รายงานวิจัยฉบับสมบูรณ์

การพัฒนาการวิเคราะห์ที่ใช้การไหล
DEVELOPMENT OF FLOW-BASED ANALYSIS

โดย รองศาสตราจารย์ ดร. เกตุ กรุดพันธ์ และคณะ

สิงหาคม 2547

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สัญญาเลขที่ RTA/08/2544

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DEVELOPMENT OF FLOW-BASED ANALYSIS

คณะผู้วิจัย	สังกัด
1. รศ. ดร. เกตุ กรุดพันธ์	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
2. ดร. จรูญ จักร์มุณี	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
3. ผศ. ดร. มงคล รายะนาคร	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
4. ดร. สุนันทา วังกานต์	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
5. ดร. สุภาภรณ์ ครัดทัพ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
6. ดร. อุไร เต็งเจริญกุล	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
7. ดร. สมพร จันทระ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
8. ดร. วินิตา บุณโยดม	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
9. ดร. สมชัย ลาภอนันต์นพคุณ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
10. ดร. พลยุทธ ศุขสมิติ	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3
11. ดร. รัตติกาล จันทิวาสน์	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี
12. รศ. ดร. ปรัชญา คงทวีเลิศ	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
13. ดร. นิสา ชวพันธ์	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
14. อ. ศุภชัย ชัยสวัสดิ์	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
15. ผศ. ดร. อรวรรณ ชัยสภากุล	คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
16. ผศ. ดร. ดวงใจ นาคะปรีชา	คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
17. รศ. ดร. ศุภลักษณ์ ศรีจารนัย	คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น
18. ผศ. ดร. รัศมี ชัยสุขสันต์	คณะวิทยาศาสตร์ มหาวิทยาลัยศิลปากร

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ขอขอบคุณ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่สนับสนุนให้ทุน โครงการวิจัยฯ ชุดวิจัยอาวุโส "การพัฒนาการวิเคราะห์ที่ใช้การไหล (Development of Flow-Based Analysis)"

โครงการวิจัยนี้สำเร็จลุล่วงไปด้วยการสนับสนุนและความร่วมมือจากหลายท่าน หลายหน่วยงาน อันได้แก่ สมาชิกเครือข่ายทั้งในและต่างประเทศทุกท่าน (อาจารย์ นักวิจัย และนักศึกษาที่มีส่วนร่วมอย่างมาก) ที่ทำให้เกิดผลงานวิจัยอันมีคุณภาพและคุณค่า ผู้ที่มีส่วนเสริมแต่เป็นกำลังสำคัญในการทำงาน (เลขานุการของกลุ่ม เจ้าหน้าที่ของ สกว. และ เจ้าหน้าที่ของสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยีที่มีส่วนเกี่ยวข้องกับโครงการนี้)

ขอขอบคุณการสนับสนุนเพิ่มเติมโดยแหล่งทุนอื่นๆที่ช่วยเสริมการทำวิจัย ทำให้มีประสิทธิภาพดียิ่งขึ้น ได้แก่ The Royal Golden Jubilee (RGJ) Ph.D. Program (TRF), Postgraduate Education and Research Program in Chemistry (PERCH), The collaborative Ph.D. Program (The Commission for Higher Education), The National Innovation Agency (NIA), The Alexander von Humboldt Foundation (AvH), Germany, Deutcher Akademischer Austausch Dienst (DAAD), Germany, The Post-graduate Education Development (PED) Program (The Commision on Higher Education), The Enhancement on the Country's Performance and Competitiveness Program (The Commission on Higher Education), Metrohm Siam/Metrohm AG, Switzerland, FIA Instruments, Co. Ltd., Japan และ Foss Tecator, Sweden

ขอขอบคุณผู้บริหารทุกท่าน ได้แก่ ผู้อำนวยการฝ่ายวิชาการ สกว. อธิการบดีมหาวิทยาลัยเชียงใหม่ ผู้อำนวยการสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี คณบดีคณะวิทยาศาสตร์ หัวหน้าภาควิชาเคมี ที่ให้การสนับสนุน

ขอขอบคุณทุกท่านที่มีส่วนร่วมในทางตรงและทางอ้อม แต่ไม่ได้เอ่ยนามในที่นี้

Every revolutionary idea in Science, Politics, Art or Whatever evokes three stages of reaction. They may be summed up by the three phases:

1. *"It is impossible, do not waste my time"*
2. *"It is possible, but it is not worth doing"*
3. *"I said it was a good idea all along"*

CLARK'S LAW OF REVOLUTIONARY IDEAS

โครงการนี้ทำให้เกิดความภาคภูมิใจและหวังเป็นอย่างยิ่งว่าเงินภาษีราษฎรในส่วนนี้ได้ตอบแทนสู่ประเทศในทางตรงและทางอ้อม

รองศาสตราจารย์ ดร. เกตุ กรุดพันธ์
หัวหน้าโครงการ

บทคัดย่อ

การพัฒนาการวิเคราะห์ที่ใช้การไหล

โครงการเมธีวิจัยอาวุโส สกว. สัญญาเลขที่ RTA/08/2544

หัวหน้าโครงการ: รองศาสตราจารย์ ดร. เกตุ กรุดพันธ์

โครงการนี้ทำการวิจัยเกี่ยวกับการพัฒนาเครื่องมือและวิธีการวิเคราะห์ทางเคมีที่ใช้เทคนิคการไหลโดยเน้นการคำนึงถึงค่าใช้จ่ายที่ถูก แต่มีนวัตกรรม ประกอบด้วย 4 โครงการย่อย ได้แก่ การพัฒนาเครื่องมือวิเคราะห์ที่ใช้การไหล การพัฒนาการวิเคราะห์ที่ใช้การไหลที่เกี่ยวข้องกับเทคนิคทางไฟฟ้าเคมี การพัฒนาระบบการวิเคราะห์ที่ใช้การไหลเพื่อการวิเคราะห์ทางคลินิก และการพัฒนาระบบการวิเคราะห์ที่ใช้การไหลเพื่อการเตรียมตัวอย่างแบบออนไลน์ที่เกี่ยวข้องกับโครมาโทกราฟี

สำหรับโครงการย่อยที่ 1 ได้สร้างระบบ gravitational field flow-fractionation (Gr-FFF) และรวมระบบนี้กับ flow injection analysis (FIA) หรือ ETAAS สำหรับการทำ size-based speciation ได้ประกอบและทดสอบเครื่อง semi-automatic stopped-FI analyzer ได้พัฒนาระบบ FIA และ sequential injection analysis (SIA) ที่มีระบบตรวจวัดแบบต่างๆ เช่น dynamic surface tension คัลเลอริเมตรี เทคนิคต่างๆ ทางเคมีไฟฟ้า ได้เสนอระบบ FIA ที่รวมกับ bead injection เป็นครั้งแรกด้วย ได้ศึกษาระบบ FIA/SIA ที่มีหน่วยสำหรับเตรียมตัวอย่างและระบบ Lab-on-Valve (LOV) ได้เสนอระบบ SI-Lab-at-Valve (SI-LAV) ซึ่งเกี่ยวข้องกับ miniaturization micro-total analysis system (μTAS) และ microfludics

ในโครงการย่อยที่ 2 ได้ศึกษาระบบที่ใช้การไหลที่ใช้กับ Voltammetry ได้พัฒนาการวิเคราะห์เดตระไซคลินด้วย flow injection electroanalysis และได้ศึกษาการทำ speciation แบบ on-line ของสารบางชนิดโดยใช้เทคนิคทางเคมีไฟฟ้า

ในโครงการย่อยที่ 3 ได้ศึกษาระบบและวิธีการวิเคราะห์หลายรูปแบบเพื่อการหาปริมาณไอโอดีน ได้ศึกษาระบบและวิธีการวิเคราะห์ที่ใช้การไหลเพื่อการคัดกรองผู้ป่วยโรคบางชนิด เช่น ธาลัสซีเมียและมะเร็ง ซึ่งจะเกี่ยวข้องกับเทคนิค immunoassay และการติดตาม biomarker บางชนิด ได้พัฒนาการวิเคราะห์ยาเตรียมบางชนิดด้วย

สำหรับโครงการย่อยที่ 4 ได้ศึกษาระบบการวิเคราะห์ที่ใช้การไหลเพื่อทำ speciation ของ As(III)/As(V) Fe(II)/Fe(III) และ NO_2^-/NO_3^- ได้ศึกษาสารที่เป็นชีวมวลที่ปรับแต่ง (ไคติน) ที่ใช้กับระบบอย่างง่ายในการวิเคราะห์ที่ใช้การไหลเพื่อการหาปริมาณไอออนบางชนิด การศึกษาการวิเคราะห์สารลดแรงตึงผิว โดยใช้ HPLC และ mass spectrometry

งานวิจัยดังกล่าวเป็นผลงานของนักวิจัยรุ่นเก่าและรุ่นใหม่ที่ร่วมกันดำเนินการ โดยเป็นนักวิจัยมาจากสถาบันต่างๆ ทั้งในประเทศและต่างประเทศ ซึ่งทำให้เกิดความเข้มแข็งในระดับบัณฑิตศึกษาและสร้างการทำงานเป็นทีม

Keywords: flow-based analysis, analytical instrumentation, clinical analysis, novel analytical techniques, applications of flow-based analysis

ABSTRACT

DEVELOPMENT OF FLOW-BASED ANALYSIS

TRF SENIOR RESEARCH SCHOLAR GRANT CONTRACT NO. RTA 108/2544

Principal investigator: Assoc. Prof. Dr. Kate Grudpan

In this project, instrumentation, chemistry and procedures involving flow-based analysis with emphasis on cost effective benefits but novel approaches have been investigated. There were 4 subprojects, namely, development of flow-based instrumentation, development of electrochemical flow-based analysis, development of flow-based systems for clinical analysis and development of flow-based analysis systems for on-line sample pretreatment with chromatography.

For the Subproject I, systems of gravitational field-flow fractionation (Gr-FFF) were set-up and combined with flow injection analysis (FIA) or ETAAS for size-based speciation. Semi-automatic stopped-FI analyzer was assembled and tested. Various systems of FIA and sequential injection analysis (SIA) with different types of detection systems such as dynamic surface tension detectors, colorimetry, electrochemical techniques were developed. A FIA system with bead injection was for the first time proposed. FIA/SIA systems with sample pretreatment devices were investigated. So was Lab-on-Valve (LOV) system. SI-Lab-at-Valve (SI-LAV), as novel systems were proposed also for the first time. They involved miniaturization, micro-total analysis system (micro-TAS) and microfluidics.

In the Subproject II, flow systems with voltammetry were investigated. Flow injection electroanalysis for tetracycline was developed. So were speciation and on-line of some species using electrochemical techniques.

In the Subproject III, various new systems and procedures with flow analysis for iodine determination have been investigated. Some studies on flow-based systems and procedures for screening some deceases such as thalassemia, and cancers have been attempted. This involved development of immunoassay and assays of some biomarkers. Assays of some pharmaceutical preparations have been developed.

For the Subproject IV, various flow-based systems for speciation were investigated. They include As(III)/As(V), Fe(II)/Fe(III) and NO_2^-/NO_3^-. Modified biomass (Chitin) based material incorporating with a simple flow-based system for determination of some ions was studied. Analyses of surfactants using HPLC and mass spectrometry were investigated.

The research works were conducted by researchers of older and younger generations in a partnership networking of institutions in Thailand and overseas. This strengthens graduate studies and builds up team working.

Keywords: flow-based analysis, analytical instrumentation, clinical analysis, novel analytical techniques, applications of flow-based analysis

Executive Summary

TRF Senior Research Scholar Grant (No. RTA/08/2544)

1. Title: การพัฒนาการวิเคราะห์ที่ใช้การไหล

Development of Flow-based Analysis

2. Principal investigator:

รองศาสตราจารย์ ดร. เกตุ กรุดพันธ์

Associate Professor Dr. Kate Grudpan

Department of Chemistry, Faculty of Science and Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200

Tel.05391-1910 Fax: 05394-1910,05322-2268

E-mail: kate@chiangmai.ac.th

3. Research Field: Analytical Chemistry

4. Budget: 7,500,000.- Baht

5. Research period: 3 years (1 September 2001 - 31 August 2004)

6. Supports from other agencies:

1. The Royal Golden Jubilee (RGJ) Ph.D. Program, TRF
2. Postgraduate Education and Research Program in Chemistry (PERCH)
3. The collaborative Ph.D. Program (The Commission for Higher Education)
4. The National Innovation Agency (NIA)
5. The Alexander von Humboldt Foundation (AvH), Germany
6. Deutcher Akademischer Austausch Dienst (DAAD), Germany
7. The Post-graduate Education Development (PED) Program (The Commision on Higher Education)

8. The Enhancement on the Country's Performance and Competitiveness Program (The Commission on Higher Education)

7. Research Work

The research was aimed to develop flow-based analysis with emphasis on cost effective benefits. Both instrumentation and chemistry were investigated. The project consisted of 4 subprojects:

1. Development of flow-based instrumentation,
2. Development of electrochemical flow-based analysis,
3. Development of flow-based systems for clinical analysis and
4. Development of flow-based analysis systems for on-line sample pretreatment with chromatography

8. Out-puts

The research work has resulted in 26 Publications in international journals (24 papers from this project and 2 papers from relevant works), 50 presentations in international conferences/meetings while 100 presentations in national meetings. It was the work by professors, researchers and students from institutions of various parts of Thailand as well as from overseas: Chiang Mai University, Mahidol University, Chulalongkorn University, Silapakorn University, Khon Kean University, The Office of Fundamental Industry and Mining Region 3 in Thailand, and University of Washington, Seattle, (USA) (Professor Dr. Gary D. Christian, Professor Dr. Jaromir Ruzicka, Professor Dr. Robert E. Synovec), Monash University, Melbourne, (AUSTRALIA) (Dr. Ron Beckett, Assoc. Prof. Dr. Ian D. McKelvie) , Karlsruhe Research Centre, Karlsruhe, (GERMANY) (Professor Dr. Thomas Fanghaenel, Dr. Horst Geckeis), University of Plymouth, (ENGLAND) (Professor Dr. Paul J. Worsfold), Turku University, Centre for Biotechnology, (FINLAND) (Dr. Rolf Sara, Dr. Michael Vilen), Okayama University, JAPAN (Professor Dr. Shoji Motomizu, President of the Japanese Association for Flow Injection Analysis), Aichi Institute of Technology, JAPAN (Professor Dr. Tadao Sakai), Technical University of Denmark, DENMARK (Assoc. Prof. Dr. Jens Christian Tjell, Prof. Dr. Hans Mosbaek), Texas Tech University, Texas, USA (Professor Dr. Pernendu K. Dasgupta)

Industrial linkages have been established with Metrohm Siam/Metrohm AG, Switzerland, FIA Instrument, Co. Ltd., Japan and Foss Tecator, Sweden.

There were annual symposia on "TRF Senior Research Scholar on Flow-based Analysis; the 1st: 1 September 2002, the 2nd: 6 September 2003 and the 3rd: 23 September 2004. There were oversea guests to participate in the symposia. In the 3rd Symposium, Prof. E. H. Hansen (Technical University of Denmark, the co-inventor of FIA), Prof. T. Sakai (Editor, Journal of Flow Injection Analysis), Prof. S. Motomizu (President, Japanese Association for Flow Injection Analysis), leading Japanese researchers (Dr. H. Itabashi, Dr. H. Ukeda, Dr. K. Higuchi) and Prof. I.D. McKelvie (Australia) joined the Symposium.

Members of the research team have received various awards nationally and internationally. The team has engaged in various national and international activities such as serving in committees, e.g. editorial advisory boards of international journals, organizing committees for international conferences. The team honorly organized the 11th International Conference Flow Injection Analysis, including related techniques (11th ICFIA), in Chiang Mai, December 2001.

การวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล"

1. ความสำคัญและที่มาของปัญหา

ในปัจจุบันนี้ยังมีความจำเป็นในการพัฒนาวิธีวิเคราะห์ทางเคมีในแนวทางใหม่ๆ เพื่อประยุกต์ในงานด้านต่างๆ ซึ่งนอกจากต้องการให้ได้วิธีการที่ได้ผลการวิเคราะห์ถูกต้อง แม่นยำ ในระยะเวลาอันสั้นแล้ว ยังต้องคำนึงถึงค่าใช้จ่ายในการวิเคราะห์ด้วย ได้มีการพยายามหาแนวทางในการเพิ่มประสิทธิภาพในการวิเคราะห์แนวทางอันหนึ่งคือการใช้เทคนิคการวิเคราะห์ที่ใช้การไหล โดยได้มีการนำเสนอเทคนิคโฟลอินเจคชันอะนาลิซิส หรือ เอฟ ไอ เอ เป็นครั้งแรกเมื่อปี คศ. 1975 โดย Ruzicka และ Hansen ซึ่งเป็นที่ประจักแล้วว่าเทคนิคนี้มีข้อดีเด่นหลายประการ ที่สำคัญเช่น ต้องการสารตัวอย่างและรีเอเจนต์ในปริมาณน้อยมาก สามารถวิเคราะห์ได้เร็วมาก รวมถึงสามารถใช้เครื่องมือที่ไม่ซับซ้อน และมีความปลอดภัยสูงเนื่องจากทำการวิเคราะห์ในท่อซึ่งเป็นระบบปิดทำให้ได้วิธีการวิเคราะห์ที่คุ้มค่า ซึ่งกลุ่มวิจัยได้พัฒนาระบบ เอฟ ไอ เอ ที่มีการเตรียมตัวอย่างในท่อ เช่น การแยกก่อนทำการวิเคราะห์ การเพิ่มความเข้มข้น การเจือจาง เป็นต้น สามารถทำการวิเคราะห์แบบ "Real time" ด้วย เอฟ ไอ เอ สามารถนำรีเอเจนต์ที่ไม่เสถียรมาใช้ในการวิเคราะห์ได้ เอฟ ไอ เอ สามารถประยุกต์เข้ากับระบบตรวจวัดได้หลากหลาย เช่น colorimetry, AAS, radioactivity, conductivity และ ion chromatograph เป็นต้น กลุ่มวิจัยของเราได้ศึกษา เอฟ ไอ เอ ในหลายๆ ด้าน โดยคำนึงถึงความต้องการ และความเป็นประโยชน์ต่อการพัฒนาประเทศ และในขณะเดียวกันให้ความรู้ใหม่ที่เป็นประโยชน์ในระดับสากลด้วย โดยได้สนใจวิจัยในเทคนิคการวิเคราะห์ที่ใช้การไหลหลายเทคนิค

ในโครงการนี้ได้วิจัยพัฒนาการวิเคราะห์ที่ใช้การไหลที่เน้นประโยชน์ที่จะให้ได้วิธีที่มีค่าใช้จ่ายในการวิเคราะห์ไม่สูง ซึ่งจะเกี่ยวข้องกับการศึกษาทั้งในส่วนของการพัฒนาเครื่องมือวิเคราะห์และปฏิกิริยาเคมีที่สนใจ โดยจะประกอบด้วย 4 โครงการย่อย คือ

1. Development of flow-based instrumentation,
2. Development of electrochemical flow-based analysis,
3. Development of flow-based systems for clinical analysis and
4. Development of flow-based analysis systems for on-line sample pretreatment with chromatography

ทั้งนี้ ทั้ง 4 โครงการย่อยจะมีความสัมพันธ์ซึ่งกันและกันด้วย

2. การพัฒนาเครื่องมือวิเคราะห์ที่ใช้การไหล (Development of Flow-based Instrumentation)

ได้ทำการศึกษาวิจัยและพัฒนาเครื่องมือที่อาศัยหลักการไหลหลายชนิด ได้แก่

2.1 ระบบวิเคราะห์ที่ใช้หลักการของ Field-Flow Fractionation (FFF)

ซึ่งได้ศึกษาเกี่ยวกับการสร้าง/ประกอบเครื่องมือ FFF ที่มีราคาไม่สูง ใช้วัสดุหาได้ง่าย ซึ่งเป็นเครื่อง FFF แบบ Gravitational FFF (GrFFF) (ดังรายละเอียดใน reprint ในภาคผนวก ก2, ก3, ก7) และได้ศึกษาการเชื่อมต่อระบบ GrFFF เข้ากับระบบ FIA มี detection system เป็น chemiluminescence ซึ่งพัฒนาจากการใช้ commercial on-line liquid scintillation counter (Packard Radiometric Flo-one ® \ Beta Series A-100 Model A140K, Canberra) (ซึ่งได้รับการสนับสนุนจาก Alexander von Humboldt Foundation) (ดังรายละเอียดใน reprint ภาคผนวก ก3,ก7) นับเป็นครั้งแรกที่ได้มีการศึกษาทางด้านนี้และได้ศึกษาการเชื่อมต่อ GrFFF เข้ากับ detection system อื่นๆ ด้วย ได้แก่ Electrothermal Atomic Absorption Spectrometer (ETAAS) (ดังรายละเอียดใน reprint ภาคผนวก ก7) และ ICP-AES นำไปใช้ในการศึกษา size-based speciation ได้

2.2 Semi-automatic stopped-FI analyzer

ได้ศึกษาวิจัยเพื่อสร้าง semi-automatic stopped-FI analyzer โดยใช้วัสดุอุปกรณ์ที่หาได้ภายในประเทศ ได้นำเครื่องที่สร้างขึ้นนี้ใช้ในการวิจัยเพื่อหาปริมาณฟอสเฟตและซิลิเกตโดยใช้ kinetic separation (ดังรายละเอียดใน reprint ภาคผนวก ก9)

2.3 FIA/SIA System ที่มี detection system ชนิดต่างๆ

2.3.1 Dynamic Surface Tension Detector (DSTD) ได้ศึกษาวิจัยเพื่อพัฒนาระบบ FIA และ SIA ที่ใช้ระบบการตรวจวัดที่ใช้คุณสมบัติทาง dynamic surface tension หลายระบบ (ดังรายละเอียดใน reprint ภาคผนวก ก13)

2.3.2 Colorimetry ได้ศึกษาวิจัยเพื่อพัฒนาระบบ FIA และ SIA ที่ใช้ colorimetric detection system ที่ประกอบขึ้นด้วยอุปกรณ์ วัสดุที่หาได้ในประเทศรวมถึง software ในการควบคุมด้วย ซึ่งจะประกอบเป็นระบบต่างๆ เช่น stoped-FI analyzer ดังกล่าวแล้วข้างต้น (ดังรายละเอียดใน reprint ในภาคผนวก ก9) และยังใช้ในระบบ FI-bead injection system ด้วย (ดังรายละเอียด reprint ในภาคผนวก ก6, ก12)

2.3.3 **Electrochemistry** ได้ศึกษาวิจัยเพื่อพัฒนาระบบ FIA และ SIA ที่ใช้ Electrochemical detection ในแบบต่างๆ ได้แก่ voltammetry (ดังรายละเอียด reprint ในภาคผนวก ก1, ก11), amperometry (ดังรายละเอียด reprint ในภาคผนวก ก15)

2.3.4 **Light Scattering Detection** ได้ศึกษาวิจัยเพื่อการสร้าง/ประกอบ light-scattering detector ที่มีราคาถูก เพื่อใช้สำหรับ FIA-nephelometry (ดังรายละเอียด reprint ในภาคผนวก ก17)

2.3.5 **AAS** ได้พัฒนาระบบ FIA/SIA ที่ใช้กับ AAS ได้แก่ Flame-AAS (ดังรายละเอียด reprint ในภาคผนวก ก4, ก12) และ ETAAS (ดังรายละเอียด reprint ในภาคผนวก ก16)

2.4 Flow Injection – Bead Injection (FI-BI) System ได้ประสบความสำเร็จครั้งแรกในการใช้เทคนิค bead injection (BI) กับ FIA (ดังรายละเอียด reprint ในภาคผนวก ก6, ก12)

2.5 FIA systems with sample pretreatment devices ได้พัฒนาระบบ in-valve column เพื่อใช้เป็น sample pretreatment device (ดังรายละเอียด reprint ในภาคผนวก ก4, ก18) และที่เป็น on-line column (ดังรายละเอียด reprint ในภาคผนวก ก14)

2.6 Lab-on-valve analysis system ได้พัฒนาระบบ SIA-Lab-on-Valve ที่ใช้กับ bead ในการ pretreatment เพื่อใช้กับ ETAAS (ดังรายละเอียด reprint ในภาคผนวก ก16)

2.7 Lab-at-valve system ได้ทำการศึกษา Lab-at-valve (LAV) เพื่อเป็น micro total analysis system (micro-TAS) แบบใหม่ที่เสนอขึ้นเป็นครั้งแรก

2.8 Flow-based systems for titration ได้ศึกษาวิจัยเพื่อพัฒนา flow-based system ที่ใช้สำหรับ on-line titration ซึ่งใช้ระบบ SIA (ดังรายละเอียด reprint ในภาคผนวก ก16) และ SIA-LOV สำหรับ micro-titration

2.9 Miniaturized flow systems ได้ทำการศึกษาเกี่ยวกับระบบ flow system ที่ย่อขนาดให้เล็กลง (miniaturization) โดยเกี่ยวข้องกับ microfluidics, micro total analysis system (micro-TAS) และ Lab-on-chip (LOC)

3. การพัฒนาการวิเคราะห์ที่ใช้การไหลที่เกี่ยวข้องกับไฟฟ้าเคมี (Development of Electrochemical Flow-based Analysis)

3.1 การวิเคราะห์สารปริมาณน้อยๆ โดยใช้ voltammetry ได้ศึกษาวิจัยการวิเคราะห์พวกโลหะปริมาณน้อยๆ ได้แก่ Pb, Zn, Cd และ Cu โดยใช้ระบบ Flow analysis, FIA และ SIA (ดังรายละเอียด reprint ในภาคผนวก ก1, ก11)

3.2 การวิเคราะห์ Tetracycline พัฒนาระบบ FIA ที่ใช้ pulsed ampermetric detection ในการปริมาณ tetracycline ในยาเตรียม (ดังรายละเอียด reprint ในภาคผนวก ก15)

3.3 การทำ speciation โดยใช้ electrochemical techniques ได้ศึกษาวิจัยระบบ FIA/SIA ที่ใช้กับ electrochemical techniques ในการทำ specication ของสารบางชนิด เช่น As (III)/As (V), chlorate/chlorite ซึ่งได้ผลการทดลองที่น่าสนใจที่ต้องการศึกษาวิจัยต่อไป

3.4 On-line sample pretreatment ได้ศึกษาการย่อยแบบต่อเนื่องในท่อ (on-line digestion) เพื่อย่อยตัวอย่างก่อนการวิเคราะห์ (ดังรายละเอียด reprint ในภาคผนวก ก1, ก11)

3.5 การหาปริมาณ Cu (II) โดยใช้ potentiometry ได้ศึกษาพัฒนาการวิเคราะห์หาปริมาณ Cu (II) ด้วยเทคนิค potentiometry โดยใช้Naphthazarin modified carbon paste electrode ในระบบ batch และ FIA

4. การพัฒนาระบบที่ใช้สำหรับการวิเคราะห์ทางคลินิก (Development of Flow-based Systems for Clinical Analysis)

4.1 การวิเคราะห์ไอโอดีน ได้ศึกษาพัฒนาการวิเคราะห์ไอโอดีนโดยใช้ flow-based techniques เพื่อประโยชน์ทางการแพทย์ (โรคขาดไอโอดีน) โดยใช้วิธีทาง Kinetic (ดังรายละเอียด reprint ในภาคผนวก ก5) วิธีที่ใช้ระบบ/วัสดุอุปกรณ์อย่างง่ายและปฏิกิริยาเคมี/รีเอเจนต์อย่างง่าย โดยใช้ปฏิกิริยาที่เกี่ยวกับน้ำแป้ง (ดังรายละเอียด reprint ในภาคผนวก ก8, ก20, ก23)

4.2 การคัดกรองผู้ป่วยโรคต่างๆ ได้ศึกษาพัฒนาระบบ FIA อย่างง่ายเพื่อการวิเคราะห์ Hemoglobin ซึ่งมีความสัมพันธ์กับการเกิดโรคธาลัสซีเมีย ซึ่งเกี่ยวกับการใช้คอลัมน์ขนาดเล็ก ทำให้มีระบบการคัดกรองที่มีราคาถูก (ดังรายละเอียด reprint ในภาคผนวก ก14) การศึกษา Flow system เพื่อ

ใช้ immunoassay เพื่อประโยชน์ในการวินิจฉัยโรค ได้สนใจศึกษา biomarker บางชนิด เพื่อติดตามโรคบางชนิด เช่น โรคตับ โรคมะเร็ง เป็นต้น biomarker ดังกล่าว เช่น hyaluronan และ WF6

4.3 การวิเคราะห์ยา ได้ศึกษาพัฒนาการวิเคราะห์ยาบางประเภท ได้แก่ วิตามิน ซี (Ascorbic acid) โดยใช้ SIA Redox titration (ดังรายละเอียด reprint ในภาคผนวก ก10) ระบบ FIA-amperometry เพื่อการวิเคราะห์ tetracycline (ดังรายละเอียด reprint ในภาคผนวก ก15)

5. การพัฒนาระบบการวิเคราะห์ที่มีการเตรียมตัวอย่างแบบ on-line ที่เกี่ยวข้องกับ Chromatography

ได้ศึกษาการทำ speciation ของ As (III)/As (V) โดยใช้ FIA system อย่างง่าย โดยมี in-valve column ซึ่งใช้ spectrophotometry (ดังรายละเอียด manuscript ในภาคผนวก ก18) การวิเคราะห์ Fe(II)/Fe(III), speciation ของ NO_2^-/ NO_3^-

การศึกษาวิจัยเกี่ยวกับการสังเคราะห์และคุณสมบัติของ modified biomass (Chitin) ซึ่งเตรียมจากวัสดุธรรมชาติ เช่น กระดองปูนา เปลือกหอย และจั๊กจั่น เพื่อนำมาเป็น dialysis membrane เพื่อการวิเคราะห์สารบางชนิด เช่น พวกเกลือแร่อิเลคโตรไลท์ในเครื่องดื่มบางชนิด โดยใช้ระบบ FIA อย่างง่าย (FIA-flame photometry)

นอกจากนี้ยังได้ศึกษาการวิเคราะห์แคทไอออนบางชนิด (Cu(II), Pb(II), Cd(II) และ Zn (II)) โดยใช้ In-valve column FIA สำหรับ sample pretreatment เพื่อการวิเคราะห์ด้วย ion-chromatograph ซึ่งมีข้อดีหลายประการ

การศึกษา on-line derivativatization ของโลหะบางชนิด เช่น Cu(II), Ni(II) และ Co(II) เป็น pre-treatment ของ HPLC (ดังรายละเอียด manuscript reprint ในภาคผนวก ก25)

การศึกษาเพื่อพัฒนาการวิเคราะห์ surfactants โดยใช้ศึกษา separation ของ surfactants ชนิดต่างๆ โดยเทคนิค HPLC และ Mass spectrometry กลุ่ม cationic surfactants ได้แก่ benzalkonium chloride domiphen bromide และ cetylpyridinium bromide กลุ่ม non-ionic surfactants เป็นพวก Triton X – 100 และกลุ่มพวก anionic surfactants ได้แก่ กลุ่ม linear alkylbenzene sulfonate โดยเฉพาะ C_{10} - C_{13} (ดังรายละเอียด manuscript reprint ในภาคผนวก ก26)

Out-put ที่ได้รับจากโครงการ

จากผลงานวิจัยที่ได้กล่าวมาข้างต้นได้ตีพิมพ์ในวารสารวิชาการนานาชาติแล้ว จำนวน 26 เรื่อง (ซึ่งเป็นผลงานทางวิชาการวิจัยโดยตรง 24 เรื่อง เกี่ยวข้องกับทางวิชาการ 2 เรื่อง ดังแสดงในตาราง 1) ซึ่งเป็นผลจากโครงการนี้โดยตรง รวมถึงผลงานวิจัยโครงการที่เกี่ยวข้องจากโครงการอื่นอีก 9 เรื่อง (ดังในตาราง 2) และได้นำเสนอในการประชุมวิชาการนานาชาติประมาณ 50 เรื่อง ในการประชุมวิชาการในประเทศประมาณ 100 เรื่อง

ผลงานวิจัยเกิดจากการทำงานเป็นทีมโดยการพยายามทำให้เกิดเครือข่ายของความร่วมมือของบุคลากรของหน่วยงานต่างๆ ทั้งในประเทศและต่างประเทศ ได้ มหาวิทยาลัยเชียงใหม่ (คณะวิทยาศาสตร์ และคณะอื่นๆ) มหาวิทยาลัยมหิดล จุฬาลงกรณ์มหาวิทยาลัย มหาวิทยาลัยศิลปากร มหาวิทยาลัยขอนแก่น สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3 ทั้งที่เป็นคณาจารย์ นักวิจัยและนักศึกษา ซึ่งอยู่ในระดับปริญญาโท-ปริญญาเอก รวมถึงนักศึกษาจากต่างประเทศด้วย) จำนวนทั้งสิ้น 70 คน (ดังตาราง 5) ได้มีความร่วมมือกับ สถาบันในต่างประเทศ 9 แห่ง ได้แก่

1. University of Washigton, Seattle, USA (Professor Dr. Gary D. Christian, Professor Dr. Jaromir Ruzicka, Professor Dr. Robert Synovec)
2. Monash University, Melbourne, AUSTRALIA (Dr. Ron Beckett, Assoc. Prof. Dr. Ian D. McKelvie)
3. Karlsruhe Research Centre, Karlsruhe, GERMANY (Professor Dr. Thomas Fanghenel, Dr. Horst Geckeis)
4. University of Plymouth, ENGLAND (Professor Dr. Paul J. Worsfold)
5. Turku University, Centre for Biotechnology, FINLAND (Dr. Rolf Sara, Dr. Michael Vilen)
6. Okayama University, JAPAN (Professor Dr. Shoji Motomizu, President of the Japanese Association for Flow Injection Analysis)
7. Aichi Institute of Technology, JAPAN (Professor Dr. Tadao Sakai)
8. Technical University of Denmark, DENMARK (Assoc. Prof. Dr. Jens Christian Tjell, Prof. Dr. Hans Mosback)
9. Texas Tech University, Texas, USA (Professor Dr. Pernendu K. Dasgupta)

นอกเหนือจากการสนับสนุนของทุนเมธีวิจัยอาวุโส ของ สกว. นี้แล้ว ยังได้รับการสนับสนุนจากองค์กรต่างๆ ทั้งในประเทศและต่างประเทศได้แก่

1. The Royal Golden Jubilee (RGJ) Ph.D. Program, TRF
2. Postgraduate Education and Research Program in Chemistry (PERCH)
3. The collaborative Ph.D. Program (The Commission for Higher Education)
4. The National Innovation Agency (NIA)
5. The Alexander von Humboldt Foundation (AvH), Germany
6. Deutcher Akademischer Austausch Dienst (DAAD), Germany
7. The Post-graduate Education Development (PED) Program (The Commision on Higher Education)
8. The Enhancement on the Country's Performance and Competitiveness Program (The Commission on Higher Education)

ได้มีความร่วมมือกับภาคเอกชนทั้งในและนอกประเทศ ได้แก่

1. Metrohm Siam/Metrohm AG, Switzerland
2. FIA Instrument, Co. Ltd., Japan
3. Foss Tecator, Sweden

ได้รับการสนับสนุนในด้านต่างๆ รวมถึงเครื่องมือที่ใช้ในการวิจัยด้วย ได้แก่

1*	ชุด Ion Chromatograph จาก Alexander von Homboldt (AvH) Foundation, Germany	มูลค่าประมาณ 800,000.- บาท
2	ชุดทำน้ำบริสุทธิ์ (Milli Q Water Purification Set) จากโครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)	มูลค่าประมาณ 270,000.- บาท
3	ชุด Voltammeter จากโครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)	มูลค่าประมาณ 500,000.- บาท
4	ชุด Liquid/solution propelling system จาก Metrohm Siam/Metrohm AG Switzerland	มูลค่าประมาณ 100,000.- บาท

*หมายเหตุ * แจ้งในการขอสนับสนุนต่อ AvH ว่าจะนำมาใช้ในโครงการวิจัยฯ โดยตรง*

บุคลากรได้รับรางวัลและเกียรติยศ ในช่วงที่ได้รับทุนเมธีวิจัยอาวุโส นี้ ดังสรุปในตาราง 3 คณาจารย์ นักวิจัยได้รับทุนในการทำวิจัยดังได้สรุปในตาราง 4 ซึ่งรวมถึงได้รับเกียรติในการจัดงาน (11th ICFIA), 11th International Conference on Flow Injection Analysis, including related techniques ที่เชียงใหม่ (ดังได้รับการเผยแพร่ใน Meeting Report ในวารสารวิชาการนานาชาติ Trends in Analytical Chemistry (ดังรายละเอียด reprint ในภาคผนวก ก21 และ ข1) และหัวหน้าโครงการได้รับเกียรติและรางวัลต่างๆ ทั้งในระดับประเทศและนานาชาติ นอกเหนือไปจากการได้รับเชิญบรรยายนำในการประชุมวิชาการนานาชาติและที่ปรึกษาในกองบรรณาธิการวารสารวิชาการนานาชาติ) เช่น รางวัลนักวิทยาศาสตร์ดีเด่น พ.ศ. 2544 และรางวัลบุคคลดีเด่นของชาติ สาขาวิทยาศาสตร์และเทคโนโลยี (ด้านเคมีวิเคราะห์) พ.ศ. 2547 โดยสำนักงานเสริมสร้างเอกลักษณ์ของสำนักนายกรัฐมนตรี และ ดร. จรูญ จักร์มุณี นักวิจัยในทีมได้รับรางวัลนักวิทยาศาสตร์รุ่นใหม่ ของมูลนิธิส่งเสริมวิทยาศาสตร์และเทคโนโลยีในพระบรมราชูปถัมภ์ พ.ศ. 2546 ทั้งนี้ รศ. ดร. เกตุ กรุดพันธ์ ได้รับเกียรติในรางวัลและโครงการต่างๆ เช่น "2003 FIA Scientific Award" ซึ่งเป็นรางวัลสูงสุดของ Japanese Association for Flow Injection Analysis (JAFIA) แห่งประเทศญี่ปุ่น ได้รับเกียรติให้ทำงานในวารสารวิชาการนานาชาติ เช่น ใน Advisory Board ของ วารสาร Talanta (Elsevier Publisher), เป็น Associate Editor ของวารสาร Water Research (The International Water Association/Elsevier Publisher), Advisory Board ของวารสาร Journal of Flow Injection (JAFIA) รวมถึงเป็นกรรมการและวิทยากรบรรยายนำในการประชุมวิชาการนานาชาติ เช่น Flow Analysis Conference IX (Geelong, Australia), 12th International Conference on Flow Injection Analysis and Related Techniques (Merida, Venezuela), Pacifichem 2005 (Hawaii, USA) (ตาราง 3)

ในการจัดประชุมวิชาการประจำปีของกลุ่มวิจัยได้ดำเนินการจัดเป็น Annual Symposium on TRF Senior Research Scholar on Flow-based Analysis

ครั้งที่ 1 วันที่ 1 กันยายน 2545

ครั้งที่ 2 วันที่ 6 กันยายน 2546

ครั้งที่ 3 วันที่ 23 กันยายน 2547

ในแต่ละครั้งได้รับการสนับสนุนจากหน่วยงานอื่นๆ ด้วย จึงทำให้มีผู้เข้าร่วมประชุมจากต่างประเทศด้วย และมีผู้ประชุมมากกว่า 70 – 80 คนในแต่ละครั้ง ในการประชุมครั้งที่ 3 นี้ Prof. Dr. E.H. Hansen (Technical University of Denmark) ซึ่งเป็น co-inventor ของ FIA ได้ให้เกียรติบรรยายนำพร้อมด้วย Prof. Dr.T. Sakai (Editor, Journal of Flow Injection Analysis), Prof. Dr. S. Motomizu (President, Japanese Association for Flow Injection Analysis) และนักวิจัยชั้นแนวหน้า

ทาง flow-based analysis จากประเทศญี่ปุ่นอีก 3 ท่าน (Dr. H. Itabashi, Dr. H. Ukeda, Dr. K. Higuchi) และนักวิจัยแนวหน้าทางด้านนี้จากประเทศออสเตรเลีย (Prof. Dr. Ian McKelvie) (ดังกำหนดการของการประชุมทั้ง 3 ครั้ง ในภาคผนวก ง และได้รายงาน (Meeting Report) ใน Journal of Flow Injection Analysis (ในภาคผนวก ก24))

Selected Presentations

1. Grudpan K (2001) **Invited paper**: Cost-Effective Flow Injection Systems for Environmental Analysis. *International Congress on Analytical Science (ICAS)*, Tokyo, Japan.
2. Grudpan K (2002) Invited paper: Some Recent Development in Automation: Flow Injection Analysis and Related Techniques for Various Degrees of Automation. *The Asia Pacific Conference on Analytical Science and the 18th Philippine Chemistry Congress, Manila.*
3. Grudpan K (2002) **Invited paper**: Some Recent Development on Flow-based Analysis. *PACCON 2002: International Conference & Exhibition on Pure and Applied Chemistry* 2002, Bangkok, Thailand.
4. Grudpan K (2001) **Plenary lecture**: Recent Development of Flow-Based Analytical Techniques: An Example of Sustainable Research. *27th Congress on Science and Technology of Thailand, Lee Garden Plaza Hotel, Hat Yai, Songkhla.*
5. Hartwell SK, Jitmanee K, Ampan P, Lapanantnoppakhun S, Sooksamiti P, Jayavasti S, Jakmunee J, Ruzicka J, Christian G and Grudpan K (2003) Simple flow-injection, bead injection system for trace ion determination. *225th ACS National Meeting, New Orleans, LA, United States.*
6. Tanikkul S, Jakmunee J, Rayanakorn M, Grudpan K, Marquardt BJ, Gross GM, Prazen BJ, Burgess LW, Christian GD and Synovec RE (2003) Raman Liquid–core Waveguide Sensor for Preconcentration Characterization and Quantitation. *Flow Analysis IX conference, Geelong, Australia.*
7. Pathimapornlert L, Jakmunee J and Grudpan K (2003) Micro-titration with sequential injection using lab-on-valve for acidity in fruit juice. *Flow Analysis IX conference, Geelong, Australia.*
8. Grudpan K (2003) **Plenary lecture**: Some Developments of Flow Injection and Related Techniques: Some Contributions from a Research Group in Thailand, *20th Anniversary of JAFIA and 44th Semi-Annual Meeting JSAC*, Okayama University, Japan, 6-7 November 2003
9. Grudpan K (2003) **Plenary lecture**: Some Recent Development on Cost-Effective Flow-Based Analysis, *12th ICFIA*, Merida, Venezuela, December 2003.

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
1	**Jakmunee J, Suteerapataranon S, Vaneesorn Y**	Determination of Cadmium, Copper, Lead and Zinc by Flow Voltammetric Analysis	Analytical Sciences 17 (2001), i399-i401.	1.14
	and **Grudpan K**			
2	**Chantiwas R, Jakmunee J,** Beckett R and	A Cost-Effective Gravitational Field-Flow Fractionation System	Analytical Sciences 17 (2001), i1419-21.	1.14
	Grudpan K			
3	**Chantiwas R, Jakmunee J,** Beckett R, McKelvie I	Combination of Field-Flow Fractionation and Flow Injection Analysis	Analytical Sciences 17 (2001), i423-4.	1.14
	and **Grudpan K**			
4	**Jakmunee J, Sooksamiti P,** Geckies H and	Flow Injection Sample Pretreatment for Determination of Lead by Flame Atomic Absorption	Analytical Sciences 17 (2001), i1415-18.	1.14
	Grudpan K	Spectrophotometry.		
5	**Nacapricha D,** Ratanawimarnwong N,	Kinetic Determination of Iodine in Urine Using Stopped-Flow Injection.	Analytical Sciences 17 (2001), i33-6.	1.14
	Suwannachoat S, Wilairat P, Shiowatana J			
	and **Grudpan K**			
6	Jitmanee K, **Kradtap Hartwell S, Jakmunee J,**	A Simple Flow Injection System with Bead Injection for Trace Iron Determination.	Talanta 57 (2002), 187-92.	2.091
	Jayasvasti S, Ruzicka J and **Grudpan K**			

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
7	**Chantiwas R, Beckett R, Jakmunee J,** McKelvie I.D.	Gravitational Field-Flow Fractionation in Combination with Flow Injection Analysis or	Talanta 58 (2002), 1375-83.	2.091
	and Grudpan K	Electrothermal AAS for Size-Based Iron Speciation of Particles.		
8	Choengchan N, Uraisin K, Choden K, Veerasai W,	Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt.	Talanta 58 (2002), 1195-201.	2.091
	Grudpan K and Nacapricha D			
9	**Grudpan K, Ampan P, Udnan Y, Jayasvati S,**	Stopped-flow Injection Simultaneous Determination of Phosphate and Silicate Using	Talanta 58 (2002), 1319-26.	2.091
	Lapanantnoppakhun S, Jakmunee J, Christian GD	Molybdenum Blue.		
	and Ruzicka J			
10	**Lenghor N, Jakmunee J,** Vilen M, Sara R, Christian	Sequential Injection Redox or Acid-Base Titration for Determination of Ascorbic Acid or	Talanta 58 (2002), 1139-44.	2.091
	GD and **Grudpan K**	Acetic Acid.		
11	**Suteerpataranon S, Jakmunee J,** Vaneesorn Y	Exploiting Flow Injection and Sequential Injection Anodic Stripping Voltammetric Systems	Talanta 58 (2002), 1235-42.	2.091
	and **Grudpan K**	for Simultaneous Determination of Some Metals.		
12	**Ampan P, Lapanantnoppakhun S, Sooksamiti P,**	Determination of trace iron in beer using Flow Injection Systems with in-valve column	Talanta 58 (2002), 1327-34.	2.091

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
	Jakmunee J, Hartwell SK, Jayasvati S, Christian	and bead injection.		
	GD and **Grudpan K**			
13	**Lenghor N, Grudpan K, Jakmunee J,** B. A.	Sequential Injection Analysis with Dynamic Surface Tension Detection: High Throughput	Talanta, 59 (2002), 1153 – 63.	2.091
	Staggemeier, W.W.C. Quigley, Bryan J. Prazen ,	Analysis of the Interfacial Properties of Surface Active Samples.		
	Christian GD, Ruzicka J. and Synovec RE			
14	**Srisawang B, Kongtawelert P, Hartwell S K,**	A Simple Flow Injection – Reduced Volume Column System for Hemoglobin Typing.	Talanta, 60 (2003), 1163 – 70.	2.091
	Jakmunee J and Grudpan K			
15	Palaharn S, Charoenraks T, Wangfuengkanagul N,	Flow Injection Analysis of Tetracycline in Pharmaceutical Formulation with Pulsed	Anal. Chim. Acta, 499 (1-2) (2003), 191-7.	2.21
	Grudpan K and Chailapakul O	Amperometric.		
16	**Pattanapong A,** Ruzicka J, Atallah R, Christian G D,	Exploiting Sequential Injection Analysis with Bead Injection and Lab-On-Valve for	Anal. Chim. Acta, 499 (1-2) (2003), 167-72	2.21
	Jakmunee J and Grudpan K	Determination of Lead Using Electrothermal Atomic Absorption Spectrometry.		
17	**Jakmunee J,** Udnan Y, Morrison R, Beckett R,	A Low-cost Light-Scattering Detector for the Flow-Injection Nephelometric Determination	Analytical Science, 19 (11) (2003), 1495 – 9	1.14
	Mckinnon I and **Grudpan K**	of Sulfate.		

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
18	**Grudpan K**, Worokijcharoenchai N, **Sooksamiti P,**	Flow Injection Spectrophotometric Determination of As (III) and As (V) using Molybdate	Indian J. Chem, sec A (2003) 2939 - 44	0.489
	Jakmunee J and Christain G D	reagent with Solid Phase Extraction In-valve Column		
19	Burakham R, Duangthong S, Patimapornlert L,	Flow Injection and Sequential Injection Determination of Paracetamol in Pharmaceutical	Analytical Sci. 20 (5) (2004), 837 - 40	1.14
	Lenghor N, Kasiwad S, Srivichai L,	Preparations using Nitrosation Reaction		
	Lapanantnoppakhun S, Jakmunee J and Grudpan K			
20	**Nacapricha D**, Uraisin K, Ratanawimarnwong N,	Simple and Selective method for determination of iodide in pharmaceutical products by	Analytical and Bioanalytical Chemistry	1.715
	Grudpan K	Flow Injection Analysis using the iodine-starch reaction	378 (3) (2004), 816-21	
21	**Grudpan K**	Editorial	Talanta 58 (2002), 1013-4	2.091
22	**Grudpan K**	Development of Cost-Performance Flow-based Chemical Analysis Systems	Journal of Flow Injection Analysis, 20 (2)	-
			(2003), 153	
23	Choengchan N, Uraisin K, Choden K, Veerasai W,	Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt	Journal of Flow Injection Analysis, 20 (2)	-
	Grudpan K and Nacapricha D		(2003), 161	

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
24	**Grudpan K**	Report the 2nd Annual Symposium on TRF Senior Research Scholar on Flow-based	Journal of Flow Injection Analysis, 20 (2)	-
		Analysis, Chiang Mai, Thailand	(2003), 226	
25	**Srijaranai S, Chanpaka S, Kukusamude C**	Flow-injection in-line complexation for ion-pair reversed phase high performance liquid	Manuscript	
	and **Grudpan K**	chromatography of some metal-4-(2-pyridylazo) resorcinol chelates		
26	**Wangkarn S, Soisungnoen P,**	Determination of Linear Alkylbenzene Sulfonates in Water Samples by Liquid	Submitted to Talanta	
	Rayanakorn M and Grudpan K	Chromatography-UV Detection and Confirmation by Liquid Chromatography-Mass Spectrometry		

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
1	**Nacapricha D,** Muangkaew S, Ratanawimarnwong N,	Continuous and Stopped Flow Injection for Cataytic Determination of Total Iodine in Urine	Analyst 126, 121 - 6	2.251
	Shiowatana J and **Grudpan K**			
2	Tipparat P, **Lapanantnoppakhun S, Jakmunee J and**	Determination of Ethanol in Liqour by Near Infrared Spectrophotometry with Flow Injection	Talanta 53 (2001), 1199 - 204	2.091
	Grudpan K			
3	**Jakmunee J and Grudpan K**	Flow Injection Amperometry for the Determination of Iodate in Iodized Table Salt	Anal. Chim. Acta 438 (2001), 299 - 304	2.21
4	Quigley WWC, Nabi A, Prazen BJ, Lenghor N,	Dynamic Surface Tension Analysis of Dodecyl Sulfate Association Compiexes	Talanta 55 (2001), 551 - 60	2.091
	Grudpan K and Synovec RE			
5	**Nacapricha D,** Ratanawimarnwong N, Suwannachoat S,	Kinetic Determination of Iodine in Urine Using Stopped-Flow Injection	Analytical Science 17 (2001), i33 - 6	1.14
	Wilairat P, Shiowatana J and **Grudpan K**			
6	Tipparat P, **Lapanantnoppakhun S, Jakmunee J and**	Determination of Diphenhydramine hydrochoride in some single tertiary alkylamine	J. Pharmaceutical and Biomedical Analysis	1.425
	Grudpan K	pharmaceutical preparations by flow injection spectrophotometry	30 (2002), 105-12	
7	Muangkaew S, McKelvie ID, Grace MR, **Rayanakorn M,**	A Reverse-Flow Injection Analysis Method for the Determination of Dissolved Ozygen in	Talanta 58 (2002), 1285 - 91	2.091

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
	Grudpan K, Jakmunee J and Nacapricha D	Fresh and Marine Waters		
8	Sakai T, Piao S, Teshima N, Kuroishi T and **Grudpan K**	Flow Injection System with In-line Winkler's procedure using 16-way valve and	Talanta 63 (2004), 893 - 8	2.091
		Spectrofluorimetric Determination of Dissolved Ozygen in Environmental Waters		
9	**Tanikkul S, Jakmunee J, Rayanakorn M, Grudpan K,**	Characterization and use of a Raman liquid-core wavegruide sensor using preconcentration	Talanta 59 (2003), 809-16	2.091
	Marquardt BJ, Gross GM, Pruzen BJ, Burgess LW,	principles		
	Christian GD and Synover RE			

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

1	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	JAFIA Scientific Award สำหรับ "Development of Cost-Performance Flow-based Chemical Analysis Systems"
	ชื่อโครงการ	Japanese Associate for Flow Injection Analysis (JAFIA) ประเทศญี่ปุ่น
	ปีที่ได้รับ	6 พฤศจิกายน 2546
2	ชื่อนักวิจัย	ผศ. ดร. ดวงใจ นาคะปรีชา
	ได้รับรางวัล/เกียรติยศ	2003 JAFIA Best Article Award สำหรับ "Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt"
	ชื่อโครงการ	Japanese Associate for Flow Injection Analysis (JAFIA) ประเทศญี่ปุ่น เป็นผลงานตีพิมพ์วารสารวิชาการนานาชาติในโครงการวิจัยนี้
	ปีที่ได้รับ	6 พฤศจิกายน 2546
3	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี
	ได้รับรางวัล/เกียรติยศ	รางวัลนักวิทยาศาสตร์รุ่นใหม่ ประจำปี พ.ศ. 2546
	ชื่อโครงการ	มูลนิธิส่งเสริมวิทยาศาสตร์และเทคโนโลยีในพระบรมราชูปถัมภ์
	ปีที่ได้รับ	พ.ศ. 2546
4	ชื่อนักวิจัย	นางสาวสุมาลี ธนิกกุล (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Award" for a student
	ชื่อโครงการ	the International Conference on Flow Analysis IX, Geelong, Australia
	ปีที่ได้รับ	17 - 20 กุมภาพันธ์ 2546

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

5	ชื่อนักวิจัย	นางสาวสิริพัชร์ สุธีรภัทรานนท์ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students The TRF-RGJ Congress IV
	ชื่อโครงการ	โครงการปริญญาเอกกาญจนาภิเษก สกว.
	ปีที่ได้รับ	25 - 27 เมษายน 2546
6	ชื่อนักวิจัย	นางสาวสิริพัชร์ สุธีรภัทรานนท์ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Conference II (Oral Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	11 - 14 พฤษภาคม 2546
7	ชื่อนักวิจัย	นางณรงค์ เล่งอ้อ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Conference II (Oral Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	11 - 14 พฤษภาคม 2546
8	ชื่อนักวิจัย	นางสาวบุญรักษา ศรีสว่าง (นักศึกษาปริญญาโท)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Conference II (Oral Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	11 - 14 พฤษภาคม 2546

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

9	ชื่อนักวิจัย	นางสาวรัตติกาล จันทิวาสน์ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Conference II (Poster Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	11 - 14 พฤษภาคม 2546
10	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Editorial Board, Talanta Elsevier publisher
	ชื่อโครงการ	
	ปีที่ได้รับ	2543 - ปัจจุบัน
11	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Guest Editor Special Issue "A collection of papers presented at the 11th International Conference on Flow Injection Analysis (11 ICFIA
	ชื่อโครงการ	Chiang Mai, Thailand, 16 - 20 December 2001" Talanta vol. 58 no. 6, December 2002
	ปีที่ได้รับ	
12	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Associate Editor, Water Research (International Water Research Association/Elsevier)
	ชื่อโครงการ	
	ปีที่ได้รับ	2545 - 2547

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

13	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	International Advisory Board, Journal of Flow Injection Analysis (JFIA) (Japanese Assoication for Flow Injection Analysis)
	ชื่อโครงการ	
	ปีที่ได้รับ	
14	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Editorial Board, Science Asia (สมาคมวิทยาศาสตร์แห่งประเทศไทย)
	ชื่อโครงการ	
	ปีที่ได้รับ	2544 - ปัจจุบัน
15	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Organizer, 11th International Conference on Flow Injection Analysis, Chiang Mai, THAILAND
	ชื่อโครงการ	
	ปีที่ได้รับ	16 - 20 ธันวาคม 2544
16	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Committee, International Conference on Flow Analysi IX, Geelong Australia
	ชื่อโครงการ	
	ปีที่ได้รับ	17 - 20 กุมภาพันธ์ 2546

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

17	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Steering Committee, 12th International Conference on Flow Injection Analysis, Merida Venezuela
	ชื่อโครงการ	
	ปีที่ได้รับ	7 - 12 ธันวาคม 2546
18	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Co-organizer, Symposium # 16, Flow-Based Analysis: State of the Art Flow Methods in Analytical Chemistry (PACIFICHEM 2005)
	ชื่อโครงการ	Honoluu, Hawaii, USA
	ปีที่ได้รับ	15 - 20 ธันวาคม 2548
19	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Plenary lectue "SOME RECENT DEVELOPMENT ON COST-EFFECTIVE FLOW-BASED ANALYSIS. "
	ชื่อโครงการ	12th International Conference on Flow Injection Analysis (12ICFIA)
	ปีที่ได้รับ	7 - 12 ธันวาคม 2546
20	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Plenary lecture " Some developments of Flow Injection and related techniques: Some contributions from a research group in Thailanc
	ชื่อโครงการ	The 20th Anniversary of JAFIA Foundation, and the 44th Semi-Annual Meeting
	ปีที่ได้รับ	6 - 7 พฤศจิกายน 2546

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

21	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Plenay lecture "Some Recent Development on Flow-based Analysis"
	ชื่อโครงการ	International Conference & Exhibition on Pure and Applied Chemistry PACCON 2002
	ปีที่ได้รับ	29 พฤษภาคม 2545
22	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Plenary lecture "Recent Development of flow-based analysis techniques: An Example of Sustainable Research "
	ชื่อโครงการ	ประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 27 หาดใหญ่ สงขลา
	ปีที่ได้รับ	16 - 18 ตุลาคม 2544
23	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Invited lecture "Some Recent Development in Automation: Flow Injection Analysis and Related techniques for Various Degrees of Aut
	ชื่อโครงการ	Asia-Pacific Conference on Analytical Science, Manila ประเทศฟิลิปปินส์
	ปีที่ได้รับ	19 - 23 กุมภาพันธ์ 2545
24	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Invited lecture "NANO-AND MICRO-SCALE CHEMICAL ANALYSIS USING FLOW-BASEDTECHNIQUES "
	ชื่อโครงการ	ประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 29 มหาวิทยาลัยขอนแก่น
	ปีที่ได้รับ	21 - 23 ตุลาคม 2546

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

25	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี
	ได้รับรางวัล/เกียรติยศ	Invited lecture "Development of Flow-based Electroanalysis: an example of sustainable research"
	ชื่อโครงการ	ประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 29 มหาวิทยาลัยขอนแก่น
	ปีที่ได้รับ	21 - 23 ตุลาคม 2546
26	ชื่อนักวิจัย	นางสาวอรวรรณ ถือเงิน (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Congress III (Oral Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	9 - 12 พฤษภาคม 2547
27	ชื่อนักวิจัย	นางสาวรจนา บุระคำ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	"The Best Presentation Awards" for students, The PERCH Congress III (Oral Presentation)
	ชื่อโครงการ	โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH)
	ปีที่ได้รับ	9 - 12 พฤษภาคม 2547

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

1	ชื่อนักวิจัย	รศ. ดร. ปรัชญา คงทวีเลิศ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุน basic research สกว.
	ชื่อโครงการ	การวิเคราะห์อิพิโทปของสายคอนดรอยติน 6-ซันเฟตซึ่งถูกจดจำโดยโมโนโคลนอล แอนติบอดีย์ WF-6 และการนำไปประยุกต์ใช้
	ปีที่ได้รับ	2546
2	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนเมธีวิจัย สกว.
	ชื่อโครงการ	การพัฒนาเครื่องมือและวิธีทางเคมีไฟฟ้าสำหรับการวิเคราะห์อาหารและเครื่องดื่ม
	ปีที่ได้รับ	สิงหาคม 2546 - สิงหาคม 2549
3	ชื่อนักวิจัย	ผศ. ดร. อรวรรณ ชัยลภากุล (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนเมธีวิจัย สกว.
	ชื่อโครงการ	การวิเคราะห์ทางเคมีไฟฟ้าทางสารประกอบอินทรีย์ โดยใช้ฟิล์มบางของโบรอน/โดปโดมอน ประยุกต์กับโฟลอินเจคชัน
	ปีที่ได้รับ	กันยายน 2544 - สิงหาคม 2546
4	ชื่อนักวิจัย	ผศ. ดร. ดวงใจ นาคะปรีชา (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนเมธีวิจัย สกว.
	ชื่อโครงการ	การวิเคราะห์ปริมาณไอโอดีนอาศัยจลนศาสตร์โดยทำเป็นโฟลอินเจคชัน
	ปีที่ได้รับ	1 พฤศจิกายน 2544
5	ชื่อนักวิจัย	ดร. สุภาภรณ์ ครัดทัพ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนพัฒนานักวิจัย สกว.

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

	ชื่อโครงการ	การพัฒนาเทคนิคการวิเคราะห์แบบโฟลอินเจคชัน และซีเควนเชียลอินเจคชัน เพื่อใช้สำหรับบีตอิมมุโนแอสเส
	ปีที่ได้รับ	กรกฎาคม 2545 - มิถุนายน 2547
6	ชื่อนักวิจัย	ดร. รัตติกาล จันทิวาสน์ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนพัฒนานักวิจัย สกว.
	ชื่อโครงการ	โครงการเตรียมตัวอย่างโดยใช้เทคนิคทางวิเคราะห์ที่ใช้การไหลโดยเกี่ยวข้องกับอนุภาค
	ปีที่ได้รับ	กรกฎาคม 2546 - มิถุนายน 2548
7	ชื่อนักวิจัย	รศ. ดร. เกตุ กรุตพันธ์ (หัวหน้าโครงการร่วม), ผศ. ดร. ดวงใจ นาคะปรีชา (หัวหน้าโครงการร่วม)
	ได้รับรางวัล/ทุนวิจัย	โครงการส่งเสริมสมรรถนะและขีดความสามารถในการแข่งขันของประเทศ ของสำนักงานคณะกรรมการอุดมศึกษา
	ชื่อโครงการ	การพัฒนาเครื่องมือและวิธีสำหรับวิทยาศาสตร์การวิเคราะห์ในระดับไมโครและนาโนเพื่อใช้ในทางสุขภาพและสิ่งแวดล้อม
	ปีที่ได้รับ	ตุลาคม 2546 - กันยายน 2551
8	ชื่อนักวิจัย	ดร. สุภาภรณ์ ครัดทัพ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนสนับสนุนอาจารย์ใหม่ โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี
	ชื่อโครงการ	การพัฒนาเทคนิคโฟลอินเจคชัน-บีตอินเจคชัน เพื่อเพิ่มความเข้มข้นในการวิเคราะห์เหล็กและทองแดง
	ปีที่ได้รับ	มกราคม 2544 - ธันวาคม 2545
9	ชื่อนักวิจัย	ดร. สมชัย ลาภอนันต์นพคุณ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนสนับสนุนอาจารย์ใหม่ โครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี
	ชื่อโครงการ	การพัฒนาวิธีโฟลอินเจคชันสำหรับหาปริมาณอะไมโลสในข้าว
	ปีที่ได้รับ	มกราคม 2546 - ธันวาคม 2547

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

10	ชื่อนักวิจัย	ดร. วินิตา บุณโยดม (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ (MTEC) สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ
	ชื่อโครงการ	พอลิเอสเทอร์ที่สามารถสลายตัวทางชีวภาพตัวใหม่สำหรับประยุกต์เป็นท่อนำเส้นประสาท
	ปีที่ได้รับ	กุมภาพันธ์ 2546 – มกราคม 2549
11	ชื่อนักวิจัย	ผศ. ดร. อรวรรณ ชัยลภากุล (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนรัชดาภิเษก
	ชื่อโครงการ	การวิเคราะห์ทางเคมีไฟฟ้าในระบบโฟลอินเจคชันของสารประกอบยาโดยใช้ขั้วไฟฟ้าฟิล์มบางของเพชรซึ่งโดปด้วยโบรอน
	ปีที่ได้รับ	ตุลาคม 2544 - ตุลาคม 2545
12	ชื่อนักวิจัย	ดร. สุนันทา วังกานต์ (ผู้ร่วมวิจัย)
	ได้รับรางวัล/ทุนวิจัย	ฝ่ายเทคโนโลยีโลหะและวัสดุ สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ
	ชื่อโครงการ	การพัฒนากระดาษสาให้มีคุณสมบัติทนไฟ
	ปีที่ได้รับ	พฤศจิกายน 2545 – ตุลาคม 2546

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล" หัวหน้าโครงการ: รศ. ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
1 เกตุ กรุดพันธ์	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	หัวหน้าโครงการ	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	หัวหน้าโครงการ
2 จรูญ จักร์มุณี	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
3 สุภาภรณ์ ครัดทัพ	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
4 มงคล รายะนาคร	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
5 สุนันทา วังกานต์	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
6 อุไร เด็งเจริญกุล	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
7 สมพร จันทระ	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
8 วินิตา บุณโยคม	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
9 พลยุทธ ศุขสมิติ	นักวิทยาศาสตร์	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3	นักวิจัย	นักวิทยาศาสตร์	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3	นักวิจัยในโครงการ
10 วัลศิกาล จันทิวาสน์ (*,1-2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	นักศึกษาปริญญาเอก	นักวิจัย	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มช.	นักวิจัยในโครงการ
11 ปริญญา คงทวีเลิศ	รศ.	ภาควิชาชีวเคมี คณะแพทยศาสตร์ มช.	นักวิจัย	รศ.	ภาควิชาชีวเคมี คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
12 คุภชัย ชัยสวัสดิ์	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัย	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
13 นิสา ชวพันธุ์	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัย	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
14 ดวงใจ นาคะปรีชา	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักวิจัยในโครงการ
15 อรวรรณ ชัยลภากุล	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักวิจัยในโครงการ
16 คุภลักษณ์ ศรีจารนัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักวิจัยในโครงการ
17 วัลมี ชัยสุขสันต์	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศิลปากร	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศิลปากร	นักวิจัยในโครงการ

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล" หัวหน้าโครงการ: รศ. ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
18 สมชาย ลาภอนันต์นพคุณ	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	ผู้ช่วยนักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	ผู้ช่วยนักวิจัยในโครงการ
19 วเรศ วีระสัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	ผู้ช่วยนักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	ผู้ช่วยนักวิจัยในโครงการ
20 กาญจนา วัดละเอียด (**,1-2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศิลปากร	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
21 บุญรักษา ศรีสว่าง (**,1-2)		ภาควิชาเคมี คณะวิทยาศาตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
22 ยุทธพงษ์ อุดแน่น (*,2-3)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยนเรศวร	อาจารย์
23 พัฒนพงษ์ ย่ำพันธุ์ (*,1-2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ สถาบันเทคโนฯ พระนครฯ	อาจารย์
24 กนกพรรณ ปทานนท์ (*,2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	-
25 ดพ ปฏิมาพรเลิศ(*,2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	-
26 เสาวภา เมืองแก้ว (2,5)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
27 ณรงค์ เล่งฮ้อ (1-2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		Okayama University	Post-doc
28 สิริพัชร์ สุธีรภัทรานนท์ (1-2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง	อาจารย์
29 ขวัญจิต มณีย่อน (2,5)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
30 สุพรรณี ดวงทอง (2,4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
31 สุมาลี ธนิกกุล (2-3)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
32 ทินกร กันยานี (2,5)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
33 อรวรรณ ถือเงิน (2-3)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
34 รจนา ปุระคำ (2-3)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล" หัวหน้าโครงการ: รศ. ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
35 ดวงพร โสมประยูร (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
36 สุภาภรณ์ กสิวัฒน์ (2,4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
37 สราวุฒิ สมนาม (2,4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
38 รักสกุล แก่นแรญ (2,4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
39 อภิชาต บุญมาลัย (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
40 วศิน วงศ์วิไล (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
41 พิพูน บุญเป็ง (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
42 Thu Giang Ton (7)		Environmental Science Program คณะวิทยาศาสตร์ มช.	นักศึกษาปริญญาโท (ES)		Environmental Science Program คณะวิทยาศาสตร์ มช	นักศึกษาปริญญาโท (ES)
43 ลลิดา ศรีวิชัย (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
44 เสาวรัตน์ ทับใจ (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	-
45 พิมพา สร้อยสูงเนิน (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
46 ภูษิตา คู่ชัยภูมิ		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
47 ปัญชลี ศรีอุทธวงศ์		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
48 เสาวลักษณ์ งานรุ่งเรือง		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
49 ปานหทัย ทองเวส		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
50 ศิลา กิตติวัชนะ (4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
51 ชนิสร เหง้าจำปา (6)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล" หัวหน้าโครงการ: รศ. ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
52 ฐาปนนท์ เศรษฐีวรฤทธิ์ (6)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
53 พิสันต์ เกตุโกมล (6)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
54 อัครวิช ปองทอง		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
55 ทัชชาวัตน์ ชวัญยืน		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
56 ทิพย์สุดา บุญญากุล		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
57 วัลยา แสงจันทร์		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
58 วัลมี สิทธิขันแก้ว		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาตรี
59 สายฝน จันผกา (*,2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		-	-
60 ณัฐวุฒิ เชิงชั้น (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก
61 มะลิวรรณ อมตชัยโชย (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก
62 กาญจนา อุไรสิทธิ์ (**,2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท		Okayama University, ทุนมอนบูโช	นักศึกษาปริญญาเอก
63 นวลละออ รัตนวิมานวงศ์ (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก
64 เบญจมภรณ์ ชมทอง (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท
65 สนิท ผลาหาญ (*)		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		-	-
66 ธีระภรณ์ เจริญรักษ์		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท
67 ณัฐกานต์ หวังเฟื่องคณากุล		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก
68 ชาคร ชินวงศ์อมร		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ที่ใช้การไหล" หัวหน้าโครงการ: รศ. ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล		เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
		ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
69	กนกพร บุญทรง		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก
70	ศราวดี ทองศรีสกุล		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท

* นักศึกษาสำเร็จการศึกษาแล้ว

** นักศึกษาที่ศึกษาต่อ

1 = นักศึกษาทุน คปก.

2 = นักศึกษาทุนโครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี

3 = นักศึกษาทุนโครงการปริญญาเอกร่วมสถาบันฯ ของ สกอ. (ทบวงมหาวิทยาลัย)

4 = นักศึกษาทุนโครงการ พสวท.

5 = นักศึกษาทุนพัฒนาอาจารย์ UDC

6 = นักศึกษาโครงการเด็กอัจฉริยะ

7 = นักศึกษาชาวเวียดนาม ทุนกรมวิเทศสหการ

หมายเหตุ

สังกัด หมายถึง ภาควิชา/คณะ/มหาวิทยาลัย ในกรณีที่อยู่หน่วยงานอื่น ให้ใส่รายละเอียดที่อยู่ต้นสังกัด

สถานภาพปัจจุบัน หมายถึง ยังอยู่ในโครงการหรือไม่ หรือหากออกจากโครงการแล้ว ขณะนี้สังกัดหน่วยงานใด หรือย้ายไปทำงานที่ใด

ภาคผนวก ก

ผลงานวิจัย

ผลงานวิจัย ก1

ANALYTICAL SCIENCES 2001, VOL.17 SUPPLEMENT

Determination of Cadmium, Copper, Lead and Zinc by Flow Voltammetric Analysis

Jaroon JAKUMNEE,† Siripat SUTEERAPATARANON, Yuthsak VANEESORN, and Kate GRUDPAN

Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand (E-mail: scijkmn@chiangmai.ac.th)

Flow analysis with voltammetric system for determination of cadmium, copper, lead and zinc has been developed. Mercury film working electrode (MFE) was prepared by on-line mercury deposition on a glassy carbon electrode (GCE). Cadmium, copper, lead and zinc were monitored simultaneously by anodic stripping voltammetry (ASV) after a sample was flowed through the electrochemical cell for deposition of the metals on mercury film. Detection limits for cadmium, copper, lead and zinc were 4, 7, 1 and 15 ppb, respectively, for deposition time of 20 s. Linear range of calibration graph for all metals was up to 100 ppb. The precisions (%RSD, n=11) for 25 ppb of the metals were 2-6%. The system has been applied to determine the trace metals in drinking water and wastewater samples.

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Trace heavy metals are very important in the environment due to their serious toxicity although presenting at very low concentrations. They may accumulate in food chain. The development of new methods for quantifying trace metals is required and challenged. Most of the sensitive and selective methods recently available such as ICP-MS, ICP-AES and GF-AAS are too expensive and are not practically applied in a developing country, i.e. Thailand. Alternative cost-effective methods based on combination of flow techniques for sample pretreatmeet e.g. preconcentration/separation with some spectrometric detection systems have been proposed.[1-3]

Voltammetry, especially anodic stripping voltammetry is a promising technique for the determination of trace elements.[4] It is relatively low-cost but provides high sensitivity and can be simultaneous determination of multi-elements. However, batch voltammetry consists of a time consuming and a tedious analysis procedure. Flow techniques have been applied to improve the performance of voltammetry.[5,6] For example, lead in blood was determined by hydrodynamic voltammetry in flow system which could be automated and easy to use.[7] Trace copper in water has been determined in a flow system after on-line extraction as its diethyldithiocarbamate into toluene.[8]

In this work, attempts have been made to develop a flow anodic stripping voltammetry for the simultaneous determination of cadmium, copper, lead and zinc. Conditions of the system were investigated. Several advantages were gained such as better sensitivity and peak resolution, lesser sample and reagent consumption, and faster than a batch procedure. Mercury film electrode can be repeatedly employed for more than 80 times. The proposed method has been applied to determine the trace metals in drinking water and wastewater samples.

Experimental

Chemicals

All chemicals were of analytical reagent grade and Milli Q water (Millipore) was used throughout for preparing of solutions. Acetate buffer (1 M, pH 4.6) was prepared by dissolving 68.04 g of sodium acetate trihydrate (Merck, Darmstadt, Germany) in water and glacial acetic acid (Merck) was added until pH of solution was 4.6, then diluting to 500 ml with water. The solution was then made up to 1000 ml with water. Other concentrations of buffer solutions were prepared by diluting 1 M buffer with water. Mercuric nitrate (Merck) was used to prepare a plating solution. Oxygen free nitrogen gas (99.9995 % N_2) was used for purging dissolved oxygen from a solution. A mixed standard stock solution of 100 ppm of cadmium, copper, lead and zinc were prepared from a 1000 ppm standard solutions for AAS of each ion (Merck). Other concentrations of mixed standard solutions were prepared by appropriate diluting the stock solution. Bottled drinking water samples were purchased from locally department stores.

Flow manifold

Two lines manifold was designed as shown in Fig. 1. Sample and acetate buffer solution were pumped with the same flow rate to merge together and flowed further to a mixing coil and then to a flow through electrochemical cell. Three electrodes cross flow cell (BAS, Indiana, USA) was employed. A voltammograph (BAS CV-50W, BAS) was used for square wave anodic stripping voltammetric analysis. All potentials were measured with respect to a Ag/AgCl reference electrode. A potential of working electrode, mercury thin film coated on a glassy carbon electrode,

was set at −1100 mV for a specified (deposition) time while the sample was flowing through the electrochemical cell. Then the flow was stopped and a square wave potential waveform (−1100 mV to +280 mV) was applied to the working electrode with a scan rate of 75 mV min^{-1} while current was measured, resulting a voltammogram. The flow was started again to clean the electrode and the line, and to prepare for the next sample. For electrochemical cleaning, the potential of the working was set to 0 mV for 20 s.

