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DEVELOPMENT OF FLOW-BASED ANALYSIS SYSTEM FOR DETERMINATION OF SELENIUM SULFIDE IN COSMECEUTICAL PRODUCTS

Miss Suprawee Wongsuchoto

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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Thesis Title	DEVELOPMENT OF FLOW-BASED ANALYSIS SYSTEM FOR DETERMINATION OF SELENIUM SULFIDE IN COSMECEUTICAL PRODUCTS
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สุประวีณ์ วงศ์สุโชโต : การพัฒนาระบบวิเคราะห์แบบไหลสำหรับการตรวจวัดซีลีเนียมซัลไฟด์ในผลิตภัณฑ์เวชสำอาง (DEVELOPMENT OF FLOW-BASED ANALYSIS SYSTEM FOR DETERMINATION OF SELENIUM SULFIDE IN COSMECEUTICAL PRODUCTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.ปกรณ์ วรานุศุภากุล, 69 หน้า.

งานวิจัยนี้ได้พัฒนาระบบการสกัดแบบไหลสำหรับตรวจวัดปริมาณซีลีเนียมซัลไฟด์ในผลิตภัณฑ์เวชสำอาง โดยอาศัยหลักการไอโอโดเมตรี ซึ่งซีลีเนียม(IV)จะถูกรีดิวซ์เป็นธาตุซีลีเนียม (0) ด้วยไอโอไดด์เพื่อสร้างเป็นไอโอดีน ต่อมาไอโอดีนจะถูกสกัดอย่างต่อเนื่องไปยังสารละลายอินทรีย์ และถูกตรวจวัดด้วยเครื่องตรวจวัดเส้นใยแก้วนำแสงสเปกโทรโฟโตมิเตอร์ที่ความยาวคลื่น 521 นาโนเมตร ศึกษารูปแบบต่างๆของหน่วยการสกัดโดยใช้พอลิโพรพิลีนเมมเบรนเส้นใยกลวงในการแยกเฟส ซึ่งรูปแบบหน่วยสกัดแบบท่อในรูปแบบตั้งจะให้ศักยภาพที่ดีที่สุด สำหรับตัวทำละลายที่ใช้สกัดคือเฮกเซนซึ่งสามารถใช้แทนคลอโรฟอร์ม โดยสารละลายตัวอย่างจะถูกผสมกับไอโอไดด์ที่มากเกินพอก่อนจะเติมเข้าไปในท่อแก้วของหน่วยการสกัด ขณะที่เฮกเซนจะถูกปั๊มเข้าไปในเมมเบรน ทำการศึกษาและหาภาวะที่เหมาะสมของความเข้มข้นของไอโอไดด์, เวลาในการสกัด, ความยาวเมมเบรน, การล้างระบบ, การใช้เมมเบรนซ้ำและผลของเมทริกซ์ของสารตัวอย่าง ขบวนการทั้งหมดยกเว้นการล้างจะถูกควบคุมโดยเครื่องควบคุมขนาดเล็กด้วยคอมพิวเตอร์ พบว่าช่วงการใช้งานของซีลีเนียมที่ได้รับ คือ 80 ถึง 373 มิลลิกรัมต่อลิตร เมื่อสกัดเป็นเวลา 60 วินาที และให้จำนวนตัวอย่างต่อชั่วโมงคือ 20 ตัวอย่างต่อชั่วโมง (รวมการล้าง) สำหรับการตรวจวัดซีลีเนียมในรูปของซีลีเนียมไดออกไซด์และซีลีเนียมซัลไฟด์ พบว่าให้ค่าการคืนกลับอยู่ในช่วง 97 ถึง 106 เปอร์เซ็นต์ ที่มีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์อยู่ในช่วง 1 ถึง 4 เปอร์เซ็นต์และ 95 ถึง 105 เปอร์เซ็นต์ ซึ่งมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์อยู่ในช่วง 1 ถึง 3 เปอร์เซ็นต์ ตามลำดับ และได้นำวิธีนี้ไปประยุกต์สำหรับหาปริมาณซีลีเนียมในตัวอย่างแชมพูขจัดรังแคและตัวอย่างเวชสำอางที่ได้รับจากบริษัท แพน ราชเทวี กรุ๊ป จำกัด และพบว่าผลที่ได้รับจากวิธีที่พัฒนาจะมีค่าใกล้เคียงกับฉลากที่ระบุไว้บนผลิตภัณฑ์ซึ่งมีค่าความคลาดเคลื่อนน้อยกว่า 2 เปอร์เซ็นต์ โดยเฉพาะอย่างยิ่งความคลาดเคลื่อนที่ได้รับยังมีค่าน้อยกว่าวิธีมาตรฐานไทเทรตอีกด้วย

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SUPRAWEE WONGSUCHOTO : DEVELOPMENT OF FLOW-BASED ANALYSIS SYSTEM FOR DETERMINATION OF SELENIUM SULFIDE IN COSMECEUTICAL PRODUCTS.
ADVISOR : ASST.PROF.PAKORN VARANUSUPAKUL, Ph.D., 69 pp.

Flow-based extraction system was developed for determination of selenium sulfide in cosmeceutical products. The method was based on iodometry, where selenium(IV) was reduced to selenium(0) by iodide ion producing iodine that was then on-line extracted into the organic solvent and detected by the fiber optic spectrophotometer at wavelength 521 nm. Several extraction units using polypropylene hollow fiber membrane as a phase separator were investigated. The tubular extraction unit with vertical setup provided the best performance. The extracting solvent was hexane that could be used in place of chloroform. The sample solution was first mixed with excess iodide and filled in the glass tube of the extraction unit while the hexane was pumped inside the membrane. The iodide concentration, extraction time, the length of the membrane, the cleaning system, reuse of membrane and matrix effect of sample were studied and optimized. All processes except washing were controlled by a microcontroller on a computer. The working range of 80 to 373 mg L^{-1} selenium was obtained with 60 sec extraction time providing sample throughput of 20 samples hr^{-1} (included washing). The recovery for determination of selenium in the forms of selenium dioxide and selenium sulfide were 97-106% with 1-4%RSD and 95-105% with 1-3%RSD, respectively. This method was applied for determination of selenium sulfide in anti-dandruff shampoo and cosmeceutical samples obtained from Pan Rajthevee Group Public Company Limited. The results obtained from the developed method were similar to those labeled on the products with the relative error less than 2%; especially, it showed less error than the standard titration method.

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Academic Year 2010

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LIST OF ABBREVIATIONS AND SYMBOLS

AU	Absorbance Unit
AW	Atomic Weight
cm	centimeter
g	gram
hr	hour
ID	Inner Diameter
L	Liter
mg	milligram
mg L^{-1}	milligram per liter
min	minute
mL	milliliter
mL min^{-1}	milliliter per minute
mm	millimeter
MW	Molecular Weight
nm	nanometer
OD	Outer Diameter
R^2	correlation coefficient
RSD	Relative Standard Deviation
sec	second
µm	micrometer
w/v	weight per volume

CHAPTER I

INTRODUCTION

1.1 Motivation of Proposer

Selenium is an essential element, which is important to living organisms. However, the excess level of selenium can cause toxicity in the body [1-2]. Selenium can be formed with oxygen, sulfur, metals and halogens as selenium compounds. Moreover, selenium compounds have been used in various industrials such as glass, rubber and electronic equipment etc. The most widely used selenium compounds are selenium dioxide (SeO_2) as a catalyst in reaction of organic compounds, selenium oxychloride ($SeClO_2$) as a solvent and selenium sulfide (SeS_2) as an anti-dandruff agent in shampoos [2].

Selenium disulfide is sometimes called selenium sulfide, which is bright orange colored powder. Selenium disulfide is listed in both the United States Dispensatory and the Compendium of Pharmaceuticals and Specialties (Canada) as a treatment for seborrheic dermatitis, common dandruff and tinea versicolor (a type of fungus infection of the skin), which caused by Malassezia genus fungi [2]. Selenium sulfide inhibits the growth of yeast and fungi, reduces the rate of cell turnover on the scalp, helps regulate the excessive peeling on epidermal and hair follicles cells, and inhibits the development of dermatophytes, which causes mycoses of epidermis, hair and nails [3-6].

Cosmeceutical products are the combinations of cosmetics and drugs, which not only focus on the beauty but also emphasize on the effectiveness of the drug in the treatment. In general, products containing selenium sulfide such as shampoo, cream and lotion must be controlled for appropriate quality [2-4, 7]. The quantity of selenium sulfide in cosmeceutical products recommended by the United States, are 1% strength that is available over the counter, and 2.5% strength that is available with doctor's prescription for treatment of tinea versicolor [2, 8]. Thus, determination of selenium sulfide in cosmeceutical products is important because it can indicate the quality of cosmeceutical products for treating skin diseases.

Sample preparation is one important step in the analytical processes, which lead to reliable results. It depends on the sample, the matrix and the concentration level at which the analysis needs to be carried out. Generally, sample preparation for quantitative determination of selenium in medicine, shampoo, food and soil has been prepared by acid digestion with various acids, such as nitric acid, perchloric acid or aqua regia. Moreover, there are many methods for the digestive process, such as Kjeldahl [9], microwave [10-11] and hot plate [7, 12-13]. The simple and inexpensive equipment easily found in the laboratory is hot plate.

Selenium can be determined by several methods such as hydride generation – atomic absorption spectrometry (HG-AAS) [11, 14], inductively coupled plasma - optical emission spectrometry(ICP-OES) [15] and inductively coupled plasma - mass spectrometry (ICP-MS) [16] etc. These methods require advanced analytical instrument, which are expensive and need skill, but they provide good sensitivity that is suitable for measuring selenium at trace level. Therefore, these methods may not be necessary for such a high selenium level content in cosmeceutical products.

One alternative method that is simple and cheap is iodometric titration. The analyte in oxidizing form quantitatively reacts with the excess iodide (I^-) generating iodine (I_2) that can then be titrated with thiosulfate using starch solution as indicator [7]. However, this method is time-consuming, labor-intensive and subjective because it is hard to tell the color changes at the end point (brown color became orange color). In addition to the titration method, the iodine can be extracted with organic solvent yielding purple color solution, which is detected and quantified by the visible spectrophotometric method [13]. The procedure is highly selective for iodine and no interferences. Although the problem regarding the subjectiveness has been eliminated, it is still time-consuming and labor-intensive that it would not be suitable for routine analysis. Therefore, the flow-based technique is interesting to apply for reducing labor in the analysis. It is based on the injection of sample solution into a carrier solution that later merges with a reagent and moves towards a detector. It can also be either automated or semi-automated, which is suitable for routine analysis.

Incorporation of extraction with flow-based analysis must have an extraction part and a phase separator, which must be designed and suitably chosen. There are several types of phase separators such as T-type separator, gravitational-based separator and membrane separator. The membrane separator has become popular among phase separators because membrane can do both extraction and phase

separation [17]. There was an application of using hollow fiber membrane as a phase separator for determination of copper in water by continuous liquid-liquid extraction in flow system [18]. In this work, the hollow fiber membrane has been used in extraction and as a phase separator. The extraction involves two phases that are aqueous sample solution containing analyte and organic extracting phase impregnated and located inside the hollow fiber membrane, called microporous membrane liquid-liquid extraction (MMLLE) [19-24]. Because of its hydrophobicity and porosity, it can be used as a phase separator.

In this research, an on-line liquid liquid extraction system using hollow fiber membrane as a separator coupled with a flow based iodometric method has been developed for determination of selenium sulfide in cosmeceutical products.

1.2 Objective

To develop a flow-based method with an on-line liquid liquid extraction system using hollow fiber membrane as a separator for determination of selenium sulfide in cosmeceutical products

1.3 Scopes of this research

The method for determination of selenium is developed in flow based on iodometric method with the iodine extraction instead of titration. Polypropylene hollow fiber membrane is used for on-line extraction of iodine as well as a phase separator in the flow based system. A spectrophotometric method is used for detection and determination of iodine extract. Parameters that may affect extraction efficiency or sensitivity are studied and optimized. The method is applied for determination of selenium in cosmeceutical samples after acidic digestion. The results between our developed method and the titration method (standard method) are compared.

1.4 The benefit of this research

A new method for determination of selenium is obtained, that is simple, convenient and reliable providing high sample throughput, which is suitable for routine analysis and quality control laboratory of cosmeceutical products.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Iodometric method

The method that iodine is used as a major substance for quantitative analysis has two categories which are iodometric and iodimetric methods [25]. Iodimetric method is the method for the determination of the quantity of iodine, which is stoichiometrically used in a reaction. Meanwhile, iodometric method is a quantification method that is applied for determination of amount of iodine formed from a reaction between analyte in sample and excess iodide. The amount of the obtained iodine is stoichiometrically related to the analyte in the sample. However, IUPAC (International Union of Pure and Applied Chemistry) system currently defines both methods to be similarly called “the iodimetric method”.

The concentration of iodine can be determined by titration with sodium thiosulfate solution in neutral or diluted acidic condition with starch solution as an indicator. The thiosulfate solution is first standardized by titration with iodine generated from the oxidation reaction of primary standard of acidic potassium dichromate (Eq.1) or acidic potassium iodate (Eq.2) with an excess potassium iodide.

$$Cr_2O_7^{2-}{}_{(aq)} + 6I^-_{(aq)} + 14H^+_{(aq)} \rightleftharpoons 2Cr^{3+}_{(aq)} + 3I_{2(aq)} + 7H_2O_{(l)} \quad (1)$$

$$IO_3^-{}_{(aq)} + 5I^-_{(aq)} + 6H_3O^+_{(aq)} \rightleftharpoons 3I_{2(aq)} + 9H_2O_{(l)} \quad (2)$$

The quantity of iodine directly influences the quantity of the consumed primary standard solution. There may be some systematic errors during iodometric titration, where iodide in acidic solution can be oxidized by oxygen in air to slowly generate iodine (Eq.3) or iodine can react with iodide solution to form triiodide ion (I_3^-) (Eq.4). Therefore, titration should be quickly done for decreasing of the oxidation of iodide.

$$4I^-_{(aq)} + O_{2(aq)} + 4H_3O^+_{(aq)} \rightleftharpoons 2I_{2(aq)} + 3H_2O_{(l)} \quad (3)$$

$$I^-_{(aq)} + I_{2(aq)} \rightleftharpoons I_3^-{}_{(aq)} \quad (4)$$

As mentioned above, iodometric method is based on the reaction between the analyte and a slight excess of iodide ion to produce iodine, which is determined by titration with thiosulfate solution. The amount of generated iodine is stoichiometrically related to the amount of analyte originally present in the solution. In case of determination of selenium, selenium(IV) is reduced with excess iodide ion to form selenium(0) and produce iodine (Eq.5) that can be determined by titration with thiosulfate (Eq.6).

$$SeO_3^{2-}{}_{(aq)} + 4I^-_{(aq)} + 6H^+_{(aq)} \rightleftharpoons Se_{(s)} + 2I_{2(aq)} + 3H_2O_{(l)} \quad (5)$$

$$I_{2(aq)} + 2S_2O_3^{2-}{}_{(aq)} \rightleftharpoons 2I^-_{(aq)} + S_4O_6^{2-}{}_{(aq)} \quad (6)$$

In addition, the quantity of iodine can be determined by another method such as extraction with organic solvent and determination the colored extract with spectrophotometric detection.

2.2 Liquid-liquid extraction

Liquid-liquid extraction (LLE) [26-27], sometimes called solvent extraction, is the separation of the constituents of liquid solution (sample) by contact with another insoluble liquid. It usually based on two different immiscible liquids, water and an organic solvent. Generally, they are mixed using a separatory funnel and after a certain time, partitioning of the analyte between two phases reaches equilibrium. This traditional method is mostly widespread and is a conventional technique of extraction. Although it is simple, easy to use, and employing inexpensive equipment, it has many disadvantages such as tediousness, laborious procedure, consumption of a large quantity of organic solvent, which is often highly toxic to human, and environment, difficulties to separate when emulsion is formed, and uncomfortableness to handling.

