รายงานวิจัยฉบับสมบูรณ์

การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล

DEVELOPMENT OF MICRO-AND NANO-SCALE ANALYSIS BY FLOW-BASED TECHNIQUES

โดย ศาสตราจารย์ ดร. เกตุ กรุดพันธ์ และคณะ

สิงหาคม 2550

สัญญาเลขที่ RTA4780010

รายงานวิจัยฉบับสมบูรณ์

การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล

DEVELOPMENT OF MICRO-AND NANO-SCALE ANALYSIS BY FLOW-BASED TECHNIQUES

คณะผู้วิจัย	สังกัด
1. ศ. ดร. เกตุ กรุดพันธ์	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
2. ดร. จรูญ จักร์มุณี	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
3. รศ. ดร. สุภาภรณ์ ครัดทัพ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
4. ผศ. ดร. ดวงใจ นาคะปรีชา	คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
5. รศ. ดร. อรวรรณ ชัยลภากุล	คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
6. ดร. รัตติกาล จันทิวาสน์	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี
7. รศ. ดร. ศุภลักษณ์ ศรีจารนัย	คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น
8. รศ. ดร. ปรัชญา คงทวีเลิศ	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
9. อ. ศุภชัย ชัยสวัสดิ์	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
10. ผศ. ดร. สุนันทา วังกานต์	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
11. ดร. สมชัย ลาภอนันต์นพคุณ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
12. ผศ. ดร. ศิริวรรณ องค์ไชย	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
13. ดร. พลยุทธ ศุขสมิติ	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3
14. อ. ดร. นิสา ชวพันธ์	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
15. อ. ดร. วรรณจันทร์ แสงหิรัญ ลี	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
16. ดร. ปิยรัตน์ นิมมานพิภักดิ์	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
17. ผศ. ดร. จีรยุทธ ไชยจารุวณิช	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
18. ผศ. ดร. สุคนธ์ ประสิทธิ์วัฒนเสรี	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ขอขอบคุณ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่สนับสนุนให้ทุน โครงการวิจัยเมธีวิจัยอาวุโส “การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล (Development of Micro-and Nano-scale Analysis by Flow-based Techniques)” 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), Center of Excellence for Innovation in Chemistry (PERCH-CIC), The Commission on Higher Education, The Alexander von Humboldt Foundation (AvH), Germany, Deutcher Akademischer Austausch Dienst (DAAD), Germany.

ขอขอบคุณผู้บริหารทุกท่าน ได้แก่ ผู้อำนวยการฝ่ายวิชาการ สกว. อธิการบดีมหาวิทยาลัยเชียงใหม่ ผู้อำนวยการสถาบันวิจัยวิทยาศาสตร์และเทคโนโลยี คณบดีคณะวิทยาศาสตร์ หัวหน้าภาควิชาเคมี ที่ให้การสนับสนุน

ขอขอบคุณทุกท่านที่มีส่วนร่วมในทางตรงและทางอ้อม แต่ไม่ได้เอ่ยนามในที่นี้

ในการพัฒนาซึ่งจะทำให้เกิดการเปลี่ยนแปลง มักจะมีขั้นตอนต่างๆ ซึ่งเป็นที่น่าสังเกตุเป็นไปตาม CLARK’S LAW OF REVOLUTIONARY IDEAS ซึ่งกล่าวดังนี้

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”

ในฐานะหัวหน้าโครงการฯ มีความรู้สึกว่าโครงการฯ นี้ทำให้เกิดความภาคภูมิใจและคาดว่าผลลัพธ์ที่ได้รับจากโครงการฯ นี้ได้มีความคุ้มทุนจากเงินภาษีราษฎรซึ่งจะมีผลกระทบต่อประเทศในทางตรงและทางอ้อม

ศาสตราจารย์ ดร. เกตุ กรุดพันธ์
หัวหน้าโครงการ

บทคัดย่อ

การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล

โครงการเมธีวิจัยอาวุโส สกว. สัญญาเลขที่ RTA4780010

หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

โครงการนี้ดำเนินการวิจัยต่อเนื่องจากโครงการเมธีวิจัยอาวุโส สกว. “การพัฒนาการวิเคราะห์ที่ใช้การไหล” ในการวิจัยเกี่ยวกับการพัฒนาเครื่องมือและวิธีการวิเคราะห์ทางเคมีที่ใช้เทคนิคการไหลสำหรับการวิเคราะห์ระดับไมโครและนาโน โดยเน้นการคำนึงถึงค่าใช้จ่ายที่ถูก แต่มีนวัตกรรม ประกอบด้วย 4 โครงการย่อย

โครงการย่อยที่ 1: Development of instrumentation ดำเนินการวิจัยอย่างต่อเนื่องในการพัฒนาระบบเครื่องมือต่าง ๆ รวมถึง micro-total analysis system (micro-TAS) เป็นการพัฒนาต่อเนื่องในเทคนิค Sequential Injection Analysis with Lab-at-Valve (SIA-LAV) ซึ่งเป็นการนำเสนอเทคนิคนี้ครั้งแรกโดยเป็นผลงานวิจัยจากโครงการนี้ นอกจากนี้ยังได้พัฒนาต่อเนื่องในระบบการวิเคราะห์ที่มีราคาถูก (cost-effective) และพัฒนาระบบตรวจวัดแบบต่าง ๆ รวมถึง spectrophotometric detection systems และ electrochemical detection systems.

โครงการย่อยที่ 2: Novel approaches for flow based analysis รวมถึงได้ดำเนินการวิจัยเพื่อลดขนาดระบบการวิเคราะห์โดยเน้น sequential injection analysis (SIA) และ flow injection analysis (FIA) รวมถึงแนวทางใหม่ในเทคนิค stopped flow injection systems (stopped FI) ได้พัฒนาระบบ Lab-on-Valve (LOV) systems และ Bead injection (BI) ได้เสนอแนวทางใหม่ที่เรียกว่า Lab-at-Valve (LAV) systems ซึ่งได้ทำการวิจัยพัฒนาควบคู่ไปกับการพัฒนาเครื่องมือ (ในโครงการย่อยที่ 1 ข้างต้น) ได้นำมาใช้ในการศึกษาทาง Chemical kinetics ด้วย ยังมีการศึกษาเกี่ยวกับการเตรียมตัวอย่างเพื่อใช้ใน Chromatography ด้วยนอกจากนี้ ยังได้ศึกษาแนวทางใหม่ทาง chemometrics ด้วย

โครงการย่อยที่ 3: New materials employed in chemical analysis ได้วิจัยเพื่อการเตรียมตัวอย่างโดยพัฒนาถึงการแยก (sample separation) โดยใช้ on-line mini-column separation โดยใช้ Packing material ชนิดต่าง ๆ รวมถึง monolithic column ด้วย ได้ทำการวิจัยต่อเนื่องเพื่อหา reagent ใหม่ ๆ เช่น สาร WF6 เพื่อการหาปริมาณ chondroitin sulfate proteoglycans ได้ศึกษาพัฒนาต่อเนื่องในการวิเคราะห์แอมโมเนีย/แอมโมเนียม โดยใช้ปฏิกิริยาใหม่แต่เป็นแนวทางของ Berthelot reaction

โครงการย่อยที่ 4: Application of flow-based analysis ได้ดำเนินการต่อเนื่องในการมุ่งเน้นเพื่อการวิเคราะห์ตัวอย่างทางการแพทย์โดยสนใจศึกษาในการทำคัดกรองของโรคบางชนิด เช่น

โรคมะเร็ง โรคตับ โรคข้อ เป็นต้น ทางสิ่งแวดล้อม ทางการเกษตรและอุตสาหกรรมการเกษตร การวิเคราะห์อาหาร

งานวิจัยดังกล่าวเป็นผลงานของนักวิจัยรุ่นเก่าและรุ่นใหม่ที่ร่วมกันดำเนินการโดยเป็นนักวิจัยมาจากสถาบันต่างๆ ทั้งในประเทศและต่างประเทศ ซึ่งทำให้เกิดความเข้มแข็งในระดับบัณฑิตศึกษาและสร้างการทำงานเป็นทีม

Keywords: Microaanalysis, Nanoanalysis, flow-based analysis, instrumentation, analytical procedure development

ABSTRACT

DEVELOPMENT OF MICRO-AND NANO-SCALE ANALYSIS BY FLOW-BASED TECHNIQUES

TRF SENIOR RESEARCH SCHOLAR GRANT CONTRACT NO. RTA4780010

Principal investigator: Professor Dr. Kate Grudpan

In this project a continued investigation from the previous TRF Seniors scholor Project on "Development of Flow-Based Analysis", instrumentation, chemistry and procedures involving flow-based techniques for micro-and nano-analysis, with emphasis on cost effective benefits but novel approaches have been investigated. It was composed of 4 subprojects.

Subproject 1: Development of instrumentation: Various instrument systems/ components have been continuously developed, including micro-total analysis system (micro-TAS). It has been continuously developed as Sequential Injection Analysis with lab-at-Valve (SIA-LAV) which has been introduced under this project for the first time. Also cost-effective analytical instrumentation and detection devices, including spectrophotometric, electrochemical, have been investigated.

Subproject 2: Novel approacheds for flow based analysis: Down-scaling analytical instrumentation with emphasis on novelty in sequential injection analysis (SIA) and flow injection analysis (FIA) has been explored. This included stopped flow injection systems (stopped FI), Lab-on-Valve (LOV) and Bead injection (BI). A novel approach so-called "lab-at-Valve (LAV)" system has been developed together with instrumentation development (in subproject 1). This has been employed for chemical kinetics study. Chemometrics has also been studied.

Subproject 3: New materials employed in chemical analysis: Sample preparation with on-line mini-column, employing some packing materials, including monolithic column has been investigated. New reagents have been explored for chemical analysis such as WF6 for the assay of chondroitin sulfate proteoglycans. Some Berthelot reactions were investigated for ammonia/ammonium determination.

Subproject 4: Application of flow-based analysis: Applications on flow-based techniques to medical/clinical analysis were aimed to deceases screening such as liver cancer, liver decease, bone & joint decease. Applications to environmental, agricultural, agroindustrial and food analysis have been studied.

The research works were conducted by various generations of researchers in partnership network of various institutions in Thailand and overseas.

Keywords: Microaanalysis, Nanoanalysis, flow-based analysis, instrumentation, analytical procedure development

Executive Summary

TRF Senior Research Scholar Grant (No. RTA4780010)

1. Title: การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล

Development of Micro-and Nano-scale Analysis by Flow-based Techniques

2. Principal investigator:

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3. Research Field: Analytical Chemistry

5. Research period: 3 years (3 August 2004 - 2 August 2007)

6. Supports from other agencies:

1. The Royal Golden Jubilee (RGJ) Ph.D. Program, TRF
2. The Commission for Higher Education
3. Postgraduate Education and Research Program in Chemistry (PERCH)
4. The Alexander von Humboldt Foundation (AvH), Germany
5. Deutcher Akademischer Austausch Dienst (DAAD), Germany

7. Research Work

This project was conducted in continuation from the TRF senior Research Scholar Grant on “Development of Flow-Based Analysis” The research was aimed to develop novel approaches

on flow-based techniques for micro-and nano-analysis with emphasis on cost effective benefits. Both instrumentation and chemistry were investigated. The project consisted of 4 subprojects:

1. Development of instrumentation
2. Novel approaches for flow-based analysis
3. New materials employed in chemical analysis
4. Applications of flow-based analysis

8. Out-puts

The research work has resulted in 68 Publications in international journals (39 papers from this project and 29 papers from relevant works), 57 presentations in international conferences/meetings while 23 presentations in national meetings. It was the work by professors, researchers and students from institutions of various geographical parts of Thailand as well as from overseas: Chiang Mai University, Mahidol University, Chulalongkorn 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, 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), The University of Texas at Arlington, Arlington, Texas, USA (Professor Dr. Pernendu K. Dasgupta)

During the period of this project, continuous series of annual symposia has been organized since 2002 (the first year of TRF Senior Research Scholarship to the Group), in 2005 and 2006 The 4th and 5th Annual Symposia on TRF Senior Research Scholar on Development of Micro-and Nano-Scale Analysis by Flow-based Techniques I and II" were organized respectively. In 2007, the 6th Annual Symposium on TRF Senior Research Scholar and Research on Innovation on Analytical Instrumentation CHE: "Development of Flow-based Analysis for Life Quality" was organized. In the Symposia, internationally recognized scientists in the field, were invited to give planetary/ invited lectures. The Symposia activities were reported in international journals.

It was for the first time that US-NSF Co-funded with TRF and CHE for a workshop in Thailand “Interfaces of Analytical Sciences: Workshop of US and Thai Analytical Scientists”, in January 2006. More than 10 top US analytical scientists attended the workshop, to exchange information and create and/or strengthen collaboration between US-Thai Scientists. The event was reported in Analytical Chemistry, the top in analytical chemistry international journals.

The above Symposia/workshop have created networking.