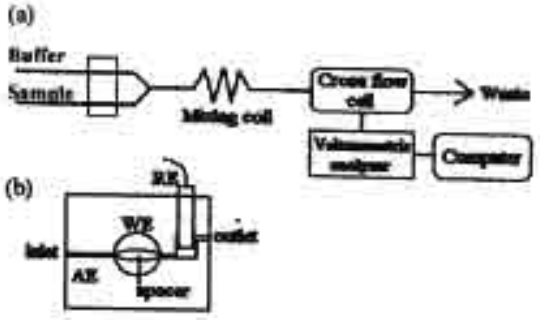

Fig. 1 Flow diagram of flow voltammetric system: (a) flow manifold and (b) details of flow cell; AE = auxiliary electrode, WE = working electrode and RE = reference electrode.

Analysis of samples

The drinking water samples were degassed by purging with oxygen free nitrogen gas before subjecting to analyze by the proposed system. The samples were also analyzed by graphite furnace atomic absorption spectrometry (GF-AAS) as a standard method for comparison. The recommended conditions for GF-AAS were employed.[9] Wastewater samples were digested before taking to analyze by the above methods. UV digestion was carried out by adding hydrogen peroxide (30%w/v/) to the sample and irradiating by UV in a home-made UV digestion unit for 6 h. The digested solution was put in a 50 ml volumetric flask and diluted to the mark with Milli Q water.

Results and discussion

Preparation of mercury film electrode

Mercury film coated on a glassy carbon electrode (GCE) was used as a working electrode (WE). Coating of Hg on a GCE was flowing a plating solution (500 ppm Hg^{2+} in a 0.1 M HNO_3) carried out by applying potential of -800 mV to GCE while through the flow cell. The resulted WE was tested for the determination of 100 ppb Cd standard solution. The WE could be repeatedly used for at least 80 analysis cycles, without the deviation of the peak height obtained more than 2% as shown in Fig. 2. The electrochemical cleaning of WE by applying a potential of 0 mV for 20 s after each analysis run was necessary for preventing of cross contamination of the metals.

Optimization

Flow rate. Flow rates of buffer and sample solutions were set to be equal. Total flow rate of the system was optimized in order to get higher peak current and good reproducibility. It was found that the higher flow rate provided the higher sensitivity and sample through put, but for a flow rate of more than 0.5 ml min^{-1}, the deterioration of mercury film was observed and leading to low reproducibility of the results. A total flow rate of 0.5 ml min^{-1} was chosen for further studies.

Concentration of buffer solution. Acetate buffer concentrations (0.2-1.0 M) were studied. The effect of buffer concentration on peak current of 70 ppm Cd and Pb was shown in Fig. 3. 0.5 M acetate buffer was chosen because the higher sensitivity and reproducibility of peak heights were obtained.

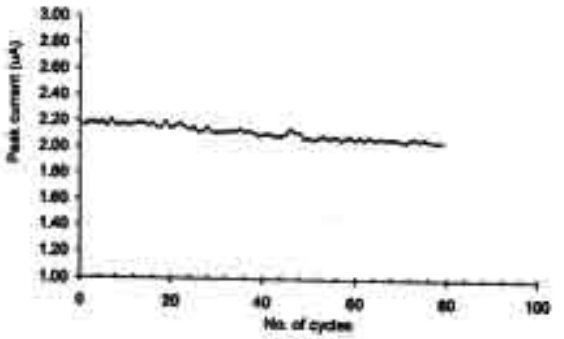

Fig. 2 Peak current of 100 ppb Cd for several analysis runs using the same mercury film.

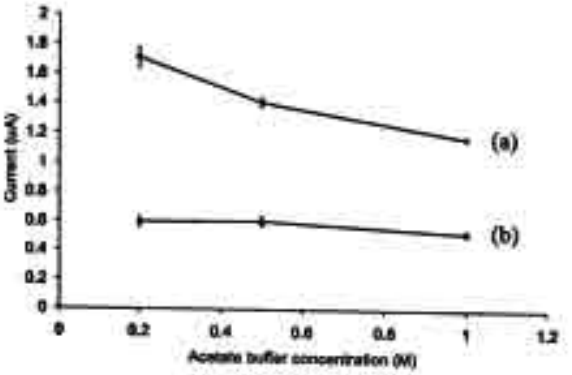

Fig. 3 Effect of buffer concentration on peak current of: (a) Cd and (b) Pb.

Mixing coil length. Mixing coils of 0, 50, 100 and 150 cm long were tried. The length of mixing coil did not much affect the peak height, but did affect the precision of the analyses. The mixing coil of 100 cm long was chosen due to the lowest %RSD of peak heights for all metals and rapid analysis were obtained.

The optimum conditions for flow anodic stripping voltammetry were summarized in Table 1.

Table 1 Optimum conditions for flow voltammetric system for the determination of Cd, Cu, Pb, and Zn.

Mercury plating	500 ppm Hg(II); -800 mV;10 min
Electrolyte	0.5 M acetate buffer pH 4.6
Flow rate (total)	0.5 ml min^{-1}
Mixing coil length	100 cm
Electrolysis potential	-1100 mV vs Ag/AgCl
Stripping (sweep) potential	-1100 to 280 mV
Sweep mode	Square wave
Sweep rate	75 mV s^{-1}

Calibration graph and precision

Using the conditions in table 1, a series of mixed standard solutions of cadmium, copper, lead and zinc (5, 20, 50, 70 and 100 ppb) was performed. The voltammograms obtained are depicted in Fig. 4. A calibration graph of each metal was constructed by

plotting peak currents obtained (μA) versus concentrations (ppb). The linear ranges, calibration equations, R^2 and detection limits (3σ) are presented in Table 2.

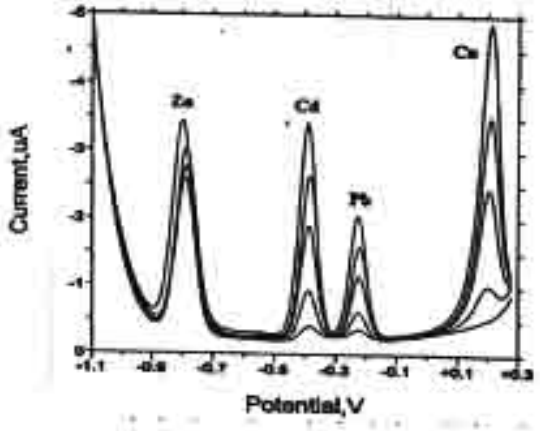

Fig. 4 voltammograms obtained from flow-ASV system; 5, 20, 50, 70 and 100 ppb each element.

Table 2 Calibration data for various elements.

Element	Linear range	Equation	R^2	Detection limit (ppb)
Cd	5-100	y=0.032x + 0.05	0.9956	4
Cu	10-100	y=0.043x + 0.50	0.9982	7
Pb	5-100	y=0.017x + 0.02	0.9986	1
Zn	20-100	y=0.007x + 1.20	0.9440	15

Precision of the method was evaluated by 11 replicate determinations of 25 ppb each element in a mixed metals standard solution. The relative standard deviations of Cd, Cu, Pb and Zn were found to be 3.8, 6.2, 3.6, and 3.0, respectively.

Analysis of samples

The results of the analyses of drinking water samples are presented in Table 3. Concentrations of the metals in bottled drinking water samples were less than detection limits, except Zn in Mont Fleur drinking water.

Table 3 Concentrations of metals in drinking water samples.

Sample	Concentration found (ppb)			
	Cd	Cu	Pb	Zn
Cooly fresh	-	-	-	-
Aura	-	-	-	-
Polaris	-	-	-	-
Mont Fleur	-	-	-	112
Hawiian	-	-	-	-
Minere	-	-	-	-
Vittel	-	-	-	-
Perrier	-	-	-	-
S. Pellegrino	-	-	-	-

- = below detection limits.

Concentrations of Cd and Pb in digested wastewater samples are summarized in Table 4. The results obtained agreed with those obtained from GF-AAS method.

Table 4 Concentrations of Cd and Pb in digested wastewater samples.

Sample	Concentration found (ppb) by method		
	GF-AAS	Flow-ASV	%different
Cadmium			
S1	25	26	4
S2	50	54	8
S3	7	4	-42
Lead			
S1	100	92	-8
S2	200	196	-2
S3	20	28	40

A flow voltammetric system was developed. Under suitable conditions, the system could be applied for the simultaneous determination of trace cadmium, copper, lead and zinc using square wave anodic stripping voltammetric mode. The sensitivity and selectivity (peak resolution) were improved from the conventional batch voltammetric system because a large area and thin mercury film electrode was employed, respectively. The same mercury film could be repeatedly used for at least 80 analysis runs. The proposed procedure consumed less sample and reagent, and was faster than a batch method. The system provided opportunity for automation and incorporation of on-line sample pretreatments.

Acknowledgements

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ผลงานวิจัย ก2

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A Cost-Effective Gravitational Field-Flow Fractionation System

Rattikan CHANTIWAS,[1] Jaroon JAKMUNEE,[1] Ron BECKETT,[2] and Kate GRUDPAN[1]†

† *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand (E-mail: kate@chiangmai.ac.th).*
2 *Water Studies Centre, Department of Chemistry, Monash University, Clayton, Vic., Australia.*

Various possibilities for cost-effective field-flow fractionation (FFF) systems are proposed. Different components of the Gravitational FFF (GrFFF) systems are discussed. A simple GrFFF channel could be made of a cut plastic sheet of transparency and sandwiched by two lucite pieces. A gas pressure system from a gas cylinder (e.g. N_2) can be an alternative means for pumping. HPLC UV/Vis detector can be used for monitoring the particles. The read-out can be either a chart recorder or a simple computer-interfacing device. The proposed cost-effective system can be applied to separate Silica Gel 60G.

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Various separation techniques have been applied to different situation such as environment, pharmaceuticals, biotechnology and industry. Those include liquid chromatography, gas chromatography, electrophoresis, size-exclusion chromatography and field-flow fractionation.[1] The latter is relatively new. Field-flow fractionation (FFF) is commonly used for separation and characterization of various particles and macromolecules.[2,3] FFF instrumentation may be similar to HPLC. Although in some cases, FFF systems may be more complicated. There are now FFF instruments commercially available,[4,5] but rather expensive, especially in Thailand, therefore we are interested in making use of existing components, not only for basic research but also in term of instrumental development. We have experienced in development of low cost instrumentation.[6-8] In this paper, we outline the possibilities of cost-effective set-ups for gravitational FFF (GrFFF).

Experimental

A GrFFF system usually consists of components, represented schematically in Fig. 1.[9-12]

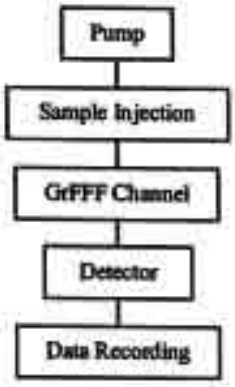

Fig. 1 Schematic diagram of a simple GrFFF system.

Possibilities of Components of a GrFFF system

Pumping system. A propelling device may be one of the following:

A gas pressure device: gas pressure based propulsion may utilize a cylinder of an inert gas e.g. N_2 to propel a carrier solution.[6,13]

A peristaltic pump: a peristaltic pump may be used.

A HPLC pump: a HPLC pumping system, a reciprocating pump, a syringe pump or a displacement pump, may be employed.[14]

Sample injection system.

Homemade injection device: an injection port can be homemade. A sample is injected into carrier stream via silicone rubber septum by a syringe with a hypodermic needle, through the port (Fig. 2) made of a plastic block.

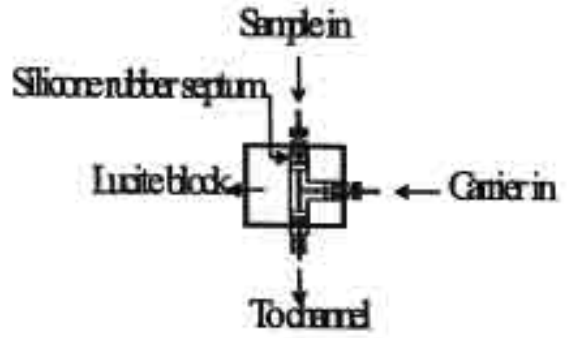

Fig. 2 A homemade injection port.

6-port injection valve: this type of valve is commercially available from various suppliers.

FFF separation unit

GrFFF channel: A simple channel can be assembled by using an overhead transparency sheet and two pieces of lucite blocks. The transparency sheet, usually 0.01 cm thick, can be cut to be a spacer and the lucite pieces (approximately 1 cm thick each)

are served as two walls. The spacer is sandwiched in between the two lucite walls and tightened with the bolts. The channel is shown in Fig. 3.

A detector. The following detectors may be employed: an UV/Vis detector, a fluorescence detector or a light scattering detector.

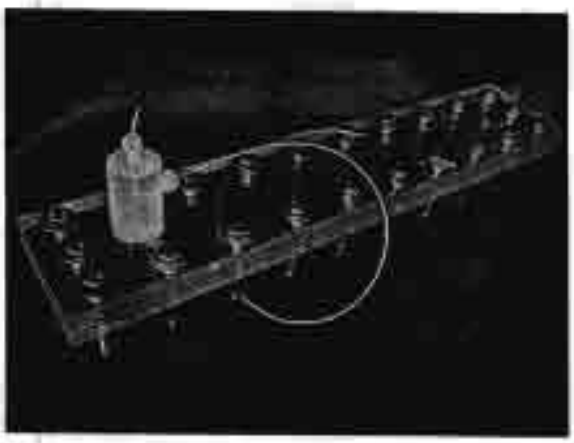

Fig. 3 GrFFF channel.

Data recording. Acquisition/read-out unit could be: a chart recorder, low-cost interfacing device (simple computer-interfacing device such as Pocket Sampler, which is commercially available (Dickamith, Australia)) and the LabVIEW (National Instruments™, USA).

Results and Discussion

A simple GrFFF system may compose of five components (Fig. 1). We set up a system by using alternative devices for each part.

As an alternative, using a cylinder gas, which can be generally available in any laboratory, N_2 gas-pressured propulsion to deliver the carrier was tried. The gas pressure produced pulse-free flow. Other alternatives, a peristaltic pump or a HPLC pump is rather relatively more expensive, though they are efficient and most widely employed in FIA and HPLC works.

A simple introducing device for sample was a homemade injection port (Fig. 2). The low concentration loaded sample is usually considered to avoid overloading effect. A small syringe with a needle (25µl) could be used. Also using 6-port valve is common for sample introduction and provides better reproducibility.

An UV/Vis detector, which was a commonly available in the analytical laboratory, is employed in this work.

A chart recorder was used. The low-cost interfacing device (Pocket Sampler) may be employed if digitized conversion was needed. A Pocket Sampler with software supplied is handy and easy for operating. The more powerful LabVIEW is also an option, however it is rather more expensive.

The GrFFF system and its application as a teaching tool.

A simple GrFFF consisting of N_2-gas pressurized pump, a homemade injection system, a GrFFF channel, an UV/Vis detector and the chart recorder was tested by using Silica Gel 60G (Merck, Germany). Results are represented in Fig. 4. A sample of thin layer chromatographic Silica Gel 60G (5-40 µm) was employed for the GrFFF's demonstration. The broad silica size ranges of 5-10 µm and 15-25 µm was prepared from Silica Gel 60G (5-40 µm) by repeated settling.[15] From the results obtained (Figs. 4A, 4B), the longer retention time, the smaller sizes of silica (5-10 µm) eluted and the flow rate effect of water carrier: the higher flow rate, the lower retention time; effect of relaxation time (stopped flow of carrier after the suspension was injected): the longer stop time the higher retention time obtained (Fig. 4C).[16]

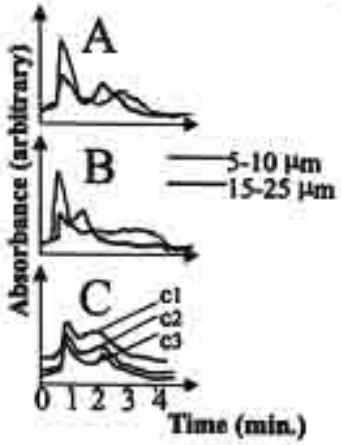

Fig. 4 The fractograms of Silica Gel samples. (A) Flow rate of 1.5 ml/min, stopped flow for 60 sec. (B) Flow rate of 4 ml/min, stopped flow for 60 sec. (C) Flow rate of 2 ml/min, stopped flow for (c1) 5, (c2) 10 and (c3) 30 sec.

In conclusion, various possibilities of simple gravitational field-flow fractionation set-ups are described. The components are cost-effective and easily available in a laboratory. Separation can be demonstrated for Silica Gel 60G of different sizes.

Acknowledgements

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ผลงานวิจัย ก3

ANALYTICAL SCIENCES 2001, VOL.17 SUPPLEMENT

Combination of Field-Flow Fractionation and Flow Injection Analysis

Rattikan CHANTIWAS,[1] Jaroon JAKMUNEE,[1] Ron BECKETT,[2] Ian MCKELVIE,[2] and Kate GRUDPAN[1]†

† *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200 Thailand. (E-mail: kate@chiangmai.ac.th)*

‡ *Water Studies Centre, Department of Chemistry, Monash University, Clayton, Vic., Australia.*

This paper presents a combination of field-flow fractionation (FFF) with flow injection analysis (FIA). FFF is a novel powerful separation technique for particles, while FIA having various advantages. Instrumentation of the set of the combination of gravitational FFF and FIA with chemiluminescence detection is proposed. Its possible application to silica sorbed iron of different particle sizes as a model is demonstrated.

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Field-flow fractionation (FFF) is a technique for size separation and characterization of particles and macromolecules. Separation by FFF takes place in a thin channel by applying an external driving force perpendicular to laminar flow of the carrier liquid.[1,2] Various driving forces have been employed in FFF such as centrifugal, flow, thermal gradients, electrical and gravitational. Gravitational field-flow fractionation (GrFFF) could be the most cost-effective and simple set up.[3-4]

Combination of FFF with one of other analytical tools such as GFAAS[3], ICP-AES or ICP-MS[4-6] results in size-based element distributions.

Flow injection analysis (FIA) has been demonstrated to provide several advantages, including simple instrumentation and economic aspects.[10-14] FIA has been combined with other analytical techniques but not yet with FFF. We have then attempted to hyphenate FFF with FIA for size-based element distribution.

In this paper, we demonstrate the combining gravitational field-flow fractionation (GrFFF) with reverse flow injection analysis with chemiluminescence (FIA-CL). The FFF is for separation of particle sizes of silica coated with goethite.[15] The FIA-CL is for iron determination which is based on catalytic reaction of alkali luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and hydrogen peroxide.[16-21]

Experimental

Instrumentation for Gravitational Field-Flow Fractionation-Reserve Flow Injection with Chemiluminescence

The GrFFF instrument equipped with the reverse FIA with chemiluminescence detection is depicted in Fig. 1. An isocratic pump (SpectraSERIES, USA) was used to propel a carrier in GrFFF system. The particles suspension samples were injected into the FFF channel (10 µl) and monitored for mass distribution by an UV/Vis detector (Linear Instruments Model 200 detector, USA) at a wavelength of 254 nm. The eluting particles from GrFFF flowed directly into the FIA-CL system for monitoring of iron in each fraction.

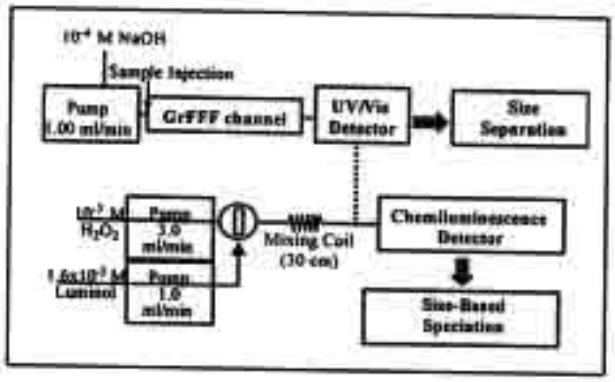

Fig. 1 Schematic diagram of the GrFFF equipped with FIA chemiluminescence detection.

For reverse FIA, luminol solution was injected, via a six-port injection valve (Upchurch, USA), into a stream of the mixture containing luminol and hydrogen peroxide which was delivered by a peristaltic pump (Eyela SPM 23, Japan) before merging with the line from GrFFF and finally entering into the CL. The chemiluminescence was monitored by a modified commercial liquid scintillation counter, with a spiral flow cell (Packard Radiometric Flo-one®\Beta Series A-100 Model A140K)(Canberra, USA).

Performance test was made by using model samples consisting of 5 µm and 10 µm silica particles coated with goethite (FeOOH).

Results and Discussion

Conditions used in the experiments are summarized in Table 1.

Table1 Experimental conditions used.

GrFFF set-up	
Concentration of sodium hydroxide carrier/ M	10^{-4}
Flow rate/ ml/min	1.00
Channel volume/ ml	0.57
Sample concentration/ mg/ml	2.0
Sample injection/ µl	10
FIA-CL set-up	
Luminol concentration/ M	1.6×10^{-3}
Hydrogen peroxide concentration/ M	10^{-1}
Flow rate of luminol/ ml/min	1.0
Flow rate of hydrogen peroxide/ ml/min	3.0
Injection volume of luminol/ µl	80
Mixing coil length/ cm	30

Fig. 2 shows the GrFFF fractogram (UV detector response for mass versus elution time) for the mixture of 5 µm and 10 µm silica coated goethite. Good resolution was obtained with a run of about 8 min. This would be a steric/hyperlayer GrFFF mode: the larger particles, the quicker elution observed.[4,26]

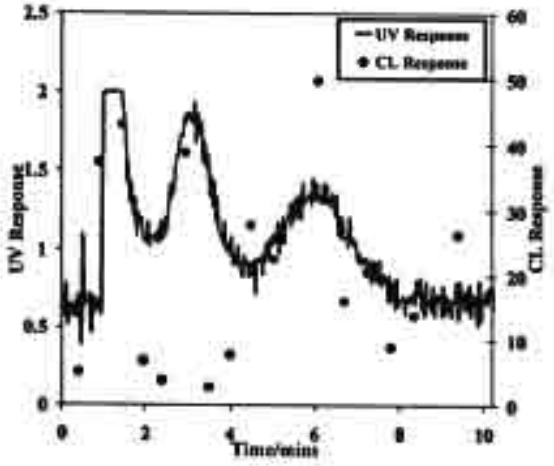

Fig. 2 UV absorbance and FIA-CL response based fractograms from 5 µm and 10 µm silica particles coated with goethite (FeOOH) obtained using GrFFF with FIA-CL for iron speciation.

Also shown in Fig. 2, the chemiluminescence responses (points) obtained from a series of injections of luminol/H_2O_2 into the GrFFF eluent prior to flowing into the FIA-CL system. It can be observed that the response due to the 5 µm fraction was higher than that of 10 µm fraction. The response corresponds to iron coated on silica. Since there was no digestion step in this experiment, it is likely that the CL responses should relate to the iron sorbed on surface area of the particles and not the total iron content in the goethite coatings. The nature of the CL responses and the possibility of including on-line digestion have been under investigation.

The combination of field-flow fractionation (FFF) with flow injection analysis (FIA) has been demonstrated for size based speciation.

Acknowledgements

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Flow Injection Sample Pretreatment for Determination of Lead by Flame Atomic Absorption Spectrophotometry

Jaroon JAKMUNEE,[1†] Ponlayuth SOOKSAMITI,[2] Horst GECKIES,[3] and Kate GRUDPAN[1]

[1†] *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand (E-mail: scijjkmn@chiangmai.ac.th)*

[2] *Office of the Mineral Resource Region III(Chiang Mai), Chiang Mai 50200, Thailand*

[3] *Institut fuer Nukleare Entsorgungstechnik, Forchungszentrum Karlsruhe, Karlsruhe,Germany*

Flow injection sample pretreatment for determination of lead by flame atomic absorption spectrophotometry has been developed. Utilizing an in-valve mini-column, packed with a solid phase extraction resin (Sr.spec™), lead was preconncentrated and separated from the matrix. The conditions for sorption and desorption of lead were investigated. A 1 M nitric acid solution and 0.005 M EDTA solution of pH 7 were suitable for soeption and desorption step respectively. A concentration efficiency of 11 min^{-1} was achieved. A linear calibration was obtained for 0.1-5.0 μg Pb and a single standard calibration can be applied. The method has been applied to the determination of lead in certified reference materials, leachates and digests of tin tailing samples. The system allows rapid analysis of very small sample volumes.

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Lead has toxicity effects to human health. Accordingly, the development of methods for its determination in variuos biological and environmental samples is both necessary and challenging. Several sensitive methods exist for lead quantitation such as GFAAS, ICP-MS, but a high instrumentation and operating cost are limitted the applications. Stripping voltammetric techniques have been widely used, but they suffer from interferences from metals presented in large amounts in mineral samples.[1] Other methods, for instance, FAAS, spectrophotometry are not sensitive or selective enough, so the preconcentration and separation step are required. Various techniques such as solvent extraction,[2] ion exchange,[3,4] extraction chromatography,[5-7] and precipitation[8] in combination with flow injection analysis have been developed for determination of lead in various samples, gaining several advantages over the conventional preconcentration/separation methods: rapid measurement, high sample throughput, fewer reagents and glass ware, avoidance of tedious work and prevention of contamination. However, conventional ion exchangers are not selective enough for application with a mineral sample which contains high concentrations of interfering ions and are ineffective for highly acidic sample solution. Recently, a novel extraction chromatographic resin (Sr.spec™) that exhibits an extremely strong retention of lead under acidic condition was developed.[9] This resin has been used in in-valve column for determination of lead in soil[10] samples by FAAS.

In this work, the conditions for separation/preconcentration of lead by flow injection in-valve mini-column packed with Sr. spec™ resin prior to the determination by AAS were investigated. EDTA solution was found to be the most effective eluent for eluting of sorbed lead from the resin. Relatively low concentration of EDTA (0.005 M) could be used, thus avoiding of burner clogging and high background signals. A single standard calibration of up to 5.0 μg Pb with a detection limit of 0.09 μg Pb was achieved. The method was applied for the determination of lead in some certified reference materials and in leachates and digests of tin tailing samples.

Experimental

Chemicals

All chemicals used were of analytical-reagent grade, except nitric acid and hydrofluoric acid (Merck, Damstadt, Germany) were of suprapure grade and sulfuric acid (Merck) was ultrapure grade. Deionized water was used for preparing of all solutions. Citric acid monohydrate, oxalic acid, tartaric acid, sodium citrate, ammonium oxalate monohydrate and ethylenediaminetetraacetic acid, disodium salt (EDTA) were obtained from Merck. Humic acid, sodium salt was obtained from Aldrich. Lead nitrate standard solution for ICP-AES (1000 ppm) was also from Merck. Sr.spec™ resin (80-100 μm grain diameter) was from Eichrome, Darien, USA. Certified reference materials (CRMs) of sediment and soil were obtained from Community Bureau of Reference (BCR) and International Atomic Energy Agency (IAEA), respectively.

Preparation of solutions

Lead standard solution. 100 ppm lead was prepared by diluting 1000 ppm lead standard with 0.1 M HNO_3. A series of

lead standard solutions were prepared by diluting the 100 ppm lead standard solution into a solution of 1 M HNO_3.

Eluent solutions. To obtain 0.5 M concentration of various eluents, the following amounts of chemicals were dissolved and made up to 1 l volume: EDTA: 1.88 g, ammonium oxalate: 7.141 g, sodium citrate: 17.85 g, tartaric acid: 7.542 g, citric acid: 10.559 g, and oxalic acid: 6.335 g.

FI manifold

Flow injection system (FIAS-400, Perkin Elmer) and atomic absorption spectrophotometer (AA 3300, Perkin Elmer) were used. A flow diagram of the system was designed as shown in Fig. 1.

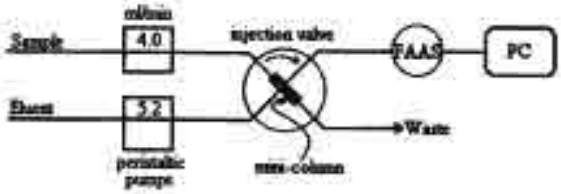

Fig. 1 Manifold for FI-in-valve mini-column determination of lead: FAAS = flame atomic absorption spectrophotometer and PC = personal computer.

Sample and eluent were propelled via peristaltic pump 1 and 2 respectively. An in-valve mini-column packed with Sr.Spec™ resin was incorporated within the injection valve, replacing one of the sample loops. While the valve was in the INJECT position, the eluent solution passed through the column to admit the sample into the detector. When the injection valve was turned to the LOAD position, a sample solution in 1 M HNO_3 passed through the column in an opposite direction, thus avoiding tighten packing of the column. After a specified loading time, the valve was automatically turned back to INJECT position and accumulated lead was eluted downstream for continuous monitoring by FAAS.

The detector conditions used were: wavelength set to 217.0 nm, fuel (C_2H_2) flow rate 3.7 l min^{-1}, air flow rate 10 l min^{-1}. A fuel flow rate, a hollow cathode lamp and burner position were adjusted until the maximum sensitivity of 10 ppm Pb aspirated was obtained. An aspiration rate was 5.0 ml min^{-1}.

Samples preparation

Digested tin tailing samples. Tin tailings were dissolved by microwave digestion system (MLS 1200 mega, Milestone, USA). 100 mg of sample was accurately weighed and put in a Teflon container. 2 ml conc. HNO_3 and 5 ml conc. HF were added. The sample containers were closed and assembled on a container block and then were digested in a microwave oven at about 100 °C for 30 min and at about 120 °C for 30 min. The digested solution and the remaining residue were transferred to another Teflon container. A 5 ml conc. H_2SO_4 was added and treated further open digestion on a hotplate in a fume cupboard for a few hour to evaporate HF. After cooling, the solution was transferred to a 50 ml volumetric flask and the volume is made up to the mark with 0.1 M HNO_3.

CRMs. The procedure used for digestion of CRMs was already reported elsewhere.[10]

Leached tin tailings. Ammonium acetate was used for leaching of exchangeable ions from the particles.[11] 1 g of tin tailing was weighed and put in a 25 ml polyethylene vial. 20 ml of 1 M ammonium acetate were added and the vial lid was tightly closed. The vials were shaken using an end-over-end shaker with rotating at 30 rpm for 6 h. After the particles were settled, the solution was transfered into a centrifuge tube. The solution was separated by centrifuging at 8000 rpm for 20 min. 15 ml of upper clear solution was taken into a 25 ml vial which contained 2 ml of conc. HNO_3 and 3 ml of deionized water.

Analysis of Samples

The above sample solutions were diluted with a 1 M HNO_3 before commencing to the determination of lead by the proposed system. For comparison purpose, inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6000, Perkin Elmer, USA) was also used to analyze the samples. A sample was prepared by transferring 1 ml of sample solution into a sample tube, adding 4 ml of 1% HNO_3 solution and 50 ul of an internal standard solution (Rh-103). The standard solution were prepared by diluting of a multi-element standard solution to suitable concentrations. The conditions of the instrument: plasma power 1050 kW, radio frequency 40 MHz, argon gas for plasma, auxiliary and nebulizer of 15.0, 0.5 and 1.0 l min^{-1}, respectively, autolensing of ion lens and sample aspiration rate of 1.0 ml min^{-1} were employed.

Results and Discussion

Concentration of nitric acid as sample medium

It was found that a peak height of 0.8 μg Pb^{2+} did not significantly increase for increasing a concentration of nitric acid to higher than 1 M. This agreed with the previous report which Pb^{2+} can be sorbed efficiently on the Sr.Spec™ resin from a medium of 0.8-2.0 M HNO_3.[10] The 1 M HNO_3 was utilized in further studies.

Flow rate of eluent

Since the aspiration rate of a nebulizer was 5.0 ml min^{-1}, the lower flow rate of the eluent caused air bubble in the flow channel leading to low reproducibility of the results. The higher flow rate caused low sensitivity that could be due to low nebulization efficiency. The eluent flow rate of 5.2 ml min^{-1} was chosen.

Investigation for eluent

The peak heights for elution of 0.8 μg sorbed Pb by 0.05 M of the various eluents of different pH values are displayed in Fig. 2. It was found that EDTA was the best one as determined by peak height and RSD of triplicate injections. However, citrate and oxalate were also quite effective. The elution mechanism would be the strong formation of a complex with Pb^{2+} in the eluent solution. The EDTA solution of pH 7 was used in subsequent analyses because it provided the better precision, narrow and high peaks, and moderate residence time compared to the higher pH solution.

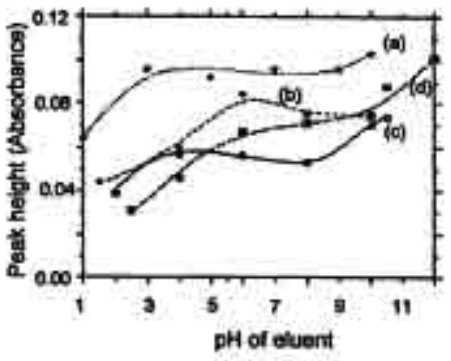

Fig. 2 Effect of various eluents of different pH : (a) EDTA, (b) oxalic acid, (c) tartaric acid and (d) citric acid.

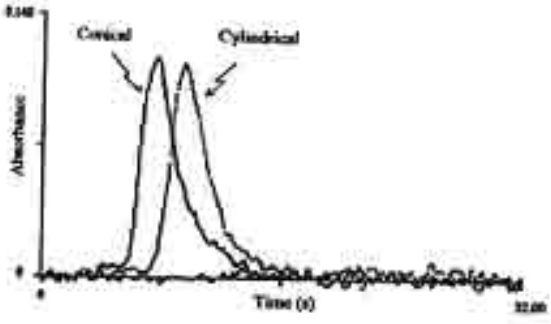

Fig. 3 Effect of column designs.

Concentration of EDTA

EDTA solution (pH 7) of different concentrations (0.00-0.01 M) were used as eluent for elution of 2 μg sorbed Pb^{2+} on the Sr.Spec™ resin. It was found that an EDTA concentration of 0.001 M (30 fold higher than Pb^{2+} concentration by mole) or more can elute the adsorbed Pb^{2+} effectively. The 0.005 M EDTA was chosen in order to ensure the completeness of elution and also to save the reagent.

Column designs

Two column designs: a cylinder (2 cm long, 3 mm i.d.) and a truncated cone (1 cm long, 2 and 4 mm i.d. at ends) were tried. The results are depicted in Fig. 3. The column dimension did not significantly affect the peak height (0.114±0.001 and 0.119±0.004 for cylinder and cone shaped column, respectively), but did affect the residence time in case of loading and elution with the same flow direction. This implied that the adsorption of lead on the column occurred in a small region at the beginning of the column. The distribution coefficient for lead extraction from 1 M HNO_3 solution into the resin was high enough to retain lead in a small area of the column. The eluent was effective enough to desorb accumulated lead from the column and had only small effect on the dispersion of the elution profiles.

Interferences

Effect of some possible interfering substances in environmental and mineral samples are shown in Table 1. The tolerance limit was defined as the concentration of the interfering ions that cause 5% deviation in peak height of 0.2 ppm Pb^{2+}. EDTA and humic acid were selected as representative of the complexing agents in the environment. Humic acid up to 20 ppm had no effect on lead sorption, but the concentration of higher than 10 ppm humic acid did cause problems with column blocking. Other interfering ions have been studied using a similar system.[10] It has been reported that Fe^{3+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Sr^{2+}, Cu^{2+} and Cd^{2+} do not interfere at concentrations up to 50 ppm.

Calibration graph and precision

Both normal calibration (plots of the ppm Pb versus the corresponding peak height) and single standard calibration (plot of μg Pb versus peak height obtained) can be used. The μg Pb can be varied by using only one standard solution of Pb^{2+} and varying the preconcentration time, so the μg Pb were calculated from the loading flow rate (q, ml min^{-1}), the preconcentration time (t, min) and the concentration of standard solution (C, μg ml^{-1}) : μg Pb equivalent = q (t) (C). The single standard calibration graph is shown in Fig. 4. The calibration equation (up to 5 μg Pb): μg Pb = 0.070 (peak height, A) + 0.007; r = 0.9995 was obtained. The detection limit (3σ) was 0.09 μg Pb and the relative standard deviations of 12 replicate injections of

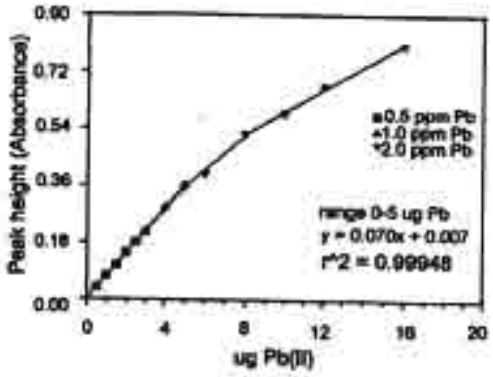

Fig. 4 Single standard calibration graph; three concentrations of lead standard solutions (0.5, 1.0 and 2.0 ppm Pb) were loaded.

Table 1 Tolerance limits of some interfering substances.

Interfering substances	Tolerance limit
EDTA	0.01 M
Humic acid	10 ppm
NaCl	0.25 M
$MgCl_2$	1.0 M
PO_4^{3-}	5000 ppm
Fe(III)	750 ppm

0.8 μg Pb and 1.0 μg Pb were 5.2 and 2.3 %, respectively.

Analysis of samples

Certified reference materials of soil and sediment, and tin tailing samples were analyzed by the developed method. The single standard calibration was applied for determining the concentrations of the samples. The results obtained are shown in Tables 2 and 3. The t-test was used to compare the results obtained from the proposed method and those obtained from the ICP-MS method or the certified values. It indicated that no significant difference was found at a 95% confidence level.

Table 2 Contents of lead in certified reference materials found by the FI-FAAS method.

Samples	Certified value (μg g^{-1})	Concentration found (μg g^{-1})	% difference
IAEA soil-7	60 (55-71)	58.4±3.2	-2.3
CRM-320 sediment	42.3±1.6	43.4±2.0	2.6

Table 3 Lead contents in tin tailing samples.

Samples*	Lead contents found (ppb) by		
	ICP-MS	FI-FAAS	% difference
1	128.7	124.0	-3.7
2	77.9	79.2	1.6
3	91.8	79.1	-13.8
4	97.7	98.3	0.6
5	255.8	257.6	0.7
6	239.0	254.0	6.3
7	194.8	212.0	9.2
8	188.6	186.2	-1.3
9	10.5	11.6	10.3
10	3.5	3.6	3.1

* 1-8 : digested tin tailing samples (1 min preconcentration)
9-10 : tin tailing leachates (2 min preconcentration).

It can be concluded that flow injection sample pretreatment system utilizing in-valve mini-column packing with Sr.Spec™ resin for the determination of lead in a complex matrix samples by flame atomic absorption spectrophotometry has been developed. Lead was sorbed on the resin from a 1 M HNO_3 medium and then eluted by a 0.005 M EDTA solution. The single standard calibration method can be applied for determining of lead in the amounts up to 5 μg, with a detection limit of 0.09 μg and precision of 5.2% (0.8 μg Pb, n=12). Concentration efficiency for the preconcentration was 11 min^{-1}. The proposed system has been applied for analysis of digest and leachate of tin tailing samples, and certified reference materials. The results obtained agreed well with those by ICP-MS method and the certified values, but the developed method is relatively economic.

Acknowledgements

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Kinetic Determination of Iodine in Urine Using Stopped-Flow Injection

Duangjai NACAPRICHA,[†1,2] Nuanlaor RATANAWIMARNWONG,[1] Supasin SUWANNACHOAT,[1] Prapin WILAIRAT,[1] Juwadee SHIOWATANA,[1,2] and Kate GRUDPAN[3]

[1] *Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Raod, Bangkok 10400 Thailand. (E-mail: scdnc@mahidol.ac.th)*

[2] *Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Nakorn Pathom 73170 Thailand.*

[3] *Department of Chemistry, Faculty of Science, Chiang Mai University, Huay Kaew Road, Chiang Mai 50200 Thailand.*

A flow injection system in the stopped mode (stopped-FI) will be presented for determination of urinary iodine based on the catalytic effect of iodide on the redox reaction between Ce(IV) and As(III). Since the reaction is a first order process, kinetic determination of the catalyst using the stopped-FI can be carried out in two ways. The iodine content could be measured based on a linear plot between the logarithm of the absorbance of Ce(IV) taken at a fixed time against iodine concentration. For this type of calibration, the use of an automatically controlled stopped-FI should diminish the errors in the conventional batch method, which often arise from the imprecision of timing. With this procedure, the detection limit (3σ) of 3 μg l^{-1} and a sample throughput of 35 samples h^{-1} were achieved. The calibration can also be made using the relationship between the rate constant and the catalyst concentration. In this case the flow was stopped for, say 25 minutes depending on the iodine concentration, to record the kinetic profile of the disappearance of Ce(IV). The stopped-FI system is an alternative tool in the status assessment of iodine deficiency disorder.

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Iodine is an essential element for human. Contents of iodine in urine are normally used as a marker for status assessment of iodine deficiency disorder (IDD). The following values are guide for IDD status: <20 μg l^{-1} (severe); 20-49 μg l^{-1} (moderate); 50-100 μg l^{-1} (mild) and >100 μg l^{-1} (normal).[1] Since iodine concentration in urine samples is considerably low, it is necessary to use sensitive methods for this application.

There have been some publications that described methods for determination of urinary iodine. The methods concern techniques such as high performance liquid chromatography (HPLC),[2-5] ion-selective method[6] and ICP-MS.[7]

Amongst other methods, kinetic methods are also suitable for general trace analysis[8] and usually give a high sensitivity with sometimes cost-effective. The most common method for determination of urinary iodine is a kinetic spectrometric method with catalytic effect of iodide on a redox reaction between Ce(IV) and As(III). Sandell and Kolthoff first described analytical application of this reaction to determine iodide catalyst in 1934.[9] Possible mechanisms of the reaction were proposed in 1969.[10] Some applications of the Sandell and Kolthoff method were later described.[11-15] for determination of iodine using its kinetic effect. Nevertheless the classical method for urine was provided by Dunn et al.,[1,15] The method is carried out batchwise and requires sample digestion since urine contains varieties of species, which interfere the kinetics. The Sandell and Kolthoff method applied in flow based techniques,[16-20] including flow injection (FI) technique[21] have been described for this application as alternative procedure to the batch method. The main purpose of these automation techniques is for convenience and a better precision in taking reading signal at a fixed-time.

Recently our laboratories have proposed a method based on flow injection.[22] The method is based on the batch procedure of Dunn et al.'s[1,15] which requires choloric digestion of samples prior kinetic determination. After the digestion, iodine species are oxidised to iodate ion,[23] which is not the catalyst for Sandell and Kolthoff method. However non-published experimental results[24] have demonstrated that under the condition used, iodate ion is reduced to iodide ion in the presence of arsenious acid and chloride ion. Our results have shown in a batch system that the kinetics is a first-order type.

For first-order kinetics, in this case, disappearance of Ce(IV) is exponential, that is

$$[Ce^{IV}]_t = [Ce^{IV}]_0 e^{-kt} \quad (1)$$

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

Therefore,

$$-\ln[Ce^{IV}]_t = -\ln[Ce^{IV}]_0 + tk_{uncatalysed} + tk_{catalysed}[I^-] \quad (2)$$

$[Ce^{IV}]_0$ and $[Ce^{IV}]_t$ are the concentrations of Ce(IV) at the original and at time t respectively. $k_{uncatalysed}$ is the rate constant in the absence of iodide ion. $k_{catalysed}$ is the rate constant due to the concentration of the iodide catalyst, $[I^-]$. According to Beer's law, equation (2) can be written as

$$-\ln A_t = -\ln A_0 + tk_{uncatalysed} + tk_{catalysed}[I^-] \quad (3)$$

where A_0 and A_t are the absorbance of $[Ce^{IV}]_0$ and $[Ce^{IV}]_t$ respectively. Thus a linear calibration plot can be obtained as written in equation (4)

$$-\ln A_t = \text{slope}[I^-] + \text{intercept} \quad (4)$$

In this work, two kinds of kinetic method which are fixed-time and rate constant methods, were employed with stopped-flow injection (stopped-FI) for iodine. With the use of stopped-FI, kinetics profile of the disappearance of Ce(IV) can be studied. Under the condition in the flow injection technique that also provides a type of pseudo first-order kinetics, calibration for iodine can be made based on equation (4). This method is a fixed-time method. Another kinetic method, which utilized measuring of the first-order rate constant, was studied in the stopped-FI system.

Experimental

Flow injection manifold

Figure 1 schematically illustrates the set up of the flow injection system used. Optimization was described in the previous work.[22] 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. 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 the 100 cm reaction coil. 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.

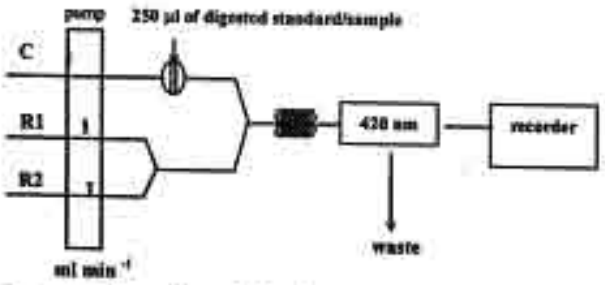

Fig. 1 The flow injection manifold for kinetic determination of iodine in urine. [C: water carrier, R1: 8.0×10^{-3} M Ce(IV) in 1.75 M H_2SO_4 , R2: 1.0×10^{-1} M As(III) + 0.8 M NaCl in 0.5 M H_2SO_4].

Reagents

All chemicals used in this work were AR grade, supplied by Merck (Germany). 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 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.

Ceric ammonium sulfate 0.008 M (R1)

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.

Arsenious acid 0.1 M (R2)

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 as reagent stream (Fig. 1).

Sample, standard and digestion

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 digestion. The employed digestion method of samples and standards was a modified Dunn et al.'s.[22]

Procedure

After digestion, samples or standards were injected into the FI system depicted in Fig. 1 for determination of iodine.

Rate constant method

At the time of 41-second after sample injection into the manifold in Fig. 1, when the peak bottom rose in the usual continuous mode, the flow was stopped. Absorbance of Ce(IV) at 420 nm was continuously recorded until constant reading was reached (complete reduction to Ce(III)).

Fixed-time method

Operating procedure for this method is similar to that described for the rate constant method. At 41 seconds after injection, the flow was stopped for a period (1 min). The signal was then 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. In the calibration, the stopped interval must be fixed for all working solutions and the samples.

Results and Discussion

Rate constant method

Fig. 2 shows kinetics of the reduction of Ce(IV) to Ce(III) which were monitored using the stopped-FI manifold in Fig. 1.

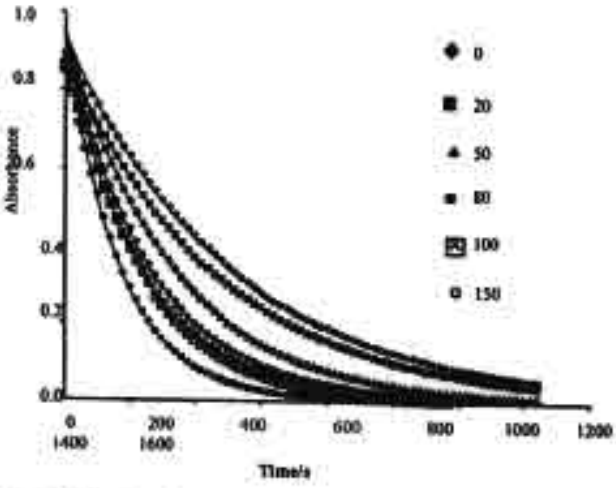

Fig. 2 Kinetics obtained from the stopped-FI system, showing reduction of Ce(IV) at different concentrations of iodine in sample plug from 0 to 150 µg l^{-1} (top to bottom). Full lines represent the exponential fittings.

Kinetic analysis was carried out using commercial software, ENZFITTER. The results in Fig. 2 show that each set of data agrees well with the theoretical exponential fit (with standard error < 10%). This indicates that in the monitoring plug, the process of decreasing in Ce(IV) concentration is a pseudo first-order type for the range of iodine concentration used. The plot between rate constant (RC), given by the software, and iodine concentration was linear ($RC = 2.1 \times 10^{-3}$ [I] + 1.43, $r^2 = 0.998$). This plot was used as the calibration for measuring iodine content in urine samples.

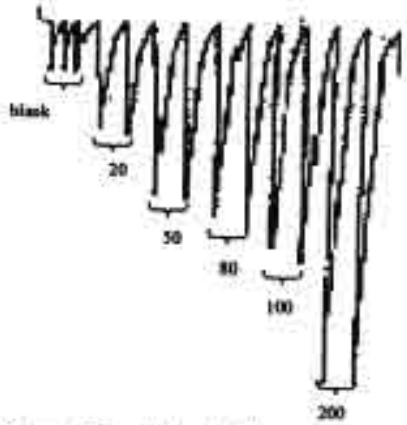

Fig. 3 Stopped-FI profiles of iodine standards (μg I l^{-1}) for the fixed-time kinetic method.

Fixed-time method

Results in Fig. 2 have demonstrated that the kinetics of disappearance of Ce(IV) are of first-order type over the concentration range of iodine catalyst of 0 to150 μg I l^{-1}. Thus, we should be able to use the relationship in equation (4) for the fixed-time method under the same condition in the stopped-FI manifold.

Figure 3 represents some of the signal profiles obtained from the stopped-FI manifold for iodine standards ranging from 0 to 200 μg l^{-1}. Linear calibration plot, made between the logarithm of Ce(IV) absorbance and iodine concentration, was satisfactorily achieved ($\ln A_t = 1.7 \times 10^{-3}$[I] + 2.41×10^{-1}, $r^2 = 0.994$). This calibration was used in determination of iodine in urine samples.

Method validation

Firstly, application of both the kinetic methods were tested using the stopped-FI. Analysis of twelve urine samples was carried out. According to linear regression test, there was no significant difference between the results given by the two methods at 95% confidence (slope = 0.880 ± 0.26 and intercept = 4.92 ± 23.3).

Although the rate constant method can be used in this application however the experiment takes a much longer time than the fixed-time method for a sample. The time taken to achieve a complete kinetics can be up to 25 min for the rate constant method whereas only 4 min is required to get a complete signal profile, in the fixed-time method. Hence, the latter method is more practical for real use and therefore selected for further investigation.

The stopped-FI manifold was used with fixed-time method to analyse another set of eleven samples. The results were compared with an inductively-coupled plasma mass spectrometric method[22] (ICP-MS). A regression line of y = 0.754±0.25x + 0.533±15.9 (x is ICP-MS and y is the stopped-FI) was obtained. This indicates that results of the two methods are not significantly different at 95% confidence. However it is observed that values of iodine contents given by the kinetic method is often lower than the ICP-MS method. This could possibly due to loss of iodine during acid digestion for the kinetic method whereas this is not likely to occur for the ICP-MS method since only dilution of urine was required.

General feature of the stopped-FI with fixed-time method

Some characteristic of the fixed-time method with the flow injection approach is summarized in Table 1.

Table 1 Feature of the stopped-FI with fixed-time method

FI parameter	Value
Detection limit (3σ)	3 μg l^{-1}
Sample throughput	35 samples h^{-1}
Reproducibility (100 μg l^{-1})	0.25%

Conclusion

This work demonstrated that kinetic methods, both the rate constant and the fixed-time methods, could be operated using flow injection technique. The calibrations are satisfactorily linear according to the experimental condition giving first-order kinetics. However for real application, the fixed-time method is more preferable. The procedure is suitable for large number of samples and can be alternative to the conventional batch method. The stopped-FI method is readily applicable for automation.

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A simple flow injection system with bead injection for trace iron determination

K. Jitmanee [a], S. Kradtap Hartwell [a], J. Jakmunee [a], S. Jayasvasti [b], J. Ruzicka [c], K. Grudpan [a,*]

[a] *Department of Chemistry, Faculty of Science and Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*

[b] *Department of Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

[c] *Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA*

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Abstract

A simple and low cost flow injection (FI) system with bead injection (BI) was developed for determination of low concentration (μmol l^{-1}) of iron in water samples. Chelex-100 chelating resin beads, trapped in a jet ring cell, were employed. The intensity of red complex of 1,10-phenanthroline with Fe^{2+} was monitored using colorimetric detector with a LED green light source. Amount of total Fe (Fe^{2+} and Fe^{3+}) and Fe^{2+} can be evaluated by with and without reduction of Fe^{3+} using ascorbic acid. Lowest detectable levels of Fe^{2+} were 0.90 and 0.45 μmol l^{-1} for sample loading time of 3 and 5 min, respectively. Working range was up to 3.90 μmol l^{-1} using 0.3% w/v 1, 10-phenanthroline. Percent recoveries of spiked water samples (0.90–2.33 μmol l^{-1} of Fe^{2+}) were 100–110%.

Keywords: Flow injection; Bead injection; Trace iron determination; 1,10-Phenanthroline

1. Introduction

The concept of bead injection (BI), where functionalized beads are used as a solid surface to accommodate chemical reaction and to accumulate and retain the analyte, was first introduced to use with sequential injection technique [1–5]. Use of bead as a replaceable stationary phase helps to improve sensitivity, minimizes dilution and reduces cross contamination between analysis runs [5,6]. Sequential injection analysis (SIA) offers many advantages such as precise automatic control of reaction conditions and microfluidic manipulation of samples and reagents [1–5,7]. It also operates at microlitre scale, saving reagents while generating very small volumes of waste. On the other hand, while classical FIA uses much larger volumes of solutions, it can be fabricated from less expensive components and operated manually as demonstrated in this work. Flow injection (FI)

* Corresponding author. Tel.: +66-53-943-345x157; fax: +66-53-222-268.

E-mail address: kate@chiangmai.ac.th (K. Grudpan).

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is a well established technique that allows solutions flow for online reaction and detection [8–13]. Many tedious steps in manual batch wise operation such as mixing reagents in beakers can be eliminated. Even though FIA is not as sophisticated as SIA but its simplicity and low cost become the main benefits [14–18].

Since low-cost instrumentation is attractive to many laboratories, we have decided to develop a novel combination of FIA with BI technique. Such a home made FI–BI system has the advantage of using beads as replaceable solid phase. Iron and Chelex-100 are chosen as a model analyte and sorption beads, respectively, to demonstrate the system. Sorption of iron ions on Chelex-100 chelating resin beads reaches a maximum above pH 4, but the elution of sorbed iron is difficult. However, the transparent of Chelex-100 resin allows the transmittance detection on beads bed to be possible. The chemistry used in the present work is a simple detection of color intensity of the red complex $[(C_{12}H_8N_2)_3Fe]^{2+}$ at 524 nm [19,20]. The complex is developed when 1,10-phenanthroline reacts with Fe^{2+} that has been trapped on the resin beads. Attempts have been made to develop a procedure to determine low concentration of iron with a simple FI–BI system.

2. Experimental

2.1. Apparatus

The diagram of the system used in this work is represented in Fig. 1(a). Two peristaltic pumps (Ismatec) were employed, one for beads loading (16 ml min^{-1}) and another for buffer and sample pumping (3 ml min^{-1}). Tygon pump tubings and three way valves (Upchurch) were used for setting up the manifold. To save color reagent solution, 1,10-phenanthroline was introduced using a six port injection valve (FIAlab) instead of pumping it with buffer solution

The reaction cell (jet ring cell) was laboratory made and the detail of the structure is as shown in the diagram Fig. 1(a). One side of the cell was connected to LED green light source of 524 nm with a 47 nm spectrum half width. While another side having a photo transister (PT). This detector unit (Fig. 1(b)) gives a peak that reaches the highest point when there is the highest light absorption. Therefore, after the signal reaches the steady state for a few seconds, it is automatically adjusted back to the base line, and a peak is resulted. The detector was connected to a chart recorder (Perkin–Elmer model 056-1002) that was set for a 10 V fsd.

2.2. Reagents

Beads of chelating resin (iminodiacetic acid, Chelex-100) was from Sigma, Germany (Bio-Rad Laboratories). Their sizes were varied in the range of 50–100 dry mesh but only-35 wet mesh beads (wet bead size smaller than 35 mesh) were collected by sieving and used.

All other following reagents were purchased from Merck, Germany. Acetate buffer solution (0.1 M pH 4.5) was prepared from sodium acetate trihydrate and glacial acetic acid in de-ionized water. Standard Fe^{2+} solutions (0.45–9.00 μmol l^{-1}) were prepared from ammonium ferrous sulfate $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$. Standard Fe^{3+} solutions (0.45–3.60 μmol l^{-1}) were prepared from ferric nitrate. Color reagent used for Fe^{2+} was 1,10-phenanthroline-monohydrate prepared to a desired concentration in the acetate buffer solution. Ascorbic acid solution was a reducing agent to convert Fe^{3+} to Fe^{2+}.

2.3. Operation steps

Buffer solution was pumped through three way connector T and valves V2 and V4 into the jet ring cell at all time. Iron solution was also pumped all the time because it was connected to the same pump, see Fig. 1(a). However, the valve V1 and the tubing were connected in the way that a standard; sample solution containing Fe^{2+}, was sent back to the sample container when it was not a standard/sample loading step. This way, a standard/sample solution and number of pumps used can be reduced.

First, beads were loaded into the cell by pumping of bead suspension through valves V3 and V4

for 20 s. Then, the pump for bead suspension was stopped and V3 and V4 were switched off. Second, a standard/sample solution was loaded via valves V1 and V2 to the cell for the desired period of time and then the valves were switched off. Only the buffer solution was run for 1 min to wash all the iron in the flow line to the cell. Finally, 0.3% w/v 1,10-phenanthroline solution was introduced using a six port valve (V2) with 210 μl injection loop in order to consume only necessary amount of reagent. This color reagent reacts with Fe^{2+} captured on the beads and shows red color that can be monitored using a green light LED colorimeter. After each analysis, used beads can be discarded by flushing them out of the cell with the buffer solution.

3. Results and discussion

3.1. Characteristics of signal

As previously mentioned, the detection unit was built to automatically adjust the signal back to the base line after it reaches the maximum steady state for a few seconds. Therefore, there were two peaks obtained from each analysis run, as in Fig. 2. The first peak corresponded to bead loading.

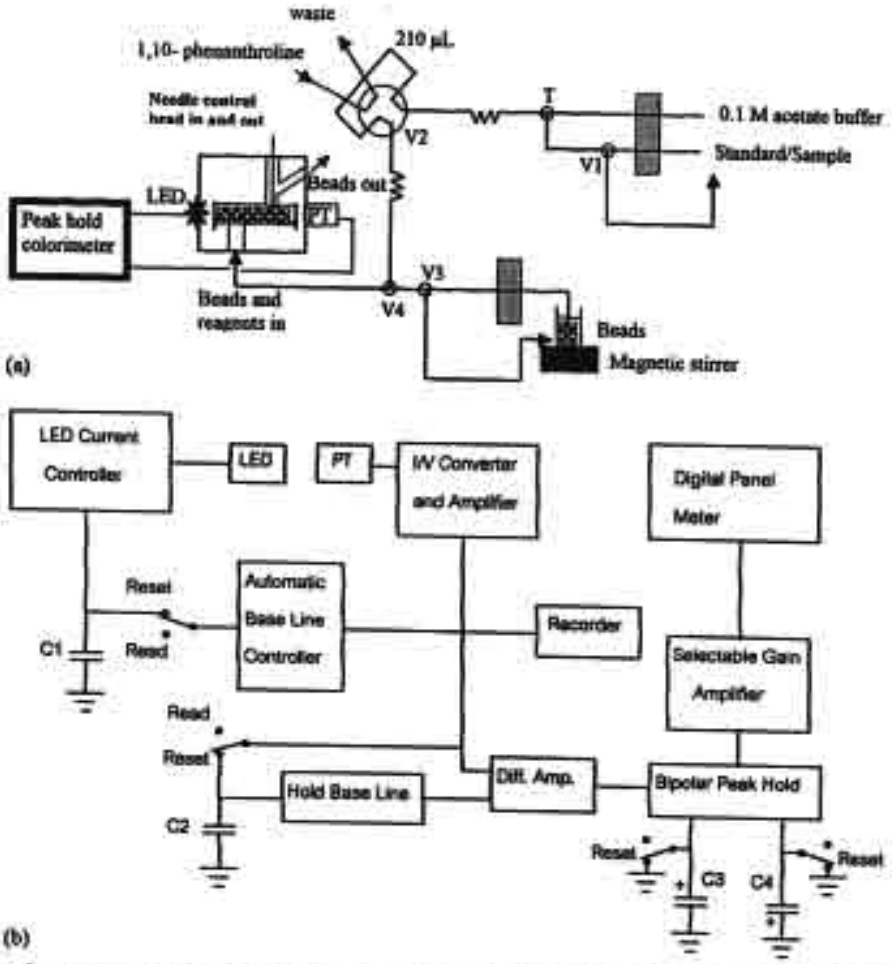

Fig. 1. (a) Flow injection set-up with bead injection for determination of iron (V1, V3 and V4 are three way valves; V2 is a six port injection valve and T is a three way connector). (b) Block diagram of the peak hold detector (LED, Light emitting diode; PT, Phototransistor).

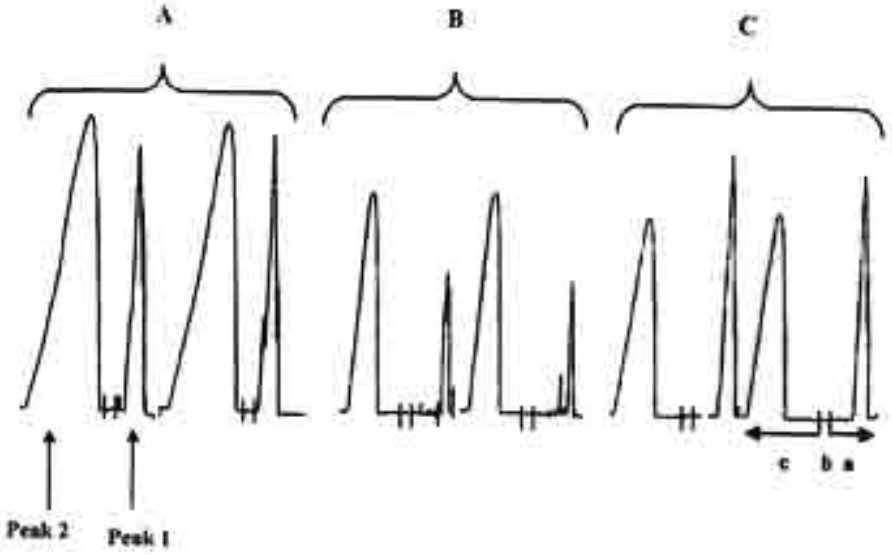

Fig. 2. Effect of bead loading time. Signals of sets A, B and C are of 9.00 μmol l^{-1} Fe^{2+} with 0.5% w/v 1,10-phenanthroline using a fixed sample loading time of 1 min and varied bead loading time of 20, 10 and 30 s, respectively. Signals from each analysis run consist of peak 1 and peak 2 that correspond to bead loading peak and analytical Fe peak, respectively, (see text). a, bead loading starts until the peak goes back to the base line; b, chart recorder is stopped; c, chart recorder starts before analytical peak appears until the peak goes back to the base line.

When beads entered the cell, they blocked the light source. As the signal reached the maximum (the cell was filled with beads), the signal went back down to the base line. The second peak was formed because of the maximum color intensity of the iron complex obtained when the color reagent solution reacted with iron trapped on the beads. This peak height directly relates to iron (Fe^{2+}) concentration on the beads and in the solution. At very high concentration of Fe^{2+}, the peak decay rate to the base line is slow. Thus, we observed the wide base peak at high concentration of iron.

3.2. Packing of beads in cell

It is important to control the homogeneity of resin bead suspension during different analysis run. Here bead suspension was stirred using magnetic stir bar at the fix rate so that at certain pump flow rate and fixed loading time, approximate same numbers of beads were introduced to the cell. Flow rate for bead loading step needs to be quite high (16 ml min^{-1}) to be able to transport these beads at the constant rate. This flow rate could be varied depending on size of beads used.

The uniformity of beads is also another important parameter. If they are too much different in size, beads may be packed in the cell differently each time. Amount of iron trapped may be different and would result in low precision. In addition, suitable and constant bead loading time is essential for good precision and for obtaining optimum analytical signals. The results shown in Fig. 2 were obtained from the fix loading time of 1 min of 9.00 μmol l^{-1}standard solution while the bead loading times were varied at 10, 20 and 30 s. This home made jet ring cell has approximated volume of about 50 μl. It was experimentally found that at 16 ml min^{-1} flow rate, the suitable bead loading time was 20 s. At shorter bead loading time (10 s) analytical signals (the second peaks) decreased due to less number of beads to trap iron. At longer bead loading time (30 s), analytical signals also decreased. There were excess beads outside the cell that could trap iron but

they were not detected because they were out of detection window. The reproducibility of the bead loading peak height was 4% R.S.D. from ten replicates.

3.3. Optimization of color reagent concentration

The experimental results (varying 1,10-phenanthroline concentrations of 0.05, 0.1, 0.3 and 0.6% w/v) showed that slightly excess concentration of 0.3% w/v of 1,10-phenanthroline offers optimum results for the determination of 9.00 μmol l^{-1} Fe^{2+} standard solution. At a higher concentration of 1,10-phenanthroline (up to at least 0.6% w/v), signals were not increased. At lower concentrations (0.1 and 0.05% w/v), signals were low because of insufficient amount of color reagent presented. We are interested in natural presented level iron (μmol l^{-1} or lower). Therefore, 0.3% w/v 1,10-phenanthroline was chosen for further experiments to save the reagent.

3.4. Calibration graph and effect of sample loading time

Sensitivity of the determination can be improved by using longer sample loading time. However, it is limited by the amounts of beads that cell can accommodate. Calibrations were obtained: $Y_1 = 0.0069X_1 - 0.542$, $(R^2 = 0.9999)$; $Y_2 = 0.0132X_2 - 0.3638$, $(R^2 = 0.9974)$; $Y_3 = 0.0144X_3 - 0.0831$, $(R^2 = 0.9452)$, where X and Y being μmol l^{-1} Fe^{2+} and peak height (V), for sample loading time of 1, 3 and 5 min, respectively. They show the significant changes in slopes between 1 and 3 min for sample loading time but only slight improve for 5 min loading time comparing with the 3 min one. The system allows 0.90 and 0.45 μmol l^{-1} Fe^{2+} to be determined for sample loading time periods of 3 and 5 min, respectively. Working range was about two order of magnitudes (up to 3.90 μmol l^{-1}).

3.5. Total iron determination

To determine Fe^{3+} and total iron using 1,10-phenanthroline reagent with the detection at 524 nm, Fe^{3+} needs to be prior reduced to Fe^{2+}. Although reducing agents, hydroxylammonium chloride, quinol or ascorbic acid can be used [13], ascorbic acid was chosen in this work because of its readily availability and its less toxicity. To test if ascorbic acid can be used effectively, peak heights obtained from analysis of 3.60 μmol l^{-1} Fe^{3+} with an addition of 5% w/v ascorbic acid were compared with those of Fe^{2+} of the same concentration. A mixture of Fe^{2+} and Fe^{3+} (1.80 μmol l^{-1} each) in the present of 5% w/v ascorbic acid was also analyzed and compared. It was confirmed that Fe^{3+} and total Fe could be determined utilizing ascorbic acid as a reducing agent. Optimum ascorbic acid concentration, from the concentrations investigated (0.5–5% w/v) for the particular concentration range of iron used in this work, was found to be 1% w/v. Too low ascorbic acid concentrations yielded low signals due to inefficient reduction of Fe^{3+} to Fe^{2+}.

3.6. Determination of iron in water samples

Table 1 summarizes the results of total iron determination and percent recoveries of spiked

Table 1
Percent recoveries of spiked iron in tap, pond and drinking water samples

Sample	[Fe] originally found (μmol l^{-1})	Spiked iron (μmol l^{-1})	Total [Fe] found (μmol l^{-1})	% Recovery of total Fe
Tap water	Fe^{3+} 0.82 ± 0.00	Fe^{2+} 1.80	2.78 ± 0.04	109
Tap water	Fe^{3+} 0.82 ± 0.00	Fe^{2+} 2.33	3.38 ± 0.04	110
Pond water	Fe^{3+} 0.64 ± 0.00 and total Fe 0.70 ± 0.00	Fe^{2+} 0.90	1.62 ± 0.19	100
Drinking water	Total Fe 0	Fe^{3+} 0.90 and Fe^{3+} 0.90	1.90 ± 0.10	106

iron in tap water, pond water and drinking water samples. The tap water sample did not contain Fe^{2+} in the level that can be detected. Iron in the form of Fe^{3+} is predominant. There are both Fe^{2+} and Fe^{3+} in the pond water sample. Fe^{3+} was estimated by subtraction of originally presented Fe^{2+} from total Fe. The drinking water sample did not show either Fe^{2+} or Fe^{3+}. Accuracy of the method was studied by spiking the samples, percent recoveries were found to be 100–110%. The positive recovery may be due to the interference from other elements in the samples.

4. Conclusion

A simple FI–BI system is proposed. A determination of trace iron in water samples using 1,10-phenanthroline with Chelex-100 resins was demonstrated. This inexpensive and manually easy to operate system offers online preconcentration for determination of low concentration ion. It improves analysis time because replaceable beads make elution steps unnecessary as compared with other preconcentration columns. This also reduces the possibility of cross contamination between runs. However, its drawbacks are that FI–BI uses large volumes of reagents and requires operators skill to carry out the assays in reproducible manner. It is hoped that this work on a model system will encourage others to exploit advantages of BI technique in combination with FI. Further investigation on online speciation of Fe^{2+} and Fe^{3+} or of other ions is in progress.

Acknowledgements

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Gravitational field-flow fractionation in combination with flow injection analysis or electrothermal AAS for size based iron speciation of particles

R. Chantiwas [a], R. Beckett [b], J. Jakmunee [a], I.D. McKelvie [b], K. Grudpan [a,*]

[a] *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[b] *Water Studies Centre, Department of Chemistry, Monash University, Clayton, Vic. 3800, Australia*

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Abstract

A simple gravitational field-flow fractionation (GrFFF) system was used for size separation of micron sized silica particles coated with hydrous iron oxide (geothite). The amount of iron on the particles was monitored either on-line by reverse-flow injection analysis (r-FIA) with chemiluminescence detection using luminol or off-line by electrothermal atomic absorption spectrophotometry (ETAAS). The combination of GrFFF with reverse FIA or with ETAAS has been demonstrated to be a cost-effective tool for size based iron speciation of particles.

Keywords: Gravitational field-flow fractionation; Flow injection analysis; Field-flow fractionation; Chemiluminescence; Iron speciation; Size based speciation

1. Introduction

There is currently much activity on the development of analytical methods for elemental speciation [1]. This is being applied in diverse fields such as medicine, agriculture, industry and the environment. While most work concentrates on determining the chemical forms of elements in samples there is also considerable interest in size based speciation. This involves determining the distribution of elements across the size range of the sample. In the field of environmental science this is important as particles size greatly influences the transport and fate of the elements associated with particles in air, soil and aquatic environments.

Size based speciation can be performed by combining separation and analytical methods. Recent examples include the off-line determination of metals (Cu, Fe, Mn) in beer by electrothermal atomic absorption spectrophotometry (ETAAS) after separation of metal complexes into different molecular weight fractions by size-exclusion chromatography (SEC) [2]. Inductively coupled plasma-mass spectrophotometry (ICP–MS) was employed for selenium speciation after capillary

* Corresponding author. Tel.: +66-53-943341; fax: +66-53-222268
E-mail address: kate@chiangmai.ac.th (K. Grudpan).

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zone electrophoresis (SEC–CZE) of selenized yeast [3]. Sedimentation field-flow fractionation has been used on-line with ICP–MS for characterization of environmental colloids [4,5]. Flow FFF–ICP–MS was also used for determination of the size distribution and trace metal speciation of aquatic colloidal material [6,7]. Determination of size based elemental distributions of proteins [8] and trace metal analysis of humic acid from different origins in municipal waste were also performed by flow FFF–ICP–MS [9].

Natural aquatic and soil particles are thought to possess a coating of hydrous iron oxide and natural organic matter [10]. These coatings influence the uptake of contaminants such as trace metals, nutrients and biocides [11–13]. Thus the size distribution of readily released iron is of some importance in assessing the potential fate of some pollutants.

Field-flow fractionation (FFF) is a technique for size separation and characterization of particles and macromolecules [14]. FFF instrumentation is similar to that used in liquid chromatography. FFF takes place in a thin rectangular open channel and the separation is carried out by applying an external driving force perpendicular to the laminar flow of the carrier liquid [14]. Various driving forces can be employed in FFF (e.g. centrifugal, flow, thermal gradients, electrical and gravitational). This work used gravitational FFF (GrFFF) due to the simple experimental set up and low cost [15]. GrFFF can be set up by replacing the column in a standard HPLC system with the GrFFF channel. GrFFF is capable of separating particles in the diameter range of 2–50 μm and has been employed for biological samples such as red blood cells, parasites, wine yeast, and wheat starch [16–19]. The application of GrFFF for industrial particles has involved coal, silica and clay minerals [20–24].

GrFFF channel and separation mechanisms are illustrated in Fig. 1 [25]. A liquid carrier is pumped through the channel and a suspension of sample is injected (Fig. 1a). For micron size particles, larger particles elute earlier than the smaller ones and the elution mechanism involved is often called the 'steric elution mode'. In GrFFF, the hydrodynamic lift force can significantly affect the motion of particles [26]. Therefore, the GrFFF separation mechanism can also be referred to as the 'steric/hyperlayer elution mode'.

Combination of FFF with various analytical techniques has gained considerable interest recently, [14]. This has led to size based element distributions [5] and adsorption distributions [27,28]. In this paper we report work on the hyphenated method involving gravitational steric/hyperlayer FFF and flow injection (FI) with chemiluminescence (CL) detection (GrFFF–FIA–CL), which was first reported by Chantiwas et al. [29]. A schematic diagram of the GrFFF–FIA–CL instrument is given in Fig. 2a. The CL detection method used is based on the catalytic reaction of alkali luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and hydrogen peroxide. Trace amounts of iron catalyse the luminol oxidation, which emits light. Determination of iron based on this reaction has been reported previously, employing either normal or reverse FI [30–38]. In addition electrothermal AAS (ETAAS) analysis for iron was performed on size fractions collected from GrFFF as represented in Fig. 2b.

The objective of this paper is to demonstrate the effectiveness of these two new and relatively simple hyphenated analytical techniques (GrFFF–FIA–CL and GrFFF–ETAAS) for generating size based speciation data of iron. ETAAS was used for determination of the 'total' iron content of each fraction collected and the chemiluminescence reverse FI method was used to detect 'easily released' iron on the particles. The size separation and iron speciation was tested using model samples consisting of 5 and 10 μm HPLC silica particles coated with a thin layer of goethite (FeOOH).

