gave high signal at lowest donor flow rate was chosen.

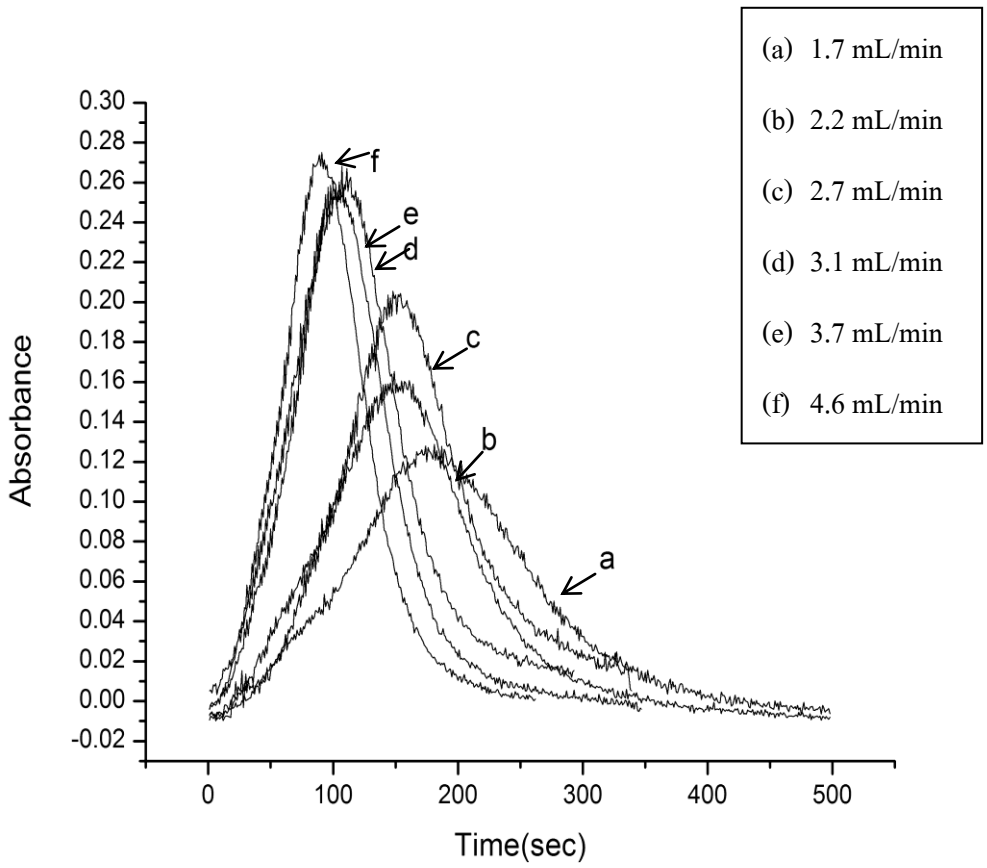

Figure 3.5 The signal of the Fe(II) 1,10-phenanthroline complex in the acceptor solution after on-line dialysis screening at various donor flow rates. (Sample = 20 mg/L standard Fe(II)solution; loading volume = 1.5 mL; acceptor flow rate = 0.6 mL/min)

3.3.2.3. ***Stopped flow mode***

The driving force of membrane dialysis process is concentration gradient between the donor and acceptor solution, thus the better way to increase flux is to keep the concentration gradient at maximum by filling the sample in the donor chamber and let the mass transfer proceed at a certain period of time. This method is also called stopped flow mode. The signal obtained from the stopped flow mode appeared to be much better than the continuous flow mode. The mass transfer was calculated from the absorbance of the analyte after dialysis divided by the absorbance of non-dialysis standard solution. The mass transfer of the stopped flow mode was 31% compared to only 14% in the continuous flow mode. In the stopped flow mode, the stopped times varied from 10 – 300 sec were investigated. The absorbance of the

Fe(II)-1,10-phenanthroline complex increased when the stopped time increased from 10 to 60 sec, nevertheless the absorbance was not increased any further when increased the stopped time up to 300 sec (Figure 3.6), supposed that the equilibrium had been reached at 60 sec of stopped time. However at the stop time 30 sec gave the highest change of sensitivity, thus a 30 sec stopped time was selected for further study.

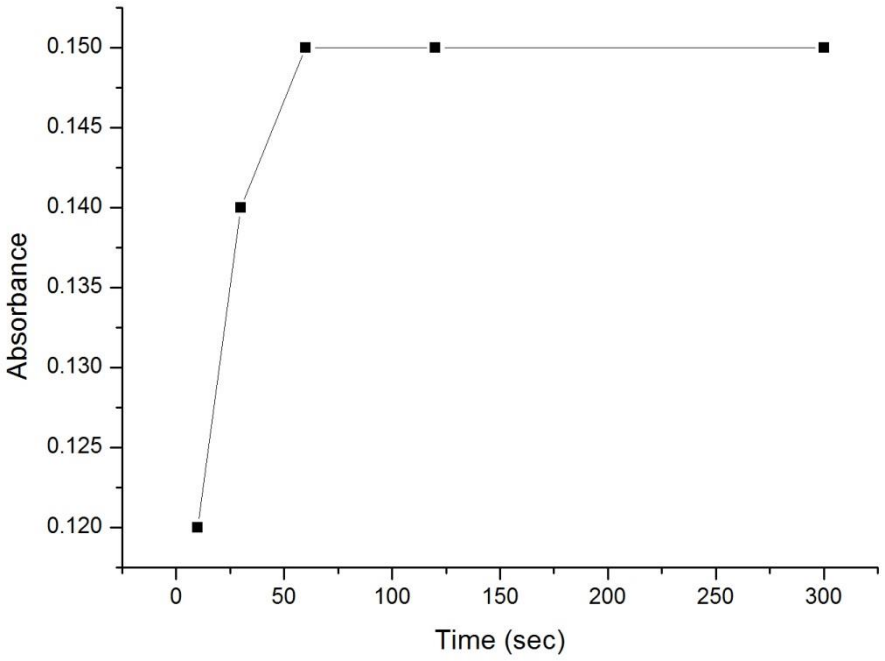

Figure 3.6 The signal of the Fe(II) 1,10-phenanthroline complex in the acceptor solution after on-line dialysis screening of a 1.5 mL loading of a 10 mg/L standard Fe(II)solution at 10–300 sec stop times and with an acceptor flow rate of 0.6 mL/min

3.3.2.4. *Acceptor flow rate*

The effect of the acceptor flow rates on the absorbance of Fe(II)-1,10-phenanthroline complex was shown in Figure 3.7. In the stopped flow mode, the acceptor flow rate would affect a longitudinal diffusion caused to dilution of detection, thus when increased the acceptor flow rate the sensitivity also increased. From the result, the peak height increased when the acceptor flow rate was increased from 0.7 mL/min until 1.7 mL/min and stable, thus 1.7 mL/min of acceptor was chosen due to giving highest sensitivity.