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.

การวิจัย “การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล”

1. ความสำคัญและที่มาของปัญหา

การวิเคราะห์ที่มีประสิทธิภาพแต่มีค่าใช้จ่ายไม่สูง เป็นสิ่งสำคัญที่ต้องคำนึงถึงเสมอ ไม่เฉพาะสำหรับในสถานที่ห่างไกลความเจริญซึ่งมีงบประมาณไม่มาก แต่รวมถึงการใช้งานทั่วๆ ไปด้วยในการเลือกรูปแบบการวิเคราะห์ นอกจากจะต้องคำนึงถึง ความถูกต้อง ความแม่นยำ ความไว และความจำเพาะ ซึ่งเป็นลักษณะทั่วไปที่ดีของเทคนิคการวิเคราะห์แล้ว ความสามารถในการวิเคราะห์แบบ real time และค่าใช้จ่ายของการวิเคราะห์ก็เป็นปัจจัยที่สำคัญด้วยเช่นกัน

เมื่อพิจารณาถึงค่าใช้จ่ายในการวิเคราะห์ จะหมายรวมถึงเวลาที่ต้องใช้ในการวิเคราะห์สารเคมีที่ต้องใช้ในปฏิกิริยา และราคาของเครื่องมือที่เกี่ยวข้อง ซึ่งราคาของเครื่องมือนี้คือราคาของระบบรวมถึงค่าใช้จ่ายในการทำงานของเครื่อง และค่าใช้จ่ายในการดูแลรักษาด้วย

เทคนิคการวิเคราะห์ที่ใช้การไหล (Flow-based analytical techniques) มีข้อดีหลายประการและข้อเด่นของเทคนิคนี้คือ ความสามารถในการวิเคราะห์สารได้หลายตัวอย่างในเวลาอันสั้น โดยใช้สารตัวอย่าง และสารเคมีปริมาณน้อย อีกทั้งใช้เครื่องมือที่ง่าย ไม่ซับซ้อน การวิเคราะห์ที่ใช้การไหลจึงเป็นเทคนิคการวิเคราะห์ที่เหมาะสมในการวิเคราะห์ ตามข้อที่ควรพิจารณาดังที่กล่าวถึงข้างต้น [2-28]

ได้มีการใช้เทคนิคโฟลอินเจคชันอะนาไลซิส (Flow injection analysis (FIA)) ในการวิเคราะห์แบบ routine แล้วมากมาย ซึ่งบ่งบอกถึงความคุ้มค่า และ ประสิทธิภาพของระบบนี้ได้เป็นอย่างดี

ระบบซีเควนเชียลอินเจคชันอะนาไลซิส (Sequential injection analysis (SIA)) เป็นระบบมีความเป็นอัตโนมัติ แต่ไม่ซับซ้อนมากนัก เมื่อเทียบกับเทคนิคอื่นๆ

มีความจำเป็นต้องพัฒนาระบบการวิเคราะห์ในระดับไมโครและนาโน เพื่อใช้ในการศึกษาด้านต่าง ๆ จึงสนใจที่จะทำการศึกษาต่อยอดงานที่ได้ทำมาโดยใช้ประสบการณ์ที่ได้รับโดยเฉพาะจากงานที่ทำในโครงการเมธีวิจัยอาวุโสที่ผ่านมา (เมธีวิจัยอาวุโส สกว.(ในโครงการที่ได้รับครั้งแรก), วุฒิเมธีวิจัย สกว., เมธีวิจัย สกว., ทุนวิจัยหลังปริญญาเอกและ ทุนปริญญาเอก คปก.)

โครงการนี้มีจุดมุ่งหมายที่จะพัฒนาการวิเคราะห์ในระดับไมโครและนาโน โดยการใช้พื้นฐานเทคนิคการวิเคราะห์ที่ใช้การไหล โดยจะทำการศึกษาทั้งในด้านเครื่องมือและด้านเคมี โครงการนี้ประกอบด้วยโครงการย่อย ดังนี้

1. Development of instrumentation
2. Novel approaches for flow-based analysis

3. New materials employed in chemical analysis
4. Applications of flow-based analysis

ทั้งนี้ ทั้ง 4 โครงการย่อยจะมีความสัมพันธ์ซึ่งกันและกันด้วย

2. การพัฒนาระบบเครื่องมือ (Development of instrumentation)

ได้พัฒนาระบบเครื่องมือที่เกี่ยวข้องกับ flow-based analysis หลายชนิด โดยมุ่งเน้นให้มีราคาไม่สูง (cost-effective)(ดังรายละเอียดใน reprint ในภาคผนวก ก1) ซึ่งรวมถึงการพัฒนาระบบ field-flow fractionation เพื่อการศึกษา size-based speciation (ดังรายละเอียดใน reprint ในภาคผนวก ก12 และ ก31) การพัฒนาระบบตรวจวัด (detection device/ system) ที่เป็น electrochemical detector (ดังรายละเอียดใน reprint ในภาคผนวก ก22, ก29, ก30, ก38) ระบบการตรวจวัดแบบ dynamic surface tension detector (ดังรายละเอียดใน reprint ในภาคผนวก ก8 และ ก26) การลดขนาดของระบบวิเคราะห์ (ดัง reprint ในภาคผนวก ก28)

3. การพัฒนาแนวใหม่ๆ ในการวิเคราะห์ที่อาศัยหลักการไหล (Novel approaches for flow-based analysis)

ได้พัฒนาระบบ bead injection เข้ากับ flow injection (ดังรายละเอียดใน reprint ในภาคผนวก ก2, ก11) การไตเตรดในระดับไมโครโดยใช้ Lab-on-Valve (ดังรายละเอียดใน reprint ในภาคผนวก ก 13) การเสนอเทคนิค Lab-at-Valve โดยใช้ poteutiometric detection (ดังรายละเอียดใน reprint ในภาคผนวก ก7) และใช้ spectrometry ในการตรวจวัด (ดังรายละเอียดใน reprint ในภาคผนวก ก17 และ ก27) ทั้งนี้ เทคนิค Lab-at-Valve(LAV) เป็นเทคนิคที่เสนอขึ้นโดยกลุ่มวิจัยนี้

ได้มีการพัฒนาระบบ Stopped flow injection analysis เพื่อใช้กับเทคนิค amperometry (ดังรายละเอียดใน reprint ในภาคผนวก ก16) ได้ศึกษาการทำ sample pretreatment ซึ่งเป็นแบบ on-line (ดังรายละเอียดใน reprint ในภาคผนวก ก23, ก25,ก31) ได้มีการพัฒนาการใช้คณิตศาสตร์และสถิติพร้อมด้วยโปรแกรมคอมพิวเตอร์อย่างง่าย (package program) เพื่อช่วยให้ประสิทธิภาพการวิเคราะห์ทางเคมีดีขึ้น (ดังรายละเอียดใน reprint ในภาคผนวก ก30)

4. วัสดุใหม่ๆ เพื่อใช้ในการวิเคราะห์ทางเคมี (New materials employed in chemical analysis)

ได้ศึกษาถึง on-line sample pretreatment ซึ่งได้ศึกษาการใช้ monolith ด้วย (ดังรายละเอียดที่ได้เสนอในการประชุมวิชาการ (ภาคผนวก 9) นอกเหนือไปจากการใช้ resin (ดังรายละเอียดใน reprint ในภาคผนวก ก4) ได้เสนอการใช้รีเอเจนต์ที่หาได้ง่ายจากธรรมชาติ (เช่น natural regent) เช่น ใช้ใน

ฝรั่งเพื่อหาปริมาณเหล็กโดย FIA (รายละเอียดใน reprint ในภาคผนวก ก18) การใช้ยาแก้ปวดศีรษะ (Salicylate reagent) สำหรับการหาประมาณเหล็กโดย FIA (reprint ในภาคผนวก ก3) การศึกษา Berthelot reaction ในการศึกษาเพื่อการวิเคราะห์หาปริมาณในแอมโมเนีย/ แอมโมเนีย ด้วย flow system. การใช้ WF6 เป็นรีเอเจนต์ในการปริมาณ chondroitin sulfate (รายละเอียด reprint ในภาคผนวก ก35)

5. การประยุกต์ flow-based analysis ในด้านต่างๆ

ได้พัฒนาอย่างต่อเนื่องในการวิเคราะห์ตัวอย่างทางการแพทย์เพื่อศึกษาในการคัดกรองโรคบางชนิด เช่น ธาลัสซีเมีย (ดัง reprint ในภาคผนวก ก10) โรคมะเร็งและโรคตับ (reprint ในภาคผนวก ก9) โรคข้อ (reprint ในภาคผนวก ก32) โรคเบาหวาน (reprint ในภาคผนวก ก33, ก36) การวิเคราะห์ทางสิ่งแวดล้อม (reprint ในภาคผนวก ก20, ก21, ก15, ก2) การวิเคราะห์ยา (reprint ในภาคผนวก ก5, ก6, ก18) การวิเคราะห์ทางอุตสาหกรรมเกษตร (reprint ในภาคผนวก ก24) การวิเคราะห์ในอาหารและเครื่องดื่ม (reprint ในภาคผนวก ก34 และ ก39)

Out-put ที่ได้รับจากโครงการ

การตีพิมพ์ในวารสารวิชาการนานาชาติ (ที่มี impact factor) จำนวน 68 เรื่อง (เป็นผลงาน 39 เรื่องจากทุนโครงการวิจัยฯ นี้) มี 3 papers ได้ปรากฏใน 25 hottest articles on Science Direct.com โดยที่ 2 papers อยู่ในวารสาร Talanta และ 1 paper ในวารสาร Analytica Chimica Acta

ได้รับเกียรติในการจัด “Interfaces of analytical Sciences: Workshop of US and Thai analytical scientists” โดยการสนับสนุนของ NSF (สหรัฐอเมริกา), สกว., สกอ. และ PERCH มีนักวิทยาศาสตร์ชั้นนำ 11 คน จากสหรัฐฯ มาประชุมร่วมกับนักวิทยาศาสตร์ไทย (ประมาณ 60 คน) เพื่อหาแนวทางความร่วมกัน (มีบทความในวารสาร Analytical Chemistry (มี impact factor 5.635) Government and Society: Thailand and US strengthen analytical ties, vol.79 No.3, Feb 1, 2007 การประชุมดังกล่าวนอกเหนือจะจัดที่มหาวิทยาลัยเชียงใหม่แล้วยังได้จัดที่สำนักงาน สกว.ด้วย (มีผู้ร่วมประชุมประมาณ 100 คน)

การพัฒนานักวิจัย

(ก) ในประเทศ

มีคณาจารย์และนิสิต/ นักศึกษาจาก 4 มหาวิทยาลัยต่างๆ เข้าร่วมในโครงการ (รวม อาจารย์ 19 คน นักศึกษาปริญญาเอก 25 คน ปริญญาโท 28 คน ปริญญาตรี 18 คน และปริญญาโทจากประเทศพม่า 1 คนและเนปาล 1 คน) ดังนี้

- **มหาวิทยาลัยเชียงใหม่**

มีอาจารย์ 15 คน (ภาควิชาเคมี 8 คน, ภาควิชาสถิติ 1 คน, ภาควิชาวิทยาการคอมพิวเตอร์ 1 คน, ภาควิชาชีวเคมี (คณะแพทย์ฯ 2 คน), ภาควิชารังสีรักษา (คณะแพทย์ฯ 2 คน))

มีนักศึกษาปริญญาเอก 18 คน ปริญญาโท 16 คน (และปริญญาโทจากประเทศพม่า 1 คนและเนปาล 1 คน) ปริญญาตรี 18 คน

- **มหาวิทยาลัยมหิดล**

มีอาจารย์ 1 คน* นักศึกษาปริญญาเอก 2 คน ปริญญาโท 3 คน

(*อนึ่งในช่วงที่ผ่านมา ผศ.ดร.ดวงใจ นาคะปรีชาได้เริ่มฟอร์มกลุ่มวิจัยเริ่มเป็นกลุ่มใหม่ (FIRST labs) โดยการรวบรวมศิษย์เก่าที่ไปเป็นอาจารย์ในมหาวิทยาลัยต่างๆ (ศรีนครินทรวิโรฒ, อุบลราชธานี, บูรพา, สถาบันเทคโนโลยีพระจอมเกล้า พระนครเหนือ) ซึ่งบางคนเคยได้รับการสนับสนุนในจากโครงการเมธีวิจัยอาวุโสฯ นี้)

- **จุฬาลงกรณ์มหาวิทยาลัย**
  มีอาจารย์ 1 คน มีนิสิตปริญญาเอก 2 คน ปริญญาโท 1 คน
- **มหาวิทยาลัยขอนแก่น**
  มีอาจารย์ 2 คน มีนักศึกษาปริญญาเอก 1 คน ปริญญาโท 7 คน

(ข) ต่างประเทศ

มีนักศึกษาปริญญาโทจาก Okayama University ประเทศญี่ปุ่น 2 คน มาทำวิจัยที่เชียงใหม่ 4 เดือน

