A KINETIC FLOW INJECTION METHOD FOR DETERMINATION OF IODATE IN IODIZED SALT

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Thesis
Entitled

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Kanokrat Lukkanakul

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ABSTRACT

A kinetic flow injection (FI) method for measuring iodine content in iodized salt was developed. This system was modified from the FI system for analysis of iodine in drinking water that was developed by N. Choengchan. The analytical method is based on the catalytic effect of iodide for the reaction between Ce (IV) and As (III). In Thailand, supplement of iodine is carried out using the iodate form especially in table salt. Therefore, prior to the catalytic analysis, iodate must be first reduced to iodide.

Arsenous acid, which already exists in the system, was employed as the reducing agent for iodate. The optimization of the FI system was studied to gain an in-line complete reduction of iodate. Three operational methods were investigated and developed. These methods are (a) full kinetic stopped-FI method, (b) the fixed time stopped-FI method and (c) kinetic continuous-FI method. All methods gave linear calibration plots in the working range of 100 to 6,000 μg I/L.

For the full kinetic stopped-FI method, the detection limit was 7.5 mg I/kg salt. Average total recovery was 107 % (n=13) with RSD (n=3) of 0.83 %. For the fixed time stopped-FI method, the limit of detection (3σ) was 1.1 mg I/kg salt. The method gave average total recovery as 114 % (n=13) and provided satisfactorily low % RSD (n=10) of 0.63 %. For the kinetic continuous-FI method, the detection limit (3σ) was found to be 1.3 mg I/kg salt. Average total recovery was 111 % (n=13). This technique gave reasonably good repeatability in term of RSD as 0.17 % (n=10).

Method validation for ten iodized salt samples and three synthetic samples showed no significant difference between iodine contents obtained from four methods (i.e. the full kinetic stopped-FI method, the fixed time stopped-FI method, the kinetic continuous-FI method and the colorimetric-FI method). This agreement implied that all proposed operational methods developed in this work are applicable. However, for real application in commercial iodized salt, the kinetic continuous-FI method was chosen. This method provided the highest sample throughput of 90 samples/h.

KEY WORDS : IODATE / IODINE / FLOW INJECTION / SANDELL AND KOLTHOFF METHOD/ IODIZED SALT

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การพัฒนาระบบโฟลอินเจคชันเพื่อใช้ในการหาปริมาณไอโอดีนในเกลือเสริมไอโอดีน
(A KINETIC FLOW INJECTION METHOD FOR DETERMINATION OF IODATE IN IODIZED SALT)

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บทคัดย่อ

งานวิจัยนี้ได้เสนอระบบโฟลอินเจคชันที่อาศัยวิธีทางจลนศาสตร์เพื่อหาปริมาณไอโอดีนในเกลือเสริมไอโอดีน ระบบโฟลอินเจคชันดังกล่าวได้ถูกปรับปรุงมาจากระบบโฟลอินเจคชันสำหรับหาปริมาณไอโอดีนในน้ำดื่มที่พัฒนาโดยนายณัฐวุฒิ เชิงชั้น ปฏิกิริยาที่ใช้ในการวิเคราะห์ไอโอดีน คือ ปฏิกิริยารีดอกซ์ระหว่างซีเรียม (IV) และอาร์เซนิก (III) ซึ่งมีไอโอไดด์เป็นตัวเร่งปฏิกิริยา อย่างไรก็ตาม การผลิตเกลือเสริมไอโอดีนในประเทศไทยนั้น พบว่านิยมเติมไอโอดีนในรูปของไอโอเดตลงในเกลือบริโภค ดังนั้น ก่อนที่จะทำการวิเคราะห์ด้วยวิธีที่นำเสนอข้างต้น ไอโอเดตจะต้องถูกรีดิวซ์กลายเป็นไอโอไดด์ก่อน

อาร์เซนิก (III) ซึ่งมีอยู่ในระบบโฟลอินเจคชัน ได้ถูกนำมาใช้เป็นตัวรีดิวซ์สำหรับไอโอเดต โดยทำการศึกษาแบบ in-line ที่ทำให้เกิดการรีดักชันของไอโอเดตไปเป็นไอโอไดด์ได้อย่างสมบูรณ์ นอกจากนี้ยังได้ทำการศึกษา และพัฒนากระบวนการวิเคราะห์แบบโฟลอินเจคชัน ได้แก่ (ก) กระบวนการโฟลอินเจคชันแบบที่มีการหยุดไหลชั่วขณะโดยใช้วิธี full kinetic (ข) กระบวนการโฟลอินเจคชันแบบที่มีการหยุดไหลชั่วขณะโดยใช้วิธี fixed-time (ค) กระบวนการโฟลอินเจคชันที่มีการไหลอย่างต่อเนื่องและใช้วิธี fixed-time วิธีการที่นำเสนอทั้งหมดนี้ ให้กราฟมาตรฐานแบบเส้นตรงอยู่ในช่วงความเข้มข้นของไอโอเดตคือ 100 ถึง 6,000 ไมโครกรัมต่อลิตร

วิธีแบบ full kinetic stopped-FI ให้ค่าขีดจำกัดต่ำสุดของการวิเคราะห์ เท่ากับ 7.5 มิลลิกรัมต่อกิโลกรัม, ค่าร้อยละของการคืนกลับเฉลี่ยเป็น 107 % (n=13) และมีค่าความแม่นยำของการวิเคราะห์ คือ RSD (n=3) เท่ากับ 0.83 % สำหรับวิธีแบบ fixed time stopped-FI ให้ค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (3σ) เท่ากับ 1.1 มิลลิกรัมต่อกิโลกรัม, ค่าร้อยละของการคืนกลับเฉลี่ยเป็น 114 % (n=13) และให้ความแม่นยำของการวิเคราะห์เป็นที่น่าพอใจ คือ RSD (n=10) เท่ากับ 0.63 % ส่วนวิธีแบบ kinetic continuous-FI ให้ค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (3σ) เป็น 1.3 มิลลิกรัมต่อกิโลกรัม, ค่าร้อยละของการคืนกลับเฉลี่ยเป็น 111 % (n=13) และให้ค่าความแม่นยำของการวิเคราะห์ที่ดี คือ RSD (n=10) เป็น 0.17 %

เมื่อเปรียบเทียบปริมาณไอโอดีนที่วิเคราะห์ได้ในตัวอย่างเกลือ จำนวนสิบสามตัวอย่าง ด้วยวิธีทางสถิติแบบ ANOVA พบว่าผลที่ได้จากวิธีวิเคราะห์ทั้งสี่ซึ่งได้แก่ วิธีแบบ full kinetic stopped-FI, วิธีแบบ fixed time stopped-FI ,วิธีแบบ kinetic continuous-FI และวิธีแบบ colorimetric-FI ให้ผลวิเคราะห์ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นเท่ากับ 95 % ผลดังกล่าวนี้ แสดงให้เห็นว่ากระบวนการที่นำเสนอในงานนี้ทั้งสามวิธี สามารถนำมาใช้วิเคราะห์ไอโอดีนในตัวอย่างเกลือได้ อย่างไรก็ตาม วิธีแบบ kinetic continuous-FI ได้ถูกเลือกสำหรับการนำไปใช้ประโยชน์จริงในการหาปริมาณไอโอดีนในเกลือเสริมไอโอดีน วิธีดังกล่าวนี้สามารถวิเคราะห์จำนวนตัวอย่างได้มากที่สุด คือ 90 ตัวอย่างต่อชั่วโมง

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LIST OF ABBREVIATIONS

Abbreviation	Definition
i.d.	Inner Diameter
mm	Millimeter
cm	Centimeter
nm	Nanometer
g	Gram
µg	Microgram
mg	Milligram
µl	Microliter
ml	Milliliter
L	Liter
s	Seconds
min	Minute
hr	Hour
^{o}C	Temperature in Degree Celsius
M	Molarity
FIA	Flow Injection Analysis
RSD	Relative Standard Deviation
ANOVA	Analysis of Variance
et al.	Et. Alli(Latin), and others.

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THE RELEVANCY OF THE RESEARCH WORK TO THAILAND

Iodine deficiency disorder (IDD) is a common problem in Thailand. Insufficient intakes of iodine in people result in impaired mental development and goiter. An adequate consumption of iodized salt per day can prevent IDD. A convenient and suitable analytical method for controlling of iodine quantity in cooking salt is therefore necessary. In this work, a kinetic flow injection method was developed to use for determination of iodine content in local iodized salt.

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CHAPTER I
INTRODUCTION

It is well - known that iodine is an essential trace element for human life. Its one function is to participate in the synthesis of the hormone thyroxine by the thyroid gland. In human, dietary iodine in the form of iodide is absorbed in the small intestine. One third of this iodide is selectively absorbed by the thyroid cells and the remaining parts is usually excreted in the urine within 2 to 3 days [1]. More than one billion people worldwide are risk of iodine deficiency disorder (IDD) because their soil lacks iodine. This disorder is cause of goiter, mental retardation and decrease of survival among children [2].

Many methods have been employed to increase iodine intake that include the iodine supplement into various foods such as table salt, drinking water, milk and bread etc.

IDD can be easily protected by consuming adequate amounts of salt fortified with iodine during the salt refining process. Iodine in form of iodide or iodate was supplemented into table salt. Iodized salt has been widely used to avoid IDD because (a) it can be produced by applying the simple method available (b) it is a condiment that employed for daily cooking.

Usually, sufficient level of iodine for the prevention of IDD is required as 150 μg per day [3] and the recommended value for iodine supplement is 50 mg I/kg [4].

In the moist tropics including Thailand, addition of iodine as iodate to common salt was superior to iodide [3]. The sufficient method has been required to check quantity of iodine in those foods.

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CHAPTER II
OBJECTIVES

Various methods have been presented for determination of iodine such as automation method [5], gas chromatography [6,7], cathodic stripping square wave voltammetry [8,9], ICP-MS [10] and Sandell-Kolthoff method [11-17]. However the latter method has been widely used for iodine quantity measurement more than the others because of its high sensitivity and low cost of instrument.

The Sandell-Kolthoff method which is based upon the iodide-catalytic on the reduction of Ce (IV) by As (III) was first reported by EB Sandell and IM Kolthoff [11] in 1934. This reaction was also found by P. Rodriquez and H. Pardue [18] that iodine in the form of only iodide accelerates the indicator reaction between As (III) and Ce(IV).

Iodine fortification in some food has been employed to solve the IDD problem. Usually, in Thailand, iodine as iodate species is favored for addition into the dietary because it is less soluble and more stable than iodide species. Therefore, to determine iodine concentration in those dietaries by using its catalytic effect on the Ce (IV) – As (III) reaction, iodate form must be converted into iodide form prior to kinetic analysis.

S. Suwannachoat [19] investigated the reduction of iodate to iodide by As (III) solution containing sodium chloride. The results showed that, under optimized batch condition, iodate ion can be completely changed to iodide within 1 min at room temperature and gave the kinetic behavior after reduction as same as the kinetic behavior of iodide ion. This condition was used for batch kinetic determination of iodine content based on the Sandell-Kolthoff method, in drinking water and iodized salt. The batch method risked to error in iodine determination because of imprecision

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of a technician in the exact timing during operation. Flow injection analysis was an alternative method, instead of batch procedure. Recently, N. Choengchan [20] employed this method for determination of iodine in drinking water.

In this work, flow injection system for kinetic determination of iodine in commercial table salt was developed. The method was based on the Ce (IV) –As (III) reaction with catalytic effect of iodide.

The aims of this research are as follows:

1. To optimize the FI system for the condition in which pseudo-first order kinetic is obtained and an in-situ reduction of iodate by As(III) solution containing sodium chloride is obtained at 100 % conversion. Full kinetic stopped-FI method was employed for this study.

2. To find the appropriate operation method amongst the full kinetic stopped-FI method, fixed time kinetic stopped-FI method and kinetic continuous-FI method for iodine quantity measurement of commercial iodized salt.

3. To validate the proposed kinetic method against another method which is a colorimetric-FI method [4] for the same commercial iodized salts.

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CHAPTER III
LITERATURE REVIEW

3.1 Analytical Methods for Quantitative Determination of Iodine

Many methods have been established to apply for specification and determination of iodine in various matrices. In this following sections, these methods will be described.

3.1.1 Spectrophotometric Methods

In 1978, VW. Truesdale presented the automated spectrophotometric determination of iodate and total iodine in seawater by using a Technicon Auto-Analyzer [5]. Triiodide (I_3^-), that is the product of the reaction between iodate, as an analyte, with acid and excess iodide was spectrophotometrically detected at about 350 nm. Total iodine was detemined by the same procedure but iodide in the sample must be oxidized to iodate before.

In 2000, the same reaction of above paragraph was also employed by AA.Ensafi [21].

Determination of iodate by applying the oxidation of iodate to periodate was established by MS. Elshahawi [22, 23]. After oxidation, periodate was extracted into organic solvent and then detected its absorbance.

A spectrophotometric method was developed by A. Afkhami in 2001 for measurement of iodine in the form of iodate and periodate to apply in the analysis of tap water and spring water [24].

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In 2002, N. Choengchan et al. developed a flow injection system for determination of iodate in iodized salt [4]. The method was carried out by utilizing the reaction between iodate (IO_3^-) and excess iodide (I^-) to generate tri-iodide (I_3^-) species. In the presence of starch, I_3^- - starch complex was formed and then detected its blue color at 590 nm.

3.1.2 Gas Chromatographic Methods

The GC method that was proposed by L. Maros in 1989 [6] was utilized for monitoring iodide in rainwater, river water and seawater. The method involved the conversion of iodate to iodo-acetone derivatives and their subsequent detection by electron capture.

Later in 1996, HS. Shin et al. used gas chromatography-mass spectrometry to determine trace amounts of iodine, iodide and iodate in commercial drinking water [7]. This method has a pre-derivatization of these analyte into 4-iodo-2,6-dimethylphenol and extracted with diethyl ether.

3.1.3 Electrochemical Methods

In 1988 by GW. Luther et al. [8] and in 1995 by RC. Tian et al. [9] employed cathodic stripping square wave voltammetry to direct determination of iodide in fresh water and seawater.

Sometimes electrochemical methods were coupled with FI systems to estimate iodine species in water samples. One of this method was reported in 1985 by E. Nakayama et al. [25].

In 2001, J. Jakmunee and K. Grupan [26] developed flow injection amperometric system for measurement of iodate quantity in iodized salts. The method was based on the reaction between iodate and excess iodide in acidic medium to generate iodine species. Iodine (I_2) was reduced at glassy carbon working eletrode. A

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potential of the working electrode at a cross flow electrochemical cell was controlled at +200 mV versus Ag/AgCl electrode as reference electrode.

3.1.4 Catalytic Methods

The catalytic effect of iodide ion on the reduction of Ce (IV) by As (III) in sulfuric acid solution has been widely used to detect and estimate iodine in the form of iodide. This method was first reported in 1934 by EB Sandell and IM Kolthoff [11]. The reaction can be applied such as determination of iodine in fresh water (proposed in 1982 by SD. Jones et al. [12]), in seawater (proposed in 1998 by TS Edmonds et al.[13]), in urine by using stopped-FI system (proposed in 2001 by D. Nacapricha et al.[14]), in biological samples (proposed in 1998 by SP Mushtakora [15]) and in iodized salt (proposed in 1953 by M. Dubravcic[16]).

The catalytic method for measurement of iodine quantity based on its catalysis on the reaction between As (III) and Ce (IV) is recommended in Standard Method for the Examination of Water and Wastewater [17] for samples containing less than 80 μg I/L.

Recently, a batch kinetic method was presented for determination of iodine in drinking water and iodized salt by S. Suwannachoat [19]. A kinetic FI method for monitoring of iodine level in commercial drinking water by N. Choengchan [20] were established. These works were performed by employing the Sandell-Kolthoff reaction.

Catalytic effects of iodide on other reactions have been employed for kinetic determination of iodine. The colorimetic determination of iodine in seawater by means of catalytic effect of iodide on the oxidation of ferric thiocyanate in nitric acid was proposed by N. Yonehara in 1963 [27]. This reaction was also applied for determination of iodide in natural water samples by RE. Moxan [28] and T. Koh et al. [29] in 1984 and 1988, respectively.

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The estimation of iodide in rainwater by applying the iodide-catalysed reaction of mercury (II)-4-(2-pyridylazo)resorcinol with 1,2-cyclohexane diamine-N,N,N',N'-tetraacetic acid was reported by S. Funahashi et al. [30].

Sanchez-Pedreno et al [31] proposed a method which is a combination between both kinetic and potentiometric features for the determination of iodide. This technique is based upon the use of a coated wire Sb (V) ion-selective electrode to study the iodide-catalysed reaction between hexachloroantimonate(V) and hydroxylamine.

The catalytic effect of iodide on the reaction between chlorpromazine and hydrogen peroxide was reported by T. Tomiyasu et al. [32]. In addition, the oxidation of chlorpromazine by bromate which has an iodide catalyst, has been also used in a measuring of iodide amount. The later reaction was proposed by P. Vinus [33]. MS Garcia et al. [34] presented the kinetic determination of iodide in pharmaceutical and food samples by using its inhibitory effect on the Pd (II) catalysed reaction between ethylenediaminetetraacetic acid (EDTA-Co(III)) and the hypophosphite ion.

Some of these presented catalytic methods for the determination of iodine is described in details in Section 3.2.

3.1.5 Other Methods

Chromatography with conductometric detection was purposed by SD Kumar et al.[35] for quantitative determination of iodate in table salt.

Probably the most promising method in terms of sensitivity and minimum sample pretreatment are ICP-MS and HPLC presented by VV. Salov et al. [10]. This method was used for determination of iodine in drinking water, in soup and in human urine.

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X-ray fluorescence spectroscopy (XRF) as presented by T. Wakisaka et al. [36] was utilized for monitoring of iodine level in brine sample.

ICP-AES method with a membrane gas-liquid separator was employed to determine iodine in ground water. This method was proposed by MR Cave et al.[37].

3.2 Information of Method based on Iodide - Catalysed Reaction

3.2.1 Catalytic Effect of Iodide on the Reaction between Chlorpromazine (cp) and Hydrogen Peroxide

The oxidation reaction of chlorpormazine (2-chloro-10-(3-dimethylaminopropyl) phenothiazene ($C_{17}H_{19}ClN_2S$) by hydrogen peroxide in sulfuric acid [38] which has the iodide catalyst can be used for kinetic determination of trace iodide amount. The indicator reaction is :

$$2\ C_{17}H_{19}ClN_2S + 3H_2O_2 + 4H^+ \longrightarrow 2\ C_{17}H_{19}ClN_2SOH_2^{\cdot 2+} + 4H_2O \quad (3.1)$$

The formation of the red free radical is accelerated by iodide. This product was utilized for iodide determination. The color formation reaction was monitored by measuring the increasing of absorbance at wavelength of 525 nm.

3.2.2 Iodide-Catalysed Reaction between Hexachloroantimonate (V)-Hydroxylamine Reaction

A coated-wire Sb (V) ion-selective electrode was employed in the iodide-catalysed reation between hexachloroantimonate (V) and hydroxylamine [31]. The net reaction can be written as:

$$2\ I^- + SbCl_6 \longrightarrow I_2 + Sb^{(III)}\ \text{product} \quad (3.2)$$

$$I_2 + NH_2OH \longrightarrow 2\ I^- + \text{hydroxylamine product} \quad (3.3)$$

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The potential of $SbCl_6^-$ shows a steady increase as a result of the iodide-catalyst.

3.2.3 Pd (III) – Catalysed Reaction between EDTA - Co(III) and Hypophosphite

The inhibitory effect of iodide on the Pd (III)-catalysed reaction between ethylenediaminetetraacetic acid (EDTA-Co(III)) and the phosphite ion has been employed to determine iodine amount. The rate of reaction is decreased in proportion to the concentration of iodide. Colour-fading of EDTA-Co(III) complex was monitored by measuring its absorbance at 540 nm [34].

3.2.4 The Sveikina Method

This method has been utilized for kinetic determination of iodide. It is based on the catalytic effect of iodide on decreasing of the iron (III)-thiocyanate complex in the presence of NO_2^- and NO_3^- [39]. This reaction can be written as :

$$2CNS^- + 3NO_3^- + 2H^+ \longrightarrow 2CN^- + 2SO_4^{2-} + 6\,NO + H_2O \qquad (3.4)$$

3.2.5 Iodide-Catalysed Reaction of Mercury (II)-4(2-Pyridylazo) Resorcinol Complex and 1,2-Cyclo-Hexanediamine-N,N,N',N'-Tetraacetic Acid

Iodide has been determined by applying the method based on the catalytic effect of iodide on the substitution reaction of mercury (II)-4(2-pyridylazo) resorcinol complex and 1,2-cyclo-hexanediamine-N,N,N',N'-tetraacetic acid (CyDTA). This reaction can be written as :

$$\text{Hg (II)-PAR} + \text{CyDTA} \longrightarrow \text{Hg(II)-CyDTA} + \text{PAR} \qquad (3.5)$$

The reaction was monitored by record the decreasing of absorbance of Hg (II)-PAR at 500 nm.

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3.2.6 The Sandell-Kolthoff Method

The method was first reported and studied in some detail by EB Sandell and IM Kothoff [11] in 1934. This method is based upon the catalytic effect of iodide on the oxidation-reduction between As (III) and Ce (IV). For many years, it has been widely utilized for kinetic determination of ultra trace amounts of iodide because of its high sensitivity.

One such as a fixed time kinetic method has been used to quantitative determination of iodide by measuring the diminishing of absorbance of Ce (IV) at a fixed time. Figure 3.1 shows absorption spectra of 1.6 x 10^{-4} M Ce (IV) in 0.1 M H_2SO_4.

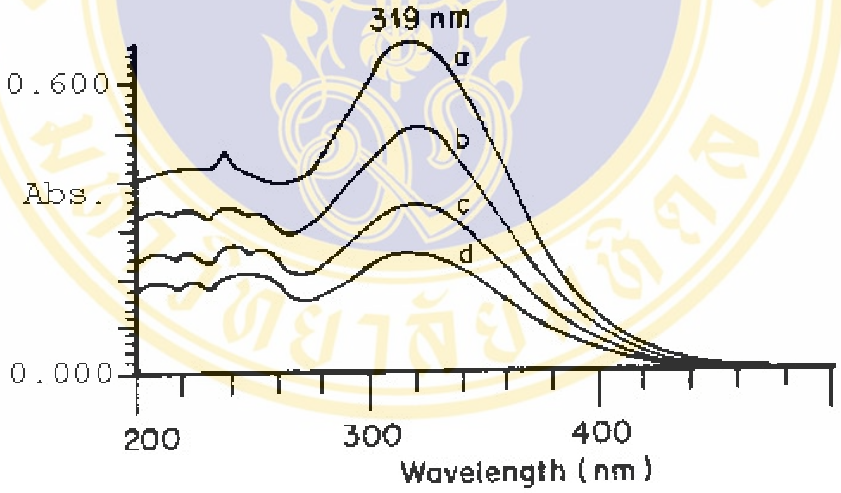

Figure 3.1 Absorption spectra of 1.6 x 10^{-4} M Ce (IV) containing 0.1 M H_2SO_4 in (a) no As (III) and no iodide catalyst and (b), (c) and (d) contained 1.6 x 10^{-4} M As (III) with reaction time of 2, 4 and 6 min, respectively.

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As (III) reduces Ce (IV) to the colorless Ce (III) . The indicator reaction is :

$$2Ce\ (IV) + As\ (III) \longrightarrow 2Ce\ (III) + As\ (V) \qquad (3.6)$$

yellow colorless

Usually the redox reaction in Equation 3.6, proceeds very slowly without the iodide catalyst. P. Rodriquez and H. Pradue [18] presented a possible mechanism that provides a rate law, which satisfied the experimental observations. The mechanism proposed is provided below (Equation 3.7-3.11).

$$I^- + Ce\ (IV) \xrightarrow{k_1} Ce(III) + I^0 \qquad (3.7)$$

$$I^0 + As\ (III) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Intermediate \qquad (3.8)$$

$$Intermediate + Ce(IV) \xrightarrow{k_3} As\ (IV) + Ce(III) + I^- \qquad (3.9)$$

$$I^0 + Ce(IV) \xrightarrow{k_4} Ce(III) + I^+ \qquad (3.10)$$

$$I^+ + As\ (III) \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} As\ (V) + I^- \qquad (3.11)$$

They observed that the rate of reduction of Ce (IV) is first order in iodide for certain conditions.

According to P. Rodriquez and H. Pradue [18], reaction 3.7-3.9 and reaction 3.7,3.10 and 3.11 show two different catalytic cycles. Neither pathway 3.7, 3.8, 3.9 nor 3.7, 3.10 3.11 taken alone sufficiently describes all their experimental data. For the more complicated branched mechanism, a steady-state condition can be employed to the species, I^0, I^-, I^+ and Int (Intermediate).

$$\frac{d[I^0]}{dt} = \frac{d[I^-]}{dt} = \frac{d[I^+]}{dt} = \frac{d[Int]}{dt} = 0 \qquad (3.12)$$

where $$I_{total} = I^0 + I^- + I^+ + Int \qquad (3.13)$$

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A rate expression for the [Ce (IV)] appling equation 3.7 – 3.11 can be derived [18]. The rate expression is

$$\text{Rate} = -\frac{d[Ce(IV)]}{dt} \quad (3.14)$$

$$\text{Rate} = \left(\frac{C_1[Ce(IV)][As(III)][C_2[Ce(IV)] + C_3[As(III) + C_4]}{D} \right] [I]_{tot} \quad (3.15)$$

where $D = C_5[As(III)]^2 + C_6[As(III)] + C_7[As(III)][Ce(IV)] + C_8[Ce\ (IV)]$
$+ C_9[Ce(IV)]^2 + C_{10}[As(V)]\{C_{11}[As(III)] + C_{12}[Ce(IV)] + C_{13}\}$ (3.16)

The constant C_1-C_{13} are related to the specific rate constants as presented in Table 1.1

Table 3.1 The meaning of the constants C_1-C_{13} [40]

Constants	Specific rate constants
C_1	$2k_1k_5$
C_2	k_3k_4
C_3	k_2k_3
C_4	k_2k_4
C_5	$k_2k_5\ (k_1 + k_3)$
C_6	$k_2k_5\ (k_1 + k_4)$
C_7	$k_3k_5\ (k_1 + k_4)$
C_8	$k_1k_2k_4$
C_9	$k_1k_3k_4$
C_{10}	k_5
C_{11}	k_2k_3
C_{12}	k_3k_4
C_{13}	k_2k_4

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When the initial rates are being considered and no As (V) had been added initially, the last term in Equation 3.16 was very small. The simplified rate expression then becomes [18].

$$\text{Rate} = \left(\frac{C_1[Ce(IV)][As(III)][C_2[Ce(IV)] + C_3[As(III) + C_4]}{D'} \right] [I]_{tot} \quad (3.17)$$

where $D' = C_5[As(III)]^2 + C_6[As(III)] + C_7[As(III)][Ce(IV)] + C_8[Ce\ (IV)] + C_9[Ce(IV)]^2$ (3.18)

In 2000, P. Tongkaew [40] investigated the kinetics of the iodide catalysed oxidation-reduction of As(III) and Ce (IV) in sulfuric acid by using a conventional stopped flow apparatus [48]. By applying the steady state approximation method, the rate expression for the changing of Ce(IV) concentration for the same mechanism (Equation 3.7-3.11) [40] was derived and is presented in Equation 3.19. In the study, the Ce(IV) concentration was very small compared to the As(III) concentration. By varying total [Ce(IV)], [As(III)] and [I⁻], but keeping [H_2SO_4] at 0.25 M (at constant temperature at 30.0 ^{o}c), the following rate law was pseudo-first order process dependence on Ce (IV) concentration.

$$\text{Rate} = -\frac{d[Ce(IV)]}{dt} = \frac{1.79 \times 10^{-4}[As(III)][Ce(IV)]}{([As(III)] + (1.06 \times 10^{-4}))} [I]_{total} \quad (3.19)$$

From Equation 3.19, P. Tongkaew summarized that the dependence on Ce (IV) concentration is first order throughout the concentration range studied ([Ce(IV)] = 0.0-0.6 x 10^{-3} M). The dependence on As(III) is zero order when [As(III)]>>1.06 x 10^{-4} M and the iodide dependence is first order throughout the range examined ([I⁻] = 0.08-0.48 μM or 10-60 ppb). The author [40] has also shown that expression Equation 3.19 is consistent with the mechanism of Rodriquez and Pardue [18]. Moreover using the values of the specific rate constants as determined by Rodriquez and Pardue [18], their rate expression Equation 3.17 is in good agreement with the values in Equation 3.19.