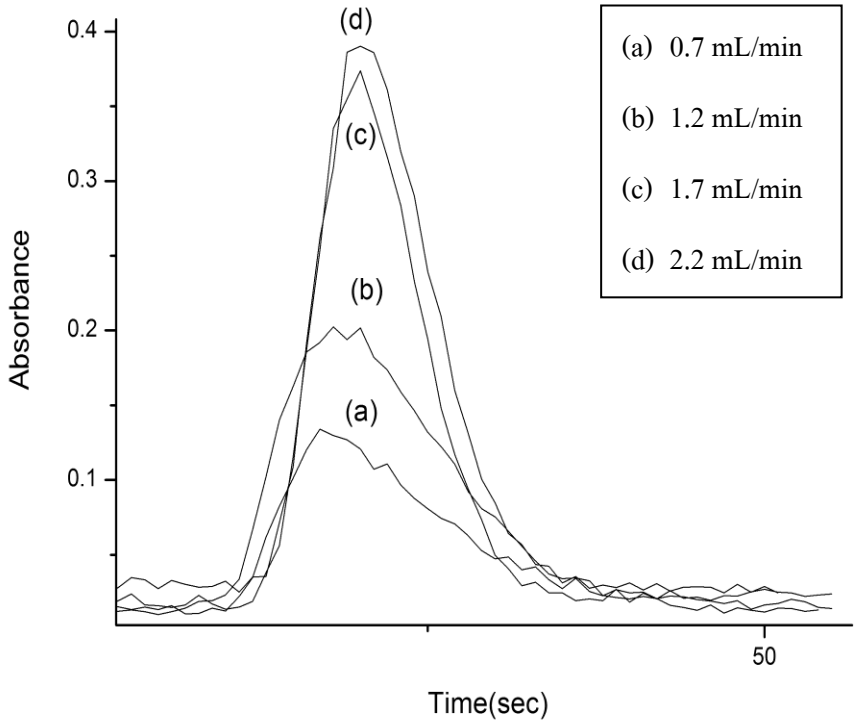

Figure 3.7 The signal of the Fe(II)-1,10-phenanthroline complex in the acceptor solutions at various acceptor flow rates after on-line dialysis screening of a 1.5 mL loading of a10 mg/L standard Fe(II)solution for with a 30 sec stopped time.

3.3.2.5. ***Flow direction***

The sensitivity of the system was compared at different flow directions; i.e., concurrent and counter current. The result showed no difference in sensitivity of detection between two-flow directions. For the further study, the concurrent flow was chosen because it generated lower pressure in the system.

3.3.2.6. ***The interference study***

The results of interference study were summarized in Table 3.2. The ion interference did not interfere in our method, where the percent recoveries were not different than 10 percent from 100 percent recovery. Only the Zn(II) and Na(I) ion might interfere at 500 mg/L and 1000 mg/L, respectively. Zn(II) ion gave low recover

because it may precipitate 1,10-phenanthroline [38]. Moreover, Na(I) ion gave high percent recovery. The explanation has not yet been known.

Table 3.2 Interference study of some major mineral ions from grape juice samples spiked with Fe(II)10 mg/L

Interference ion	Additional level (mg/L)	Fe(II)found (mg/L± SD,%RSD)	%Recovery
Na(I)	500	10.1±1.01, 10.0% (N= 3)	101.4
	1000	12.9±0.09, 0.7% (N= 3)	128.5
K(I)	500	9.7±0.24, 2.5% (N= 3)	96.6
	1000	9.4±0.51, 5.5% (N= 3)	94.2
Ca(II)	500	10.2±0.57, 5.6% (N= 3)	102.5
	1000	10.7±0.28, 2.6% (N= 3)	107.4
Mg(II)	500	9.9±0.95, 9.5% (N= 3)	99.3
	1000	10.8±0.42, 4.0% (N= 3)	107.5
Zn(II)	100	9.7±0.35, 3.6% (N= 3)	96.8
	500	7.9±0.70, 8.8% (N= 3)	79.3

3.3.3. Performance of the system

3.3.3.1. *The method performance at optimized condition*

The system was tested at the optimized condition; stopped flow mode; sample loading volume = 1.5 mL, stopped time = 30 sec, acceptor flow rate = 1.7 mL/min and loading flow rate = 3.1 mL/min. The result is shown in Table 3.3.

Table 3.3 The method performance for determination of Fe(II) in grape juice samples

Parameter	Performance
Linear range	3-30 mg/L
Linear least squares equation	0.049x+ 0.020
Coefficient of determination (R^2)	0.9965
Limit of detection (S/N= 3)	0.6 mg/L
Sample throughput	24 samples/hr
Repeatability (spiked Fe(II)10 mg/L)	2% (N=8)

3.3.3.2. ***The percent recovery of spiked Fe(II) in fruit juice samples***

The percent recovery of spiked Fe(II) in sample was shown in Table 3.4. The recovery of known concentration of spiked Fe(II) in grape juice samples was about 90%. The relative standard deviation of three replicates was less than 2%.

Table 3.4 The percent recovery of spiked Fe(II) in fruit juice samples

Spiked level (mg/L)	Found±SD (mg/L), %RSD	% Recovery
3	2.8±0.04, 1.5% (N= 3)	91.7%
5	4.4±0.09, 2.0% (N= 3)	88.5%

3.3.3.3. ***The comparison between on-line dialysis and conventional method***

The on-line dialysis method was compared to the conventional method (wet acid digestion). The amounts of iron found in the samples were reported in Table 3.5.

Table 3.5 The real sample analysis

Real sample analysis (grape juice samples)	Found±SD (mg/L)
Conventional wet acid digestion	4.5±0.25
On-line dialysis	2.4±0.19
On-line dialysis with hydroxylamine	3.8±0.31

The result showed that an on-line dialysis system gave lower amount of Fe(II) (2.4 mg/L) than the conventional method (4.5 mg/L). In the conventional method (wet acid digestion), hydroxylamine was added into the sample after digestion to convert all iron species; i.e., Fe(II), Fe(III) into Fe(II) ion before complex with 1,10 phenanthroline reagent but in an on-line dialysis, hydroxylamine was not used, so only Fe(II) in the sample was detected causing to lower amount of iron found. When hydroxylamine was added into our method, the amount of iron found in the sample increased from 2.4 mg/L to 3.8 mg/L because Fe(III) ion in the sample was converted to Fe(II). However, the amount of iron found was still lower than the conventional method. The hypothesis based on this result might be whether hydroxylamine is effective enough to convert all Fe(III) into Fe(II). Thus, to prove this hypothesis, the percent recovery has been compared between with and without reducing agent in 10 mg/L of Fe(III) and Fe(II) solution (Table 3.6).

Table 3.6 The effect of hydroxylamine on percent recovery of Fe(II) and Fe(III) spiked in fruit juice sample.

