รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาระบบสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะแบบใหม่

โดย ดร. ดวงใจ นาคะปรีชา และคณะ

กรกฎาคม 2543

รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาระบบสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะแบบใหม่

โดย ดร. ดวงใจ นาคะปรีชา และคณะ

กรกฎาคม 2543

สัญญาเลขที่ PDF/65/40

รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาระบบสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะแบบใหม่ โดยอาศัยเทคนิค Flow Injection Analysis

คณะผู้วิจัย	สังกัด
1. ดร. ดวงใจ นาคะปรีชา	มหาวิทยาลัยมหิดล
2. รศ. ดร. เกตุ กรุดพันธ์	มหาวิทยาลัยเชียงใหม่
3. รศ. ดร. ยุวดี เชี่ยววัฒนา	มหาวิทยาลัยมหิดล
4. รศ. ดร. ประพิณ วิไลรัตน์	มหาวิทยาลัยมหิดล
5. ดร. กฤษณเดช เจริญสุธาสินี	มหาวิทยาลัยวลัยลักษณ์
6. นางสาวเสาวภา เมืองแก้ว	มหาวิทยาลัยมหิดล (อดีตนักศึกษาระดับปริญญาโท)
7. นางสาวนวลละออ รัตนวิมานวงศ์	มหาวิทยาลัยมหิดล (อดีตนักศึกษาระดับปริญญาโท)
8. นายมานะ อมรธำมรงค์	มหาวิทยาลัยมหิดล (อดีตนักศึกษาระดับปริญญาโท)

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

ชุดโครงการวิจัยหลังปริญญาเอก

กิตติกรรมประกาศ

ขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่ได้ให้โอกาสในการสร้างงานวิจัยชิ้นนี้ โดยให้การสนับสนุนเป็นเงินทุนวิจัยหลังปริญญาเอก ขอขอบคุณคณะวิทยาศาสตร์ มหาวิทยาลัยมหิดลที่ได้ให้ทุนสนับสนุนเพื่อสมทบทุนวิจัยที่ได้จาก สกว. และขอขอบคุณ ศูนย์เทคโนโลยีอิเลคทรอนิกส์และคอมพิวเตอร์แห่งชาติ ที่ได้ให้ทุนสำหรับการทำงานบางส่วนในการศึกษาการเชื่อมต่อสัญญาณจากเครื่องตรวจวัดเข้าคอมพิวเตอร์ (computer interface)

ขอขอบคุณ รศ. ดร. เกตุ กรุดพันธ์ และ รศ. ดร. ขุวดี เชี่ยววัฒนา ที่ได้รับเป็นนักวิจัยที่ปรึกษา และให้ข้อคิดและคำเสนอแนะต่าง ๆ เพื่อการนำผลงานไปตีพิมพ์ ขอขอบคุณ รศ. ดร. ประพิณ วิไลรัตน์ในคำวิจารณ์และข้อเสนอแนะที่เป็นประโยชน์โดยเฉพาะในเรื่องของ kinetic studies ขอขอบคุณ ดร. กฤษณะเดช เจริญสุธาสินี ผู้ร่วมวิจัยที่ได้เสนอความคิดและร่วมวิจัยในส่วนของการทำ computer interface ขอบคุณนักศึกษาระดับปริญญาโทที่อยู่ในคณะวิจัยทุกคนที่ได้อุทิศตนทำงานอย่างเต็มที่ ทำให้งานวิจัยและการเสนอผลงานในระดับต่าง ๆ ประสบความสำเร็จด้วยดี

Abstract

Project Code: PDF/65/40

Project Title: Development of a novel system for urinary iodine determination based on flow injection analysis

Investigators: Duangjai Nacapricha[a], Kate Grudpan[b], Juwadee Shiowatana[a], Prapin Wilairat[a], Krisanadej Jareonsutasinee[c], Saowapha Muangkaew[a], Nuanlaor Ratanawimarnwong[a] and Mana Amornthammarong[a]
[a]Mahidol University, [b]Chiang Mai University, [c]Walailak University

Email Address: scdnc@mahidol.ac.th

Project Period: 1 August 1997 to 31 July 2000.

Objectives: 1. To develop a flow injection (FI) system for determination of iodine in urine.
2. To validate the FI system with another technique of determination.
3. To gain some international publications
4. To interface the spectrophotometer, which is the detector used in the FI system, with a personal computer. The results will be used in the design of a commercial FI system in future.

Methodology: Development of the FI system was carried out using the method of Sandell and Kolthoff with the catalytic effect of iodide. Three types of on-lined treatment was studied for urine prior to determination in the FI system. However the three on-lined methods were not appropriate. Finally a continuous-FI system was developed for an off-lined urine digestion. The system was later modified to a stopped-FI system, which can be used in alternative to the continuous system. The two systems of FI were validated with the standard batch method and a method with different detection for iodine that is inductively coupled plasma mass spectrometry.

As well as the development of the FI systems, interfacing of spectrophotometer to a PC was studied.

Results: Two FI systems which are continuous and stopped FI systems, were developed and were validated. Results of urine analysis between all the methods gave statistically good agreement. A method for interfacing a spectrophotometer to a PC was developed.

Discussion Conclusion: The validation results have shown that the two FI systems are suitable to use in determination of urinary iodine. The systems provide satisfactory sensitivities and detection limits. The methods gave better control of analytical precision than the standard batch method. The method is cost effective comparing to other methods proposed by some authors. Also the methods are readily available for automation.

The method developed for interfacing a spectrophotometer to a PC was novel because there have never been reported that an audio card was used in this purpose.

Suggestion/Further Implication/Implementation:
The method of interfacing the instrument giving DC output can be used for future design of a commercially available FI instrument for determination of urinary iodine.

Keywords: iodine, urine, determination, interface, catalytic determination

บทคัดย่อ

รหัสโครงการ: PDF/65/40

ชื่อโครงการ: การพัฒนาระบบสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะแบบใหม่โดยอาศัยเทคนิค Flow Injection Analysis

ผู้ทำการวิจัย: ดวงใจ นาคะปรีชา เกตุ กรุดพันธ์ ยุวดี เชี่ยววัฒนา ประพิณ วิไลรัตน์ กฤษณะเดช เจริญสุธาสินี เสาวภา เมืองแก้ว นวลละออ รัตนวิมานวงศ์และ มานะ อมรธำมรงค์

Email: scdnc@mahidol.ac.th

ระยะโครงการ: 1 สิงหาคม 2540 ถึง 31 กรกฎาคม 2543

วัตถุประสงค์: 1. เพื่อพัฒนาระบบโฟลอินเจคชันสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะ
2. เพื่อนำเทคนิคที่พัฒนาได้มาพิสูจน์ความเหมาะสมในการใช้งานจริงโดยเทียบกับเทคนิคที่ใช้อยู่และเทคนิคอื่น ๆ
3. เพื่อผลิตผลงานวิจัยคุณภาพ สำหรับตีพิมพ์ในวารสารระดับนานาชาติ
4. เพื่อศึกษาวิธีการเชื่อมต่อสัญญาณจากเครื่องสเปคโตรโฟโตมิเตอร์เข้ากับคอมพิวเตอร์ ทั้งนี้จะได้ใช้เป็นแนวทางเพื่อการออกแบบและพัฒนาระบบโฟลอินเจคชันดังกล่าว

วิธีการทดลอง: ได้ทำการพัฒนาระบบโฟลอินเจคชันซึ่งอาศัยการตรวจวัดโดยวิธีที่เสนอโดย แซนเดลและโคลธอฟ ซึ่งใช้การเร่งของไอโอไดด์ เริ่มด้วยการทดสอบการใช้ระบบ on-lined สำหรับการ treat ตัวอย่างก่อนนำเข้าทำปฏิกิริยา พบว่าระบบทั้ง 3 ระบบที่เลือกศึกษาไม่เหมาะสม จึงได้ใช้วิธีการย่อยแบบ off-lined แทน จากนั้นได้พัฒนาระบบโฟลอินเจคชันที่ใช้กับวิธีย่อยตัวอย่างดังกล่าวขึ้น 2 ระบบ และได้ทดสอบผลที่ได้จากการนำระบบทั้งสองใช้วิเคราะห์โดยเทียบผลวิเคราะห์ที่ได้กับผลที่ได้จากเทคนิคอื่น ๆ คือ เทคนิคมาตรฐาน ซึ่งเป็นแบบ batch และ เทคนิคอินดักทีฟลีคัพเพิลด์พลาสมา

นอกจากงานพัฒนาเทคนิควิเคราะห์ดังกล่าว ยังได้ศึกษาถึงวิธีการเชื่อมต่อเพื่อนำสัญญาณจากสเปคโตรโฟโตมิเตอร์เข้ากับเครื่องคอมพิวเตอร์ด้วย

ผลการทดลอง: ได้ระบบโฟลอินเจคชันสำหรับใช้วิเคราะห์ปริมาณไอโอดีนในน้ำปัสสาวะ 2 ระบบ ผลจากการทดสอบทางสถิติพบว่า ผลลัพธ์วิเคราะห์ที่ได้จากระบบทั้งสองให้ค่าที่สอดคล้องกับผลลัพธ์วิเคราะห์ที่ได้จากเทคนิคอื่น ๆ และนอกจากนี้ยังได้วิธีการเชื่อมต่อเพื่อนำสัญญาณจากสเปคโตรโฟโตมิเตอร์เข้ากับเครื่องคอมพิวเตอร์ด้วย

วิจารณ์และสรุป: จากผลการทดสอบและเปรียบเทียบกับเทคนิคอื่น ๆ พบว่าระบบโฟลอินเจคชันที่พัฒนาได้ทั้งสองระบบเหมาะสมจะใช้งานได้จริง และระบบให้ค่าความไวและขีดต่ำสุดสำหรับการวิเคราะห์เป็นที่น่าพอใจ การใช้ระบบจะทำให้มีการควบคุมความแม่นยำในการวิเคราะห์มากกว่าวิธีที่ใช้ในปัจจุบันซึ่งเป็นแบบ batch นอกจากนี้ค่าใช้จ่ายในการวิเคราะห์ก็ไม่สูงด้วยเมื่อเทียบกับเทคนิคอื่น ๆ ที่มีผู้เคยเสนอมา อีกทั้งระบบโฟลอินเจคชันทั้งสองก็เป็นระบบที่สามารถนำไปปรับเป็นระบบวิเคราะห์แบบอัตโนมัติได้ทันที

สุดท้ายงานดังกล่าวได้เสนอวิธีใหม่สำหรับการเชื่อมต่อเพื่อนำสัญญาณจากสเปคโตรโฟโตมิเตอร์เข้ากับเครื่องคอมพิวเตอร์ด้วย เทคนิคนี้ใช้ audio card เป็นอุปกรณ์หลัก ซึ่งเป็นเทคนิคใหม่

ข้อเสนอแนะหรือการนำไปใช้ประโยชน์: วิธีการเชื่อมต่อสัญญาณเข้ากับอุปกรณ์ตรวจวัดที่ให้ dc output นี้จะเป็นประโยชน์แก่การออกแบบระบบโฟลอินเจคชันสำหรับวิเคราะห์ปริมาณไอโอดีนในปัสสาวะ ที่จะคาดว่าจะปรับปรุงเป็นระบบ commercial ได้ต่อไป

Keywords: ไอโอดีน ปัสสาวะ การวิเคราะห์เชิงปริมาณ การเชื่อมต่อ การวิเคราะห์แบบคะตะไลติก

Executive summary

1. การปรับวัตถุประสงค์โครงการและแผนการวิจัย

หัวหน้าโครงการมีความจำเป็นจะต้องปรับวัตถุประสงค์ของโครงการพร้อมปรับเปลี่ยนแผนการวิจัย เพื่อให้สอดคล้องกับผลการทดลองที่ได้ และเพื่อให้มี output ของโครงการ ทัดเทียมกับที่ได้เคยเสนอไว้ในโครงร่างงานวิจัย โดยสรุปการเปลี่ยนแปลงดังกล่าวในตารางที่ 1

ตารางที่ 1 สรุปการปรับเปลี่ยนวัตถุประสงค์การวิจัย

วัตถุประสงค์เดิม	วัตถุประสงค์ใหม่
1. เพื่อพัฒนาระบบโฟลอินเจคชันสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะ	1. คงเดิม
2. เพื่อพัฒนาระบบโฟลอินเจคชันสำหรับวิเคราะห์ไอโอดีนในน้ำปัสสาวะ ที่มีการพ่วง on-lined UV digester เพื่อจะได้ฉีดปัสสาวะเข้าระบบโดยตรง*	2. เพื่อนำเทคนิคที่พัฒนาได้มาพิสูจน์ความเหมาะสมในการใช้งานจริงโดยเทียบกับเทคนิคที่ใช้อยู่และเทคนิคอื่น*
3. เพื่อผลิตผลงานวิจัยคุณภาพ สำหรับตีพิมพ์ในวารสารระดับนานาชาติ	3. คงเดิม
	4. เพื่อศึกษาวิธีใหม่สำหรับการเชื่อมต่อสัญญาณจากเครื่องสเปกโตรโฟโตมิเตอร์เข้ากับเครื่องคอมพิวเตอร์

หมายเหตุ * สาเหตุที่ต้องปรับเปลี่ยนวัตถุประสงค์โครงการวิจัยเพราะไม่สามารถทำให้วัตถุประสงค์ที่ 2 เดิมนั้นสัมฤทธิ์ผลได้ จึงได้ปรับเป็นวัถุประสงค์ที่ 2 อันใหม่ และได้เพิ่มเติมวัตถุประสงค์ที่ 4 เป็นการทดแทน

ทั้งนี้หัวหน้าโครงการได้ปรับเปลี่ยนแผนการวิจัยไว้ดังได้เสนอในรายงานความก้าวหน้า (18 และ 30 เดือน และรายงานประจำปีที่ 2)

2. Output ของโครงการ

2.1 Output ของโครงการในเรื่องของการตีพิมพ์สรุปได้ดังต่อไปนี้

2.1.1 M. Amornthammarong, K. Jareonsutasinee and **D. Nacapricha**, "Interfacing of spectrophotometric detector to a personal computer via an audio card for a flow injection system, Lab. Robotics and Automation, 2000, **12**, 138-141. (ภาคผนวก ก)

2.1.2 **D. Nacapricha**, S. Muangkaew, N. Ratanawimarnwong, J. Shiowatana and K. Grudpan, "Continuous and stopped flow injection for catalytic determination of iodine in urine" submitted to The Analyst by August 2000 (ทางสำนักบรรณาธิการวารสารได้แจ้งการรับบทความเพื่อพิจารณาแล้ว เมื่อวันที่ 4 ก.ย. 43) (ภาคผนวก ข)

2.1.3 **D. Nacapricha**, N. Ratanawimarnwong, S. Suwannachoat, P. Wilairat, J. Shiowatana and K. Grudpan, "Stop flow injection with the catalytic effect of iodide for kinetic determination of iodine" (manuscript in preparation and to be submitted either to Talanta or to Anal. Chim. Acta. by October 2000.