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EB Sandell and IM Kolthoff [11] also observed that the Ce (IV) - As(III) reduction is obey in a first order kinetic in Ce (IV) concentration under some condition. From Equation 3.19 can be re-written as

$$-d[Ce(IV)]/dt = k[Ce(IV)] \quad (3.20)$$

where $$k = [I^-]k_{catalysted} + k_{uncatalysed} \quad (3.21)$$

Change of Ce(IV) concentration with time is presented in Equation 3.22

$$[Ce(IV)]_t = [Ce(IV)]_o . e^{-kt} \quad (3.22)$$

where $[Ce(IV)]_t$ is the concentration of Ce (IV) at any time.

$[Ce(IV)]_o$ is the initial concentration of Ce(IV).

k is the pseudo-first order rate constant.

The reduction of Ce (IV) by As (III) proceeds extremely, slowly when iodide is absent. In contrast, in the presence of iodide, the rate of the reaction is enormously increased. This result is directly proportional to the iodide concentration.

3.3 Kinetic Determination for Catalytic Analyte

The term *catalytic methods* here denotes chemical determination based directly or indirectly on monitoring the rate of a catalyzed reaction. The criteria for investigation of a substance to act as catalytic in the reaction is [41] :

1. At the end of the reaction, the catalyst is unchanged chemically.
2. A small quantity of catalyst is often sufficient to bring about a considerable amount of reaction.
3. The position of equilibrium in a reversible reaction is not change when the catalyst presents in the reaction.

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Primary analytical effect are those in which the rate of a given indicator reaction(the main reaction in which the monitored species participates) is greatly increased by the presence in the system of the catalyst. It is such effects that are the basis of the most catalytic determinations.

The rate of any catalyzed reaction can be shown to be directly (or nearly) proportional to the initial concentration of catalyst as a result of the occurrence of catalytic cycles. This relationship is obeyed most exactly in certain reaction which the conditions are met, for example, keeping at a constant level all variables affecting the rate (temperature, ionic strength, solvent used, solution background). The concentration of all reactants, other those of the catalyst and the species whose change in concentration is monitored. Also, the reaction involving the considering species is adjusted to give a pseudo-first order dependence.

In this work, the Sandell-Kolthoff reaction [11] that is the pseudo-first order in iodide concentration under some condition was applied to kinetic determination of iodide in commercial iodized salt. Two kinetic method were used to get the iodine concentration.

The first method, full kinetic method, quantitative analysis of iodide is obtained from investigation on the rate expression in Equation 3.21. The plot between rate constant and iodide concentration gives a linear relationship. Therefore, By using this calibration plot, level of iodine in sample can be determined.

The second method, which is denoted as the fixed time method, only the value of [Ce (IV)] at a fixed time t after the start of the reaction (t=0) is employed to calculate the iodine concentration. Equation 3.20 can be written as

$$\ln[Ce(IV)]_t = \ln[Ce(IV)]_o - kt \quad (3.23)$$

where $k = k_{catalysed}[I^-] + k_{uncatalysed}$ (3.24)

Therefore, $-\ln[Ce(IV)]_t = -\ln[Ce(IV)]_o + t\,k_{catalysed}[I^-] + tk_{uncatalysed}$ (3.25)

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$[Ce(IV)]_o$ and $[Ce(IV)]_t$ are the concentrations of Ce (IV) at the beginning of the reaction and at time t, respectively. $k_{uncatalysed}$ is the rate constant in the absence of iodide species and $k_{catalysed}$ is the rate constant due to catalytic of iodide.

From Beer's law, $A \alpha C$ (3.26)

where A is absorbance and C is concentration. Therefore, Equation 3.25 can be written as

$$-\ln A_t = -\ln A_o + tk_{uncatalysed} + t\, k_{catalysed}[I^-] \quad (3.27)$$

where A_o and A_t are the absorbance of $[Ce(IV)]_o$ and $[Ce(IV)]_t$, respectively. Thus, a linear calibration plot can be obtained as follow :

$$-\ln A_t = \text{slope } [I^-] + \text{intercept} \quad (3.28)$$

For the fixed time method, the relationship in Equation 3.28 or plotting between the logarithm of absorbance of Ce(IV) at time t and iodide concentration, was used for quantitative analysis of iodide.

3.4 Flow Injection Analysis

Flow injection analysis (FIA or FI) was first reported by J. Ruzicka and EH Hansen in 1975 [42]. This method was carried out by the injection of a liquid sample into a moving, nonsegmented continuous carrier stream of an appropriate liquid. The injected sample generates a zone, which is transported to a detector that continuously records the physical property of analyte such as absorbance, electrode potential or others, as it continuously changes as the results of the passing of the sample zone through the flow cell in the detector. This technique is concern with sample injection, controlled dispersion and reproducible timing [43].

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The simplest flow injection system is presented in Figure 3.2.

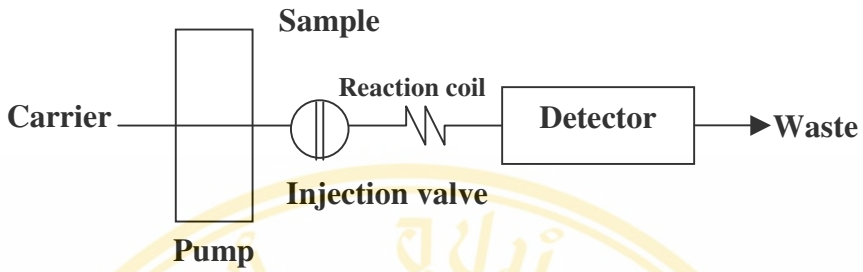

Figure 3.2 A simple flow injection system.

It consists of a pump, which is employed to propel the carrier stream through a narrow tube, an injection valve, reaction coils and detector. An injection valve was used to transfer the sample into the system. A reaction coil was employed for mixing the reagent carrier and the injected sample zone. This coil may not be necessary for some FI system. A detector which responses to considered analyte is utilized for data monitoring.

Merits of the FI system :

- Simple and easy for understanding of the fundamental principles.
- Instrumentation can be rapidly assembled from simple, inexpensive and off the shelf components.
- High sampling rates
- It provides a simple means of automating most manual procedures of wet analysis.

Some mode of the FI techniques that was employed for this work was described in the following section 3.4.1 and 3.4.2.

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3.4.1 Continuous-FI Mode

A continuous-FI is a first generation of FI mode. Many laboratories employed this FI method to determine iodine such as :

A. Cerda et al. [44] proposed the determination of iodide in table salt by flow injection analysis using Pyrocatechol Violet. The method was carried out by injection of 100 µl of sample into the optimized FI system as present in Figure 3.3

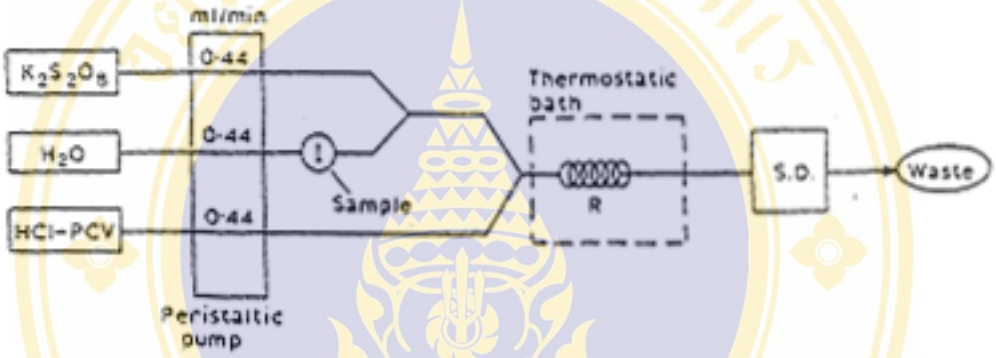

Figure 3.3 FI manifold employed for the determination of iodide. I, sample injection; S.D., spectrophotometric detector (λ = 550 nm); R, reaction coil (3 m); $[K_2S_2O_8]$ = 12 g/l; [HCl] = 3 M; [Pyrocatechol Violet] = 6.0 x 10^{-4} M; T = 55 ^{o}C.

A decreasing in the absorbance of pyrocatechol violet that was result from catalytic effect of iodide on the oxidation of pyrocatechol violet by persulphate ion was recorded at 550 nm. The working range of iodide was 0.5-5 mg I/L and the precision of this method for 1 mg I/L was 4% (n=10). The detection limit was 0.3 mg I/L. The method has a high sampling rate as 60 samples per hour. Sample pretreatment was not required.

J.T. Hakedal et al [45] reported the determination of iodide in brines by using a flow injection method. The method was based on the oxidation of iodide to become iodine by dichromate. Then, iodine diffused through a PTFE membrane and was detected spectrometrically at 350 nm. This system gave a detection limit as 0.2 mg I/l.

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A flow injection system for analysis of iodate and iodide in seawater was presented by K. Oguma et al. [46]. This method used the catalytic effect of either iodate or iodide on the reaction between iron (III) thiocyanate and nitrite to monitor iodine concentration. The system allows the determination of iodate and total iodine by inserting column in the flow system, respectively. Iodide concentration was calculated from the different concentration of total iodine and iodate. Level of iodate-iodine and total iodine in seawater was found in the range of 0.75-150 mg I/L. Analysis times for iodate-iodine and total iodine were 20 and 9 min, respectively. The precision as shown in term of RSD was within 1.3 % for both iodate and iodide in seawater.

A flow injection system for quantitative determination of iodate and iodide based on the reaction of iodate and iodide in acidic medium was presented by O.F. Kamson [47]. Iodine as the product of this reaction was spectrometrically measured at 350 nm. Iodate and iodide which have the concentration in range of 1-6 x 10^{-5} M and 2-8 x 10^{-3} M can be determined by this technique.

D. Nacapricha et al. [14] proposed a flow injection technique for determination of iodide in urine. The method was based on the Sandell-Kolthoff reaction, which has the iodide catalysed. Pretreatment of urine sample was required before injection into the system. The color-fading of Ce(IV) in reaction zone was monitored at 420 nm. A thermostated bath was employed to increase the sensitivity. This method provided the detection limit (S/N = 3) as 2.3 μg I/L and the sample throughputs as 35 samples/h. The FI manifold was shown in Figure 3.4.

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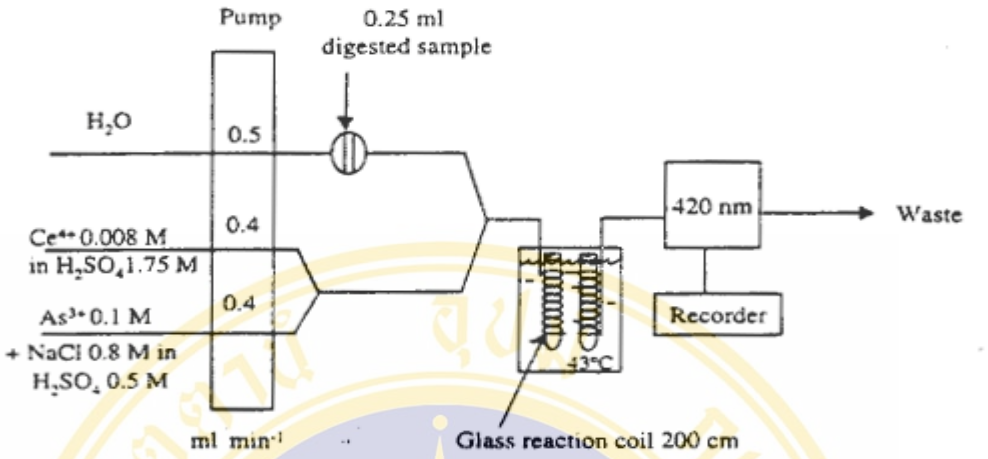

Figure 3.4 The FI manifold of the continuous-FI used for determination of iodine in urine [14].

3.4.2 Stopped-Flow Injection (Stopped-FI) Mode

This technique involves the stopping of sample zone, carrier and reagent streams in the FI system. The reaction zone is arrested inside the detector cell. Increasing in the residence time usually enhance the degree of chemical reaction without increasing the dispersion. Three different purposes for operating a FIA system in the stopped-FI mode are summarized as the following [42]:

(a) To increase the sensitivity of measurement by increasing the residence time. In this way, the conversion of a measured species increases. Usually this is applied with a catalysed reaction.

(b) To determine the reaction rate which can serve as one of the analytical data.

(c) To compensate for variations in background signal producted by the potential interference or differences in the sample matrix.

In this work, the stopped-FI technique was used for two types of kinetic method, full kinetic method and fixed time method, (see Section 3.3), for determination of iodine level in table salt. The full kinetic stopped-FI method and the

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fixed time stopped-FI method are described in Section 6.2.2.1 and Section 6.2.2.2, respectively.

The stopped-FI procedure may be an ideal method to measure reaction rate and to study rate law, provided that an experimental approach could be designed to allow for resolving the individual contribution of chemical kinetics.

This approach to kinetic determination is more convenient than the use of a conventional stopped-flow apparatus (see Appendix I) changing over from sample to sample.

A simple stopped-flow FIA manifold is presented in Figure 3.5

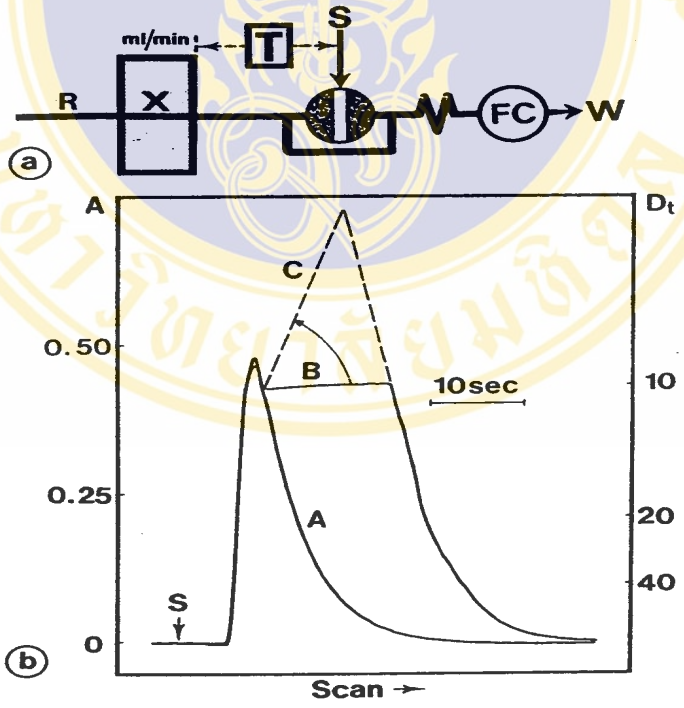

Figure 3.5 The principle of the stopped-flow FIA manifold as demonstrated by injecting a dyed sample solution (S) into a colorless carrier stream and recording the absorbance by means of a colorimetric flow through cell. (a) the FI gram that was obtained from the continuous-FI mode and (b) an example of the FI profile given by the stopped-FI mode (pump for 9 s, stop for 14 s, continue pump).

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The rising linear portion represented the reaction evaluation during the stop time. The slope of this portion is found to be proportional to the analyte concentration in some applications [49].

3.5 Review of the Previous Work

Some colleagues in our laboratory have developed some methods based on the Sandell and Kolthoff method, for determination of iodine in different samples. These methods are summarized in Table 3.2.

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Table 3.2 Summary of the previous work in our laboratory that employed the oxidation-reduction of As (III) and Ce (III) for iodine amount measurment.

Year	Author	Publication/Thesis	Title	Sample	Detection limit
1998	W. Chantore et al.	LRA, **11**, 37.	Flow injection for iodide determination at the ppb level	-	0.8 µg I/L
2001	D. Nacapricha et al.	Analyst, **126**, 121.	Continuous and stopped flow injection for catalytic determination of total iodine in urine	Urine	2.3 µg I/L for the continuous-FI method and 3 µg I/L for the stopped-FI method
2001	D. Nacapricha et al.	Anal. Sci., **17**, i33.	Kinetic determination of iodine in urine using stopped-flow injection	Urine	3 µg I/L

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Table 3.2 (Continued).

Year	Author	Publication/Thesis	Title	Sample	Detection limit
2001	S. Suwannachoat	M.S. Thesis in Analytical and Inorganic Chemistry, Mahidol University.	Investigation of reduction of iodate to iodide prior to kinetic determination of iodine using Ce (IV)-As (III) reaction	Drinking water and iodized salt	-
2002	N. Choengchan	M.S. Thesis in Analytical and Inorganic Chemistry, Mahidol University.	A flow injection system for determination of iodine in drinking water	Drinking water	9.3 µg I/L

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CHAPTER IV
MATERIALS AND METHODS

4.1 Instrument

These followings are list of general instruments employed for this thesis:

4.1.1 A Precisa 40SM-2000A analytical balance (Switzerland) was used for weighing chemicals in the preparation of potassium iodate solution and potassium iodide solution, including the other reagent solution.

4.1.2 An Eyela cool ace CA-1111 low temperature circulator cooling aspiration (Japan) was utilized in conjunction with the Peltier system in 4.1.3.

4.1.3 A Peltier temperature programmer (model PTP-1, USA) was applied to control temperature of a flow cell at 25^{o} C during the kinetic studies.

4.2 Equipment and Apparatus Used in the FI System

The followings are list of the equipment for construction of the studied FI system for kinetic analysis (Figure 6.1 and 6.8).

4.2.1 An Autosampler AS-90 series (USA) was utilized to automatically transfer standard iodine solutions or sample solutions into a 250 µl PTFE loop (1.0 mm i.d.).

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4.2.2 A FIAS pump module (FIAS-300, USA) consisted of two peristaltic pumps and a FIAS injection valve. The FIAS injection valve was used to inject standard iodine solutions or sample solutions into the system. The peristaltic pump was employed to propel all reagents and sample zone through the detector. Three channels for reagents were fitted with magazines in which holding Tygon pump tubes (i.d. 0.89 mm, wall 0.86 mm). The other channel used for sample injection employed a Tygon pump tube with i.d. 2.06 mm and wall 0.86 mm.

4.2.3 A Perkin Elmer chemifold is a block made of clear plastic with holes for inserting tube connectors. Reagents were pumped through the chemifold and were merged before mixing in the mixing coils.

4.2.4 A Lambda 25 UV/Vis spectrophotometer (USA) was used to measure the absorbance of Ce (IV) at wavelength of 420 nm. A Phillips flow through cell (USA) (10 μl) was used.

4.2.5 A UV-FIAS software was used to control the proposed FI method and to collect the data from the detector. The protocol for operating this software is shown in Appendix II)

All flow injection manifolds reported in this work were established using the equipment listed in Section 4.2.1 to 4.2.5. All PTFE tubings used in the FI systems were of Perkin Elmer with 0.5 mm i.d

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4.3 Equipment and Apparatus of the Colorimetric-FI System

The followings are list of equipment and apparatus used for constructing the colorimetric-FI system. This system was used as a validating method according to the previous work [4].

4.3.1 An Ismatec peristaltic pump (model IS7610, Switzerland) was used for propelling reagents. Two channels were fitted with cartridges in which holding Tygon pump tubes (i.d.0.95 mm, wall 0.9 mm).

4.3.2 Two Rheodyne injection valves (model 5020, USA) were employed. One was fitted with a 250 μl PTFE loop (1.0 mm i.d.) for injection of all standard and sample solutions. The other was fitted with a 100 μl PTFE loop (1.0 mm i.d.) for injection of cleaning solution.

4.3.3 A Jenway spectrophotometer (model 6405, UK), fitted with a Philips flow cell of 10 μl volume, was used for monitoring the changing of absorbance at 590 nm.

4.3.4 An Alltech chart recorder (model LR 93025, USA) was used for signal recording.

For this system, PTFE tubing with i.d. of 0.5 mm was used throughout.

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4.4 Chemicals

All chemicals used in this work were of AR grade. Deionized water obtained from a Milli-Q-system, was used for preparing the reagents. List of chemicals and their suppliers are summarized in Table 4.1.

Table 4.1 List of chemicals and their suppliers

Chemicals	Formula	Supplier
Arsenic trioxide	As_2O_3 (s)	Univar (Australia)
Ceric sulfate	$Ce(SO_4)_2.4H_2O$ (s)	E. Merck (Germany)
Potassium iodate	KIO_3 (s)	Univar (Australia)
Potassium iodide	KI (s)	Univar (Australia)
Sodium chloride	NaCl (s)	E. Merck (Germany)
Sulfuric acid	H_2SO_4 (l)	E. Merck (Germany)
Starch	-	E. Merck (Germany)
Sodium thiosulfate pentahydrate	$Na_2S_2O_3.5H_2O$ (s)	E. Merck (Germany)

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4.5 Preparation of Reagent Solutions

4.5.1 Modification and Optimization of the Proposed FI System

Reagents were prepared as described in the following sections.

4.5.1.1 Standard Iodine Solutions

Solids of potassium iodate (KIO_3) and potassium iodide (KI) were oven dried at 120^{o} C for 2 hr and stored in a desiccator until used (> 48 hr).

(i) Stock Solution of Iodine Standard (1000 mg I/L)

For standard iodate stock solution, 0.1686 g of KIO_3 was dissolved in deionized-distilled water to 100.0 ml.

For standard iodide stock solution, 0.1308 g of KI was dissolved in deionized-distilled water to 100.0 ml.

(ii) Secondary Stock Solution of Iodine Standard (10 mg I/L)

1.0 ml of the stock solution (1000 mg I/L), either iodide or iodate solutions, was first diluted to 100.0 ml with deionized-distilled water to give a standard iodine concentration of 10 mg I/L.

(iii) Working Iodine Solutions

(a) Iodine Standards in 2% (w/v) NaCl

For calibration, the working solutions (iodide or iodate) containing 100, 500, 1000 and 2000 μg I/L were prepared by respectively transferring 0.25, 1.25, 2.5 and 0.5 ml of the secondary stock solution (10 mg I/L) into

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25.0-ml volumetric flasks. Then, 4.17 ml of 12% (w/v) sodium chloride solution was added into these volumetric flasks and making up to 25.0 ml with deionized-distilled water.

(b) Iodine Standards in 3% (w/v) NaCl

For calibration, the working solutions of iodine (iodide or iodate) were 100, 2000, 4000 and 6000 μg I/L.

The procedure for preparing the standard iodine solutions at the concentration of 100 and 2000 μg I/L was similar to the method described in Section 4.5.1.1 (iii a). However, in order to obtain 3% (w/v) sodium chloride concentration (NaCl), the aliquots of 6.25 ml of 12% (w/v) sodium chloride solution was transferred into the volumetric flask instead.

Iodine standard solutions of 4000 and 6000 μg I/L were prepared by respectively transferring 0.1 and 0.15 ml of stock standard iodine solution (1000 mg I/L) into 25.0-ml volumetric flask and then added 6.25 ml of 12% (w/v) sodium chloride solution into these flasks. These mixture solutions were diluted to 25.0 ml with deionized-distilled water.

(c) Iodine Standards in 6% (w/v) NaCl

The concentrations of iodine solutions in the range of 100, 500, 1000, 2000, 4000 and 6000 μg I/L were prepared in 6% (w/v) NaCl.

The method for preparing of the iodine solutions of 100 to 2000 were similar to the method described in Section 4.5.1.1 (iii a). The other iodine concentrations which are 4000 and 6000 μg I/L were prepared as same as the procedure presented in Section 4.5.1.1 (iii b). However, to get a 6% (w/v) NaCl,

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the volume of 12.5 ml of 12% (w/v) sodium chloride solution was added into each volumetric flasks before making the volumes up to 25.0 ml.

4.5.1.2 Sodium Chloride Solutions

(i) 12% (w/v) Sodium Chloride Solution

Sodium chloride solid (NaCl) 30 g was dissolved in 250 ml of deionized-distilled water.

(ii) 2% (w/v) Sodium Chloride Solution

5 g of solid sodium chloride (NaCl) was dissolved in 250 ml of deionized-distilled water.

(iii) 3% (w/v) Sodium Chloride Solution

The preparation was similar to the procedure described in Section 4.5.1.2 (i) but employed sodium chloride solid 7.5 g instead.

(iv) 6% (w/v) Sodium Chloride Solution

The preparation of this FI carrier solution was similar to the method described in Section 4.5.1.2 (i) but used solid sodium chloride approximately 15 g instead.

4.5.1.3 Arsenious Acid Solution (0.048 M) Prepared in 0.53 M H_2SO_4 and 1.0 M NaCl

Arsenic trioxide powder (As_2O_3) 2.3741 g, sodium hydroxide pellet (NaOH) 3.6 g and solid sodium chloride (NaCl) 29.8 g were dissolved together in approximately 100 ml of water. When the solution was clear,

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14.4 ml of concentrated sulfuric acid (conc.H_2SO_4) was added to this solution. This mixture solution was made up to 500.0 ml with deionized-distilled water.

4.5.1.4 Ceric Sulfate Solution (0.006 M)

Solid ceric sulfate ($Ce(SO_4)_2.4H_2O$) 1.2127 g was dissolved in approximately 100 ml of water. After this solution was clear, 12.2 ml of concentrated sulfuric acid (conc. H_2SO_4) was added (to obtain 0.45 M sulfuric acid). This mixture was brought up to 500.0 ml using deionized-distilled water.