2. Experimental

2.1. Gravitational field-flow fractionation

The components of the GrFFF instrument are represented in Fig. 2. The GrFFF system was low cost and easy to assemble. An overhead transparency sheet was cut to form the spacer. The channel dimensions were $0.095 \times 300 \times 20$ mm^3 for the

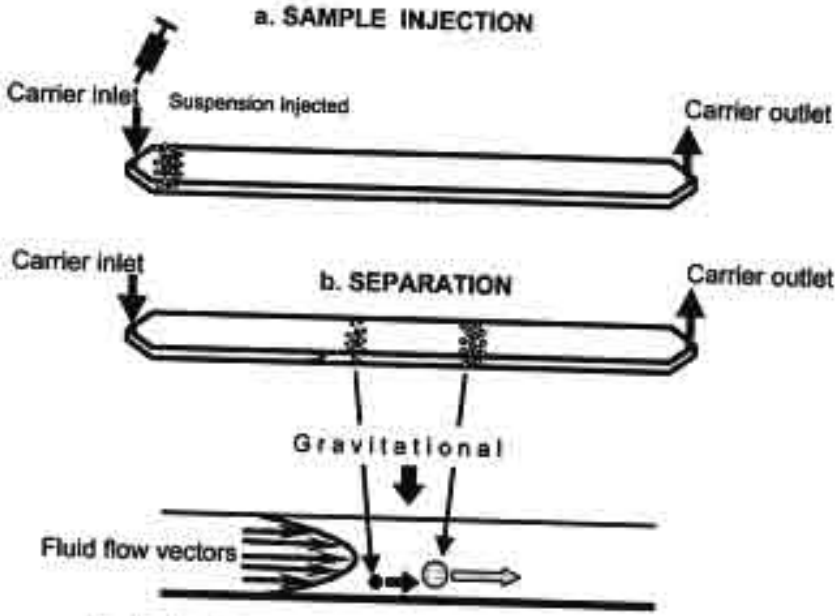

Fig. 1. GrFFF channel and steric hyperlayer separation mechanism.

channel thickness, length and breadth, respectively. The spacer was clamped between two of pieces of lucite plastic. An isocratic pump (SpectraSERIES, USA) was used to deliver the carrier solution. Particle suspensions were injected into the carrier stream with a hypodermic syringe (40 μl) through a home made injection port [39]. The concentration of particles in the FFF eluent was monitored by a UV detector (Linear Instruments Model 200 detector, USA) at an operating wavelength of 254 nm.

2.2. The electrothermal atomic absorption spectrophotometry

The ETAAS instrument was a Perkin–Elmer Model 5100 (Norwalk, CT) equipped with Zeeman correction, an HGA-600 graphite furnace and AS-60 autosampler. A pyrolytically coated graphite tube with L'vov platform was used. The ETAAS technique was used for trace iron determination of the eluted particles from the GrFFF system that were collected by a fraction collector (ISCO, Inc. Model RETRIEVER 500, Lincoln). Particle fractions were directly introduced as a slurry off-line into the ETAAS system for iron determination. ETAAS analysis was performed using the temperature program in Table 1 and with a wavelength of 248.3 nm, operating lamp current of 30 mA and sample volume of 10 μl was used. All fractions were homogenised by a vortex mixer before introducing into the atomiser. The modifier used for Fe determination was 1% of HNO_3 (5 μl). The calibration graph obtained using Fe standard solutions (10, 20, 40, 60 $\mu g\,l^{-1}$) was $Y = 0.00806X$, $r^2 = 0.997$.

2.3. Reverse-flow injection analysis with chemiluminescence detection (r-FIA–CL)

A luminol solution (8×10^{-6} M, 80 μl) was injected via a six-port injection valve (Upchurch, USA) into a stream hydrogen peroxide of (1×10^{-3} M) which was delivered by a peristaltic pump (Eyela SPM 23, Japan). After merging with an iron solution (standard/sample), the merged solutions were passed through a 30 cm mixing coil before finally entering the CL detector. The chemiluminescence intensity was monitored by a modified commercial liquid scintillation

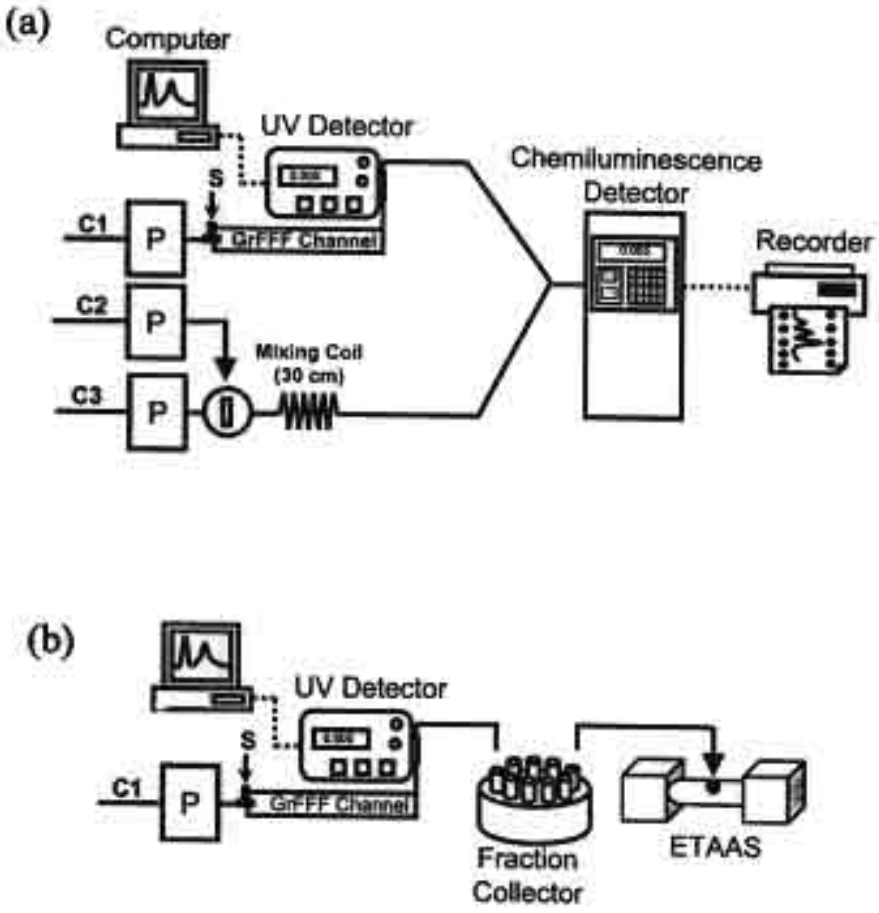

Fig. 2. Schematic diagram of the GrFFF coupled with (a) rFIA with chemiluminescence detection for iron determination. The reagents used were C1: 10^{-4} M NaOH (Flow rate 1.00 ml min^{-1}), C2: 8×10^{-6} M Luminol (Flow rate 1.0 ml min^{-1}), C3: 10^{-3} M H_2O_2 (Flow rate 3.0 ml min^{-1}) and (b) ETAAS.

counter, with a spiral flow cell (Packard Radiometric Flo-one®\Beta Series A-100 Model A140K, Canberra). The flow cell, Quick Change Flow Cell (serial number 7144) had a spiral shape and was

Table 1
Graphite furnace temperature program used for Fe analysis

Step	Furnace temperature (°C)	Time (s)		Internal Ar gas flow (ml min^{-1})
		Ramp	Hold	
1	110	1	15	300
2	130	7	5	300
3	20	7	10	300
4	1000	1	10	300
5	2400	2	15	0
6	2600	1	5	300

sandwiched between the two photomultiplier tubes. The volume of the flow cell was 200 μl. The calibration line for iron standards (10–80 $\mu g\,l^{-1}$) was $Y = 29.68X + 1373$, $r^2 = 0.996$.

2.4. Chemicals

All chemicals were analytical grade and were used without further purification. Water was obtained from a Milli-Q system (Milford, Massachusetts, MA). A 0.01 M solution of luminol was prepared by dissolving 5-Amino-2,3-dihydro-1,4-phthalazinedione (Sigma-Aldrich, Germany) (0.1772 g) in 100 ml of phosphate buffer. The buffer (pH 11) was prepared by dissolving Na_2HPO_4 (E. Merck, Germany) (0.7477 g) in 1000 ml of water and the pH was adjusted to be 11 with 2 M NaOH. Luminol solutions of appropriate concentrations were further diluted with the phosphate buffer. Hydrogen peroxide solution (0.001 M) was obtained by diluting H_2O_2 (30% volume) (Carlo Erba, Italy). An AAS iron (III) standard solution (1000 $mg\,l^{-1}$) (Merck, Germany) was diluted to appropriate concentrations. All reagents were freshly prepared and degassed for 12–15 min before use by using a sonication bath.

2.5. Silica particles

Chromatographic silica (5 and 10 μm) was obtained from used HPLC columns (CN packing from PARTISPHERE RTF Columns, WHATMAN, UK). The 5 μm particles were spherical and the 10 μm particles had an irregular shape.

2.6. Preparation of the goethite coated silica particles

The goethite coated silica was synthesized by a method developed based on the results reported by Stumm and O'Melia. It was used previously for the production of goethite coated kaolinite [40,41]. A solution of iron, (3.5 mM $Fe(NO_3)_3$) was gradually added to 10 ml of a suspension of silica particles (2 $mg\,ml^{-1}$). The pH was continually adjusted to 3.3 with 2 M HCl. The Fe solution addition rate was 1 $ml\,h^{-1}$ for the first 2 ml and 6 $ml\,h^{-1}$ for the remaining 12 ml. The mixture was continuously stirred. The suspension was then centrifuged and the silica residue was washed 4 times with Milli-Q water. The final suspension was dispersed in 10^{-4} M NaOH solution and heated in an oven at 60 °C for 24 h.

3. Results and discussion

3.1. Fe content of the Fe coated silica particles

The amount of iron on the coating silica was measured by ETAAS after being digested with *aqua regia* (HCl:HNO_3, v/v, 3:1). The concentration of iron was found to be 12.1 ± 0.2 $mg\,g^{-1}$ and 13.7 ± 0.7 $mg\,g^{-1}$ for the 5 and 10 μm particles, respectively. This indicates that the iron content of the two samples should be very similar.

Analysis of the Fe in the coated silica particles after acid dissolution (HCl:HNO_3, v/v, 3:1) was compared with slurry sampling (suspension of particles in Milli-Q water with the same dilution). It was found that the Fe content obtained from the slurry sampling method was lower than that measured after acid digestion. The recoveries were found to be $64 \pm 6\%$ and $59 \pm 10\%$ for the 5 and 10 μm particles, respectively.

Chen and Beckett reported almost complete recovery of Fe in a colloid sized (<1 μm) soil sample analysed by sedimentation FFF–ETAAS [41]. This suggests that the release of Fe coatings from these larger 5 and 10 μm sized particles is less efficient.

3.2. Thickness of the FeOOH layer on the silica particles

The thickness of the FeOOH coating on the particles (t) can be estimated if we assume the particle shape is spherical and coating layer is uniform. It is given by

$$t = \frac{\rho_{SiO_2} m_{FeOOH} d}{24\, \rho_{FeOOH} m_{SiO_2}} \quad (1)$$

where m_{SiO_2} is the known amount of sample mass injected, d is the diameter of the particles (5 μm),

ρ_{SiO_2} is the density of the silica (2.3 g ml^{-1}). We assume the iron coating on the particle is goethite (FeOOH) which has a density, ρ_{FeOOH}, of 3.8 g ml^{-1}. m_{FeOOH} is the mass of goethite on the coatings. The value of m_{FeOOH} was obtained from Fe based fractogram using the Fe content found in peak eluted between 3.75 and 7.75 min.

The calculated thickness of the goethite coating on the spherical 5 μm silica particles is found to be 5.1 nm, which is quite thin compared to the diameter of the silica particles of 5000 nm. The Fe content in the 10 μm sample peak was very similar, suggesting that the FeOOH coating thickness on the 10 μm particles could be approximately twice that of the 5 μm. However, the 10 μm particles are not spherical which could increase the specific surface area to some extent thus decreasing the actual coating thickness.

3.3. Separation of the Fe coated silica particles by GrFFF

Fig. 3 shows the GrFFF fractogram of a mixture of the 5 and 10 μm silica particles. The first peak is the void, which contains unretained particles. The 10 μm silica eluted before the 5 μm particles thus separation occurred by the steric/hyperlayer elution mechanism. Optical microscope pictures of the fractions collected at the maximum of the eluting peaks (Fig. 3) confirm the good separation obtained. The diameter scale was obtained using the empirical calibration expression:

$$\log t_r = -S_d \log d \quad (2)$$

and measuring the elution time (t_r) at the peak maximum of the 5 and 10μm particles. In Eq. (2) d is the diameter of the particles and S_d is the size based selectivity [42].

It was found by optical micrographs that there was a high proportion of small particles (< 1 μm) present in the 10 μm sample. These submicron particles would not be relaxed under the run condition performed here and would thus be eluted in the unretained void peak. This was confirmed by optical micrographs of the void peak fraction.

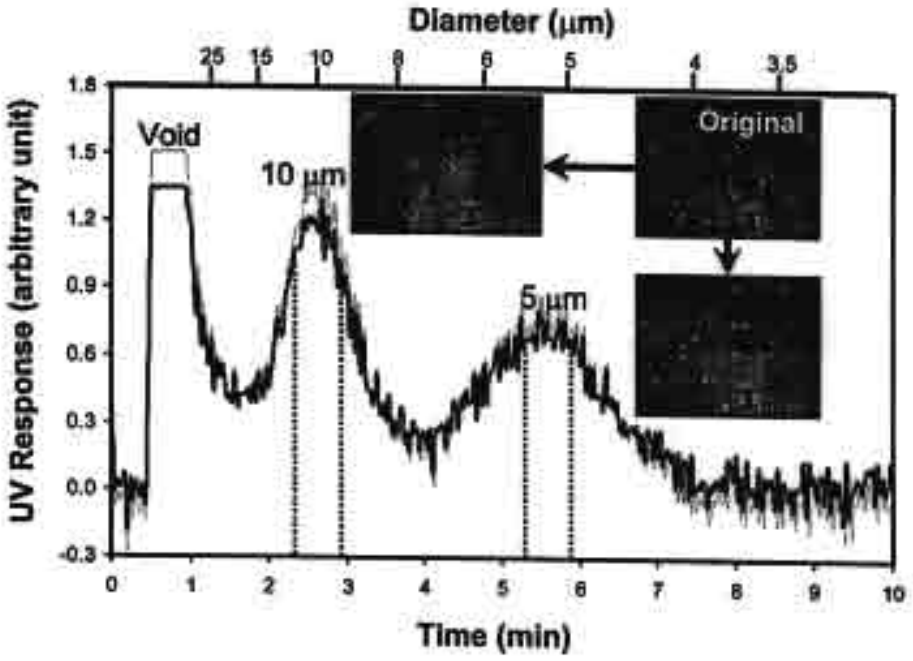

Fig. 3. The fractogram of a mixture of 5 and 10 μm goethite coated silica particles and the optical microscope pictures of the original mixture and fractions collected at the two peak maxima.

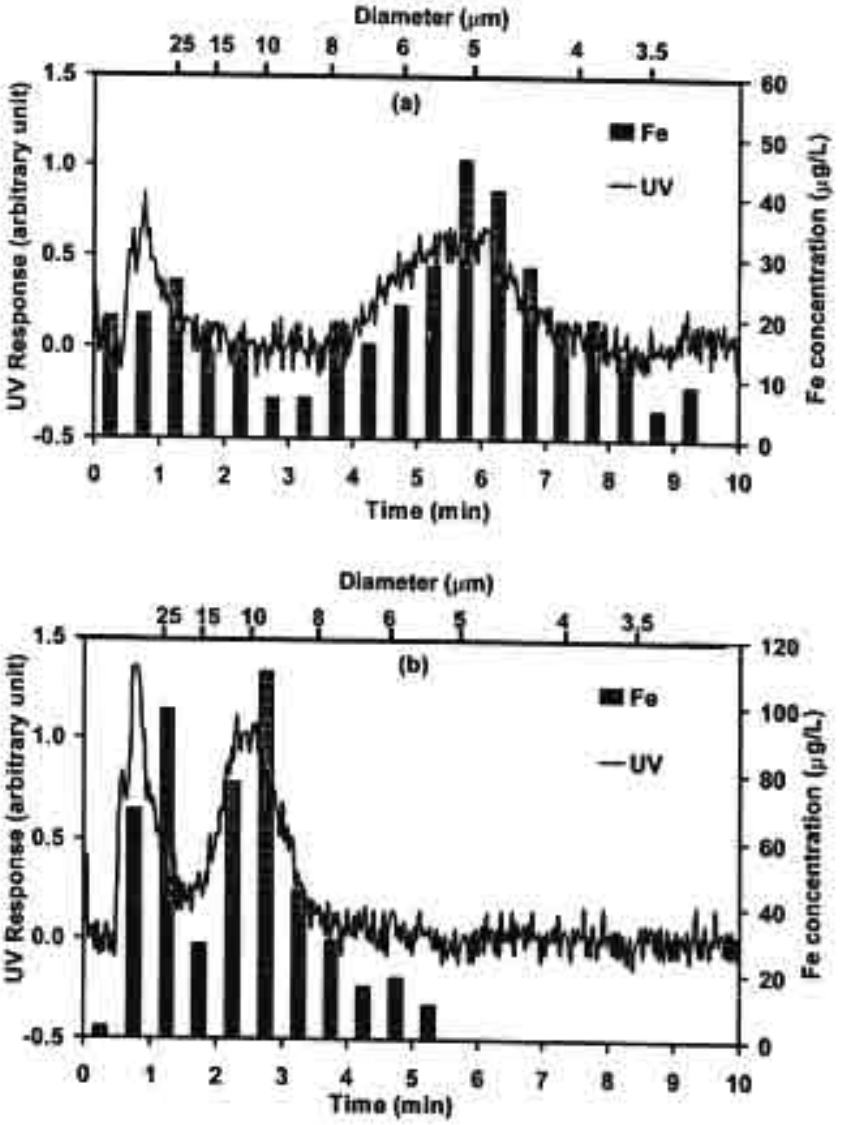

Fig. 4. UV detector fractograms and iron content of the fractions collected from GrFFF and determination by off-line ETAAS, (a) 10 μm, (b) 5 μm.

3.4. GrFFF–ETAAS of the Fe coated silica particles

Fig. 4 shows the fractograms of individual GrFFF runs of the 10 (Fig. 4a) and 5 μm (Fig. 4b) silica samples. The bar graphs represent the iron content in the collected fractions, which were determined off-line by ETAAS with slurry injection. It can be seen that the Fe concentration of each fraction follows quite closely the UV fractograms.

Computing the area under the Fe based fractograms ([Fe] vs elution time) including the void peak and comparing this with the Fe content of the whole sample digested with *aqua regia*, showed that 71 and 88% of the Fe on the particles was detected by slurry ETAAS, for the 5 and 10 μm samples, respectively. As noted above the recovery

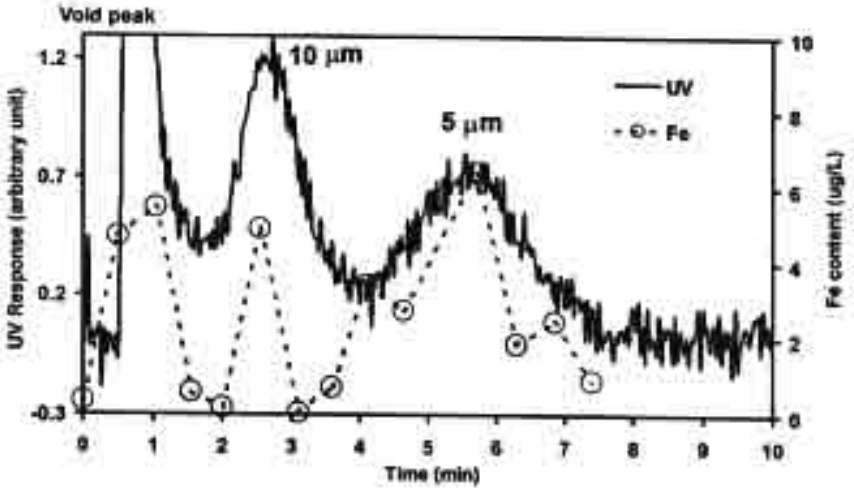

Fig. 5. UV (solid line) and Fe concentration (open circles with dashed line) fractogram of the mixture of 10 and 5 µm particles. The Fe concentration was determined on-line from the GrFFF combined with FIA–CL detection.

efficiency appears for these larger particles seems to be less than that found by Chen and Beckett for submicron particles (close to 100%) [41]. For the 10 µm particles a significant proportion of the Fe is associated with the fine particles found in the void peak which may be responsible for the higher proportion recorded by ETAAS (88%).

3.5. GrFFF–FIA–CL of the mixture of 5 and 10 µm of Fe coated silica particles

Fig. 5 shows the GrFFF fractogram for the mixture of 5 and 10 µm silica as well as the results of the online Fe analysis by r-FIA–CL. The eluant from the GrFFF was continuously flowed into the r-FIA–CL for Fe analysis. Each data point of the iron content corresponds to a signal obtained from an injection of the mixture of luminol and H_2O_2 into a flowing steam of the GrFFF eluent before entering into the CL detector.

The area of under the Fe profile ([Fe] vs elution time) was used to calculate the iron content of each individual peak. From the calculation, the Fe content in the 5 and 10 µm particles were found to be 0.57 and 0.14 mg g^{-1}, respectively. The iron content present in the 5 µm particles is higher than that in the 10 µm particles. This could suggest that the Fe content is related to the particle surface area. This is different from the trend in the Fe content obtained by GrFFF–ETAAS and after *aqua regia* digestion of the whole sample, where the Fe content in the two samples is approximately the same. It is also apparent that the r-FIA–CL method detects only a small proportion of the total Fe in the sample particles. In the case of the 10 µm particles, this is only about 1% of the total Fe obtained after *aqua regia* digestion.

4. Conclusion

In this work, we have attempted to apply simple GrFFF combined with FIA–CL or ETAAS as an approach to size based element speciation. We have tested this approach by employing two model silica particles which were coated with hydrous iron oxide (geothite). We have found that the simple GrFFF can be employed to separate micron sized particles, and when combined with another analytical device, such as FIA–CL or ETAAS, is a promising and cost-effective tool for size based element speciation of particles.

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Simple flow injection system for colorimetric determination of iodate in iodized salt☆

N. Choengchan [a], K. Uraisin [a], K. Choden [a], W. Veerasai [a,b], Kate Grudpan [c], D. Nacapricha [a,b,*]

[a] Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand
[b] The Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Nakorn Pathom 73170, Thailand
[c] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

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Abstract

This work presents a flow injection (FI) system that was developed for determination of iodate. The system utilizes the oxidation of iodide by the analyte to iodine, which subsequently forms tri-iodide. In the presence of starch, the blue I_3^-–starch complex is developed within the sample zone and can be colorimetrically detected at 590 nm. Optimization was carried out to make the system suitable for quantitating iodate added to table salts. To prevent accumulation of the blue complex residue on walls of tubing and the flow cell, a port was placed in the system for injection of 10^{-3} M thiosulfate plug (100 μl). An injection of this cleaning solution after each sample injection is recommended to avoid positive baseline shift. By means of the paired *t*-test, the amounts of iodine (mg I kg^{-1}) were statistically compared with the results determined by titration and by iodide ion selective electrode. No significant disagreement at 95% confidence was observed. The proposed system is very simple, uses common chemicals and provides rapid analysis (65 injections per h) with high precision (R.S.D. = 0.66%, $n = 10$). A detection limit of 2 mg I kg^{-1} salt can be achieved.

Keywords: Iodate; Iodized salt; Tri-iodide; Starch; Flow injection; ISE

1. Introduction

Addition of iodine to table salts is part of a preventive program for iodine deficiency disorder in many countries. Salts are commonly iodized with potassium iodide. However, for some tropical countries, including Thailand, salts are supplemented with potassium iodate for a longer shelf life. The recommended value for iodine supplement is 50 mg I kg^{-1}. Most, available methods for measuring iodine in iodized salt are for those supplemented with potassium iodide [1–4]. An ion chromatographic method is available for determination of iodate in iodized common salt [5], but sample throughput is considered low. Recently

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* Corresponding author. Tel.: +66-2-201-5127; fax: 66-2-245-8332
E-mail address: scdnc@mahidol.ac.th (D. Nacapricha).

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there was a report of an amperometric method for the iodate supplement [6], which provides a considerably high sample throughput at 35 injections per h. The method utilized the flow injection (FI) technique [7].

This work describes use of the well-known iodine chemistry, that is the Dushman reaction [8], for quantifying iodate content in iodized salt. Analysis is based on FI technique. Tri-iodide which is formed in the sample zone, after the reduction of iodate, intercalates inside starch molecules to produce a blue colored complex of tri-iodide starch (I_3^-–starch). Thus, instead of employing UV detection (I_3^-, 350 nm), the detection wavelength is shifted to the visible region (I_3^-–starch, 590 nm). This makes the system attractively economical. The method is new particularly for this application. Although some authors have used FI systems with detection of the starch complex, the systems were for other applications [9–11]. These works adapted detection of decolorization of the complex in a reagent stream, as determined by the concentration or activity of analytes. Examples of the works were the determination of amylase [9,10] and penicillin [11]. A group of authors [10] recommended pumping 0.2 M sodium hydroxide solution through the system to clean out the complex deposited on tubing wall. In this paper a more convenient way of cleaning is presented. The developed method was compared with the conventional titration method and with a potentiometric method.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade. Distilled water was used throughout. A stock of iodate standard was prepared by dissolving 2.1402 g of potassium iodate crystal (Univar, Australia) in water to 100.00 ml. Appropriate dilutions in 6% (w/v) sodium chloride were made in the preparation of working solutions.

The carrier stream was made by dissolving 2 g of potassium iodide(Univar, Australia) in a solution of 6% (w/v) sodium chloride and making up to 500 ml. The starch stream of 0.1% (w/v) starch solution containing 0.01 M sulfuric acid was daily prepared. Starch (0.5 g) (Merck, Germany) was mixed with a few milliliters of water to make slurry. The slurry was added to 500 ml of boiling water. After cooling, 5 ml of 1 M sulfuric acid (diluted from conc. H_2SO_4, Lab-scan, Ireland) was added to this solution.

The solution of 0.1 M thiosulfate was prepared by dissolving 2.5 g of solid sodium thiosulfate pentahydrate (Merck, Germany) in water and making up to 100 ml. This solution was diluted with water to obtain thiosulfate 1.0×10^{-3} M.

2.2. Sample

All samples were oven dried at 120 °C overnight and stored in desiccator until constant weight. A sample solution was prepared by dissolving an accurate weight of 30 g of salt in water and making up to 250.00 ml (note: filtration may be necessary for some sample solutions that contain particulate matter). By this preparation, a sample solution would contain approximately 12% (w/v) NaCl. The samples were analyzed directly using the titration method. For the developed FI and the potentiometric method, the liquid samples were diluted from 12 to 6% (w/v) salt before analysis.

2.3. Flow injection system

A FI manifold (Fig. 1) was set up and studied. An Ismatec peristaltic pump (model IS7610, Switzerland) was used for propelling reagents. Two Rheodyne injection valves (model 5020, USA) were used as V1 and V2 in the manifold. A Jenway spectrophotometer (model 6405, UK), fitted with a Philips flow cell of 10 μl volume, was used for monitoring the change of absorbance at 590 nm. PTFE tubing with i.d. of 0.5 mm was used throughout. An Alltech chart recorder (model LR 93025, USA) was used for recording signal.

2.4. Titration method

A 10.00 ml aliquot of 12% (w/v) salt solution was transferred into an iodine stoppered flask. To

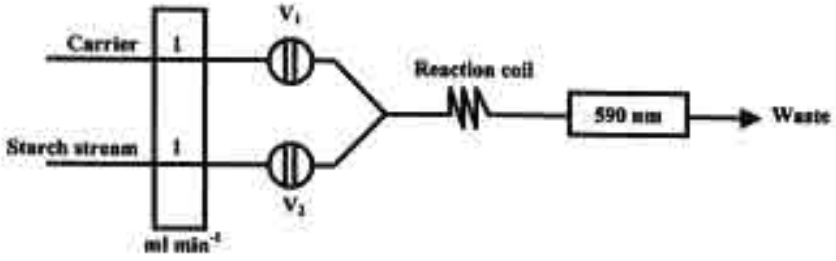

Fig. 1. The FI manifold for determination of iodate in iodized salt. Carrier: 2.5×10^{-2} M KI in 6% (w/v) NaCl, starch stream: 0.1% starch in 1.0×10^{-2} M H_2SO_4, V_1: 250 μl injection of sample solution or standard, V_2: 100 μl injection of 1.0×10^{-3} M $Na_2S_2O_3$.

the sample, 5 ml of 1 M H_2SO_4 and 10 ml of 10% (w/v) KI were added. The mixture was titrated against a standardized $Na_2S_2O_3$ solution (about 2.4×10^{-4} M), using 1% (w/v) starch as indicator.

2.5. Potentiometric method

Accurate 5.00 ml of a sample solution was directly transferred into a 50 ml volumetric flask which contained 12 ml of 2% (w/v) ascorbic acid. The volume was made up to the mark with water. After mixing, the solution was measured for the potential developed across the iodide-ISE and the saturated calomel electrode. Calibration was carried out with standard solution made from potassium iodate (1.0×10^{-6}–1.0×10^{-2} M) prepared to have the same liquid matrix as the sample (1.2% (w/v) NaCl and 0.5% (w/v) ascorbic acid).

3. Results and discussion

3.1. Manifold design

3.1.1. Background

Our present FI manifold (Fig. 1) was in fact modified form a previous manifold, which consisted of three channels. Recently, the three-channel manifold [14] was used in the validation of another FI system reported for determination of iodine in table salt using amperometric detection [6]. The system in Fig. 1 is made simpler than the previous system by combining together some of the reagents in the manifold and thus reduced to two-channel. Dispersion of an injected zone is less in the current manifold ($D = 2.6$) as compared with the former system ($D = 3.8$). The sensitivity (calibration slope) is increased by a factor of 1.5.

Levels of iodine found in iodized samples can be lower or higher than the recommended value. However, it is appropriate to prepare samples by dissolution to roughly 6% (w/v) salt before direct injection. Thus, the carrier stream of the system in Fig. 1 was designed to contain 6% (w/v) NaCl to prevent the Schileren effect [12]. Standard iodate solutions were also prepared in 6% (w/v) NaCl solution. The solid NaCl used in these preparations, can be of general grade or even sea salt (non-iodized).

3.1.2. Elimination of baseline shift

It was observed during analysis that there was accumulation of tri-iodide starch complex on tubing wall situated between the stream confluence and the flow cell. There may also be the deposition inside the flow cell. These resulted in positive shift of baseline. To diminish the problem, a valve (V2) was inserted in the system for injection of a plug of cleaning solution. A 100 μl injection of sodium thiosulfate, 1.0×10^{-3} M, was selected for washing off the starch complex. The complex is decolorized by thiosulfate to produce iodide, which does not form a colored complex with amylose molecule. An injection of the thiosulfate was carried out after every sample injection. Injection of sample was carried out approximately at 30 s intervals.

3.2. Optimization of the FI manifold

The objective of the experiment was to achieve a suitable signal reading (0.5–0.6 a.u.) for $2.5 \times$

10^{-5} M standard iodate solution. This concentration correspond to the recommended value at 50 mg I kg^{-1} of salt. Contents of iodine in real samples could be below or above this value.

Concentrations of reagents were adapted from the previous work of Jakmunee and Grudpan [6]. Three physical parameters, viz., injection volume at V1, flow rate and mixing coil length, were optimized one at a time. Repetitive injections of 2.5×10^{-5} M iodate solution were carried out in triplicate whereas concentrations of reagents were fixed at the values given in Fig. 1.

It was observed that increasing the volume to 250 μl raised up the signal to the desired absorbance value. This volume was chosen for optimization of the other parameters.

Flow rates were varied from 0.5 to 2.5 ml min^{-1}. The results showed that increasing the flow rate increased the sensitivity. This is probably due to the better mixing achieved at the confluence point in Fig. 1. In this work, the flow rate of 1 ml min^{-1} was selected for each flow channel in Fig. 1.

The system was most sensitive when no coil was used. The absorbance decreased from 0.615 to 0.485 a.u. when the coil length was increased from 0 to 1.5 m. In fact, any length could have been chosen but for this manifold a 120 cm coil was employed so that the absorbance for the highest concentration of iodate standard (4.0×10^{-5} M) was within the range of the detector.

3.3. Calibration

The calibration graph obtained for iodate concentration ranging from 5.0×10^{-6} to 4.0×10^{-5} M was linear: peak height (absorbance) = $25520[IO_3^-] - 0.052$, $r^2 = 0.999$ ($n = 4$). It was observed that the intercept was never at the origin. This may be due to the limitation of the reaction kinetics in the region of low concentrations or it could be the limitation of the dynamic range of detector.

3.4. Recovery

An experiment was carried out on four samples to evaluate the recovery of this method. Addition of potassium iodate standards (5.0×10^{-6}, 1.0×10^{-5} and 2.0×10^{-5} M) was made for a sample solution. The results given in Table 1 demonstrate that the method provides satisfactorily good recovery of the signal.

3.5. Effect of foreign ions

Normally our iodized salts are produced using sea salt. Five foreign ions that are present in seawater, viz. the remaining two halides and three major cations, were studied for possible affect on the analysis. As shown in Table 2, a standard solution, 2.0×10^{-5} M, was prepared containing, species of ions at concentration level normally found in a sample of sea salt (providing the salt was prepared from complete evaporation). The levels were in fact calculated based on the elemental abundance in seawater [13]. The results in Table 2 indicate that none of these ions gave rise to signal alteration of the pure standard outside ± 5%. Thus, it can be concluded that these foreign ions do not interfere with the proposed method.

3.6. Tolerance to pH of sample

Since iodized salts are produced using raw materials from various sources, a study was made to see if pH of sample solution has any effect on the FI signal. The experiment was carried out similarly to the optimization of parameters using injections of the same concentration of iodate standard. pH of the standard solution was adjusted (with HCl or NaOH) to 1.99, 5.15, 5.96, 7.05 and 9.09. The signal was constant for pH 5.15–9.09 (0.646–0.621 a.u.). The signal dropped to 0.502 a.u. for the lowest pH. This indicates that in acidic medium the yield of complex is less than at higher pHs. All of the salt samples used in this work did not have their solution pH outside the range of 6–8. Thus the method should be practical for most salts.

3.7. Application to real samples and validation

Analysis for iodine contents in nine samples was first carried out. The results were compared with those given by the titration method as shown in Table 3. According to paired t-test, no significant

Table 1
Recovery study of iodate added to samples

Sample	Sample concentration (M)	Added (M)*	Found (M)	Percentage recovery
S1	7.5×10^{-6}	5×10^{-6}	1.3×10^{-5}	110
		1×10^{-5}	1.9×10^{-5}	115
		2×10^{-5}	2.9×10^{-5}	107
S2	8.8×10^{-6}	5×10^{-6}	1.5×10^{-5}	124
		1×10^{-5}	1.9×10^{-5}	102
		2×10^{-5}	2.8×10^{-5}	96
S3	1.5×10^{-5}	5×10^{-6}	2.1×10^{-5}	120
		1×10^{-5}	2.6×10^{-5}	110
		2×10^{-5}	3.6×10^{-5}	105
S4	1.8×10^{-5}	5×10^{-6}	2.3×10^{-5}	100
		1×10^{-5}	2.9×10^{-5}	110
		2×10^{-5}	3.9×10^{-5}	105

* Concentration of standard iodate added to sample solutions.

Table 2
Effect of foreign ions on alteration of the FI signal of standard potassium iodate 2.0×10^{-5} M prepared in 6% (w/v) NaCl

Foreign species	Added as	Concentration added (M)*	Signal alteration (%)
F^-	NaF	1.4×10^{-4}	0
Br^-	NaBr	2.0×10^{-3}	0
K^+	KCl	2.0×10^{-2}	−1.2
Ca^{2+}	$CaCl_2 \cdot 2H_2O$	2.0×10^{-2}	−2.3
Mg^{2+}	$MgCl_2 \cdot 2H_2O$	1.0×10^{-1}	+3.2

* Based on normal levels present in sea salt.

Table 3
Iodine contents in table salts obtained from the colorimetric FI method and the titration method

Sample	Iodine content (mg I kg^{-1})±S.D.	
	Colorimetric FI ($n=3$)	Titration ($n=3$)
S5	16.5±0.1	18.5±0.2
S6	16.9±0.2	18.4±0.5
S7	24.3±0.2	24.2±0.2
S8	31.1±0.2	30.1±0.4
S9	31.5±0.1	33.5±0.4
S10	34.0±0.1	34.5±0.5
S11	42.2±0.2	40.6±0.6
S12	70.3±0.2	65.0±0.5
S13	71.9±0.1	65.8±0.8

different was found between the results of both methods at 95% confidence limit ($t_{observed} = 0.89$, $t_{critical} = 2.31$).

A further 12 samples of table salts were determined for iodine contents using the FI method and the method employing an iodide-ISE. The latter method requires addition of ascorbic acid into salt solution to reduce iodate ion to iodide ion before potentiometric detection. Iodine contents summarized in Table 4 demonstrate good agreement between the methods. The difference between both methods was not significant at 95% confidence ($t_{observed} = 0.77$, $t_{critical} = 2.20$).

3.8. Analytical features and advantage over other methods

The present method satisfactorily provides rapid analysis (65 injections per h) and thus is more rapid than the former FI method presented

Table 4
Iodine contents in table salts obtained from the colorimetric FI method and the potentiometric method (ISE)

Sample	Iodine content (mg I kg^{-1})±S.D.	
	Colorimetric FI (n = 3)	Potentiometric method (n = 3)
S14	15.7±0.1	18.7±1.9
S15	15.8±0.1	19.3±2.1
S16	16.6±0.3	18.8±2.1
S17	17.3±0.4	21.2±2.0
S18	18.8±0.3	25.4±2.2
S19	29.4±0.1	28.3±2.0
S20	33.3±0.1	30.3±2.1
S21	33.5±0.4	29.8±1.9
S22	35.2±0.3	30.7±2.0
S23	38.0±0.3	33.7±2.2
S24	55.4±0.4	48.8±2.2
S25	56.5±0.3	47.5±2.1

recently with use of amperometric detection (35 injections per h) [6]. The method is much faster and more convenient than the conventional titration method. The FI method also gave higher sample throughput than the potentiometric method, which has the throughput of only seven measurements per h. As the iodide-required approximately 4–5 min for stable reading.

The detection limit of the FI method (3 S/N, $n = 10$) was 2 mg I kg^{-1}. The method has high precision with the R.S.D. of 0.5% (ten injections of the 2.0×10^{-5} M standard).

4. Conclusions

A FI system was developed and is suitable for determination of iodate in table salts. The system requires spectrometric detection in the visible region of the blue I_3^-–starch complex formed in sample zone. The FI detection, which exploited this complex formation, has not been reported elsewhere. There are a few systems that utilized similar chemistry for the detection [9–11] but their monitorings are based upon decoloration of the complex. Initially detection of the I_3^-–starch complex gave some problems (e.g. continuous shift of baseline, irreproducible signal after some injections). This was due to the adsorption of I_3^-–starch mainly on tube walls. The present use of an injection of thiosulfate solution, prior to injection of sample, eliminated this contamination. Air–oxygen oxidation of iodide, which tends to occur in batch operation, is easily prevented in this technique. The present method was successfully validated against the titration and the potentiometric methods.

Features of the system meet all requirements in terms of simplicity, rapidity, precision, linear working range, detection limit and economy. As the chemistry takes place in a close system, no blank signal was observed.

Acknowledgements

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Stopped-flow injection simultaneous determination of phosphate and silicate using molybdenum blue☆

K. Grudpan [a,b,*], P. Ampan [a], Y. Udnan [a], S. Jayasvati [b,c], S. Lapanantnoppakhun [a], J. Jakmunee [a,b], G.D. Christian [d], J. Ruzicka [d]

[a] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand
[c] Department of Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[d] Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA

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Abstract

Kinetic information for the phosphate–molybdate–ascorbic acid reaction can be obtained by making use of a very simple manually operated stopped-flow injection (FI) system. Various parameters (concentrations of reagents, flow rate, mixing coils, and volume of flow cell) were investigated for determination of phosphate. A stopped-FI system should be arranged for low degree of mixing (of reactants) and low dispersion so that good signals of rate changes will be observed. Simultaneous determination of phosphate and silicate by the stopped-FI technique is proposed, using a laboratory-made semi-automatic stopped-FI Analyzer with LED-based photometer. It is based on kinetic separation of phosphate and silicate using molybdenum blue. The proposed procedure has been demonstrated for the application to water samples. The results obtained agree with that of a standard method.

Keywords: Stopped-FI; Simultaneous determination; Phosphate; Silicate; Molybdenum blue; Kinetics

1. Introduction

Since the first application by Ruzicka and Hansen [1] for the determination of phosphate using molybdenum blue, a number of flow injection (FI) techniques have been reported for phosphate and silicate. They involve a continuous mode, such as on-line microwave digestion [2], determination of phosphate in silicate rocks by adding tartaric acid [3], on-line column separation [4–7]. Sequential injection analysis (SIA) has been applied to determine phosphate and silicate simultaneously by forming vanadomolybdo complexes [8], or using the molybdenum blue [9]. SIA with lab-on-valve using a stopped flow mode for determination of phosphate has been proposed [10,11].

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* Corresponding author. Tel.: +66-53-943-345x157; fax: +66-53-222-268
E-mail address: kate@chiangmai.ac.th (K. Grudpan).

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Kanaya and Hiromi [12] used stopped-flow to record the progress curve for the formation of the colored complex of 12-molybdophosphate with malachite green. Linares et al. [13] described the fluorometric differential kinetic determination of silicate and phosphate using two measurements at different times. Yoza et al. [14] determined ortho-, di-, and triphosphates using stopped-flow, based on substitution reactions with colored metal complexes. Lacy et al. [15] used sorbent extraction on a hydrophobic sorbent and optosensing measurement to determine mixtures of phosphate and silicate, based on differences in the reduction rates of the heteropoly complexes, using partial least squares analysis.

In this work, we employ stopped-FIA with simple instrumentation for kinetic information on phosphate and silicate using the molybdate and ascorbic acid reactions. Simultaneous determination of phosphate and silicate based on kinetic separation, using a simple semi-automatic stopped-FI Analyzer is proposed.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were analytical grade unless otherwise stated. Deionized water was used throughout. A stock phosphate standard solution (1000 μg ml^{-1}) was prepared by dissolving potassium dihydrogen phosphate (Merck, 0.4390 g) in a portion of water before making up to a volume of 100.00 ml. A commercial silicate standard solution of 1000 μg ml^{-1} (Merck) was used. Working phosphate and silicate standard solutions were obtained freshly by appropriate dilutions of the stock solutions.

A sodium molybdate solution (0.6% w/v) was prepared by dissolving sodium molybdate (Merck, 1.512 g) in 100 ml water, and 3.25 ml of conc. HNO_3 before making to a volume of 250.0 ml with water.

An ascorbic acid solution (0.5% w/v) was prepared freshly from 2.5 g of L-ascorbic acid (Fluka) which was dissolved in 500-ml water.

2.2. FI manifolds with manual operation

Two FI manifolds for manual operation were designed, as depicted in Fig. 1. In manifold I, a standard/sample solution (S) was injected via an injection valve (FIA-lab, USA, with a sample loop of 100 μl) into a molybdate line with a mixing coil (MC1) before merging with the ascorbic acid stream, passing through another mixing coil (MC2) before entering into a flow cell in a spectrometer (Spectronic 21, Spectronic Instruments, USA), connected to a chart recorder (R100 A, Perkin-Elmer, USA). Manifold II represents a different arrangement, in which a standard/sample solution (S) was injected into a merged stream of molybdate and ascorbic acid via an injection valve placed between mixing coils (MC3 and MC4).

2.3. Semi-automatic FI Analyzer

The laboratory-made system (Fig. 2) consists of a controller via a microprocessor to control a peristaltic pump (S-mini, Alitea) to propel re-

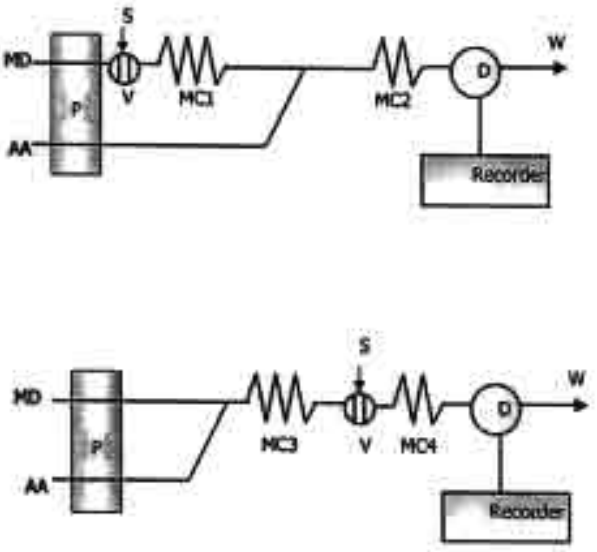

Fig. 1. FI manifolds with manual operation for stopped-FIA for phosphate, *top*: manifold I, S injected into MD before merging with AA; *bottom*: manifold II, S injected into a stream of mixed MD and AA; MD = molybdate solution, AA = ascorbic acid solution, P = peristaltic pump, MC = mixing coil, D = spectrometer, V = injection valve, W = waste.

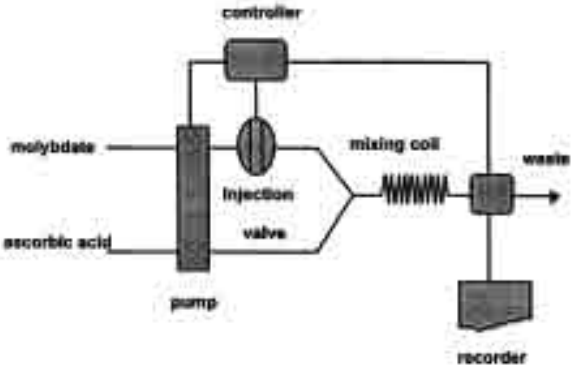

Fig. 2. Semi-automatic FI Analyzer.

agents, to switch an injection valve (V-451, Upchurch), and to set a photometer for data acquisition and to evaluate the signals. The photometer is a laboratory-design, using a LED red light source, with a peak height holding feature, similar to the ones previously reported [16–18]. The output signals can be via built-in digital read-out and/or a chart recorder (Philips, PM 8251, Holland). Signal profiles are illustrated in Fig. 3. The controller can be used to preset the traveling time (T), the period of flow between the point of injection to the point at which the flow is stopped for monitoring the reaction development at the flow-cell; the stopping time (S), the period during the flow stopped; and the washing time (W), the period when the stream is re-started to flow (after stopping) until this operation cycle ends and is ready for the next cycle. I and D, the signals at the first and last stopped points, respectively, will be given as digital read-outs on the photometer. Fig. 3(b–d) demonstrate stopped-FI recordings for phosphate, silicate, and mixtures of the standard solutions.

3. Results and discussion

3.1. Continuous FIA for phosphate

In preliminary investigation, it was found that the reactions to form the molybdenum blue product are reversible and will be in equilibrium. For continuous FI determination of phosphate, manifold II should be employed. Manifold II resulted in higher FI responses compared to the peaks obtained by using manifold I. Calibrations (a plot of peak height vs concentration) were: $y_1 = 65.08x_1 + 9.76$, $r^2 = 0.998$ and $y_2 = 91.93x_2 + 15.39$, $r^2 = 0.991$ for the manifolds I and II, respectively (0.5–3 μg P ml^{-1}). The reactions for molybdenum blue may involve [19]:

$$\text{heptomolybdate} + \text{phosphate} \overset{\text{fast}}{\rightleftharpoons} \text{yellow heteropoly complex} \quad (1)$$

$$\underset{\text{(Mo (VI))}}{\text{yellow heteropoly complex}} + \text{ascorbic acid} \overset{\text{slow}}{\rightleftharpoons} \underset{\text{(Mo(V)–Mo(III))}}{\text{molybdenum blue}}. \quad (2)$$

Using manifold II with a premixed stream of molybdate and ascorbic acid, the reactive intermediate of the reduced form of molybdate will be readily available and reactive to the injected phosphate, to yield the molybdenum blue very fast (on the order of seconds), whereas in the manifold I, phosphate injected into molybdate stream will form an observed yellow product of phosphomolybdate which is not as reactive, with slow reduction to yield molybdenum blue (on the order of minutes). This was found in the batch molybdenum blue method for determination of phosphate. It should be noted that for batch analysis, if molybdate was premixed with ascorbic acid before adding phosphate, no molybdenum blue was observed at all.

Using manifold I with MC1 and MC2 being 25 and 50 cm, respectively, and ascorbic acid solution being 0.5% w/v, molybdate concentrations were varied (0.1–1.2% w/v). It was observed (Fig. 4(a)) that the same steady state of the system should be established in the molybdate concentrations in the range of 0.5–1% w/v. Similar observation was found for the stopped-FIA studies (Fig. 4(b)). A lower molybdate concentration would cause slower approach to equilibrium. Disproportionation of Mo(VI) and Mo(III) yielding Mo(V) may occur if a higher concentration of molybdate is used. This shifts the equilibrium in Eq. (2), resulting in less phosphomolybdenum(V) blue.

Similarly, a study on the effect of ascorbic acid concentrations (Fig. 5(a and b)) indicated that the

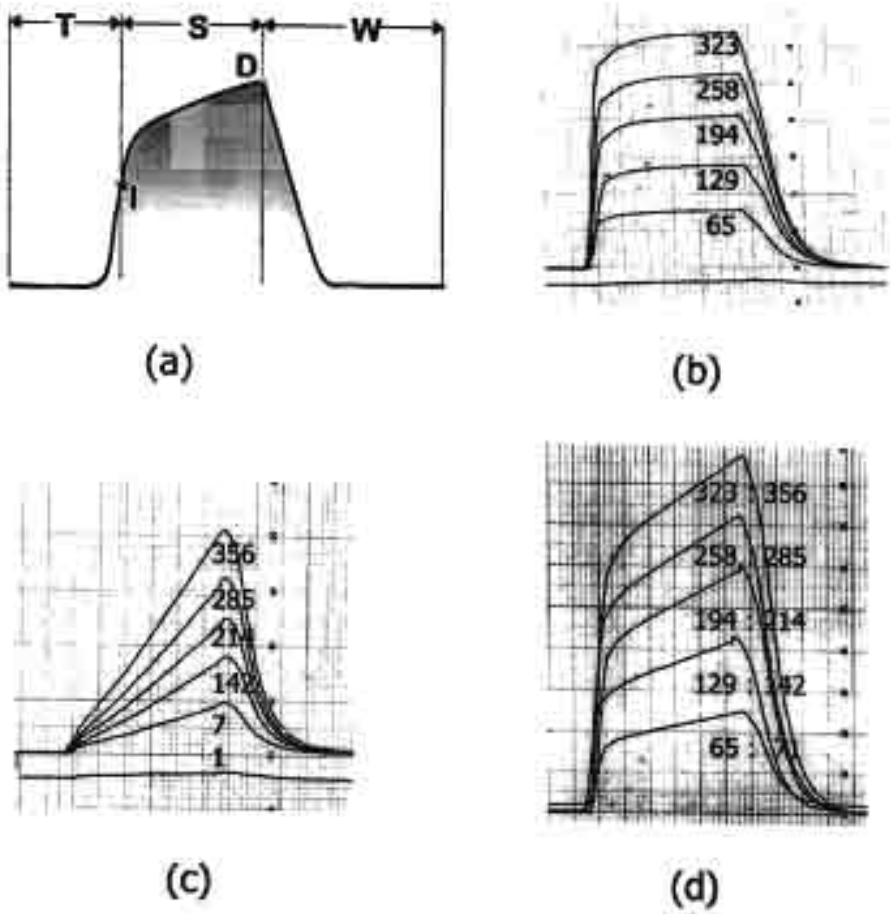

Fig. 3. Stopped-FI profiles obtained (a) in general, (b) phosphate, (c) silicate, (d) mixture of phosphate and silicate: T = traveling time, S = stop time, W = washing time, I = signal at the first stopped point, D = signal at the last stopped point before resuming flow. The numbers refer to concentrations [μM].

system with 0.3–0.8% w/v ascorbic acid resulted in reaction rates at steady state for the molybdate used (0.8% w/v). With lower concentrations of ascorbic acid, different rates were observed.

3.2. Stopped-FI determination of phosphate

At the flow-cell, in the stream, a bolus of the mixture (phosphate, molybdate and ascorbic acid) is stopped, and an increase in signal is observed for a period before gradually becoming constant (Fig. 3(b)). The signal represents the rate of the reactions, which come into equilibrium as discussed earlier.

A manifold used for stopped-FIA should provide both a low degree of axial mixing of reactants and low dispersion, to provide a suitable bolus of reaction mixture stopped at the flow cell for monitoring the reaction progress.

It was found that the manifold I (Fig. 1) without mixing coil and using a 8-μl flow cell is more suitable, compared to manifold II for stopped-FI determination of phosphate (Fig. 6). A dispersion study using a dye (bromothymol blue) [20] for manifold I is summarized in Table 1. As expected, apart from the effect of mixing coil length on dispersion, a higher volume flow cell creates more dispersion. It should also be noted that a signifi-

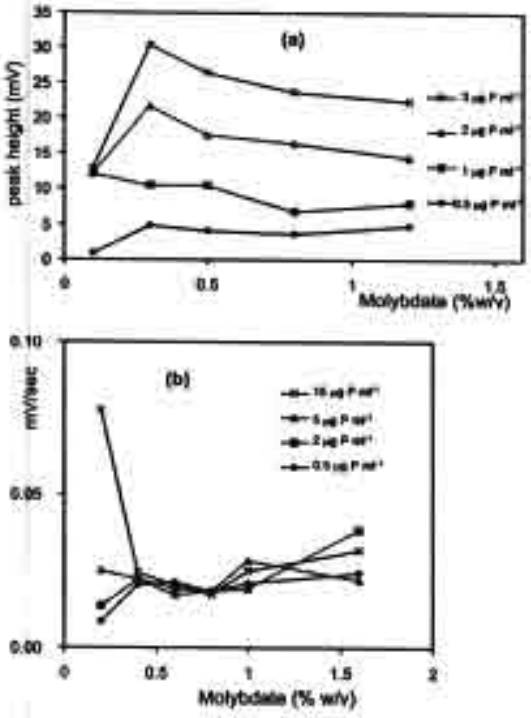

Fig. 4. Effect of molybdate: (a) continuous-FI; (b) stopped-FI.

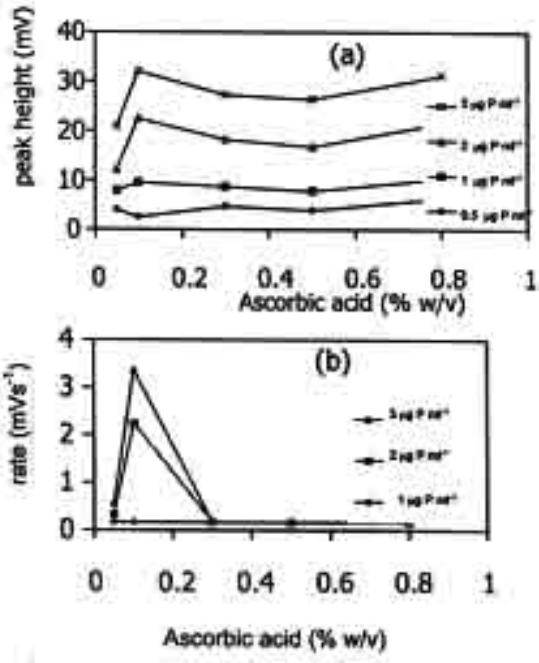

Fig. 5. Effect of ascorbic acid, (a) continuous-FI; (b) stopped-FI.

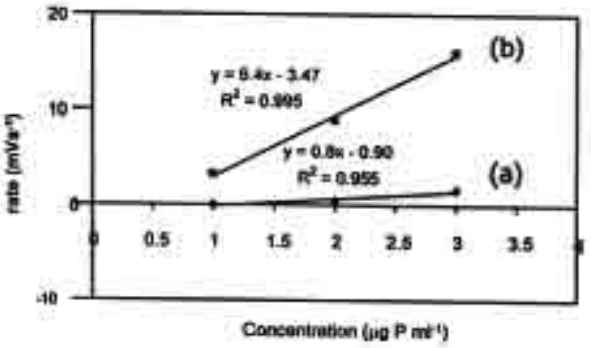

Fig. 6. Plot of reaction rates and phosphate (as P) concentrations using manifold I: (a) with and (b) without mixing coil.

cant effect of flow rate on dispersion was not observed.

3.3. Kinetic information obtained by the stopped-FIA

For a general reaction:

$$A + B \rightarrow C \quad (3)$$

with a rate expression: $r = d[C]/dt = k[A]^n$, where r = rate, k = rate constant, n = order of reaction, or $\ln r = \ln(k[A]^n)$, then:

$$\ln r = \ln k + n\ln[A] \quad (4)$$

From Eq. (4), a plot of $\ln r$ vs $\ln[A]$, the order of the reaction can then be obtained from the slope (n), and the rate constant (k) can be evaluated from the y-intercept.

From the stopped-FI experiments for phosphate (Table 2), the rate (mV s^{-1}) was obtained from each stopped-FI recording for each phosphorous (phosphate) concentration (i.e., slope of the signal). By assuming that the reagents (molybdate and ascorbic acid) were in large excess, then the rate of product formation should be proportional to the phosphorous (phosphate) concentration. A plot of ln(rate) and ln(phosphorous concentration) was found to be linear: $y = 1.3x - 3.99$, $r^2 = 0.992$ (Fig. 7). The slope of 1.3 reflects the order of the reaction under the experimental conditions to be close to unity, in agreement with other reports [21,22]. The rate constant obtained was 1.9×10^{-2} µM s^{-1}.

Table 1
Studies on dispersion in the manifold I

Total flow rate (ml min^{-1})	Dispersion (C_o/C_{max})			
	With mixing coils[a]		Without mixing coil[b]	
	8-μl flow-cell	80-μl flow-cell	8-μl flow-cell	80-μl flow-cell
2	3.1	4.9	1.2	2.8
3	2.9	4.7	1.3	2.8
4	2.7	4.1	1.1	2.8

[a] MC1 = 25 cm, MC2 = 50cm.
[b] No MC1 and MC2.

Table 2
Experiments for kinetic information on the reactions of phosphate with molybdate and ascorbic acid

Phosphate (P) concentration		ln P	Rate of reaction[a]	Ln (rate)
(μg P ml^{-1})	(μM)		(mV s^{-1})	
0.5	16.4	2.80	8.0×10^{-1}	-2.2×10^{-1}
1	32.8	3.49	1.6	4.70×10^{-1}
2	65.6	4.18	4.4	1.48
3	98.4	4.59	8.0	2.08

[a] d[Product]/dt.

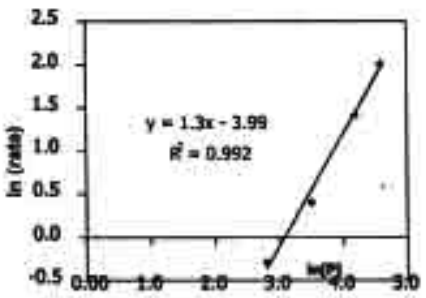

Fig. 7. Plot of ln(rate) vs ln[Phosphate].

3.4. Simultaneous determination of phosphate and silicate by stopped-FIA

The rate of the phosphate–molybdate–ascorbic acid reaction is faster than that of the silicate–molybdate–ascorbic acid reaction. It is then possible to determine phosphate and silicate simultaneously using the kinetic separation approach.

Fig. 3(b–d) show stopped-FIA recordings of phosphate, silicate, and a mixture of the two. By using the semi-automated stopped-FI Analyzer (Fig. 2), concentrations of phosphate and silicate in a mixture can be evaluated from I_P, I_{Si} and I_{mix} (signals at the first stopped points for phosphate, silicate and mixture, respectively, see Fig. 3), and D_P, D_{Si} and D_{mix} (signals at the last stopped points for phosphate, silicate and mixture, respectively, see Fig. 3).

Employing the conditions summarized in Table 3, calibration plots were obtained from the experimental results (Fig. 3). For phosphate standards (up to 15 μg P ml^{-1}):

$$I_P = 13.31[P] + 9.20,\ r^2 = 0.986 \quad (5)$$

Table 3
Conditions for simultaneous determination of phosphate and silicate with molybdate using the semi-automatic stopped-FI Analyzer

Condition	Value
Molybdate concentration, % w/v in 0.18 M HNO_3	0.6
Ascorbic acid concentration, % w/v	0.5
Injection volume, μl	35
Mixing coil length, cm	50
Flow rate (each line), ml min^{-1}	3.2±0.2
Travel time, s	2.5
Stop time, s	20
Wash time, s	15
Light source	Red LED (630 nm)

Table 4
Determination of phosphate and silicate by the proposed stopped-FI method and the standard method [23]

Water sample	Phosphate (μg P ml^{-1})		Silicate (μg Si ml^{-1})		Phosphate added[a] (μg P ml^{-1}) (*C*)	% Recovery of phosphate[b]	
	Stopped-FI method (*A*)	Standard method (*B*)	Stopped-FI method	Standard method		Stopped-FI method (*D*)	Standard method (*E*)
Reservoir	n.d.	n.d.	8.2	8.6	–	–	–
	1.6	1.8[a]	9.4	8.7	2.0	80	90
Pond	n.d.	n.d.	7.6	7.9	–	–	–
	5.7	3.5[a]	7.5	8.0	4.0	143	88
Irrigation canal	n.d.	n.d.	8.3	8.2	–	–	–
	6.5	5.7[a]	7.8	8.3	6.0	108	95
Moat	n.d.	n.d.	8.1	9.0	–	–	–
	6.3	7.9[a]	7.3	8.9	8.0	79	99
Drainage (from a dormitory)	1.1	1.2	11.9	13.9	–	–	–
	3.1	3.1[a]	11.2	13.9	2.0	100	95

[a] Total of present+added.
[b] % Recoveries evaluated by: $D = (A/C) \times 100$ and $E = (B/C) \times 100$.

For correlation of D_P (mV) and I_P (mV):

$$D_P = 4.65I_P - 3.38,\ r^2 = 0.998, \quad (6)$$

For silicate standards (up to 15 μg Si ml^{-1}):

$$D_{Si} = 35.82[Si] + 30.55,\ r^2 = 0.999. \quad (7)$$

The concentration of phosphate in a mixture or a sample is obtained from the calibration plot of phosphate standards (Eq. (5)) by using I_{mix} from the mixture or the sample for I_P (as at the first stopped point, there should be no contribution due to silicate). Then D_P is calculated from Eq. (6). In the signal profile of the mixture/sample, calculation for signal due to silicate at the last stopped point, $D_{Si,mix}$ is made from the correlation:

$$D_{Si,mix} = (D_{mix} - D_{P,mix}) + I_P. \quad (8)$$

$D_{P,mix}$ is the contributed signal due to phosphate in the mixture and can be evaluated from the Eq. (6). The last term (I_P) is for contribution from the initial (background) signal due to phosphate. Substituting the value of $D_{Si,mix}$ for D_{Si} in Eq. (7), one obtains the concentration of silicate in the mixture/sample.

The proposed procedure has been applied to several natural water samples. The results are compared to that obtained by the standard method [23], as summarized in Table 4.

4. Conclusion

A stopped FI system offers kinetic information on the phosphate–molybdate–ascorbic acid reaction. Simultaneous determination of phosphate and silicate in a mixture/sample is proposed by employing kinetic separation using molybdenum blue, and using a laboratory-made semi-automatic stopped-FI Analyzer with a microprocessor to control the system and for signal read-out. The proposed procedure has been demonstrated for its application to water samples by validation with a standard method.

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Sequential injection redox or acid–base titration for determination of ascorbic acid or acetic acid☆

Narong Lenghor[a], Jaroon Jakmunee[a,*], Michael Vilen[b], Rolf Sara[b], Gary D. Christian[c], Kate Grudpan[a]

[a] *Department of Chemistry, Faculty of Science, Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*

[b] *Turku Center for Biotechnology, Abo Akademi University and University of Turku, PO Box 123, BioCity, Turku FIN-20521, Finland*

[c] *Department of Chemistry, Center for Process Analytical Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA*

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Abstract

Two sequential injection titration systems with spectrophotometric detection have been developed. The first system for determination of ascorbic acid was based on redox reaction between ascorbic acid and permanganate in an acidic medium and lead to a decrease in color intensity of permanganate, monitored at 525 nm. A linear dependence of peak area obtained with ascorbic acid concentration up to 1200 mg l^{-1} was achieved. The relative standard deviation for 11 replicate determinations of 400 mg l^{-1} ascorbic acid was 2.9%. The second system, for acetic acid determination, was based on acid–base titration of acetic acid with sodium hydroxide using phenolphthalein as an indicator. The decrease in color intensity of the indicator was proportional to the acid content. A linear calibration graph in the range of 2–8% w v^{-1} of acetic acid with a relative standard deviation of 4.8% (5.0% w v^{-1} acetic acid, $n = 11$) was obtained. Sample throughputs of 60 h^{-1} were achieved for both systems. The systems were successfully applied for the assays of ascorbic acid in vitamin C tablets and acetic acid content in vinegars, respectively.

Keywords: Sequential injection; Titration; Spectrophotometry; Ascorbic acid; Acetic acid

1. Introduction

Fast, economical and automated methods are especially required for routine analysis and process control. Flow injection analysis (FIA) has widely been used as it provides high sample throughput, low sample and reagent consumption, high reproducibility and simple automated operation [1]. More recently, sequential injection analysis (SIA)

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* Corresponding author. Tel.: +66-53-943-3415x157; fax: +66-53-22-2268

E-mail address: scijjkmn@chiangmai.ac.th (J. Jakmunee).

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based on the similar principles as FIA has been introduced [2]. Normally FIA uses a multi-channel pump and unidirectional forward flow; in contrast SIA uses a single-channel pump to move the fluid zones in forward and reverse steps through a system consisting of a holding coil (HC), a multi-position valve and a detector. The multi-position valve acts as a central distributor through which required volumes of liquid segments are sequenced by aspiration into the HC and then flushed by a flow reversal into the detector. As only one pump is used to move the composite zone through the system, the sampling frequency of SIA is generally lower than the multi-channel pump FIA method. However, the SI system uses a smaller number of moving parts than a comparable FIA system and uses at least an order of magnitude less of reagents, on the order of microliters. Manipulation of solutions in an SIA system can be made via a computer keyboard using appropriate software.

Different methods were reported for the determination of ascorbic acid [3]. Most of them utilized the reducing property of ascorbic acid. A simple FI redox or acid–base titration of ascorbic acid with potassium permanganate or ammonia was proposed [4]. SI redox titration of ascorbic acid with cerium(IV) in sulfuric acid was applied for pharmaceutical products [5]

Acetic acid in vinegar was determined by a FI pervaporation system with acid–base titration [6]. FI titration of acetic acid could be performed under both non-steady and steady state conditions [7]. In the steady state condition, a high degree of mixing between the analyte and reagents is required which may be made on-line by use of a mixing chamber, and thus involves long analysis time. Monosegmented flow titration for determination of total acidity of vinegar with a sample throughput of 30 h^{-1} was proposed [8]. SI acid–base titration was developed to determine strong and weak acids [9].

In this work, SIA procedures for the determinations of ascorbic acid and acetic acid employing simple reagents with spectrophotometric detection have been developed. Both titrations were performed under a non-steady state condition, so they do not require a special mixing part apart from a mixing coil, and so provide short analysis times. The SIA system has robust hardware and flexible control software, which enables convenient optimization and operation of the system. On switching from the determination of ascorbic acid to acetic acid, no change in hardware configuration was required, only the program sequence for control of the instrument was modified. An analysis can be made rapidly with computerized control. Sample throughput of 60 h^{-1} was obtained for both systems. Sample, reagents and waste can be minimized.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical reagent grade, and deionized water was used throughout. Potassium permanganate, sulfuric acid, L-ascorbic acid, acetic acid, ethanol and phenolphthalein were obtained from Merck (Merck, Germany). Sodium hydroxide (AKZO Nobel, Sweden) was used. Vitamin C tablets and vinegar samples were from a local market.

A 0.1 M potassium permanganate solution was prepared by dissolving 1.58 g of potassium permanganate in 0.1 M H_2SO_4 solution and making up into a 100 ml volume. This stock solution was then further diluted for appropriated concentrations. Stock standard ascorbic acid solution (2000 mg l^{-1}) was prepared by dissolving 0.5000 g of ascorbic acid in deionized water in a 250 ml volumetric flask. Working standard ascorbic acid solutions were freshly prepared by diluting the stock solution.

The 0.2% w v^{-1} phenolphthalein in 50% v v^{-1} ethanol solution was prepared by dissolving 0.50 g of phenolphthalein in 50% v v^{-1} ethanol and made to a volume of 250 ml. This stock solution was then used to prepare various concentrations of phenolphthalein solutions. Standard acetic acid solution (20% w v^{-1}) was prepared by weighing 50.00 g of glacial acetic acid into a 250 ml volumetric flask and diluting it to the mark with deionized water. Working standard acetic acid solutions were freshly prepared by diluting the stock solution.

2.2. SIA manifold for determination of ascorbic acid

Fig. 1(a) shows a diagram of the SIA manifold used. A laboratory made SIA analyzer (Center for Biotechnology, University of Turku, Finland) consisting of a syringe pump, a HC (200 cm long), two six port selection valves, a reaction coil (100 cm long), a simple spectrophotometer (Spectronic 21, Bausch & Lomb, USA) with a 10 mm path length flow through cell (Hellma, Germany) and a personal computer was used. All tubings were 0.6 mm i.d. Teflon. An in-house made software (ANALYSIA, Center for Biotechnology, University of Turku, Finland) was used to control the system and collect data from the detector via a plug-in interface card (Lab PC+, National instruments, USA). The analysis was performed automatically following sequences that had been written as a computer program called ANALYSIA SCRIPT LANGUAGE (ASL).

After all lines were filled with water (also used as carrier), the standard/sample and reagents were sequentially aspirated into a HC. The sequence of solutions in the HC is shown in Fig. 1(b). Aspiration volumes of each solution and other conditions are summarized in Table 1. The zones

Table 1
The SIA conditions for ascorbic acid determination

Parameter	Studied range	Selected conditions
Concentration of potassium permanganate (mM)	1.0–5.0	5.0
Aspiration volume of acidic potassium permanganate (μl)	100–220	180
Aspiration volume of sample/standard ascorbic acid (μl)	80–120	80
Aspiration volume of 0.1 M sulfuric acid (μl)	–	50
Flow rate (μl s^{-1})	50–250	150
Analytical wavelength (nm)	–	525

were propelled through a mixing coil to the detector, while transmission of light at a selected wavelength was monitored (arbitrary units). The peak area was evaluated from the SIA profile obtained. A calibration graph is a plot of the peak area versus ascorbic acid concentration.

2.3. SIA manifold for determination of acetic acid

The same configuration manifold as for ascorbic acid determination was utilized, except with different standard/sample and reagents as shown in

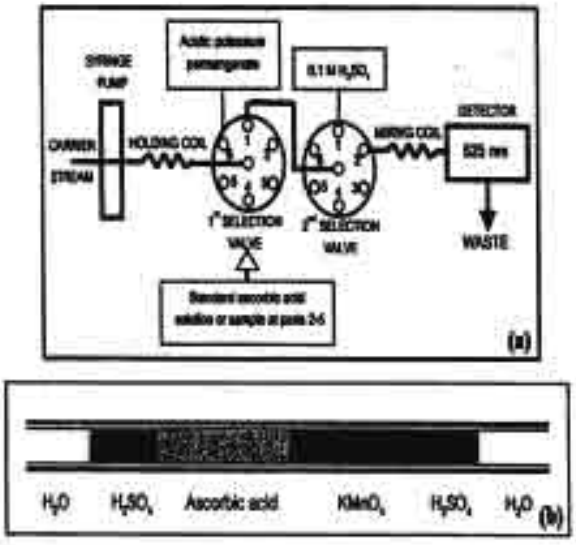

Fig. 1. (a) A schematic diagram of the SIA system for ascorbic acid determination; (b) a schematic diagram of order of the segments for ascorbic acid determination.

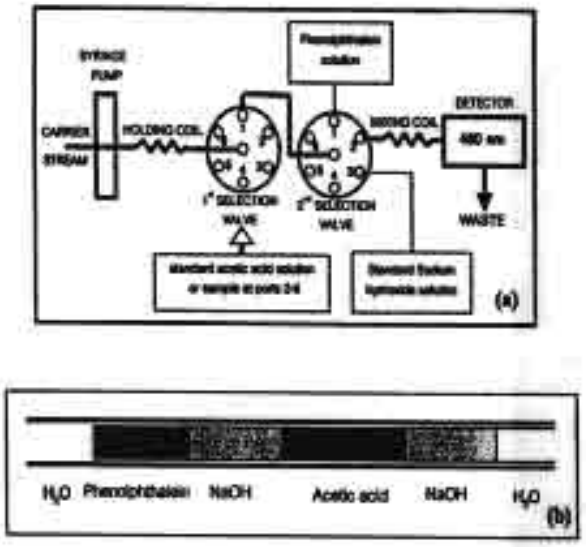

Fig. 2. (a) A schematic diagram of the SIA system for acetic acid determination; (b) a schematic diagram of the order of the segments for acetic acid determination.

Fig. 2(a). A similar operational sequence as above was also written as an ASL. The sequence of solutions in the HC is represented in Fig. 2(b). The aspiration volume of each solution and the other selected conditions are presented in Table 2.

3. Results and discussion

3.1. Optimization of SIA system for determination of ascorbic acid

A redox reaction of ascorbic acid with potassium permanganate was utilized in this system. The permanganate ion was also used as a color indicator, which was monitored for absorbance at 525 nm. A 0.1 M sulfuric acid solution was employed to prevent the conversion of permanganate to manganese dioxide precipitate. Various parameters such as concentration and aspiration volume of potassium permanganate, volume of sample, and flow rate of solution during propelling through the flow cell were investigated for the determination of ascorbic acid in range of 0–1200 mg l^{-1}. This concentration range was suitable for analysis of vitamin C tablets without difficulty in sample preparation. The studied ranges and the selected conditions are summarized in Table 1. The selected conditions were judged from a good slope and linearity of the calibration graph obtained, and a reasonable analysis time.