2.2 Output ของโครงการในเรื่องของผลการวิจัยสรุปได้ดังต่อไปนี้

2.2.1 ได้ระบบ 2 ระบบ ที่เป็นระบบวิเคราะห์แบบ flow injection (FI) สำหรับใช้เพื่อวิเคราะห์ปริมาณไอโอดีนในน้ำปัสสาวะ ซึ่งเหมาะสมที่จะใช้สำหรับการสำรวจการขาดแร่ธาตุไอโอดีนในประชากร ซึ่งมักจะต้องวิเคราะห์ตัวอย่างน้ำปัสสาวะครั้งละหลาย ๆตัวอย่างพร้อมกัน ระบบที่พัฒนาได้นั้นให้ความแม่นยำที่ดีกว่าวิธีเดิมที่ใช้กันในปัจจุบัน เพราะสามารถควบคุมความแม่นยำในการวัดได้ และมีความสะดวกกับผู้ใช้มากกว่าวิธีเดิมมาก ระบบพร้อมที่จะปรับใช้เป็นแบบอัตโนมัติได้ หากมีเครื่องมือ FIA ที่มีจำหน่ายอยู่แล้ว ระบบที่พัฒนาได้นี้มีค่าใช้จ่ายในการวิเคราะห์ต่ำมาก เมื่อเทียบกับเทคนิคที่เป็น gold standard อื่น ๆ เช่น Inductively coupled plasma mass spectrometry ซึ่งทดสอบทางสถิติแล้วพบว่าให้ผลการวิเคราะห์ที่ไม่แตกต่างอย่างมีนัยสำคัญ

2.2.2 ได้วิธีใหม่ในการเชื่อมต่อ spectrophotometer เข้ากับคอมพิวเตอร์เพื่อนำสัญญาณไปประมวลต่อไป ซึ่งเทคนิคดังกล่าวได้ใช้ audio card เป็นอุปกรณ์สำคัญในการเชื่อมต่อ วิธีดังกล่าวเป็นวิธีใหม่ และจะได้ทดลองนำวิธีนี้ไปใช้ในอนาคตซึ่งมีโครงการที่จะขยายผลงานวิจัยเพื่อปรับระบบวิเคราะห์ให้เป็นแบบ commercial หากได้รับการสนับสนุน

2.2.3 โครงการวิจัยนี้ได้เอื้อให้มีการผลิตมหาบัณฑิต ทางด้านเคมีวิเคราะห์และเคมีอินทรีย์ประยุกต์ จำนวน 3 คน

2.2.4 งานวิจัยในส่วนที่เป็นการศึกษาการเชื่อมต่อสัญญาณ spectrophotometer เข้ากับคอมพิวเตอร์ ซึ่งได้รับทุนสนับสนุนบางส่วนจาก ศูนย์เทคโนโลยีอิเลคทรอนิกส์และคอมพิวเตอร์แห่งชาติ (NECTEC) นั้น งานดังกล่าวนอกจากจะเป็นจุดเริ่มให้มีโอกาสได้ทำงานร่วมกับอาจารย์ในสาขาฟิสิกส์ (ดร. กฤษณะเดช เจริญสุธาสินี) แล้ว นักศึกษาที่ดูแลศึกษาเรื่องดังกล่าวได้รับรางวัลที่ 3 ประเภทซอฟต์แวร์ที่เป็นเครื่องมือและซอฟต์แวร์อื่น ๆ จาก NECTEC เมื่อวันที่ 13 กุมภาพันธ์ 2541

เนื้อหางานวิจัย (ดูหัวข้อ 1 ถึง 5)

1 Introduction

Iodine contents in urine have been widely used as a marker for status assessment of iodine deficiency disorder (IDD). In 1992, the ICCIDD/WHO/UNICEF[1], gave the following values as a guide for IDD status: <20 μg I l^{-1} (severe); 20-49 μg I l^{-1} (moderate); 50-100 μg I l^{-1} (mild) and >100 μg I l^{-1} (normal). There have been a number of documents that describe methods for determination of inorganic iodine content and total content in urine.

For the quantitation of free iodide, different modes of high performance liquid chromatography (HPLC) such as ion[2] and ion-pair reversed-phase[3,4,5] were proposed with either electrochemical detection[3,4,5] or post-column reaction.[2] Yabu et al. have proposed a method to measure iodide content in urine using iodide-selective electrode.[6]

Total content of iodine can be determined by using a hyphenated technique such as inductively coupled plasma mass spectrometry (ICP-MS). Usually dilution of sample with water[7] or other types of reagent such as nitric acid[7,8] and ammonia[9] is adequate for this technique. Variations in the signals of the detection mass, are corrected by addition of some internal standards, e.g., europium[8] rhodium or indium.[7] An ICP-MS for determination of urinary iodine using isotope dilution with iodine-129 was presented by Haldimann et al.[9]

Surrounded by other methods, the most common method used in laboratories dealing with monitoring of IDD status is a spectrometric method with catalytic effect of iodide on a redox reaction between Ce(IV) and As(III).[1,10] The method is more preferable than other methods because it is more practical as a survey tool. Sandell and Kolthoff first described the use of this reaction for determination of trace iodide catalyst in 1934.[11] Rodriguez and Pardue proposed some possible mechanisms based on their experimental data achieved in 1969.[12] The net reaction is

$$\underset{\text{yellow}}{2Ce(IV) + As(III)} \longrightarrow \underset{\text{colorless}}{2Ce(III) + As(V)} \qquad \text{.....(1)}$$

The rate of reaction is directly proportional to the iodide concentration when other factors are fixed.

Mushtakova et al.[13] proposed a Sandell and Kolthoff method for determination of free iodide in urine. Their method requires no step of urine digestion prior to spectrometric detection of Ce(IV). However extensive investigation of interfering effect from potential species in urine is yet required for this method.

Description of the classical method was provided by Dunn et al.[1,10] The procedure is usually carried out batchwise. Interferences to the redox reaction in urine must be eliminated prior to the step of spectrometric measurement. Digestion with chloric acid is more often used than the alkaline ashing method.[14] For chloric acid digestion, iodine species are oxidised to iodate ions after digestion. Unpublished results[15] apparently indicated that chloride ion added in the arsenious solution helps in conversion of iodate to iodide within less than a minute. The results have also demonstrated that the disappearance of Ce(IV) is a first-order process under the condition of the conventional method.

After chloric acid digestion, the sample is added with As(III) solution which contains sodium chloride. Finally after an accurate volume of Ce(IV) solution was added to this solution the reading of absorbance (405-420 nm) must be taken at a fixed time for all the samples and the standards. Hence technician error can easily occur in the conventional batch method. Automation of the procedure has been reported through the use of autoanalyser,

which is based on air-segmented flow (ASF) technique.[4,16,17,18,19] The use of flow injection (FI) technique offers various advantages.[20] Nevertheless, there has been no report of a FI system to use in conjunction with this type of sample preparation.

In 1996, Yaping et al.[21] have proposed a FI method for determination of iodine in urine. The method employed Sandell and Kolthoff reaction but with the use of different type of reagent (H_2SO_4+$KMnO_4$+$K_2Cr_2O_7$) for on-line digestion. With this mixture of oxidant, and with the detection wavelength at 480 nm, spectral interferent is at risk especially from $KMnO_4$. Another disadvantage of the method is that the method unnecessarily hired a toxic brucine as a reagent stream.

In this work, two methods developed for determination of urinary iodine based on flow injection techniques are described. The first method is proposed to use in the normal continuous mode. The system can be modified to operate under a stopped-FI mode.[20] Both methods are readily applicable for automation to reduce technician error, which often arise in the convention batch method.

Also the application of stopped FI was used to study kinetics of Sandell and Kolthoff reaction and the interferences of the kinetics which are caused by other species normally present in urines.

2 Experimental

2.1 Instruments

A Boekel digital dry block heater, models 111002, USA, was used for the digestion of all standards for calibration and urine samples. Two block modules were used to accommodate 12 rest tubes (20 mm x 150 mm) for a digestion run.

A PMC hot plate, model 501, USA, was used for heating arsenious acid solution during the preparation.

A precisa 40SM-200A analytical balance, Switzerland, was used for weighing chemicals in the preparation of potassium iodate stock solution and of reagent solutions.

An Elan 6000 inductively couple plasma-mass spectrometer, Canada, was used for monitoring ratio of ^{127}I to ^{115}In in method comparison.

An ORION ion selective electrode, 9653 ionplus™ series, USA, was used for measuring iodine concentration in urine for method comparison.

A Waters Sep-Pak cartridge, W9349J2, USA, was used for trapping organic substances in urine to determine inorganic iodine content.

2.2 Apparatus for the FI systems

2.2.1 Continuous FI system

The followings include the equipment for the construction of the continuous FI system used in this work. The FI manifold is shown in Fig. 1.

An Ismatec peristaltic pump, model IS7610, Switzerland, was used for propelling all reagents and injectates. Three out of four channels of the pump were fitted with cartridges each holding Tygon pump tubes i.d. 0.95 mm, wall 0.90 mm.

A Rheodyne injection value, model 7125, USA, fitted with a Teflon loop (1.0 mm i.d.) was employed for injection of all standards and samples. Interchangeable loop allows for different size of samples to be injected by simply changing the loop length.

A Shimadzu spectrophotometer, UV-2-01, Japan, with a tungsten lamp and a Philips flow cell of 10 μL volume was used for monitoring any changes in transmittance of Ce(IV) at a wavelength within the range 410-430 nm.

A PolyScience water bath, model 2L-M, USA, was used for controlling the temperature of glass reaction coil immersed in the bath.

An Altech chart recorder, model LR 93025, USA, was used for recording the signal from the Shimadzu spectrophotometer. The recorder was set to either 20 or 50 mV f.s.d..

The FI chemifold consisted of a body made of Perspex plastic with holes for inserting tube connectors. Reagents were pumped through the chemifold and were merged before mixing in mixing coils.

Glass reaction coils of different size length, 50 and 100 cm, were made of glass tubing with i.d. 1.0 mm by curling this glass tube into a spiral shape around an opened glass cylinder i.d. 1.5 cm and 17.5 cm in length. These two sizes of reaction coil were connected using pieces of Tygon tube to make larger coils of desirable lengths.

All flow injection manifolds reported in this work were constructed using the equipment listed above. Cole Parmer tygon tubings with i.d. of 0.75 mm, 2.25 mm o.d. and 0.75 mm wall, were used for the FI assembly and for making mixing coil.

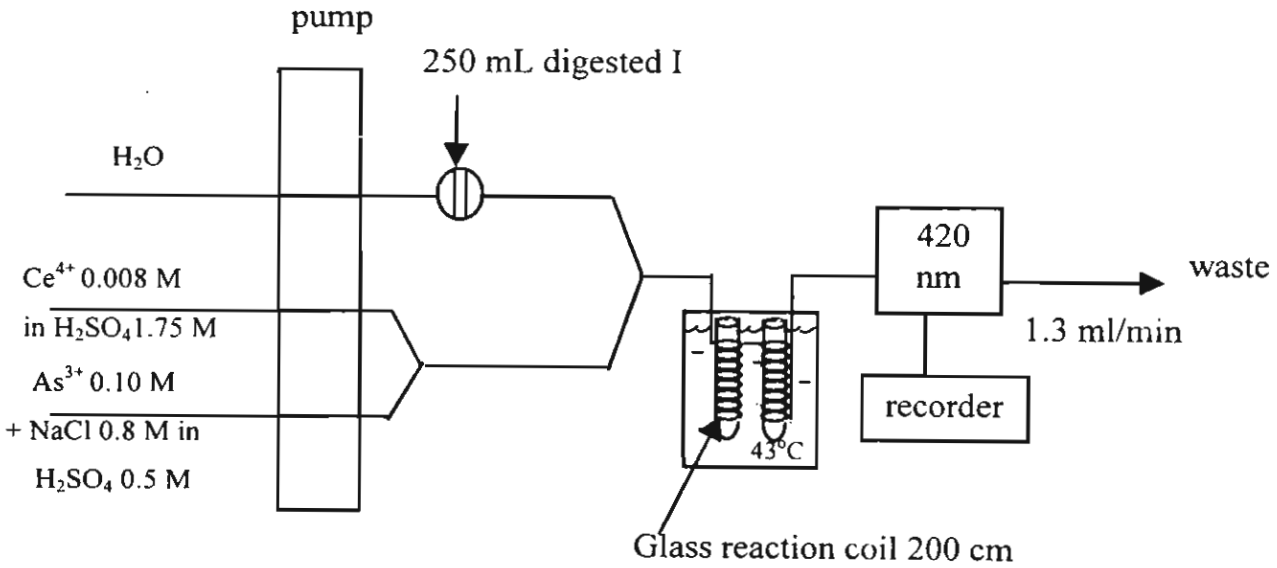

Figure 1 Schematic diagram of optimum FI system for urinary iodine

2.2.2 Stopped-FI system

The system of the continuous-FI shown in Fig. 1 was modified to operate under the stop mode for determination of urine with chloric acid digestion. In this mode, the glass coils were replaced by a coil made of Tygon tube (100 cm). The pumping rate for each channel is approximately 1 ml min-1.

2.3 Preparation of solutions

All chemicals used in this work were AR grade, supplied by Merck (Germany) except indium nitrate (Fluka, Switazerland). Deionized-distilled water was obtained from a Milli-Q system, Millipore, USA, and was used throughout.

Standard iodine solutions

a) Primary stock concentration of 1000 mg I/L

For potassium iodate (KIO_3), the solid potassium iodate 0.1865 g was dissolved with deionized-distilled water in a 100.00 mL volumetric flask.

For potassium iodide (KI), the preparation procedure was similar to what described above except that the weight of KI was 0.1310 g.

b) Working iodine standard

Both solutions KIO_3 and KI were diluted in similar way as following. 0.1 mL of the primary stock was diluted to 100.00 mL with deionized-distilled water to give a standard having iodine concentration of 1.0 mg I/L that was used as the secondary stock for preparations of standard iodine for calibration.

Iodine standards for calibration, containing 50, 80, 100, 150, 200 μg I/L were prepared by respectively transfer aliquots of the secondary stock (1mμg I/mL) at the volume of 2.5, 4.0, 5.0, 7.5 and 10.0 mL into 100.00 mL volumetric flasks. These aliquots were then diluted to mark with deionized-distilled water.

KIO_3 solutions were employed as the standard solutions in this studies in Sections 3.1, 3.3 to 3.5. KI solution was also employed as standard solutions for the studies in Sections 3.2.

Chloric acid solution (28% w/v)

250 g of potassium chlorate ($KClO_3$) was dissolved in 455 mL of deionized-distilled water in an Erlenmeyer flask. The mixture was heated until clear solution is attained. 187.5 mL of 70% (v/v) perchloric acid ($HClO_4$) was slowly added into this solution with constant stirring. This solution was stored in freezer overnight and was filtered through a filter paper (Whatman no.1 or equivalent), preferably on a Buchner funnel. The final volume of filtrate was approximately 850 mL. This solution containing approximately 28% (w/v) of chloric acid solution has been used for digestion of all urine samples and the standard. The solution was stored at 4°C in a refrigerator.

Arsenious acid solution (0.08M)

Arsenic acid solution of 0.08 M was used in kinetic and gas diffusion studies. 8.0320 g of As_2O_3 and 12.0 g of NaOH were dissolved together in approximately 400 mL of deionized-distilled water. To this solution, 40.0 mL of concentrated H_2SO_4 was added. The solution was then diluted to 1000.00 mL with deionized-distilled water. This arsenious solution is stable for several months at room temperature.

Arsenious acid solution (0.10M)

Approximately 10 g of As_2O_3 and 47 g of NaCl were dissolved with heating in 500 mL of deionized-distilled water on a hot plate. The solution was cooled to room temperature. Deionized-distilled water was added to this solution to make the final volume of approximately one litre.

The solution was used in the continuous- and stop-FI systems.

Arsenious acid solution (0.025 M)

The preparation procedure of arsenious acid solution 0.025 M was similar to what described in the previous system, except that the weight of As_2O_3 and NaCl were approximately 5 and 25 g respectively. This solution was used in the batch method described in Sections 3.1.

Ceric sulfate solution (0.01M)

Ceric sulfate solution of 0.01 M was used in kinetic and gas diffusion studies. The preparation was performed by dissolving 4.0010 g of $Ce(SO_4)_2.4H_2O$ in one litre of 0.9 M H_2SO_4. This solution is stable for several months at room temperature.

Ceric ammonium sulfate solution (0.008 M)

Approximately 5 g of $Ce(NH_4)_4(SO_4)_4.2H_2O$ was dissolved in 1.75 M H_2SO_4 to approximately one litre. This solution of ceric ammonium sulfate was used in the continuous and stop-FI systems.

Ceric ammonium sulfate solution (0.038 M)

This solution was used in the batch method described in Section 3.1. The preparation procedure was similar to that described in the previous Section for the FI methods, except for the weights of $Ce(NH_4)_4(SO_4)_4.2H_2O$, which was weighted at approximately 24 grams.

Feric ammonium sulfate solution (0.1M)

Approximately 24 g of $NH_4Fe(SO_4)_2.12H_2O$ was dissolved in 500 mL of 5 M HNO_3.

Potassium thiocyanate (0.002 M)

Solid potassium thiocyanate of approximately 1 g was dissolved with deionized-distilled water to approximately 500 mL.