4.5.2 Determination of Iodine in Iodized Salt by Using Kinetic-FI Methods

4.5.2.1 Working Iodine Solutions

For calibration curve, concentrations of iodine solution in 3% (w/v) sodium chloride solution were employed at 100, 500, 1000, 2000, 4000 and 6000 μg I/L.

The preparation method for these iodine solutions was similar to the procedure described in Section 4.5.1.1 (iii c). However, in order to obtain 3% (w/v) sodium chloride solution, 6.25 ml of 12%(w/v) sodium chloride solution was added into volumetric flasks. Deionized-distilled water was used to make the volume to 25.0 ml.

4.5.2.2 Sodium Chloride Solution 3% (w/v)

This reagent was used as the carrier solution (Figure 6.8). Solid sodium chloride 7.5 g was dissolved in 250 ml of deionized-distilled water.

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4.5.2.3 Arsenious Acid Solution (0.048 M) Prepared in 0.53 M H_2SO_4 and 1.0 M NaCl

This method was carried out as same as the procedure described in Section 4.5.1.3.

4.5.2.4 Ceric Sulfate Solution (0.006 M)

This reagent was prepared as same as the solution described in Section 4.5.1.4.

4.5.3 Reagent Used in the Colorimetric-FI Method

In Section 4.5.3.1 to 4.5.3.5 describe chemical preparations of the colorimetric-FI method [4]. These reagents are used in the FI system presented in Figure 4.1.

4.5.3.1 Standard Iodate Solutions

Solid potassium iodate (KIO_3) was oven dried at 120^o C for 2 hr and then stored in a desiccator till used (> 48 hr).

(i) Stock Solution of Iodine Standard (1000 mg I/L)

For standard iodate stock solution, 0.1686 g of KIO_3 was dissolved in deionized-distilled water to 100.0 ml.

For standard iodide stock solution, 0.1308 g of KI was dissolved in deionized-distilled water to 100.0 ml.

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(ii) Secondary Stock Solution of Iodate Standard (18 mg I/L)

0.9 ml of the stock solution of iodate standard (1000 mg I/L) was first diluted to 50.0 ml with deionized-distilled water to give a standard iodine containing 18 mg I/L.

(iii) Working Iodine Solutions

Concentrations of the iodate standards (900, 1800, 3600 and 7200 μg I/L in 6% (w/v) NaCl) for the calibration plot were prepared by respectively pipetting of 1.25, 2.5, 5.0 and 10.0 ml of the secondary stock (18 mg I/L), into 25.0 ml-volumetric flasks. 12.5 ml of 12% (w/v) sodium chloride solution was transferred into these flasks. These were made to 25.0 ml using deionized-distilled water.

4.5.3.2 Sodium Chloride Solution (6% w/v)

The solution was used for preparing all working solutions and the carrier stream (Figure 4.1). It was made by dissolving 30 g of solid sodium chloride in deionized-distilled water to approximately 500 ml.

4.5.3.3 Carrier Solution

The carrier solution of the FI-system in Figure 4.1 (validated method)was made by dissolving 2 g of potassium iodide crystal (KI) in a solution of 6% (w/v) sodium chloride solution and making the volume to approximately 500 ml.

This solution contains of 2.5 x 10^{-2} M KI and 6% (w/v) NaCl.

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4.5.3.4 0.1% (w/v) Starch Solution in 0.01 M Sulfuric Acid

This solution was always prepared daily. It was made by mixing 0.5 g of starch with a few milliliters of deionized-distilled water to make slurry. This slurry was transferred into 500 ml of boiling deionized-distilled water. After cooling, 5 ml of 1 M sulfuric acid (H_2SO_4) was added into this solution.

For the preparation of 1 M H_2SO_4, 5.4 ml of conc. H_2SO_4 was transferred into 70 ml of deionized-distilled water and was finally brought up to 100 ml.

4.5.3.5 Thiosulfate Solutions

(i) Thiosulfate Solution (0.1 M)

2.5 g of solid sodium thiosulfate pentahydrate was dissolved in 100 ml of deionized-distilled water.

(ii) Thiosulfate Solution (1×10^{-3} M)

This solution was used for cleaning the FI-system (Figure 4.1). It was prepared by diluting of 1 ml of the 0.1 M thiosulfate solution with deionized-distilled water to 100 ml.

4.5.4 Determination of the Analytical Recovery (Proposed FI System)

Recovery of real commercial iodized salts and synthetic salt samples were analyzed. 12.5 ml of stock sample solutions (12%(w/v) were pipetted into 50.0-ml volumetric flasks. Salt samples (3%(w/v)) were spiked with 5.0 ml of the stock standard iodate solution (10 mg I/L) in the flasks. Then, the mixtures were made to the mark using deionized-distilled water. Sample recovery was calculated according to:

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$$\text{Recovery (\%)} = \left(\frac{[I]_{\text{spiked sample}} - [I]_{\text{sample}}}{[I]_{\text{add}}} \right) \times 100 \qquad (4.1)$$

where, $[I]_{\text{spiked sample}}$ is iodine concentration in the iodine spiked sample (mg I/L).

$[I]_{\text{sample}}$ is iodine concentration in the sample (mg I/L)

$[I]_{\text{add}}$ is iodine concentration that was added into the sample (mg I/L).

In this work, there was a particularly high iodine content in a sample (S3). When making to 3% (w/v) salt solution, the sample contains iodine of more than 5000 μg I/L. The sample was spiked similarly to the above procedure. However for this sample, 2.5 ml of 10 mg I/L of standard iodate solution was added into the sample instead using 5.0 ml.

4.5.5 Sample Preparation

4.5.5.1 Commercial Salt Samples

Ten iodized table salts were purchased from local markets and department stores. All samples were oven dried at 120 ^{o}C overnight and stored in desiccator until constant weight.

(i) Stock Sample Solutions (12% w/v)

An accurate weight of 30.xxxx g of a table salt was dissolved in deionized-distilled water and was made up to 250.0 ml. If some sample solutions are not clear, they were filtered through filter paper (Whatman No.1) before the analysis.

(ii) Sample Solutions (3% w/v)

In the FI analysis, the samples were made to contain 3% (w/v) salt solutions. This was to match with the FI carrier which is 3% (w/v) NaCl.

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Thus, for the proposed method, 12.5 ml of stock sample solutions (12% w/v) were diluted in deionized-distilled water to 50.0 ml.

(iii) Sample Solutions (6% w/v)

In contrast to the proposed FI system (this work), sample solutions for colorimetric-FI analysis required 6% (w/v) sample solutions. They were prepared as same as the procedure described in Section 4.5.5.1 (ii), but an amount of 25.0 ml of stock sample solutions (12% w/v) were transferred into 50.0-ml volumetric flask instead and were used deionized-distilled water to make volumes.

4.5.5.2 Synthetic Samples

Solids of potassium iodate (KIO_3) was oven dried at 120^o C for 2 hr and stored in a desiccator until used (> 48 hr).

The following synthetic samples were prepared.

.

(i) Synthetic Sample S1

0.1 ml of the standard iodate stock solution (1000 mg I/L) was pipetted into a 50.0-ml volumetric flask. Then, 25.0 ml of 12% (w/v) NaCl was added into the same flask. Deionized-distilled water was added to the mark. After mixing, S1 contains iodine 2 mg I/L and 6% (w/v) NaCl.

(ii) Synthetic Sample S2

This solution was prepared by addition of 0.25 ml of the standard iodate stock solution (1000 mg I/L) and 25.0 ml of 12% (w/v) NaCl into a 50.0-ml volumetric flask. The mixture solution was diluted to 50.0 ml with deionized-distilled water. After mixing, S2 contain iodine 5 mg I/L and 6% (w/v) NaCl.

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(iii) Synthetic Sample S3

The method for preparation was similarly to the procedure described in Section 4.5.5.2 (i) but employed 0.5 ml of the standard iodate stock solution (1000 mg I/L) instead. After mixing, S3 contain iodine 10 mg I/L and 6% (w/v) NaCl.

(iv) Synthetic Sample S1 Diluted to 3 % (w/v) NaCl Solution

Before directly injection into the kinetic FI manifold (Figure 6.8), the sample S1 (contained 6 % (w/v) NaCl) was 50 % diluted to contain 3 % (w/v) NaCl. This was carried out by transferring 25.0 ml of the solution prepared in Section 4.5.5.2 (i) into a 50.0-ml volumetric flask. This mixture solution was made up to 50.0 ml with deionized-distilled water.

(v) Synthetic Sample S2 Diluted to 3 % (w/v) NaCl Solution

Before directly injection into the kinetic FI manifold (Figure 6.8), the sample S2 (contained 6 % (w/v) NaCl) was 50 % diluted to contain 3 % (w/v) NaCl. The preparation was carried out by transferring 25.0 ml of the solution described in Section 4.5.5.2 (ii) into a 50.0-ml volumetric flask. This mixture solution was brought up to 50.0 ml using deionized-distilled water.

(vi) Synthetic Sample S3 Diluted to 3 % (w/v) NaCl Solution

Before directly injection into the kinetic FI manifold (Figure 6.8), the sample S3 (contained 6 % (w/v) NaCl) was 50 % diluted to contain 3 % (w/v) NaCl. The preparation was similar to the method presented in Section 4.5.5.2 (v) but pipetted 25.0 ml of the solution described in section 4.5.5.2 (iii) into a 50.0-ml volumetric flask instead.

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4.5.6 Analysis of Sample Solutions

For kinetic analysis using the FI manifold in Figure 6.8 , injection of 3 % (w/v) salt solution was carried out. This applied to all types of sample including commercial samples (Section 4.5.5.1 (ii)) and the synthetic samples (Section 4.5.5.2 (iv) to Section 4.5.5.2 (vi).

In the colorimetric FI analysis (for validation), 6 % (w/v) salt solution is preferable. Thus, to determine iodine in the commercial samples, sample solutions obtained from Section 4.5.5.1 (iii) were directly injected into the manifold in Figure 4.1. Synthetic salt solutions described in Section 4.5.5.2 (i) to Section 4.5.5.2 (iii) that contain 6 % (w/v) NaCl were also directly injected.

4.6 Description of the Colorimetric-FI System Used for Method Validation

The FI-manifold of the colorimetric method [4] is shown in Figure 4.1

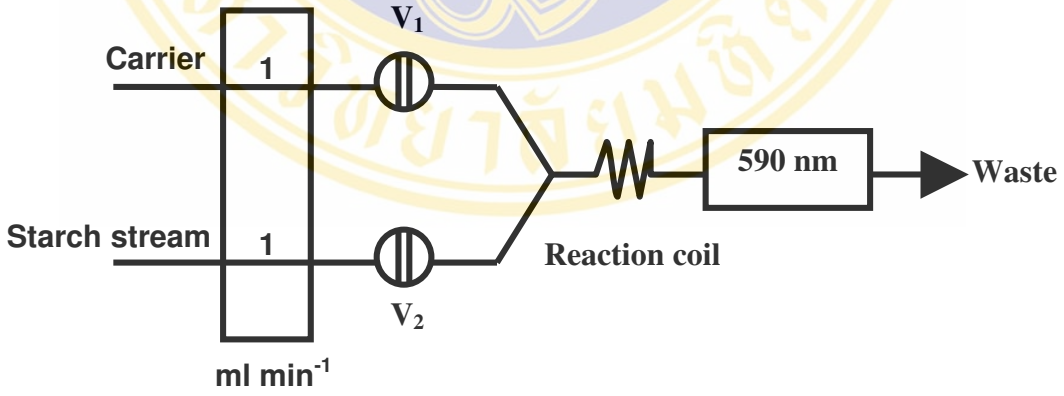

Figure 4.1 The FI manifold for determination of iodate in iodized salt. Carrier: 2.5 x 10^{-2} M KI in 6% (w/v) NaCl, starch stream: 0.1% starch in 1.0 x 10^{-2} M H_2SO_4, V_1: 250 µl injection of standard or sample solution, V_2: 100 µl injection of 1.0 x 10^{-3} M $Na_2S_2O_3$.

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(i) Method for Determination of Iodine Content

The following procedure was carried out in continuous-FI mode.

- 250 µl of standard iodate solutions (900-7200 µg I/L in 6% (w/v) NaCl) were injected into the system

- The absorbance reading of I_3^--starch at 590 nm was made.

- The cleaning solution was injected after an approximately 30 seconds of one injection of sample.

- The calibration was a plot between absorbance (at a top peak) and iodine concentrations (mg I/L).

4.7 Analysis of Kinetic Data

Fitting error (%) and rate constant (s^{-1}), presented in Section 6.1.2 and Section 6.1.3 were criteria for consideration of degree of iodate conversion to iodide. These parameters were obtained by using the SigmaPlot 2000 program [51]. The procedure was carried out by fitting of kinetic data with the theoretical curve provided by the software based on single exponential function accordingly to

$$A_t = A_f + A_o e^{-kt} \qquad (4.2)$$

where, A_t is the absorbance of Ce (IV) at any time t.

A_f is the final absorbance of Ce (IV).

A_o is the initial absorbance of Ce (IV).

k is the pseudo-first order rate constant (s^{-1}).

t is the time t (s).

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From Equation 4.2, the rate constant is displayed in term of k. The fitting error or coefficients of variation (%CV) were determined by using Equation 4.3.

$$\text{Fitting error (\%)} = \frac{(\text{standard error of the given rate constant} \times 100)}{\text{rate constant}} \qquad (4.3)$$

Kinetic behavior of the iodide catalyzed Ce (IV) and As (III) reaction is a pseudo first-order reaction in some conditions. The fitting error given by iodide is less usually than 1%. Thus, if iodate is completely reduced to iodide, fitting error provided by iodate should not exceed 1%. Examples for completeness and non-completeness of iodate reduction are presented in Figure 4.2 (a) and Figure 4.2 (b), respectively.

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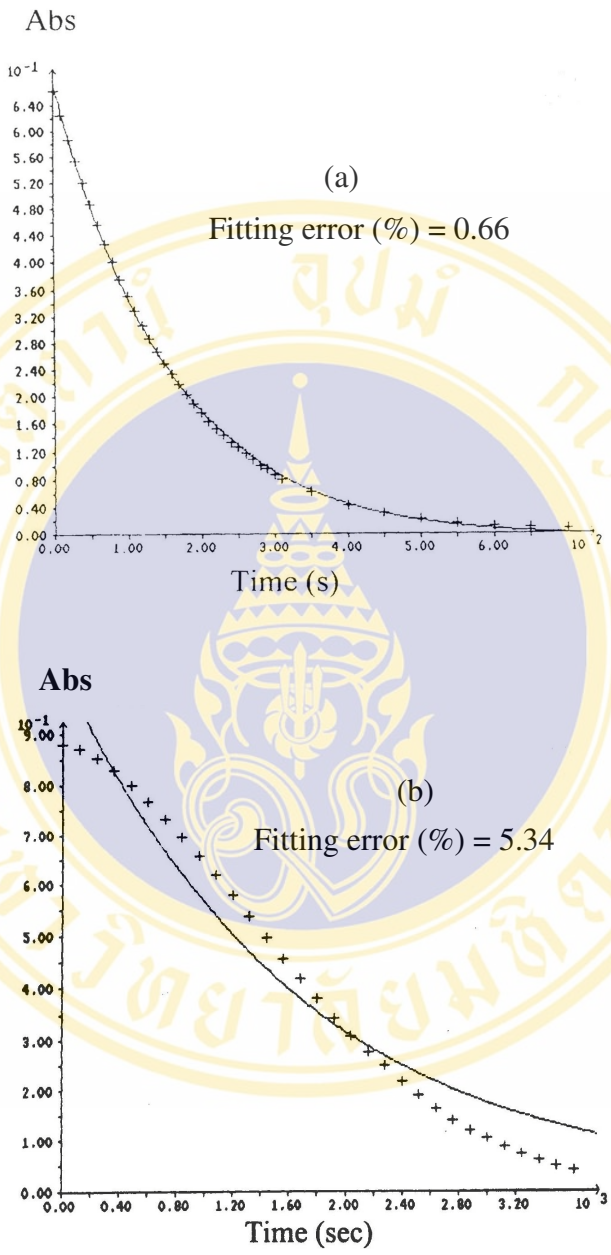

Figure 4.2 Examples of (a) good fitting or complete reduction of iodate and (b) poor fitting or non-complete reduction of iodate of the kinetic data (+) with the theoretical fit (——).

The rate constant given by iodate is equal to that given by iodide at the same concentration of I when iodate is absolutely converted to iodide. Thus, the rate constant of the indicator reaction should be the same between use of iodide and iodate.

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4.8 Determination of Sample Throughput

A sample throughput (samples/h) is determined by using the following equation,

$$\text{Sample throughput} = \frac{1\ (\text{h}) \times 60\ (\text{min} / \text{h}) \times 60\ (\text{s} / \text{min})}{t} \qquad (4.4)$$

where, t is the time interval (s) from injection to the maximum drop of the signal.

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CHAPTER V
RESULTS

5.1 Modification and Optimization of the FI System

The following sections are results obtained from the FI manifold in Figure 6.1 operated using the full kinetic stopped-FI mode, except for Section 5.1.1 which employed, the continuous-FI technique.

5.1.1 Effect of Mixing Geometry 'B'

This work was to find a suitable mixing geometry 'B' (Figure 6.1) for mixing a carrier stream (R_1), 0.048 M arsenous acid solution in 1.0 M NaCl (R_2) and 0.006 M ceric sulfate (R_3). The results in Table 5.1 are analysed in Section 6.1.1.

Table 5.1 Results obtained from the variation of in length and geometry of mixing reactor 'B' studied at different concentrations of NaCl in the carrier stream.

Condition code	Mixing reactor at 'B' position	Variation of baseline noise ($x10^{-3}$)[a]		
		1% (w/v)[b]	2% (w/v)[b]	6% (w/v)[b]
A	A straight tube (i.d. 0.5 mm, 100 cm)	± 0.9	± 2.0	± 1.0
B	A mixing coil (i.d. 0.5 mm, 100 cm)	± 1.0	± 0.4	± 0.5
C	A mixing coil (i.d. 0.5 mm, 160 cm)	± 0.7	± 0.4	± 0.3
D	A mixing coil (i.d. 0.5 mm, 200 cm)	± 0.4	± 0.4	± 0.3
E	Without any mixing reactor	± 4.8	± 6.7	± 4.9

[a] Variation of absorbance of Ce (IV) measured at 420 nm.

[b] NaCl concentration in the carrier stream.

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5.1.2 Optimal Concentration of Sodium Chloride in the Carrier Stream

Concentration of NaCl solution in carrier stream was optimized for 100 % conversion of iodate to iodide. In the optimization, fitting errors (Table 5.2 and Table 5.4) and rate constants (Table 5.3 to Table 5.5) were measured. These studies employed at NaCl concentrations of 2 and 3 % (w/v). The results given in Table 5.2 to Table 5.5 are discussed in Section 6.1.2.

Table 5.2 Fitting errors (%) obtained from injection of different concentrations of iodide, using 2 and 3 % (w/v) NaCl as the carrier streams.

Iodide concentration (μg I/L)	Fitting error [a] (%) ± SD	
	R1 : 2% (w/v) NaCl	R1 : 3% (w/v) NaCl
100	0.014 ± 0.001	0.024 ± 0.002
500	0.068 ± 0.004	-
1000	0.124 ± 0.018	-
2000	0.196 ± 0.004	0.034 ± 0.005
4000	-	0.162 ± 0.078
6000	-	0.127 ± 0.008

[a] From replicate injection of standard iodine solution.

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Table 5.3 Rate constants (s^{-1}) obtained from injections of different concentrations of iodide, studied at 2 and 3 % (w/v) NaCl as the carrier streams.

Iodide concentration (μg I/L)	Rate constant [a] (s^{-1}) ± SD	
	2% (w/v) NaCl	3% (w/v) NaCl
100	4.49 (±0.03) x 10^{-3}	3.87 (±0.02) x 10^{-3}
500	1.36 (±0.01) x 10^{-2}	-
1000	2.52 (±0.01) x 10^{-2}	-
2000	4.62 (±0.00) x 10^{-2}	4.26 (±0.02) x 10^{-2}
4000	-	8.16 (±0.10) x 10^{-2}
6000	-	1.18 (±0.01) x 10^{-1}

[a] From replicate injection of standard iodine solution.

Table 5.4 Fitting errors (%) obtained from injections of different concentrations of iodate, studied at 2 and 3 % (w/v) NaCl as the carrier streams.

Iodate concentration (μg I/L)	Fitting error [a] (%) ± SD	
	R1 : 2% (w/v) NaCl	R1 : 3% (w/v) NaCl
100	0.020 ± 0.004	0.018 ± 0.009
500	0.074 ± 0.003	-
1000	0.131 ± 0.008	-
2000	0.246 ± 0.008	0.056 ± 0.013
4000	-	0.164 ± 0.076
6000	-	0.164 ± 0.010

[a] From replicate injection of standard iodine solution.

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Table 5.5 Rate constants (s^{-1}) obtained from injections of different concentrations of iodate, studied at 2 and 3 % (w/v) NaCl as the carrier streams.

Iodate concentration	Rate constant [a] (s^{-1}) ± SD	
(μg I/L)	R1 : 2% (w/v) NaCl	R1 : 3% (w/v) NaCl
100	4.34 (±0.05) x 10^{-3}	3.83 (±0.01) x 10^{-3}
500	1.36 (±0.01) x 10^{-2}	-
1000	2.40 (±0.01) x 10^{-2}	-
2000	4.60 (±0.01) x 10^{-2}	4.08 (±0.03) x 10^{-2}
4000	-	8.03 (±0.02) x 10^{-2}
6000	-	1.16 (±0.01) x 10^{-1}

[a] From replicate injection of standard iodine solution.

5.1.3 Appropriate Length of Tubing 'A'

Length of the straight tubing 'A' (Figure 6.1) was optimized for complete reduction of iodate. Straight tube (2 mm i.d.) was used at position 'A' at various lengths of 0, 5, 10, 15, 30 cm. The carrier stream was fixed at 3 % (w/v) NaCl. Fitting errors and rate constants obtained are displayed in Table 5.6 to Table 5.9 and Figure 5.1. Those results are discussed in Section 6.1.3.

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Table 5.6 Fitting errors (%) obtained from various lengths of straight tube 'A' (2 mm i.d.), studied using injection of iodide standards.

Iodide concentration	Fitting error [a] (%) ± SD				
(µg I/L)	0 cm [b]	5 cm [b]	10 cm [b]	15 cm [b]	30 cm [b]
100	0.007 ± 0.000	0.012 ± 0.003	0.024 ± 0.002	0.005 ± 0.001	0.008 ± 0.001
2000	0.024 + 0.002	0.020 + 0.000	0.034 + 0.005	0.111 + 0.002	0.164 + 0.018
4000	0.125 ± 0.084	0.063 ± 0.000	0.162 ± 0.078	0.243 ± 0.007	0.338 ± 0.036
6000	0.141 ± 0.005	0.110 ± 0.006	0.127 ± 0.008	0.444 ± 0.019	0.527 ± 0.050

[a] from replicate injection of standard iodine solution.

[b] Length of 'A' (cm).

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Table 5.7 Rate constants (s^{-1}) obtained from various lengths of straight tube 'A' (2 mm i.d.), studied using injection of iodide standards.

Iodide concentration	Rate constant [a] (%) + SD				
(μg I/L)	0 cm [b]	5 cm [b]	10 cm [b]	15 cm [b]	30 cm [b]
100	4.22 (±0.01) x 10^{-3}	4.11 (±0.05) x 10^{-3}	3.87 (±0.02) x 10^{-3}	3.53 (±0.00) x 10^{-3}	3.28 (±0.00) x 10^{-3}
2000	5.07 (+0.04) x 10^{-3}	4.70 (+0.00) x 10^{-2}	4.26 (+0.02) x 10^{-2}	3.60 (+0.02) x 10^{-2}	3.06 (+0.01) x 10^{-2}
4000	9.85 (±0.01) x 10^{-2}	9.12 (±0.05) x 10^{-2}	8.16 (±0.20) x 10^{-2}	6.88 (±0.01) x 10^{-2}	5.82 (±0.00) x 10^{-2}
6000	1.39 (+0.00) x 10^{-1}	1.32 (+0.00) x 10^{-1}	1.18 (+0.01) x 10^{-1}	9.90 (+0.05) x 10^{-2}	8.37 (+0.00) x 10^{-2}

[a] from replicate injection of standard iodine solution.

[b] Length of 'A' (cm).

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Table 5.8 Fitting errors (%) obtained from various lengths of straight tube 'A' (2 mm i.d.), studied using injection of iodate standards.

Iodate concentration	Fitting error [a] (%) +SD				
(μg I/L)	0 cm [b]	5 cm [b]	10 cm [b]	15 cm [b]	30 cm [b]
100	0.015 ± 0.001	0.008 ± 0.002	0.018 ± 0.001	0.006 ± 0.000	0.008 ± 0.001
2000	0.028 + 0.007	0.031 + 0.001	0.056 + 0.013	0.088 + 0.002	0.130 + 0.020
4000	0.066 ± 0.008	0.081 ± 0.017	0.164 ± 0.076	0.201 ± 0.016	0.244 ± 0.016
6000	0.133 + 0.022	0.161 + 0.024	0.164 + 0.010	0.480 + 0.008	0.425 + 0.015

[a] from replicate injection of standard iodine solution.

[b] Length of 'A' (cm).

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Table 5.9 Rate constants (s^{-1}) obtained from various lengths of straight tube 'A' (2 mm i.d.), studied using injection of iodate standards.

Iodate concentration	Rate constant [a] (%) ± SD				
(µg I/L)	0 cm [b]	5 cm [b]	10 cm [b]	15 cm [b]	30 cm [b]
100	4.21 (±0.00) x 10^{-3}	4.09 (±0.02) x 10^{-3}	3.83 (±0.00) x 10^{-3}	3.59 (±0.03) x 10^{-3}	3.29 (±0.00) x 10^{-3}
2000	4.99 (±0.01) x 10^{-2}	4.67 (±0.02) x 10^{-2}	4.08 (±0.03) x 10^{-2}	3.61 (±0.01) x 10^{-2}	3.04 (±0.01) x 10^{-2}
4000	9.26 (±0.10) x 10^{-2}	9.14 (±0.10) x 10^{-2}	8.03 (±0.02) x 10^{-2}	6.93 (±0.03) x 10^{-2}	5.77 (±0.10) x 10^{-2}
6000	1.33 (±0.01) x 10^{-1}	1.31 (±0.00) x 10^{-1}	1.16 (±0.01) x 10^{-1}	9.86 (±0.01) x 10^{-2}	8.25 (±0.07) x 10^{-2}

[a] from replicate injection of standard iodine solution.

[b] Length of 'A' (cm).