2.3 Membrane extraction

Membrane extraction was introduced in 1999 by Jönsson et al [28]. A membrane is applied as a selective barrier between two phases; one is called the donor phase, the other is called the acceptor phase. The membrane facilitates the two phases coming into contact with each other without direct mixing; moreover, it can also help eliminating problems such as emulsion formation and high solvent usage. The membrane functions are a separator of two phases and control the mass transfer between them. The factors affecting mass transfer across the membrane are the types of membrane extraction and the driving force of the extraction process. Therefore, it needs to choose a suitable type of membrane extraction. The species of the analyte of interest move through it by diffusion and are driven by a concentration (ΔC), a pressure (ΔP) or an electrical potential (ΔE) gradient which depends on each process [20, 24]. The process of separation is shown schematically in Figure 2.1.

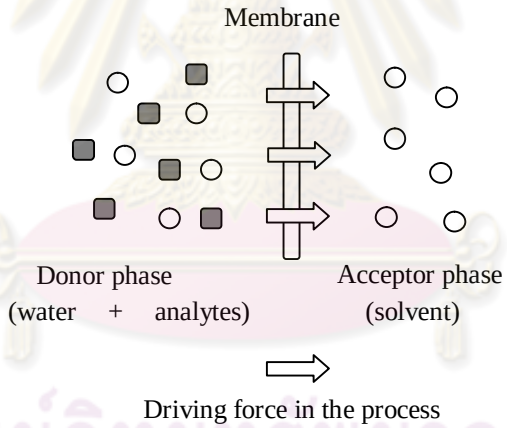

Figure 2.1 Schematic representation of the transport through membranes (adapted from [24])

The membrane is a synthetic product of different chemical natures exhibiting different properties. Generally, membrane characterization is based on its porosity, which can be porous and non-porous membrane [20-21, 24, 28]. In porous membrane, analyte is partitioned from one phase to second phase by moving through the porous membrane. The separation is based on size-exclusion; therefore, only particles smaller than the pore size can pass through the membrane, which leads to clean up matrix from sample. Hence, the size, shape and distribution of pores in the membrane and

size of analyte molecules play an important role in mode of this separation. Porous membranes are used in dialysis, microfiltration and reverse osmosis process.

On the other hand, non-porous membranes have been widely used for extraction. Non-porous membranes do not have pores in their structures. The operation is based on the differences in solubility and diffusion coefficient of individual analyte in the membrane material. Non-porous membranes act as interface between two liquid solutions, which can be a liquid or a solid phase. A liquid-impregnating porous membrane may be used either two-phase extraction called microporous membrane liquid-liquid extraction (MMLLE) or three-phase extraction called supported liquid membrane (SLM). In addition, absolute solid membranes are also available, which are made of monolithic material and silicone rubber.

2.3.1 Membrane-based LLE

The microporous membrane liquid-liquid extraction (MMLLE) is a two-phase system. The acceptor phase is an organic phase immobilized in hydrophobic membrane pores. The donor phase may be water or sample solution containing the analyte of interest [19-20, 22-24]. In MMLLE, almost the same extraction principle as LLE can be applied. The analyte is extracted from an aqueous solution into an organic solvent, which is illustrated in Figure 2.2.

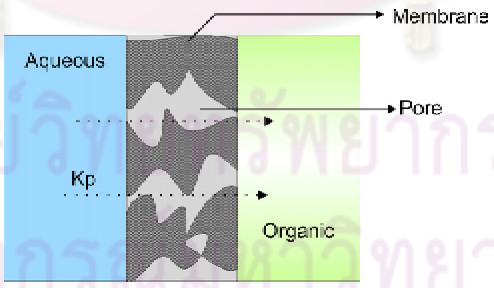

Figure 2.2 Schematic representation of MMLLE when analyte diffuses across a microporous membrane and partition coefficient (*K*) of the analyte of two phases [20].

In this technique, analyte to be efficiently extracted should have low solubility in the aqueous phase. The moving of analyte molecules from aqueous to organic phase is driven by the concentration gradient of the analyte and is limited by

its partition coefficient. MMLLE is typically used for neutral and/or more hydrophobic organic compounds.

The partition coefficient (*K*) controls the diffusion of analyte molecules across the membrane. The partition coefficient is defined as the ratio of the concentration of analyte in the membrane to the concentration of analyte in the matrix, which is shown in (Eq.7).

$$K = \frac{C_o}{C_w} \qquad (7)$$

Where C_o and C_w represent the analyte concentrations in the organic and aqueous phases, respectively at the equilibrium stage.

Extraction efficiency can be considered from percentage of extraction, which may be called percentage of recovery. Extraction percentage may be defined as the ratio of the analyte concentration in the acceptor phase after extraction, C_a to the analyte concentration in the initial donor (the sample solution), C_d, which is shown in (Eq.8).

$$\% \text{ Extraction} = (C_a/C_d)*100 \qquad (8)$$

2.3.2 Mode of extraction

Mode of extraction can be classified into two modes: a static mode and a dynamic mode. In the static extraction, the analyte is only exposed to one batch of acceptor phase, and the maximum amount of extracted analyte will be limited by its distribution constant between the two phases. In dynamic extraction, it is possible to pass a continuous stream of fresh extracting phase through the system, so increasing the amount of extracted analyte. Although acceptor phase is fresh all the time for the dynamic mode, the static mode is easier.

2.3.3 Kinds of membranes

Membranes may be classified into several groups depending on a mode of classification [20-21, 24], which are listed in Figure 2.3.

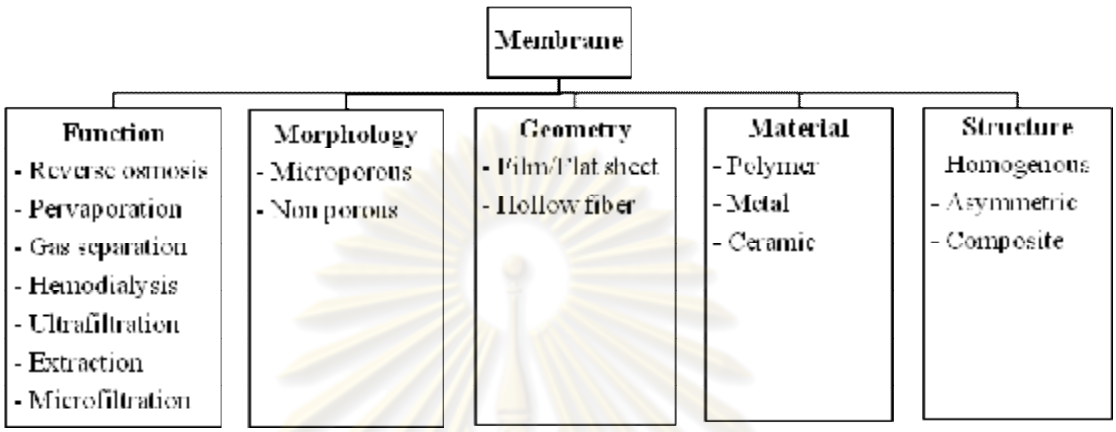

Figure 2.3 Membranes classifications (adapted from [20]).

Membranes can be used in several separation functions, which depend on the samples (e.g. aqueous, non-aqueous, air, etc.,) and the properties of the analyte. Morphology of membranes refers to the quantity, size and distribution of pores in the membrane. They can be divided into two types: porous membrane and non-porous. Geometrically membranes may be classified into film/flat sheet or hollow fiber. Flat sheet membrane is flat as a sheet of paper, which needs the holder to keep it in place. Typically it is thin sheet, which is less than 1 μm thickness shown in Figure 2.4. Hollow fiber or tubular membrane is tube like structure as illustrated in Figure 2.5. Donor and acceptor solutions can be flowed through both inside and outside of the membrane depending on the design of the extraction system. Generally, the hollow fiber membrane provides much higher surface area per unit volume than the flat one.

Figure 2.4 Flat sheet membranes [29].

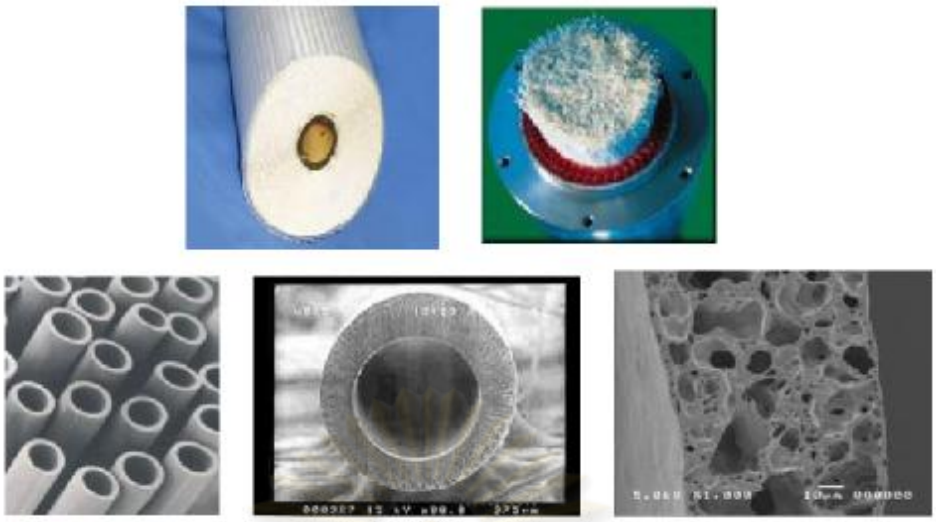

Figure 2.5 Hollow fiber membranes [30]**.**

In addition, membranes can be made from several materials such as polymer, metal and ceramic. Typical polymer materials used in fabricating hollow fiber membranes are polypropylene (PP), polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), silicone, and polysulfone (PS). These materials are stable at all pH ranges, inert to many other chemicals and resistant to high temperature [20, 24]. However, it must be selected upon usage. Moreover, membranes can be classified into three types: homogenous, asymmetric, and composite based on the structures. Structure refers to the uniformity, degree of pores and the membrane material. Homogenous membranes are uniform throughout having variable pore sizes, where the size cited is normally an average. They are usually used for extraction, reverse osmosis and pervaporation. Homogenous membranes, shown in Figure 2.6, consist of microporous and non-porous dense membranes.

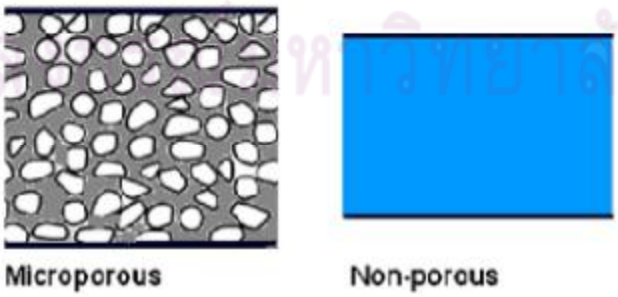

Figure 2.6 Homogenous membranes [20].

2.4 On-line liquid liquid extraction flow analysis system

On-line liquid liquid extraction is the introduction of the extraction combined with flow system [17, 31-33]. Flow injection analysis (FIA or FI) was firstly presented in 1975 by Ruzicka and Hansen [34]. This method is based on the injection of sample into a carrier solution and later merged with the reagent solution. The reaction developed along reaction coil (R) is transported towards a detector that continuously records the absorbance, electrode potential or the other physical parameters as it continuously changes due to a passage of the sample material through the flow cell. The signal output is a peak recorded as a function of time. The height (H), width (W), or area (A) is proportional to the concentration of the analyte present in the samples. The time spent between the sample injection (S) and the highest of peak is the residence time (T) (the time that chemical reaction takes place). The basic components of a flow system can be demonstrated in Figure 2.7.

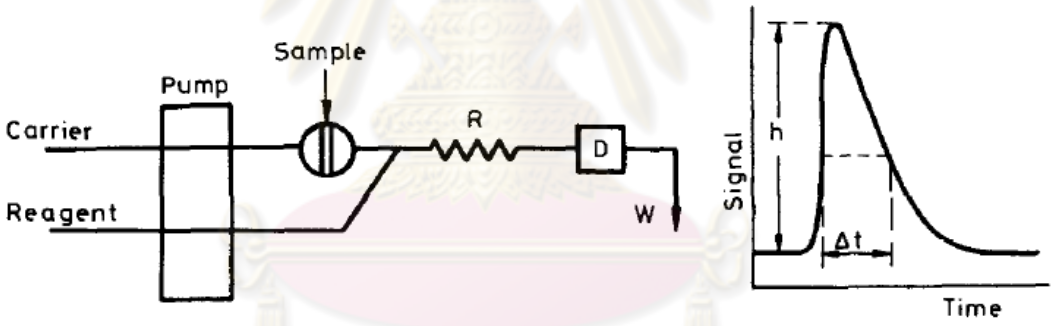

Figure 2.7 Schematic diagram of a flow injection system**.** R, reaction coil; D, detector; W, waste [35].

Typical flow injection system consists of a pump, an injection valve, a reaction coil and a detector. The carrier solution and the sample are introduced into the system with the pump and the injection valve, respectively. Some reaction coil and connector may be necessary for mixing reagent carrier and the injected sample zone. The detector is used for data monitoring. In addition, mode of FI has two modes: continuous and stopped-flow. The advantage of the stopped-flow mode is that the residue time is increased resulting to higher sensitivity of measurement [34].

The introduction of flow based liquid–liquid extraction was purposed by Karlberge and Thelander [32]. This on-line extraction needs the extraction section and the phase separation section within the system. In general, on-line LLE consists of three essential parts: a segmentor, an extraction coil and a phase separator [17, 33]. A typical on-line liquid-liquid extraction flow analysis system is depicted in Figure 2.8. The operation of each is described:

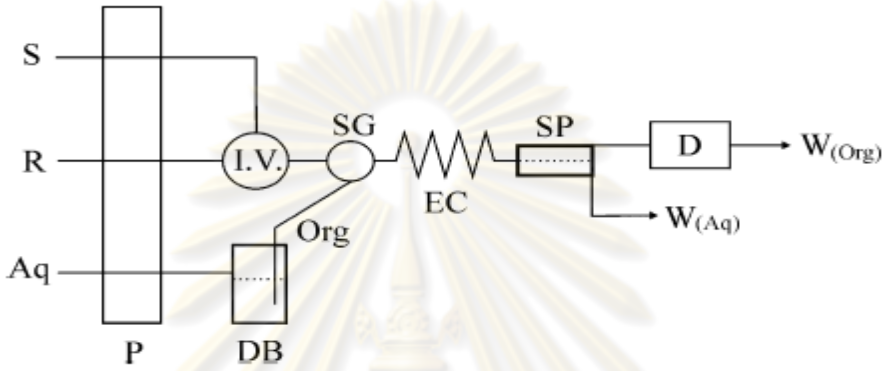

Figure 2.8 Schemes of liquid-liquid extraction flow analysis system manifold. S, sample; R, reagent; P, peristaltic pumps; I.V., injection valve; Aq, aqueous phase; Org, organic phase; DB, displacement bottle; SG, segmentor; EC, extraction coil; SP, phase separator; D, detector; W, waste [32].

1. The segmentor is the unit providing alternate and regular segments of the two immiscible phases converging on a single channel/ a minichamber or confluent point. The segmentor has several configurations based on three different designs (Y, T and W). These types are used to implement the segmentation-mixing process. They are made of homogenous materials e.g., glass, fluoroplastic, stainless steel and Teflon. The aqueous and organic phase stream can merge frontally or laterally at different angles, while the segmented phase can leave from the central or one of the side openings. Choosing the type of segmentor depends on the density ratio between two phases.