Table 2
The SIA conditions for acetic acid determination

Parameter	Studied range	Selected conditions
Concentration of sodium hydroxide (M)	0.2–1.0	0.8
Aspiration volume of sodium hydroxide solution (μl)	50–90	70
Concentration of phenolphthalein (% w v^{-1})	0.008–0.08	0.02
Aspiration volume of phenolphthalein solution (μl)	50–80	70
Aspiration volume of sample/standard acetic acid (μl)	40–70	50
Flow rate (μl s^{-1})	75–200	125
Analytical wavelength (nm)	–	480

Under the selected conditions, a linear calibration graph up to 1200 mg l^{-1} ascorbic acid ($y = -0.121x + 242.3$; $R^2 = 0.9933$) and relative standard deviations for 11 replicate determinations of 400 and 1200 mg l^{-1} ascorbic acid of 2.9 and 1.8%, respectively, were achieved. SIA profiles of a series of standard ascorbic acid solution are illustrated in Fig. 3. A sample throughput of 60 h^{-1} was obtained.

3.2. Analysis of vitamin C tablets

The system was applied to the determination of ascorbic acid in locally commercial vitamin C tablets. Twenty tablets of a vitamin C sample were weighed and ground in a mortar. After that a portion of the ground sample equal to about one tablet was weighed and then it was dissolved with deionized water to 1 l. The solution was then aspirated into the system using the same conditions as a standard. A titrimetric method [10] for reference purposes was also carried out. The results are summarized in Table 3. The comparison of the results obtained by the SIA method was evaluated by t-test [11]. The calculated t-test value was 0.67. The critical value of the t-test was 2.26 (9° of freedoms) at 95% confidence interval. Since the calculated t-test value is less than the critical value, the results from the two recommended methods were not significantly different.

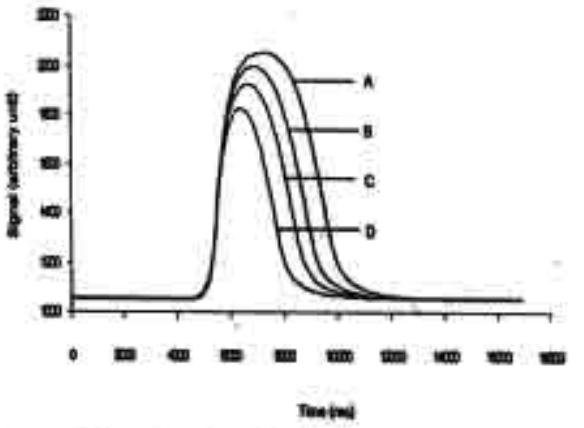

Fig. 3. SIA peaks for calibration graph of ascorbic acid determination; (A) blank, (B) 400 (C) 800 and (D) 1200 mg l^{-1} of standard ascorbic acid.

Table 3
Determination of ascorbic acid in vitamin C tablet samples

Sample code	Labeled amount (mg per tablet)	Ascorbic acid found (mg l^{-1})			
		Titrimetric method		SIA method	
		Milligram per tablet	Percentage label	Milligram per tablet	Percentage label
A	1000	1022	102	1014	101
B	1000	1013	101	892	89
C	500	477	95	520	104
D	100	101	101	101	101
E	60	69	115	72	120
F	1000	957	96	951	95
G	100	95	95	104	104
H	500	459	92	512	102
I	50	49	98	48	96
J	100	103	103	103	103

3.3. Optimization of SIA system for determination of acetic acid

Determination of acetic acid was based on acid–base titration of the acid with sodium hydroxide using phenolphthalein as an indicator. The physical configuration of the SIA system was the same as above for determination of ascorbic acid. The solutions around the selection valves and the computer program were changed (see Fig. 2). A similar optimization as in Section 3.1 was carried out for the determination of acetic acid in a range of 2–8% w v^{-1}. The studied ranges and the selected conditions are summarized in Table 2. The selected conditions were judged from a good slope and linearity of the calibration graph obtained with a reasonable analysis time.

Under the selected conditions, a linear calibration graph for 2–8% w v^{-1} acetic acid ($y = -37.709x + 390.9$; $R^2 = 0.9989$) was obtained. Relative standard deviations for 11 replicate determinations of 5 and 4% w v^{-1} acetic acid were 5.0 and 6.3%, respectively. Sample throughput of 60 h^{-1} was achieved.

3.4. Analysis of vinegar samples

The optimized procedure was applied to vinegar samples without any sample pretreatment. The acetic acid contents obtained from this procedure and titrimetry [12] are presented in Table 4. The results from the two methods were not significantly different (judged by t-test at 95% confident interval).

4. Conclusion

SI titration procedures for the determination of ascorbic acid and acetic acid have been proposed. The system is composed of robust hardware components and can be automatically controlled by a personal computer, with appropriate soft-

Table 4
Determination of acetic acid in local commercial vinegar samples

Sample code	Concentration of acetic acid (% w v^{-1}) by	
	Titrimetric method	SIA method
A	5.2	5.1
B	5.1	5.5
C	5.1	5.4
D	5.1	5.5
E	5.1	5.5
F	5.3	6.0
G	5.2	5.7
H	5.1	5.1
I	4.9	4.4
J	4.8	4.5
K	5.0	5.1

ware. Optimization of the systems can be conveniently carried out. No physical configuration changes of the system are required, but new programming sequences are needed in application of the system to determine a new parameter. Both procedures employed simple reactions with inexpensive and available chemicals. Chemical consumption was lower compared with batch and flow injection procedures, leading to lesser waste and more economical determinations.

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Exploiting flow injection and sequential injection anodic stripping voltammetric systems for simultaneous determination of some metals☆

Siripat Suteerapataranon, Jaroon Jakmunee*, Yuthsak Vaneesorn, Kate Grudpan

Department of Chemistry, Faculty of Science, and Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50202, Thailand

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Abstract

Flow injection (FI) and sequential injection (SI) systems with anodic stripping voltammetric detection have been exploited for simultaneous determination of some metals. A pre-plated mercury film on a glassy carbon disc electrode was used as a working electrode in both systems. The same film can be repeatedly applied for at least 50 analysis cycles, thus reducing the mercury consumption and waste. A single line FI voltammetric system using an acetate buffer as a carrier and an electrolyte solution was employed. An injected standard/sample zone was mixed with the buffer in a mixing coil before entering a flow cell. Metal ions were deposited on the working electrode by applying a potential of −1.1 V vs Ag/AgCl reference electrode. The stripping was performed by anodically scanning potential of working electrode to +0.25 V, resulting a voltammogram. Effects of acetate buffer concentration, flow rate and sample volume were investigated. Under the selected condition, detection limits of 1 μg l^{-1} for Cd(II), 18 μg l^{-1} for Cu(II), 2 μg l^{-1} for Pb(II) and 17 μg l^{-1} for Zn(II) with precisions of 2–5% ($n = 11$) were obtained. The SI voltammetric system was similar to the FI system and using an acetate buffer as a carrier solution. The SI system was operated by a PC via in-house written software and employing an autotitrator as a syringe pump. Standard/sample was aspirated and the zone was then sent to a flow cell for measurement. Detection limits for Cd(II), Cu(II), Pb(II) and Zn(II) were 6, 3, 10 and 470 μg l^{-1}, respectively. Applications to water samples were demonstrated. A homemade UV-digester was used for removing organic matters in the wastewater samples prior to analysis by the proposed voltammetric systems.

Keywords: Flow injection; Sequential injection; Anodic stripping; Voltammetry; Cadmium; Copper; Lead; Zinc

1. Introduction

The determination of trace heavy metals in natural water and wastewater has been of concern because of their toxicities on man and the envir-

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* Corresponding author. Tel.: +66-53-943341-5x157; fax: +66-53-222268

E-mail address: scijjkmn@chiangmai.ac.th (J. Jakmunee).

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onment. Anodic stripping voltammetry (ASV) has been widely used recently due to its speed, simplicity, low cost, high sensitivity and capability of multielement determination [1–3]. However, batch voltammetry consumes more time and chemicals. Therefore, flow techniques have been developed for voltammetric analysis to solve those drawbacks [4,5]. Differential pulse ASV in a flow system for determination of copper, lead, cadmium and zinc has been developed [6]. In our previous work [7], an on-line voltammetric system was set up for simultaneous determination of cadmium, copper, lead and zinc. A sample and electrolyte buffer were mixed together prior to aspiration into a flow cell where metals were in-situ pre-concentrated on the working electrode during the deposition step. The proposed system provides low detection limits and good precision but it concerns long analysis time and high reagent volumes.

Flow injection (FI) and sequential injection (SI) methods are more rapid, precise and consume lesser amounts of reagents [8]. FI with differential pulse voltammetric (DPV) detection using a static mercury drop electrode (SMDE) for determination of Cd(II) and Pb(II) was reported [9]. FI stripping voltammetry with wall-jet detector was applied for lead determination in blood [10]. SI-ASV was demonstrated for determination of copper in tap water. SI provided a convenient way to perform a medium exchange procedure for the stripping step, increasing selectivity of the method [11]. SI-ASV for determination of copper, lead, cadmium and zinc in river sediment extracts was reported [12]. SI with adsorptive stripping voltammetry was developed for determination of riboflavin and some heavy metals [13]. A mercury film electrode (MFE) is usually employed for ASV in flow systems due to that it provides better stability, well defined peaks of the voltammogram with high sensitivity and reduces risk of exposure to mercury vapour. The mercury film (MF) is usually plated in-situ with analytes during deposition step. However, the MFE preparation by the in-situ method produces mercury-contaminated waste.

In this work, FI and SI voltammetric systems using a pre-plated MFE as a working electrode were developed for simultaneous determination of cadmium, copper, lead and zinc. The same MF can be repeatedly used for several cycles, thus reducing the amount of mercury used. Peaks of metal ions obtained in a voltammogram were well resolved. Detection limits of a few $\mu g\ l^{-1}$ can be achieved. The proposed FI and SI systems were easily operated and required less sample and reagent than the batch and on-line systems. In addition, the systems are more rapid with high degrees of automation.

2. Experimental

2.1. Chemicals

Acetate buffer solution (pH 4.6), prepared from 100% glacial acetic acid (BDH, England) and sodium acetate 3-hydrate (BDH, England), was used for preparation of an electrolyte solution. Mercury(II) solution was prepared by dissolving Hg(II) nitrate (AJAX, Australia) in 0.1 M nitric acid (Merck, Germany). Metal standard solutions were prepared by diluting standard stock solution (1000 $mg\ l^{-1}$) (atomic absorption spectrometric grade, Merck, Germany). Water used throughout was from a Milli-Q water system (Millipore, Sweden). Nitrogen gas (99.999%) was used for purging oxygen in a solution.

2.2. Flow injection voltammetric (FIV) system

The FIV manifold is shown in Fig. 1(a). The system consists of a peristaltic pump (EYELA, Japan), a six-port injection valve (FIALab, USA), a mixing coil (PTFE, i.d. 0.8 mm) and a voltammograph with an electrochemical flow-cell (Bioanalytical System, USA). The flow-cell composes of a glassy carbon-working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE). After the MF was coated on the GCE, the de-aerated electrolyte solution was pumped through the mixing coil and the flow-cell. A de-gassed standard solution or sample was injected into the carrier stream by the injection valve. When the sample zone reached the flow-cell, the potential of −1.1 V vs Ag/AgCl RE was applied to deposit the metals onto the MFE. After

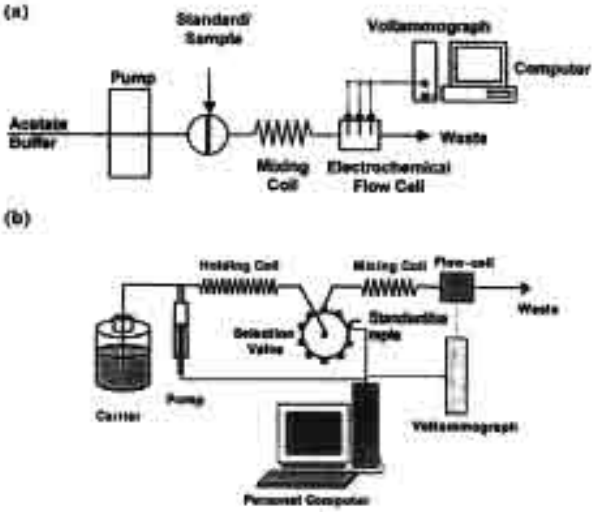

Fig. 1. Manifolds for simultaneous determination using voltammetric: (a) FI; and (b) SI.

the deposition step, the flow was briefly stopped to strip the metals out of the MFE by anodically scanning the potential to 0.25 V. The electrode was cleaned for 20 s by applying a potential of 0 V prior to performing the next analysis cycle.

2.3. Sequential injection voltammetric (SIV) system

The components of the SIV system (as shown in Fig. 1(b)) were similar to that of the above described FIV system except for the pump and valve. An autotitrator (Dosimat 765, Metrohm, Switzerland) was used as a syringe pump. A 10-port selection valve (Valco Instruments Co., Texas) was used. The pump and valve were controlled by a computer via an in-house written software based on LabVIEW® program (National Instruments Co., USA). The SIV flow conditions were set to be similar to the FIV system. Operation sequences were as followed: (1) 130 μl of standard/sample was aspirated into a holding coil; (2) the standard/sample zone was propelled through a mixing coil to a flow cell; (3) when the zone reached the flow cell, a potential of −1.1 V vs Ag/AgCl electrode was applied to the WE in order to deposit metals on the MFE; (4) the flow was stopped while a buffer was in the flow cell and stripping of the metals out of MFE was carried out; (5) finally, a potential of 0 V was applied for 20 s with the solution flowing to clean the MFE.

2.4. MF preparation

Mercury(II) 500 mg l^{-1} in 0.1 M HNO_3 solution was aspirated at a flow rate of 0.5 ml min^{-1} through the electrochemical flow-cell. A potential of −0.8 V vs Ag/AgCl RE was applied to the GCE for 10 min. The MF was then obtained on the GCE. Only 5 ml of the mercury solution was used for this purpose. The mercury solution from the waste out-let was collected for recycling.

2.5. A home-made UV-digestion unit

A home-made UV digester consists of two UV-C (180–280 nm) lamps, 15 W each, as depicted in Fig. 2. Aluminium foil, covered inside the digester house, was used for preventing the UV radiation. Sample trays were made by cutting a brown-coloured glass bottle into halves, which would increase the area of irradiation to sample in the tray. The trays were stored in nitric acid and rinsed with Milli-Q water before use. Hydrogen peroxide solution (30% v v^{-1}, 10 μl), as an oxidant, was

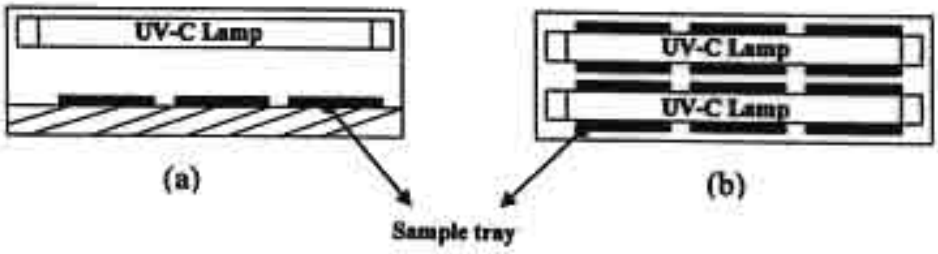

Fig. 2. A home-made UV-digestion unit: (a) side view; and (b) top view.

added to 10 ml of sample in the tray. The sample trays were then put in the UV digester.

3. Results and discussion

3.1. Study of MF preparation

Mercury(II) concentration, deposition potential and deposition time were considered for MF preparation. Mercury(II) concentrations of 500 and 1000 mg l^{-1} were tested. MF was obtained fully on the GCE by using 500 mg l^{-1} Hg(II), whereas using 1000 mg l^{-1} Hg(II) gave the film only on some area of the GCE. Deposition potentials of −0.8, −0.9 and −1.0 V were studied. It was found that mercury was adsorbed on the GCE at a potential of −0.8 V vs Ag/AgCl RE. On the other hand, at the potential of −0.9 and −1.0 V, mercury could not be coated on the GCE because H^+ in the solution could be reduced more at the GCE, at higher negative potentials. Higher peak currents (for the metals) were obtained when using the MF plated with a deposition time of 10 min compared with that obtained by using a 5 min deposition time. However, MF deterioration was observed. This should be due to that mercury did not adhere strongly to the glassy carbon and resulted in rapid decrease in sensitivity. Therefore, the MF was cleaned electrochemically at a potential of 0 V for 20 s before performing the next run. The proposed MF has been experimentally proven for reusing for more than 50 deposition-stripping cycles (as shown in Fig. 3), in comparison to the other reports that the MF was used only for one determination [10–12].

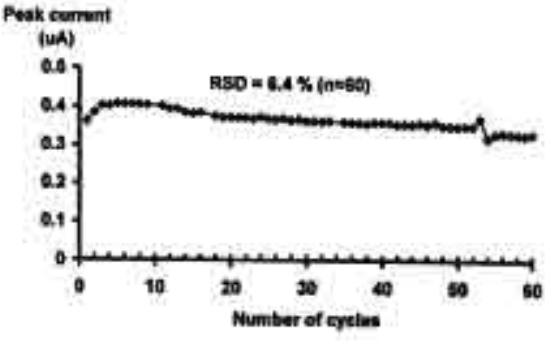

Fig. 3. Stability of the MF using electrochemical cleaning (60 analysis cycles of 10 μg l^{-1} Cd(II)).

3.2. Optimization of FIV system

3.2.1. FI parameters

3.2.1.1. Acetate buffer concentration. Electrolyte concentration affects the ionic strength of the solution and hence the peak currents of metals. Concentrations of acetate buffer (0.1, 0.2, 0.3, 0.5, 0.7 and 1.0 M) were varied while the flow rate, the sample volume and the length of mixing coil were fixed at 0.5 ml min^{-1}, 30 μl and 50 cm, respectively. It was found that the peak currents were fluctuated at an acetate buffer concentration less than 0.5 M. This could be due to that ionic strength of a lower concentration was not sufficient. A concentration of 0.6 M acetate buffer was chosen.

3.2.1.2. Flow rate. As expected, less dispersion was observed at higher flow rate. Peak currents of metals increased when the flow rate increased. However, at a higher flow rate than 0.5 ml min^{-1}, the deterioration of the MFE was obtained. Therefore, the flow rate of 0.5 ml min^{-1} was used in this FIV system.

3.2.1.3. Sample volume. A sample volume is related to the deposition time. At a deposition time of 20 s and a flow rate of 0.5 ml min^{-1}, approximately 167 μl of sample should be required. Injection volumes of 30, 80 and 130 μl were tested. It was found that the sensitivity obtained by using a volume of 30 μl were not different from that obtained by using a volume of 80 μl. However, using an injection volume of 130 μl yielded the worse precision. The precision of the currents obtained by using 30 μl injection volume was found to be the best. This could probably because less sample volume would provide better mixing pattern of sample and reagent and thus better reproducibility for the current observed. Using a sample volume of 30 μl also resulted in satisfactory current. Therefore, an injection volume of 30 μl was chosen.

Table 1
The voltammetric parameters used for the FIV and SIV system

Parameter	Condition
Deposition potential	−1.1 V
Deposition time	20 s
Sweep potential	−1.1–+0.25 V
Sweep mode	Square wave
Sweep rate	75 mV s^{-1}

3.2.1.4. Mixing coil length. For the low flow rate employed (0.5 ml min^{-1}), a length of 50 cm was used so that sample throughput and reproducibility of signal could be compromised.

3.2.2. Voltammetric parameters

The electrochemical parameters are represented in Table 1. Deposition times of 10, 20 and 30 s were tested. It was found that the longer deposition time, the higher peak current obtained, as expected. However, the deposition time of 20 s was chosen because it was faster and yielded enough sensitivity.

3.2.3. Characteristics of the developed FIV system

Voltammograms of metal standards obtained by the FIV system are depicted in Fig. 4. Calibration data, detection limits and precisions for the metal ions (Cd(II), Cu(II), Pb(II) and Zn(II)) are summarized in Table 2. The results illustrate that simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) can be performed by using the developed FIV system with a sample throughput of 20 h^{-1} and with better sensitivities than in other previous reports [6,10–13].

3.2.4. Applications to real samples

Six drinking water samples were analyzed by injecting them into the system. The metals in those samples were found to be below the detection limits. It indicated that the metal concentrations in the drinking water samples were below the values of maximum allowance of 0.010 mg l^{-1}, 1.0 mg l^{-1}, 0.1 μg l^{-1} and 500 mg l^{-1} for Cd(II), Cu(II), Pb(II) and Zn(II), respectively, which is announced by the Department of Environment Quality Standard, Ministry of Science, Technology and Environment of Thailand [14]. Wastewater

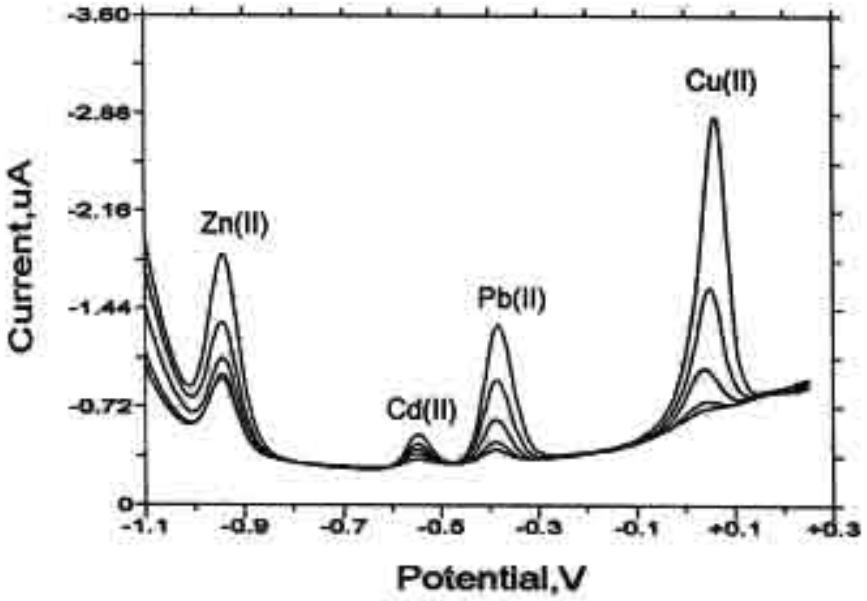

Fig. 4. Voltammograms of metal standards obtained from the proposed FIV system. (Concentrations of Zn(II), Pb(II), Cu(II): 10, 20, 50, 100 and 200 μg l^{-1}, and Cd(II): 5, 10, 15, 20 and 30 μg l^{-1}).

Table 2
Characteristics of the FIV system

Metal	Dynamic range (μg l^{-1})	Equation	R^2	Detection limit (μg l^{-1})	% RSD, $n = 11$
Cd	10–30	$y = 6.667x + 0.0235$	0.9990	1	3.6 (at 10 μg l^{-1})
Cu	20–200	$y = 11.569x - 0.1860$	0.9962	18	4.4 (at 75 μg l^{-1})
Pb	10–100	$y = 5.466x + 0.0171$	0.9997	2	1.8 (at 80 μg l^{-1})
Zn	50–200	$y = 4.338x - 0.0497$	0.9971	17	4.3 (at 100 μg l^{-1})

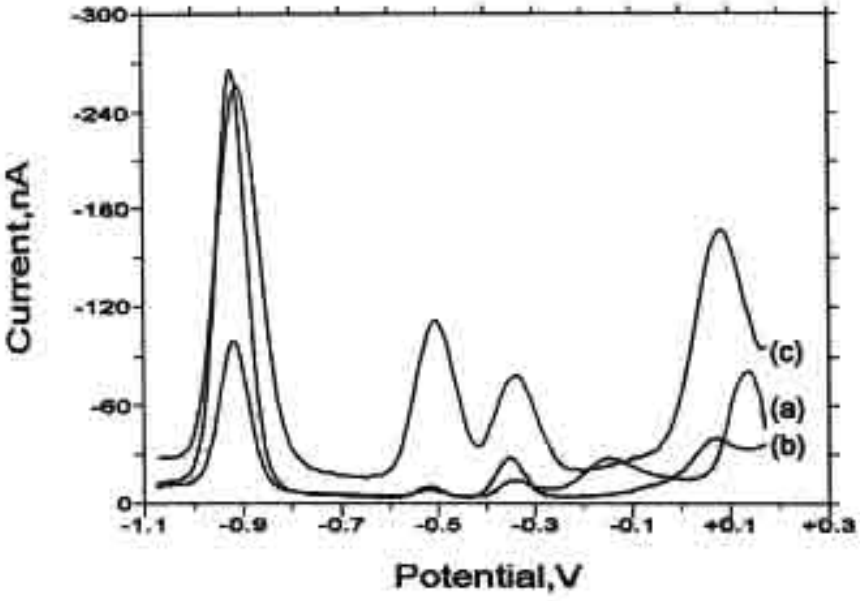

Fig. 5. Voltammograms of wastewater sample: (a) without; (b) with UV-digestion; and (c) a standard solution.

samples were also analyzed. The samples were digested for 2 h by using the home-made UV digester. UV-digestion can reduce some interferences in such a sample as illustrated in Fig. 5. Well-defined voltammetric peaks of metals were obtained for a sample with UV-digestion. Results are summarized in Table 3. The samples were also analyzed by ET-AAS [15]. The results obtained from the FIV method agreed with that by ETAAS. Zn and Cu may form an intermetallic Cu–Zn

Table 3
Examples of analysis of wastewater samples by the developed FIV system

Sample	Concentration found (μg l^{-1})							
	Cd		Cu		Pb		Zn	
	FIV	ETAAS	FIV	ETAAS	FIV	ETAAS	FIV	ETAAS
1	26	25	60	75	92	95	154	142
2	54	50	115	153	196	204	74	155
3	4	7	27	22	28	24	45	25

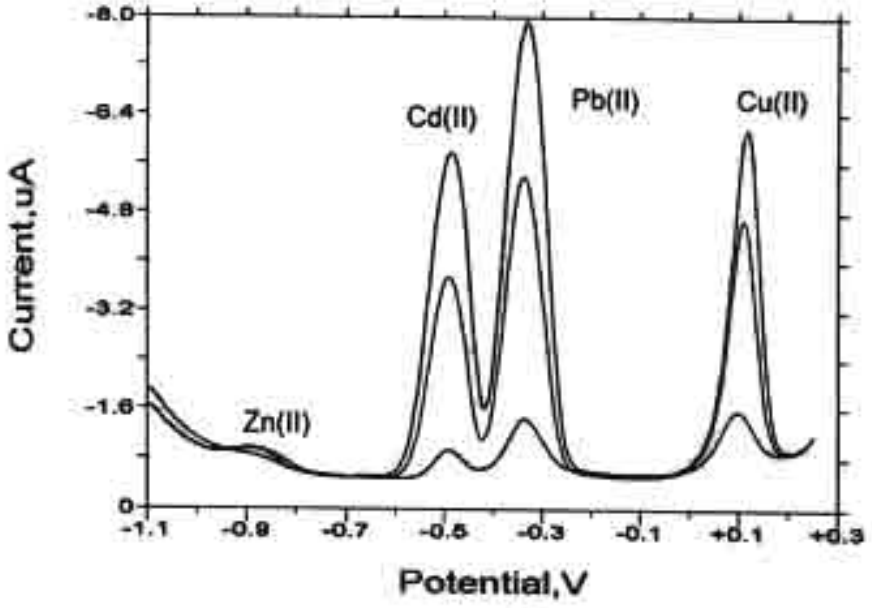

Fig. 6. Voltammograms of metal standards obtained from the propcsed SIV system. (Concentrations of Zn(II): 100, 700 and 1000 μg l^{-1}; Cd(II), Pb(II): 10, 70 and 100 μg l^{-1}; and Cu(II): 50, 150 and 200 μg l^{-1}).

phase in MF which may cause variation in FIV-results of these metals [12].

3.3. Preliminary study of SIV

The SI system with acetate buffer 0.6 M as carrier, was carried out for testing the performance of the system. By applying the experiences in the FIV system, similar conditions as employed in the FIV system were applied in the SIV system. Due to that the minimum pipette volume of the autotitrator used is 100 μl, a sample volume of 130 μl was aspirated into the SIV system. Dispensing volume was studied because it concerned the time to start the deposition step. Starting the deposition step at a volume of 0.30 ml or at a time of 36 s after starting dispensing the sample zone to the detector, the highest peak current was observed. Simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) can be performed by using the SIV system. The voltammograms of the metal ions are illustrated in Fig. 6. The results in Table 4 showed that the SIV system provided good precisions, linear calibrations and low detection limits for the metals, except the detection limit for Zn was high because of its low sensitivity and less reducibility. Due to the discontinuous nature of SI operation, reagent consumption and waste generation can be minimized and become advantageous. Further investigation on incorporation of on-line sample pre-treatment to the SIV system is in progress.

Table 4
Characteristics of the preliminary SIV system

Metal	Dynamic range (μg l^{-1})	Equation	R^2	Detection limit (μg l^{-1})	% RSD, $n=3$
Cd	10–70	$y=37.47x-0.051$	0.9976	6	9.8 (at 10 μg l^{-1})
Cu	50–200	$y=29.22x-0.537$	0.9999	3	1.0 (at 50 μg l^{-1})
Pb	10–100	$y=62.90x-0.191$	0.9946	10	2.6 (at 40 μg l^{-1})
Zn	470–700	$y=0.26x-0.0006$	0.9904	470	0.5 (at 700 μg l^{-1})

4. Conclusion

FI and SI systems with anodic stripping voltammetric detection for determination of some metal ions have been exploited. Employing the proposed conditions for making a pre-plated MFE as a working electrode, which can be utilized for several analysis cycles (at least 50 cycles), the mercury consumption can be minimized. The mercury waste solution was collected for recycling. The systems, therefore, produced less mercury waste. Detection limits of a few $\mu g\ l^{-1}$ of the metal ions can be achieved. The proposed single-line FI voltammetric system is cost-effective, very simple and easy to handle but achieves a high sensitivity simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II). The proposed SI voltammetric system, using an autotitrator as a syringe pump and a PC with an in-house software program, is an alternative for cost-effective higher degree of automation.

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Determination of trace iron in beer using flow injection systems with in-valve column and bead injection☆

P. Ampan[a], S. Lapanantnoppakhun[a], P. Sooksamiti[b], J. Jakmunee[a,c], S. Kradtap Hartwell[a], S. Jayasvati[c,d], G.D. Christian[e], K. Grudpan[a,c,*]

[a] *Faculty of Science, Department of Chemistry, Chiang Mai University, Chiang Mai50200, Thailand*
[b] *Office of Mineral Resources Region III (Chiang Mai), Chiang Mai50200, Thailand*
[c] *Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai50200, Thailand*
[d] *Faculty of Medicine, Department of Radiology, Chiang Mai University, Chiang Mai 50200, Thailand*
[e] *Department of Chemistry, University of Washington, Box 351700, Seattle, WA98195-1700, USA*

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Abstract

Three flow injection (FI) systems were investigated for the determination of trace iron in beer: an FI-in-valve column-flame atomic absorption spectrophotometry (FI-FAAS) system, a spectrophotometric FI system with a column placed at the detection point, and an FI-spectrophotometric system with bead injection (FI-BI). Cationic exchange resin Dowex 50W X8 and iminodiacetate chelating resin, Chelex-100, were employed for the FI-spectrophotometric and FI-FAAS systems, respectively. The FI-in-valve column, packed with the resin, enhances the FAAS performance. The spectrophotometric FI system with a column (packed with Chelex-100) placed at the detection point (in a cell holder of a spectrophotometer) is based on the formation of iron (II)–1,10-phenanthroline complex sorbed onto the resin. No eluent has been found to be suitable. The FI-BI for renewable microcolumn has been proven to be an alternative. The FI-FAAS and FI-BI procedures provide online sample preseparation and preconcentration for the determination of iron in beer. Both are simple, rapid, and economical. The procedures also involve sample preparation (decarbonation and suppression of tannin interference by adding ascorbic acid) and standard addition. The results obtained by FI-FAAS and FI-BI agree with those of AOAC spectrophotometric method.

Keywords: Iron; Beer; Flow injection; Bead injection; Atomic absorption spectrometry; Absorptiometry; Spectrophotometry

1. Introduction

According to the AOAC standard method for the analysis of beer quality [1], various determinations are involved, namely for physical properties: color, total haze, refractive index, calories content, total acidity, foam collapse rate, beer bitterness,

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* Corresponding author. Present address: Faculty of Science, Department of Chemistry, Chiang Mai University, Chiang Mai50200, Thailand. Tel.: +66-53-943-345x157; fax: +66-53-222-268

E-mail address: kate@chiangmai.ac.th (K. Grudpan).

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and for chemical assays: iron, copper, calcium, carbon dioxide, sulfur dioxide, alcohol, ethanol, glycerol, protein, starch, and proteolytic chill-proofing enzymes.

Iron influences beer quality such as taste [2], haze, the growth of yeast, the foaming quality, the flavor enhancement [3], and exhibiting of antioxidant activity [4]. Iron in beer is thus an important trace element to be assayed.

A standard method for the determination of iron in beer using conventional spectrophotometry based on complexation to 1,10-phenanthroline or 2,2′-bipyridine [1] is time consuming, with high chemical consumption and is tedious. Higher background from beer color (golden brown or black beer) and relatively high detection limits are found to be the drawbacks of the method.

Although using electrothermal atomic absorption spectrometry will improve sensitivity in terms of limit of detection [3], disadvantages of this method include relatively more expensive instrumentation, skill of operator required, matrix chemical modifiers needed, and complicated temperature programming.

Using a flow injection (FI) in-valve column-flame atomic absorption spectrometry (FI-FAAS) [2], flame interference by the matrix can be reduced by introducing low quantities of sample (200 μl of sample injection), and the problem of burner clogging could be overcome. Iron and some other elements such as Ag, Cd, Cu, In, Mn, Pb, Tl, and Zn were determined using an integrated-atom-trap system mounted on a standard atomic absorption air–acetylene flame burner [5]. Based on ion-exchange separation and size exclusion chromatography, copper, iron, and manganese in beer were investigated using electrothermal atomic absorption spectrometry [3].

Column techniques have been extensively utilized for metal preconcentrations. Even a conventional cation exchange resin used for online preconcentration of iron with spectrophotometric detection was described [6]. An iminodiacetate chelate resin such as Chelex-100 and Muromac A-1 played the role of high efficient solid-phase extractant for transition metals [7–9]. In addition, other packing materials in a sorption mechanism have been used for iron preconcentration; for instance, resin-immobilized 8-hydroxyquinoline [10], a cationic exchanger paper [11], C_{18}-bonded silica with 5,7-dichlorooxine [12], and Amberlite XAD-4 functionalized by *N*-hydroxyethylethylenediamine [13].

An in-valve column exhibited practical and convenient use for loading and eluting for metal preconcentrations [14–16]. Flow-based analysis with bead injection (BI) described as a renewable microcolumn has been applied for many detection systems such as amperometry [17], fluorescence [18–20], and UV–visible absorptiometry [21]. BI spectroscopy exhibited outstanding usefulness in terms of a renewable, small, and disposable sensing layer [22]. A simple FI-spectrophotometric system with bead injection (FI-BI) has recently been achieved for trace iron determination in water samples [23].

In this study, an FI-FAAS system with in-valve column, a spectrophotometric FI system with a column placed at the detection point, and an FI-BI system were investigated. The FI-FAAS and FI-BI systems were found to be useful for online separation and preconcentration of iron in beer so that the sensitivity and selectivity would be improved with possibility for automation. The in-valve column FI was coupled to flame atomic absorption spectrometry and a conventional cationic exchange resin was utilized. FI-BI was used for absorptiometry using 1,10-phenanthroline in conjunction with a renewable microcolumn packed with chelating resin.

2. Experimental

2.1. Materials and reagents

Two types of packing materials, conventional cationic exchange resin, Dowex 50W X8, 50–100 mesh (Fluka) and iminodiacetate chelating resin, Chelex-100, 50–100 dry mesh or 16–50 wet mesh (Bio-Rad) were used. By sieving the chelating resin, beads in the range 35–50 wet mesh were employed. For the FI system with in-valve column, the former was chosen in accordance with commonly used and inexpensive resins. The latter was used for both FI with column at detection

point and FI-BI due to its transparent characteristic.

Acetate buffer solution, 0.1 M, was prepared by dissolving 3.85 g ammonium acetate in water and adjusting to pH 4.5 by adding 2 ml of glacial acetic acid. Iron(III) stock solution, 1000 μg ml^{-1}, was prepared by dissolving 0.8634 g $NH_4Fe(SO_4)_2 \cdot 6H_2O$ (Merck) in water and adding 1 ml 4.5 M H_2SO_4 before making up to a volume of 100 ml with water.

Iron(II) stock solution, 1000 μg ml^{-1}, was obtained by dissolving 0.7020 g $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (BDH) in water and adding 1 ml 4.5 M H_2SO_4, then making up to volume of 100 ml with water.

Ascorbic acid 10% (w v^{-1}), using L-ascorbic acid (Merck) was freshly prepared. 1,10-Phenanthroline, 0.3% (w v^{-1}) in the acetate buffer solution was prepared by using solid chemical (Merck).

2.2. Sample preparations

Local commercial beer samples were taken for analysis. A sample was transferred into a larger container for decarbonation by stirring for 1 h. A portion of ascorbic acid solution (10%, w v^{-1}) was added to a known volume of the sample to give a final concentration of 1000 μg ml^{-1} ascorbic acid. A series of solutions for standard addition measurements for a sample was made to contain 0, 0.1, 0.25, 0.5 and 1.0 μg ml^{-1} Fe(II) standard added.

2.3. FI-FAAS with in-valve column

An acrylate column (0.3 mm i.d. and 40 mm long) was used in place of an injection loop in a 6-port valve (V-451, Upchurch). The column was packed with Dowex 50W X8 resins having Teflon wool as frits at both the ends.

As depicted in Fig. 1, the FI system with in-valve column consists of a peristaltic pump (FIAlab, USA), a 6-port valve, and a flame atomic absorption spectrometer (AA-670, Shimadzu, Japan). The FAAS conditions followed those recommended by the manufacturer with some adjusted parameters (burner height, ratio of fuel, and oxidant). After pumping water for conditioning the column, the three-way valve (V1) was switched to let standard/sample load onto the column at a certain loading flow rate and for a desired loading time. When reaching the duration of loading, V1 was switched back to the position that allowed water to flow in order to wash the column. By switching the 6-port valve (V2), sorbed iron will be released from the column by the eluent, countercurrent to the loading step. Opposite flow direction of loading and elution avoids problems due to tendency of clogging, which would affect column performance, as well as maximizes preconcentration [15]. The series of the standard addition set of solutions for a sample prepared as described earlier was injected in the FI manifold (Fig. 1) by employing the conditions in Table 1.

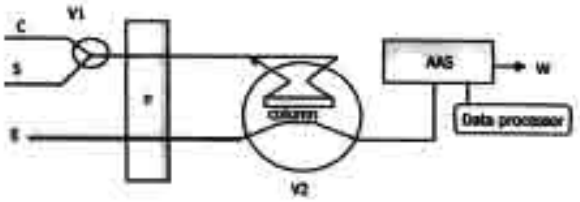

Fig. 1. Manifolds with in-valve column for FAAS; C = carrier (water or acetate buffer pH 4.5), S = standard or sample, E = eluent, P = peristaltic pump, AAS = atomic absorption spectrometer, V1 = three-way valve, V2 = 6-port valve, W = waste.

2.4. FI spectrophotometric system with bead injection

The instrumental setup for FI-BI was similar to that previously described [23]. Modification was made by inserting a three-way valve (V1) (Fig. 2) to get rid of pulsating and fluctuating signals caused by back-pressure. The peak-hold colori-

Table 1
Proposed method for FI system with in-valve column

Conditions	Studied range	Suggested value
Loaded solution (M HNO_3)	0.05–1.00	0.05–0.15
Eluent (M HNO_3)	1–6	4
Eluent flow rate (ml min^{-1})	3–8	4
Loading time (min)	15 s–10 min	15 s–10 min
Loading flow rate (ml min^{-1})	2–8	2–8

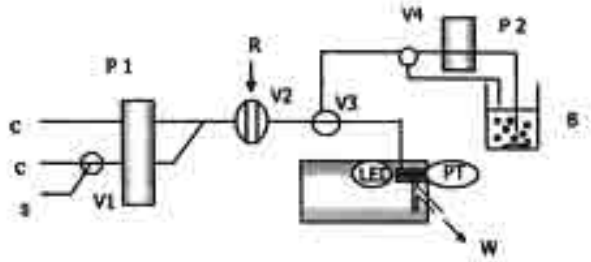

Fig. 2. FI-BI system; C = carrier (acetate buffer pH 4.5), S = standard or sample, P1 and P2 = peristaltic pumps 1 and 2, B = bead suspension, R = 1,10-phenanthroline, LED = light emitting diode, PT = phototransistor, V1, V3 and V4 = three-way valves 1, 3, and 4, V2 = 6-port valve, W = waste.

meter has also been modified so that a longer period (up to 5 min) for the maximum height of the output signal could be held for reading. After introducing the bead suspension at 16 ml min^{-1} for 15 s, acetate buffer as a carrier was propelled at 3 ml min^{-1} for 30 s to place beads into the flow cell. A standard/sample solution was pumped at 3 ml min^{-1} for a certain time. The beads in the cell were washed with the carrier, before 1,10-phenanthroline solution (0.3%, w v^{-1}) was injected via the injection valve V2 [23]. Signal due to increase in the color intensity of the Fe(II)–1,10-phenanthroline complex was monitored. Finally, the beads were discarded out of the cell. The set of standard addition solutions for a beer sample (described above) was analyzed by using a loading time of 30 s with a flow rate of 3 ml min^{-1}.

3. Results and discussion

3.1. Optimization of FI system with in-valve column (FI-FAAS)

Using the manifold in Fig. 1 and the range of conditions in Table 1, acidity of the loading iron(III) solution and of the eluent were studied, as summarized in Figs. 3 and 4. Lower acidity was suitable for loading, while higher acidity was proper for eluting conditions. This agrees with previous findings for the relationship of distribution ratio (D) and acidity [24]. The effect of eluent flow rate was found to be similar to the previous report [15] that a flow rate of eluent higher than an

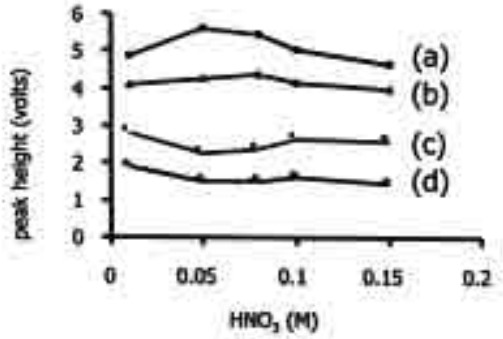

Fig. 3. Effect of acidity of loading solution for (a) 3 μg Fe, (b) 2 μg Fe, (c) 1 μg Fe, and (d) 0.5 μg Fe.

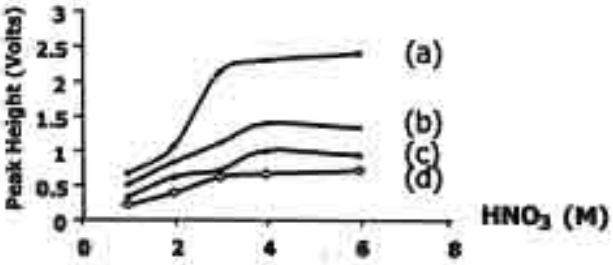

Fig. 4. Effect of acidity of eluent for (a) 1.2 μg Fe, (b) 0.4 μg Fe, (c) 0.08 μg Fe, and (d) blank.

uptake rate of FAAS (3.5 ml min^{-1}) would provide better signals. An eluent flow rate of 4 ml min^{-1} should be employed. Using the combined set of conditions in Table 1, single standard calibration could also be successfully applied (see Fig. 5). The maximum volume for sample loading was 80 ml. The detection limit was found to be 0.1 μg Fe (1.2 ng ml^{-1}; 80 ml loading).

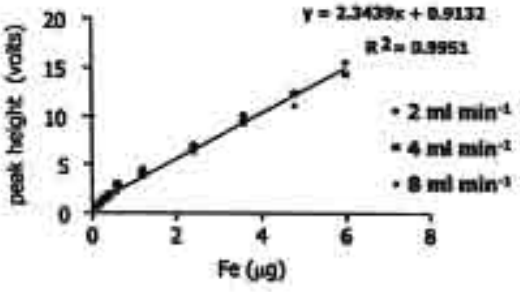

Fig. 5. Single standard calibration for FI-FAAS by volume-based loading (various time, and flow rate).

3.2. Spectrophotometric FI system with a column placed at detection point

A spectrophotometric FI system should also be an alternative for simple instrumental iron determination. Such a system was, therefore, investigated. In order to decrease the degree of dispersion of sorbed iron from elution, a resin was placed at the detection point in the spectrophotometer instead of at the injection valve as previously described. A glass column (3 mm i.d. × 40 mm long) was inserted at the position of the cell holder of a spectrophotometer (Spectronic 21, Spectronic Instruments, USA). The column was packed with the transparent chelating resin, Chelex-100. The chelating resin was expected to retain the iron complex. After passing a solution containing iron through the column (loading), 1,10-phenanthroline solution was injected via the injection valve into the flow, and the reagent formed a complex with the iron. Intensity of the colored complex (red) was continuously monitored at 505 nm.

Some eluents, namely solutions of NaCl (0.1 and 1 M), HNO_3 (2 M), EDTA (1×10^{-3}, 1×10^{-2}, and 1×10^{-1} M), and EDTA in 2 M HNO_3 (1×10^{-3}, 1×10^{-2}, and 1×10^{-1} M) were tried for eluting the complex from the column. None of the eluents was found to be suitable. The (sorbed) iron–1,10-phenanthroline complex retained very strongly on the column (see Fig. 6). Although HNO_3 (2 M) could elute free iron ions sorbed on Chelex-100 as reported previously [9], it is not an effective eluent to desorb the iron–1,10-phenanthroline complex. Even using another eluent, EDTA, which forms a more stable complex with iron(II) (the overall stability constant, $\log \beta = 21.3$ [25]) than iron(II)–1,10-phenanthroline (having $\log K_1 \approx \log \beta = 14.33$), the complex cannot be removed from the column.

3.3. Flow injection spectrophotometric system with bead injection

To overcome problem in not having a suitable eluent as described above, the FI-BI system (Fig. 2) with similar conditions for the determination of iron in water samples [23] was adopted for the determination of iron in beer. Beads were loaded into a cell for 15 s with 1.5% bead (Chelex-100) suspension at a flow rate of 16.0 ml min^{-1}.

Single standard calibrations were obtained by volume-based sample loading at two flow rates (3 or 6 ml min^{-1}) with the period of time 5–20 min or 150 s–10 min, respectively (Fig. 7). A detection limit (3σ) [26] was found to be 0.01 μg or 0.2 ng ml^{-1} with 60 ml sample loading.

3.4. Interference study

It was found in preliminary studies using iron spiked beer when applying without any sample preparation, very low recoveries, 10 and 2%, were obtained by using FI-FAAS and FI-BI, respectively. Non-retained iron from the column of FI-FAAS was found to be 90%. This indicated interference from sample matrices.

Some metal ions (Na^+, K^+, Ca^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Mg^{2+}) and some organic compounds (ethanol, cysteine and gallic acid) were studied for interference and found to have no effect in the range of concentrations presented in beer. Tannic acid was found to affect strongly the sorption efficiency of iron onto cationic exchange resin. There was a report [27] that gallic acid can be used as a standard for a spectrophotometric determination of tannic acid. Iron(II) and iron(III) can form complexes with both gallic acid and tannic acid at pH 4.5. Both complexes absorb at 595 nm. The former retains on the cation exchanger column whereas the latter does not. Iron–gallic acid complex could present in positively charged form [28] while iron–tannic acid complex should exhibit negative charge [3]. In this work, tannic acid cannot be replaced by gallic acid to represent an interference study by tannic acid.

Mixtures of ascorbic acid, 1000 μg ml^{-1}, and iron solutions spiked in beers resulted in better recoveries of iron: 30–45 and 60–66% for FI-FAAS and FI-BI, respectively. Using hydroxylamine could also yield 65% recovery of iron in the FI-BI procedure. Ascorbic acid could reduce iron(III) and/or tannic acid. Also ascorbic acid could possibly replace EDTA and form complex with iron [29].

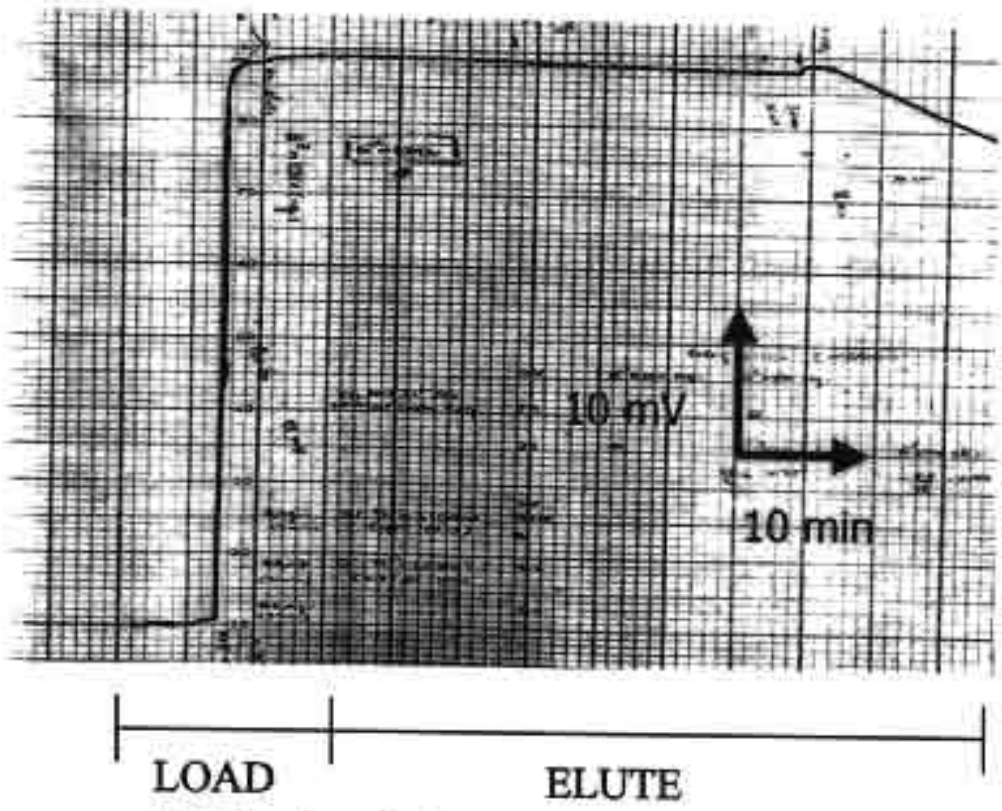

Fig. 6. Absorption profile of iron–1,10-phenanthroline (1 μg Fe ml^{-1}).

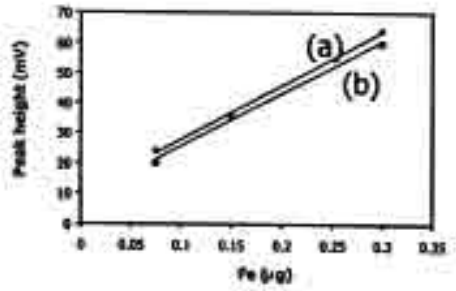

Fig. 7. Single standard calibration for FI-BI using iron(II) standard solution, 5 μg ml^{-1}: (a) at 6 ml min^{-1} for various loading times (2 min 30 s, 5 min, and 10 min); (b) at 3 ml min^{-1} for various loading times (5, 10, and 20 min).

In the FI-FAAS system, both Fe(III) in 0.1 M HNO_3 and Fe(II) in acetate buffer resulted in similar sorption efficiencies by the resin column. Tannic acid affected both iron(II) and iron(III) similarly. Therefore iron(II) can be used as standard iron for FI-FAAS method.

3.5. Determinations of iron in beer samples

Following the procedure for sample preparation, five beer samples were analyzed by the proposed FI-FAAS and FI-BI methods. The AOAC standard method [1] was also performed. All the methods were employed by using standard addition. Graphs of the standard addition method were plotted, of peak height (mV) or absorbance versus iron added (μg ml^{-1}). Iron contents in beer samples were calculated by extrapolating. The results are summarized in Table 2. The results obtained by the proposed FI-FAAS and FI-BI for traces of iron agree reasonably well with those of the AOAC method, being within tolerances required for the beer analysis. The color of a sample would affect the results obtained by the AOAC method because the absorbance measurement was performed using the whole solution while the resin column in the FI-BI suppresses the contribution

Table 2
Determination of iron contents in beer samples by FI and AOAC standard methods (all using standard addition)

Beer sample	Iron content (μg ml^{-1})		
	FI-BI	AOAC	FI-FAAS
Singha (Thailand)	0.089	0.067	0.103
Heineken (France)	0.111	0.085	0.086
Amstel (Holland)	0.021	0.035	0.039
Beck's (Germany)	0.130	0.101	0.103
Black Beer (Thailand)	0.071	0.174	0.117

due to the sample color. Both FI-FAAS and FI-BI can serve as alternatives for the assay of the iron content in beer. Although standard addition is still to be performed, in comparison to the AOAC spectrophotometric method [1], the procedures required much smaller volumes/amounts of sample and reagents. The operation time for an analysis is also reduced (24 and 30 injections h^{-1} for FI-FAAS and FI-BI, respectively). Less glassware is used. Both procedures offer a very cost-effective way to determine trace iron.

4. Conclusion

Three FI systems for trace iron determination were investigated: FI-in-valve column-FAAS, spectrophotometric FI with a column placed at the detection point, and FI-BI. FI-FAAS and FI-BI can be alternative procedures to assay iron contents in beer, which are cost-effective, rapid, and sensitive, especially in comparison with the AOAC standard method. Interference due to tannin is suppressed by adding ascorbic acid. The procedures incorporate standard addition technique.

Acknowledgements

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Sequential injection analysis with dynamic surface tension detection
High throughput analysis of the interfacial properties of surface-active samples☆

Narong Lenghor [a], Kate Grudpan [a], Jaroon Jakmunee [a], Bethany A. Staggemeier [b], Wes W.C. Quigley [b], Bryan J. Prazen [b], Gary D. Christian [b], Jaromir Ruzicka [b], Robert E. Synovec [b,*]

[a] *Department of Chemistry, Faculty of Science and Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*

[b] *Department of Chemistry, Center for Process Analytical Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA*

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Abstract

A sequential injection analysis (SIA) system is coupled with dynamic surface tension detection (DSTD) for the purpose of studying the interfacial properties of surface-active samples. DSTD is a novel analyzer based upon a growing drop method, utilizing a pressure sensor measurement of drop pressure. The pressure signal depends on the surface tension properties of sample solution drops that grow and detach at the end of a capillary tip. In this work, SIA was used for creating a reagent concentration gradient, and for blending the reagent gradient with a steady-state sample. The sample, consisting of either sodium dodecyl sulfate (SDS) or poly(ethylene glycol) at 1470 g mol^{-1} (PEG 1470), elutes with a steady-state concentration at the center of the sample plug. Reagents such as Brij®35, tetrabutylammonium (TBA) hydroxide and β-cyclodextrin were introduced as a concentration gradient that begins after the sample plug has reached the steady-state concentration. By blending the reagent concentration gradient with the sample plug using SIA/DSTD, the kinetic surface pressure signal of samples mixed with various reagent concentrations is observed and evaluated in a high throughput fashion. It was found that the SIA/DSTD method consumes lesser reagent and required significantly less analysis time than traditional FIA/DSTD. Four unique chemical systems were studied with regard to how surface activity is influenced, as observed through the surface tension signal: surface activity addition, surface activity reduction due to competition, surface activity enhancement due to ion-pair formation, and surface activity reduction due to bulk phase binding chemistry.

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* Corresponding author. Tel.: +1-206-685-2328; fax: +1-206-685-8665.
E-mail address: synovec@chem.washington.edu (R.E. Synovec).

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Keywords: Sequential injection analysis; Dynamic surface tension detector; Concentration gradient

1. Introduction

Multidimensional dynamic surface tension detection (DSTD) is based upon measuring drop pressure with a pressure sensor as a function of time during the formation of repeating drops with continuous flow [1–5]. Hence, DSTD measures the pressure across the air–liquid interface of drops that repeatedly grow and detach from the end of a capillary tip. The pressure signal is dependent upon surface tension properties of the solution. DSTD was first used with flow injection analysis (FIA) [1] for studying the characteristics of industrially relevant samples. A variety of useful information on dynamic surface tension was provided, such as the surface tension at the liquid–air interface and adhesion at the solid–liquid interface, for the rapid characterization of complex samples. DSTD has been coupled with hydrophobic interaction chromatography for determination of surface-active species in protein formulations [2]. A technique for calibration of dynamic surface tension by application of the Young–Laplace equation was recently created [3] facilitating the ease of application to FIA and HPLC. That work demonstrated that FIA/DSTD has promise as an investigative tool for the study of complex biological and industrial samples. The calibration procedure has been subsequently improved for rapid on-line calibration of DSTD [4]. The on-line calibration procedure is utilized in this paper. The new calibration procedure enables the measurement of a given surfactant solution in a flowing system without the need for optical measurements. These improvements to DSTD increase its potential as a detector for flow-based analytical techniques such as FIA and HPLC.

Because nearly all practical applications of surfactants involve the use of surfactant mixtures, the study of mixed surfactant solutions is of great interest [6–12]. In previous work, DSTD was used with FIA to study the interfacial properties of various surface-active analytes in mixtures [5]. A large volume of carrier (mobile phase) was needed for these experiments due to the continuous flow nature of the FIA technique. Also, individual concentrations of analytes were separately injected and processed, which was time consuming. From an analytical perspective it is desirable to further improve the implementation of DSTD to provide the desired analytical information while reducing the carrier (mobile phase) consumption and reducing analysis time. To this end, we report herein the integration of sequential injection analysis (SIA) with DSTD to study the interfacial properties of surface-active analytes in various concentrations of reagents. A concentration gradient of reagent was generated using SIA. In concert, a steady-state concentration of surface-active analyte was injected to blend with the reagent concentration gradient. Hence, a single concentration of analyte in various concentrations of reagent was achieved, analogous to running several reagent-to-analyte concentration blends in a relatively short period of time. Only a single concentration of reagent and sample was prepared instead of multiple concentrations as done with the typical FIA approach. In this manner, time, reagents, waste, and steps of data processing were reduced.

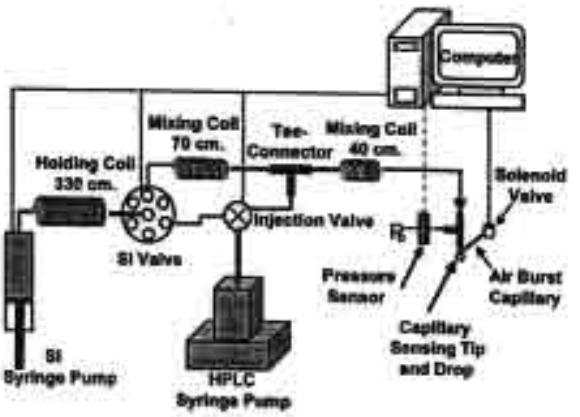

Fig. 1. The schematic diagram of SIA system with the dynamic surface tension detector.

To demonstrate the attributes of the SIA/DSTD system (Fig. 1), the concentration gradient of reagent was made using the SIA system, and the steady-state concentration of surface-active sample was injected to blend with the reagent gradient. A concentration gradient in the range of zero to the prepared concentration occurred by the SIA dilution phenomenon. After the blending of the reagent and sample (analyte) zones at a junction, the on-line mixture was blended and dispensed to DSTD to detect the surface pressure of the mixture. In this work, four unique chemical systems were selected and studied. A surface tension addition effect will be seen with a sodium dodecyl sulfate (SDS) sample mixed with a Brij®35 reagent gradient. A competition surface tension effect will be observed with a poly(ethylene glycol) at 1470 g mol^{-1} (PEG 1470) sample and a Brij®35 reagent gradient. A surface tension enhancement effect will be reported with an SDS sample combined with a tetrabutylammonium (TBA) hydroxide reagent gradient. Finally, a binding effect is observed with the SDS sample and a β-cyclodextrin (β-CD) reagent gradient. All experiments consumed lesser reagent and generated less waste, while requiring significantly less analysis time than traditional FIA/DSTD.

2. Theory

DSTD is a growing drop method utilizing a pressure sensor. The pressure signal measured is dependent on the surface tension properties of a sample solution drop that grows at the end of a capillary tip. The pressure sensor measures the internal pressure of a growing drop relative to atmospheric pressure as described in previous work [4]. Surface pressure measurements were related by the Young–Laplace equation. The time-dependent Young–Laplace equation [3] is shown in Eq. (1):

$$P(t) = \frac{2\gamma(t)}{r(t)} + P_C, \quad (1)$$

where P is the pressure signal, γ the surface tension, r the drop radius, and P_C accounts for viscous losses in the capillary tubing and the relative position of the sensor from the capillary tip. Note that P, γ, and r are all a function of time, t. Pneumatic detachment is used to remove the drops from the sensor capillary tip before gravity elongates the drop and reduces the applicability of the Young–Laplace equation [4,5]. Drops are detached in a size regime in which they are essentially spherical so one radius of curvature is sufficient to apply the Young–Laplace equation. The pneumatic detachment method will be explained in Section 3. The calculation of the dynamic surface pressure from the measured pressure signals has also been described in previous work [4,5]. First, the dynamic surface pressure of an analyte, $\pi(t)_A$, can be defined as the surface tension of an analyte, $\gamma(t)_A$, subtracted from the surface tension of the carrier (mobile phase), $\gamma(t)_M$. Thus, by application of Eq. (1), the dynamic surface pressure of an analyte in the mobile phase can be shown to be

$$\pi(t)_A = \gamma(t)_M - \gamma(t)_A = \frac{r(t)[P(t)_M - P(t)_A]}{2}. \quad (2)$$

To calibrate DSTD, a standard solution with a known surface tension is measured to collect the $P(t)_S$ signal. So, an equation similar to Eq. (2) can be written for a standard solution $\pi(t)_S$. Finally, the dynamic surface pressure of an analyte and standard solution can be related to the drop pressure data as shown in Eq. (3):

$$\pi(t)_A = \frac{\pi(t)_S[P(t)_M - P(t)_A]}{P(t)_M - P(t)_S}. \quad (3)$$

By using Eq. (3), the dynamic surface pressure of an analyte can be obtained from the drop pressure, $P(t)$ profile data from the mobile phase, M, the analyte, A, and the standard solution, S, of known surface pressure, $\pi(t)_S$. The high reproducibility of the experimentally determined surface pressure plots obtained using DSTD, either with or without kinetic effects observed, indicates that radius growth is very reproducible and thus Eq. (3) can be used to factor out the radius dependence term, $r(t)$. In previous work, we rigorously evaluated the time dependence of $r(t)$ for the mobile phase, typical analyte solution, and standard solution.

The results of the study indicated that $r(t)$ was essentially a constant function under a broad range of experimental conditions employed using pneumatic drop detachment [5]. In this regard, the role of convection is also factored out in the comparison of pressure signals of mobile phase, standard, and sample drops. On the other hand, convection is taking place with DSTD similar to the dropping mercury electrode (DME) experiment. While with DSTD convection of either analyte or standard occurs from within the growing drop and with the DME convection occurs from outside the growing drop, in both experiments useful diffusion and adsorption information is obtained in the presence of convection, since the convective process is reproducible.

Water was used as mobile phase, and 5% glacial acetic acid solution with surface pressure of 10.3 dyne cm^{-1} [13] was used as standard solution since it has a constant surface tension throughout drop growth [4]. Fig. 2A shows the drop pressure profile data of the mobile phase, $P(t)_M$, standard solution, $P(t)_S$, and analyte, $P(t)_A$. For this example, 3 mM SDS was used as the analyte. The three profiles shown in Fig. 2A were then used to calculate the dynamic surface pressure of 3 mM SDS as shown in Fig. 2B by application of Eq. (3). The resulting surface pressure plot in Fig. 2B for SDS is constant over the drop growth, indicating that the SDS surface activity is a fast process with no kinetic hindrance [5]. This rapid, on-line calibration technique enables the measurement of surfactant solutions in a flowing system without the need of cumbersome optical measurements and without the need for independently measuring the pressure offset, P_C.

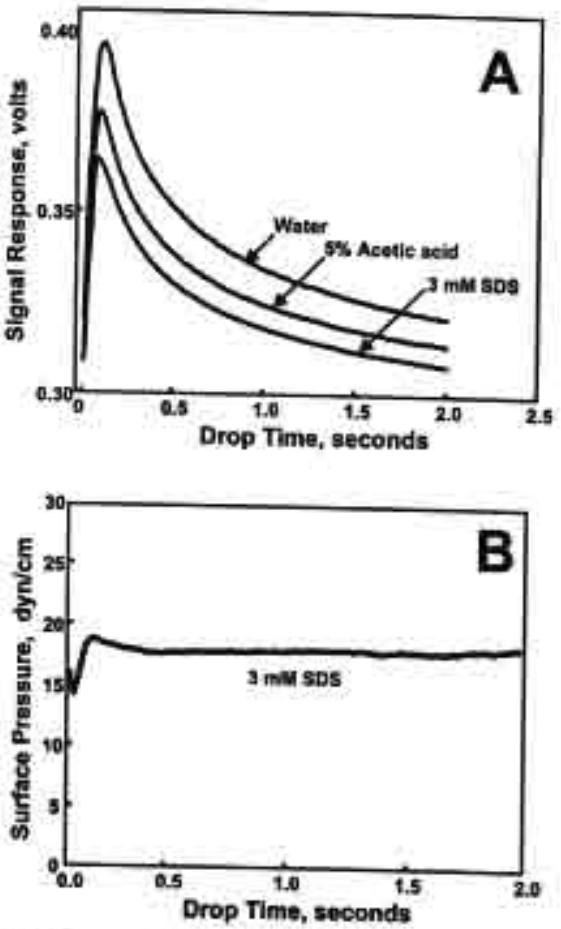

Fig. 2. Drop profile pressure data and application of the calibration procedure. (A) Overlay plots of individual drop profiles of water (mobile phase carrier), 3 mM SDS solution (analyte), and 5% (w/w) acetic acid (standard). (B) The dynamic surface pressure plot of 3 mM SDS solution after application of Eq. (3) to the drop profile data in (A).

3. Experimental

3.1. Materials

SDS (98% purity) and Brij®35 were obtained from Aldrich (Aldrich Chemical Co., Milwaukee, WI). Freshly prepared SDS solutions were used without further purification. SDS solutions handled by this procedure were previously found to produce DSTD surface pressure results that agreed with the literature [4]. PEG with an average molar mass of 1470 g mol^{-1} ($M_n = 1470$, $M_w/M_n = 1.02$) was obtained from Polymer Laboratories (Amherst, MA). Tetrabutylammonium hydroxide was obtained from Dionex (Dionex Corporation, Sunnyvale, CA). Acetic acid (glacial), of reagent grade quality, was obtained from Baker (J.T. Baker, Inc., Phillipsburg, NJ). All chemicals were used as received. Deionized (DI) water, demineralized to greater than 18 MΩ with a Millipore system (Millipore, Bedford, MA), was used in the preparation of all solutions. The water was degassed by using an ultrasonic bath (Cole-

Parmer Instrument Company, Vernon Hills, IL) for 15 min prior to use.

3.2. Instrumentation

A schematic diagram of the sequential injection dynamic surface tension detector (SIA/DSTD) analyzer is shown in Fig. 1. The SIA/DSTD system operates with three major operational stages. First, the SIA system produces the reagent concentration gradient and loads the sample solution into the sample loop. Second, the sample introduction system injects a plug of sample into the reagent concentration gradient. Last, DSTD collects dynamic surface tension information on each drop eluting from the system. A detailed diagram of DSTD can be seen in previous reports [4,5].

The SIA system employed consisted of a syringe pump (XP-3000 Syringe Pump with a valve; Cavro, San Jose, CA) and a six-port Lab on valve (FIA Lab Instrument, Bellevue, WA). For the flow lines, PTFE tubing of 0.76 mm i.d. and 1.59 mm o.d. was used. The holding coil was 330 cm and the two mixing coils were 70 and 40 cm. The first mixing coil was used for mixing the reagent concentration gradient and the second was used for mixing the concentration gradient and sample solution together before the mixed solution was detected by DSTD. A Tee connector (P-715; Upchurch, Oak Harbor, WA) was used to overlap the concentration gradient and sample solution before passing through the second mixing coil.

A syringe pump in the sample introduction system (Isco μLC-500; Lincoln, NE) was used to deliver the carrier, i.e. mobile phase (water) and sample solution to the system. Sample solutions were injected with a LabPRO two-position ten-port fluid processor (PR 700-102-01; Rheodyne, Cotati, CA). An injection volume of 500 μl was made from poly(etheretherketone) (PEEK) tubing (Upchurch, Oak Harbor, WA). Sufficient volume was injected so that the sample plug zone was not diluted by the water mobile phase and could be overlapped with the whole reagent concentration gradient zone. In all experiments, this pump was run at 1 μl s^{-1} unless otherwise stated.

In the DSTD portion of the system, the pressure sensor (Validyne P30 SD-20-2369; Northridge, CA) was configured with a sensing membrane (Validyne diaphragm 3-36) that was optimized for the measurements of interfacial kinetics at 120 μl min^{-1} total volumetric flow. The sensor capillary tip was made from a short piece of PEEK tubing, 457 μm i.d. and 635 μm o.d. Pneumatic drop detachment was performed at a rate of 0.5 Hz, corresponding to 2 s (i.e. 4 μl) drops at a volumetric flow rate of 120 μl min^{-1}. A skinner solenoid valve (MBD 002; Skinner valve, New Britain, CT) controlled by a computer did the pneumatic detachment. To record the concentration of any non-surface-active solution, a refractive index detector (HP 1047A; Hewlett-Packard Company, Germany) was used.

Data collection and instrument control were both done with a personal computer (600 MHz Pentium®; Intel Corporation, Santa Clara, CA) equipped with a data acquisition card (AT-MIO-16XE-50; National Instruments, Austin, TX). The data were averaged down to 100 points s^{-1} prior to saving. Data collection and instrument control were completed using LABVIEW (version 6; National Instrument, Austin, TX) with programs written in-house. Subsequent data analysis was performed using MATLAB 6.0 (Math Works, Natick, MA).

3.3. Procedure

The SIA/DSTD system was constructed as shown in Fig. 1. Deionized water was used as carrier solution (mobile phase). A reagent concentration gradient was introduced using the SIA system and passed through the first mixing coil to increase dilution, and then stopped in the front of the Tee connector. The sample was then introduced to the sample loop using the SIA system. After the reagent concentration gradient was achieved and the sample in the loop was ready, the injection valve was switched to inject the sample into the system and the syringe pump was started to push the reagent concentration gradient. The concentration gradient and sample were then overlapped at the Tee connector and transported to the second mixing coil to increase mixing. Finally, the mixed solution was dispensed

Table 1
SIA/DSTD method operations

Steps	SI valve*	Position of syringe pump	Operation	Injection valve position	Commentary
1	–	Valve in	Aspirate: 500 μl	Load	
2	2	Valve out	Dispense: 500 μl	Load	Flushes sample loop
3	5	Valve out	Aspirate: 700 μl	Load	Loads sample into sample loop
4	–	Valve in	Fill syringe	Load	Fills pump with water
5	2	Valve out	Dispense: 700 μl	Load	Fills the sample into loop
6	3	Valve out	Dispense: 300 μl	Load	Pushes solution residue to waste
7	4	Valve out	Aspirate	Load	Loads reagent
8	–	Valve in	Fill syringe	Load	Fills pump with water
9	1	Valve out	Dispense: 200 μl, 20 μl s^{-1}	Load	Makes a reagent dilution gradient
10	–	Valve in	Fill syringe	Load	Fills pump with water
11	1	Valve out	Dispense: 1000 μl, 1 μl s^{-1}	Inject	Detects the surface pressure
12	1	Valve in	Fill syringe	Inject	Fills pump with water
13	1	Valve out	Dispense: 1000 μl, 20 μl s^{-1}	Inject	Flushes system
14					Repeats n times from step 1

* SI valve positions: (1) to detector; (2) to sample loop; (3) to waste; (4) reagent; and (5) sample solution.

to DSTD for surface tension detection. A complete SIA cycle is described in Table 1.

The solution flowed out the end of the tip at a flow rate of 2 μl s^{-1} (each syringe pump was run with a flow rate of 1 μl s^{-1}). The key steps of the procedure are described in Table 1 and summarized here. In steps 1–6, the sample solution is loaded in the 500 μl sample loop by aspiration of 700 μl sample solution into the holding coil with SIA syringe pump. Then, the aspirated sample solution is dispensed to the sample loop. Thus, sample solution in the loop is ready and waiting for the injection step. In steps 7–10, the desired aspiration volume of reagent is aspirated to the holding coil and then the solution in the holding coil is dispensed through the first mixing coil with a flow rate of 20 μl s^{-1} and stopped in front of the Tee connector. Then, in step 11, multiple-drop pressure measurement of the solution is obtained. In this step, the SIA syringe pump with 1000 μl gradient solution at flow rate of 1 μl s^{-1} is started and the injection valve is switched to the injection position simultaneously, to allow the sample solution to overlap with the concentration gradient at the Tee connector. This mixture is then passed through the second mixing coil and then through DSTD. Finally, in steps 12 and 13, flushing of the flow lines is done with 1000 μl of deionized water used to flush the system.