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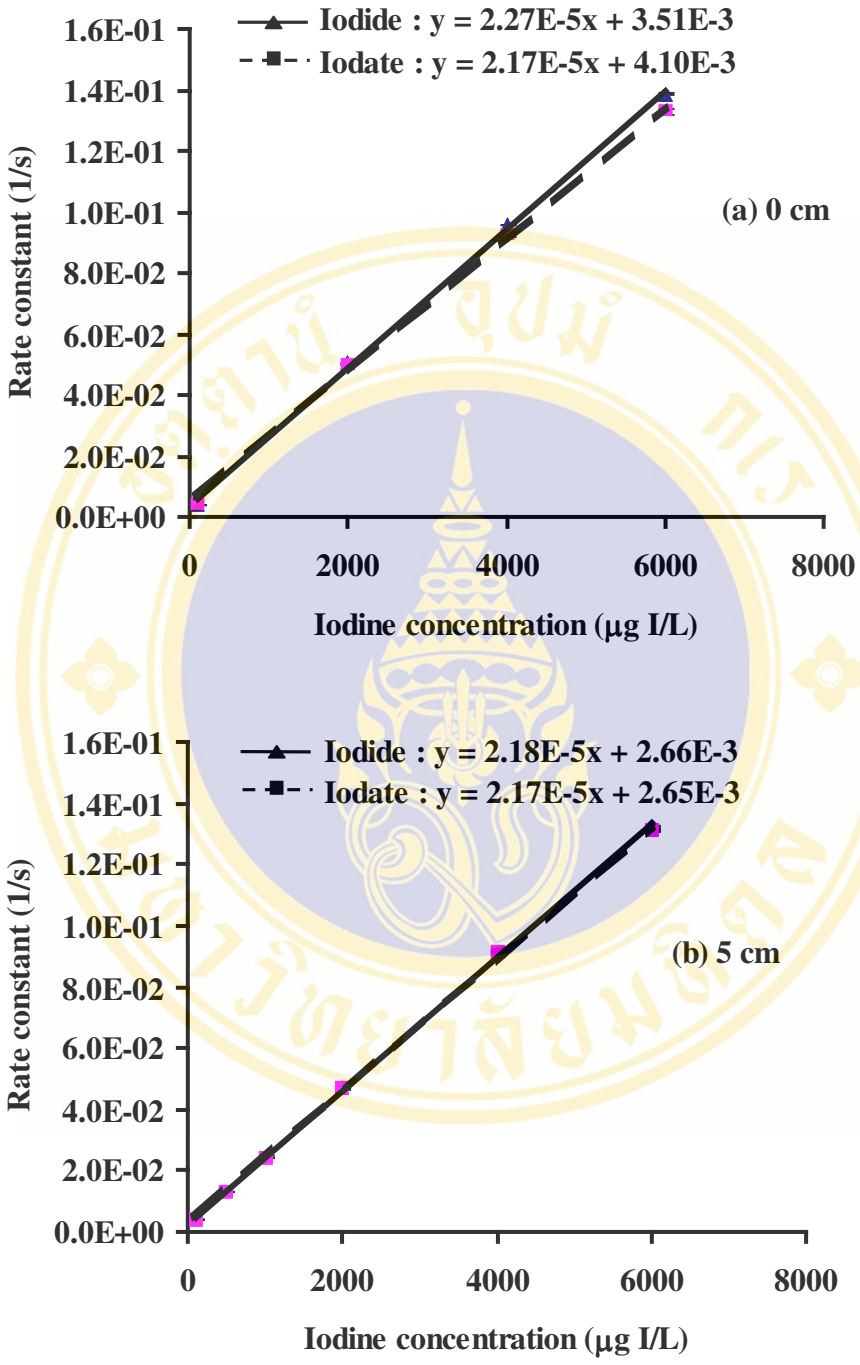

Figure 5.1 The standard plots of the pseudo-first order rate constant and the iodine concentration obtained from different length of 2 mm i.d. straight tube 'A': (a) 0 cm, (b) 5 cm, (c) 10 cm, (d) 15 cm and (e) 30 cm.

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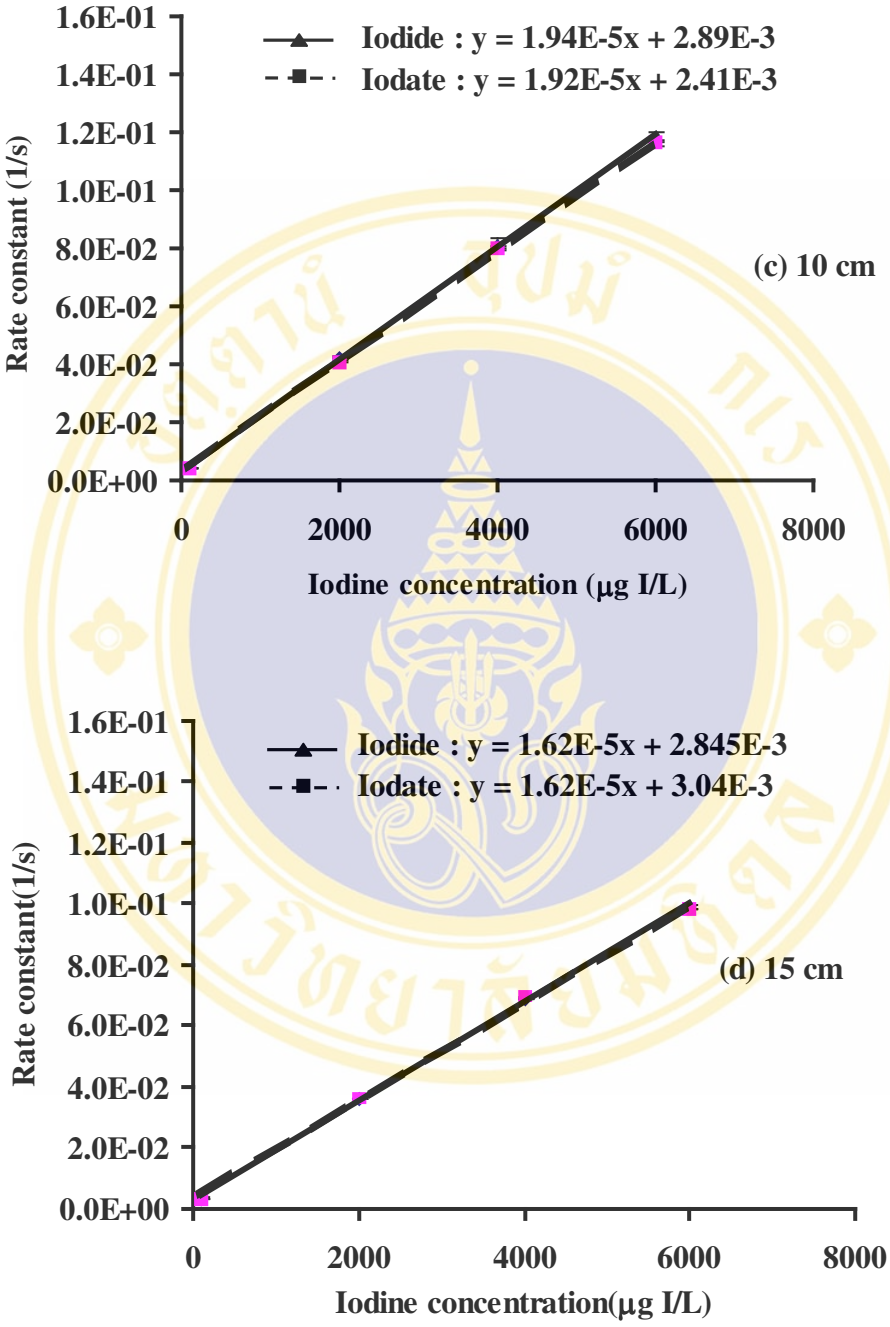

Figure 5.1 (Continued)

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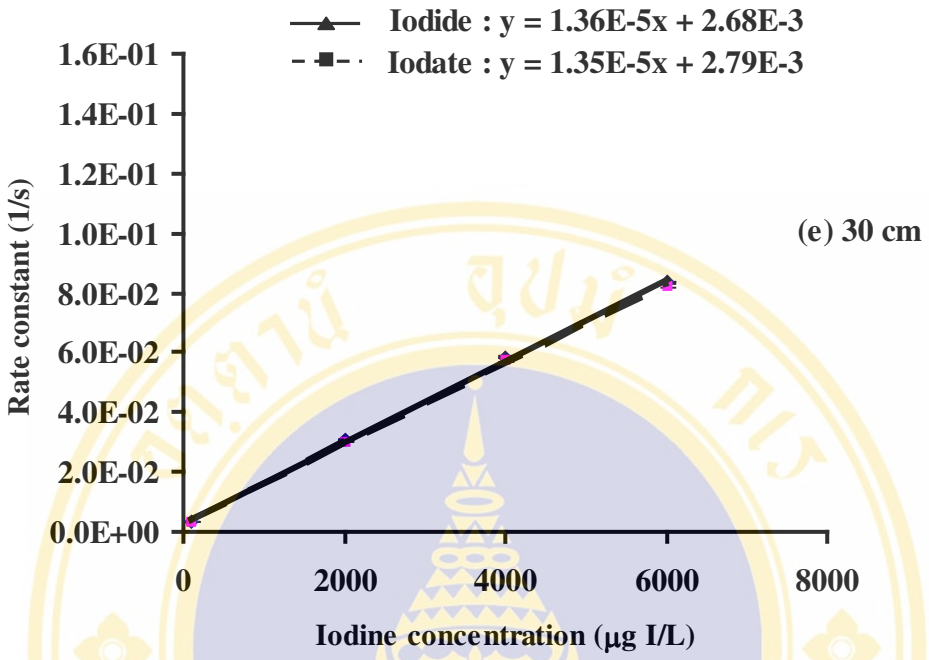

Figure 5.1 (Continued)

5.2 Data Obtained in the Calibrations of the Present FI System

Three operational methods which are the full kinetic stopped-FI method, the fixed time stopped-FI method and the kinetic continuous-FI method were investigated for their characteristic calibration equations. The calibration results obtained from injections of iodate standards are tabulated in Table 5.10 to Table 5.12 as well as shown in Figure 5.2 to Figure 5.4. These results are discussed in Section 6.2.2.

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Table 5.10 Calibration results for the full kinetic stopped-FI method.

Concentration of standard iodate (μg I/L)	Rate constant (s^{-1})
100	$3.80 (\pm 0.06) \times 10^{-3}$
500	$1.06 (\pm 0.08) \times 10^{-2}$
1000	$1.94 (\pm 0.05) \times 10^{-2}$
2000	$3.70 (\pm 0.06) \times 10^{-2}$
4000	$7.78 (\pm 0.04) \times 10^{-2}$
6000	$1.20 (\pm 0.04) \times 10^{-1}$

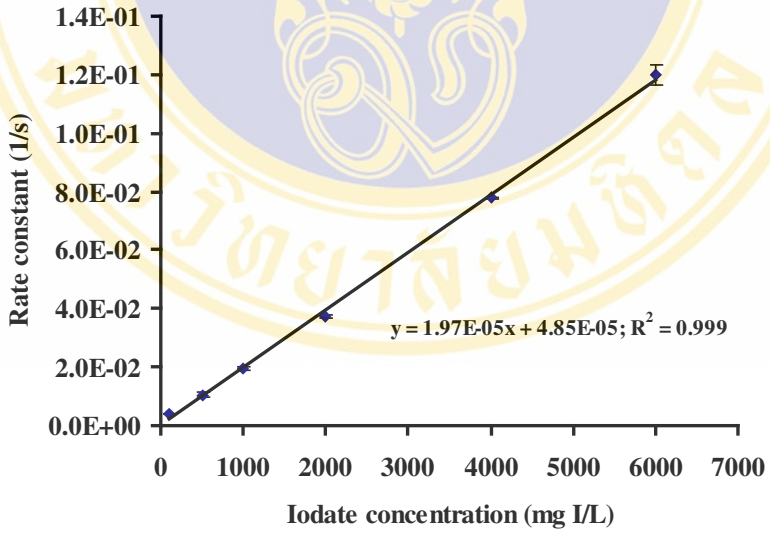

Figure 5.2 Calibration plot for the full kinetic stopped-FI method.

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Table 5.11 Calibration results for the fixed time stopped-FI method.

Concentration of standard iodate (µg I/L)	-ln (A_{8sec})
100	0.232 ± 0.002
500	0.370 ± 0.002
1000	0.527 ± 0.003
2000	0.852 ± 0.005
4000	1.411 ±0.011
6000	1.915 ± 0.017

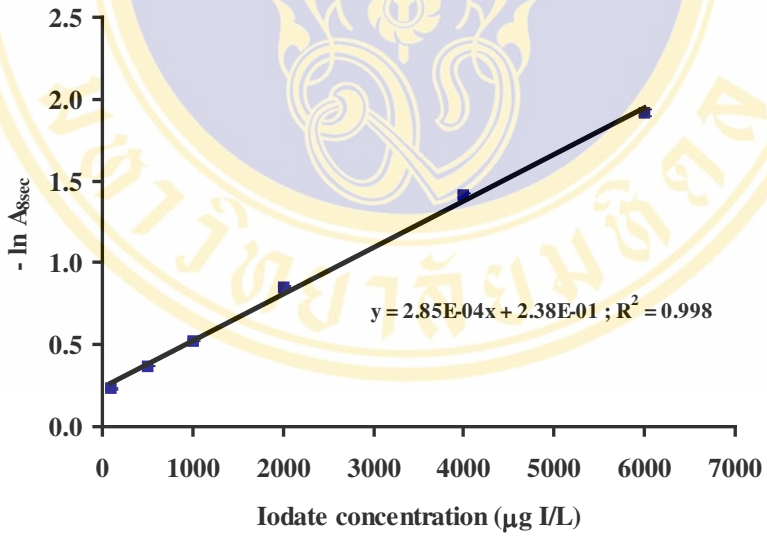

Figure 5.3 Calibration plot for the fixed time stopped-FI method.

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Table 5.12 Calibration results for the kinetic continuous-FI method.

Concentration of standard iodate (μg I/L)	-ln A
100	0.183 ± 0.005
500	0.258 ± 0.002
1000	0.356 ± 0.002
2000	0.559 ± 0.000
4000	0.942 ± 0.000
6000	1.279 ± 0.008

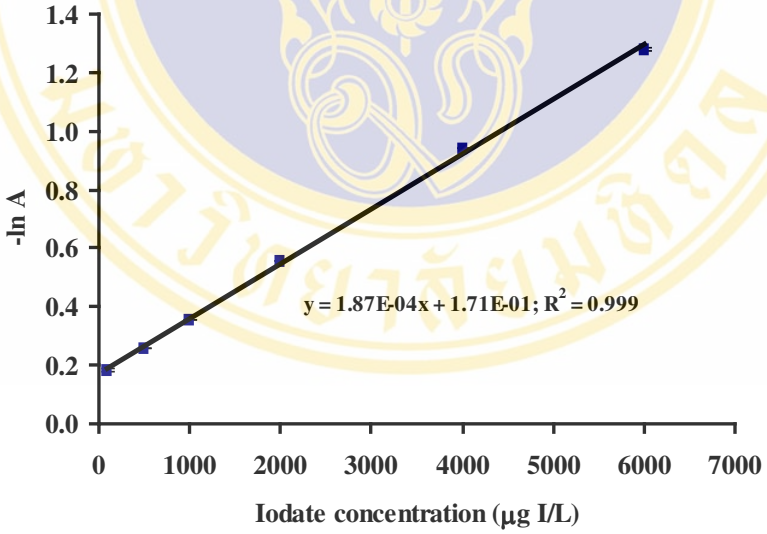

Figure 5.4 Calibration plot for the kinetic continuous-FI method.

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5.3 Application to Iodized Salt Samples

5.3.1 Test of Kinetic Interferences

Effect of kinetic interferences in ten various commercial iodized salts (T1 to T10) to the Sandell-Kolthoff reaction which was utilized for measurement of iodine quantity in these samples was considered. This work was studied under the optimum condition and used only the full kinetic stopped-FI technique. The fitting errors are shown in Table 5.13.

Table 5.13 Interference studies carried out on ten commercial iodized salts (T1 to T10). This investigation employed the fitting error based on first-order kinetics. The results in this table are analyzed in Section 6.3.1.

Sample code	Fitting error [a] (%)
T1	0.090 ± 0.005
T2	0.072 ± 0.042
T3	0.022 ± 0.005
T4	0.078 ± 0.056
T5	0.035 ± 0.008
T6	0.037 ± 0.021
T7	0.038 ± 0.009
T8	0.405 ± 0.017
T9	0.019 ± 0.000
T10	0.285 ± 0.103

[a] From triplicate injection of the sample.

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5.3.2 Analytical Recovery

Recoveries of iodine level were studied in ten iodized salt samples (T1 to T10) and in three synthetic samples (S1 to S3). Those studied employed (a) the full kinetic stopped-FI method, (b) the fixed time stopped-FI method and (c) the kinetic continuous-FI method. The results are shown in Table 5.14 to Table 5.16.

Table 5.14 Analytical recovery of the full kinetic stopped-FI method, obtained from iodized salts. Each experiment was carried out using triplicate injections. (These recoveries are discussed in Section 6.3.2.)

Sample code	Original content in sample solution (μg I/L)	Standard addition		Recovery [a] (%)
		Added (μg I/L)	Found (μg I/L)	
T1	469.9 ± 8.46	1000	1479 ± 6.65	90.7 ± 0.9
T2	934.1 ± 24.1	1000	2085 ± 39.1	113 ± 3.9
T3	1326 ± 12.4	1000	2496 ± 49.5	114 ± 4.0
T4	1565 ± 23.9	1000	2666 ± 25.1	108 ± 3.8
T5	2093 ± 2.47	1000	3149 ± 21.0	103 ± 2.3
T6	2335 ± 15.5	1000	3453 ± 35.6	107 ± 2.8
T7	2301 ± 25.9	1000	3471 ± 31.5	112 ± 3.8
T8	2241 ± 43.0	1000	3037 ± 23.4	72 ± 4.6
T9	2586 ± 5.51	1000	3652 ± 48.7	104 ± 4.7
T10	2752 ± 17.0	1000	3826 ± 40.2	105 ± 5.6
S1	1174 ± 11.3	1000	2518 ± 33.4	128 ± 2.1
S2	2910 ± 30.6	1000	4208 ± 29.5	124 ± 1.0
S3	5792 ± 41.2	500.0	6294 ± 32.8	110 ± 9.4

T : commercial iodized salt

S : synthetic solution

[a] From triplicate injections of the sample

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Table 5.15 Analytical recovery of the fixed time stopped-FI method, obtained from iodized salts. Each experiment was carried out using triplicate injections. (These recoveries are discussed in Section 6.3.2.)

Sample code	Original content in sample solution (μg I/L)	Standard addition		Recovery [a] (%)
		Added (μg I/L)	Found (μg I/L)	
T1	578.7 ± 8.00	1000	1702 ± 4.16	111 ± 1.9
T2	966.2 ± 6.75	1000	2192 ± 8.30	121 ± 5.1
T3	1308 ± 6.45	1000	2486 ± 54.2	116 ± 3.5
T4	1586 ± 4.04	1000	2840 ± 23.1	124 ± 1.1
T5	2006 ± 7.85	1000	3128 ± 21.7	111 ± 2.5
T6	2205 ± 9.64	1000	3345 ± 37.2	112 ± 1.9
T7	2214 ± 12.8	1000	3341 ± 39.8	111 ± 2.7
T8	2148 ± 12.5	1000	3194 ± 11.1	103 ± 2.8
T9	2483 ± 22.7	1000	3684 ± 29.4	119 ± 0.3
T10	2966 ± 33.2	1000	4268 ± 48.1	129 ± 0.8
S1	1251 ± 22.9	1000	2459 ± 35.8	119 ± 1.5
S2	3074 ± 28.3	1000	4293 ± 46.1	120 ± 7.3
S3	5037 ± 57.2	500.0	5448 ± 75.5	88.3 ± 4.1

T : commercial iodized salt

S : synthetic solution

[a] From triplicate injections of the sample.

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Table 5.16 Analytical recovery of the kinetic continuous-FI method, obtained from iodized salts. Each experiment was carried out using triplicate injections. (These recoveries are discussed in Section 6.3.2.)

Sample code	Original content in sample solution (μg I/L)	Standard addition		Recovery [a] (%)
		Added (μg I/L)	Found (μg I/L)	
T1	536.9 ± 6.96	1000	1682 ± 17.3	113 ± 1.5
T2	990.8 ± 4.18	1000	2279 ± 10.9	117 ± 1.4
T3	1336 ± 4.50	1000	2522 ± 23.0	108 ± 2.3
T4	1529 ± 0.00	1000	2679 ± 31.8	114 ± 3.1
T5	1982 ± 10.3	1000	3190 ± 23.8	110 ± 1.3
T6	2231 ± 14.3	1000	3384 ± 11.9	105 ± 0.3
T7	2203 ± 14.2	1000	3396 ± 20.7	109 ± 3.0
T8	2076 ± 24.6	1000	3101 ± 17.2	101 ± 3.0
T9	2429 ± 14.9	1000	3709 ± 36.7	120 ± 3.5
T10	2913 ± 12.3	1000	4013 ± 7.70	109 ± 0.5
S1	1123 ± 11.9	1000	2320 ± 5.60	117 ± 0.7
S2	2720 ± 16.0	1000	3837 ± 26.9	109 ± 3.1
S3	5401 ± 17.4	500.0	5937 ± 29.3	109 ± 4.5

T : commercial iodized salt

S : synthetic solution

[a] From triplicate injections of the sample.

5.3.3 Method Validation

The full kinetic stopped-FI method, the fixed time stopped-FI method and the kinetic continuous-FI method were applied to determine iodine quantity in ten commercial iodized salts and in three synthetic salt samples.

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Table 5.17 Iodine content in iodized salt determined by using the different analysis methods.

Sample code	Iodine content [a] (mg I/kg salt)			
	Full kinetic Stopped-FI	Fixed Time stopped-FI	Kinetic continuous-FI	Colorimetric-FI [b]
T1	15.6 ± 0.3	19.3 ± 0.3	17.9 ± 0.2	18.3 ± 0.2
T2	30.3 ± 0.8	31.4 ± 0.2	32.2 ± 0.1	36.3 ± 0.5
T3	43.0 ± 0.4	42.5 ± 0.2	43.4 ± 0.2	40.2 ± 0.3
T4	52.0 ± 0.8	52.7 ± 0.1	50.8 ± 0.0	61.2 ± 0.4
T5	67.6 ± 0.1	64.7 ± 0.3	64 ± 0.3	71.8 ± 0.5
T6	75.6 ± 0.5	71.4 ± 0.3	72.2 ± 0.5	76.6 ± 1.2
T7	75.0 ± 0.8	72.2 ± 0.4	71.9 ± 0.5	78.3 ± 1.6
T8	74.7 ± 1.4	71.6 ± 0.4	69.2 ± 0.8	82.4 ± 0.2
T9	86.0 ± 0.2	82.6 ± 0.8	80.8 ± 0.5	84.2 ± 5.4
T10	91.6 ± 5.7	98.7 ± 1.1	96.9 ± 0.4	112.3 ± 1.6
S1	37.7 ± 1.2	41.7 ± 0.8	37.0 ± 0.4	37.7 ± 1.2
S2	97.0 ± 2.0	102.5 ± 0.9	89.8 ± 0.5	96.6 ± 0.6
S3	193.1 ± 1.4	204.2 ± 2.0	187.2 ± 0.6	179.7 ± 3.9

[a] From triplicate injections of the sample.

[b] This method was carried out by N. Choengchan [4].

T: commercial iodized salt sample.

S: synthetic salt sample.

Iodine content in the samples analyzed by the proposed kinetic methods were validated by means of the statistical ANOVA test with those given by the colorimetric-FI method [4]. The results are discussed in Section 6.3.3.

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5.3.4 Precision of the Developed Methods

Precisions of the developed methods (the full kinetic stopped-FI method, the fixed time stopped-FI method and the kinetic continuous-FI method) were compared with the precisions of the colorimetric-FI method [4]. The studied were made using ten commercial iodized salts and three synthetic samples. In Table 5.18, the results in term of RSD of each method are presented (data to be analysed in Figure 6.11 Section 6.3.4). These results are also discussed in Section 6.3.4 to gain the suitable kinetic method for real application in commercial iodized salt.

Table 5.18 Precision (RSD) of four different methods for determination of iodine content in iodized salt samples (T1 to T10) and synthetic salt samples (S1 to S3).

Sample code	RSD [a] (%)			
	Full kinetic stopped-time	Fixed time stopped-FI	Kinetic continuous-FI	Colorimetric-FI [b]
T1	1.9	1.6	1.1	1.1
T2	2.6	0.6	0.3	1.4
T3	0.9	0.5	0.5	0.7
T4	1.5	0.2	0.0	0.7
T5	0.1	0.5	0.5	0.7
T6	0.7	0.4	0.7	1.6
T7	1.1	0.6	0.7	2.0
T8	1.9	0.6	1.2	0.2
T9	0.2	1.0	0.6	6.4
T10	2.3	1.1	0.4	1.4
S1	1.0	1.9	1.1	3.2
S2	2.1	0.9	0.6	0.6
S3	0.7	1.0	0.3	2.2

[a] From triplicate injections of the sample.

[b] This of analysis was carried out by N. Choengchan [4].

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5.4 Limit of Detection

5.4.1 The Kinetic Continuous-FI Method

The following results were used to determine the detection limit of the kinetic continuous-FI method in Section 6.4.1.

Table 5.19 Results for measuring of the limit of detection of the kinetic continuous-FI method.

No.[a]	1	2	3	4	5	6	7	8	9	10
-ln A	0.170	0.173	0.168	0.171	0.167	0.168	0.164	0.166	0.171	0.168

[a] No. of injection of blank (3% (w/v) NaCl).

5.4.2 The Fixed Time Stopped-FI Method

The absorbances of Ce (IV) in Table 5.20 are employed for calculation of detection limit of the fixed time stopped-FI method. The detection limit of this method is presented and discussed in Section 6.4.2.

Table 5.20 Results for measuring of the limit of detection of the fixed time stopped-FI method.

No.[a]	1	2	3	4	5	6	7	8	9	10
$-\ln A_{8sec}$	0.300	0.302	0.296	0.296	0.305	0.305	0.301	0.298	0.297	0.296

[a] No. of injection of blank (3% (w/v) NaCl).

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5.4.3 The Full Kinetic Stopped-FI Method

In Table 5.21, it shows original results for evaluation of the detection limit of the full kinetic stopped-FI method that is discussed in Section 6.4.3.

Table 5.21 Results for measuring of the limit of detection of the full kinetic stopped-FI method.

Concentration of standard iodate (μg I/L)	Rate constant (s^{-1}) (n=2)
100	3.56 (±0.02) x 10^{-3}
500	9.50 (±0.17) x 10^{-2}
1000	1.63 (±0.02) x 10^{-2}
2000	2.91 (±0.21) x 10^{-2}
4000	5.34 (±0.11) x 10^{-2}
6000	7.82 (±0.23) x 10^{-1}

These results were acquired by replicate injection of standard iodate solution (100 to 6,000 μg I/L) into the optimized FI system and employing the full kinetic stopped-FI method to measure the absorbance of Ce (IV) at 420 nm with time. To obtain the rate constant, the SigmaPlot 2000 software was used to fit theoretical single exponential decay with experimental data.