Spiked	Spiked Found±SD (mg/L),%RSD	%Recovery
Fe(II) 10 mg/L	10.0±0.83, 8.3% (N= 3)	99.8
Fe(II) 10 mg/L + hydroxylamine	10.0±0.33, 3.2% (N= 3)	100.2
Fe(III) 10 mg/L	Not found	-
Fe(III) 10 mg/L + hydroxylamine	10.0±0.61, 6.1% (N= 3)	100.5

From the result, the hydroxylamine could reduce Fe(III) ion to Fe(II) ion completely. It means that hydroxylamine is effective enough to convert all Fe(III) to Fe(II) ion. However the amount of iron found in the sample was still lower than that found by the conventional method, so another reason might be attributed to the membrane dialysis that allow only free iron ions to transfer across while in the

membrane but not other forms of iron such as bound iron that might be bigger size than the pores of the membrane, so they could not be transferred across the membrane. The conventional method, all the iron forms including bound iron were digested and reduced to Fe(II) ion.

3.4. Conclusion

On-line dialysis system can be used for determination of free iron ions in the fruit juice samples. Our system can analyse Fe(II) ions that added in the sample without further sample preparation step. The on-line membrane dialysis can remove interference that cause high background signal in spectrophotometer. In addition, our on-line membrane dialysis unit can be used for more than 100 times per one setup. The membrane unit provides many advantages such as cost effective, simple setup and easy to clean. Our system can be used for determination of any ion that might be contained in the sample by flow-based colorimetric method with chromogenic reagent.

CHAPTER IV
THE HYBRID FLOW ANALYZER FOR AUTOMATIC SINGLE STRAND HOLLOW FIBER MEMBRANE FOR LIQUID-PHASE MICROEXTRACTION WITH IN-LINE MEMBRANE REGENERATION

4.1. Introduction

Liquid phase microextraction techniques (LPME) have been more popular now a day and become an alternative extraction technique to the conventional liquid-liquid extraction (LLE) due to its many advantages such as less use of organic solvent, simple setup and high enrichment factor. LPME techniques include single-drop microextraction, dispersive LPME, solidification of organic drop and hollow fiber LPME (HF-LPME). In the case of HF-LPME, the hollow fiber membrane is used to support an organic solvent in its lumen and pores, and then immersed into the donor solution for extraction. The target analytes are transferred from the donor solution across the membrane into the acceptor solution, i.e., aqueous donor to organic acceptor called two-phase system; or aqueous donor to organic extractor to aqueous acceptor called three-phase system.

Typically, the HF-LPME procedure is performed in batch wise format, where the membrane is manually prepared for single use to assure reproducibility and avoid carry over. Recently, there are some development of HF-LPME in on-line format, where the hollow fiber membrane units have been designed to connect with or incorporate into an on-line analysis system such as microfluidic device [26] or flow through configuration [17]. Nevertheless, the hollow fiber membrane is still manually prepared, connected, disconnected as well as regenerated, which may not be convenient for routine analysis because the system must be stopped each time for cleaning and regenerating the membrane prior to the next run. In this work, we are interested in development of automated on-line HF-LPME system using a hybrid flow analyzer, which is capable of membrane cleaning and liquid membrane regeneration. The performance of the system would be tested on analysis of Cr(VI) in environmental samples.

4.2. Experiment

4.2.1. Chemical

All chemicals and reagents were analytical grade and summarized in Table 4.1

Table 4.1 Chemicals and reagents

Chemical	Supplier
Methytrioctyl ammonium chloride (Aliquat 336©)	Sigma-Aldrich (Steinheim, Germany)
Kerosene	Sigma-Aldrich (Steinheim, Germany)
Potassium dichromate ($K_2Cr_2O_7$)	Panreac, Barcelona, Spain
1,5-diphenylcarbazide (DPC)	Panreac, Barcelona, Spain
Ethanol	Sigma-Aldrich (Steinheim, Germany)
Sulfuric acid (H_2SO_4)	Sigma-Aldrich (Steinheim, Germany)
Acetic acid (CH_3COOH)	Sigma-Aldrich (Steinheim, Germany)
Sodium acetate (CH_3COONa)	Fluka, Steinheim, Germany
Sodium phosphate dibasic (Na_2HPO_4)	Sigma-Aldrich (Steinheim, Germany)
Sodium phosphate monobasic (NaH_2PO_4)	Sigma-Aldrich (Steinheim, Germany)
Sodium tetraborate ($Na_2[B_4O_5(OH)_4]\cdot 8H_2O$)	Sigma-Aldrich
Hydrochloric acid (HCl)	Sigma-Aldrich
A reference soil material (SRM 2701) contained hexavalent Cr 551.2 ± 17.2 mg/kg	NIST
Sodium hydroxide (NaOH)	Sigma-Aldrich
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)	Scharlau, Barcelona
Sodium carbonate (Na_2CO_3)	Sigma-Aldrich

4.2.1. Preparation of solution

4.2.1.1. *Stock standard Cr(VI) solution; 1000 mg/L*

A 0.2826 g of potassium dichromate was dissolved in a 100 mL of Milli-Q water.

4.2.1.2. ***DPC solution; 0.01 % (w/v)***

A 0.025 g of DPC was dissolved in a 25 mL of mixture of 70% ethanol and 1.0 mol/L H_2SO_4.

4.2.1.3. ***Organic extracting phase***

The organic extracting phases were prepared by dissolving Aliquat 336 with kerosene in various ratios (%V/V).

4.2.2. **Alkaline digestion of soil sample**

The reference soil was digested follow by slightly modification of USEPA method 3060A. Sample was digested with a mixture of 0.28 mol/L sodium carbonate and 0.5 mol/L sodium hydroxide. A 0.84 g of magnesium chloride was added for suppress the oxidation of Cr(III) to Cr(VI). The digested solution was filtered with 0.45-μm nylon syringe filter and adjusted pH with phosphate buffer before analysis.

4.2.3. **The instrument and analysis system**

The adjustable concentric hollow fiber membrane unit (see Figure 2.6) with a polypropylene hollow fiber membrane (Accurel PP Q3/2, Wuppertal, Germany internal diameter 600 nm, wall thickness 200 um, pore size 0.2 nm, length 10 cm) was used. The hybrid flow analyzer system, which combined the liquid delivery systems and valve system in FIA and SIA, was developed (Figure 4.1). In the system, the multi syringe pump was used for delivery of the carrier solution and DPC solution as an acceptor solution. The hollow fiber membrane unit was connected to a selection valve and a three-way valve for extraction, stripping, detection, membrane cleaning and membrane regeneration, successively.