Sodium nitrite solution (0.3 M)

Approximately 5 g of $NaNO_2$ was dissolved in deionized-distilled water to approximately 250 mL.

The solutions, which were described in last three Sections, were used as reagent streams in the on-line dialysis studied in Section 3.3.3.

Sample

Casual urine samples were collected from students and were selected to cover to range of iodine concentration studied. The samples were frozen and thaw before use.

3. Results and discussion

3.1 Examination of content ratio between the free iodide to total iodine in urines

Some authors have reported that approximately 90 % of iodine are present mainly in the form of iodide.[1] Other forms of iodine existing in urine are thyroxine, tyrosine. In this study, the content ratios of inorganic to total iodine in urine samples were re-investigated. Determination of contents of inorganic iodine and total iodine were carried out in ten urine samples. The samples were digested, using the method described by Dunn et al., with and without sample pretreatment before batch analysis for iodine. The iodine content measured in the eluate collected through the C-18 cartridges was counted as the inorganic iodine (non-bound), which is most likely present in the free iodide form. Total content of iodine (inorganic + organic forms) was the amount derived form direct determination without sample clean up. The results calculated as the percentage of inorganic iodine to total iodine are displayed in Table 1.

Table 1 Percentage (w/w) of inorganic iodine calculated based on total iodine found in urine samples.

Sample no.	% inorganic iodine ± SD (n = 4)
1	68.0±5.6
2	69.3±0.9
3	79.4±1.2
4	81.2±13
5	81.8±7.2
6	87.9±5.2
7	90.6±3.3
8	96.5±4.1
9	97.8±4.9
10	98.0±3.0

According to the percentages of inorganic iodine (free iodide) reported in Table 1, there were some samples, which were found to contain free iodide lower than the value reported in the IDD news[1] at 99%. Out of ten samples, three sample (30%) contained free iodide less than 80% (w/w) of the total iodine. However, for these three urines, some of the free iodide may have been slightly trapped in the layer of pigments or other organic

substances that were retained in C-18 column. Nevertheless, it can be observed from these results that urinary iodine presents mainly in the form of free iodide.

3.2 Kinetics studies using stopped FI technique

3.2.1 Determination of rate constant

Kinetics of the reaction between Ce(IV) and As (III), with iodide catalyst, were studied batchwise. The kinetic results have demonstrated that the reaction is first order in Ce (IV) concentration[15]. Therefore the relationship between the absorbance of Ce^{4+} and time is an exponential decay as expressed in equation 2.

$$[Ce^{4+}]_t = [Ce^{4+}]_o e^{-kt} \quad (2)$$

The linear relation between the rate of reaction (k) and concentration of iodide is:

$$k = k_{uncatalyst} + k_{catalyst}[I^-] \quad (3)$$

where k is rate constant of reaction which is a result of the uncatalysed and catalysed reaction, $k_{uncatalyst}$ is rate constant without the catalyst, $k_{catalyst}$ is rate constant of reaction in which the catalyst is present, $[I^-]$ is iodide concentration.

The kinetic study was then repeated using a stop-FI technique. The system, shown in Figure 4, was modified from system developed by W. Chantore et al., 1998.[22] Various concentration ranging from 50 to 200 µg I/L were injected in duplicate into the FI system. The temperature of glass reaction coil was controlled equally to the room temperature at 25°C. The flow was stopped when the zone of standard iodide reached the detector. Kinetic measurements were then taken. The results are shown in Figure 2.

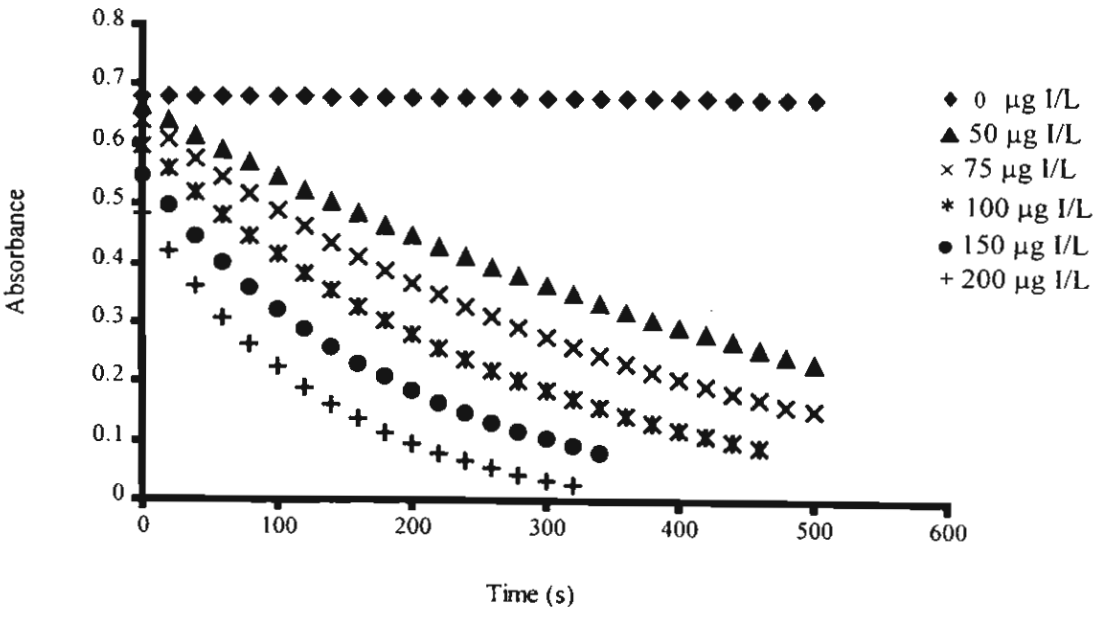

Figure 2 Decreasing of Ce^{4+} absorbance with time (s)

The results in Figure 2 were fitted with a non-linear curve fit program called ENZFITTER using single exponential equation. The profile of the curve fitting of 100 µg I/L is depicted as an example in Figure 3

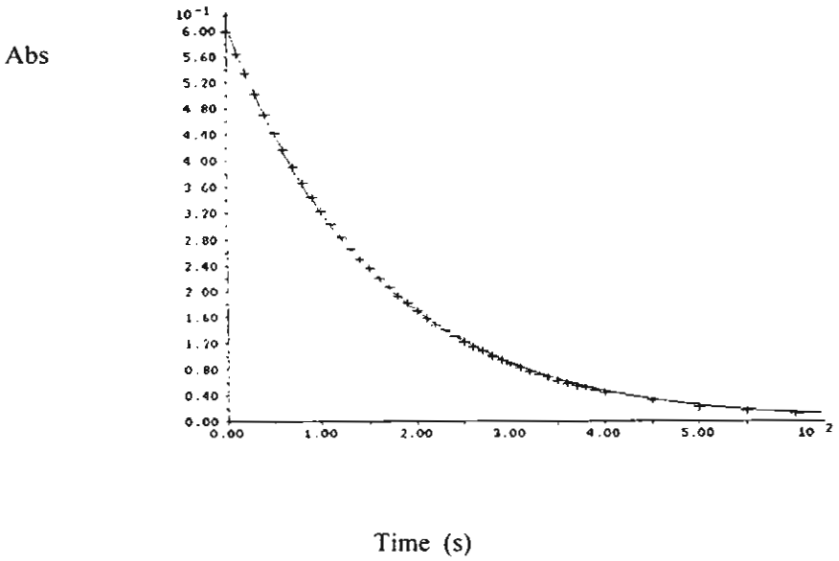

Figure 3 Fitting curve of experimental data with single exponential equation (100 μg I/L).

In Figure 3, the single exponential curve fits well with the experimental data obtained from 100 μg I/L. The results obtained from another concentration (see Figure 2) are also fitted well with the exponential equation. However the rate changed depending upon iodide concentration. These results indicated that the order of the reaction studied by using the proposed stop-FI method was first-order in Ce(IV) concentration, similar to the results obtained in the previous study by batch analysis.

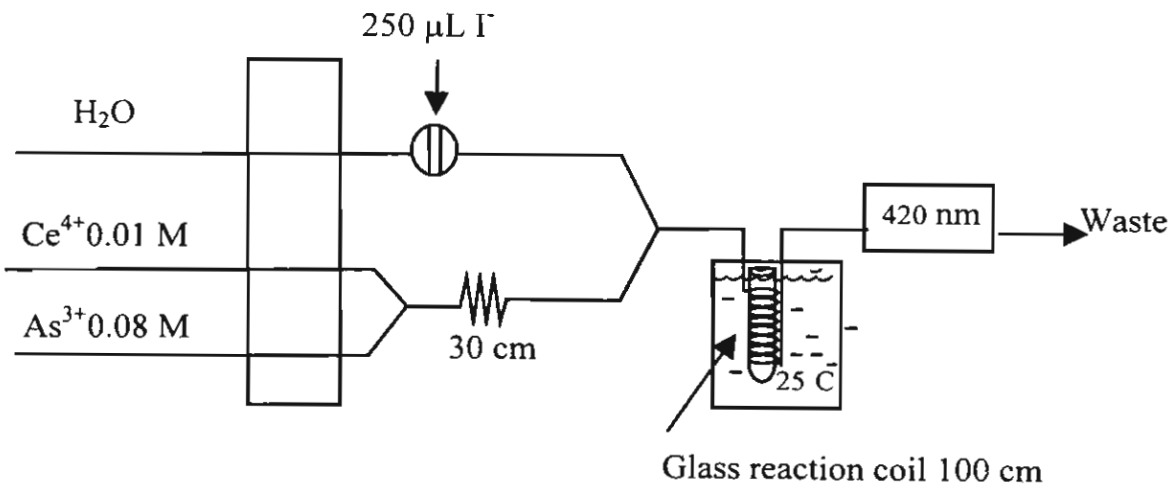

Figure 4 Schematic diagram of the FI system used in the kinetic study. The system was operated in a stopped-FI mode.

3.2.2 Determination of iodide concentration using rate constant

The relationship between absorbance representing Ce^{4+} concentration and time was exponential similar to the results obtained from the batch method. From the first order fitting, the rate constant (k) resulted from at each concentration, the rate of reactions (k) were obtained and taken to linear plot with iodide concentration (equation 3). The linear relationship was then obtained as shown in Figure 5.

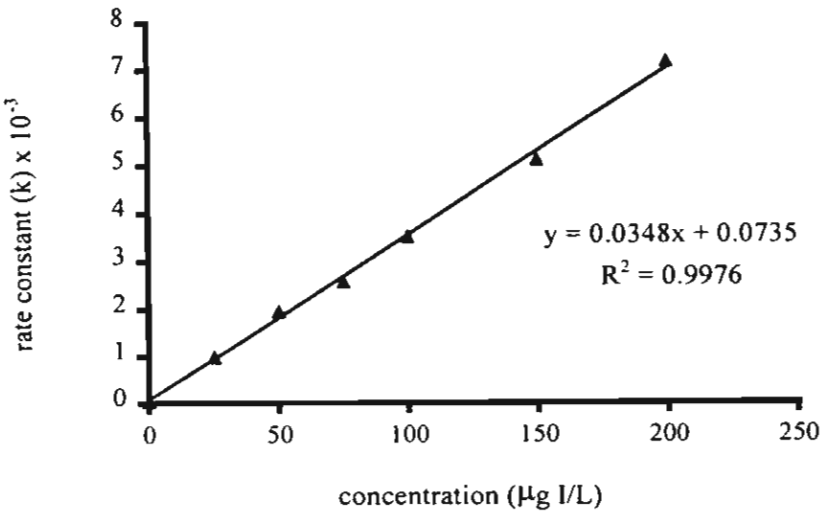

Figure 5 Linear relationship between the rate constant (k) and concentration of iodide (μg I/L).

Figure 5 shows a good linear curve fitting which agrees with the relationship written in equation 3. The results show that iodide concentration can be determined using the rate constant measured by the stopped-FI technique.

The results in Section 3.2.1 and 3.2.2 also demonstrate that the stopped-FI technique can be used as an alternative technique for kinetic studies. The system was used to study interference effect in the next section.

3.2.3 The use of stopped-FI technique for studying interferences of the kinetic pattern of Sandell and Kolthoff reaction

The investigation of interferent effect was carried out using the stopped-FI mode based on exponential kinetic profile of the reaction. Three urine samples were injected in duplicate into the FI system shown in Figure 4. Kinetic studies were carried out in the same way as that described in section 3.2.1. The theoretical first order curves fitting (full time) are depicted together with the data (+) in Figure 6.

The results appear in Section 3.1 have demonstrated that for most samples, urinary iodine is present in the form of iodide. Determination of iodide in a urine using the catalytic reaction may be possible without sample digestion provided that there is no interferent in that sample. Interferent of a catalytic determination can be either enhance or inhibit the catalysis.

In Figure 6, it can be observed that the theoretical curves did not fit well with the three sets of data point. This means that there are some interferent species present in urines and these results are in agreement with that reported by other authors.[1,17,18,21] Sample treatment is thus necessary when the redox reaction of Ce^{4+} and As^{3+} is being employed in determination of UI.

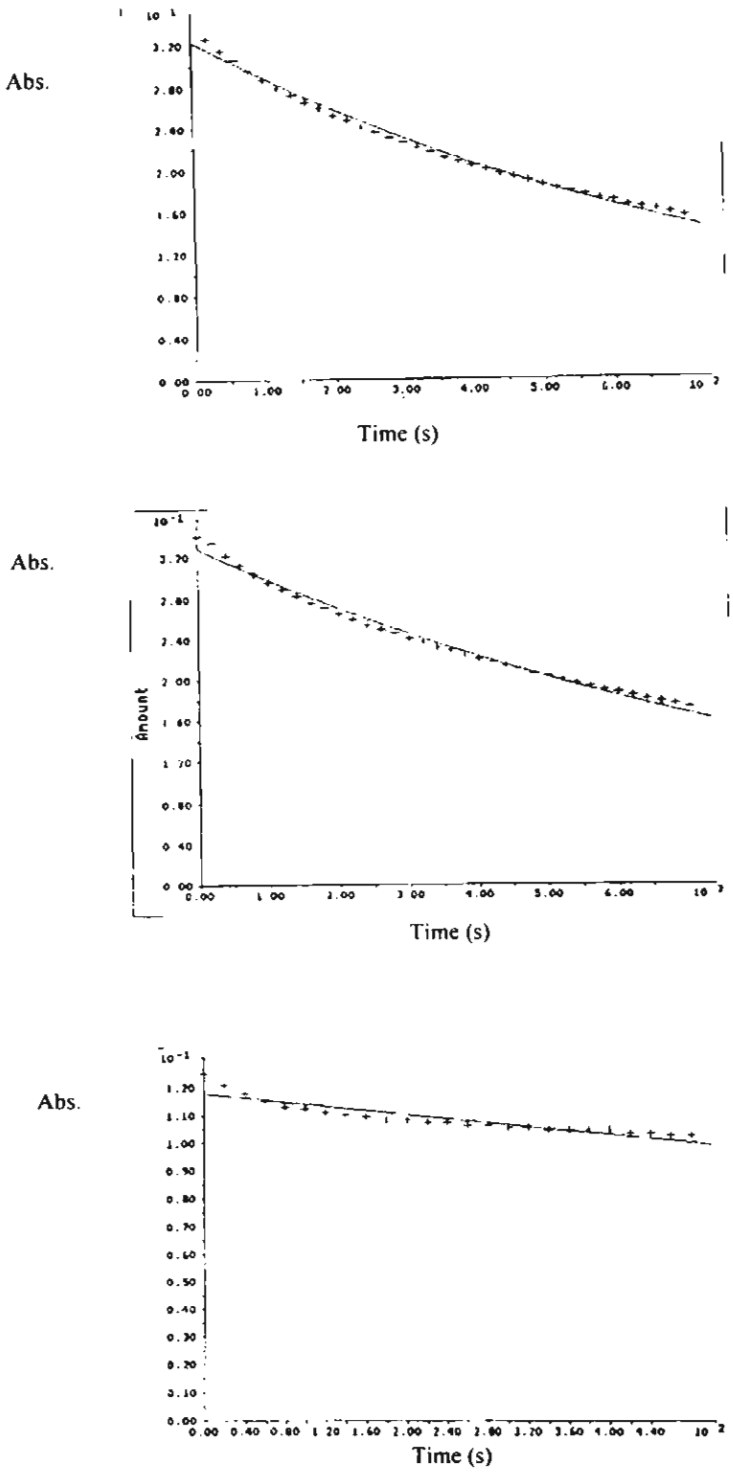

Figure 6 Kinetic of the disappearance of Ce^{4+} obtained from three urine samples: (+ data, - theoretical single exponential fitting).