5.5 Repeatability of FI Method

Repeatability of the kinetic continuous-FI method and the fixed time stopped-FI method were studied by using ten replicate injections of standard iodate solution (2000 μg I/L). For the full kinetic stopped-FI, determination of repeatability was carried out by triplicate injections of standard iodate solution (2000 μg I/L). The results as depicted in Table 5.22 are analyzed in Section 6.5.

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Table 5.22 Results for determination of repeatability of the kinetic continuous-FI method, the fixed time stopped-FI method and the full kinetic stopped-FI method.

No. of injection	$A_{Ce(IV)}$		Rate constant (s^{-1})
	Kinetic continuous-FI method [a]	Fixed time stopped-FI method [b]	
1	0.498	0.430	4.06×10^{-2}
2	0.498	0.425	4.10×10^{-2}
3	0.497	0.429	4.08×10^{-2}
4	0.498	0.425	-
5	0.498	0.429	-
6	0.497	0.429	-
7	0.496	0.431	-
8	0.498	0.433	-
9	0.498	0.427	-
10	0.499	0.432	-

[a] $A_{Ce(IV)}$ is absorbance of Ce(IV) at a maximum drop which was detected at wavelength of 420 nm.

[b] $A_{Ce(IV)}$ is absorbance of Ce(IV) at 8 second after switching off the peristaltic pump (P1). The absorbance was measured at 420 nm.

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CHAPTER VI
DISCUSSION

A kinetic flow injection method for determination of iodate in iodized salt was developed by employing the Sandell-Kolthoff method. This work was modified from a flow injection system for determination of iodine in drinking water that was developed by N. Choengchan [20].

Prior to determination of iodate in iodized salt by using the Sandell-Kolthoff method, iodate must be completely reduced to iodide. Arsenous acid was used as the reducing agent for iodate [19] because it already exists in the system. An in-line FI system for reduction of iodate to iodide was optimized by using stopped-FI mode. Standard iodide (control) and iodate solutions in the range of 100 to 6000 μgI/L (all prepared in 3% (w/v) NaCl) were injected into the proposed FI system (Figure 6.1). The decrease in absorbance of Ce (IV) was measured at 420 nm with time. The SigmaPlot 2000 software was used to calculate the rate constant and the fitting error (%) based on the pseudo-first order kinetics. If reduction of iodate to iodide is complete, the kinetic behavior given by iodate will be the same as the kinetic behavior given by the iodide control for equal concentrations of I. Two parameters, fitting error and rate constant (k), were investigated for the optimization of the FI system. The following are criteria, were employed for optimization of the system.

(a) Fitting Error for Pseudo-First Order Kinetics

The Ce (IV)-As (III) reaction with iodide as the catalyst, can be made a pseudo first-order reaction in cerium (IV), with the observed first order rate constant linear with iodide concentrations. [18, 40]. Being a first order kinetics, the relationship between absorbance of Ce (IV) and time is an exponential function as given by equation 6.1.

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$$A_t = A_o . e^{-kt} \quad (6.1)$$

where, A_o is the absorbance of Ce (IV) at time t=0.

A_t is the absorbance of Ce (IV) at any time t

k is the pseudo-first order rate constant of the reaction

Usually, the fitting error (%) is less than 1 for iodide. Therefore, if iodate is absolutely reduced to iodide, it should provide the fitting error (%) of less than 1.

(b) Rate Constant

Since the Sandell-Kolthoff reaction gives first order kinetic in some conditions. Thus, it can be applied for quantification of iodide by plotting the rate constant (s^{-1}) against the iodide concentration for the calibration. This plot is a linear relationship. For this work, the reaction was used for iodine measurement in iodized salt. However, iodine supplement in table salt is commonly added in the form of iodate. Therefore, iodate must be reduced to iodide before the analysis of iodine content. If reduction of iodate into iodide by arsenous acid was accomplished, the linear equations obtained from iodide and iodate will not be significant .

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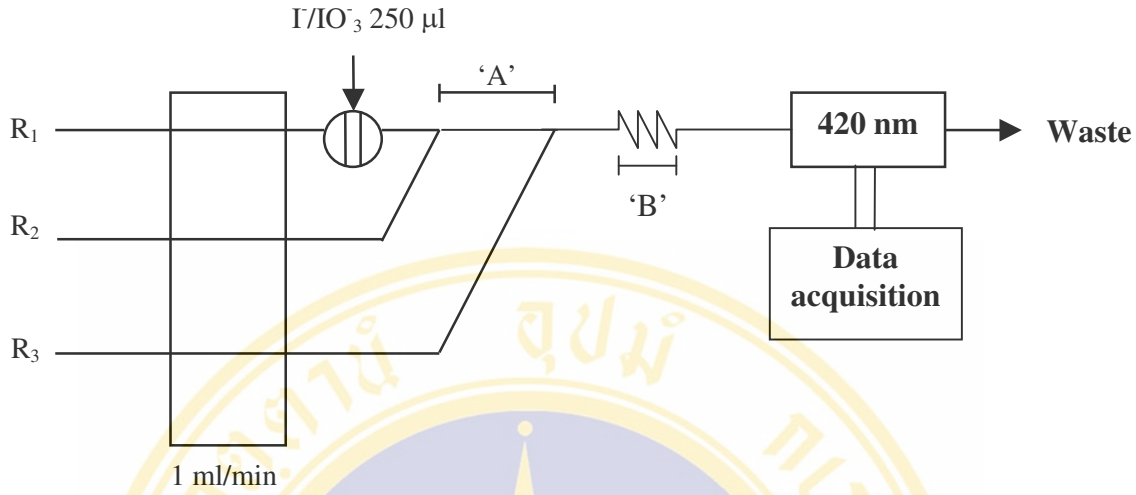

Figure 6.1 The FI manifold used in the optimization for iodized salt. Straight tube 'A': i.d. 2 mm, 10 cm; mixing coil 'B': i.d. 0.5 mm, 160 cm; R_1: NaCl carrier solution; R_2: 0.048 M As (III) in 0.53 M H_2SO_4 containing 1.0 M NaCl and R_3 : 0.006 M Ce (IV) in 0.45 M H_2SO_4.

6.1 Modification and Optimization of the FI System

N. Choengchan developed a FI-system (Figure 6.2) for determination of iodate in bottled drinking water. The system employed the Sandell-Kolthoff method [20].

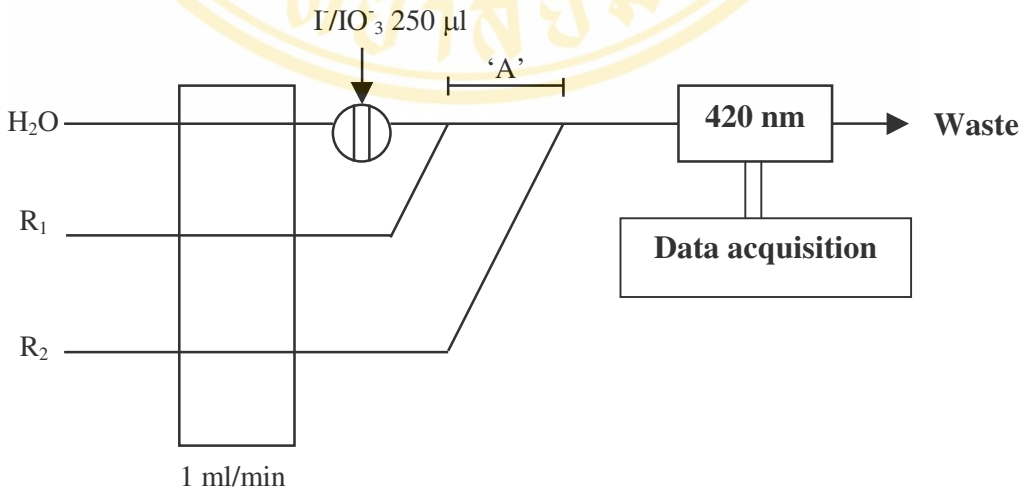

Figure 6.2 The FI manifold presented by N. Choengchan for determination of iodate in drinking water [20]. Straight tube 'A': i.d. 2 mm, 15 cm; R_1 : 0.04 M As (III) in 0.53 M H_2SO_4 containing 1.0 M NaCl; R_2 : 0.005 M Ce (IV) in 0.45 M H_2SO_4.

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To apply this kinetic method to other samples, iodized salt was chosen. However, commercial drinking water and iodized salt have different levels of iodine concentrations. The iodine concentration supplemented in bottled drinking waters is 100 μg I/L. Iodine is added into salt at the nominal level of 50 mg I/kg salt. For a 3% salt solution, iodine is present at 1500 μg I/L. Drinking water and salt solution also differ in their matrices. Thus, it is necessary to first examine if the kinetic method is still suitable for the level of iodine in table salt (whether the assumptions are valid). The FI-system should be optimized for iodized salt.

The FI system employed in this work (Figure 6.1) was modified from the system in Figure 6.2, developed for drinking water. When an iodate solution is injected into the system, iodate is aimed to be reduced to iodide by arsenous acid in tube 'A'. The reduction of iodate should be completed inside this tube prior to the reaction with Ce (IV) in coil 'B'. The 'A' and 'B' tubes were optimized. The concentration of sodium chloride in the carrier stream (R_1) was also optimized.

6.1.1 Effect of Mixing Geometry 'B'

In this work, the length of mixing coil 'B' was varied to obtain an appropriate mixing between the carrier stream (R_1) and the reagents (R_2 and R_3). This studied were made at 3 conditions of R_1 (i.e., 1%, 2% and 6% (w/v) NaCl solution). By employing the FI system in Figure 6.1, the procedure was carried out by varying the length and mixing geometry 'B' and then monitoring the baseline of the absorbance of Ce (IV). The results are presented in Figure 6.3.

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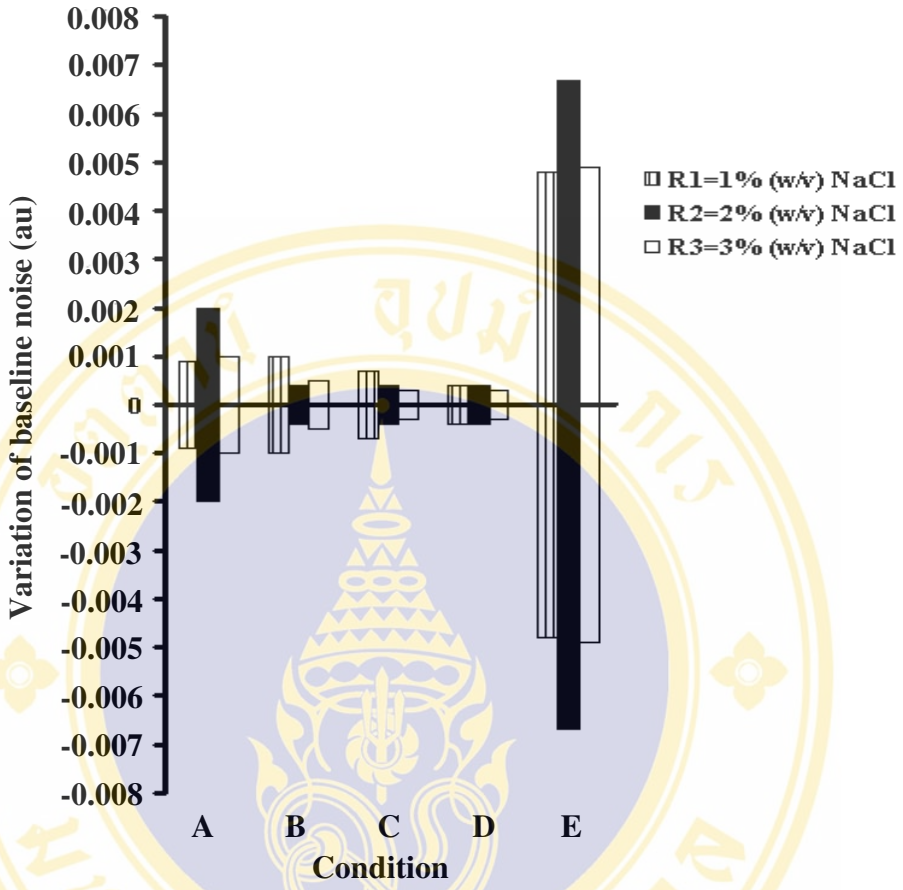

Figure 6.3 Relation between variation of baseline noise (absorbance of Ce(IV)) measured at different conditions of mixing reactor 'B'. Condition A: a straight tube with i.d. 0.5 mm, 100 cm; Condition B: a mixing coil with i.d. 0.5 mm, 100 cm; Condition C: a mixing coil with i.d. 0.5 mm, 160 cm; Condition D: a mixing coil with i.d. 0.5 mm, 200 cm and Condition E: without mixing reactor at position 'B'.

It was found in Figure 6.3. that increasing the length of tube 'B' brought to decreasing in the variation of absorbance. Moreover, changing the geometry 'B' from straight tube to mixing coil can reduce the variation in baseline noise. Most conditions are appropriate, except condition E, because this condition provides the highest variation. In addition, heterogeneous mixing of reagents (R_1, R_2 and R_3) was visually observed in the flow cell when condition E was used. Therefore, it can be concluded that without any mixing at position'B', the FI system is not appropriate. The mixing coil with i.d. 0.5 mm and 160 cm in length (condition C) was

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chosen for this work because it gave a low variability of the baseline absorbance which is an indicator of a proper mixing of reagents.

6.1.2 Optimal Concentration of Sodium Chloride in the Carrier Stream

To avoid the "Schleren effect" [43], sodium chloride solution was chosen as the carrier stream for the system in Figure 6.1. The concentration of sodium chloride should be made the same as the salt concentration.

S. Suwannachoat [19] reported that the reduction of iodate to iodide reached its completion faster as chloride concentration was increased.

In this work, the concentration of NaCl in the carrier was optimized and studied at conditions I, II and III, as written in Table 6.1.

Table 6.1 Summary of the studied conditions: carrier stream, sample dissolution, expected concentration of iodine in the sample solution and suitable calibration range.

	Condition		
	I	II	III
1. Carrier solution	2 % (w/v) NaCl	3 % (w/v) NaCl	6 % (w/v) NaCl
2. Sample	2 % (w/v) salt	3 % (w/v) salt	6 % (w/v) salt
3. Expected concentration of iodine in the sample solution (from 2.)	600-1,600 μg I/L	900-2,400 μg I/L	1,800-4,800 μg I/L
4. Suitable calibration range	100-2,000 μg I/L	100-6,000 μg I/L	100-6,000 μg I/L

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Since when a table salt is dissolved in deionized water, prior to the FI system analysis, different concentrations of salt will give different iodine contents. Thus, the calibration range will differ depending upon the sample preparation (Table 6.1). For example, the suitable calibration range for 6% (w/v) salt solution is 100-6,000 μg I/L.

At condition III, standard iodine solutions, i.e., 100, 500, 1000, 2,000, 4,000 and 6,000 μg I/L all prepared in 6% (w/v) NaCl solution, were injected into a carrier (R_1) of 6% (w/v) NaCl solution. It was noticed that precipitation of salt often appeared inside the flow cell when the system was operated continuously in the stopped-FI mode for about 1 h. This event caused positive baseline shift as shown in Figure 6.4. Therefore, condition III is not suitable due to the high concentration level of salts. However, no salt precipitation was observed for conditions I and II.

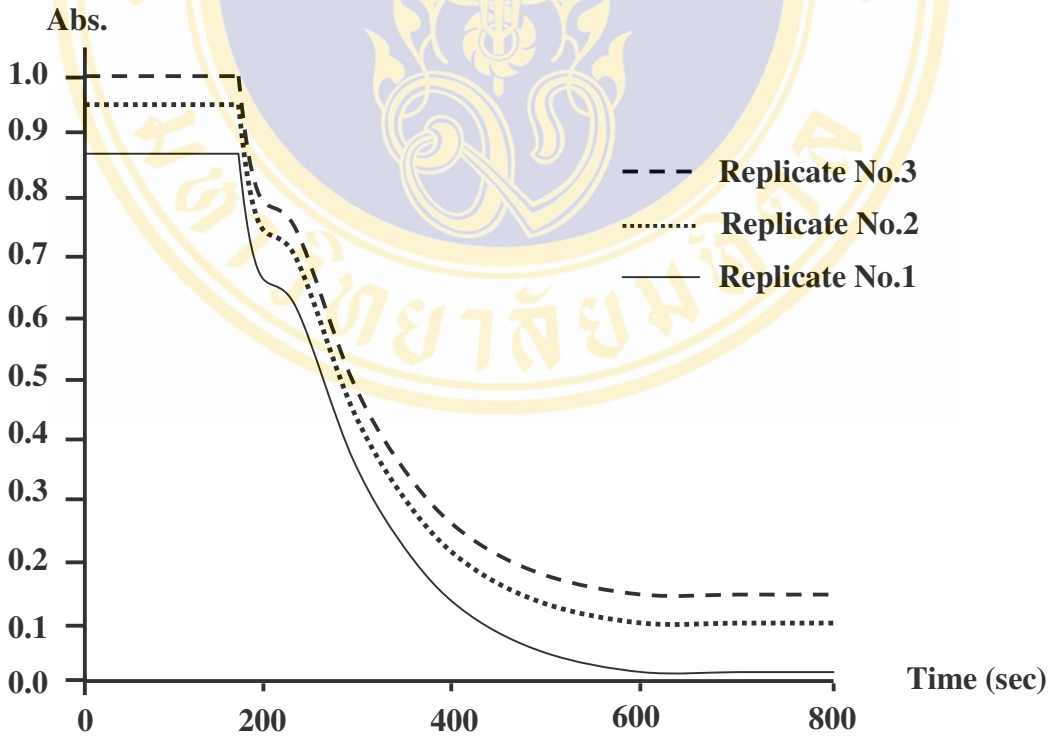

Figure 6.4 Effect of too high concentration level of NaCl (6% (w/v)) causing baseline shift. Curve plotted between the absorbance of Ce(IV) at 420 nm and time (s) when 500 μg I/L iodide standard was injected into the FI system (Figure 6.1).

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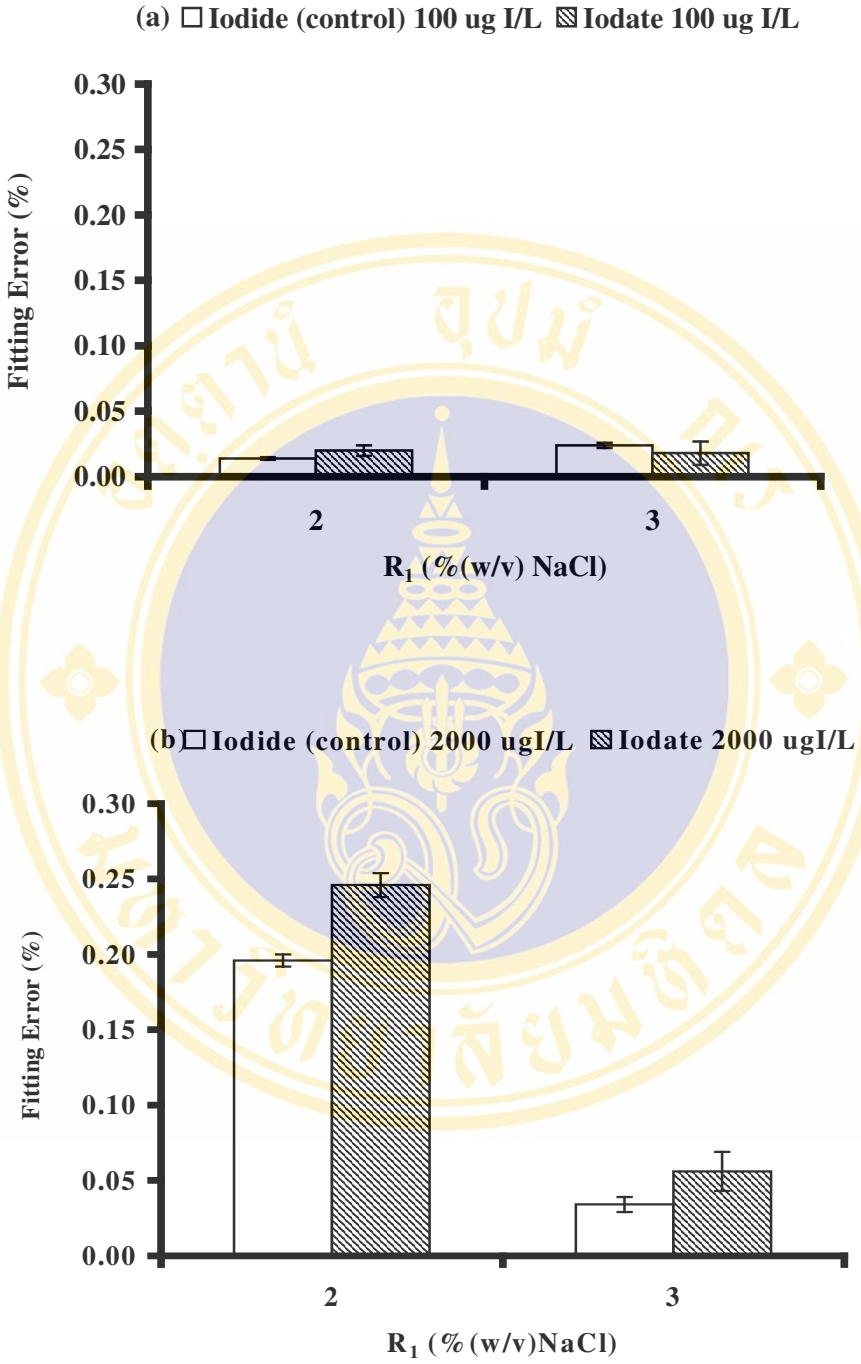

Figure 6.5 Effect of chloride concentration on the fitting errors for pseudo-first order kinetics.

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To evaluate condition I and II, fitting errors and rate constants were used as the criteria. The kinetics of all iodine concentrations in each calibration range were investigated. However, all fitting errors measured at every condition studied were below 1. Thus, in this section, the fitting errors obtained from only the minimum and the maximum concentrations of iodine were presented in Figure 6.5.

In Figure 6.5, the fitting errors, obtained from 100 μg I/L (Figure 6.5(a)) and from 2,000 μg I/L (Figure 6.5(b)), were less than the acceptable limit of 1 when condition I and II were operated. This indicated that the required conversion of iodate to iodide were complete. The fitting errors for other iodine concentrations are presented in Section 5.1.2.

The next parameter for consideration was rate constant. Calibration plots between rate constant (s^{-1}) and standard iodine concentration were contrived for condition I and II, as shown in Figure 6.6a and Figure 6.6b, respectively.

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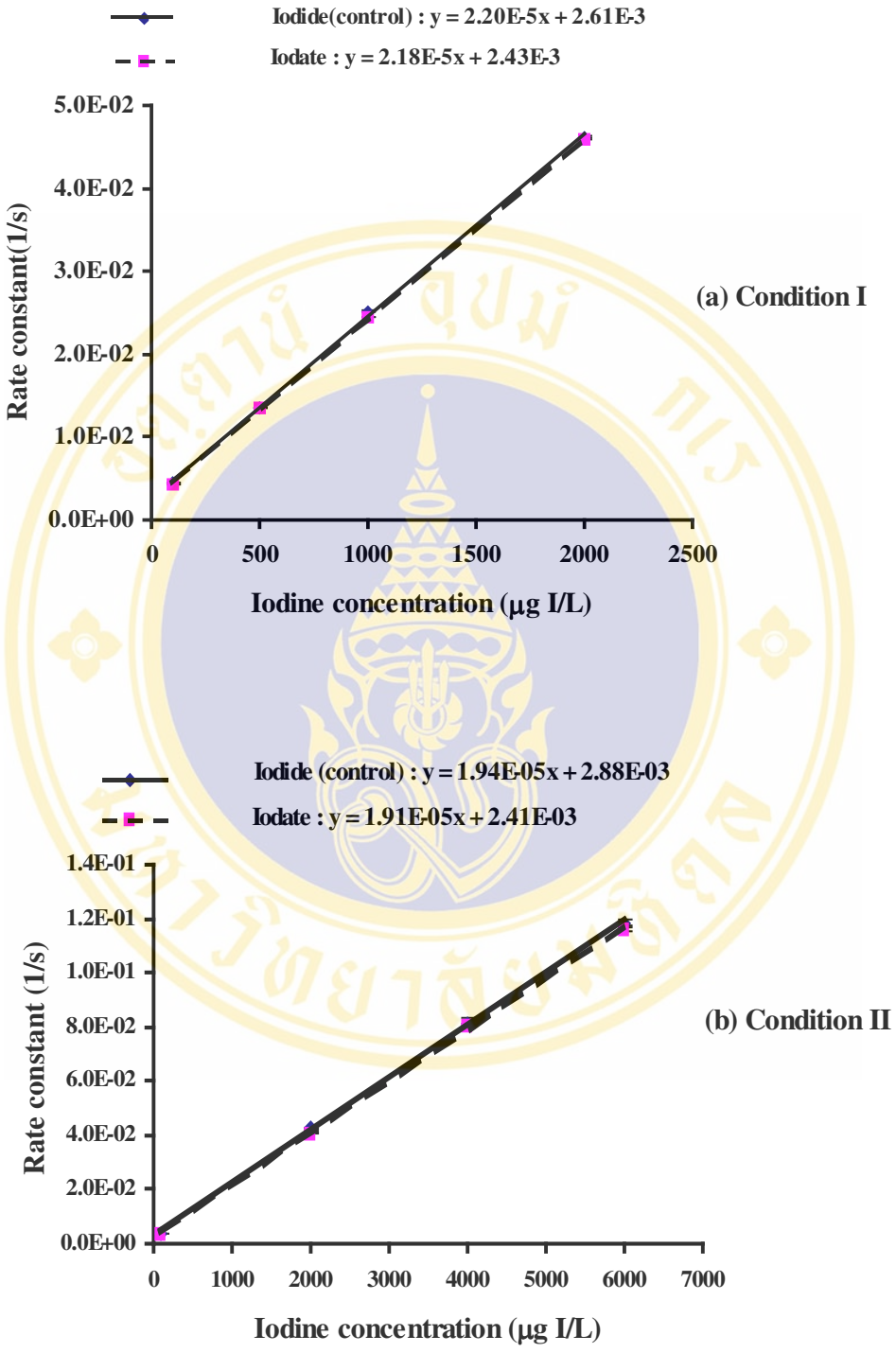

Figure 6.6 Calibrations obtained from standards iodate and iodide studied at two conditions of the carrier stream (Figure 6.1); (a) Condition I: 2 % (w/v) NaCl solution as the carrier stream and (b) Condition II: 3 % (w/v) NaCl solution as the carrier stream.