4. Results and discussion

4.1. Kinetic surface pressure behavior of SDS with a Brij®35 concentration gradient

The development of SIA/DSTD for studying the interfacial properties of surface-active analytes and various mixture chemistries was investigated. The developed method was initially applied for the study of the interfacial properties of a surfactant mixture containing SDS and Brij®35. The signal profile results of Brij®35 concentration gradient mixed with an SDS sample are shown in Fig. 3. In Fig. 3A, surface pressure plots at drop detachment of water baseline, 2 mM SDS alone, 200 ppm Brij®35 alone, SIA on-line blending of SDS and Brij®35, and the signal addition of SDS and Brij®35 are shown. In this experiment, SDS elutes as a sample plug and contains a steady-state region in the center of the plug. Brig®35, on the other hand, elutes as a concentration gradient that begins after the SDS plug. Now, discussion of Fig. 3A is facilitated by dividing the plot into four time periods. In the first period, 0–200 s, the signal shows the dynamic surface pressure of water only, since neither the injection plug nor the concentration gradient has reached the detector. In the second period, 200–320 s, the initial SDS injection plug zone passes through the capillary tip and the

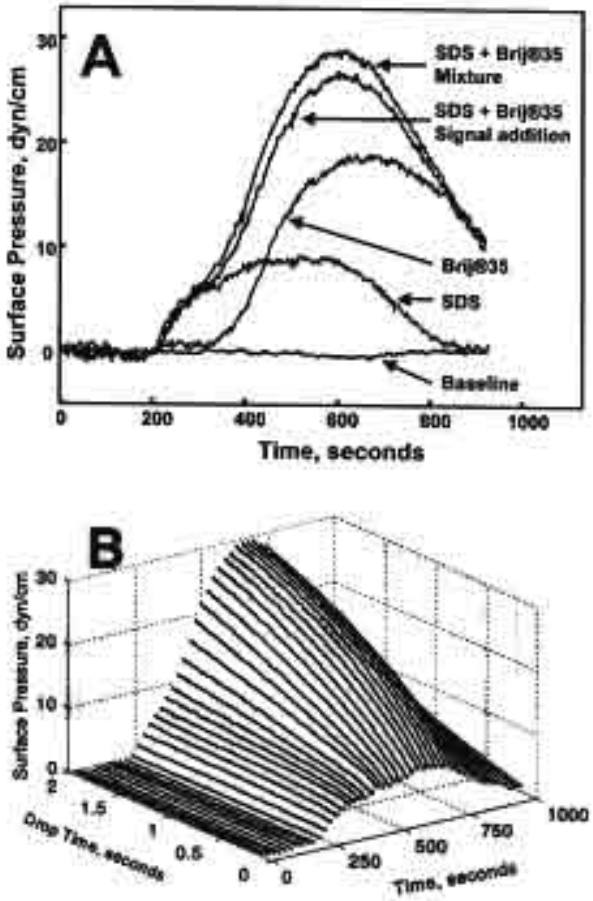

Fig. 3. The dynamic surface pressure plots of a 400 μl, 200 ppm Brij®35 concentration gradient mixed with a 500 μl, 2.0 mM SDS injection plug. (A) The overlay plots of the SIA plots for the average surface pressure at 1.8–2.0 s in the drop profile: baseline—dynamic surface pressure of water as baseline signal; SDS—dynamic surface pressure of SDS injection; Brij®35—dynamic surface pressure of Brij®35 gradient; SDS+Brij®35 mixture—dynamic surface pressure of SDS injection blended on-line with Brij®35 gradient; and SDS+Brij®35 signal addition—dynamic surface pressure of signal addition of individual SDS and Brij®35 signals. (B) Three-dimensional dynamic surface pressure plot of Brij®35 concentration gradient mixed with SDS injection. The drop time axis shows the surface pressure during the growth of individual drops (2 s each). For visual clarity only every tenth drop profile on the elution time axis was plotted.

sensor detects the surface activity of SDS only. In the third period, 320–600 s, the surface pressure plot shows the signal of the mixture, which consists of both the essentially steady-state concentration of SDS and the Brij®35 concentration gradient. In this region, various concentrations of Brij®35 are blended with the 2 mM SDS solution. In the last of the four time periods, 600–900 s, the surface pressure was returning back to baseline, indicating that the injection plug and gradient had both passed. With this particular mixture chemistry, the SDS and Brij®35 blend exhibits an additive effect of surface activity due to the presence of both analytes. That is, the signal of the SDS/Brij®35 mixture mimics that of the SDS signal added to the Brij®35 signal. This additive interaction has been observed in previous work [3] and is one type of interaction that is observed [14] in steady-state surface tension measurements. However, this surface tension interaction has never been observed with a high throughput system that measures multiple concentration combinations of two or more chemical components. The surface pressure at drop detachment of the mixture increased proportionately as the concentration of Brij®35 increased. By blending the steady-state concentration of 2 mM SDS as an injection plug zone with Brij®35, a concentration gradient zone in the concentration range 0–200 ppm is created. The kinetic surface pressure behavior of SDS mixed with various concentrations of Brij®35 is observed in Fig. 3B. The kinetic surface activity signal due to Brij®35 can be clearly delineated in the three-dimensional plot in Fig. 3B. The kinetic signal of Brij®35 becomes more pronounced when the concentration of Brij®35 in the mixture is increased. This behavior and the degree of interaction between SDS and Brij®35 can be explained from the compatibility of their molecular structures. SDS and Brij®35 both contain the same dodecyl hydrophobic chain. As the molecules align at the surface of the liquid–air interface, this hydrophobic region allows them to pack similarly alongside each other. In addition, the electrostatic repulsion forces between the anionic head groups of dodecyl sulfate at the drop liquid–air interface are minimized by the presence of the uncharged Brij®35 polar regions.

4.2. Kinetic surface pressure behavior of PEG 1470 with a Brij®35 concentration gradient

Using the same procedure and conception as the experiment of Brij®35 with SDS, the interfacial

properties of Brij®35 with PEG 1470 were studied. The signal profile results of Brij®35 concentration gradient blending with the PEG 1470 steady-state plug zone are shown in Fig. 4A and B. In Fig. 4A, the overlay plots are shown of surface pressure signal of baseline water, 50 ppm PEG 1470 alone, 100 ppm Brij®35 alone, PEG 1470 blending with the Brij®35 concentration gradient, and the signal addition of the individual Brij®35 and PEG 1470 signals. In this experiment, during the Brij®35 gradient that occured during the 320–600 s time period, the surface tension at drop detachment shows a competition effect between Brij®35 and PEG 1470. The signal for the mixture follows that of PEG 1470 alone until the point in time where the Brij®35 signal alone exceeds the PEG 1470 signal alone. At this point, the mixture signal follows that of Brij®35. The competition effect can be explained by molecular structure [3], since both hydrophobic chain groups are derived from the same PEG. Thus, when Brij®35 is mixed with PEG 1470 together in the system, the hydrophobic chain of Brij®35 (lauryl group) orients at the surface of drops. The kinetic surface pressure behavior of the mixture is shown in the three-dimensional plot in Fig. 4B. The increase in surface pressure during each growing drop shows the kinetic behavior of Brij®35 due to the dilation rate of the drop surface area being faster than the transportation rate of the Brij®35 molecule from the bulk solution to the surface of the drop. The kinetic surface pressure behavior effect of Brij®35 can be further evaluated by raising the concentration of Brij®35. These results are not shown for brevity.

4.3. Kinetic surface pressure behavior of SDS with TBA concentration gradient

The surface pressure plot results from the interfacial properties' study of SDS and TBA solutions are shown in Fig. 5A and B. A 400 μl, 1.0 mM TBA solution was delivered into the system to make the concentration gradient, and 500 μl of 1.0 mM SDS was injected to blend with TBA concentration gradient zone. By blending the two zones, the high throughput experiment region of various concentrations of TBA in 1.0 mM SDS was achieved. TBA alone is essentially non-surface active, as shown in Fig. 5A, and the counter-ion of TBA is hydroxide. SDS is an anionic surfactant, with the dodecyl sulfate (DS) acting as anionic group. When the TBA solution is mixed with the SDS solution in the system, DS and TBA can exhibit a kinetic surface pressure behavior, since

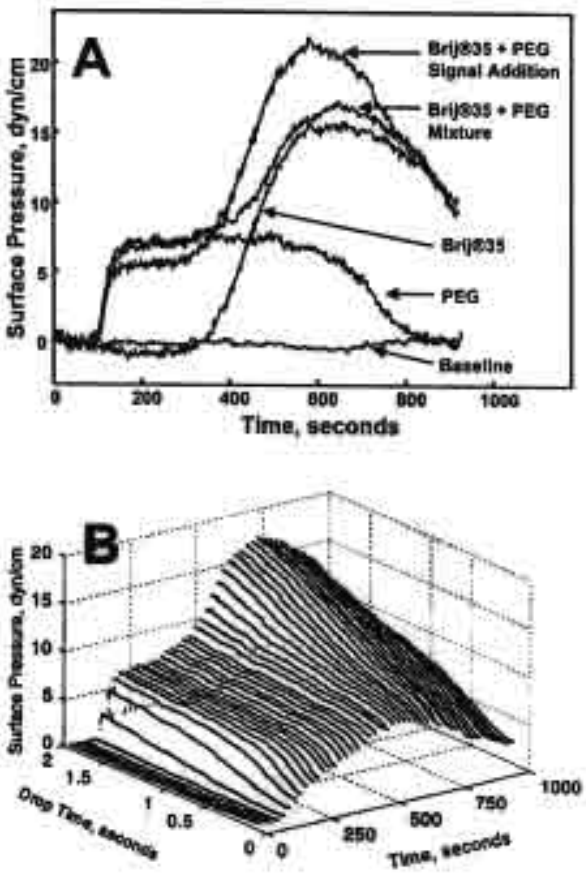

Fig. 4. The dynamic surface pressure plots of a 400 μl, 100 ppm Brij®35 concentration gradient mixed with a 500 μl, 50 ppm PEG 1470 injection plug. (A) The overlay plots of the SIA plots for the average surface pressure at 1.8–2.0 s in the drop profile: baseline—dynamic surface pressure of water baseline signal; PEG—dynamic surface pressure of PEG 1470 injection; Brij®35—dynamic surface pressure of Brij®35 gradient; PEG+Brij®35 mixture—dynamic surface pressure of PEG 1470 injection blended on-line with Brij®35 gradient; and PEG+Brij®35 signal addition—dynamic surface pressure of signal addition of individual PEG and Brij®35 signals. (B) Three-dimensional dynamic surface pressure plot of Brij®35 concentration gradient mixed with PEG 1470 injection. For visual clarity only every tenth drop profile on the elution time axis was plotted.

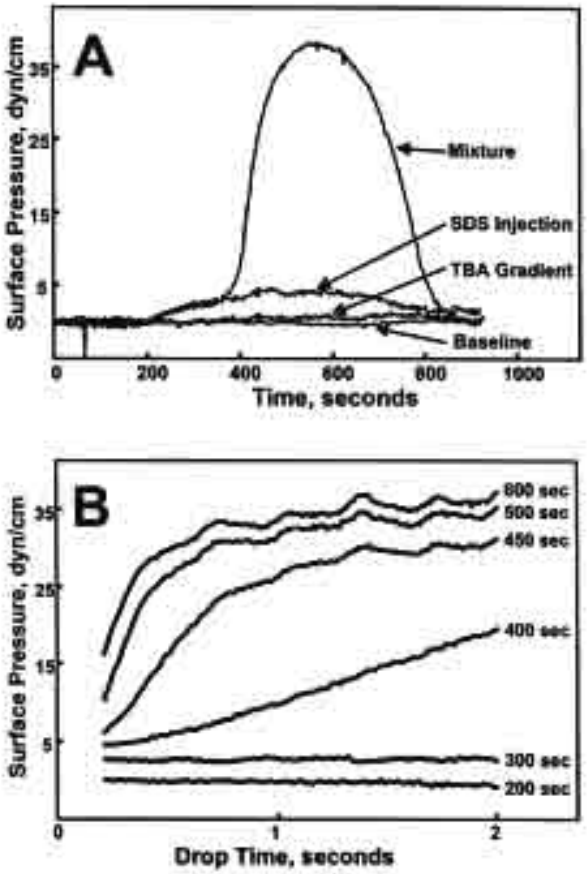

Fig. 5. Dynamic surface pressure plots of a 200 μl, 2.0 mM TBA concentration gradient mixed with a 500 μl, 1.0 mM SDS injection plug. (A) The overlay plots of the SIA plots for the average surface pressure at 1.8–2.0 s in the drop profile: baseline—dynamic surface pressure of water as baseline signal; TBA gradient—dynamic surface pressure of TBA gradient; SDS injection—dynamic surface pressure of SDS injection; and mixture—dynamic surface pressure of SDS injection mixed with TBA gradient. (B) Dynamic surface pressure plots for various elution times of SDS injection mixed with TBA gradient shown in (A), for elution times at 200, 300, 400, 450, 500, and 600 s.

an ion-pair of DS can form with TBA, similar to the other coordination surfactant systems. The enhancement of the surface activity of SDS by TBA was shown, in the time period between 350 and 600 s, in Fig. 5A. As the TBA concentration increases in 1.0 mM SDS solution, the surface pressure of each drop increases considerably. The change in the signal as the TBA concentration increases is shown in Fig. 5B. The SDS alone does not show the kinetic signal. The mixture of SDS and TBA does give a kinetic signal. The kinetic signal of the mixture is seen in the time window as the DSTD drop grows because of the higher packing of surfactant, DS, with TBA at the surface. Higher packing of SDS when TBA is present is indicated by the increase in surface pressure at drop detachment. Because the equilibrium surface concentration is higher when TBA is present, it takes longer to reach the equilibrium surface pressure [5]. The enhancement effect can be increased when the concentration of TBA is increased with time, as shown in both Fig. 5A and B.

4.4. Kinetic surface pressure behavior of SDS with a β-CD concentration gradient

Fig. 6 illustrates the surface pressure profiles of SDS alone, an on-line blending of SDS with a concentration gradient of β-CD, and the refractive index response of the β-CD concentration gradient alone. Since β-CD has no surface activity, DSTD was replaced with the refractive index detector to record the changes in β-CD concentration. The SDS profile shows the 500 μl, 3 mM SDS injection plug zone, and the β-CD profile shows the concentration gradient of 400 μl, 3 mM β-CD. When SDS and β-CD were mixed together in the system, it was shown that the surface pressure of the mixture decreases with an increase in β-CD concentration. This demonstrates that β-CD and SDS form an inclusion complex, and the inclusion complex does not respond to DSTD. When β-CD was present with SDS, the inclusion complex formed remains in the bulk drop solution. Thus, some fraction of the surfactant was essentially removed from the liquid–air interface. As seen in Fig. 6, as the β-CD concentration increases, the larger is the decrease in the surface activity of SDS present in the bulk solution. The surfactant/β-CD inclusion complex has been described in previous work [15–21]. It is well known that β-CD easily complexes with dodecyl sulfate, binding with a 1:1 mole ratio. β-CD is a cyclic oligosaccharide consisting of seven glucose units, and has a lipophilic cavity. When the surfactant binds with β-CD, methylene groups of a fully extended

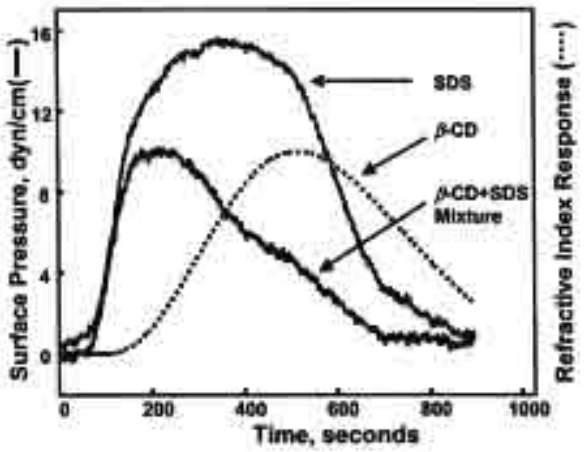

Fig. 6. The surface pressure overlay plots of a 400 μl, 3.0 mM β-CD concentration gradient mixed with a 500 μl, 3.0 mM SDS injection plug. The surface pressure signal from DSTD is shown as a solid line and the refractive index response from a refractive index detector is shown as a dashed line. The overlay plots of the DSTD data are for the average surface pressure at 1.8–2.0 s in the drop profile: SDS—surface pressure of SDS injection; β-CD—refractive index response of β-CD gradient; and β-CD + SDS mixture—surface pressure of SDS injection mixed with β-CD gradient.

hydrocarbon chain of surfactant are included in the cavity [19], thus, masking its surface hydrophobic character from exhibiting surfactant properties. From data, as shown in Fig. 6, one can readily extract the binding constant for the chemistry involved. In this case, the binding constant with dodecyl sulfate and β-CD was determined to be approximately 2500 M^{-1}. This method can be used in a general way to do drug binding studies with analytes such as proteins. Studies such as these are underway in our laboratory.

5. Conclusions

The study of interfacial properties of SDS in a Brij®35 gradient, PEG 1470 in a Brij®35 gradient, SDS in a TBA gradient, and SDS in a β-CD gradient was demonstrated experimentally with SIA/DSTD. An addition effect was observed with the SDS sample and a Brij®35 reagent gradient. A competition effect was observed with a PEG 1470 sample and a Brij®35 reagent gradient. An enhancement effect was observed with an SDS sample combined with a TBA reagent gradient. Finally, a bulk phase binding effect was observed with an SDS sample and a β-CD reagent gradient. SIA/DSTD introduces a high throughput, fast analysis method that does not require individual injections for each concentration of mixture samples. In this system, only a single concentration of sample and reagent was used to study the effect of sample in different concentrations of reagent. Thus, it consumed less reagent, produced less waste, and required significantly less analysis time than traditional FIA/DSTD.

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A simple flow injection-reduced volume column system for hemoglobin typing

Boonraksa Srisawang [a], Prachya Kongtawelert [b], Supaporn Kradtap Hartwell [a,*], Jaroon Jakmunee [a], Kate Grudpan [a]

[a] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

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Abstract

A flow injection (FI)-reduced volume column system was developed for hemoglobin (Hb) typing to be used as an initial screening method for thalassemia. The column was packed with 140 μl diethylaminoethyl (DEAE)-Sephadex A-50 ion exchange beads. Hb can be separated using Tris–HCl buffer solution with pH gradient 8.5–6.5 and then monitored spectrophotometrically at 415 nm. The hemolysate of 40 blood samples from packed red cells were screened for thalassemia by determining the amount of HbA_2 and HbE present. The proposed system was able to predict positive test results from those samples with β, E-trait and EE homozygous thalassemia, Hb types that were independently identified following the conventional method at the hospital laboratory. Advantages of the proposed system over the conventional column technique include low amount of reagents and blood sample needed, short analysis time and low cost. Each analysis required only 80 μl of 50 times diluted packed cells, which is equivalent to 1.6 μl undiluted packed cells, and it can be completed in only 35 min. This simple FI-reduced volume column system was demonstrated to be an economic alternative system for Hb typing to initially screen some types of thalassemia such as β-trait, E-trait and EE-homozygous which are commonly found in Thailand.

Keywords: Flow injection; Hemoglobin typing; Reduced volume column

1. Introduction

Mutations of protein structure of the hemoglobin (Hb) are inherited through ancestor genes and cause many blood-related disorders such as Sickle Cell Anemia, and thalassemia. In Southeast Asia, Africa and Middle East, thalassemia trait is commonly found [1,2]. People with thalassemia trait are generally without health problems. However, if both parents have Hb variants (i.e. thalassemia trait), there is 25% chance that the baby will have homozygous variant (i.e. thalassemia disorder) [3].

Techniques commonly employed in the hospital to indicate the existence of thalassemia in patients are cellulose electrophoresis, micro-column chromatography and HPLC [4,5]. Electrophoresis can

* Corresponding author. Fax: +66-5-322-2268.
E-mail address: kradtas@yahoo.com (S.K. Hartwell).

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qualitate but cannot conveniently quantitate for different types of Hb. It is used to find out the exact type of thalassemia after the indication of having thalassemia was found. On the other hand, HPLC technique can be used to qualitatively and quantitatively analyze Hb but it requires an expensive instrumentation. Separation of Hb using DEAE-Sephadex column is well established and it is normally done to screen for thalassemia before performing further examinations. However, the conventional column technique involves analyzing many fractions of eluate collected batch-wise leading to long time, high amount of reagents and sample consumption. This work attempts to develop a simple, low cost and fast system to perform Hb typing.

In the proposed system, flow injection (FI) analysis coupled with a reduced volume chromatographic column for Hb separation has been developed. A flow based-system dramatically decreases the analysis time and can be automated [6,7]. Its closed system also reduces the possibility of sample contamination [8,9]. Hb typing is achieved using a reduced volume micro-DEAE-Sephadex ion exchange column that requires very small volume of diluted blood, which in turn generates only small amount of biological hazardous waste. The amount of Hb can be spectrophotometrically monitored at 415 nm. The technique is used to screen for some types of thalassemia based on abnormally high ratio of HbA_2 and HbE relatively compared with total amount of Hb as percentage. Comparison of the results with those obtained from the larger conventional column technique, indicates the possibility of applying the proposed system to initially screen for some types of thalassemia such as β-trait, E-trait and EE-homozygous before further conducting more expensive and conclusive testing.

2. Experimental

2.1. Combination of methods for thalassemia diagnosis

In regions where routine thalassemia testing is most needed due to its prevalence in the populations, such as in Southeast Asia and Africa, it is often also the case that economic restrictions on the medical systems prevent the use of the latest technologies that exist. In the laboratories that are not well equipped with more technically complicated instrumentation such as HPLC, diagnosis of Hb type is normally accomplished by conducting several different tests in combination, namely Osmotic Fragility Test (OFT), DEAE-column separation, HbE screening test (E-screen) and Polymerase Chain Reaction (PCR). OFT is a preliminary test based on the slower rupture rate of red blood cells of patients with thalassemia (positive) in hypotonic salt solution as compared with normal red blood cells (negative). DEAE-column separation can estimate percentage of HbA_2+HbE (positive when higher than 3.5%). E-screen is similar to the DEAE-column technique but the working buffer has precise pH that is more specific for HbE separation. Normal blood has less than 10% HbE (negative). Blood samples that have 25–30% HbE are considered as having HbE trait (positive) while those that have 70–90% HbE are identified as EE homozygous (positive). PCR-fluorescence spectrophotometry is used to indicate α-thalassemia gene in the patients who have normal level of HbA_2 with negative E-screen test. The relationship between results of each test and the diagnosis of Hb type is summarized as shown in Table 1.

2.2. Materials and apparatus

All pump tubings were tygon tubings (Saint-Gobain Performance Plastics, USA). The rest of the flow lines were assembled from PTFE tubings (Cole Parmer, USA). A peristaltic pump (FIA-lab, USA) was used to deliver buffer solutions. A reduced volume micro-column was made of acrylic piece into the dimension of 3 mm i.d. and 2 cm long. This dimension is much smaller than conventional column, which is about 1 cm i.d. and 5 cm long. It was packed with 140 μl of 40–120 μm DEAE-Sephadex A-50 beads (Phamasia Biotech, Sweden) which was about 20 times less than the amount of beads needed for packing a larger conventional column. Both sides of the column were sealed with cotton wool. Samples were

Table 1
Summarization of test results with diagnosis of Hb type

OFT	HbA_2 (conventional micro column)	E-screen	PCR	Hb type
+	–	–	–	A_2 normal
+	+ (>3.5%)	–	–	β-trait
+	+ (>60%)	+ (70–90%)	–	EE homozygous
+	+ (>3.5%)	+ (25–30%)	–	E-trait
+	–	–	+	α

introduced by means of a six port injection valve with an 80 μl sample loop. The flow through cell (HELLMA, Germany) with 1 cm path length was placed in the Spectronic 21 (Spectronic Instrument, USA). The detection of Hb was done at 415 nm. The absorbance data were converted to voltage and recorded with a computer software (Metex Corp., USA) installed in a personal computer (Compaq Presario 425). These data were transferred and integrated with Microsoft Excel (Microsoft Corp., USA).

2.3. Reagents

Tris–HCl 0.05 M buffer solutions of different pH values were prepared by dissolving 6.057 g Tris (hydroxymethyl) aminomethane (UBS, USA) and 0.1 g KCN (Riedel-De Haen Ag Seelze-Hannover, Germany) in 1000 ml distilled water and adjusted to the desired pH of 8.5, 7.5 and 6.5 with HCl (Merck, Germany). These three different pH buffers were used to create the pH gradient between 8.5 and 6.5.

2.4. Samples

Total of 40 blood samples (packed cells) were obtained from the Thalassemia Research Laboratories, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University where all subjects were routinely checked up. Each blood sample was hemolysed and diluted 50 times with Tris buffer pH 8.5 prior to use. The result from each sample was compared with the diagnosis result by the routine procedures of the hospital laboratory. The analytical procedures were independently run.

2.5. Manifold and operation steps

The diagram of a simple manifold used is shown in Fig. 1. Buffer solution pH 8.5 was pumped through the unloaded column to condition the column at a flow rate of 0.8 ml min^{-1}. Blood sample (80 μl) was injected into the system through a six port injection valve (V2). After the first peak of co-eluted HbA_2 and HbE appeared, the pH 8.5 buffer solution was replaced with pH 7.5 buffer solution by switching the valve V1. In between the gradient of pH 8.5 and 7.5, the HbA was eluted. Via the valve V1 then the buffer solution was again changed to pH 6.5 to elute out HbF. As a precaution the valve V3 can be used to drive off any observed air bubbles before they can get into the column.

3. Results and discussion

3.1. Elution profiles of hemoglobin

DEAE-Sephadex bead has diethylaminoethyl ($-OCH_2CH_2N^+H(CH_2CH_3)_2$) functional group that interacts with anionic groups on Hb and thus can retain all types of Hb. Different types of Hb contain different amount of net negative charge and, therefore, can be separated with the pH gradient elution. Upon elution using the more acidic buffer, containing more HCl, Hb becomes less negative and thus anionic groups of Hb captured by DEAE-bead can be exchanged with Cl^-. The order of Hb eluted from a DEAE-Sephadex column is HbA_2 co-eluted with HbE, then HbA and finally HbF [10,11].

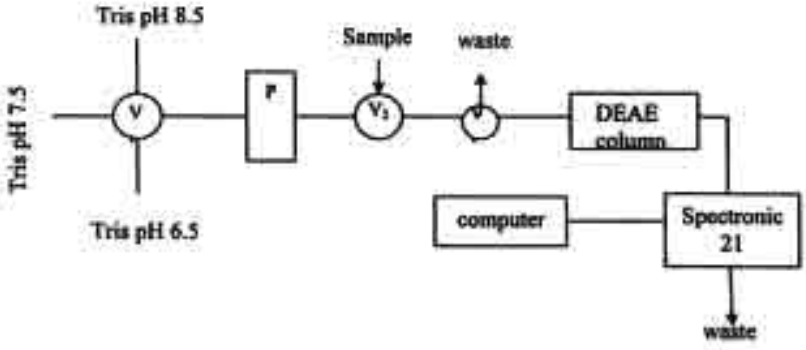

Fig. 1. Diagram of the simple FI manifold for Hb typing. V1, 4-ways valve; V2, six port injection valve; V3, 3-ways valve; P, peristaltic pump.

Examples of elution profiles of normal blood sample and that of EE-homozygous thalassemia patient obtained from the proposed system are illustrated in Fig. 2a and b, respectively. It should be noted that the *Y*-axis is in voltage that relates to transmittance and, therefore, peak height decreases with increasing of absorbance. The patterns of both elution profiles are similar but different in ratio of peak areas. Thalassemia patients have HbE that is coeluted with HbA_2 and, therefore, an abnormal blood sample shows larger first peak as compared with a normal blood sample.

3.2. Ratio of HbA_2 and HbE

Composition of Hb types in blood can vary depending on patient's age. Normal adult blood has been known to contain approximately 95–98% HbA, 2–3% HbA_2, and 0.8–2% HbF [12]. An individual with β-thalassemia trait usually has an elevated level of HbA_2, HbE and sometimes HbF along with the evidence of microcytosis and distinctive abnormal facial feature [2,4]. Patients with α-thalassemia, on the other hand, usually have HbA_2 and the same level as normal people have. Therefore, ratio of HbA_2 and HbE to total Hb cannot pinpoint what type of thalassemia the patient has without further examination.

From the elution profiles of blood samples, areas under each peak were integrated and the sum of those areas was counted as total Hb. The percentage of HbA_2+HbE was calculated from the ratio of area under HbA_2+HbE peak to the total area. Examples of calculation are also summarized in Fig. 2a and b. Even though ratio of HbA_2+HbE to total Hb cannot predict exactly what type of thalassemia the patient has, it can help indicate the existence of some types of thalassemia (i.e. β, EE homozygous, E trait types). The aim here is to utilize the benefits of the flow

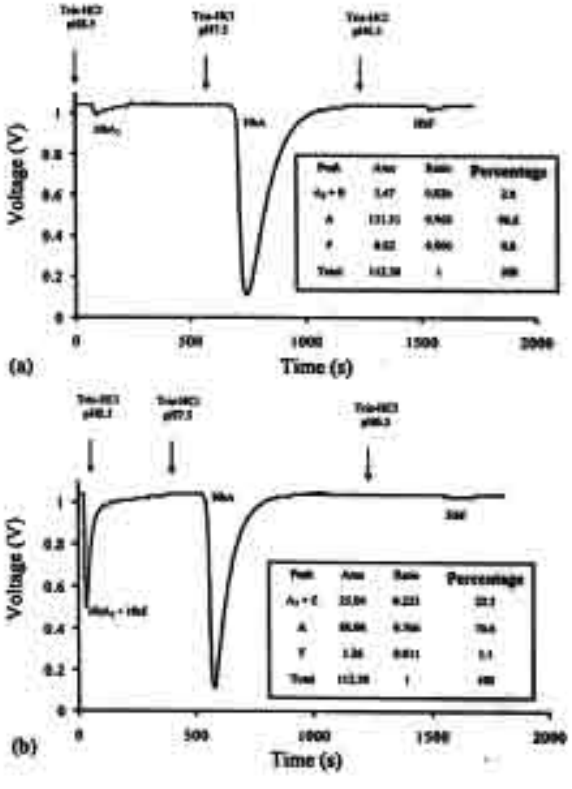

Fig. 2. FI-elution profiles of Hb in blood samples from (a) a normal adult and (b) an EE-homozygous thalassemia patient.

system combining with a DEAE-column technique and to show that the proposed FI-reduced volume column system can be used to initially screen for patients with some types of Thalassemia.

3.3. Evaluation of the proposed FI-system

In this work 40 adult blood samples (packed red cells) with positive OFT were examined without the prior knowledge of the type of Hb of each sample. Related to the hospital diagnostic results which were independently run, the correlation plot between the two systems, $R = 0.9271$, was obtained as shown in Fig. 3. The calculated percentages of HbA_2+HbE obtained from the proposed FI-reduced volume column system along with the results from a conventional column, E-screen and PCR are shown in Table 2(a) with a summary in Table 2(b). Two samples (one β-trait (18.4%) and one α-trait (17%)) were statistically eliminated from Table 2 because they gave results much higher than their standard ranges (β-trait 4–8%, α-thal-1-trait 2.5–3.5%) that are normally obtained using the HPLC. Because of the differences in the system characteristics and column size, the averages of HbA_2+HbE amounts obtained from different cases of thalassemia using the proposed system are lower than those obtained when using the conventional system, especially for the cases of E-trait and EE homozygous. The cause of deviation is currently under further investigation. However, the proposed system can differentiate normal and α-thal-trait samples (HbA_2+HbE < 3.5%) from abnormal samples (> 3.5%) and was still able to yield accurate positive test results. The level of HbA_2+HbE in EE homozygous samples was higher than in E-trait and β-trait samples, respectively, the same trends as indicated in the conventional column results.

For other types of Hb such as HbS, no testing could be conducted as there were no samples available in Thailand. However, according to a

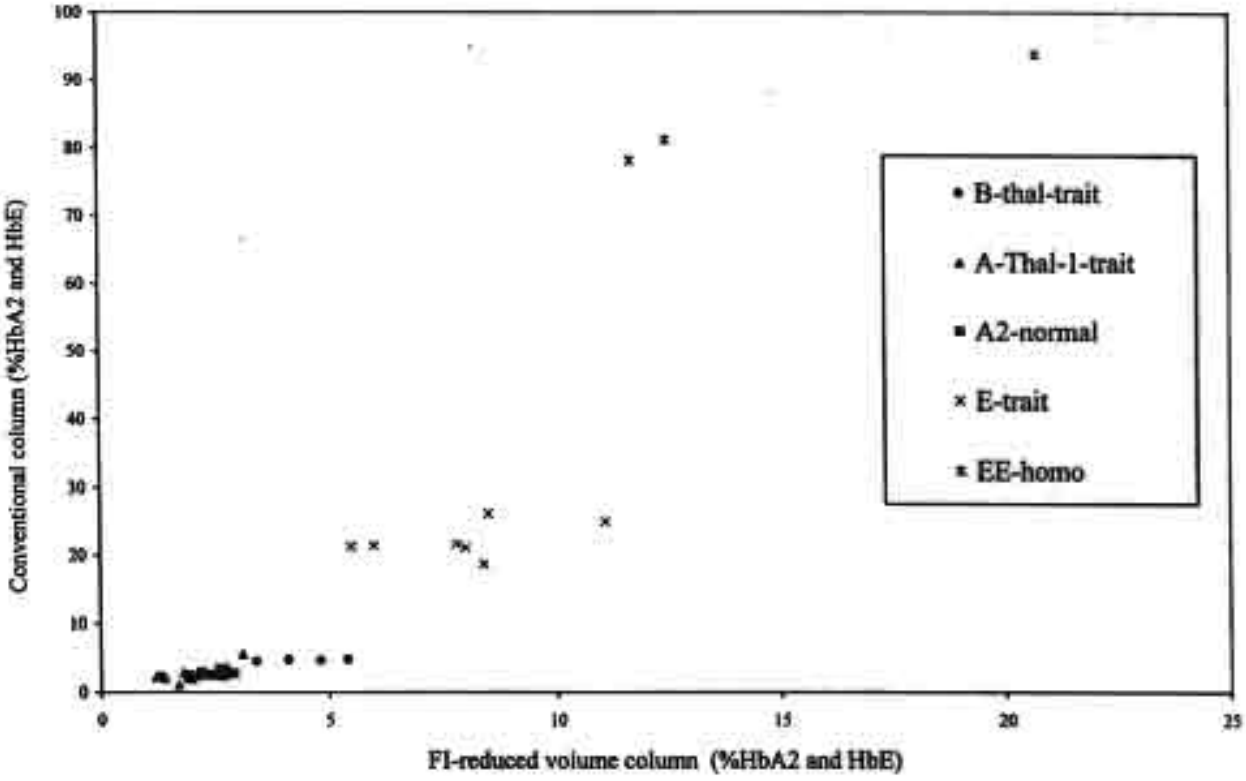

Fig. 3. Correlation plot between percentages of HbA_2+HbE obtained from the proposed FI-reduced volume column system and those obtained from the conventional column system.

Table 2
Comparison of calculated percentages of HbA_2 + HbE peak areas from the proposed FI-reduced volume column system* and from the conventional column system*

Sample	Percentages HbA_2 + HbE			E-screen*	PCR*	Hb type
	FI-red. V column*	Conventional column*	Ratio, FI-red. V: convention			
(a)						
A4	2.6	2.9	1:1.1	−	−	A_2-Normal
B6	1.3	2.2	1:1.7	−	−	A_2-Normal
B11	2.2	2.9	1:1.3	−	−	A_2-Normal
B13	1.9	2.5	1:1.3	−	−	A_2-Normal
B15	2.6	3.4	1:1.3	−	−	A_2-Normal
B16	2.7	3.4	1:1.3	−	−	A_2-Normal
C3	1.9	2.5	1:1.3	−	−	A_2-Normal
C25	2.8	2.8	1:1.0	−	−	A_2-Normal
D17	2	2.3	1:1.2	−	−	A_2-Normal
E6	2.6	2.4	1:0.9	−	−	A_2-Normal
E25	2.3	2.6	1:1.1	−	−	A_2-Normal
F18	2.4	2.5	1:1.0	−	−	A_2-Normal
C8	2.9	2.7	1:0.9	−	−	A_2-Normal
C5	3.4	4.5	1:1.3	−	−	β-Thal-trait
D3	5.4	4.8	1:0.9	−	−	β-Thal-trait
D9	4.1	4.7	1:1.1	−	−	β-Thal-trait
D18	7.3	18.4	1:2.5	−	−	β-Thal-trait
E24	4.8	4.6	1:1.0	−	−	β-Thal-trait
A9	1.8	2.9	1:1.6	−	+	α-Thal-1-trait
A8	3.1	5.5	1:1.8	−	+	α-Thal-1-trait
A28	2	2.5	1:1.3	−	+	α-Thal-1-trait
B2	2.2	2.5	1:1.1	−	+	α-Thal-1-trait
C2	1.7	1.1	1:0.6	−	+	α-Thal-1-trait
C11	1.4	2	1:1.4	−	+	α-Thal-1-trait
C12	1.2	2.2	1:1.8	−	+	α-Thal-1-trait
C16	2.7	2.4	1:0.9	−	+	α-Thal-1-trait
D2	1.3	2.2	1:1.7	−	+	α-Thal-1-trait
E7	1.9	2.1	1:1.1	−	+	α-Thal-1-trait
E10	2	1.9	1:1.0	−	+	α-Thal-1-trait
E20	8.5	17	1:2.0	−	+	α-Thal-1-trait
A1	5.5	21.3	1:3.9	+	−	E-trait
A23	8	21.1	1:2.6	+	−	E-trait
B4	8.4	18.7	1:2.2	+	−	E-trait
C13	6	21.4	1:3.6	+	−	E-trait
D20	11.1	25	1:2.3	+	−	E-trait
E19	8.5	26.1	1:3.1	+	−	E-trait
C7	7.8	21.7	1:2.8	+	−	E-trait
A39	12.5	81.2	1:6.5	+	−	EE-homo
C24	11.7	78.2	1:6.7	+	−	EE-homo
A37	20.7	93.8	1:4.5	+	−	EE-homo

Table 2 (*Continued*)

Hb type	HbA_2+HbE percentage				
	FI-reduced volume column*		Conventional larger column*		Standard range (HPLC)*
	Range	Average ± S.D.	Range	Average	
(*b*)					
A_2-normal (13)*	1.3–2.9	2.3 ± 0.1	2.2–3.4	2.7	2.5–3.5
α-thal-1-trait (11)*	1.2–3.1	1.8 ± 0.1	1.1–2.9	2.5	2.5–3.5
β-thal trait (4)*	3.4–5.4	4.1 ± 0.2	4.5–4.8	4.6	4.0–8.0
E-trait (7)*	5.5–11.1	8.0 ± 0.1	18.7–26.1	22.2	> 10
EE-homo (3)*	11.7–20.7	14.3 ± 0.9	78.2–93.8	84.5	> 60

(a) Results from each samples are shown along with the results from E-screen*, PCR-fluorescence spectrometry* and Hb Type diagnosis that were done by the Thalassemia Research Laboratories, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University (+ is positive result and − is negative result). (b) Summarization of ranges of HbA_2+HbE percentages and average values obtained when using the proposed and conventional column systems. *Independently run. Note: there were no S.D. data for the results obtained from the conventional column.

* Number of samples for each Hb type.

previous study by Dozy et al. [5], the DEAE column can separate Hb by descending pH gradient between 8.5 and 7.0. The order of Hb eluted from the column were HbA_2, HbS, HbA and finally HbF, respectively, in that study. Similarly, in this proposed study, HbA_2, HbA and HbF were eluted in the same order based on a similar pH range. Therefore, this proposed method should be able to screen for HbS if it is contained within the sample.

Reproducibility of the system was tested by running seven replicates of three samples (normal, β-trait and E-trait) each of which represented different levels of HbE. R.S.D. of the method was found to be 3.4% for normal sample and 4.6% for both β-trait and E-trait samples.

3.4. Advantages and disadvantages

The DEAE column technique has two main disadvantages. Neither the proposed nor the conventional column systems can point out patients with α-thalassemia due to non-differentiated level of HbA_2+HbE from normal people, in which case PCR result is needed. Also, both the proposed reduced volume column and the conventional column systems have another similar inconvenience in that they have to be repacked after each run due to constriction of the flow caused by coagulation of some matrices in blood. However, using a fresh column each time will eliminate the carry over effect from the previous run.

In the proposed FI-reduced volume column system, the improvement comes from the reduction in the amount of beads used and the time it takes for column packing, both of which are minimized as compared with those of the conventional column. The proposed system offers other advantages such as ease of operation, low cost, small amount of sample, and fast analysis time. The analysis time using the FI-reduced volume column is about 35 min per sample as compared with 4 h using a conventional column. Amount of sample for each run was as little as 80 μl of 50 times diluted sample, a major improvement as compared with the 2 ml of undiluted packed cell that is needed for a conventional column technique.

4. Conclusion

The FI-reduced volume column system for Hb typing was developed. The system was used as an initial screening for some types of thalassemia such

as β-thal-trait, E-trait and EE-homozygous which are commonly found in Thailand. It was demonstrated that the proposed system could differentiate normal blood samples from abnormal ones. Although the cause of deviation between results from the proposed system and those from the larger conventional column technique normally performed in the hospital needs further investigation, the proposed system was still able to predict positive test results. This preliminary study shows that the proposed system offers some advantages over the conventional column technique, including a simpler instrumentation with ease of operation, shorter analysis time and lower amounts of sample and reagents needed. These benefits will help reduce the overall analysis cost and should be useful as an economic alternative technique for routine thalassemia screening involving a large number of blood samples.

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Flow injection analysis of tetracycline in pharmaceutical formulation with pulsed amperometric detection

Sanit Palaharn [a], Thiraporn Charoenraks [a], Nattakarn Wangfuengkanagul [a], Kate Grudpan [b], Orawon Chailapakul [a,*]

[a] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[b] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

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Abstract

A flow injection method with pulsed amperometric detection (PAD) was proposed for the determination of tetracycline in pharmaceutical formulations. Tetracycline was also studied at a gold rotating disk electrode with cyclic voltammetry as a function of the pH of supporting electrolyte solution. The well-defined cyclic voltammogram, providing the highest peak current at 1.15 V versus Ag/AgCl, was obtained when using potassium dihydrogen phosphate solution at pH 2. The optimized pulsed amperometric detection conditions were 1150 mV (versus Ag/AgCl) detection potential (E_{det}) for 600 ms (500 ms delay time and 100 ms integration time), 1600 mV (versus Ag/AgCl) oxidation potential (E_{oxd}) for 150 ms oxidation time (t_{oxd}) and 100 mV (versus Ag/AgCl reference electrode) reduction potential (E_{red}) for 300 ms reactivation time (t_{red}). The optimized PAD waveform was applied to the determination of tetracycline standard solution and tetracycline in pharmaceutical formulations. The sensitivity of this method was found to be 13.7 μA/mM. The determination of tetracycline in commercially available tablet dosage forms by the proposed method (254.3 ± 9.3 mg per capsule) was comparable to those labeled (250 mg per capsule).

Keywords: Tetracycline; Gold electrode; Pulsed amperometric detection; Flow injection analysis

1. Introduction

Tetracycline is an antibiotic with a broad spectrum of activity against bacteria. It is used for many different infections, such as respiratory tract infections, urethritis and severe acne. It also has a role in the treatment of multidrug resistant malaria. Adverse effects include gastrointestinal disturbances, renal dysfunction, hepatotoxicity, raised intracranial pressure and skin infections, such as rosacea and perioral dermatitis. Various methods have been used for the determination of this compound. Spectrophotometric [1,2], fluorimetric [3–7], chemiluminometric [8–10], microbiological [11–13] and electrochemical [14–19] methods have been suggested. Electrochemical methods are of more interest than the others due to simplicity, less time analysis and low cost. Flow injection analysis can be applicable to electrochemical method, such as flow injection amperometric detection based on ion transfer across water-solidified nitrobenzene interface to determine tetracycline [18]. The results showed very low detection limit of 20 ng and the linear concentration range of 0.002–0.2 mM. Polarographic methods offer high sensitivity but their drawback is the use of mercury and they may not be practical for many applications, including flow injection. Although

* Corresponding author. Tel.: +66-2-218-7615; fax: +66-2-254-1309.
E-mail address: corawon@chula.ac.th (O. Chailapakul).

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voltammetry and amperometry also offer high sensitivities, their major disadvantage is deposition of the detection products and/or solution impurities on the electrode surface. Thus mechanical polishing is used to reactivate electrode surface and this is known to temporarily alter the response of a working electrode, often requiring 60–90 min before a stable baseline is obtained [20]. Therefore, pulsed amperometric detection (PAD) at a noble metal electrode which combines amperometric detection with alternated anodic and cathodic polarization to clean and reactivate the electrode surface, has been introduced to overcome this problem [21–24]. In contrast to simple amperometric detection, PAD offers the possibility to clean and regenerate the electrode surface effectively after each measuring cycle without the need for mechanical polishing. In the simplest implementation of PAD, the potential of the working electrode is stepped between the potentials for detection, E_{det}, cleaning, E_{oxd}, and reactivation, E_{red}. The cleaning and reactivating pulses maintain constant responsivity from injection to injection. All three variants of PAD require the following: (a) the analytes are oxidized during the detection step; (b) the oxidation products are removed from the electrode surface at E_{oxd}; (c) the oxide film, which inhibits the oxidation of analytes, is reduced at E_{red} [25]. Pulsed amperometric detection at gold or platinum electrodes has been used for the sensitive detection of numerous polar compounds [26–34] and metal species [35]. The difficulties resulting from electrode fouling are also avoided. To obtain fast and reproducible results, many reports also introduced flow injection analysis for the determination of tetracycline [36,37].

The goal of this work is optimization of the PAD waveform for the determination of tetracycline in pharmaceutical formulations. Furthermore, flow injection analysis, which provides fast, repetitive and reproducible analysis, has been combined with PAD to reduce the analysis time and to obtain a lower detection limit.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical grade and all solutions were prepared by using deionized water. Phosphate solutions for (for pH 2, 2.5, 3, 3.5, 4, 4.5) were prepared from 0.1 M potassium dihydrogen phosphate (Merck) and adjusted to the desired pH using 85% phosphoric acid (J.T. Baker) solution in 0.1 M sodium hydroxide solution (for pH 5, 5.5, 6, 6.5, 7, 8, 9, 10). Standard tetracycline hydrochloride (Sigma–Aldrich) solutions were freshly prepared in 0.1 M potassium dihydrogen phosphate solution prior to use.

A stock solution containing 481 $\mu g\,ml^{-1}$ (1 mM) of tetracycline hydrochloride in 0.1 M potassium dihydrogen phosphate solution (pH 2) was used to prepare standards in four 10 ml volumetric flasks. The final concentrations of the standard solutions were 24.1, 48.1, 96.1, and 144.3 $\mu g\,ml^{-1}$, respectively.

All solutions were protected from exposure to light with aluminium foil and stored at <4 °C in an ice bath.

2.2. Sample preparation

Tetracycline hydrochloride capsules (250 mg TC-mycin, Vesgo, USA) were used in this study.

The powder from 10 capsules was dissolved in 0.1 M potassium dihydrogen phosphate solution (pH 2) in a 1000 ml volumetric flask, and then filtered through a 0.45 μM nylon membrane syringe filter. The filtrate was further diluted with 0.1 M potassium dihydrogen orthophosphate solution (pH 2) to obtain a final concentration of 0.2 mM (calculated from the label value). This sample preparation has been repeated for five times.

2.3. Electrode

The gold rotating disk electrode (Au RDE, Metrohm, Switzerland) and gold disk electrode (Bioanalytical System, West Lafayete IN, USA) were pretreated by polishing with 0.05 μm of alumina/water slurries on a felt pad, followed by rinsing with ultra pure water prior to use.

2.4. Rotating disk voltammetry

Electrochemical measurements were carried out in a single compartment three electrode glass cell. The rotation speed was held at 250 rpm. A Ag/AgCl electrode and a platinum electrode were used as

the reference and auxiliary electrodes, respectively. Cyclic voltammetry was performed with an Autolab Potentiostat 100 (Metrohm, Switzerland).

2.5. Flow injection analysis with pulsed amperometric detection

The flow injection analysis system consisted of a thin-layer flow-through electrochemical cell (Bioanalytical System, Inc.), an injector port (Rheodyne 7125) with a 20 μl sample loop, a peristaltic pump (BIO-RAD) and an electrochemical detector (PG 100). The carrier solution, 0.1 M potassium dihydrogen phosphate, was regulated at a flow rate of 1.0 ml min^{-1}. The thin-layer flow, through electrochemical cell consisted of a silicone rubber gasket as a spacer, a gold disk electrode as the working electrode, a Ag/AgCl electrode as the reference electrode and a stainless steel tube as the auxiliary electrode and the outlet from the flow cell. The experiments were performed in a Faraday cage to reduce the electrical noise. The PAD waveform used to obtain the FI–PAD response is depicted in Fig. 1.

The FI–PAD response was monitored for independent variation of all potential and time parameters. The electrode was conditioned in a solution of 0.1 M potassium dihydrogen phosphate solution and pumped through the flow system at a constant flow rate of 1.0 ml min^{-1} with the selected PAD waveform until a stable baseline was established. The sample was then injected into the flow injection system via an injection valve equipped with a fixed sample loop of 20 μl and the resulting peaks were recorded.

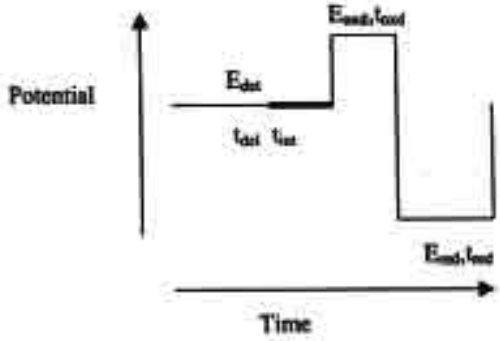

Fig. 1. Typical PAD waveform.

3. Results and discussion

3.1. pH dependence study

The electrochemical behavior of tetracycline was studied at the Au RDE in 0.1 M potassium dihydrogen orthophosphate solution of pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9 and 10. It was found that the best-resolved anodic signal for oxidation of tetracycline was obtained at pH 2. Therefore, this pH was used for the rest of the experiments.

3.2. Cyclic voltammetry

The cyclic voltammetric (I–E) response is shown in Fig. 2 for the Au RDE in 0.1 M potassium dihydrogen orthophosphate solution with and without 1 mM tetracycline. The background response for the supporting electrolyte exhibits an anodic wave on the positive scan in the region of ca. +0.8 to +1.2 V versus Ag/AgCl (wave A). This background response corresponds to charging of the interfacial double layer and formation of a small amount of surface oxide. The cathodic peak obtained on the negative scan in the region of ca. +0.7 to +0.4 V versus Ag/AgCl (wave B) corresponds to dissolution of the surface oxide formed on the positive scan. In the presence of tetracycline, the two-step anodic signal for oxidation of tetracycline was observed on the positive scan beginning at ca. 0.6 V versus Ag/AgCl. The first and second steps was occurred in the region of ca. +0.6 to +0.9 V versus Ag/AgCl (wave C) and +0.95 to +1.15 V versus Ag/AgCl (wave D), respectively. The anodic response for tetracycline on the positive scan was sharply inhibited by the onset of surface oxide formation at potentials greater than ca. +1.2 V versus Ag/AgCl. The decrease of signal on the subsequent negative scan in the region of ca. +1.25 to +0.8 V versus Ag/AgCl indicates the reduction of activity for the oxide covered gold surface.

3.3. PAD waveform optimization

The PAD waveform used in this experiment is described in Fig. 1. E_{det} is the detection potential applied for the time period t_{det} ($t_{det} = t_{del} + t_{int}$), and the electrode current is sampled by electronic integration over the time period t_{int} following a delay of t_{del}

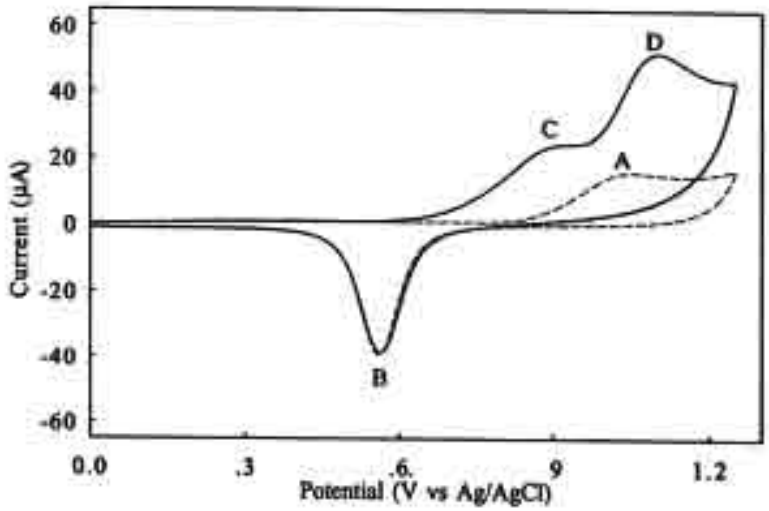

Fig. 2. Cyclic voltammetric response for 1 mM tetracycline in 0.1 M potassium dihydrogen orthophosphate solution (pH 2) at the Au RDE (0.07 cm^2). Condition: 250 rpm rotation speed; 50 mV s^{-1} scan rate. The background cyclic voltammogram is also shown (dash line).

to allow the charging current to diminish to a negligible value. A positive cleaning potential (E_{oxd}) that removes the oxidizable contaminant on the electrode surface is applied for the time period t_{oxd} following E_{det}. A negative reactivating potential (E_{red}) that dissolves the inert oxide product on the electrode surface is applied for the time period t_{red} following E_{oxd}. The optimization of each waveform parameter carried out in the FI system was studied while the other parameters were held constant. The average peak currents for each parameter were plotted versus the varied parameter. Observations of each parameter are discussed later.

3.4. Optimization of E_{det}, and t_{det}

Fig. 3a shows the FI–PAD response variations for 1 mM tetracycline according to E_{det} variation in the range +0.8 to +1.2 V versus Ag/AgCl in intervals of 0.5 V. The potential range used for E_{det} optimization was chosen from the potential region in the cyclic voltammogram (Fig. 2) where oxidation of tetracycline occurred. Clearly, the maximum current response is obtained at $E_{det} = 1.15$ V versus Ag/AgCl and application of this value in the PAD waveform was used. For this value, only a small contribution from surface oxide formation exists.

Fig. 3b shows the response for 1 mM tetracycline with t_{del} variation. It can be seen that the current is increased from 100 to 500 ms of delay time and the response decays beyond 500 ms. The t_{del} value of 500 ms is recommended as the optimal value.

Fig. 3c shows the FI–PAD response for 1 mM tetracycline with variation of t_{int} from 40 to 140 ms in intervals of 20 ms. On the basis of these results, a value of $t_{int} = 100$ ms was chosen as the optimal value.

3.5. Optimization of E_{oxd} and t_{oxd}

Fig. 3d shows the FI–PAD response for 1 mM tetracycline as a result of the variation of t_{oxd} from 30 to 180 ms at intervals of 30 ms for several values of E_{oxd} in the range +1.2 to +1.6 V versus Ag/AgCl in intervals of 0.1 V. The clean electrode becomes progressively fouled by the detection products during application of E_{det} and E_{oxd} was applied to clean the surface of electrode. For the values of E_{oxd} shown, the value of $t_{oxd} = 150$ ms is sufficient for the oxidative cleaning of the electrode surface. For each value of t_{oxd}, the highest current signals were obtained at $E_{oxd} = 1.6$ V versus Ag/AgCl. Hence, the values for $E_{oxd} = 1.6$ V versus Ag/AgCl and $t_{oxd} = 150$ ms are recommended as optimal.

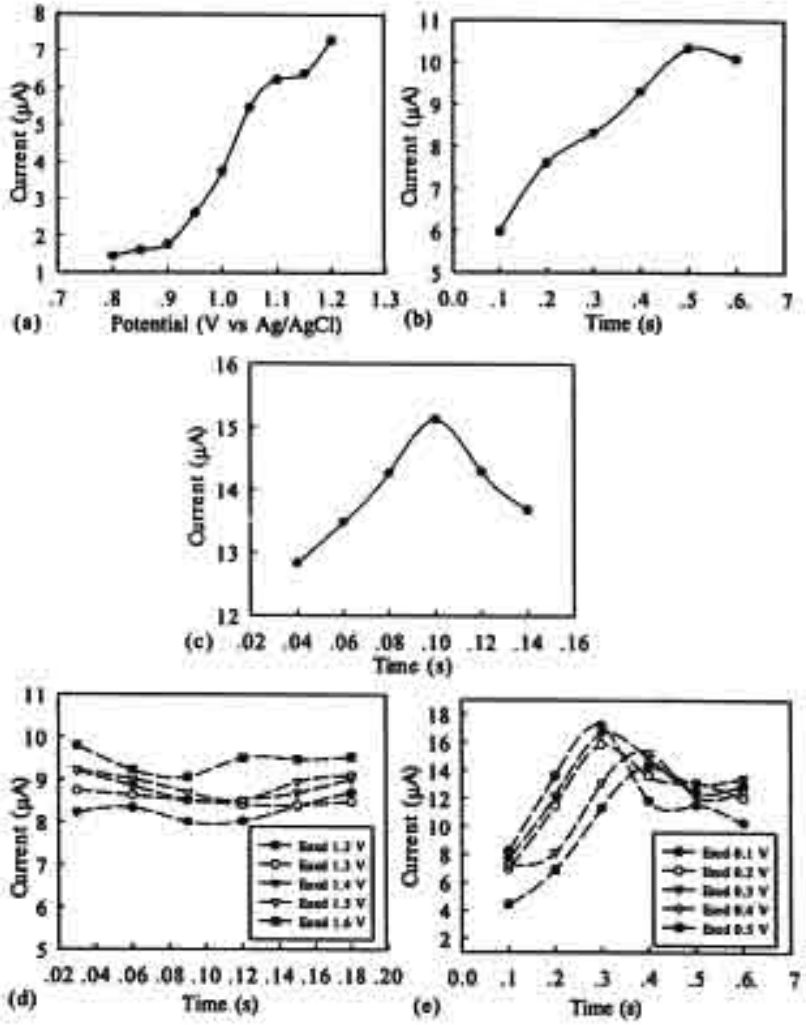

Fig. 3. FI–PAD response as a function of (a) E_{det}, (b) t_{det}, (c) t_{int}, (d) E_{oxd} and t_{oxd} and (e) E_{red} and t_{red} for tetracycline in 0.1 M potassium dihydrogen orthophosphate solution (pH 2) at the Au RDE (0.07 cm^2).

3.6. Optimization of E_{red} and t_{red}

The formation of surface oxide at the electrode surface, which reduced the electrode surface activity, occurred during the application of E_{oxd}. Therefore, it is essential that the values of E_{red} and t_{red} are chosen to achieve complete reductive dissolution of the surface oxide. Fig. 3e shows the FI–PAD response of 1 mM tetracycline with variation of t_{red} from 100 to 600 ms in intervals of 100 ms for several values of E_{red} in the range +0.1 to +0.5 V versus Ag/AgCl in intervals of 0.1 V. For the values of E_{red} shown, the highest current signals were obtained at the value of $t_{red} = 300$ ms. For each value of t_{red} +0.1 V is chosen as the optimum value for E_{red}. Therefore, the values of $E_{red} = 0.1$ V versus Ag/AgCl and $t_{red} = 300$ ms are recommended as the chosen values for the PAD waveform.

3.7. Linear range and the detection limit

Fig. 4 shows a series of repetitive 20 μl injections of tetracycline in 0.1 M potassium dihydrogen orthophosphate solution pH 2 under the optimized PAD waveform parameters described above. Well-defined signals

were obtained at all concentration from 5 μM to 2 mM. The current signal increased with increase in concentration. The calibration curve for tetracycline standard solutions was obtained from the pulsed amperometric responses. A linear response range between 5 μM and 0.6 mM tetracycline with sensitivity of 13.7 μA mM^{-1} and a correlation coefficient of 0.993 was obtained. The regression equation was $Y = 13.71X + 0.23$, where Y is the current response (μA) and X the concentration (mM). Interestingly, the detection with a S/N > 3 was obtained at a concentration of 1 μM of tetracycline.

3.8. Repeatability

The R.S.D. value was used to define the repeatability. Under the optimal PAD waveform parameters, a 0.5 mM of tetracycline solution in 0.1 M potassium hydrogen phosphate was injected 10 times at a flow rate of 1 ml min^{-1}. The %R.S.D., from the results was 3.5%.

3.9. Drug analysis of tetracycline capsules

The proposed PAD method for tetracycline was applied to the determination of tetracycline capsules. Using the regression equation (in linear range and the detection limit section) for the calibration plot, the amount of tetracycline hydrochloride was obtained to be a value of 254.3±9.3 mg per capsule ($n = 5$). Good agreement was obtained between the value obtained by the PAD method and the labeled value (250 mg per capsule). The anodic detection of tetracycline compound is concomitant with the anodic formation of oxide at the Au electrode and so could cause some error from interference products during the oxidative step. However, pulsed amperometric detection of tetracycline with FI still gives a low detection limit and a wide dynamic linear range.

4. Conclusion

This is the first investigation of tetracycline using pulsed amperometry applied to a flow injection system. The optimized conditions, such as pH and the various potentials were examined. The results show that FI–PAD with the optimized conditions can be used to determine tetracycline in the concentration range of 0.005–0.6 mM with a slope of 13.7 μA/mM. FI–PAD provided very low detection limit (10 ng). In comparison to the other amperometric method reported in the literature [18], this proposed method gives lower detection limit, wider working concentration range. The proposed method is simple and time saving because the cleaning step occurs simultaneously during the measurement. The determination of tetracycline in commercially available tablet dosage forms indicates that this proposed method is precise and accurate.

Acknowledgements

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Exploiting sequential injection analysis with bead injection and lab-on-valve for determination of lead using electrothermal atomic absorption spectrometry

Pattanapong Ampan[a], Jaromir Ruzicka[b], Raja Atallah[c], Gary D. Christian[b,*], Jaroon Jakmunee[a], Kate Grudpan[a]

[a] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA
[c] Department of Environmental Health, University of Washington, Box 357234, Seattle, WA 98195-7234, USA

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Abstract

Sequential injection (SI) with bead injection (BI) and lab-on-valve (L-O-V) was exploited for determination of trace lead using electrothermal atomic absorption spectrometry (ETAAS). A renewable microcolumn incorporated within the L-O-V system was investigated by using Sephadex G-25 impregnated by dithizone. Lead solution was passed through the impregnated beads. The beads were directly propelled into a graphite tube where they were pyrolyzed and lead ions were subsequently atomized. Conditions of the ETAAS measurement were studied including chemical modifiers (palladium, molybdenum and tartaric acid). The SI system for trapping of lead on the beads in the L-O-V could be operated in parallel to the ETAAS operation.

Keywords: Sequential injection; Bead injection; Lead; Electrothermal atomic absorption spectrometry

1. Introduction

Electrothermal atomic absorption spectrometry (ETAAS) is conventionally employed for trace metal analysis. Lead has been assayed in application to various types of samples, such as wine [1], sugars [2], fish samples [3], and blood [4,5]. Effects due to the matrix of samples must usually be eliminated. Besides addition of matrix modifiers, sample-pretreatment using column techniques has been widely used. Flow injection-ETAAS has been achieved for determination of lead in many kinds of samples, such as estuarine water and fertilizers [6], blood samples [7], and some high-purity reagents [8].

FI minicolumns (in-valve or in-line) are useful, but factors such as column back-pressure, efficiency of eluent, eluent volume, and elution time must be dealt with, and may influence precision and accuracy. Sequential injection (SI) was first introduced to reduce reagent consumption and perform operations readily with automation via a computer [9].

SI with a bead injection (BI) renewable microcolumn for preconcentration and pre-separation has been used for determination of copper [10]. The measurement is carried out after the analyte is sorbed onto beads, and then the used beads are discarded. A

* Corresponding author. Tel.: +1-206-543-1635; fax: +1-206-685-3478.
E-mail address: christian@cbem.washington.edu (G.D. Christian).

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renewable microcolumn loaded with SP Sephadex C-25 cation exchange resin was used for on-line preconcentration and separation before determination of nickel by ETAAS [11,12]. The ion sorbed beads [11] or the eluate from the beads [12] could be transferred into the graphite atomizer. A similar system was applied for the determination of trace bismuth in urine and river sediment [13].

In this paper, attempts were made to exploit SI with BI and the lab-on-valve (L-O-V) [14] for sample-pretreatment for trace lead determination using beads (Sephadex G-25 impregnated with dithizone) as renewable carrier. The bead sorbed lead was directly transported into the graphite atomizer.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer Model 5100 PC atomic absorption spectrometer equipped with Zeeman background correction (Perkin-Elmer, Norwalk, CT, USA) with an AS-60 autosampler and HGA-600 graphite furnace were employed. Measurements were performed using a hollow cathode lamp operated at a wavelength of 283.3 nm, with a bandwidth of 0.7 nm and a current of 14 mA. A pyrolytic graphite platform was used for atomization. Argon served as the inert gas at 300 ml min^{-1}, except in the atomization step during which the flow was halted.

The ETAAS was coupled with an SI system (FIAlab-3500, FIAlab Instruments, Medina, WA, USA) consisting of a syringe pump (1000 μl volume), a six-port selection valve, and a unidirectional peristaltic mini-pump. The six-port selection valve was mounted with an L-O-V, which served as an integrated microsystem for this application (see Fig. 1).

The L-O-V was designed to match a six-port valve. This unit was made of Perspex (diameter 50 mm, thickness 10 mm), having five ports and one-flow-through port, in addition to in and out ports for loading the sample. Two of the channels served as microcolumns (referred to as C1 and C2 in Fig. 1 and Table 1). The outlets of these channels were inserted with rods, small pieces of rigid PEEK tubing, id 0.0635 mm (Upchurch, part number 1560), in order to entrap beads into the channel but to allow liquid through. (See Refs. [11–13] and http://www.flowinjection.com for further details of the L-O-V and BI system.)

2.2. Chemicals and reagents

All reagents were of analytical grade. A series of lead standard solutions was prepared from 1000 μg ml^{-1} lead standard, AAS grade (Merck, Germany). Beads were of Sephadex G-25, 50–150 μm

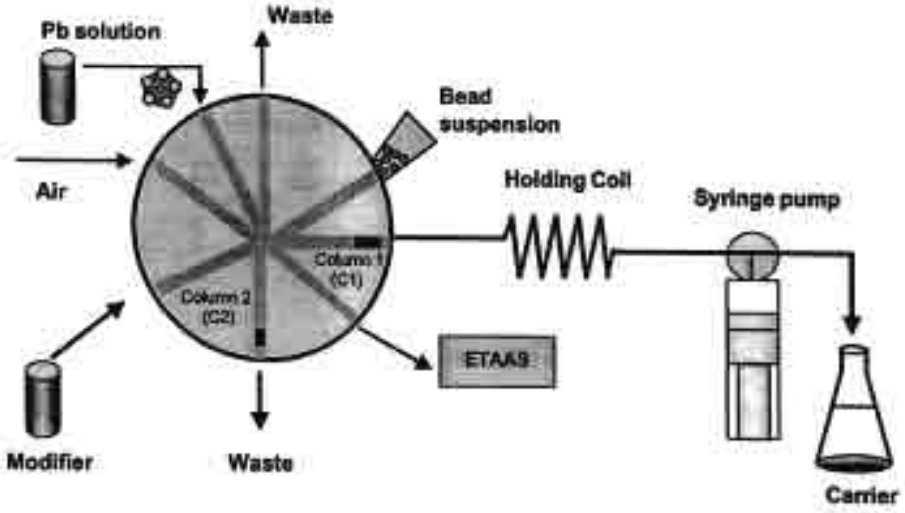

Fig. 1. SI system with L-O-V device mounted on the selection valve.

Table 1
Sequence of operating of the selection valve (SL valve), the syringe-pump valve (SP valve), and sample/reagents for 500 μl of sample loading

Step	Description	Port of SP valve	Port of SL valve	Flow rate (μl/s)	Action of flow	Volume (μl)
1	Washing					
	(a) Empty column 2 (C2)	Out	Column 2	25	Dispense	Empty
	(b) Fill syringe	In	Column 2	200	Aspirate	750
	(c) Washing C2	Out	Column 2	100	Dispense	650
2	Introducing beads					
	(a) Beads to column 1 (C1) (preventing to HC)	Out	Bead	10	Aspirate	10
	(b) Beads from C1 to C2	Out	Column 2	10	Dispense	100
3	Filling carrier	In	Column 2	200	Aspirate	400
4	Preconcentration					
	(a) sample into HC	Out	Std/sample	200	Aspirate	500
	(b) Sample passing through beads in column 2	Out	Column 2	10	Dispense	500
5	Washing sample behind C2					
	(a) Inserting air	Out	Air	100	Aspirate	50
	(b) Washing column 2	Out	Column 2	10	Dispense	50
6	Getting air	Out	Air	50	Aspirate	500
7	Drawing beads	Out	Column 2	200	Aspirate	100
8	Filling modifiers	Out	Modifier	10	Aspirate	10
9	Sending beads	Out	Detector	200	Dispense	100
		Out	Detector	50	Dispense	400

(SIGMA, USA). Dithiophenylcarbazone (Merck, Germany) was prepared in pH 12 solution (Table 4).

2.3. Procedure

The sequence for the SI–BI–L-O-V system was performed using a software "Program A", as summarized in Table 1. A mini-motor stirrer was used to assist homogenizing the bead suspension (1:20) during the bead introduction into the system. The conditions and temperature program of the ETAAS measurement are shown in Table 2.

Table 2
The graphite furnace program for determination of lead with Sephadex G-25 beads

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate ($ml\,min^{-1}$)
Preheating	70	5	10	300
Drying-1	120	1	10	300
Drying-2	300	10	20	300
Pyrolysis	1200	20	40	300
Cooling	20	1	15	300
Atomization	1800	0	5	0
Cleaning	2600	1	5	300

The cycle was set so that when the beads with the sorbed lead had just been introduced into the graphite tube of the ETAAS, the next sample-pretreatment by the SI system was started in parallel to the ETAAS detection process.

3. Results and discussion

3.1. Optimization of conditions

Because the lead sorbed on Sephadex is to be detected by ETAAS, lead measurement with the beads as matrix was investigated. During the atomization step, the beads should be sufficiently decomposed, to decrease the background signal due to the beads.

The effect of the pyrolysis temperature was studied by starting at a temperature of 850 °C and using $Mg(NO_3)_2$ and $NH_4H_2PO_4$ as chemical modifiers, which are recommended by the manufacturer for a sample with organic matter. The results (in Fig. 2) indicated that 1050 °C should be used, but high background was still observed. The temperature of 1200 °C would be preferred, but the lead is lost. The effect of pyrolysis time was then investigated for the

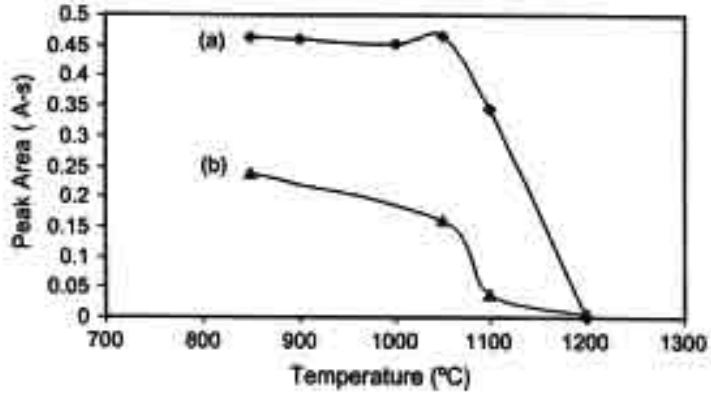

Fig. 2. Effect of pyrolysis temperature (pyrolysis time of 40 s): (a) 2 ng Pb-sorbed on beads; (b) beads alone.

temperature of 1050 °C. The results (Fig. 3) indicated that prolonged pyrolysis time resulted in loss of lead.

Various chemical modifiers (Table 3) were then considered, to suppress loss of lead, employing a pyrolysis time of 40 s. This time assured adequate pyrolysis and recovery under the optimum conditions. While we did not explore shorter pyrolysis times, it is possible pyrolysis can be achieved with somewhat shorter times. Under pyrolysis at 850 °C using magnesium chloride and ammonium dihydrogen phosphate as modifiers (lower temperature conditions) it was observed that carbonaceous residue built up on the graphite tube. For the lower temperature conditions, the lifetime of a graphite tube was about 20 runs whereas it was roughly 40 runs for the higher temperature conditions (1200 °C with modifiers used). It was found that a mixture of palladium chloride, ammonium heptamolybdate and sodium tartrate (3 μg:20 μg:400 μg) should be the most appropriate, as it resulted in the lowest background, the least loss, and longer lifetime of the graphite tube. With this, the recovery of 2 ng lead was 95% and the net background signal was 0.012 compared to 0.1 for the lead. The H_2O_2 and HNO_3 mixture, which was suitable to determination of Pb in honey [15], was not satisfactory here. Pd has been employed in lead determination in honey [15], blood and urine [16], and geological samples [17,18], but Pd alone was not suitable here either. Neither was its mixture with H_2O_2.

The effects of loading flow rate and the flow rate for bead transfer were investigated. High flow rates for loading may cause beads to escape from the cell

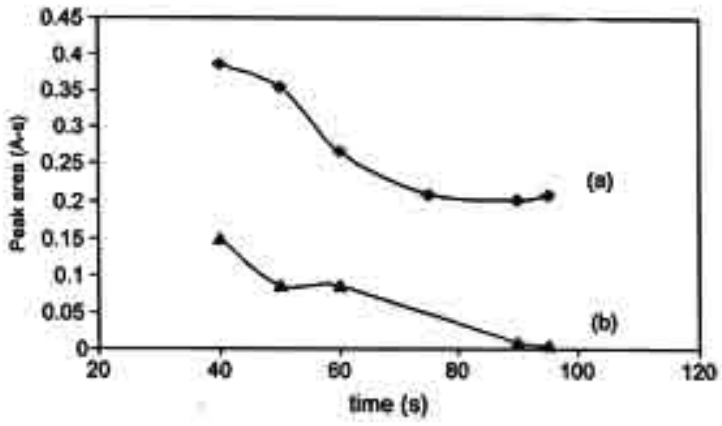

Fig. 3. Effect of pyrolysis time (pyrolysis temperature of 1050 °C): (a) 2 ng Pb sorbed on beads; (b) beads alone.

Table 3
Determination of lead added (2 ng) onto Sephadex beads in the presence of various modifiers

Modifiers (in 10 μl)	Pyrolysis temperature (°C)	Background signal (Abs-s)	Lead found (ng)	Relative loss (%)
$Mg(NO_3)_2$ (0.01 mg) + $NH_4H_2PO_4$ (0.2 mg)	850	0.036	1.9	5
H_2O_2 (3%, v/v)	850	0	1.0	50
H_2O_2 (3%, v/v) + HNO_3 (1%, w/v)	850	0.01	1.1	45
$Mg(NO_3)_2$ (0.01 mg) + $NH_4H_2PO_4$ (0.2 mg) + H_2O_2 (3%, v/v)	850	0.042	1.8	10
$PdCl_2$ (3 μg)	1200	0.025	1.8	10
$PdCl_2$ (3 μg) + H_2O_2 (3%, v/v)	1200	0.019	1.7	15
$PdCl_2$ (3 μg) + $(NH_4)_6Mo_7O_{24}$ (20 μg) + $C_4H_4Na_2O_6$ (400 μg)	1200	0.012	1.9	5
$PdCl_2$ (3 μg) + $(NH_4)_6Mo_7O_{24}$ (20 μg) + $C_4H_4Na_2O_6$ (400 μg) + H_2O_2 (3%, v/v)	1200	0.057	1.8	10

(microcolumn) via the very small space between a column plug and the inner wall of the channel of the L-O-V [14]. No significant difference was found for a loading flow rate of 1–20 $\mu l\,s^{-1}$ (step 4(b) in Table 1). The flow rate for transferring beads from the cell (column 1) of the L-O-V to the ETAAS was 200 $\mu l\,s^{-1}$, followed by 50 $\mu l\,s^{-1}$ as they reached the atomizer.

The choice of buffer and effect of pH of the carrier were studied. Tartrate buffer should not be used because tartrate is one of the chemical modifiers used, and the amount of tartrate would affect the signal obtained. Potassium hydrogen phthalate (KHP) was employed in this work by using a carrier with various compositions for pH 3–5 as described in Table 4. It was found that pH of the carrier was critical. A carrier of phthalate buffer with pH 5 gave the highest signal (orange color of beads was observed) (Fig. 4). By using buffer pH <5 as carrier, the signals tremendously deteriorated (purple color of beads was observed) and the signals decreased when using buffer pH >5 as carrier (with an observed diminishing of the orange color of the beads). Therefore the phthalate buffer pH 5 was selected.