มี Postdoc จากประเทศสหรัฐอเมริกา มาทำวิจัยที่เชียงใหม่ 2 เดือน (สืบเนื่องจากโครงการในข้อ 2 ข้างต้น (สนับสนุนจาก NSF))

Assoc. Prof. Dr. Norio Teshima จาก Aichi Institute of Technology ประเทศญี่ปุ่น มาทำวิจัยที่เชียงใหม่ 2 เดือน

ความร่วมมือกับต่างประเทศ

- มีความร่วมมือกับ 6 มหาวิทยาลัย/ สถาบัน ในต่างประเทศ (5 ประเทศ)
- จากความร่วมมือในข้อ 4.1 ซึ่งเริ่มที่มหาวิทยาลัยเชียงใหม่ได้ขยายไปยังมหาวิทยาลัยอื่นๆ (มหิดล, จุฬา, ขอนแก่น)
- มีความร่วมมือกับ Aichi Institute of Technology (AITech) (ผ่านโครงการ Frontier Research Project ของ AITech ที่ได้รับการสนับสนุนจากรัฐบาลญี่ปุ่น) มีอาจารย์ในกลุ่ม 1 คนไปทำ postdoc และอาจารย์จาก AITech ได้มาทำวิจัยที่เชียงใหม่ 2 เดือน (ดังกล่าวข้างต้น)

รางวัล/ เกียรติยศที่ได้รับ

- เป็นครั้งแรกในการจัดการประชุมที่สนับสนุนโดย NSF ในประเทศไทย (ต้องเขียน proposal โดย Professor Gary D. Christian (University of Washington, Seattle) ร่วมเป็น co-organizer กับเกตุ กรุดพันธ์) (ดังกล่าวในข้อ 2)
- การทำงานให้กับวารสารนานาชาติ

(1) Associate Editor ของวารสาร Water Research (Elsevier) (มี impact factor = 3.019) (เกตุ กรุดพันธ์ ตั้งแต่ 2003-ปัจจุบัน)

(2) Advisory Editorial Board ของวารสาร Talanta (Elsevier) (มี impact factor = 2.391) (เกตุ กรุดพันธ์ (2000-2006) และดวงใจ นาคะปรีชา (2007-2009))

- **รางวัลที่ได้รับโดยอาจารย์และนักศึกษา**

(1) ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

บุคคลดีเด่นของชาติ สาขาวิทยาศาสตร์และเทคโนโลยี (ด้านเคมีวิเคราะห์) จากสำนักงานเสริมสร้างเอกลักษณ์ของชาติ สำนักนายกรัฐมนตรี (พ.ศ. 2547)

(2) ศาสตราจารย์ ดร.เกตุ กรุดพันธ์

รางวัลเมธีวิจัยอาวุโส สกว. 2547-2548 สาขาเคมีวิเคราะห์ ประจำปี 2547 จากสำนักงานกองทุนสนับสนุนการวิจัย (สกว.)

(3) รองศาสตราจารย์ ดร. สุภาภรณ์ ครัดทัพ

รางวัลนักวิจัยรุ่นใหม่ที่มีผลงานตีพิมพ์จากสกว.และสกอ. สำหรับผู้รับทุนพัฒนาศักยภาพการทำงานของอาจารย์รุ่นใหม่ (พ.ศ. 2548)

(4) ดร. จรูญ จักรมุณี

รางวัล “นักวิจัยรุ่นใหม่ดีเด่น” สาขาวิทยาศาสตร์และเทคโนโลยี รางวัลมหาวิทยาลัยเชียงใหม่ “ช้างทองคำ” (พ.ศ. 2549)

(5) ผู้ช่วยศาสตราจารย์ ดร. ดวงใจ นาคะปรีชา

Model Lecturer of the Year 2005 คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

(6) น.ส.จันทร์เพ็ญ ครุวรรณ์ (นักศึกษาปริญญาโท)

The Award of Excellence for great poster presentation (12th International Symposium on Advanced Technology and Application, Korea (พฤศจิกายน 2549))

(7) น.ส. กาญจนา อุไรสิน (นักศึกษาปริญญาเอกไทย เรียนที่ Okayama University)

The Best Poster Award (13 ICFIA, Las Vegas, USA)

(8) ศาสตราจารย์ ดร.เกตุ กรุดพันธ์

รางวัล “นักวิจัยดีเด่น” สาขาวิทยาศาสตร์และเทคโนโลยี รางวัลมหาวิทยาลัยเชียงใหม่ “ช้างทองคำ” ประจำปี 2550

- **การมีส่วนร่วมในงานประชุมนานาชาติทาง flow analysis**

(1) Co-organizer and invited speaker, , PACIFICHEM 2005 -Symposium #16: Advances in flow-based analytical techniques, Honolulu, USA (เกตุ กรุดพันธ์)

(2) Steering committee and invited speaker, 13th International Conference on Flow Injection Analysis, April 2005, Las Vegas, USA (เกตุ กรุดพันธ์)

(3) Internation advisory committee and invited speaker, 10th International Flow Analysis Conference, September 2006, Porto, Portugal (เกตุ กรุดพันธ์)

(4) Steering committee and invited speaker, 14th International Conference on Flow Injection Analysis, September 2007, Berlin, Germany (เกตุ กรุดพันธ์)

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 Alexander von Humboldt Foundation (AvH), Germany
5. Deutcher Akademischer Austausch Dienst (DAAD), Germany
6. The Post-graduate Education Development (PED) Program (The Commision on Higher Education)
7. 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

ในการจัดประชุมวิชาการประจำปีของกลุ่มวิจัยได้ดำเนินการจัดเป็น

ครั้งที่ 1 The 4th Annual Symposium on TRF Senior Research Scholar on "Development of Micro-and Nano-scale Analysis by Flow-based Techniques I" ณ ห้องสัมมนา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ วันที่ 19 กันยายน 2548

ครั้งที่ 2 The 5th Annual Symposium on TRF Senior Research Scholar on "Development of Micro-and Nano-scale Analysis by Flow-based Techniques II" ณ ห้องสัมมนา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ วันที่ 12 สิงหาคม 2549

ครั้งที่ 3 The 6th Annual Symposium: TRF Senior Research Scholar and Research Group on Innovation on Analytical Instrumentation CHE: "Development of Flow-based

Analysis for better life quality” ณ ห้องสัมมนา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ วันที่ 16 สิงหาคม 2550

และ

ครั้งที่ 4 Interfaces of Analytical Sciences: Workshop of U.S. and Thai Analytical Scientists ณ โรงแรมอมารีรินคำ เชียงใหม่ จ.เชียงใหม่ วันที่ 4-8 มกราคม 2549

ในแต่ละครั้งได้รับการสนับสนุนจากหน่วยงานอื่นๆ ด้วย จึงทำให้มีผู้เข้าร่วมประชุมจากต่างประเทศด้วย และมีผู้ประชุมมากกว่า 70 – 80 คนในแต่ละครั้ง

Selected Presentations

เสนอผลงานวิจัยในการประชุมวิชาการนานาชาติ จำนวน 57 เรื่อง

(ทั้งที่ได้รับเชิญเป็นวิทยากรบรรยายนำ และการเสนอผลงานตามปกติ)

1. Amatotongchai M, Chailapakul O, Nacapricha D, Wilairat P and Grudpan K (2004) NEW APPROCH FOR CHROMATOGRAPHEIC DETECTION OF IODIDE IN PHARMACEUTICAL PRODUCTS USING THE BORON DOPED-DIAMOND THIN FILM ELECTRODE. PBA 2004 Symposium, Florence, Italy.
2. Chantiwas R, Ngangrungreung S, Kongtawelert P and Grudpan K (2004) A SIMPLE FLOW SYSTEM FOR REPID BRADFORD PROTEIN ASSAY IN MILKS SAMPLES. PBA 2004 Symposium, Florence, Italy.
3. Chantiwas R, Kongtawelert P, Kradtap S, Jakmunee J and Grudpan K (2004) THE FLOW-MICROPARTICLES BASED IMMUNOASSAY SYSTEM. PBA 2004 Symposium, Florence, Italy.
4. Grudpan K (2004) NOVEL APPROACHES IN ANALYTICAL INSTRUMENTATION AND PROCEDURES FOR WATER MONITORING
5. Burakham R, Oshima M, Grudpan K and Motomizu S (2004) MONITORING WATER SAMPLES FOR NITRITE AND NITRATE USING FLOW INJECTION WITH A NOVEL SPECTROPHOTOMETRIC REACTION SYSTEM. The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety, Songkla, Thailand
6. Jakmunee J , Somnam S, and Grudpan (2004) NOVEL STOPPED FLOW INJECTION IODOMETRY FOR DETERMINATION OF CHLORATE IN SOIL. The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety, Songkla, Thailand
7. Burakham R, Oshima M, Grudpan K and Motomizu S (2004) MONTORING WATER SAMPLES FOR NITRITE AND NITRATE USING FLOW INJECTION WITH A NOVEL SPECTROPHOTOMETRIC RECTION SYSTEM. The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety, Songkla, Thailand
8. Lapanantnoppakhun S, Jitmanee K, Jakmunee J, Jayasvasti S, Motomizu S, Sakai T and Kate Grudpan (2004) DETERMINATION OF TRACEAMOUNT OF

ALUMINIUM IN WATER BY FLOW INJECTION WITH SEMI-AUTOMATIC IN – VALVE PRECONCENTRATOR SYSTEM. *The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety*, Songkla, Thailand

9. Tue –Ngeun Orawan, Ellis P, Ian D. McKelvie, Faul Worsfold, Jakmunee J and Grudpan K (2004) FLOW INJECTION WITH ON – LINE UV PHOTO-OXIDATION FOR SPECTROPHOTOMETRIC DETERMINATION OF DISSOLVED REACTIVE PHOSPHORUS(DOP) IN NATURAL WATER. *The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety*, Songkla, Thailand
10. Dungthong S, Hans Mosbaek and Grudpan K. (2004) Cr(III)/Cr(VI) SPECIATION BY USING AN AUTOMATER ON – LINE SOLVENT EXTRACTION COUPLING WITH FLAME ATOMIC ABSORPTION SPECTROMATRY. *The 2 rd Asian International Conference on Ecotxiclogy and Environmrnt Safety*, Songkla, Thailand.
11. Burakham R, Jakmunee J and Grudpan K (2005) Sequential Injection Lab-at-Valve System for Solvent Extraction. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
12. Jakmunee J, Somnam S and Grudpan K (2005) New Approach Cost Effective Sequential Injection (SI) System: Speciation of Iron(II) and Iron(III) by Manual SI. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
13. Grudpan K and Jakmunee J (2005) Miniaturization for cost-effective analysis: Some aspects in flow-based analysis. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
14. Uraisin K, Nacapricha D, Lapanantnoppakhun S, Grudpan K and Motomizu S (2005) Determination of Trace Amounts of Bromide by Flow Injection/Stopped-Flow Detection Technique Using Kinetic-Spectrophotometric method. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
15. Preechaworapun A, Chuanuwatanakul S, Einaga Y, Grudpan K, Motomizu S and Chailapakul O (2005) Electroanalysis of sulfanomides by flow injection system/high-performance liquid chromatography coupled with amperometric

detection using boron-doped diamond electrode. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.

16. Setheevorawit T, Hartwell S.K, Christian G.D and Grudpan K (2005) Exploiting of Guava Leaf Extract as an Alternative Natural Reagent for Flow Injection Determination of Iron. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
17. Chantiwas R, Kongtawelert P, Kradtap S and Grudpan K (2005) Development of Sequential Injection Lab-at-Valve-Bead immunoassay System for Chondroitin 6-Sulfate. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
18. Synovec R, Christian G, Maneejamnong P, Khamphachua J, Chaijaruwanich J, Prasitwattanaseree S, Lenghor N, Jakmunee J, Nimmanpipug P, Sanghiran V and Grudpan K (2005) Flow injection system with dynamic interfacial pressure detection for unleaded gasoline. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
19. Lapanantnoppakhun S, Kasiwad S, Ganranoo L, Jakmunee J and Grudpan K (2005) Flow Injection Determination of Chromium(III) using EDTA after On-line Preconcentration. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
20. Ganranoo L, Lapanantnoppakhun S, Jakmunee J and Grudpan K (2005) Flow Injection and Sequential Injection Systems for the Determination of Tetracyclines by Employing Diazotization Using Sulfanilic Acid and Nitrite. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
21. Tue-Ngeun O, Jakmunee J and Grudpan K (2005) A Novel Stopped Flow Injection-Amperometric Procedure for Chlorate Determination. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
22. Jakmunee J, Somnam S, Jayasvati S and Grudpan K (2005) Determination of Dissolved Phosphate in Fertilizer and Soil Samples by Electronically Controlled Stopped-Flow Injection System. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.

