

**DETERMINATION OF IODIDE IN PHARMACEUTICAL  
SAMPLES BY GAS DIFFUSION FLOW INJECTION USING  
IODINE-STARCH REACTION**



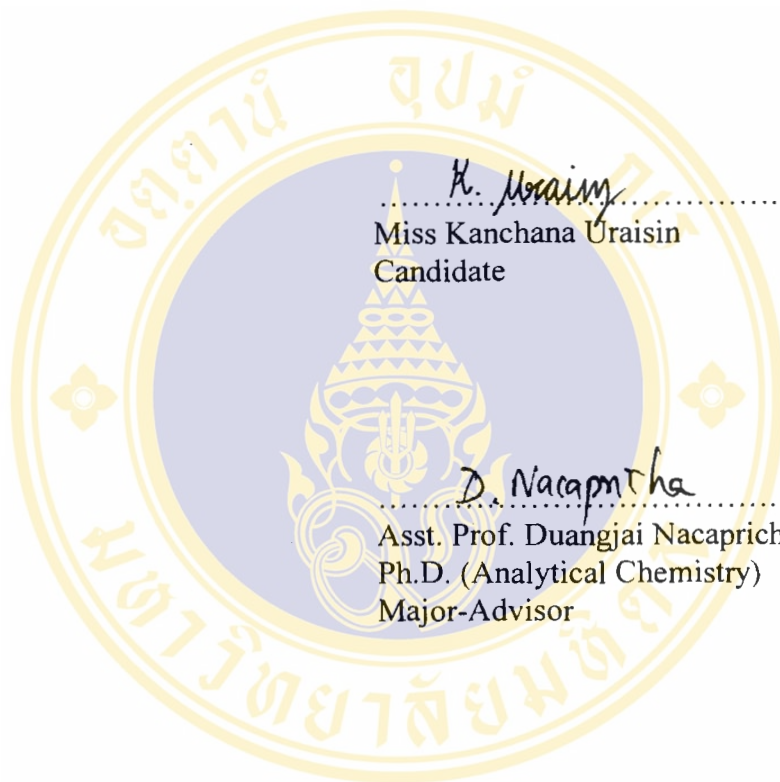
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IODINE-STARCH REACTION**



*K. Uraisin*  
.....  
Miss Kanchana Uraisin  
Candidate

*D. Nacapricha*  
.....  
Asst. Prof. Duangjai Nacapricha,  
Ph.D. (Analytical Chemistry)  
Major-Advisor

*P. Wilairat*  
.....  
Assoc. Prof. Prapin Wilairat,  
Ph.D. (Physical Chemistry)  
Co-Advisor

*Rassmidara Hoonsawat*  
.....

Assoc. Prof. Rassmidara Hoonsawat,  
Ph.D.  
Dean  
Faculty of Graduate Studies

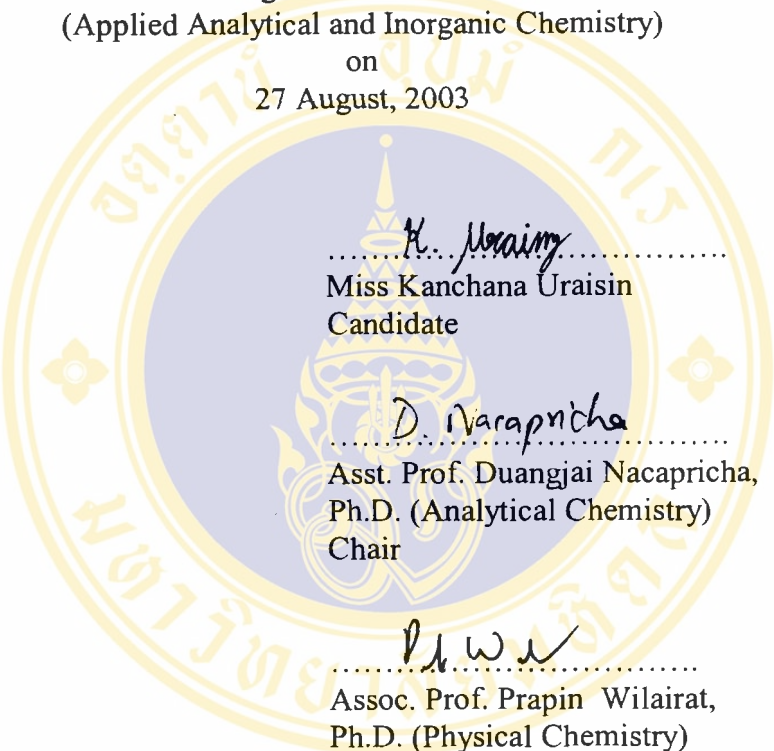
*Laddawan Pdungsap*  
.....

Assist. Prof. Laddawan Pdungsap,  
Ph.D.  
Chair  
Master of Science Programme in Applied  
Analytical and Inorganic Chemistry  
Faculty of Science

Thesis  
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27 August, 2003



*K. Uraisin*  
.....  
Miss Kanchana Uraisin  
Candidate

*D. Nacapricha*  
.....  
Asst. Prof. Duangjai Nacapricha,  
Ph.D. (Analytical Chemistry)  
Chair

*P. Wilairat*  
.....  
Assoc. Prof. Prapin Wilairat,  
Ph.D. (Physical Chemistry)  
Member

*Orawon Chailapakul*  
.....  
Asst. Prof. Orawon Chailapakul,  
Ph.D. (Analytical Chemistry)  
Member

*Rassmidara Hoonsawat*  
.....

Assoc. Prof. Rassmidara Hoonsawat,  
Ph.D.  
Dean  
Faculty of Graduate Studies  
Mahidol University

*Amaret Bhumiratana*  
.....

Prof. Amaret Bhumiratana,  
Ph.D.  
Dean  
Faculty of Science  
Mahidol University

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DETERMINATION OF IODIDE IN PHARMACEUTICAL SAMPLES BY GAS  
DIFFUSION FLOW INJECTION USING IODINE-STARCH REACTION

KANCHANA URAISIN 4336621 SCAI/M

M.Sc. (APPLIED ANALYTICAL AND INORGANIC CHEMISTRY)

THESIS ADVISORS: DUANGJAI NACAPRICHA, Ph.D. (ANALYTICAL CHEMISTRY),  
PRAPIN WILAIRAT, Ph.D. (PHYSICAL CHEMISTRY)

ABSTRACT

This work presents the development of gas diffusion (GD) flow injection (FI) methods for the determination of iodide in pharmaceutical samples by using the common iodine-starch reaction. An iodide standard or a sample is injected into a carrier stream in which iodide is oxidized to iodine. Some of the liberated iodine permeates through the hydrophobic membrane fitted inside the gas diffusion unit into an acceptor stream containing iodide and starch. Within the permeate zone, the blue-colored complex of  $I_3^-$ -starch is developed and can be detected at 590 nm by a colorimeter. After a standard or sample injection, injection of a cleaning solution is necessary. Separation of the analyte from the matrix via the use of gas diffusion increases the selectivity and allows for the analysis of colored samples.

Three GD-FI systems were investigated. System 1 is optimized to give 100 % in-line oxidation of I to  $I_2$ . The system proved its accuracy in determination of total iodine in synthetic samples of  $I_{(aq)} + I_{2(aq)}$ . System 1 is quite versatile in terms of use as a real application. The system is perfectly suitable for the analysis of KI tablets sold in the case of a nuclear emergency. Due to its working concentration range (50 to 300 mg I/L), dilution (1/100) is required for an asthma medicine (Mixt. Stramonium Co., ca. 9,000 mg I/L).

For direct injection of the medicine (Mixt. Stramonium Co.), a modification of System 1 was made and the optimized system was called System 2. Coupling of a dialysis unit to the FI System 1 increased the working range from 6,000 to 10,000 mg I/L. The liquid sample can be directly injected into the system without employing a complicated nanolitre injection. Similar to System 1, System 2 provided satisfactorily high precision with an RSD of 1.44 % (RSD for System 1 = 1.27 %). System 2 has a good potential for incorporation into the manufacturing process for on-line analysis.

The developed systems provide rapid analysis of 30 and 20 samples/h for System 1 and 2, respectively. These are much faster than the use of a batch ion selective electrode (8 samples/h). Also one of the developed methods (System 2) should be more practical for use in process control.

Limit of detection (LD) was not the key parameter for development of System 1 and 2. LDs of System 1 and 2 were 1 and 200 mg I/L, respectively. These systems are surely not suitable for samples containing a low level of iodine. System 1 was therefore modified for analysing multivitamin tablets. The resulting System 3 with a LD of 0.035 mg I/L and dynamic range of 0.5 to 3.0 mg I/L, was tested for Centrum extract. However, System 3 was not suitable for the sample gave negative results with a poor recovery (about 80 %). Solid phase extraction using  $C_{18}$  Sep-Pak did not improve the method's accuracy.

KEY WORDS: FLOW INJECTION/ GAS DIFFUSION/ DIALYSIS/ IODIDE/  
IODINE-STARCH

การวิเคราะห์ไอโอไดด์ในผลิตภัณฑ์ยาด้วยระบบโพลินเจกชันร่วมกับการใช้ก๊าซคิฟฟิวชันโดยอาศัยปฏิกิริยาของไอโอดีนและน้ำแป้ง

(DETERMINATION OF IODIDE IN PHARMACEUTICAL SAMPLES BY GAS DIFFUSION FLOW INJECTION USING IODINE-STARCH REACTION)

กาญจนา อูโรสินทร์ 4336621 SCAI/M

วท.ม. (เคมีวิเคราะห์และเคมีอินทรีย์ประยุกต์)

คณะกรรมการควบคุมวิทยานิพนธ์ : ดวงใจ นาคะปรีชา, Ph.D. (Analytical Chemistry),  
ประพิณ วิไลรัตน์, Ph.D. (Physical Chemistry)

#### บทคัดย่อ

งานวิจัยนี้ได้พัฒนาระบบโพลินเจกชันเชื่อมกับก๊าซคิฟฟิวชันชนิดสำหรับการวิเคราะห์ไอโอไดด์ในตัวอย่างยาโดยอาศัยปฏิกิริยาอย่างง่ายของไอโอดีนและน้ำแป้ง สามารถฐานไอโอไดด์หรือตัวอย่างถูกฉีดเข้าไปในกระแสตัวพาซึ่งไอโอไดด์สามารถถูกออกซิไดซ์เป็นไอโอดีน โดยไอโอดีนที่เกิดขึ้นจะแพร่ผ่านไฮโดรโฟบิกเมมเบรนซึ่งอยู่ภายในก๊าซคิฟฟิวชันชนิดไปสู่กระแสตัวรับซึ่งประกอบด้วยไอโอไดด์และน้ำแป้งเกิดสารประกอบเชิงซ้อนสีน้ำเงินขึ้น และสามารถทำการตรวจวัดสีที่ 590 นาโนเมตร โดยหลังจากทำการฉีดสารมาตรฐานหรือตัวอย่างจำเป็นต้องทำการฉีด cleaning solution ทุกครั้ง การแยกสารสนใจออกจากสารรบกวนโดยอาศัยก๊าซคิฟฟิวชันชนิดส่งผลให้เพิ่มความเฉพาะเจาะจงและสามารถทำการวิเคราะห์ตัวอย่างที่มีสีได้

ระบบโพลินเจกชันเชื่อมกับก๊าซคิฟฟิวชัน 3 ระบบได้ถูกเสนอขึ้น โดยระบบที่ 1 สามารถทำให้มีประสิทธิภาพสูงสุดโดยการให้เกิด 100 เปอร์เซ็นต์ออกซิเดชันของไอโอไดด์เป็นไอโอดีนได้ภายในระบบ และสามารถพิสูจน์จากความต้องการโดยการวิเคราะห์ปริมาณไอโอดีนรวมในสารผสมสังเคราะห์ระหว่างไอโอไดด์และไอโอดีน ระบบนี้สามารถประยุกต์ใช้ได้จริงในการวิเคราะห์ KI tablet ซึ่งเป็นยาที่ใช้ในกรณีที่เกิดภาวะฉุกเฉินเกี่ยวกับปอด และยารักษาโรคหอบหืด (Mixt. Stramonium Co. มีความเข้มข้นประมาณ 9,000 มิลลิกรัมต่อลิตร) ซึ่งต้องเจือจางตัวอย่าง 100 เท่า ก่อนทำการวิเคราะห์ เนื่องจากระบบนี้สามารถทำการวิเคราะห์ในช่วง 50 – 300 มิลลิกรัมต่อลิตรเท่านั้น

ระบบที่ 2 ได้ทำการปรับปรุงขึ้นจากระบบที่ 1 เพื่อที่จะสามารถทำการฉีดยา Mixt. Stramonium Co. ได้โดยตรง การนำเอาไอโอดีนชนิดต่อเข้ากับระบบที่ 1 ทำให้สามารถทำการวิเคราะห์ในช่วงที่สูงขึ้นคือ 6,000 - 10,000 มิลลิกรัมต่อลิตร ตัวอย่างสามารถฉีดเข้าสู่ระบบได้โดยตรงโดยปราศจากการฉีดในระดับนาโนลิตรที่สูงยาก เช่นเดียวกับกับระบบที่ 1 ระบบนี้ให้ความแม่นยำสูง คือ RSD เท่ากับ 1.44% ขณะที่ระบบที่ 1 มี RSD เท่ากับ 1.27% ระบบที่ 2 มีความสามารถนำไปใช้เป็นประโยชน์สำหรับการควบคุมคุณภาพในระบบการผลิตได้

ระบบที่พัฒนาขึ้นสามารถทำการวิเคราะห์ได้อย่างรวดเร็ว คือ 30 และ 20 ตัวอย่างต่อชั่วโมงสำหรับระบบที่ 1 และ 2 ตามลำดับ ซึ่งวิเคราะห์ได้รวดเร็วกว่า batch ion selective electrode (8 ตัวอย่างต่อชั่วโมง) นอกจากนี้ระบบที่ 2 สามารถใช้งานได้อย่างจริงสำหรับควบคุมคุณภาพการผลิต

ขีดจำกัดต่ำสุดในการวิเคราะห์ (LD) ไม่ได้เป็นสิ่งสำคัญสำหรับการพัฒนาระบบที่ 1 และ 2 โดย LD ของระบบที่ 1 และ 2 คือ 1 และ 200 มิลลิกรัมต่อลิตร ซึ่งเป็นที่แน่นอนว่าระบบเหล่านี้ไม่เหมาะสมกับตัวอย่างที่มีความเข้มข้นของไอโอดีนในระดับต่ำ ดังนั้นจึงได้ทำการปรับปรุงระบบที่ 1 เป็น ระบบที่ 3 โดยมีวัตถุประสงค์เพื่อที่จะสามารถทำการวิเคราะห์ในตัวอย่างวิตามินรวมได้ LD ของระบบที่ 3 คือ 0.035 มิลลิกรัมต่อลิตร และสามารถทำการวิเคราะห์ในช่วง 0.5 – 3.0 มิลลิกรัมต่อลิตร และได้ทำการทดสอบระบบกับสารสกัดจากวิตามิน Centrum พบว่าระบบนี้ยังไม่เหมาะสมกับตัวอย่างประเภทนี้ถึงแม้ว่าตัวอย่างได้ผ่านการปรับปรุงโดยใช้ C18 Sep-Pak ก็ไม่ได้ทำให้ระบบมีความถูกต้องขึ้นโดยให้ผลวิเคราะห์ในทางลบ และให้ค่าร้อยละของการคืนกลับที่ต่ำประมาณ 80 เปอร์เซ็นต์

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## LIST OF ABBREVIATIONS

Abbreviation	Definition
ng	Nanogram
$\mu\text{g}$	Microgram
mg	Milligram
kg	Kilogram
$\mu\text{L}$	Microliter
mL	Milliliter
L	Liter
M	Molarity
mg I/L	Milligram iodine per liter
ppm	Part per million
v/v	Volume by volume
w/v	Weight by volume
nm	Nanometer
mm	Millimeter
cm	Centimeter
i.d.	Internal diameter
s	Second
min	Minute
h	Hour
%	Percentage
$^{\circ}\text{C}$	Temperature in Degree Celsius
keV	Kilo electron volt
mV	Millivolt
a.u.	Absorbance Unit

## LIST OF ABBREVIATIONS (CONTS.)

Abbreviation	Definition
IDD	Iodine Deficiency Disorder
FI	Flow Injection
GD	Gas Diffusion
PTFE	Polytetrafluoroethylent
ISE	Ion-selective electrode
UV	Ultraviolet
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
SD	Standard Deviation
RSD	Relative Standard Deviation
et al.	Et. Alli (Latin), and other

## THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Iodine compounds are often used in preparations of some pharmaceutical products. In patients with hyperthyroidism, iodine rapidly inhibits the synthesis of thyroid hormones and this affects metabolism and growth. The level of iodine should be used with extreme caution in case of patients who are markedly sensitive to iodine. Usually the levels of iodide present in pharmaceutical products are quite large. Determination does not necessary require a very sensitive method of detection. However selectivity is more concerned. The simple detection method using the iodine-starch reaction may be good enough and as well selective for iodide determination. This type of detection although not new but has not much been exploited for flow-based analysis method. Therefore the objective of this research is to develop a simple, accurate and very selective method available to determine iodine content in pharmaceutical preparations. The method should be very cost-effective and suitable for drug analysis including Thai pharmaceutical preparation.

## CHAPTER I

### INTRODUCTION

Iodine is an important microconstituent in human body. The highest concentration of iodine in human body is found in the thyroid gland, which contains 70-80 % of the total iodine content (15-20 mg) [1]. It is necessary for the biosynthesis of the thyroid hormones thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) through the precursor protein thyroglobulin and the action of enzyme thyroid peroxidase within the thyroid gland. These play an important role in human development, growth and metabolism. The average daily requirement of iodine is about 150  $\mu\text{g}$ . When iodine requirements are not adequately met, a range of deficiency disorders can develop. Iodine intakes consistently lower than 0.050 mg/day usually result in thyroid hypertrophy (endemic goiter) and severe and prolonged iodine deficiency may result in hypothyroidism.

Prevention of iodine deficiency disorder (IDD) is to add iodine, as supplement, into food and drinking water. It should be noted that the levels of iodine present in food are at trace levels. Iodine content in some foods have been reported, for example, 200-1000  $\mu\text{g}/\text{kg}$  for seafood, 20-70  $\mu\text{g}/\text{litre}$  for cow's milk, 10-50 mg/kg for salt and 4-18  $\mu\text{g}/\text{litre}$  for drinking water [2].

Furthermore, iodine compounds are often used in preparations of some pharmaceutical products. These products have a range of iodine levels. Iodine in the form of tri-iodide is an antiseptic and disinfectant while iodide have been prescribed for respiratory problems such as asthma and chronic bronchitis by acting as an expectorant [3]. In patients with hyperthyroidism, iodide rapidly inhibits the synthesis of thyroid hormones. Iodine is also useful as a protectant of the thyroid gland following radiation exposure. It is accumulated in thyroid gland and blocks the uptake of radioactive iodine isotopes by the thyroid gland thereby minimizing the risk of radiation-induced thyroid neoplasms.

## CHAPTER II

### OBJECTIVES

Iodide is often found in some pharmaceutical products. Sometime, these samples contain iodide at approximate 10,000 mg I/L and have dark color. Other samples such as KI tablets contain iodide at levels of 65 to 130 mg I/tablet. These samples are considered as having high concentrations of iodide. On the other hand, vitamins contain iodide in the range of 100 to 150  $\mu\text{g}$  I/tablet.

In 1992, S. Motomizu et al. reported a method employing use of property of halogens which can be made a gaseous form with subsequent determination at room temperature [4]. In 1997, J.T. Hakedal and P.K. Egeberg [5] developed a flow injection (FI) system, which employed a gas diffusion unit, fitted with PTFE membrane. In this work, iodine ( $\text{I}_2$ ), from the oxidation of the iodide analyte, permeates through the membrane into a stream of iodide. In the work of J.T. Hakedal and P.K. Egeberg, absorbance of tri-iodide ( $\text{I}_3^-$ ) was measured in the UV region (350 nm). In such system, the volatile analyte can be separated from interferences in sample via diffusion across the hydrophobic membrane. This is a form of an on-line clean up system. Nevertheless, this system can be made simpler by using colorimetric detection instead of detection in the UV region.

In a previous work, N. Choengchan [6] developed a FI system for determination of iodate in salt. The detection utilizes measurement of absorbance of the blue  $\text{I}_3^-$ -starch complex and can be detected in the visible region at 590 nm. Employment of this blue complex provides a more sensitive spectrometric detection than the use of the absorbance of the tri-iodide (with twice the molar absorptivity, compared to  $\text{I}_3^-$ ). However, the work was employed for iodate quantitation.

In this work, an on-line gas diffusion flow injection system was developed for colorimetric determination of iodide by detecting the  $I_3^-$ -starch complex. Applications were focussed on pharmaceutical products.

The followings are the aims of this research.

1. To further explore the use of  $I_3^-$ -starch complex for determination of iodide (this method was previously proposed for iodate).
2. To develop a gas diffusion flow injection (GD-FI) system for determination total iodine in the following samples:
  - 2.1 A highly colored sample containing  $I^-$ .
  - 2.2 KI tablet samples
  - 2.3 Multi-vitamins
  - 2.4 Sample containing  $I_2$  or a mixture containing  $I^-$  and  $I_2$ .
3. To develop an on-line dilution method for samples having high iodide contents.
4. To validate the developed method.

