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SAMPLE PREPARATION USING MEMBRANE DIALYSIS FOR DETERMINATION OF IODIDE ION IN MILK AND EGG SAMPLES

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A Thesis Submitted in Partial Fulfillment of the Requirements
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กนกวรรณ จันทร์โฮง : การเตรียมตัวอย่างด้วยเมมเบรนไดแอลิซิสสำหรับการตรวจวัดไอโอไดด์ไอออนในตัวอย่างนมและไข่. (SAMPLE PREPARATION USING MEMBRANE DIALYSIS FOR DETERMINATION OF IODIDE ION IN MILK AND EGG SAMPLES)

อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. ปกรณ์ วรานุศุภากุล, 83 หน้า.

งานวิจัยนี้ได้พัฒนาการเตรียมตัวอย่างด้วยเมมเบรนไดแอลิซิสเพื่อวิเคราะห์หาปริมาณไอโอไดด์ในนมและไข่ โดยใช้วิธีไทโอนิตในการตรวจวัด ได้ศึกษารูปแบบการไดแอลิซิสแบบต่างๆ ด้วยเมมเบรนเส้นใยกลวงชนิดพอลิซัลโฟน โดยให้สารละลายป้อน (สารละลายไอโอไดด์) หรือสารละลายรับ (น้ำปราศจากไอออน) ไหลวนผ่านด้านในของเมมเบรนที่แช่อยู่ในสารละลายอีกชนิดหนึ่ง พบว่าให้เปอร์เซ็นต์ไดแอลิซิสค่อนข้างสูง แต่ใช้เวลาไดแอลิซิสนาน และพบผลจากการกรองเกิดขึ้น เมื่อให้สารละลายป้อนและสารละลายรับไหลวนสวนทางกันผ่านเมมเบรน พบว่าให้ผลใกล้เคียงกับรูปแบบที่ผ่านมา แต่เมื่อบรรจุสารละลายป้อนภายในเมมเบรนและให้สารละลายรับไหลผ่านด้านนอกโดยไม่มีการไหลวน พบว่าตัวอย่างเพียง 0.2 มิลลิลิตร สามารถให้เปอร์เซ็นต์ไดแอลิซิสเพียงพอสำหรับการตรวจวัดที่เวลาไดแอลิซิสน้อยกว่า 10 นาที และไม่พบผลจากการกรองเกิดขึ้น นอกจากนี้ที่อัตราการไหลของสารละลายรับ 1 มิลลิลิตรต่อนาที จะให้เปอร์เซ็นต์ไดแอลิซิสเพียงพอสำหรับการตรวจวัดในระยะเวลาที่สั้น จากนั้นปรับปรุงองค์ประกอบของสารละลายป้อน โดยใช้สารละลายผสมของโซเดียมคาร์บอเนต และโซเดียมไฮโดรเจนคาร์บอเนตที่ความเข้มข้นต่างๆ พบว่าเปอร์เซ็นต์ไดแอลิซิสเพิ่มขึ้นอย่างมีนัยสำคัญ เนื่องจากผลของความแรงไอออนที่มีต่อกลไกการไดแอลิซิส แต่เมื่อเพิ่มความเข้มข้นของสารละลายเกลือเปอร์เซ็นต์ไดแอลิซิสกลับลดลง เนื่องจากสารละลายป้อนอาจมีความหนืดเพิ่มขึ้น ทำให้ไอโอไดด์ไอออนแพร่ได้ช้าลง วิธีนี้ให้ช่วงความเข้มข้นในการตรวจวัดที่เป็นเส้นตรงระหว่าง 0 ถึง 480 ไมโครกรัมต่อลิตร ขีดจำกัดต่ำสุดในการตรวจวัดที่ 32.9 ไมโครกรัมต่อลิตร ขีดจำกัดต่ำสุดในการวิเคราะห์ปริมาณที่ 97.7 ไมโครกรัมต่อลิตร และค่าการคืนกลับที่ได้จากการตรวจวัดสารอ้างอิงมาตรฐานนมไขมันต่ำ 89.7 เปอร์เซ็นต์ ที่ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ 9 เปอร์เซ็นต์ นำวิธีการนี้ไปใช้ในการเตรียมตัวอย่างนมและไข่ พบว่าในตัวอย่างนมที่มีการเติมไอโอไดด์ ให้ค่าการคืนกลับอยู่ในช่วง 100 ถึง 115 เปอร์เซ็นต์ ที่ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ 4 ถึง 22 เปอร์เซ็นต์ และในตัวอย่างไข่ที่มีการเติมไอโอไดด์ ให้ค่าการคืนกลับอยู่ในช่วง 100 ถึง 112 เปอร์เซ็นต์ ที่ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ 15 ถึง 22 เปอร์เซ็นต์

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KANOKWAN CHUNHONG : SAMPLE PREPARATION USING MEMBRANE DIALYSIS FOR DETERMINATION OF IODIDE ION IN MILK AND EGG SAMPLES. ADVISOR : ASSIST.PROF.PAKORN VARANUSUPAKUL, Ph.D., 83 pp.

The sample preparation using membrane dialysis for iodide determination in milk and egg samples was developed. Thio-nit method was used for iodide detection. Several dialysis configurations with polysulfone hollow fiber membrane were investigated. When donor solution (iodide solution) or acceptor solution (deionized water) was recirculated through the membrane which was immersed in the other solution, high %dialysis was obtained, however, dialysis time was quite long and filtration effect was found. The similar effects were observed when the donor and acceptor solutions were recirculated countercurrently across the membrane. When the donor solution was fixed inside the membrane while the acceptor was flown outside in non-recirculation system, only 0.2 mL of sample could provide sufficient %dialysis for dialysis time less than 10 min and the filtration effect was not found. Furthermore, it was found that 1 mL/min of acceptor flow rate could provide sufficient %dialysis in short dialysis time. The donor composition was optimized using the mixture of Na_2CO_3 and $NaHCO_3$. It was observed that the %dialysis was significantly increased due to the ionic strength effect on the dialysis mechanism. However, as higher salt concentration, the % dialysis was decreased. Because of the solution may become more viscous, the diffusion of iodide ion would be slower. The linear dynamic range was 0 to 480 µg/L. The limit of detection and the limit of quantitation were 32.9 µg/L and 97.7 µg/L, respectively. The recovery of 89.7% was obtained from skim milk powder standard reference material with 9 %RSD. This method was applied to milk and egg samples. The recovery of spiked milk and egg samples were in the range of 100-115 % with 4-22 %RSD and 100-112% with 15-22 %RSD, respectively.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abs	Absorbance
AU	Atomic Unit
^{o}C	degree Celcius
cm	centimeter
g	gram
hr	hour
ID	Inner Diameter
kDa	kiloDalton
kg	kilogram
L	Liter
LOD	Limit of Detection
LOQ	Limit of Quantitation
ng	nanogram
M	Molar
mm	millimeter
mM	millimole
mL	milliliter
mg	milligram
min	minute
OD	Outer Diameter
R	correlation
RSD	Relative Standard Deviation
sec	second
SD	Standard Deviation
µg	microgram
µm	micrometer
µL	microliter

CHAPTER I

INTRODUCTION

Iodine is an essential nutrition for all animal species, including human. The human body contains about 25 mg of iodine, 10 mg of which is in the thyroid gland [1]. It is an integral component of the thyroid hormones that play an important role in the brain development, physical and mental growth, several organs function, circulatory activity and the metabolic processes. In food, iodine is present in the forms of iodide salts or iodate salts. Iodine deficiency can cause irreversible health problems including goiter, mental retardation, cretinism and childhood mortality [1-2]. Generally, the amount of iodine daily intake of 150 µg of iodide is required for adult but it should not exceed 1 mg of iodide/day (WHO). To prevent iodine deficiency disorder, many food products have been fortified with iodine, such as drinking water, tablets, salts, milks, as well as eggs. Therefore, determination of iodine content in iodine fortified food is important for controlling the quality of food products and providing information about iodine content to the consumer.

Sample preparation is currently regarded as one of the crucial steps in the analytical process as well as the bottleneck in several analytical methodologies. It depends on the sample, the matrix, and the concentration level at which the analysis needs to be carried out. Generally, quantitative analysis of iodine in food employs sample preparation for destruction of organic matter. The liberated iodine may be converted into an appropriate form prior to iodine determination.

Several sample preparation techniques for determination of iodine have been used. The technique most commonly employed is alkaline dry ashing [3-6]. Ashing aids such as potassium carbonate and zinc sulphate were added into food samples such as milk, egg and fish. The mixture was dried overnight and ignited approximately 2.5 hr. Then, zinc sulphate was added to the charred residue and the drying and ashing procedure was repeated. The cool ash was transferred to centrifuge tube with distilled water, spun for 5 min and decanted about half of the solution for analysis of total iodine by semi-automatic catalytic method [3]. In this technique, the

organic matter in sample was destructed and the liberated iodine was analyzed. Although it is simple and has productivity potential, it is time-consuming because of involving many steps. The major drawbacks of this method are the loss of some iodine by volatilization, contamination of sample by airborne dust, and irreversible sorption of iodine into the wall of vessel. In addition, particles generated within the muffle furnace may be the cause of high and variable blanks that can not be tolerated when the concentration iodide found in the blank and the sample are similar [7].

Schöninger combustion was applied to the sample preparation for determination of iodine [5, 8]. The biological materials sample such as serum, milk, plants was wrapped in filter paper, ignited and quickly introduced into the flask. The sample burned in the oxygen atmosphere while volatile iodine formed was collected by sodium hydroxide as receiver. Then, the flask was cooled down in approximately 60 min, opened and rinsed with water. The solution was determined by inductively coupled plasma mass spectrometry (ICP-MS) [9]. This approach was not suitable for performing in a large number of samples, because a cooling down period was needed for each sample. In addition, their limit of detections were limited by the small maximum sample size combusted and the contamination of sample by the filter paper, so the trace-O-Mat oxygen combustion apparatus was used for solving these problems [10]. Nutritional and biological samples such as oyster tissue, milk powder, and egg were mineralized by trace-O-Mat oxygen combustion. The sample was pressed into a pellet and placed on a sample holder, to be incinerated by two infrared radiators within oxygen stream. A residue of nonvolatile elements remained in the burning chamber while the volatile products of combustion were condensed against the walls of the overlying cooling unit. When the combustion was completed, the sample holder was turned upside down to lay at the bottom that was filled with amine solution. Infrared radiators were used to boil the reagent under reflux for 30 min, thus collecting both volatile and nonvolatile elements in the dissolved phase. Total iodine was determined by ICP-MS. This equipment mineralized as much as 1 g of sample and eliminated the need for wrapping the sample in the filter paper; however, trace-O-Mat oxygen combustion was inconvenienced by the use of liquid nitrogen for cooling system. The disadvantages of this technique were the high ratio of the flask surface to the sample amount resulting in analytical errors by adsorption and

desorption effect, including the use of unique device that was not available in common laboratory.

Liquid extraction and microwave-assisted digestion were also used as sample preparation technique for iodine measurement [8]. For liquid extraction, milk powder or bovine liver in closed vessel spiked with ^{129}I-enriched iodate was extracted with tetramethylammonium hydroxide at 90 ℃ for 3 hr. For microwave-assisted digestion, the sample in vessel spiked with ^{129}I-enriched iodate was digested with $HClO_4/HNO_3$ mixture for 45 min. Both extracts were diluted with water and measured for isotope ratio $^{129}I/^{127}I$ by ICP-MS [11]. Comparing to microwave-assisted digestion, liquid extraction was simpler with respect to the instrument but normally more time-consuming and could not really guarantee a 100% iodine extraction in all cases. Although microwave-assisted digestion had advantages of fast and complete decomposition of samples, the use of acid and microwave energy together for sample decomposition must be operated carefully.

More recently, membranes have gained widespread use in sample preparation as they are selective separation, low energy consumption, and simple operation with gentle condition. Dialysis is a sampling technique based on size selective diffusion driven by concentration gradient across a semi permeable membrane. The analytes whose sizes are smaller than the pores of the membrane would diffuse across the membrane whereas the larger size matrices would not. As there is no applied pressure, membrane fouling didn't occur in this process. In addition, dialysis is simple, fast, low energy consumption due to not involving phase inversion and cost effective process for a wide range of sample volumes [12-14].

There were reports for determination of ion using dialysis as sample preparation technique [15-16]. A manually operated flow injection dialysis (FID) system was combined with an automated ion chromatography (IC) system equipped with conductivity detector for analysis of standard inorganic anions in natural water samples [17]. The samples were injected into the donor stream where it was combined with 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ as a modifier and passed through a mixing coil and then into the dialysis unit. The dialysis cell consisted of two symmetric acrylic blocks that engraved for groove-path of solution, where a wrapping foil as dialysis membrane was sandwiched. The analytes were allowed to permeate

the membrane and it was injected into the IC system for analysis. FID provided simple on-line separation and dilution of the analytes from matrix especially from some species such as proteins, surfactant, particulates which might cause damage to the IC columns. The life-time of the IC columns which were usually expensive was prolonged. In addition, a wider concentration range could be obtained with high reproducibility. On-line microdialysis ion chromatography was used for the determination of inorganic anions (chloride, nitrite, nitrate, phosphate and sulphate) in olive-oil mill effluents [18]. To made homogenous sample, the wastewater was sonicated at room temperature for 10 min, then diluted and microdialized. The sandwich type dialyzer fitted with a cellulose triacetate membrane with molecular weight cut-off (MWCO) 100 kDa was used. After 10 min the acceptor solution was on-lined transferred to IC equipped with conductivity detector. Most of organic contents in the effluents were removed within a few minutes, while soluble anion quantity remained unaffected. When overcoming a 10 min of microdialysis time, 96-104% of spike recoveries and an average of 4% of relative standard deviations were obtained for all the anions.

In addition, microdialysis has also been applied to the determination of ions in environmental, food and industrial samples [19]. Anions and cations in various matrices were determined by using on-line dialysis as a sample preparation technique for ion chromatography. The method was based on stopped-flow dialysis, where the diluted samples were continuously dialyzed for 10 min while the acceptor solution was stationary within the recipient channel. The dialysis cell was made of two Plexiglas blocks where a cellulose acetate membrane with pore size = 0.2 μm was sandwiched. Deionized water was used as acceptor solution for the anions analysis and 2 mM HNO_3 was used for cations analysis to minimize any potential complexing between the analytes and organic solutes present in the sample matrix. This system was applied to the analysis of real samples such as milk, untreated waste water, engine coolant and a multivitamin tablet without additional sample treatment. The analyte recoveries ranged from 87-106% and repeatability were found in the range of 0.2 to 4%. In addition, it was found that the analysis of these samples did not influence the column lifetime and macromolecular components remained on the donor side of dialysis membrane.

On-line dialysis system coupled to a flow injection analysis-capillary electrophoresis (FIA-CE) interface was presented for the treatment of complex matrices (milk, juice, slurry and liquors from pulp and paper industry) [20]. The sample was continuously pumped into the dialysis unit which consisted of a sandwich type dialyzer with a Cuprophan dialysis membrane. The analyte anions penetrated the membrane and entered into the acceptor stream of water. A representative sample was injected into an electrolyte stream which continuously passed through the FIA-CE interface into which the end of a capillary had been inserted. A small fraction was electrokinetically introduced into the capillary where separation took place. The repeatability was in the range 1.6-3.3% and a wide range of real samples with complicated matrices was analyzed without any off-line pretreatment.

Several dialysis techniques for the determination of drug in egg were described in the literatures [21-22]. The combination of on-line continuous flow dialysis, on-line pre-concentration and high-performance liquid chromatography analysis was applied for determination of meticlorpindol in whole egg, egg white and yolk. The sample was diluted, homogenized for 30 sec and centrifuged to minimize the danger of blocking the continuous flow part for 10 min. The homogenized sample was dialyzed on-line against water using a dialyzer of sandwich type. The dialysate was concentrated on-line on a short reversed-phase column and then was transferred to the reversed-phase analytical column by means of the mobile phase. When egg extract was mixed with NaOH before dialysis, the dialysis recovery was raised from 5% to around 17%. In addition, the reproducibility was about 2% and linear calibration graphs were obtained in the range 40-900 ng/g in whole egg and egg white (detection limit 10 ng/g) and 80-1800 ng/g in yolk (detection limit 20 ng/g) [23].

An automated analytical method was used for simultaneous determination of all tetracyclines and metabolites in whole egg, egg yolk, egg white and blood plasma of the hens [24]. This method used a hyphenated dialysis with solid-phase extraction for clean-up based on the automated sequential trace enrichment of dialysates (ASTED) system and high-performance liquid chromatography (HPLC) with UV, fluorescence or tandem mass spectrometry (MS–MS) detection. Sample was homogenized, diluted with buffer, pH 5.75 for keeping egg protein dissolved and then injected into the donor side of the dialysis cell. The dialysis unit was composed of two

dialyzer blocks and a Cuprophan dialysis membrane with 15 kDa MWCO. While the sample was held in the static mode for 15.5 min in the donor channel, the same buffer as the acceptor solution was transported through the acceptor channel in four pulses. Effective dialysis time was 12.5 min. After dialysis, dialysate was transferred to trace enrichment column, and then the enriched analytes were flushed onto analytical column. The limit of detection and quantitation were between 11 and 15 µg/kg or 34 and 45 µg/kg respectively and the relative recoveries were between 90% and 99% for all analytes.