23. Jakmunee J, Tue-ngeun P, Nimmanpipug P, Sanghiran V, Chijaruwanich J and Grudpan K (2005) Flow injection differential pulse voltammetry with principal component regression technique for simultaneous of chlorate and chlorite. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
24. Jitmanee K, Jakmunee J, Lapanantnoppakhun S, Wangkan S and Grudpan K (2005) Application of derivatization employing a commercial simple package software program to batch and flow analyses: A technical note. *13th International Conference on Flow Injection Analysis, Including Related Techniques,* Las Vegas, Nevada USA.
25. Jakmunee J, Siriangkhawat W and Grudpan K (2005) Sequential injection with UV digestion and anodic stripping voltammetry for speciation of some metals in water. International chemical congress of pacific basin societies, Honolulu, Hawaii.
26. Jakmunee J, Wongwilai W, Jayasvasit S and Grudpan K (2005) Determination of trace orthophosphate in natural water by flow injection amperometry with electronic control pretreatment column. International chemical congress of pacific basin societies, Honolulu, Hawaii.
27. Grudpan K, Hartwell SK, Somprayoon D, Kongtawelert P and Jakmunee J (2005) On-line bone-alkaline phosphatase assay system based on a simple bead injection-flow injection technique. International chemical congress of pacific basin societies, Honolulu, Hawaii.
28. Lapanantnoppakhun S, Jakmunee J, Poouthree K and Grudpan K (2005) On-line sample pre-separation for flow injection spectrophotometric assays of ascorbic acid. International chemical congress of pacific basin societies, Honolulu, Hawaii.
29. Ohno S, Teshima N, Sakai T, Grupan K and Polasek M (2005) Simultaneous spectrophotometric determination of trace amounts of copper and iron by sequential injection lab-on-valve technique. International chemical congress of pacific basin societies, Honolulu, Hawaii.
30. Chailapakul O, Dungchai W, Wangfuengkanagul N, Chuanuwatanakul S, Einaga Y, Motomizu S and Grudpan K (2005) FIA-electrocatalytic determination of hydrogen peroxide at chromium (III) hexacyanoferrate (II) modifies boron-doped

diamond electrodes. International chemical congress of pacific basin societies, Honolulu, Hawaii.

31. Jakmunee J, Somnam S and Grudpan K (2005) Novel approach for simple sequential injection (SI) system: Development of an electronically controlled SI for speciation of iron(II) and iron(III). International chemical congress of pacific basin societies, Honolulu, Hawaii.
32. Grudpan K, Hartwell S.K, Jakmunee J and Christian G.D (2005) Titration using flow-based techniques. International chemical congress of pacific basin societies, Honolulu, Hawaii.
33. Burakham R, Jakmunee and Grudpan K (2005) Micro-total analysis system using sequential injection analysis with lab-at-valve (SIA-LAV) for on-line micro extraction determination of anionic surfactants. International chemical congress of pacific basin societies, Honolulu, Hawaii.
34. Rodjana, Supalax Srijaranai, Kate Grudpan (2006) ON-LINE DERIVATIZATION AND DETERMINATION OF SOME HEAVY METALS BY COUPLING OF SEQUENTIAL INJECTION WITH ION-PAIR REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY . 10th International Conference on Flow analysis, Porto, Portugal
35. Tinakorn Kanyanee, Walter L. Borst, Jaroon Jakmunee, kate Grudpan, Jianzhong Li ,Purnendu K. Dasgupta (2006) A FLOW SYSTEM WITH SOAP BUBBLE FOR GAS SAMPLING DEVICE. 10th International Conference on Flow analysis, Porto, Portugal
36. Jaroon Jakmunee,Somchai Lapanantnoppakhun,Gary D. Christian, Kate Grudpan (2006) FLOW INJECTION AND SEQUENTIAL INJECTION EMPLOYING STOPPED FLOW. 10th International Conference on Flow analysis, Porto, Portugal
37. Tadao Sakai, Yoshikazu Kito, Norio Teshima, Shuji Kato, and Kate Grudpan (2006) FLOW INJECTION ANALYSIS FOR THE DETERMINATION OF PRO-TEIN IN BIOLOGICAL SAMPLES USING NONIONIC SURFACTANT. 10th International Conference on Flow analysis, Porto, Portugal
38. Kate Grudpan,Jaroon Jakmunee, Supaporn Kraptap, Somchai Lapanantnoppakhun, Rodjana Burakham EXPLOITING FLOW ANALYSIS WITH LAB-AT-VALVE

(LAV) APPROACH. 10th International Conference on Flow analysis, Porto, Portugal

39. Jaroon Jakmunee, Sarawut Somnam, Somchai Lapanantnoppakhun, Narong Lenghor, Shoji Motomizu, Kate Grudpan (2006) DEVELOPMENT OF HYDRODYNAMIC FLOW ANALYTICAL SYSTEMS. 10th International Conference on Flow analysis, Porto, Portugal
40. Wasin Wongwilai,Jaroon Jakmunee, Somchai Lapanantnoppakhun, Shoji Motomizu, Tadao Sakai ,Kate Grudpan (2006) FLOW INJECTION AMPEROMETRIC SYSTEM WITH SAMPLE PRETREATMENT COLUMN FOR DETERMINATION OF TRACE ORTHOPHOSPHATE. 10th International Conference on Flow analysis, Porto, Portugal
41. Jaroon Jakmunee, Natsuki Miyoshi, Watsaka Siriangkhawut,Somchai Lapanantnoppakhun, Shoji Motomizu, Kate Grudpan (2006) DEVELOPMENT OF FLOW INJECTION VOLTAMMETRIC SYSTEM WITH SAMPLE PRETREATMENT COLUMN FOR THE DETERMINATION OF CADMIUM , LEAD, COPPER AND ZINC. 10th International Conference on Flow analysis, Porto, Portugal
42. Kate Grudpan, Kanchana Watlaiad, Takashi Suekane, Narong Lenghor, Somchai Lapanantnop pakhun, Jaroon Jakmunee,Tadao Sakai, Shoji Motomizu (2006) DEVELOPMENT OF FLOW SYSTEMS FOR THE DETERMINATION OF AMMONIA. 10th International Conference on Flow analysis, Porto, Portugal
43. Duangjai Nacapricha, Chanpen Karuwan, Benjamapawn Promthong, Thitirat Mantim, Orawon Chailapakul, Kate Grudpan , Einaga Yasuaki (2006) USE OF BORON-DOPED DIAMOND THIN FILM AS SENSOR FOR FLOW ANALYSIS OF BETA-AGONISTS. 10th International Conference on Flow analysis, Porto, Portugal
44. Kate Grudpan, Supaporn Karadtap Hartwel, Duangporn Somprayoon, Somchai Lapanantnop pakhun, Siriwan Ongchai, Prachya Kongtawelert (2006) FLOW INJECTION-BEAD INJECTION SYSTEM FOR THE ASSAY OF BONE ALKALINE PHOSPHATASE. 10th International Conference on Flow analysis, Porto, Portugal

45. Duangjai Nacapricha, Benjamapawn Promthong Prapin Wilairat, Piyada Jittangprasert, Orawon Chailapakul,Kate Grudpan (2006) APPLICATION OF BORON-DOPED DIAMOND THIN FILM AS SENSOR FOR ANALYSIS OF INORGANIC ANIONS IN FLOW INJECTION AND ION CHROMATOGRAPHY. 10th International Conference on Flow analysis, Porto, Portugal

46. Kate Grudpan (2006) Some Approaches in Cost-effective (Flow) Analysis for Development in Environment. Symposium on Frontier Research Project: Materials for the 21st Century-Materials Development for Environment, Energy and Information. Aichi Institute of Technology, Toyota, Japan, 11-13 October 2006.

47. Christian, Gary D., Grudpan, Kate (2006) Analytical chemistry in Thailand: Opportunities for U.S. electrochemists and analytical scientists. Abstracts of Papers, 232nd ACS National Meeting, San Francisco, CA, United States, Sept. 10-14, 2006.

48. Kate Grudpan, Kritsana Jitmanee, Somchai Lapanantnoppakhun, Jaroon Jakmunee, Tadao Sakai and Norio Teshima (2007), "Flow-based Analytical Techniques: Some Developments for Materials and Environment", In "Frontier Research Project : Materials for the 21st Century-Materials Development for Environment,Energy and Information" ,Aichi Institute of Technology,Japan,pp 104-107.

49. Kate Grudpan, Jaroon Jakmunee, Somchai Lapanantnoppakhun, Supaporn Kradtap Hartwell, Kritsana Jitmanee, Wasin Wongwilai and Gary D Christian (2007) Flow Injection Analysis and Related Techniques: How Related are They? (Keynote Lectures). Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007

50. Kancha Watla-iad, Kate Grudpan and Shoji Motomizu (2007) A simple flow injection simultaneous determination of nitrate and nitrite in real water samples using 1-naphthol. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007

51. Sarawut Somnam, Jaroon jakmunee, Kate Grudpan and Shoji Motomizu (2007) An automated hydrodynamic sequential sequential injection system for

determination of phosphate and silicate in waste water. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007

52. Watsaka Siriangkhawut, Muriel Bouby, Horst Geckeis, Jaroon Jakmunee and Kate Grudpan (2007) A flow-based analytical method to study kinetics of metal-humic acid complexes. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007
53. Wasin Wongwilai, Jaroon Jakmunee, Somchai Lapanantnoppakhun and Kate Grudpan (2007) Flow Injection Spectrometric determination of Ascorbic Acid using Methylene Blue. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007
54. Rodjana Burakham and Kate Grudpan (2007) Miniaturized spectrophotometric determination of amoxicillin using sequential injection lab-om-valve. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007
55. Kuruna Jainontee, Vannajan Sanghiran Lee, Sukon Prasitwattanaseree, Jeerayut Chaijaruwanich, Jaroon Jakmunee and Kate Grudpan (2007) Spectrophotometric analysis of component colors in colorant with the aids of principal component regression and partial least squares. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007
56. Somchai Lapanantnoppakhun, Lucksagoon Ganranoo, Wasin Wongwilai, Jaroon Jakmunee, Prasak Thavornyutikarn and Kate Grudpan (2007) Sequential Injection Chromatography for Some Quinine Alkaloids. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007
57. Lucksagoon Ganranoo, Somchai Lapanantnoppakhun, Wasin Wongwilai, Jaroon Jakmunee and Kate Grudpan (2007) Sequential Injection Chromatography of Calcium and Magnesium using Murexide Reagent. Fourteenth International Conference on Flow Injection Analysis (ICFIA 2007) ,Berlin, Germany September 3-7,2007

การเสนอผลงานวิจัยในการประชุมวิชาการภายในประเทศ จำนวน 23 เรื่อง
(ทั้งที่ได้รับเชิญเป็นวิทยากรบรรยายนำ และการเสนอผลงานตามปกติ)

1. Watla-iad K, Chantiwas R, Jakmunee J and Grudpan K (2005) Determination of Milk Protein by Using a Developed Sequential Injection System with Bradford Method. *RGJ-Ph.D. Congress VI*, Jomtien Palm Beach Resort, Pattaya, Chonburi, 215.
2. Grudpan K, Jakmunee J, Lapanantnoppakhun S, Kradtap S, Jitmanee K, Chantiwas R, Nacapricha D, Srijaranai S, Burakham R and Chailapakul O (2005) Some Aspects for Miniaturization in Chemical Analysis. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-L4.
3. Siriangkhawut W, Suteerapataranon S, Jakmunee J and Grudpan K (2005) Development of On-line Dilution Procedure Using Sequential Injection-Monosegmented Fiow for Voltammetric Determination of Some Heavy Metals. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-O2.
4. Watla-iad K, Chantiwas R, Jakmunee J and Grudpan K (2005) Development of Sequential Injection Analysis for Protein Assay Using Bradford Method. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-O4.
5. Somnam S, Jakmunee J and Grudpan K (2005) Determination of Fe(II) and Fe(III) by a Novel Hydrodynamic Flow Injection System. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-O6.
6. Burakham R, Motomizu S and Grudpan K (2005) Flow Injection with a Novel Spectrophotometric Reaction System for water Monitoring of Nitrite and Nitrate. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-P20.
7. Somprayoon D, Hartwell S.K and Grudpan K (2005) Flow Injection-Bead Injection System for Determination of Bone Alkaline Phosphatase. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-P24.
8. Boomalai A, Hartwell S.K and Grudpan K (2005) Development of Flow Injection-Capillary Immunoassay System. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-P30.