3.3 Studies of FI on-line techniques for sample treatment.

3.3.1 On-lined UV digestion

An on-lined UV digestion system was set up for a continuous flow injection system as shown in Fig 7.

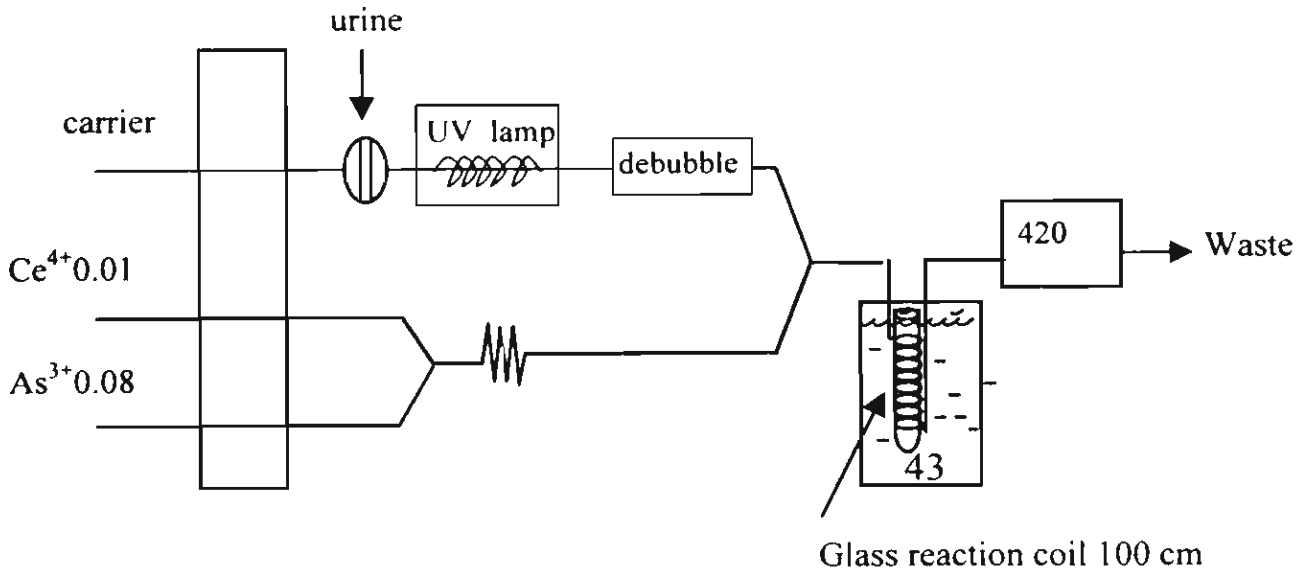

Figure 7 The manifold of the on-lined UV-FI system used for determination of iodine in urine.

It was found that the operation of the system was having the problem of too much air bubbles was being generated through the UV digestion. Some attempts have been made to de-bubble, i.e., using a PTFE porous coil and using a home made de-bubble to get rid of the air. However the problem still persisted. Thus the system was not used for further studies. Other types of sample treatment were then designed as discussed in sections 3.3.2 and 3.3.3.

3.3.2 On-lined gas-diffusion

A FI system in conjunction with a gas-diffusion unit was used to carry out the on-line treatment of urine for determination of iodine. In practice, urinary iodine, in form of iodide ion, will be oxidized to iodine (I_2), which can then permeate through a PTFE membrane into a reagent stream. This idea was taken from the work introduced in 1987 by J. T. Hakedal and P. K. Egberg[24]. However in this work, the detection was not based on detection of triiodide. The acceptor stream used contained Ce^{4+} mixed with As^{3+} in acid media, so that the reaction was catalyzed and the decreasing of Ce^{4+} can be measured spectrophotometrically at 420 nm. However the form of iodine initially present in the acceptor stream was not I^-, however, I_2, after permeation, is reduced to $2I^-$ by As^{3+}. Figure 8 shows the manifold was used in this study.

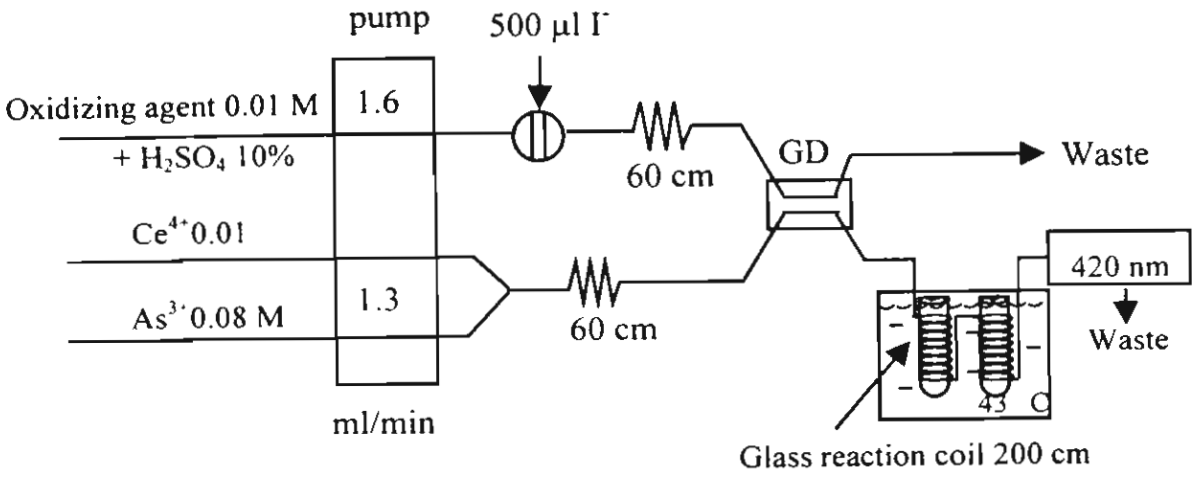

Figure 8 FI manifold of an on-lined gas diffusion system used in this study GD: gas diffusion unit.

Two different of oxidizing reagents, KIO_3 and $K_2Cr_2O_7$, were used to oxidize various standard iodide concentration ranging from 50 to 200 µg I/L. Two sets of calibration were attained from these two oxidizing agents. The results are shown in Figure 8

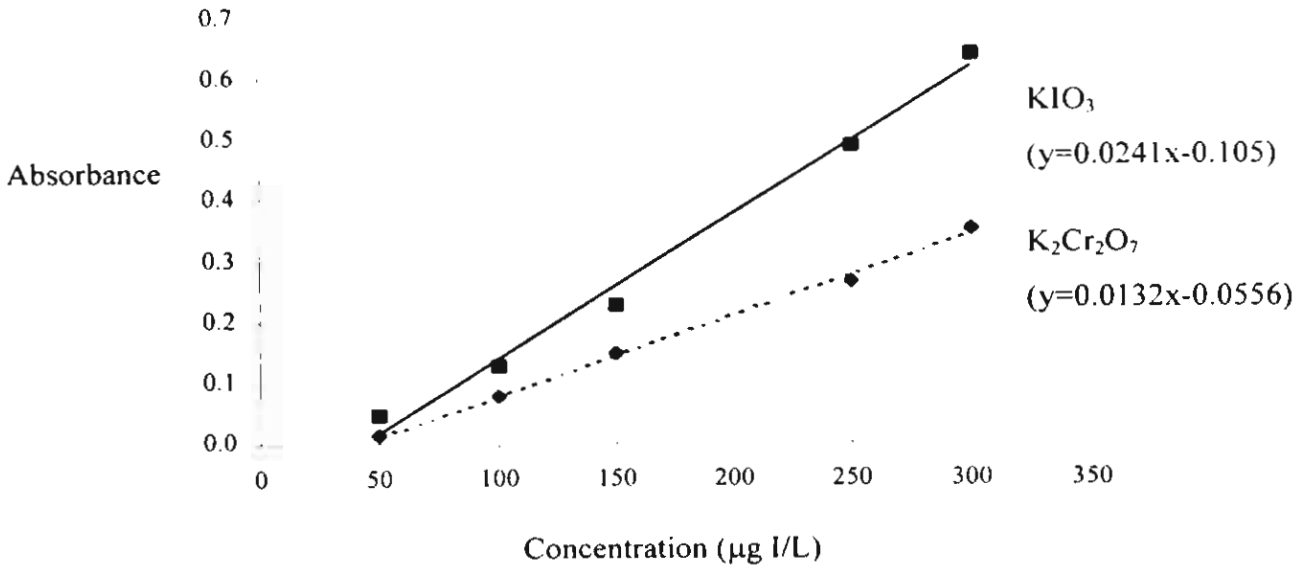

Figure 9 Effect of two different oxidizing agents, 0.01M KIO_3 and $K_2Cr_2O_7$, on sensitivity of FI with gas diffusion method.

Results in Figure 9 indicated that using 0.1 M KIO_3 as the oxidant, a better sensitivity was achieved over the use of 0.1 M $K_2Cr_2O_7$ in term of both signal size and the slope of calibration. The greater sensitivity due to excess iodine produced from the reduction of KIO_3 as the following reaction

$$IO_3^- + 6H^+ + 5e \longrightarrow \tfrac{1}{2}I_2 + 3H_2O \qquad (4)$$

In extending this method to urine samples, the procedure had to be modified by trial-and-error but the oxidizing agents used were found unsuitable. The oxidizing agents used in the carrier stream were 0.01 M KIO_3, 0.01 M $K_2Cr_2O_7$ and 0.01 M $KMnO_4$. $KMnO_4$

was finally used in addition to the other two oxidants for trial purpose. The recovery obtained from using KIO_3 was not too high (15%) when iodide standard was added to sample (100 μg I/L). IO_3^- perhaps does not react only with I^- but also with other reducing substances in the sample and therefore produces some extra amount of I_2. For 0.01M $K_2Cr_2O_7$ and 0.01M $KMnO_4$, there were not signal obtained although when the sample was added with standard iodide to contain 1000 μg I/L. These may be due to both of these oxidizing agents under the condition used were not strong enough to oxidize all of organic substances and the reducing agents (including iodide) in urine.

3.3.3 On-lined dialysis

On-line sample treatment for measuring UI by using dialysis was studied. Urine samples were dialyzed and then directly analyzing iodide in the analysate. Because the urine contains interference substances such as thiocyanate, ascorbic acid or chloride ion (Cl^-), which their molecular weight were 258, 176 and 35 respectively. These substances can penetrate the dialysis membrane (molecular cut-off = 10,000 Dalton) to interfere Sandell-Kolthoff reaction. To avoid this problem, the catalytic effect of iodide on destruction of the organic iron (III) thiocyanate by nitrite ion was used instead of Sandell-Kolthoff reaction in this study. The manifold used was shown in Figure 9. However, a lot of air bubbles have been occurring from this system and the reason for this is not known.

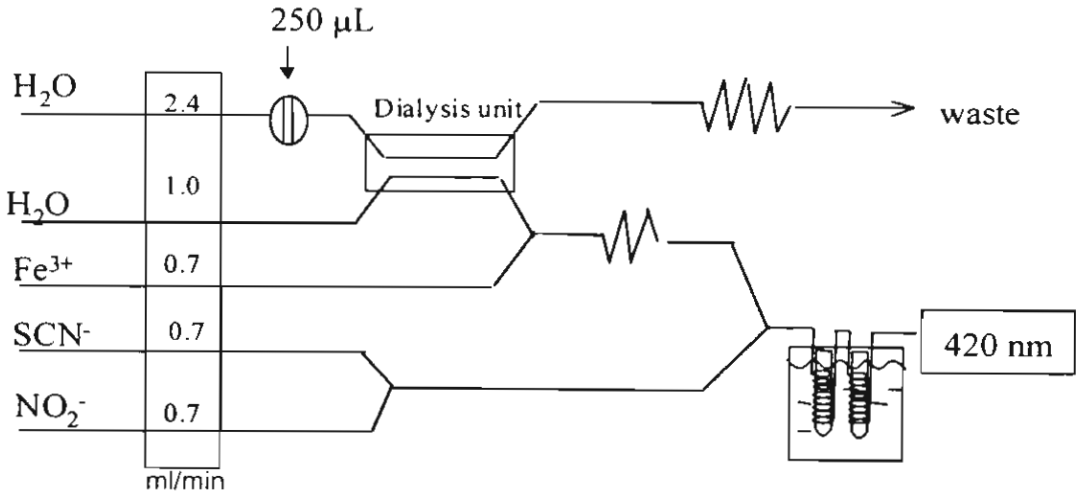

Figure 10 Manifold of FIA corporating with dialysis unit used in this study.

From Section 3.3.1 and 3.3.2, the results demonstrate that there are not great possibilities for performing on-line sample treatment by using gas diffusion and dialysis units. So acid digestion was still used to eliminate the matrix interferents in further experiments.

5.3.4 Continuous and stopped flow injeciton for catalytic determination of iodine in urine

ดูรายละเอียดใน ภาคผนวก ข.

5.3.5 Interfacing of spectrophotometric detector to a personal computer via an audio card for a flow injection system

This work was added in to substitute the work that was proposed for development of an on-lined UV-digestion.

ดูรายละเอียดใน ภาคผนวก ก.

4 Conclusions

The results in section 3.1 have shown that iodine usually present in urines as iodide. The results agree with that reported by other authors.[1] Thus determination of free iodide in urine may be adequate for the use as an index in evaluating of IDD status. However determination of iodide in urine using the Sandell and Kolthoff method without sample treatment is not appropriate (Section 3.2) because it was found that some urines contain species which interfere the kinetics measurement of the method. Therefore if the urines samples were to be determined using the Sandell and Kolthoff method which is a low cost method, then sample treatment is necessary.

In this work, three methods of sample treatments were studied (Section 3.3) by coupling the treatments to the FI system formerly developed.[22] The on-lined methods are UV-digestion, gas diffusion and dialysis. However those sample treatment did not show to be appropriate with the application. Therefore, two FI systems were developed to use in conjunction with a sample digestion method used by Dunn et al.[1,10]

Two FI procedures are described in the report for determination of iodine in urine. The principle which is based on catalytic effect of iodide in the reaction between Ce(IV) and As(III) was adopted from the common batch method used in monitoring IDD status. Continuous-FI and stopped-FI modes can be applied to a routine method and applicable for automation. Technician error, which often arises in the batch method from the reading of absorbance at a fixed time, can be quite a problem when there are hundreds of samples collected from a subject area. This error is eliminated through the use of flow injection technique because addition and mixing of reagents is always reproducible by the control of pumping rate. Analysis time per sample is much shorter for the FI methods when comparing to the conventional method.

It was found that the calibration plot is linear for both the FI systems. The signal measured at a fixed time is therefore directly proportional to the rate of reaction.

The sensitivity and sample throughput given by the two systems was not much different. The systems although gave satisfactorily low value of detection limit (≤ 3 μg I l^{-1}), determination of samples having the contents below 20 μg I l^{-1}(severe IDD) can be erratic. However the systems are still suitable for screening these types of samples from the samples above the margin of severe IDD status. If necessary the samples containing this very low level of iodine can be re-determined in the stopped mode by increasing the stopped time.

For this work, condition for urine digestion was developed by modifying the former condition[1,10] to reduce the amount of chloric acid. The proposed condition improves effect of interferent and provides complete digestion.

Apart from the use in the quantitation purposes, the stopped-FI system can be used to study kinetic profiles of the reaction. Some results have shown that the kinetic is first-order in Ce(IV) concentration under the condition used.

One additional work to replace the development of an on-lined UV-digestion in FI system is the development of an interface of spectrophotometer to a PC via an audio card which is a novel technique for interfacing an instrument giving a dc output to the PC.