The diphasic dialysis based on the liquid–liquid extraction, with non-polar solvents, of low molecular mass analytes contained in an aqueous phase was used as extraction and purification method for the analysis of the fluoroquinolones enrofloxacin and ciprofloxacin in eggs [25]. The eggs were homogenized and mixed with buffer, pH 6 to obtain neutral molecules in an Erlenmayer flask. To avoid hydrogen bonding between membrane and analytes, the mixture was mixed again with isopropanol. Then, a dialysis tubing of regenerated cellulose (MWCO 12-14 kDa) containing dichloromethane with isopropanol was introduced into the Erlenmeyer flask. Extractions were performed by stirring at 37 ◦C for 4.5 hr in a shaker incubator. The content of the dialysis tubing was evaporated to dryness and redissolved with water and injected into HPLC–MS system. The method was found wider concentration range with high repeatability and reproducibility. The recovery percentages were in the 70–104% range for enrofloxacin, and 55–97% for ciprofloxacin. The limit of quantitation of 2 and 4 ng/g for enrofloxacin and ciprofloxacin in egg were obtained respectively. The use of diphasic dialysis for the extraction allowed to reduce the LOD because of a great amount of sample could be extracted. Additionally, this method did not require the operator to perform much work and requires a low volume of organic solvents, mainly because extraction and purification of the extracts were performed in a single step.

According to the review of literatures, dialysis offered several advantages including simple, fast, highly efficient sampling and capability for clean-up sample with complicated matrices. So, dialysis is interesting technique and should be further applied to various samples. Since sample preparation step for determination of iodide in water, salt and tablet is relatively simple, while in complex matrices such as milk

and egg are quite complicated, tedious and time-consuming. For this reason, development of the sample preparation method for determination of iodide in complex matrices such as milk and egg are challenging.

In the present work, the sample preparation based on hollow fiber membrane dialysis has been developed for determination of iodide ion in milk and egg samples.

CHAPTER II

THEORY

2.1 Dialysis

Dialysis is one of techniques based on barrier separation processes. The separation of species depends on the difference between their rates of transfer across a semi permeable membrane located between the two liquids [26].

Dialysis processes can be classified according to the dynamic state which one or two phases involved. The process may or may not be allowed to develop until mass transfer equilibrium is reached. If the two phases are quiescent, then the process must develop to equilibrium, however, if one or both phases are in motion or if either is stopped over a given interval to increase the process efficiency – equilibrium is never reached and precise timing must be used. In the latter case, the process can be carried out with agitation of a constant volume of the two phases or by using streams of the two phases that may flow concurrently. Static dialysis which very popular in laboratory bench dialysis is less efficient than dynamic dialysis. In dynamic dialysis, the two phases move at various velocities across the membrane surface helping to minimize boundary layering of retained solutes or particulates from accumulating on the surface of the membranes (fouling) [13, 26].

Dialysis can be achieved in four modes; the first and the last mode are the most widely used.

1. Passive or conventional dialysis
2. Active dialysis in the Donnan mode
3. Active dialysis in the electrodialysis mode
4. Microdialysis (MD), which is also a passive mode.

The passive process involves diffusion of particles within a given range of molecular weight across a neutral membrane. Donnan dialysis involves the transfer of ions with a given type of charge across an ion-exchange membrane. Ions of appropriate charge from the acceptor solution, of smaller volume and higher ionic

strength are transported into the sample solution as a result of the existing ionic strength gradient, while co-ions from the sample solution, including analyte ions, diffuse in the opposite direction in order to maintain electroneutrality. In electrodialysis, the solutes (electrolytes) are transferred across a membrane on application of electrical energy. Unlike passive dialysis, electrodialysis and Donnan dialysis also afford preconcentration of the analyte. However, electrodialysis requires complicated instruments and Donnan dialysis needs the acceptor solution of high extremely ionic strength [27-28].

2.2 Passive dialysis [12-13, 26-33]

Conventional, passive dialysis is the separation process involves diffusion of solute across the semi permeable membrane from a liquid with higher concentration to liquid with lower concentration. The driving force of process is the concentration gradient between two liquids on each side of membrane. This technique is typically used in analytical chemistry to separate small molecules from macromolecules, colloidal matters and suspended particles.

2.2.1 Equipment

The heart of dialysis process is dialyzer and membrane. The dialyzer module or probe is connected with the conduits or other units to perform the separation process.

2.2.1.1 Dialyzer

2.2.1.1.1 Dialyzer module

Dialyzer modules are accessible in variety designs according to their origin and application. They can be classified into two main categories: sandwich type and tubular or hollow fiber type.

Sandwich (parallel plate) type is conventional dialyzer that made of Teflon, Acrylic glass, Aluminum or other chemically and mechanically resistant materials. It consists of two blocks which are mirror images. Each block is engraved with semi circular, triangular or rectangular groove (usually 0.1-1 mm deep and 0.5-2 mm wide) as shown in Figure 2-1 and the path lengths could vary between 25 mm and 600 mm. The membrane is placed between the two blocks which are tightly screwed to each other to prevent leakage. The dialysis efficiency is increased with increased exchange surface, although the increase in dispersion or dilution of analyte is found. In both channels, the solutions move at various velocities in parabolic flow due to the frictions against the channel walls, thus beads are filled in the channels to reduce their dispersion and to also support the membrane. The beads are made from of inert material (usually glass) and the size of them (0.15-0.25 mm in diameter) depends on the depth and width of the channels. To keep the beads in position, polypropylene nets are inserted at the inlets and the outlets of the channels. Generally, the donor chamber is above the acceptor chamber in order to favor mass transfer. The dialyzer of sandwich type with different channel designs was displayed in Figure 2-2.

Figure 2-1 Cross-sectional view of the most common types of channels used.

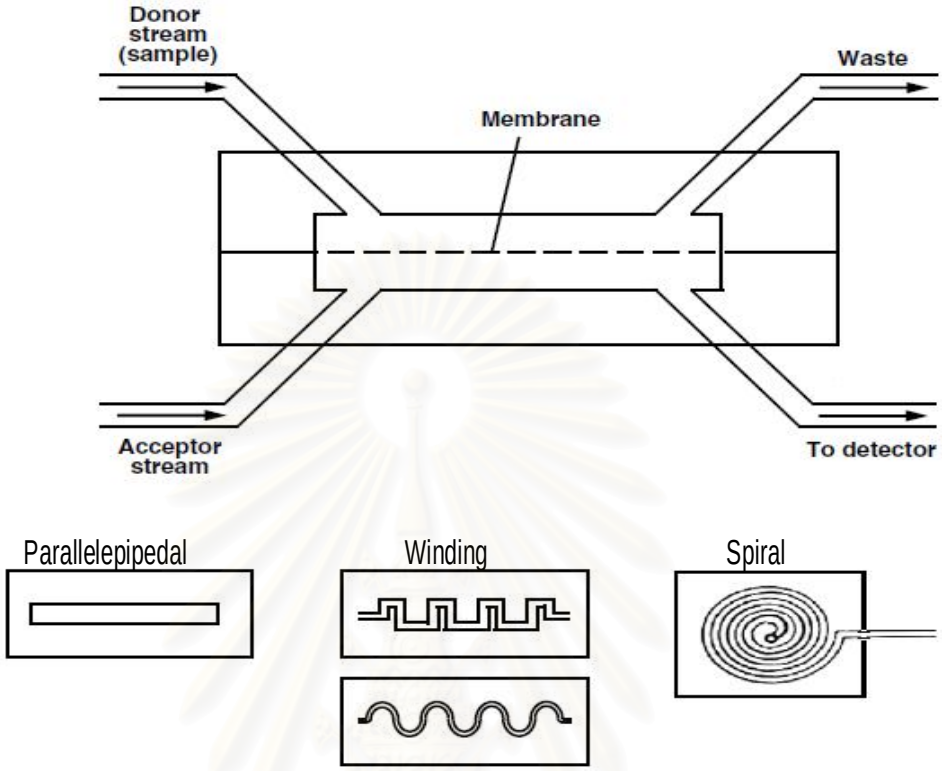

Figure 2-2 Scheme of sandwich type dialyzers with different chamber designs [27].

Tubular module comprises the two concentric tubes. The inner tube is porous tube of an appropriate polymer (Teflon). The sample solution flows inside porous tube while species which have suitable size for the pores of this tube diffuse into the acceptor solution that flows outside or vice versa. The diameter tubes ranges between 0.3-1.0 mm (inner tube) and 0.5-2.0 mm (outer tube), while their lengths range 3-15 cm. The tubular module was shown in Figure 2-3.

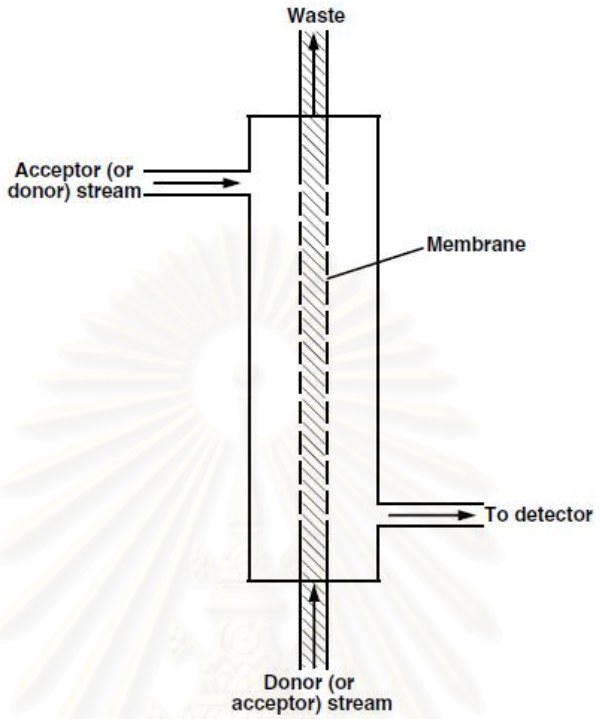

Figure 2-3 Scheme of a tubular or hollow fiber type dialyzer [27].

These dialyzers are manufactured as single device or part of continuous analyzers. Continuous analyzers are often used in clinical or environmental laboratories due to that they are included in standard method. According to the different demands, researchers most frequently design their own modules. This can lead to the development of dialyzers with different design.

2.2.1.1.2 Microdialysis probe

Microdialysis probes are commonly used in microdialysis sampling and made of stainless steel, fused silica or the combination of two materials. Generally, the outer diameter of probe is about 300 μm and the active window (membrane window) ranges from 4-10 mm. The small size of them can reduce the

disturbance to target tissues or surrounding. Microdialysis probes can separate into four types as being shown in Figure 2-4.

1. Stainless steel, concentric cannula
2. Flexible, side-by-side cannula
3. Linear
4. Flow-through, shunt or by-pass

The concentric cannula, also known as “pin-style, rigid cannula” (Figure 2-4a) is comprised of an inner and an outer length of stainless steel tubing. The inner cannula extends beyond the outer and is covered with membrane. This type of probe is mechanical stand and suitable to fix in the brain, but not proper for implantation in peripheral tissue.

The side-by-side cannula or the pin-style flexible cannula is used for implantation in intravenous (Figure 2-4b). This probe modified from the concentric cannula consists of fused silica tubes attached side-by-side to each other. The one piece of fused silica extends beyond the other and is covered with a dialysis membrane. According to the flexible design, it is allowed to bend as the animal moves without any structural damage.

For the sampling in peripheral tissue, the linear probe is often used (Figure 2-4c). The hollow fiber membrane is connected to the small bore tubings that made of Teflon, polyaryletheretherketone, polyethylene or fused silica. The one tubing forms an inlet of sample and the other forms an outer. The length of dialysis membrane can be stretched as it is more flexible than fused silica. This probe can be threaded through the target tissue.

The flow-through probe, as shown in Figure 2-4d is used for sampling in the bile duct. The polyethylene tubing functions as a shunt to bring the biological fluid past the dialysis membrane while the perfusate flow through the dialysis membrane. Thus, the analyte diffused into the perfusate, the separation is occurred.

Microdialysis probes are commercially available in the market under different trade names.

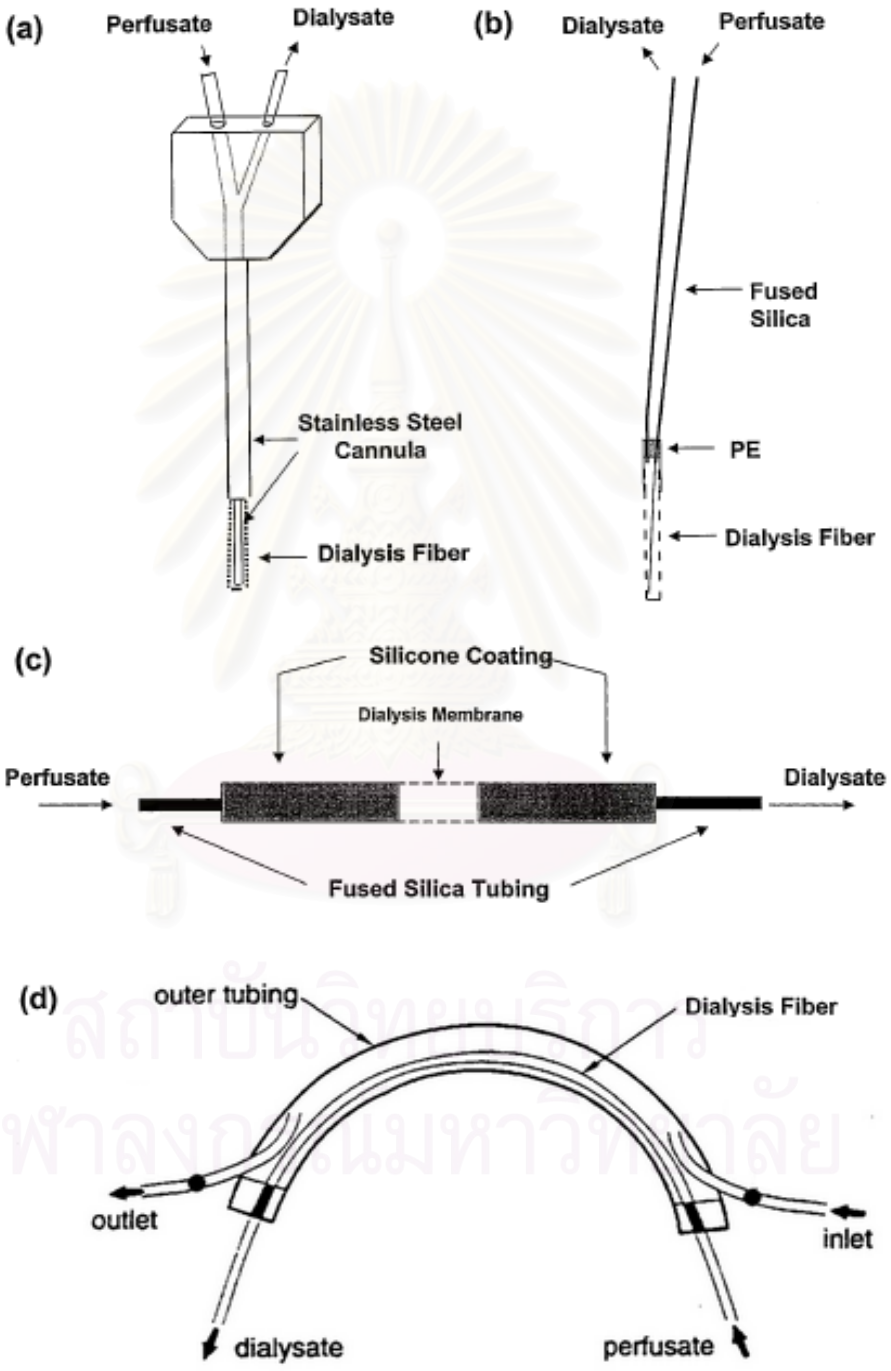

Figure 2-4 Schemes of different types of microdialysis probes: (a) Concentric cannula; (b) side-by-side cannula; (c) Linear; and (d) Flow-through [31-32].

2.2.1.2 Membrane

In general, dialysis membranes are usually hydrophilic membrane with MWCO range of 3-30 kDa and are classified base on the structure as microporous, homogenous or ion-exchange membranes.