9. Ganranoo L, Lapanantnoppakhun S and Grudpan K (2005) Development of Flow Injection Spectrophotometric for Determination of Tetracyclines. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-P31.
10. Wongwilai W, Jakmunee J and Grudpan K (2005) Investigation on Electrochemical Reactions for Determination of Phosphate. *PERCH congress IV*, Jomtien Palm Beach Resort, Pattaya, Chonburi, AC-P44.
11 Wasin Wongwilai, Jaroon Jakmunee, Somchai Lapanantnopakhun and Kate Grudpan (2006) Sequential Injection Analysis for Fluorometric Determination of Aluminium. 32nd congress on Science and Technology of Thailand, Queen Sirikit National Convention Center (QSNCC), Bangkok, C1_C0180
12. Lucksagoon , Somchai Lapanantnopakhun ,Jaroon Jakmunee and Kate Grudpan (2006) Sequential Injection Spectrophotometry for The Determination of Tetracyclines. 32nd congress on Science and Technology of Thailand, Queen Sirikit National Convention Center (QSNCC), Bangkok, C1_C0154
13. Rodjana Burakham, Supalax Sijaranai and Kate Grudpan (2006) On- Line Derivatization Determiantion of Metal Ions Using Sequential Injection High Performance Liquid Chromatography. 32nd congress on Science and Technology of Thailand, Queen Sirikit National Convention Center (QSNCC), Bangkok, C1_C0073
14. Lapanantnoppakhun S, Ganranoo L, Kasiwad S, Jakmunee J and Grudpan K (2006) Development of Simple Systems with Usage of Simple Reagent. *การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว.*, Regent Hotel, Cha Am, Phetchaburi, O-S1F-16.
15. Jakmunee J and Grudpan K (2006) Development of Instrument and Methods for Coulometric and Amperometric Analysis of Some Foods and Beverages. *การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว.* , Regent Hotel, Cha Am, Phetchaburi, P-S1E-12.
16. Kradtap-Hartwell S, Pathanon K, Fongmoon D, Grudpan K and Kongtawelert P (2006) Simple Assay of Chondroitin Sulfate Proteoglycans Using a Flow Injection System with Mini-Immunoaffinity Chromatographic Column. *การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว.*, Regent Hotel, Cha Am, Phetchaburi, P-S1E-17.

17. Jakmunee J, Kradtap-Hartwell S, Lapanantnoppakhun S, Jitmanee, K and Grudpan K (2006) **Plenary Lecture:** Development of Flow-Based Analysis in Thailand, PRECH-CIC Congress V, Jomtien Palm Beach Resort Pattaya, PL7
18. Sarawut Somnam, Jaroon Jakmunee, Kate Grudpan and Shoji Motomizu (2007) Development of an Automated Hydrodynamic Sequential Injection System for determination of Phosphate and Silicate. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007
19. Napaporn Wannaprom, Supaporn Kradtap Hartwell, Prachya Kongtawelert, Jaroon Jakmunee and Kate Grudpan (2007) Preliminary Studies on Development of Capillary Immunoassay for Biomarker of cancer. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007
20. Supada Khonyoung, Supaporn Kradtap Hartwell, Prachya Kongtawelert, Somchai Lapanantnopakhun, Jaroon jakmunee and Kate Grudpan (2007) Immobilization of Chondroitin Sulfate Proteoglycan on Glass Capillary for its use in Immunoassay. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007
21. Pipoon Bunpeng, Jaroon jakmunee and Kate Grudpan (2007) Development of Flow Injection-Amperometric method for Determination of Ascorbic Acid in Fruit Juice Samples. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007
22. Kraingkrai Ponhong, Jaroon Jukmunee and kate Grudpan (2007) Cost-Effective Amperometric-Iodometric Titration for Determination of Some Reducing Agents. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007
23. Nuanlaor Ratanawimarnwong, Saowapak Teerasong Kate Grudpan and Duangjai Nacapricha (2007) Determanation of Formaldehyed contaminated in food using Hybrid-Flow Analysis. 33rd Congress on Science and Technology of Thailand.(STT.33) , Walailak University ,Nakhon Si Thammarat, Thailand. October 18-20, 2007

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
1	Hartwell S Kradtap, Grudpan K and Christian G D	Bead injection with a simple flow-injection system: an economical alternative for trace analysis.	Trends in Analytical Chemistry (2004), 23(9) , 619-623.	5.068
2	Grudpan K	Some recent developments on cost-effective flow-based analysis.	Talanta (2004), 64(5), 1084-1090.	2.810
3	Udnan Y, Jakmunee J, Jayasavati S, Christian GD, Synovec RE, Grudpan K	Cost-effective flow injection spectrophotometric assay of iron content in pharmaceutical preparations using salicylate reagent.	Talanta (2004) , 64(5), 1237-1240.	2.810
4	Tanikkul S, Jakmunee J, Lapanantnoppakhun S, Rayanakorn M, Sooksamiti P, Synovec RE, Christian GD, Grudpan K	Flow injection in-valve-mini-column pretreatment combined with ion chromatography for cadmium, lead and zinc determination.	Talanta (2004), 64(5), 1241-1246.	2.810
5	Charoenraks T, Palaharn S, Grudpan K, Siangproh W, Chailapakul O	Flow injection analysis of doxycycline or chlortetracycline in pharmaceutical formulations with pulsed amperometric detection.	Talanta (2004), 64(5), 1247-1252.	2.810

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
6	Chailapakul O, Amatatongchai M, Wilairat P, Grudpan K, Nacapricha D	Flow-injection determination of iodide ion in nuclear emergency tablets, using boron-doped diamond thin film electrode.	Talanta (2004), 64(5), 1253-1258.	2.810
7	Jakmunee, J, Patimapornlert L, Suteerapataranon S, Lenghor N, Grudpan K	Sequential injection with lab-at-valve (LAV) approach for potentiometric determination of chloride.	Talanta (2005), 65(3), 789-793.	2.810
8	Lenghor N, Staggemeier BA, Hamad ML, Yuthapong U, Tanikkul S, Jakmunee, J, Grudpan K, Prazen BJ and Synovec RE	A dynamic liquid-liquid interfacial pressure detector for the rapid analysis of surfactants in a flowing organic liquid.	Talanta (2005), 65(3), 722-729.	2.810
9	Hartwell S Kradtap, Srisawang B, Kongtawelert P, Jakmunee Jand Grudpan K	Sequential injection-ELISA based system for online determination of hyaluronan.	Talanta (2005), 66(2), 521-527.	2.810

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
10	Hartwell S Kradtap, Srisawang B, Kongtawelert P, Christian GD and Grudpan K	Review on screening and analysis techniques for hemoglobin variants and thalassemia.	Talanta (2005), 65(5), 1149-1161.	2.810
11	Hartwell S Kradtap, Boonmalai A, Jayasvati S, Lapanantnoppakhun S, Jakmunee J and Grudpan K	Simple Bead Injection – Flow Injection System for Determination of Copper.	Anal. Science (2005) , 21(4), 437-439.	1.589
12	Chantiwas R, Beckett R, Grudpan K	Size-based speciation of iron in clay mineral particles by gravitational field-flow fractionation with electrothermal atomic absorption spectrometry.	Spectrochimica Acta Part B (2005), 60(1), 109-116.	3.092
13	Jakmunee J, Pathimapornlert L, Hartwell SK and Grudpan K	Novel approach for mono-segmented flow micro-titration with sequential injection using a lab-on-valve system: a model study for the assay of acidity in fruit juices.	Analyst (2005), 130(3), 299-303.	3.198
14	Udnan Y, McKelvie ID, Grace MR, Jakmunee J and Grudpan K	Evaluation of on-line preconcentration and flow-injection amperometry for phosphate determination in fresh and marine waters.	Talanta (2005), 66(2), 461-466.	2.810

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
15	Wangkarn S, Soisungnoen P, Rayanakorn M and Grudpan K	Determination of linear alkylbenzene sulfonates in water samples by liquid chromatography-UV detection and confirmation by liquid chromatography-mass spectrometry.	Talanta (2005), 67(4), 686-695.	2.810
16	Tue-Ngeun O, Jakmunee J, Grudpan K	A novel stopped flow injection-amperometric procedure for the determination of chlorate.	Talanta (2005), 68(2), 459-464.	2.810
17	Burakham R, Lapanantnoppakhun S, Jakmunee J and Grudpan K	Exploiting sequential injection analysis with lab-at-valve (LAV) approach for on-line liquid-liquid micro-extraction spectrophotometry.	Talanta (2005) , 68(2), 416-421.	2.810
18	Settheeworrarit T, Hartwell SK, Lapanatnoppakhun S, Jakmunee J, Christian GD and Grudpan K	Exploiting guava leaf extract as an alternative natural reagent for flow injection determination of iron.	Talanta (2005), 68(2), 262-267.	2.810

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
19	Manee-on K, Lapanantnoppakhun S, Jakmunee J and Grudpan K	A simple flow injection assay of Ca(II) in mineral supplement using Mg(II)-EDTA.	Journal of Flow Injection Analysis (2005), 22(1), 9-10.	
20	Suteerapataranon, Siripat; Bouby, Muriel; Geckeis, Horst; Fanghaenel, Thomas; Grudpan, Kate.	Interaction of trace elements in acid mine drainage solution with humic acid.	Water Research (2006), 40(10), 2044-2054.	2.459
21	Grace, Michael; Udnan, Yuthapong; McKelvie, Ian; Jakmunee, Jaroon; Grudpan, Kate.	On-line Removal of Sulfide Interference in Phosphate Determination by Flow Injection Analysis.	Environmental Chemistry (2006), 3(1), 19-25.	0.814
22	Preechaworapun, Anchana; Chuanuwatanakul, Suchada; Einaga, Yasuaki; Grudpan, Kate; Motomizu, Shoji; Chailapakul, Orawon.	Electroanalysis of sulfonamides by flow injection system/high-performance liquid chromatography coupled with amperometric detection using boron-doped diamond electrode.	Talanta (2006), 68(5), 1726-1731.	2.810
23	Srijaranai, Supalax; Chanpaka, Saiphon; Kukusamude, Chutima; Grudpan, Kate.	Flow-injection in-line complexation for ion-pair reversed phase high performance liquid chromatography of some metal-4-(2-pyridylazo) resorcinol chelates.	Talanta (2006), 68(5), 1720-1725.	2.810

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
24	Jakmunee, Jaroon; Rujiralai, Thitima; Grudpan, Kate.	Sequential injection titration with spectrophotometric detection for the assay of acidity in fruit juices.	Analytical Sciences (2006), 22(1), 157-160.	1.589
25	Lapanantnoppakhun, Somchai; Kasuwas, Supharphorn; Ganranoo, Lucksagoon; Jakmunee, Jaroon; Grudpan, Kate.	Simple spectrophotometric flow injection system with an in-valve minicolumn for enhancement during the determination of chromium(III) using EDTA.	Analytical Sciences (2006), 22(1), 153-155.	1.589
26	Lenghor, Narong; Jakmunee, Jaroon; Prazen, Bryan J.; Synovec, Robert E.; Christian, Gary D.; Grudpan, Kate.	Simple sequential injection analysis systems with a dynamic surface tension detector.	Analytical Sciences (2006), 22(1), 147-151.	1.589
27	Burakham, Rodjana; Jakmunee, Jaroon; Grudpan, Kate.	Development of sequential injection-lab-at-valve (SI-LAV) micro-extraction instrumentation for the spectrophotometric determination of an anionic surfactant.	Analytical Sciences (2006), 22(1), 137-140.	1.589

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
28	Kate Grudpan, Supada Khonyoung, Supaporn Kradtap Hartwell, Somchai Lapanantnoppakhun and Jaroon Jakmunee .	Down scaling : From operation on lab bench space to manipulation at a valve.	Flow Injection Anal (2006), 23(2), 94 – 101	
29	Chanpen Karuwan, Thitirat Mantim, Patcharin Chaisuwan, Prapin Wilairat, kate Grudpan, Piyada Jittangprasert, Yasuaki Einaga, Orawon Chailapakul, Leena Suntornsuk, Oraphan Anurukvorakun and Duangjai Nacapricha.	Pulsed Amperometry for Anti-fouling of Boron-doped Diamond in Electroanalysis of β-Agonists : Application to Flow Injection for Pharmaceutical Analysis.	Sensors (2006), 6, 1837-1850.	1.373
30	Kritsana Jitmanee, Jaroon Jakmunee, Somchai Lapanantnoppakhun, Sunanta Wangkarn, Norio Teshima, Tadao Sakai, Gary D. Christian and Kate	Enhancing chemical analysis with signal derivatization using simple available software packages.	Microchemical Journal (2007), 8(2), 195-203	1.558
31	Apichai Santalad, Rodjana Burakham, Supalax Srijaranai and Kate Grudpan	Field-amplified sample injection and in-capillary derivatization for capillary electrophoretic analysis of metal ions in local wines.	Microchemical Journal (2007), 86(2), 209-215	1.558