## CHAPTER III

### LITERATURE REVIEW

#### 3.1 Analytical Methods for Determination of Iodine

Several methods are available for identification and quantitation of iodine species in various matrices. In this section, these methods are described.

##### 3.1.1 Chromatographic Method

In 1991, a HPLC with electrochemical (EC) detection was employed for determination of iodide that formed inorganic iodine in aqueous solution. The sensitivity was  $5 \times 10^{-8}$  M [7].

In 1992, derivatization of iodide into 4-iodo-2,6-dimethylphenol with UV detection was found to be a suitable method for the sensitive detection of iodide [8]. The detection limit was 0.5 ng of iodide.

Ion chromatographic method, with isocratic separation and micro-membrane suppression was proposed for determination of iodide in soils and ground water by H.L. Tucker and R.W. Flack in 1998. The method detection limit was 0.3 mg/L [9].

In 2001, a novel method for determination of iodide by size exclusion chromatography was established. Iodide was converted to iodine, then sequestered with starch and separated from matrix using a Shim-pack DIOL-150 size-exclusion column with methanol mobile phase and UV detection at 224 nm. The calibration curve was linear from 1.0 ng/mL to 100.0 ng/mL and detection limit was 0.2 ng/mL [10].

An electrostatic ion chromatographic method for rapid and direct determination of iodide in seawater was reported by W. Hu et al. in 2002. Separation was achieved using a reversed-phase ODS packed column modified by coating with Zwittergent-3-4 micelles and using UV detection at 210 nm. The detection limit was 0.011 M [11].

Gas chromatography is an indirect method for determination of trace iodine because a sample preparation step is necessary for example derivatization before detection. In 1996, gas chromatography-mass spectrometry was proposed for the determination of iodine, iodide and iodate in aqueous solution. The sample preparation consists of the derivatization of iodine with 2,6-dimethylphenol followed by a single step extraction of the derivative with diethyl ether [12].

The gas chromatographic method has been applied to a large range of water samples with conversion of iodide to acetone derivative and its subsequent detection by electron capture detector [13]. The technique was used for measuring iodide in rainwater, drinking water, river water and seawater.

### 3.1.2 Electrochemical Method

Cathodic stripping square wave voltammetry provides a direct determination of iodide in fresh water and seawater. The methods were proposed by G.W. Luther et al. in 1988 [14] and R.C. Tian et al. in 1995 [15]. Chronopotentiometry and square wave voltammetry was proposed for simultaneous coulometric determination of iodide, bromide and chloride in aqueous samples. The method was proposed by H. Parham and B. Zargar in 2002 [16].

Differential pulse cathodic stripping voltammetry for determination of trace iodine in food samples and salt have been reported by S. Yang et al. [17]. The iodine was preconcentrated and measured at glassy carbon disk working electrode.

There have been some reports of the electrochemical method for the determination of iodine using ion-selective electrode. A potentiometric flow injection system was developed in 1990 for determination of iodide and iodine in pharmaceutical sample [18]. Iodine can be determined by reduction to iodide with sodium metasilphite. The method has been applied for sample with the range of  $10^{-6}$  to  $10^{-1}$  M and  $10^{-5}$  to  $10^{-3}$  M for iodide and iodine, respectively.

Flow injection (FI) amperometry [19] was used to measure level of iodate in table salt where the method involved reduction of iodate to iodine with excess iodide in acidic media and the iodine thus formed was reduced at a glassy carbon electrode.

### 3.1.3 Spectrophotometric Method

There are many detection systems proposed for determination of iodine such as UV-Vis spectrophotometry, spectrofluorometry and chemiluminescence.

In 1988, a colorimetric method was developed by T. Koh et al. for determination of iodide in which iodide is separated from other species by oxidation and solvent extraction, followed by back-extraction of the iodine into the aqueous phase and extraction of the methylene blue-iodide ion pair into 1,2-dichloroethane. The absorbance of methylene blue-iodide ion pair was measured at 657 nm [20].

C.G. Taylor et al. [21] reported the study on distribution of potassium iodide in charcoals. An aqueous extract of iodide in the charcoal was converted to tri-iodide with iodate in acidic media followed by spectrophotometric measurement at 460 nm. The other tri-iodide detection has been proposed in 1997 by J.T. Hakedal and P.K. Egeberg [5] where iodide was oxidized to iodine with acidic dichromate. The liberated iodine was then permeated through a PTFE membrane within gas diffusion unit into an acceptor stream containing iodide, tri-iodide was formed and measured at 350 nm. This method was applied to brine solutions.

In 1992, batch method for determination of iodine using leucocrystal violet as a reagent was reported [22]. This method based on the oxidation of iodine to iodate with bromine water and the liberation of free iodine from the iodate by addition of potassium iodide in acidic medium. This iodine selectively oxidises leucocrystal violet to form the crystal violet dye. The dye was further extracted in chloroform and followed by measurement at 588 nm. This method was applied to marine water sample.

M. Yaqoob et al. [23] proposed a FI system which was coupled with spectrofluorimetric and spectrophotometric detection for determination of iodide and iodate/iodide respectively. Iodide was determined using a reaction based on the catalytic reduction of Ce(IV) to fluorescent Ce(III) ( $\lambda_{\text{ex}} = 260 \text{ nm}$ ,  $\lambda_{\text{em}} = 350 \text{ nm}$ ). Iodide, after oxidized by hydrogen peroxide to iodate, was determined spectrophotometrically by a sensitive color reaction with p-aminophenol to form indamine, which has an absorbance maximum at 540 nm.

Other spectrophotometric methods for determination of iodide involve catalytic effect of iodide ion using some redox reactions, in this approach, determinations were aimed for trace levels of analyte. The catalytic action of iodide in redox reaction between Ce(IV) and As (III) has been widely used in trace level determination of iodine [24, 25]. These methods measured the decrease in the absorbance of Ce(IV) at fixed time with increasing iodide concentrations.

Iodide can be determined by another catalytic effect using the kinetic effect on the color fading of Fe(III)-thiocyanate complex in nitric acid solution. The rate of the color-fading reaction is in proportion to the concentration of iodide [26].

In 1995, O.V. Zui and A.V. Terletskaia reported the chemiluminescence detection of trace iodate in river water [27]. This method was based on the reaction of iodate with an excess of iodide in acidic solution, gas extraction of the iodine formed and detection in the stream of carrier gas by alkaline luminol solution. Another application of chemiluminescence detection was proposed by J.L. Burguera [28] in

1996. A head-space FI was coupled with chemiluminescence detection for determination of iodide in urine. The iodide was converted to iodine by potassium dichromate under stirring in closed head-space vial. After trapping iodine with iodide solution, an aliquots of iodine was introduced to FI system and reacted with luminol and cobalt (II). The emission intensity was at 425 nm.

**Table 3.1** Summarizes the work concerning development of methods for iodide determination.

Methods	Sample	Detection species	Working range	Authors
<b>Chromatography</b>				
1. HPLC-EC	Aqueous solution	I <sup>-</sup>	NR	R.O. Rahn, 1991
2. IC	Ground water and soil	I <sup>-</sup>	0.5 – 10 mg/L	H.L. Tucker et al., 1998
3. Size exclusion chromatography	Seawater	I <sub>2</sub> -starch	1 – 100 µg/L	F. Chen et al., 2001
4. Electrostatic IC	Seawater	I <sup>-</sup>	Up to 1.5 µM	W. Hu et al., 2002
5. GC	Drinking water	I <sub>2</sub> -2,6-dimethylphenol	1 – 200 µg/L	S.H. Shin et al., 1996
<b>Electrochemistry</b>				
1. Coulometry	Mineral water	I <sup>-</sup>	NR	H. Parham et al., 2002
2. ISE-FIA	Pharmaceutical product	I <sup>-</sup>	10 <sup>-6</sup> – 10 <sup>-3</sup> M	D.E. Davey et al., 1990
<b>Spectrometry</b>				
1. Batch	Seawater	I <sub>2</sub> -leucocrystal violet	0.04 – 0.36 mg/L	V.K. Gupta et al., 1999

NR: No report

**Table 3.1** Summarizes the work concerning development of methods for iodide determination. (Continued)

Methods	Sample	Detection species	Working range	Authors
2. Membrane-permeable FIA	Brine	I <sub>3</sub> <sup>-</sup> (350 nm)	50 – 400 mg/L	J.T. Hakedal et al., 1997
3. Stopped-FI	Urine	I <sup>-</sup> and Ce (IV)- As(III) (420 nm)	50 – 200 µg/L	D. Nacapricha et al., 2001
4. Batch	River water	I <sub>2</sub> -luminol (425 nm)	NR	O.V. Zui et al., 1995
5. Head-space FI	Urine	I <sub>2</sub> -luminol (425 nm)	0.5 – 5 mg/L	J.L. Burguera et al., 1996
6. FIA	Iodized salt	I <sub>3</sub> <sup>-</sup> -starch	0.6 – 5 mg/L	N. Choengchan et al., 2002

NR: No report

### 3.1.4 Other Methods

Neutron activation has been used for determination of iodide in seawater. This method was proposed by G.T.F. Wong and P.G. Brewer in 1976 [29]. Iodide was precipitated with palladium before analysis using neutron activation by counting the <sup>128</sup>I 442.7 keV photopeak.

Flow injection with microwave induced plasma atomic emission spectroscopy reported by F. Camuna et al. in 1993 [30] for determination of iodide.

ICP-MS has been used for determination of iodine in food and biological sample by H. Vanhoe et al. in 1993 [31] and later by P.A. Fecher et al. in 1998 [32].

## 3.2 Tri-iodide Starch Complex

### 3.2.1 Iodometric Titration

It is well known that iodometric titration method is the titration of iodine liberated in chemical reaction [33]. In most iodometric titration, when an excess of iodide ion is present, the tri-iodide ( $I_3^-$ ) ion is formed.

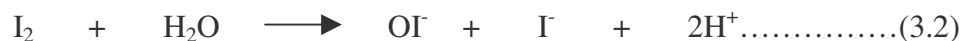


Actually, a solution of iodine in aqueous iodide has an intense yellow to brown color. Thus, iodine can serve as its own indicator in the titration with reducing agent. The test is made visually easier by using a solution of starch as indicator. Starch reacts with iodine in the present of iodide to form an intensely blue-color complex, which is visible at very low concentrations of iodine.

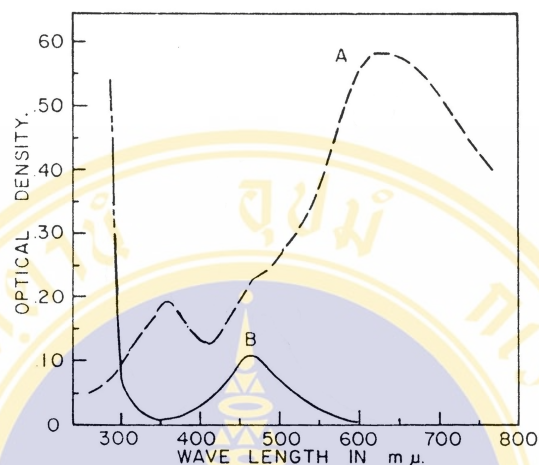
Starches can be separated into two major components, amylose and amylopectin, which exist in different proportions in various plants. Amylose, which is a straight-chain compound and is abundant in potato starch, gives a blue color with tri-iodide and the chain assumes a spiral or helical form. Amylopectin, which has a branched-chain structure, forms a red purple product, probably by adsorption [33].

### 3.2.2 Starch-iodine-iodide Interaction

In 1960, J.A. Thoma et al. [34] reported starch-iodine-iodide interaction by using spectrophotometric method. This study found that iodide is necessary for the formation of blue starch-iodine complex in aqueous solution. Thoma studied formation of iodide by the hydrolysis of iodine ( $I_2$ ) when suppressed by the addition of  $HIO_3$  to an amylose-iodine solution.



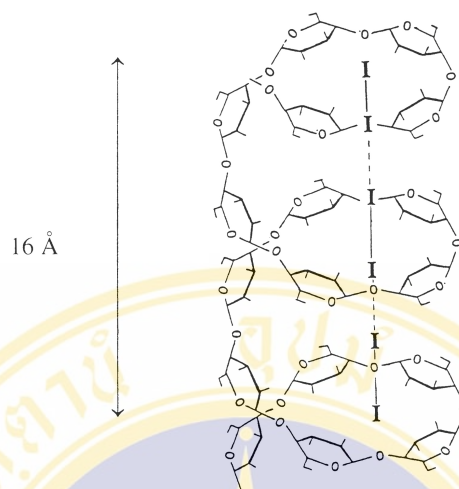
The resulting spectrum was essentially identical with that of iodine in  $\text{HIO}_3$  (Figure 3.1, curve B). When production of iodide was not suppressed the characteristic blue starch-iodine complex resulted (curve A).



**Figure 3.1** Ultraviolet and visible absorption of amylose. Solution contains 0.08% amylose,  $5 \times 10^{-5}$  M  $\text{I}_2$  (curve A) with iodide formation from hydrolysis of iodine; 0.08% amylose,  $1.3 \times 10^{-4}$  M  $\text{I}_2$ , 0.2 M  $\text{HIO}_3$  (curve B). Reagent blank contained equivalent amounts of amylose in water [34].

In aqueous solution the amylose molecule are present in helical configuration. In the amylose-iodine reaction, iodine molecules occupy the central cavity of the helix of amylose. It has been noted that small amount of iodide ion is needed for this reaction to yield the blue color complex in aqueous solution [35].

In 1999, V.T. Calabrese and A. Khan [36] synthesized amylose-iodine (AI) complex without iodide. Under the suitable condition, the blue color complex with helix configuration amylose was obtained. Calabrese observed that the complex is due to an  $\text{I}_6$  unit stabilized within the amylose helix cavity as shown in Figure 3.2.



**Figure 3.2** Schematic left-handed amylose helix enclosing the chromophore of six iodine atoms ( $3I_2$ ) within the cavity. A minimum of 12 anhydroglucose units (AGUs,  $C_6H_{10}O_5$ ) within the amylose segment of  $16 \text{ \AA}$  covers the full length ( $15 \text{ \AA}$ ) of the chromophore [36].

Calabrese et al. [36] proposed dissociation mechanism of amylose-iodine (AI) complex as shown in following equation.



It seems that a rigid helix of AI complex must be in equilibrium with a less rigid helix coil of amylose so that the complex can release iodine molecule. In the present of iodine molecule in solution (unbound or loosely bound), many amylose coils will convert back to the AI complex, shifting the equilibrium to the far left, thus providing a significant concentration of the AI complex. As these iodine concentrations decrease during titration with reducing agent, the equilibrium becomes favored to the right, releasing more iodine molecules from the complex. This can give a mild color of blue complex. This indicated that amylose can be used an indicator for iodometric titration.

### 3.2.3 The Use of $I_3^-$ -starch Complex for Colorimetric Analysis

Tri-iodide starch complex detection is the most widely used for determination of amylose in starches or flours with its advantages of a simple colorimetric measurement [37, 38]. The blue color complex was measured at 620 nm. The standard calibration curve was obtained from 10 – 100% of amylose [37].

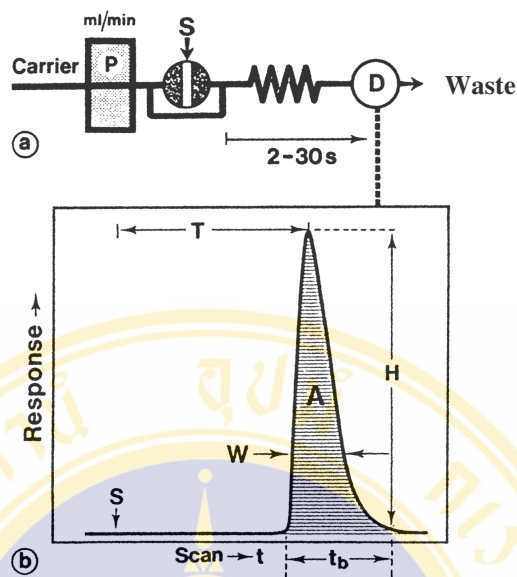
Recently in 2002, N. Choengchan et al. reported the use of  $I_3^-$ -starch detection to flow injection system for determination of iodate in salt [6]. The system utilizes the oxidation of iodide by iodate to iodine, which subsequently forms  $I_3^-$ . In the present of starch, the blue complex was developed and can be monitored at 590 nm.

### 3.3 Flow Injection Analysis

Flow injection analysis (FIA), as developed by J. Ruzicka and E.H. Hansen [39] in 1975, has been widely used for automated continuous analysis in various fields. The low sample and reagent consumption, good sensitivity, reproducibility and high sample throughput are considered to be the main advantages of flow injection method.

FIA is based on the injection of liquid sample into a moving nonsegmented continuous carrier stream of suitable liquid. The injection sample form a zone, which is then transported towards a detector that continuously records the absorbance, electrode potential or the physical parameter as it continuously changes due to the passage of sample material through the flow cell.

The simplest flow injection analyzer (Figure 3.3 a) consists of a pump, which is used to propel the carrier stream through a narrow tube. An injection port, by mean of which a well-defined volume of sample solution is injected into the carrier stream in a reproducible manner, and a micro-reactor in which the sample zone disperses. Reaction with the component in carrier stream, forming a species that is sensed by flow through detector and recorded.



**Figure 3.3** (a) The simplest single line FIA manifold utilizing a carrier stream of reagent, S: sample injection port, D: detector. (b) The analog output has the form of peak. This figure is taken from “Flow Injection Analysis” by J. Ruzika and E.H. Hansen, p. 20 [39].

A typical recorder output has form of peak (Figure 3.3 b), the height,  $H$ , width,  $W$ , or area,  $A$ , of which is related to the concentration of the analyte. The time span between the sample injection  $S$  and peak maximum, which yields an analytical read out as peak height is the residence time during which the chemical detection take place.

FIA is an automated microchemical technique, capable of having a high sampling rate and a minimum sample and reagent consumption. FIA method is based on a combination of three principles.

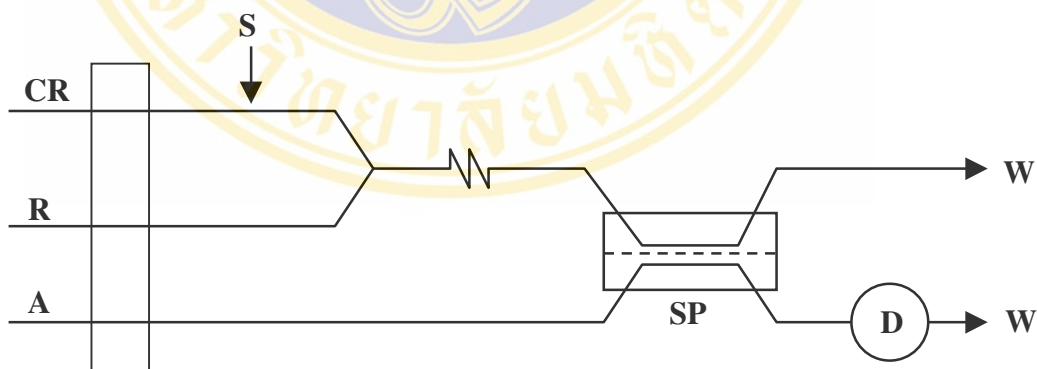
- a) Reproducible sample injection.
- b) Controlled precise dispersion of the injected sample zone.
- c) Reproducible timing of its movement from the injection point toward the detector.

### 3.4 FIA Determination Based on Separation Process

Many analytical procedures involve a separation step, the purpose of which is to increase the selectivity of determination by separating the analyte from an interfering matrix. In batch analysis such separations are always time consuming, laborious and difficult to perform in microscale. It is not practical to automate them by a batchwise mode, and a continuous flow approach is therefore the only way to automate solvent extraction, gas diffusion and dialysis [40]. Thus the advent of the FIA techniques has allowed significant advances in the automation for these separation techniques.

#### 3.4.1 Gas Diffusion

Figure 3.4 is a schematic diagram of a typical FI manifold for gas diffusion separations. A gas diffusion technique is used in a FI system to transfer a gaseous compound from one to another stream. The two streams are separated by a suitable gas permeable membrane (or hydrophobic membrane).



**Figure 3.4** Schematic diagram of a typical FI manifold with gas diffusion. CR: carrier stream, S: sample, R: reagent for formation of volatile analyte, SP: membrane gas diffusion separator, A: acceptor stream, D: detector and W: waste outlet for donor and acceptor streams. This figure is taken from “Flow Injection Separation and Preconcentration” by Z. Fang, p. 138 [40].

In FIA, volatile analytes may be separated from interference in an ill-defined sample stream and transplanted into a liquid or gaseous acceptor stream with well-defined composition. The advantage of gas diffusion process is that it removes the analyte of interest from a matrix. The analyte is transferred to a new matrix, which contains no interference. Reaction conditions and detection of the separated species may be optimized, often greatly enhancing the selectivity of the determinations.

According to the literature survey cited by Z. Fang [40], factors influencing the mass transfer and performance of FI gas diffusion separation systems was studied by B. Karlberg and G.E. Pacey [41] using chlorine dioxide as the gaseous analyte. The important observations are summarized as followed.

- a) Mass transfer of gaseous analyte increases with a decrease in sample / carrier flow rate, particularly when the gaseous analyte is transformed irreversibly into another chemical species in the acceptor solution.
- b) Slightly higher peak signals are obtained with the donor and acceptor streams flowing countercurrently than flowing concurrently. But the enhancement is mainly due to a change in peak form and not to an increase in the mass transfer.