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The results in Figure 6.6 show that the linear equations obtained from iodate species did not differ from those obtained from the iodide species (control), for both conditions. Moreover, by means of regression test at confidential limit of 95% [50], the slopes and the intercepts given by each condition (Table 6.2) were within 1.000 and 0.000, respectively. These results indicated that complete reduction of iodate to iodide took place for all conditions studied.

Table 6.2 Slopes (a) and y-intercepts (c) obtained by using the Regression test in the comparison between the calibration given by iodate standards as compared to iodide.

Condition	Parameter	
	$a \pm s_a$* (minimum to maximum)	$c \pm s_c$* (minimum to maximum)
I	0.9937 ± 0.0549 (0.9388 to 1.0486)	-0.0002 ± 0.0015 (-0.0017 to 0.0013)
II	0.9861 ± 0.0341 (0.9520 to 1.0202)	-0.0004 ± 0.0026 (-0.0030 to 0.0021)

* S_a and S_c are standard deviations of slope and intercept, respectively.

However, 3% (w/v) of salt are chosen as the sample preparation for this work (condition II). At this concentration of salt sample, the concentration of iodine is not as low as in condition I and thus gives faster analysis time for the full kinetic stopped-FI mode.

6.1.3 Appropriate Length of Tubing 'A'

This work was aiming to study effect of length of tubing 'A' on the completeness of reduction of iodate to iodide by arsenic (III).

N. Choengchan had studied this effect in his FI-system which was applied for drinking water samples [20]. It was observed that the reduction of iodate

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could be accomplished when employing any length of tubing 'A' from 15 to 60 cm (2 mm i.d.).

For straight tube 'A', a 2 mm i.d. was investigated at 5, 10, 15, 30 cm in length or not employing the tube at all (without 'A'). Standard iodine solutions (100, 2000, 4000 and 6000 μg I/L in 3% NaCl) were injected in duplicate into the FI system in Figure 6.1. The carrier stream (R_1) was 3% (w/v) NaCl solution.

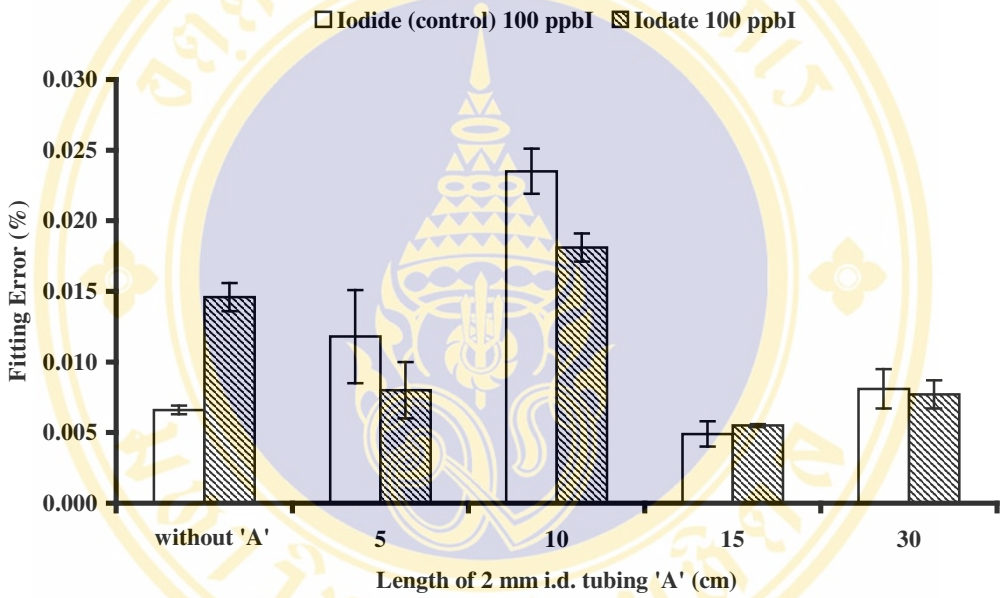

Figure 6.7 Effect of length of straight tubing 'A' on the first order behavior of the indicator reaction.

Fitting error was investigated for the kinetic behavior given by iodate compared to that given by the iodide control. In Figure 6.7, the results are presented only for one concentration of iodine (100 μg I/L). It was found for all length of straight tubing 'A' that iodate gave the fitting errors less than 1%. This indicated that the kinetics in iodate condition were pseudo-first order type as well as in the condition employing iodide. The fitting errors of other iodine concentrations are shown in Section 5.1.3.

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Linear calibration plots between rate constant (s^{-1}) and iodine concentration (iodate compared to iodide) are also investigated when the length of tubing 'A' was varied. The comparison against the control (iodide) employs both the slopes and y-intercepts. The linear equations that were obtained from the calibration plots were tabulated in Table 6.3.

Table 6.3 Linear equations obtained from the plots between rate constant and iodine concentration when the length of straight tubing 'A' was varied.

Length of tubing 'A' (cm)	Linear equation and regression coefficient	
	Iodide (control)	Iodate
Without 'A'	$k = 2.28 \times 10^{-5} (\pm 4.8 \times 10^{-7}) [I] + 3.51 \times 10^{-3} (\pm 1.8 \times 10^{-3})$ $R^2 = 0.999$	$k = 2.17 \times 10^{-5} (\pm 6.2 \times 10^{-7}) [I] + 4.10 \times 10^{-3} (\pm 2.3 \times 10^{-5})$ $R^2 = 0.998$
5	$k = 2.18 \times 10^{-5} (\pm 3.3 \times 10^{-7}) [I] + 2.66 \times 10^{-3} (\pm 1.2 \times 10^{-3})$ $R^2 = 0.999$	$k = 2.17 \times 10^{-5} (\pm 4.5 \times 10^{-7}) [I] + 2.65 \times 10^{-3} (\pm 1.7 \times 10^{-3})$ $R^2 = 0.999$
10	$k = 1.94 \times 10^{-5} (\pm 3.1 \times 10^{-7}) [I] + 2.89 \times 10^{-3} (\pm 1.1 \times 10^{-3})$ $R^2 = 0.999$	$k = 1.92 \times 10^{-5} (\pm 2.7 \times 10^{-7}) [I] + 2.41 \times 10^{-3} (\pm 1.0 \times 10^{-4})$ $R^2 = 0.999$
15	$k = 1.62 \times 10^{-5} (\pm 3.2 \times 10^{-7}) [I] + 2.85 \times 10^{-3} (\pm 1.2 \times 10^{-3})$ $R^2 = 0.999$	$k = 1.62 \times 10^{-5} (\pm 4.0 \times 10^{-7}) [I] + 3.04 \times 10^{-3} (\pm 1.5 \times 10^{-3})$ $R^2 = 0.999$
30	$k = 1.36 \times 10^{-5} (\pm 3.3 \times 10^{-7}) [I] + 2.68 \times 10^{-3} (\pm 1.2 \times 10^{-3})$ $R^2 = 0.999$	$k = 1.35 \times 10^{-5} (\pm 3.0 \times 10^{-7}) [I] + 2.79 \times 10^{-3} (\pm 1.1 \times 10^{-3})$ $R^2 = 0.999$

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No significant difference (in terms of slope and intercept) was observed at any length of straight tube 'A' from 5 to 30 cm, when comparing the use of iodide or iodate standards. However, for the condition without any 2 mm i.d. straight tube at position 'A', the linear fititngs were different between the conditions of iodate and iodide standards. This result indicated that iodate was not completely converted to iodide in this condition.

For each length of straight tube 'A', rate constants obtained from iodate solutions were statistically compared with rate constants of the iodide solutions (control). The regression test [50] was used in this comparison (see Appendix III). At the confident limit of 95%, the slopes and y-intercepts lied within the values of 1.000 and 0.000, when 5, 10, 15 and 30 cm in length of 2 mm i.d. straight tube 'A' were applied. However, the calibrations are significantly different, without a 2 mm i.d. tube at position 'A'.

It was noticed in Table 6.3 that the sensitivity dropped when the length of tubing 'A' was longer. In this work, 10 cm (2 mm i.d. of straight tube) was chosen as the optimal condition. Below this length, it was not suitable to apply.

6.2 The Present FI System : Optimal and Operational Conditions

In this section, the optimal condition of the FI system is summarized. The operational method for determination of iodine in iodized salt by using iodide catalysed reaction of Ce (IV) – As (III) is also presented.

6.2.1 The Optimal Condition

The optimized FI system for kinetic analysis of iodine in iodized salt is depicted in Figure 6.8.

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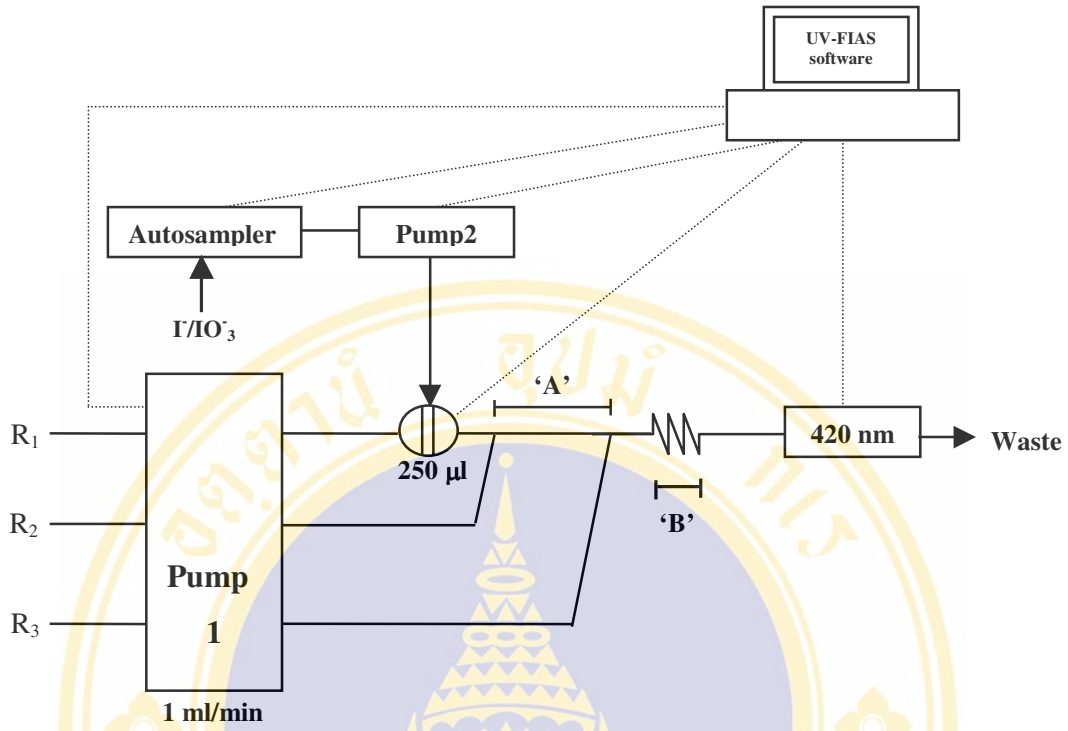

Figure 6.8 The optimum FI manifold used for iodine quantitation in iodized salt based on the iodide catalysed reaction of Ce (IV)-As (III). Straight tube 'A' : i.d. 2 mm, 10 cm; mixing coil 'B' : i.d. 0.5 mm, 160 cm; R_1 : 3% (w/v) NaCl, R_2 : 0.048 M As (III) in 0.53 M H_2SO_4 prepared in 1.0 M NaCl and R_3 : 0.006 M Ce (IV) in 0.45 M H_2SO_4. All standard iodate solutions are prepared in 3% (w/v) NaCl. The FI system was a FIAS 300 of Perkin Elmer, controlled by UV-FIAS software. The spectrophotometer used was a Lambda 25 UV/VIS spectrophotometer of Perkin Elmer.

6.2.2 Operational Condition

To look for an appropriate performance on the FI system, three operational methods were considered which are

1. Full kinetic stopped – FI
2. Fixed time stopped – FI
3. Kinetic continuous – FI

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These methods were operated by using UV-FIAS program (the protocol is described in Appendix II).

6.2.2.1 Full Kinetic Stopped – FI Method

The operational step used in this method is tabulated as shown in Table 6.4.

Table 6.4 Method for iodine analysis by employing the full kinetic stopped-FI.

Step	Time interval(s)	Pump1 (rpm)	Pump2 (rpm)	Valve	Data collection time (s)
1	0	0	0	Fill	0
2	17	28	120	Fill	0
3	40	28	0	Fill	0
4	40	28	0	Inject	2500

In summary, The peristaltic pump P2 was switched on to fill a sample for 17 s into the injection loop. Accurately 40 s after injection of a sample, the peristaltic pump P_1 is switched off to keep the reaction zone inside the flow cell. Ce (IV) is recorded for its absorbance at 420 nm, every 10 s. The obtained kinetic data are then fitted with an exponential equation via SigmaPlot 2000 software. The calculated rate constants obtained from a series of standard iodate (μg I/L) are plotted against the concentration. This plot is used in the calibration for determination of iodine in table salt. The working range of this method is from 100 to 6000 μg I/L. A representative standard equation is k = (1.973E-5)[I] + (4.854E-3): $R^2 = 0.999$.

The benefit obtained from this method is that the kinetics is gained. This information can be used to study sample interferences. However, the method takes quite a long time to achieve one full kinetic exponential curve. For

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example, it takes 35 min for the iodate standard of 100 μg I/L. The period is shorter with increasing in iodate concentration.

6.2.2.2 Fixed Time Stopped-FI Method

The operational step employed in this method is described in Table 6.5.

Table 6.5 Method for determination of iodine by using the fixed time stopped-FI.

Step	Time interval(s)	Pump1 (rpm)	Pump2 (rpm)	Valve	Data collection time (s)
1	0	0	0	Fill	0
2	17	28	120	Fill	0
3	40	28	0	Fill	0
4	40	28	0	Inject	147
5	8	0	0	Inject	0
6	99	28	0	Inject	0

From Table 6.5, The peristaltic pump P2 was used for 17 s to fill a sample into the injection loop. Then, following an injection of a sample at 40 s, a reaction zone is kept for 8 s in the flow cell to monitor the absorbance of Ce (IV) (420 nm), every 1 s. Calibration plot between the –(logarithm of the absorbance) and the concentration of standard iodate solution (μg I/L) is made. Iodate concentrations are employed in range of 100 to 6000 μg I/L. A representative of the standard equation is $-\ln (A)_{8s} = (2.854E\text{-}4)[I] + (2.373E\text{-}1)$: $R^2 = 0.998$.

Example of the kinetic profile of this technique is presented in Table 6.10 Section 6.6.

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In Table 6.6, it is observed that when the stopped period is prolonged, the sensitivity (slope) increased. However this also increased the analysis time per one injection.

Table 6.6 Calibration equations of the fixed time stopped-FI method obtained at different stopped-time. These calibrations were obtained from used of iodate standards.

Stopped-time (s)	Calibration equation	Sample throughput (samples/h)	R^2
2	$-\ln A_{2s} = 2.21 \times 10^{-4} (\pm 3.1 \times 10^{-6}) [I] + 1.986 \times 10^{-1} (\pm 9.6 \times 10^{-3})$	85	0.999
8	$-\ln A_{8s} = 3.03 \times 10^{-4} (\pm 5.0 \times 10^{-6}) [I] + 2.21 \times 10^{-1} (\pm 1.6 \times 10^{-2})$	75	0.999
12	$-\ln A_{12s} = 3.59 \times 10^{-4} (\pm 6.8 \times 10^{-6}) [I] + 2.39 \times 10^{-1} (\pm 2.1 \times 10^{-2})$	69	0.999
30	$-\ln A_{30s} = 5.61 \times 10^{-4} (\pm 2.3 \times 10^{-5}) [I] + 3.63 \times 10^{-1} (\pm 7.2 \times 10^{-2})$	51	0.993
60	$-\ln A_{60s} = 7.11 \times 10^{-4} (\pm 7.4 \times 10^{-5}) [I] + 6.999 \times 10^{-1} (\pm 2.3 \times 10^{-1})$	36	0.959

For this work, 8 s was chosen as the optimal stopped-time, because this period provided an acceptable sensitivity and a reasonably fast analysis (sample throughput is at 75 samples/h.)

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6.2.2.3 Kinetic Continuous FI Method

The operational step of this method is presented in Table 6.7.

Table 6.7 Method for iodine measurement by applying the kinetic continuous-FI.

Step	Time interval (s)	Pump1 (rpm)	Pump2 (rpm)	Valve	Data collection time (s)
1	0	0	0	Fill	0
2	17	28	120	Fill	0
3	40	28	0	Fill	0
4	99	28	0	Inject	150
5	51	28	0	Inject	0

For this FI method, the reaction zone is continuously pumped through the detector. Changing of absorbance of Ce (IV) (420nm) in this zone is measured per 5 s. Iodate quantity in iodized salt can be measured by plotting the –logarithm of the absorbance of Ce (IV) at the maximum drop against concentration of standard iodate solution. The working range of iodate concentration is 100 to 6,000 μg I/L. A representative standard equation is $-\ln A = (1.881E\text{-}4)[I] + (1.682E\text{-}1)$; $R^2 = 0.9990$. For this method, the sample throughput is at 90 samples/h.

6.3 Application to Iodized Salt Samples

6.3.1 Test of Kinetic Interferences

Ten commercial iodized salts were first investigated for possible interferences to the Sandell-Kolthoff reaction. This work was studied in the term of fitting error. The procedure was carried out by using the full kinetic stopped-FI

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method. Each sample solution was injected in triplicate into the FI system shown in Figure 6.8.

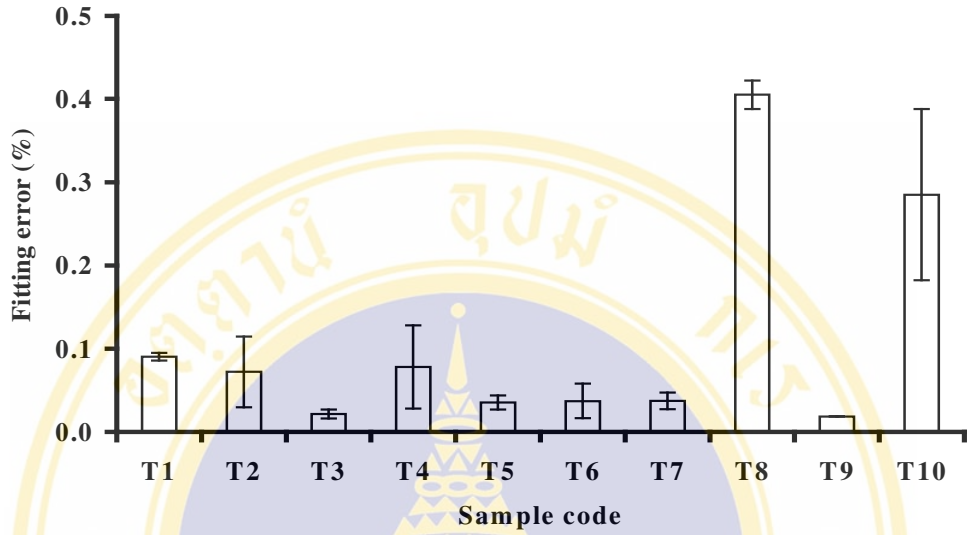

Figure 6.9 Interference studies carried out on ten commercial iodized salts (investigated in the term of the fitting error based on first-order kinetics).

From Figure 6.9, fitting error (%) obtained from all iodized salt samples (T1 to T10) were all less than the acceptable limit of 1. These results indicated that the considered samples have no interferences that affect to the Sandell-Kolthoff method. Thus, sample pretreatment was not required.

6.3.2 Analytical Recovery

The three operational methods, i.e., the full kinetic stopped-FI, the fixed time stopped-FI and the kinetic continuous-FI were employed in this work.

Recoveries of iodine analysis were studied by using the previous ten iodized salt samples (T1 to T10) and three synthetic samples (S1 to S3). The experiment was carried out by adding known quantity of iodate into the salt solutions. All of the samples were added to contain extra iodate of 1000 μg I/L except for the synthetic S3 (extra iodate of 500 μg I/L). The recoveries obtained from the kinetic

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continuous-FI method are shown in Table 6.8. Similar results of the full kinetic stopped-FI method and of the fixed time stopped-FI are presented in Section 5.3.2.

Table 6.8 Analytical recovery of the kinetic continuous-FI method, obtained from iodized salts. Each experiment was carried out using tripicate injections.

Sample code	Original content in sample solution (μg I/L)	Standard addition		Recovery (%)
		Added (μg I/L)	Found (μg I/L)	
T1	536.9 ± 6.96	1000	1682 ± 17.3	113 ± 1.5
T2	990.8 ± 4.18	1000	2279 ± 10.9	117 ± 1.4
T3	1336 ± 4.50	1000	2522 ± 23.0	108 ± 2.3
T4	1529 ± 0.00	1000	2679 ± 31.8	114 ± 3.1
T5	1982 ± 10.3	1000	3190 ± 23.8	110 ± 1.3
T6	2231 ± 14.3	1000	3384 ± 11.9	105 ± 0.3
T7	2203 ± 14.2	1000	3396 ± 20.7	109 ± 3.0
T8	2076 ± 24.6	1000	3101 ± 17.2	101 ± 3.0
T9	2429 ± 14.9	1000	3709 ± 36.7	120 ± 3.5
T10	2913 ± 12.3	1000	4013 ± 7.70	109 ± 0.5
S1	1123 ± 11.9	1000	2320 ± 5.60	117 ± 0.7
S2	2720 ± 16.0	1000	3837 ± 26.9	109 ± 3.1
S3	5401 ± 17.4	500.0	5937 ± 29.3	109 ± 4.5

T: commercial iodized salt

S: synthetic solution

According to Table 6.8, the kinetic continuous FI method gave sample recovery (%) between 101 to 120 (the average was 110.8%). These recoveries are acceptable for an analytical method.

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Also recoveries of the other two methods (the full kinetic stopped-FI method and the fixed time stopped-FI method) are summarized in Table 6.9.

Table 6.9 Recoveries obtained from all three proposed kinetic methods, as compared to the previously developed colorimetric-FI method.

Method	Recovery (%)	
	range	average
Kinetic continuous-FI (n=13)	101-120	111
Fixed time stopped-FI (n=13)	88-129	114
Full kinetic stopped-FI (n=13)	72-128	107
Colorimetric-FI* (n=12)	96 - 124	109

* The method was a stoichiometric analysis, previously presented by N. Choengchan [4].

In Table 6.9, the recoveries given by all of the proposed kinetic methods are satisfied and are comparable with the recovery obtained from the colorimetric-FI which had been employed in this work for validations.

These results indicated that the methods, based on Ce (IV) – As (III) reaction, could be an alternative for determination of iodine quantity in iodized salt. The results also confirm the results in Section 6.3.1 that sample pretreatment was not necessary.

6.3.3 Method Validation

This topic focused on the accuracy of the present methods (kinetic continuous-FI, the fixed time stopped-FI and the full kinetic stopped-FI). The experiments were carried out using triplicate injections per one sample. Ten iodized salts of different brands and three of synthetic samples were used.

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To consider the accuracy, the results obtained from (a) the kinetic continuous-FI, (b) the fixed time stopped-FI and (c) the full kinetic stopped-FI were validated against the colorimetric-FI [4] (Figure 6.10) using the statistical ANOVA test [50].

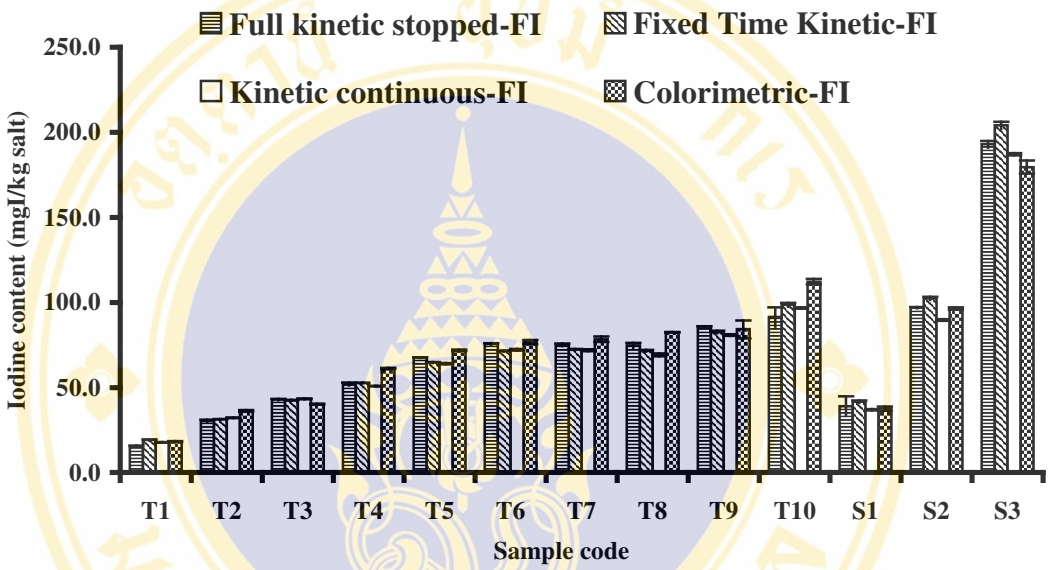

Figure 6.10 Iodine contents, obtained from four analytical methods (the full kinetic stopped-FI, the fixed time stopped-FI and the kinetic continuous-FI), in iodized salt (T1 to T10) and synthetic salt sample (S1 to S2).

According to the ANOVA test, the results provided by all methods were not significantly different at 95% confidence limit because their F value (0.044) is less than the F critical (2.798).

6.3.4 Precision of the Developed Methods and the Method Chosen for Real Application

The kinetic methods, that are continuous-FI, the fixed timed stopped-FI and the full kinetic stopped-FI, were considered for their precisions. The data obtained from triplicate injections of a sample are used for this consideration.

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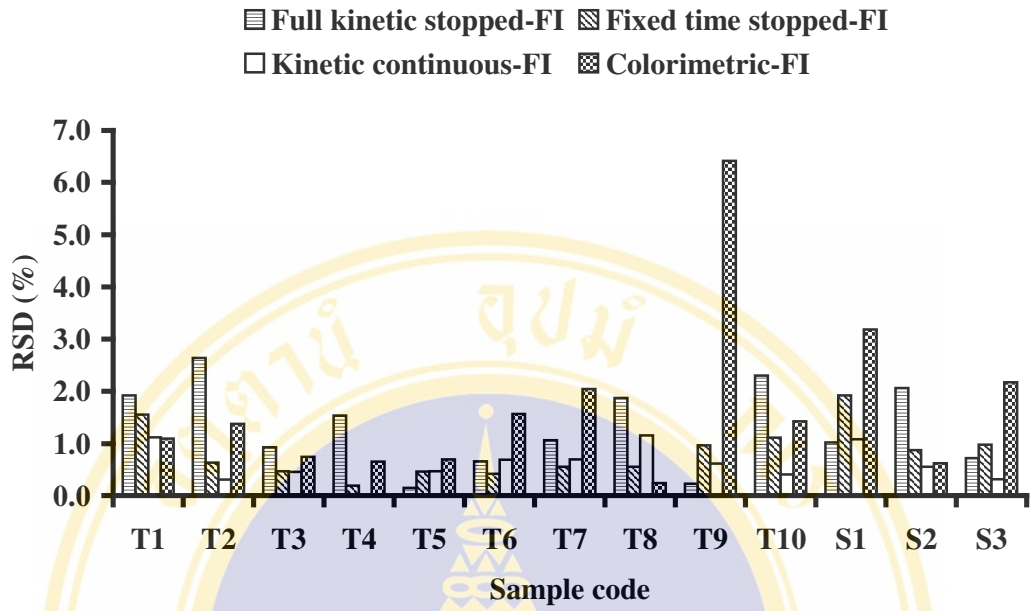

Figure 6.11 Precisions (RSD) of the four different methods used in iodine determinations carried out on iodized salts (T1 to T10) and synthetic samples (S1 to S3).