Microporous membranes are similar to conventional filter in structure and principle operation. Microporous membranes are rigid with high void volume and the range of pore sizes are 1-10 nm which is a lot smaller than the conventional filters. The mass transfer through the membrane depends mainly on different pore sizes. Particles with a size larger than the largest pore size of the membrane are retained while particles smaller than the smallest pore size move through easily. Intermediate particles are only partly allowed to move through if they find the correct pore size that distribute throughout the membrane. Typically, these membranes are made of polytetrafluoroethylene, cellophane, cellulose acetate, polycarbonate, polysulfone, polyacetal, polyacrylate, copolymer of acrylonirile and vinyl chloride, polyelectrolyte complexes, cross-linked polyvinyl alcohol and acrylic copolymers. They are filled with both donor and acceptor solutions and the molecules of appropriate sizes can be separated from the other.

Homogenous or nonporous membranes are homogenous films with interfaces distributed uniformly through the membrane. Mass transfer across membrane occurs via molecular diffusion and dialysis efficiency depends on the solubility and the diffusion of analyte across the membrane interface. So, homogenous membranes permit same size particles to be separated if their solubilities in membrane are sufficiently different.

Ion-exchange membranes have a sub-microporous structure and no conventional macroscopic pores. These membranes consist of film forming polymers with positively or negatively charged ions fixed to the pore walls, hence, they function as anion or cation exchangers respectively. An ionic reaction/diffusion makes a mass transfer across

membrane and the separation efficiency not only depends on their solubility and diffusion but also upon the exclusion of membrane co-ions on the same charged species.

Membrane can be classified as flat sheet and hollow fiber based on the geometry. As name suggests, flat sheet membranes are flat as paper sheet which are made as thin as less than 1 μm. They need the holders to hold them in place. Hollow fiber or tubular membranes are tubes which inner diameter ranges from 200 to 500 μm. Donor and acceptor solutions can be flown through or flown over hollow fiber membrane. Although flat sheets are easy to design and construct the dialyzer, hollow fibers are self-supported and provide larger surface area per unit volume and high packing density. A large number of fibers can be parallel packed into a small volume.

2.2.2 Factors influencing dialysis efficiency [7, 12, 26-31]

Mass transfer efficiency of dialysis is expressed as the percentage of dialysis as follows:

$$\% \text{ Dialysis} = (C_d/C_s)*100$$

where C_d is the analyte concentration found in the dialysate solution and C_s is the initial analyte concentration in the sample or donor solution. In order to achieve high efficiency of mass transfer, the variables could be properly optimized. Two major parameters involved with the dialysis efficiency are the membrane properties and experimental parameters.

2.2.2.1 Membrane variables

2.2.2.1.1 Membrane composition

Nature of semi permeable membranes can affect performance of dialysis process. The exact composition of most membrane materials is not known owing to Patent, so it is difficult to decide the membrane performance due to their composition. However, the properties of the same type of membranes such as cellophane and cellulose acetate are similar in the mass transfer efficiency due to they are also made from regenerated natural cellulose.

2.2.2.1.2 Membrane path length

Efficiency of mass transfer is increased with increased dialyzer path length. In flow injection dialysis, the best results can be obtained with path length nearly 300 mm. If the path length is longer than 300 mm, the flow dynamic may have changed unusually as the differential pressure changes resulting to an impact on the mass transfer efficiency. However, shorter path length can increasingly make a difficulty to detect analyte in dialysate, especially when low analyte concentration of donor solution was used.

2.2.2.1.3 Membrane porosity

In dialysis process, mass transfer occurs through diffusion across the membrane, so the pore size of membrane has an important role in the diffusion of species. Generally, dialysis efficiency tends to increase when pore size is increased. However, the selection of the suitable pore size leads to appropriate

separation between macromolecular matrices and analyte in short time. For homogenous membranes, though they actually exist as a gel, they act as a membrane with fixed pore size. Thus, any stress on the membrane should be avoided to keep a constant porosity.

2.2.2.1.4 Membrane thickness

When membrane thickness is decreased, efficiency of mass transfer is increased. However, the membrane life is decreased with decreased the membrane thickness. It should be compromise between the mass transfer and the membrane life.

2.2.2.1.5 Membrane geometry

Mass transfer efficiency may depend on membrane geometry in the meaning of different contact area. It supposes that the increased membrane contact area leads to improve the dialysis efficiency. Consequently, hollow fiber membrane which provides larger surface area per unit volume than flat sheet membrane could enhance the dialysis efficiency. In addition, the dialysis efficiency can be improved by increasing in diameter and/or length of membrane.

2.2.2.2 Experimental parameters

2.2.2.2.1 Flow direction

Dynamic dialysis is utilized in two modes of operation, namely concurrent and countercurrent flow. Concurrent flow is defined as a dynamic condition which donor and acceptor solutions pass dialyzer in same direction while in countercurrent

flow donor and acceptor solutions flow in opposing direction. The two modes of dynamic dialysis are shown in Figure 2-5.

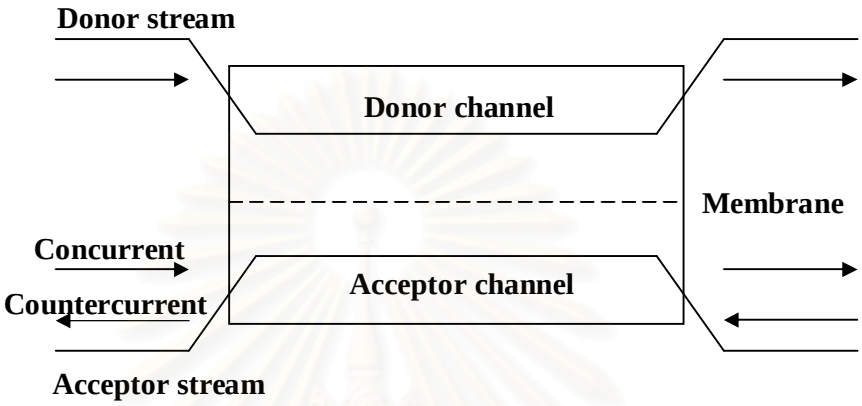

Figure 2-5 Scheme of dynamic dialysis in concurrent and countercurrent modes.

Flow direction of solution streams is one of factors that affect on dialysis performance. When donor and acceptor solutions flow in concurrent mode the equilibrium time is shorter than in countercurrent mode and less cross-contamination between samples is found due to more efficient washing. The concurrent flow offers smaller differential pressure across the membrane and smaller dispersion in a long dialyzer. However, the countercurrent flow provides higher efficiency than the concurrent flow.

2.2.2.2.2 Flow rate

Mass transfer efficiency is increased with decreased flow rate of solutions due to the rise in contact time between membrane surface and analytes. However, lower flow rate may affect precision of results.

2.2.2.2.3 Composition of donor and acceptor solutions

Composition of donor and acceptor solutions is important factor that leads to successful dialysis process. Nevertheless, there is no generalization to choose the composition due to that it depends on the nature of separated analytes.

2.2.2.2.4 Temperature

Temperature has an influence on mass transfer efficiency and could therefore be controlled in order to get reproducible results. There has been a study of the temperature change; it was found that the temperature change of 0.1 °C would result to efficiency loss of 0.26%.

2.3 Catalytic colorimetric method

Several methods have been used for determination of iodine, including ICP-MS, spectrometry, chromatography, electrochemical, neutron activation and colorimetric methods [34-40]. The catalytic colorimetric method has been widely used for determination of iodide in biological, clinical and food samples .There are two main catalytic colorimetric methods that are employed. The Sandell and Kolthoff method (SK method) is based on the catalytic effect of iodide on the reduction of yellow colored cerium (IV) to colorless cerium (III) by arsenious acids. The other method is the Sveikina method (Thio-nit method) that is based on the iodide catalytic action on the color destruction of iron (III) thiocyanate by nitrite. Both methods have been used for determination of iodide at µg/L level and offer high sensitivity, easy operation and cost-effectiveness. However, the SK method gives a curve of calibration graph while the Thio-nit method gives a straight-line calibration curve. Moreover, the Thio-nit assay provides better precision and sensitivity [3-4].

2.4 The Thio-nit method [41]

In this method, iodide ion catalyses the decomposition of iron (III) thiocyanate complex (red) by nitrite in acid and the fade out of the complex can be determined the absorbance of light at 450 nm. The Sveikina reaction is autocatalytic in nitrous acid and depends on iodide concentration. The equation of reaction is as follows:

$$2SCN^-_{(aq)} + 3NO_2^-{}_{(aq)} + 3NO_3^-{}_{(aq)} + 2H^+_{(aq)} \longrightarrow 2CN^-_{(aq)} + 2SO_4^{2-}{}_{(aq)} + 6NO_{(g)} + H_2O_{(l)}$$

There were several reports for determination of iodine using the Thio-nit method [3-4, 41]. A flow injection method based on catalytic action of iodide was presented and utilized for determination of iodine in milk samples [5]. After organic matter was destructed, the sample was injected to the flow injection system to determine iodine concentration. This method offered the linear response up to 100 µg/L with the correlation, R = 0.9996. The detection limit was 0.99 µg/L and the recoveries ranges between 94.5 and 105%. In addition, the Thio-nit method was also applied for determination of iodine in fortified culinary products [42]. The presented method provided the linear calibration range from 0 to 12 µg/L with $R^2 > 0.99$. The iodine recoveries were in the range of 100 ± 10% and the limit of quantitation was about 2 mg/kg of product. The iodine concentrations obtained from this method were similar to those by ICP-MS assay. From these reports, the Thio-nit method is a sensitive method that can be used for determination of iodine. Moreover, this method is easy to operate and requires only simple laboratory equipments.

CHAPTER III

EXPERIMENTAL

3.1 Instruments and Equipments

1. UV-visible spectrophotometer, HP 8453 (Hewlett Packard, USA)
2. Syringe pump (Prosense B.V., USA)
3. Peristaltic pump (Masterflex, USA)
4. Stirrer (Fisher scientific, USA)
5. Polysulfone hollow fiber membrane with ID 1100 µm, wall thickness 270 µm and porosity 30% (Vifil 4040, ultrapure, Thailand)
6. Home-made dialyzers for Dialysis system 2 consisted of a plastic tubing (10.0 mm OD x 0.57 mm ID x 26.7 cm length) with both ends were attached to three-way pipes by using two-way pipes as connectors and all linking parts were sealed with glue to prevent leakage.
7. Home-made dialyzers for Dialysis system 3 consisted of a glass tubing (5.10 mm OD x 3.25 mm ID x 30 cm length) with both ends were attached to three-way pipes by using polyvinyl chloride tubing as connectors and all linking parts were sealed with glue to prevent leakage.
8. Tubing with ID 0.8 mm (Tygon, precision tubing, Masterflex)
9. Flexible polyvinyl chloride aquarium tubing (purchased locally)
10. Epoxy glue gun
11. Plastic syringes 3 mL and 20 mL (BD, Singapore)
12. Hypodermic needles with ID x OD (mm): 0.5 x 0.8, 0.6 x 0.9 and 0.8 x 1.2 (NIPRO, Japan)
13. Autopipettes and tips (Eppendorf, Germany) 10 and 100 µL (BRAND, Germany) 1000 and 10000 µL
14. Volumetric flasks 10, 25, 50,100, 250, 500 and 1000 mL (class A, witeg, Germany)

15. Beakers 10, 25, 50, 100, 150, 500 and 1,000 mL (SCHOTT DURAN, Germany)
16. HDPE bottles with screw cap 10, 50 and 1000 mL (NALGENE, USA)
17. Conical centrifuge tube 10 mL
18. Magnetic bar

3.2 Chemicals and Reagents

1. Potassium iodide (CARLO ERBA, France)
2. Potassium thiocyanate (CARLO ERBA, France)
3. Iron(III) ammonium sulfate (Ajax Finechem, Australia)
4. Sodium nitrite (CARLO ERBA, France)
5. Nitric acid 95% (MERCK, Germany)
6. Ethanol 99.9% (MERCK, Germany)
7. Sodium hydrogen carbonate (MERCK, Germany)
8. Sodium carbonate (J.T.Baker, USA)
9. 1-Pentanol (FLUKA, USA)
10. Standard Reference Material, BCR-151 (Institute for Reference Material and Measurements, Belgium)
11. Milk and egg samples (purchased locally)

3.3 Preparation of chemical solution

3.3.1 Stock standard iodide solution

The stock standard iodide solution of 1000 mg/L was prepared by dissolving 32.7 mg of potassium iodide in deionized water and diluting the solution in a 25 mL volumetric flask.

3.3.2 Reagents for colorimetric method

3.3.2.1 Intermediate standard iodide solution

The intermediate standard iodide solution of 2 mg/L was prepared daily by pipetting 50 µL of the stock standard iodide solution into and adjusting to 25 mL with deionized water.

3.3.2.2 Working standard iodide solutions

Working standard iodide solutions of 4, 8, 12, 16 and 20 µg/L were made from the intermediate standard iodide solution of 2 mg/L. A 100, 200, 300, 400 and 500 µL of the intermediate solution were transferred into five 50 mL volumetric flasks respectively and then, the volumes were made up to the mark with deionized water.

3.3.2.3 Potassium thiocyanate solution 0.023% (m/V)

A 0.23 g of potassium thiocyanate (KSCN) was dissolved in deionized water and diluted to 1.0 L in a volumetric flask.

3.3.2.4 Sodium nitrite solution 2.07% (m/V)

Sodium nitrite ($NaNO_2$) solution was freshly prepared by dissolving 2.07 g $NaNO_2$ in deionized water and diluting to 100 mL in a volumetric flask.

3.3.2.5 Ammonium iron (III) sulphate reagent

A 77 g of ammonium iron(III) sulphate [$NH_4Fe(SO_4)_2.12H_2O$] was dissolved in approximately 400 mL of deionized water and a 167 mL of

concentrated nitric acid (65%) was added and diluted to 1.0 L in a volumetric flask.

3.3.3 Reagents for dialysis sampling

3.3.3.1 Intermediate standard iodide solution

The intermediate standard iodide solution of 40 mg/L was prepared daily by pipetting 1000 µL of the stock standard iodide solution and adjusting to 25 mL with deionized water.

3.3.3.2 Working standard iodide solutions

For Dialysis system 1 and 2, working standard iodide solution of 180 µg/L was prepared by transferring 9 µL of the stock standard iodide solution into 50 mL HDPE bottle which contained 50 mL of deionized water. For Dialysis system 3, a series of 160, 240, 320, 400 and 480 µg/L of standard iodide solutions were prepared by pipetting 100, 200, 300, 400 and 500 µL of the intermediate standard iodide solution into five volumetric flasks respectively and making up to 50 mL with deionized water.

3.3.3.3 Reagents for modification of the donor solutions

3.3.3.3.1 Mixture of sodium hydrogen carbonate 0.0022 M and sodium carbonate 0.0028 M

The mixture of salts was prepared by weighing 0.18 g sodium hydrogen carbonate ($NaHCO_3$) and 0.30 g sodium carbonate (Na_2CO_3), dissolving each component with deionized water. Then, the two solutions were mixed together and diluted to 1.0 L in a volumetric flask.

3.3.3.3.2 Mixture of Sodium hydrogen carbonate 0.022 M and Sodium carbonate 0.028 M

A 1.85 g of sodium hydrogen carbonate and a 2.98 g of sodium carbonate were dissolved in deionized water. After that, mixed the solution of each component together and diluted to a volume of 1.0 L.

3.4 Catalytic colorimetric method

Catalytic colorimetric method was used for determination of iodide. The procedure was as followings: a 1.5 mL of standard iodide solution or sample solution was pipetted into centrifuge tube. Then, 375 µL of 0.023% (m/V) potassium thiocyanate solution, 750 µL of ammonium iron (III) sulphate in nitric acid and 375 µL of deionized water were added, successively. Then, a 375 µL of $NaNO_2$ was added to the solution. The solution was shaken and waited for 15 min. The absorbance of the fading color was measured by the UV-VIS spectrophotometer at 450 nm. A series of the solutions could be prepared in the same way. A 375 µL of $NaNO_2$ was added to each solution for every 2 min interval and each solution was measured for the absorbance every 15 min.

3.4.1 Calibration and linearity for catalytic colorimetric method

The standard iodide solutions of 4, 8, 12, 16 and 20 µg/L were used for calibration. The measurement was done in duplicate. The calibration curves were plotted between the absorbance and the concentrations. The linear regression method was used to obtain slope, intercept and R^2.

3.5 Dialysis sampling

3.5.1 Preparation of membranes

The polysulfone hollow fiber membrane was first washed and filled the lumen of the fiber with deionized water. Then, it was soaked in deionized water so that the pores would be filled. Prior to the dialysis sampling, the fiber was cut into desired length and washed again by pumping deionized water through the lumen of the fiber for 5 min after that it was emptied by mild flow of air**.**

3.5.2 Design and setup of dialysis system

3.5.2.1 Dialysis system 1

The Schematic diagram of Dialysis system 1 was illustrated in Figure 3-1. It consisted of a magnetic stirrer and a peristaltic pump which was used to deliver the donor solution at the required flow rate. To connect the fiber with tubing, the needles were used. The needle and the fiber were sealed to prevent leaking with glue, and then a 50 mL of standard iodide solution was recirculatory pumped via 15-cm hollow fiber membrane which was immersed in an equal volume of deionized water. The acceptor solution was agitated throughout the process. The process was performed until the desired degree of % dialysis was achieved.