Table 4
Preparation of buffer solutions with various pH values by mixing solutions A and B [18]

pH	Solution A	Solution B
1	25 ml of 0.2 M KCl	67.0 ml of 0.2 M HCl
2	25 ml of 0.2 M KCl	6.5 ml of 0.2 M HCl
3	50 ml of 0.1 M KHP	22.3 ml of 0.1 M HCl
4	50 ml of 0.1 M KHP	0.1 ml of 0.1 M HCl
5	50 ml of 0.1 M KHP	22.6 ml of 0.1 M NaOH
6	50 ml of 0.1 M KH_2PO_4	5.6 ml of 0.1 M NaOH
7	50 ml of 0.1 M KH_2PO_4	29.1 ml of 0.1 M NaOH
8	50 ml of 0.1 M KH_2PO_4	46.1 ml of 0.1 M NaOH
9	50 ml of 0.025 M borax	4.6 ml of 0.2 M HCl
10	50 ml of 0.025 M borax	18.3 ml of 0.1 M NaOH
11	50 ml of 0.05 M Na_2HPO_4	4.1 ml of 0.1 M NaOH
12	25 ml of 0.2 M KCl	6.0 ml of 0.2 M NaOH

A study on the effect of pH of the standard solution (prepared in the buffers listed in Table 4) was made. No significant effect of pH of the standard solution (pH 3–8) was found when using the phthalate buffer at pH 5 as carrier.

Dithizone was selected as the chelating agent for concentrating lead since it is a common reagent for the solvent extraction of lead [19]. A potential advantage of using a chelating agent in lieu of cation exchange is less effect of high concentrations of other cations, e.g., sodium, on the equilibrium. The effect of dithizone concentration was investigated. Sephadex G-25 beads were soaked in dithizone at pH 12, then equilibrated

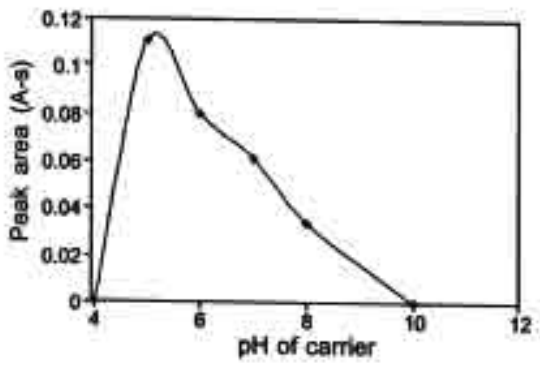

Fig. 4. Effect of pH of carrier.

at ambient temperature and kept in a photo-protected area for 30 min. The impregnated beads had to be freshly prepared. A suitable concentration of dithizone was 0.01% (w/v). The lead responses obtained from using various concentrations of dithizone (0.004, 0.01, 0.03, and 0.05%, w/v) were not significantly different. At 0.01% dithizone, it was easily sorbed in a reasonable time and the intensity of the dye was not too pale, assuring by visual inspection that an adequate amount had been sorbed.

3.2. Calibration

Using the proposed conditions of the ETAAS and SI system, a single standard calibration graph (plot of ng vs peak area) was obtained by loading 10 $\mu g\,l^{-1}$ Pb in pH 5 buffer with different loading times (100, 200, 400 μl; $Y = 0.082X - 0.047$; $R^2 = 0.992$). Loading 2000 μl of 1 and 2 $\mu g\,l^{-1}$ yielded the same results as loading 10 $\mu g\,l^{-1}$ for the same amount of Pb (ng). The linear regression line was: $Y = 0.080X - 0.052$; $R^2 = 0.991$. The relative standard deviation for 2 ng was 1.9% ($n = 3$). The detection limit was estimated at 0.3 ng (signal three times the standard deviation of the blank). The measured working range was 1–4 ng, which can be adjusted by the volume of sample loaded. The enrichment factor was 39. The detection limit is about an order of magnitude higher than the 0.02 ng obtained by Wang and Hansen [11] for nickel with an enrichment factor of 72. The difference was probably due to differences in backgrounds as well as in enrichment factors for the different elements.

4. Conclusion

A SI–BI–L-O-V system was exploited to determine trace lead by sorbing the lead on to microbeads for ETAAS measurement. Dithizone impregnated Sephadex G-25 beads were employed in a renewable microcolumn in the L-O-V system. The Pb-sorbed beads were directly transferred from the L-O-V into a graphite tube of the ETAAS where beads were pyrolyzed and lead ions were atomized subsequently, using a pyrolysis temperature of 1200 °C for 40 s, with chemical modifiers (Pd, Mo, and tartaric acid).

The SI–BI–L-O-V process for lead sorption onto the beads could be run in parallel to the ETAAS operation. This leads to a high sample throughput (12 determinations h^{-1}). This system should allow rapid separation and preconcentration of lead from samples with high matrix background.

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A Low-Cost Light-Scattering Detector for the Flow-Injection Nephelometric Determination of Sulfate

Jaroon JAKMUNEE,*1,*2† Yuthpong UDNAN,*1 Richard MORRISON,*3 Ronald BECKETT,*3,*4 Ian MCKINNON,*3 and Kate GRUDPAN*1,*2

*1 *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
*2 *Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*
*3 *School of Chemistry, Monash University, Clayton, Victoria 3800, Australia*
*4 *Water Studies Centre, Monash University, Clayton, Victoria 3800, Australia*

A simple low-cost flow-through light-scattering detector was developed for determining the particle mass concentration in colloidal suspensions. Employing a laser pointer as a light source and a photodiode IC as a light sensor, the detector was shown to have good sensitivity, yet was small and battery operated. The detector was demonstrated to be effective for the flow-injection nephelometric determination of sulfate by precipitation as barium sulfate.

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Introduction

Light-scattering detection has been applied in flow analysis for either the determination of size or mass concentration of particles.[1] Multiangle light-scattering detectors can be combined with particle separation techniques, such as field-flow fractionation (FFF)[2] and size-exclusion chromatography (SEC),[3] for size characterization. The mass concentration of particles can be determined by either multiangle or simple uniangle detectors.

The determination of a single analyte by uniangle light-scattering detection in flow injection (FI) and sequential injection (SI) analysis has been widely investigated. Either nephelometric (detection of scattered light at an angle of 90° to the incident beam) or turbidimetric (detection at 0°) methods are usually used. Although turbidity can be easily measured using a spectrophotometer, other phenomena (*e.g.* light absorption or light diffraction) may interfere. Nephelometric measurements are also more sensitive than turbidimetric measurements.

An SI system for the determination of chloride based on precipitation with silver ions has been proposed.[4] Phosphate can be determined by an SI turbidimetric method based on calcium phosphate precipitation.[5] A stopped FI turbidimetric immunoassay using the interaction of concanavalin A (antibody) and yeast mannan (antigen) has been investigated.[6] FI systems based on precipitation reactions have also been widely applied for the determination of drugs, such as thiamine,[7] promethazine,[8] amitriptyline,[9] and chlorhexidine.[10]

The determination of sulfate using barium chloride as a precipitating agent has previously been applied in both FI[11-14] and SI[15,16] systems. A liquid-drop windowless optical cell has been developed for FI turbidimetric or nephelometric determination of sulfate by precipitation with barium ion.[17]

In this work, a simple and low-cost flow-through light scattering detection system for determining the particle mass concentration was developed. It is based on nephelometric detection, using a laser pointer as a light source and a photodiode IC as a light sensor. The detector was utilized for the flow injection determination of sulfate by precipitation as barium sulfate.

Experimental

Chemicals

Deionized water (Milli Q, Millipore) was used throughout. All reagents were of analytical grade, unless otherwise stated. Sodium sulfate (Merck) was used to prepare a standard sulfate stock solution of 1000 mg SO_4^{2-}/L. Solutions of 1.2% w/v $BaCl_2$ and 0.1% w/v polyvinyl alcohol (PVA) in 0.05 M HCl were prepared from chemicals from Merck. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA, Merck) was used to prepare 0.2% w/v EDTA. A polystyrene latex bead suspension (particle diameter 0.144 μm) was obtained from Seradyn (IN, USA).

Flow injection apparatus

The flow-injection system used for the determination of sulfate is illustrated in Fig. 1. The system was assembled using a peristaltic pump (FIAlab Instruments, USA), a 6-port injection valve (Upchurch Scientific, USA), a 100 cm mixing coil and a flow-through light-scattering cell with a home-built detection system. All connections were made with 0.8 mm i.d. PTFE tubing.

Light scattering detector

A cross-section of the cell is shown in Fig. 2. A perspex plastic block was drilled in order to insert a straight glass tube

† To whom correspondence should be addressed.
E-mail: scijjkmn@chiangmai.ac.th

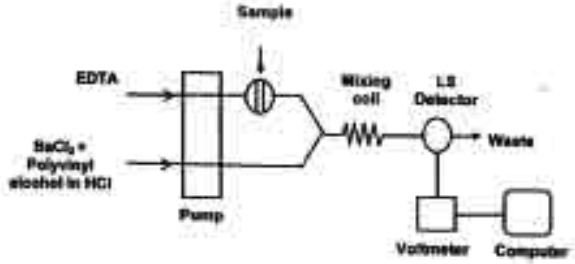

Fig. 1 Flow-injection nephelometric system for the determination of sulfate. Solutions were 0.2% w/v EDTA (or 0.0060 M) and 1.2% w/v $BaCl_2$ (or 0.058 M) plus 0.1% w/v PVA in 0.05 M HCl.

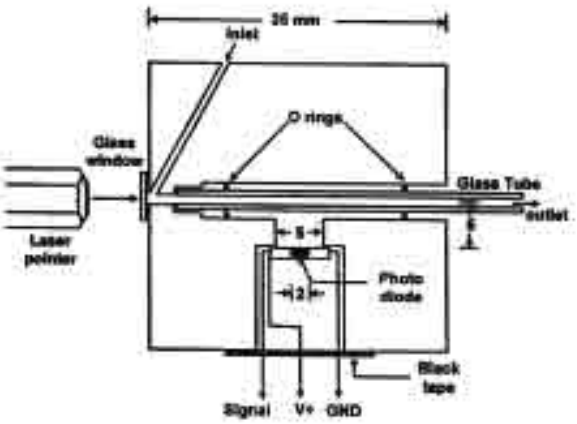

Fig. 2 Schematic diagram of the flow-through light scattering cell.

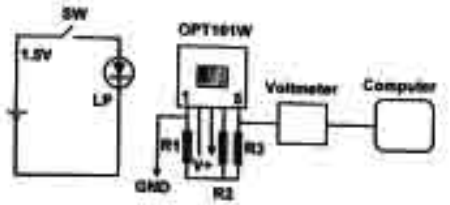

Fig. 3 Light-scattering detector circuit. R1, R2 and R3 are resistors of 3.3 kΩ, 10 MΩ, 100 kΩ, respectively.

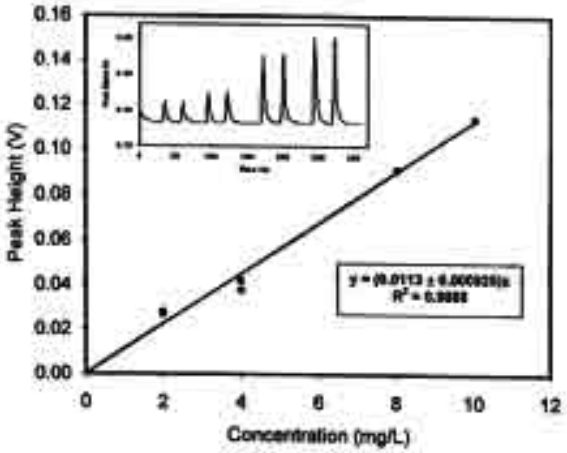

Fig. 4 Calibration plot of the detector voltage *versus* the polystyrene (monodisperse 0.144 μm beads) concentration fitted to a straight line through the origin.

(2 mm i.d.) with o-ring sealing. A light sensor (described below) was mounted at right angles to the tube axis, as shown. A glass microscope cover slide, attached to the block using epoxy glue, acted as a window and sealed the flow-through cell. The block was painted black to minimize any stray light. Finally, the flow cell, the light source and the detector were placed inside a black box

The detection system is depicted in Fig. 3. A red laser diode pointer (680 nm) was used as a light source. A 1.5 V AAA size alkaline battery was sufficient to power the laser diode for at least 3 h. The optical sensor which converts light intensity to voltage was an OPT101W (Burr-Brown Corporation), which has an active area of 2.3 × 2.3 mm and a peak spectral response at 850 nm, where the responsivity was 0.6 A/W. The useable working range of this detector was 300 nm to 1 μm.

The OPT101W is housed in a 5-pin, single, in-line package (SIP) containing an integral photodiode and current amplifier. Three external resistors, connected in a "tee" network, were added, as shown in Fig. 3. This configuration[18] results in an effective feedback resistance exceeding 300 MW, giving an effective gain of 3×10^8 V/A. While the frequency response of the detector is significantly reduced when operating at such a high gain, we estimate its value to be about 40 - 50 Hz, which is more than adequate for the experiments described here. The output of the OPT101W appears as a voltage on pin 5, which was measured by a digital multimeter (Dick Smith Electronics, Australia) interfaced to a computer (Compaq, USA).

Owing to the high circuit gain the input offset current of the OPT101W's internal op-amp produces a small, constant offset voltage (<100 mV) that contributes to the baseline signals in the experiments reported here.

As shown in Fig. 2, the 2.3 mm square detector is positioned 5 mm from the center of the glass tube through which samples flow. This geometry results in the collection of light scattered through the range 90 ± 40°. Experiments performed using a much smaller aperture (2 mm) immediately in front of the detector resulted in much weaker signals. The use of a higher powered light source would allow the collection angle to be better defined while maintaining a useful scattering signal.

Procedure for sulfate analysis

A standard or sample (100 μL) was injected into a stream of 0.2% w/v EDTA pumped at 1.5 mL/min using a peristaltic pump (see Fig. 1), which then passed through a mixing coil to merge with a stream of 1.2% w/v $BaCl_2$ and 0.1% w/v polyvinyl alcohol (PVA) in 0.05 M HCl before entering the flow-through cell. Light scattering caused by the barium sulfate precipitate was monitored by the detector and recorded on a computer. An FIAgram (a plot of output voltage (linearly proportional to the light scattering intensity) *vs.* time) was obtained for each injection. Data for a series of standard sulfate solutions were plotted (peak height *versus* sulfate concentration), and the resulting calibration graph was used to determine the concentration of sulfate in the unknown water samples.

Results and Discussion

Testing of FI-LSD instrument

A series of 0.144 μm polystyrene (PS) latex suspensions with concentrations in the range of 2 – 10 mg/L were injected into the FI system and allowed to flow-through the LS detector. The

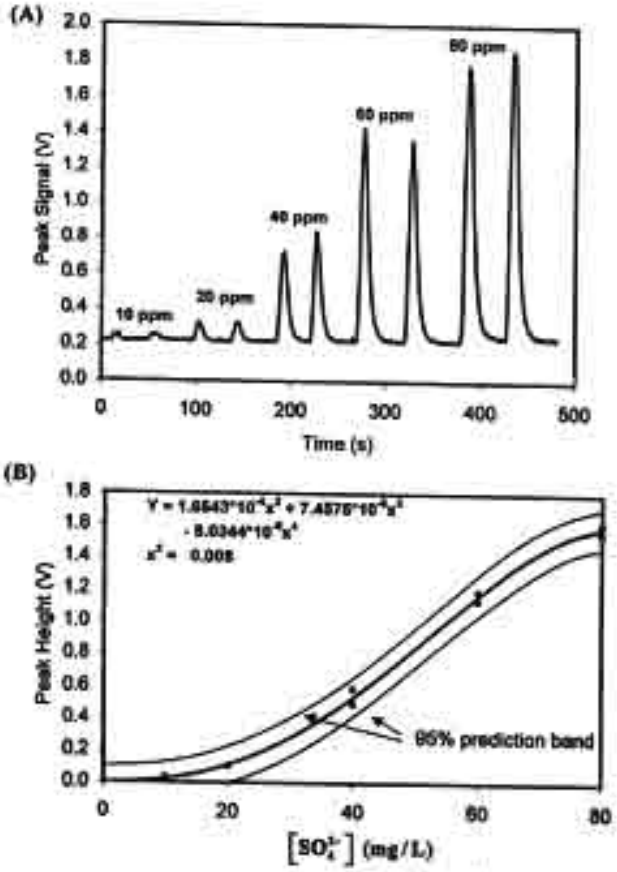

Fig. 5 (A) FIAgrams obtained with the FIA-LS system shown in Fig. 1 using 100 µL injections of sulfate of various concentrations. (B) Calibration plot of the detector voltage *versus* the sulfate concentration fitted to a truncated quartic equation, as explained in the text. x^2 = sum of squares of deviations of the points from the line. 95% prediction band such that 95% of the measured points are expected to lie within the band.

maximum difference between replicates of the resulting FIAgrams (detector voltage *versus* time) was 10%. The pooled standard deviation between replicates was 1 mV or 2% at the mid-range of the data. The resultant calibration line is plotted in Fig. 4. The data demonstrate the linearity of the detection system. The uncertainty in the slope of the line (the standard error as determined by a least-squares fitting procedure), was ±1%.

Flow-injection nephelometric determination of sulfate

Using the FI manifold shown in Fig. 1, the effects of various measurement conditions, concentrations of reagents (EDTA, $BaCl_2$, polyvinyl alcohol), flow rates, injection volume and mixing coil length were studied. From this information the run conditions selected were: Na_2EDTA concentration 0.2% (w/v) or 0.0060 M, $BaCl_2$ concentration 1.2% (w/v) or 0.058 M, PVA concentration 0.1% (w/v), sample injection volume 100 µL, mixing coil length 100 cm and flow rate for each line 1.5 mL/min.

The carrier stream of alkaline EDTA acts as a wash solution as well as binding some metal ions in the sample.[14] Sulfate reacts with the excess barium ions to form a precipitate before reaching the light-scattering cell. The polyvinyl alcohol would help to stabilize the precipitate by forming a protective layer on the particles.

Table 1 Sulfate contents of natural water samples using the FIA-LS system

Sample	Peak height/V			Sulfate/mg L^{-1}	
	(i)	(ii)	Mean	Mean	95% confidence
A	0.378	0.362	0.370	30.3	±3
B	0.614	0.511	0.5625	41.8	±3
C	1.005	1.14	1.0725	58.7	±3
D	0.138	0.124	0.131	19.1	±6
E	0.549	0.491	0.52	34.8	±3
F	0.189	0.189	0.189	21.1	±5
G	0.104	0.102	0.103	18.7	±9

The FIAgrams obtained for a series of sulfate injections in the concentration range 10 – 80 mg/L are shown in Fig. 5A. No baseline drift was observed for at least 50 injections. The reproducibility of the peak heights was generally not as good as that for the PS latex sample, with the maximum variation between replicates being 17% and the pooled standard deviation ($f = 5$) over all the samples being 40 mV or 10% at mid-range. This may be due to the variation in the particle size of the precipitate formed, which would affect the scattering of light.[14]

The data are plotted in Fig. 5B and are clearly nonlinear. In the absence of any theoretical model for describing the non-linearity, several functions (exponential, sigmoid, polynomial) were evaluated (using the software package Igor Pro[15]) for use as an interpolation function. The simplest function that adequately represented the data was a truncated quartic,

$$y = k2x2 + k3x3 + k4x4, \quad (1)$$

The fitted curve is shown in Fig. 5B together with the prediction bands drawn such that 95% of measured points are expected to lie within the band.

The FIA-LS system was then utilized to analyze sulfate in some natural water samples. The reproducibility is similar to that of the sulfate standards: the maximum difference between replicates was 0.1 V or 17% and the pooled standard deviation over all the samples was 0.05 V ($f = 7$).

Using the quartic calibration curve obtained with the sulfate standards, the concentration of sulfate in each natural water was estimated. The results are given in Table 1 together with the 95% confidence limits, determined as follows.

$$95\%\ \text{confidence limit} = 0.5 \times \text{the width of the 95\% prediction band}/(\text{number of replicates})^{1/2} \quad (2)$$

Conclusion

A simple low-cost flow-through light scattering detector was developed for use in FI systems, where precipitates are generated and the resulting turbidity is used as the basis for chemical analysis. The detector employed a readily available laser pointer as a light source and photodiode IC as a light sensor. The detector was tested using polystyrene latex beads and found to be quite sensitive and reliable.

In order to demonstrate the applicability of the LS detector, it was incorporated into an FI system, which was designed to measure the sulfate concentrations using an approach based on the standard turbidimetric method of barium sulfate precipitation. Reasonable results were obtained considering the

known limitations of this method, which result from variations in the particle size of the precipitate. We believe that this method has considerable promise due to the very reproducible mixing conditions that can be obtained in FI systems. Improvements in the accuracy of the analysis should be possible by optimizing the precipitation conditions to obtain more precise control of the particle size. Work is ongoing in our laboratories to produce a method for sulfate analysis which is more precise and efficient than the commonly used batch turbidimetric procedure.

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Flow injection spectrophotometric determination of As(III) and As(V) using molybdate reagent with solid phase extraction in-valve column

Kate Grudpan[1]*, Ngarmsri Worakijcharoenchai[1,2], Ponlayuth Sooksamiti[3], Jaroon Jakmunee[1] & Gary D Christian[4]

[1]Department of Chemistry, Faculty of Science and Institute of Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand
E-mail: kate@chiangmai.ac.th

[2]Permanent address: Department of Industrial Works, Ministry of Industry, Bangkok 10400, Thailand
[3]Office of the Mineral Resources, Region III (Chiang Mai), Chiang Mai 50200, Thailand
[4]Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700 USA

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Flow injection (FI) spectrophotometry for speciation of As(III) and As(V) has been investigated. As(III) in a mixture of AsO_2^- and AsO_4^{3-} merging with IO_3^- will convert into As(V). The total AsO_4^{3-} forms heteropoly acids with molybdate reagent. Mo(VI) is subsequently reduced to Mo(V). The compound in the stream flows into an in-valve microcolumn packing with C_{18} resin. The sorbed As-complex is eluted and continuously monitored by an LED colorimeter. The signal corresponds to the sum of AsO_2^- and AsO_4^{3-}. A signal obtained without merging with IO_3^- is that of the AsO_4^{3-} alone in the mixture. Optimization for the conditions has been investigated. Single standard As(V) calibration is possible. Application to a sample free from phosphate such as water leachates of zinc ores has been demonstrated.

Arsenic compounds are used in agriculture as insecticides, herbicides and also in veterinary medicine. Arsenic acid is used as desiccant for the defoliation of cotton boll prior to harvesting and for the preparation of wood preservative salts. Arsanilic acid is used as a feed additive for poultry. Arsenic alloyed with aluminium, gallium or indium forms III-V semiconductors for integrated circuit, diode, infrared detector and laser technology. The toxicity of arsenic depends on its chemical state. Trivalent arsenic compounds (As_2O_3) are usually more toxic to mammalian tissues than the pentavalent compounds (As_2O_5)[1-3]. Various techniques have been proposed for the determination of arsenic, for example, spectrophotometry[4,5], HG-AAS[6,7], ICP-AES[8], ICP-MS[9] and voltammetry[10]. Flow injection (FI) procedures for arsenic determination and arsenic speciation have been reported[11-17]. An FI manifold was reported for sequential determination of AsO_2^- and AsO_4^{3-} for 0.6-18.7 and 1.9-4.75 mg/l respectively. It is based on the reaction of AsO_4^{3-} with ammonium molybdate to form molybdoarsenate which is then reduced to the "molybdenum blue". Oxidation with KIO_3 for conversion of As(III) to As (V) is for the determination of total arsenic[12]. An FI system with a column packed with a resin provides not only on-line preconcentration and separation but also possibility for single standard calibration[18-20]. It also offers speciation such as Fe(II)/Fe(III)[21] and Cr(III)/Cr(VI)[22].

In this work, attempts have been made to develop an FI system comprising simple and low-cost components with C18 solid phase extraction in-valve for preconcentration and separation and for speciation of As(III) and As(V) by sequential determination. AsO_4^{3-} forms heteropoly acids with molybdate reagent. Mo(VI) is subsequently reduced to Mo(V) which is sorbed onto a C18 in-valve column. The sorbed As-complex is eluted and continuously monitored by an LED colorimeter. As(III) in the mixture of AsO_2^- and AsO_4^{3-} is in-line oxidised to As(V), leading to the total As, and As(III) can be obtained by the difference. Optimisation of conditions was investigated. Single standard calibration was studied. Application to water leachate of zinc ores has been demonstrated.

Materials and Methods

All chemicals were analytical reagent grade except where otherwise stated, and de-ionised water was used.

A stock standard As(III) (1000 mg/l) solution was prepared by dissolving $NaAsO_2$ (Carlo Erba) (0.1734 g) in water and diluted to 100.0 ml. A stock standard As(V) (1000 mg/l) solution was prepared similarly from $Na_2HAsO_4.7H_2O$ (BDH) (0.4165 g). Further appropriate dilutions were freshly made. Ammonium molybdate [0.20% (*w/v*)] in sulphuric acid solution (0.25 *M*) was prepared by dissolving $(NH_4)_6Mo_7O_{24}.4H_2O$ (BDH) (2.0 g) in sulphuric acid (0.25 *M*, 1 litre) and kept in a polyethylene bottle to prevent introduction of silica. Ascorbic acid solution [6.0% (*w/v*)] was freshly prepared before use by dissolving ascorbic acid (Roche) (30.0 g) in 500 ml of water and kept in a brown glass bottle. Potassium iodate solution (0.05 *M*) was daily prepared by dissolving KIO_3 (10.7 g) in 1 litre of water.

C18 SPE in-valve column

The laboratory made microcolumn similar to that previously reported[22] was a cylindrical Perspex drilled for 25 mm × 3 mm i.d. The column was filled with the C18 resin (Lichrolut® RP-C18, Merck). The two ends of the column were plugged with porous Teflon frits and covered with fittings for PTFE tubing (0.8 mm i.d.). The column was connected with the injection valve (to replace a sample loop). Connecting of tubing to the valve is designed for reverse flow directions of the standard/sample loading and of the elution to prevent blockage in the column which may be caused by accumulation of the resin at the one end of the column if the loading and elution passed through the column in the same direction[19].

FI manifold

Figure 1 depicts the flow system used. A four channel peristaltic pump (Ismatec, MC-MS/CA 4/6) was used to propel standard/sample solution(s), water/oxidizing agent (KIO_3) (R1) stream, ammonium molybdate in acid solution (R2) and ascorbic acid solution (R3). A three-way valve (Connecta ®, Sweden, normally used for clinical purposes) was used to select the H_2O or R1 stream. RC1 and RC2 were mixing coils for the merged streams of S and H_2O or R1 and the R2 and R3, respectively. The mixing coil RC3 was immersed in a water bath (controlled temperature ±2°C) and connected to the injection valve (V2) (FIAlab-2000, USA), having the in-valve column (C18) as described above. When switching the valve to the loading position, an eluent was pumped by another peristaltic pump (P1) (Eyela, Japan) to elute the sorbed As-complex from the column and pass it through the mixing coil, RC4, before entering into a flow-through cell (Hellma, 1 cm, Suprasil 1 window) in a spectrophotometer (Ismatec, red LED for 820 nm). FIAgrams were recorded by a chart recorder (Philips PM 8521). All connecting tubings were of Teflon (0.8 mm i.d.).

Procedure for sequential determination of As(III) and As(V)

As(V) alone in the mixture was determined first by merging the sample with a water stream controlled by valve V1. The merged stream was further merged with the colouring stream R2. The blue molybdoarsenic acid complex was retained on the C18 microcolumn and eluted by changing the position of the column on the injection valve V2, and the eluted product flowed further through RC4 to the detector (820 nm).

As(III) in the mixture was determined by switching the three way valve, V1, so that the KIO_3 solution (R1) flowed to merge with the sample containing As(V) and As(III). The total As(V) [the original As(V) contained in the sample plus the oxidized As(III)] was merged with ammonium molybdate and ascorbic acid (R2). The sum of As(V) and As(III) was obtained from the signal.

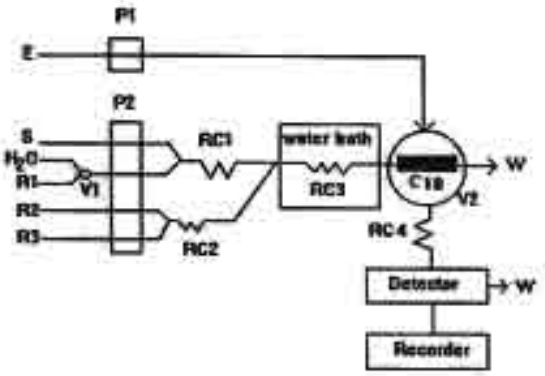

Fig. 1—Flow diagram system for speciation of As(III) and As(V) [P1, P2 = peristaltic pumps, V1 = three-way valve, S = sample, E = eluent, R1 = oxidizing agent (KIO_3), R2 = ammonium molybdate in acid solution, R3 = ascorbic acid, RC = reaction coil, V2 = rotary injection valve, C_{18} = in-valve microcolumn on V2, W = waste].

Results and Discussion

Optimization of the FI manifold

The parameters kept constant were: R1 (KIO_3, 0.05 *M*); R2 [ammonium molybdate (0.10% (*w/v*)] in H_2SO_4 (0.25 *M*); R3 [ascorbic acid [6.0% (*w/v*)]]; RC1 (40 cm); RC2 (80 cm); RC3 (200 cm); RC4 (75 cm); flow rates of R1, R2, R3 and the standard/sample were 1.8 ml/min; eluent (NaOH, 0.10 *M*) with a flow rate of 3.5 ml/min; and a water bath temperature of 55±2°C. The effect of ammonium molybdate concentration in 0.25 *M* H_2SO_4, presented in Fig. 2, indicates that if the concentration of ammonium molybdate was too high the net peak height due to As(V) decreased with increase in the contribution from the blank. An ammonium molybdate concentration of 0.20% (*w/v*) was chosen.

The acid concentration must be controlled. If the acidity is too low, silicate or even molybdate alone will give a blue colour; and if it is too high, the colour due to arsenic is decreased in intensity[20]. A series of calibration graphs [0.10-0.25 mg/l As(V)] was obtained using 0.20, 0.25, 0.30, 0.40 and 0.50 *M* H_2SO_4: y=236x+4.4, y=239x−0.5, y=247x−4.5, y=192x−1.3, y=165x+0.1 with r^2 values of 0.9791, 0.9994, 0.9900, 0.9892, and 0.9946 respectively. The results indicated that maximum slope was obtained in the presence of 0.30 *M* H_2SO_4 but the correlation coefficient was poorer than that obtained in the presence of 0.25 *M* H_2SO_4. A concentration of 0.25 *M* H_2SO_4 was chosen. At this concentration, a linear calibration was obtained passing close to the origin.

When concentrations of ascorbic acid were varied from 2.0-10.0% (*w/v*), an increase in the concentration of ascorbic acid caused an increase in slope of the calibration graph up to a concentration of 6.0% (*w/v*). Increases in concentration above 6.0% (*w/v*) did not alter the slope significantly; therefore for economical reasons, 6.0% (*w/v*) ascorbic acid was selected.

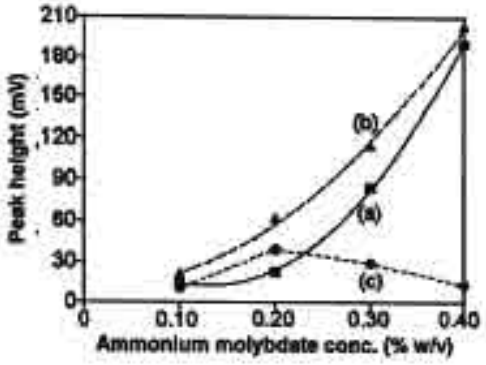

Fig. 2—Effect of ammonium molybdate concentration [(a) blank, (b) 0.25 mg/l As(V) and (c) corrected for blank].

In preliminary studies, various eluents including methanol, ethanol, borax (0.10 *M*) solution (*p*H 9.0) and sodium hydroxide (0.10 *M*) solution, were examined for suitability. The effect of concentration of sodium hydroxide was investigated by varying its concentration between 0.05-0.50 *M* NaOH. It was found that 0.20 *M* sodium hydroxide solution was suitable for further studies.

The effect of eluent flow rate was also studied. It was found that the higher the flow rate, the higher the peak. The flow rate of 4.4 ml/min was chosen as a compromise between peak height and rate of sample injection. This rate is within a region where small variations in flow rate does not alter the peak height significantly.

The effect of flow rate of reagents and sample indicated that peak height increased with increase in flow rate up to a rate of at least 1.8 ml/min. It was noted that above a flow rate of 2.2 ml/min leakage from connections occurred. A flow rate of 1.8 ml/min was therefore chosen.

Peak heights were practically constant for the RC2 (10-80 cm), so 10 cm of RC2 should be used. The optimum RC3 length is 100 cm. The peak height decreased with increase in the RC4 lengths (15-100 cm), so a length of 15 cm should be suitable.

When increasing the KIO_3 concentration, the percent oxidation was increased but peak heights were decreased (Table 1). And if KIO_3 concentration was higher than 0.05 *M*, iodine precipitated in Teflon tubing. An optimum concentration of 0.05 *M* of KIO_3 was chosen.

Table 1—Effect of KIO_3 concentration

KIO_3 conc. (*M*)	Peak height (mV) Blank	Corrected for blank of 0.25 mg/l As(V) (*a*)	Corrected for blank of 0.25 mg/l As(III) (*b*)	Oxidation* (%)
0.025	24	59	26	44
0.05	25	47	39	83
0.06	23	41	34	83
0.07	21	37	32	87
0.08	23	33	28	85
0.10	24	25	25	100

*% oxidation = (*b*/*a*) × 100

The effect of water bath temperature on peak height indicated that peak height increased with increase in temperature up to 55°C. If the temperature exceeded 65°C, air bubbles interfered. Water bath temperature should be maintained at 55°C.

Loading time for As(V) preconcentration

The loading time for 0.01-0.25 mg/l As(V) was varied from 30 to 180 s and with increase in loading time, the slope of the calibration increased. But long loading times decreased the rate of sample throughput. The loading time of 60 s should be appropriate.

The optimum conditions for the sequential determination of As(III)/As(V) are summarized in Table 2.

Analytical characteristics

Using the proposed conditions (Table 2), calibrations were performed as follows: (a) A plot of peak height versus As(V) concentration with the water stream in operation which is used for As(V) determination alone; (b) a plot of peak height with the oxidizing agent stream in operation versus As(V) concentration; and (c) a plot of peak height versus As(III) concentration with the oxidizing agent stream. The three plots were used for evaluation of As(III) and As(V) concentrations in a mixture. From the plots, detection limits (3σ) were estimated[24], as 0.02 mg/l As(V), 0.04 mg/l As(V) and 0.03 mg/l As(III), respectively (a, b, c). Relative standard deviations for As(V) and As(III) were 3.6 and 7.4% [n = 11, 0.1 mg/l for both As(V) and As(III)] respectively (b and c).

Interference study

The effect of interfering ions was studied by loading a blank, 0.10 mg/l As(V) in water stream and 0.10 mg/l As(III) in 0.05 *M* KIO_3 stream in presence of interfering ions. The concentration of an ion is considered to be interfering when peak height lies outside the range $x\pm3\sigma$ where x is the response due to arsenic alone. Results indicate that silicate (as Na_2SiO_3) above 1.0 mg/l, chromium(III) (as $CrCl_3$) and chromium(VI) (as $K_2Cr_2O_7$) above 0.5 mg/l positively interfere. Phosphate seriously interferes due to phosphomolybdenum blue production. Dasgupta *et al.*[25,26] discuss different methods for removal of phosphate and other interferences in similar arsenomolybdate-based measurements.

Table 2—Conditions for the sequential determination of As(III)/As(V)

Reagent R1	0.05 *M* KIO_3
Reagent R2	0.20% (w/v) ammonium molybdate in 0.25 *M* H_2SO_4
Reagent R3	6.0% (w/v) ascorbic acid
Eluent	0.20 *M* NaOH
Flow rate of samples, R1, R2 and R3	1.8 ml/min
Flow rate of eluent	4.4 ml/min
RC1 dimension	80 cm 0.8 mm i.d.
RC2 dimension	10 cm 0.8 mm i.d.
RC3 dimension	100 cm 0.8 mm i.d.
RC4 dimension	15 cm 0.8 mm i.d.
Temperature of water bath	55±2 °C
C_{18} column dimension	2.5 cm 3.0 mm id
Loading time	60 s
LED colour	red
Sensitivity of recorder	200 mV
Chart speed of recorder	0.5 cm/min

Single standard solution calibration for As(V) determination

Standard solutions containing 0.01, 0.05, 0.10, 0.20 and 0.25 mg/l As(V) were loaded at a constant flow rate of 1.7 ml/min through the C18 column, varying the preconcentration times at each concentration. The peak heights of each time were recorded. The results led to five conventional calibrations (a plot of peak height vs μg/ml As), one for each preconcentration time. A plot of μg of As(V) against peak height yields a single line: μg As(V)=flow rate (1.7 ml/min) × [As(V)] (μg/ml) × preconcentration time (min). This indicates that a single standard calibration is linear up to 0.85 μg As ($y=153x+0.74$; $r^2=0.982$).

Analysis of mixtures

The proposed conditions (Table 2) were applied to determine As(III) and As(V) in mixtures. The signals obtained when using a water stream were due to As(V) only; when the oxidizing agent (0.05 *M* KIO_3) stream was used the signals were due to the sum of As(III) and As(V). Percentage recoveries were evaluated. The results are displayed in Table 3. It was found that the recoveries were 96 to 110 and 80 to 100 percent for As(V) and As(III), respectively.

Evaluation of arsenic concentration

Arsenic(V)—As(V) concentration, x_1, can be directly evaluated from the expression:

Table 3—Determination of As(III) and As(V) in mixtures (mean of duplicate injections)

No.	Conc. present (mg/l)		Peak height (mV)		Conc.* found (mg/l)		Recovery (%)	
	As(V)	As(III)	Water stream	Oxidizing stream	As(V)	As(III)	As(V)	As(III)
1	0.10	0	34.0	22.0	0.10	0	100	-
2	0.20	0	64.0	42.0	0.20	0	100	-
3	0	0.08	0	16.0	0	0.08	-	100
4	0	0.15	0	28.0	0	0.15	-	100
5	0.10	0.10	37.0	39.0	0.11	0.08	110	80
6	0.15	0.05	51.0	43.0	0.15_5	0.04	106	80
7	0.04	0.10	12.5	28.0	0.04	0.10	100	100
8	0.25	0	78.0	49.0	0.24	0	96	-
9	0.10	0.08	35.0	37.0	0.10_7	0.07	107	88
10	0	0.25	0	43.0	0	0.24	-	96

*Calculation described in the text.

$y_1 = a_1x_1 + b_1$ ... (i)
or $x_1 = (y_1 - b_1)/a_1$

where y_1 = sample peak height, a_1 = a constant, and b_1 = the peak height of the blank.

Arsenic(III)—The concentrations of As(V), x_2, and As(III), x_3, are evaluated using the oxidizing agent stream from the expressions:

$y_2 = a_2x_2 + b_2$ ... (ii)
$y_3 = a_3x_3 + b_3$ ... (iii)

where y_2 and y_3 are the peak heights of As(V) and As(III), respectively, a_2 and a_3 are constants and b_2 and b_3 are the corresponding blank contributions.

If As(V)=0, $[As(III)]=x_3=(y_3-b_3)/a_3$ ... (iv)

If As(V) ≠ 0 , [As(III)] can be calculated from Eqs (ii) and (iii) as follows:

Peak height due to total As, $h = y_2 + y_3$
$=(a_2x_2+b_2)+(a_3x_3+b_3)$
$=(a_2[As(V)]+b_2)+(a_3[As(III)]+b_3)$
hence, $[As(III)]=\{h-a_2[As(V)]-b_2-b_3\}/a_3$ ... (v)

From the calibrations we obtained:

$y_1 = 315.2(x_1) + 1.141$, $y_2 = 218.2(x_2) + 0.5747$, and $y_3 = 173.4(x_3) + 1.445$

For sample no. 1 in Table 3
As(V) is calculated from $y_1 = 315.2(x_1) + 1.141$

Table 4—Comparative determination of arsenic in ore leaching water samples by proposed method and HG-AAS

Sample No.	As (mg/l)			
	HG-AAS	FIA		
		As(V)	As(III)	Total As
1	0.50± 0.06	0.54	ND*	0.54
2	0.21± 0.02	0.22	ND	0.22
3	2.51± 0.03	2.37	ND	2.37
4	0.75± 0.04	0.84	ND	0.84
5	1.20± 0.03	1.58	ND	1.58

* ND = not-detected

$y_1 = 34.0$ mV hence, $34.0 = 315.2[As(V)] + 1.141$
$[As(V)] = 0.10$ mg/l
For sample no. 3, $y_3 = 16.0$ mV (peak height using water stream = 0)
[As(III)] is calculated from $y_3=173.4[As(III)]+1.445$

hence, $[As(III)] = (16.0-1.445)/173.4=0.08$ mg/l

For sample no. 5, $y_1 = 37.0$ mV
and h (peak height using KIO_3 stream) = 39.0 mV

$[As(V)] = (37.0-1.141)/315.2 = 0.11$ mg/l
$[As(III)]=\{39.0-218.2(0.11)-0.5747-1.445\}/173.4$
$=0.08$ mg/l

Determination of As(III) and As(V) in water samples

The recommended conditions (Table 2) were used to determine As(III) and As(V) in leaching solutions from a zinc ore dump. The results are represented in Table 4. A comparative determination of As(V) by HG-AAS was also carried out. The differences

between the means obtained from the proposed method and the reference method (HG-AAS) were evaluated by a t-test. The calculated t-test value of the proposed-FIA and HG-AAS methods is 0.89. The critical value of t-test is 2.13 (4 degrees of freedom) at the confidence interval of 90%, indicating that the results obtained by the recommended method are comparable to those obtained by the reference method.

Conclusion

A flow injection system with in-valve C18 SPE column for speciation of arsenic(III) and arsenic(V) by sequential determination, based on the formation of a heteropoly acid with molybdate reagent in acid solution and subsequent reduction of Mo(VI) to Mo(V)(molydenum blue), is proposed. As(III) in a mixture of AsO_2^- and AsO_4^{3-} is merged with an oxidizing agent solution(KIO_3) and thereby converted to As(V). The total AsO_4^{3-} is preconcentrated onto a C18 in-valve microcolumn. The sorbed As-complex is eluted and continuously monitored by an LED colorimeter. The signal corresponds to the sum of As(V) and As(III). A signal obtained using water instead of the KIO_3 stream gives the As(V) concentration alone in the mixture.

The method has been applied to a sample free from phosphate, such as water leachates from zinc ores.

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Flow-Injection and Sequential-Injection Determinations of Paracetamol in Pharmaceutical Preparations Using Nitrosation Reaction

Rodjana BURAKHAM,* Supunnee DUANGTHONG,* Lop PATIMAPORNLERT,* Narong LENGHOR,* Suphaphorn KASIWAD,* Lalida SRIVICHAI,* Somchai LAPANANTNOPPAKHUN,*, Jaroon JAKMUNEE,*,** and Kate GRUDPAN*,**†**

**Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
***Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand*

A simple FI and two different SI systems have been investigated for the determination of paracetamol by employing a simple reagent for a nitrosation reaction. It is based on the on-line nitrosation of paracetamol with sodium nitrite in an acidic medium. The formed nitroso derivative species reacts further with sodium hydroxide to convert it to a more stable compound. The yellow product is continuously monitored at 430 nm. The FI system is very simple and cost effective for fast manual operation (60 injections/h; $y = 0.268x + 44.314$, $r^2 = 0.9910$ for 400 - 1000 mg/l and $y = 0.1687x + 145.72$, $r^2 = 0.9970$ for 1000 - 2500 mg/l). The two SI systems with different components and configurations are automated and optimized for the conditions for which no extra dilution is to be required for sample handling: one with a syringe pump and two selection valves (60 samples/h; $y = 0.1488x - 4.7297$, $r^2 = 0.9946$ for 400 - 1000 mg/l and $y = 0.0858x + 63.933$, $r^2 = 0.9849$ for 1000 - 2500 mg/l); the other is simpler and more cost-effective, with an autoburette and only one selection valve (15 samples/h; $y = 0.0072x + 1.1467$, $r^2 = 0.9977$ for 200 - 1000 mg/l and $y = 0.0028x + 5.4699$, $r^2 = 0.9879$ for 1000 - 2500 mg/l). They have all been applied to assay paracetamol in pharmaceutical preparations. The obtained results agree with those by the US Pharmacopeia method.

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Introduction

Paracetamol is an extensive analgesic and antipyretic drug. Several batch methods have been reported for the determination of paracetamol in pharmaceutical preparations, such as spectrophotometry,[1-6] reflectance near-infrared spectroscopy,[7] chemiluminescence[8] and liquid chromatography.[2] A number of flow-injection (FI) methods have also been reported for the determination of paracetamol, such as FI-spectrophotometry, using different on-line derivatization reactions. However, the control of such reactions and/or manifolds is still complicated.[9-12] Some methods, such as FI-FTIR[13] and FI with a boron-doped diamond thin film electrode,[14] involve relatively higher cost instruments.

A further generation of FIA, SIA is a great potential technique for chemical analysis due to much less reagent consumption, its simplicity and convenience with which manipulation can be automated. So far, an SI procedure for paracetamol determination has been reported. It is based on a reaction with hexacyanoferrate(III), followed by a reaction with phenol at elevated temperature in aqueous ammonia.[13]

Simple and inexpensive spectrophotometric flow-injection and sequential-injection systems for the determination of paracetamol using simple reagents based on preliminary concepts introduced at the ICFIA 2001 Conference,[15] are presented. The procedures employ the reaction of paracetamol with nitrous acid at room temperature, producing a derivative.

Experimental

Chemicals and reagents

All of the reagents used were of analytical reagent grade. Deionized water was used throughout the experiments. A stock solution (5000 mg/l) of 4-acetaminophenol standard (paracetamol, Fluka, Switzerland), which was assayed using the USP method,[16] was prepared by dissolving 0.5146 g of the standard in water and diluting to the mark in a 100 ml volumetric flask. Working standards were freshly prepared by diluting the stock solution with water to obtain appropriate concentrations.

Sample preparation

Some locally commercial pharmaceutical preparations were taken as samples to be assayed. For tablet samples, 20 tablets were accurately weighed and finely powdered. An amount of the powder equivalent to a tablet was dissolved in water. After stirring for 15 min at a temperature of 60 - 70°C, the volume was made to 250 ml. The solution was then filtered before analysis. Further appropriate dilutions for FIA procedures were made using water. For a syrup sample, an aliquot corresponding to 200 mg of paracetamol was diluted to 100 ml

† To whom correspondence should be addressed.
E-mail: kate@chiangmai.ac.th

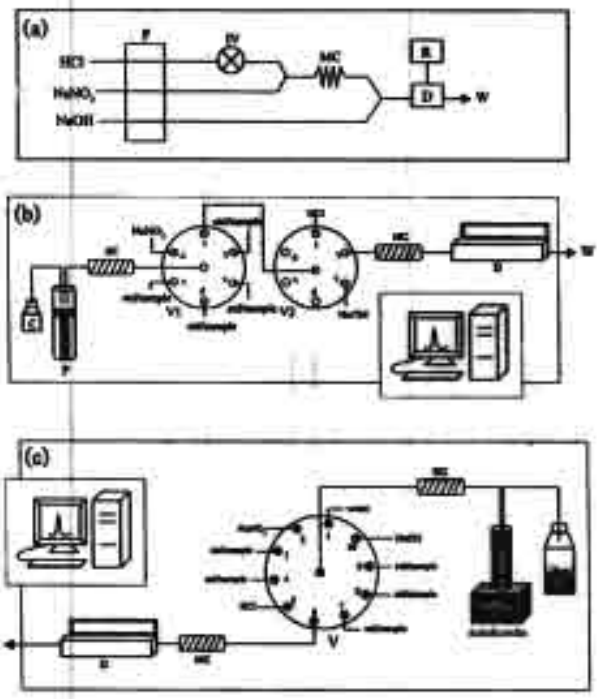

Fig. 1 Schematic diagrams of FI/SI systems for determination of paracetamol: (a) FIA, (b) SIA-I and (c) SIA-II. P, pump; C, carrier; HC, holding coil; MC, mixing coil; IV, injection valve; V, selection valve; D, detector; R, recorder; W, waste.

and then filtered.

FI system

The FI system, similarly to the previously reported one,[16] which is more simple than that described by Knochen *et al.*,[19] consisted of a peristaltic pump (Ismatec, Switzerland), a six-port injection valve (V-451, Upchurch), a reaction coil, a colorimeter (Cole Parmer, USA) with a flow through cell (Hellma, Germany) of 1 cm light path and a recorder (Philips PM 8251, Holland), as shown in Fig. 1(a). Through an injection valve, a solution containing paracetamol was injected into a HCl carrier and merged with $NaNO_2$ to form the nitroso derivative, which was subsequently stabilized with NaOH. The absorbance of the formed product was continuously recorded at a wavelength of 430 nm, and the peak height was used for an evaluation.

SI systems

Two SI systems were investigated. Both systems were aimed for operation with minimum steps in sample handling, such as no requirement for further dilution of the sample stock solution. The first one (SIA-I) was a SI Analyser (Laboratory made, Turku Center for Biotechnology, University of Turku and Åbo Akademi University, Finland) consisting of a 2500 µl syringe pump (Cavro), two six-port selection valves (Cavro), a holding coil (200 cm × 0.6 mm i.d.), a reaction coil (100 cm × 0.6 mm i.d.) and using a Spectronic 21 (Bausch & Lomb, USA) as a detector with a flow-through cell (Hellma, Germany) of 1 cm light path, as shown in Fig. 1(b). A personal computer was used for instrumental control, data acquisition and evaluation *via* a Lab PC+ interface card (National Instruments) and an AnalySIA program (The Biosense Team).

The second SI system (SIA-II) used is schematically depicted in Fig. 1(c). It consisted of an autoburette Dosimat 765 (Metrohm, Switzerland) equipped with a 10 ml exchange unit, for a pumping system, and connected to a personal computer *via* an RS232C interface, a 10-position selection valve VICI with a microelectric actuator (Valco Instruments, USA) and a Spectronic21 (Bausch & Lomb, USA) detector with a flow-through cell (Hellma, Germany) of 1 cm light path. The autoburette was connected to the center of the selection valve by means of a holding coil (250 cm × 1.27 mm i.d. Tygon tubing). A mixing coil (30 cm × 0.79 mm i.d. PTFE tubing) was placed between the selection valve and the detector. Both instrumental control and data acquisition were manipulated *via* software using LabVIEW, developed in-house and using a CYDAS ULV interfacing board (CyberResearch, USA). This software provided control of the volume to be dispensed or aspirated by the autoburette, flow rate, selection of the different valve positions and performed data acquisition. The data processing was computed by using MATLAB.

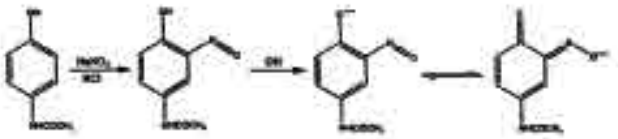

Fig. 2 Schematic diagram of the nitrosation reaction of paracetamol.[1]

The SI operation steps were as follows: firstly, the manifold lines were washed with water, and all of the reagents were filled into the ports of the selection valves. Then, suitable volumes of the reagents were sequentially aspirated and stacked as zones in a holding coil. Finally, these zones were propelled through a reaction coil. A zone penetration occurred. The absorbance of a product zone was continuously monitored at a wavelength of 430 nm.

Results and Discussion

The FIA

The flow system and chemical variables were investigated. Optimization studies were carried out for each individual variable and optimum values were selected. The effect of the mixing coil length, which influenced both the sensitivity (*i.e.* slope of a plot of concentration (mg/l) of paracetamol *vs.* peak height) of the method and the sampling frequency, was studied. The lengths of mixing coils between 50 - 280 cm were examined. A longer mixing coil resulted in increasing the sensitivity as a result of promoting better mixing of the sample and reagent. A mixing coil of 200 cm was selected for further experiments due to the linearity and sensitivity.

By fixing the flow-system variables (mixing coil length, 200 cm; flow rate, 2.0 ml/min and sample volume, 70 µl), the influence of the chemical variables was studied by varying the concentrations: NaOH (0.03 - 0.10 mol/l), HCl (0.03 - 0.15 mol/l) and $NaNO_2$ (0.01 - 0.14 mol/l). The conditions were selected to be 0.10, 0.07 and 0.07 mol/l for NaOH, HCl and $NaNO_2$, respectively.

The SIA-I

By using the SIA-I system, the sequence order of aspiration was firstly optimized to ensure good mixing of the sample zone with all of the reagents involved. Several sequence orders were examined and a suitable one that provided a good peak shape and high sensitivity (better response) was selected. The selected

Table 1 Selected conditions of the SIA-I system for the determination of paracetamol

Sequence	Valve no.	Position	Volume/μl	Description
1	2	1	200	Aspirate 0.05 mol/l HCl into HC
2	1	6	75	Aspirate 0.1 mol/l $NaNO_2$ into HC
3	1	2 - 5	10	Aspirate sample into HC
4	2	1	100	Aspirate 0.15 mol/l NaOH into HC
5	2	3	250	Aspirate 0.05 mol/l HCl into HC
6	1, 2	1, 2	2500	Pump to detector with flow rate of 9 ml/min

sequence of the sample and the reagent zones was created by first aspirating.

HCl into a holding coil, then, $NaNO_2$ and the standard/sample were inserted to the acid zone to increase mixing of the sample and the reagents. In this step, a nitroso derivative compound was formed. NaOH was finally aspirated to stabilize the product before detection of the signal.

Although some reported that the reaction product was a nitro derivative,[17,19] there has been a report mentioning the product being a nitroso derivative.[1] A study on the formation of metal chelates of such a reaction product indicated that the formation of a nitroso derivative of acetaminophen is more probable than the nitro derivative.[1]

According to the nitrosation reactions[1] of paracetamol, as shown in Fig. 2, the nitroso derivatization of paracetamol and $NaNO_2$ should take place under an acidic condition. Therefore, the $NaNO_2$ zone should overlap with the sample and the HCl zone to ensure an acidic medium for efficient forming of the nitroso compound before being stabilized by the NaOH zone to convert it into another species, to be monitored for absorbance. It was found that the best sequence was to sandwich the sample and the $NaNO_2$ zones with two HCl zones.

The concentrations of the three reagents ($NaNO_2$, HCl and NaOH) involved in the reactions were optimized. A series of concentrations of a single reagent was varied, while the others were kept constant. The reagent concentrations giving high sensitivity and good linearity were selected by considering a regression equation (*i.e.* slope and r^2-value) for a plot of the concentration of the paracetamol *vs.* peak area. The trends of the reagent concentrations were quite similar to those obtained from the FI system. However, it is not convenient to work with high concentrations of NaOH, since the Schlieren effect would have a great influence.

The effects of the reagent and sample volumes were evaluated by varying the volumes of NaOH (100 - 300 μl), HCl (100 - 350 μl), $NaNO_2$ (50 - 200 μl) and the sample (5 - 50 μl). An increase in the reagent and sample volumes resulted in increased sensitivity. However, a poor sensitivity was obtained by using a very high volume, probably due to less zone overlap.

In addition, the flow rate was optimized to achieve a good analytical signal and sensitivity. The selected conditions are summarized in Table 1.

SIA-II

Although the SIA-I system could be successfully applied to the determination of paracetamol, another SIA set-up with more cost effective considerations was investigated. Instead of a syringe pump, an autoburette was used, and only one 10-port selection valve was employed. Optimization was studied. The optimum conditions are described in Table 2.

Table 2 Selected conditions of the SIA-II system for the determination of paracetamol

Sequence	Valve position	Mode	Volume/μl	Description
1	5	PIP[a]	300	Aspirate 0.05 mol/l HCl into HC
2	6	DIS C[b]	110	Dispense HCl
3	2	PIP	110	Aspirate 0.10 mol/l $NaNO_2$ into HC
4	3 - 4, 7 - 9	PIP	20	Aspirate sample into HC
5	6	PIP	110	Re-aspirate HCl (sequence 2)
6	10	PIP	250	Aspirate 0.20 mol/l NaOH into HC
7	6	DIS C	5000	Dispense to detector with flow rate of 30 ml/min

a. Pipetting.
b. Cumulative dispensing.

Table 3 Analytical characteristics of the proposed methods

Parameter	FIA	SIA-I	SIA-II
Linear range (mg/l), r^2	400 - 1000, 0.9991 1000 - 2500, 0.9970	400 - 1000, 0.9946 1000 - 2500, 0.9849	200 - 1000, 0.9977 1000 - 2500, 0.9879
%RSD (n = 11)	2[a]	3[a,b]	4[a,b]
Detection limit (mg/l)	45	70	65
Sampling frequency (h^{-1})	60	60	15

a. At 2000 mg/l.
b. Without extra dilution for sample handling.

Analytical characteristics

An evaluation of the analytical characteristics of all the proposed procedures was carried out by studying the linear range, precision, detection limit and sample frequency, as summarized in Table 3.

The precision of each proposed procedure was studied. When using a standard (2000 mg/l), the RSDs for SIA procedures with no dilution for sample handling were less than 4%. RSDs of less than 1% for a particular case could be obtained by altering some conditions, which should be useful for the routine quality control of a particular formula of pharmaceutical preparation. The precision of such a procedure was tested daily for 5 days. It was found that the RSDs were also within 4%.

By using the FI method, the effect of foreign compounds that can be found in pharmaceutical preparations containing paracetamol was also studied. Chlorpheniramine maleate with a concentration of up to 80 mg/l was tested for the determination of paracetamol (2000 mg/l). The results showed that the presence of chlorpheniramine did not interfere with the determination of paracetamol.

The accuracy of the proposed methods was evaluated by analyzing real samples. The results agreed with those obtained

Table 4 Determination of paracetamol in some pharmaceutical preparations

Sample	Type of preparation	%Label			
		FIA	SIA-I	SIA-II	Standard method[18]
1	Tablet	99	97	97	92
2	Tablet	98	98	98	92
3	Tablet	102	101	101	94
4	Tablet	95	100	100	93
5	Tablet	100	105	105	96
6	Tablet	107	90	90	98
7	Tablet	104	98	98	91
8	Tablet	107	99	99	94
9	Tablet	106	98	103	92
10	Suspension	108	102	95	92
11	Syrup	105	102	101	92

by the standard method (Table 4).[18]

Application to samples

The three proposed systems (FI, SIA-I and SIA-II) were applied to the determination of paracetamol in pharmaceutical preparation samples. Each sample was prepared to obtain solutions having concentrations of *ca.* 700 and 2000 mg/l of paracetamol. For SIA procedures, no sample dilution was made. The obtained results are summarized in Table 4. The evaluation by a t-test at the 95% confidence level indicates that there is no significant difference in the results obtained by the proposed and standard methods.

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ORIGINAL PAPER

D. Nacapricha · K. Uraisin · N. Ratanawimarnwong
K. Grudpan

Simple and selective method for determination of iodide in pharmaceutical products by flow injection analysis using the iodine–starch reaction

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Abstract This work exploited the well-known iodine–starch reaction for development of a simple flow-injection (FI) method for determination of iodide in pharmaceutical samples. Iodide in an injected zone was oxidized to iodine. A gas diffusion unit enables selective permeation of iodine through a hydrophobic membrane. Detection was made very selective for elemental iodine by employing formation of the I_3^-–starch complex. The detection limit ($3S/N$) of the system was 1 mg $I\,L^{-1}$. For a liquid patent medicine used for asthma treatment we suggested modification of the system. Direct injection of this sample, which contains a particularly high concentration level of iodide (ca. 9000 mg $I\,L^{-1}$), can be achieved by coupling a dialysis unit to the FI system. This has increased the working range to 6000–10,000 mg $I\,L^{-1}$ without employing complicated nanoliter injection.

Keywords Flow injection · Gas diffusion · Dialysis · Iodide · Iodine–starch

Introduction

Iodine compounds are often used in pharmaceutical products. Iodine in the form of tri-iodide is an antiseptic and disinfectant and potassium iodide is thought to act as an expectorant. In patients with hyperthyroidism iodide rapidly inhibits the synthesis of thyroid hormones. In some countries potassium iodide tablets are sold in drug stores for thyroid protection in the event of nuclear emergency. However, iodide should be used with extreme caution when patients are markedly sensitive to the element [1]. Therefore it is essential to have an accurate and precise method available to determine the iodine content of pharmaceutical preparations.

Several methods are available for determination of iodide at different levels. For determination of microgram and nanogram amounts of iodide, the most frequently used method is chromatography [2, 3, 4, 5]. Iodide can be determined by spectrometric [6], catalytic spectrometric [7, 8], and potentiometric [9, 10] methods. However, most available methods are suitable for trace levels of iodide [2, 3, 4, 5, 6, 7, 8].

Mixt. Stramonium Co. is a liquid patent medicine sometimes used in patients suffering from asthma [11]. The level of iodide in this drug is quite high, ca. 9000 mg $I\,L^{-1}$. Apart from potassium iodide 12 g, other ingredients are present in one liter: hyoscyamus tincture 134 mL, stramonium tincture 20 mL, and liquorice liquid extract 34 mL. The liquid medicine has a very dark brown color. It would be quite difficult to measure the iodide content using a common colorimetric method.

Usually a gas diffusion (GD) unit, as applied to flow injection analysis, is a form of an on-line clean-up system. Volatile analytes can be separated from interference in the sample by permeation across a hydrophobic membrane. This process is fairly selective, because few species are turned into gaseous form at room temperature [12]. Employment of a GD unit enables colored samples, or samples which differ in refractive index from the reagent, to be analyzed without optical interference. Motomizu and his group reported a number of GD applications in flow injection (FI) analysis [12, 13, 14, 15, 16, 17] including application to iodide and other halogens [15]. In 1997, Hakedal and Egeberg [18] proposed an FI system for determination of iodide in brine. This system employed a

D. Nacapricha (✉) · K. Uraisin · N. Ratanawimarnwong
Department of Chemistry, Faculty of Science,
Mahidol University, Rama 6 Rd, 10400 Bangkok, Thailand
e-mail: scdnc@mahidol.ac.th

D. Nacapricha
Institute of Science and Technology for Research and Development, Mahidol University,
Salaya, 73170 Nakorn Pathom, Thailand

K. Grudpan
Department of Chemistry, Faculty of Science,
Chiang Mai University, 50200 Chiang Mai, Thailand

K. Grudpan
Institute of Science and Technology Research and Development,
Chiang Mai University, 50200 Chiang Mai, Thailand

GD unit fitted with a PTFE membrane. Iodine (I_2), from oxidation of the iodide analyte permeated through the membrane into a stream of iodide. Absorbance of tri-iodide (I_3^-) was measured in the UV region for calibration.

In this work we incorporated a GD unit into a colorimetric FI system. Detection is by simple measurement of the absorbance of the I_3^-–starch complex, recently presented by our group [19]. The system was primarily employed to analyze pharmaceutical products containing iodide, for example KI tablets. These samples were dissolved in water and the iodide content of filtered samples could be determined directly by use of our system. However, for Mixt. Stramonium Co. the sample much be diluted before analysis. Staden et al. recently adopted dialysis for simultaneous dilution of a sample [20]. For our work on Mixt. Stramonium Co. a dialysis unit was coupled to the GD unit and was used as an on-line dilutor. The use of a GD unit with detection based on I_3^-–starch has made the system very selective and simple. The sensitivity in the visible range is twice that of the spectrometric detection of I_3^- in the UV region [18].

Experimental

Reagents and solutions

Flow injection

All chemicals were analytical reagent grade. Deionized–distilled water was used for chemical preparation. A stock solution of standard iodide (ca. 20,000 mg L^{-1}) was prepared by dissolving approximately 26.16 g (accurate weight) potassium iodide crystals (Merck, Germany) in 1 L water. Working solutions of iodide were obtained by appropriate dilution with water.

The oxidant was prepared by dissolving 3 g potassium dichromate (Univar, Australia) in 1 L of 10% (*v/v*) sulfuric acid (Labscan, Ireland).

The acceptor stream was a solution of 0.016 mol L^{-1} potassium iodide and 0.1% (*w/v*) starch. This mixture was first made by mixing 1 g starch (Merck) with a few milliliters of water to form a slurry. The slurry was added to 1 L boiling water. This resulted in a starch solution of 0.1% (*w/v*). Potassium iodide (2.6 g) was then dissolved in this starch solution. This mixed solution was always prepared daily, because of deterioration of starch molecule.

Thiosulfate solution (0.1 mol L^{-1}) was prepared by dissolving 2.5 g sodium thiosulfate pentahydrate (Merck) in 100 mL water. This solution was diluted with 0.1% (*w/v*) starch to give 2×10^{-3} mol L^{-1} of thiosulfate. The solution was used to remove the starch complex or iodine residues deposited on tube walls.

Potentiometric analysis

Potassium iodide standards for calibration were prepared from the same stock iodide solution prepared for FI analysis (20,000 mg L^{-1}). Sodium nitrate (5 mol L^{-1}), the ionic strength adjustor, was prepared by dissolving 42.5 g crystalline sodium nitrate (Fluka, Switzerland) in 100 mL water.

Samples

Commercial potassium iodide tablets were used for method validation. NO-RAD (65 mg KI per tablet) is a product of Body Gold, USA. IOSAT (130 mg KI per tablet) is distributed by Anbex (NY, USA). These sample tablets were dissolved in deionized–distilled

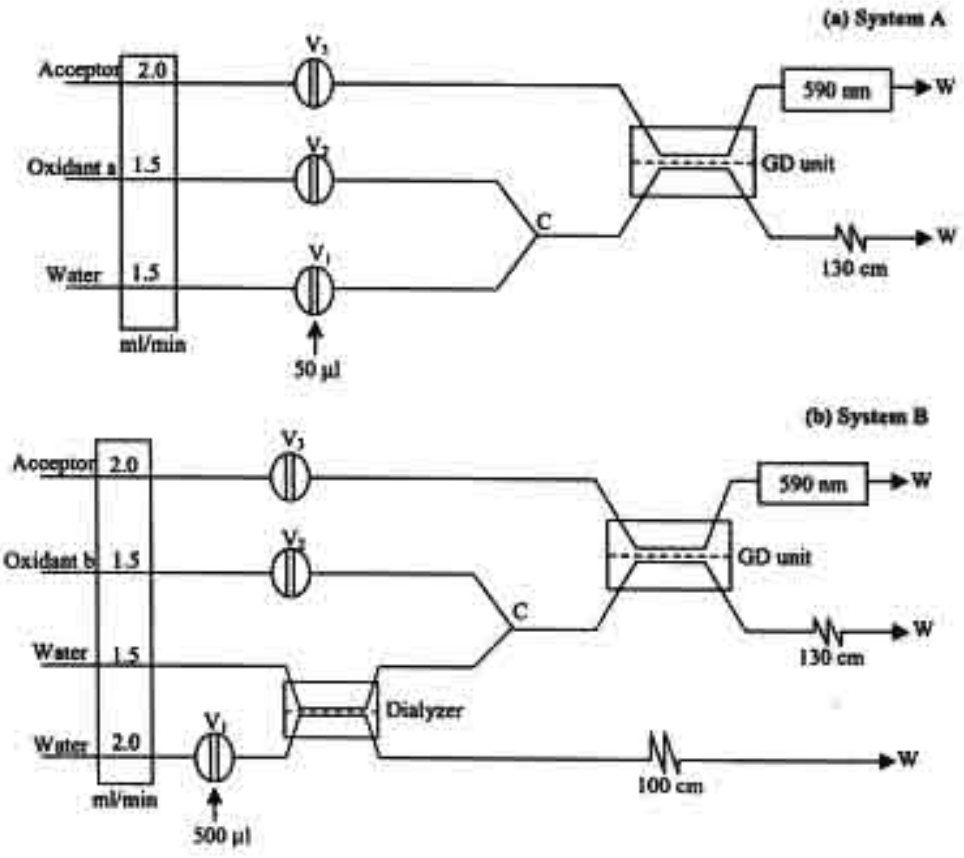

Fig. 1 The flow-injection systems used in this work for determination of iodide. Acceptor, 0.016 mol L^{-1} KI in 0.1% (*w/v*) starch; oxidant a, 0.01 mol L^{-1} $K_2Cr_2O_7$ in H_2SO_4 of different concentrations (2, 4, 6, 8, and 10%, *v/v*); oxidant b, 0.01 mol L^{-1} $K_2Cr_2O_7$ in 10% (*v/v*) H_2SO_4. *C*, a confluence point; V_1, sample injection port (under the optimized conditions), V_2 and V_3, 250 µL injection of cleaning solution (2×10^{-3} mol L^{-1} $Na_2S_2O_3$ in 0.1% (*w/v*) starch); *W*, waste

water. Suspension was eliminated by filtration through Whatman filter paper No. 1 before injection into the FI systems.

The medicinal sample Mixt. Stramonium Co. is produced by the Government Pharmaceutical Organization, Bangkok, Thailand. It is a dark-brown liquid.

FI apparatus

Figure 1 depicts two FI systems, A and B, which were used for method development. Each manifold employed an Ismatec peristaltic pump (model IS 7610, Switzerland) for propelling reagents. Three Rheodyne injection valves (model 5020, USA) were used. A Metrohm gas diffusion unit (model 754, Switzerland), fitted inside with a circular PTFE membrane (47 mm i.d. with pore size 0.45 µm; Sartorius, Germany), was employed. The unit consists of two Perspex blocks, each with a concentric spiral groove (2 mm× 300 mm×0.2 mm, width×length×depth). System A, shown in Fig. 1a, was used in the preliminary studies. The system was later modified as shown in Fig. 1b (System B), by incorporating a dialysis unit for on-line dilution of samples containing high levels of iodide.

The homemade dialysis unit used in System B (Fig. 1b) consisted of two half-Perspex blocks. Each block had a straight groove (1.5 mm×120 mm×1 mm, width×length×depth). A cellulose acetate membrane (10 mm wide with molecular weight cut-off from 12,000 to 14,000 Dalton; Thomas Science, USA) was placed inside the dialysis unit.

A Jenway spectrophotometer (model 6405, UK), fitted with a Philips flow cell of 0.01 mL volume, was used for monitoring the absorbance at 590 nm. PTFE tubing of i.d. 0.5 mm was used for construction of the two FI systems. An Alltech chart recorder (model LR 93025, USA) was employed for recording the signal.

Table 1 is the summary of the operating steps of the FI systems shown in Fig. 1.

Potentiometric method

Sample solution (30.0 mL, accurately measured) was transferred into a 50-mL beaker. To this sample sodium nitrate solution (5 mol L^{-1}, 0.6 mL) was added for control of the ionic strength. The solution was analyzed by measuring the potential developed across the Orion (USA) iodide-ISE (model 9453) and an Orion saturated calomel electrode. A digital Orion Ionanalyzer (model 601A) was used for this measurement. The operation of this technique was performed in accordance with the instruction manual [21]. Calibration was carried out with standard solutions (1 to 1000 mg I L^{-1}) prepared from potassium iodide stock solution (20,000 mg I L^{-1}). According to the specification, the linearity of the iodide electrode is from 10^{-1} to 10^{4} mg I L^{-1}.

Table 1 Summary of the operating steps for the FI manifolds shown in Figs. 1a and 1b (Injections: V_1, sample; V_2 and V_3, cleaning solution)

Step	Valve position			Duration (s)	
	V_1	V_2	V_3	Fig. 1a[a]	Fig. 1b[b]
1	Inject	Load	Load	45	110
2	Load	Inject	Inject	75	75

[a]Analysis time per injection run is 45+75 s,=2 min
[b]Analysis time per injection run is 110+75 s,=3 min

Results and discussion

Manifold design

GD-FI system

First, the manifold of the gas-diffusion flow injection (GD-FI) system, shown in Fig. 1a, was examined. Introduction of a liquid sample was carried out via injection through V_1. In this manifold, iodide in sample plugs is oxidized after mixing with the stream of oxidant, acidic potassium dichromate. Liberated iodine (I_2), which diffuses through the PTFE membrane, forms the tri-iodide–starch complex in the acceptor stream. Detection of the blue complex zone and calibration are similar to previous work with iodized salt [19].

System cleaning

When measuring the tri-iodide–starch complex, a problem often arises from deposition of the complex on tube walls and the flow-cell. Injection of pure thiosulfate solution to remove the adsorbed starch complex [19] was found effective only for the acceptor stream (Fig. 1a). The species in the donor stream expected to be adsorbed on the manifold walls are $I_{2(aq)}$ and/or $I_3^-{}_{(aq)}$. However, the solution found suitable for removing the complex in this stream is a mixture of thiosulfate and starch (2×10^{-3} mol L^{-1} $Na_2S_2O_3$ in 0.1%(*w/v*) starch). Addition of starch into the previously employed cleaning solution [19] is necessary. It was observed that reduction of iodine by $S_2O_3^{2-}$ was easier (or more rapid) for the iodine complex than for the non-complex form. However, this was true for deposits on tube walls and is different from what is normally used and observed in titration [22].

The cleaning solution was injected at two positions (V_2 and V_3) as shown in Fig. 1a. The cleaning steps and sample injections suitable for this manifold are summarized in Table 1.

Appropriate concentration of sulfuric acid in the oxidant

For oxidation of iodide in a homogeneous system, H^+ must be present at sufficient concentration [18]. The system employed is based on non-homogeneous mixing and spectrometric detection was not at reaction equilibrium. For such a system concentrations of chemicals must be optimized. For the system in Fig. 1a different concentrations of sulfuric acid in the oxidant (2 to 10%, *v/v*) were investigated for the concentration of potassium dichromate selected, 0.01 mol L^{-1}.