In Figure 6.11, the precisions were investigated in the term of RSD (%). T1 to T10 represented different brands of iodized salt.

The results show that the RSD, provided by all methods are acceptable (< 4%), barring the RSD of T9 analysed by the colorimetric-FI. The chemical reaction employed in colorimetric-FI method is based on detection of the tri-iodide starch complex. It is necessary to inject a plug of cleaning solution to wash off the FI system before the next analysis. This may cause poor precision in some measurements and may be the cause of the rather distinctively high RSD in T9 for the colorimetric FI method.

Results in Figure 6.11 indicated that all of the proposed kinetic methods can be used for determination of iodine (IO^-_3 form) in iodized salt. However, for real application in commercial iodized salt, the kinetic continuous-FI method is

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chosen. This method is the most convenient kinetic FI method and provides the highest sample throughput.

6.4 Limit of Detection

6.4.1 The Kinetic Continuous-FI Method

The limit of detection of this method was calculated based on three times the standard deviation of the mean of blank signals (n=10). The detection limit (3δ) of the kinetic continuous-FI method was 1.3 mg I/kg salt.

6.4.2 The Fixed Time Stopped-FI Method

Determination of the limit of detection was the same as that already defined in section 6.4.1. For this work, the detection limit was found to be 1.1 mg I/kg salt.

6.4.3 The Full Kinetic Stopped-FI Method

The method used to determine the limit of detection of this analytical method was based on that given by J.N. Miller [50]. That is the sum of the calculated intercept (a) and three times of the standard deviation of y-residuals ($s_{y/x}$):

$$\text{Limit of detection} = a + 3s_{y/x} \qquad (6.2)$$

where, $s_{y/x} = \sqrt{\frac{\Sigma(y_i - \hat{y}_i^2)}{n - 2}}$.

$\hat{y}_i$ = The points on the calculated regression line corresponding to the individual x-values.

y_i = The analytical signal.

n = Numbers of sample.

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From the calculation, the limit of detection was found to be 7.5 mg I/kg salt.

Normally iodine concentrations in Thai supplemented salts are in the range of 30 to 50 mg I/kg salt [19]. Therefore, these levels of detection limits (0.24 to 7.5 mg I/kg salt) implies that the proposed methods are applicable.

6.5 Repeatability of FI Method

Evaluation of the repeatability was carried out. The relative standard deviation (RSD) was used. Ten replicate injections of a standard iodate solution (2000 μg I/L) were made using the FI system in Figure 6.8. The RSD found for the kinetic continuous-FI was equal to 0.17 %. The fixed time stopped-FI method gave the RSD of 0.63 %. For determination of the repeatability of the full kinetic stopped-FI method, standard iodate solution (2000 mg I/L) was triplicate injected into the FI system (Figure 6.8). The RSD obtained by the full kinetic stopped-FI method was 0.83 %. These results show reasonably good repeatabilities.

6.6 Feature Comparison amongst the Three Kinetic Methods

The characteristics obtained from the three proposed operational methods are summarized and compared in Table 6.10.

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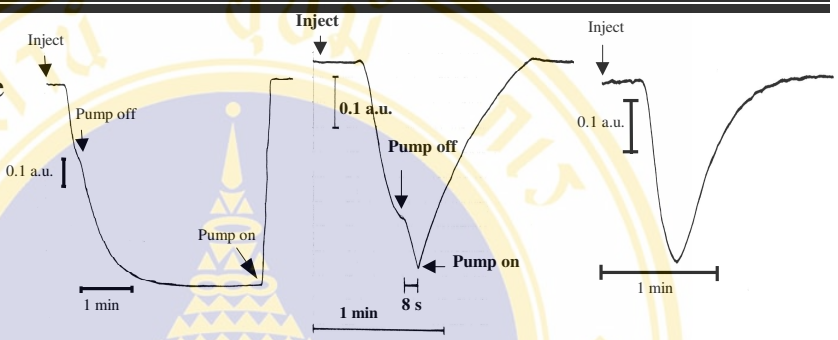
Table 6.10 Feature comparison of the following proposed methods: (a) the full kinetic stopped-FI, (b) the fixed time stopped-FI and (c) the kinetic continuous-FI method.

	Operational method		
	Full kinetic stopped-FI	Fixed time stopped-FI	Kinetic continuous-FI
Example of kinetic profile given by iodate (2000 μg I/L)			
Sample throughput	Depends on the concentration of iodine e.g. 1.7 samples/h[a]	75 samples/h	90 samples/h

[a] For example, the analysis time obtained from an iodate 100 μg I/L is 35 min.

[b] obtained from iodate concentrations in range of 100 to 6,000 μg I/L.

[c] Reported in the term of RSD (%). Determinations of the precision of three presented methods are presented in Section 6.3.4.

[d] The definitions of detection limit of each method are described in Section 6.4.

[e] Reported in the term of RSD (%). The values obtained from the kinetic continuous-FI method and the fixed time stopped-FI method using ten replicate injections of standard iodate solution 2,000 μg I/L and that obtained from the full kinetic stopped-FI method using triplicate injections of standard iodate solution 2,000 μg I/L.

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Table 6.10 (continued).

	Operational method		
	Full kinetic stopped-FI	Fixed time stopped-FI	Kinetic continuous-FI
Provide kinetic data and simultaneously check for presence of interference giving rise to non-exponential	Yes	No	No
Linear equation [b]	k = 1.25E-3 [I] + 3.23E-3	$-\ln A_{8sec}$ = 3.03E-4 [I] + 2.21E-1	-ln A = 1.88E-4 [I] + 1.68 E-1
Precision [c]	< 2.6 %	< 1.9 %	< 1.2 %
Limit of detection [d]	7.5 mg I/L	1.1 mg I/L	1.3 mg I/L
Repeatability [e]	0.83 %	0.63 %	0.17 %

[a] For example, the analysis time obtained from an iodate 100 μg I/L is 35 min.

[b] obtained from iodate concentrations in range of 100 to 6,000 μg I/L.

[c] Reported in the term of RSD (%). Determinations of the precision of three presented methods are presented in Section 6.3.4.

[d] The definitions of detection limit of each method are described in Section 6.4.

[e] Reported in the term of RSD (%). The values obtained from the kinetic continuous-FI method and the fixed time stopped-FI method using ten replicate injections of standard iodate solution 2,000 μg I/L and that obtained from the full kinetic stopped-FI method using triplicate injections of standard iodate solution 2,000 μg I/L.

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FI profiles obtained from these operational methods are different. The three operational methods have adequate sensitivity for analysis iodine content in commercial iodized salt. However, the full kinetic stopped-FI method gives a longer analysis time than the other two methods.

For the methods of fixed time stopped-FI and the kinetic continuous-FI, the latter method is more convenient. The continuous FI does not required resting time of the reaction zone inside the detector. This therefore brings in a faster analysis time (90 samples/hour) than the fixed-time method (75 samples/hour).

It can be concluded that the kinetic continuous-FI method is the most suitable method for routine application of iodized table salt. However all of the kinetic methods developed in this work were used in the analysis of 13 samples for validations.

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CHAPTER VII
CONCLUSION

The work presented in this thesis can be summarized as the followings.

(a) A kinetic flow injection method for the quantitation of iodine in commercial iodized salt was developed. The FI system was modified and optimized from the system applied in drinking water developed by N. Choengchan's [20]. This kinetic FI method for salt employed the well-known method presented by Sandell-Kolthoff. Kinetics of the indicator reaction between Ce (IV) and As (III) was exploited in flow injection manner. In this work, both continuous and stopped-flow modes were exploited.

(b) Similar to the method previously presented for drinking water, the FI system for salt was optimized to gain a condition in which the reactor kinetics between Ce (IV) and As (III) is first order in iodide concentration. In the optimization the FI system was operated in stopped-mode in order to obtain the kinetic data. For this, the absorbance of Ce (IV) of an arresting plug inside the flow cell was monitored until zero absorbance was reached. SigmaPlot software was used to obtain an exponential fitting and the rate constant. Quality of the curve fitting, presented as 'fitting error', was used throughout the work to evaluate the kinetic results.

(c) To determine iodine in the form of iodate, present in local iodized salts, the FI system was assembled to allow in-line reduction of iodate to iodide prior the kinetic analysis within the system. Iodate itself can interfere this method of analysis and thus it is necessary to guarantee for 100 % reduction to iodide. This work utilized arsenous acid for the reduction [19]. The FI system was optimized to give and in-line 100 % conversion of iodate to iodide.

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(d) This work presents three possible operational methods of the developed system for iodine quantitation. These techniques are full kinetic stopped-FI, fixed time stopped-FI and kinetic continuous-FI. All methods provided linear calibration plots in the working range of 100 to 6,000 μg I/L with linear least squares of 0.99xx. Amongst the three methods, the kinetic continuous-FI gave a highest sample throughput (90 samples/h) whereas the full kinetic stopped-FI exhibits the lowest sample throughput.

(e) All of the FI methods developed were successfully validated. The system provides satisfactorily good recoveries and precisions.

(f) Amongst the proposed operational methods, the kinetic continuous-FI method was chosen for real application in commercial iodized salt. Arresting the reaction zone in detector cell to improve the sensitivity is not essential for iodine levels found in commercial iodized salt. The kinetic continuous-FI method is based on the basis of fixed-time analysis.

(g) Detection limit of the kinetic continuous-FI was 1.3 mg I/kg salt. This value was less than the range of iodine concentration that is usually found in commercial iodized salt (30-50 mg I/kg salt). Therefore, the kinetic continuous-FI can be useful for quantitative measurement of iodine in table salt.

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APPENDIX A

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APPENDIX A

Conventional stopped-flow technique

This appendix is taken from the Physical Chemistry with Biochemical Application written by K. J. Laidler [48] The first difficulty can sometimes be surmounted by using special techniques for bringing the reactants very rapidly into the reaction vessel and for mixing them very rapidly. Normally, with the use of conventional techniques, it takes from several seconds to a minute to bring solutions into a reaction vessel, and to have them completely mixed at the temperature of the surroundings. This time can be reduced greatly by using a rapid flow, and flow techniques are frequently employed for rapid reactions. One particular modification of these methods is the stopped-FI technique, shown schematically in Figure A1. This particular apparatus is designed for the study of a reaction between two substances in solution. A solution of one of the substances is maintained initially in syringe A, and a solution of the other in syringe B. The plungers of the syringes can be forced down rapidly, perhaps by mechanical means and a rapid stream of the two solutions passes into the mixing system. This is designed in such a way that jets of the two solutions impinge on one another and give very rapid mixing; with a suitable design of the mixing chamber it is possible for mixing to be essentially complete in one-thousandth of a second. From this mixing chamber the solution passes at once into the reaction cuvette. Alternatively, the two may be combined.

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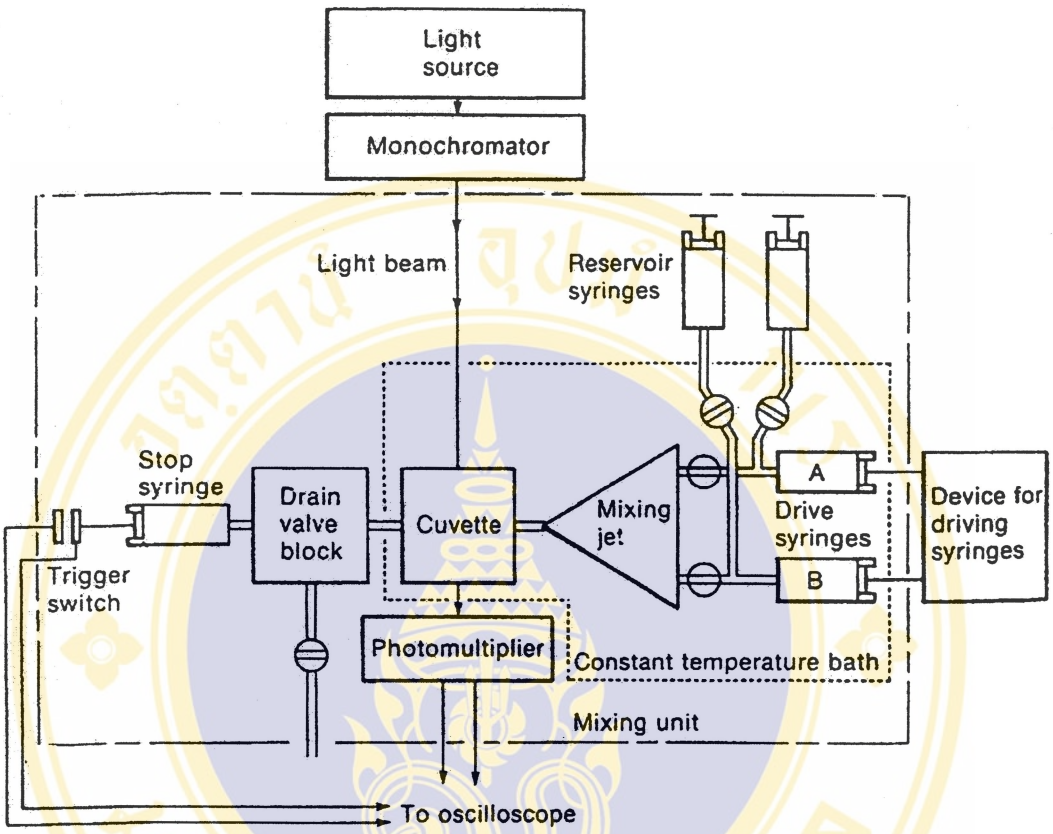

Figure A1 Schematic diagram of stopped-flow apparatus. Solutions in the two drive syringes A and B are rapidly forced through a mixing jet into a cuvette. When the flow is stopped, the oscilloscope is triggered and records light absorption as a function of time [48].

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APPENDIX B

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APPENDIX B

User's Guide for Flow Injection Analysis System

Instruments as shown in Figure A2 were used for this thesis.

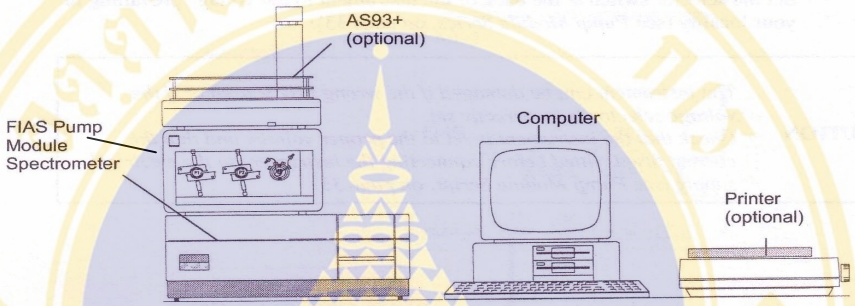
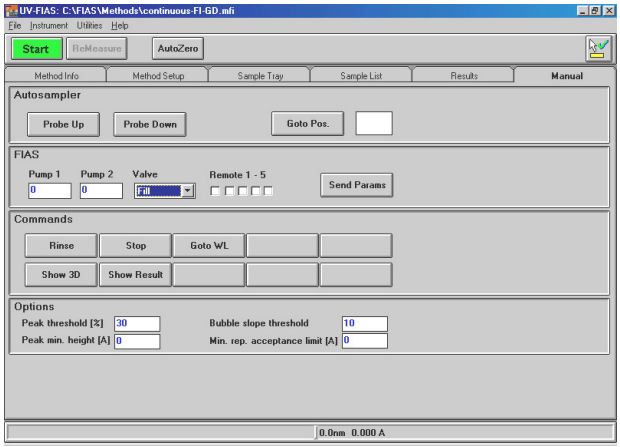


Figure A2 Typical Lamba FIAS system.

System Sartup

1. Switch on a Lambda 25 UV/VIS spectrometer, FIAS pump module (FIAS-300), PC and printer, respectively.
2. Open **UV-Fias** software.
3. Set up the tubing and magazines on the FIAS pump module.
4. Connect the drain tubing to the empty waste container.
5. Check the alignment of a flow cell in the sample beam.
6. Click the **Manual** tab



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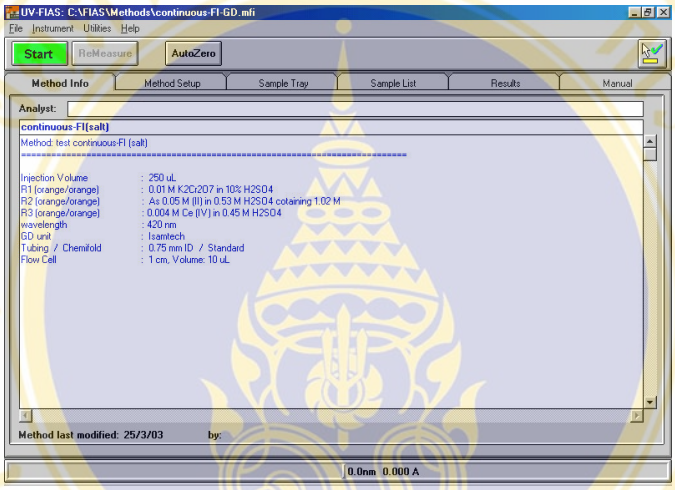
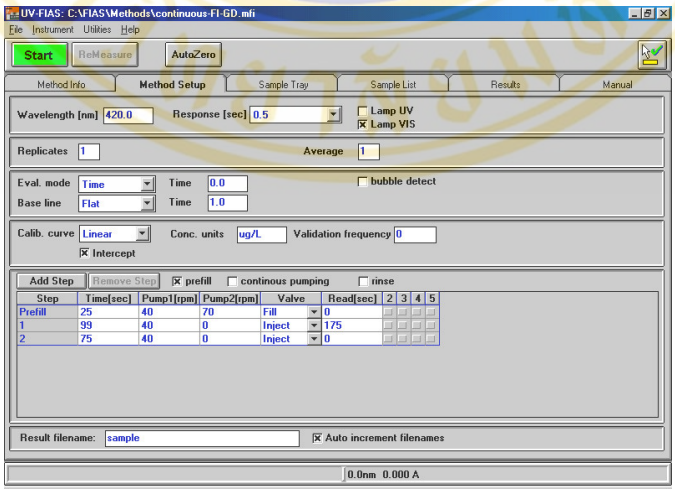
7. Set the pump speed of **P1** to measure the flow rate of carrier and reagent solutions or **P2** to determine the flow rate of sample solution.
8. Click the **Send Params** (Send Parameter) tab.
9. Select the **Stop** tab when determination of the flow rates were succeeded.

Running Analyses with UV-FIAS Software

1. Select the **Method Info** tab and type a short detail of the methodology.



2. Click the **Method Setup** tab to create the operational step.



3. Click **File** menu and then select **Method Save**.
4. Select the **Manual** tab.
5. Adjust the zero absorbance by set the pump speed of **P1** and select the **Send Parameter** to pump carrier and/or reagent solution into the system.

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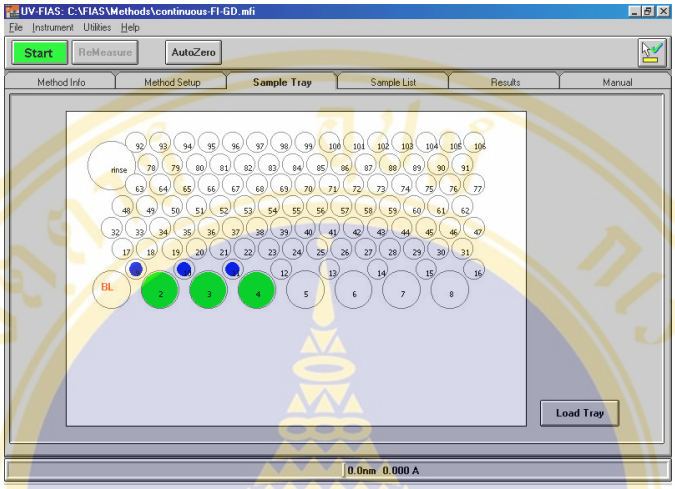
6. Click **Autozero** when the absorbance becomes constant.
7. Insert blank, standard and sample solution into the AS93+ Autosampler.
8. Select the **Sample Tray** tab.



Define : a) **Holding SHIFT** and **Left-clicking** on the corresponding circle to define the blank tube. The text BL is displayed over the desired circle.

b) **Holding CTRL** and **Left-clicking** on the corresponding circle to define the standard tubes. These tubes are shown as green color over the desired circle.

c) **Left-clicking** on the corresponding circle to define the sample tube. These tubes are displayed as blue color over the desired circle.

Deselect : a) **Holding SHIFT** and **right-clicking** on the desired circle to cancel the blank tube.

b) **Holding CTRL** and **right-clicking** on the desired circle to cancel the standard tube.

c) **Right-clicking** on the desired circle to cancel the sample tube.

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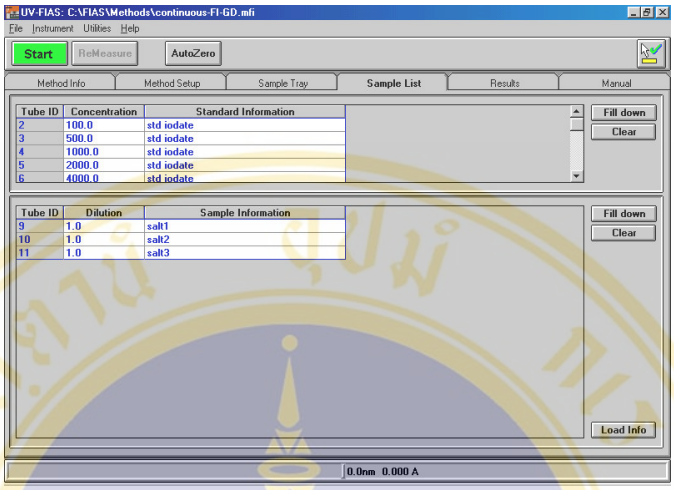
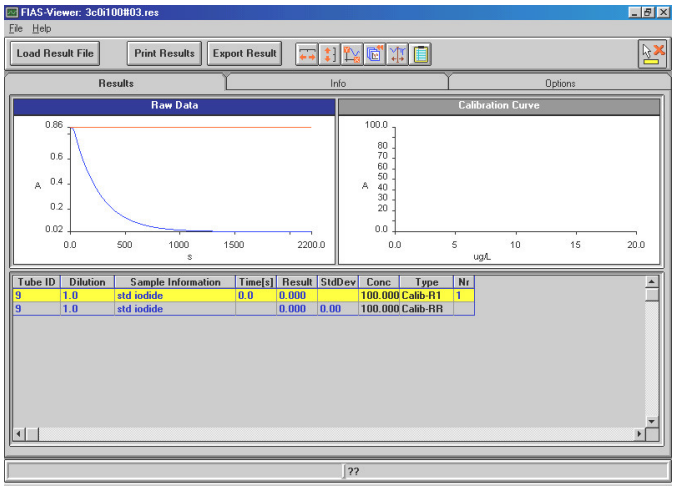
9. Select the **Sample List** tab. This part describes information of the standards and samples on the tray.



10. Type the details of each standard and sample tube.
11. Check the method settings on the **Method Setup** tab.
12. Click **Start** to begin the FIAS method.
13. Check the results by clicking the **Results** tab. If addition of sample information is required. Wait for the FIAS analysis to complete or press **Stop** to end the run prematurely.
14. Export the results to Excel or print them by selecting the **UV-FIAS Viewer** program when the process is finished.
15. Click **Load Result File**. Select a wanted file and click **open**.
16. Clicking on a row in the grid shows a graph of the data in the graph window, a yellow bar will be displayed.



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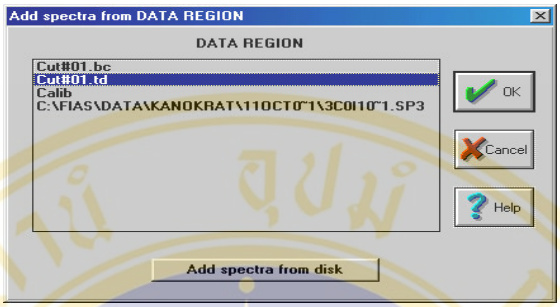
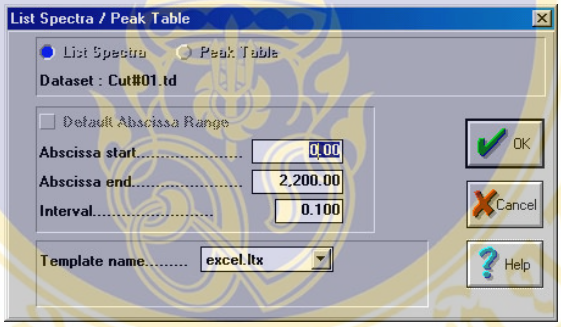
17. Select the **Lambda 25** software.
18. Click **View** menu and then select **Add spectrum**.
19. Select **Cut#01.td** and click **OK**.



20. Click **File** menu, select **Save As** to save the spectrum.
21. Click **Data handling** menu.
22. Click **List** and select Template name = excel.ltx.



23. Click **File** menu, select **Save As** to save the data.

Switching off the System

1. Select the **Manual** tab in the UV-Fias program.
2. Rinse the system with a suitable solution by set the pump speed **P1** and **P2**.
3. Click **Send Parameter**.
4. Set the pump speed P1 and P2 as zero, when a constant absorption is displayed.
5. Click **Send Parameter**.
6. Release the pressure at the pump tubing by pushing the magazines.
7. Exit the **UV-Fias program**, the **UV-Fias View** program and the **Lambda 25** program.

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8. Switch off the system.

Information in Method Setup Tab

Parameter	Description
Wavelength	Wavelength is measured in nm.
Response	Response time in s. This function suppresses the noise.
Lamps	Select the lamps in operation. ☒ = Lamp on ☐ = Lamp off Swich off the lamp to conserve lamp lifetime, if it is not required. However, the lifetime of the deuterium lamp will be reduced markedly, if it is often switched on and off. Therefore, switch off the deuterium lamp only when it is not required for a prolonged period.
Replicates	Number of replication determination per one sample.
Acceptance limit	The tolerance value in percentage for the standard deviation of replicate measurement.
Average	Selects the number of sample measurements to be used for the calculation of mean and standard deviation.