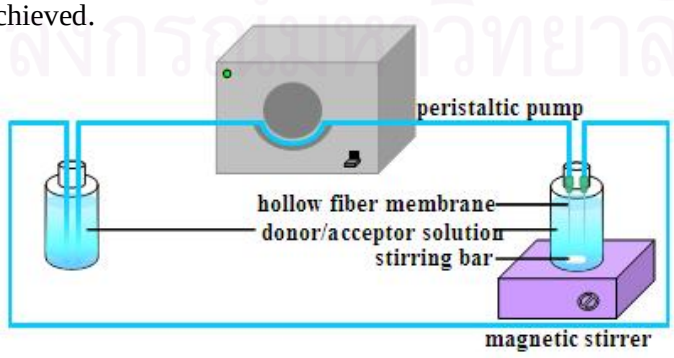

Figure 3-1 Schematic diagram of the Dialysis system 1.

3.5.2.2 Dialysis system 2

In this system, the homemade dialyzer was constructed. It consisted of a plastic tubing (10.0 mm OD x 0.57 mm ID x 26.7 cm length) with both ends were attached to three-way pipes. A 31-cm hollow fiber membrane was inserted into the dialyzer. The needle was used to connect the fiber with the tubing together where they were sealed with glue. Then, a 50 mL of standard iodide solution and an equal volume of deionized water were recirculatory pumped in opposite direction inside the dialyzer at the flow rate of 0.5 mL/min. The process was performed until the desired degree of %dialysis was obtained. The Schematic diagram of Dialysis system 2 was illustrated in Figure 3-2.

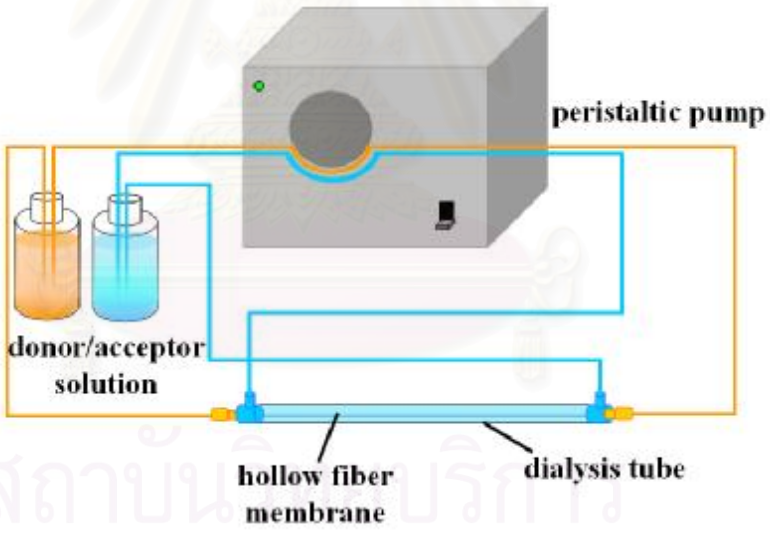

Figure 3-2 Schematic diagram of the Dialysis system 2.

3.5.2.3 Dialysis system 3

The Schematic diagram of dialysis sampling system 3 was illustrated in Figure 3-3. A 36-cm hollow fiber membrane was gradually filled with a 0.2 mL of standard iodide solution after that the end of the fiber was sealed with glue. The fiber was inserted into the dialyzer which was constructed of a glass tubing (5.10 mm OD x 3.25 mm ID x 30 cm length) with both ends were attached to three-way pipes, then the other end of the fiber was sealed. A 4 mL of deionized water was slowly pumped through as an acceptor phase at the flow rate of 1 mL/min via a syringe pump. The dialysate was collected for subsequent determination of iodide content.

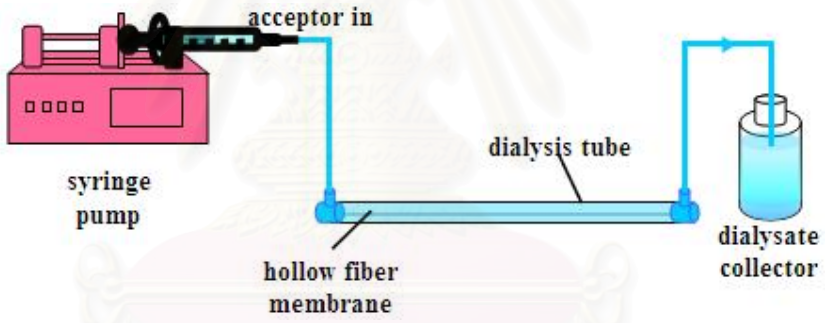

Figure 3-3 Schematic diagram of the Dialysis system 3.

3.5.3 Cleaning the dialyzer

The used dialyzers were cleaned by thoroughly flowing of tap water overnight. Then, the clean dialyzers were rinsed with deionized water and 99.9% ethanol to prevent algae growing.

3.5.4 Optimization of %dialysis

3.5.4.1 Dialysis time

As analytes in donor solution may need time to diffuse across membrane into acceptor solution, dialysis time must be optimized for sufficient transfer of analytes in short time. This experiment was studied for the appropriate time of dialysis sampling. The dialysis was performed by pumping the donor solution and the acceptor solution with various periods of time. The time that gave a sufficient %dialysis for iodide detection in a short time was considered as optimum dialysis time.

3.5.4.2 Flow rate

Flow rate of donor solution and acceptor solution dictates contact time between solutions and membrane. The long contact time can cause more analyte diffused to acceptor solution, so the flow rate of donor solution and acceptor solution was optimized to obtain sufficient membrane-solution contact in short time. The dialysis was performed by pumping the donor solution and the acceptor solution with various flow rates. The flow rate that gave a sufficient %dialysis in a short time was considered as optimal.

3.5.4.3 Component of donor solution and acceptor solution

The presence of ionic salts in donor and acceptor solutions may effect on diffusion of the analyte, so the composition of both solutions were adjusted to get highest mass transfer. The dialysis was performed using the donor solutions and the acceptor solutions modified with varying concentration of salts. The condition that gave maximum %dialysis was considered as optimal.

3.5.5 Method evaluation for dialysis sampling

3.5.5.1 Calibration curve for quantitative determination of iodide

The 50 mL of 160, 240, 320, 400 and 480 µg/L standard iodide solutions were used for calibration. As 1-pentanol was added to the samples to reduce foaming, 2 drops of 1-pentanol was also added to the standard solutions for obtaining similarity matrices. Each concentration of standard iodide solution was dialyzed in duplicate, and then the dialysate was determined the absorbance of light. The calibration curves were plotted between the absorbance and the initial iodide concentrations. The linear regression method was used to obtain slope, intercept and R^2.

3.5.5.2 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) was defined as the concentration giving a signal of $Y_B + 3S_B$, where Y_B was blank signal and S_B was standard deviation of blank signal. The corresponding concentration was then calculated from the calibration equation. The limit of quantitation (LOQ) was defined as the concentration giving a signal of $Y_B + 10S_B$.

3.5.5.3 Precision and Accuracy

The accuracy and precision of the method were evaluated by determination of recovery and relative standard deviation of the repeat analysis of standard reference material and food samples.

3.5.5.3.1 Standard Reference Material

The skim milk powder standard reference material (BCR 151) which provided certified value for iodine content of 5.35 mg/L, ± 0.14 was analyzed to test the accuracy and precision of dialysis method. A 1.5 g milk powder was accurately weighed into 25 mL volumetric flask containing about 10 mL of deionized water and briskly stirred for 10-15 min to fully wet and mix milk powder. One drop of 1-pentanol was added to the solution to reduce foaming. The solution was diluted to the volume with deionized water and ready for dialysis sampling.

3.5.5.3.2 Milk samples

Liquid milk samples were purchased from a local market. About 20 mL of sample was transferred to a 25 mL volumetric flask and then directly spiked with standard iodide solution at desired concentration level. One drop of 1-pentanol was added to reduce foaming, after that the volume was made up to the mark with the sample.

3.5.5.3.3 Egg samples

Egg samples were purchased from a local market. The whole egg including its shell was weighed but only egg was used. The egg was quickly stirred to allow homogenization for 10-15 min. A 12.5 g of homogenized sample was accurately weighed into 25 mL volumetric flask and spiked with iodide at desired concentration level, and then 1 drop of 1-pentanol was added to reduce foaming. The solution was diluted to the volume with deionized water and ready for dialysis sampling.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Catalytic colorimetric method

4.1.1 Catalytic colorimetric method for determination of iodide

Several methods of iodide determination have been proposed [34-40]. In this experiment, the catalytic colorimetric method was selected because of their productivity, sensitivity, easy operation and a use of UV-visible spectrometry which was small and inexpensive equipment for detection. The method linearity between the signal responses of iodide and iodide concentrations ranging from 0 to 20 µg/L was verified. Figure 4-1 showed the plot of the absorbance versus iodide concentrations. A working range of 0 to 20 µg/L was observed and was represented by the linear regression equation: $y = -0.0246x + 0.6855$ with correlation coefficient, $R^2 = 0.9999$.

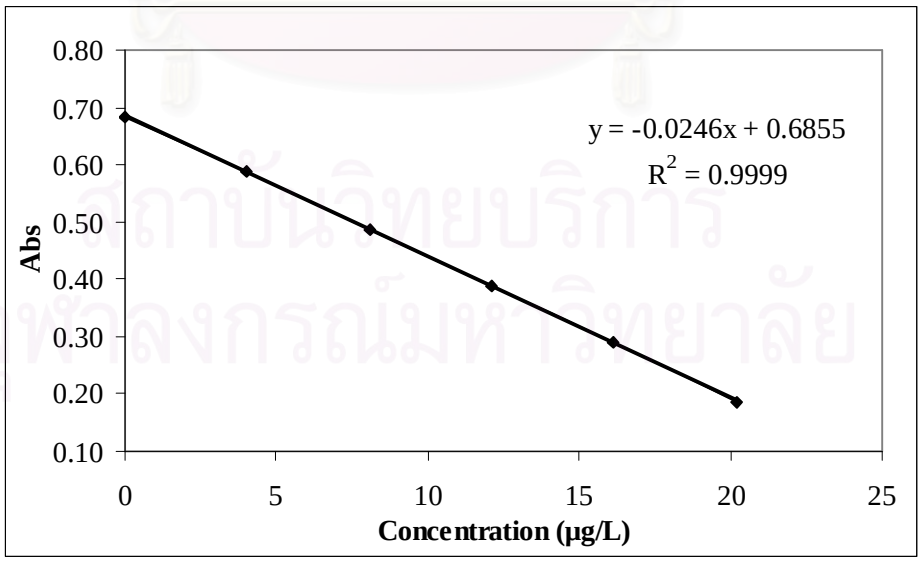

Figure 4-1 Working range of iodide determined by catalytic colorimetric method.

4.2 Dialysis systems

Polysulfone membrane has been employed for dialysis sampling in several reports [16, 43]. It provides many advantages, namely, its resistance in extreme pH conditions, its high-strength, its thermal stability, as well as its hydrophilic property can help minimize membrane fouling [28, 44-45]. In this work, polysulfone hollow fiber membrane was asymmetry porous structure which offered very low protein binding and allowed higher analyte flux than nonporous structure [13, 45]. Furthermore, the tubular geometry could enhance the dialysis efficiency due to large surface area per unit volume [7, 29].

The %dialysis was defined as the ratio percentage of the iodide concentration found in the dialysate, C_d to the initial iodide concentration in the sample (donor) solution, C_s. The equation of dialysis percentage was shown below.

$$\% \text{ Dialysis} = (C_d/C_s)*100$$

4.2.1 Design and setup of dialysis system

4.2.1.1 Dialysis system 1

There have been several reports of using concentric or side-by-side type of probe for sampling in sample solutions [46-47]. These probes consisted of two tubes, where one tube extended beyond the other and was covered with dialysis membrane (see Fig. 2-4a and 2-4b in Chapter II). The probe was put into a sample solution while perfusate was flown on the other side of the membrane. From the probe design, it seemed that two tubes were linked by using hollow fiber membrane as a connector. So, this design presented a simple dialysis configuration base on the function of probe. In case Dialysis system 1, the hollow fiber membrane was arranged in u-shape and immersed in either donor or acceptor solution, while the other solution was flown through the membrane. This configuration, there was no need of probe construction and longer

membrane length could be applied because no tubing was needed to hold the membrane.

Firstly, the sample or iodide solution was recirculatory pumped via hollow fiber membrane which was immersed in deionized water as an acceptor solution. This format was called Dialysis system 1.1 (see Fig. 3-1 in Chapter III). When a 180 µg/L of standard iodide solution as donor solution was dialyzed at the flow rate of 1 mL/min for 1 hr, it was found that about 3% of dialysis was obtained (Table 4-1). Since this dialysis system was a closed recirculating system, a longer dialysis time might be needed to obtain more mass-transfer; subsequently, higher %dialysis might be achieved. The results showed that the %dialysis was increased to about 13% and 14% for 20 hours and 47 hours, respectively (Table 4-1). Although %dialysis was increased, dialysis times were getting too long. If the dialysis process should have reached equilibrium, it could take much longer dialysis time. Therefore, this dialysis process may not necessarily reach equilibrium but other factors may be optimized to get high %dialysis. It was first speculated that there might be some difficulty of analyte diffusion through the membrane. Therefore, the membrane structure was examined.

Figure 4-2 illustrated the Scanning Electron Micrograph (SEM) of the hollow fiber membrane used in this experiment. It indicated that the membrane was asymmetric porous membrane, where the inner pores of the hollow fiber membrane were smaller than the outer pores. This membrane structure could have affected how well the analyte diffused from inside to outside of the membrane. Therefore, if the acceptor and the donor streams were switched by flowing the acceptor solution inside and the donor solution outside of the membrane, the analyte transfer might be increased.

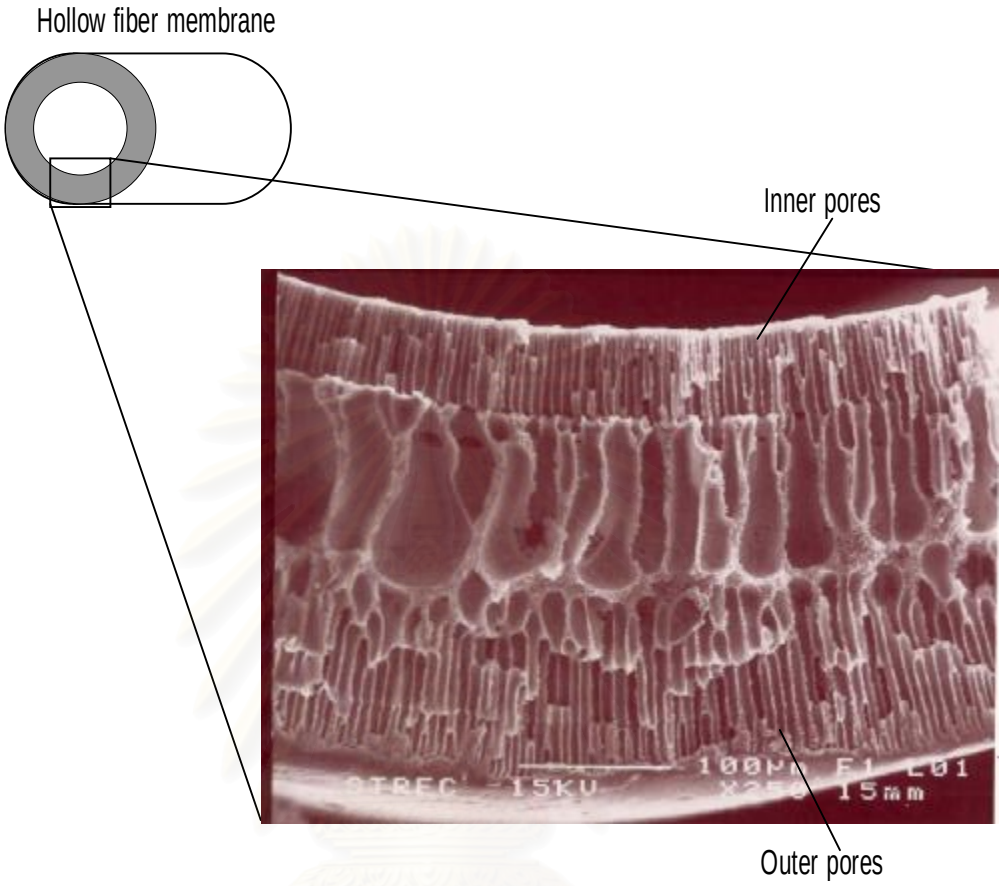

Figure 4-2 Scanning Electron Micrograph of the cross-sectional view of the polysulfone hollow fiber membrane.

Thus the format of dialysis sampling was slightly changed by switching between the acceptor stream and the donor stream. The acceptor solution was pumped into the lumen of the fiber while the iodide solution was agitated outside. This format was called Dialysis system 1.2 (Fig. 3-1 in Chapter III). The results showed that when 1 hour dialysis time was applied to the same concentration of iodide solution (180 µg/L), around 4 %dialysis was obtained (Table 4-1).