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
32	Supaporn Kradtap Hartwell, Duangporn Somprayoon, Prachya Kongtawelert, Siriwan Ongchai, Olarn Arpporchayanon, Lucksagoon Ganranoo, Somchai Lapanantnoppakhun and Kate Grudpan	Online assay of bone specific alkaline phosphatase with a flow injection-bead injection system.	Analytica Chimica Acta (2007), 600(1-2), 188-193	2.894
33	Tadao Sakai, Yoshikazu Kito, Norio Teshima, Shuji Katoh, Kanchana Watla-lad and Grudpan	Spectrophotometric Flow Injection Analysis of Protein in Urine Using Tetrabromophenolphthalein Ethyl Ester and Triton X-100.	Flow Injection Anal (2007), 24(1), 23-26	
34	Rodjana Burakham, Supalax Srijaranai and Kate Grudpan	High-Performance Liquid Chromatography with Sequential Injection for On-line Pre-column Derivatization of Some Heavy Metals.	Journal of Separation Science (2007), 30 (16), 2614-2619	2.535
35	Supaporn Kradtap Hartwell, Kanokphan Pathanon, Duriya Fongmoon, Prachya Kongtawelert and Kate Grudpan	Exploiting flow injection system with mini-immunoaffinity chromatographic column for chondroitin sulfate proteoglycans assay.	Analytical and Bioanalytical Chemistry (2007), 388 (8), 1839-1846	2.591
36	Watla-iad Kanchana, Tadao Sakai, Norio Teshima, Shuji Katoh and Kate Grudpan	Successive determination of urinary protein and glucose using spectrophotometric sequential injection method.	Analytica Chimica Acta (2007) , 604(2) 139-146	2.894

ตาราง 1 output จากกลุ่มวิจัยจากโครงการวิจัยฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
37	Beckett, R., Sharma, R., Andric, G., Chantiwas, R., Jakmunee, J., Grudpan, K.	Illustrating some principles of separation science through gravitational field-flow fractionation.	Journal of Chemical Education (2007), 84 (12), 1955-1962	0.439
38	Chaisuksant, R., Pattanarat, L., Grudpan, K.	Naphthazarin modified carbon paste electrode for determination of copper(II).	Microchimica Acta Article (2007), 162, 181-188	1.237
39	Orawon Chailapakul, Sarawadee Korsrisakul, Weena Siangproh and Kate Grudpan	Fast and simultaneous detection of heavy metals using a simple and reliable microchip-electrochemistry route: An alternative approach to food analysis.	Talanta (2008), 74(4), 683-689	2.810

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
1	Burakham R, Higuchi K, Oshima M, Grudpan K, Motomizu S	Flow injection spectrophotometry coupled with a crushed barium sulfate reactor column for the determination of sulfate ion in water samples.	Talanta (2004) , 64(5), 1147-1150.	2.810
2	Burakham R, Oshima M, Grudpan K, Motomizu S	Simple flow- injection system for the simultaneous determination of nitrite and nitrate in water samples.	Talanta (2004), 64(5), 1259-1265.	2.810
3	Tue-Ngeun O, Ellis P, McKelvie ID, Worsfold P, Jakmunee J and Grudpan K	Determination of dissolved reactive phosphorus (DRP) and dissolved organic phosphorus (DOP) in natural waters by the use of rapid sequenced reagent injection flow analysis.	Talanta (2005), 66(2), 453-460.	2.810
4	Uraisin K, Nacapricha D, Lapanantnoppakhun S, Grudpan K. and Motomizu S.	Determination of trace amounts of bromide by flow injection/stopped-flow detection technique using kinetic-spectrophotometric method.	Talanta (2005), 68(2), 274-280.	2.810
5	Rujiwatra A, Phueadpho M and Grudpan Kate	Selective synthesis of zeolitic phillipsite and hibschite hydrogarnet from lignite ash employing calcium hydroxide under mild conditions.	Journal of Physics and Chemistry of Solids (2005), 66(6), 1085-1090	1.164

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
6	Orawan Tue-Ngeun, Richard C. Sandford, Jaroon Jakmunee, Kate Grudpan, Ian D. McKelvie, Paul J. Worsfold.	Determination of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in freshwaters by sequential injection spectrophotometry with on-line UV photo-oxidation,	Analytica Chimica Acta (2005), 554, 17-24.	2.894
7	Kanyanee, Tinakorn; Borst, Walter L.; Jakmunee, Jaroon; Grudpan, Kate; Li, Jianzhong; Dasgupta, Purnendu K.	Soap Bubbles in Analytical Chemistry. Conductometric Determination of Sub-Parts Per Million Levels of Sulfur Dioxide with a Soap Bubble.	Analytical Chemistry (2006), 78(8), 2786-2793.	5.646
8	Ohno, Shinsuke; Teshima, Norio; Sakai, Tadao; Grudpan, Kate; Polasek, Miroslav.	Sequential injection lab-on-valve simultaneous spectrophotometric determination of trace amounts of copper and iron.	Talanta (2006), 68(3), 527-534.	2.810
9	Natchanon Amornthammarong, Jaroon Jakmunee, Jianzhong Li, and Purnendu K. Dasgupta	Hybrid Fluorometric Flow Analyzer for Ammonia.	Anal. Chem (2006) , 78(6), 1890 -1896.	5.646
10	McBrady, Adam D.; Chantiwas, Rattikan; Torgerson, Ana Kristine; Grudpan, Kate; Synovec, Robert E.	An absorbance-based micro-fluidic sensor for diffusion coefficient and molar mass determinations.	Analytica Chimica Acta (2006), 575(2), 151-158.	2.894

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
11	Kritsana Jitmanee, Norio Teshima, Tadao Sakai and Kate Grudpan	DRC™ ICP-MS coupled with automated flow injection system with anion exchange minicolumns for determination of selenium compounds in water samples.	Talanta (2007), 73(2),352-357	2.810
12	Pothacharoen P, Siriaunkgul S, Ong-chai S, Supabandhu J, Kumja P, Wanaphirak C, Sugahara K,	Raised Serum Chondroitin Sulfate Epitope Level in Ovarian Epithelial Cancer	J. Biochem. 140, 517–524. (2006)	2.160
13	Srijaranai S, Sririangkhawut W, Srijaranai S, Ruksakulpiwat C, Deming RL	Effect of bound copper(II) on the LC separation of selected phenols using an aminophosphonic acid silica stationary phase	Anal. Lett 37 (2004) 2577-2594	1.036
14	Santalad A, Teerapornchaisit P, Burakham R, Srijaranai S	Capillary zone electrophoresis of organic acids in beverages	LWT Food Sci. Technol ' (2007), 40(10),1741-1746	1.155
15	Amornthammarong N., Anujaravat P.,Sereenonchai K., Chaisuwan P., Sastranurak P., Wilairat P. and Nacapricha D.	Method development based on all injection analysis for determination of phosphorus in soil and sediment extracts.	Talanta 2005: **68**: 480-487	2.391

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
16	Choengchan N., Mantim T., Wilairat P., Dasgupta P. K., Motomizu S., Nacapricha D.	A membraneless gas diffusion unit: design and its application to determination of ethanol in liquors by spectrophotometric flow injection.	Anal. Chim. Acta 2006: 579: 33-37	2.760
17	**Nacapricha D.**, Amornthammarong N., Sereenonchai K., Anujaravat P., Wilairat P.	Low cost telemetry with PC sound card for chemical analysis applications	Talanta 2007: **71**: 605–609	2.391
18	Amatatongchai M., Hofmann O., Nacapricha D., Chailapakul O., and J. deMello A.	A microfluidic system for evaluation of antioxidant capacity based on a peroxyoxalate chemiluminescence assay.	Anal. Bioanal. Chem. 2007: 387: 277–285	2.695
19	Nimmanpipug, P.; Jitonnom, J.; Ngaojampa, C.; Lee, V. S.	H5N1 Neuraminidase Implicit Molecular Dynamics Model and Its Binding Interactions with Commercial Drugs.	*Molecular Simulations* 2007, *in revision*.	1.345
20	Yeap, B.; Othman, A. S.; Lee, V. S.; Lee, C.	Molecular phylogenetics of the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and the Philippines milk termite, *Coptotermes vastator* Light (Isoptera: Rhinotermitidae)	*Journal of Economic Entomology* (Household and Structural Insects) 2007, *in press* .	1.205

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
21	Junkaew, A.; Jitonnom, J.; Nimmanpipug, P.; Lee, V. S.	Polymerizability of lactones calculated by molecular mechanics, semiempirical and density functional theory methods.	Chiang Mai Journal of Science 2007, 34(1), 1-9.	
22	Aruksakunwong, O.; Wittayanarakul, K.; Sompornpisut, P.; Sanghiran, V.; Parasuk, V.; Hannongbua, S.	"Structural and Dynamical Properties of Different Protonated States of Mutant HIV-1 Protease Complexed with the Saquinavir Inhibitor Studied by Molecular Dynamics Simulations"	Journal of Molecular Graphic and Modelling 2006, 25, 324-332.	2.333
23	Wittayanarakul, K.; Aruksakunwong, O.; Sompornpisut, P.; Lee, V. S.; Parasuk, V.; Pinitglang, S.; Hannongbua, S.	Structure, Dynamics and Solvation of HIV-1 Protease/Saquinavir Complex in Aqueous Solution and their Contributions to Drug Resistance: Molecular Dynamic Simulations.	Journal of Chemical Information and Modeling 2005, 45, 300-308.	2.923
24	Promsri, S.; Chuichay, P.; Lee, V. S.; Parasuk, V.; Hannongbua, S.	Molecular and Electronic Properties of HIV-1 Protease Inhibitor C60 derivatives as Studies by the ONIOM Method.	Journal of Molecular Structure: THEOCHEM 2004, 715, 47-53.	1.045
25	Nimmanpipug, P.; Tashiro, K; Rangsiman, O.	Factors Governing the Three-Dimensional Hydrogen-Bond Network Structure of Poly(*m*-Phenylene Isophthalamide) and a Series of Its Model Compounds (4): Similarity in Local Conformation and Packing Structure between a Complicated Three-Arm Model Compound	J. Phys. Chem. 2006; 110B; 20858-20864	4.033

ตาราง 2 output จากกลุ่มวิจัยที่ไม่ได้ร่วมในโครงการฯ : ผลงานตีพิมพ์

ลำดับ	ผู้ร่วมวิจัย	ผลงานตีพิมพ์	วารสารวิชาการ	Impact Factor
26	Metawee Srikummool, Jeerayut Chaijaruwanich, Jatupol Kampuansai, Daoroong Kangwanpong	Minimal Y-chromosomal Haplotype Selection for Phylogenetic Study using the Bootstrapped DTI Method	Science Asia , 2006, Vol.32, 361-364	
27	Traisathit P, Le Cœur S, Mary JY, Kanjanasing A, Lamlertkittikul S, Lallemant M.	Gestational age determination and prevention of human immunodeficiency virus perinatal transmission.	IJGO 2006; 92:176-180.	1.147
28	Briand N, Le Cœur S, Traisathit P	Growth of HIV-uninfected children exposed to perinatal zidovudine for the prevention of mother-to-child HIV transmission.	PIDJ. 2006; 25:325–332.	2.262
29	Briand N, Lallemant M, Jourdain G, Traisathit P	Haematological Parameters in HIV-1 Pregnant Women Exposed to Perinatal Zidovudine in Thailand: Secondary Analysis of a Randomized Trial.	PLoS Clinical Trials (2007) Vol. 2, No. 4, e11	

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

1	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	บุคคลดีเด่นของชาติ สาขาวิทยาศาสตร์และเทคโนโลยี (ด้านเคมีวิเคราะห์)
	ชื่อโครงการ	จากสำนักงานเสริมสร้างเอกลักษณ์ของชาติ
	ปีที่ได้รับ	2547
2	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	เมธีวิจัยอาวุโส สกว.
	ชื่อโครงการ	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ปีที่ได้รับ	2547-2550
3	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	เป็นผู้ประสานงานฝ่ายไทยในการจัด Joint Workshop on Modern Analytical Science ระหว่างสหรัฐ-ไทย
	ชื่อโครงการ	ได้รับทุนของ NSF, สกอ.และ สกว.
	ปีที่ได้รับ	
4	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	นักวิจัยดีเด่น
	ชื่อโครงการ	รางวัลมหาวิทยาลัยเชียงใหม่ ‘ ช้างทองคำ ’
	ปีที่ได้รับ	2550

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

5	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	กรรมการสภาวิจัยแห่งชาติ สาขาวิทยาศาสตร์เคมีและเภสัช
	ชื่อโครงการ	สำนักงานคณะกรรมการวิจัยแห่งชาติ
	ปีที่ได้รับ	(2545-ปัจจุบัน)
6	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะอนุกรรมการสำนักงานคณะกรรมการอุดมศึกษาในการจัดทำ software เกี่ยวกับข้อมูลในการวิจัย
	ชื่อโครงการ	สำนักงานคณะกรรมการการอุดมศึกษา
	ปีที่ได้รับ	(2547-ปัจจุบัน)
7	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะอนุกรรมการสำนักงานคณะกรรมการอุดมศึกษาเกี่ยวกับการจัดการทุนการศึกษาชั้นสูงเชิงกลยุทธ์ เพื่อสร้างเครือข่ายวิจัยระดับแนวหน้า
	ชื่อโครงการ	สำนักงานคณะกรรมการการอุดมศึกษา
	ปีที่ได้รับ	(2547-ปัจจุบัน)
8	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ผู้ประสานงานศูนย์ความเป็นเลิศทางวิชาการโครงการพัฒนาการลดขนาดเพื่อการวิเคราะห์ระดับไมโครและนาโน
	ชื่อโครงการ	คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
	ปีที่ได้รับ	