The most frequently used detector in FI systems with gas diffusion separation is the spectrophotometer. Quite often the gas diffusion process offers sufficient selectivity to allow relatively non-specific chemical reactions in the acceptor stream to detect analyte. Sulfur dioxide and ammonia can be determined using suitable acid-base indicators in appropriate buffer solution, used as the acceptor stream [42, 43]. The determination was based on a protolytic reaction, with the pH variations in the acceptor stream indicated by a suitable acid-base indicator.

When additional selectivity is required in the detection, more specific reagents are then used in the acceptor streams. In 1985 [44], G.E. Pacey et al. proposed the method for determination of residual aqueous ozone using the redox

reagent potassium indigo trisulfonate and bis (terpyridine) iron (II) after gas diffusion separation which also transferred ozone across the membrane. The reagents showed sufficient specificity for ozone.

S. Motomizu and his group has reported a number of GD applications used for flow injection (FI) analysis [4, 45-49] including the application for iodide as well as other halogens [4].

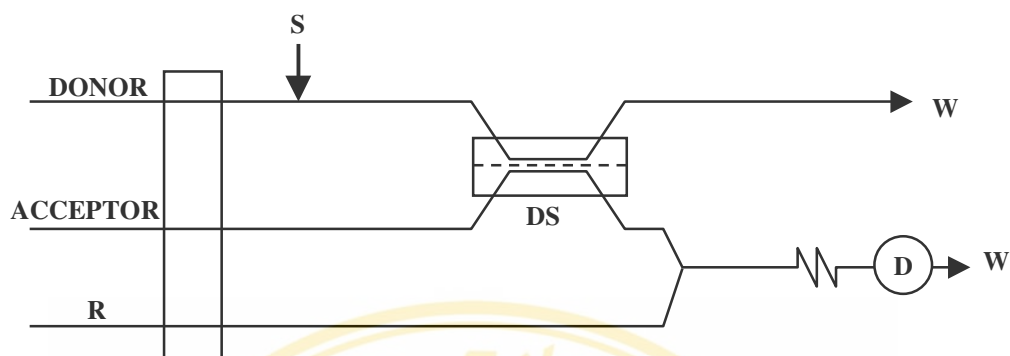
In 1997 [5] J.T. Hakedal and P.K. Egeberg developed a gas diffusion FI system for determination of iodide in brine. The procedure was based on the oxidation of iodide to iodine, which later permeated into a specific collector stream containing iodide to forming tri-iodide. This species could be measured spectrometrically at 350 nm. This method is suitable for the analysis of high salinity waters and brines with high selectivity for iodide, as no matrix effects by sulfide were found.

### 3.4.2 Dialysis

FI systems comprising dialysis employ modules similar to those used for gas diffusion, the hydrophobic microporous membrane being replaced by a hydrophilic dialysis membrane such as cuprophane, cellulose acetate and cellulose nitrate.

The technique is based on differences in mobility of ionic or molecule constituents in a liquid phase during their transport across a semipermeable membrane into the second liquid phase.

Figure 3.5 shows basic configuration of FI manifold for on-line dialysis. The manifold differs from gas diffusion manifolds in that usually no reagents are added to the donor stream while most applications involve merging of a reagent to the acceptor stream to transform the dialysate into a detectable species.



**Figure 3.5** Schematic diagram of a typical FI manifold for on-line dialysis. S: sample DS: membrane dialyzer, R: reagent, D: detector, W: waste. This figure is taken from “Flow Injection Separation and Preconcentration” by Z. Fang, p. 164 [40].

The dialysis technique is often used for removal of interference in the sample matrix. The phenomenon allows compound of a low molecular weight to be separated from larger molecule by using a membrane filter.

Dialysis can also be used for diluting samples. Bringing a flowing sample solution into contact with a dialysis membrane causes some ions or molecules present in the sample to diffuse across the membrane, thereby acting as donors, provided that an acceptor solution (or pure solvent) is placed on the other side. Typically small amount of solute that crosses the membrane (3-30 %) undergoes effective dilution [50]. The extent of dilution achieved will be determined not only by the nature of membrane (pore size, contact surface) and sample (nature and size of the solute), but also by such working conditions as the contact time of the donor and acceptor solution with the membrane.

## CHAPTER IV

### MATERIALS AND METHODS

#### 4.1 Instruments

These following is the list of general instruments employed in this work.

4.1.1 A Precisa of 40SM-200A analytical balance (Switzerland) was used for weighing chemicals in the preparation of potassium iodide and of reagent solutions.

4.1.2 A PMC hot plate, (model 501, USA), was used to prepare starch solution.

4.1.3 A Lamda 25 UV/Vis spectrophotometer (USA) was used to scan spectrum of  $I_3^-$  and  $I_3^-$ -starch complex.

4.1.4 A Hettich Universal II Centrifuge, (model D7200, Germany), was used to separate binding substances in vitamin tablet sample from supernatant.

4.1.5 A Branson Ultrasonic bath (USA) was used for degassing in the preparation of mixture solution of potassium iodide, starch and methanol.

4.1.6 A Waters Sep-Pak cartridge, (W9349J2, USA), was used for trapping organic substances in vitamin tablet sample to determine iodide content.

4.1.7 A digital Ionalyzer of Orion, (model 601A, USA), was employed to measure the changes in electrode potential. The electrodes used are:

An Orion iodide-ISE, (model 9453, USA), was used as an indicator electrode.

A Calomel reference electrode, (4092-H10 double junction type, USA), was used as a reference electrode. This electrode contains a calomel element in an inner section filled with saturated KCl solution (TOA Electronic Ltd., Japan). This inner section connects with the outer section that is filled with 0.1 N KNO<sub>3</sub> solution. Junction with sample is made through a ceramic plug on the tip. The outer solution is chosen to avoid precipitation or contamination in solution incompatible with chloride ion.

4.1.8 Magnetic stirrer, (IKA colorsquid, Germany), was used for stirring the solutions during the measurement of e.m.f.

## 4.2 Apparatus for the FI System

The following include the equipment for the construction of the FI system in this work.

4.2.1 An Ismatec peristaltic pump, (model IS7610, Switzerland), was used for propelling all reagents carriers and injectates. Each channel of carrier was fitted with cartridges each holding Tygon pump tubes i.d. 0.95 mm, wall 0.91 mm.

4.2.2 Three Rheodyne injection valves, (model 5020, USA), fitted with a Teflon loop (i.d. 1.0 mm) were used. One valve used for injection of standard and sample solutions. Two valves for injection of washing solution.

4.2.3 A Metrohm, (model 754, Switzerland) gas diffusion unit, fitted inside with a circular PTFE membrane, (47 mm i.d. with pore size 0.45 μm: Sartorius, Germany), was employed. The membrane was mounted between two perspex blocks. The block has a concentric spiral groove with a width of 2 mm, a depth of 0.2 mm and a length of 300 mm.

4.2.4 The homemade dialysis unit consisted of two half-perspex blocks. Each block had a straight groove width of 1.5 mm, a depth of 1 mm and a length of 120 mm. The membrane tape used for the dialysis unit was cellulose acetate (10 mm width with molecular weight cutoff from 12,000 to 14,000 Dalton: Thomas Science, USA)

4.2.5 A Jenway spectrophotometer, (model 6450, UK), fitted with a 1 cm path length Phillip flow cell (USA) or fitted with a 5 cm path length Hellma flow cell, was used for monitoring the absorbance at 590 nm.

4.2.6 An Alltech chart recorder, (model LR93025, USA), was employed for recording the signal from the Jenway spectrophotometer. The recorder was set to 1 V.

Flow injection manifolds reported in this work were constructed using the equipment listed above. All connecting tubing was PTFE with i.d. 0.5 mm.

### 4.3 Reagents

All chemicals used in this work were AR grade. Deionized-distilled water, obtained from a Mili-Q-system, was used for preparation of reagent solutions. List of chemicals and their suppliers is summarized in Table 4.1.

**Table 4.1** List of chemicals and their suppliers.

Chemical	Formula	Supplier
Iodine	$I_{2(s)}$	BDH (England)
Methanol	$CH_3OH_{(l)}$	Lab-scan (Ireland)
Potassium dichromate	$K_2Cr_2O_{7(s)}$	Univar (Australia)
Potassium iodate	$KIO_{3(s)}$	Univar (Australia)
Potassium iodide	$KI_{(s)}$	E. Merck (Germany)
Sodium nitrate	$NaNO_{3(s)}$	E. Merck (Germany)
Sodium thiosulfate pentahydrate	$Na_2S_2O_3 \cdot 5H_2O_{(s)}$	E. Merck (Germany)
Starch	-	E. Merck (Germany)
Sulfuric acid	$H_2SO_{4(l)}$	Lab-scan (Ireland)

## 4.4 Preparation of Iodine Solutions

Crystal potassium iodide and potassium iodate were oven dried at 120°C for 2 hours then stored in the desiccator until used.

### 4.4.1 Standard Iodide Solutions

#### (a) Stock Solution of Iodide Standard (20,000 mg I/L)

Potassium iodide 26.1417 g was dissolved and diluted with deionized-distilled water into a 100 mL volumetric flask.

#### (b) Secondary Stock Solution of Iodide Standard (10,000 mg I/L)

1.00 mL of stock solution (20,000 mg I/L) was diluted to 100.0 mL with deionized-distilled water to give a standard concentration of 10,000 mg I/L.

#### (c) Tertiary Stock Solution of Iodide Standard (100 mg I/L)

10.00 mL of secondary stock solution (10,000 mg I/L) was diluted to 100.0 mL with deionized-distilled water to give a standard concentration of 100 mg I/L.

### 4.4.2 Standard Iodate Solutions

#### (a) Stock Solution of Iodate Standard ( $1 \times 10^{-2}$ M)

Potassium iodate 0.2140 g was dissolved and diluted with deionized-distilled water into a 100 mL volumetric flask.

#### (b) Secondary Stock Solution of Iodate Standard ( $1 \times 10^{-4}$ M)

500  $\mu\text{L}$  of  $1 \times 10^{-2}$  M potassium iodate was diluted to 50.0 mL with deionized-distilled water to give a standard iodate concentration of  $1 \times 10^{-4}$  M.

#### 4.4.3 Saturated Iodine Solution

This solution was prepared for synthetic samples containing of  $\text{I}_2$  and/or  $\text{I}^-$  as mentioned in Section 4.7.3.

##### (a) Stock Solution of Saturated Iodine

Solid iodine 0.5 g was dissolved in deionized-distilled water to approximately 500 mL. The iodine solution was kept in glass-bottle. To obtain saturated iodine solution, this solution was stored in dark place at 1 night.

##### (b) Standardizing Iodine Solution

Iodine solution was standardized against sodium thiosulphate solution, which has been recently standardized against potassium iodate according to the following procedures.

25.00 mL of  $1 \times 10^{-4}$  M potassium iodate standard (as mentioned in Section 4.4.2 (b)) was transferred into a 250 mL conical flask followed by adding of 2 g of potassium iodide and 10 % (v/v) sulfuric acid. The liberated iodine was titrated with the sodium thiosulfate solution ( $2 \times 10^{-3}$  M). When the colour of the liquid has become a pale yellow, add 2 mL of 1 % (w/v) starch solution and continue the titration until the colour changes from blue to colourless.

For the preparation of sodium thiosulfate solution approximately concentration of  $2 \times 10^{-3}$  M, 0.5 g of sodium thiosulfate pentahydrate was dissolved in deionized-distilled water to approximately 1 L.

For standardized iodine solution, transfer 25.00 mL of saturated iodine solution to 250 mL conical flask, add 2 g of potassium iodide and standardized against thiosulfate solution using 1 % (w/v) starch as an indicator.

#### 4.5 Batch Analysis for $I_3^-$ and $I_3^-$ -starch Complex Studies

In order to control the same concentration of  $I_2$  for investigation of  $I_3^-$  and  $I_3^-$ -starch detection. Dushman's reaction [51] was proceeded to obtain the same equivalent of  $I_2$  in  $I_3^-$  and  $I_3^-$ -starch.

Reagents were prepared as the followings.

(i) For the preparation of 0.5 M sulfuric acid, Approximately 2.5 mL of concentrated sulfuric acid was slowly added into a beaker containing 100 mL of deionized-distilled water.

(ii) For the preparation of 0.08 M potassium iodide, solid potassium iodide of approximately 0.6 g was dissolved with deionized-distilled water to approximately 50 mL.

(iii) For the preparation of 0.5 % (w/v) starch, dissolved 0.5 g of starch with a few milliliters of water to form slurry. The slurry was added to 100 mL of boiling water.

To obtain iodine containing 3, 9, 15, 21 and  $27 \times 10^{-6}$  M, standard iodate concentration at 1, 3, 5, 7 and  $9 \times 10^{-6}$  M in volumetric flask were used for construction the calibration. The following procedures were carried out.

##### 4.5.1 $I_3^-$ Detection by Batch Method

Pipette 50, 150, 250, 350 and 450  $\mu$ L of  $1 \times 10^{-4}$  standard iodate solution (in Section 4.4.2 (b)) into 5 mL volumetric flask. Add 100  $\mu$ L of 0.5 M sulfuric acid and

1 mL 0.08 M potassium iodide. These aliquots were then diluted to mark with deionized-distilled water.

#### 4.5.2 I<sub>3</sub><sup>-</sup>-starch Detection by Batch Method

Pipette 50, 150, 250, 350 and 450  $\mu\text{L}$  of  $1 \times 10^{-4}$  standard iodate solution (in Section 4.4.2 (b)) into 5 mL volumetric flask. Add 100  $\mu\text{L}$  of 0.5 M sulfuric acid, 1 mL 0.08 M potassium iodide and 1 mL of 0.5 % (w/v) starch solution. These aliquots were then diluted to mark with deionized-distilled water.

After shake well, the absorption spectrum of reagent in Section 4.5.1 and 4.5.2 were recorded from 200 to 750 nm. A blank experiment was also performed by adding deionized-distilled water instead of standard iodate solution.

### 4.6 Sample Preparations

#### 4.6.1 Mixt. Stramonium Co.

This medicine (the Government Pharmaceutical Organization, Thailand) is in a liquid form with some solid suspension (brown in colors for both the liquid and the suspended particles). The sample was filtered through a Whatman Paper No. 2 before used.

#### 4.6.2 KI Tablets

NORAD (Body Gold, Inc., USA) and IOSAT (ANBEX Inc., USA) were the KI tablet samples used for this work. These tablets contain iodide 49.7 (NORAD) and 99.5 (IOSAT) mg I/tablet. Each tablet was added into approximately 20 mL of deionized-distilled water. Dissolution was made as well as filtering through a Whatman paper No. 1. The final volume of dissolved matters was brought up to 500.0 mL. These were all clear solutions.

### 4.6.3 Multi-vitamin Tablet

Centrum (Interthai Pharmaceutical manufacturing Ltd., Thailand) was the vitamin chosen for this work. Ground Centrum vitamin (2.7xxx g) was placed into a 250 mL beaker. 100.00 mL of deionized-distilled water was added to the beaker. This mixture was stirred for 30 minutes. The suspended particles (possibly binder plus oil-soluble vitamins) was separated by centrifugation at 300 rpm for 15 minutes. The supernatant was filtered through 0.45  $\mu\text{m}$  cellulose acetate membrane filter. This is called vitamin extract.

## 4.7 GD-FI System 1

The followings are descriptions of chemical preparations and experiments for the first FI system namely GD-FI System 1.

### 4.7.1 Reagents Used in the GD-FI System 1

#### (a) Working Iodide Solutions

Iodide standard for calibration, containing 50, 100, 150, 200, 250 and 300 mg I/L, were prepared by pipetting the secondary iodide solution (10,000 mg I/L) at the volume of 250, 500, 750, 1,000, 1,250 and 1,500  $\mu\text{L}$  into 50 mL volumetric flasks. These aliquots were then diluted to mark with deionized-distilled water.

#### (b) Cleaning Solution of $2 \times 10^{-3}$ M Sodium Thiosulfate in 0.1 % (w/v) Starch

Thiosulfate solution (0.1 M) was prepared by dissolving 2.5 g of sodium thiosulfate pentahydrate in 100 mL of water. This solution was diluted with 0.1 % (w/v) starch to give  $2 \times 10^{-3}$  M of thiosulfate.

**(c) Acceptor Solution of 0.016 M Potassium Iodide in 0.1 % (w/v) Starch**

This mixture was first made by mixing 1 g of starch with a few milliliters of water to form slurry. The slurry was added to 1 L of boiling water. This resulted in a starch solution of 0.1 % (w/v). 2.6 g of potassium iodide was then dissolved in this starch solution. This solution mixture was always prepared daily due to deterioration of starch molecule.

**(d) Oxidant Solution of 0.01 M Potassium Dichromate in Various Concentration of Sulfuric Acid**

Solid potassium dichromate 0.7354 g was dissolved in approximately 100 mL of deionized-distilled water. Add 5.00, 10.00, 15.00, 20.00 and 25.00 mL of concentrated sulfuric acid (to obtain 2, 4, 6, 8 and 10 % (v/v) sulfuric acid in oxidant solution). This mixture was brought up to 250.0 mL using deionized-distilled water.

**4.7.2 Investigation for Appropriate Concentration of Sulfuric Acid**

All of oxidant solution in Section 4.7.1 (d) was used to investigate for appropriate concentration of sulfuric acid. To find the appropriate condition for complete oxidation, the oxidation between in-line and off-line oxidation was compared. The results are discussed in Section 5.1.4.3.

**(a) In-line Oxidation**

Standard iodide containing 300 mg I/L was prepared by pipetting 10,000 mg I/L (obtained in Section 4.4.1 (b)) at the volume of 1.5 mL into 50 mL volumetric flask. This aliquot was then diluted to mark with deionized-distilled water.

This standard iodide was injected in GD-FI (System 1) as shown in Figure 5.4.

### (b) Off-line Oxidation

The following procedure was carried out.

300  $\mu\text{L}$  of standard iodide 10,000 mg I/L (from Section 4.4.1 (b)) was pipetted into a glass vial. 9.50 mL of 10 % (v/v) sulfuric acid was added followed by 200  $\mu\text{L}$  of 0.25 M potassium dichromate in 10 % (v/v) sulfuric acid into the vial. Cover the glass vial with septum and shake well. This solution was immediately injected into the GD-FI (System 1) in Figure 5.4.

This solution was contained concentration of iodide as same as the procedure presented in Section 4.7.2 (a).

### 4.7.3 Synthetic Sample for Determination of Total Iodine

The following synthetic samples were prepared. Sample in Section 4.7.3 (a) to 4.7.3 (c) were determined by complete oxidation condition (10 % (v/v) sulfuric acid in oxidant solution) while sample in Section 4.7.3 (d) were determined by non-complete oxidation condition (4 % (v/v) sulfuric acid in oxidant solution).

#### (a) Synthetic Sample of Iodide (I)

**Table 4.2** Preparation of synthetic samples containing purely iodide species.

Sample code	Volume of iodide standard <sup>a</sup> / $\mu\text{L}$	Calculated iodine content <sup>b</sup> / mg I/L
Syn 1	250	50
Syn 2	500	100
Syn 3	750	200
Syn 4	1,000	300
Syn 5	1,250	400
Syn 6	1,500	500

<sup>a</sup>Standard iodide 10,000 mg I/L (Section 4.4.1(b)).

<sup>b</sup>Final concentration of iodide in a 50 mL volumetric flask.

**(b) Synthetic Sample of Iodine (I<sub>2</sub>)****Table 4.3** Preparation of synthetic samples containing purely iodine species.

Sample code	Volume of iodine standard <sup>a</sup> / mL	Calculated iodine content <sup>b</sup> / mg I/L
Syn 7	10.00	66.3
Syn 8	12.00	79.6
Syn 9	15.00	99.5
Syn 10	18.00	119.4
Syn 11	20.00	132.7
Syn 12	22.00	145.9
Syn 13	25.00	165.8

<sup>a</sup>The concentration of iodine solution was 165.8 mg I/L (Section 4.4.3 (a)).

<sup>b</sup>Final concentration of iodine (I) in a 25 mL volumetric flask.

**(c) Synthetic Mixture of Iodide (I<sup>-</sup>) and Iodine (I<sub>2</sub>) for Complete Oxidation Condition****Table 4.4** Preparation of synthetic mixtures of iodide and iodine.

Sample code	Volume of iodide standard used / $\mu\text{L}$ <sup>a</sup> (contributed iodide concentration / mg I/L)	Volume of iodine standard used / mL <sup>b</sup> (contributed concentration / mg I/L)	Calculated iodine content <sup>c</sup> / mg I/L
Syn 14	0 (0)	12.50 (82.6)	82.6
Syn 15	50 (20)	12.50 (82.6)	102.6
Syn 16	75 (30)	12.50 (82.6)	112.6
Syn 17	100 (40)	12.50 (82.6)	122.6
Syn 18	250 (100)	12.50 (82.6)	182.6
Syn 19	500 (200)	12.50 (82.6)	282.6
Syn 20	750 (300)	12.50 (82.6)	382.6

<sup>a</sup>Standard iodide 10,000 mg I/L (Section 4.4.1 (b)).

<sup>b</sup>The concentration of iodine solution was 165.3 mg I/L. (Section 4.4.3 (a))

<sup>c</sup>Final concentration of total I (as I) in a 25 mL volumetric flask.