Although the donor and the acceptor solutions were switched, it seemed that the obtained %dialysis did not differ from the previous format. This indicated that the membrane structure might not be the

main obstacle that caused low %dialysis of iodide. However, there was another important factor that involved mass transfer efficiency, namely flow rate of solution. Typically, the slower the flow rate, the better the contact of both solutions, enhancing the mass transfer [29].

In the previous experiments, the flow rate of 1 mL/min was used. This flow rate might not be able to provide enough contact time for more analyte to diffuse across the membrane. In order to get higher %dialysis, the lower flow rate of 0.5 mL/min (the lowest flow rate for the peristaltic pump used in the experiment) was used for acceptor solution. It was observed that about 8% dialysis was achieved at 3-hr dialysis time (Table 4-1). In addition, the longer dialysis time of 74-hr and 121-hr were also attempted. The results showed that not much higher %dialyses were obtained, namely 16% (Table 4-1). Although the flow rates were decreased and the dialysis times were increased, the obtained %dialysis was quite similar to the previous studies. It was suggested that our lowest flow rate might not be low enough and our dialysis times might not be long enough to significantly enhance the mass transfer of the analyte to the acceptor phase. The overall results were summarized in Table 4-1.

Table 4-1 The %dialysis of iodide of various flow rates of donor or acceptor solutions and dialysis time in the Dialysis system 1.

Dialysis system	Concentration of iodide in donor solution (µg/L)	Flow rate of donor or acceptor solutions (mL/min)	Dialysis time (hr)	%Dialysis	%Mass transfer*
1.1	180	1	1	3	3
			20	13	13
			47	14	14
1.2	180	1	1	4	4
		0.5	3	8	8
			74	16	16
			121	16	16

Dialysis system 1.1 = the donor solution was pumped via the fiber which was immersed in the acceptor solution.

Dialysis system 1.2 = the acceptor solution was pumped via the fiber which was immersed in the donor solution.

*%Mass transfer of iodide = ratio percentage of the mass of iodide found in the dialysate to the initial mass of iodide in the donor solution.

Noted that it was clearly observed that the volume of the acceptor or the donor solution that flew outside the membrane (Dialysis system 1.1 and 1.2, respectively) was increased when the process was performed over a long period of time (more than 3-hr). During the process of Dialysis system 1.1 or 1.2, the solution that flew inside the membrane could flow out due to the result of the pressure gradient, while iodide simultaneously diffused across the membrane. Next, the experiment was designed to test this hypothesis.

Dialysis system 1.1 was performed with 180 µg/L of iodide concentration in donor solution at 0.5 mL/min for 263 hr. It was found that the concentration of iodide that was calculated by the iodide content of dialysate per the increased volume of the acceptor solution was quite

similar to the initial iodide concentration in the donor solution. It was suggested that the mechanism of this process might have involved the filtration effect. Then, Dialysis system 1.2 was carried out. If the mechanism of the process mainly involved filtration, small amount of iodide could be found in the dialysate due to the acceptor solution was filtrated into the donor solution, while iodide which diffused against the pressure into the acceptor solution was difficult to occurr. On the other hand, if the mechanism was the combination of filtration and dialysis, iodide contents of the dialysate could be similar to the result from Dialysis system 1.1 because the analyte transfer also depended on the concentration gradient. The dialysis sampling was operated in Dialysis system 1.2 for 282 hr. The final volume of acceptor solution that flew inside a hollow fiber membrane was also decreased. Interestingly, the iodide content of the dialysate was similar to the result from Dialysis system 1.1. It indicated that the acceptor solution which flown inside the hollow fiber membrane was filtrated into the donor solution, while iodide ions in the donor solution diffused in the opposite direction into the acceptor solution. Consequently, the mechanism of process was the combination of dialysis effect and filtration effect. The explanation of the phenomena in Dialysis system 1.1 and 1.2 was illustrated in Figure 4-3.

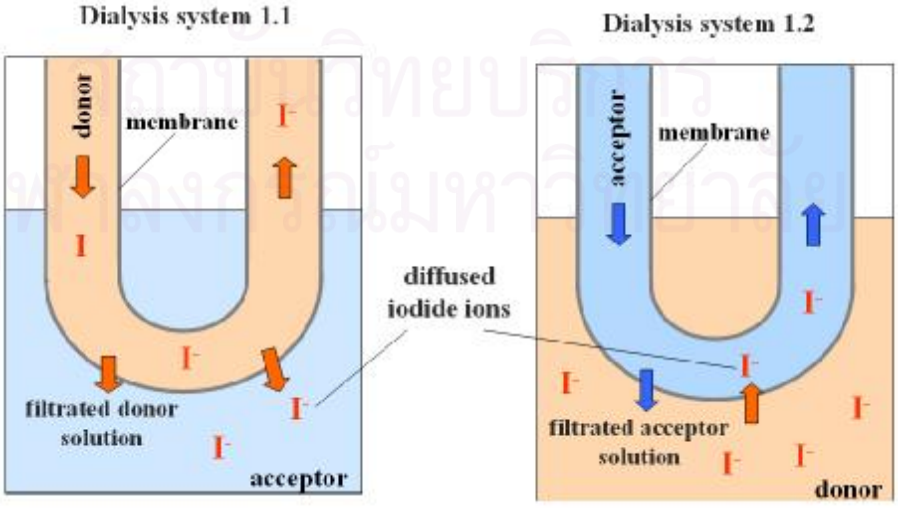

Figure 4-3 The mechanism of iodide transfer in Dialysis system 1.1 and 1.2.

It has been mentioned that filtration effect due to the pressure gradient from the pumping can be observed during dialysis process [13]. Especially, membrane exhibiting molecular weight cut-off (MWCO) > 30 kDa could generate the filtration effect [33, 48]. Since, the MWCO of the membrane used in this experiment was 50 kDa the filtration effect might have possibly occurred. The filtration effect that was generated by the pressure inside the hollow fiber membrane from pumping could be affected the accuracy and precision of the results. Moreover, the u-shape hollow fiber membrane might cause the membrane to be folded which might have obstructed the flow of solution as well as it might be torn by agitation of solution. Although this system offered high %dialysis, dialysis time was too long and the loss of iodide might have occurred by light or heat from the stirrer.

4.2.1.2 Dialysis system 2

For the above reasons, Dialysis system 2 was developed to solve the problems. The longer membrane (31 cm) was used to increase the surface area of mass-transfer and inserted into a dialyzer made of a plastic tube without folding the membrane. To reduce the filtration effect, the needle was adjusted to a larger diameter (from 0.8 mm became 0.9 mm) for matching with the fiber and the tubing diameters. Furthermore, countercurrent flow was used in order to maintain the highest analyte concentration gradient across the surface of the dialysis membrane and the higher dialysis efficiency might be resulted[13, 26].

At first, standard iodide solution at 180 µg/L which was used as donor solution was pumped into the lumen of the hollow fiber membrane at the flow rate of 0.5 mL/min while the acceptor solution, which was deionized water, was countercurrent flown outside the membrane at the same flow rate. This configuration was a closed recirculating system and called Dialysis system 2.1. It was found that about 5% of dialysis was obtained for 1-hr and about 15% for 24-hr. The

obtained %dialysis was similar to those using previous dialysis systems. Then the flow directions of the solutions were switched; the acceptor solution was flown inside the membrane while the donor solution was flown outside. This configuration was called Dialysis system 2.2. It was observed that about 4% of dialysis was gained for 1-hr and about 13% for 2-hr. When 67-hr was applied for dialysis time, it offered about 16% of dialysis. The overall results were summarized in Table 4-2.

Table 4-2 The %dialysis of iodide of various dialysis time in the Dialysis system 2.

Dialysis system	Concentration of iodide in donor solution (µg/L)	Flow rate of donor and acceptor solutions (mL/min)	Dialysis time (hr)	%Dialysis	%Mass transfer
2.1	180	0.5	1	5	5
			24	15	15
2.2	180	0.5	1	4	4
			2	13	13
			67	16	16

Dialysis system 2.1 = the donor solution was flown inside the membrane while the acceptor solution was flown outside.

Dialysis system 2.2 = the acceptor solution was flown inside the membrane while the donor solution was flown outside.

From these experiments, the obtained %dialysis was not different from Dialysis system 1. The explanation might be expressed in the Figure 4-4. The analyte transfer in the Dialysis system 1 occurred as a result of agitation while in the Dialysis system 2, the analyte transfer occurred as a result of the flow. If the mass transfer occurred near the membrane, any of dialysis system should be given the similar result. Therefore, the %dialysis from the dialysis system 2 was quite similar to that from the Dialysis system 1.

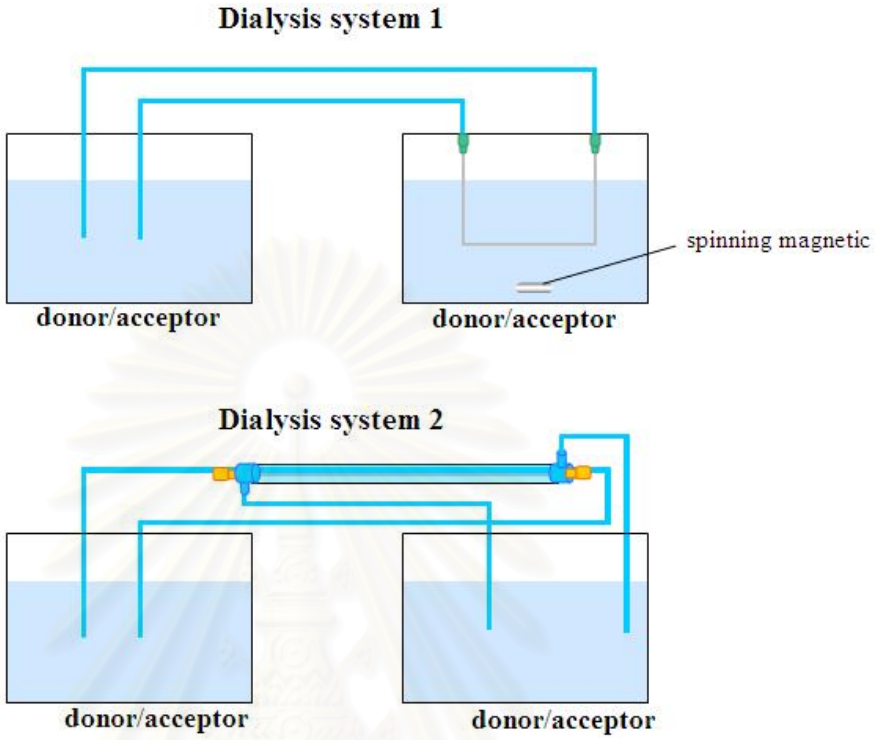

Figure 4-4 The comparison of designs between Dialysis system 1 and 2.

Nevertheless, the filtration effect was still found even if the needle was adjusted to match with the fiber and the tubing inner diameters. To minimize the filtration effect, Dialysis system 3 was developed.

4.2.1.3 Dialysis system 3

In the Dialysis system 3, the constant volume of the donor solution was fixed inside the lumen of the hollow fiber membrane. Since there was no flow of solution, the pressure gradient across the membrane was not occurred, so the filtration effect might be eliminated. In the present design, the longer membrane (36 cm) was used and only 0.2 mL of sample was fixed inside the lumen while 4 mL of the acceptor solution, which was sufficient for duplicate determination of iodide, was flown outside in the non-recirculation system, so the fresh acceptor solution

always maintained the highest analyte concentration gradient (driving force) across the membrane. As mass-transfer equilibrium was never been reached, the analyte diffusion into the acceptor solution could continuously occurred. Thus, the %dialysis could be possibly improved.

For acceptor solution flow rate of 1 mL/min and the iodide concentration of donor solution of 160 µg/L, 3.2% of dialysis was obtained. Although it seemed that the obtained %dialysis did not differ from the previous system, the %mass transfer was quite high, namely 64.3%. Considering the previous dialysis systems, donor and acceptor solutions was used in equal volume, so the calculated percentage of mass transfer was equal to the %dialysis. The %mass transfer in the previous formats was quite low, although the very long period of dialysis time was used. This indicated that the Dialysis system 3 offered high dialysis efficiency because the high concentration gradient could be always maintained by the fresh acceptor solution, even through only 0.2 mL of sample was used. In addition, the dialysis system 3 offered less than 10 min dialysis time, longer membrane for more surface area was allowed; furthermore, the filtration effect might be eliminated. Therefore, the Dialysis system 3 was suitable to develop for sampling iodide from milk and egg samples.

4.2.2 Optimization of %dialysis

4.2.2.1 Acceptor flow rate and dialysis time

In this experiment, the flow rates of the acceptor solution were varied because the efficiency of dialysis sampling depended on the contact time between donor solution and acceptor solution as above mentioned. In Dialysis system 3, the volume of the acceptor solution kept constant (4 mL), so dialysis time was a function of the acceptor flow rate. In addition, syringe pump was used in this experiment as it could provide lower flow rate than that obtained from the peristaltic

pump. Using 240 µg/L of the iodide concentration of donor solution, it was found that 3.8% and 3.3% of dialysis were obtained at acceptor flow rate of 0.1 mL/min and 1 mL/min, respectively. Compared to 1 mL/min, the dialysis time using 0.1 mL/min was 4.5 times slower while the %dialysis using 0.1 mL/min was only 0.15 times higher. So, 1 mL/min was more suitable for providing sufficient %dialysis for dialysis time less than 10 min.

4.2.2.2 Type of modifiers

Ionic strength is a characteristic of an electrolyte solution. It is a key factor in the mass transfer mechanism due to attracting and repelling of ions in the solution to each other. These interactions influence ions behavior, including the rates at which ions move across the membrane. There has been a report on use ionic salts to enhance efficiency of dialysis because the diffusion of the analyte may be influenced by the presence of them or their ionic strength of the donor solution [17].

According to the Fick's law

$$J = -D(A/t)(\mathrm{d}c/\mathrm{d}x)$$

the diffusion rate J (mol/s) of the analyte depends on its diffusion coefficient D (m^2/s), on the concentration gradient $-\mathrm{d}c/\mathrm{d}x$ (mol/m^4), and on the effective membrane area A (m^2). The factor τ, the tortuosity of the membrane, is a constant that takes all other membrane parameters such as porosity, pore size, membrane thickness, etc., into account. The diffusion coefficient D can be expressed by the Stokes-Einstein relationship

$$D = kT/6phr$$

where k is the Boltzmann constant (J/K), T the absolute temperature (K), h the solvent viscosity (kg m^{-1} s^{-1}), and r the radius of the analyte molecule. The diffusion rate at constant T and h depends only on the analyte diameter. Consequently, the estimation of diffusion rate for various analytes can be based on respective ionic or molecular size values. Small inorganic anions are expected to have several times higher transport efficiency than large organic anions [20].

For the above reason, the selected ionic salts should be comprised of small cations to obtain high mobility, while anions should be large or bulky in order to be difficult to transfer into the acceptor solution. Thus, iodide ion, which is small, should move quickly across the semi permeable membrane. Accordingly, ionic salts such as phosphate salts, borate salts or carbonate salts can be used as the modifier for the donor solutions. In this work, carbonate salts (a mixture of Na_2CO_3 and $NaHCO_3$) were selected for this purpose. In addition, sodium chloride, also known as common salt was selected as benchmark for this experiment.

Firstly, the modifiers were checked for the effect on the autocatalytic reaction that was used for determination of iodide by plotting the calibration curve between the absorbance and the concentrations of modified standard iodide solutions. The results were shown in Figure 4-5 and Figure 4-6.

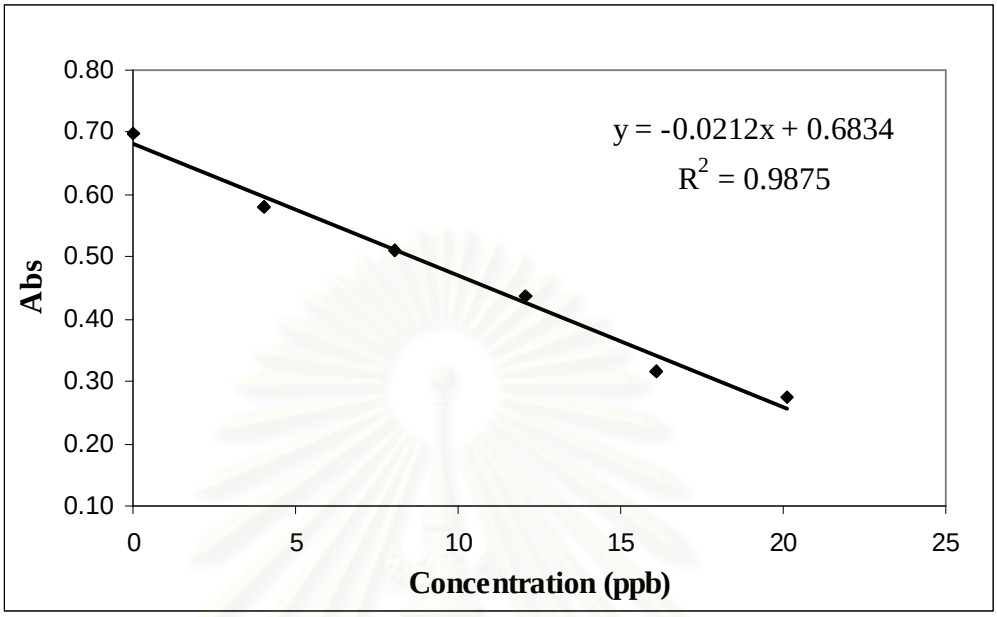

Figure 4-5 Relationship between the absorbance and the concentrations of standard iodide solutions modified with 0.01 M NaCl.