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

9	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ผู้ประสานงานห้องปฏิบัติการวิจัยเพื่อพัฒนาเครื่องมือวิเคราะห์
	ชื่อโครงการ	สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเชียงใหม่
	ปีที่ได้รับ	
10	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะกรรมการผู้ทรงคุณวุฒิพิจารณาตำแหน่งทางวิชาการสภามหาวิทยาลัยแม่โจ้
	ชื่อโครงการ	มหาวิทยาลัยแม่โจ้
	ปีที่ได้รับ	(2549-ปัจจุบัน)
11	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ผู้ประสานงานโครงการวิจัยร่วมมือกับสถาบันในต่างประเทศ เช่น (Karlsruhe Research Center (เยอรมนี); University of Washington, Seattle
	ชื่อโครงการ	
	ปีที่ได้รับ	
12	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ที่ปรึกษาเครือข่าย สวทช. ภาคเหนือ
	ชื่อโครงการ	สวทช.
	ปีที่ได้รับ	(2545-ปัจจุบัน)

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

13	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ที่ปรึกษาการวิจัยคณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี
	ชื่อโครงการ	มหาวิทยาลัยอุบลราชธานี
	ปีที่ได้รับ	
14	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะกรรมการพิจารณาทุนมหาวิทยาลัยเชียงใหม่ เพื่อสนับสนุนการไปศึกษาต่อระดับปริญญาเอก ณ ต่างประเทศ
	ชื่อโครงการ	มหาวิทยาลัยเชียงใหม่
	ปีที่ได้รับ	
15	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะทำงานเพื่อพัฒนารูปแบบและระบบการจัดสรรทุนการผลิตและพัฒนาอาจารย์ในสถาบันอุดมศึกษา
	ชื่อโครงการ	สำนักงานคณะกรรมการการอุดมศึกษา
	ปีที่ได้รับ	2549 – ปัจจุบัน
16	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะทำงานสรรหาโครงการวิจัยด้านวิทยาศาสตร์เคมีและเภสัช
	ชื่อโครงการ	สำนักงานคณะกรรมการวิจัยแห่งชาติ (วช.)
	ปีที่ได้รับ	

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

17	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะกรรมการผู้ทรงคุณวุฒิพิจารณาตำแหน่งทางวิชาการสภามหาวิทยาลัยนเรศวร
	ชื่อโครงการ	มหาวิทยาลัยนเศวร
	ปีที่ได้รับ	(2549-ปัจจุบัน)
18	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ผู้ประสานงานโครงการเพิ่มสมรรถนะความแข่งขันกับต่างประเทศของ สกอ.
	ชื่อโครงการ	ในโครงการ “การพัฒนาเครื่องมือและวิธีสำหรับวิทยาศาสตร์การวิเคราะห์ในระดับไมโครและนาโนเพื่อใช้ในทางสุขภาพและสิ่งแวดล้อม”
	ปีที่ได้รับ	(2546 – 2549)
19	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Editorial Advisory board
	ชื่อโครงการ	ของวารสาร Talanta (Elsevier Publish)
	ปีที่ได้รับ	(2543 - ปัจจุบัน)
20	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Associate Editor
	ชื่อโครงการ	ของวารสาร Water Research (Elsevier Publish)
	ปีที่ได้รับ	(2546 - ปัจจุบัน)

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

21	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Editorial board
	ชื่อโครงการ	ของวารสาร Science Asia
	ปีที่ได้รับ	2544 – 2547
22	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Referee พิจารณา manuscript เพื่อตีพิมพ์
	ชื่อโครงการ	ในวารสาร เช่น Analytica Chimica Acta, Talanta, Journal of Pharmaceutical and Biomedical Analysisและ Analytical Chemistry ซึ่ง
	ปีที่ได้รับ	
23	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Member, Steering Committee
	ชื่อโครงการ	13th International Conference on Flow Injection Analysis
	ปีที่ได้รับ	24 – 29 เมษายน 2548.
24	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	Co-organizer,
	ชื่อโครงการ	Symposium on Flow based analysis, Pacifichem 2005, Honolulu, USA.
	ปีที่ได้รับ	ธันวาคม 2005,

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

25	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	ได้รับเชิญเป็นคณะกรรมการในการจัดงานประชุม รวมทั้งเป็นผู้บรรยายรับเชิญ และนำเสนอผลงาน
	ชื่อโครงการ	การประชุมวิชาการนานาชาติ The 14th International Conference on flow Injection Analysis(ICFIA 2007) and including related
	ปีที่ได้รับ	ระหว่างวันที่ 3 – 7 กันยายน 2550
26	ชื่อนักวิจัย	ศ. ดร. เกตุ กรุดพันธ์
	ได้รับรางวัล/เกียรติยศ	คณะกรรมการและวิทยากรบรรยายนำ
	ชื่อโครงการ	ในการ ประชุม 10th International Conference on Flow analysis, Porto, Portuga
	ปีที่ได้รับ	
27	ชื่อนักวิจัย	ดร.จรูญ จักร์มุณี
	ได้รับรางวัล/เกียรติยศ	นักวิจัยรุ่นใหม่ดีเด่นสาขาวิทยาศาสตร์และเทคโนโลยี
	ชื่อโครงการ	มหาวิทยาลัยเชียงใหม่
	ปีที่ได้รับ	2549
28	ชื่อนักวิจัย	ผศ.ดร.ศิริวรรณ องค์ไชย
	ได้รับรางวัล/เกียรติยศ	เป็นที่ปรึกษาวิทยานิพนธ์ได้อย่างมีคุณภาพ ทำให้นักศึกษาได้รับวิทยานิพนธ์ดีมาก ของ มหาวิทยาลัยเชียงใหม่ ปี 2549
	ชื่อโครงการ	Investigation of the epitope in chondroitir-6-sulfate recognized by monoclonal antibody W-F-6
	ปีที่ได้รับ	15 มกราคม 2550
	นักศึกษา	นางสาวพีรพรรณ โปธาเจริญ

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

29	ชื่อนักวิจัย	น.ส.รจนา บุระคำ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/เกียรติยศ	Best Presentation Award (Oral presentation)
	ชื่อโครงการ	RERCH Congress III, Chonbari Thailand.
	ปีที่ได้รับ	2547
30	ชื่อนักวิจัย	ผู้ช่วยศาสตราจารย์ ดร.สุภาภรณ์ ครัดทัพ
	ได้รับรางวัล/เกียรติยศ	รางวัลนักวิจัยรุ่นใหม่ที่มีผลงานตีพิมพ์ดี จาก สกว.และสกอ.
	ชื่อโครงการ	ทุนพัฒนาศักยภาพการทำงานวิจัยของอาจารย์รุ่นใหม่
	ปีที่ได้รับ	2548
31	ชื่อนักวิจัย	ผู้ช่วยศาสตราจารย์ ดร.สุภาภรณ์ ครัดทัพ
	ได้รับรางวัล/เกียรติยศ	นักศึกษาในความดูแลได้รับรางวัลชนะเลิศการเสนอผลงานแบบโปสเตอร์
	ชื่อโครงการ	ในงานวันเสนอผลงานทางวิชาการของนักศึกษาปี 4
	ปีที่ได้รับ	2548
32	ชื่อนักวิจัย	รศ.ดร. อรวรรณ ชัยลภากุล (หัวหน้าโครงการ)
	ได้รับรางวัล/เกียรติยศ	Best article Award สำหรับ"Cost-effective flow cell for the determination of malachite green and leucomalachite green at a
		diamond thin-film electrode"
	ชื่อโครงการ	Development of Boron-doped Diamond Thin film by Anodization
	ปีที่ได้รับ	พฤศจิกายน /2006

ตาราง 3 นักวิจัยในโครงการที่ได้รับรางวัลและเกียรติยศในระหว่างรับทุนส่งเสริมกลุ่มวิจัย

33	ชื่อนักวิจัย	ผศ. ดร. ดวงใจ นาคะปรีชา
	ได้รับรางวัล/เกียรติยศ	Exampler Lecturer of the Year 2005
	ชื่อโครงการ	Faculty of Science, Mahidol University
	ปีที่ได้รับ	2548
34	ชื่อนักวิจัย	นางสาวจันทร์เพ็ญ ครุวรรณ์ (นักศึกษาปริญญาโท)
	ได้รับรางวัล/เกียรติยศ	"The Award of Excellence" for great poster presentatior
	ชื่อโครงการ	the 12th International Symposium on Advanced Technology and Application, Masan, Korea
	ปีที่ได้รับ	2-11 พฤศจิกายน 2549

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
1	ชื่อนักวิจัย	ศ.ดร.เกตุ กรุดพันธ์ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	ได้รับทุน คปก. รุ่นที่ 7
	ปีที่ได้รับ	2548
2	ชื่อนักวิจัย	ศ.ดร.เกตุ กรุดพันธ์ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	ได้รับทุน คปก. รุ่นที่ 8
	ปีที่ได้รับ	2549
3	ชื่อนักวิจัย	ศ.ดร.เกตุ กรุดพันธ์ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	ได้รับทุน คปก. รุ่นที่ 9
	ปีที่ได้รับ	2550
4	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	การพัฒนาเครื่องมือและวิธีทางเคมีไฟฟ้าสำหรับการวิเคราะห์อาหารและเครื่องดื่ม
	ปีที่ได้รับ	2546-2549
5	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	คณะวิทยาศาสตร์, มหาวิทยาลัยเชียงใหม่
	ชื่อโครงการ	การพัฒนาขั้วไฟฟ้าอ้างอิงและขั้วจำเพาะคลอไรด์สำหรับประยุกต์ในอุตสาหกรรม
	ปีที่ได้รับ	2547

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
6	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	คณะวิทยาศาสตร์, มหาวิทยาลัยเชียงใหม่
	ชื่อโครงการ	ทุนสนับสนุนการวิจัยจากเงินรายได้คณะวิทยาศาสตร์ เรื่อง การสร้างและทดสอบประสิทธิภาพของระบบย่อยสลายสารด้วยคลื่นอัลตราไวโอเลต
	ปีที่ได้รับ	2547
7	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สวทช.
	ชื่อโครงการ	ทุนโครงการนำร่องเพื่อส่งเสริมการวิจัยด้านเทคโนโลยีสะอาด
	ปีที่ได้รับ	2549
8	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	ทุนเพิ่มขีดความสามารถด้านการวิจัยของอาจารย์รุ่นกลางในสถาบันอุดมศึกษา ประจำปี พ.ศ. 2549
	ปีที่ได้รับ	2549-2552
9	ชื่อนักวิจัย	ดร. จรูญ จักร์มุณี (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	ได้รับทุน คปก. รุ่นที่ 9
	ปีที่ได้รับ	2549

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
10	ชื่อนักวิจัย	ดร. สมชัย ลาภอนันต์นพคุณ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	The Postgraduate Education and Research Program in Chemistry (PERCH)
	ชื่อโครงการ	Determination of Flow Injection Procedure for Determination of Amylose in Rice
	ปีที่ได้รับ	2546-2547
11	ชื่อนักวิจัย	ดร. สมชัย ลาภอนันต์นพคุณ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	คณะกรรมการการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	Development of Simple System with simple Reagent
	ปีที่ได้รับ	2547-2549
12	ชื่อนักวิจัย	ดร. สมชัย ลาภอนันต์นพคุณ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ (สวทช.ภาคเหนือ)
	ชื่อโครงการ	Development of test kit for trans fatty acid in edible oi
	ปีที่ได้รับ	
13	ชื่อนักวิจัย	ผู้ช่วยศาสตราจารย์ ดร.สุภาภรณ์ ครัดทัพ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	
	ชื่อโครงการ	การพัฒนาระบบ FIA/SIA สำหรับบีดอิมมูโนแอสเสย์
	ปีที่ได้รับ	2545-2547
14	ชื่อนักวิจัย	ผู้ช่วยศาสตราจารย์ ดร.สุภาภรณ์ ครัดทัพ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)
	ชื่อโครงการ	การพัฒนาระบบวิเคราะห์ขนาดเล็กสำหรับสารบ่งชี้ทางชีวภาพ
	ปีที่ได้รับ	2548-2550