**(d) Synthetic Mixture of Iodide (I<sup>-</sup>) and Iodine (I<sub>2</sub>) for a Non-complete Oxidation Condition**

**Table 4.5** Preparation of synthetic mixtures of iodide and iodine.

Sample code	Volume of iodide standard used / $\mu\text{L}^{\text{a}}$ (contributed iodide concentration / mg I/L)	Volume of iodine standard used / $\text{mL}^{\text{b}}$ (contributed concentration / mg I/L)	Calculated iodine content <sup>c</sup> / mg I/L
Syn 21	125 (50)	15.00 (105.7)	155.7
Syn 22	375 (150)	15.00 (105.7)	255.7
Syn 23	625 (250)	15.00 (105.7)	355.7
Syn 24	875 (350)	15.00 (105.7)	455.7
Syn 25	1,125 (450)	15.00 (105.7)	555.7

<sup>a</sup>Standard iodide 10,000 mg I/L (Section 4.4.1 (b)).

<sup>b</sup>The concentration of iodine solution was 176.2 mg I/L. (Section 4.4.3 (a))

<sup>c</sup>Final concentration of total I (as I) in a 25 mL volumetric flask.

#### 4.7.4 Application on Real Samples

##### (a) Sample Preparation and Analysis

2.50 mL of the filtered Mixt. Stramonium Co. (Section 4.6.1) was pipetted into 250 mL volumetric flask. These were made to mark with deionized-distilled water. These sample solutions were analyzed using GD-FI System 1 (Figure 5.4) and iodide-ISE measurement as described in Section 4.10.

Extracted solution of KI tablets (NORAD and IOSAT) in Section 4.6.2 were analyzed using GD-FI System 1 (Figure 5.4) and iodide-ISE measurement as described in Section 4.10.

##### (b) Analytical Recovery

Recovery was first determined using a pharmaceutical sample. 2.50 mL of Mixt. Stramonium Co. (prefiltered through Whatman No. 2) was pipetted into 250 mL volumetric flask. Standard iodide 2.50 mL of 10,000 mg I/L was pipetted into the flask (obtained 100 mg I/L standard addition). Then the mixtures were made to the mark using deionized-distilled water. The same analysis was performed on this sample but added 2.50 mL deionized-distilled water to the sample instead of standard iodide solution.

For KI tablets, 24.00 mL of extracted solution (in Section 4.6.2) was pipetted into 25 mL volumetric flask. Standard iodide 250  $\mu$ L of 10,000 mg I/L was pipetted in to the flask (obtained 100 mg I/L) standard addition). Then the mixture were made to the mark using deionized-distilled water. The same analysis was performed on this sample but added 250  $\mu$ L deionized-distilled water to the sample instead of standard iodide solution.

The recovery was determined and discussed in Section 5.2.5, the whole procedural method was calculated based on the percentage of the recoverable amount of iodine added to a sample.

## **4.8 GD-FI System with an On-line Dilution (System 2)**

The followings are descriptions of chemical preparations and experiments for the FI system namely GD-FI System 2.

### **4.8.1 Reagent Used in the GD-FI System 2**

#### **(a) Working Iodide Solutions**

Iodide standard for calibration, containing 6,000, 7,000, 8,000, 9,000 and 10,000 mg I/L, were prepared by respectively transfer aliquots of the 20,000 mg I/L iodide standard solution at the volume of 15.00, 17.50, 20.00, 22.50 and 25.00 mL into

50 mL volumetric flask. These aliquots were then diluted to mark with deionized-distilled water.

**(b) Cleaning Solution of  $2 \times 10^{-3}$  M Sodium Thiosulfate in 0.1 % (w/v) Starch**

This reagent was prepared as same as the solution described in Section 4.7.1 (b).

**(c) Acceptor Solution of 0.016 M Potassium Iodide in 0.1 % (w/v) Starch**

This reagent was prepared as same as the solution described in Section 4.7.1 (c).

**(d) Oxidant Solution of 0.01 M Potassium Dichromate in 10 % (v/v) Sulfuric Acid**

Dissolving approximately 3 g of potassium dichromate in 1 L of 10 % (v/v) sulfuric acid.

#### **4.8.2 Real Application to Pharmaceutical Samples**

**(a) Sample Preparation**

Mixt. Stramonium Co. samples were filtered through Whatman filter No. 2. These sample solutions can be directly by injected into the GD-FI system as described in Figure 5.11 without prior dilution, while prior dilution with water (1/100) of these samples was done before analysis using iodide-ISE measurement as described in Section 4.10.

### **(b) Analytical Recovery**

4.00 mL of Mixt. Stramonium Co. (prefiltered through Whatman No. 2) was pipetted into 5 mL volumetric flask. Standard iodide 875  $\mu\text{L}$  of 20,000 mg I/L was pipetted into the flask (obtained 3,500 mg I/L standard addition). Then the mixtures were made to the mark using deionized-distilled water. The recovery was determined and discussed in Appendix C. In determination of iodide content, samples were used in the same way as previously described without the addition of iodide.

## **4.9 GD-FI System for Sample with Low Concentration Levels (System 3)**

The followings are descriptions of chemical preparations and experiments for the FI system namely GD-FI System 3.

### **4.9.1 Reagent Used in the GD-FI System 3**

#### **(a) Working Iodide Solutions**

Iodide standard for calibration, containing 0.5, 1.0, 2.0, 2.5 and 3.0 mg I/L, were prepared by respectively transferring aliquots of the 100 mg I/L iodide standard solution at the volumes of 0.50, 1.00, 2.00, 2.50 and 3.00 mL into 100 mL volumetric flask. These aliquots were then diluted to mark with deionized-distilled water.

#### **(b) Cleaning Solution of $2 \times 10^{-3}$ M Sodium Thiosulfate in 0.1 % (w/v) Starch**

This reagent was prepared as same as the solution described in Section 4.7.1 (b).

**(c) Acceptor Solution of 0.016 M Potassium Iodide in 0.1 % (w/v) Starch in Various Concentration of Methanol**

Solid potassium iodide 0.6640 g was dissolved in 100.00 mL of 0.25 % (w/v) starch solution, add 0, 25 and 50 mL of methanol. These solutions were sonicated for 20 minutes and then make up using deionized-distilled water to 250 mL in volumetric flask. This solution was 0.016 M potassium iodide in 0.1 % (w/v) starch in 0, 10 and 20 % (v/v) methanol respectively.

**(d) Oxidant Solution of 0.01 M Potassium Dichromate in 10 % (v/v) Sulfuric Acid**

This reagent was prepared as same as the solution described in Section 4.8.1 (d).

**4.9.2 Application to Sample Containing ca. 2.7 mg I/L**

**(a) Sample Preparation**

**(i) Centrum Vitamin Tablet**

Extracts of Centrum multi-vitamin (Section 4.6.3) were placed onto C<sub>18</sub> Sep-Pak column to trap organic compound in this sample. C<sub>18</sub> Sep-Pak cartridges were activated by passing 5 mL of methanol and 5 mL of deionized-distilled water respectively through the packing material. This was carried out using a vacuum manifold. Then 5 mL of sample was poured onto each column. The first 1 mL of eluate were discarded and the others were collected.

**(ii) Mixt. Stramonium Co.**

1.00 mL of filtered Mixt. Stramonium Co. (Section 4.6.1) was first diluted to 100.0 mL with deionized-distilled water. This solution was then further diluted as the previous procedure described in Section 4.7.4 (a).

The samples were analyzed using the GD-FI System 3 (Figure 5.13) and the iodide-ISE measurement as described in Section 4.10.

### **(b) Analytical Recovery**

20 mL of a vitamin extract obtained from Section 4.9.2 (a), was pipetted into a 25 mL volumetric flask. 125  $\mu$ L of 100 mg I/L standard iodide was added into the flask. This solution was brought up to 25.0 mL using deionized distilled water (contributed 0.5 mg I/L standard addition). In determination of iodide content, samples were used in the same way but this time without the addition of iodide.

## **4.10 Potentiometric Method for Analysis of Iodide**

30.00 mL of each standard potassium iodide concentration 0.1, 1, 10, 100 and 1,000 mg I/L was pipetted into a 50 mL beaker. 0.6 mL of 5 M sodium nitrate (used as the ionic strength adjuster) was added to each beaker and the mixture were stirred thoroughly. Iodide-ISE was rinsed with deionized-water and was placed into the beaker. During the measurement, the solution was continuously stirred with a magnetic bar. The reading in millivolts was recorded when the signal was stable. In this direct measurement, a calibration curve was obtained by plotting the electrode potential (mV) against the logarithm of the iodide concentration. The same analysis was performed on the sample but 30.00 mL of sample was pipetted instead of standard solution. The concentration of iodide in sample was obtained from direct calibration curve prepared as above.

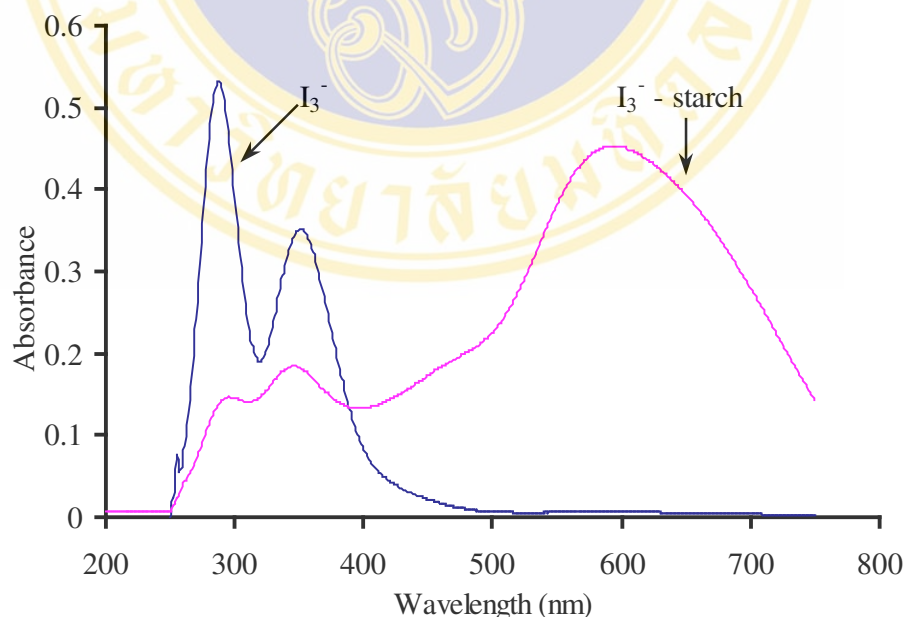
## CHAPTER V

### RESULTS AND DISCUSSION

#### 5.1 Investigation of Colorimetric Detection of $I_3^-$ -starch Using Gas Diffusion Flow Injection (GD-FI) for Determination of Iodide

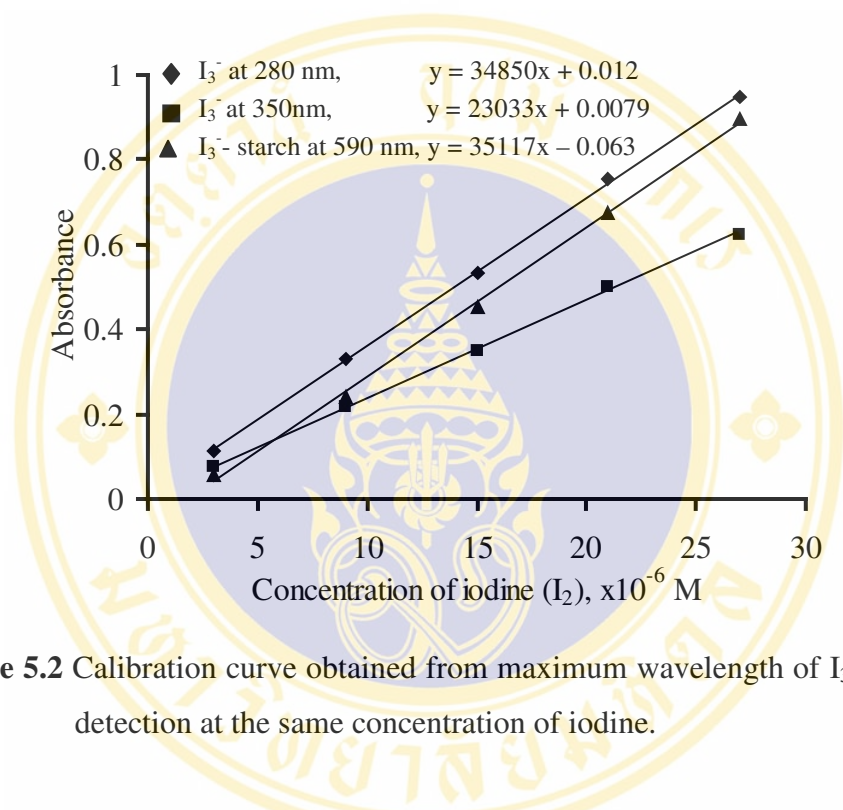
##### 5.1.1 Detection of $I_3^-$ -starch Complex

Iodine in the presence of iodide and starch forms the blue complex of tri-iodide-starch ( $I_3^-$ -starch) [37, 38], which has a maximum wavelength in the visible region at 590 nm [6]. The absorption spectrum of  $I_3^-$  and  $I_3^-$ -starch obtained from an experiment (Section 4.5) are shown in Figure 5.1.



**Figure 5.1** Absorption spectrum of  $I_3^-$  and  $I_3^-$ -starch at the same concentration of iodine  $1.5 \times 10^{-5}$  M.

In this work, sensitivities for detection of  $I_3^-$  and  $I_3^-$ -starch were investigated by using batch analysis, as described in Section 4.5. The detection wavelengths were at 280 and 350 nm for  $I_3^-$ ; and at 590 nm for  $I_3^-$ -starch. Examples of the calibration are shown in Figure 5.2. The slopes of calibration curve for  $I_3^-$  were 0.34 (280 nm) and 0.23 (350 nm) a.u./ $\mu\text{M}$ . The slope for  $I_3^-$ -starch was 0.35 a.u./ $\mu\text{M}$ .



**Figure 5.2** Calibration curve obtained from maximum wavelength of  $I_3^-$  and  $I_3^-$ -starch detection at the same concentration of iodine.

Results in Figure 5.2 indicated that using  $I_3^-$ -starch for detection (590 nm) provided a better sensitivity than the use of  $I_3^-$  for the detection at 350 nm.

$I_3^-$  also has a maximum absorption wavelength in the UV region at 280 nm (Figure 5.1) and the sensitivity at this wavelength is comparable with the use of the  $I_3^-$ -starch for the detection at 590 nm (Figure 5.2). However, this work employed the detection of  $I_3^-$ -starch complex for the analysis of iodide since it is more convenient and simpler than the detection in the UV range.

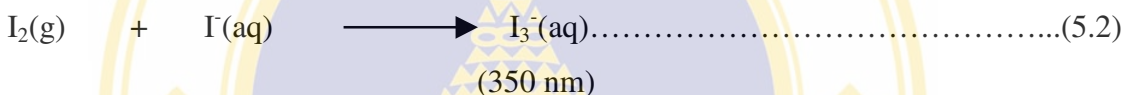
### 5.1.2 Applying the Use of $I_3^-$ -starch for a Flow Injection System

In 1997, J.H. Hakedal and P.K. Egberg [5] reported a flow injection (FI) system for determination of iodide in brine samples. This system employed gas diffusion (GD) unit, fitted with PTFE membrane as shown in Figure 5.3. Iodide was determined via the following reactions.

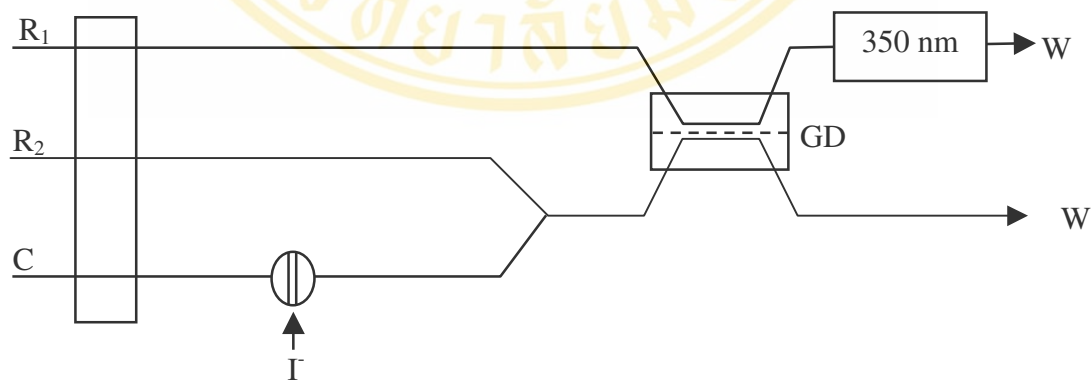
**Donor stream:**



**Acceptor stream:**

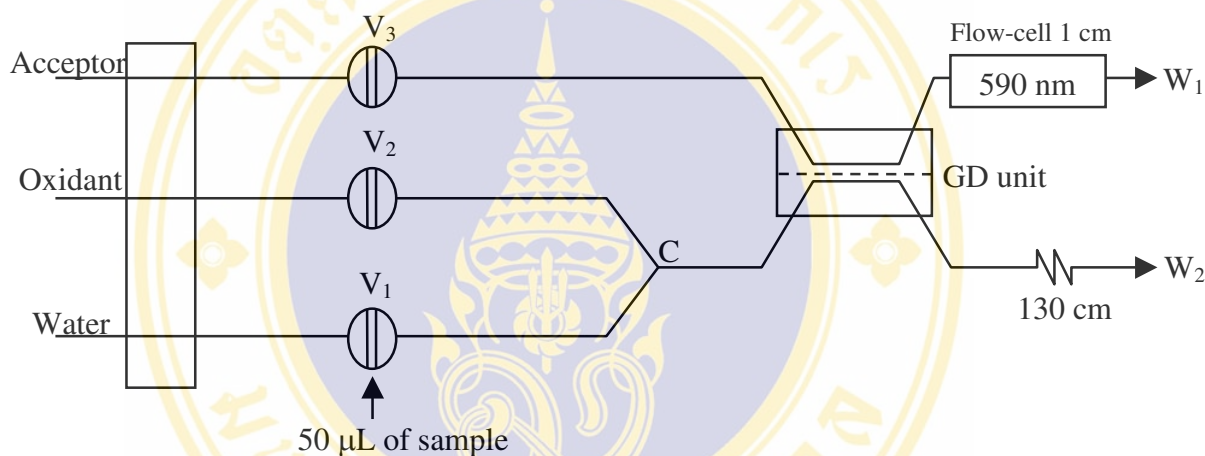


The standard or sample was injected into a carrier stream and merged with acidic dichromate, oxidizing iodide to iodine ( $\text{I}_2$ ). The iodine permeates through the membrane into an acceptor stream containing iodide. Absorbance of tri-iodide ( $\text{I}_3^-$ ) was measured at of 350 nm for the calibration.



**Figure 5.3** Schematic diagram of the flow injection manifold proposed by J.T. Hakedal and P.K. Egberg [5] for determination of iodide in brine. C: carrier,  $R_1$ : 0.016 M KI,  $R_2$ : 0.15 M  $\text{K}_2\text{Cr}_2\text{O}_7$  in 5 % (v/v)  $\text{H}_2\text{SO}_4$ , GD: gas diffusion unit, W: waste.

As already discussed in Section 5.1.1 about the advantage in sensitivity and convenience, the colorimetric detection of  $I_3^-$ -starch was therefore applied to the system presented by J.T. Hakedal and P.K. Egberg. The modified system is shown in Figure 5.4. Solution of 0.016 M potassium iodide in 0.1 % (w/v) starch was used as an acceptor stream solution. Potassium dichromate 0.01 M was used as the oxidant. These reagent concentrations were adapted from the GD-FI system for determination of iodide in urine by catalytic reaction between Ce(IV) and As(III) that present by N. Ratanawimarnwong [52].



**Figure 5.4** The GD-FI System 1 for determination of iodide. The followings are the optimized condition. Acceptor: 0.016 M KI in 0.1 % (w/v) starch. Oxidant: 0.01 M  $K_2Cr_2O_7$  in 10 % (v/v)  $H_2SO_4$ . C: confluence point.  $W_1$ : acceptor flow rate at 2 mL/min and  $W_2$ : donor flow rate at 3 mL/min.  $V_1$ : sample injection port.  $V_2$  and  $V_3$ : 250  $\mu$ L injection of cleaning solution ( $2 \times 10^{-3}$  M of  $Na_2S_2O_3$  in 0.1 % (w/v) starch).

### 5.1.3 Cleaning of System

When measuring the complex formation of tri-iodide starch, problem often arises from deposition of the complex on tube walls and on the flow-cell. Injection of pure thiosulfate solution for removing the adsorbed starch complex [6] was found effective only for the acceptor stream (Figure 5.4). The species in the donor stream, expected to adsorb on the manifold walls are  $I_{2(aq)}$  and/or  $I_{3(aq)}$ . The solution that was

found suitable for removing the complex in this stream is a mixture of thiosulfate and starch ( $2 \times 10^{-3}$  M  $\text{Na}_2\text{S}_2\text{O}_3$  in 0.1 % (w/v) starch). Addition of starch into the previously employed the cleaning solution [6] is necessary. It was observed that reduction of iodine by  $\text{S}_2\text{O}_3^{2-}$  was easier (or more rapid) for the iodine complex than for the non-complex form. However, this was true for deposits on tube walls and is different to what is normally used and observed in titration [33].