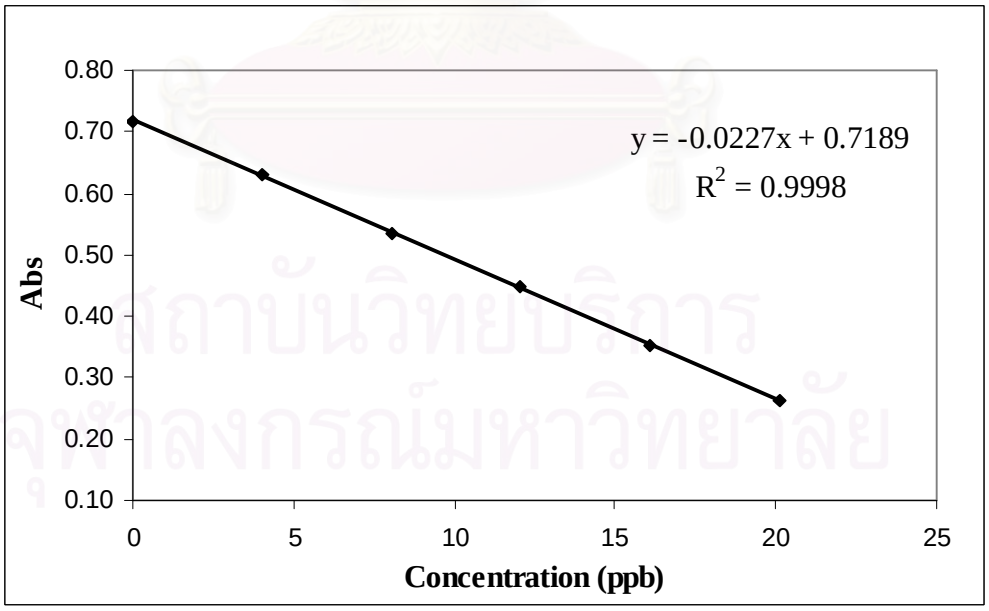

Figure 4-6 Relationship between the absorbance and the concentrations of standard iodide solutions modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$.

The results, which was shown in Figure 4-5 and 4-6, indicated that mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ less affected to the determination of iodide concentration, but that NaCl at 0.01 M gave correlation coefficient, R^2 =0.9875. It was possible that 0.01 M NaCl could affects the autocatalytic reaction. In addition, the mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ could act as a buffer, pH 10 against the oxidation of iodide to iodine [49]. For above reasons, they were used as the modifier of donor solutions. The %dialysis of various initial iodide concentrations in unmodified donor solutions and donor modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ were shown in Table 4-3.

Table 4-3 The average %dialysis of various initial iodide concentrations in unmodified donor solutions and donor modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$.

Concentration of iodide in donor solutions	Donor solutions modified with			
	Deionized water		0.0028 M Na_2CO_3+0.0022 M $NaHCO_3$	
(µg/L)	%Dialysis	%RSD	%Dialysis	%RSD
160	3.2	30 (N=18)	4.4	38 (N=27)
240	3.3	15 (N=23)	3.6	22 (N=26)
320	3.0	25 (N=21)	3.5	16 (N=27)
400	3.0	18 (N=21)	3.3	13 (N=26)
480	3.1	14 (N=21)	3.2	11 (N=27)

As expected, the %dialyses of iodide at various concentration from the donor modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ were higher than those from donor solution with deionized water. The added salts caused ionic strength gradient that had an impact on the dialysis mechanism. Sodium ions that have higher mobility than carbonate ions moved through semi permeable membrane into the acceptor solution, while carbonate ion that was bulky ion moved slower. The different mobility of added ions caused difference function potential

between the donor solution and the acceptor solution, so the iodide ion from the donor solution would diffuse in order to maintain electroneutrality [27].

To proof this concept, the mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was added to acceptor solutions and the result was shown in Figure 4-7. Sure enough, the iodide concentration in acceptor solutions that modified with the mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was lower than other conditions.

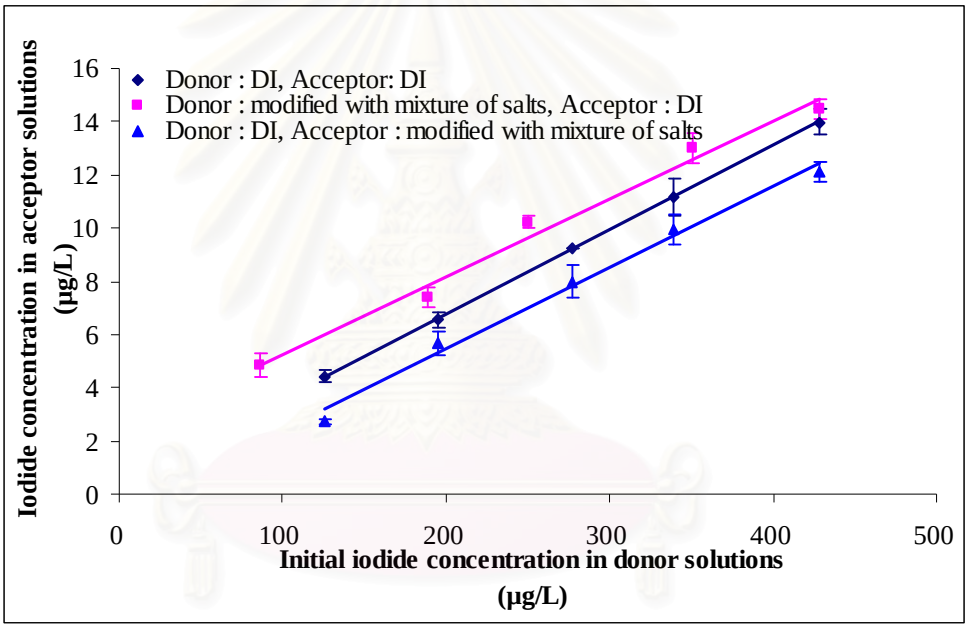

Figure 4-7 The average iodide concentrations in the acceptor solutions with various conditions of modifier.

4.2.2.3 Modifier concentration

As the salt concentration in the donor solution was increased, the ionic strength was also increased so that it would have an effect on the %dialysis of iodide. As usual, the mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ and the mixture of 0.28 M Na_2CO_3 and 0.22 M $NaHCO_3$

were checked the effect on the reaction. The results were shown in Figure 4-8 and Figure 4-9.

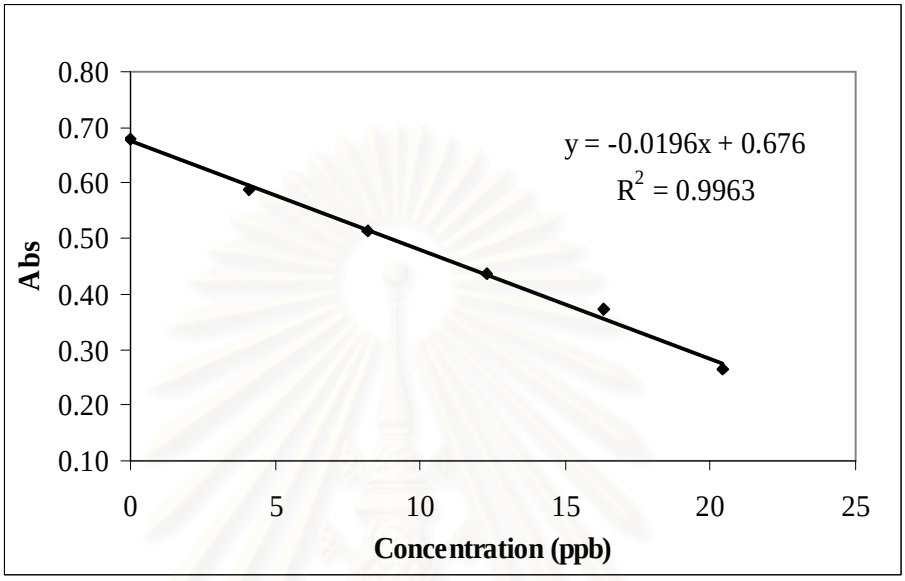

Figure 4-8 Relationship between the absorbance and the concentrations of standard iodide solutions modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$.

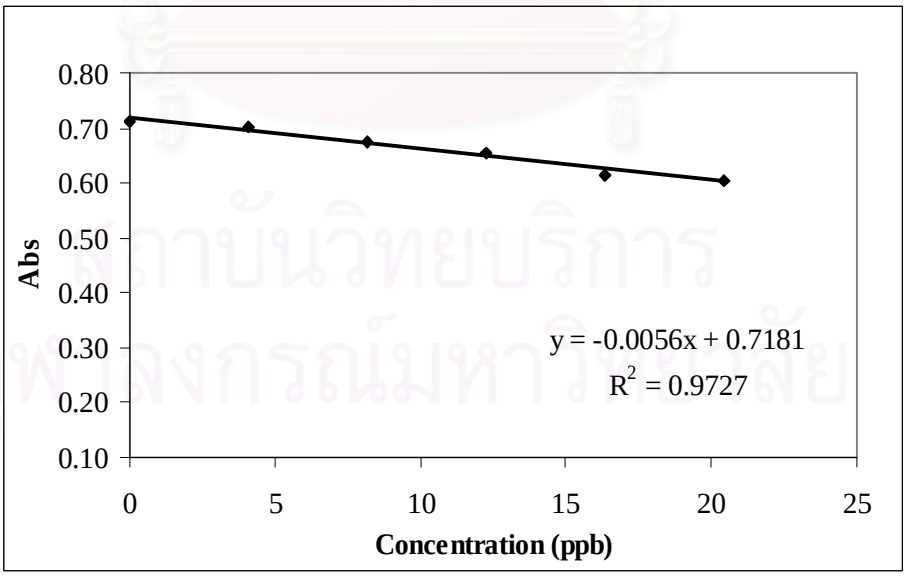

Figure 4-9 Relationship between the absorbance and the concentrations of standard iodide solutions modified with mixture of 0.28 M Na_2CO_3 and 0.22 M $NaHCO_3$.

Figure 4-9 indicated that the mixture of 0.28 M Na_2CO_3 and 0.22 M $NaHCO_3$ had an affect on the reaction. This might be due to the electrolyte effect or salt effect. The ions of salt have electrostatic attraction and repulsion to the ions involved in the reaction, so each ion from the dissociated reactant was surrounded by a layer of salt ions of the opposite charge. This charged layer makes ions involving in the reaction less positive or negative than those in the absence of salts. This shielding effect decreases overall attraction between ions concerned in the reaction and it becomes greater as the concentration of salts become higher [50]. For this reason, the reaction rate was decreased with increased salts concentration and the slope of the calibration curve was so low that could impact the sensitivity of method. However, the mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ less affected to the determination of iodide concentration as shown in Figure 4-8, so this mixture was used to modify the donor solutions and the results was shown in Table 4-4.

Table 4-4 The average %dialysis of various initial iodide concentrations of donor solutions modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$.

Concentration of iodide in donor solutions (µg/L)	Donor modified with			
	0.0028 M Na_2CO_3 + 0.0022 M $NaHCO_3$		0.028 M Na_2CO_3 + 0.022 M $NaHCO_3$	
	%Dialysis	%RSD	%Dialysis	%RSD
160	4.4	38 (N=27)	4.3	35 (N=15)
240	3.6	22 (N=26)	3.6	13 (N=14)
320	3.5	16 (N=27)	3.4	14 (N=15)
400	3.3	13 (N=26)	3.3	16 (N=14)
480	3.2	11 (N=27)	3.0	15 (N=15)

As the salt concentration in the donor solution was increased, the %dialysis of iodide was not increased or even lower. The reason might be that as higher salt concentration, the ions of salt are denser and closer together that cause strong electrostatic attractions among the ions. The solution may become more viscous so that the diffusion of iodide would be slower [51].

The overall results of the optimization of component of donor solution were presented in Figure 4-10. As shown in Figure 4-10, the %dialysis of iodide from the donor modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ offered the highest %dialysis. In addition, it was observed that %RSD was high. This could be due to the problem of the catalytic colorimetric method. When applied as a manual procedure, the rapid rate of decoloration made it difficult to obtain repeatable results. Small deviation in time and temperature during the reaction significantly influence the absorbance measurement [42]. According to a small slope of calibration curve, if the absorbance shifted, usually not more than 0.02 AU, it could have made the concentration of iodide be deviated around 1 µg/L. In addition, each manual dialysis sampling of iodide standard solution also offered different means of dialysate signal about 0.02 AU that was equal to iodide concentration about 1 µg/L. So, the iodide concentration of the dialysate that was used for calculation of %dialysis was affected by these factors. Furthermore, it was noted that %RSD was decreased with increased iodide concentration. When the donor solution was spiked with a large amount of iodide, it made more difference between background signal and response signal of dialysate that improved the precision of results. However, most of %RSD were acceptable according to the AOAC Peer-Verified methods (November 1993) specified that it must not be exceed 21% at 10 µg/L concentration levels.

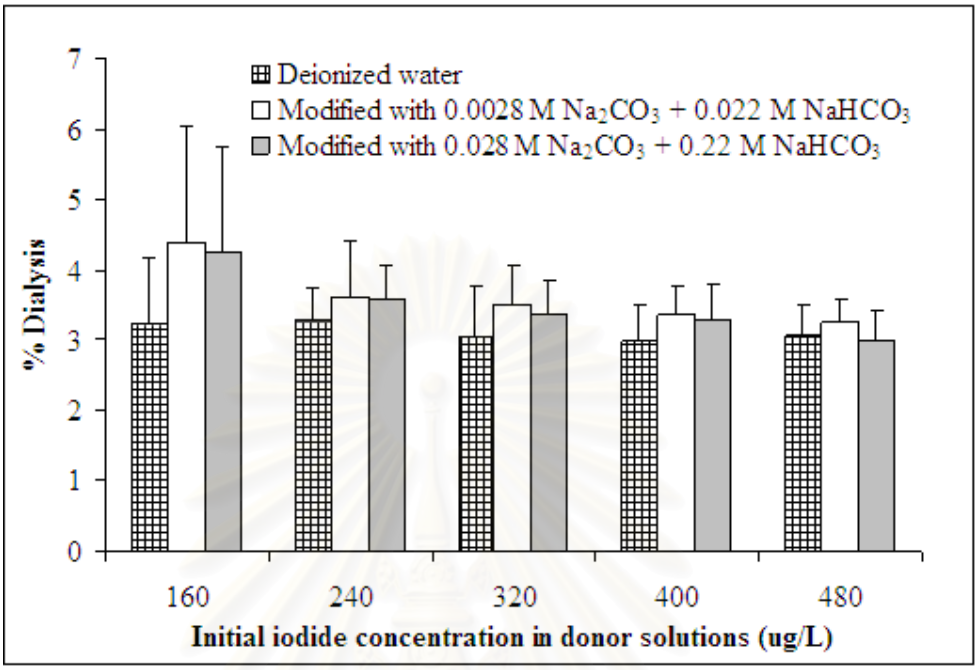

Figure 4-10 The average %dialysis of iodide from the donor solutions modified with mixture of salts.

According to the results, two concerned factors were the initial iodide concentrations in the donor solutions and the modifiers. Two-way analysis of variance (ANOVA) was used to test the effects of two independent variables on %dialysis. The %dialyses of iodide from the unmodified donor solution were the lowest and statistically different among the others ($P<0.05$, $N=14$), while no difference was found in their %dialysis when the donor solutions were modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ or 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$. It indicated that the dialysis mechanism was strongly influenced by the addition of salts.

The effect of modifiers at the same initial iodide concentration was tested by using one-way ANOVA. The result of statistical analysis was shown in Table 4-5. No significantly difference ($P>0.05$, number of samples was shown in Table 4-5) was found at every initial iodide concentration except at 480 µg/L. At this iodide concentration, the %dialysis of iodide from the donor modified with mixture of

0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was significantly higher than that from donor solution modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$. The explanation was that the viscosity of the solution modified with higher salt concentration had become more significant effect on iodide diffusion at higher initial iodide concentration. As a large amount of iodide was influenced by this effect, the content of iodide diffused into the acceptor solution was quite low compared to the other modifier.

Table 4-5 The average %dialysis of various initial iodide concentrations of donor solutions modified with mixture of salts.