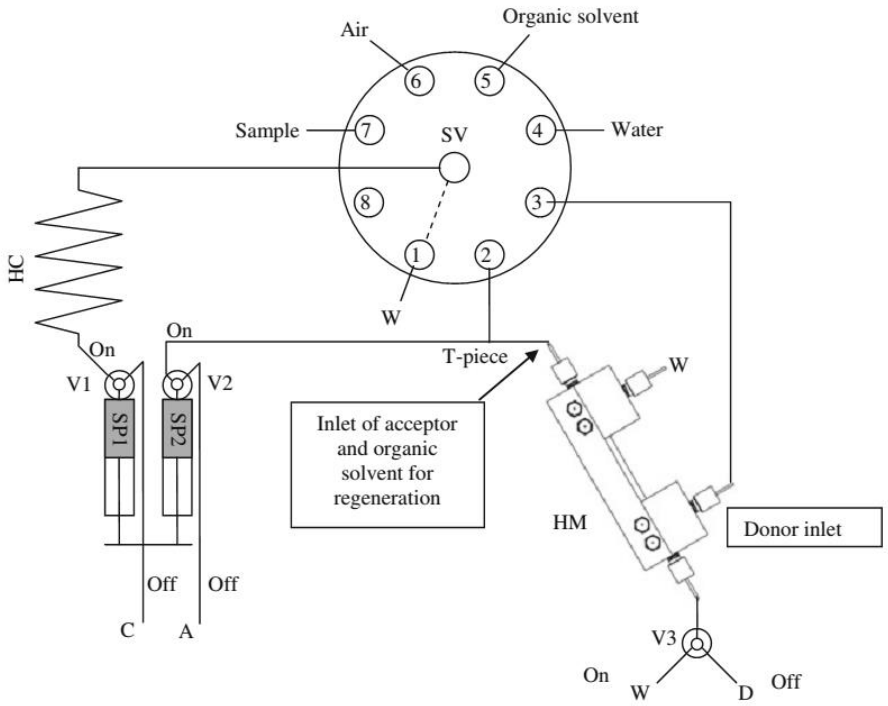

Figure 4.1 Schematic diagram of the hybrid flow analyzer with in-line HF-LPME platform, Holding coil (HC), solenoid valve (V), syringe pump (SP), carries solution (C), acceptor solution (A), holding coil (HC), selection valve (SV), waste (W), HF-LPME platform (HM), miniaturized spectrophotometer (D)

The method was divided into three main steps; pretreatment/regeneration, extraction and detection. In the pretreatment/regeneration step, a 120 µL of extracting organic solvent was pumped through the lumen of the hollow fiber membrane and cleaned with MilliQ water afterward. In this step, the membrane pores were completely impregnated with the extracting organic solvent. Then, the lumen of the hollow fiber membrane was filled with the acceptor solution via the multi syringe pump. In the extraction step, a 1.4 mL of sample solution was pumped into the sample chamber (outside the hollow fiber membrane) and drawn into the waste afterward. The extraction step was repeated twice. In this step, the Cr(VI) in sample was extracted from sample solution into the acceptor phase via the liquid membrane which, the Cr(VI) was complexed with the DPC in the acceptor solution. In the detection step, the acceptor solution, where the Cr(VI)-DPC was formed, was then pumped through the detector for measurement at 540 nm. Finally, the hollow fiber membrane was rinsed with 2 mL of acceptor solution to remove the remaining

analyte to prevent carry over before the next analysis. The operating steps were programmed for automation, which were summarized in Figure 4.2.

Table 4.2 Operating steps of automatic single strand hollow fiber membrane for LPME with in-line membrane regeneration

Operational step	Valve position			Direction	SV position	Flow rate (mL/min)	Volume (µL)
	V1	V2	V3				
Membrane pretreatment/regeneration							
- Fill the donor chamber with MilliQ water	On	Off	Off	Dispense	3	6.0	1500
- Draw the organic solvent into HC	On	Off	Off	Aspirate	5	1.5	120
- Fill the lumen of the membrane with the organic solvent	On	Off	On	Dispense	2	0.3	120
- Halt the flow for membrane impregnation	Off	Off	Off	-	2	-	-
- Empty the lumen of the membrane	On	Off	On	Dispense	2	0.3	500
- Empty the donor chamber	On	Off	Off	Aspirate	3	1.0	1500
- Flush to waste	On	Off	Off	Dispense	1	2.5	2000
- Fill the lumen with the DPC solution	Off	On	Off	Dispense	-	0.1	40
Sample extraction/clean-up							
- Draw the sample into HC	On	Off	Off	Aspirate	7	5.0	1400
- Pump the sample through the HF lumen	On	Off	Off	Dispense	3	1.0	1400
- Retrieve the sample volume	On	Off	Off	Aspirate	3	1.0	1400
- Flush to waste	On	Off	Off	Dispense	1	5.0	1400
- Repeat steps 9-12 twice							
Detection							
- Bring the preconcentrated Cr-DPC complex to the detector	Off	On	Off	Dispense	-	0.4	200
- Rinsing of the lumen of the membrane	Off	On	On	Dispense	-	2.0	1000

4.3. Results and discussion

4.3.1. Preliminary study

The extraction conditions were adapted and modified from the work done by Castillo et al. [38]. Three-phase LPME was set, where Aliquat in kerosene was used as the organic extracting solvent and DPC in ethanol was used as the acceptor solution. Aliquat performed as an anion exchanged carrier, where the chromate ion was extracted. The chromate ion was stripped off and transferred into the acceptor phase by forming Cr-DPC complex. It was observed that Cr-DPC complex could be partitioned into the kerosene phase during extraction. For this reason, the Cr-DPC complex must be completely eluted from the system to prevent carryover to the next run. To ensure complete stripping of Cr-DPC complex from the membrane, high percent ethanol in DPC solution; i.e., 80% v/v was prepared.

4.3.2. The extraction flow mode

Practically, the extraction efficiency in HF-LPME technique is based on rate of mass transfer across the membrane, which depends on diffusion by concentration gradient at the adjacent phases (diffusion zone) and convection from the bulk solution. For our in-line HF-LPME setup, convection by moving the donor solution may increase the mass transfer of the analyte from the bulk solution to the diffusion zone. Dynamic flow mode was investigated. In our experiment, three different modes; i.e., stopped flow mode, forward flow mode, and forward and backward flow mode, were studied at equivalent extraction time (5 min). The signals of Cr-DPC in the acceptor solutions obtained from three different modes were compared in Figure 4.2 .