2. The extraction coil is the unit that the extraction takes place by transferring of analyte from one phase to the other. However, some extent extraction can also be done in the segmentor or phase separator. Efficient extraction depends on the sample residence time within the extraction coil, which is affected from length and inner diameter of the extraction tube and the flow rate. The efficiency of extraction is

increased with decreasing the inner diameter of the tube. The length of the tube should be sufficiently long to prevent transfer kinetics being the limiting factor, which is usually a helically coiled tube. The material of coiled tube is commonly constructed by either glass or Teflon.

3. The phase separator is the important component of on-line extraction systems. The phase separation process involves a partition of the segmented phase and transfer of analyte containing organic phase to the detector. The phase separators can be classified into three broad categories according to their operational principle, which also dictates their internal shape [17, 33].

(1) Gravity-based separators

This is the simplest type of separator, which formally resembles a separatory funnel but it is miniature. The segmented flow from the top or through one of its side is received with a minichamber and a separation is established from density differences. The various models of phase separator depend on gravity shown in Figure 2.9, where the last two forms are used with liquid-liquid extractors incorporated into FIA manifold. The flow of the heavier phase leaves the minichamber from the bottom, while the lighter phase emerges from the top or one side. However, this type has two disadvantages that are the poorer reproducibility and the larger volume resulted in outdatedness.

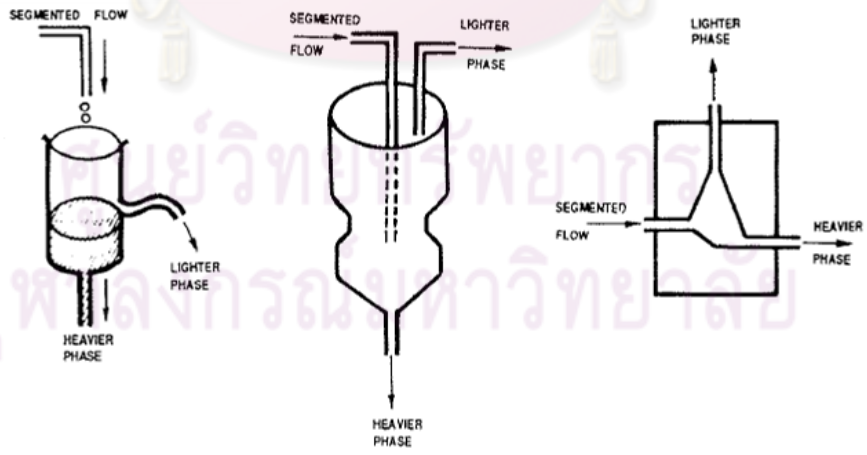

Figure 2.9 Scheme of different types of gravity-based separation [17].

(2) T-type separators

This separator type is constructed from Technicon connectors. The efficiency can be increased by inserting a Teflon piece or a hydrophobic paper into the tube end, through which organic solvent emerges. The segmented flow enters through one side of the separator and the phases are continuously separated at the confluent point. There are many models for T-type separators shown in Figure 2.10, which first design (Figure 2.10A) is general T-type while the last two designs have been used in the incorporation of a LLE system into an automatic continuous segmented-flow. Phase separator should remove air that may be formed in the segmented flow and introduce the solution into the outgoing organic solvent. They are applied according to the relative density of the two phases. Figure 2.10B is used when organic solvent is lighter than aqueous phase, while the Figure 2.10C is used in the opposite case.

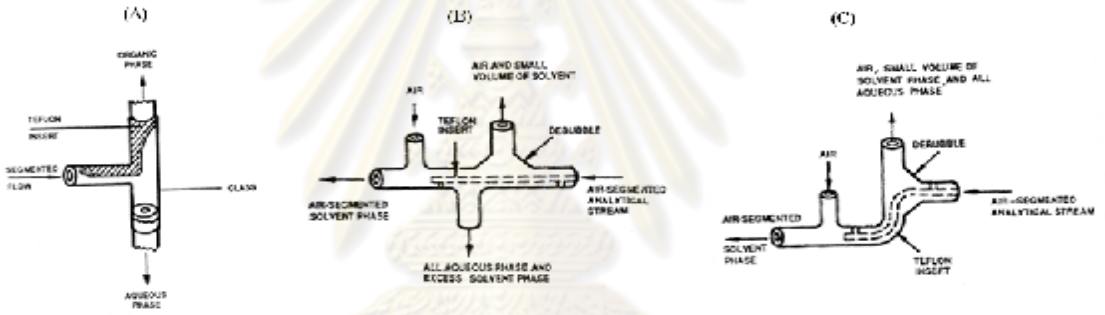

Figure 2.10 Scheme of T-type phase separator [17].

(3) Membrane separators

In general, this type of segmented liquid-liquid flow separator is similar to most gas-diffusion and dialysis units as it is based on the permeability of membrane that is wetted by one phase. Sandwich type phase separator consists of two parallelepiped, cylindrical, or round blocks of Teflon grooved with holes that allow entry and exit of the organic and aqueous phase flows. The membrane is inserted between the two blocks, which are tightly squeezed together by screws in order to avoid leakage (Figure 2.11). Normally, this type uses a hydrophobic membrane, which is compatible to the non-polar organic solvent. The membrane separator is easy to use and inexpensive so that it is much more frequently used at present.

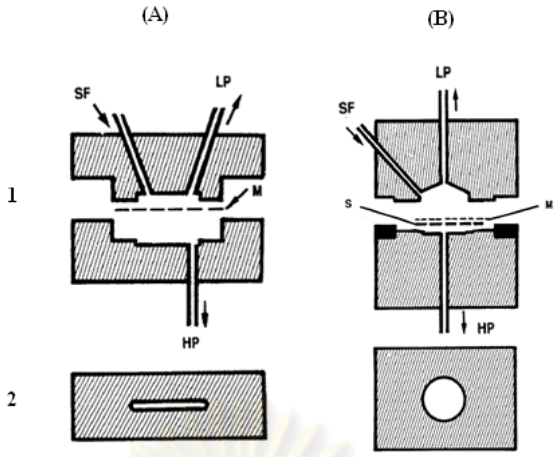

Figure 2.11 Scheme of sandwich type membrane phase separators. (A) grooved chamber and (B) cylindrical chamber by (1) side view and (2) top view: LP, lighter phase; HP, heavier phase; M, membrane ; S, membrane support [17].

2.5 Factors affecting to the extraction efficiency

Efficiency of extraction is defined as the percentage of extraction as follows:

$$\% \text{ Extraction} = (C_a/C_d)*100 \qquad (9)$$

Where C_a is the concentration of analyte in the acceptor phase after extraction and C_d is the initial concentration of analyte in the sample or donor solution. Several factors influencing the extraction efficiency must be optimized.

2.5.1 Selection of membrane properties

It is necessary to select the appropriate membrane properties in order to get good extraction efficiency. Selection of membrane is based on the properties of the analyte (polar or non-polar); otherwise, the analyte cannot be properly extracted.

2.5.2 Selection of extracting solvent

The selection of extracting solvent is of major importance in liquid membrane extraction in order to obtain an efficient extraction. Choosing a suitable organic solvent is based on like-dissolved-like in order to reduce the risk of losing analyte.

2.5.3 Extraction time

Mass transfer efficiency is increased with extraction time due to the rise in contact time between donor solution and acceptor solution. However, it must be compromised between time providing good sensitivity and sample throughput (number of samples hr^{-1}).

2.5.4 Flow rate

Generally, mass transfer is increased with slow flow rate of solution owing to the rise in contact time between the membrane surface and the analyte. However, the lower flow rate may affect the analytical precision because analyte may be extracted back and solvent may leak out.

2.6 Literature review

Sample preparation is the first procedure that leads to the correct result. Selenium sulfide cannot dissolve in the water, so it is usually prepared by acid digestion using various acids such as nitric acid, perchloric acid, mixed between sulphuric and hydrochloric acid or aqua regia. Furthermore, there are many methods for digestive process such as Kjeldahl [9], microwave-assisted wet digestion [10-11] and hot plate [7, 12-13]. The simple method for digestion is hot plate, which is easy to use, inexpensive and the equipment is commonly available in the laboratory.

The selection of the method for determining the analyte of interest is necessary. The method should provide accuracy, precision and reliable results; besides, time, complexity of the method, cost and concentration range should also be

considered. Several methods for determination of selenium have been used. One typical method was based on catalytic kinetic spectrophotometry, where the selenium ion was the catalyst and the color of complex solution was measured with a spectrophotometer. These methods were based on the catalytic effect of selenium on the reduction of nile blue [36], azure A [12], mixilon blue-SG [37], toluidene blue [38] or sulfonazo [39] with sulfide ion. These methods have been applied for determination of selenium in water, Kjeldahl tablets and health care products. Although these methods were simple giving good accuracy and sensitivity, they were complicated because the working temperature must be controlled.

Another method was the hydride generation method, which could be measured with various detectors, such as atomic absorption spectrometer (AAS) [11, 14, 40], inductively coupled plasma - optical emission spectrometer (ICP-OES) [15] and inductively coupled plasma - mass spectrometer (ICP-MS) [16] etc. These methods provide good sensitivity that is suitable for measuring at trace level of selenium but they require advanced analytical instruments that are expensive and need skills. However, such sensitive methods may not be necessarily applied for high level of selenium content samples such as cosmeceutical products.

Furthermore, iodometric method is an alternative method for determination of selenium. Typically, selenium(IV) is first reduced to selenium(0) with iodide to generate iodine, and determined instead of selenium(IV), where the amount of iodine quantitatively refers to the amount of selenium(IV) initially being present in the sample. In iodometric titration, the selenium solution reacts with excess iodide generating iodine that is immediately titrated with sodium thiosulfate [7]. The titration is labor-intensive and subjective that may be not suitable for routine analysis.

Another method based on iodometry is that the generated iodine is obtained for measurement by liquid-liquid extraction. Somer and Ekmekci [13] introduced the extraction method for the determination of selenium in anodic slime. Selenium was reduced with iodide ion to generate iodine that was extracted with chloroform. The extracts were collected and measured by a spectrophotometer. It was found that the concentration range was 10^{-2} – 5×10^{-6} mol L^{-1} and the relative error was 1-4 %. Although the procedure is highly selective for iodine, no interferences and uses relatively inexpensive reagents, this method is usually a batch method that is time-consuming and labor-intensive so that it would not be applicable for routine analysis.

According to the disadvantages of extraction procedure mentioned above, the flow analysis system may be able to help resolving this weak point because it can be automated, provides relatively high sample throughput and may be suitable for routine analysis. Flow injection systems have been applied for the determination of selenium. One was based on the catalytic reduction of thionine with sulfide ion in the presence of selenium [9]. Thionine was fed and mixed with sulfide in the tubing and merged with the selenium solution in another tube. After that the solution was pushed into the UV-Vis spectrophotometer. All processes might be temperature controlled. The method has been applied for the determination of selenium in anti-dandruff shampoo samples. This method was rapid giving high sensitivity. The sample throughput of 25 – 30 samples hr^{-1} was obtained. Another was based on the reaction between 4-aminoantipyrine (4-AAP) and N-(naphthalene-1-yl)ethane-1,2-diamine dihydrochloride (NEDA) by oxidizing with selenium ion [41]. A 4-AAP was fed and merged with selenium ion in tubing and mixed with NEDA in another tube. After that the purple solution was carried to the UV-Vis spectrophotometer. All processes must also be temperature controlled. This method has been applied for determination of selenium in vitamin, mineral, natural water and soil. The method provided high precision, accuracy and sample throughput. According to these works, the flow-based analysis system could be used to solve problems involving time-consumption and labor-intensiveness.

Incorporation of the extraction technique into a flow injection system for determination of selenium must have the extraction section and the phase separator section. Several researches employ hollow fiber membrane in the extraction section because it was easy to use and inexpensive and could be used in various styles such as one phase, two phases or three phases. Microporous membrane liquid-liquid extraction (MMLLE) is a two-phase membrane extraction. A hydrophobic membrane made of polytetrafluoroethylene (PTFE) or polypropylene separates the sample solution and organic solvent, which inside the membrane is impregnated with organic solvent. Membrane extractions have been applied for both on-line and off-line mode. For on-line mode, the combination of membrane extraction with capillary gas chromatography for studying model compounds in blood plasma sample. The extraction unit consisted of two titanium blocks and a porous PTFE flat sheet membrane that was placed between the both blocks for blood plasma samples [42]. An aqueous sample (blood plasma) was fed to the donor side of the hydrophobic

microporous membrane while an organic solvent (hexane) was in the membrane pores as an acceptor solution. The analyte in the sample was extracted into the organic acceptor phase which was transferred directly to the injection loop in the gas chromatographic system. Another application was membrane extraction for determination of organotin compounds prior to analysis by GC-MS [43]. The extraction unit was similar to the previous one but the blocks were made of PTFE. For off-line mode, the hollow fiber membrane was impregnated with the organic phase (2-heptanone) and was placed into the aqueous sample for extraction and was shaken for 7 hrs. After the extraction, the solution the hollow fiber membrane was placed into a GC vial for further analysis [44]. The results from these methods were good which demonstrated that membrane could efficiently be used for extraction of interest analyte.

Phase separators are available in several types such as T-connector [45], glass gravitational phase separator [46] and membrane separator [17, 33]. Nevertheless, membrane separator has become the most popular phase separator. There was an application of using hollow fiber membrane as a phase separator for determination of copper in water by continuous liquid-liquid extraction in flow system [18]. After extraction process, the sample zone reached the phase separator unit where the colored complex contained in the organic phase passed through the membrane to reach the acceptor phase of pure dichloroethane (DCE). The process was operated in the stopped flow mode. The phase separator consisted of a 75-cm length of polytetrafluoroethylene hollow fiber membrane inserted into a helically coiled glass tube containing one inlet and one outlet for the flow of the DCE acceptor stream. It was found that this method was simple, rapid, yielding high accuracy and precision. Therefore, the membrane, especially hollow fiber membrane could excellently be used as a phase separator.

According to the reviewed literatures, both the extraction technique and the flow system have several advantages. From this reason, the flow analysis system along with the extraction is interesting technique. This research attempts to develop an automated analysis method for determination of selenium in cosmeceutical products by incorporation of on-line liquid-liquid extraction using the hollow fiber membrane extraction with flow-based analysis system.

CHAPTER III

EXPERIMENTAL

3.1 Instrument and equipment

1. Spectrophotometer model V-325-XS (Shanghai LW scientific, China)
2. Fiber optic UV-Visible spectrophotometer with micro flow Z-cell (path length 10 cm) (Aventes BV, the Netherlands)
3. Syringe pump (Prosense B.V, USA)
4. Peristaltic pump (Cole-parmer, USA)
5. Stabilizer (LEONICS)
6. Polypropylene hollow fiber membrane Accurel® PP Q3/2 with ID 600 µm, wall thickness 200 µm and pore size 0.2 µm (Membrana, Wuppertal, Germany)
7. Tubing with 0.8 mm (Tygon, precision tubing, Masterflex)
8. Tubing with ID × OD (mm): 1.0 × 1.58 (Teflon, Upchurch Scientific)
9. Glass syringes 10 mL (Magyar)
10. Hypodermic needles with OD × length (mm): 0.55 × 25 and 0.9 × 40 (NIPRO, Japan)
11. Autopipettes and tips 1000 µL and 10 mL (BRAND, Germany)
12. Hot plate (Sci Lution, Germany)
13. Stirrer (IKA, Germany)
14. Volumetric flasks 25, 50, 100,250, 500 and 1000 mL (class A, witeg, Germany)
15. Flasks 150 and 250 mL
16. Burette 50 mL (Witeg, Germany)

3.2 Chemicals and Reagents

1. Selenium dioxide (SeO_2) (FLUKA, USA)
2. Selenium sulfide (SeS_2) (Aldrich, USA)
3. Potassium iodide (KI) (QReC, New Zealand)
4. Sodium thiosulfate ($Na_2S_2O_3$) (Ajax Finechem, Australia)

5. Sodium carbonate (Na_2CO_3) (J.T. Baker, USA)
6. Sodium hydrogen carbonate ($NaHCO_3$) (CARLO ERBA, France)
7. Potassium dichromate ($K_2Cr_2O_7$) (CARLO ERBA, France)
8. Urea (Sigma-Aldrich, USA)
9. Hexane (MERCK, Germany)
10. Nitric acid 65% (MERCK, Germany)
11. Hydrochloric acid (MERCK, Germany)
12. Starch (purchased locally)
13. Cosmeceutical samples (bead samples) (obtained from PAN Rajdhevee Group Company)
14. Shampoo (Selsun, purchased pharmacy)

3.3 Experiment

3.3.1 Determination of selenium by iodometric titration method

3.3.1.1 Preparation of chemical solutions

3.3.1.1.1 Stock standard selenium dioxide solution

The stock 100 mg L^{-1} standard selenium dioxide solution was prepared by digesting 0.0141 g of selenium dioxide with 25 mL of the conc. nitric acid (65%). The solution was boiled about 50-90 min, transferred to a 100-mL volumetric flask and made to the volume with deionized water.