5 References

1. J. T. Dunn, IDD newsletter, 1993, 9, 5. Accessed 26th January 1999 from http://avery.ined.virgima.edu/~jtd/iccidd/idddocs/idd196htm.
2. W. Buchberger and K. Winsauer, *Anal.Chim. Acta.*, 1985, **3**, 347.
3. J. Odink, J. J. P. Bogaards and H. Sandman, *J. Chromatogr.*, 1988, **431**, 309.
4. J. Rendl, S. Seybold and W. Borner, *Clin. Chem.*, 1944, **40**, 908.
5. F. Moussa, M. C. R. Demy, F. Veinberg, F. Depasse, R. Gharbi, J. Y. Hautem and P. Aymard, *J. Chromatogr. B*, 1995, **667**, 69.
6. Y. Yabu, K. Miyai, S. Hayashizaki, Y. Endo, N. Hata, Y. Iijima and R. Fushimi, *Endocrinol. Japon.*, 1986, **33**, 905.
7. V. Poluzzi, B. Cavalchi, A. Mazzoli, G. Alberini, A. Lutman, P. Coan, I. Ciani, P. Trentini, M. Ascanelli and V. Davoli, *J.A.A.S.*, 1996, **11**, 731.
8. P. Allain, Y. Mauras, C. Douge, L. Jaunault, T. Delaporte and C. Beaugrand, *Analyst*, 1990, **115**, 813.
9. M. Haldimann, B. Zimmerli, C. Als and H. Gerber, *Clin. Chem.*, 1998, **44**, 817.
10. J. T. Dunn, H. E. Crutchfield, R. Gutekunst and A. D. Dunn, *Thyroid*, 1993, **3**, 119.
11. E. B. Sandell and I. M. Kolthoff, *J.A.C.S.*, 1934, **56**,1426.
12. P. A. Rodriguez and H. L. Pardue, *Anal. Chem.*, 1969, **41**, 1369.
13. S. P. Mushtakova, L. F. Kozhina, L. M. Ivanova, A. K. Myshikina and O. A. Rytova, *J. Anal. Chem.*, 1998, **53**, 214.
14. G. Aumont and J. C. Tressol, *Analyst*, 1986, **111**, 841.
15. S. Suwannachoat, P. Wilairat and P. Tongkaew, unpublished work.
16. P. J. Garry, D. W. Lashley and G. M. Owen, *Clin. Chem.* 1973, **19**, 950.
17. W. May, D. Wu, C. Eastman, P. Bourdoux and G. Maberly, *Clin. Chem.*, 1990, **36**, 865.
18. K. Tsuda, H. Namda, T. Nomura, N. Yokoyama, S. Yamashita, M. Izumi and S. Nagataki, *Clin. Chem.*, 1995, **41**, 581.
19. S. Pino, S. L. Fang and L. E. Braverman, *Clin. Chem.*, 1996, **42**, 239.
20. J. Ruzicka and E. M. Hansen, *Flow Injection Analysis*, Wiley, New York, 2nd edn., 1988.
21. Z. Yaping, Y. Dongxing, C. Jixiang, L. Tianshiu and C. Huiqin, *Clin. Chem.*, 1996, **42**, 2021.
22. W. Chantore, S. Muangkaew, J. Shiowatana and D. Nacapricha, *Lab. Robotics and Automation*, 1999, 11,37.
23. J. C. Miller and J. N. Miller, *Statistics for analytical chemistry*, Ellis Horwood, New York, 3rd edn., 1993, p. 120.
24. J. T. Hakedal and P. K. Egeberg, Analyst, 1997, 123, 1151.

Output

1 M. Amornthammarong, K. Jareonsutasinee and **D. Nacapricha**, "Interfacing of spectrophotometric detector to a personal computer via an audio card for a flow injection system, Lab. Robotics and Automation, 2000, **12**, 138-141.

2 **D. Nacapricha**, S. Muangkaew, N. Ratanawimarnwong, J. Shiowatana and K. Grudpan, "Continuous and stopped flow injection for catalytic determination of iodine in urine" submitted to The Analyst by August 2000 (ทางสำนักบรรณาธิการวารสารได้แจ้งการรับบทความเพื่อพิจารณาแล้ว เมื่อวันที่ 4 ก.ย. 43)

3 **D. Nacapricha**, N. Ratanawimarnwong, S. Suwannachoat, P. Wilairat, J. Shiowatana and K. Grudpan, "Stop flow injection with the catalytic effect of iodide for kinetic determination of iodine" manuscript is in prepraration for the submission to either to Talanta or to Anal. Chim. Acta. by 1st October 2000.

ภาคผนวก ก

สำเนา reprint

M. Amornthammarong, K. Jareonsutasinee and **D. Nacapricha**, "Interfacing of spectrophotometric detector to a personal computer via an audio card for a flow injection system, Lab. Robotics and Automation, 2000, **12**, 138-141.

LRA, Vol. 12, pp. 138–141

Interfacing of Spectrophotometric Detector to a Personal Computer via an Audio Card for a Flow Injection System

Mana Amornthammarong,[1] Knadej Jareonsutasinee,[2] and Duangjai Nacapricha[1]

[1]*Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400 Thailand*
[2]*Institute of Science, Walailak University, Tasala, NakhonSiThammarat 80160 Thailand*

Received 1 September 1999

ABSTRACT: This work describes the use of an audio card for interfacing a spectrophotometer with a personal computer (PC). Digital recording on the computer was carried out using a voltage-controlled oscillator (VCO) and an audio card primarily installed in a multimedia PC. The output voltage of the spectrophotometer was transformed to frequency by the VCO. The audio card, linked to the VCO, converts the VCO output to digital data. An executable program named SigREC was written in C language using Microsoft Visual C++ 5.0 compiler to record the data.

The interfacing system was tested and compared with a chart recorder to record signals from a flow injection system with a spectrophotometer as a detector. The audio card system gave a better recording precision (RSD = 1.8%) when compared to the chart recorder (RSD = 2.5%). © 2000 John Wiley & Sons. Inc. Lab Robotics and Automation 12:138–141, 2000

Correspondence to: Duangjai Nacapricha.
Contract Grant Sponsor: National Electronics and Computer Technology Center (NECTEC).
Contract Grant Sponsor: Thailand Research Fund (TRF).

INTRODUCTION

Interfacing of an analytical instrument to a personal computer (PC) is usually carried out by using an analog-to-digital conversion card (A/D card) [1, 2]. The purpose of the interface is either for recording of the signal or for controlling the instrument, or both.

TABLE 1. The List of Electronic Parts Used in the Construction of the VCO Shown in Figure 1

Item	Description
IC1	LM13600 dual OTA
C1	400 pF capacitor
R1, R5	10K (1/4 watt, 5%)
R2	22K (1/4 watt, 5%)
R3	4.7K (1/4 watt, 5%)
R4	47K (1/4 watt, 5%)

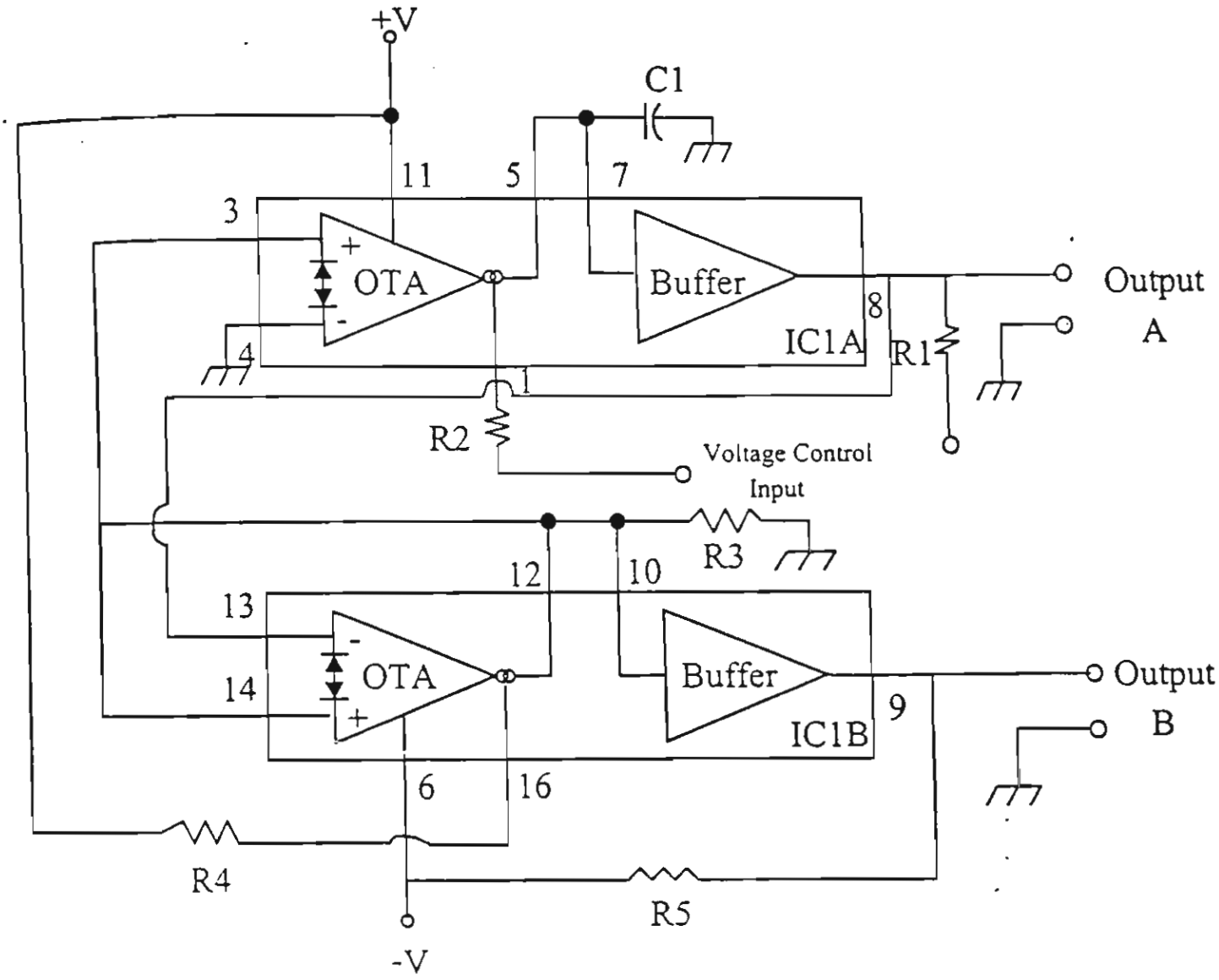

Figure 1. The circuit diagram of a VCO.

A modern computer is fully equipped with several multimedia devices, for example, speakers, microphone, CD-ROM drive, and an audio card or a so-called sound card. The audio card is a very efficient interfacing card for sound processing.

In this work, the use of an audio card for recording signal from a spectrophotometer was developed. A computer program was written to control the recording of the signal. The final system, including a voltage-to-frequency converter for the audio card and the computer program, has been tested with a spectrophotometer used in a flow injection (FI) system.

HARDWARE

Computer System

An IBM-compatible personal computer, with accessories for multimedia work, was used. The computer had the following specifications: Intel Pentium 133 MHz CPU; 16 MB RAM; 1.2 GB hard disk, Sound Blaster 16 Wavetable audio card, and 6x speed CD-ROM drive.

Computer Interface

For interfacing via audio card, the input dc voltage from an instrument (a spectrophotometer in this work) must first be transformed to an oscillating signal (ac voltage). A voltage-controlled oscillator (VCO) was thus constructed and tested for the conversion of dc signals to audio-type signals. A power supply was also constructed, giving voltages ranging from 0 to 30 volts, to generate dc voltages to test the interface system.

Figure 1 shows the circuit of a VCO assembled for this purpose. The list of electronic parts is shown in Table 1.

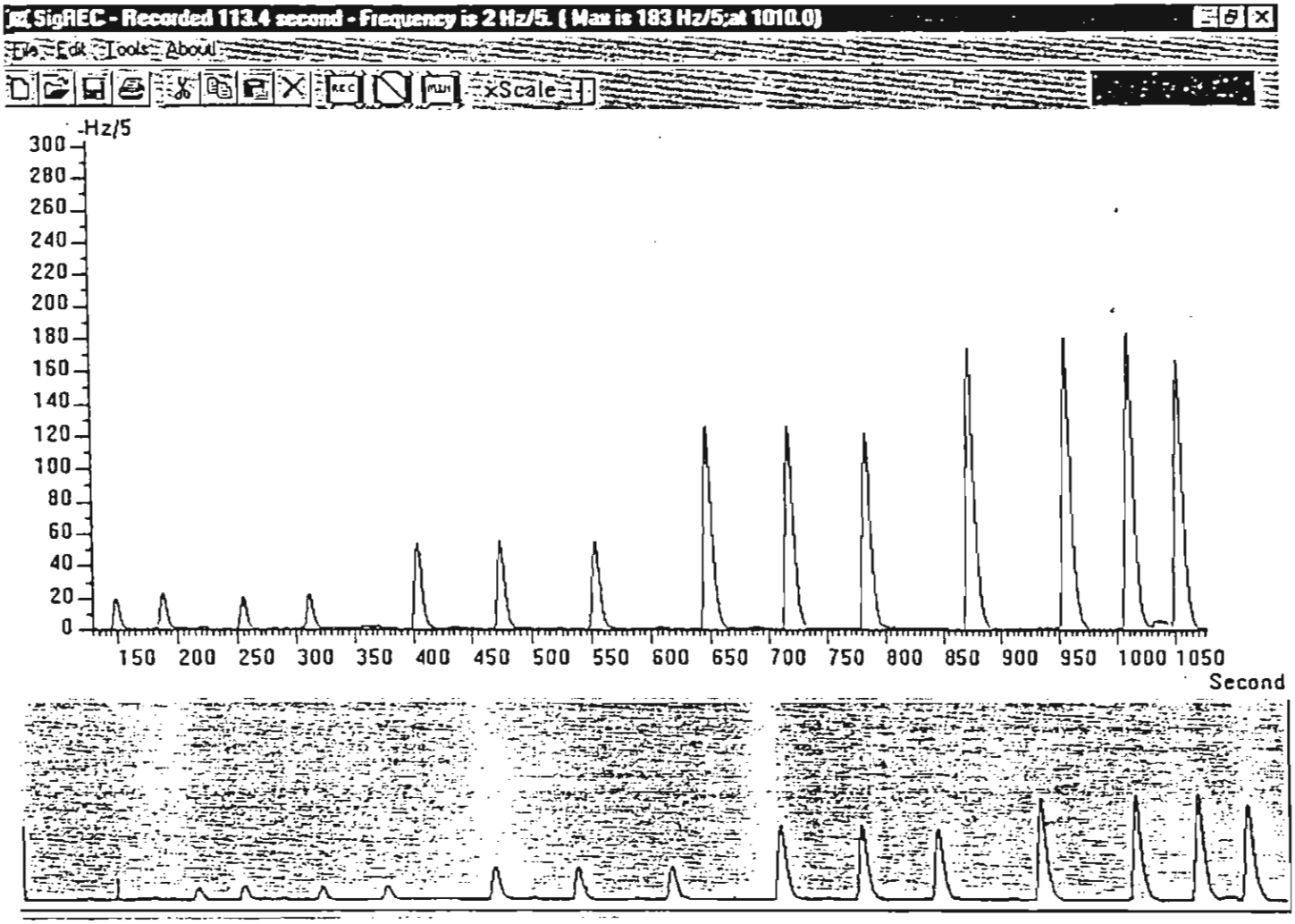

Figure 2. Window display of the SigREC and an example of signals recorded from a flow injection system.

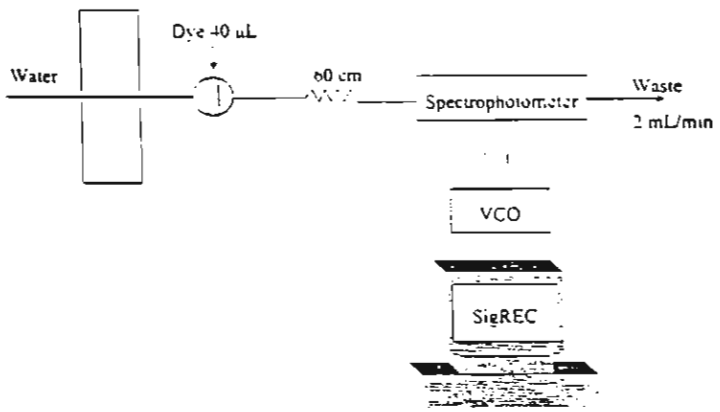

Figure 3. Schematic diagram of the flow injection system that employed the interface system.