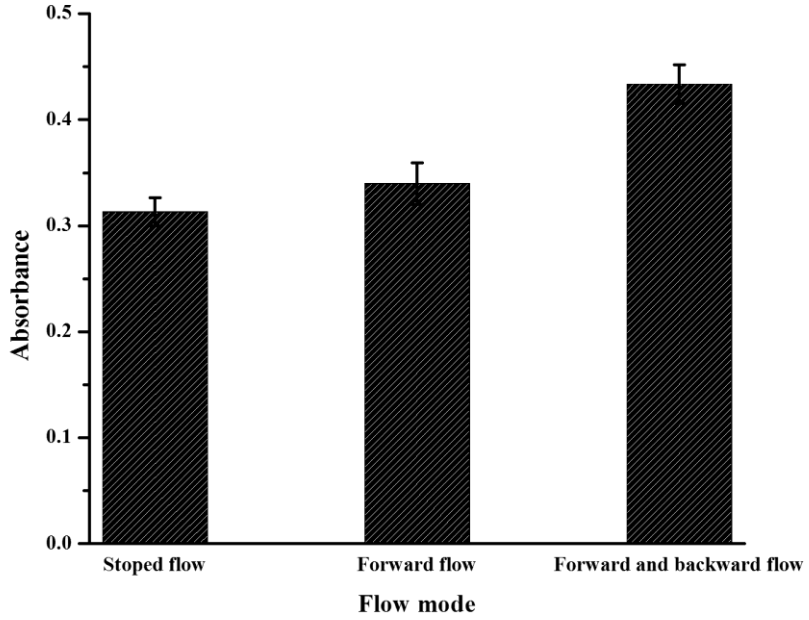

Figure 4.2 The signals of Cr-DPC in the acceptor solutions obtained from of automatic single strand hollow fiber membrane for LPME with in-line membrane regeneration at three different flow modes

The result showed that the stopped flow mode exhibited the lowest sensitivity due to no convection of the donor solution. Therefore, the mass transfer of the analyte was limited only by the concentration gradient in the diffusion zone. In the dynamic flow modes, the mass transfer of the analyte in the bulk solution could be enhanced by the convection of the donor solution so that the analyte in the diffusion zone could be replenished. The forward and backward flow mode yielded higher sensitivity than the forward flow mode because the back and forth flow direction created more turbulence providing more intimate contact of the donor solution with the supported liquid membrane. For this reason, the forward and backward flow mode was selected for the next experiment.

4.3.3. The factorial design for method optimization

The experimental factors such as Aliquat concentration, donor flow rate and pH were investigated using factorial design. Each factor was studied at three levels with three replicates of the center point. The standard Cr(VI) solution of 250 µg/L was tested. The Aliquat concentrations were studied in the range from 0.1 to 0.3 according to Castillo et al. [39]. The flow rates were varied in the range of 0.3 – 2.0

mL/min. The pH was varied in the range of 5 – 9. The results (Table A.1) were calculated and visualized by statistical program (StatGraphics Centurion XV, Stat Point Inc., Herndon, VA, 2005) to build the first order factorial design and estimate factors interactions model (pareto chart) as shown in Figure 4.3

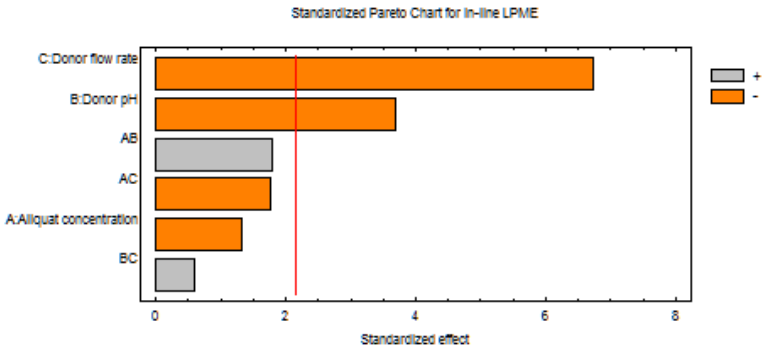

Figure 4.3 Pareto chart of the main effects on HF-LPME parameters

The analysis of the results yielded Pareto chart of the main effects that facilitate ascertainment of the relevance of three-factor interactions. Pareto chart is a histogram where the length of each bar is proportional to the absolute value of the estimated effect, namely, Aliquat concentration (A), pH (B), donor flow rate and interaction between them (AB), (AC), (BC), on the absorbance of the Cr-DPC complex in the acceptor solution. The cross vertical line indicates the significance of each factor at the 95% confidence level. An effect exceeding this vertical line should be considered as statistically significant regarding to the absorbance of the Cr-DPC complex in the acceptor solution. Pareto chart for HF-LPME revealed that sample flow rate and sample pH were statistically significant parameters at the 95% confidence level, corresponding in our case to a Student's *t* of 2.36 for seven degrees of freedom. Nevertheless, there were no significant interactions between factors for chromium extraction ($p > 0.05$). Therefore, the methods can be readily optimized using one-at-a-time approaches without risks of biased.

According to the Pareto chart (Figure 4.3), the lower flow rate gave better extraction efficiency because it provided longer contact time between the donor and the supported liquid membrane. The flow rate of 1.25 mL/min was selected because it gave shorter analysis time (20 min per cycle) compared to the flow rate of 0.3 mL/min (30 min per cycle) without significant different sensitivity. At the donor pH 5, the chromium was available in the form of $HCrO_4^-$ (pH 4-6) giving higher diffusion coefficient ($HCrO_4^-$ 1.95×10^{-7} m/s) while at the higher pH (pH > 6), the chromium

was available in the form of CrO_4^{-2} having somewhat lower diffusion coefficient (1.31×10^{-7} m/s) [39]. To ensure that $HCrO_4^-$ was the dominant species, the donor pH was adjusted to 4. In addition, the method was further modified for double extraction, where two portions of the sample were extracted to achieve sufficient detection limit at the chromium concentration below 50 µg/L (maximum contaminant level in drinking water by WHO provisional guideline).

4.3.4. The effect of in-line membrane regeneration

Figure 4.4 showed the signals of Cr-DPC after performing in-line HF-LPME with and without membrane regeneration using the same membrane. The signals of Cr-DPC obtained from in-line HF-LPME without membrane regeneration was decreasing while the signals of Cr-DPC obtained from in-line HF-LPME with membrane regeneration could be maintained. The reason could be that the Aliquat may be lost during each extraction, whereas with membrane regeneration, the Aliqaut was replenished for each extraction cycle. It has been demonstrated that membrane regeneration in HF-LPME is necessary. Furthermore, our hybrid flow analyzer has offered both automatic in-line extraction and automatic in-line membrane regeneration making continuous analysis without human attention and field application possible.

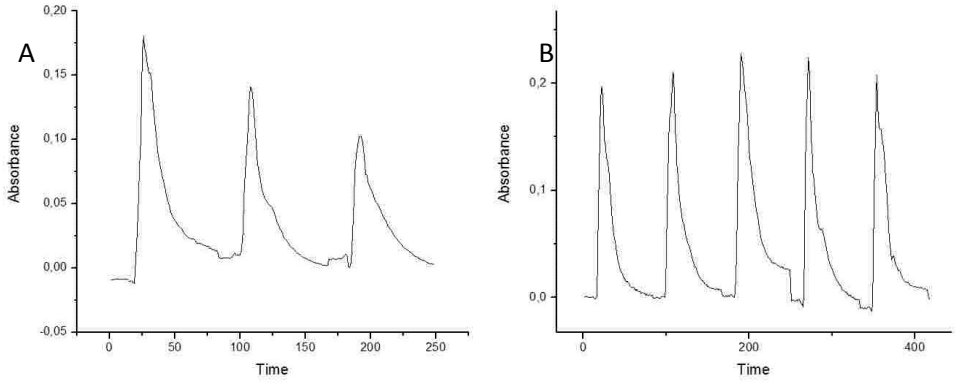

Figure 4.4 Signals of 200 µg/L Cr-DPC after in-line HF-LPME. A) Without membrane regeneration B) With membrane regeneration

4.3.5. The system performance

The system performance was evaluated using standard 250 µg/L chromium (VI) solution. The figures of merit were summarized in Table 4.3. The limit of detection (LOD) and quantization (LOQ) of the method were 4 µg/L and 15 µg/L based on three times and ten times standard deviation of blank signal respectively. The enrichment factor of approximately 11 was obtained. The linear working

concentration range was 30 - 500 µg/L giving r^2 greater than 0.99. The method showed good repeatability and reproducibility, where % RSD was less than 10%.