3.3.1.1.2 Stock selenium sulfide solution

The stock 100 mg L^{-1} selenium sulfide solution was prepared by digesting 0.0181 g of selenium sulfide with 25 mL of the conc. HNO_3 (65%). The solution was boiled about 50-90 min, transferred to a 100-mL volumetric flask and made to the volume with deionized water.

3.3.1.1.3 0.005 mol L^{-1} Sodium thiosulfate solution

The 0.005 mol L^{-1} sodium thiosulfate solution was prepared by dissolving 1.24 g sodium thiosulfate and 0.05 g of sodium carbonate in 1 L of cooled boiled deionized water. The exact concentration of sodium thiosulfate solution was determined by standardization with potassium dichromate as described in 3.3.1.2.

3.3.1.1.4 10% (w/v) Potassium iodide solution

The 10% potassium iodide solution was prepared daily by dissolving 25 g potassium iodide in 250 mL deionized water.

3.3.1.2 Standardization of sodium thiosulfate solution with potassium dichromate

A 0.01 g of potassium dichromate was dissolved with 30 mL of deionized water in a conical flask. A 1 g of sodium hydrogen carbonate was added. The solution was shaken until it was completely dissolved. Then, 5 mL of 6 mol L^{-1} hydrochloric acid and 5 mL of 10% potassium iodide solution was added, respectively. The solution was kept in the dark for 10 min and then immediately titrated with the standard thiosulfate solution. When the solution became a pale yellow color, a few drops of starch solution were added (the blue color appeared), and continued titrating until the blue color disappeared. The equation of reaction was described in (Eq.1) and (Eq.6) in chapter II, which the concentration of sodium thiosulfate solution was calculated as follows.

$$[S_2O_3^{2-}]\,\text{mol}\,L^{-1} = \frac{6\,\text{mole}\,S_2O_3^{2-}}{1\,\text{mole}\,K_2Cr_2O_7} \times \frac{\text{g of}\,K_2Cr_2O_7 \times 1000}{MW\,K_2Cr_2O_7 \times \text{mL of}\,S_2O_3^{2-}}$$

Where MW $K_2Cr_2O_7$ = 294.19 g mol^{-1}

3.3.1.3 Determination of selenium

A 10 mL of sample solution was pipetted into a conical flask. A 2 g of urea was added and boiled to eliminate excessive acid in the solution. After it was cooled, a few drops of starch solution and 5 mL of 10% potassium iodide solution were added, respectively. The solution was immediately titrated with the thiosulfate solution until a dark brown colored solution became a bright orange colored solution. The concentration of selenium was calculated as follows.

$$[Se^{4+}]\,mg\,L^{-1} = \frac{1\,mole\,Se^{4+}}{4\,mole\,S_2O_3^{2-}} \times \frac{[S_2O_3^{2-}]\,mol\,L^{-1} \times mL\ of\ S_2O_3^{2-}}{mL\ of\ Se^{4+}} \times AW\,Se \times \frac{1000\,mL}{1\,L}$$

Where AW Se = 79.1 g mol^{-1}

3.3.2 Determination of selenium by iodometric extraction method

3.3.2.1 Preparation of chemical solutions

3.3.2.1.1 Stock selenium solution

The 100 mg L^{-1} stock selenium solutions of both selenium dioxide solution and selenium sulfide were prepared as described in 3.3.1.1.1 and 3.3.1.1.2, respectively.

3.3.2.1.2 Working standard selenium dioxide solution

The working standard selenium solutions of 13, 25, 38, 50, 63, 75, 88 and 100 mg L^{-1} were prepared by pipetting 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mL of 100 mg L^{-1} stock selenium dioxide solution into each vial and made up the volume to 2 mL with deionized water.

3.3.2.1.3 Potassium iodide solution 0.10 mol L^{-1}

The 0.10 mol L^{-1} solution was prepared daily by dissolving 1.66 g of potassium iodide in 100 mL of deionized water.

3.3.2.2 Determination of selenium

A 1 mL sample was pipetted into 12-mL vial. A 1 mL of deionized water and 1 mL of 0.1 mol L^{-1} of iodide solution was added, respectively. A 3 mL of organic solvent was immediately added after adding iodide solution. The vial was capped, shaken, and kept in the dark for 4 min. An aliquot of organic solvent was taken for measurement by spectrophotometer at 511 nm for chloroform and 521 nm for hexane. The amount of selenium was determined by using linear regression method.

3.3.2.2.1 Calibration curve and linearity

The linear calibration curve between the absorbance and the concentrations of selenium was established for the concentrations ranging from 13 – 100 mg L^{-1}. The linear regression method was used to obtain slope, intercept and R^2.

3.3.2.3 Types of organic extracting solvents

In this work, hexane has been tested as an alternative solvent to chloroform because it is more environmental friendly than chloroform. Both chloroform and hexane were investigated for extraction efficiency. The chloroform extract was measured at 511 nm while hexane extract was measured at 521 nm.

3.3.3 Determination of selenium by flow based iodometric extraction method

3.3.3.1 Preparation of chemical solutions

3.3.3.1.1 Stock standard selenium dioxide solution

The stock 400 mg L^{-1} standard selenium dioxide solution was prepared by digesting 0.0562 g of selenium dioxide with 20-30 mL of the conc. nitric acid (65%). The solution was boiled about 50-90 min, transferred to a 100-mL volumetric flask and made to the volume with deionized water.

3.3.3.1.2 Stock selenium sulfide solution

The stock 200 mg L^{-1} selenium sulfide solution was prepared by digesting 0.0362 g of selenium sulfide with 20-30 mL of the conc. HNO_3 (65%). The solution was boiled about 50-90 min, transferred to a 100-mL volumetric flask and made to the volume with deionized water.

3.3.3.1.3 Working standard selenium dioxide solution

The working standard selenium solutions of 80, 107, 160, 213, 268, 320 and 373 mg L^{-1} were prepared by pipetting 0.3, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mL of 400 mg L^{-1} stock selenium dioxide solution into each vial and made up the volume to 1.5 mL with deionized water.

3.3.3.2 Selection of membrane

In several reports, membrane has been used for the extraction [43-44] and phase separator [18]. Polymer membrane has many advantages such as its stability in all pH ranges, its inertia to many other chemicals and its thermal resistance. In this work, polypropylene hollow fiber membrane, which is hydrophobic and suitable for extraction of non-polar compound with organic solvent, was used as a

phase separator. The membrane was impregnated with organic solvent prior uses with syringe pump.

3.3.3.3 Signal acquisition

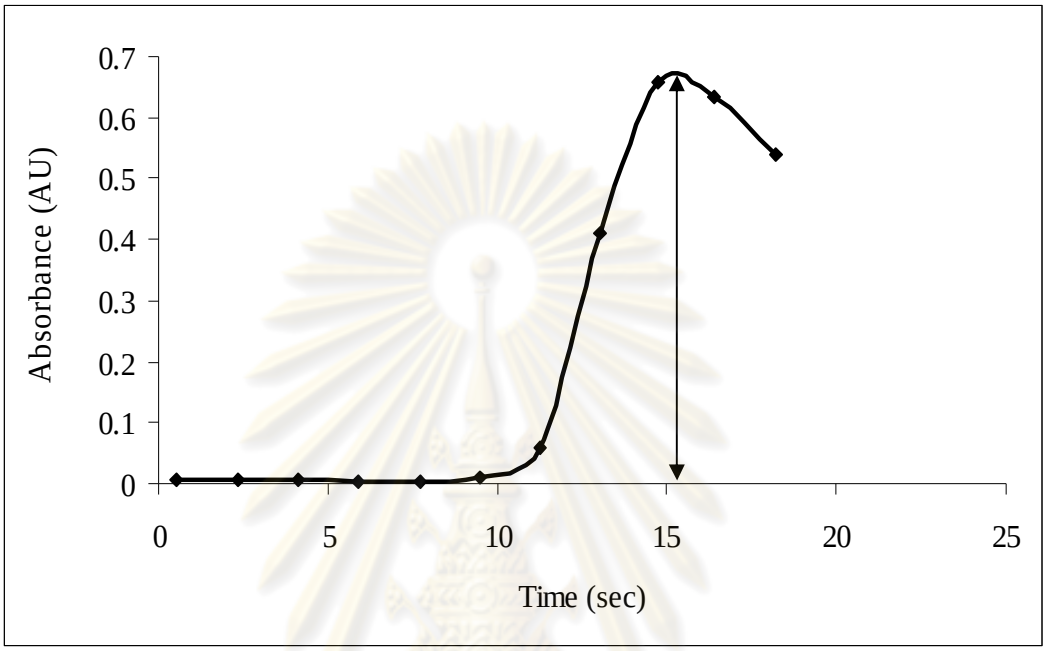

Figure 3.1 Typical signal profile obtained from the fiber optic spectrophotometer.

Figure 3.1 showed a typical signal profile obtained when the purple colored extract was carried to the fiber optic spectrophotometer. The highest absorbance was acquired and processed by using spread sheet software such as Microsoft Excel or Origin software.

3.3.3.4 Design and setup of the extraction unit

3.3.3.4.1 The U-type extraction unit

The U-type extraction unit consisted of a vial and an open-hole screw cap with silicone septum. Two needles were pierced through the septum. The membrane was attached to both ends of the needles (U-type). The sample solution was contained in the vial closed with the membrane-attached cap. One needle was connected to the syringe pump to carry an organic extracting solvent. The other was

connected to the fiber optic spectrophotometer. There were two lengths of membrane studied; 2 cm and 32 cm. The solution was agitated with stirrer while an acceptor phase (hexane) was gradually flowed through the membrane to the detector for measurement at 521 nm with syringe pump. The schematic diagram of the U-type extraction unit was illustrated in Figure 3.2.

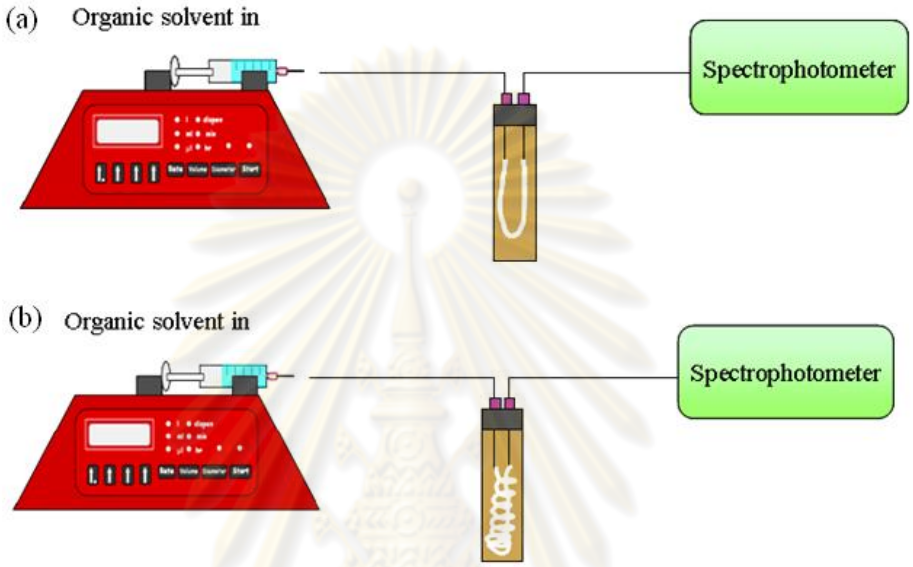

Figure 3.2 Schematic diagram of the U-type extraction unit. (a) 2-cm; (b) 32-cm.

3.3.3.4.2 The Tubular type extraction unit

The tubular type extraction unit consisted of glass tube where both ends were appended with T-connectors. The hollow fiber membrane was inserted through the straight side of the T-connectors and the glass tube. At both ends of the T-connectors were sealed as illustrated in Figure 3.3. There were two setups; horizontal (Figure 3.3a) and vertical (Figure 3.3b). The specification of each setup was summarized in Table 3.1. The sample solution was pumped into the tubing (outside the membrane) by peristaltic pump while the acceptor phase (hexane) was gradually flowed through the membrane to the fiber optic spectrophotometer for measurement at 521 nm with syringe pump.

Table 3.1 Specification of tubular extraction units.

	Horizontal setup	Vertical setup
Glass tube		
- OD (mm)	5.1	6.1
- ID (mm)	3.25	3.8
- Length (cm)	30	4.4
Total membrane length (cm)	36	15.5
Sealed with T-connector	Epoxy glue	Nut and ferrule

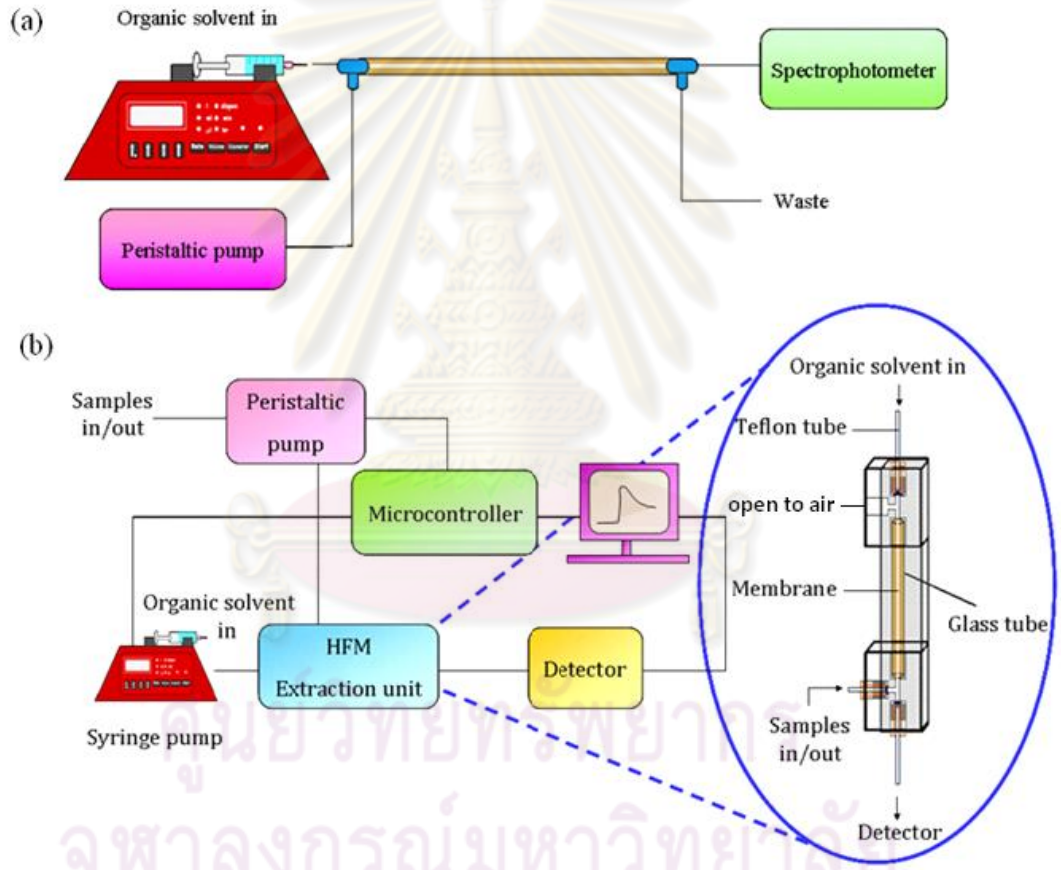

Figure 3.3 Schematic diagram of the tubular type extraction unit. (a) Horizontal; (b) Vertical.