The output from the VCO is then sent to the audio card for digital conversion and subsequent recording by the PC. A non-inverting buffer amplifier, consisting of an operational amplifier (UA 741 CN), was also assembled to amplify the output voltage from the spectrophotometer to match the input working range of the VCO.

SOFTWARE

A program was written to control the recording of the signal and to utilize some functions of the audio card. The program source code was written in C language using the compiler of Microsoft Visual C++ 5.0 to produce the executable program, named SigREC. The SigREC program provides a fixed sampling rate of 5 Hz. The system records frequency versus time and can continuously record for up to 4 hours. The data are saved in the format of a text file (.txt), with a maximum size of 65,536 bytes, which is readily available for further processing or analysis by other programs such as Microsoft Excel or Mathematica. The window display is user friendly, as shown in Figure 2.

Figure 2 shows an example of signals recorded from a flow injection experiment with spectrometric detection. At the top of SigREC window, some information of the data file is shown, including time of the recording, the signal frequency, where the cursor is placed, and the maximum signal measured. The signal display is in units of Hz/5. The bottom section of the window shows the complete recording. A sam-

ple of the section can be marked for enlargement and displayed in the top part of the window.

APPLICATION

The interface system, consisting of the VCO, the audio card, and the SigREC program, was used to monitor dc signals from a spectrophotometer (Shimadzu Model UV-120–1, Japan) used as a light absorbance detector of a FI system. Figure 3 shows the FI system. The system consists of a modified peristaltic pump of Ismatec Model IS7610 (Switzerland), a Rheodyne injection valve Model 7125 (USA), and a Philips flow-through cell of 10 mm path length. All manifold tubings were poly(tetrafluoroethylene) (PTFE) with i.d. 0.8 mm. An Ismatec Tygon tube with three stops (i.d. 0.95 mm) was used as the pump tube.

The transient absorbance signal of the FI system as recorded by the PC was compared with signals recorded on a strip-chart recorder (Barnstead Model 1201, U.K.). Ten replicate injections of a dye were made with the FI system. It was found that RSDs of the peak height were 1.8% for the digital recording and 2.5% for the chart recorder.

CONCLUSIONS

Most PC systems have multimedia capability with an audio card. Thus, the PC system can be used to record dc signal, which has been first converted to frequency, from an instrument without the necessity of purchasing an A/D card. Results from the flow injection experiment have shown that the interfacing system provides a better precision in signal recording than the strip-chart recorder.

The interface system developed has a linear relationship between the applied dc voltage and frequency, over a wide range of dc voltage (0 to 25 V). The system can also be used to record signal from other instruments with higher range of dc output than a spectrophotometer (0–2 V).

ACKNOWLEDGMENTS

The authors thank Dr. Prapin Wilairat for editing the manuscript and Dr. Kate Grudpan for advice.

REFERENCES

[1] Derenzo, S. E. Interfacing: A Laboratory Approach Using the Microcomputer for Instrumentation, Data Analysis and Control; Prentice- Hall: London, 1990.

[2] Mustafa, M. A. Microcomputer Interfacing and Applications, 2nd edition; Newnes: Oxford, 1994.

ภาคผนวก ข

สำเนา submitted manuscript

D. Nacapricha, S. Muangkaew, N. Ratanawimarnwong, J. Shiowatana and K. Grudpan, "Continuous and stopped flow injection for catalytic determination of iodine in urine" submitted to The Analyst by August 2000 (ทางสำนักบรรณาธิการวารสารได้แจ้งการรับบทความเพื่อพิจารณาแล้ว เมื่อวันที่ 4 ก.ย. 43)

Continuous and stopped flow injection for catalytic determination of iodine in urine

Duangjai Nacapricha*[a], Saowapha Muangkaew[a], Nuanlaor Ratanawimarnwong[a], Juwadee Shiowatana[a]and Kate Grudpan[b]

[a]*Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400 Thailand*
E-mail: scdnc@mahidol.ac.th
[b]*Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand*

This paper describes the use of flow injection (FI) techniques for the determination of iodine in urine, based on the catalytic effect of iodide in the redox reaction between Ce(IV) and As (III). The proposed procedures minimize errors in the conventional batch method arising from the reading of absorbance at a fixed time after addition of Ce(IV) reagent. Two FI systems, for the continuous and stopped modes of operation were assembled. In the continuous-FI system, a thermostated bath was used to increase the sensitivity. However this is not necessary for the stopped-FI system. The two systems are comparable in terms of sensitivity, sample throughput and detection limit. The continuous-FI and the stopped-FI exhibited detection limits (3σ) of 2.3 and 3 μg I l^{-1} respectively. Both systems have equal samples throughputs of 35 samples h^{-1}. Calibration plots for both techniques are linear. The FI procedures provide very short analysis time compared to the batch procedure. Using the linear regression test, there is no significant difference between the results from the four methods, i.e., continuous-FI, stopped-FI, conventional method and ICP-MS. The proposed methods are readily applicable for automation and can be an alternative to the conventional procedure for the survey of the iodine deficiency disorder.

A condition for sample digestion is also proposed to reduce the amount of chloric acid for a complete digestion. Kinetic information of the reaction can also be obtained from the stopped flow mode.

Keywords: *Iodine, iodate, urine, urinary iodine, flow injection, stoppedf flow injection, catalytic determination, inductively coupled plasma mass spectrometry*

Introduction

Iodine contents in urine have been widely used as a marker for status assessment of iodine deficiency disorder (IDD). In 1992, the ICCIDD/WHO/UNICEF[1], gave the following values as a guide for IDD status: <20 μg I l^{-1} (severe); 20-49 μg I l^{-1} (moderate); 50-100 μg I l^{-1} (mild) and >100 μg I l^{-1} (normal). There have been a number of documents that describe methods for determination of inorganic iodine content and total content in urine.

For the quantitation of free iodide, different modes of high performance liquid chromatography (HPLC) such as ion[2] and ion-pair reversed-phase[3,4,5] were proposed with

either electrochemical detection[3,4,5] or post-column reaction.[2] Yabu et al. have proposed a method to measure iodide content in urine using iodide-selective electrode.[6]

Total content of iodine can be determined by using a hyphenated technique such as inductively coupled plasma mass spectrometry (ICP-MS). Usually dilution of sample with water[7] or other types of reagent such as nitric acid[7,8] and ammonia[9] is adequate for this technique. Variations in the signals of the detection mass, are corrected by addition of some internal standards, e.g., europium[8] rhodium or indium.[7] An ICP-MS for determination of urinary iodine using isotope dilution with iodine-129 was presented by Haldimann et al.[9]

Surrounded by other methods, the most common method used in laboratories dealing with monitoring of IDD status is a spectrometric method with catalytic effect of iodide on a redox reaction between Ce(IV) and As(III).[1,10] The method is more preferable than other methods because it is more practical as a survey tool. Sandell and Kolthoff first described the use of this reaction for determination of trace iodide catalyst in 1934.[11] Rodriguez and Pardue proposed some possible mechanisms based on their experimental data achieved in 1969.[12] The net reaction is

$$\underset{\text{yellow}}{2Ce(IV) + As(III)} \longrightarrow \underset{\text{colorless}}{2Ce(III) + As(V)} \qquad (1)$$

The rate of reaction is directly proportional to the iodide concentration when other factors are fixed.

Mushtakova et al.[13] proposed a Sandell and Kolthoff method for determination of free iodide in urine. Their method requires no step of urine digestion prior to spectrometric detection of Ce(IV). However extensive investigation of interfering effect from potential species in urine is yet required for this method.

Description of the classical method was provided by Dunn et al.[1,10] The procedure is usually carried out batchwise. Interferences to the redox reaction in urine must be eliminated prior to the step of spectrometric measurement. Digestion with chloric acid is more often used than the alkaline ashing method.[14] For chloric acid digestion, iodine species are oxidised to iodate ions after digestion. Unpublished results[15] apparently indicated that chloride ion added in the arsenious solution helps in conversion of iodate to iodide within less than a minute. The results have also demonstrated that the disappearance of Ce(IV) is a first-order process under the condition of the conventional method.

After chloric acid digestion, the sample is added with As(III) solution which contains sodium chloride. Finally after an accurate volume of Ce(IV) solution was added to this solution the reading of absorbance (405-420 nm) must be taken at a fixed time for all the samples and the standards. Hence technician error can easily occur in the conventional batch method. Automation of the procedure has been reported through the use of autoanalyser, which is based on air-segmented flow (ASF) technique.[4,16,17,18,19] The use of flow injection (FI) technique offers various advantages.[20] Nevertheless, there has been no report of a FI system to use in conjunction with this type of sample preparation.

In 1996, Yaping et al.[21] have proposed a FI method for determination of iodine in urine. The method employed Sandell and Kolthoff reaction but with the use of different type of reagent (H_2SO_4+$KMnO_4$+$K_2Cr_2O_7$) for on-line digestion. With this mixture of oxidant, and with the detection wavelength at 480 nm, spectral interferent is at risk especially from $KMnO_4$. Another disadvantage of the method is that the method unnecessarily hired a toxic brucine as a reagent stream.

In this article, two methods developed for determination of urinary iodine based on flow injection techniques are described. The first method is proposed to use in the normal continuous mode. The system can be modified to operate under a stopped-FI mode.[20] Both

methods are readily applicable for automation to reduce technician error, which often arise in the convention batch method.

Experimental

Manifolds for the continuous and stopped flow injection

The configuration of the FI system used in the continuous mode was modified from the system reported in the previous work[22] and is shown in Fig.1. Concentrations of Ce(IV) and As(III) were optimized from the conventional method used by Dunn et al.[1,10] An Ismatec peristaltic pump, model IS7610 was used for propelling reagents and sample plug. A Rheodyne injection valve, model 7125, fitted with Teflon loop (1.0 mm i.d.) was employed for injections of standards and samples. A glass reaction coil, 100 cm, was made of glass tubing with i.d. 1.0 mm by curling this glass tube into a spiral shape around an opened glass cylinder (i.d. 1.5 cm and 17.5 cm in length). Two pieces of the coil were connected together to make 200 cm coil length. Cole Parmer tygon tubings with i.d. of 0.75 mm, 2.25 mm o.d. and 0.75 mm wall, were used for the FI assembly. A PolyScience water bath, model 2L-M, was used for controlling the temperature of the glass coil immersed in the bath. A Shimadzu spectrophotometer, model UV-2-01, Japan, with a tungsten lamp and a Philips flow cell of 0.01 ml volume was used for monitoring the absorbance of Ce (IV) at 420 nm. An Alltech chart recorder, model LR 93025, USA, was used for recording the signal from the spectrophotometer. The recorder was set at 50 mV f.s.d.

The system of the continuous-FI shown in Fig. 1 was modified to operate under the stopped-FI mode. In the stopped mode, the glass reaction coil was replaced by a coil made of Tygon tube (100 cm). The water bath was not used in this system. The pumping rate for each channel was 1 ml min^{-1}.

Reagents

All chemicals used in this work were AR grade, supplied by Merck (Germany) except indium nitrate. Deionized-distilled water was obtained from a Milli-Q system, Millipore, USA, and was used throughout.

Iodine standards

A stock of iodine standard (1,000 mg I l^{-1}) was prepared by dissolving 0.1685 g of potassium iodate in water and making up to mark of a volumetric flask (100 ml). Further dilutions were made for working solutions of appropriate concentrations.

Chloric acid 28% w/v

Potassium chlorate (250 g) was dissolved in 455 ml of deionized-distilled water in an Erlenmeyer flask. The mixture was heated until clear solution is accomplished. 187.5 ml of perchloric acid 70% v/v was slowly added into this solution with constant stirring. This solution mixture was stored in a freezer overnight and was filterred through a filter paper (Whatman no.1 or equivalent), into a Buchner funnel.[1,10] The final volume of filtrate was approximately 850 ml. This solution contained approximately 28% w/v of chloric acid and

has been used for digesting all urine samples and iodine working solutions. The solution of chloric acid was stored at 4 °C in a refrigerator.

Arsenious acid 0.05 and 0.1 M

As_2O_3 (10 g) and NaCl (47 g) were dissolved in 500 ml of water with heating on a hot plate. After cooling to the room temperature, 27.8 ml of conc. H_2SO_4 was added to this solution followed by dilution with water to one litre. This 0.1 M arsenious acid solution was used in the FI methods. Arsenious acid 0.05 M was prepared in similar way except that the weights of As_2O_3 and NaCl were respectively reduced to 5 and 25 g. This more diluted solution of arsenious acid was used throughout the batch method.

Ceric ammonium sulfate 0.008 and 0.038 M

Ceric ammonium sulfate 0.008 M was used as a reagent stream in the FI methods. This solution was prepared by dissolving 5 g of $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ in 1 l of 1.75 M H_2SO_4. Ceric ammonium sulfate 0.038 M was prepared in similar way using 24 g of $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ dissolved in 3.5 M H_2SO_4. This latter solution was used for determination of urinary iodine in the batch method.

Indium nitrate 1 μg In l^{-1}.

Indium nitrate (Fluka, Switzerland) 0.655 g was dissolved and diluted to 250.00 ml in 2% v/v nitric acid. This solution was used as the internal standard for determination of iodine in urine by inductively coupled plasma mass spectrometry (ICP-MS).

Sample

Casual urine samples were collected from students and were selected to cover to range of iodine concentration studied. The samples were frozen and thaw before use.

Determination of urinary iodine by catalytic methods

Digestion of urine samples

The general method for acid digestion of urine,[1,10] was used with slight modification. The volume ratio of chloric acid solution to urine was reduced from conventionally 3+1 to be 1.2+1.

4.00 ml of urine or working iodine standard was pipetted into a test tube, which contained 4.8 ml of 28% w/v chloric acid. After one-hour digestion in a heating block at 105-110 °C, the solution was transferred into a volumetric flask and was made up to 10.00 ml with water. The reagent blank was prepared in similar way using deionized-distilled water. There is also enough volume of a liquid for replicate injections in the FI methods.

To avoid any course of error, calibration of samples were always made against the set of iodine standards digested in the same run. Working range of standards are the same for the batch and the flow methods, which ranged from 20 to 200 μg I ml^{-1}.

Batch method

Aliquots of 1.0 ml of the digested solutions were transferred into separate test tubes. Determination of iodine was carried out using the method described by Dunn et al.[1,10]

Continuous-FI

Operation of the system in Fig.1 for optimization and analysis was carried out in the usual manner by injecting the liquid sample into a continuous flowing stream of water carrier. The sample zone, after being merged and mixed with streams of arsenious acid and ceric ammonium sulfate, was transported to the detector which recorded profiles of the signal. Besides the catalytic effect of iodine, the reaction zone was also thermally catalysed in the glass coil immersed in the 43 °C bath. Signal profiles of the continuous mode are depicted in Fig. 2a. The drop in absorbance reading was due to the color fading of Ce(IV). For this set up, the maximum drop was achieved at 100 seconds after injection.

Stopped-FI

For this mode, it is convenient to operate the system at room temperature (26 °C), thus, the bath (Fig. 1) was not used to raise up the temperature. Injection of a sample was carried out in similar way to the continuous mode. At the time of 41-second, when the peak bottom rose in the usual continuous mode, the flow was stopped for a period (1 min). The signal was continually recorded during the stopped-flow period. The flow was restarted again at time t' (101 seconds after injection) to push away the sample zone resulted in the rise of absorbance back to the baseline. Some of the stopped-FI profiles are depicted in Fig. 2b. In the calibration, the stopped interval must be fixed for all working solutions and the samples.

Interferent studies

Interferences of diverse species which potentially exist in urines (Table 2: Cl^-, SO_4^{2-}, HCO_3^-, Ca^{2+}, K^+, Mg^{2+}, SCN^-, Fe^{3+}, F^-, Mn^{2+}, $(NH_4)_2HPO_4$, glucose, urea, oxalic acid, uric acid and ascorbic acid) were examined.