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Parameter	Description
Eval. Mode	**Auto** Automatic search for peak maxima over the total measurement range. A baseline point is measured at the start of the measurement time. **Time** Evaluation at a set time point. This is suitable for the gradient dilution technique and stopped flow technique. Further parameters: Time: in s Base line: no = no baseline correction Flat=1 baseline point Slope=2 baseline points Time/range=1or 2 timepoints **Peak Max/Min** Peak height. Further parameters as baseline. **Area** Calculates area below the spectral curve. Further parameters as baseline. **Bubble detect** Function to recognize air bubbles. ☐ all peaks are treated as normal signal. ☒ all peaks with steep flanks are treated as air peaks, the measure ment is repeated and an error

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Parameter	Despcription
	message is shown
Calib. Curve	Selects the mathematical function, which determines the number of data points (reference solutions) required to establish the calibration curve. **Conc. Units** Enter the units to be appended to values in the results tab and file. **Validation** After the entered number of measurements, the first standard is measured. If the relative difference is greater than the acceptance limit (below), a recalibration is performed. **Acceptance limit** Maxima relative difference, in percent.
Add Step/Remove step	Adds or removes a step at the highlighted line. If no line is highlighted, a step is added at the end.
Prefill	☒ Adds a prefill step to the grid. ☐ Removes prefill step.

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Parameter	Description
Continuous pumping	☒ Pump 1 continues pumping at the speed of the last step. ☐ Pump 1 stops between measurements. Pump 2 always stops between measurements. At the end of the run, both pumps are stopped.
Rinse	☒ After each measurement, the system rinses at 100 rpm for the specified number of seconds. This avoids carry over between measurements. ☐ No rinsing.
Step	This is the FIAS step number. If a prefill step is present and the number of replicates is greater than one, the prefill is only carried out when a new tube is introduced, before the first measurement.
Time	The duration of the step, in seconds.
Valve	Fill : Fills the sample loop. Inject: Injects the sample into the carrier stream.
Pump1and Pump 2	The speed of pump 1 or pump 2 in rpm. The recommended initial value is 50 rpm; the flow rate also depends on the diameter of the tube. Pump 2 is generally selected as the sample pump. Only start this when the valve is set to Fill.

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Parameter	Description
Read	Measurement time in seconds. **0** : no measurement. The measurement time runs from the start of the step. It can be longer or shorter than the step time. The measurement time should start from the injection of the sample. Only one read step is allowed per analysis, and this should start at the point of injection at the latest.
Result filemane	Enter a path and filename for the results. UV-FIAS saves two files, an Excel-compatible ASCII file and a binary .sp3 raw data file.
Auto increment filename	UV-FIAS intelligently increments the filenames for data. Enter a filename with five cahracters in the Result filename box, and UV-FIAS will add, for example, 001. If this number already exists, it will use the next available unique suffix.

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Operational methods of the full kinetic stopped-FI method, the fixed time stopped-FI method and the kinetic continuous-FI method for iodine quantity measurement in commercial iodized salt are presented in Figure A3, A4 and A5, respectively.

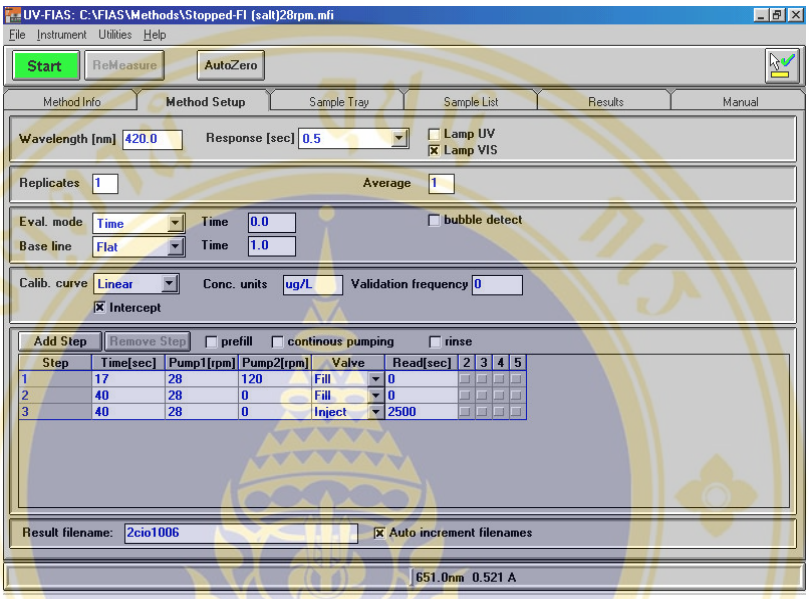

Figure A3. Determination of iodine content in iodized salt by using the full kinetic stopped-FI method.

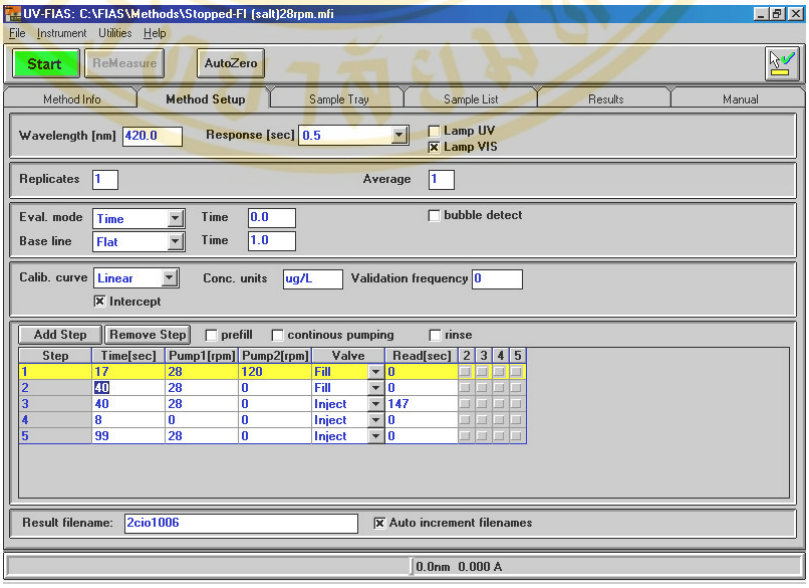

Figure A4. Determination of iodine content in iodized salt by employing the fixed time stopped-FI method.

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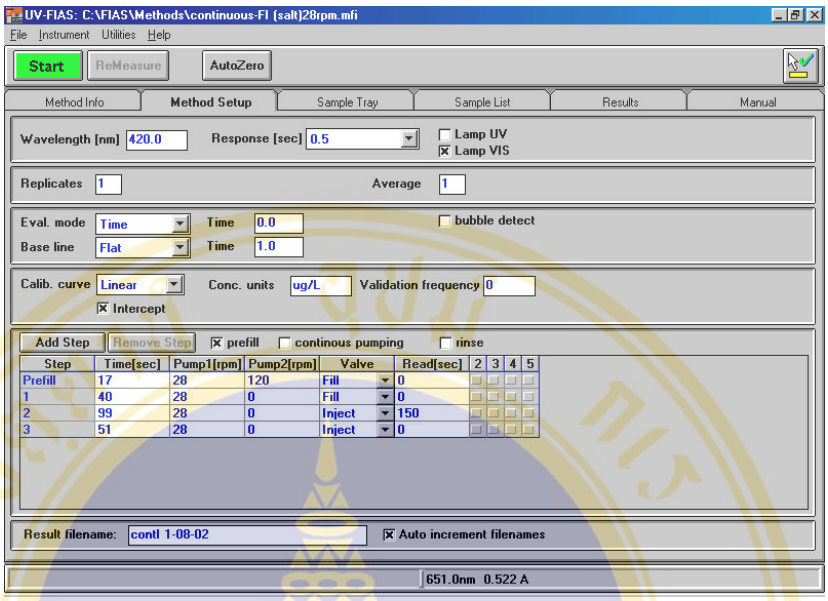

Figure A5. Determination of iodine content in iodized salt by using the kinetic continuous-FI method.

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APPENDIX C

Effect of length of straight tube 'A'

The statistical regression test [50] was also used for investigation of the reduction of iodate to iodide by arsenous acid when length of 2 mm i.d. straight tube at position 'A' (Figure 6.2) was varied at 5, 10, 15 and 30 cm and without tubing 'A'. By means of this statistic, if iodate is absolutely converted to iodide, rate constant given by iodate is equal to that given by iodide as the same iodine concentration. Therefore, slope and intercept of graph plotting between the rate constants provided by iodate (y-axis) against those provided by iodide (x-axis) are 1.000 and 0.000, respectively.

Table A1.Comparison between the calibrations given by iodate standards as compared to iodide.

Length of 'A' (cm)	Parameter	
	$a \pm s_a$ (minimum to maximum)	$c \pm s_c$ (minimum to maximum)
0	0.9561 ± 0.0312	0.0007 ± 0.0028
	(0.9249 to 0.9874)	(-0.0020 to 0.0035)
5	0.9927 ± 0.0245	0.0002 ± 0.0021
	(0.9682 to 1.0173)	(-0.0019 to 0.0022)
10	0.9861 ± 0.0341	-0.0004 ± 0.0026
	(0.9520 to 1.0202)	(-0.0030 to 0.0021)
15	0.9971 ± 0.0241	0.0002 ± 0.0015
	(0.9730 to 1.0212)	(-0.0013 to 0.0017)
30	0.9858 ± 0.0302	0.0002 ± 0.0006
	(0.9741 to 1.0160)	(-0.0005 to 0.0008)

s_a and s_c are standard deviations of slope and intercept, respectively.

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In Table A1, at confidential limit of 95 %, the slopes and the intercepts of the calibration plots obtained from using of straight tube ‘A’ (2mm i.d.) at 5, 10, 15 and 30 cm in length were within 1.000 and 0.000, respectively. These indicated that completeness of conversion of iodate to iodide was achieved when length of 2 mm i.d. straight tube ‘A’ was employed at 5, 10, 15 and 30 cm. However, when not employing any tube at position ‘A’ (Figure 6.2), the slope and the intercept obtained from this condition were not within 1.000 and 0.000, respectively. In this case, it implied that iodate was not completely reduced to iodide.

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APPENDIX D

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APPENDIX D

Studied of effects of some reducing species on the kinetic Sandell and Kolthoff method

1. Introduction

Some colleague in our laboratory developed a FI system for determination of total iodine in urine by using the Sandell-Kolthoff reaction [14].

It is well-know that iodide is the catalyst of the Ce (IV)-As (III) reaction. Catalysis, iodide is reduced to I^0 by Ce^{4+}. However, not only iodide ion can reduce Ce (IV) to Ce (III) but also other reducing species. Thus, presence of other reducing species in the samples can interfere the determination by most likely giving positive.

In this work, interference effect on kinetic determination of iodide based on Ce (IV)-As (III) reaction was studied. Thiocyanate and ascorbic acid as reducing agents were the interferents of interest. They are sometimes found in urine sample.

2. Materials and Methods

2.1 Instrument

2.1.1 A Hewlett-Packard 8453 UV-Visible spectrometer (USA) was used for study of interference effect.

2.1.2 A thermostated bath circulation of NESLAB RTE-111 (USA) was used for temperature control of the reagents and the cuvette during study on interference effect.

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2.2 Preparation of Reagent Solutions

2.2.1 Reduction of Ce (IV) by Thiocyanate and a Ascorbic Acid as compared to the Reduction by Iodide

2.2.1.1 Iodide Solutions

(i) Stock Solution of Iodide (1.0 M)

16.6080 g of KI was dissolved in deionized water and made to 100.0 ml.

(ii) Secondary Stock Solution of Iodide (0.005 M)

0.5 ml of the stock solution (1.0 M) was transferred into a 100.0-ml volumetric flask and was made to the mark using deionized-distilled water.

2.2.1.2 Thiocyanate solutions

(i) 1.0 M Thiocyanate Solution (SCN^-)

This solution was prepared by dissolving 9.7180 g of solid potassium thiocyanate in deionized water and made up to 100.0 ml.

(ii) 0.005 M Thiocyanate Solution

0.5 ml of 1.0 M SCN^- was delivered into 100.0-ml volumetric flask. This solution was made up to 100.0 ml with deionized water.

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2.2.1.3 Ascorbic acid

(i) 1.0 M Ascorbic Acid ($C_6H_8O_6$)

To prepare this solution, 17.6130 g of ascorbic acid was dissolved in deionized-distilled water and made up to 100.0 ml.

(ii) 0.005 M Ascorbic Acid

0.5 ml of 1.0 M ascorbic acid was pipetted into 100.0-ml volumetric flask and was made to the mark using deionized-distilled water.

2.2.1.4 Ceric Sulfate Solution (0.005 M)

Solid ceric sulfate ($Ce(SO_4)_2.4H_2O$) 2.0369 g was dissolved in approximately 100 ml of deionized water. After the solution was transparent, 25 ml of concentrated sulfuric acid was added to the solution. Then, this solution was made to 1000.0 ml with deionized-distilled water to acquire 0.45 M H_2SO_4.

2.2.2 Investigation of the Interference Effect on the Sandell-Kolthoff Method

2.2.2.1 Iodide solutions

(i) Stock Solution of Iodide (1000 mg I/L)

For iodide stock solution, 0.1308 g of KI (s) was dissolved in deionized-distilled water to 100.0 ml.

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(ii) Secondary stock solution of iodide (10 mg I/L)

1.0 ml of the stock iodide solution (1000 mg I/L) was diluted to 100.0 ml using deionized-distilled water.

(iii) Iodide Solution (1000 μg I/L)

To prepare this solution, 10.0 ml of the secondary stock solution (10 mg I/L) was pipetted into a 100.0-ml volumetric flask. This solution was made to mark using deionized-distilled water.

2.2.2.2 Thiocyanate solutions

(i) 0.01 M Thiocyanate Solution (SCN^-)

For preparation of this solution, 1.0 ml of 1 M SCN^-, which was described in Section 2.1.2 (i), was delivered into 100.0-ml volumetric flask. This solution was then diluted to 100.0 ml with deionized-distilled water.

(ii) 3.2×10^{-5} M Thiocyanate Solution

80 μl of 0.01 M SCN^- was transferred into 25.0-ml volumetric flask and was made to mark using deionized-distilled water.

(iii) 8×10^{-5} M Thiocyanate Solution

The method for preparation of this solution was similar to the procedure described in Section 2.2.2 (ii) but used 0.2 ml of 0.01 M SCN^- instead.

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(iv) 1.6 x 10^{-4} M Thiocyanate Solution

0.4 ml of 0.01 M SCN^- was added into 25.0-ml volumetric flask and then used deionized-distilled water to make volume.

(v) 3.2 x 10^{-4} M Thiocyanate Solution

he preparation method for this solution was as same as the procedure that was offered in Section 2.2.2 (ii). However, 0.8 ml of 0.01 M SCN^- was used instead.

(vi) 8 x 10^{-4} M Thiocyanate Solution

2.0 ml of 0.01 M SCN^- was pipetted into 25.0-ml volumetric flask and was then diluted with deionized-distilled water to 25.0 ml.

2.2.2.3 Ascorbic acid

(i) 0.01 M Ascorbic Acid ($C_6H_8O_6$)

This solution was prepared by transferring of 1.0 ml of 1 M ascorbic acid that was obtained from section 4.5.6.1.3 (i), into the 100.0-ml volumetric flask and was made volume to 100.0 ml with deionized-distilled water.

(ii) 8x10^{-5} M Ascorbic Acid

To prepare this solution, 0.2 ml of 0.01 M ascorbic acid was delivered into 25.0 ml volumetric flask and was diluted into 25.0 ml with deionized-distilled water.

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(iii) 8×10^{-4} M Ascorbic Acid

The procedure was similar to the preparation in Section 2.2.3 (ii), but 2.0 ml of 0.01 M ascorbic acid was used instead.

(iv) 3.2×10^{-3} M Ascorbic Acid

The method for preparation of this solution as same as the procedure in Section 2.2.3 (ii), but 8.0 ml of 0.01 M ascorbic acid was employed instead.

(v) 4.8×10^{-3} M Ascorbic Acid

This solution was prepared by using the preparation method as same as the procedure that was described in Section 2.2.3 (ii), but 12.0 ml of 0.01 M ascorbic acid was applied instead.

(vi) 7.2×10^{-3} M Ascorbic Acid

The method for preparation of this solution was similar to the procedure in Section 2.2.3 (ii). However, 18.0 ml of 0.01 M ascorbic acid was pipetted instead.

2.2.2.4 Arsenious Acid Solution (0.04 M) in 0.36 M H_2SO_4

Arsenic trioxide powder (As_2O_3) 4.0225 g and sodium hydroxide pellets (NaOH) 6 g were together dissolved in approximately 100 ml of deionized water. After the mixture was clear, 20 ml of conc. H_2SO_4 was added into this solution. This was made the volume to 1000.0 ml with deionized water.

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2.3 Method for studying of interference effect

All following sections were carried out by batch analysis.

2.3.1 Reduction of Ce (IV) by thiocyanate and ascorbic acid as compared with to the reduction by iodide.

(a) Investigation on reaction between Ce (IV) and a true iodide catalyst

- Pipette 0, 50, 100, 150 and 200 µl of 0.005 M potassium iodide solution, respectively, into a quartz cuvette and add deionized-distilled water to make the volume into 2.25 ml and shake well. For example, 50 µl of 0.005 M potassium iodide was transferred into a quartz cuvette. Then, 2.2 ml of deionized-distilled water was added into the same cuvette and shake well.

- Add 250 µl of 0.005 M ceric sulfate solution, and shake well.

- Exactly 15 second after addition of Ce (IV), the absorbance at wavelength at 380 nm was measured.

- An absorbance of Ce (IV) and a iodide concentration (M) was plotted for this work.

(b) Investigation on reaction between Ce (IV) and some reducing agent

The method for this work was similar to the procedure described in Section 3.1 (a), but used 0, 25, 50, 100 and 200 µl of 0.005 M potassium thiocyanate solution or 0, 25, 50, 100 and 200 µl of 0.005 M ascorbic acid, respectively, instead of iodide.

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2.3.2 Investigation of the interference effect on the Sandell-Kolthoff method

Concentrations of thiocyanate solution that were prepared in Section 2.2.2 (ii) to 2.2.2 (vi) and concentrations of ascorbic acid that were obtained from Section 2.2.3 (i) to 2.2.3 (vi) were used for this work.

- Pipette 125 µl of thiocyanate solution or ascorbic acid into a quartz cuvette. Then, add 250 µl of deionized water and shake well.

- Add 125 µl of iodide solution (1000 µg I/L) and shake well.

- Add 1.0 ml of 0.04 M arsenious acid solution and shake well.

- Add 1.0 ml of 0.005 M ceric sulfate solution and shake well.

- After addition of Ce (IV) at exactly 15 seconds, the absorbance of Ce (IV) at 420 nm was recorded, every 20 seconds. The experiment was carried out by fixing the reduction time at 1 min. The SigmaPlot2000 software was employed to fit theoretical exponential equation with the experimental data plotted between the absorbance and time.

Blank of this method was performed as same as this above procedure, but 375 µl of deionized water was used instead of the first step.

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3. Results and Discussion

3.1 Reduction of Ce (IV) by Thiocyanate and Ascorbic Acid as compared to the Reduction by Iodide.

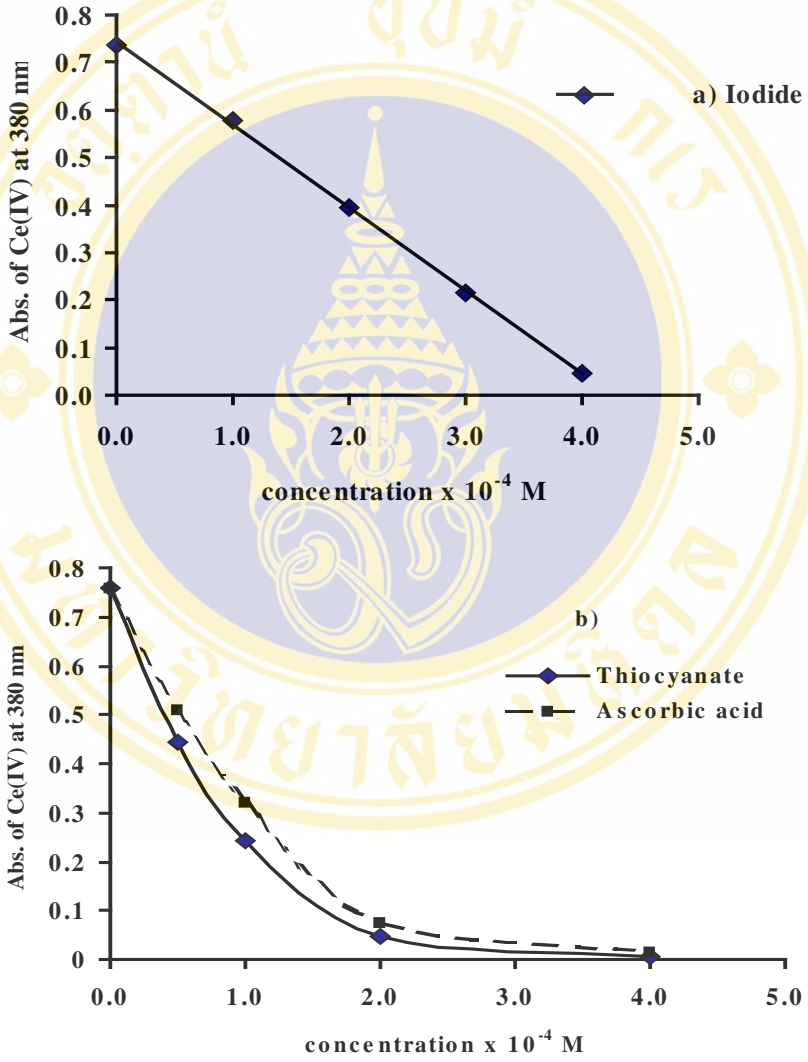

Figure A6 Absorbance change of Ce(IV) with (a) concentration of iodide and with (b) concentrations of thiocyanate and ascorbic acid. The concentration of Ce (IV) was fixed at 5 x 10^{-4} M (in cuvette).

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This experiment was carried out, according to that described in Section 3.1 (a) and Section 3.1 (b) by adding a reducing species and 250 µl of Ce (IV) solution (5 x 10^{-3} M) into a quartz cuvette. The absorbance of Ce (IV) at 380 nm was measured. This wavelength was not interfered by iodine that is the product in the reaction of iodide and Ce (IV). It was found that the absorbance of Ce (IV) did not change with time after mixing. The net reaction is

$$2Ce\ (IV) + 2I^- \longrightarrow 2Ce\ (III) + I_2 \qquad (6.3)$$

At higher concentration of iodide, 4 x 10^{-4} M for example, I_3^- was formed from I_2 product because of the excess iodide. This product interferes with the absorbance measurement. Therefore, the experiment was no longer carried out at the concentrations higher than 4 x 10^{-4} M of iodide.

According to Figure A6(b), the absorbance of Ce (IV) decreased with increasing concentrations of both thiocyanate and ascorbic acid. As expected, these two species, like iodide (Figure A6(a)), reduce Ce (IV) to Ce (III). Thus, they can interfere this analytical method if they are present in the samples.

3.2 Investigation of the Interference Effect on the Sandell-Kolthoff Method.

The experiment was carried out by allowing to have thiocyanate or ascorbic acid present in the same mixture of the Ce (IV)-As (III) and iodide . An attempt has been made to fit the kinetic data with the theoretical first order curve. Fitting errors (%), giving by the software, SigmaPlot 2000, were plotted against the concentration of each reducing agent as shown in Figure A7.

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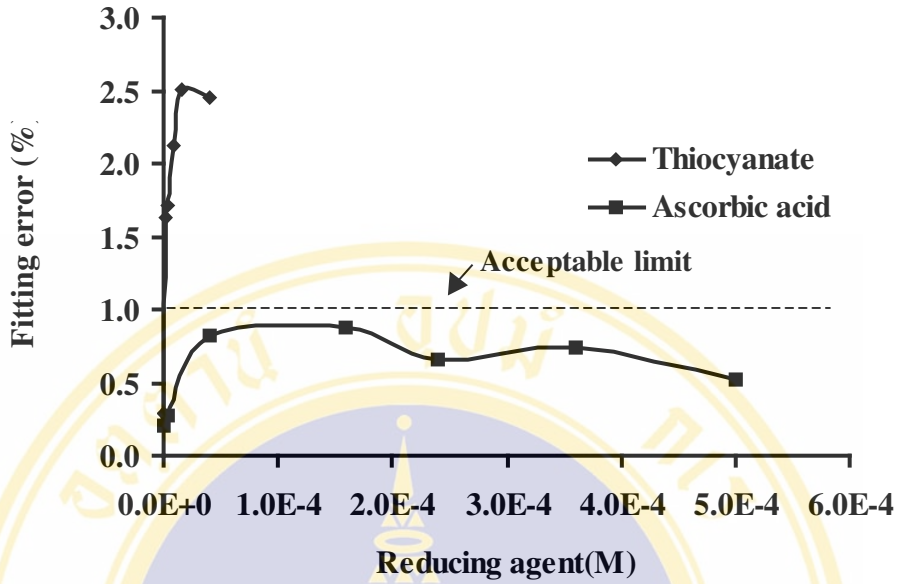

Figure A7 Effect of reducing species on the character of the first order kinetics for Ce (IV)- As (III) reaction which are represented by fitting error (%). Experimental condition: $[I^-] = 3.9 \times 10^{-7}$ M, [Ce (IV)] = 2×10^{-3} and [As (III)] = 1.6×10^{-2} M (in cuvette).

Figure A7, clearly demonstrates that the fitting error (%) increased with increasing in concentration of thiocyanate and ascorbic acid. However, thiocyanate has more effect than ascorbic acid. Increasing in fitting error (%) caused by thiocyanate, reflects that the catalysed reaction has lost the character of being the first order process (its kinetic profile was not a single exponential) decay. Ascorbic acid used in the concentration range shown in Figure 8A did not interfere the kinetics of the Sandell-Kolthoff method. If thiocyanate is present in the samples, it could cause an error for the rate constant determination by giving positive error (according to the results in Figure A7).

It can conclude that the presence of some reducing species in samples can cause deviations of the first-order kinetics and bring up the positive error in the quantitation of iodine.

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APPENDIX E

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APPENDIX E

Analytical Recovery

The acceptable recovery for analysis proposed by AOAC Peer Verified Methods Program [52] is presented in Table A2.

Table A2 Analytical recovery at different concentration.

Analyte concentration	Mean recovery (%)
100 %	98-102
10 %	98-102
1 %	97-103
0.10 %	95-105
100 ppm	90-107
10 ppm	80-110
1 ppm	80-110
100 ppb	80-110
10 ppb	60-115
1 ppb	40-120

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