3.3.3.5 Determination of selenium

The hollow fiber membrane extraction unit was constructed and installed in the flow analysis system as illustrated in Figure 3.3b. The sample (donor solution) was pipetted into 4-mL vial and made up to1.5 mL with deionized water. A 0.5 mL of 0.1 mol L^{-1} iodide solution was added and mixed well. An aliquot of 0.5 mL was immediately pumped to fill the glass tubing (outside the membrane) with peristaltic pump. The iodine generated from the reaction was extracted into the hexane (acceptor) that was filled in the membrane turning into the purple colored solution. After desired extraction time, both pumps were turned on. The extract was carried to the fiber optic spectrophotometer for measurement while the sample solution in glass tubing was carried to waste. All processes except washing were controlled by a computer using LabView. The amount of selenium obtained was determined using linear regression method.

3.3.3.6 Cleaning the system

Between runs, the sample line and the extraction line were cleaned by flushing with deionized water and hexane, respectively. The tubing of the sample line was also cleaned by thoroughly flushing with tap water overnight after finishing all the analyses.

3.3.3.7 Method optimization

3.3.3.7.1 Concentration of iodide

This method, iodine was determined instead of selenium(IV). The amount of iodide should be in excess so that the amount of iodine determined was proportional to the amount of selenium. Too much amount of iodide might react with some iodine generated to form triiodide, resulting in that less amount of iodine was extracted into the organic solvent. Various concentrations of iodide were studied. The iodide concentration that gave maximum of response factor was considered as optimal.

3.3.3.7.2 Extraction time

The amount of iodine extracted into organic solvent depends on the contact time or extraction time between donor phase and acceptor phase, which is corresponding to the sensitivity of method. Therefore, the extraction time was investigated. Thus, longer extraction time would increase a contact time between donor phase and acceptor phase resulting in more iodine was extracted into the organic solvent. The time that gave both sufficient signals and more sample throughput was considered as optimum extraction time.

3.3.3.7.3 Size of extraction unit

The length of membrane might affect extraction efficiency because the different length provided different contact area between donor phase and acceptor phase, which corresponding to the amount of iodine extracted into the organic solvent. The longer membrane might allow more mass transfer of iodine into the organic solvent but the signal obtained might be broader than the shorter membrane. The size of extraction unit that gave the sufficient signal was considered as optimal.

3.3.3.8 Method evaluation for extraction

3.3.3.8.1 Calibration curve and linearity

The linear calibration curve between the absorbance and the concentrations of selenium was established for the concentrations ranging from 80 – 373 mg L^{-1}. The linear regression method was used to obtain slope, intercept and R^2.

3.3.3.8.2 Precision and accuracy

The 200 mg L^{-1} of selenium in the forms of selenium dioxide and selenium sulfide was determined. It was prepared by pipetting 0.75 mL of 400 mg L^{-1} stock selenium dioxide solution and 1.5 mL of 200 mg L^{-1} of selenium sulfide into each vial and made up the volume to 1.5 mL of deionized water. Then, 0.5 mL of

0.1 mol L^{-1} iodide solution was pipetted into the vial of selenium solution prior to analysis. The recoveries and the standard deviation of replicate analyses were reported.

3.3.3.9 Real samples

3.3.3.9.1 Shampoo sample (2.5% SeS_2)

Shampoo sample (Selsun) was purchased from local store. The sample was weighed and digested with 65% nitric acid. After it was cooled, it was transferred to a 100-mL volumetric flask and made up to the volume with deionized water.

3.3.3.9.2 Cosmeceutical samples (Bead samples) (1.02%SeS_2)

Cosmeceutical samples were obtained from Pan Rajdhevee Group Company Limited. The sample was first homogenized, weighed and digested with 65% nitric acid. After it was cooled, it was transferred to a 100-mL volumetric flask and made up to the volume with deionized water.

3.3.3.9.3 Calculation of selenium sulfide contenting in samples

The %Selenium sulfide (SeS_2) obtained was calculated as follows.

$$\%SeS_2 = \frac{[Se^{4+}]_{curve}\ \text{mg L}^{-1} \times 100\,\text{mL} \times \frac{1\,\text{L}}{1000\,\text{mL}} \times \frac{MW\,SeS_2}{AW\,Se}}{\text{weight sample mg}} \times 100\%$$

Where MW SeS_2 = 143.1 g mol^{-1}

AW Se = 79.1 g mol^{-1}

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The iodometric titration method

The method was applied for determination of selenium in the forms of selenium dioxide and selenium sulfide. Both selenium solutions were treated with the same process. Table 4.1 summarized the results obtained from determining 100 mg $L^{-1}$of both selenium solutions.

The %recovery was explained as the ratio of percentage of the final selenium concentration found (C_f) to the initial selenium concentration (C_i). The equation of recovery percentage was shown below.

$$\%Recovery = (C_f/C_i)*100$$

Table 4.1 The average amount of selenium obtained from the titration method

	Selenium dioxide (SeO_2)	Selenium sulfide (SeS_2)
Concentration (mg L^{-1})	102	100
Found (mg L^{-1})	96	98
% Recovery	95	98
%RSD	2 (N=21)	3 (N=21)

The recoveries obtained from both selenium solutions were less than 100%, probably due to that the titration was operated in an opening system where the iodine generated might be affected from oxygen in the air, light or unsuitable acidity in the selenium solution. Although less than 100% of recoveries were obtained, they were in the acceptable range, which should be in the range of 90 to 107% and 5.3%RSD [47].

4.2 The iodometric extraction method

Extraction method was another method based on iodometry. The principle was similar to the titration method. The iodine generated was extracted into an organic solvent producing the purple colored extract that could be measured with the fiber optic spectrophotometer. Typically, the extraction method employed chloroform as an extracting solvent. In this research, hexane was studied as an alternative extracting solvent. The extraction efficiency was compared with that using chloroform. The calibration curves of selenium ranging from 13-100 mg L^{-1} using both organic solvents as extracting solvent were established and compared in Figure 4.1. The linear regression equation when using chloroform and hexane as extracting solvent was: $y = 0.0115x - 0.0013$ and $y = 0.009x - 0.0039$, respectively, with correlation coefficient (R^2) > 0.99.

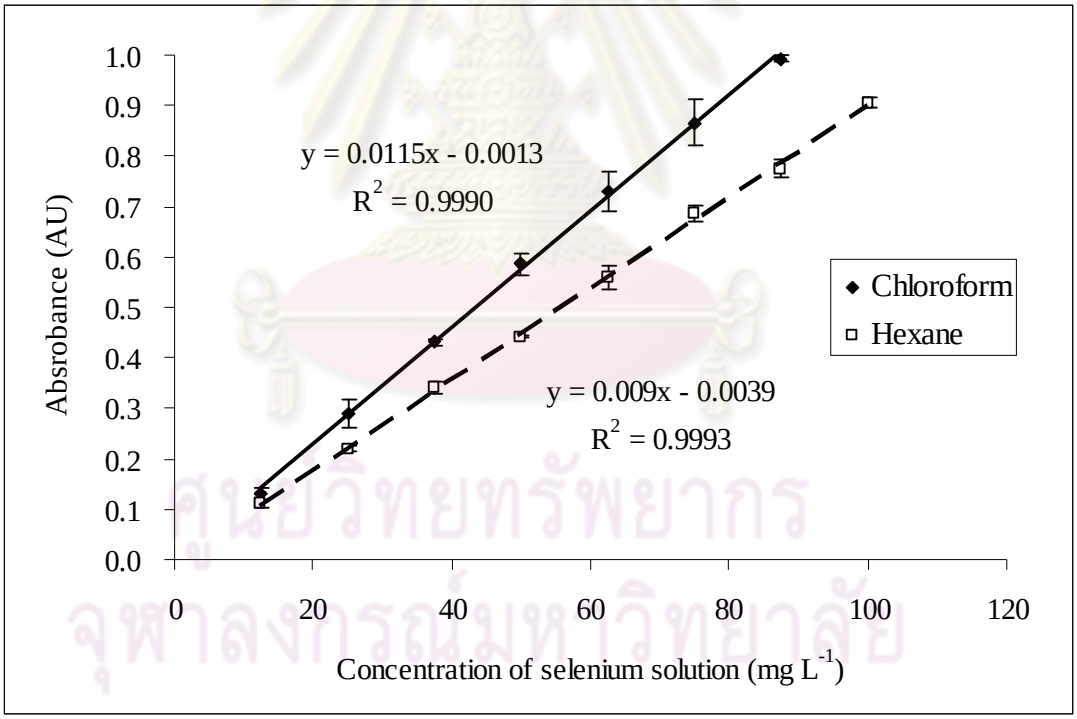

Figure 4.1 Calibration curves for determination of selenium when using chloroform and hexane as extracting solvents.

Although extraction with hexane exhibited less sensitivity, it provided good linearity range. Alternatively, hexane might be used in place of chloroform.

This method was applied for determination of selenium in the form of selenium dioxide and selenium sulfide. Both selenium solutions were treated with the same process. The results obtained when using chloroform and hexane as extracting solvents were summarized in Table 4.2.

Table 4.2 The average amount of selenium obtained when using chloroform and hexane as extracting solvents

		Chloroform	Hexane
SeO_2	Concentration (mg L^{-1})	51	51
	Found (mg L^{-1})	51	50
	% Recovery	101	98
	%RSD	4 (N=9)	3 (N=9)
SeS_2	Concentration (mg L^{-1})	50	50
	Found (mg L^{-1})	54	53
	% Recovery	108	105
	%RSD	7 (N=8)	3 (N=8)

The recoveries obtained using chloroform and hexane as extracting solvents were in acceptable range and not significantly different ($P>0.05$, Paired t-Test). Therefore, hexane was chosen in place of chloroform because it was more environmental friendly than chloroform.

4.3 The Flow based iodometric extraction method

4.3.1 Designs and setup of extraction unit

4.3.1.1 The U-type extraction unit

The U-type extraction unit was operated in continuous flow mode for extraction of 100 mg L^{-1} of selenium solution. The extracting solvent was continuously pumped into the inside membrane at 0.5 mL min^{-1}. First the 2-cm length of membrane was tested for 20 min. It was found that the color of the extracted solution was not significantly turned purple, probably because there was not enough

iodine extracted into the hexane. Apparently, the membrane was too short that there was less contact area and the iodine was slightly extracted into the membrane. Therefore, the longer membrane was employed in order to increase the contact area for extraction. The 32-cm length of membrane was used. Since it was so long that it could not fit in the vial as a U-shape. The setup was coiled and used for extraction for 15 min. The extracted solution turned much purple. Apparently the longer membrane provided the more contact area allowing the more iodine to be extracted. However, leakage of hexane and air bubbles were observed. The coiled membrane might have caused fracture of membrane and high inner pressure. This model suggested that the longer membrane could enhance the extraction efficiency, but it should not be coiled. The tubular type extraction unit was investigated.

4.3.1.2 The Tubular type extraction unit

The tubular type extraction unit was first operated in a continuous flow mode. The sample was pumped into the glass tube (outside the membrane) at 1 mL min^{-1} while the hexane was pumped into the membrane (inside the membrane) at 0.5 mL min^{-1}. The membrane length was 36 cm. The extraction time was about 15 min. Despite of the fact that the extracted solution turned purple, there was still some drawbacks. The membrane could be swollen when contacted with organic solvent and might have caused leakage since it was contracted and touched the glass tube wall. Use of the epoxy glue for sealing the membrane with T-connector might be difficult to stretch the membrane after swollen. Moreover, there were air gaps found during feeding the donor solution to the glass tube since there were differences in inner diameters between the tubing and the glass tube. The extraction unit was modified using ferrules and nuts that made it easily to adjust the membrane after swelling. In addition, the extraction was done in stopped flow mode and the donor solution was filled the glass tube in the vertical direction to avoid the leakage.

4.3.2 Optimization of extraction efficiency

Several parameters affecting the extraction efficiency or sensitivity were investigated and optimized.

4.3.2.1 Calibration curve

Although selenium sulfide is the compound we intend to measure, but preparation of the standard requires treatment with acid. Normally, selenium dioxide has been used in preparing standard selenium solution because it is easily dissolved in water. Therefore, in this experiment, calibration curves constructed from selenium dioxide and selenium sulfide were compared in Figure 4.2. The results presented that the calibration curves using selenium dioxide and selenium sulfide, both of which were digested with the acid, were not different. Therefore, in this experiment, the calibration curve for determination of selenium might be constructed using selenium dioxide digested with acid.

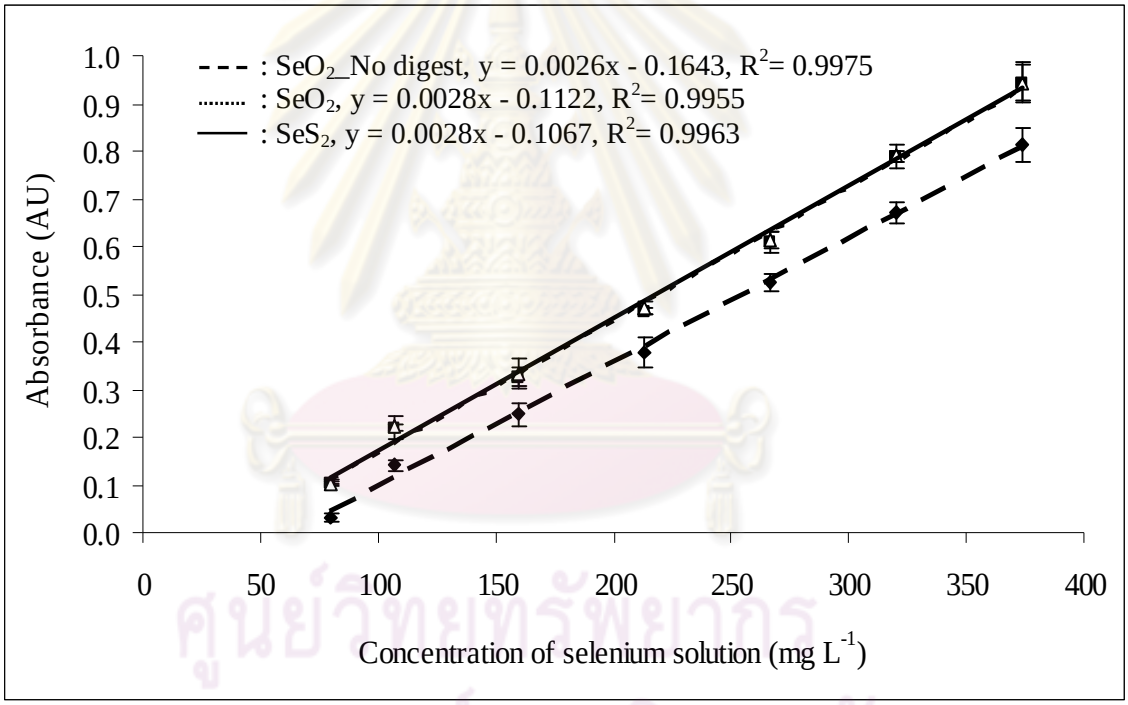

Figure 4.2 Calibration curves for determination of selenium using various types of selenium standard solutions**.**

4.3.2.2 Flow rate of the solution

Since the extraction was occurred in a stopped flow mode, the flow rate of both donor and acceptor solution might not affect extraction efficient but it might affect the pressure when feeding donor or acceptor solution. Flow rate of both

solutions was studied in order to avoid a high pressure during loading the donor solution into the glass tube with a peristaltic pump and carrying the acceptor solution to the inside of the membrane with a syringe pump. A 100 mg L^{-1} of selenium solution extracted for 9 min was determined. A 1.95, 3.10, 4.70 and 5.56 mL min^{-1} of the donor solution were investigated and flow rate of the acceptor solution was studied at 0.75, 1.00, 1.13 and 1.25 mL min^{-1} when used a peristaltic pump and syringe pump, respectively. Figure 4.3 and Figure 4.4 showed the absorbance obtained from 100 mg L^{-1} selenium solution at various flow rates of the donor and acceptor solution, respectively. Figure 4.3 showed that the increased flow rates did not affect to the amount of iodine extracted into the hexane that was held in the membrane. On the other hand, the higher flow rate of the donor solution could cause some air bubbles while loading the solution due to the higher inner pressure. Therefore, the 4.70 mL min^{-1} of the donor solution was employed because it was maximum flow rate that there was no bubble.