To optimize the acid concentration a standard iodide solution of 300 mg I L^{-1} was chosen. By repeated injection of this iodide solution it was shown that absorbance increased dramatically with increasing concentration of sulfuric acid from 2 to 6% (*v/v*) before approaching a plateau at

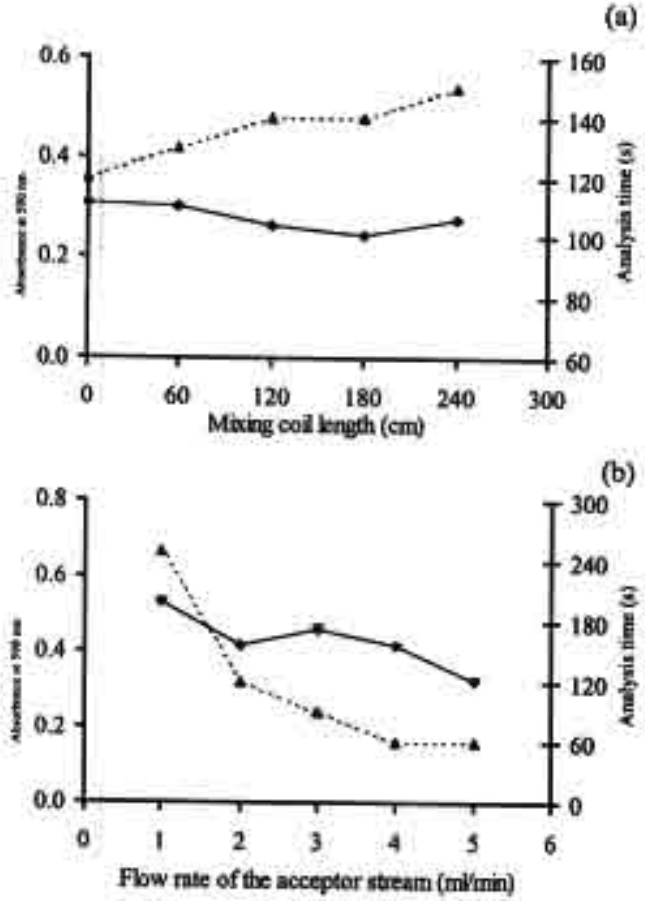

Fig. 2 Effect on analytical signal (*diamonds*) and analysis time (*triangles*) of variations in (a) coil length (between C_1 and GD unit) in System A (Fig. 1a) and (b) acceptor flow rate

8% (*v/v*). The highest concentration of sulfuric acid (10%, *v/v*) was chosen as the optimum.

System A

Optimization

Preliminary optimization was carried out using repeated injection (n=5) of 200 mg I L^{-1} standard iodide solution, to study two aspects of the manifold in Fig. 1a. The objective was to get a compromise between analytical signal and analysis time. "Analysis time" is defined as the time taken from injection of sample until the cycle of system operation is complete (for a run of 1 injection).

Mixing coil of different lengths, from 0 to 240 cm, were inserted between the confluence point (C) and the GD unit (Fig. 1a). According to the results in Fig. 2a a slight difference in the signals was observed with variation of coil length. The shortest analysis time was achieved when no mixing coil was used. Thus, this condition was chosen.

The flow rate of the acceptor stream (Fig. 1a) was varied from 1 to 5 mL min^{-1}. As expected, the absorbance decreased as the flow rate increased (Fig. 2b). This was because of the decrease in the yield of the reaction between tri-iodide and starch. At an acceptor flow rate of 1 mL min^{-1} it was observed that the system was too sensitive for samples containing high iodine concentrations (>300 mg I L^{-1}). However, too rapid a flow rate would lead to high consumption of reagent. We chose to set the flow rate at 2 mL min^{-1}. The analysis time of 2 min per injection was obtained at this flow rate.

Analytical features and applications of the GD-FI manifold (The System A)

To conclude, the optimized conditions for System A employ 10%(*v/v*) H_2SO_4 as the oxidant and an acceptor stream flow rate of 2 mL min^{-1}. System characteristics are summarized in Table 2.

Three samples were analyzed for iodide content. The results were compared with values obtained by using an ion-selective electrode (Table 3). The iodide content as determined by the two methods agreed significantly with each other and with the nominal values. A paired t-test was employed to compare the results in terms of the concentration (mg I L^{-1}) of the injected liquid samples. No significant difference was found between the two analytical methods (P=0.01). This good agreement demonstrates that the (GD-FI manifold in Fig. 1a) is suitable for these samples. No evidence was found that these pharmaceutical products cause interference.

System B

The GD-FI system with on-line dilution by dialysis

The level of iodide present in the liquid Mixt. Stramonium Co. medicine is particularly high (ca. 9,000 mg I L^{-1}) compared with KI tablets. Dissolution of a tablet in 500 mL of

Table 2 Analytical performance of GD-FI system

Analytical feature	GD-FI (System A, Fig. 1a)	GD-FI with on-line dilution (System B, Fig 1b)
1. Working range (mg I L^{-1})	50 to 300	6000 to 10,000
2. Standard equation	PH=3.4×10^{-3}[I$^-$]+9.1×10^{-2}; r^2=0.999	PH=8.3×10^{-5}[I$^-$]–2.3×10^{-1}; r^2=0.999
3. Precision (*RSD*)	1.27% (for 100 mg I L^{-1}, n=10)	1.44% (for 9000 mg I L^{-1}, n=25)
4. Sample throughput (injections h^{-1})	30	20
5. Limit of detection (mg I L^{-1}; 3*S*/*N*)	1	200

PH: peak height (absorbance)

Table 3 Determination of iodide in pharmaceutical products by this method and the ISE method, compared with the nominal content. The means and standard error were from a set of three samples of the same product

Trade name	Sample type	Concentration unit	Iodide content		
			Labeled	GD-FI (Fig. 1a)	ISE
1. NORAD	KI tablet	mg I per tablet	49.7	51.6±7.3	57.9±7.3
2. IOSAT	KI tablet	mg I per tablet	99.5	101.2±3.5	102.8±5.6
3. Mixt. Stramonium Co.	Liquid patent medicine	mg $I L^{-1}$	9,181	8,926±170	9,312±335

Table 4 Results obtained by use of different injection volumes when working on the GD-FI system coupled with a dialysis unit (System B, Fig. 1b). Calibration ranged from 6000 to 10,000 mg $I L^{-1}$

Injection volume (μL)	Signal range (a.u.)	Standard equation[a]	r^2
250	0.106–0.279	$PH=4.3\times10^{-5}[I^-]-1.5\times10^{-1}$	0.999
350	0.169–0.390	$PH=5.5\times10^{-5}[I^-]-1.6\times10^{-1}$	0.999
500	0.249–0.603	$PH=8.9\times10^{-5}[I^-]-2.9\times10^{-1}$	0.995
750	0.361–0.725	$PH=9.1\times10^{-5}[I^-]-1.9\times10^{-1}$	0.997

PH: peak height (absorbance)
[a]Iodide standards were 6,000, 8,000 and 10,000 mg $I L^{-1}$.

water will bring the concentration to approximately 100 or 200 mg $I L^{-1}$, much lower than in Mixt. Stramonium Co. For this medicine prior dilution with water (1:100) was necessary before the analysis using System A.

On-line dilution in flow-injection analysis is usually achieved by merging and mixing the sample zone with a suitable carrier. This technique is not always applicable when high dilution is required, unless a special mechanism for injecting very small samples is used.

When dialysis [23, 24] is applied to flow injection for pretreatment, analyte concentration is often quite dilute after diffusion through the membrane. Although in our work the sample (Mixt. Stramonium Co.) did not require removal of interferences by use of a dialysis membrane, we employed dialysis for the purpose of dilution by using System B (Fig. 1b).

To obtain the optimized conditions, summarized in Fig. 1b, the effect of injection volume was first studied. A suitable volume should give a signal reading between 0.2 and 0.8 a.u. for the desired calibration range of 6000 to 10,000 mg $I L^{-1}$. Replicate injections of iodide standards were performed using the manifold shown in System B. Results in Table 4 show that absorbance was not appropriate when the injection volume was below 500 μL. The sensitivity and signal range given for injection volumes of 500 and 750 μL were both applicable. However, because small volumes are simpler to handle, a volume of 500 μL was selected for further study.

Performance and application of the FI system with on-line dilution

Figure 1b depicts the manifold developed for final application in analysis of Mixt. Stramonium Co. We can inject the liquid medicine directly into the system without a need for off-line dilution. A summary of the analytical features is shown in Table 2. On-line dilution via dialysis has made the system capable of working at a much higher range than previously (up to 6000 to 10,000 mg $I L^{-1}$).

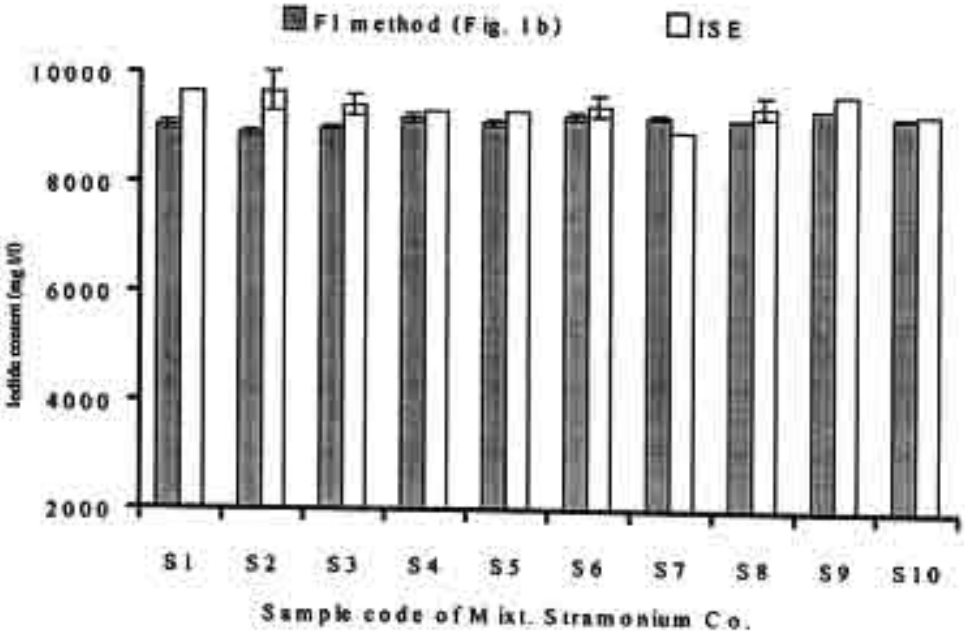

Fig. 3 Comparison of the iodide content determined by the FI method using System B (Fig. 1b) (n=3) and by the potentiometric method (n=3)

The on-line dilution manifold was used to determine iodide in Mixt. Stramonium Co. using direct injection without prior dilution. The results were compared with those determined by use of the iodide-ISE (Fig. 3). A paired *t*-test showed there was no significant disagreement at 99% confidence ($t_{observed}$=2.82; $t_{critical}$=3.25).

Recovery

Recovery was studied for all types of sample, for both FI manifolds. The results showed recovery was satisfactory – from 93.8 to 104%. This implies the samples contain no analytical interferences under the conditions employed for both systems.

Advantage of the proposed methods

For the ISE, which is operated batchwise, it can take longer than 1 min to obtain a stable reading. Electrode washing to ensure no cross-contamination between samples requires 5 to 6 min at least. One should also allow for the time required for transfer of sample. Thus the dominant advantage of our method is probably the throughput of samples. The ISE method enables analysis of approximately eight samples per hour whereas our methods enable throughput of 30 and 20 samples per hour for manifolds A and B, respectively.

For liquid samples containing 6500 to 9500 mg $I L^{-1}$ System B has a good potential for integration into the process for manufacture of the pharmaceutical preparation. Maintenance should be simpler than using an iodide-selective electrode.

Coupling of the dialyzer for analysis of the Mixt. Stramonium Co. sample is useful for two reasons. Dialysis enables on-line dilution of the analyte, which is convenient and enables direct injection of the medicine. Sample matrix is also diluted through the dialyzer membrane. Mixt. Stramonium Co. contains 10% (*v*/*v*) ethanol. Dilution of this alcohol will help maintain proper function of the hydrophobic membrane (PTFE) in the GD unit.

Conclusions

We have developed two flow-injection systems, both employing a gas-diffusion unit, for selective detection of iodide. The principle of detection is very simple, based on formation of the I_3^-–starch complex. However, this complex is sparingly soluble in water and one must be careful not to exceed the appropriate working concentration range of iodide, otherwise clogging of the inside of the tube or deviation from Beer's law will occur. The first system (System A) is recommended for determination of KI tablets. The sample, after dissolution and normal filtration, can be analyzed directly. Use of this FI system for more concentrated samples requires dilution. For our liquid medicine that contains ca. 9000 mg $I L^{-1}$, System B is more suitable. After direct injection, the sample is sequentially diluted and analyzed for iodide content. In fact, the latter system can be adapted for monitoring iodide content in the manufacturing process of the medicine.

Acknowledgements This work was supported by grants from the Thailand Research Fund and the Postgraduate Education and Research Program in Chemistry. The authors would like to thank Associate Professor Prapin Wilairat for editing this paper.

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ผลงานวิจัย ก21

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Editorial

The 11th International Conference on Flow Injection Analysis (ICFIA 2001) was held in Chiang Mai, Thailand, December 16–20, 2001. This conference started informally in the USA in 1987, organized by Gary Christian, Gil Pacey and Jarda Ruzicka. The Conference grew and attracted numerous international participants and was changed to the ICFIA in 1995. At the same time, the Japanese Association for Flow Injection Analysis (JAFIA) was invited to join the Conference and they have held their semi-annual meeting jointly with ICFIA since. ICFIA 2001 was held jointly with the 38th Semi-annual meeting of JAFIA. An international steering committee is chaired by Gary Christian and includes Jose Luis Burguera (Venezuela), Kate Grudpan (Thailand), Bernard Lendl (Austria), Ian McKelvie (Australia), Shoji Motomizu (Japan, JAFIA), Jarda Ruzicka (USA), Tadao Sakai (Japan, J. Flow Injection Anal.), Rolf Sara (Finland), and Koos van Staden (South Africa), with Sue Christian as Advisor.

ICFIA 2001 was hosted by Chiang Mai University and organized by a team in the Department of Chemistry. The Conference was sponsored and supported by Chiang Mai University, Thailand, the JAFIA, the British Council, the Chemical Society of Thailand, the Post-graduate Education and Research Program in Chemistry (PERCH), the Science Society of Thailand (Chemistry and Northern Divisions), the Thai Ministry of University Affairs, the Thailand Research Fund (TRF), Constellation Technology, Corp., FIAlab Instruments, Inc., Foss Tecator, Metrohm Siam Co., Ltd, Perkin Elmer, Inc., and Thai Unique Co., Ltd, (Lachat).

There were 120 participants from 21 countries. Opening the conference was announced by the traditional striking of a Gong, by the President of Chiang Mai University, and was followed by traditional dancing and a drum show. Social programs included a wonderful Thai culture evening with a Khan Toke dinner, Traditional Northern Thai Music and hill tribe dancing; and an afternoon touring and sightseeing with all the participants invited to an elephant camp, and the Queen Sirikit Botanic Garden, followed by an evening with refreshments at the Chiang Mai University, Science Faculty Observatory.

The Conference was devoted exclusively to flow injection analysis and related techniques. Thirty-six papers and 72 posters were presented over three and a half days. Collaborations among the researchers and users of such techniques exit in—and across—various geographical parts of the globe. Some of the collaborations were initiated from the previous ICFIA conferences. Strengthening of the collaborative activities has been observed.

This special issue of Talanta includes manuscripts from the conference, representing state-of-the-art research and application from academic, government and industrial laboratories.

The Steering Committee has selected the next venue for the 12th ICFIA (ICFIA 2003) to be Los Andes University in Merida, Venezuela. Pioneering FIA colleagues Joes Luis Burguera and Marcella Burguera have graciously agreed to organize and host the conference. Professors J.F.

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(Koos) van Staden and Raluca-Ioana Stefan from the University of Pretoria will be co-organizers, in charge of the technical program. The scheduled date is 7–11 December, 2003, a very pleasant time of the year in this beautiful sub-tropical country. Information is posted at www.up.ac.za/academic/chem/analytical/first.pdf, and may be accessed at www.flowinjection.com. Direct contacts are burguera@ciens.ula.ve and koos.vanstaden@chem.up.za.

Those who shared in the contributions to the success of ICFIA 2001 are here thanked.

Kate Grudpan
Department of Chemistry,
Faculty of Science,
Chiang Mai University, 50200 Chiang Mai,
Thailand
E-mail: kate@chiangmai.ac.th

Editors' note: The Conference was proclaimed in honor of Kate Grudpan, in recognition of his being named the Outstanding Scientist of the Year in Thailand for 2001, the first time in the 19-year history of the award that it was given to a scientist from outside Bangkok.—GDC and J-MK.

ผลงานวิจัย ก22

Kate Grudpan

Associate Professor and Senior Research Scholar of the Thailand Research Fund

1953: born, 1974: B.S., Chiang Mai University, 1981: Ph.D., Liverpool John Moores University, UK, 1983-4: Post doctorate at the University of Karlsruhe/Karlsruhe Research Center, Germany, 1991-2: Alaxander von Humboldt Research Fellow 1999: Outstanding Researcher Award of Thailand, 2000-present: Member, Editorial Advisory Board, Talanta, 2001: Senior Research Scholar Award of the Thailand Research Fund, 2001: Outstanding Scientist Award of Thailand, 2002–present: member, International Advisory Board, JFIA

Development of Cost-Performance Flow-Based Chemical Analysis Systems

Various alternatives for instrumentation with parts/components are summarized. Some recent developments are briefed. They include uses of cost-effective reagents, FI, BI SI, LOV, LAV as well as a new concept for flow-based analysis using a stopped FI analyzer.

1.Instrumentation/parts/components of a beginner and/or a person with budget constrain. Various alternatives for cost performance have been proposed for FIA and relative techniques such as pumping system [1-4], injection devices [1-4], mixing devices [2,4,17], detection systems [1-17], and some on-line sample separation/treatment devices [4-7,10,16,18,19].

2.Hyphenated techniques/detection systems. FIA and related techniques have been hyphenated and/or connected to other techniques and devices such as a simple colorimeter [3,9-11,18,30-35], radiometry [1,2], chemiluminescence [11], NIR [36], Raman spectroscopy [37], conductometry [9,10], amperometry [38], voltammetry [15], IC [13] and DSTD[18, 19].

3.Cost-effective reagents. Unstable reagents [7,11,22] can be employed,e.g. permanganate, murexide.

4.Some recent developments. Size-based speciation using FFF has been reported [17]. Bead injection (BI) was for the first time coupled to FI [14,19], SI systems have been proposed: with modified autoburet for reversal flow [12], titration [11],: SI with standard addition-voltammetry. Lab-on-Valve (LOV) systems including micro-titration has been proposed. Novel SI with "Lab-at-Valve (LAV)" systems are being investigated. New concept for flow-based analysis using a stopped-FI analyzer for various analytes without changing any hardware parameters is introduced.

5.Applications have been aimed for various fields including environmental analysis [5,14,18,21], clinical/medical analysis [19-21,25], pharmaceutical [7,11,26],and agro-industry/agriculture [8, 11].

6.Networking. Some of the developments have been under active collaboration in various geographical areas [27].

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Title: Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt

Journal: Talanta, 58, 1195-1201 (2002)

Authors: Nathawut Choengchan[a], Kanchana Uraisin[a], Kezang Choden[a], Weret Veerasai[a], Kate Grudpan[b] and Duangjai Nacapricha[a]

([a]Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400 Thailand and [b] Department of Chemistry, Faculty of Science, Chiang Mai University, 6 Road, Chiang Mai 10400 Thailand)

Summary

Complex formation of iodine-starch has long been used to indicate the end-point for titration in iodine analysis. The complex is formed by intercalation of tri-iodide (I_3^-) inside starch molecules. Since the complex absorbs visible light (λmax = 590 nm), we propose a simple colorimetric detection of this blue complex as applied to flow injection (FI).

This work demonstrates use of iodine-starch in flow injection for analysis of iodine. The application was focussed on determination of iodate in iodized salt. In Thailand, for a longer shelf life, salts are supplemented with potassium iodate, not potassium iodide. The recommended level of iodine in iodized salt is at 50 mg I/kg.

There have been some methods for quantitation of iodine in iodized salts but mostly for the supplementation with potassium iodide [1-4]. A flow injection (FI) method was presented for salts, which are iodized with potassium iodate. The FI method employed amperometric detection and provided a throughput of sample at 35 injections/h [5]. In this work, we present a different concept of detection that is the colorimetric analysis, which is much simpler than the former amperometric method.

We have utilized the Dushman reaction [6] by formation of tri-iodide (I_3^-) when there is a limited amount of iodate and excess amounts of iodide and hydronium ion. In a condition in which starch is present, the blue complex of I_3^--starch then forms. Linear calibration, based on the Beer's law, can be obtained from a plot of the relationship between the absorbance of the complex and the iodate concentration.

In our FI method, a sample plug of salt solution (made to approximately 6% (w/v)) is injected directly into a carrier stream which consists of 2.5×10^{-3} M KI in 6% (w/v) NaCl. The zone in the carrier line is then merged and mixed with a continuous flowing stream that contains starch and acid. Detection of the blue product in the FI manifold employs a colorimeter. In fact, a yellow LED can be an alternative light source, which will make the method even more, cost effective.

In the FI manifold, there are two injection ports. One is for injecting the liquid salt samples and standards. We use another injection valve for injecting a cleaning solution into the manifold. We observed that there was accumulation of tri-iodide starch complex on walls of tubing and the flow cell. This used to cause shift of baseline. We therefore present a very simple idea of cleaning off this deposit on the walls by injecting a small plug of thiosulfate solution. The purpose is to decolorize the blue complex deposit, by reducing tri-iodide (which intercalates inside the starch molecules) to iodide ion. We recommend an injection of thiosulfate solution after every sample injection. Injection of sample is usually carried out at approximately at 30 s intervals.

At the optimized condition, the calibration is perfectly linear in the range of 5.0×10^{-6} to 4.0×10^{-5} M: absorbance = $25{,}520[IO_3^-] - 0.052$, $r^2 = 0.999$. It is suspected that negative intercept may be caused by limitation in the reaction kinetics at low concentrations or it could be due to restriction of the dynamic range of the detector.

The proposed method although is simple but very efficient and precise (RSD = 0.66%). With this principle of detection, the method provides more rapid analysis than the former amperometric FI method. Our sample thoughput is 65 injections/h. A detection limit as low as 2 mg I/kg is achieved. Successful validation of our method was accomplished using other two analytical methods, which are the conventional titration method and the potentiometric method that uses an iodide-selective elective electrode.

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Report on the 2nd Annual Symposium on TRF Senior Research Scholar on Flow-based Analysis, Chiang Mai, Thailand, 6 September 2003

A-one-day symposium was organized with objectives: (1) to report research progress in the past years, especially the last year of the grant on the Thailand Research Fund (TRF) Senior Research Scholar (Kate Grudpan) on "Development of Flow-based Analysis" and (2) to open opportunities for discussion/exchange ideas and information in the research field among those who are in-and outside the group of the TRF Senior Research Scholar Grant.

The symposium invited 2 plenary lectures: "Flow injection analysis for determination of trace air pollutants" by Prof. Dr. Tadao Sakai, Editor-in-chief, JAFIA, Aichi Institute of Technology and "Ultratrace and trace determination with flow-based techniques" by Prof. Dr. Shiji Motomizu, President of JAFIA, Okayama University. There were 70 participants including faculty members, researchers and students from 10 universities with 30 contribution papers which were presented in poster format. Some of the authors were invited to present orally for brief concepts. This was aimed for more discussion and interaction among those who are in the same interests to meet maximum benefit at posters.

Some of the presentations were given by graduate students (master and doctoral). Some recent works on clinical analysis were reported, for examples, flow based ion exchange micro-column system for screening of thalassemia and hemoglobinopathies, a flow-based system to assay specific proteoglycans. Some developments on instrumentation were discussed. They included automated systems for paracetamol; bead injection combining with flow injection system ; an economical alternative for determination of some trace metals, boron doped -diamond thin film electrode in FI system; pervaporation for high speed GC for volatile organic compounds, and dynamic surface tension detector for flow analysis. Various applications were involved such as release of metal ions from contaminated soil in mining area to environment by humic acid colloids; phosphate contents in fertilizer and soil by stopped FI-Analyzer, iodide/iodine contents in various types of samples.

The enjoying and easy environment with good academic atmosphere encouraged the interaction among students, newer and older generations leading to build up relationship for the young and more senior researchers.

Kate Grudpan
Flow-based Analysis Research Group
Chiang Mai, Thailand

ผลงานวิจัย ก25

Flow-injection in-line complexation for ion-pair reversed phase high performance liquid chromatography of some metal-4-(2-pyridylazo) resorcinol chelates

Supalax Srijaranai [a,*], Saiphon Chanpaka [a],
Chutima Kukusamude [a] and Kate Grudpan [b]

[a] *Department of Chemistry, Faculty of Science, Khon Kaen University, 40002, Thailand.*
[b] *Department of Chemistry, Faculty of Science, Chaing Mai University, 50002, Thailand.*

Abstract

Flow injection (FI) was coupled to ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) for the simultaneous analysis of some metal-4-(2-pyridylazo) resorcinol (PAR) chelates. The reverse flow injection (rFI) was used as an in-line complexation of metal-PAR chelates prior to their separation by ion pair reversed phase HPLC. The lab made rFI was coupled to HPLC via switching valve and the performance of the system was fully manually operated. The rFI conditions were injection volume of PAR 85 μL, flow rate of metal stream 4.5 $mLmin^{-1}$, concentration of PAR $1.8x10^{-4}$ $molL^{-1}$ and the mixing coil length of 150 cm. IP-RPHPLC was carried out using a C_{18} μBondapak column with the mobile phase containing 37% acetonitrile, 3.0 $mmolL^{-1}$ acetate buffer pH 6.0 and 6.2 $mmolL^{-1}$ tetrabutylammonium bromide (TBABr) at a flow rate of 1.0 $mLmin^{-1}$ and visible detection at 530 and 440 nm. The analysis cycle including in-line complexation and separation by IP-RPHPLC was 16 minutes which able to separate Cr(VI) and the PAR chelates of Co(II), Ni(II) and Cu(II).

Keywords: Flow injection; In-line complexation; Ion pair reversed phase high performance liquid chromatography; Metal-PAR chelates

* Corresponding author. Tel.: +66-43-202222/41x2243; fax: +66-43-202373.
E-mail address: supalax@kku.ac.th (S. Srijaranai).

1. Introduction

Liquid chromatography has been widely recognized as one of the methods for multi-element and sensitive analysis of trace metals. Various modes of liquid chromatography have been used, including normal phase [1-3], reversed phase and ion exchange chromatography [4-10]. Since the introduction of ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) [11-12] for the separation of charged solutes. IP-RPHPLC has gained wide acceptance as an alternative method to ion exchange chromatography (IEC) for charged analytes, including metal ions. IP-RPHPLC offers multi-element detection capacity, selectivity and sensitivity of analysis. Moreover, the reversed-phase stationary phase has the benefit of lower cost compared to the IEC stationary phase.

Most of the reports on IP-RPHPLC for metal analysis [13-16] are based on the separation as their chelate ions. Pre-complexation of metal ions with appropriate ligands has many advantages such as increasing selectivity between metal ions, the ability to determine speciation, and, for chelates with high absorptivity, increasing sensitivity. Among the many ligands successfully used for IP-RPHPLC separation of metal ions, the azo dye, 4-(2-pyridylazo) resorcinol (PAR) is one of the most widely used ligands for the spectrometric determination of over 40 different metals [17]. PAR forms ionic complexes with large absorptivity (~ 10^4 $L.cm^{-1}.mol^{-1}$) [18] at about 500 nm. It has been shown to be an effective reagent for the determination of metals using HPLC with either pre-column [19] or post column complexation techniques [20-21].

Typically, complexation of metals is performed by batch or external to the chromatographic system before injection. External complexation is time consuming and the large amounts of chemicals used mean more waste to discharge. It is prone to contamination, especially for trace level determinations. Nowadays, the main consideration include automation of the method, low operating costs, less waste as well as high sample throughput.

Flow injection (FI) has been known with features of a simple operational basis, using inexpensive hardware, straightforward thus leading to convenient operation, high sample throughput, cost effective performance and versatility. FI has been

widely used as an analytical tool and also as a complement to the other analytical techniques.

Flow injection (FI) coupled with HPLC systems, reviewed by Luque de Castro M.D. and Valcacel M.[22], is usually intended to improve general features of the analytical process such as sensitivity, precision, rapidity, cost, etc. FI coupled with HPLC is used in two different modes i.e., pre- or post-column arrangements. For the pre-column arrangement, as in the present study, the FI system is placed before the HPLC. The specific objectives of pre-column coupling are automation of sample clean-up and/or preconcentration steps, automatic implementation of derivatization reactions and saving reagents. Two methods have been used to couple FI as precolumn of HPLC. The first method, the sample plug is injected through the FI valve and then passed through HPLC loop. In the second method, the sample from the FI system is retained in a precolumn placed in HPLC loop.

Previous work in this laboratory has involved metal analysis by IP-RPHPLC via batch complexation with PAR [23]. In the work described here, a simple FI system was developed as the in-line precolumn for complexation of some metal-PAR chelates before being analysed by IP-RPHPLC. The FI part is operated in the "reverse mode" that is a metal solution is the flowing stream and the PAR reagent solution is injected into it. A portion of the PAR-metal mixture zone is then sampled with the HPLC injection valve for subsequent separation and further detection.

2. Experimental

2.1. Chemicals and Reagents

All the reagents used were of analytical reagent (AR) grade, 4-(2-pyridylazo) resorcinol and tetrabutylammonium bromide (TBABr) were purchased from Fluka (Switzerland). 2-diethylaminoethanol was obtained from Merck (Germany). Methanol and acetonitrile were of HPLC grade and were obtained from Lab-Scan (Thailand). The atomic absorption standard solutions (1000 mgL^{-1}) of Cu(II), Cd(II), Co(II), Hg(II), Zn(II), Fe(III) and Pb(II) were obtained from Ajax Finechem (Australia) whereas Ni(II) was purchased from BDH (England). Cr (VI) oxide was obtained from Merck (Germany). Aqueous solutions were prepared with deionized

water obtained from RiOs™ type I simplicity 185 (Millipore Waters, USA) throughout the experiment.

2.2. Instruments

A schematic representation of the reverse flow coupled with the high performance liquid chromatographic system is shown in Figure 1.

The configuration of the injection for the reverse flow injection system, using a 505s 505LA peristaltic pump (Watson Marlow, England). PFA Teflon tubes (1.5 mm i.d.) were employed for the reaction coils and were connected to a six-port low pressure injection valve, four way switching valve (Upchurch, USA) was used to allow the metal-chelates flow to HPLC system. A stop watch (Casio, Japan) was used for time control.

The chromatographic set-up consisted of a Waters 6000A Dual Pump, a Rheodyne injector with 20 μL sample loop and a Waters 484 Tunable Absorbance Detector (Waters, USA) equipped with Waters 740 Data Module Integrator (Waters, USA), A 996 photodiode array (Waters, USA) and the Millinium 32 Software data aquisition system was also used for the study of interferences. A C_{18}-μBondapak (3.9 x 300 mm i.d.) coupled to a guard column (Waters, USA) was used as the stationary phase.

The spectra of the metal chelates in batchwise experiments were obtained with a Agilent 8453 (USA) UV-visible spectrophotometer equipped with a 1 cm quartz cell. The pH measurements were performed on a SP-701 pH meter (Suntex, Taiwan). Micropipette Nichiyo 500 DG (Nichiyo, Japan) and Disposable syrige (1 mL) (Nipro, Thailand) were used.

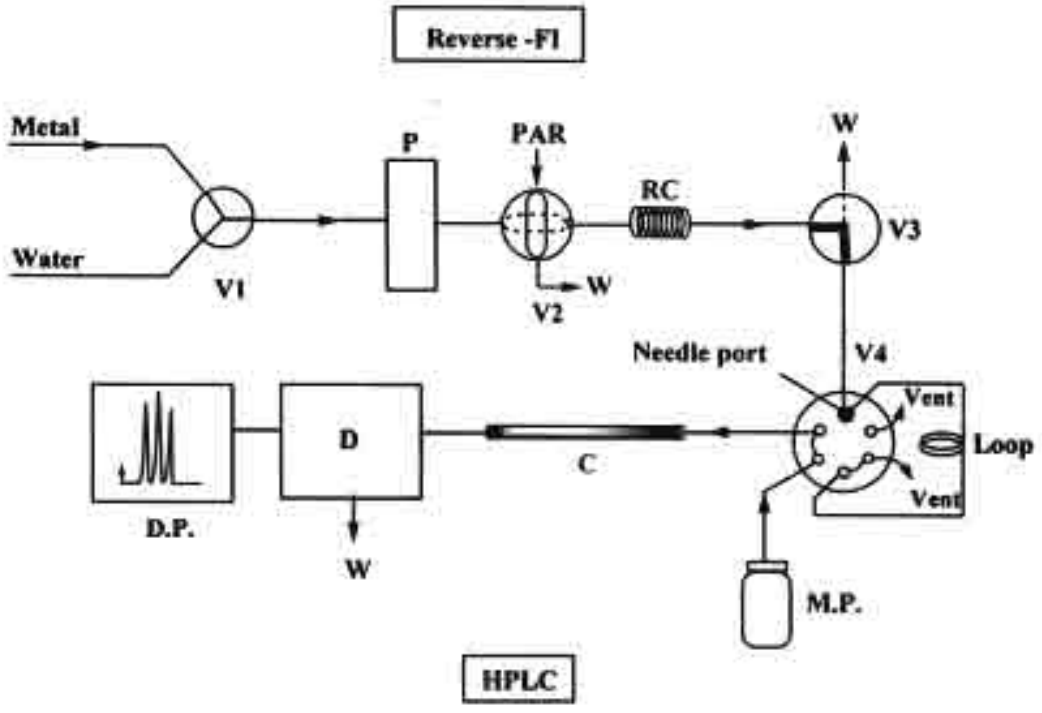


Figure 1 Flow diagram of FI-HPLC in-line derivatization system : P = peristaltic pump ; RC = reaction coil ; V1 = three way valve ; V2 = low pressure injection valve ; V3 = switching valve ; V4 = high pressure injection valve; C = analytical column ; M.P = mobile phase ; D = UV-visible detector/photodiode array detector ; D.P. = data processor ; W = waste

2.3 Procedure

2.3.1. Preparation of standard metal solution

Standard solutions of metal ions were prepared daily by stepwise dilution of 1000 mgL^{-1} stock solutions by deionized water.

Standard solution of Cr(VI) 1000 mgL^{-1} was prepared from chromium oxide.

2.3.2. Preparation of PAR solution

Stock standard solution (0.001 $molL^{-1}$) of PAR was prepared by dissolving an accurately weighed amount of 4-(2-pyridylazo)resorcinol in deionized water and stored in dark bottle. Working solution was prepared daily with deionized water and appropriate volume of 2-diethylaminoethanol was added to make the concentration of 2.5×10^{-4} $molL^{-1}$ in PAR solution.

2.3.3. Optimization of the reverse flow injection (rFI)

Factorial design was used to investigate the influence of the reverse flow injection (rFI) system on the peak height (absorbance). The four variables studied were flow rate of metal ions stream, injection volume of PAR, length of the mixing coils and concentration of PAR. A factorial design for four variables at two levels (2^4 resolution, 16 experiments) was performed. According to the results obtained from the factorial design, the chosen parameters to be optimized were the concentration of PAR, injection volume of PAR solution and flow rate of metal stream. The variable size simplex was then employed for optimization. The mixing coil length of 150 cm was used throughout the experiment.

3. Results and Discussion

3.1. Coupling of rFI to IP-RPHPLC

In-line complexation of metal-PAR chelates was performed using the rFI which the optimum conditions were mixing coil length of 150 cm, injection volume of PAR 85 µL, flow rate of metal stream 4.5 $mLmin^{-1}$ and concentration of PAR 1.8 $x10^{-4}$ $molL^{-1}$. The PAR chelates were then separated via IP-RPHPLC. The rFI was chosen instead of normal FI because of its low background noise for HPLC baseline as well as lower reagent consumption for the expensive ligand, PAR. The rFI was coupled to the HPLC by switching valve (V3) shown in the diagram in Figure 1.

Synchronization of the FI manifold and the HPLC is very important to achieve good performance of the coupling system. The time intervals and valve positions of the rFI-HPLC were investigated using the results obtained from the study of the optimization of the reverse flow injection . Manual operation of the rFI-HPLC system was sufficient for good precision. Each step was performed using a stopwatch as the timer control.

Once the baseline of the HPLC was steady, a complete cycle (4 steps) of the rFI-HPLC manifold was started. The 4 steps include prefill, complexation, separation and washing. In the first (prefill) step, the aqueous solution containing metal ions was pumped through the rFI manifold for 30 s, this period was long enough to fill the

transmission line with metal solution. The HPLC was in the load mode throughout this step to maintain a steady baseline of solvent.

During the prefill step, an aliquot of PAR was filled into the loop connected to V2 (at LOAD position).

Step 2, the complexation step, was started by switching V2 to INJECT position. The metal ions merged with PAR and complexation occurred during their passage through the reaction coil (RC). To avoid a dilution edges of the zone and to allow only the middle zone of the PAR chelates to pass into the HPLC-loop, the valve V3 was switched after 8 seconds of injection of PAR. The subsequent time period of 2 s, was enough to rinse and fill the HPLC-loop (20 µL).

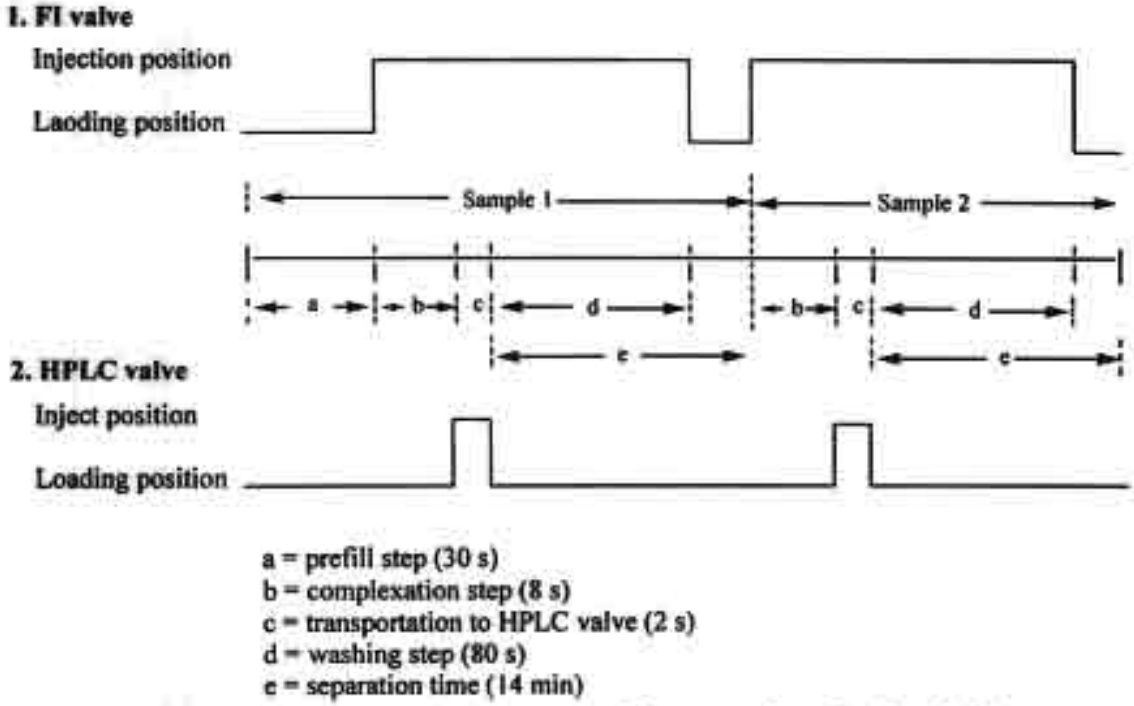
Then, step 3 (separation step), was initiated via HPLC-valve. The PAR-chelates were introduced and then separated in the HPLC system.

Finally, step 4 (washing step), was to wash the rFI and HPLC-loop for the next analysis. While separation was taking place on the HPLC column.

The complete cycle of rFI coupling to IP-RPHPLC was 120 seconds, where as the analysis time of the HPLC was 14 minutes. Operating periods and valve position for rFI –IPRPHPLC are summarized in Table 1 and Figure 2.

Table 1 Valves positions and operating times for rFIA -IPRPHPLC

Step	Time (s)	Valve position				In-line complexation operation	
		V1	V2	V3	V4	Medium pump	Stage of operation
Sample 1							
1	30	To V2	Load	To waste	Load	Metals	Prefill
2	38	To V2	Inject	To V4	Load	Metals	Complexation
3	40	To V2	Inject	To V4	Inject	Metals	Separation
4	120	To V2	Load	To V4	Load	Water	Washing
Sample 2							



a = prefill step (30 s)
b = complexation step (8 s)
c = transportation to HPLC valve (2 s)
d = washing step (80 s)
e = separation time (14 min)

Figure 2 Schematic diagram of timing control for operation of valves in the FI-HPLC system.

3.2. IP-RPHPLC of metal-PAR chelates

In IP-RPHPLC, the metal chelates which are successfully separated have to be stable and kinetically inert [24]. Typically, PAR forms anionic chelates with metals at the metal to ligand ratio of 1:2 [25]. It is known that the retention behavior of chelates in IP-RPHPLC depends strongly on complex composition (metal:ligand) which is governed by the nature of the central metal ion. The mobile phase composition is also govern the separation. The principal parameters of interest in the mobile phase are pH, buffer (type and concentration), organic modifier and ion pairing agent (long-chain alkyl ions with a charge opposite that of analytes). There are several mechanisms [26-28] explaining the retention behavior of IP-RPHPLC, such as the ion-exchange mechanism, the solvophobic theory and dynamic equilibrium.

The optimum mobile phase obtained by slightly adjusting the one obtained in our previous work [23]. The mobile phase composition was 37% acetonitrile, 6.2 $mmolL^{-1}$ TBABr and 3.0 $mmolL^{-1}$ acetate buffer pH 6.0.

Baseline separation of four metal-PAR chelates was achieved within 14 minutes, with the elution order of Co(II)-PAR, Ni(II)-PAR and Cu(II)-PAR. The excess PAR was detected at the retention time of 9.6 minutes. The chromatogram is shown in Figure 3.

Surprisingly that under the condition used and the detection at 440 nm, Cr(VI) was retained shortly (4.9 min) after unretained peak (at 3.3 min), as shown in Figure 4. The spectrochromatogram corresponding to Figure 4 is shown in Figure 5. Although, Cr(VI) does not form complex with PAR but it oxyanion ($HCrO_4^-$) could interacted with ion pairing agent in the same manner to the anionic chelates. The peak at 4.9 minutes which was identified as Cr(VI) gives the absorption spectrum (as shown in Figure 6.) identical to the spectrum of Cr(VI) detected by UV-vis spectrometer. The resolutions between pairs were as follow: 1.4 for Cr(VI) and Co(II)-PAR, 1.0 for Co(II)-PAR and Ni(II)-PAR, 2.4 for Ni(II)-PAR and excess PAR, and 1.3 for excess PAR and Cu(II)-PAR.

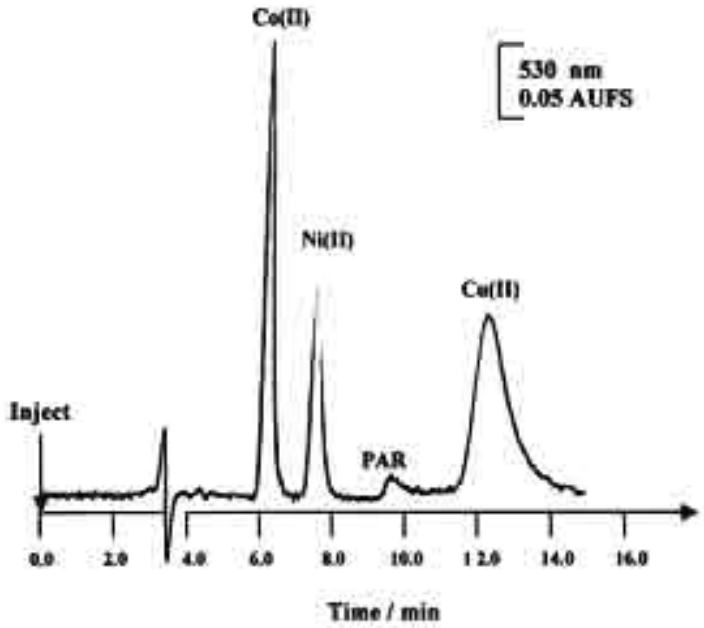


Fig. 3. Chromatogram of metal-PAR chelates : condition ; C_{18} column, mobile phase 37 % acetonitrile, 6.2 $mmolL^{-1}$ TBABr and 3.0 $mmolL^{-1}$ acetate buffer pH 6.0, flow rate of mobile phase 1.0 $mLmin^{-1}$ visible detection at 530 nm: peak ; 0.10 μgmL^{-1} Co(II), 0.20 μgmL^{-1}Ni(II), excess PAR and 0.80 μgmL^{-1} Cu(II)

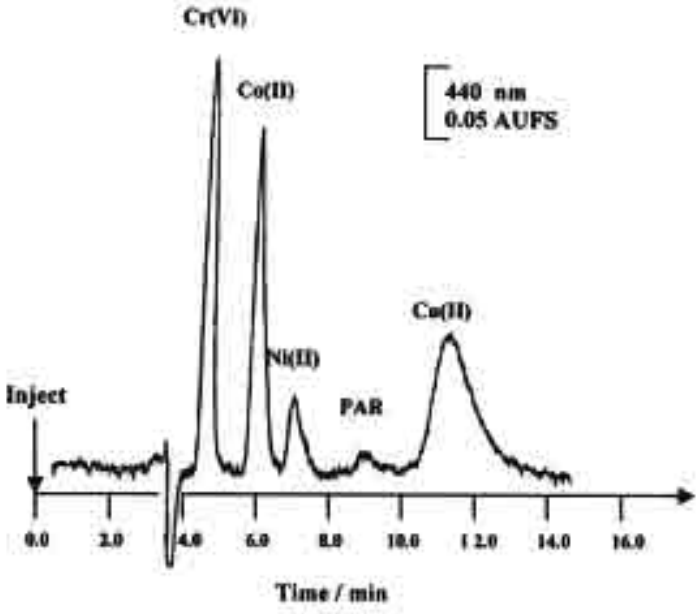


Figure 4. Chromatogram for Cr(VI) and metal-PAR chelates : peak ; 5.0 μgmL^{-1} Cr(VI), 0.10 μgmL^{-1} Co(II), 0.10 μgmL^{-1} Ni(II), excess PAR and 0.40 μgmL^{-1} Cu(II) (condition as described in Figure 3, except visible detection at 440 nm)

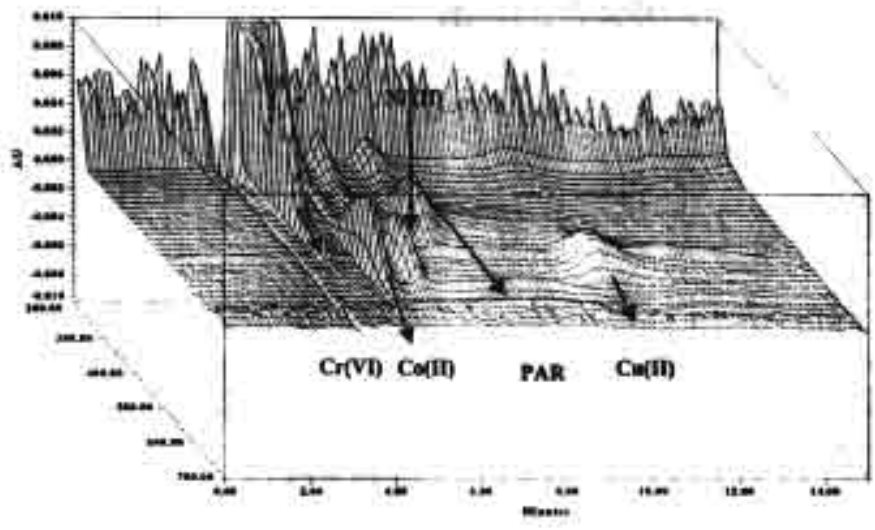


Figure 5. 3D plot of Cr(VI) and metal-PAR chelates of chromatogram in Figure 4.

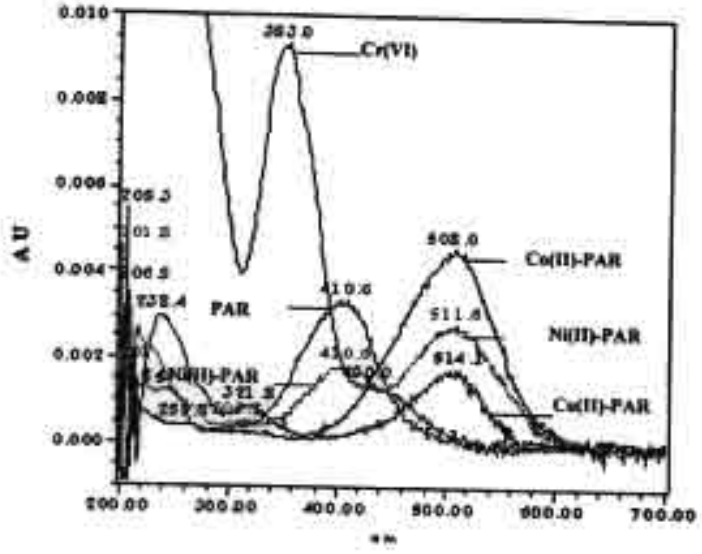


Figure 6. The absorption spectra of chromatogram in Figure 4.

3.3. Performance of rFI-IP-RPHPLC

Quantitative features including linearity and reproducibility for retention time and peak area were studied using the optimum condition. Calibration graphs were prepared by plotting the concentration of each metal ion (μgmL^{-1}) against the peak area. The limit of detection (LOD) was deduced based on 3 times of baseline signal. The calibration equation, coefficient of correlation (r^2), recovery, reproducibility and LOD are summarized in Table 2.

Table 2

The quantitative features of rFIA-HPLC

Chelates	Concentration ($\mu g\ mL^{-1}$)	Linear equations Y = AX + C	Correlation Coefficient (r^2)	R.S.D* (%) (n =11)		LOD (3σ) ($\mu g\ mL^{-1}$)	% Recovery
				t_R	Area		
Cr(VI)	1.00 - 6.00	473.51X – 0.17	0.99614	2.01	0.94	1.0	113
Ni(II) –PAR	0.010 – 0.20	341.66X – 4.73	0.99835	2.07	0.91	0.015	84
Co(II) –PAR	0.010 – 0.40	547.21X – 0.61	0.99516	0.56	0.97	0.030	96
Cu(II) –PAR	0.05 – 0.60	293.80X+ 5.49	0.99864	2.43	0.71	0.150	102

* concentration of each metal was described in Figure 4

3.4. Interferences

The effects of interferences on the chromatography of metal-PAR chelates was investigated. The chosen ions are the ions able to form chelate with PAR including Cd(II), Cr(III), Hg(II), Mn(II), Fe(III), Pb(II) and Zn(II). These metal ions were individually injected into the rFI-HPLC. All of the studied ions could not formed chelates with PAR under the condition used. Only peak which was identified as PAR (9.6 min) was observed.

The study on tolerance level of the metal ions which could not form chelates with PAR was studied by individually spiking the metal ions at difference amounts (ranging from 0.5-10.0 μgmL^{-1}) into the mixture of 0.10 μgmL^{-1} Co(II), 0.20 μgmL^{-1} Ni(II) and 0.40 μgmL^{-1}Cu(II). It was found that the presence of the foreign ions did not affect the retention time of the PAR chelates of Co(II), Ni(II) and Cu(II). However, the quantitative signals (both peak height and peak area) were affected by the addition of the foreign ions. Cu(II)-PAR was strongly influenced when the concentrations of the foreign ion increase to 2.5 times resulted in either decreased or increased of peak height and peak area. The effect on Ni(II)-PAR was observed when the foreign ion increase to 5 times greater than Ni(II), resulted in the decreasing of peak height and peak area. This effect was also observed for Co(II) when the concentration of the foreign ion was 10 times to Co(II). The peak area of Cr(VI) was not affected by the addition of the foreign ion. However, the obtained spectra and the 3-D plots (results not shown) revealed that neither PAR chelates of the foreign ions nor the ternary complexes was formed. According to the obtained results indicating that PAR was enough for all of metal ions. Furthermore, to ensure the excess amount of PAR, ten times higher concentration of PAR i.e. 1.0×10^{-3} $molL^{-1}$ was used. Similar results were obtained and large peak of excess PAR overlapped the analyte peaks. The effect of interference on the present method was obviously seen when compared to the previous work on precomplexation of metal-PAR chelates by batch method prior to the analysis by IP-RPHPLC. This may attribute to the nature of the flow system which a short time that stream of reagents are reacted. Neither physical equilibrium nor chemical equilibrium (i.e. the completeness of reaction) has been attained by the time it was detected.

3.5. Analysis of real sample

According to the study it is possible to analyse Cr(VI) simultaneously with Ni(II). The present method was applied to the analysis of chrome plating waste water. The samples were collected from chrome plating plant in Khon Kaen and were analysed after dilution, pH adjustment and filtration through 0.45 μm membrane. Data obtained are list in Table 3 which were in good agreement with atomic spectrometry.

Table 3

Result of analysis of chrome plating waste water

Metal	Concentration ($\mu g\ mL^{-1}$)	
	Sample 1	Sample 2
Cr(VI)	984.5 ± 6.48[b]	69.3 ± 10.74[b]
Co(II)	N.D.[a]	N.D.[a]
Ni(II)	1574.6 ± 10.25[b]	121.4 ± 7.42[b]
Cu(II)	N.D.[a]	N.D.[a]

[a] Not detected, [b] S.D. (n = 3)

5. Conclusion

In the present study, a simple combination of reverse flow injection and HPLC resulted in a powerful technique for simultaneous analysis of metal ion as their PAR chelates. The reversed flow injection (rFI) was coupled to HPLC and the performance of the system was fully manually operated. Using the developed rFI for in-line complexation gives benefit of less PAR consumption, less analysis time and less waste disposed comparison to the batch derivatization. The analysis cycle was 16 minutes including in-line complexation (ca. 2 minutes) and separation by IP-RPHPLC (14 minutes). The method was successfully applied for the separation of Co(II), Ni(II) and Cu(II) as their PAR chelates.

Acknowledgements

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ผลงานวิจัย ก26

Determination of Linear Alkylbenzene Sulfonates in Water Samples by Liquid Chromatography-UV Detection and Confirmation by Liquid Chromatography-Mass Spectrometry

Sunanta Wangkarn*, Pimpa Soisungnoen, Mongkon Rayanakorn and Kate Grudpan
Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

Abstract

A high performance liquid chromatography (HPLC) method was developed for the separation and determination of individual (C_{10} – C_{13}) linear alkylbenzene sulfonates (LAS). A new set of conditions has been established for routine analysis of individual chemical forms of four LAS surfactants, i.e. C_{10} - C_{13} LAS. The mobile phase containing 1.5 mM ammonium acetate in methanol/water mixture of 78 to 22 (v/v) was used. Under the optimum condition, detection limits obtained were in the range 1.43 pg μl^{-1} (for C_{10} LAS) to 11.35 pg μl^{-1} (for C_{13} LAS). This method offers the advantages of significant improvement in resolution, short separation time and using less amount of common salt under isocratic condition. In addition, the use of simple mobile phase containing a simple low amount of salt cannot deposit at the entrance of mass spectrometric detector. The method is applicable to the simultaneous determination of LAS surfactants in various water samples. LAS surfactants presented in these samples were also successfully confirmed by using electrospray mass spectrometry.

Keywords: anionic surfactant, linear alkylbenzene sulfonates, HPLC, mass spectrometry, water

* Corresponding author: Tel.: +6653-943341; fax: +6653-892277
E-mail address: sunanta@chiangmai.ac.th

1. Introduction

Linear alkylbenzene sulfonates (LAS), synthetic anionic surfactants, have been used in household laundry and dishwashing detergents [1]. The commercial product is a mixture of homologues containing carbon atoms between 10 and 13 atoms. Each of these homologues consists of positional isomers resulting from the attachment of the phenyl ring to the carbon atoms of the linear alkyl chain (Fig. 1) [2, 3]. They are rapidly biodegraded under aerobic conditions. LAS-containing detergents are used in large quantities and are therefore released in the environment. LAS homologues with alkyl chain lengths from C_{10} to C_{13} have been found in municipal wastewaters and sediments at the ppm levels [4-6]. It has been reported that LAS and their degradation products can affect membrane permeability, enzyme and lysosomal activity [7, 8]. The toxicity of the LAS containing 13 carbon atoms to the microalgae namely *Chaetoceros gracitis* was found to be greater than that of the C_{11} LAS [9]. For these reasons, the identification and quantification of individual LAS species are invaluable for estimating the environmental impact and potential health effects of LAS species.

The standard methylene blue method has long been used for determining total amounts of sulfonate and sulfate based anionic surfactants in wastewater [10]. The method cannot differentiate individual anionic surfactant. The method is sensitive. It is time consuming and is often interfered by sample matrix, i. e. organic sulfonates, sulfates, carboxylates and phenol. This method also requires a large quantity of the toxic solvent for extraction such as chloroform.

A number of methods have been developed for identifying and quantifying individual chemical forms of anionic surfactants. Chromatographic techniques like gas chromatography (GC) [4, 11-15], electrophoresis [1] and high performance liquid chromatography (HPLC) [16-18] are efficient separation methods for the analysis of LAS mixture. Due to their low volatility and anionic form, derivatization of these compounds is necessary when GC-based method is used [19].

HPLC is currently a suitable method for the determination of LAS. Reversed-phase HPLC provides a good separation of LAS mixture when using various chromatographic detectors, e. g. ultraviolet (UV) [16-18], fluorescence [2, 20, 21] and mass spectrometry (MS) [22-24]. However, most existing methods and procedures are still far from being considered suitable for the routine determination of individual chemical forms of LAS. Most HPLC methods with UV detector require the mobile phase containing either sodium perchlorate [22]

or additive mixture such as triethylamine and acetic acid [23, 24], trifluoroacetic acid (TFA) and tetrabutylammonium dihydrogenphosphate (TBA-H_2PO_4) [25] or cetyltrimethylammonium ($CTMA^+$) ions [26] in order to resolve LAS homologues under gradient conditions. In practical application of the mobile phase containing high amount of those compounds, particularly sodium perchlorate (10 g l^{-1}) can shorten the column life and can also clog the capillary when mass spectrometric detector is used. In addition, the complicated mass spectra of LAS homologues would be obtained. This makes the identification of individual LAS in environmental samples so difficult.

As reported earlier by other workers, LAS compounds containing 10 to 13 carbon atoms are used in large amounts and are therefore released in the environment [1, 4-6]. The toxicity of surfactant to aquatic organisms increases with increasing of carbon atom [9].

The main purpose of this study was to develop HPLC method that would allow for routine analysis of LAS mixture, particularly in respect to reducing analysis time, improving separation efficiency for all the four LAS surfactants, precision and accuracy under isocratic condition. As mentioned earlier, several publications have been reported to use complicated mixtures as mobile phase under gradient conditions for separating some LAS surfactants. These approaches can also cause either capillary blockage or additional spectral interferences when a mass spectrometric detector is used for confirmation results. Therefore, common salts, i. e. sodium chloride, sodium acetate and ammonium acetate added into mobile phase were chosen because of their suitability of identifying LAS in water samples using mass spectrometric detection.

2. Experimental

2.1 Chemicals

Linear alkylbenzene sulfonates in the forms of sodium salts were obtained from Henkel (Germany). HPLC grade methanol was purchased from BDH (Poole, England). Sodium chloride, sodium acetate and ammonium acetate were AnalaR grade and purchased from Carlo Erba (Barcelona, Spain). Milli-Q water was used in this study.

2.2 Instrumentation

A HP 1100 high performance liquid chromatograph (Agilent Corp., Wilmington, USA) consisting of an Agilent 1100 quaternary pump and an Agilent 1100 UV detector (224 nm) was employed. An inlet frit with 2 μm pore size was placed between the injector and

HPLC column. A Zorbax Eclipse XDB C_8 column (Agilent Corp., USA), 15 cm x 4.6 mm i. d., containing 5 μm diameter packing material was used.

Samples were injected onto this column via an injection valve filled with 20 μl loop. The mobile phase system was the mixture of methanol/water containing various amounts of sodium chloride, sodium acetate or ammonium acetate at flow rate 1.0 ml min^{-1}. All chromatographic elutions were isocratic and carried out at room temperature.

A HP 1100 series mass-selective detector single quadrupole instrument equipped with the orthogonal spray-ESI (Agilent, USA) interface was used for these investigations. The fragmentor voltage, nebulizer pressure, drying gas flow rate, drying gas temperature and capillary voltage were set to 150 volts, 20 psi, 10 l min^{-1}, 350 °C and 3,500 volts, respectively.

2.3 Sample preparation

Prior to HPLC analysis, water samples were subjected to purification and preconcentration on the Sep-Pak C_{18} cartridge (Waters, USA). The cartridge was preconditioned with 7 ml methanol, followed by 7 ml of deionised water and then the sample was passed through the cartridge. The cartridge was washed with 6 ml of the mixture of MeOH-H_2O (30:70, v/v) and was eluted with 3 ml of methanol.

3. Results and discussion

The optimization of LAS separation using reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection at 224 nm was achieved, employing Eclipse XDB C_8 column with 15 cm x 4.6 mm dimension and 5 μm film thickness. Optimum separation of LAS homologues containing 10-13 carbon atoms was obtained by appropriately adjusting the composition of mobile phase, type and the concentration of salt. The emphasis was placed on the use of common salts (sodium chloride, sodium acetate and ammonium acetate), instead of using sodium perchlorate, in order to avoid capillary blockage and high background signal when using mass spectrometric detection.

3.1 Effect of mobile phase composition

Mobile phase compositions in the range of 70-80% (v/v) methanol in water were investigated. Separation of a mixture of C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS was carried out as depicted in Fig. 2. LAS compounds were separated using 70% methanol in water as mobile phase. However, most peaks obtained were broad, particularly C_{13} LAS. It was also observed that the LAS compounds containing 10 and 11 carbon atoms were not resolved completely when using 75% methanol. No separation was observed when using the amounts of methanol exceeding 80%. It is evident from these chromatograms that the composition of mobile phase affects peak resolution and peak shape significantly. It was noticed that peak resolution deteriorated with increasing methanol content. This could be explained that surfactants are hydrophobic in nature. The hydrophobic characteristic of long chain surfactants is suppressed by increasing methanol resulting in reducing retention time [27].

3.2 Effect of type and concentration of salt

Preliminary experiments were undertaken in an attempt to find suitable salt adding into mobile phase for improving LAS separation. Sodium chloride was common salt used for separations of LAS mixture as shown in Fig. 3. Four LAS compounds were successfully resolved within 6 min when using the 80/20 (v/v) mixture of methanol and water containing 3.5 mM NaCl. When using the 75/25 (v/v) mixture of methanol and water containing 3.5 mM NaCl the same four LAS compounds were separated in over 12 min. It was also observed that C_{10} LAS and C_{11} LAS were partially resolved when using the amounts of methanol exceeding 85%. The mixture of methanol/water (80/20, v/v) was therefore selected for further method development and applications.

As demonstrated earlier, the selection of a suitable common salt is a critical factor in obtaining optimum resolution and short separation times. Three types of common salts, i. e., sodium chloride, sodium acetate and ammonium acetate were investigated at the concentrations ranging from 1 to 10 mM adding into the mixture of methanol/water (80/20, v/v) a long with a mobile phase flow rate 1.0 $ml.min^{-1}$. It was observed that the resolution and their retention time increased with the concentration of salt (Figs. 4-6). The minimum concentrations of sodium chloride, sodium acetate and ammonium acetate that could be used to separate the four LAS compounds (resolution ≥ 1.5) in approximately 5 min under isocratic condition were 1, 2 and 1.5 mM, respectively. In comparing the data obtained in this study to the data reported elsewhere [28] indicated that the developed method provided a

significantly improved resolution, peak shape, particularly C_{12} LAS, short analysis time and simple approach for confirmation results by mass spectrometric detector.

3.3 Linearity, accuracy and detection limit

All experiments were carried out under the optimum mobile phase containing 80% methanol and 1.5 mM ammonium acetate in water. Linearity, accuracy and detection limit for individual LAS compounds are summarized in Table 1. The accuracy expressed in terms of percentage recovery was done by spiking various amounts of LAS standard (1 ng μl^{-1}) into the water samples collected from wastewater in Chiang Mai and Utraradit, Thailand. The percentage recoveries of this method for C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS were found to be between 91-101 (n = 3), 92-99 (n = 3), 95-99 (n = 3) and 94-102 (n = 3), respectively. Satisfactory recovery was obtained. The C_{10} LAS had the lowest detection limit of 1.43 pg μl^{-1}. The C_{13} LAS, which is the last compound to elute under the conditions employed, had the highest detection limit value of 11.35 pg μl^{-1}. From the observation, the peak shape of C_{13} LAS is rather broader than that of C_{10} LAS.

3.4 Analysis of LAS surfactant in real water samples using HPLC-UV

In order to demonstrate that the method developed in this study is suitable for LAS separation in "real" samples, several natural water and wastewater extracts were analysed. As depicted in Fig. 6(b), an increase of methanol from 75 to 80% is expected to cause C_{10} LAS and C_{11} LAS to elute close to some interferences present in sample extracts (Fig. 7). The mobile phase containing 1.5 mM ammonium acetate in methanol/water mixture of 78 to 22 (v/v), instead of the ratio of 80 to 20 (v/v) methanol/water, was used in order to avoid matrix effects arising from the water extracts. These effects cause a 2 minute increase in the separation time of LAS compounds. Under the proposed condition, the LAS compound concentrations in various water samples determined using a Zorbax Eclipse XDB C_8 column in combination with the methanol/water mixture containing ammonium acetate, were reported in Table 2.

3.5 Identification of LAS surfactants in water extract using LC-ES-MS

It is well known that the identification of LAS compounds using chromatographic techniques is based solely on retention time matching. As a consequence, errors can result from using this approach, especially in the case of co-eluting compounds. Also of particular

interest is the numerous unknown anionic surfactants that have been found in environmental samples when analysing them using HPLC-UV [17-19].

To overcome such problems, the negative-ion electrospray (ES) – mass spectrometry was used for confirmation of LAS compounds in water samples. The mass spectra of water extracts (Fig. 8) show the *m/z* 183 ion common to LAS compounds. In addition, the high intensity of the molecular ions observed at *m/z* 297, 311, 325 and 339 originating from the water extracts are similar to those originating from the LAS standards (Fig. 9). These ions correspond to C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS, respectively.

4. Conclusion

The proposed method offers superior performance characteristics, i.e. a simple method, significant improvement in resolution ($R > 1.5$, indicating a complete separation), short analysis time (7 min) and using less amount of common salt (1.5 mM ammonium acetate) under isocratic condition. With this regard, it is easy to use this method with a mass spectrometric detector without any blockage of MS capillary. In addition, the use of low amounts of salt also increases the column's life and only requires very short re-equilibration time between each injection. Overall, these features demonstrate that the method is suitable to be used for routine analysis for both the identification and quantification of individuals of C_{10} – C_{13} LAS surfactants in various water samples.

Acknowledgements

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Table 1 Analytical merits of the proposed method (linearity, accuracy and detection limit)

Compound	Concentration range ($pg\ \mu l^{-1}$)	R^2	Linearity cut-off ($pg\ \mu l^{-1}$)x10^3	%Recovery	Detection limit* ($pg\ \mu l^{-1}$)
C_{10} LAS	6-310	0.9984	41	91-101	1.43
C_{11} LAS	20-1030	0.9997	137	92-99	7.92
C_{12} LAS	20-1070	0.9994	143	95-99	7.07
C_{13} LAS	10-590	0.9979	78	94-102	11.35

*Calculation based on three times the background standard deviation.

Table 2 Concentration of LAS compounds in water samples (mean ± *s*; n = 3)

Type of water	Concentration (pg μl^{-1})			
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS
W1	115.3 ± 2.7	303.2 ± 7.9	184.4 ± 8.0	81.9 ± 2.2
W2	310.0 ± 0.3	1173.4 ± 4.6	1145.0 ± 12.9	424.4 ± 5.0
W3	5.0 ± 0.1*	23.4 ± 0.5	26.5 ± 1.1	21.1 ± 1.5
W4	n.d.	n.d.	13.0 ± 0.9	14.8 ± 0.5
W5	n.d.	n.d.	n.d.	n.d.
W6	3.4 ± 0.1*	12.8 ± 0.9	15.1 ± 1.3	16.7 ± 1.6
W7	54.1 ± 1.9	120.1 ± 5.5	69.1 ± 4.8	36.6 ± 5.2
W8	n.d.	n.d.	n.d.	n.d.
W9	n.d.	n.d.	n.d.	n.d.
W10	8.8 ± 0.3*	30.9 ± 0.7	33.5 ± 1.7	25.2 ± 1.7
W11	14.5 ± 0.8*	48.0 ± 2.4	46.9 ± 3.5	28.9 ± 2.8
W12	6.5 ± 0.4*	27.8 ± 1.9	30.3 ± 2.1	22.6 ± 1.3

n.d.: not detected. (less than the detection limit value)

* preconcentration as described in Section 2.3.

W1: Drainage water from student dormitory, Chiang Mai University.

W2: Wastewater from Center of Medical Sciences, Ministry of Public Health.

W3: Wastewater from Khuy Hospital, Utraradit Province.

W4: Domestic wastewater released into Thorn canal, Utraradit Province

W5: Natural water in Mae-Ping River, Chiang Mai Province.

W7: Wastewater in Mae-Kha canal, Chiang Mai Province.

W8: Natural water in Ang-Kaew reservoir, Chiang Mai University.

W9: Water in Chiang Mai Moat, Chiang Mai Province.

W6 and W10 – W13: Natural water from irrigation canal, Chiang Mai Province.