The cleaning solution was injected at two positions ( $V_2$  and  $V_3$ ) as shown in Figure 5.4. It was observed that without use of cleaning solution injected via  $V_2$ , the reproducibility was very poor (RSD = 18.67 % as determined from 10 injections of a standard iodide containing 100 mg I/L). Without use of the cleaning solution at  $V_3$ , positive shift of baseline was continually observed. The appropriate cleaning steps and sample injections are summarized in Table 5.1.

**Table 5.1** Summary of the operation steps for the FI manifold shown in Figure 5.4 [Injection;  $V_1$ : sample,  $V_2$  and  $V_3$ : cleaning solution ( $2 \times 10^{-3}$  M  $\text{Na}_2\text{S}_2\text{O}_3$  in 0.1 % (w/v) starch)].

Step	Valve position			Duration (s)
	$V_1$	$V_2$	$V_3$	
1	Inject	Load	Load	45
2	Load	Inject	Inject	75

Analysis time per injection  $45+75$  s = 2 min.

According to Table 5.1, the operation was carried out by injection of a sample via valve  $V_1$ , until the sample zone reached the flow cell (step 1), injection of cleaning solution in valve  $V_2$  and  $V_3$  were made for washing off the complex from tube walls and the flow-cell (step 2). Analysis time per injection was the summation of timing from step 1 to step 2.

### 5.1.4 Optimization

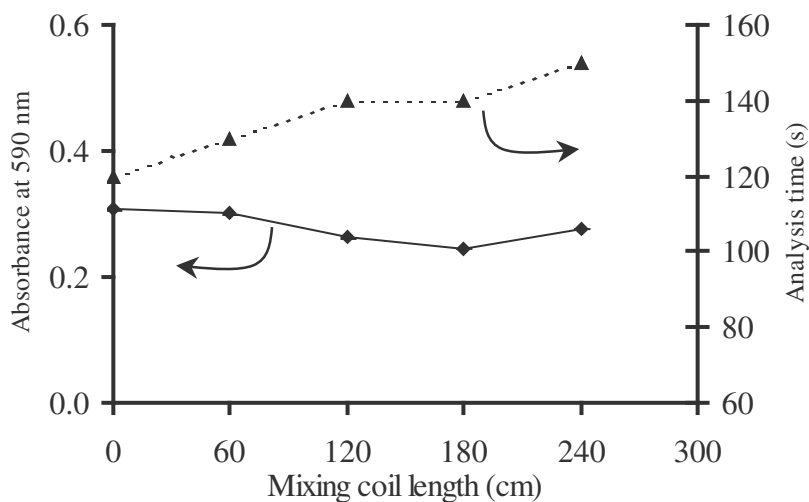
The optimization was preliminary carried out to study two parameters of the manifold in Figure 5.4. The objective of the experiments was to get a compromise between analytical signal and analysis time.

In this work, 'analysis time' is defined as the time taken from injection of sample until the cycle of operation is completed (for 1 injection). The following parameters (Section 5.1.4.1 and 5.1.4.2) were optimized by using repetitive injections of 200 mg I/L iodide standard solution (n=5).

#### 5.1.4.1 Effect of Mixing Coil Length

The most frequent parameter for optimizing a FI system is coil length.

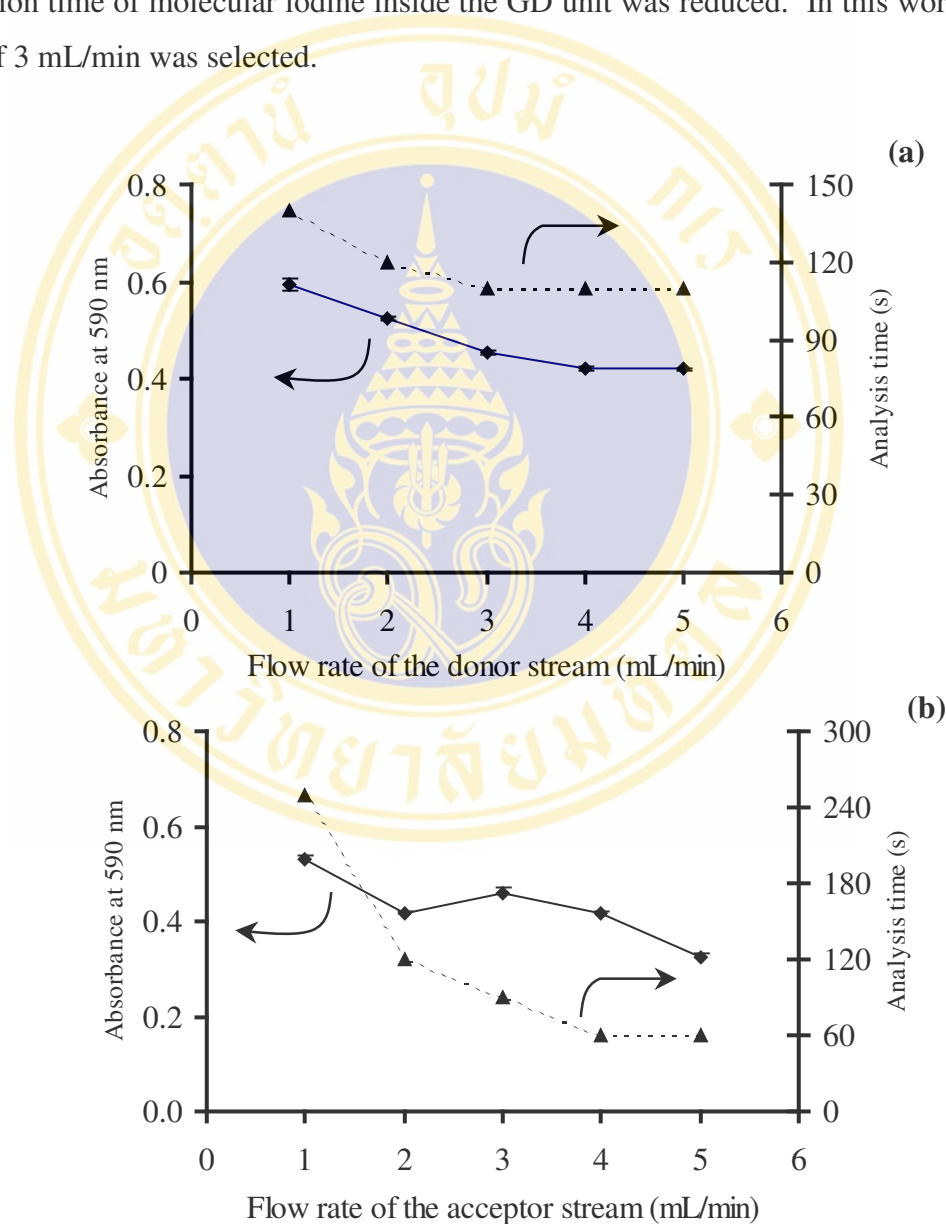
In the experiment, mixing coil of different lengths, from 0 to 240 cm, were inserted between the confluence point (C) and the GD unit (Figure 5.4). According to Figure 5.5, variation of mixing coil length resulted in slight difference in analytical signals. Analysis time increased from 120 to 150 s when the coil length increased from 0 to 240 cm. The shortest analysis time was achieved when no mixing coil was used. Thus, this condition was chosen for further studies.



**Figure 5.5** Influences on analytical signal (◆) and analysis time (▲) from the variation in coil length, between C and GD unit in Figure 5.4.

### 5.1.4.2 Effect of Flow Rate

The influence of donor flow rate at the outlet ( $W_2$  in Figure 5.4) was studied on both the sensitivity and the analysis time (Figure 5.6 a). The results indicated that increasing flow rate has decreased the analytical signal because the diffusion time of molecular iodine inside the GD unit was reduced. In this work, flow rate of 3 mL/min was selected.



**Figure 5.6** Influences of the donor flow rate (a) and acceptor flow rate (b) on analytical signal (◆) and an analysis time (▲).

The flow rate of acceptor stream ( $W_1$  in Figure 5.4) was varied from 1 to 5 mL/min. As expected, the absorbance decreased as the flow rate increased (Figure 5.6 b). This was due to the decrease in yield of the reaction between tri-iodide and starch. It was observed that the system was too sensitive at the acceptor flow rate of 1 mL/min. However, too much of a flow rate would lead to over consumption of reagent. The flow rate at 2 ml/min was therefore chosen. The analysis time of 2 minutes per injection (Table 5.1) was obtained at this flow rate.

#### 5.1.4.3 Investigation for Appropriate Concentration of Sulfuric Acid

Normally, it is not necessary in a flow injection system to oxidize iodide to molecular iodine completely. However it is our special interest to find a condition for the complete oxidation to iodine, so that the GD-FI system is feasible for determination of total content of iodine e.g., in iodine ( $I_{2(aq)}$ ) solution or in mixture containing iodine ( $I_{2(aq)}$ ) and iodide ( $I_{(aq)}$ ). An optimization was carried out aiming for complete oxidation of iodide to iodine. The oxidation between off-line mixing ( $I^-$  + oxidant) and in-line mixing was compared, as represented in a term called degree of oxidation.

In a proper experiment, oxidizing iodide ion off-line is considered as a complete reaction condition. In this work, degree of oxidation that took place in-line was evaluated, using the same concentration a standard iodide, according to

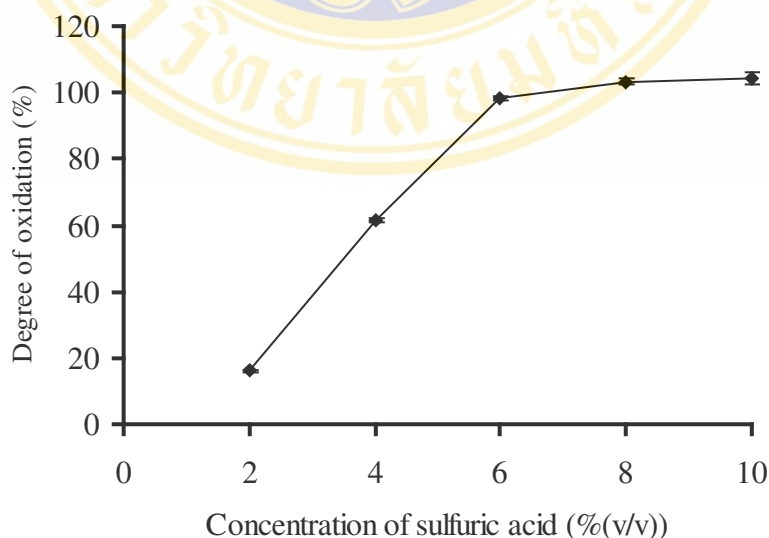
$$\text{Degree of oxidation (\%)} = \frac{S_{\text{in-line}}}{S_{\text{off-line}}} \times 100 \dots \dots \dots (5.3)$$

$S_{\text{off-line}}$  is the FI signal obtained from the injection of an iodide standard, which was homogeneously mixed with the oxidant until the reaction reached the equilibrium.  $S_{\text{in-line}}$  is the signal obtained by injecting the same mole quantity of iodide directly into the FI (no pre-oxidation). Section 4.7.2 described the practical experiment.

For the oxidation of iodide in a homogeneous system,  $H^+$  must be present at an adequate amount [5]. The system employed is based upon non-homogeneous mixing and the spectrometric detection was not at the reaction equilibrium. For such a system, the concentration of  $H^+$  ( $H_2SO_4$ ) should be optimized.

To optimize the acid concentration of the oxidant stream in Figure 5.4, a standard iodide solution of 300 mg I/L (maximum concentration in the calibration) was injected into the FI system in Figure 5.4. The signals obtained in-line were compared with the injections of  $I_{2(aq)}$  that were obtained from off-line oxidation.

According to Figure 5.7, increasing the concentration of sulfuric acid, from 2 to 6 % (v/v) has brought the system to reach the 100 % in-line oxidation of iodide above 8 % (v/v). The consideration to choose the concentration of sulfuric acid is based on the required complete oxidation of iodide and sufficient oxidation of interference (reducing species) in a sample. In this work, the highest concentration of sulfuric acid (10 % (v/v)) was chosen as the optimum.



**Figure 5.7** Effect of concentration of  $H_2SO_4$  on degree of oxidation. Standard iodide 300 mg I/L was injected repetitively (n=3).

## 5.2 The GD-FI System 1

### 5.2.1 The Optimized Condition

Regarding the optimization studies discussed in Section 5.1.4.1 to 5.1.4.3, the optimal condition for the GD-FI System 1 was achieved. Figure 5.4 presents the optimized condition. The operational steps are shown in Table 5.1.

### 5.2.2 Analytical Feature of System 1

In this work, there are all together three GD-FI systems investigated. The performance of System 1 is summarized together with the modified system (System 2) are shown in Section 5.5.

### 5.2.3 Application for Determination of Total Iodine

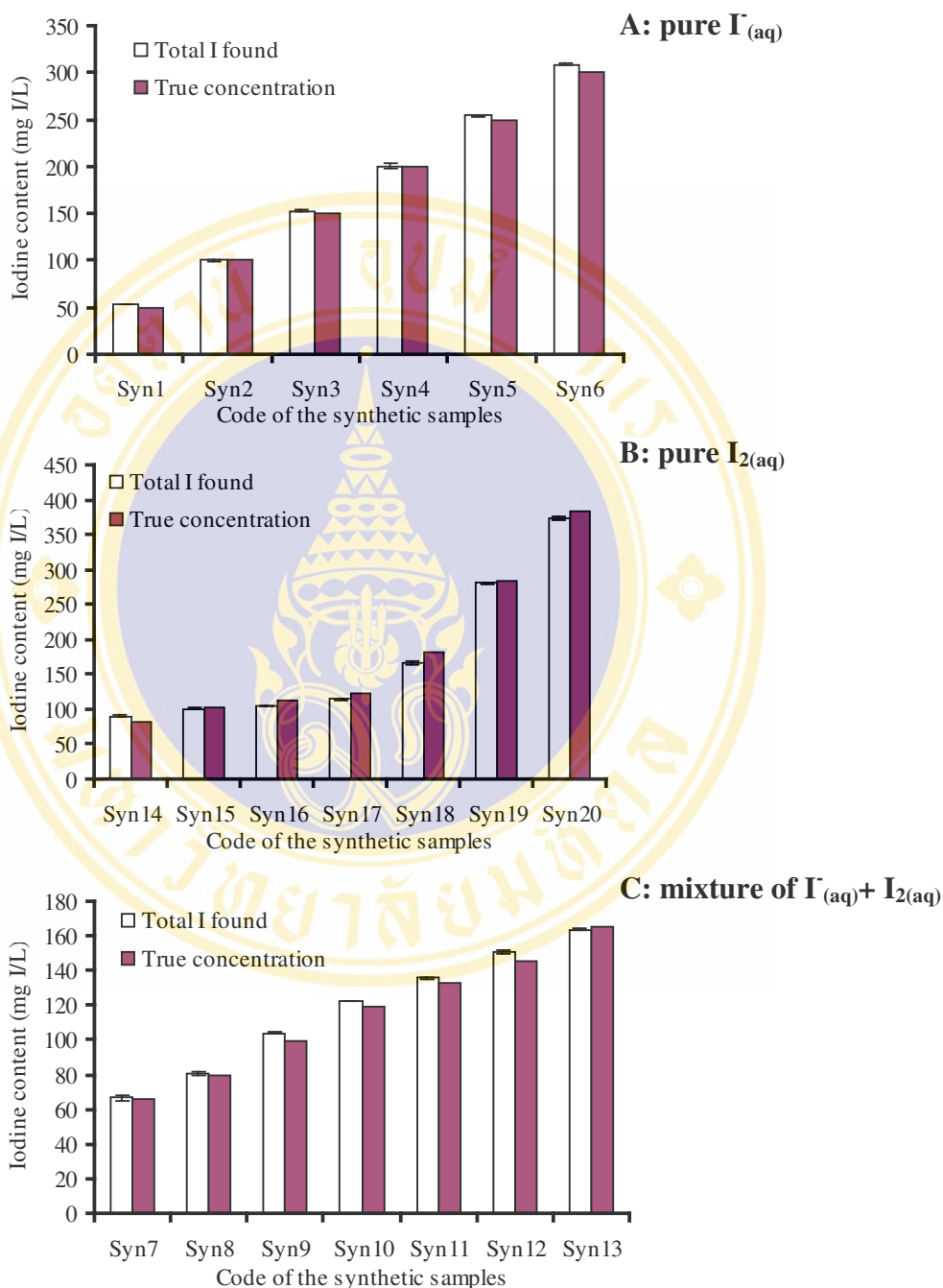
The general objective of the GD-FI development is that it should be able to be used in determination of iodide in pharmaceutical products, because iodide is the usual present form. However, it is a special interest to examine the use of this GD-FI system for determination of total iodine in some samples as well. In the optimized condition, iodide should be 100 % oxidized to iodine ( $I_2$ ) and therefore the calibration curve obtained using series of standard iodide can be used for determination of total iodine ( $I$ ).

Quantitative analysis for total iodine was tested using in (a) synthetic iodide solution ( $I_{(aq)}$ ), (b) synthetic iodine solution ( $I_{2(aq)}$ ) and (c) synthetic mixture containing iodide and iodine ( $I_{(aq)} + I_{2(aq)}$ ).

#### 5.2.3.1 Applicability as Evaluated Using Synthetic Samples

The results in Figure 5.8 clearly demonstrated the effectiveness of the developed system for determination of iodine when there is a mixture of  $I_2$  and  $I$ .

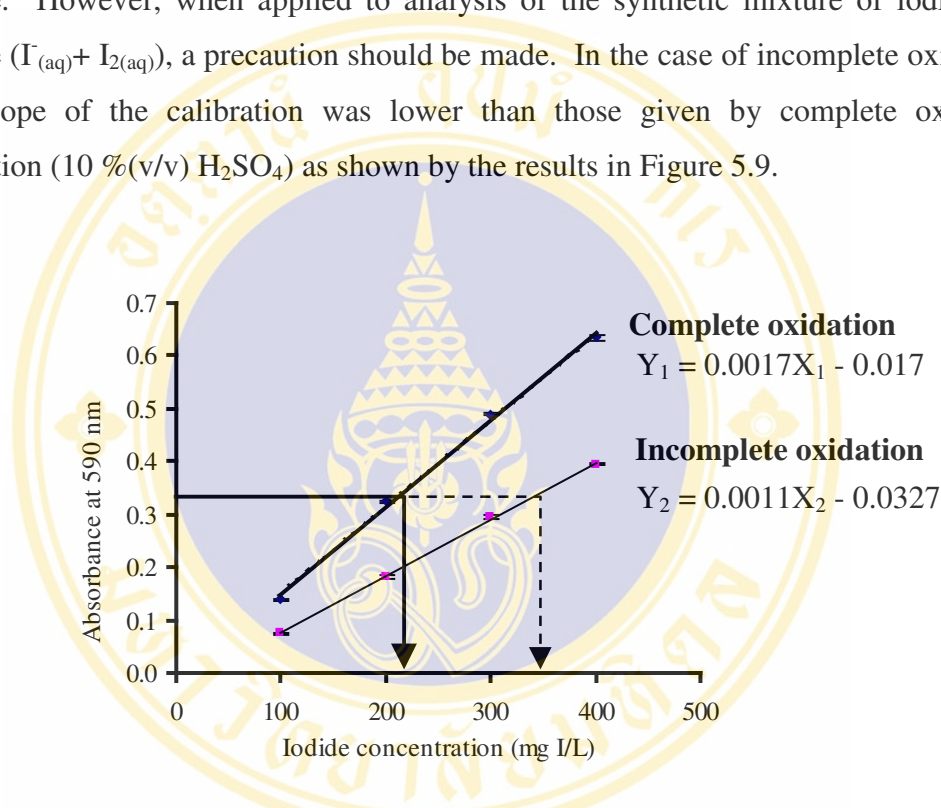
The paired *t*-test [53] indicated no difference between the experimental results and the calculated value (95 % confidence).



**Figure 5.8** Iodine contents in synthetic aqueous samples as determined by using the GD-FI System 1 ( $n=3$ ) compared with the expected values. A: synthetic  $I$  ( $t_{\text{observed}} = 2.48$ ,  $t_{\text{critical}} = 2.57$ ), B: synthetic  $I_2$  ( $t_{\text{observed}} = 2.18$ ,  $t_{\text{critical}} = 2.45$ ), C: synthetic mixture of  $I$  and  $I_2$  ( $t_{\text{observed}} = 1.92$ ,  $t_{\text{critical}} = 2.45$ ). The oxidant stream (Figure 5.4) was 0.01 M  $K_2Cr_2O_7$  in 10% (v/v)  $H_2SO_4$ .