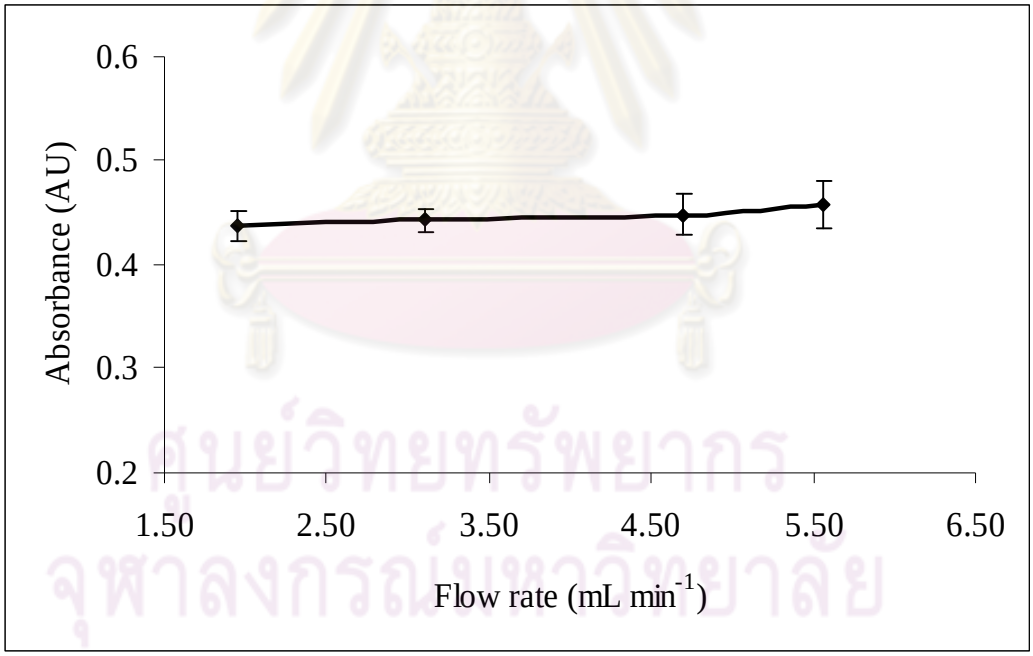

Figure 4.3 Relationship between the absorbance obtained from 100 mg L^{-1} selenium solution and various flow rates of the donor solution when extracted for 9 min and using 1 mL $min^{-1}$of acceptor solution.

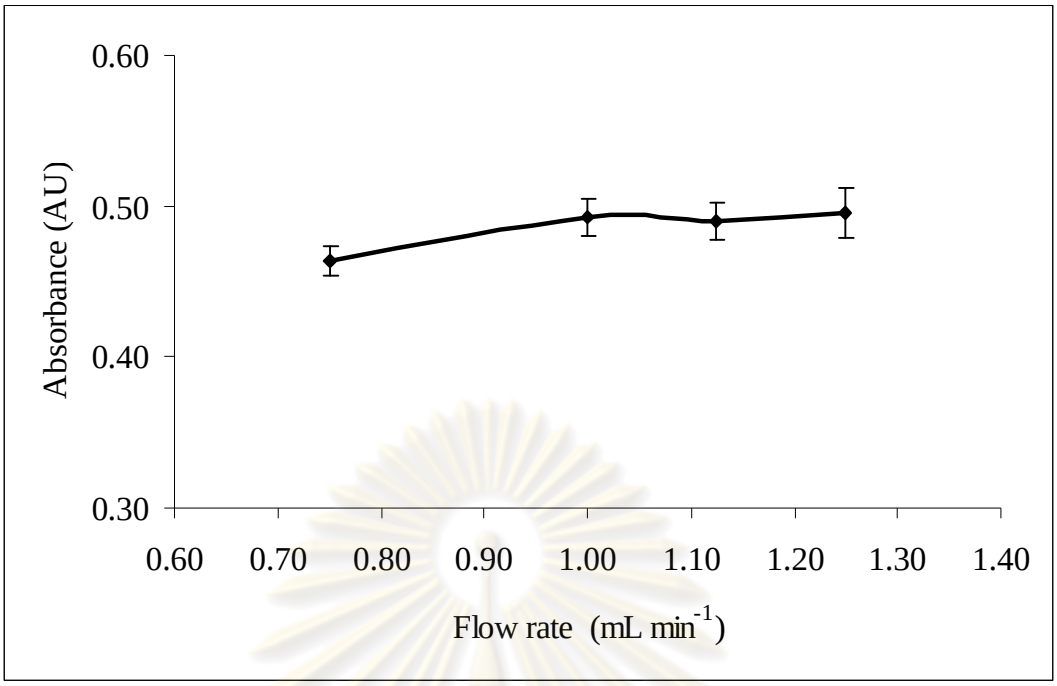

Figure 4.4 Relationship between the absorbance obtained from 100 mg L^{-1} selenium solution and various flow rates of the acceptor solution when extracted for 9 min and using 4.70 mL min^{-1} of donor solution.

Figure 4.4 showed that the flow rate of the acceptor solution of more than 1.0 mL min^{-1} could be used. The higher flow rate could produce high pressure leading to leakage of hexane out of membrane, while the lower flow rate could cause broad signal resulting in decreased sensitivity. Hence, the acceptor flow rate of 1.0 mL min^{-1} was employed for further studies.

4.3.2.3 Concentration of iodide

Iodide plays an important role in the iodometry, where iodide reduces selenium(IV) to selenium(0) and iodine. The amount of iodide should be in excess so that the amount of iodine obtained is proportional to the amount of selenium refer to (Eq.5) in chapter II. Nevertheless, excess iodide might react with iodine to form triiodide (seeing (Eq.4) in chapter II) resulting in less amount of iodine extracted into the organic solvent.

Effect of iodide concentration on response factor (the ratio of absorbance to concentration of selenium) at each selenium concentration was illustrated in Figure 4.5. The response factors were increased as increasing selenium

concentrations. As the fixed volume of iodide solution was added to various concentrations of selenium solutions, the mole ratio of iodide to selenium was decreased as increasing selenium concentrations; consequently, the formation of triiodide was lessened resulting in that the more iodine could be extracted into the hexane. The 0.1 mol L^{-1} iodide concentration provided sufficient iodide. Too much iodide concentrations could increase the chance for triiodide formation. Therefore, 0.1 mol L^{-1} iodide solution was chosen in the extraction.

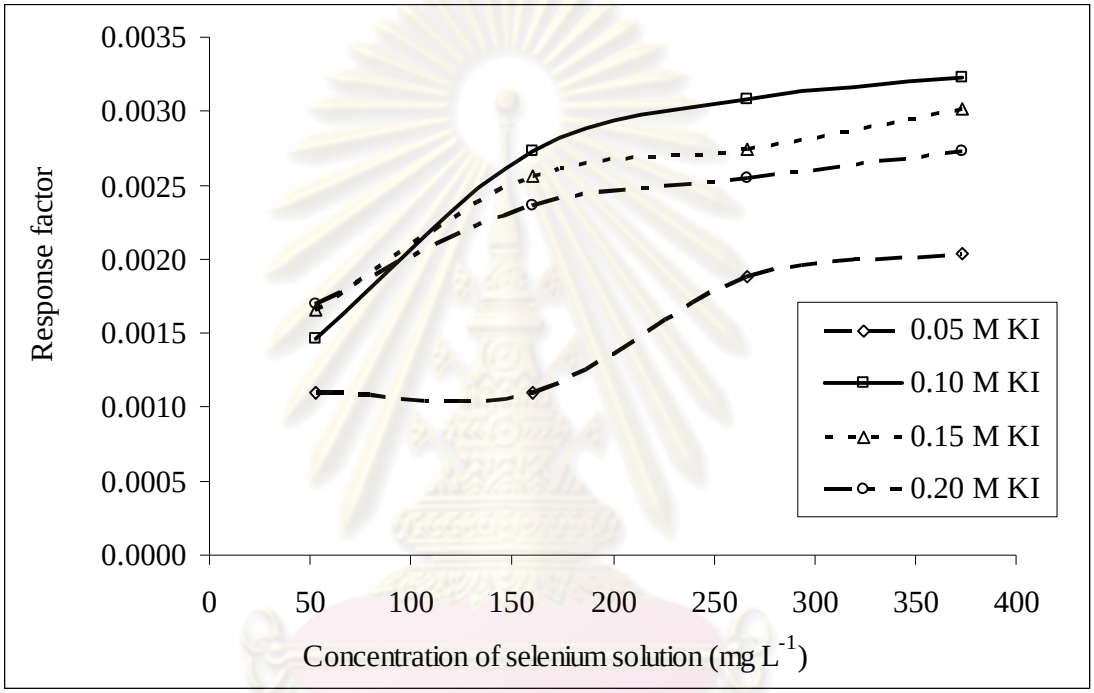

Figure 4.5 The response factor when using iodide at various concentrations for the determination of selenium.

4.3.2.4 Extraction time

The extraction time was another main parameter that had to be investigated. Since the extraction efficiency depended on the contact time between donor solution and acceptor solution, a long time of stopped flow resulted in that more iodine could diffuse into the hexane. The amount of iodine extracted was corresponding to the sensitivity of the method. Extraction times of 30, 60, 120, 180 and 300 sec were investigated, which was shown in Figure 4.6. The longer extraction time exhibited the more sensitivity (slope) but it provided smaller working range. On

the other hand, shorter extraction time showed wider working range while it gave relatively less sensitivity. In addition, another factor that should be taken into account was the sample throughput. The relationship between the sensitivity, extraction time and sample throughput was shown in Figure 4.7. Evidently, about 90 sec was the optimum extraction time owing to that the sensitivity and the sample throughput were compromised. For the quality control of cosmeceutical products, which usually contain high selenium content, the extraction time of 60 sec might be chosen. Despite it provided somewhat less sensitivity, the sample throughput was considerably useful for routine analysis.

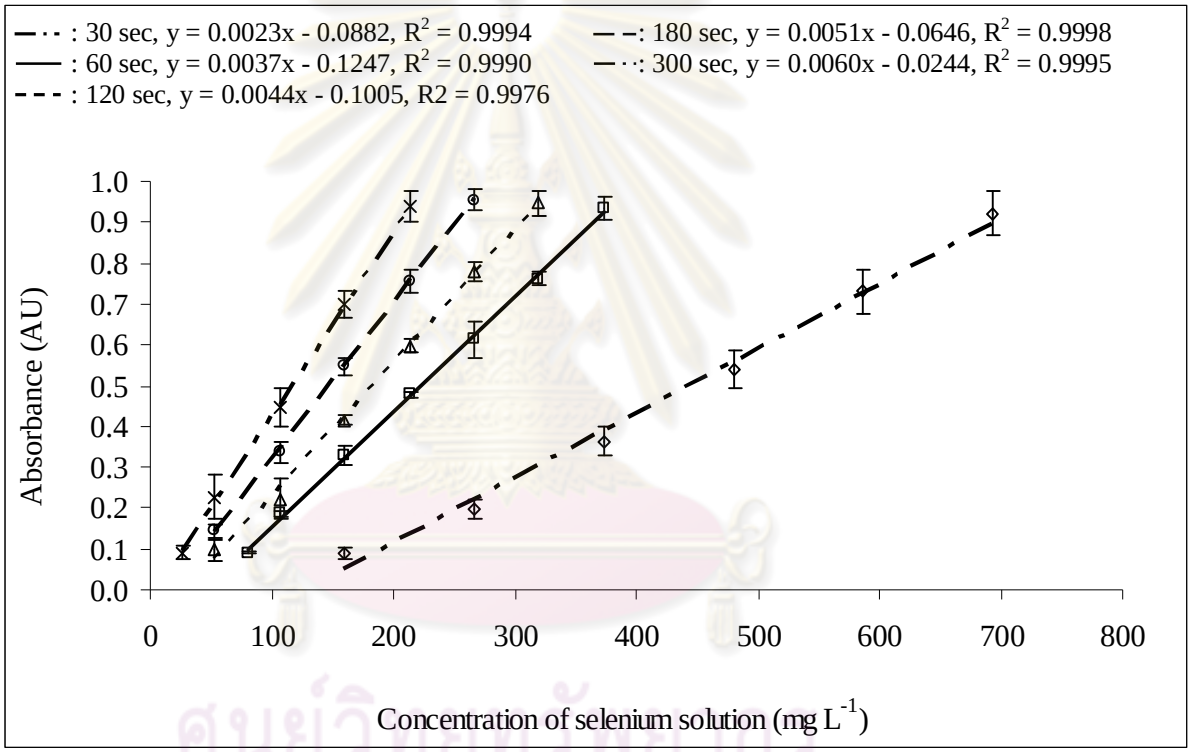

Figure 4.6 Concentration ranges for determination of selenium at various extraction times.

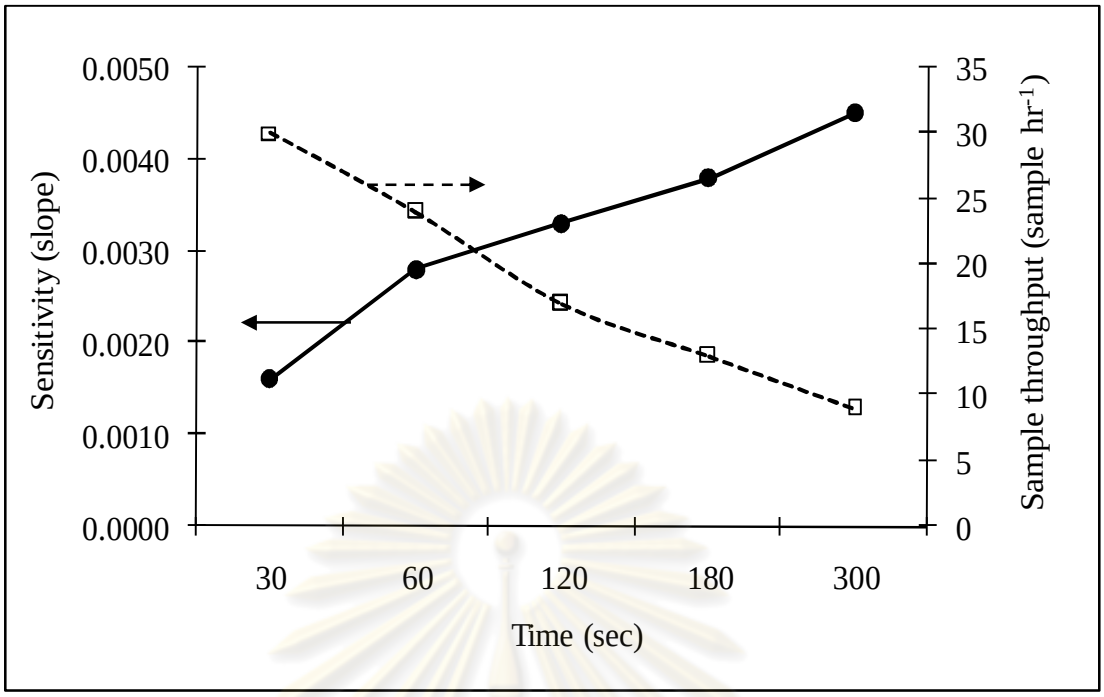

Figure 4.7 Sensitivity and sample throughput at various extraction times.