Modifier	%dialysis at initial iodide concentration (µg/L)				
	160	240	320	400	480
0.0028 M Na_2CO_3+ 0.0022 M $NaHCO_3$	4.4(N=27)[a]	3.6(N=26)[a]	3.5(N=27)[a]	3.3(N=26)[a]	3.2(N=27)[a]
0.028 M Na_2CO_3+ 0.022 M $NaHCO_3$	4.3(N=15)[a]	3.6(N=14)[a]	3.4(N=15)[a]	3.3(N=14)[a]	3.0(N=15)[b]

[a, b]Same superscripts in the same column indicate no significant difference ($P<0.05$).

The effect of concentration on %dialysis within each modifier was statistically analyzed by using one-way ANOVA. It was found that the dialysis percentage of each iodide concentration in unmodified donor was no statistically difference ($P>0.05$, number of samples was shown in Table 4-3 and Table 4-4) while significantly difference was found when the mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ or 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ were used. In case of the unmodified donor, no trend was observed, however, between the dialysis percentage and the iodide concentration. The downward trend in the %dialysis of iodide was found when both of the mixtures of salts were used as modifier. When initial iodide concentration was increased, the iodide

transfer rose, owing to increased concentration gradient across the dialysis membrane.

According to the %dialysis equation

$$\% \text{ Dialysis} = (C_d/C_s)*100$$
$$= [(N_d*V_s)/ (N_s*V_d)]*100$$

where C_d is the detected iodide concentration in the dialysate solution (µg/L), C_s the initial iodide concentration in the sample solution (µg/L), N_d the iodide content in the dialysate (g), N_s the initial iodide content in the sample (g), V_d volume of the dialysate and V_s volume of the sampler. In this system, the factor V_d and V_s are 4 and 0.2 mL respectively, so the equation is as follows.

$$\% \text{ Dialysis} = (0.2N_d/ 4N_s)*100$$

When N_s was increased, N_d must be high enough when compared with N_s to get higher dialysis percentage. In this case, the transferred iodide could not high enough to make any difference. Accordingly, the dialysis percentage was decreased with increased initial concentration of iodide.

4.2.2.4 Modification of acceptor solution

According to the catalytic colorimetric method for iodide determination, the dialysate was reacted with reagents before absorbance measurement. This might not be suitable for the modification of acceptor solution that affected the reaction, so only deionized water was used as acceptor solution for dialysis.

The optimal parameters for dialysis sampling were summarized in Table 4-6.

Table 4-6 Optimal parameters for dialysis sampling.

Parameter	Optimum
Volume of donor solution (mL)	0.2
Volume of acceptor solution (mL)	4
Flow rate of acceptor solution (mL/min)	1
Component of donor solution	0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$

4.2.3 Method evaluation for dialysis sampling

4.2.3.1 Calibration curve for quantitative determination of iodide

The method linearity between the absorbance response of iodide in dialysates and initial iodide concentrations ranging from 0 to 480 µg/L was studied. Figure 4-11 showed the plot of the absorbance versus initial iodide concentrations. A linear dynamic range of 0 to 480 µg/L was observed with correlation coefficient, R^2 =0.9987. This calibration curve was used to quantitative determination of iodide in sample. The corresponding concentration was then calculated from the calibration equation.

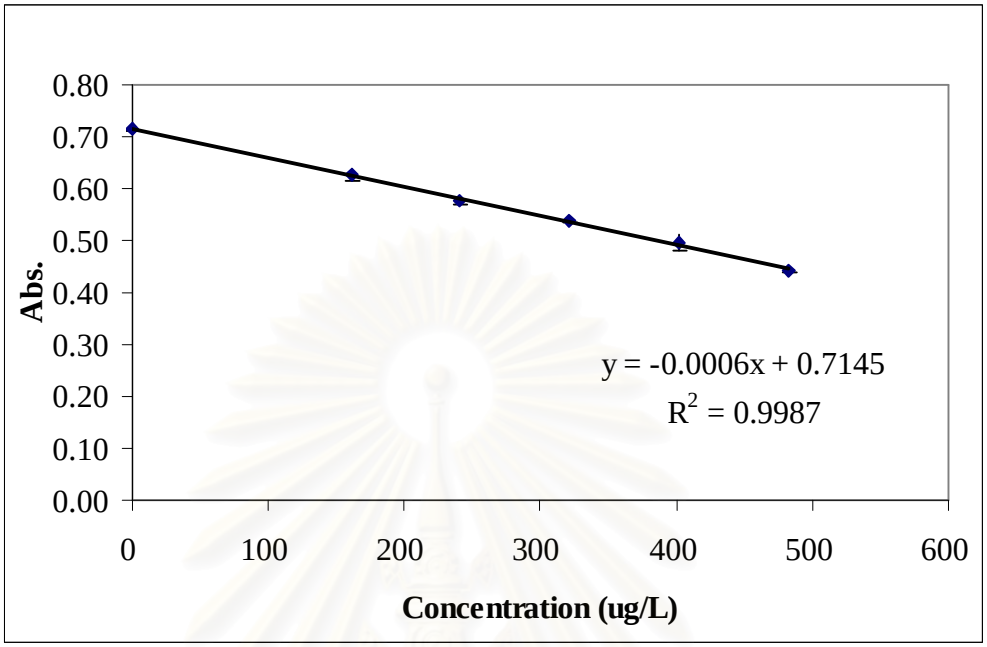

Figure 4-11 Linear dynamic range of the dialysis sampling for determination of iodide.

4.2.3.2 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) was calculated from the equation in subtopic of 3.5.5.2 of Chapter III. The corresponding concentration was then calculated from the calibration equation as shown in Figure 4-11. The limit of detection of 32.9 µg/L and the limit of quantitation of 97.7 µg/L were determined from 46 replicate dialyses of reagent blanks.

4.2.3.3 Precision and Accuracy

To evaluate the precision and accuracy of dialysis sampling, a standard reference material was determined by using the proposed sampling method followed with catalytic colorimetric method. The results were shown in Table 4-7.

Table 4-7 Iodide concentration determined in standard reference materials by using dialysis sampling as sample preparation step.

Sample	Certified value (mg/kg)	Found value (mg/kg)	%Recovery	%RSD	N
Skim milk powder (SRM 151)	5.35 ± 0.14	4.80 ± 0.43	89.7	9	19

Since some of iodine in sample might be found in protein-bonded form, the sampling technique could not draw this species from the sample. This could have an affect on the accuracy of the results. However, the accuracy was acceptable according to the AOAC criteria which specified that %recovery must not be exceeding the range of 80-110% at 1 mg/kg concentration level.

4.3 Application of the dialysis sampling for determination of iodide in food samples

As examples to show the applicability of the developed dialysis sampling, milk and egg samples was sampled for determination of iodide content. The recoveries of spiked iodide samples were shown in Table 4-8.

Table 4-8 Average recovery of spiked iodide from milk and egg samples.

Sample	Original iodide concentration (µg/L)		Iodide spiked (µg/L)	Iodide found (µg/L)		%Recovery**	
	Mean	%RSD		Mean	%RSD	Mean	%RSD
Milk 1	385.7	2 (N=3)	161.4	559.6	1 (N=3)	107.7	4 (N=3)
Milk 2	375.2	6 (N=3)	159.5	533.9	3 (N=3)	99.5	11 (N=3)
Milk 3	349.4	1 (N=3)	160.5	534.4	8 (N=4)	115.3	22 (N=4)
Egg 1	468.2*	3 (N=3)	160.5	634.7*	5 (N=3)	103.4	18 (N=3)
Egg 2	296.4*	4 (N=3)	241.4	566.3*	7 (N=3)	111.7	15 (N=3)
Egg 3	386.9*	9 (N=3)	241.0	626.1*	8 (N=3)	99.8	22 (N=3)

*Iodide concentration of egg solution in 25 mL.

**%Recovery = ratio percentage of the mass of iodide found in spiked sample subtracted by the original mass of iodide found in sample to the spiked mass of iodide.

As shown in Table 4-8, recoveries (%) were in the range of 100-115% with 4-22% RSD for milk samples and 100-112% with 15-22% RSD for egg samples. It can be seen that %RSD of some data was high. The reason of this might be attributed to the imprecision of manual procedure of detection technique. In addition, another factor affected on the results was the magnification of working range of dialysis sampling. According to the iodide dilution of dialysis sampling, the working range of 0-20 µg/L as shown in Figure 4-1 was magnified to 0-480 µg/L that caused more difference between iodide concentrations in the same sample. Therefore, the %RSD was affected by a combination of these factors. However, they were acceptable at this concentration level. Therefore, dialysis sampling was successfully applied for determination of iodide in milk and egg samples.

CHAPTER V

CONCLUSION AND SUGGESTION OF FUTURE WORK

5.1 Conclusion

The sample preparation using hollow fiber membrane dialysis was developed for determination of iodide ion in milk and egg samples. The design of dialysis systems was studied. When the standard iodide solution as donor solution or deionized water as acceptor solution was recirculatory pumped via the polysulfone hollow fiber membrane which was immersed in the other one, the high %dialysis was obtained, however, the dialysis time was too long. In addition, the mechanism of the process involved the filtration effect that could be affected the accuracy and precision of the results. When the donor and acceptor were recirculated countercurrently across the membrane, the similar %dialysis was achieved and the filtration effect was still found. To minimize the filtration effect, the constant volume of sample was fixed inside the lumen while the acceptor solution was flown outside in the non-recirculation system. It was found that this design offered higher dialysis efficiency than previous systems and the filtration effect was not found. In addition, the sample volume was greatly reduced providing sufficient %dialysis for dialysis time less than 10 min. Therefore, this dialysis system was chosen for this work.

The acceptor flow rate was optimized. When the acceptor flow rate was decreased from 1 mL/min to 0.1 mL/min, the dialysis time was 4.5 times slower while the %dialysis was only 0.15 times higher. So, 1 mL/min was selected due to providing sufficient %dialysis for short dialysis time.

Furthermore, the composition of the donor solution was studied. It was found that the %dialysis from the donor modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was higher than that from donor solution with deionized water due to the effect of ionic strength on the dialysis mechanism. However, as the salt concentration in the donor solution was increased, the %dialysis of iodide was not increased or even lower. It was possible that the solution may become more viscous

that caused slower diffusion of iodide. Consequently, the mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was used for modification of the donor solution.

The dialysis sampling was evaluated for the effectiveness of method. It was found that the linear dynamic range of 0 to 480 µg/L was observed with R^2 =0.9987. The detection limit and the quantitation limit were found to be 32.9 µg/L and 97.7 µg/L respectively. The recovery of 89.7% with 9% RSD was obtained from 19 replicate dialysis of skim milk powder standard reference material, which is considered to be acceptable values for food analysis.

The dialysis method was applied to milk and egg sample. The recovery percentages were found in the range of 100-115% (4-22% RSD) for milk samples and 100-112% (15-22% RSD) for egg samples. Although %RSD of some data was high, they were acceptable at this concentration level. From the results, dialysis sampling was successfully applied for determination of iodide in milk and egg samples.

5.2 Suggestion of future work

Since, the Dialysis system 3 has been the system of choice and some parameters involving the dialysis efficiency have been studied, there are a few parameters that might affect the dialysis efficiency may be studied, such as membrane types, other type of ionic salts and temperatures. Furthermore, the dialysis sampling can be developed into an automatic system with flow injection analysis (FIA) for more precise and accurate results.

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APPENDICES

APPENDIX A

Table A-1 The %dialysis of iodide of various initial iodide concentrations of unmodified donor solution.

Modifier	%Dialysis at initial iodide concentration (µg/L)				
	160	240	320	400	480
Deionized water	2.7	2.6*	2.7	2.9	3.2
	3.1	3.1	2.9	3.0	3.8*
	3.1	3.0	2.8	2.3*	2.8
	1.8*	3.1	4.7*	3.8*	4.0*
	2.6	2.9	4.6*	4.0*	3.0
	2.7	3.3	4.0*	3.8*	3.0
	2.8	3.2	3.0	2.3*	2.5*
	3.1	4.2*	2.1*	2.7	2.7
	2.9	3.9	2.3*	2.5	2.7
	3.0	2.5*	2.7	2.7	3.3
	2.2	3.0	2.5	2.7	2.9
	2.0*	3.8*	2.8	3.0	2.9
	3.4	3.2	3.0	2.2*	2.7
	3.6	3.5	1.9*	2.7	2.4*
	4.5	2.7*	1.9*	2.4	2.4*
	5.0*	3.0	3.5	3.5	3.1
	4.3	2.6*	3.3	3.1	3.3
	5.1*	3.5	3.3	3.6*	3.4*
	-	3.3	3.2	3.1	3.3
	-	4.0*	3.6	3.1	3.4
	-	4.1*	3.0	3.2	3.7*
	-	3.4	-	-	-
	-	3.5	-	-	-

* The data was not used for statistically analysis by using two-way ANOVA with replication.

Table A-2 The %dialysis of iodide of various initial iodide concentrations of donor solution modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$.

Modifier	%Dialysis at initial iodide concentration (µg/L)				
	160	240	320	400	480
0.0028 M Na_2CO_3 +	7.5*	5.0*	4.5*	4.1*	2.9*
0.0022 M $NaHCO_3$	7.8*	4.6*	4.3*	3.3	2.9*
	7.8*	4.5*	3.6	3.4	2.9*
	3.5	3.4	3.4	3.3	3.0
	3.5	3.5	3.0*	3.0*	3.0
	4.1	3.3	2.9*	3.4	2.6*
	2.5*	2.1*	2.9*	2.9*	3.4
	2.5*	2.9*	3.1	3.4	3.5*
	2.8*	3.2	3.2	3.2	3.4
	5.2	3.6	4.2*	3.8*	3.3
	6.0*	4.0	4.0*	3.6	3.5*
	4.8	4.1	3.7	3.8*	3.2
	5.1	3.9	3.7	3.3	3.3
	6.9*	4.7*	3.8	3.3	3.0
	6.0*	4.8*	3.8	3.9*	3.5*
	3.8	3.0*	4.0*	4.0*	3.3
	4.6	4.2	4.0*	2.9*	3.7*
	5.0	4.5*	4.6*	4.0*	4.1*
	2.7*	2.4*	2.6*	2.5*	3.2
	2.2*	2.4*	2.4*	2.4*	3.0*
	3.0*	2.5*	3.0*	2.9*	2.7*
	3.5	3.5	3.6	3.4	3.8*
	3.2	3.6	3.3	3.1	3.7*
	2.8*	3.1	3.3	3.4	3.0
	3.9	3.7	3.2	3.6	3.3
	3.9	3.5	3.2	3.2	3.2
	3.9	-	3.5	-	3.1

* The data was not used for statistically analysis by using two-way ANOVA with replication.

Table A-3 The %dialysis of iodide of various initial iodide concentrations of donor solution modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$.

Modifier	%Dialysis at initial iodide concentration (µg/L)				
	160	240	320	400	480
0.028 M Na_2CO_3 +	4.1	3.5	2.6*	4.3	2.5
0.022 M $NaHCO_3$	3.2	3.8	2.9	3.8	2.4*
	3.3	3.3	3.1	2.8	2.7
	3.4	3.3	3.2	2.5	2.8
	2.2	2.8	3.0	2.7	2.6
	4.8	3.5	3.5	3.1	2.8
	3.3	3.0	2.9	2.9	3.1
	3.2	3.1	3.2	2.9	2.9
	3.5	4.4	3.3	3.2	2.5
	6.8	4.2	3.9	3.4	3.5
	8.0*	3.4	4.0	3.1	3.4
	3.7	4.0	3.6	3.1	3.2
	4.7	4.0	3.5	3.9	3.1
	5.0	4.2	4.0	4.0	3.7
	4.6	-	4.0	-	3.7

* The data was not used for statistically analysis by using two-way ANOVA with replication.

Table A-4 The %dialysis of iodide at various initial iodide concentrations with various modifiers.

Modifier	Initial iodide concentration in donor solutions (µg/L)	%Dialysis	%RSD	N
H_2O	160	3.2	30	18
	240	3.3	15	23
	320	3.0	25	21
	400	3.0	18	21
	480	3.1	14	21
0.0028 M Na_2CO_3+	160	4.4	38	27
0.0022 M $NaHCO_3$	240	3.6	22	26
	320	3.5	16	27
	400	3.3	13	26
	480	3.2	11	27
0.028 M Na_2CO_3+	160	4.3	35	15
0.022 M $NaHCO_3$	240	3.6	13	14
	320	3.4	14	15
	400	3.3	16	14
	480	3.0	15	15

Table A-5 Average iodide concentrations in the acceptor solutions at various initial iodides with various conditions of modifier.

Set of experiment	Condition		Iodide concentration in acceptor solutions at initial iodide concentration (µg/L)				
	Donor	Acceptor	160	240	320	400	480
1*	modified with salts	DI	4.8	7.4	10.2	13.0	14.5
	DI	DI	4.4	6.6	9.3	11.2	14.0
	DI	modified with salts	2.7	5.7	8.0	10.0	12.1
2**	modified with salts	DI	-	-	-	-	-
	DI	DI	4.2	6.8	8.1	11.3	12.0
	DI	modified with salts	2.4	5.2	6.9	7.7	10.0

*N=2 observations per mean.