### 5.2.3.2 Precaution in the Construction of Calibration Graph

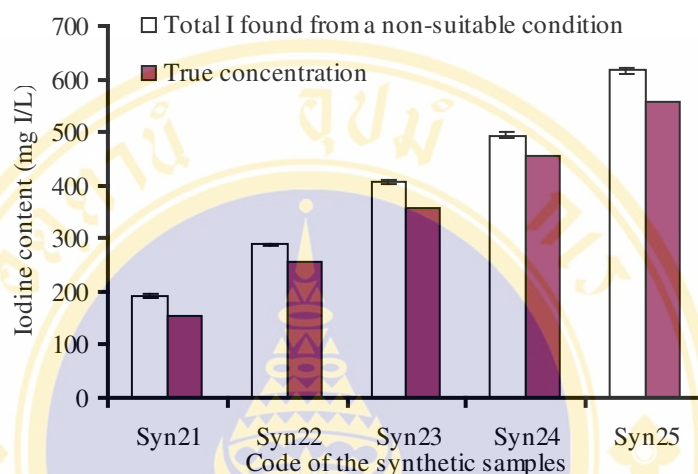
One can actually use the FI system in Figure 5.4 for quantitation of iodide in a sample in which iodide is the only species. Calibration using iodide standards is always applicable and does not rely on the complete oxidation of iodide to iodine. However, when applied to analysis of the synthetic mixture of iodide and iodine ( $I_{(aq)} + I_{2(aq)}$ ), a precaution should be made. In the case of incomplete oxidation, the slope of the calibration was lower than those given by complete oxidation condition (10 % (v/v)  $H_2SO_4$ ) as shown by the results in Figure 5.9.



**Figure 5.9** Difference in the calibration parameters for the complete and for the incomplete oxidation conditions. The oxidant stream (Figure 5.4) was 0.01 M  $K_2Cr_2O_7$  in 10 % (v/v)  $H_2SO_4$  (complete oxidation) and 4 % (v/v)  $H_2SO_4$  (incomplete oxidation).

According to Figure 5.9, positive error will occur when utilizes the calibration of the incomplete oxidation (dotted line). Therefore, using this calibration is not appropriate for the quantitation of total iodine (as I) in a mixture sample, containing ( $I_{(aq)} + I_{2(aq)}$ ). It is recommended to use the calibration constructed under the condition of complete oxidation.

Figure 5.10 demonstrates the disagreement between the results and the expected values, when working in an incomplete oxidation condition. The values obtained from the GD-FI system, employing not enough acid for oxidation (4 % (v/v)  $\text{H}_2\text{SO}_4$ ) were all higher than the true values (as expected, in Figure 5.9).



**Figure 5.10** Iodine content in synthetic aqueous mixture samples of  $\text{I}^-$  and  $\text{I}_2$  as determined by using the GD-FI System 1 ( $n=3$ ) compared with the expected value ( $t_{\text{observed}} = 8.82$ ,  $t_{\text{critical}} = 2.78$ ). The oxidant stream (Figure 5.4) was 0.01 M  $\text{K}_2\text{Cr}_2\text{O}_7$  prepared in 4 % (v/v)  $\text{H}_2\text{SO}_4$  (whereas 10 % (v/v)  $\text{H}_2\text{SO}_4$  is recommended).

#### 5.2.4 Application on Real Samples and Validation of System 1

Three pharmaceutical samples were analysed for iodide contents. The results were compared with the values according to the labels (Table 5.2). The contents of iodide as determined by the GD-FI System 1 agreed significantly well with the labels.

Paired  $t$ -test was employed to compare the difference in the results of KI tablets, in the term of concentrations (mg I/L) of the injected liquid samples ( $n=6$ ). No significant difference was found ( $t_{\text{observed}} = 1.98$ ,  $t_{\text{critical}} = 2.57$  at  $P=0.05$ ). Ten samples of Mixt. Stramonium Co. were analysed using the FI System1 and iodide-ISE. The results from both methods are not significantly different ( $t_{\text{observed}} = 2.31$ ,  $t_{\text{critical}} = 3.25$ ).

at  $P=0.01$ ). Appendix A shows the details of the validation results. These good agreements demonstrate that the GD-FI System 1 is suitable for these samples. Thus these pharmaceutical products do not exhibit interference effect.

**Table 5.2** Determination of iodide in pharmaceutical products by using GD-FI System 1, compared to the nominal contents. The means and standard error were from a set of several samples of the same product.

Trade name	Sample type	Concentration unit	Labeled	Iodide content	
				GD-FI System 1	ISE
1. NORAD	KI tablet	mg I/tablet	49.7	$51.6 \pm 7.3$ (n=3)	$57.9 \pm 7.3$ (n=3)
2. IOSAT	KI tablet	mg I/tablet	99.5	$101.2 \pm 3.5$ (n=3)	$102.8 \pm 5.6$ (n=3)
3. Mixt. Stramonium Co.	Liquid patent medicine	mg I/L	9,181	$8,926 \pm 170$ (n=10)	$9,118 \pm 441$ (n=10)

### 5.2.5 Analytical Recovery of System 1

The recovery was first studied by addition of 100 mg I/L into the extracts obtained from KI tablets (both NORAD and IOSAT). Satisfactorily good recoveries from 99 to 103 % (n=6) were obtained.

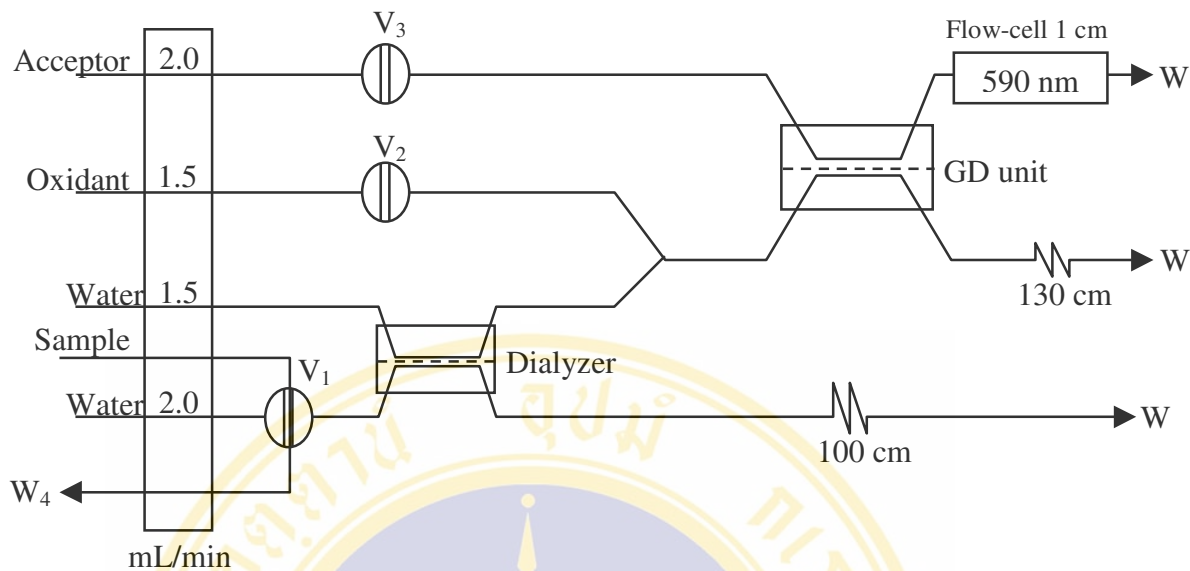
Recovery of iodide content was also studied using ten samples of Mixt. Stramonium Co. by addition of 100 mg I/L standard iodide into diluted samples (1/100). The recoveries were analyzed by using the optimal FI system shown in Figure 5.4. The recoveries were ranging from 99.6 to 116 % (n=10). Appendix C shows the details of the recovery results. These results demonstrated that components in the sample matrix did not interfere in the analysis of iodide.

### 5.3 Incorporation of an On-line Dilutor to the System 1

The level of iodide presents in the liquid Mixt. Stramonium Co. medicine is particularly high (ca. 9,000 mgI/L) compared to the KI tablets (NORAD and IOSAT). Dissolution of a tablet in 500 ml of water will bring the concentrations to approximately 100 or 200 mg I/L. These concentration levels are much lower than the concentration of iodide in Mixt. Stramonium Co. For Mixt. Stramonium Co., prior dilution with water (1/100) was necessary before the analysis using the System 1.

Usually on-line dilution in flow injection is made by merging and mixing the sample zone with a suitable carrier. This technique is not always applicable when high dilution is required, unless a special mechanics for injecting very small sample size is used.

Normally, dialysis is often used for separating analyte from the interference in sample matrix. The technique is based on differences in mobility of ionic or molecule constituents in liquid phase during their transport across a semipermeable membrane into the second liquid phase. Actually for this work, samples do not require removal of interference using dialysis unit because the separation of iodide from sample matrix is already happening via gas diffusion. However, when dialysis is applied to FI for pretreatment, analyte concentration is often quite dilute after diffusion through the membrane [40]. Therefore, we decided to employ dialysis for the purpose dilution of the medicine, Mixt. Stramonium Co. Figure 5.11 shows system configuration after incorporating a dialysis unit (dialyzer) to the GD-FI System 1.



**Figure 5.11** The GD-FI System 2: modified from System 1 for direct injection of Mixt. Stramonium Co. Acceptor: 0.016 M KI in 0.1 %(w/v) starch, oxidant: 0.01 M  $K_2Cr_2O_7$  in 10 %(v/v)  $H_2SO_4$ ,  $V_1$ : sample injection port (at the optimized conditions),  $V_2$  and  $V_3$ : 250  $\mu$ L injection of cleaning solution ( $2 \times 10^{-3}$  M of  $Na_2S_2O_3$  in 0.1 %(w/v) starch), W: waste.

### 5.3.1 Optimization of Sample Volume

The influence of injection volume on analytical signal was investigated using the FI System 2 in Figure 5.11. A suitable volume should give reading between 0.2 and 0.8 a.u. for the calibration range of 6,000 to 10,000 mg I/L. Triplicate injections of iodide standard were carried out over different injection volume ranging from 250 to 750  $\mu$ L as shown in Table 5.3. Results in Table 5.3 show that absorbance were not appropriate when the injection volume was below 500  $\mu$ L. The sensitivity and signal range given for the injection volume of 500  $\mu$ L and 750  $\mu$ L were both applicable. However, since small volume is simpler for handling the volume of 500  $\mu$ L was selected for further study.

**Table 5.3** Results obtained at various injection volumes, when working on the GD-FI System 2 which was coupled with a dialysis unit (Figure 5.11). Calibration ranged from 6,000 to 10,000 mg I/L.

Injection volume ( $\mu\text{L}$ )	Signal range (a.u.)	Standard equation <sup>a</sup>	$r^2$
250	0.106 – 0.279	$A = 4.3 \times 10^{-5}[\text{I}] - 1.5 \times 10^{-1}$	0.999
350	0.169 – 0.390	$A = 5.5 \times 10^{-5}[\text{I}] - 1.6 \times 10^{-1}$	0.999
500	0.249 – 0.603	$A = 8.9 \times 10^{-5}[\text{I}] - 2.9 \times 10^{-1}$	0.995
750	0.361 – 0.725	$A = 9.1 \times 10^{-5}[\text{I}] - 1.9 \times 10^{-1}$	0.997

<sup>a</sup>Iodide standards were 6,000, 8,000 and 10,000 mg I/L.

## 5.4 The GD-FI System 2 with On-line Dilution

An appropriate condition gained from the results discussed in Section 5.3.1 has brought up another GD-FI system, which is specifically suitable for Mixt. Stramonium Co. sample. The system has an on-line dilutor and therefore injection of the medicine can be carried out directly. The system has been optimized to give a linear working range in considerably high iodide concentration (6,000 to 10,000 mg I/L). Summary of the analytical performance will be discussed later in Section 5.5.

### 5.4.1 Steps of Operation

The steps of operation optimized for System 2 are shown in Table 5.4.

**Table 5.4** Summary of the operational steps for System 2 (Figure 5.11). Injection;  $V_1$ : sample,  $V_2$  and  $V_3$ : cleaning solution of  $2 \times 10^{-3}$  M  $\text{Na}_2\text{S}_2\text{O}_3$  in 0.1 % (w/v) starch.

Step	Valve position			Duration (s)
	$V_1$	$V_2$	$V_3$	
1	Inject	Load	Load	110
2	Load	Inject	Inject	75

Analysis time per injection is  $110+75$  s = 3 min.

Step1 Sample injection and detection of  $\text{I}_3^-$ -starch complex: Analyte in the sample zone (500  $\mu\text{L}$  injection) is dialysed before being oxidized to iodine. Iodine then permeates through the PTFE membrane in the GD unit. The time of 110 s gave the maximum reading of absorbance.

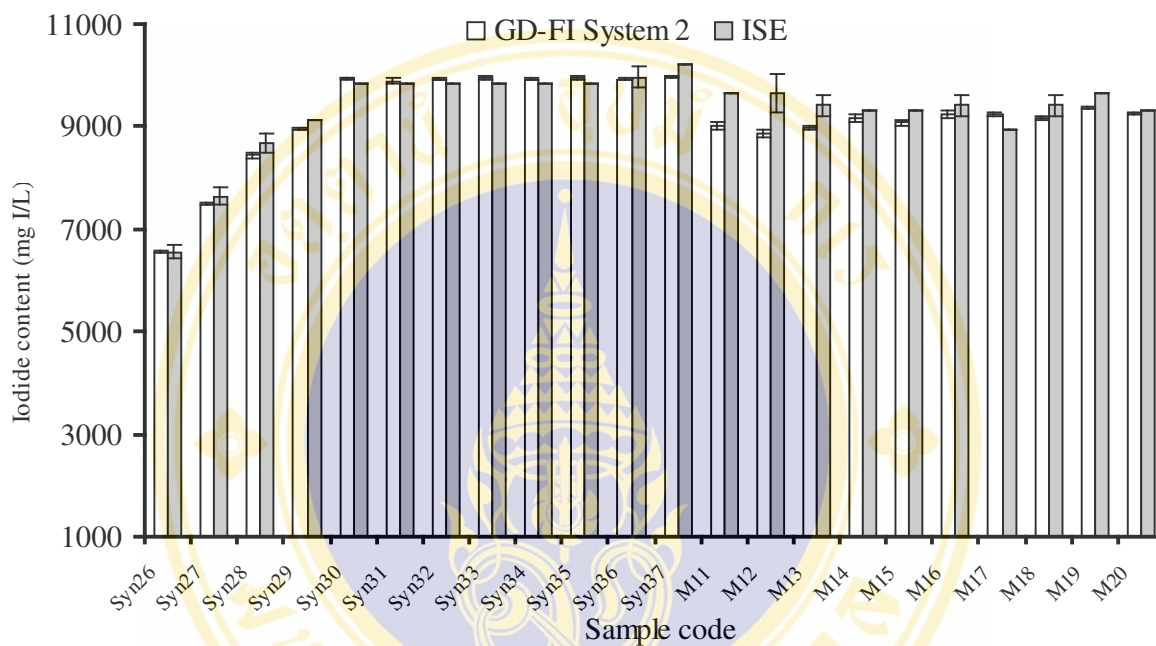
Step 2 Cleaning the system: After detection of signal, injection of the cleaning solution ( $V_2$  and  $V_3$ ) was done. The washing time was set at 75 s. This length of time was adequate for washing off  $\text{I}_3^-$ -starch complex deposited on the tube and flow-cell windows.

Analysis time per injection was a summation of the time from step 1 to step 2. Therefore the total cycle time was 3 minute per injection.

#### 5.4.2 Method Validation for System 2

The System 2 (Figure 5.11) was used to determine iodide contents in 12 synthetic samples (prepared to contain 6,500 to 9,500 mg I/L). These samples were made to contain 10 % (v/v) ethanol to imitate the solvent of medicine. Good agreement in the results of the iodide contents was obtained between the proposed method and the potentiometric method (iodide-ISE) as seen in Figure 5.12. This was confirmed by paired  $t$ -test at 95% confidence limit ( $t_{\text{observed}} = 0.54$ ,  $t_{\text{critical}} = 2.20$ ).

The System 2 in Figure 5.11, was then applied to Mixt. Stramonium Co. using direct injection. The results are compared with the concentration determined by iodide-ISE (Figure 5.12). No significant disagreement, according to paired *t*-test, was observed at 99% confidence ( $t_{\text{observed}} = 2.82$  and  $t_{\text{critical}} = 3.25$ ).



**Figure 5.12** Comparison of iodide contents in synthetic samples (Syn26 to Syn37) and in Mixt. Stramonium Co (M11 to M20) determined in triplicate by GD-FI System 2 and by the potentiometric method.

#### 5.4.3 Stability of the Calibration Plot of System 2

The System 2 has a good potential for attaching on to the production plant of the Mixt. Stramonium Co. medicine. This work therefore explored the stability of the system.

**Table 5.5** Stability of the calibration plot during 6 hours.

Time (hour)	(Slope $\pm$ SD) $\times 10^{-5}$	(Intercept $\pm$ SD) $\times 10^{-1}$	$r^2$
0 - 1	8.4 $\pm$ 0.3	2.3 $\pm$ 0.1	0.999
3 - 4	8.2 $\pm$ 0.3	1.9 $\pm$ 0.3	0.995
5 - 6	7.9 $\pm$ 0.4	2.0 $\pm$ 0.3	0.996

The system stability was evaluated during the continuous 6 hours of operation. As shown in Table 5.5, the calibration parameters, which are the slopes (sensitivity) and the intercepts, did not change significantly during the 6 hours. This implied that recalibration within this period is not necessary during a working day.

### 5.5 Analytical Features of the System 1 and System 2

System 1 and System 2 were FI systems presented for samples containing high concentration levels of iodide. System 1 is suitable for KI tablets when using the dissolution method reported in this thesis. System 2 is capable of using in the process control with on-line dilution. This allows direct injections of a liquid sample containing 6,000 to 10,000 mg I/L. The System 2 is proposed for an asthma medicine, Mixt. Stramonium Co. Table 5.6 contains summaries of the systems' performance.

**Table 5.6** Analytical performance of the proposed GD-FI systems for quantitation of iodide in KI tablets and Mixt. Stramonium Co.

	System 1	System 2
1. Recommended sample	KI tablets / solid (for nuclear emergency)	Mixt. Stramonium Co. (for asthma treatment)
2. Working range (mg I/L)	50 to 300	6,000 to 10,000
3. Standard equation	$PH = (1.5 \pm 0.2) \times 10^{-3}[I] - (4.5 \pm 0.1) \times 10^{-2}, r^2 = 0.999$	$PH = (8.3 \pm 0.3) \times 10^{-5}[I] - (2.3 \pm 0.1) \times 10^{-1}, r^2 = 0.999$
4. Precision (RSD)	1.27 % (for 100 mg I/L, n=10)	1.44 % (for 9,000 mg I/L, n=25)
5. Sample throughput (injection/h)	30	20
6. Sample preparation	Off-line dissolution and filtration	Not required (direct injection)
7. Limit of detection in mg I/L (3S/N)	1	200
8. Recovery	99 to 103	91.7 to 104.6
9. Matrix interference	None	None

PH: Peak height

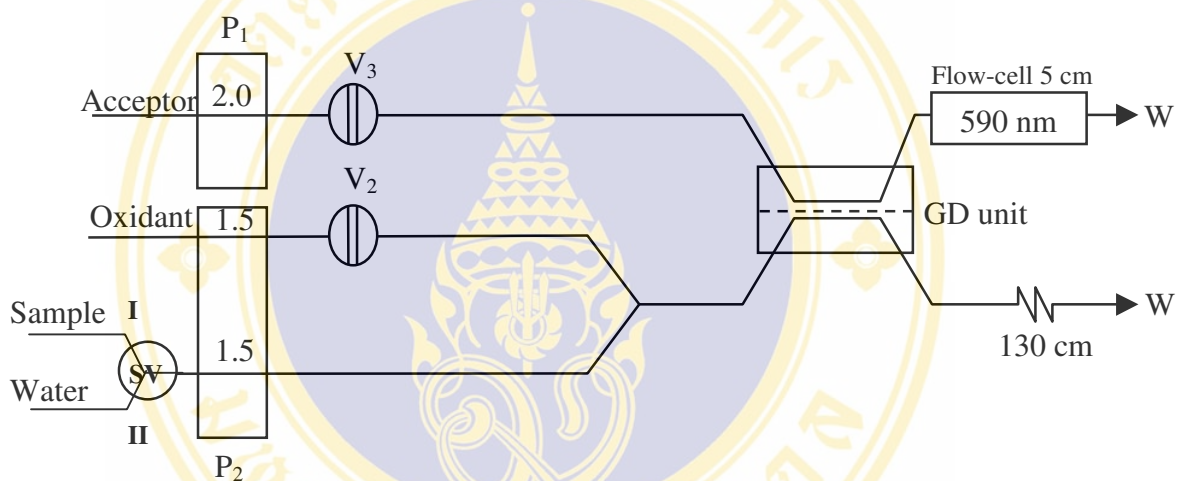
## 5.6 Development of a Suitable GD-FI System for a Multi-vitamin

Multi-vitamin tablet is one of the products that contain iodine but at considerably lower levels (0.65 to 0.1 mg I/g) compared to KI tablets and Mixt. Stramonium Co. Centrum, one brand of vitamin tablet, contains many types of vitamins and minerals (shown in Appendix F). Iodine in the form of potassium iodide is one of the ingredients. The content of iodide in this vitamin is 0.15 mg I per tablet (or 0.1 mg I/g). The iodide salt in the tablet should be soluble in water. If 1.5 g of ground sample is dissolved in 100.00 mL of water, the concentration level of iodide is approximately 1.5 mg I/L (ppm). The extracts of KI tablet samples have iodide in the range of 100 to 200 mg I/L (100 times greater). Thus, modification of the developed

GD-FI System 1 (Figure 5.4) was made to the analyse iodide in low concentration level. The calibration range is 0.5 to 3.0 mg I/L.