4.3.2.5 Cleaning the system

The system was continuously operated, so it had to be cleaned between runs to assure that there was no memory effect from the previous run. The time of washing system was investigated because it would be included in the sample throughput. The washing system was divided into two parts: the sample line and the extraction line. The sample line (outside the membrane) was studied by comparing between just soaking and rinsing the sample line before soaking with deionized water, where the soaking time was varied according to the washing time of the extraction line. The extraction line (inside the membrane) was washed with hexane for 10, 20 and 30 sec. Figure 4.8 compared the absorbance obtained from 213 mg L^{-1} selenium solution after soaking and rinsing before soaking with deionized water while the extraction line was washed with hexane at various times. The results exhibited that washing the sample line by soaking was not sufficient because there was still the memory effect, but rinsing before soaking could solve the problem. For washing the extraction line, it was found that there was no memory effect after washing for more than 20 sec. Therefore, the cleaning procedure was that the sample line was rinsed and

soaked with deionized water while the extraction line was washed with hexane for 20 sec.

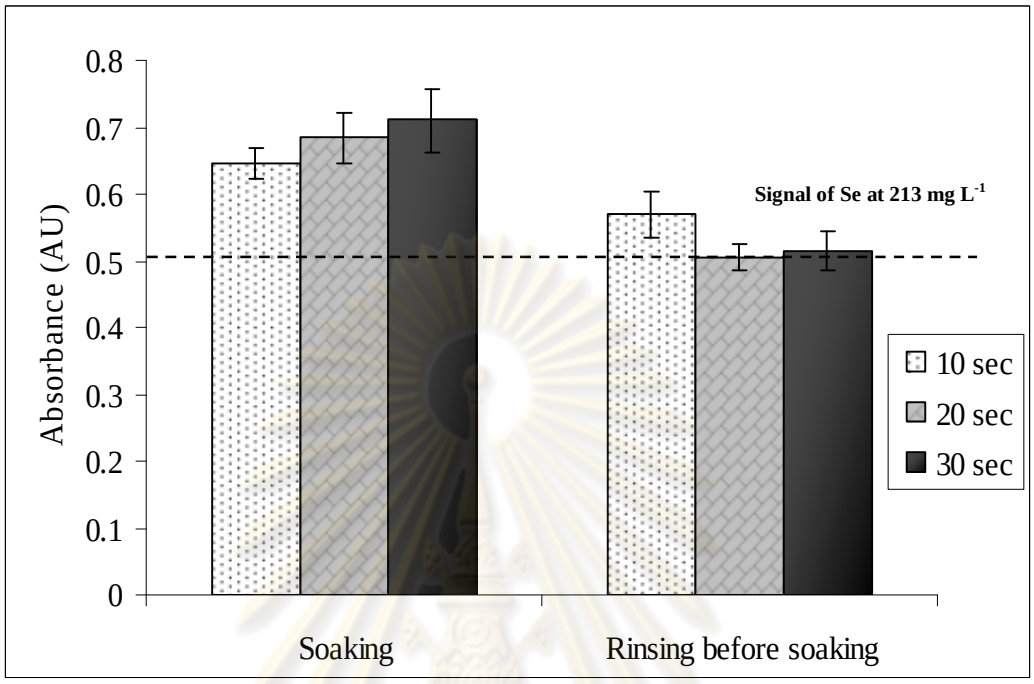

Figure 4.8 The absorbance obtained from 213 mg L^{-1} selenium solution after washing the sample line and the extraction line with deionized water and hexane, respectively.

4.3.2.6 Size of the extraction unit

The size of extraction unit might affect extraction efficiency because the longer extraction unit would increase both membrane length and donor volume leading to increased mass transfer of the analyte into organic solvent due to the more feeding analyte and the more contact area. Table 4.3 showed the characteristic of the shorter and the longer extraction units studied.

Table 4.3 The characteristic of extraction units

	Short extraction unit	Long extraction unit
1. Glass tube		
- Length (cm)	4.4	8
- ID (mm)	3.8	3.8
- OD (mm)	6.1	6.1
2. Total length of membrane	15.5	19.5
3.Volume of donor phase	0.50	0.91

The absorbance obtained for 107, 213 and 320 mg L^{-1} of selenium solution extracted with short and long extraction units for 60 sec was illustrated in Figure 4.9. The results showed that the longer extraction unit did not significantly improve the extraction efficiency. After testing with Paired t-Test, there was not significantly different ($P>0.05$) between the absorbance obtained from the longer and the shorter extraction units. Therefore, the short extraction unit was chosen because it used less membrane and shortened time for loading and washing.

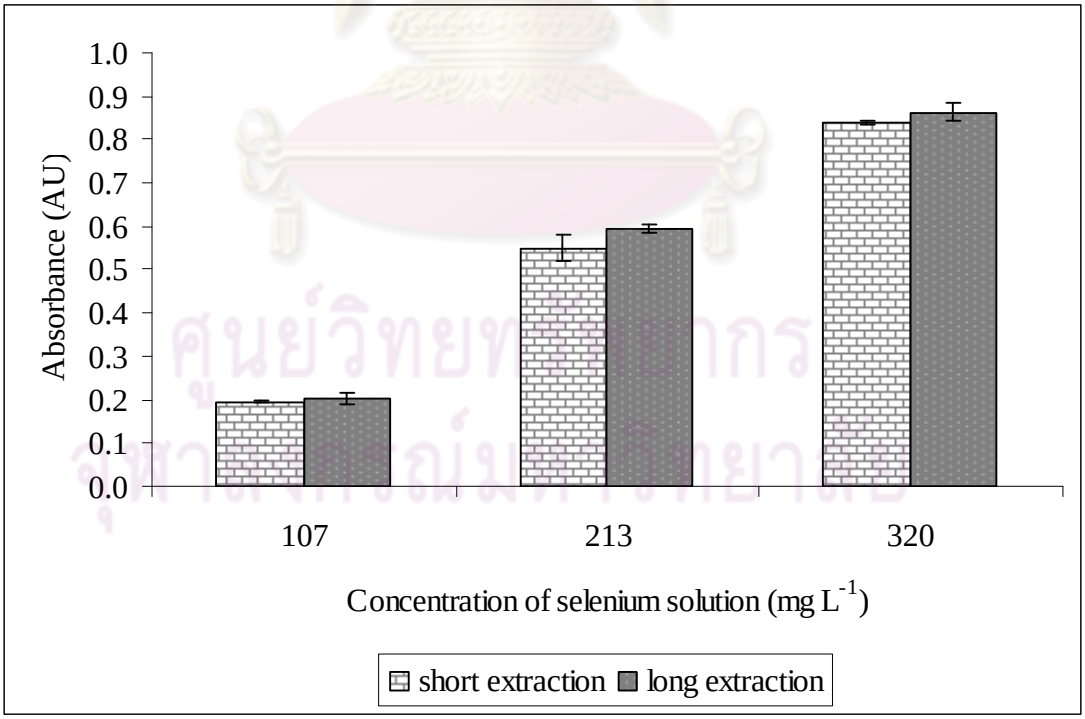

Figure 4.9 The absorbance obtained when using short and long extraction unit at various selenium concentrations.

4.3.2.7 Reuse of membrane

Reuse of membrane was another factor, which should be considered because it could save cost and time. To evaluate the reusability of the membrane, a 200 mg L^{-1} of selenium solution was extracted at 60 sec and repeated several times. The results were demonstrated in Figure 4.10. It was found that the membrane could be reused for more than 100 times without significant loss of signal.

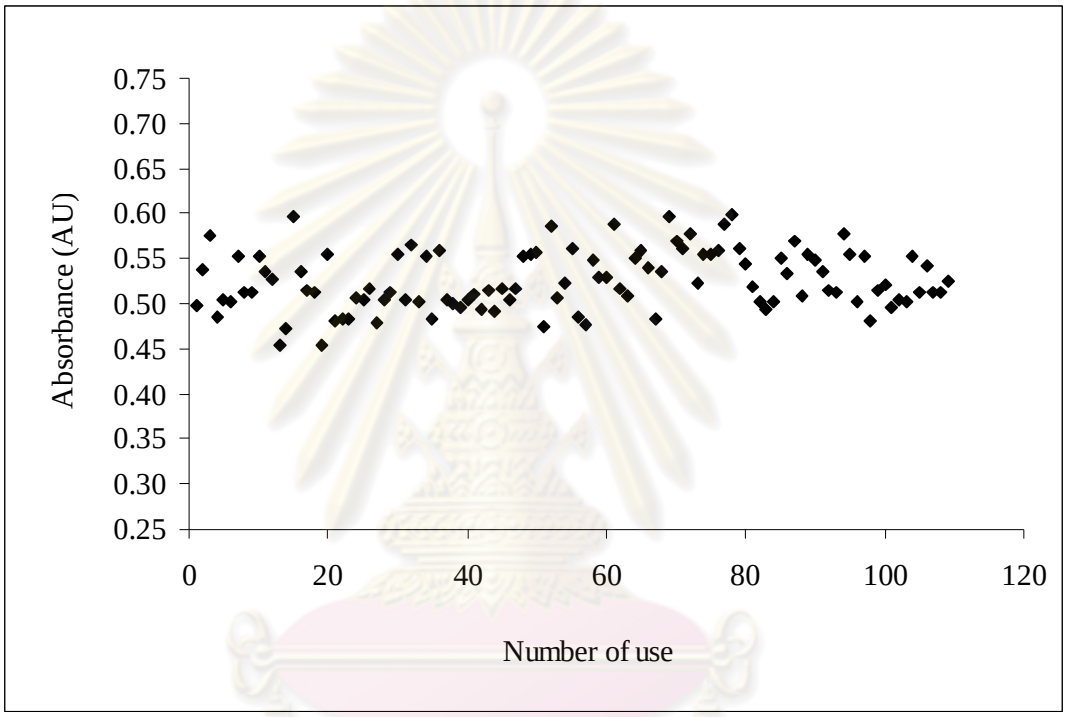

Figure 4.10 The absorbance obtained from 200 mg L^{-1} selenium solution when the reusability of the membrane.

4.3.2.8 Matrix effect

Since the cosmeceutical samples typically consisted of polymer matrices that might interfere with the analysis, therefore matrix effect should be considered. Figure 4.11 showed the calibration curve obtained when using selenium dioxide and mixture of selenium dioxide and placebo. Both types were treated with acid in the same process. The slopes obtained from two types were not different indicating that the placebo did not disturb the analysis. So the calibration curve could be prepared using selenium dioxide solution treated with acid.

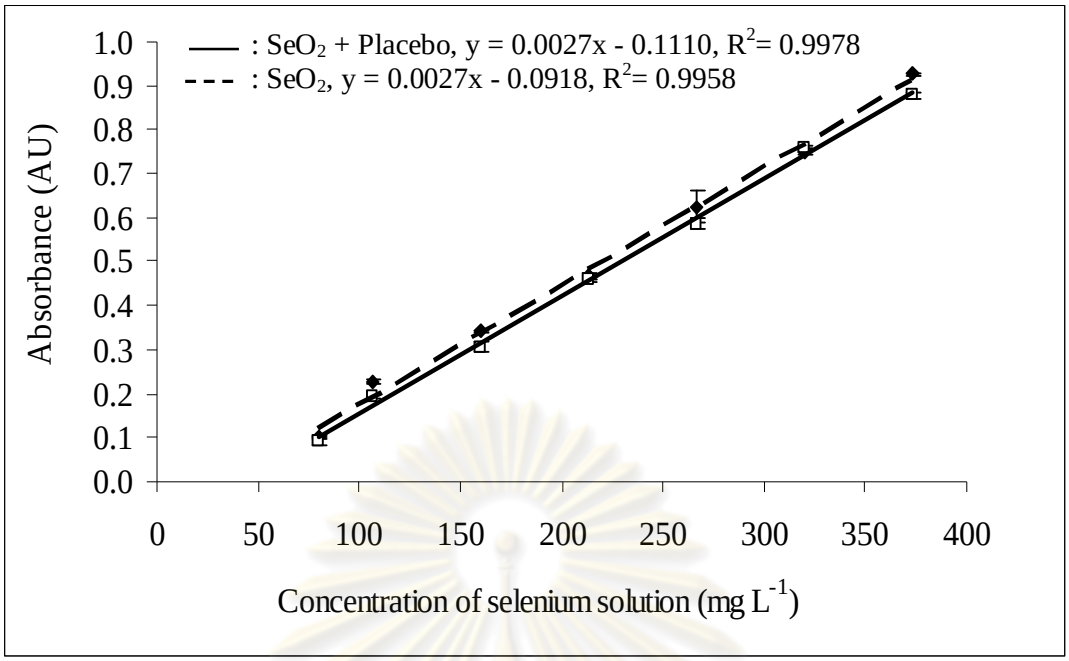

Figure 4.11 Calibration curve obtained when using selenium dioxide and mixture of selenium dioxide and placebo.

The optimal parameters of on-line hollow fiber membrane extraction iodometric method for determination of selenium were summarized in Table 4.4. The total analysis time was about 180 sec providing sample throughput of approximately 20 sample hr^{-1}.

Table 4.4 Optimum condition of flow based membrane extraction for determination of selenium.

Parameters	Optimum
Concentration of iodide solution	0.1 mol L^{-1}
Extracting solvent (acceptor solution)	Hexane
Size of extraction unit	Short
- Glass tube	6.1 mm OD × 3.8 mm ID × 4.4 cm length
- Total membrane	15.5 cm
Total analysis time	180 sec
- Sample loading time (4.7 mL min^{-1})	15 sec
- Extraction time	60 sec
- Extract discarding time to detector (1 mL min^{-1})	25 sec
- Wash the sample line with DI (rinsing before soaking)	60 sec (in and out 15 sec each)
- Wash the extraction line with hexane	20 sec

4.3.3 Method evaluation for on-line extraction

4.3.3.1 Calibration curve for determination of selenium

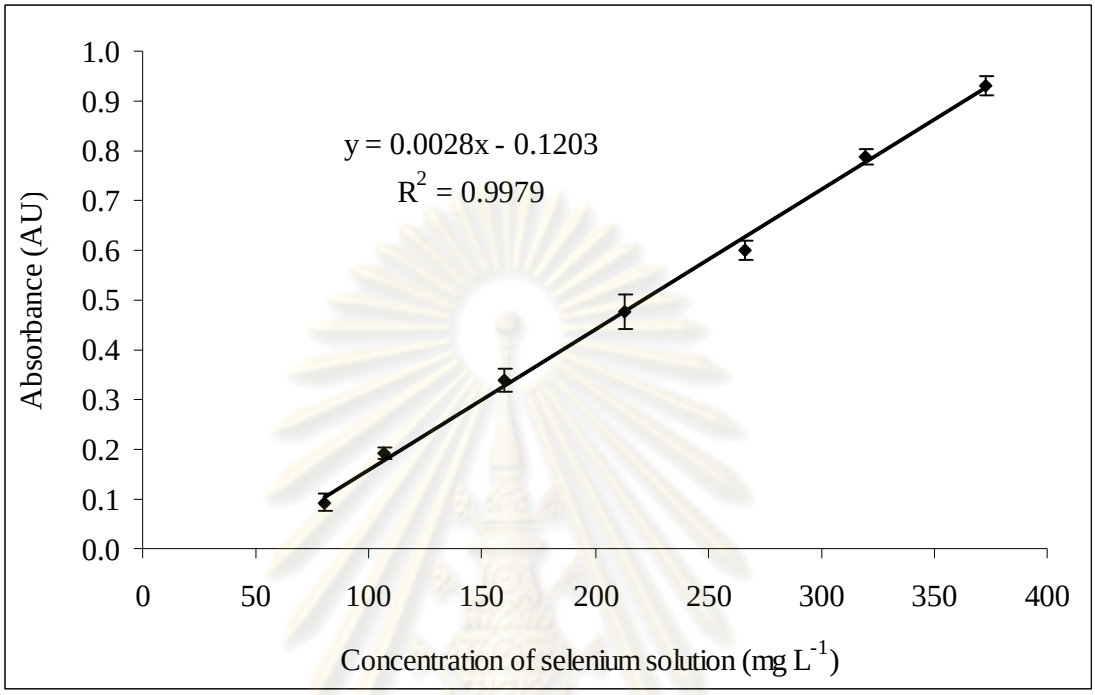

Figure 4.12 Working range of selenium determined by on-line membrane extraction iodometric method.