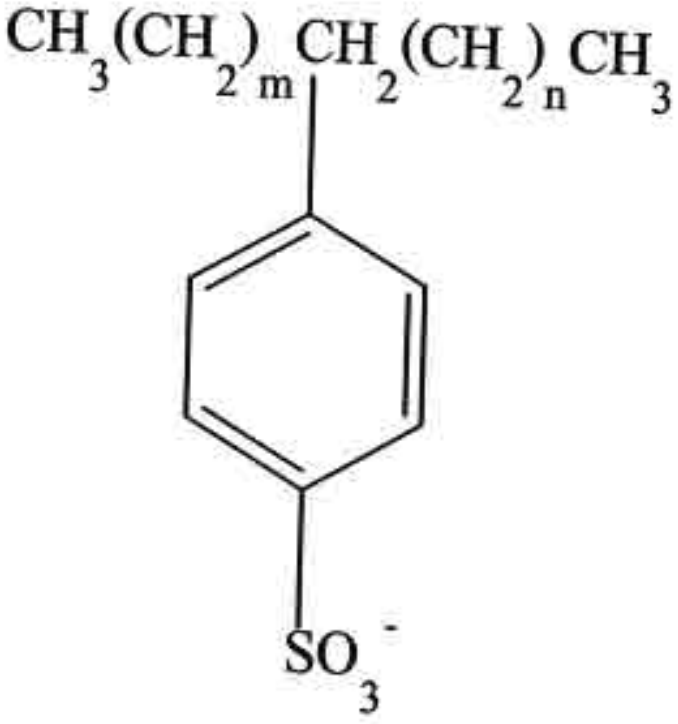

Fig. 1 General chemical structure of LAS

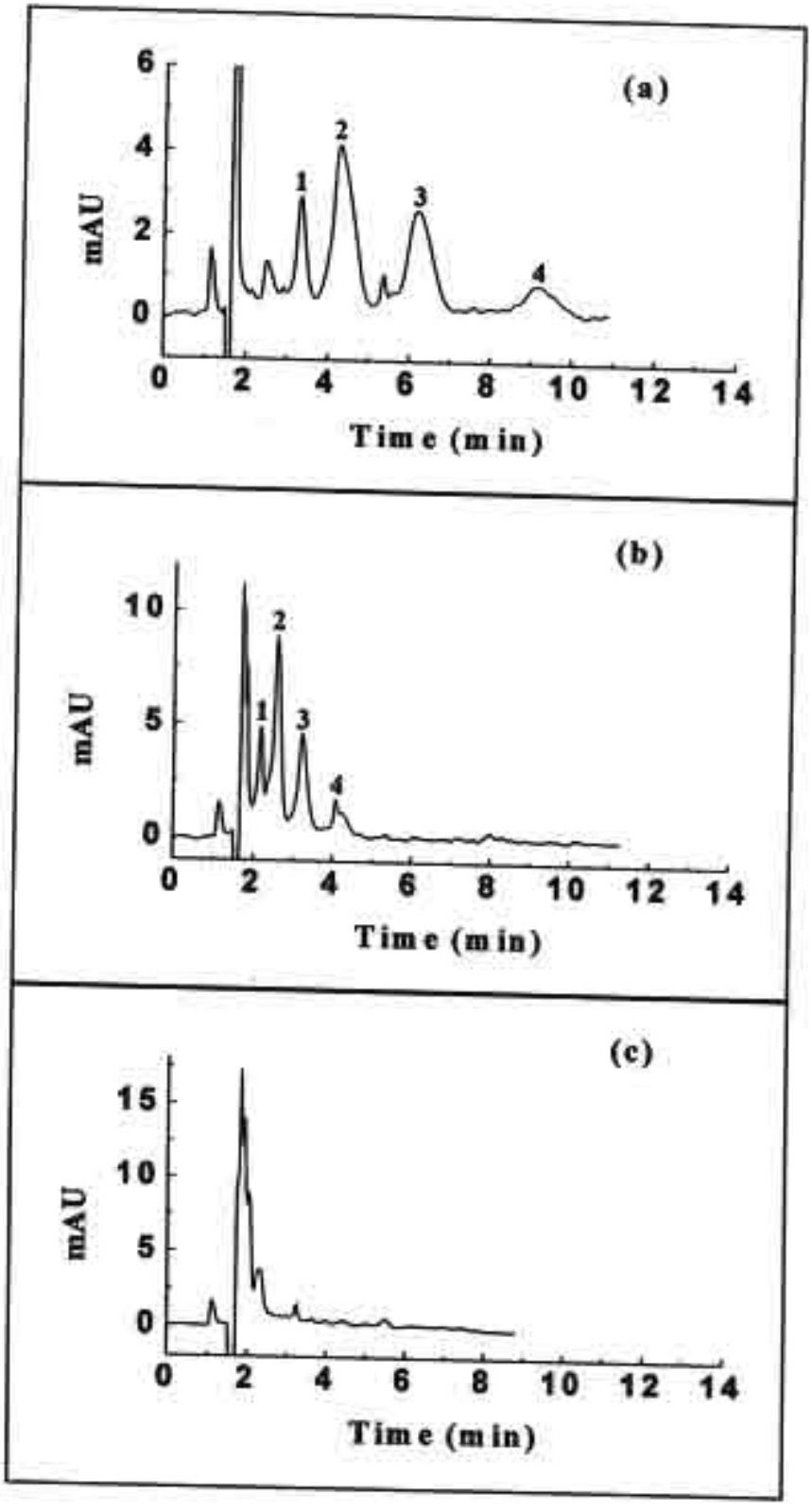

Fig. 2 Chromatograms of mixture of four LAS compounds obtained using various mobile phase compositions of MeOH-H_2O: (a) 70-30, (b) 75-25 and (c) 80-20. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

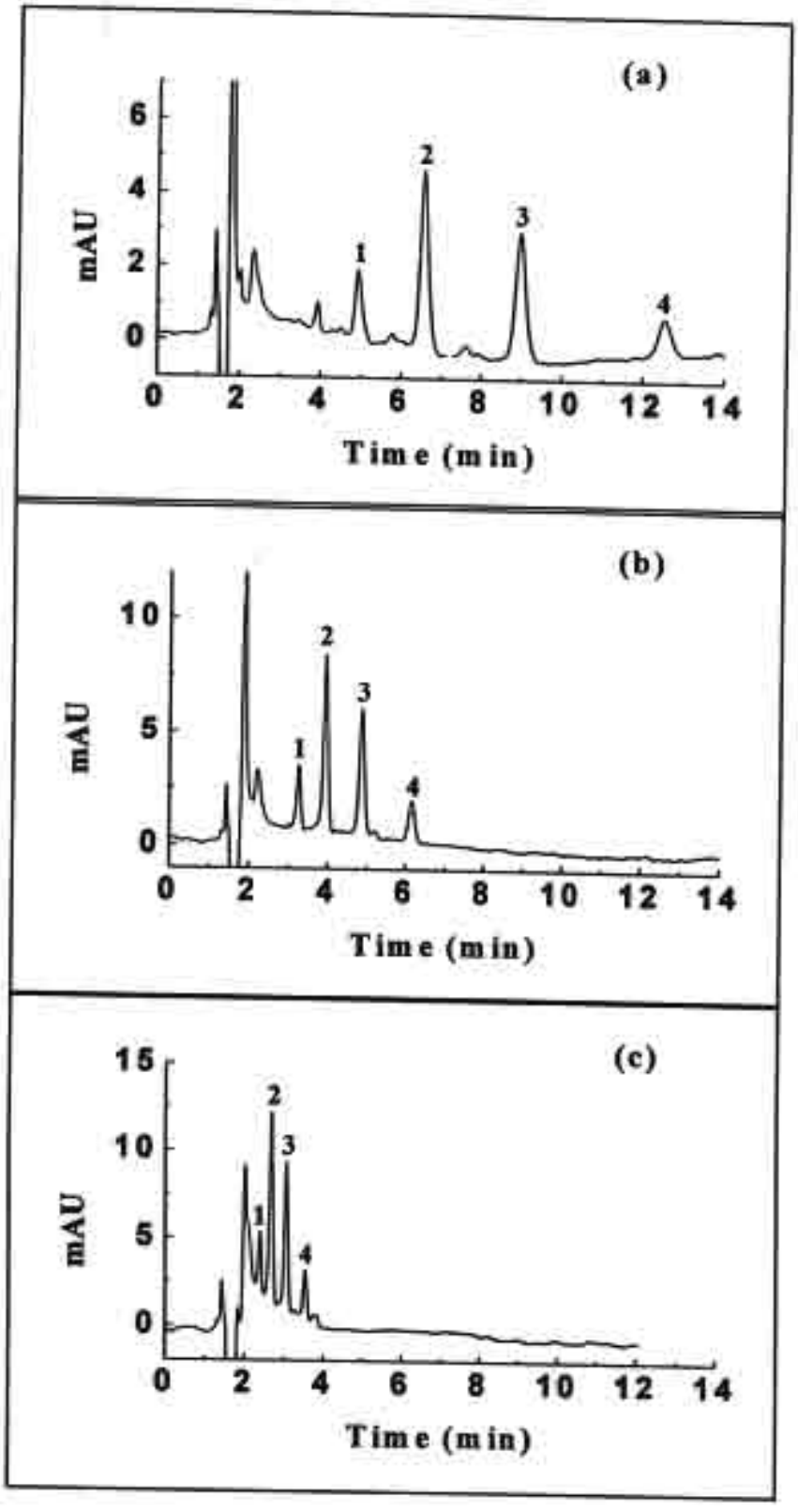

Fig. 3 Chromatograms of mixture of four LAS compounds obtained using various mobile phase compositions in the presence of 3.5 mM NaCl and MeOH-H_2O: (a) 75-25, (b) 80-20 and (c) 85-15. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

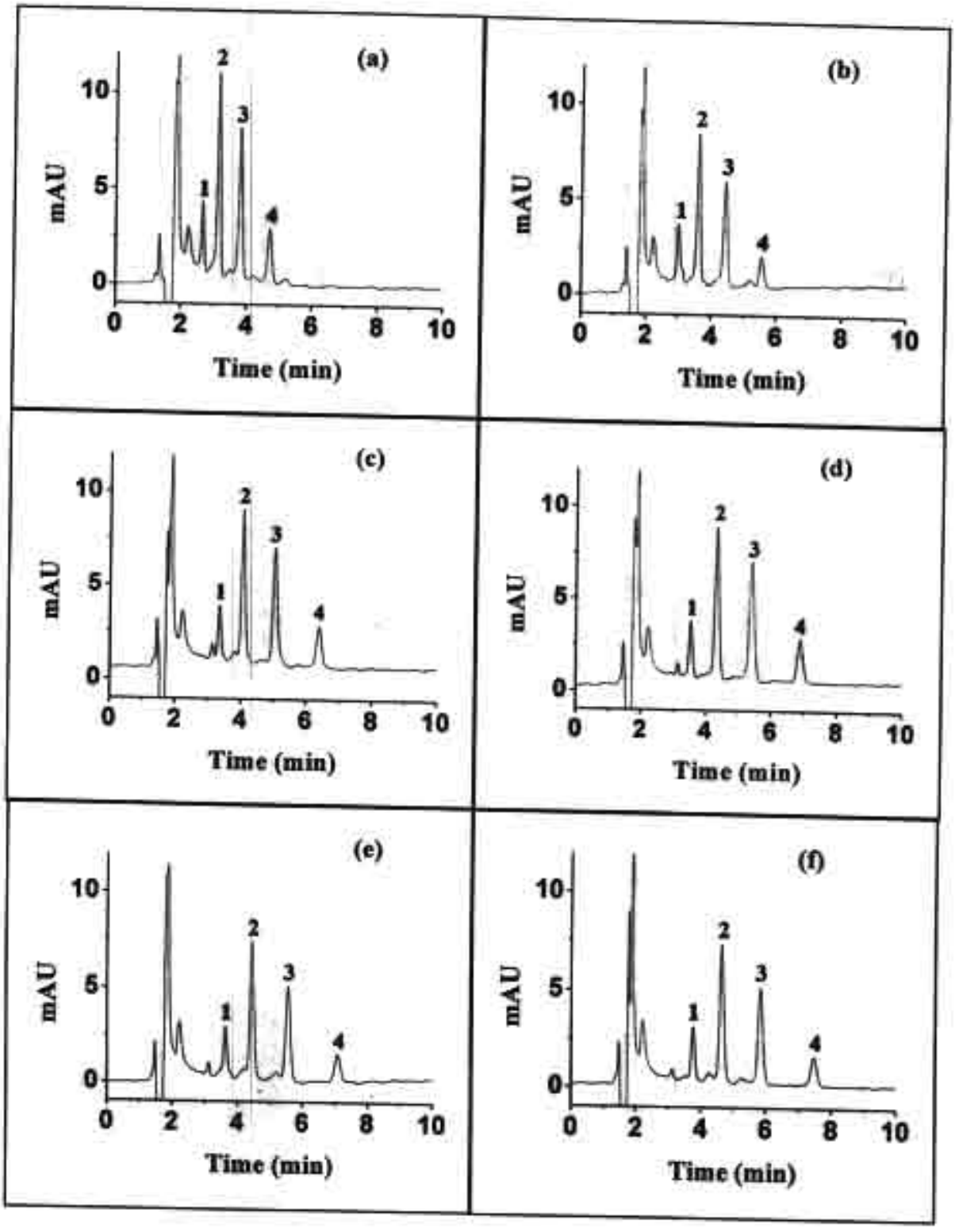

Fig. 4 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium chloride: (a) 1, (b) 2, (c) 4, (d) 6, (e) 8 and (f) 10 mM. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

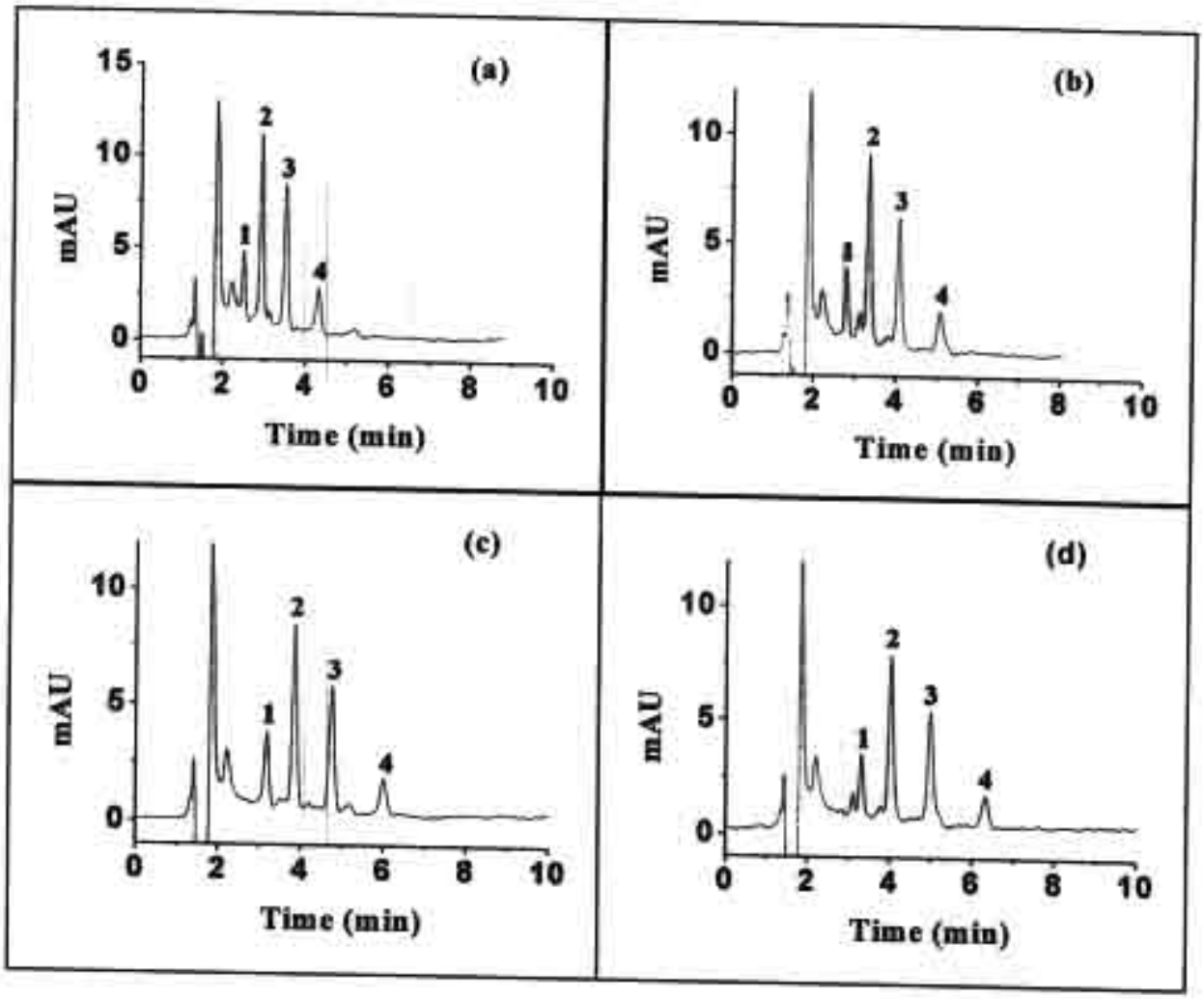

Fig. 5 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium acetate: (a) 2, (b) 4, (c) 8, and (d) 10 mM. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

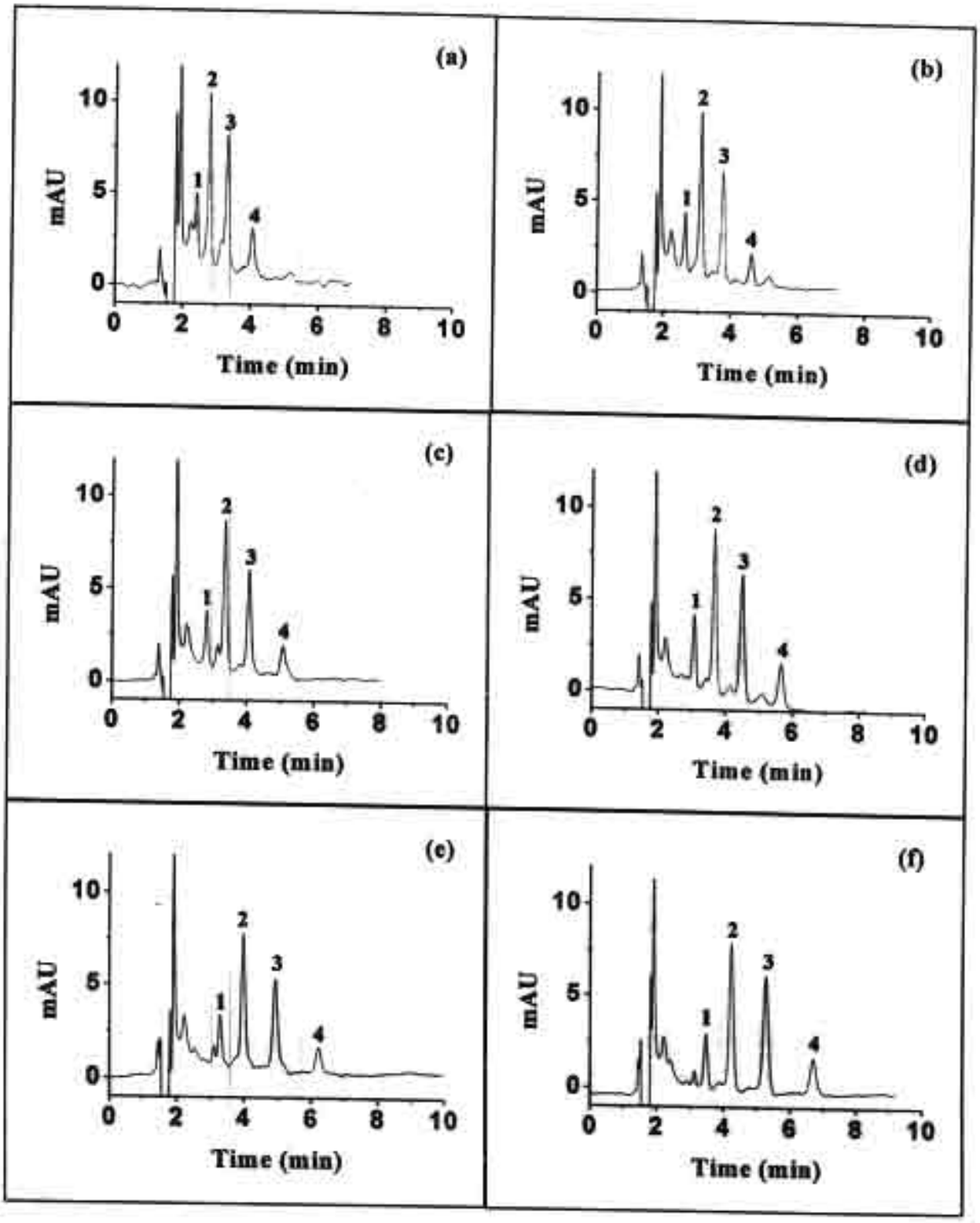

Fig. 6 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of ammonium acetate: (a) 1, (b) 1.5, (c) 2, (d) 4, (e) 6 and (f) 8 mM. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

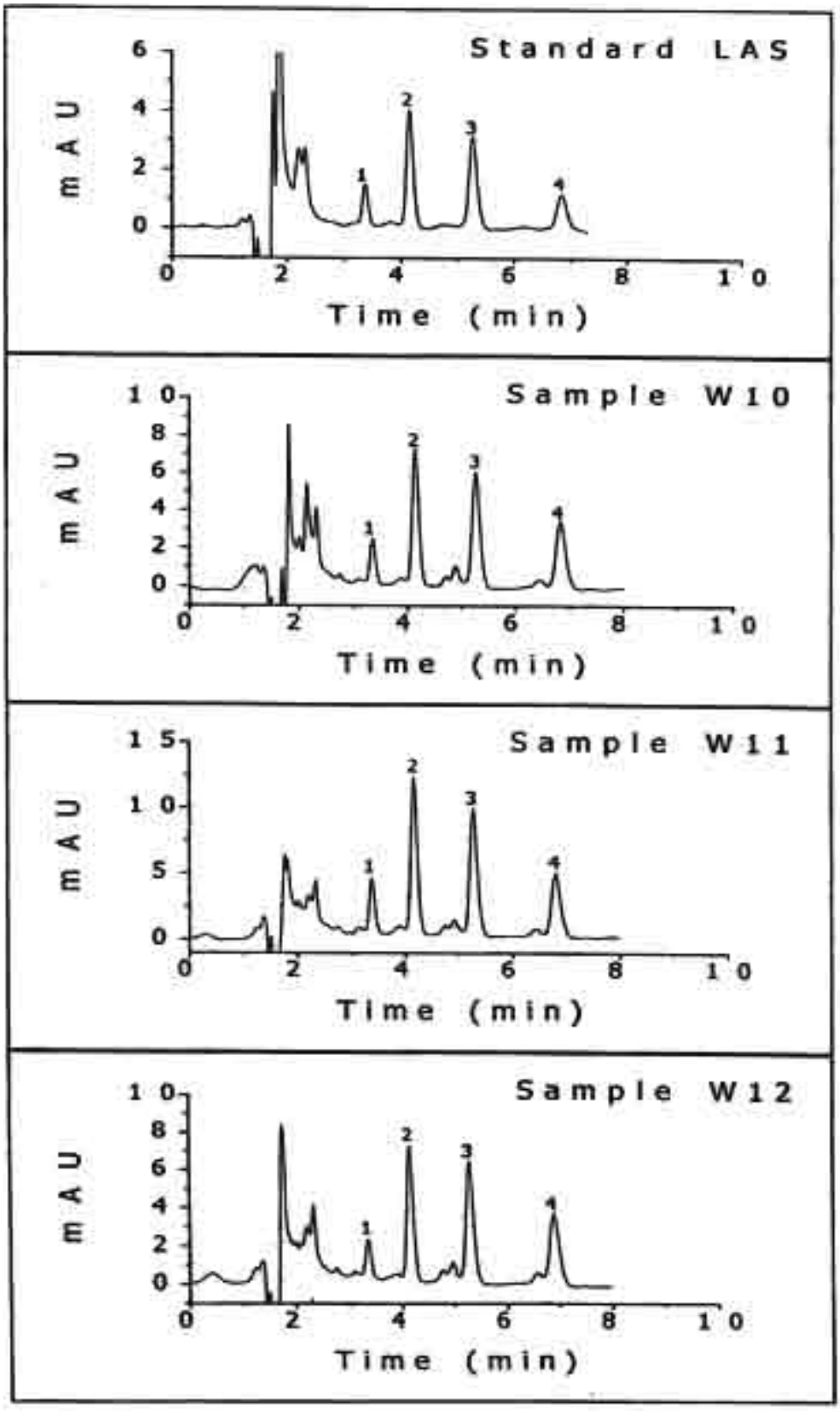

Fig. 7 Chromatograms of mixture of four LAS compounds in standard solution and water extracts obtained using 78% (v/v) methanol in water containing 1.5 mM ammonium acetate. Peak identification: (1) C_{10} LAS, (2) C_{11} LAS, (3) C_{12} LAS and (4) C_{13} LAS.

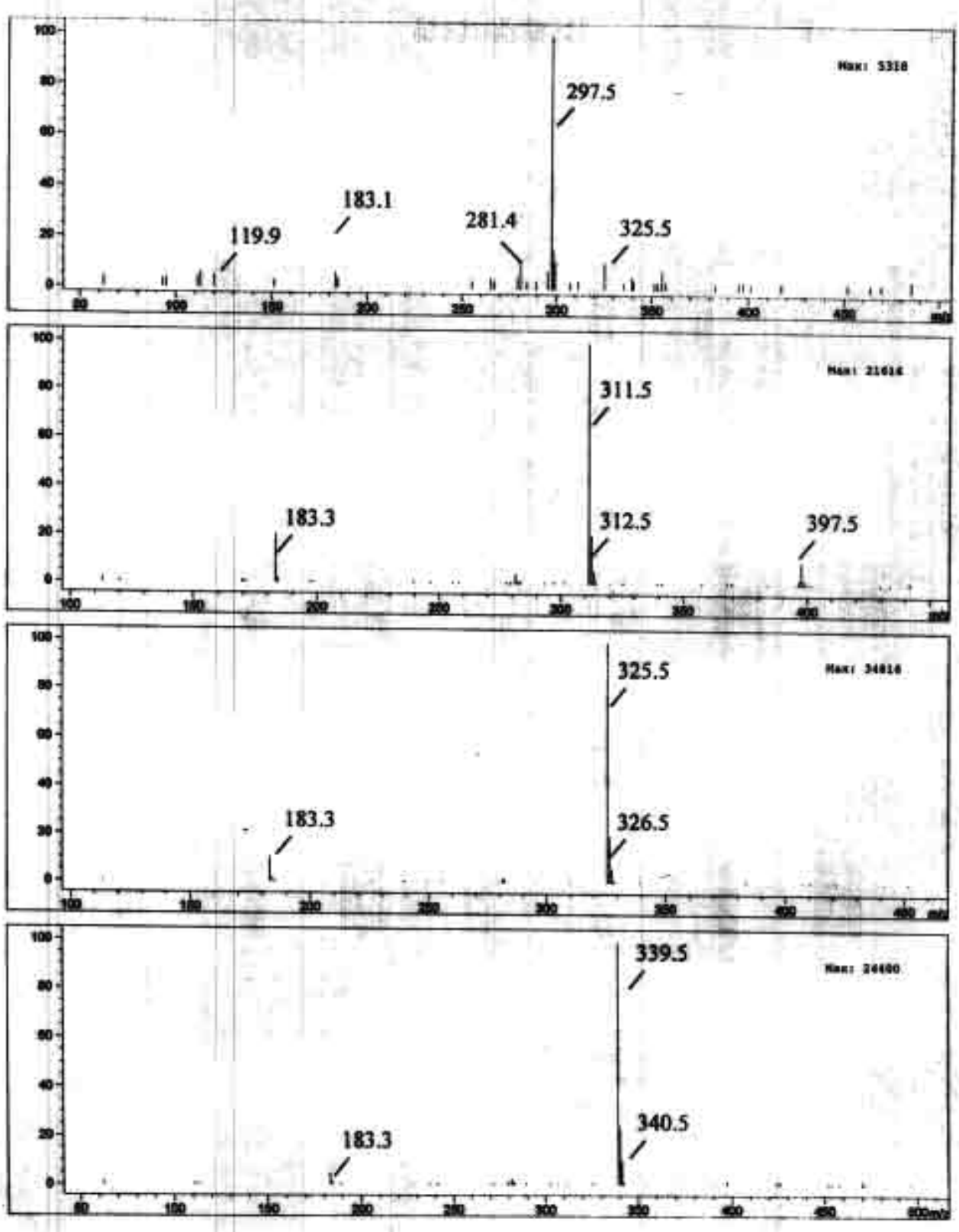

Fig. 8 Negative ion ESI mass spectra of LAS compounds originating from water extract (W 10)

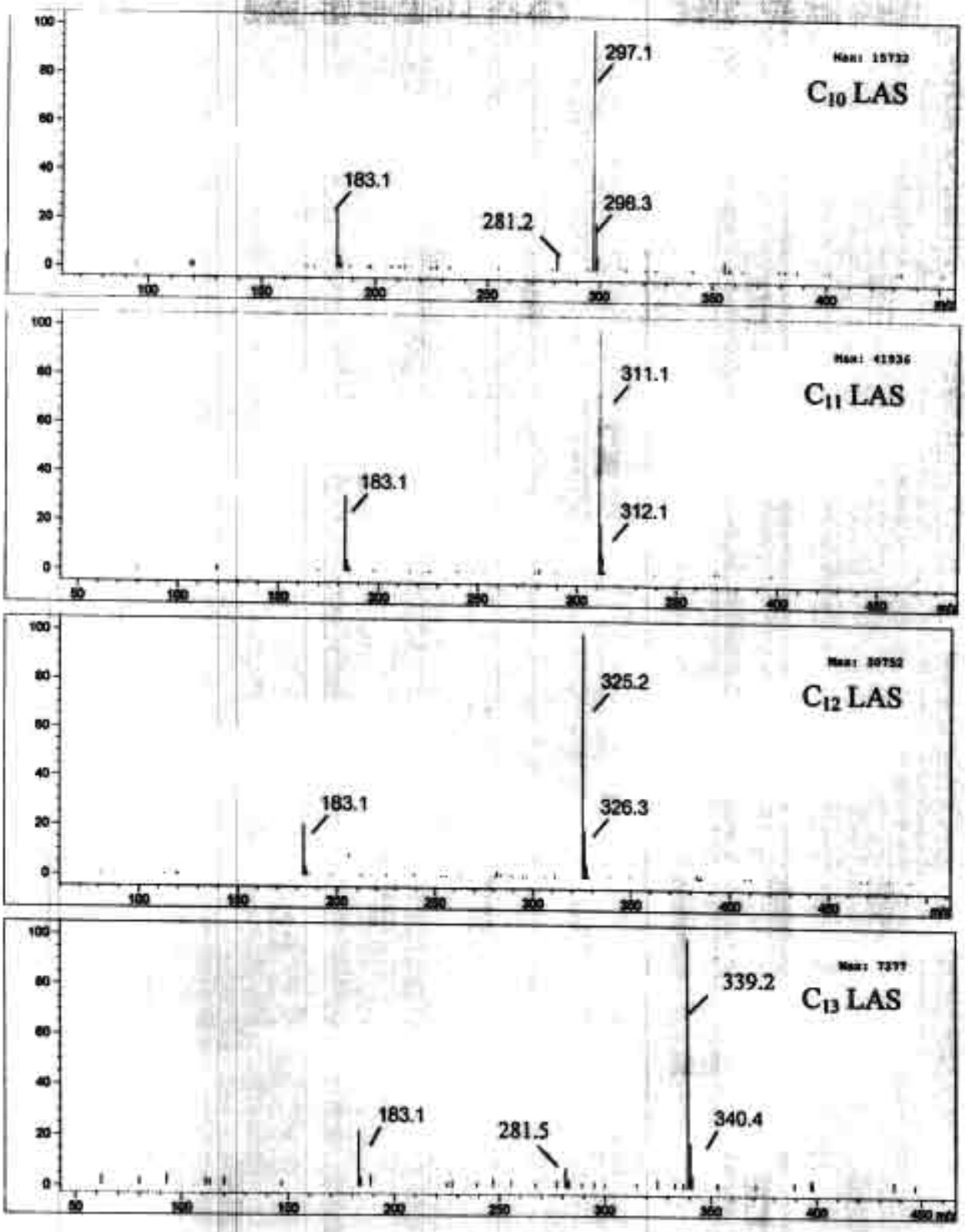

Fig. 9 Negative ion ESI mass spectra of the molecular ion originating from the mixture of LAS standard.

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Going with the flow

Gary D. Christian*
Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA

11th International Conference on Flow Injection Analysis (ICFIA 2001), Chiang Mai, Thailand, December 16–20, 2001.

1. Introduction

This conference started informally in the USA in 1987 as the Winter Conference on Flow Injection Analysis (WCFIA), organized by Gary Christian, Gil Pacey and Jarda Ruzicka. The first conference was held in Orlando, Florida, and subsequent conferences were held also in Scottsdale, Arizona, Marathon, Florida, and San Diego, California as well as Orlando.

The conference grew and attracted numerous international participants, and, in 1995, the name was changed to the International Conference on Flow Injection Analysis, with the initial ICFIA meeting being held in Seattle, Washington, in August. At the same time, the Japanese Association for Flow Injection Analysis (JAFIA) was invited to join the conference, and they have held their semi-annual meeting jointly with ICFIA since. ICFIA 2001 was held jointly with the 38th Semi-annual meeting of JAFIA.

Since 1998, the conference venue has been held outside the USA. The 10th ICFIA was held in Prague, Czech Republic, in June 1999, hosted by Charles University Faculty of Pharmacy and organized by Miroslav Polasek and Petr Solich.

An international steering committee was formed, chaired by Gary Christian, and includes José Luis Burguera (Venezuela), Kate Grudpan (Thailand), Bernard Lendl (Austria), Ian McKelvie (Australia), Shoji Motomizu (Japan, JAFIA), Jarda Ruzicka (USA), Tadao Sakai (Japan, J. Flow Injection Anal.), Rolf Sara (Finland), and Koos van Staden (South Africa), with Sue Christian as Advisor. The Steering Committee is charged with selecting international venues and dates for the conference.

ICFIA 2001 was hosted by Chiang Mai University in Northern Thailand, and was ably organized by Professor Kate Grudpan and his colleagues in the Department of Chemistry. There were 120 participants from 21 countries. President Nipon Tuwanon officially opened the conference with the traditional striking of a gong, which was followed by traditional dancing and a drum show. The conference was proclaimed in honor of Kate Grudpan, in recognition of his being named the Outstanding Scientist of the Year in Thailand for 2001, the first time in the 19-year history of the award that it was given to a scientist from outside Bangkok.

The technical program consisted of 36 oral presentations over three-and-a-half days and 72 posters. This report summarizes the oral presentations. A copy of the entire list of oral and poster presentations can be obtained by contacting Gary Christian at christian@chem.washington.edu. The E-mail addresses of authors can also be provided, if senders wish to obtain further information about a specific paper.

*Tel.: +1 (206) 543-1635; Fax: +1 (206) 685-3478.
E-mail: christian@chem.washington.edu

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2. Instrumentation

The opening lecture, "Microsequential Injection: A Versatile Approach to (Bio)chemical Assays Using Lab-on-valve System", by J. Ruzicka (University of Washington, USA), was well presented by E.H. Hansen (The Technical University of Denmark), since Professor Ruzicka was unable to attend. The applicability of the lab-on-valve system was presented, including use with bead injection.

S. Motomizu (Okayama University, Japan, and President of the JAFIA) reported on a portable microflow system capable of parts per trillion detection limits (10^{-8} *M* spectrophotometry, 10^{-9} *M* fluorometry) as a result of having a very stable pump system. Flow channels are 0.25 mm, the pump stroke is 2.5 µl, and the flow cell is 8 µl.

P. Worsfold (University of Plymouth, UK) described a portable FIA system with chemiluminescence detection for shipboard monitoring of sub-nanomolar levels of cobalt, copper, and manganese in oceanic waters. Metals were concentrated on microcolumns of immobilized 8-hydroxyquinoline to achieve selectivity. A tangential flow filter was used to filter turbid waters.

M. Zenki (Okayama University of Science, Japan) described a closed-loop, cyclic FIA system that allows recycling of reagents that are present in excess. More than 300 repetitive determinations of chloride and calcium ions gave good reproducibility and no baseline drift.

H. Itabashi (Guma University, Japan) described an all-injection analysis system in which all reagent solutions are injected into a reaction coil and all solutions are circulated for a definite time. The system minimizes reagent consumption and different analytical reaction systems can be utilized without rearranging the construction of the FIA assembly.

P. Ampan (Chiang Mai University, Thailand) described an in-valve minicolumn packed with ion-exchange resin for on-line sample preseparation and pre-concentration. The column was placed directly in the spectrometer for direct measurement of iron(II) 1,10-phenanthroline, with the resin discarded after each run. An FI system with bead injection (BI) was also developed.

K. Oguma (Chiba University, Japan) incorporated photo-induced reactions for oxidizing Fe(II) to Fe(III). Measurements before and after irradiation allowed determination of Fe(III) and Cu(II) or Pd(II) in mixture.

3. Sequential injection analysis (SIA)

J. F. van Staden (University of Pretoria, South Africa) described various configurations of SI and FI to achieve multi-component analyses. He used SIA for the simultaneous determination or speciation of metal ions, inorganic anions, and organic compounds.

T. Imato (Kyushu University, Japan) used microbeads immobilized with antibodies for the SI photometric determination of the endocrine disruptor, vitellogenin.

A. Ivaska (Åbo Akademi University, Finland) determined Fe(II) and Fe(III) in pickle baths using automated SIA.

J. Jakmunee (Chiang Mai University, Thailand) used voltammetric detection for the SIA simultaneous determination of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}.

4. Atomic spectroscopy/mass spectrometry

The separation and pre-concentration of trace metals was used by several investigators prior to measurement.

M. Burguera (Los Andes University, Venezuela) determined total and soluble silica in water by ETAAS using on-line dilution for total silica followed by precipitation with ammonium chloride and collection on the walls of a knitted coil and then dissolution to determine soluble silica.

J. L. Burguera (Los Andes University, Venezuela) developed on-line FIA microwave assisted mineralization and on-line precipitation for determination of 3 ppb Mo in 0.5 ml serum.

The enrichment in the knotted coil was 3.5-fold with a detection limit of 0.1 ppb in the sample.

Y. Zolotov (Lomonosov Moscow State University, Russia) used on-line combination of FI sample pretreatment and atomic spectroscopy determination. Solid-phase extraction was used for hydrophobic species and stable complexes. Cd, Co, Cr, Cu, Fe, Mn, Mo, Pb, V, Zn, rare earths and noble metals were determined in waters, soils, food-stuffs, ores and alloys using FIA with FAAS, FAF, and AES-ICP.

E. H. Hansen (The Technical University of Denmark) described pre-concentration/separation using liquid-liquid extraction, (co)precipitation in knotted reactors, adsorption, hydride generation, or ion-exchange columns, for measurement by ETAAS or ICP/MS.

S. Hirata (The National Institute of Advanced Industrial Sciences Technology, Japan) determined rare earth elements in seawater by on-line pre-concentration followed by elution with 0.7 *M* HNO_3 into an ICP/MS for measurement.

M. Bloomfield (GlaxoSmithKline Consumer Healthcare, UK) reported on current developments and applications for monitoring drug release, degradation products, and amine drugs, with emphasis on the use of FIA for process methodology, with coupling with mass spectrometry.

5. Electrochemistry

B. Deore (Yamaguchi University, Japan) reported on a triple-pulse amperometric detection of underivatized amino acids at a polypyrrole modified copper electrode. The partially overoxidized polypyrrole film allowed measurements in acid mobile phase, rather than the alkaline medium normally required.

N. Wangfuengkanagul (Chulalongkorn University, Bangkok, Thailand) described the determination of penicillamine using a boron-doped diamond (BDD) thin-film electrode in an FIA system. The BDD electrode provides a much larger response compared with a glassy carbon electrode.

R.-I. Stefan (University of Pretoria, South Africa) developed a high-throughput SIA screening of chiral drugs using unique potentiometric and amperometric carbon paste enantioselective membrane electrodes, based on either selective binding or catalytic selectivity.

M. Trojanowicz (Warsaw University, Poland) gave the closing lecture – on the FI amperometric determination of selected pesticides based on inhibition of immobilized acetylcholinesterases of different origin.

6. Luminescence

P. Francis (Deakin University, Australia) used the recently developed pulsed-flow chemistry approach for chemiluminescence reactions. A pulsed-flow module develops short pressure pulses to generate precisely timed bursts of solution flow, interspersed with longer periods when the solution remains static. The digital flow profile avoids flow-rate variation problems, and solution ratios are easily varied.

S. Nakano (Tottori University, Japan) determined V(IV) and total vanadium by FI chemiluminescence based on catalysis of the oxidation of purpurogallin with periodate.

T. Sakai (Aichi Institute of Technology and Editor, J. Flow Injection Anal.) reported on the analysis of trace amounts of indoor formaldehydes, using a fluorometric FI measurement.

7. FI applications

Several variations of FI were used for specific applications.

Kate Grudpan (Chiang Mai University, Thailand) emphasized the cost-effectiveness gained by employing flow-based techniques in analytical problem solving in Thailand, including Cr(III/VI) speciation, sample pretreatment, and use of unstable reagents. He gave an excellent historical account of how FIA was introduced in the South Pacific area of the world, with early contributions from his laboratory.

D. Nacapricha (Mahidol University, Bangkok, Thailand) determined total iodine using a kinetic approach, based on the catalytic effect of iodide on the Ce(IV)/As(III) reaction. In-line reduction of iodate was employed.

J. Itoh (Kitami Institute of Technology, Japan) utilized the inhibition effects of some metals on the ascorbic acid-copper-porphyrin reaction system for the sensitive kinetic determination of Cr(VI), Fe(III), V(V), and Hg(II).

N. Teshima (Aichi Institute of Technology, Japan) determined copper and iron photometrically in serum using long-path absorption cells, with damper coils placed before detection to provide a smooth baseline.

I. McKelvie (Monash University, Australia) disclosed a number of new FI techniques to study the aquatic phosphorus cycle, by determining inorganic and organic species.

J. Simon (Free University of Berlin) used chromatomembrane cells for sample pretreatment for PAH and EOX monitoring in wastewater.

8. Separations

M-R. Fuh (Soochow University, Taipei, Taiwan) determined free-form amphetamine in rat's blood by in-vivo microdialysis and liquid chromatography with fluorescence detection. The pharmacokinetics of amphetamine was examined.

P. Sutthivaiyakit (Kasetsart University, Bangkok, Thailand) determined polyphosphates in seafood by indirect spectrophotometric chromatography, using an anion-exchange column with UV measurement at 285 nm.

R. Chantiwas (Chiang Mai University, Thailand) reported on the use of gravitational field-flow fractionation in combination with FIA for sized-based speciation of silica particles. Iron on the particles was monitored by reverse-flow injection chemiluminescence or by ETAAS.

B. Karlberg (Stockholm University, Sweden) described titration and extraction in CE capillaries, in a miniaturized, monosegmented flow analysis system. Weak acids were titrated and pK_a values determined in the range 3–6, using pH indicators and spectrophotometric detection. A sample volume of less than 250 nl is required. Octanol/water partition coefficients were screened with the system using 3 µl aqueous sample and 110 nl of organic phase.

The 72 posters presented numerous examples of innovative flow techniques and applications. Titles may be found in the complete program, which is available from Gary Christian on request.

9. Social program

A wonderful Thai cultural evening was enjoyed on Tuesday evening, with a Khan Toke dinner, traditional Northern Thai music and hill tribe dancing by students from the Chiang Mai College of Dramatic Arts.

Wednesday afternoon was free for touring and sightseeing, with all participants invited to an elephant camp, and the Queen Sirikit Botanic Garden, a true gem of the country. That evening, refreshments were enjoyed at the Chiang Mai University Science Faculty Observatory, where we were able to stargaze, and plan for the next conference. Some participants were still seeing stars the next morning.

10. Publication

Manuscripts of presentations and posters submitted by participants will be reviewed for publication in a special issue of *Talanta*.

11. Sponsors and exhibitors

The conference was sponsored and supported by Chiang Mai University, Thailand, the Japanese Association for Flow Injection Analysis (JAFIA), the British Council, the Chemical Society of Thailand, the Post-graduate Education and Research Program in Chemistry (PERCH), the Science Society of Thailand (Chemistry and Northern Divisions), the Thai

Ministry of University Affairs, the Thailand Research Fund (TRF), Constellation Technology, Corp., FIAlab Instruments, Inc., Foss Tecator, Metrohm Siam Co., Ltd., Perkin Elmer, Inc., and Thai Unique Co., Ltd. (Lachat).

12. 12th ICFIA

The Steering Committee has selected the next venue for the ICFIA to be Los Andes University in Mérida, Venezuela. Pioneering FIA colleagues José Luis Burguera and Marcella Burguera have graciously agreed to organize and host the conference. Professors J. F. (Koos) van Staden and Raluca-Ioana Stefan from The University of Pretoria will be co-organizers, in charge of the technical program. The tentative date is 7–11 December 2003, a very pleasant time of year in this beautiful sub-tropical country. Information will be posted at www.flowinjection.com, and direct contacts are burguera@ciens.ula.ve and koos.vanstaden@chem.up.ac.za

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Pioneers host success high in the Andes

Report on 12th International Conference on Flow Injection Analysis (ICFIA 2003), Mérida, Venezuela, 7–13 December 2003

Gary D. Christian*

1. Introduction

Following the 11th ICFIA held in Chiang Mai, Thailand, in December 2001, the 12th ICFIA was hosted by Professors Jose Luis Burguera (Co-organizer) and Marcela Burguera (Co-secretariat) from Los Andes University (Universidad de los Andes), Mérida, Venezuela. The local organizing committee also included P. Carrero, M. Gallignani, Y. Petit, C. Rondon and M.R. Brunneto, with able assistance from their students. The conference was held jointly with the Japanese Association for Flow Injection Analysis (JAFIA). There were 18 countries from five continents represented.

2. Social events

Opening ceremonies were held on Sunday evening (7 December 2003) at the University's Aula Magna (Great Hall). Rector Genryl Vargas formally welcomed participants, and Dean of the Faculty of Sciences Patricia Rosenzweig expressed her appreciation and support for the conference. As Chair of the conference International Steering Committee, I expressed the appropriateness of holding the conference in Venezuela, since the Burgueras are pioneers in the early development of flow injection analysis (FIA). Jose Luis Burguera welcomed all to the conference and the city of Mérida. The participants were then treated to a string quintet concert before adjourning for a fine reception.

On Monday evening, we were treated to a show of Venezuelan dancers with colorful costumes.

On Wednesday, all participants went on a fascinating tour to the high country in the Andes, which at 4100 m, literally took our breath away.

A gala banquet was held Thursday evening. It began with a demonstration of Latin American dances and concluded with participants dancing to Mexican and Venezuelan music late into the evening.

Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700, USA
*Tel.: +1 (206) 543-1635; Fax: +1 (206) 685-3478;
E-mail: christian@chem.Washington.edu

3. JAFIA's 20th Anniversary and Awards

The JAFIA celebrated its 20th anniversary in 2003. Shoji Motomizu, President of JAFIA, and Tadao Sakai, Editor of the Japanese *Journal of Flow Injection Analysis* (*JFIA*) presented a pictorial history and account of the Association and important contributors to *JFIA*.

The occasion of the ICFIA was used as the venue for presenting the annual JAFIA awards (for 2003). Professor Kate Grudpan, Chiang Mai University, was awarded the JAFIA Scientific Award medal and Professor Duangjai Nacapricha, Mahidol University, received the JAFIA Best Article Award medal for a paper in *JFIA*. Professors Elo Hansen, Jacobus (Koos) van Staden, Jose Luis Burguera and I were greatly honored to receive the JAFIA Scientific Honor Award medal and certificate, which JAFIA and the Division of the Japan Society for Analytical Chemistry (JSAC) grant only every five years, and Sue Christian was specially honored with the JAFIA Special Gold Medal for her years of contributions and devotion to the Conference. These awards were particularly special as 20th anniversary recognitions.

4. Scientific program

4.1. Plenary lectures

Professor Jacobus (Koos) F. van Staden (Co-organizer) and Raluca-Iona Stefan (Co-secretariat) of the University of Pretoria arranged the scientific program that began on Monday morning and concluded Friday afternoon.

It was opened, with the first of 14 Plenary Lectures, by E.H. Hansen (Technical University of Denmark) who spoke on the impact of FIA on modern analysis.

Plenary lectures throughout the week included presentations on:

- the compact disc analyzer, by J.F. van Staden;
- development of flow-bio-detection amperometric sensors for food and cosmetics analysis, by M.I. Karayyannis (University of Ionnina);
- multi-pumping flow systems for automation, by J. L. F. Costa Lima (Universidade do Porto); and,

0165-9936/03/$ - see front matter doi:10.1016/S0165-9936(04)00205-5

- determination of trace impurities in ultrapurified waters and chemicals at parts per trillion levels by flow-based analysis, by S. Motomizu (Okayama University).

M. Burguera presented a comprehensive, informative lecture on analytical applications of organized media for on-line spectrometric determinations of parts per trillion metal ions; T. Sakai (Aichi Institute of Technology) described the development of new devices for FI detection for environmental and biological analysis. A. Ivaska (Åbo Akademi University) presented a sophisticated mathematical modeling of flow-injection systems in straight tubes, including sequential injection analysis; and, I traced the origins of analytical chemistry and the development of analytical methods and textbooks that led up to the development of FIA-based techniques.

J. van Staden presented Ralcua-Iona Stefan's Plenary Lecture in her absence; it was on the use of electrochemical sensors for multi-component analyses in flow systems, particularly for measuring enantiomers. V. Cerda (Universitat de les Iles Balears) described a flexible multi-syringe, multivariate system for multicommuted FIA (MCFIA), along with sophisticated Windows-based software for instrument control and data acquisition. K. Grudpan described novel, low-cost, flow-based systems, including the "Lab-at-Valve" concept, in which a detection device is attached to one port of a conventional multi-position selection valve. E. Zagatto (Universidade de São Paulo, Piracicaba) emphasized the importance of exploiting prior assays for reducing the number of analyses needed. For example, low molybdenum content in plants results in low nitrogen fixation. Analyzing for molybdenum only for plants with low nitrogen (an easier analysis) increases the efficiency of the laboratory.

The Plenary Lectures concluded with a comprehensive presentation of the use and power of FTIR (Fourier-transform infrared spectroscopy) in flow analysis, by M. Gallignani (University of Los Andes).

4.2. *Oral presentations*

There were 25 lectures covering various aspects of flow analysis, including novel developments in instrumentation, and spectrophotometric, luminescence, atomic spectrometric, and electrochemical applications.

4.2.1. *Spectrophotometry*. Optical methods continue to represent the majority of FIA-based applications. B. Karlberg (Stockholm University) described how IR detection in microflow systems, including capillary electrophoresis, achieved picogram detection.

F. Suliman (Sultan Qaboos University) reported on an optical sensor for micro-sequential bead injection analysis, with 9 ppb detection for aluminum.

Simultaneous determination and speciation of heavy metals in waters was presented by V. Cerdà, using on-line UV oxidation of organic matter.

Multivariate analysis was used to subtract free ligand contributions. J. van Staden used SIA (sequential injection analysis) for metal ion, inorganic anion, and organic compound speciation.

M. Almeida (Universidade Católica Portuguesa) speciated phosphorus species in environmental samples, using microwave oven digestion for total phosphorus determination.

S. Meneses (Universidade de São Paulo, Piracicaba) determined sulfate in soil extracts at the µg/L level, based on reaction with the barium-dimethylsulfanazo (III) complex.

K. Oguma (Chiba University) reported on the determination of bromate and iodate by oxidation of the iron(II)-1,10-phenanthroline complex. Bromide and iodide were differentiated by reaction with chlorite at selected pH values.

M. Bloomfield (GlaxoSmithKline Consumer Healthcare, UK) reported on the assay for peroxide in healthcare products, along with the necessary validation challenges for the industry.

M. Zenki (Okayama University of Science) described the repetitive determination of ascorbic acid in the presence of oxidants using a novel circulatory flow injection system and an inhibitor; 300 repetitive determinations could be made with a 50 mL reservoir.

J. Prior (Universidade do Porto) determined antithyroids by reaction with Pd(II), with UV detection, or by the inhibitory effect on the Pd(II) catalyzed reaction between pyronine G and hypophosphite ions, monitored at 548 nm.

H. Ukeda (Koichi University) determined the antioxidant capacity of compounds in food based on scavenging of the ABTS radical cation (ABTS= 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonate)).

J. Liu (Kitami Institute of Technology) described the kinetic determination of sulfhydryl thiols based on their catalytic effect on the reaction of the ttmap (5,10,15,20-tetrakis[4-(N-trimethylammonio) phenyl]) porphyrin with copper ions.

A. Cáceres (La Universidad del Zulia) described the on-line analysis of linear alcohols using microwave-assisted derivatization.

4.2.2. *Luminescence*. Y. Iida (Kanagawa Institute of Technology) determined urea in alcoholic beverages using an acid-urease column and fluorometric detection of isoindole derivative from the ammonia produced.

D. Nacapricha (Mahidol University) used gas-diffusion FI for the selective determination of iodide in pharmaceutical products, using the iodine-luminol reaction for detection.

T. Imato (Kyushu University) used magnetic microbeads and chemiluminescence detection for the immunoassay of the endocrine disruptor, vitellogenin, in fish.

4.2.3. Atomic spectrometry. P. Carrero (University of Los Andes) determined bismuth in urine by successive retention of Bi(III) and tetrahydroborate(III) on an anion exchange resin for hydride generation AAS.

J. Chirinos (Universidad Central de Venezuela) described fast, on-line determination of selenium by hydride generation and ICP-OES (inductively coupled plasma optical emission spectroscopy) measurement.

4.2.4. Electrochemistry. J. Jakmunee (Chiang Mai University) reported on an on-line standard addition sequential injection method for the simultaneous determination of four metal ions using anodic stripping voltammetry.

T. Nagaoka (Osaka Prefecture University) used a boron-doped, diamond-coated electrode, coated with a molecularly imprinted polymer, for the selective voltammetric determination of amino acids at +2.0 V vs. Ag/AgCl.

O. Chailapakul (Chulalongkorn University) used an anodized, boron-doped, diamond thin-film electrode to determine tetracycline antibiotics, with a 10-nM detection limit. The diamond electrodes exhibit low background and enhanced stability, as well as wider potential range, compared with glassy carbon electrodes.

K. Vytras (University of Pardubice) reported on the development of biosensors based on carbon-paste substrates modified with a manganese dioxide powder mediator for detecting H_2O_2 in enzymatic reactions. Disposable screen-printed electrodes can be prepared, with enzyme entrapped in Nafion.

I. Satoh (Kanagawa Institute of Technology) described the electrochemical removal of zinc ions from immobilized alkaline phosphatase; injection of a sample containing zinc ions regenerated the enzyme activity, and the reversible process was used for the determination of nanomolar concentrations of zinc ions, with spectrophotometric detection.

H. Tanaka (Tokushima University) described a new concept: feedback-based flow ratiometry to determine acid/base dissociation constants, using a pH glass electrode for detection.

4.2.5. Analytical Sciences Digital Library. I introduced participants to the US National Science Foundation-sponsored Analytical Sciences Digital Library (ASDL), a new website database of URLs dealing with all aspects of analytical chemistry. The websites are peer reviewed for content and relevance; they cover categories such as pedagogy, laboratory experiments, techniques, and applications. The website is free: http://www.asdlib.org/.

4.3. Poster presentations

There were over 60 poster presentations by participants form Brazil, Greece, Japan, Oman, The Philippines, South Africa, Spain, Turkey, Thailand, Uruguay, USA, and Venezuela.

The breadth of flow-based techniques was well illustrated by posters on automation, SIA, on-line pre-concentration, separation, reactions and sample clean-up, membrane separations, microwave-assisted derivatization and sample preparation, multi-component analyses, chemometrics, catalytic and kinetic methods, stopped-flow reactions in a mixing coil, electrochemical and biosensor detection, and FTIR, fluorescence, spectrophotometric, and vapor-phase atomic absorption detection. Applications to a variety of samples were reported, including pharmaceutical products, environmental samples, gasoline, oil, and soil extracts.

4.3.1. Best Poster Awards Certificates for the most outstanding posters for each of the three sessions were awarded to:

- Mürvet Volkan (Middle East Technical University, Ankara, Turkey);
- Moisés Knochen (Universidad de la Republica, Montevideo, Uruguay); and,
- Ricardo Rivas (Universidad Centro Occidental Lisandro Alvarado, Barquisimeto, Venezuela).

4.4. Publication of the Proceedings

Papers at the conference will be submitted for a Special Issue of *Talanta*, with Guest Editors J.L. Burguera and J.F. van Staden.

5. ICFIA 13

After considering several sites, members of the Steering Committee (M. Bloomfield, J.L. Burguera, K. Grudpan, S. Motomizu, T. Sakai, J. van Staden and I. and S. Christian, Advisor) selected the USA for the next conference, to be held in 2005. For details, please contact Sue Christian (E-mail: sue@flowinjection.com).

ภาคผนวก ค

ข่าวจากหนังสือพิมพ์

นักวิทยาศาสตร์มช.สร้างชื่อ JAFIAญี่ปุ่นมอบรางวัลสูงสุด

ข่าวจากมหาวิทยาลัยเชียงใหม่(มช.) แจ้งว่า สมาคมโฟลอินเจ็กชั่นและนาลิซิสแห่งประเทศญี่ปุ่น (Japanese Association for Flow Injection Analysis) หรือ JAFIA มอบเหรียญรางวัลสูงสุด พร้อมประกาศนียบัตรของสมาคมแก่ รองศาสตราจารย์ ดร.เกตุ กรุดพันธุ์ อาจารย์ภาควิชาเคมี คณะวิทยาศาสตร์ มช. จากผลงานการพัฒนาระบบวิเคราะห์ทางสังคมที่ใช้การไหลที่มีประสิทธิภาพสูงราคาประหยัด(Development of Cost-Performance Flow-based Chemical Analysis Systems) โดยจัดพิธีมอบประกาศนียบัตรในการประชุมทางวิชาการครบรอบ 20 ปี และการประชุมทางวิชาการครั้งที่ 44 ที่มหาวิทยาลัยโอกายามา เมืองโอกายามา ประเทศญี่ปุ่น

นอกจากนี้ได้จัดพิธีมอบเหรียญรางวัลในการประชุมวิชาการนานาชาติ 12 th International Conference on Flow Injection Analysis and Related Technique(12 th ICFIA) ที่เมืองเบริคา ประเทศเวเนซุเอลา รางวัลดังกล่าวถือเป็นรางวัลสูงสุดของสมาคมที่มอบให้กับนักวิทยาศาสตร์ชั้นนำในระดับนานาชาติ โดยรองศาสตราจารย์ ดร.เกตุ กรุดพันธุ์ ได้รับเชิญเป็นผู้บรรยายเรื่องพัฒนาการใหม่ ทางการวิเคราะห์ที่ใช้การไหล ที่มีราคาประหยัด (Development of Cost-Performance Flow-based Chemical Analysis Systems) ในการประชุมวิชาการครั้งนี้ด้วย

รองศาสตราจารย์ ดร.เกตุ กรุดพันธุ์ เป็นผู้มีผลงานทางวิชาการจำนวนมาก เคยได้รับรางวัลเมธีวิจัยรุ่นที่ 1 (2537-2540) รางวัลเมธีวิจัยอาวุโส(2544-2546) รางวัลนักวิจัยดีเด่นแห่งชาติ สาขาวิทยาศาสตร์ เคมีบาลัร ของสภาวิจัยแห่งชาติ เป็นนักวิจัยคนแรกของภูมิภาคที่ได้รับรางวัลนักวิทยาศาสตร์ดีเด่นจากมูลนิธิส่งเสริมวิทยาศาสตร์และเทคโนโลยีในพระบรมราชูปถัมภ์ ปี 2544 และได้รับรางวัลเกียรติยศทางวิชาการทั้งในระดับชาติ และระดับนานาชาติอีกหลายรางวัล

หนังสือพิมพ์มติชนรายวัน ประจำวันพุธที่ 25 กุมภาพันธ์ 2547 หน้า 21

ภาคผนวก ง

Program ของ Annual Symposium on TRF Senior Research Scholar on Flow-based Analysis

The 1st Annual Symposium on TRF Senior Research Scholar on Flow-Based Analysis

The 1st Annual Symposium on TRF Senior Research Scholar on Flow-Based Analysis

At Buares–Kamthong Room, Department of Chemistry, Faculty of Science, Chiang Mai University

8.30 - 9.00	**Registration**
9.00 - 9.15	**Opening session** - "Symposium Objectives" by Assoc. Prof. Dr. Kate Grudpan, TRF Senior Scholar - Welcoming address by Head of Chemistry - Address by Dean, Faculty of Science - Address by Director, Institute for Science and Technology Research and Development - Address by Director, Basic Research Division, TRF - Opening address by Director, TRF
9.15 - 10.15	**Plenary lecture** "Advanced Chemical Analyses Using Flow-Based Techniques" by Prof. Dr. Shoji Motomizu, Department of Chemistry, Faculty of Science, Okayama University and President, The Japanese Association for Flow Injection Analysis (JAFIA), JAPAN.
10.15 - 10.45	**Break**
10.45 – 11.15	"Some Recent Development on Flow-Based Analysis in Thailand : Examples from the TRF Senior Research Scholar Grant" by Assoc. Prof. Dr. Kate Grudpan
11.15 - 11.35	"Simply Tri-Iodide Starch Chemistry in Flow Injection" by Assist. Prof. Dr. Duangjai Nacapricha
11.35 - 11.55	"Flow Injection Voltammetric Systems with Repeated Use of Mercury Film for Simultaneous Determination of Cd Cu Pb and Zn" by Dr. Jaroon Jakmunee
12.00 - 13.00	**Lunch**
13.00 - 13.20	"Electrochemical Determination of Tetracycline Antibiotics by Pulsed Amperometric Detection Applied to Flow Injection System" by Assist. Prof. Dr. Orawon Chailapakul
13.20 - 13.40	"Flow Based-Reduced Volume Column System for Thalassemia Screening" by Dr. Supaporn Kardtap Hartwell
14.00 - 15.30	**Poster session / Break**
15.30 – 16.00	**Discussion / Comments**

Poster Session

ster N	Name	Title
P-01	Duangjai Nacapricha	USE OF IODINE-STARCH REACTION TO DETERMINE IODIDE IN A HIGHLY COLORED PHARMACEUTICAL PRODUCT
P-02	Kanokkarn Sa-nguanwong	DEVELOPMENT OF THE GRADIENT ELUTION FOR SEPARATION OF CHLORINE-CONTAINING ANIONS BY ION CHROMATOGRAPHY USING CONDUCTIVITY DETECTION
P-03	Kwuanjit Manee-on	DETERMINATION OF ETHANOL BY OSTERYOUNG SQUARE WAVE VOLTAMMETRY
P-04	Lop Patimaporndet	SEQUENTIAL INJECTION TITRIMETRY WITH LAB-ON-VALVE FOR ACIDITY IN FRUIT JUICE
P-05	Nathawut Choengchan	USE OF IODINE-STARCH REACTION IN FLOW INJECTION TO STDUY IODINE LOSS THROUGH THERMAL DIGESTION
P-06	Nattawan Kuppithayanant	MULTIPARAMETER OPTIMIZATION OF THE ULTRASONIC EXTRACTION FOR POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL SAMPLES
P-07	Nathawut Choengchan	USE OF IODINE-STARCH COMPLEXATION FOR DETERMINATION OF IODATE IN IODIZED SALT BY FLOW INJECTION
P-08	Nichanan Tepsuparungsikul	SEPARATION OF POLYCHLORINATED BIPHENYLS USING GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR
P-09	Orawan Tue-Ngeun	DETERMINATION OF IODATE IN IODIZED-TABLE SALTS BY USING FLOW INJECTION AMPEROMETRIC DETECTION
P-10	Parinya Masawat	FLOW INJECTION VOLTAMMETRIC DETERMINATION OF ACETAMINOPHEN IN PHARMACEUTICALS USING AN INEXPENSIVE FLOW-THROUGH CELL INCORPORATING WITH A PENCIL LEAD ELECTRODE
P-11	Pattanapong Ampan	COUPLING SEQUENTIAL INJECTION SYSTEM WITH LAB-ON-VALVE TO ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY FOR LEAD DETERMINATION BASED ON THE USE OF RENEWABLE MICROCOLUMN
P-12	Rattikan Chantiwas	SIZE-BASED IRON SPECIATION OF GOETHITE COATED SILICA PARTICLES BY GrFFF WITH FIA OR ETAAS
P-13	Rodjana Burakham	FLOW INJECTION AND SEQUENTIAL INJECTION SPECTROPHOTOMETRIC DETERMINATION OF PARACETAMOL IN PHARMACEUTICAL PREPARATIONS
P-14	Saisunee Liawruangrath	DEVELOPMENT OF A FLOW INJECTION SPECTROPHOTOMETRIC PROCEDURE FOR CADMIUM DETERMINATION
P-15	Sanit Palaharn	ELECTROANALYSIS OF TETRACYCLINE IN PHARMACEUTICAL FORMULATION BY FLOW INJECTION SYSTEM
P-16	Saowapha Muangkaew	A REVERSE FIA METHOD FOR DISSOLVED OXYGEN DETERMINATION IN WATER SAMPLE
P-17	Siripat Suteerapataranon	STUDY OF COLLOIDS RELEASED FROM A CONTAMINATED SOIL BY FIFFF-ICP-MS
P-18	Jaroon Jakmunee	SIMULTANEOUS DETERMINATION OF PHOSPHATE AND SILICATE BY STOPPED FLOW INJECTION FOR KINETIC SEPARATION
P-19	Sombat Chowwanapoonpohn	RECOVERY OF ELECTROCOAGULATED PHENOLIC COMPOUNDS
P-20	Somchai Lapanantnoppakhun	FLOW INJECTION ANLYSIS FOR TRACE IRON IN BEER SAMPLES
P-21	Sunanta Wangkarn	ELUTION PROFILES OF ANIONIC AND CATIONIC SURFACTANTS
P-22	Supalax Srijaranai	ON-LINE COMPLEXATION OF SOME METALS PRIOR TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS
P-23	Thanyarat Chaosaard1	FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF COPPER USING TETRACYCLINE
P-24	Waraporn som-aum	FLOW INJECTION ON-LINE PRECONCENTRATION OF Cr(VI) USING A COLUMN REACTOR PACKED WITH PTFE BEADS FOR THE ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION
P-25	Winita Punyodom	SOME TYPES OF CHITOSAN MEMBRANES FOR ON-LINE SEPARATION AND DILUTION
P-26	Wirat Ruengsitagoonb	FLOW INJECTION CHEMILUMINESCENCE DETERMINATION OF TETRACYCLINE
P-27	Worawit Chaeurwan1	OPTIMIZATION OF SEQUENTIAL EXTRACTION METHOD FOR THE DETERMINATION OF SOME HEAVY METALS IN THE RIVER SEDIMENT SAMPLES

The 2nd Annual Symposium on TRF Senior Research Scholar on Flow-Based Analysis

The 2nd Annual Symposium on TRF Senior Research Scholar on "Flow-based Analysis"

At Seminar Room ScB 2

Faculty of Science, Chiang Mai University

6th September 2003

8.15 - 8.30	Registration	
8.30 - 8.45	**Opening session**	
	- "Symposium Objectives" and "Progress on Development of Flow-based Analytical Techniques under the TRF Senior Research Scholar Project" by Assoc. Prof. Dr. Kate Grudpan, TRF Senior Research Scholar	
	- Opening address by the Director, TRF (Prof. Dr. Piyawat Boon-long)	
	- Address by the Director, Academic Division, TRF (Prof. Dr. Vichai Boonsaeng)	
8.45 - 9.05	"Flow Injection Analysis for Determination of Trace Air Pollutants" by Prof. Dr. Tadao Sakai, Aichi Institute of Technology, JAPAN	
9.05 - 9.25	"Ultratrace and Trace Determination with Flow-based Techniques" by Prof. Dr. Shoji Motomizu, Department of Chemistry, Faculty of Science, Okayama University, JAPAN	
9.25-9.45	**Break**	
9.45-9.55	Oral Presentation 1 (for poster nos. 1 - 3)	Dr. Supaporn Kradtap Hartwell
9.55-10.05	Oral Presentation 2 (for poster no. 20)	Miss Maliwan Amatatongchai
10.05-10.15	Oral Presentation 3 (for poster no. 21)	Miss Teeraporn Charoenraks
10.15-10.25	Oral Presentation 4 (for poster no. 16)	Dr. Jaroon Jakmunee
10.25-10.35	Oral Presentation 5 (for poster no. 22)	Asst. Prof. Dr. Supalax Srijiranai
10.35-10.45	Oral Presentation 6 (for poster no. 10)	Dr. Winita Punyodom
10.55-11.05	Oral Presentation 7 (for poster no. 7)	Miss Siripat Suteerapataranon
11.05-11.15	Oral Presentation 8 (for poster nos. 11-12)	Miss Lalida Srivichai
11.15-11.25	Oral Presentation 9 (for poster no. 9)	Mr. Narong Lenghor
11.25-12.30	Discussion at Posters	
12.30-13.30	**Lunch**	
13.30-14.30	Discussion at Posters (Continued)	
14.30-16.00	Discussion in overall	

* Note Oral presentations:

(1) Some from the all posters presented

(2) Should be within 3 slides

List of Presentations

No.	Title
PL-01	FLOW INJECTION ANALYSIS FOR DETERMINATION OF TRACE AIR POLLUTANTS
PL-02	ULTRATRACE DETERMINATION WITH FLOW-BASED TECHNIQUES
P-01	DEVELOPMENT OF FLOW BASED-ION EXCHANGE MICRO-COLUMN SYSTEM FOR SCREENING OF THALASSEMIA AND HEMOGLOBINOPATHIES
P-02	FLOW BASED ON-LINE IMMUNOASSAY FOR HYALURONAN
P-03	DETERMINING THE RELATIVE AMOUNT OF SPECIFIC PROTEOGLYCANS BY FLOW INJECTION-AFFINITY MICRO-COLUMN SYSTEM
P-04	AUTOMATED SYSTEM FOR PARACETAMOL ASSAY
P-05	BEAD INJECTION-FLOW INJECTION SYSTEM, AN ECONOMIC ALTERNATIVE FOR DETERMINATION OF IRON AND COPPER AT TRACE LEVELS
P-06	A NOVEL METHOD FOR SIZE-BASED IRON SPECIATION OF CLAY SAMPLES
P-07	THE RELEASE OF METAL IONS FROM CONTAMINATED SOIL IN MINING AREAS TO THE ENVIRONMENT BY HUMIC ACID COLLOIDS
P-08	STOPPED –FLOW INJECTION ANALYZER FOR THE DETERMINATION OF PHOSPHATE IN FERTILIZER AND SOIL SAMPLES
P-09	DEVELOPMENT OF FLOW-SYSTEMS WITH DYNAMIC SURFACE TENSION DETECTORS
P-10	DEVELOPMENT OF SOME TYPES OF CHITOSAN MEMBRANES FOR ON-LINE SEPARATION AND DILUTION
P-11	QUALITATIVE AND QUANTITATIVE ANALYSIS OF LINEAR ALKYLBENZENE SULFONATES AND TRITON X-100 IN WATER SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
P-12	THE ANALYSIS AND CHARACTERIZATION OF SOME CATIONIC SURFACTANTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
P-13	DETERMINATION OF SOME CATIONS USING FLOW INJECTION-ION CHROMATOGRAPHY
P-14	DETERMINATION OF IODIDE USING FI-HPLC
P-15	INVESTIGATION ON ELECTROPHORESIS FOR SPECIATION OF Fe(II)/Fe(III)
P-16	DEVELOPMENT OF SOME ELECTROANALYTICAL INSTRUMENTATION/ METHODS
P-17	ON-LINE DERIVATIZATION SYSTEM WITH ELECTROCHEMICAL DETECTION FOR ETHANOL DETERMINATION
P-18	SOME ELECTROANALYTICAL SET-UPS FOR STUDENTS' LABORATORY EXERCISES
P-19	DETERMINATION OF IODIDE IN PHARMACEUTICAL SAMPLES BY GAS DIFFUSION FLOW INJECTION USING IODINE-STARCH REACTION
P-20	USE OF THE BORON DOPED-DIAMOND THIN FLIM ELECTRODE FOR DETERMINATION OF IODIDE ION
P-21	FLOW INJECTION ANALYSIS OF DOXYCYCLINE AND CHLORTETRACYCLINE IN PHARMACEUTICAL FORMULATIONS WITH PULSED AMPEROMETRIC DETECTION
P-22	ANALYSIS OF METALS USING REVERSE FLOW INJECTION COUPLED TO ION-PAIR REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
P-23	A NOVEL POTENTIOMETRIC DETERMINATION OF COPPER USING NAPHTHAZARIN CARBON PASTE ELECTRODE
P-24	DETERMINATION OF COPPER (II) ION BY SEQUENTIAL INJECTION ANALYSIS COUPLED WITH LAB-ON-VALVE
P-25	DETERMINATION OF COPPER(II) BY FLOW INJECTION ANALYSIS
P-26	DETERMINATION OF SUULPHITE AND SULPHATE IN BEVERAGES BY FLOW INJECTION ANALYSIS
P-27	FLOW INJECTION SPECTROPHOTOMETRIC DETERMINATION OF ACETAMINOPHEN IN ANALGESIC
P-28	FLOW INJECTION CHEMILUMUNESCENCE DETERMINATION OF TETRACYCLINE
P-29	CONTINUOUS FLOW PERVAPORATIVE MEMBRANE SAMPLING FOR HIGH SPEED GAS CHROMATOGRAPHIC ANALYSIS OF VOLATILE ORGANIC COMPOUNDS
P-30	ARSENIC SPECIATION BY ON-LINE CONTINUOUS FLOW HYDRIDE GENERATION-INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (HG-ICP-OES)

The 3rd Annual Symposium on TRF Senior Research Scholar on Flow-Based Analysis

The 3rd Annual Symposium on TRF Senior Research Scholar on Flow-Based Analysis

At Seminar Room ScB. 2
Faculty of Science, Chiang Mai University
23rd September 2004

8.00 - 8.30	Registration
8.30 - 8.50	**Opening session** - "Symposium Objectives" and "Progress on Development of Flow-based Analytical Techniques under the TRF Senior Scholar Project" by Assoc. Prof. Dr. Kate Grudpan, TRF Senior Scholar - Opening address by The Director, Academic Division, TRF
8.50 - 9.20	"The Impact of Flow Injection on Modern Chemical analysis" by Prof. Dr. Elo Harald Hansen, Department of Chemistry, Technical University of Denmark, DENMARK
9.20 - 9.45	"Flow Injection Solvent Extraction for Some Water Monitoring" by Prof. Dr. Tadao Sakai, Aichi Institute of Technology, JAPAN
9.45 - 10.10	"Some Flow-Based Analyses for Air Monitoring" by Prof. Dr. Shoji Motomizu, Department of Chemistry, Faculty of Science, Okayama University, JAPAN
10.10 - 10.35	"Macronutrient Mapping in Estuarine and Marine Waters" by Flow Analysis" by Assoc. Prof. Dr. Ian McKelvie, Monash University, AUSTRALIA
10.35 - 10.50	*Coffee Break*
10.50 - 11.00	*Oral Presentation 1(for P-01, Dr. H. Itabashi)
11.00 - 11.10	*Oral Presentation 2 (for P-02, Dr. H. Ukeda)
11.10 - 11.20	*Oral Presentation 3 (for P-03, Dr. K. Higuchi)
11.20 - 11.30	*Oral Presentation 4 (for P-04, J. Promchan)
11.30 - 11.40	*Oral Presentation 5 (for P-05, M. Amatatongchai)
11.40 - 11.50	*Oral Presentation 6 (for P-06, T. Charoenraks)
11.50 - 12.00	*Oral Presentation 7 (for P-07-08, O. Tue-ngeun)
12.00 - 12.10	*Oral Presentation 8 (for P-09, S. Sommam)
12.10 - 12.20	*Oral Presentation 9 (for P-10-12, R. Burakham)
12.20 - 12.30	*Oral Presentation 10 (for P-13, Dr. J. Jakmunee)
12.30 - 14.00	*Lunch*
14.00 - 15.15	Discussion at Posters
15.15 – 15.30	*Coffee Break*
15.30 – 16.00	Discussion in overall

**Note: Some from the all posters presented and should be within 3 slides*

List of Presentations

No.	Title
L-01	The Impact of Flow Injection on Modern Chemical analysis
L-02	Flow injection solvent extraction for some water monitoring
L-03	Some Flow-Based Analyses for Air Monitoring
L-04	Macronutrient Mapping in Estuarine and Marine Waters by Flow Analysis
P-01	All Injection Analysis: a Simple Technique for Liquid Waste Monitoring
P-02	Flow Injection Analysis of Antioxidant Capacity Based On Scavenging of ABTS Radical Cation
P-03	Determination of Nitrogen Oxides, Sulfur Oxide and Ozone in Ambient Air by Using Portable Flow Analyzer after Collecting with Passive Sampling Devices
P-04	A Continuous-Flow Dialysis System with On-Line Electrothermal Atomic Absorption Spectrometric and pH Measurements for Evaluation of Bioavailability of Minerals
P-05	New Approach for Electrochemical Detection of Iodine in Pharmaceutical Products using the Boron Doped-Diamond Thin Film Electrode
P-06	Flow Injection Analysis of Tetracycline Antibiotics in Pharmaceutical Formulations
P-07	Flow Injection with On-Line UV Photo-Oxidation for Spectrophotometric Determination of Dissolved Reactive Phosphorus (DRP) and Dissolved Organic Phosphorus (DOP) in Natural Water
P-08	A Stopped Flow Injection-Differntial Pulse Voltammetric (sFI-DPV) System for Simultaneous Determination of Chlorate and Chlorite in Agrochemical Samples
P-09	Novel Stopped Flow Injection Iodometry for Determination of Chlorate in Soil
P-10	Micro-Total Analysis System Using the Sequential Injection for Liquid-Liquid Extraction Spectrophotometry

P-11	On-Line Nitrosation Reaction System for the Simultaneous Determination of Nitrite and Nitrate
P-12	A Crushed Barium Sulfate Reactor Column for Sulfate Determination
P-13	Lab-At-Valve (LAV):A Micro Total Analysis System Using Sequential Injection for Determination of Chloride
P-14	An Electrochemical of Chloramphenicol: Comparative Study of Boron-Doped Diamond Thin Film Electrode and Cr(III) Modified Boron-Doped Diamond Thin Film Electrode
P-15	On-Site Determination of Trace Amounts of Formaldehyde in Air by Flow-Injection Technique Coupled with Batchwise Collection Method
P-16	Flow Injection Amperometry for Phosphate Determination in High Salinity Water and On-Line Preconcentration for Low Level Phosphate Water
P-17	Speciation of Trace Amounts of As(III) and As(V) by ICP-AES and On-Line Preconcentration System
P-18	Development of the Flow Microparticle-Based Immunoassay System
P-19	Determination of Protein by Using Bradford Method with Flow Injection System
P-20	Design and Fabrication of Lab on a Chip with the Micro Flow System for Copper(II) Determination
P-21	Development of Micro Reactor for Determining Trace Fe (III) in water by Flow Analysis
P-22	Applications of Flow-Based Techniques in Clinical Analysis
P-23	Flow Injection Analysis of Cefadroxil in Pharmaceutical Preparations with Chemiluminescence Detection
P-24	Flow Injection Determination of Tetracyclines Based on Manganese(II) Enhanced Chemiluminescence Using Ru(bipy)32+ and KMnO4 System
P-25	Dynamic Surface Tension Detector (DSTD) for Flow-Based Analysis
P-26	Flow injection analysis for determination of proparacaine, procaine and tetracaine hydrochloride with chemiluminescence detection

P-27	A micro total analysis system for acid – base microtitration using SI-LOV with air segmentation
P-28	Determination of Some Heavy Metals in Soil Samples by Using an Automated On-Line Solvent Extraction with Flame Atomic Absorption Spectrometry
P-29	Sequential Injection-Column Preconcentration for Iron Determination by Using Flame Atomic Absorption Spectrometry
P-30	Fabrication of a Simple Silver-Silver Chloride Reference Electrode
P-31	Sedimentation Field-Flow Fractionation: Size Characterization of Dairy Products
P-32	GrFFF-lCPMS: An Approach for Size-Based Elemental Speciation
P-33	Analysis of C_{10}-C_{13} Anionic Surfactants Using High Performance Liquid Chromatography and Mass Spectrometry