### 5.6.1 Modification of the GD-FI System for Low Levels of Iodide

To increase the sensitivity, a flow-cell with path length of 5 cm was used instead of the 1 cm. Sample volume was increased to 4.5 mL/injection using time-based introduction. The modified GD-FI system is shown in Figure 5.13.



**Figure 5.13** The GD-FI System 3 developed for determination of iodide in the ppm levels. Acceptor: 0.016 M KI in 0.1 % (w/v) starch in 10 % (v/v) MeOH (the optimized conditions), oxidant: 0.01 M  $K_2Cr_2O_7$  in 10 % (v/v)  $H_2SO_4$ ,  $P_1$ : acceptor pump,  $P_2$ : donor pump,  $V_2$  and  $V_3$ : 250  $\mu$ L injection of cleaning solution ( $2 \times 10^{-3}$  M of  $Na_2S_2O_3$  in 0.1 % (w/v) starch). SV: selection valve. W: waste.

### 5.6.2 The Steps of Operation: Requirement of Stopped-FI Mode

The operational steps for the manifold in Figure 5.13 is shown in Table 5.7. As already mentioned that the concentration level of iodide in this type of sample is particularly much lower than before. In order to bring up the sensitivity, the system is therefore operated based on stopped-FI mode. The acceptor stream was stopped

while sample extract was pumped through the diffusion cell (GD unit). This is for the purpose of preconcentration of diffused  $I_2$  in the acceptor side (step 1).

**Table 5.7** Summary of the operational steps for System 3 (Figure 5.13).  $V_2$  and  $V_3$ : cleaning solution of  $2 \times 10^{-3}$  M  $Na_2S_2O_3$  in 0.1 % (w/v) starch.  $P_1$  and  $P_2$ : acceptor pump and donor pump.

Step	Pump operation		Valve position			Duration (s)
	$P_1$	$P_2$	$V_2$	$V_3$	SV	
1	Off	On	Load	Load	I	180 (4.5 mL sample)
2	Off	Off	Load	Load	II	5
3	On	On	Load	Load	II	15 <sup>a</sup>
4	On	On	Inject	Inject	II	120

<sup>a</sup>Time for detection of top-peak

Analysis time per injection is 5 min 20 s.

Step 1 Sample introduction: Selection valve (SV) is selected to position I, while pump  $P_1$  is stopped, sample is pumped and allowed to pass through the GD unit.

Step 2 Switch to water reservoir: After sample is pumped through for 3 min, pump  $P_2$  is stopped and the selection valve was switched to position II to feed water into the system.

Step 3 Detection of  $I_3^-$ -starch complex: Pumps  $P_1$  and  $P_2$  are started.  $I_3^-$ -starch complex is aspirated to the detector. The time of 15 s was allowed for recording of the FI profile.

Step 4 Cleaning the system: Injections of cleaning solution was done via  $V_2$  and  $V_3$ . The washing time was set at 120 s.

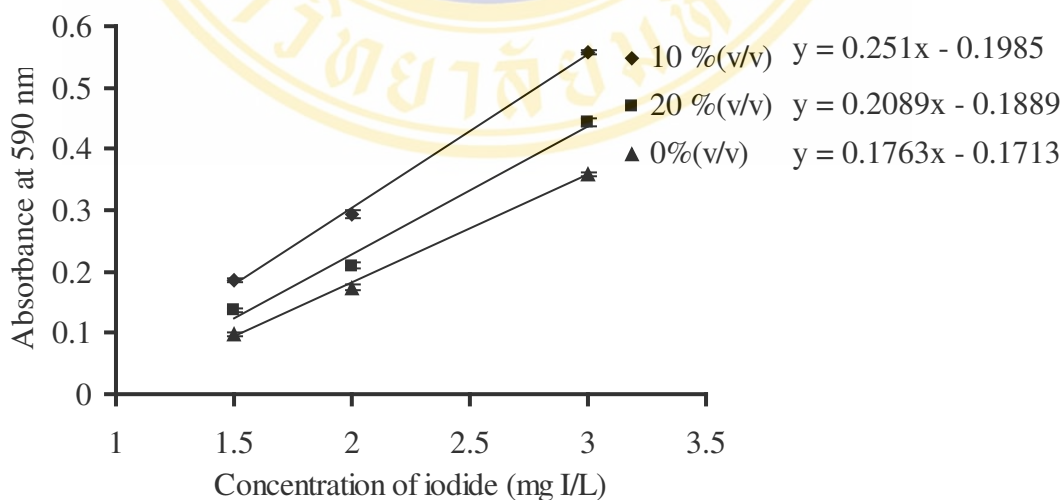
Step 1 to step 4 is repeated for the next analysis.

### 5.6.3 Optimization of Methanol Concentration in the Acceptor Stream

The destination of this study is to increase the permeation of iodine through the acceptor side, iodine ( $I_2$ ), is a non-polar molecule that can be dissolved in organic solvent. Therefore, utilization of solvent mixture in the acceptor stream may aid the solubility of iodine. Methanol was chosen for this investigation and was added to the acceptor solution.

It is known that  $I_2$  solubilizes in methanol, better than in water [54]. However, pure methanol is not suitable for using as our acceptor stream, because 100 % methanol may later cause wetting effect of membrane's pores. This will lead to malfunction of the hydrophobic membrane (water and solutes can pass through the pores).

For this work, an optimal mixture ratio of methanol and water in the acceptor stream (Figure 5.13) was studied. Polarity was optimized by varying percentage of methanol. The FI manifold used for this particular study is shown in Appendix D. The results are shown in Figure 5.14.



**Figure 5.14** Calibration curve obtained at various methanol concentrations (%(v/v)) in the acceptor stream. Each data point was from triplicate injection of standard iodide. The results were obtained from the same membrane and the experiment was carried out in sequence from 0 to 20 % methanol.

In Figure 5.14, the slope or sensitivity is greater when increasing methanol concentration from 0 to 10 %(v/v). However, the sensitivity dropped at 20 %(v/v) methanol. The drop of sensitivity is expected to be the cause of formation of  $I_3^-$ , which may differ at different solvent polarity or solvent polarity may also affect formation constant of the  $I_3^-$ -starch products. For this work, 10 %(v/v) was chosen as the optimal condition.

#### 5.6.4 Working Range and the Improved Sensitivity of System 3

The FI manifold modified for the vitamin tablet samples containing low iodide concentration level (Figure 5.13) provides a linear calibration graph over the entire range of 0.5 to 2.0 mg I/L with the equation of  $A = (5.7 \pm 0.1) \times 10^{-1} [I] - (1.9 \pm 0.1) \times 10^{-1}$ ;  $r^2 = 0.996$ , where A is absorbance. By employing the stopped-FI mode and the 5 cm flow-cell, the sensitivity was better than the System 1's to approximately 100 times.

#### 5.6.5 Application to a Vitamin Extract Sample Containing ca. 2.7 mg I/L

The FI System 3 in Figure 5.13 was applied to determine iodide content in Centrum vitamin (prepare to contain iodide 1.5 mg I/L in the extract). There were no signal obtained although the sample was pretreated using the  $C_{18}$  Sep-Pak (described in Section 4.9.2 (a)).

According to the above result, the sample extract was prepared to expectedly contain 2.7 mg I/L in the extract (using 2.7 g of ground sample to 100.00 mL of water), and C-18 pretreatment was employed. The results are compared with other methods as shown in Table 5.8.

**Table 5.8** Iodide contents found in Centrum vitamin as determined by System 1 (Figure 5.4), System 2 (Figure 5.11), System 3 (Figure 5.13), GD-FI with chemiluminescence (CL) detection (Appendix E) and by the potentiometric method, all compared to the labeled content.

Sample code	Iodide content ( $\mu\text{g I/tablet}$ )					
	System 1	System 2	System 3	GD-FI with CL	ISE	Labeled
C1	n.d.	n.d.	$68.9 \pm 1.1$	$96.7 \pm 1.7$	$145.0 \pm 7.2$	150
C2	n.d.	n.d.	$72.8 \pm 1.1$	$108.3 \pm 1.1$	$158.9 \pm 5.0$	150

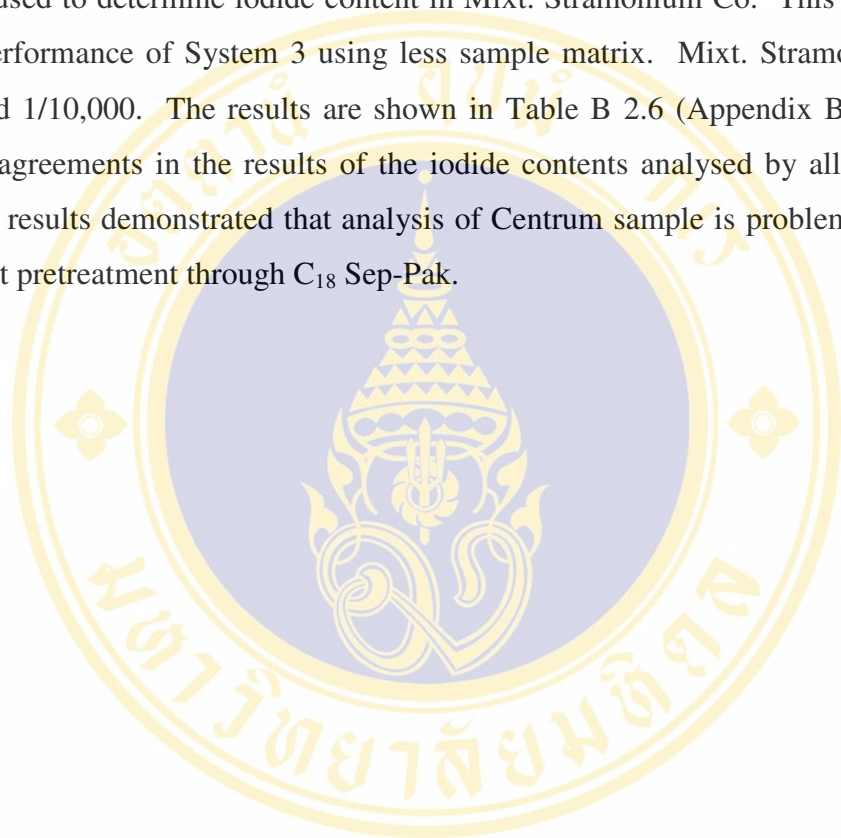
n.d.: non-detectable

The sample extracts, as expected, failed to give signal in System 1 and System 2. The results given by System 3 were lower than the labeled contents whereas the ISE results agreed well with the labels. Another GD-FI system with chemiluminescence detection, developed by N. Ratanawimarnwong (Appendix E), was also applied. The contents of iodide as determined by this system were also lower than those from the ISE method and the nominal value. When matrix interference was investigated (recovery) by adding 0.5 mg I/L of iodide standard into the sample extracts (after C<sub>18</sub> Sep-Pak, before injection). The results in recovery of Centrum sample C1 and C2 were 80.1 and 86.1 % respectively. These evidences suggest that there are some interferents in the sample extracts for all the GD-FI methods. These interferents did not allow use of direct (external) calibration to be possible. However standard addition method is not applicable since the calibration intercept is negative.

The ISE results which are close to the nominal value indicated that iodide is the most present form in the extracts. However, after oxidation to I<sub>2</sub> in the extract did not diffuse through the membrane as same as the I<sub>2</sub> in the standard. It is suspected that I<sub>2</sub> may react with some water extractable compounds containing double bonds [55] (Appendix F shown ingredients in the Centrum). This should cause the recovery of less than 100 %.

To use the developed FI system for quantitation of iodide in Centrum or other multi-vitamin tablets, a special way of sample preparation is required and needs further investigation.

Some of the GD-FI systems (System 1, System 3, GD-FI-CL) and the ISE were used to determine iodide content in Mixt. Stramonium Co. This was for testing the performance of System 3 using less sample matrix. Mixt. Stramonium Co. was diluted 1/10,000. The results are shown in Table B 2.6 (Appendix B). There were good agreements in the results of the iodide contents analysed by all the technique. These results demonstrated that analysis of Centrum sample is problematic even with extract pretreatment through C<sub>18</sub> Sep-Pak.



## CHAPTER VI

### CONCLUSION

The following are the conclusions of this research.

6.1 In this work, three FI systems namely System 1, System 2 and System 3 were developed for iodide determination. All systems employ gas diffusion (GD) unit and a simple colorimetric detection of  $I_3^-$ -starch complex.

6.2 Incorporation of GD unit with FI systems results in a selective separation of elementary iodine, which diffuses through the membrane. And for the first time that a very simple reaction based on formation of the  $I_3^-$ -starch complex was ever used with GD technique. However, this complex is sparingly soluble in water, and one must be careful not to exceed the appropriate working concentration range of iodide, otherwise clogging inside tube or deviation from Beer's law will occur.

## 6.3 Characteristics of the developed systems

**Table 6.1** Summary of the characteristics of System 1, 2 and 3 developed for determination of iodide.

Summary of the different characteristics of the three analytical systems	Type of GD-FI system developed		
	System 1 (Figure 5.4)	System 2 (Figure 5.11)	System 3 (Figure 5.13)
1. Suitable sample	1.1 KI tablets (for nuclear emergency) 1.2 Mixt. Stramonium Co. (1/100 dilution) 1.3 Mixture of $I_{(aq)}$ and $I_{2(aq)}$ (synthetic)	Direct injection of Mixt. Stramonium Co (asthma drug) after pre-filtration	NFI (for Centrum vitamin)
2. Working range (mg I/L)	50 - 300	6,000 – 10,000	0.5 – 3.0
3. Is the method validated?	YES (for KI tablets and Mixt. Stramonium Co.)	YES (for Mixt Stramonium Co.)	NFI
4. Sample throughput (injection/h)	Satisfying (30)	Satisfying (20)	Satisfying (12)
5. Level of precision	Very precise (RSD = 1.27 %)	Very precise (RSD = 1.44 %)	Very precise (RSD = 2.34 %)
6. Detection limit (mg I/L)	Suitable for KI tablet (1)	Suitable for Mixt. Stramonium Co. (200)	Considerably low (0.035)
7. Detection chemistry	Very simple	Very simple	Very simple
8. Applicable for colored samples	YES	YES	YES

NI: No investigation

NFI: Needs further investigation

**Table 6.1** Summary of the characteristics of System 1, 2 and 3 developed for determination of iodide. (Continued)

Summary of the different characteristics of the three analytical systems	Type of GD-FI system developed		
	System 1 (Figure 5.4)	System 2 (Figure 5.11)	System 3 (Figure 5.13)
9. Required stopped-FI technique?	NO	NO	YES
10. Allows for on-line dilution	NO	YES	NO
11. Applicability on determination of total iodine ( $I^- + I_2$ )	Very good	NI	NI
12. Potential for investigating the FI system to the manufacturing control	Not good	Very good	Not Good

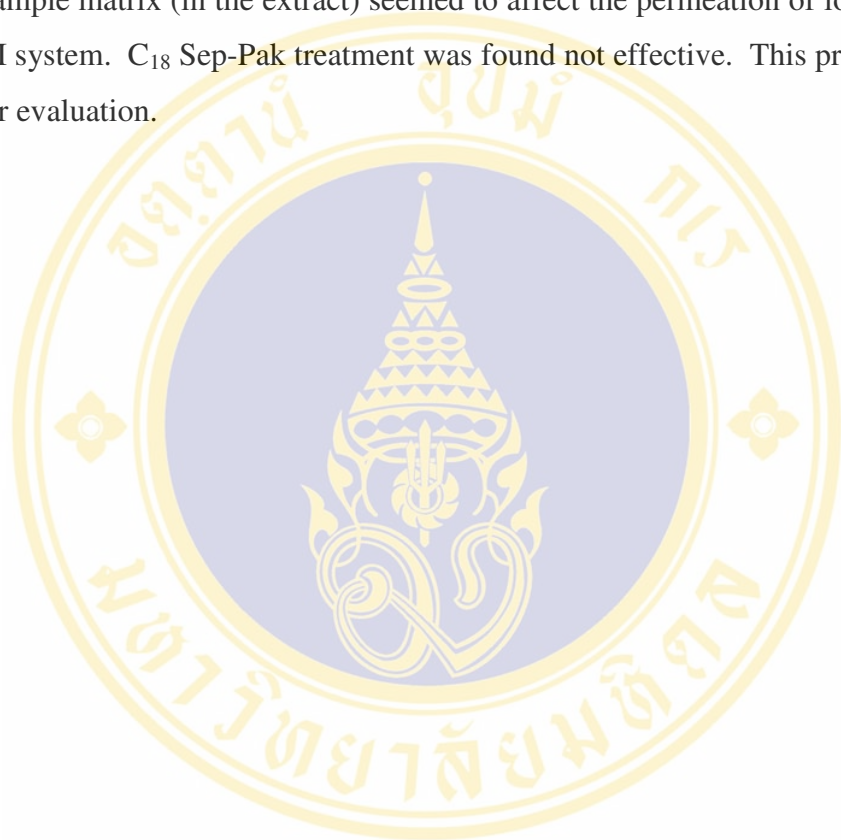
NI: No investigation  
NFI: Needs further investigation

6.4 The dominant advantage of the methods employing System 1 and 2 is probably the throughput of samples. The batch ISE method gives approximately 8 samples/h whereas these systems provide the throughput of 30 and 20 samples/h for the System 1 and 2, respectively. For liquid sample containing 6,500 to 9,500 mg I/l, the System 2 has a good potential for integrating into the manufacturing process of the pharmaceutical preparation. Maintenance should be simpler than using an iodide-selective electrode.

6.5 The GD-FI System 1 was successfully optimized and is proved suitable for the quantitation of total iodine in a sample mixture containing iodide and iodine ( $I_{(aq)}^- +$

$I_{2(aq)}$ ). The system was optimized to give an in-line 100 % oxidation of iodide to iodine.

6.6 System 3 although provides a suitable linear working range for low concentration levels of multi-vitamin tablets, but the system failed in real application. The sample matrix (in the extract) seemed to affect the permeation of iodine inside the GD-FI system.  $C_{18}$  Sep-Pak treatment was found not effective. This problem required further evaluation.



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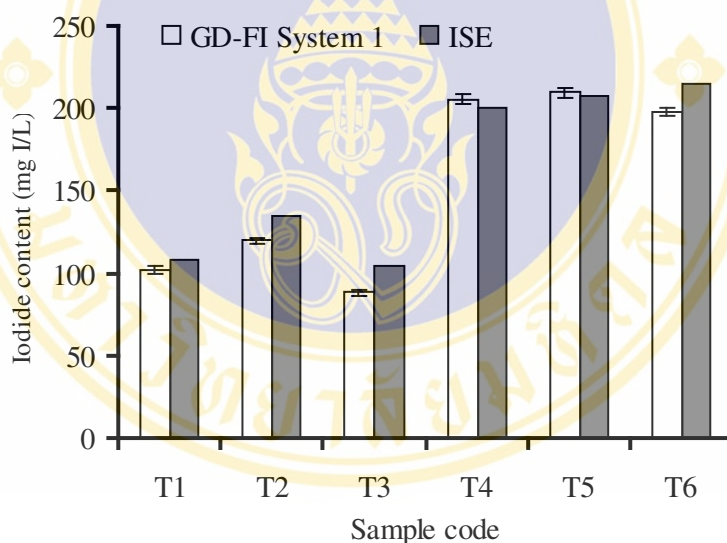


## APPENDIX A

### Method Validations (System 1)

#### 1. KI tablets

Six samples of KI tablets were dissolved in deionized-distilled water (500.0 mL) and was determined for iodide contents in each solution samples (with pre-filtration through Whatman filter paper No. 1). The GD-FI system shown in Figure 5.4 was used. Validation was carried out against the ISE method. Results are reported as the concentration of iodide in the liquid samples (Figure A 1.1).

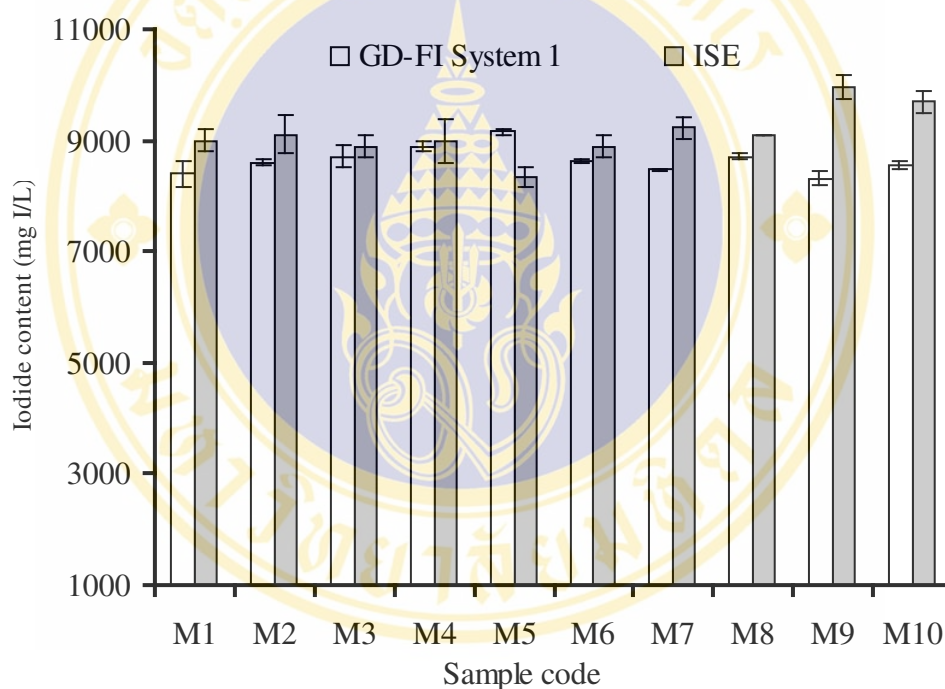


**Figure A 1.1** Comparison of iodide contents in KI tablets determined by using System 1 (Figure 5.4) ( $n=3$ ) and by the potentiometric method ( $n=1$ ).