Calibration curve was prepared from selenium dioxide solutions after on-line extraction. The method linearity between the signal responses of iodine extracted and selenium concentrations ranging from 80 to 373 mg L^{-1} was verified. Figure 4.12 showed the calibration curve of this method plotted between absorbance versus selenium concentrations. A working range of 80 to 373 mg L^{-1} was observed and illustrated by the linear regression equation: $y = 0.0028x – 0.1203$ with correlation coefficient (R^2) = 0.9979. Furthermore, the calibration curves that were established over five-day period gave the %RSD of slopes less than 3%, suggesting that the method was robust for routine analysis.

4.3.3.2 Precision and Accuracy

Evaluation of the precision and accuracy of the proposed method could be done by applying for determination of selenium(IV) in forms of selenium dioxide and selenium sulfide. Both selenium solutions were treated with the same process. The results were compared between the on-line membrane extraction (the proposed method) with the standard titration method and summarized in Table 4.5.

Table 4.5 The average amount of selenium obtained from the proposed method and the titration method.

		The proposed method	The titration method
SeO_2	Concentration (mg L^{-1})	201	102
	Found (mg L^{-1})	205	96
	Recovery (%)	102	95
	%RSD	3 (N=8)	2 (N=21)
SeS_2	Concentration (mg L^{-1})	200	100
	Found (mg L^{-1})	202	98
	Recovery (%)	101	98
	%RSD	1 (N=8)	3 (N=21)

Although the recovery obtained from the proposed method was relatively higher than that obtained from the titration method, the average recovery of both methods were within the acceptable range, which should be in the range of 90 to 107% with 5.3%RSD [47].

4.4.4 Method performance in samples application

The flow based extraction method was applied to determine selenium(IV) in the form of selenium sulfide in real cosmeceutical samples such as anti-dandruff shampoo and cosmeceutical samples. The results were compared to the results obtained by the standard titration method. These experiments were performed

in 7 replicates. The results obtained from both the proposed method and the titration method was summarized in Table 4.6.

Table 4.6 The average amount of selenium obtained in shampoo and cosmeceutical samples (bead samples) from the proposed method and the titration method.

		The proposed method	The titration method
Shampoo[a]	Found (%)	2.54	2.31
	Recovery (%)	102	93
	%RSD	1 (N=7)	3 (N=7)
Cosmeceutical samples[b]	Found (%)	1.02	0.93
	Recovery (%)	100	91
	%RSD	1 (N=7)	1 (N=7)

[a] Anti-dandruff shampoo (Selsun, contained 2.5%SeS_2)

[b] Cosmeceutical samples (PAN Rajahevee Group, contained 1.02%SeS_2)

The amounts of selenium obtained from both methods were compared with the amount of selenium labeled on shampoo and cosmeceutical product. The relative errors obtained by this proposed method were 1.6% and 0.2%, respectively for shampoo and cosmeceutical samples while the relative errors obtained by the titration method were 7.6% and 8.8%, respectively. The proposed method showed less error than the titration method. Therefore, it could be applied for determination of selenium as alternative to the titration method in quality control of selenium sulfide in cosmeceutical samples.

CHAPTER V

CONCLUSION AND SUGGESTION OF FUTURE WORK

5.1 Conclusion

A flow-based method with on-line liquid extraction was developed for determination of selenium sulfide in cosmeceutical products. The method of determination was based on iodometric method, where the amount of selenium was proportional to the amount of iodine generated from the reaction with excessive iodide. The iodine was on-line extracted based on a liquid membrane extraction with an organic solvent that was held in a polypropylene hollow fiber membrane as a phase separator. This work particularly focused on the design of the extraction unit. The U-type extraction unit and the tubular-type extraction unit with continuous and stopped flow modes were tested. The tubular-type extraction unit operated with stopped flow mode in a vertical direction was chosen. Furthermore, the extracting solvent that was used to be chloroform was alternatively replaced by hexane, which was more environmental friendly without losing much sensitivity.

Parameters that influenced the extraction efficiency or sensitivity were studied. The concentration of iodide that was pre-mixed with the sample could affect the sensitivity of the method. The amount of iodide should be in excess of the amount of selenium in the sample; however, too much amount of iodide might have reacted with the iodine generated yielding triiodide resulting in that the less iodine was extracted. The extraction time was a main parameter, which affected the sensitivity and sample throughput. The sensitivity or the extraction efficiency depended on the contact time between donor solution and acceptor solution. The longer extraction time showed the more sensitivity but it provided smaller working range. The size of extraction unit might affect extraction efficiency because the longer extraction unit would increase both the length of the membrane and the volume of the donor solution resulting in increased mass transfer of the analyte into the organic solvent. Since the system was operated continuously, it was necessary to be cleaned in order to ensure

that there was no memory effect from the previous run. Cleaning the system by rinsing and soaking the sample line with deionized water and flushing the extraction line for 20 sec with hexane was recommended to ensure that there was no memory effect. In addition, the system was tested that the hollow fiber membrane could be used for more than 100 cycles without losing any performance.

The flow-based iodometric extraction method was applied for determination of selenium sulfide in shampoo and cosmeceutical samples (bead samples). The amounts of selenium obtained were compared between the proposed method and the titration method. The proposed method provided less %relative error than the titration method. The proposed method was successfully applied for determination of selenium in shampoo and cosmeceutical products (bead samples). In addition, the proposed method was simple, inexpensive and less laborious that be suitable for routine analysis. It suggested that the proposed method could be used as alternative to the titration method in quality control of selenium in cosmeceutical samples.

5.2 Suggestion of future work

The on-line membrane extraction method was still semi-automated so it could be developed to fully automated system. It can be improved by using the switching valves to connect between the peristaltic pump and the extraction unit. The applications of hollow fiber membrane extraction unit for extraction of other compounds; i.e., metals or organic compounds could be explored. In addition, the design of the hollow fiber membrane extraction unit, the incorporation of the extraction unit with the flow-based system, and modification of the system using other desired detectors could be challenges.

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APPENDICES

APPENDIX A

Table A.1 Average amount of selenium obtained from 100 mg L^{-1} of selenium dioxide with the titration method.

	Found (mg L^{-1})	%Recovery	%RSD
SeO_2	94	93	1
	94	93	2
	94	92	3
	99	98	1
	98	98	1
	98	96	1
	97	94	1
	94	93	1
	97	94	2
	94	93	1
	95	92	1
	91	91	1
	95	94	2
	94	93	2
	97	95	1
	97	95	1
	97	95	1
	95	95	1
	97	96	1
	98	97	1

N=3 observations per mean.

Table A.2 Average amount of selenium obtained from 100 mg L^{-1} of selenium sulfide with the titration method**.**

	Found (mg L^{-1})	%Recovery	%RSD
SeS_2	94	95	1
	98	98	1
	99	97	1
	97	97	1
	104	103	1
	103	102	1
	97	96	1
	102	103	1
	97	97	1
	96	96	1
	99	99	1
	99	99	1
	98	97	1
	97	96	1
	99	99	1
	98	97	2
	98	98	1
	100	99	1
	98	98	1
	93	92	1

N=3 observations per mean.

Table A.3 The average amount of selenium obtained from 50 mg L^{-1} of selenium solution with the extraction method.

	Chloroform			Hexane		
	Found (mg L^{-1})	%Recovery	%RSD	Found (mg L^{-1})	%Recovery	%RSD
SeO_2	51	101	1	51	99	4
	55	107	1	49	96	2
	51	101	1	48	96	2
	51	101	1	52	104	7
	52	103	2	48	94	3
	52	104	2	51	101	7
	47	94	3	50	99	2
	51	101	3	48	95	1
	50	98	1	50	100	1
SeS_2	53	106	1	50	100	0
	58	114	1	53	106	3
	60	119	2	55	109	2
	55	110	1	55	110	7
	56	111	2	54	107	3
	49	97	1	52	103	8
	50	100	3	51	103	5
	52	103	1	52	103	3

N=3 observations per mean.

Table A.4 The average amount of selenium obtained from 200 mg L^{-1} of selenium solution with on-line membrane extraction method.

	Found (mg L^{-1})	%Recovery	%RSD
SeO_2	198	99	3
	206	103	4
	194	97	3
	213	106	2
	198	99	2
	210	105	3
	208	104	2
	209	104	1
SeS_2	206	103	2
	203	102	0
	198	99	3
	209	105	1
	206	103	2
	190	95	2
	197	99	3
	206	103	1

N=3 observations per mean.

Table A.5 The average amount of selenium obtained from 2.5%SeS_2 in shampoo and 1.0%SeS_2 in cosmeceutical samples solution with on-line membrane extraction method (the proposed method) and titration method.

	The proposed method			The titration method		
	%SeS_2	%Recovery	%RSD	%SeS_2	%Recovery	%RSD
Shampoo	2.5	101	2	2.3	91	1
(2.5%SeS_2)	2.5	100	3	2.2	90	1
	2.5	101	3	2.3	90	1
	2.6	104	1	2.3	94	1
	2.5	102	1	2.4	95	1
	2.6	103	1	2.3	94	1
	2.5	102	2	2.4	95	1
Cosmeceutical	1.0	100	1	0.9	91	1
sample	1.0	101	1	0.9	90	1
(1.0%SeS_2)	1.0	102	2	0.9	91	1
	1.0	100	1	0.9	91	1
	1.0	100	3	0.9	91	1
	1.0	100	0	0.9	91	2
	1.0	99	0	0.9	91	1

N=3 observations per mean.

APPENDIX B

Table B.1 A statistical test for difference between %recovery of selenium obtained from extracting with chloroform and hexane by using Paired t-Test at P=0.05.

t-Test: Paired Two Sample for Means

	SeO_2		SeS_2	
	Chloroform	Hexane	Chloroform	Hexane
Mean recovery	101.2164	98.21584	107.8171	105.1147
Variance	13.75799	10.24403	52.65629	12.95847
Observations	9	9	8	8
Pearson Correlation	-0.26657		0.697509	
Hypothesized Mean Difference	0		0	
df	8		7	
t Stat	1.634454		1.415109	
P(T<=t) one-tail	0.070402		0.099974	
t Critical one-tail	1.859548		1.894579	
P(T<=t) two-tail	0.140804		0.199948	
t Critical two-tail	2.306004		2.364624	

Table B.2 A statistical test for difference between absorbance of selenium obtained from short and long extraction unit with on-line membrane extraction method by using Paired t-Test at P=0.05.

t-Test: Paired Two Sample for Means

Selenium concentration	107 mg L^{-1}		213 mg L^{-1}		320 mg L^{-1}	
	Short	Long	Short	Long	Short	Long
Mean absorbance	0.195725	0.203673	0.550271	0.595244	0.838624	0.863377
Variance	1.67E-05	1.61E-04	8.54E-04	1.14E-04	1.87E-05	4.92E-04
Observations	3	3	3	3	3	3
Pearson Correlation	0.27023		0.000691		-0.88056	
Hypothesized Mean Difference	0		0		0	
df	2		2		2	
t Stat	-1.12554		-2.50391		-1.6453	
P(T<=t) one-tail	0.188636		0.064641		0.120823	
t Critical one-tail	2.919985		2.919985		2.919986	
P(T<=t) two-tail	0.377273		0.129282		0.241646	
t Critical two-tail	4.302652		4.302652		4.302653	

Table B.3 A statistical test for difference between %recovery of selenium obtained from selenium dioxide solutions when using the on-line membrane extraction method and the titration method by using F-Test at P=0.05.

F-Test Two-Sample for Variances

	The proposed method	The titration method
Mean SD	2.393583	0.938071
Variance	0.909957	0.563681
Observations	8	21
df	7	20
F	1.614313	
P(F<=f) one-tail	0.188662	
F Critical one-tail	2.514011	

Table B.4 A statistical test for difference between %recovery of selenium obtained from selenium dioxide solutions when using the on-line membrane extraction method and the titration method by using t-Test at P=0.05.

t-Test: Two-Sample Assuming Equal Variances

	The proposed method	The titration method
Mean recovery	101.8991	94.53282
Variance	11.62068	4.120673
Observations	8	21
Pooled Variance	6.065119	
Hypothesized Mean Difference	0	
df	27	
t Stat	7.199179	
P(T<=t) one-tail	4.82E-08	
t Critical one-tail	1.703288	
P(T<=t) two-tail	9.64E-08	
t Critical two-tail	2.05183	

Table B.5 A statistical test for difference between %recovery of selenium obtained from selenium sulfide solutions when using the on-line membrane extraction method and the titration method by using F-Test at P=0.05.

F-Test Two-Sample for Variances

	The proposed method	The titration method
Mean SD	1.663717	0.869628
Variance	0.884518	0.199702
Observations	8	21
df	7	20
F	4.429183	
P(F<=f) one-tail	0.00407	
F Critical one-tail	2.514011	

Table B.6 A statistical test for difference between %recovery of selenium obtained from selenium sulfide solutions when using the on-line membrane extraction method and the titration method by using t-Test at P=0.05.

t-Test: Two-Sample Assuming Equal Variances

	The proposed method	The titration method
Mean recovery	101.0761	97.78859
Variance	9.59839	7.155448
Observations	8	21
Pooled Variance	0	
Hypothesized Mean Difference	11	
df	2.648722	
t Stat	0.011319	
P(T<=t) one-tail	1.795885	
t Critical one-tail	0.022638	
P(T<=t) two-tail	2.200985	
t Critical two-tail	101.0761	97.78859

Table B.7 A statistical test for difference between $\%SeS_2$ in samples from on-line membrane extraction method and the titration method by using Paired t-Test at P=0.05.

t-Test: Paired Two Sample for Means

	Shampoo		Cosmeceutical sample	
	The proposed method	The titration method	The proposed method	The titration method
$\%SeS_2$ found	2.54333931	2.31438657	1.022409	0.927321
Variance	0.00098296	0.00337504	8.04E-05	2.05E-05
Observations	7	7	7	7
Pearson Correlation	0.6510732		-0.20159	
Hypothesized Mean Difference	0		0	
df	6		6	
t Stat	13.5917892		23.2287	
P(T<=t) one-tail	4.9222E-06		2.09E-07	
t Critical one-tail	1.94318027		1.94318	
P(T<=t) two-tail	9.8445E-06		4.17E-07	
t Critical two-tail	2.44691185		2.446912	

VITA

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Poster presentation and proceeding

"Semi-automated iodometric method using on-line hollow fiber membrane extraction for the determination of selenium" Suprawee Wongsuchoto, Pakorn Varanusupakul. Poster presentation and proceeding, *Pure and Applied Chemistry International Conference 2011 (PACCON 2011)*, Miracle Grand Hotel, Bangkok, Thailand, 5-7 January, 2011.

Oral presentation and proceeding

"Hollow fiber membrane extraction and phase separator for on-line determination of selenium" Suprawee Wongsuchoto, Pakorn Varanusupakul. Oral presentation and proceeding, *the 5th Srinakharinwirot Vichakarn Conference*, Srinakharinwirot University, Bangkok, Thailand, 17-18 March, 2011.