The studies were made using the continuous-FI system shown in Fig.1. The sixteen chemical species were separately added into 1 ml aliquots of 1,000 μg I l^{-1} potassium iodate solution in a volumetric flask. These solution mixtures were made up to mark with water to 10.00 ml. After the digestion, the signal obtained from these solution were compared to the signal achieved from a pure standard of potassium iodate (100 μg I l^{-1}). A species was considered to interfere if its presence resulted in a signal alteration of greater than ±10%.

Determination of urinary iodine by ICP-MS

An Elan 6000 inductively coupled plasma mass spectrometer, Canada, with the recommended operation condition was used in determination of iodine as a comparative method. Sample introduction to the nebulizer was carried out using a Gilson peristaltic pump.

In the analysis, 10-fold dilution with 0.16 M nitric acid was made on the samples. In, added as indium nitrate, was used as the internal standard to correct for non-spectral interference and for signal instability.

Results and discussion

Modification of the digestion method

For this work, the volume ratio of chloric acid solution to urine was reduced from conventionally 3+1[1,10] to be 1.2+1. The results preliminarily studied on five samples using the manifold in Fig. 1 have shown that there was no significant variation in the signals over the ratios of 4+1, 3+1, 2+1 and 1+1 (all made up with water to 5.00 ml). This indicated of complete digestion for all conditions tested.

With the current volume ratio, concentrations of iodine after digestion lie within appropriate range of standards for the FI methods as well as the batch method. There is also enough volume of a liquid for replicate injections in the FI methods.

Optimization of FI parameters

For a catalytic reaction, concentrations of reagents play an important role on the kinetic patterns. In the Sandell and Kolthoff method, it is desirable to keep the concentration of Ce (IV) well below the As (III) concentration.

Optimization of the flow injection method was carried out on the set up shown in Fig.1 using continuous mode. The concentration of Ce(IV) was varied over the range of 6.0×10^{-3} to 2.0×10^{-2} M when the concentration of As (III) was fixed at 0.1 M. Replicate injections of 100 μg $I\ l^{-1}$ solution were made. The results have shown that the size of signal increased to approximately 0.2 absorbance value as the concentration of Ce(IV) was greater up to 1.5×10^{-2} M. Above this concentration, the signal stayed fairly constant. The concentration of Ce(IV) at 8.0×10^{-3} M was chosen, because at this concentration acceptable sensitivity was obtained. Under this condition the molar ratio between Ce(IV) and As (III) was 1:12.5 (the pumping rates of these two reagent streams were equal).

Chantore et al.[22] have found that raising up the temperature of the reaction zone can increase the sensitivity. For the continuous-FI system, the effect of temperature on sensitivity was studied at two bath temperatures. Linear calibrations were obtained from both temperatures, e.g., $\Delta A = 0.0010[I] + 0.049$, $r^2 = 0.998$ (26 °C) and $\Delta A = 0.0016[I] + 0.133$, $r^2 = 0.997$ (43 °C). The intercepts represent signals of reagent blank. The results have shown that the sensitivity was better achieved at 43°C than a room temperature (26 °C), in terms of slope and intercept of calibration. Temperatures greater than 43°C although gave higher sensitivity but can decrease solubility of air and sometimes cause the air bubbles inside the glass coil. Thus for the continuous-FI, the bath temperature was fixed at 43 °C.

The set up condition of the stopped mode was similar to the condition illustrated in Fig.1 except that the flow rate could be made faster to 3 ml min^{-1}. The results in Table 1 show that prolong of the stopped time increased the sensitivity. For this work, a stopped time of 1 min was sufficient to provide reasonable absorbance readings over the working range of 50 to 200 μg $I\ l^{-1}$.

Sensitivity and sample throughput

In the studies, criteria for consideration of sensitivity are based on the slope of calibration and the calibration intercept.

Due to some differences such as configuration, the operating procedure and under the conditions studied the flow rates, the sensitivities given by the two modes, are incomparable. Nevertheless, it was observed that the sensitivity of the continuous system is greater than that obtained from the condition of the stopped mode. For examples, the regression equations are $\Delta A = 0.0016[I] + 0.133$, $r^2=0.989$ (for the continuous-FI, 43 °C) and $\Delta A = 0.0011[I] + 0.091$, $r^2= 0.996$ (for the stopped-FI, 26 °C).

In the stopped-FI mode, it is expected that the size of signal will be enlarged if the time spent by the reaction zone is prolonged. The results in Table 1 demonstrate that increasing the interval length resulted in larger size of signals including the signal of the reagent blank (intercept). Also the results have indicated that the slope became greater with time. Thus, prolong of the stopped time increases the sensitivity of stopped mode.

Besides the sensitivity, sample throughput is often a parameter used for consideration. This parameter is an indication of speed of analysis. The throughputs as determined by stopped interval are summarized in Table 1. Although the stopped time of 180 seconds has delivered the highest sensitivity, the analysis was time consuming. For this work, the stopped interval of 1 min was chosen as it resulted in a reasonable sensitivity and acceptable throughput of sample (35 samples h^{-1}). This sample throughput is approximately equal to that given by the continuous-FI system.

Limits of detection

Limits of detection were determined by ten replicate injections of a reagent blank into both systems. The limits (3σ) were found to be 2.3 and 3 $\mu g\ I\ l^{-1}$ for the continuous-FI and the stopped-FI respectively. Therefore these two systems are capable for detection iodine in severe IDD samples ($< 20\ \mu g\ I\ l^{-1}$).

Interferences

The results in Table 2 show that signal alteration of all the species were less than the margins ($\pm 10\%$), even when the first seven species were studied at remarkably high levels. The rest of foreign species that were formerly tested in another FI system were not considered to interfere in the present system. Although chloric acid was less used with this modified condition, serious interferents ever reported for this reaction such as SCN^- and ascorbic acid[17] were eliminated.

Recovery

Recovery study was made on fourteen samples using both techniques of flow injection as summarized in Table 3. From the results, recovery was ranging from 84% to 111% or 99% in average. This satisfactorily good recovery reflects that interfering species were completely eliminated during digestion. This also indicated that the modified procedure of acid digestion is applicable for this application.

Comparison between the batch and the flow methods

For the batch method, calibration is a plot between the absorbance reading against iodine concentration. The reading must be taken at a constant interval after the addition of Ce(IV) solution to the mixture of digested sample and As(III) solution.[1,10] Pino et al.[19] used the plot between the change in %transmission and the concentration. None of these calibration plots is linear.

The calibration plot as suggested to use for the continuous-FI system was the plot between the size of signal (baseline reading - peak bottom reading, ΔA) versus iodine concentration. Under the condition used, the calibration was linear which means that within the 100-seconds the size of signal was directly proportional to the rate of reaction. Similar to the continuous-FI method, linear calibration was also obtained from the stopped-FI method by plotting the signal size versus concentration. It is thus an advantage of the proposed methods.

Twenty samples of urine were determined for iodine contents using the conventional method and the continuous flow injection method. The two procedural approaches gave good agreement in the results. The plot between the mean values of both techniques showed the absence of any analytical bias. The Pearson coefficient of correlation (n= 24) was found to be 0.952 and a reasonable linear regression line was obtained ($y = 0.938x + 4.63$).

Precision of the measurement between the batch and the flow method was compared. Ten replicates of injection (100 $\mu g\ I\ l^{-1}$) were made on the continuous-FI set up. Both methods contributed satisfactorily low values of RSD (1.6% and 0.4% for the batch and the flow methods respectively).

Although the batch method did not give a prominently high value of RSD but the results could be worse in different hands of operators. The RSD results have shown that the continuous flow mode has allowed for a better control of timing than the batch method. Addition and mixing of reagents are constant and continuous within tube lines of the flow systems. The time at which the absorbance is measured is always reproducible as controlled by the pump. In this way technician error in the step of catalytic measurement is eliminated.

Repeatability of the stopped FI system was also measured and the RSD was found to be 1.2%. This RSD value was obtained through which the pump was turned on and off manually. The precision index would have been improved if the system had been fully automated.

Excluding the step of digestion, the two systems of FI have given the analysis time for a sample of approximately 1 minute and 40 seconds. The analysis time is therefore much less than the time required in the batch method (25 to 35 min per sample).

Method validation

Iodine contents in eleven samples were determined by using four different procedures: three based on Sandell and Kolthoff reaction, i.e., the conventional batch method, continuous-FI and stopped-FI methods and another by using ICP-MS. The results in Table 4 demonstrated that the results from the ICP-MS were greater than those from the catalytic methods. This perhaps suggested that there is some loss of iodine during the digestion. However, the results of all four methods are mostly consistent. It is observed for some samples, having the contents below 20 $\mu g\ I\ l^{-1}$(e.g. samples 1 and 3), that the precision of the catalytic methods

were low. These samples were below the calibration ranges of the catalytic methods. This was not found for sample 2.

Applying linear regression,[23] comparison was made for each method pair via the plot between the results achieved from the two methods. Parameters of the regression line such as intercept (a), slope (b) and correlation coefficient (r^2) were then obtained.

The correlation coefficients (r^2) shown in Table 5 indicate reasonably good correlation (r^2>0.850). The confidence limits suggest that the slope and the intercept do not differ significantly from the ideal values. Thus, there is no evidence for systematic differences between the results obtained from the four techniques, i.e., continuous-FI, stopped-FI, batch and ICP-MS. This agreement of the results shows that the two modes of flow injection can be an alternative to the conventional batch method.

Stopped-FI mode as a tool for kinetic study

With the use of stopped-FI mode, kinetic information can be fulfilled. In the stopped mode, the fading in color of Ce(IV) is recorded against time. Fig. 3 are profiles of the kinetic process which were recorded from the proposed stopped-FI system. It is observed that the data points agreed well with the exponential fitting. This indicated that under the FI condition, the disappearance of Ce(IV) is a first-order process. An investigation has been carried out in progress for using the rate constant in determination of iodine.

Conclusions

Two FI procedures are described for determination of iodine in urine. The principle which is based on catalytic effect of iodide in the reaction between Ce(IV) and As(III) was adopted from the common batch method used in monitoring IDD status. Continuous-FI and stopped-FI modes can be applied to a routine method and applicable for automation. Technician error, which often arises in the batch method from the reading of absorbance at a fixed time, can be quite a problem when there are hundreds of samples collected from a subject area. This error is eliminated through the use of flow injection technique because addition and mixing of reagents is always reproducible by the control of pumping rate. Analysis time per sample is much shorter for the FI methods when comparing to the conventional method.

It was found that the calibration plot is linear for both the FI systems. The signal measured at a fixed time is therefore directly proportional to the rate of reaction.

The sensitivity and sample throughput given by the two systems was not much different. The systems although gave satisfactorily low value of detection limit (≤ 3 μg I l^{-1}), determination of samples having the contents below 20 μg I l^{-1}(severe IDD) can be erratic. However the systems are still suitable for screening these types of samples from the samples above the margin of severe IDD status. If necessary the samples containing this very low level of iodine can be re-determined in the stopped mode by increasing the stopped time.

For this work, condition for urine digestion was developed by modifying the former condition[1,10] to reduce the amount of chloric acid. The proposed condition improves effect of interferent and provides complete digestion.

Besides, the stopped-FI system can be used to study kinetic profiles of the reaction. Some results have shown that the kinetic is first-order in Ce(IV) concentration under the condition used.

Acknowledgements

This work was supported by grants from the Thailand Research Fund and the Shell Centenary Education Fund. We would like to thank Dr. Prapin Wilairat for his useful comments.

References

1. J. T. Dunn, IDD newsletter, 1993, 9, 5. Accessed 26th January 1999 from http://avery.ined.virgima.edu/~jtd/iccidd/idddocs/idd196htm.
2. W. Buchberger and K. Winsauer, *Anal.Chim. Acta.*, 1985, **3**, 347.
3. J. Odink, J. J. P. Bogaards and H. Sandman, *J. Chromatogr.*, 1988, **431**, 309.
4. J. Rendl, S. Seybold and W. Borner, *Clin. Chem.*, 1944, **40**, 908.
5. F. Moussa, M. C. R. Demy, F. Veinberg, F. Depasse, R. Gharbi, J. Y. Hautem and P. Aymard, *J. Chromatogr. B*, 1995, **667**, 69.
6. Y. Yabu, K. Miyai, S. Hayashizaki, Y. Endo, N. Hata, Y. Iijima and R. Fushimi, *Endocrinol. Japon.*, 1986, **33**, 905.
7. V. Poluzzi, B. Cavalchi, A. Mazzoli, G. Alberini, A. Lutman, P. Coan, I. Ciani, P. Trentini, M. Ascanelli and V. Davoli, *J.A.A.S.*, 1996, **11**, 731.
8. P. Allain, Y. Mauras, C. Douge, L. Jaunault, T. Delaporte and C. Beaugrand, *Analyst*, 1990, **115**, 813.
9. M. Haldimann, B. Zimmerli, C. Als and H. Gerber, *Clin. Chem.*, 1998, **44**, 817.
10. J. T. Dunn, H. E. Crutchfield, R. Gutekunst and A. D. Dunn, *Thyroid*, 1993, **3**, 119.
11. E. B. Sandell and I. M. Kolthoff, *J.A.C.S.*, 1934, **56**,1426.
12. P. A. Rodriguez and H. L. Pardue, *Anal. Chem.*, 1969, **41**, 1369.
13. S. P. Mushtakova, L. F. Kozhina, L. M. Ivanova, A. K. Myshikina and O. A. Rytova, *J. Anal. Chem.*, 1998, **53**, 214.
14. G. Aumont and J. C. Tressol, *Analyst*, 1986, **111**, 841.
15. S. Suwannachoat, P. Wilairat and P. Tongkaew, unpublished work.
16. P. J. Garry, D. W. Lashley and G. M. Owen, *Clin. Chem.*, 1973, **19**, 950.
17. W. May, D. Wu, C. Eastman, P. Bourdoux and G. Maberly, *Clin. Chem.*, 1990, **36**, 865.
18. K. Tsuda, H. Namda, T. Nomura, N. Yokoyama, S. Yamashita, M. Izumi and S. Nagataki, *Clin. Chem.*, 1995, **41**, 581.
19. S. Pino, S. L. Fang and L. E. Braverman, *Clin. Chem.*, 1996, **42**, 239.
20. J. Ruzicka and E. M. Hansen, *Flow Injection Analysis*, Wiley, New York, 2nd edn., 1988.
21. Z. Yaping, Y. Dongxing, C. Jixiang, L. Tianshiu and C. Huiqin, *Clin. Chem.*, 1996, **42**, 2021.
22. W. Chantore, S. Muangkaew, J. Shiowatana and D. Nacapricha, *Lab. Robotics and Automation*, 1999, 11,37.
23. J. C. Miller and J. N. Miller, *Statistics for analytical chemistry*, Ellis Horwood, New York, 3rd edn., 1993, p. 120.

Figure captions

Fig. 1 The manifold of the continuous-FI system used for determination of iodine in urine.
Fig. 2 Profiles of iodine standards obtained from the (a) continuous FI system and (b) stopped-FI system.
Fig. 3 Kinetics obtained from the stopped-FI system, showing the disappearance of Ce(IV) at different concentration of iodine standard in sample zones. Full lines represent the exponential fittings.

Table 1 Sensitivity and sample throughput of the stop-FI system as determined by the stop interval

Stop time/s	Slope ($\times 10^{-4}$)	Intercept	r^2	Sample throughput/ sample hr^{-1}
0	3.0	0.019	0.999	88
20	6.0	0.039	0.999	59
40	9.0	0.067	0.999	44
60	12	0.095	0.999	35
120	17	0.188	0.996	22
180	21	0.265	0.996	16

Table 2 Effect of foreign species studied based on alteration of signal obtained from triplicate injections of iodine standard, 100 μg I l^{-1}

Foreign species	Added as	Concentration level tested /mol dm^{-3}	Signal alteration (%)
1. Cl^-	NaCl	1.5*	+8.1
2. SO_4^{2-}	Na_2SO_4	0.2*	+5.2
3. HCO_3^-	$NaHCO_3$	0.06*	+0.9
4. $(NH_4)_2HPO_4$	$(NH_4)_2HPO_4$	0.144*	-4.8
5. Ca^{2+}	$CaCl_2$	0.084*	0.0
6. Glucose	$C_6H_{12}O_6$	2.34 m*	+0.9
7. Urea	$CO(NH_2)_2$	9.9 m*	+2.2
8. K^+	KCl	9.9 m†	+0.9
9. Mg^{2+}	$MgCl_2$	20.6 m†	+1.8
10. SCN^-	KSCN	0.3 m†	+4.0
11. Fe^{3+}	$FeCl_3$	0.36 m†	+7.3
12. F^-	NaF	0.53 m†	-0.8
13. Mn^{2+}	$MnSO_4$	36.4 μ†	-0.9
14. Oxalic acid	$C_2H_6O_6$	4.0 m†	0.0
15. Uric acid	$C_5H_4O_3N_4$	11.9 m†	-4.0
16. Ascorbic acid	$C_6H_8O_6$	0.57 m†	+0.9

* 3-fold of the typical levels in urines.