Paired  $t$ -test was employed to evaluate the difference in the results of the two methods. There was no significant evidence of the difference in the results (95 % confidence;  $t_{\text{observed}} = 1.98$ ,  $t_{\text{critical}} = 2.57$ ).

## 2. Mixt. Stramonium Co.

The GD-FI system in Figure 5.4 was applied to determine iodide in ten samples of Mixt. Stramonium Co. The results were compared with the results determined by using iodide-ion selective electrode (ISE) as shown in Figure A 1.2. The experimental procedures for the ISE method are described in Section 4.10 Both methods are not significant different (99% confidence), according to the paired *t*-test [53] ( $t_{\text{observed}} = 2.31$ ,  $t_{\text{critical}} = 3.25$ ).



**Figure A 1.2** Comparison of iodide contents in Mixt. Stramonium Co., determined by using System 1 (Figure 5.4) and by the potentiometric method. Analysis was carried in triplicate.



## APPENDIX B

### Experimental Data in Determination of Iodine Content in Sample

**Table B 2.1** Experimental data of iodine content as described in Figure 5.8.

Sample code	Total I found (mg I/L) $\pm$ SD, (n = 3)	True concentration (mg I/L)
Syn 1	53.3 $\pm$ 0.6	50
Syn 2	100.0 $\pm$ 0.3	100
Syn 3	152.6 $\pm$ 1.1	150
Syn 4	200.5 $\pm$ 2.1	200
Syn 5	254.0 $\pm$ 1.4	250
Syn 6	308.7 $\pm$ 0.9	300
Syn 7	66.6 $\pm$ 1.4	66.3
Syn 8	80.5 $\pm$ 1.2	79.6
Syn 9	104.0 $\pm$ 0.5	99.5
Syn 10	122.6 $\pm$ 0.3	119.4
Syn 11	135.6 $\pm$ 0.9	132.7
Syn 12	150.7 $\pm$ 0.6	145.9
Syn 13	163.6 $\pm$ 0.8	165.8
Syn 14	90.4 $\pm$ 1.4	82.6
Syn 15	100.4 $\pm$ 1.4	102.6
Syn 16	104.6 $\pm$ 0.7	112.6
Syn 17	113.9 $\pm$ 1.2	122.6
Syn 18	165.9 $\pm$ 2.0	182.6
Syn 19	180.6 $\pm$ 1.8	282.6
Syn 20	373.7 $\pm$ 3.3	382.6

**Table B 2.2** Experimental data of iodine content as described in Figure 5.10.

Sample code	Total I found	True concentration
	(mg I/L) $\pm$ SD, (n = 3)	(mg I/L)
Syn 21	192.1 $\pm$ 1.1	155.7
Syn 22	289.4 $\pm$ 1.9	255.7
Syn 23	406.4 $\pm$ 4.1	355.7
Syn 24	494.8 $\pm$ 4.6	455.7
Syn 25	616.1 $\pm$ 4.7	555.7

**Table B 2.3** Experimental data of iodine content as described in Figure A 1.1.

Sample code	Iodide content (mg I/L) $\pm$ SD (n = 3)	
	GD-FI System 1	ISE
T1	101.9 $\pm$ 1.9	108.6
T2	119.8 $\pm$ 1.9	134.7
T3	88.4 $\pm$ 1.9	104.8
T4	205.4 $\pm$ 2.8	200.0
T5	209.4 $\pm$ 3.0	207.3
T6	197.9 $\pm$ 2.5	214.9

**Table B 2.4** Experimental data of iodine content as described in Figure A 1.2.

Sample code	Iodide content (mg I/L) $\pm$ SD (n = 3)	
	GD-FI System 1	ISE
M1	8,403 $\pm$ 231	8,995 $\pm$ 193
M2	8,603 $\pm$ 58	9,111 $\pm$ 341
M3	8,714 $\pm$ 201	8,883 $\pm$ 193
M4	8,892 $\pm$ 102	8,999 $\pm$ 394
M5	9,148 $\pm$ 51	8,346 $\pm$ 179
M6	8,641 $\pm$ 37	8,883 $\pm$ 193
M7	8,468 $\pm$ 21	9,222 $\pm$ 201
M8	8,702 $\pm$ 57	9,106 $\pm$ 0
M9	8,307 $\pm$ 128	9,939 $\pm$ 216
M10	8,567 $\pm$ 74	9,694 $\pm$ 208

**Table B 2.5** Experimental data of iodine content as described in Figure 5.12.

Sample code	Iodide content (mg I/L) $\pm$ SD (n = 3)	
	GD-FI System 2	ISE
Syn 26	6,560 $\pm$ 31	6,561 $\pm$ 145
Syn 27	7,485 $\pm$ 19	7,639 $\pm$ 169
Syn 28	8,443 $\pm$ 52	8,670 $\pm$ 189
Syn 29	8,951 $\pm$ 13	9,119 $\pm$ 0
Syn 30	9,948 $\pm$ 19	9,839 $\pm$ 0
Syn 31	9,894 $\pm$ 51	9,839 $\pm$ 0
Syn 32	9,932 $\pm$ 26	9,839 $\pm$ 0
Syn 33	9,944 $\pm$ 38	9,839 $\pm$ 0
Syn 34	9,928 $\pm$ 22	9,839 $\pm$ 0
Syn 35	9,948 $\pm$ 26	9,839 $\pm$ 0
Syn 36	9,928 $\pm$ 12	9,966 $\pm$ 220
Syn 37	9,973 $\pm$ 31	10,200 $\pm$ 0

**Table B 2.5** Experimental data of iodine content as described in Figure 5.12.  
(Continued)

Sample code	Iodide content (mg I/L) $\pm$ SD (n = 3)	
	GD-FI System 2	ISE
M11	9,027 $\pm$ 73	9,662 $\pm$ 0
M12	8,865 $\pm$ 73	9,667 $\pm$ 372
M13	8,985 $\pm$ 38	9,418 $\pm$ 210
M14	9,173 $\pm$ 81	9,297 $\pm$ 0
M15	9,090 $\pm$ 56	9,297 $\pm$ 0
M16	9,230 $\pm$ 71	9,419 $\pm$ 210
M17	9,225 $\pm$ 36	8,947 $\pm$ 0
M18	9,168 $\pm$ 33	9,419 $\pm$ 210
M19	9,368 $\pm$ 8	9,662 $\pm$ 0
M20	9,254 $\pm$ 22	9,297 $\pm$ 0

**Table B 2.6** Comparison of iodide contents in Mixt. Stramonium Co. determined by GD-FI System 1 (Figure 5.4), System 3 (Figure 5.13), GD-FI with chemiluminescence (CL) detection (Appendix E) and by the potentiometric method.

Sample code	Iodide content in the medicine (mg I/L)				
	System 1	System 3	GD-FI with CL	ISE	Labeled
M21	90.5 $\pm$ 0.5	93.6 $\pm$ 0.6	90.0 $\pm$ 0.3	100.9 $\pm$ 3.72	91.8
M22	90.2 $\pm$ 0.3	93.4 $\pm$ 0.6	91.8 $\pm$ 0.2	102.1 $\pm$ 2.2	91.8
M23	89.1 $\pm$ 1.2	93.6 $\pm$ 0.6	91.3 $\pm$ 0.5	98.4 $\pm$ 2.1	91.8



## APPENDIX C

### Experimental Data of Recovery Studies

Recovery of the iodide content measurement was studied using ten samples of Mixt. Stramonium Co. by addition of iodide standard (100 mg I/L) into the sample.

**Table C 3.1** Recovery study of iodide added to samples as determined using System 1. Mixt. Stramonium Co. was used in this study.

Sample code	Iodide initially found <sup>a</sup> (mg I/L)	Iodide added (mg I/L)	Total iodide found <sup>a</sup> (mg I/L)	Recovery (%)
M1	84.0	100	196.7	116.5
M2	86.0	100	197.7	115.5
M3	87.1	100	196.0	108.0
M4	88.9	100	191.3	101.5
M5	91.5	100	195.3	102.9
M6	86.4	100	186.5	99.6
M7	84.7	100	186.0	100.9
M8	87.0	100	188.0	100.5
M9	83.1	100	192.7	109.1
M10	85.7	100	190.0	103.8

<sup>a</sup>Mean of three replicate analysis.

Results have shown that recoveries ranged from 99.6 to 116.5 %. The results demonstrated that components in the sample matrix did not interfere in the analysis of iodide.

Recovery of the iodide content measurement was studied using ten samples of Mixt. Stramonium Co. by addition of iodide standard (3,500 mg I/L) into the sample.

**Table C 3.2** Recovery study of iodide added to samples as determination by GD-FI System 2.

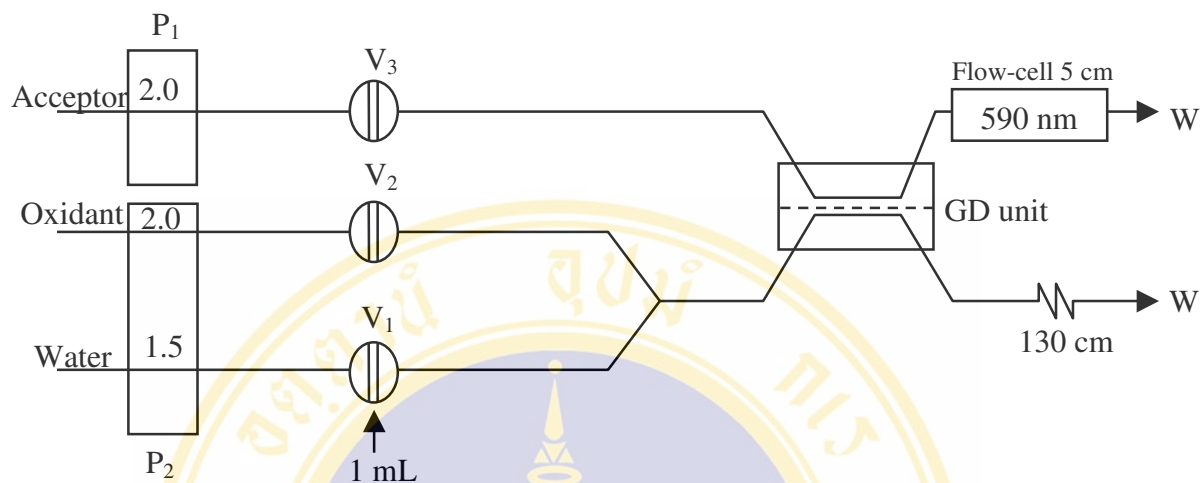
Sample	Iodide initially found <sup>a</sup> (mg I/L)	Iodide added (mg I/L)	Total iodide found <sup>a</sup> (mg I/L)	Recovery (%)
M21	5,931	3,500	9,342	93.8
M22	6,053	3,500	9,386	91.7
M23	6,075	3,500	9,653	98.4
M24	6,169	3,500	9,708	97.3
M25	6,264	3,500	9,714	94.9
M26	6,295	3,500	10,006	103.9
M27	6,567	3,500	9,939	94.4
M28	6,295	3,500	10,034	104.6
M29	6,384	3,500	9,751	94.2
M30	6,312	3,500	9,917	100.9

<sup>a</sup>Mean of three replicates analysis

Results have shown that recoveries ranged from 91.7 to 104.6 %. The results demonstrated that components in the sample matrix did not interfere in the analysis of iodide.



## APPENDIX D



**Figure D 4.1** The schematic diagram of FI system used for the study of effect of methanol concentration. Acceptor: 0.016 M KI in 0.1 % (w/v) starch in various concentration of MeOH (0, 10, 20 % (v/v)), oxidant: 0.01 M  $K_2Cr_2O_7$  in 10 % (v/v)  $H_2SO_4$ ,  $P_1$ : acceptor pump,  $P_2$ : donor pump,  $V_1$ : sample injection port: 1 mL,  $V_2$  and  $V_3$ : 250  $\mu$ L injection of cleaning solution ( $2 \times 10^{-3}$  M of  $Na_2S_2O_3$  in 0.1 % (w/v) starch). W: waste.

**Table D 4.1** Summary of the operation steps for the FI manifold shown in Figure D 5.1 (Injection;  $V_1$ : sample,  $V_2$  and  $V_3$ : cleaning solution of  $2 \times 10^{-3}$  M  $Na_2S_2O_3$  in 0.1 % (w/v) starch,  $P_1$  and  $P_2$ : acceptor pump and donor pump).

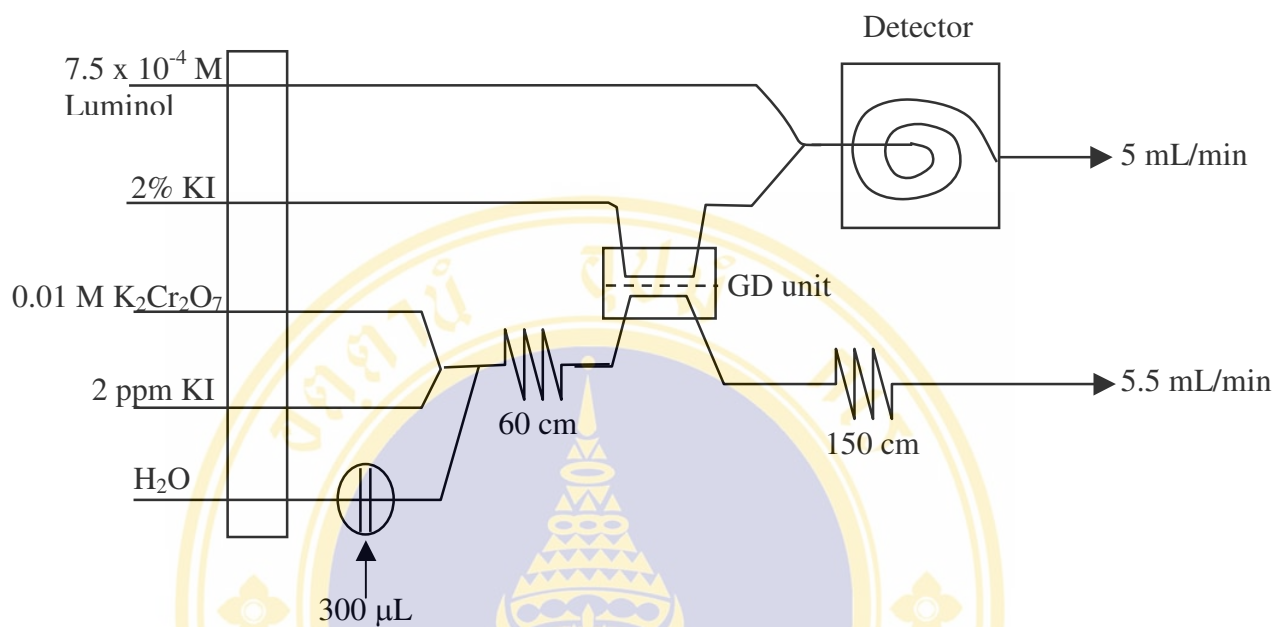
Step	Pump operation		Valve position			Duration (s)
	$P_1$	$P_2$	$V_1$	$V_2$	$V_3$	
1	Off	On	Inject	Load	Load	180
2	Off	Off	Inject	Load	Load	5
3	On	On	Inject	Load	Load	15 <sup>a</sup>
4	On	On	Load	Inject	Inject	120

<sup>a</sup>Time for detection of top-peak

Analysis time per injection is 5 min 20 s.



## APPENDIX E



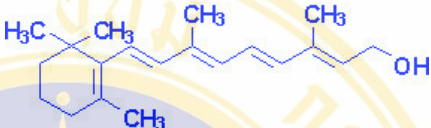
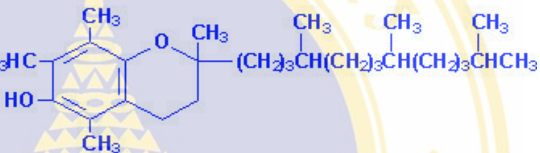
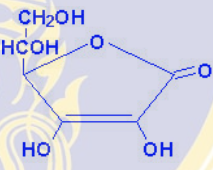
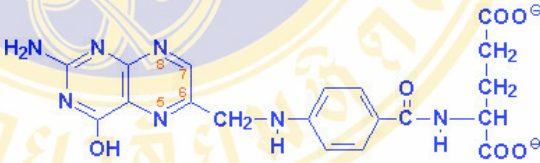
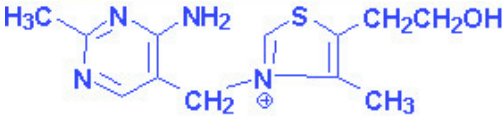
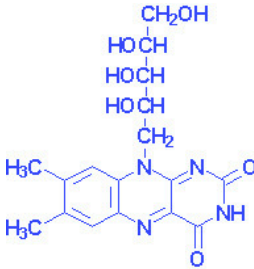
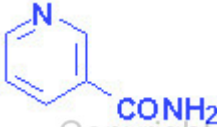
**Figure E 5.1** GD-FI system with chemiluminescence detection for determination of iodide. The system was developed by N. Ratanawimarnwong.



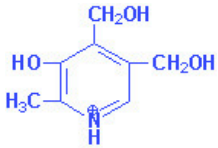
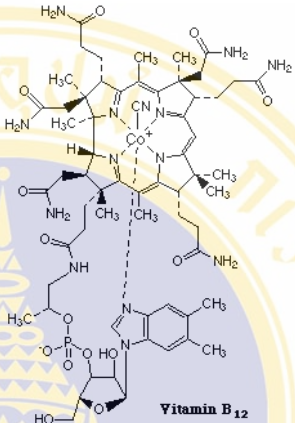
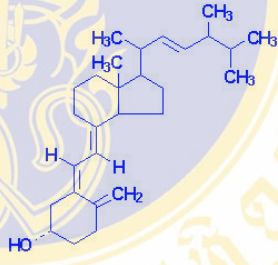
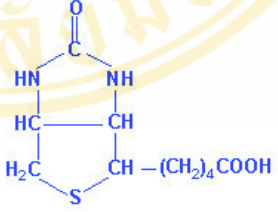
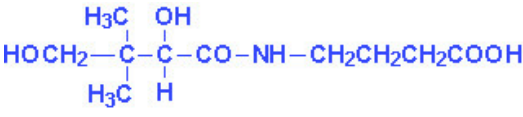
## APPENDIX F

## Ingredients in the Centrum Vitamin Tablet

Table F 6.1 Vitamins in Centrum Vitamin Tablet.

Vitamin	Structure	Content
Vitamin A (as acetate)		5,000 I.U.
Vitamin E (as dl-alpha tocopheryl acetate)		30 I.U.
Vitamin C (as ascorbic acid)		90 I.U.
Folic acid (as folacin)		0.4 mg
Vitamin B1 (as thiamine mononitrate)		2.55 mg
Vitamin B2 (as riboflavin)		2.6 mg
Nicotinamide		20 mg

**Table F 6.1** Vitamins in Centrum Vitamin Tablet. (Continued)

Vitamin	Structure	Content
Vitamin B6 (as pyridoxine hydrochloride)		3 mg
Vitamin B12 (as cyanocobalamin)		9 µg
Vitamin D		400 I.U.
Biotin		45 µg
Pantothenic acid (as calcium pantothenate)		10 mg

I.U. is International Unit

**Table F 6.2** Minerals in Centrum Vitamin Tablet.

Mineral	Formula	Equivalent to	Content
Dicalcium phosphate	Ca <sub>2</sub> PO <sub>4</sub>	Calcium	162 mg
		Phosphorus	125 mg
Potassium iodide	KI	Iodine	150 µg
Ferrous fumarate	C <sub>4</sub> H <sub>2</sub> FeO <sub>2</sub>	Iron	27 mg
Magnesium oxide	MgO	Magnesium	100 mg
Copper oxide	CuO	Copper	3 mg
Manganese sulfate	MnO	Manganese	7.5 mg
Potassium sulfate	K <sub>2</sub> SO <sub>4</sub>	Potassium	7.5 mg
Zinc sulfate	ZnSO <sub>4</sub>	Zinc	22.5 mg

**BIOGRAPHY**

<b>NAME</b>	Miss Kanchana Uraisin
<b>DATE OF BIRTH</b>	5 November 1978
<b>PLACE OF BIRTH</b>	Bangkok, Thailand
<b>INSTITUTIONS ATTENDED</b>	Mahidol University, 2000: Bachelor of Science (Chemistry with second class honor) Mahidol University, 2003: Master of Science (Applied Analytical and Inorganic Chemistry)
<b>GRANT</b>	Recipient of a Teaching Assistance Scholarship from the Department of Chemistry, Faculty of Science, Mahidol University in the academic year of 2000 and 2003 Recipient of the Higher Education Development Project: Postgraduate Education and Research Program in Chemistry, 2000 – 2002
<b>PUBLICATION</b>	“Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt” Talanta, 58 (2002) 1195-1201.
<b>HOME ADDRESS</b>	68/13-14 Soi. Nakbunrung, Bumrungruang Rd., Promprab, Bangkok 10100