Table 4.3 Figures of merit for in-line HF-LPME of Chromium (VI) in water sample

Analytical parameter	Value
Calibration curve	y=0.8881x-0.0026 (R^2 = 0.9963)
Linear working range	30 – 500 µg/L
LOD	4.6 µg/L
LOQ	15.3 µg/L
Repeatability %RSD (250 µg/L n=7)	4.2 %
Reproducibility % RSD (250 µg/L n=5 days)	9.6 %
Extraction efficiency (%)	13.2
Enrichment factor	10.9

The accuracy of the method has been investigated for potential interference; Cr(III), effect of water hardness, and several kinds of matrices such as drinking water (commercial brand), tap water, and waste water (Biological treatment plant in University of Balearic Island, Spain). The signals of spiked Cr(VI) in all kinds of matrices were not deviated more than 10% compared to those of standard Cr(VI) at the same concentration. According to Table 4.4, the average recoveries of spiked samples at two levels; 50 µg/L and 30 µg/L were in the range of 83-109%, which are acceptable (±20%) at the spiked concentration level [40]. In addition, the method was applied to the reference soil sample containing Cr(VI) 551.2 ± 34.5 mg/kg. The Cr(VI) in the digested soil sample was determined using standard addition method. The average

concentration of Cr(VI) from three replicate analyses was 545.6±56.6 mg/kg, which was not significantly different from the certified value (t-test, P>0.05).

Table 4.4 Recovery study of spiked samples in several kinds of matrices

Sample	Spiked (µg/L)	Found (µg/L)	Average recoveries (%) N=3	RSD(%)
Milli Q water + 5000 µg/L Cr(III)	50.0	50.6	101.2	4.9
Milli Q water + 180 mg/L $CaCl_2$ (hard water)	50.0	54.0	108.1	11.0
Drinking water	30.0	32.8	109.3	4.3
	50.0	47.1	94.3	9.4
Tap water 1	30.0	32.1	107.0	14.0
	50.0	46.9	93.9	18.8
Tap water 2	30.0	32.5	108.4	13.0
	50.0	53.7	107.5	4.0
Ground water	30.0	27.6	91.9	20.0
	50.0	48.2	96.4	14.9
Waste water	100.0	83.2	83.2	5.2
	200.0	188.9	94.4	13.9
	300.0	310.4	103.5	2.8

4.4. Conclusion

On-line LPME techniques using supported liquid membrane requires membrane regeneration to maintain its extraction efficiency. The hybrid flow analyzer has been developed to achieve a fully automation for both in-line membrane regeneration and membrane extraction procedures. The method was developed for the determination of Cr(VI) in several kinds of water matrices as well as soil sample showing good precision and accuracy. The system is simple, cost effective and applicable for field applications.

CHAPTER V
Conclusion

5.1. Conclusion

In this work, we developed single strand hollow fiber membrane separation units for incorporation with on-line analysis methods for determination of metal ions in complex matrix samples. Two designs of membrane separation units have been demonstrated and integrated into flow based analysis systems. In the first system, the easy assembly hollow fiber membrane unit was used for on-line dialysis screening for determination of Fe(II) ions in fruit juice samples. The Fe(II) ions can be analyzed within the range of 3 to 30 mg/L. This system was automatically operated. The samples could be analyzed within 2.5 min without further sample preparation step. The easy assembly hollow fiber membrane unit provided many advantages such as cost effective, durable and simple setup. The second system was the on-line HF-LPME system that was developed for determination of Cr(VI) ion in environmental samples. In this system, the adjustable concentric membrane unit, where the dimension of the unit can be adjusted within few minutes, was designed. The unit was well incorporated with the hybrid flow analyzer for complete automation; i.e., membrane pretreatment, sample loading, extraction, sample elution, detection, membrane cleaning and membrane regeneration for the next run. The system not only offers an automatic operation, but also allow the use of single membrane for more than 100 times.

5.2. Suggestions for the future work

In the on-line dialysis system, the system can be extended application for another ion analysis also included ions speciation application. In addition the on-line dialysis system can be cooperated with another analysis technique such as atomic absorption or ion chromatography. In the on-line LPME system, the in-line regeneration process was still slow that can be improved by change the method into one-side membrane extraction, which only the lumen side of hollow fiber was use for extraction and elution that the organic membrane can be flush out by flow organic solvent inside the hollow fiber. In the our membrane separation unit, the membrane separation unit can be operated in electromembrane mode [41] that can accelerate the extraction process in order to reduce the extraction time. The instrument size can be reduced in the future that will be useful for the field analysis.

REFERENCES

1. Ruzicka, J. and Hansen, E.H., Flow injection analyses. Part I. A new concept of fast continuous flow analysis. Analytica Chimica Acta. **78** (1975): 145-157.
2. Lima, J.L.F.C., Delerue-Matos, C., and Vaz, M.C.V.F., Automation of iron and copper determination in milks using FIA systems and colourimetric detection. Food Chemistry. **62** (1998): 117-121.
3. Ruzicka, J. and Hansen, E.H., Retro-review of flow-injection analysis. TrAC Trends in Analytical Chemistry. **27** (2008): 390-393.
4. Melchert, W.R., Reis, B.F., and Rocha, F.R.P., Green chemistry and the evolution of flow analysis. A review. Analytica Chimica Acta. **714** (2012): 8-19.
5. Mesquita, R.B.R. and Rangel, A.O.S.S., A review on sequential injection methods for water analysis. Analytica Chimica Acta. **648** (2009): 7-22.
6. van Staden, J.F. and Stefan, R.I., Chemical speciation by sequential injection analysis: an overview. Talanta. **64** (2004): 1109-1113.
7. Ruzicka, J. and Marshall, G.D., Sequential injection: a new concept for chemical sensors, process analysis and laboratory assays. Analytica Chimica Acta. **237** (1990): 329-343.
8. Miró, M., Cerdà, V.c., and Estela, J.M., Multisyringe flow injection analysis: characterization and applications. TrAC Trends in Analytical Chemistry. **21** (2002): 199-210.
9. Šatínský, D., Karlíček, R., and Svoboda, A., Using on-line solid phase extraction for flow-injection spectrophotometric determination of salbutamol. Analytica Chimica Acta. **455** (2002): 103-109.
10. Worsfold, P.J., et al., Flow injection analysis as a tool for enhancing oceanographic nutrient measurements—A review. Analytica Chimica Acta. **803** (2013): 15-40.
11. Sweileh, J.A., On-line flow injection solid sample introduction digestion and analysis: spectrophotometric and atomic absorption determination of iron, copper and zinc in multi-vitamin tablets. Microchemical Journal. **65** (2000): 87-95.
12. Tao, G. and Fang, Z., On-line flow injection solvent extraction for electrothermal atomic absorption spectrometry: Determination of nickel in biological samples. Spectrochimica Acta Part B: Atomic Spectroscopy. **50** (1995): 1747-1755.
13. Kuban, P. and Karlberg, B., On-Line Dialysis Coupled to a Capillary Electrophoresis System for Determination of Small Anions. Analytical Chemistry. **69** (1997): 1169-1173.