† These levels of foreign ions were previously tested on a continuous FI system proposed by Yaping et al.[20].

Table 3 Percentage of the recovery measured by using the flow injection systems*

Urine sample	Iodine content/ μg I l^{-1}	Added/μg I l^{-1}	Found/μg I l^{-1}	Recovery (%)
A	31±2	100.00	131±0	100
B	78±2	100.00	178±0	100
C	83±2	100.00	167±1.5	84
D	106±5	100.00	206±3	100
E	147±0	100.00	256±3	109
F	330±2	100.00	440±6	111
G	15±1	50.00	67±0.6	110
H	33±1.5	50.00	79±0.6	85
I	51±0.6	50.00	100±1	98
J	52±0.6	50.00	105±0.6	107
K	53±0.6	50.00	103±0.6	99
L	81±2.1	50.00	126±2.5	93
M	86±0.6	50.00	133±2.7	96
N	94±3.5	50.00	146±0.6	101

* Determination of samples A to F and G to N were respectively carried out using the continuous mode (n=2) and the stop mode (n=3).

Table 4 Contents of iodine in urine determined on eleven samples using four methods of analysis, i.e., continuous-FI, stop-FI, batch and ICP-MS (n=3)

Sample	Iodine content/μg l^{-1} ± SD			
	Continuous-FI	Stop-FI	Batch	ICP-MS
1	5.44 ± 22	11.2 ± 15	18.9 ± 8.1	12.1 ± 15
2	13.3 ± 6.7	14.5 ± 1.5	17.4 ± 3.6	16.9 ± 4.2
3	15.5 ± 11	13.3 ± 13	14.6 ± 19	15.5 ± 1.7
4	31.2 ± 1.1	27.1 ± 4.9	28.8 ± 8.4	38.4 ± 17
5	34.0 ± 6.1	37.1 ± 4.0	32.5 ± 3.9	42.3 ± 2.2
6	37.7 ± 2.7	35.6 ± 2.9	39.5 ± 5.3	74.5 ± 0.61
7	43.4 ± 0.7	49.9 ± 3.5	49.8 ± 4.7	55.3 ± 2.5
8	57.1 ± 4.4	52.5 ± 0.8	59.6 ± 3.7	73.4 ± 0.19
9	78.8 ± 1.3	74.1 ± 2.6	84.3 ± 7.3	87.2 ± 11
10	79.0 ± 2.6	83.9 ± 2.3	76.6 ± 3.4	97.8 ± 1.7
11	82.5 ± 1.3	84.9 ± 1.4	81.8 ± 7.5	105 ± 1.2

Table 5 Parameters of the regression lines obtained from the plot between iodine contents measured by each of both techniques. The slope (b) and the intercept (a) are shown with the 95% confidence limits (ts)

Method pair (x,y)	$b \pm ts_b$	$a \pm ts_a$	r^2
Batch, continuous-FI	1.04±0.27	-4.45±11.96	0.968
Batch, stop-FI	1.03±0.17	-4.15±8.97	0.959
Stop-FI, continuous-FI	0.981±0.11	0.860±5.74	0.982
ICP-MS, batch	0.732±0.23	4.87±15.18	0.894
ICP-MS, continuous-FI	0.784±0.27	0.797±17.6	0.923
ICP-MS, stop-FI	0.789±0.27	1.09±17.7	0.915

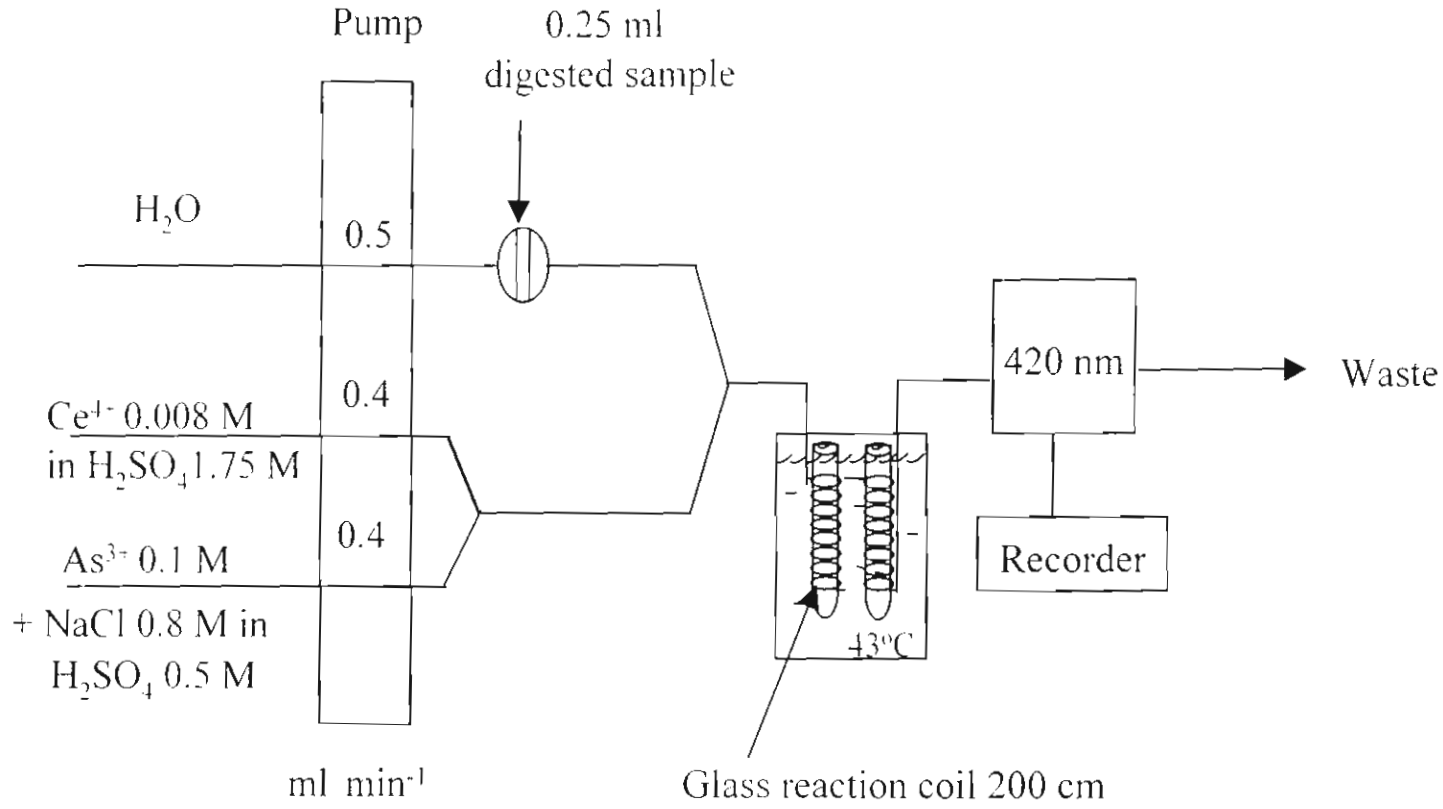
Pump
0.25 ml
digested sample
H_2O
0.5
0.4
Ce^{4+} 0.008 M
in H_2SO_4 1.75 M
0.4
As^{3+} 0.1 M
+ NaCl 0.8 M in
H_2SO_4 0.5 M
ml min^{-1}
420 nm
Waste
Recorder
43°C
Glass reaction coil 200 cm

Fig 1.

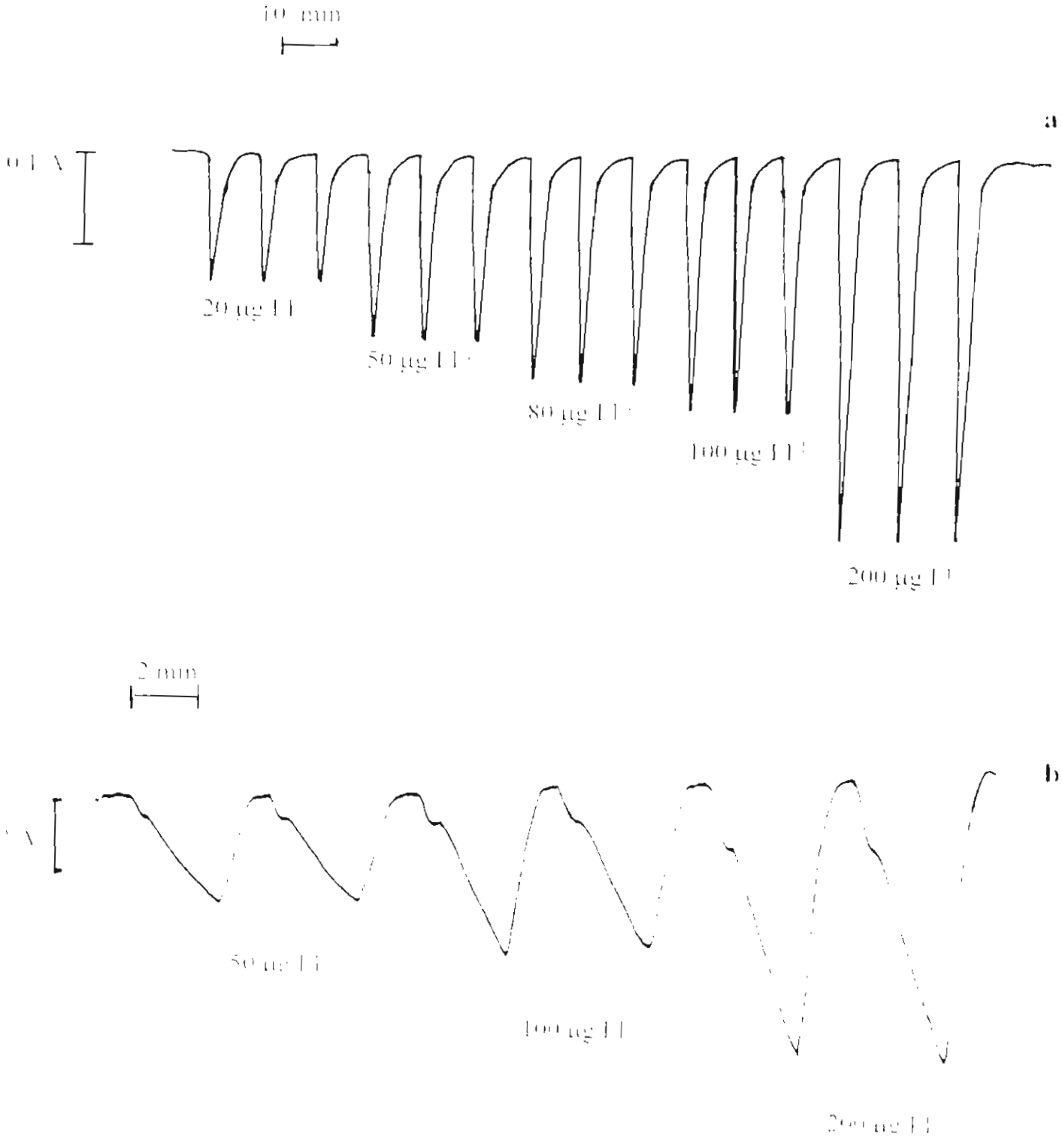
10 mm
a
0.1 V
20 µg l⁻¹
50 µg l⁻¹
80 µg l⁻¹
100 µg l⁻¹
200 µg l⁻¹
2 mm
b
50 µg l⁻¹
100 µg l⁻¹
200 µg l⁻¹

Fig. 2

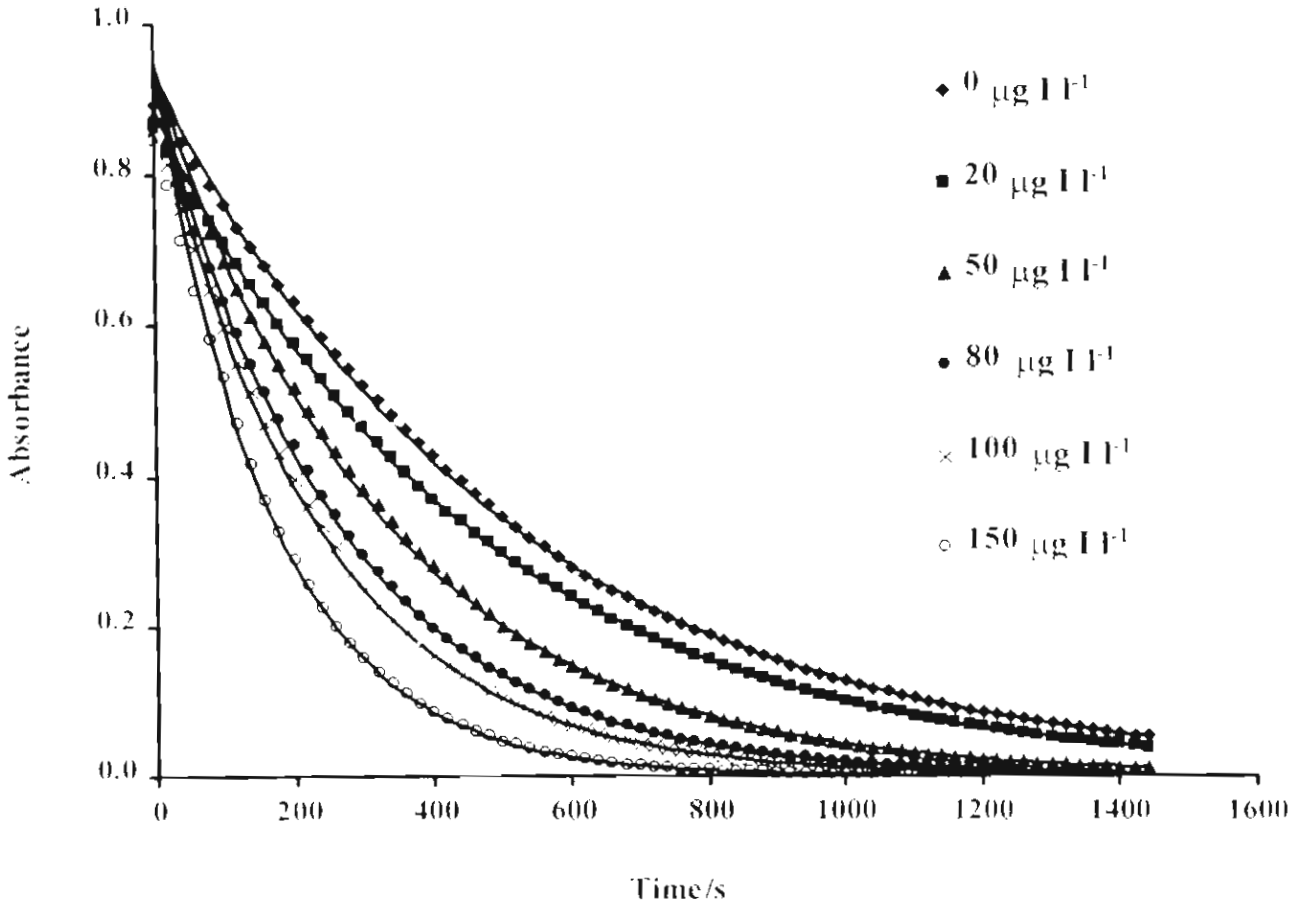
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
0
200
400
600
800
1000
1200
1400
1600
Time/s
0 μg l l-1
20 μg l l-1
50 μg l l-1
80 μg l l-1
100 μg l l-1
150 μg l l-1

Fig.3