**N=3 observations per mean.

APPENDIX B

Table B-1 A statistical test for difference between %dialysis of iodide from the unmodified donor solutions , modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ and mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ by using two-way ANOVA at P = 0.05.

ANOVA: Two-Factor with Replication

Source of Variation	SS	df	MS	F	P-value	F crit
Initial iodide concentration	12.17382	4	3.043455	12.94808	2.28E-09	2.417963
Modifier	9.82876	2	4.91438	20.90774	5.93E-09	3.04223
Interaction	3.530724	8	0.441341	1.877639	0.06544	1.986129
Within	45.8349	195	0.235051			
Total	71.3682	209				

Table B-2 A statistical test for difference between %dialysis of iodide from the unmodified donor solutions and modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ by using two-way ANOVA at P = 0.05.

ANOVA: Two-Factor with Replication

Source of Variation	SS	df	MS	F	P-value	F crit
Initial iodide concentration	5.884359	4	1.47109	10.81157	1.35E-07	2.44135
Modifier	8.671971	1	8.671971	63.73346	6.56E-13	3.913989
Interaction	2.731151	4	0.682788	5.018054	0.000858	2.44135
Within	17.68861	130	0.136066			
Total	34.97609	139				

Table B-3 A statistical test for difference between %dialysis of iodide from the unmodified donor solutions and modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ by using two-way ANOVA at P = 0.05.

ANOVA: Two-Factor with Replication

Source of Variation	SS	df	MS	F	P-value	F crit
Initial iodide concentration	5.554813	4	1.388703	4.862023	0.001097	2.44135
Modifier	5.778525	1	5.778525	20.23133	1.5E-05	3.913989
Interaction	2.413328	4	0.603332	2.112341	0.082887	2.44135
Within	37.13093	130	0.285623			
Total	50.8776	139				

Table B-4 A statistical test for difference between %dialysis of iodide from the donor solutions modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ and mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ by using two-way ANOVA at P = 0.05.

ANOVA: Two-Factor with Replication

Source of Variation	SS	df	MS	F	P-value	F crit
Initial iodide concentration	14.67383	4	3.668458	12.94155	6.73E-09	2.44135
Modifier	0.292643	1	0.292643	1.032384	0.311487	3.913989
Interaction	0.151607	4	0.037902	0.133709	0.969715	2.44135
Within	36.85025	130	0.283463			
Total	51.96833	139				

Table B-5 The statistical test for difference between %dialysis of iodide at the same initial iodide concentrations of donor solutions modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ and mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ by using one-way ANOVA at P = 0.05.

ANOVA: Single Factor

Initial iodide concentration	Source of Variation	SS	Df	MS	F	P-value	F crit
160 µg/L	Between Modifiers	0.18973	1	0.18973	0.07326	0.78804	4.08475
	Within Modifiers	103.592	40	2.58979			
	Total	103.781	41				
240 µg/L	Between Modifiers	0.00699	1	0.00699	0.01418	0.90585	4.09817
	Within Modifiers	18.7253	38	0.49277			
	Total	18.7323	39				
320 µg/L	Between Modifiers	0.13547	1	0.13547	0.47618	0.49414	4.08475
	Within Modifiers	11.3802	40	0.2845			
	Total	11.5156	41				
400 µg/L	Between Modifiers	0.052	1	0.052	0.23657	0.62948	4.09817
	Within Modifiers	8.35264	38	0.21981			
	Total	8.40464	39				
480 µg/L							
	Between Modifiers	0.60308	1	0.60308	4.16073	0.04801	4.08475
	Within Modifiers	5.79783	40	0.14495			
	Total	6.40091	41				

Table B-6 A statistical test for difference between %dialysis of iodide of each iodide concentration in unmodified donor solutions by using one-way ANOVA at P = 0.05.

ANOVA: Single Factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Concentrations	1.355594	4	0.338899	0.803196	0.525990405	2.46355
Within Concentrations	41.77184	99	0.421938			
Total	43.12743	103				

Table B-7 A statistical test for difference between %dialysis of iodide of each iodide concentration in donor solutions modified with mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ by using one-way ANOVA at P = 0.05.

ANOVA: Single Factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Concentrations	22.07568	4	5.51892	6.806577	5.33238E-05	2.442453
Within Concentrations	103.7852	128	0.810822			
Total	125.8609	132				

Table B-8 A statistical test for difference between %dialysis of iodide of each iodide concentration in donor solutions modified with mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ by using one-way ANOVA at P = 0.05.

ANOVA: Single Factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Concentrations	13.28571	4	3.321428	5.125847	0.001140035	2.506621
Within Concentrations	44.0624	68	0.647976			
Total	57.34811	72				

APPENDIX C

Table C-1 Certificated concentration and uncertainty of elements contained in Standard Reference Materials (BCR-151).

Element	Concentration (based on dry mass)	
	Certified value	Uncertainty
Cd	101 ng/g	8 ng/g
Cu	5.23 µg/g	0.08 µg/g
Fe	50.1 µg/g	1.3 µg/g
Hg	101 ng/g	10 ng/g
I	5.35 µg/g	0.14 µg/g
Pb	2.002 µg/g	0.026 µg/g

Table C-2 The recovery percentage of iodide determined in Standard Reference Materials by using dialysis sampling as sample preparation step.

Sample	Certified value of iodide (mg/kg)	Iodide found (mg/kg)	%Recovery
Skim milk powder	5.35 ± 0.14	5.11	95.5
(BCR-151)		4.37	81.6
		4.40	82.2
		4.90	91.6
		4.87	91.1
		4.38	81.9
		4.52	84.5
		4.94	92.3
		4.72	88.3
		5.22	97.5
		5.60	104.7
		4.86	90.8
		5.22	97.5
		5.25	98.1
		5.45	101.9
		4.11	76.8
		4.21	78.8
		4.56	85.2
		4.50	84.1

Table C-3 Recovery of spiked iodide from milk and egg samples.

Sample	Original iodide concentration (µg/L)	Iodide found (µg/L)	%Recovery
Milk 1	383.6	554.3	104.4
	379.4	567.8	112.8
	394.1	556.6	105.9
Milk 2	400.7	514.7	87.5
	363.5	536.4	101.1
	361.4	550.4	109.9
Milk 3	347.1	564.7	134.2
	353.1	560.5	131.5
	347.9	536.8	116.8
	-	475.5	78.6
Egg 1	288.1*	611.1*	130.2
	310.1*	549.5*	104.7
	291.2*	538.3*	100.1
Egg 2	383.9*	642.4*	106.6
	353.2*	668.8*	117.5
	423.7*	567.1*	75.3
Egg 3	461.1*	602.4*	83.3
	484.8*	661.0*	119.8
	458.6*	640.7*	107.1

*Iodide concentration of egg solution in 25 mL.

APPENDIX D

HOLLOW FIBER MEMBRANE DIALYSIS SAMPLING FOR DETERMINATION OF IODIDE IN MILK SAMPLES

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Abstract: Iodine is an essential nutrient for human. Insufficient uptake of iodine leads to iodine deficiency disorder (IDD). To ensure sufficient iodine uptake, many food products are fortified with iodine. Therefore, determination of iodine content in iodine fortified food is important for controlling the quality of food products. Nevertheless, method for determination of iodine in food sample has been an analytical problem, especially in the sample preparation step, which, in general, is tedious, time-consuming. Currently, membrane separation techniques have gained widespread uses in sample preparation as they are size selective separation and simple operation with gentle condition. In this work, the hollow fiber membrane dialyzer has been designed and developed for determination of iodide ion in milk samples. The dialyzer was consisted of polysulfone hollow fiber membrane, filled with sample as a donor phase and inserted in glass tubing, where deionized water was slowly flown through as an acceptor phase. Iodide ion was determined by catalytic colorimetric method based on thiocyanate-nitrite assay (Thio-Nit method). Our designed dialyzer provided the average % dialysis of 2.2 % (0.2 SD). In addition, the effect of donor composition was studied. The results showed that the % dialysis of iodide from the donor modified with a mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was significantly higher than that from donor solution with deionized water. The relationship between the initial iodide concentrations in the donor solutions and the iodide concentration in the acceptor solution was also studied. It found that the linear relationship was obtained. The sampling procedure was applied for determination of iodine content in milk samples. The sample preparation step was simple, inexpensive and less than 10 min compared to approximately 5-6 hours of dry ashing method.

Introduction

Iodine is an essential micronutrient. In food, iodine is present in the forms of iodide salts or iodate salts. Iodine is one of key components of thyroid hormones that play an important role in the development of brain function, cell growth and control the energy metabolism of the body. Deficiency of iodine leads to goiter, irreversible mental retardation and decrease of survival rates among children [1]. Generally, the amount of iodine daily intake of 150 µg of iodide is required for adult but it should not exceed 1 mg of iodide/day (WHO). To prevent iodine deficiency disorder, many food products are fortified with iodine. Therefore, determination of iodine content in iodine fortified food is important for controlling the quality of food products.

Despite the fact that there are several methods available for determination of iodine [2-7], sample preparation steps for food matrices, such as combustion with oxygen [5], wet ashing [6] and dry ashing [7], are still tedious, time-consuming and multiple steps. Nowadays, membrane separation techniques have gained widespread uses in sample preparation [8,9] as they are size selective separation, low energy consumption, and simple operation with gentle condition. Dialysis is a sampling technique based on size selective diffusion driven by concentration gradient across a semi permeable membrane. The analytes whose sizes are smaller than the pores of the membrane would be diffused across the membrane whereas the larger size matrices would not.

In the present work, the hollow fiber membrane has been applied for dialysis sampling of iodide ion. The hollow fiber membrane dialyzer has been designed and developed for determination of iodide ion in milk samples.

Materials and Methods

The Schematic diagram of dialysis sampling system was illustrated in Figure 1. The dialyzer was consisted of a 36-cm polysulfone hollow fiber membrane inserted in a glass tubing with one end connected to a syringe pump. The hollow fiber was first washed and soaked in deionized water so that the pores would be filled. Then, the lumen of the hollow fiber was emptied by mild flow of air prior to use. A 0.2 mL of sample was gradually filled in the lumen by the mean of syringe. Then, both ends of the fiber were sealed with glue. The fiber was inserted into the glass tubing. A 4 mL of deionized water was slowly pumped through as an acceptor phase at the flow rate of 1 $mLmin^{-1}$. The dialyzed solution was collected and determined for iodide by the catalytic colorimetric method based on thiocyanate-nitrite assay [7].

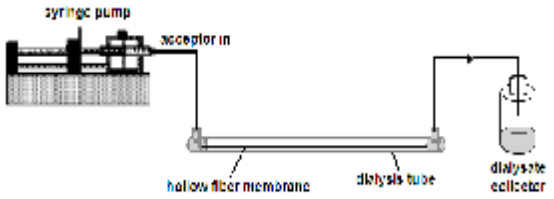

Figure 1. Schematic diagram of the hollow fiber dialysis sampling system

Results and Discussion

Firstly, the dialysis configuration was a closed recirculating system. A 50 mL of sample solution was recirculatory pumped via 15-cm hollow fiber membrane which was immersed in an equal volume of acceptor solution at the flow rate of 1 $mLmin^{-1}$. The acceptor solution was agitated through the process which was continued until the desired degree of separation was achieved. It was found that about 3% of dialysis was obtained when 1-hr was used for dialysis and increased to about 13 % at 20-hr. Although % dialysis was increased, dialysis time was too long. Moreover, the pressure that occurred inside the lumen of the hollow fiber membrane from pumping might have caused filtration effect that increased the final volume of the acceptor solution, so that the accuracy and precision of the results could be affected. For this reason, a new design of dialysis configuration was developed. In the present design, the longer membrane was used and only 0.2 mL of sample was fixed inside the lumen while the acceptor solution was flown in open system, so the fresh acceptor solution always maintained the highest driving force. As mass-transfer equilibrium was never been reached, the % dialysis could be possibly improved. In addition, the sample volume for dialysis was greatly reduced and the surface area of mass-transfer was increased by the longer membrane.

Since the diffusion of the analyte may be influenced by the presence of other ionic salts or ionic strength of the donor solution, the donor solutions containing a mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ and a mixture of 0.028 M Na_2CO_3 and 0.022 M $NaHCO_3$ were studied. Table 1 showed the % dialysis of iodide from the donor solutions modified with mixture of salts. The % dialysis of iodide from the donor modified with salts was significantly higher than that from donor solution with deionized water. This could be due to the effect of ionic strength on the dialysis mechanism. The difference mobility of added ions caused difference function potential between donor solutions and acceptor solutions. Sodium ions that have higher mobility than carbonate ions moved through semi permeable membrane into acceptor solutions, while iodide ions were brought along by charge attraction. However, as the salt concentration in the donor solution was increased, the % dialysis of iodide was not increased or even lower. The reason might be that as higher salt concentration, the solution may become more viscous so that the diffusion of iodide would be slower.

Table 1: % dialysis of iodide from the donor solutions modified with mixture of salts

Donor solution ([I] = 320 µg L^{-1})	% dialysis*	SD
Deionized water	2.2	0.2 (N=5)
Modified with 0.0028 M Na_2CO_3 + 0.0022 $NaHCO_3$	2.9	0.3 (N=14)
Modified with 0.028 M Na_2CO_3 + 0.022 $NaHCO_3$	2.8	0.3 (N=6)

*% dialysis = ratio percentage of the iodide concentration found in the acceptor solution to the initial iodide concentration in the donor solution

Figure 2 illustrated the relationship between the initial iodide concentrations in the donor solutions and the iodide concentration in the acceptor solution. The linear relationship with correlation coefficient (r) of 0.9610 was obtained. The slope of that relationship might represent average % dialysis throughout the iodide concentration ranges which was approximately 3 %. According to that relationship, it suggested that the iodide content could be determined quantitatively. However, the accuracy and precision must be improved. It was expected that the interface between donor solution and acceptor solution inside the pore of the membrane might not be in a good contact causing irregular diffusion rates.

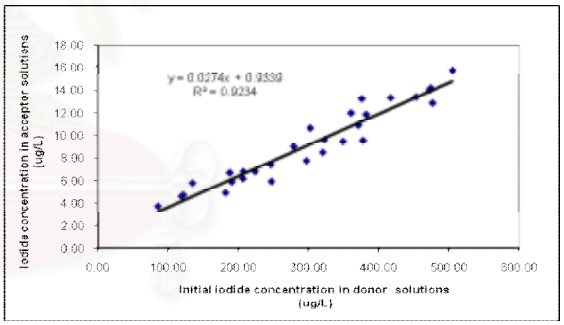

Figure 2. Relationship between the initial iodide concentration in the donor solution and the iodide concentration in the acceptor solution. (Donor phase was modified with 0.0028 M Na_2CO_3 + 0.0022 M $NaHCO_3$)

The hollow fiber dialysis sampling procedure was applied for determination of iodine content in infant milk samples. The milk samples were purchased from the local stores. The preliminary results were summarized in Table 2.

Table 2: Iodide concentration in infant milk samples

Sample	Iodine content labeled ((µg L^{-1})	Iodide concentration determined (µg L^{-1})	SD (N=3)
M1	375	276	2
M2	375	446	8
M3	375	516	28

The iodide concentrations determined by the hollow fiber dialysis sampling procedure were much different from the labeled values. The accuracy and the precision of the procedure must be further verified by employment of Certified Reference Materials or comparison of the iodide concentration with other methods.

Conclusions

According to our studies, the new designed dialyzer could greatly reduce the sample volume and increase the surface area of mass-transfer. The modification of donor solutions by adding mixture of 0.0028 M Na_2CO_3 and 0.0022 M $NaHCO_3$ was improved efficiency on dialysis. In addition, the linear relationship between the initial iodide concentrations in the donor solutions and the iodide concentration in the acceptor solution indicated that this sampling procedure could be possibly used for determination of iodide ion in milk samples. The hollow fiber dialysis sampling technique was simple, inexpensive and took less than 10 min compared to approximately 5-6 hours of dry ashing method. In the future, it could be applied for the determination of iodide ion in other food samples such as fish sauce, tablets and egg.

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Poster presentation and proceeding

"Hollow fiber membrane dialysis for sampling of iodide in food sample" Kanokwan Chunhong, Pakorn Varanusupakul. Poster presentation, *Thirty fourth Congress on Science and Technology of Thailand (STT 34)*, Queen Sirikit National Convention Center, Bangkok, Thailand, 31 October – 2 November, 2008.

"Hollow fiber membrane dialysis sampling for determination of iodide in milk samples" Kanokwan Chunhong, Pakorn Varanusupakul. Poster presentation and proceeding, *Pure and Applied Chemistry International Conference 2009 (PACCON 2009)*, Naresuan University, Phitsanulok, Thailand, 14-16 January, 2009.