14. van Staden, J.F. and Tlowana, S.I., On-line separation, simultaneous dilution and spectrophotometric determination of zinc in fertilisers with a sequential injection system and xylenol orange as complexing agent. Talanta. **58** (2002): 1115-1122.
15. Rasmussen, K.E. and Pedersen-Bjergaard, S., Developments in hollow fibre-based, liquid-phase microextraction. TrAC Trends in Analytical Chemistry. **23** (2004): 1-10.
16. Gjelstad, A., Rasmussen, K.E., and Pedersen-Bjergaard, S., *Hollow fiber liquid-phase microextraction*. 2012, Elsevier Inc. p. 475-496.
17. Jönsson, J.Å. and Mathiasson, L., Liquid membrane extraction in analytical sample preparation: I. Principles. TrAC Trends in Analytical Chemistry. **18** (1999): 318-325.
18. Jahangiri, S., Hatami, M., Farhadi, K., and Bahram, M., Hollow-fiber-based LPME as a reliable sampling method for gas-chromatographic determination of pharmacokinetic parameters of valproic acid in rat plasma. Chromatographia. **76** (2013): 663-669.
19. Yazdi, A.S., Yazdinezhad, S.R., and Akhoundzadeh, J., Simultaneous derivatization and extraction of iodine from milk samples by hollow fiber liquid-phase microextraction followed by gas chromatography- electron capture detection. Journal of the Iranian Chemical Society. **10** (2013): 643-651.
20. Zupancic, T. and Pihlar, B., Preconcentration of quinolones by dialysis on-line coupled to high-performance liquid chromatography. Journal of Chromatography A. **840** (1999): 11-20.
21. Ganeshjeevan, R., Chandrasekar, R., Yuvaraj, S., and Radhakrishnan, G., Determination of hexavalent chromium by on-line dialysis ion chromatography in a matrix of strong colourants and trivalent chromium. Journal of Chromatography A. **988** (2003): 151-159.
22. Shrivas, K. and Patel, D.K., Ultrasound assisted-hollow fibre liquid-phase microextraction for the determination of selenium in vegetable and fruit samples by using GF-AAS. Food Chemistry. **124** (2011): 1673-1677.
23. Li, L. and Hu, B., Hollow-fibre liquid phase microextraction for separation and preconcentration of vanadium species in natural waters and their determination by electrothermal vaporization-ICP-OES. Talanta. **72** (2007): 472-479.
24. Coan, F.L.A., CA, US), Schletz, Jeffrey C. (Clayton, CA, US), *INTEGRATED MEMBRANE MODULE FOR GAS DEHYDRATION AND GAS SEPARATION*. 2011, GENERON IGS, INC. (Houston, TX, US): United States.
25. Miró, M. and Frenzel, W., Automated membrane-based sampling and sample preparation exploiting flow-injection analysis. TrAC Trends in Analytical Chemistry. **23** (2004): 624-636.

26. Hylton, K. and Mitra, S., A microfluidic hollow fiber membrane extractor for arsenic(V) detection. Analytica Chimica Acta. **607** (2008): 45-49.
27. de Castro, M.D.L., Capote, F.P., and Ávila, N.S., Is dialysis alive as a membrane-based separation technique? TrAC Trends in Analytical Chemistry. **27** (2008): 315-326.
28. Hylton, K. and Mitra, S., Automated, on-line membrane extraction. Journal of Chromatography A. **1152** (2007): 199-214.
29. *SEM hollow fiber picture*. 2013 [cited 2013 30 December 2013]; Available from: http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Membranes/hollow1.htm.
30. Ibrahim, D., PIC32 Microcontroller, in Designing Embedded Systems with 32-bit PIC Microcontrollers and Mikroc. 2014, Newnes: Oxford. p. 41-90.
31. Hazrat Ali, M.D., Katsuki, A., Kurokawa, S., and Sajima, T., Design of Mechanical Actuator in 3D CAD Software. Procedia Engineering. **64** (2013): 473-478.
32. Nordmeyer, F.R. and Hansen, L.D., Automatic dialyzing-injection system for liquid chromatography of ions and small molecules. Analytical Chemistry. **54** (1982): 2605-2607.
33. Nitiyanontakit, S., Varanusupakul, P., and Ngamukot, P., Single strand hollow fiber membrane (SSHFM): An on-line sample preparation for the flow based colorimetric determination of free iron in fruit juices. Talanta. **84** (2011): 1304-1308.
34. Wonguchoto, S., Nitiyanontakit, S., and Varanusupakul, P., Flow-Based Iodometric Extraction Method by the Hollow Fiber Membrane for the Determination of Selenium Sulfide Levels in Cosmeceutical Products. Journal of Chemistry and Chemical Engineering. **6** (2012): 299-306.
35. Nitiyanontakit, S., Varanusupakul, P., and Miró, M., Hybrid flow analyzer for automatic hollow-fiber-assisted ionic liquid-based liquid-phase microextraction with in-line membrane regeneration. Analytical and Bioanalytical Chemistry. **405** (2013): 3279-3288.
36. van de Merbel, N.C., Membrane-based sample preparation coupled on-line to chromatography or electrophoresis. Journal of Chromatography A. **856** (1999): 55-82.
37. Pyenson, H. and Tracy, P.H., A 1,10—Phenanthroline Method for the Determination of Iron in Powdered Milk. Journal of Dairy Science. **28** (1945): 401-412.

38. Snell, F.D. and Snell, C.T., Colorimetric methods of analysis, including some turbidimetric and nephelometric methods. 1972, Huntington, N.Y.,: R. E. Krieger Pub. Co. v.
39. Castillo, E., Granados, M., and Cortina, J.L., Liquid-supported membranes in chromium(VI) optical sensing: transport modelling. Analytica Chimica Acta. **464** (2002): 197-208.
40. Horwitz, W., Evaluation of Analytical Methods Used for Regulation of Foods and Drugs. Analytical Chemistry. **54** (1982): 67A-76A.
41. Pedersen-Bjergaard, S. and Rasmussen, K.E., Electrical potential can drive liquid-liquid extraction for sample preparation in chromatography. TrAC Trends in Analytical Chemistry. **27** (2008): 934-941.

APPENDIX

APPENDIX A

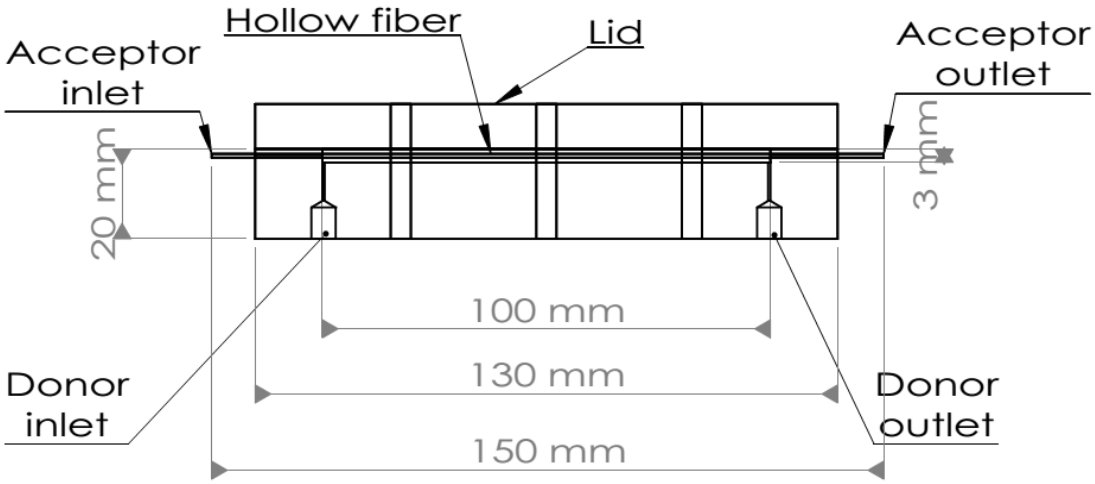

Figure A.1 The Easy assemble hollow fiber membrane unit (original version)

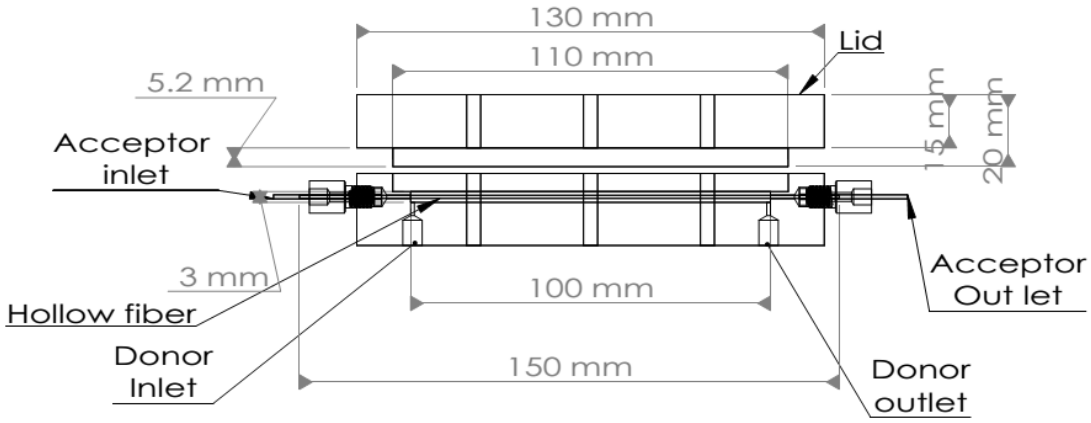

Figure A.2 The improved design of Easy assemble hollow fiber membrane unit

Table A.1 The results of the experimental design matrix

Aliquat Concentration (M)		Donor pH		Flow rate(ml/min)		Absorbance
coded	Uncoded	coded	Uncoded	coded	Uncoded	
1	0.3	1	9	1	2.0	0.0262
1	0.3	1	9	1	2.0	0.023
1	0.3	-1	5	1	2.0	0.0323
1	0.3	-1	5	1	2.0	0.0324
1	0.3	1	9	-1	0.3	0.1219
1	0.3	1	9	-1	0.3	0.1162
1	0.3	-1	5	-1	0.3	0.1685
1	0.3	-1	5	-1	0.3	0.1532
-1	0.1	1	9	1	2.0	0.0326
-1	0.1	1	9	1	2.0	0.0318
-1	0.1	-1	5	1	2.0	0.1042
-1	0.1	-1	5	1	2.0	0.1069
-1	0.1	1	9	-1	0.3	0.0938
-1	0.1	1	9	-1	0.3	0.1037
-1	0.1	-1	5	-1	0.3	0.1838
-1	0.1	-1	5	-1	0.3	0.1546
0	0.2	0	7	0	1.2	0.0345
0	0.2	0	7	0	1.2	0.0561
0	0.2	0	7	0	1.2	0.0465
0	0.2	0	7	0	1.2	0.0439
0	0.2	0	7	0	1.2	0.0539
0	0.2	0	7	0	1.2	0.0609

VITA

Acting Sub, Lt. Sira Nitiyanontakit was born on February 7, 1984 in Bangkok, Thailand. He entered the Faculty of science, Chulalongkorn University in 2004. Then he graduated with Bachelor of Science degree in Chulalongkorn University in 2006. He then entered the Department of chemistry, Faculty of Science, Chulalongkorn University (2006) and become a member of Chromatography and Separation Research Unit under the supervision of Asssistant Professor Dr. Pakorn Varanusupakul. He received the scholarship from Thailand Research Fund through Royal Golden Jubilee Ph.D. Program in 2009. He spent time doing his short research at University of the Balearic Islands under the supervision of Professor Manuel Miró Lladó from November 1, 2011 to April 30, 2012 and April 14, 2013 to August 1, 2013. Finally, he finished his Doctoral degree in Analytical Chemistry in 2014.

Poster and proceeding

"Single strand hollow fiber membrane: An on-line sample preparation for the flow based colorimetric determination of element in fruit juices" Sira Nitiyanontakit, Pakorn Varanusupakul. Poster presentation 16 th International Conference on Flow Injection Analysis Including Related Techniques, 25 - 30 April 2010, Pattaya grand sea view.

Publications

1. Nitiyanontakit, S., Varanusupakul, P., and Ngamukot, P., "Single strand hollow fiber membrane (SSHFM): An on-line sample preparation for the flow based colorimetric determination of free iron in fruit juices" Talanta. 84 5 (2011): 1304-1308.

2. Nitiyanontakit, S., Varanusupakul, P., and Miró, M., "Hybrid flow analyzer for automatic hollow-fiber-assisted ionic liquid-based liquid-phase microextraction with in-line membrane regeneration" Analytical and Bioanalytical Chemistry. 405 10 (2013): 3279-3288.