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
15	ชื่อนักวิจัย	ผู้ช่วยศาสตราจารย์ ดร.สุภาภรณ์ ครัดทัพ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สกว.และสกอ.
	ชื่อโครงการ	ทุนพัฒนาศักยภาพการทำงานวิจัยของอาจารย์รุ่นใหม่
	ปีที่ได้รับ	2548
16	ชื่อนักวิจัย	อ. ศุภชัย ชัยสวัสดิ์ (ผู้ร่วมวิจัย)
	ได้รับรางวัล/ทุนวิจัย	
	ชื่อโครงการ	การสร้างเครื่องมือควบคุมคุณภาพเครื่องล้างฟิล์มอัตโนมัติ
	ปีที่ได้รับ	2546-2547
17	ชื่อนักวิจัย	อ. ศุภชัย ชัยสวัสดิ์ (ผู้ร่วมวิจัย)
	ได้รับรางวัล/ทุนวิจัย	
	ชื่อโครงการ	การออกแบบและสร้างเครื่องมือตรวจสอบคุณภาพลำรังสีประจำวันของเครื่องเร่งอนุภาค
	ปีที่ได้รับ	2550
18	ชื่อนักวิจัย	อ.ดร.นิสา ชวพันธุ์ (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	
	ชื่อโครงการ	การออกแบบและสร้างเครื่องมือตรวจสอบคุณภาพลำรังสีประจำวันของเครื่องเร่งอนุภาค
	ปีที่ได้รับ	2550

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
19	ชื่อนักวิจัย	ดร. รจนา บุระคำ (ผู้ร่วมวิจัย)
	ได้รับรางวัล/ทุนวิจัย	ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ (สกว.)
	ชื่อโครงการ	การพัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลงเพื่อการเตรียมตัวอย่างแบบออนไลน์
	ปีที่ได้รับ	มิถุนายน 2548 - พฤษภาคม 2550
20	ชื่อนักวิจัย	ผศ.ดร.ศิริวรรณ องค์ไชย (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	The Postgraduate Education and Research Program in Chemistry (PERCH)
	ชื่อโครงการ	ผลของไพลต่อเซลล์กระดูกอ่อน
	ปีที่ได้รับ	ตุลาคม 2548 - กันยายน 2550
21	ชื่อนักวิจัย	อ. ดร. วรรณจันทร์ แสงหิรัญ ลี, อ. ดร.ปิยรัตน์ นิมมานพิภักดิ์, อ. ดร. จีรยุทธ ไชยจารุวณิช,อ. ดร. สุคนธ์ ประสิทธิ์วัฒนเสรี,อ. ภัทรินี ไตรสถิตย์ (นักวิจัย)
	ได้รับรางวัล/ทุนวิจัย	ทุนวิจัยพื้นฐานแบบกำหนดทิศทางเคมีทางยา ทุนสกว.
	ชื่อโครงการ	การใช้เทคนิคใหม่ทางสถิติเพื่อหาการยึดจับระหว่างลิแกนด์ และตัวรับ เพื่อการประยุกต์สำหรับการคัดกรองข้อมูลเพื่อค้นหายาอย่างรวดเร็ว
	ปีที่ได้รับ	2548-2551
22	ชื่อนักวิจัย	รักสกุล แก่นเรณู (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/ทุนวิจัย	Partial support from the Postgraduate Education and Research in Chemistry Program (PERCH)
	ชื่อโครงการ	Development of Flow Injection and Seqvential Injection System for the Determination of Tetracycline, Oxytetracycline and Chlortetraoycline
	ปีที่ได้รับ	2546-2548
23	ชื่อนักวิจัย	รักสกุล แก่นเรณู (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/ทุนวิจัย	Royal Golden Jubilee Ph.D. Program (RGJ)
	ชื่อโครงการ	Development of a Coat Effective Micro-Total Analysis System using Lab-at-Valve Approach
	ปีที่ได้รับ	2548-2550

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
24	ชื่อนักวิจัย	ทินกร กันยานี (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/ทุนวิจัย	Full support from The Postgraduate Education and Research in Chemistry Program (PERCH) and Development and Promotion of
		science and Technology Talent (DPST)
	ชื่อโครงการ	Scholarship from the Institute for Promotion of Teaching Science and Technology
	ปีที่ได้รับ	2546-2548
25	ชื่อนักวิจัย	น.ส.กรุณา ใจนนถีย์ (นักศึกษาปริญญาเอก)
	ได้รับรางวัล/ทุนวิจัย	โครงการพัฒนาและส่งเสริมผู้มีความสามารถพิเศษ ทางวิทยาศาสตร์ และเทคโนโลยี: พสวท
	ชื่อโครงการ	การใช้ Chemometrics กับงานทางเคมีวิเคราะห์
	ปีที่ได้รับ	2547-2552
26	ชื่อนักวิจัย	น.ส.สุภาดา คนยัง (นักศึกษาปริญญาโท)
	ได้รับรางวัล/ทุนวิจัย	Full support from the Postgraduate Education and Research in Chemistry Program (PERCH)
	ชื่อโครงการ	Development of Stopped-Flow Injection System for Screening of Thalassemia
	ปีที่ได้รับ	2546-2549
27	ชื่อนักวิจัย	น.ส.นภาพร วรรณาพรม (นักศึกษาปริญญาโท)
	ได้รับรางวัล/ทุนวิจัย	Full support from the Postgraduate Education and Research in Chemistry Program (PERCH)
	ชื่อโครงการ	
	ปีที่ได้รับ	2548-2550

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
28	ชื่อนักวิจัย	รศ.ดร. อรวรรณ ชัยลภากุล (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนเมธีวิจัย สกว.
	ชื่อโครงการ	Development of Boron-doped Diamond Thin film by Anodization
	ปีที่ได้รับ	2547-2550
29	ชื่อนักวิจัย	รศ.ดร. ศุภลักษณ์ ศรีจารนัย (หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานคณะกรรมการวิจัยแห่งชาติ หมวดเงินอุดหนุนทั่วไป มหาวิทยาลัยขอนแก่น
	ชื่อโครงการ	การศึกษาการเปลี่ยนแปลงทางธรณีเคมีของน้ำใต้ดินด้วยการจัดการการแพร่กระจายตัวของดินเค็ม-น้ำเค็มบริเวณลุ่มน้ำพอง-น้ำชี พื้นที่ผลกระทบ
		ท้ายเขื่อนอุบลรัตน์ จ.ขอนแก่น
	ปีที่ได้รับ	2547- 2548
30	ชื่อนักวิจัย	รศ.ดร. ศุภลักษณ์ ศรีจารนัย (ผู้ร่วมวิจัย)
	ได้รับรางวัล/ทุนวิจัย	สำนักงานคณะกรรมการวิจัยแห่งชาติ หมวดเงินอุดหนุนทั่วไป มหาวิทยาลัยขอนแก่น
	ชื่อโครงการ	การศึกษาผลกระทบของการชะล้างเกลือตามธรรมชาติและการผลิตเกลือต่อคุณภาพน้ำในแม่น้ำมูล-น้ำชี
	ปีที่ได้รับ	2546 - 2547
31	ชื่อนักวิจัย	ดร. ดวงใจ นาคะปรีชา (**หัวหน้าโครงการ)
	ได้รับรางวัล/ทุนวิจัย	ทุนเพิ่มขีดความสามารถด้านการวิจัยของอาจารย์รุ่นกลางในสถาบันอุดมศึกษา
	ชื่อโครงการ	การทำไอระเหยเพื่อความจำเพาะเจาะจงในการวิเคราะห์และการออกแบบอุปกรณ์
	ปีที่ได้รับ	กรกฎาคม 2548 ถึง กรกฎาคม 2551

ตาราง 4 นักวิจัยในโครงการที่ได้รับทุนวิจัยอื่นในระหว่างรับทุนส่งเสริมกลุ่มวิจัย		
32	ชื่อนักวิจัย	ดร.วรรณจันทร์ แสงหิรัญ ลี, ดร.ปิยรัตน์ นิมมานพิภักดิ์, ดร.จีรยุทธ ไชยจารุวณิช, ดร.สุคนธ์ ประสิทธิ์วัฒนเสรี, ดร.ภัทรินี ไตรสถิตย์
	ได้รับรางวัล/ทุนวิจัย	ทุนวิจัยพื้นฐานแบบกำหนดทิศทางเคมีทางยา ทุนสกว.
	ชื่อโครงการ	การใช้เทคนิคใหม่ทางสถิติเพื่อหาการยึดจับระหว่างลิแกนด์ และตัวรับ เพื่อการประยุกต์สำหรับการคัดกรองข้อมูลเพื่อค้นหายาอย่างรวดเร็ว
	ปีที่ได้รับ	30 สิงหาคม 2548-28 สิงหาคม2551
33	ชื่อนักวิจัย	ดร.ปิยรัตน์ นิมมานพิภักดิ์
	ได้รับรางวัล/ทุนวิจัย	ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ ทุนสกว.
	ชื่อโครงการ	การศึกษาการจับระหว่างสาร สกัดจากเมล็ดลิ้นจี่กับเอช ไอ วี-1 โพรทีเอส
	ปีที่ได้รับ	31 กรกฎาคม 2547-1 สิงหาคม2549

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล" หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
1 เกตุ กรุดพันธ์	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	หัวหน้าโครงการ	ศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	หัวหน้าโครงการ
2 จรูญ จักร์มุณี	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
3 ปรัชญา คงทวีเลิศ	รศ.	ภาควิชาชีวเคมี คณะแพทยศาสตร์ มช.	นักวิจัย	รศ.	ภาควิชาชีวเคมี คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
4 อรวรรณ ชัยลภากุล	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักวิจัย	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักวิจัยในโครงการ
5 ดวงใจ นาคะปรีชา	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักวิจัย	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักวิจัยในโครงการ
6 พลยุทธ ศุขสมิติ	นักวิทยาศาสตร์	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3	นักวิจัย	นักวิทยาศาสตร์	สำนักงานอุตสาหกรรมพื้นฐานและการเหมืองแร่ เขต 3	นักวิจัยในโครงการ
7 ศุภลักษณ์ ศรีจารนัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักวิจัย	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักวิจัยในโครงการ
8 สุนันทา วังกานต์	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
9 สมชัย ลาภอนันต์นพคุณ	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	นักวิจัย	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช.	นักวิจัยในโครงการ
10 สุภาภรณ์ ครัดทัพ	ผศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	รศ.	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
11 รัตติกาล จันทิวาสน์	นักวิจัย	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มช.	นักวิจัย		Postdoc Louisina State University	
12 นิสา ชวพันธุ์	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัย	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
13 ศุภชัย ชัยสวัสดิ์	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัย	อาจารย์	ภาควิชารังสีวิทยา คณะแพทยศาสตร์ มช.	นักวิจัยในโครงการ
14 ศิริวรรณ องค์ไชย	ผศ.	ภาควิชาเคมี คณะแพทย์ศาสตร์ มช	นักวิจัย	ผศ.	ภาควิชาเคมี คณะแพทย์ศาสตร์ มช	นักวิจัยในโครงการ
15 วรรณจันทร์ แสงหิรัญลี	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
16 จิรยุทธ ไชยจารุวาณิช	อาจารย์	ภาควิชาวิทยาการคอมพิวเตอร์ คณะวิทยาศาสตร์ มช	นักวิจัย	ผศ.	ภาควิชาวิทยาการคอมพิวเตอร์ คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
17 ปิยรัตน์ นิมมานพิภักดิ์	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัย	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล" หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
18 สุคนธ์ ประสิทธิ์วัฒนเสรี	อาจารย์	ภาควิชาสถิติ คณะวิทยาศาสตร์ มช	นักวิจัย	ผศ.	ภาควิชาสถิติ คณะวิทยาศาสตร์ มช	นักวิจัยในโครงการ
19 ทินกร กันยานี *		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	
20 รจนา บุระคำ *		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	-
21 ขวัญจิต มณีอ่อน *		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยทักษิณ	-
22 อรวรรณ ถือเงิน *		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยนเรศวร	-
23 สิริพัชร์ สุธีรภัทรานนท์		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง	
24 สุมาลี ธนิกุล		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี	
25 เสาวภา เมืองแก้ว		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์	
26 สุพรรณี ดวงทอง		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์	
27 ณรงค์ เล่งฮ้อ		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	ผช.กรรมการผู้จัดการ	บริษัท ไทยยูนีค จำกัด	
28 กาญจนา วัดละเอียด*(1,2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง	
29 สราวุฒิ สมนาม*(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยราชภัฏเชียงใหม่	
30 วศิน วงศ์วิไล*		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท	นักวิจัย	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มช.	นักวิจัยในโครงการ
31 วัสกา ศิริอังคาวุธ (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
32 รักสกุล แก่นเรณู (1)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
33 จรูญ จันทร์สมบูรณ์ (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
34 เกรียงไกร พลหงษ์ (1)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล" หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
35 สำอาง ศุภฤกษ์ (1)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
36 สายฝน จันผกา (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
37 กรุณา ใจนนถีย์ (4)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
38 สุภาดา คนยัง **(2,3)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาเอก
39 พิพูน บุญเป็ง* (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	สำเร็จการศึกษาแล้ว
40 ศิลา กิตติวัชนะ **		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		Bristol University, UK	นักศึกษาปริญญาเอก
41 อภิชาต บุญมาลัย*(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	สำเร็จการศึกษาแล้ว
42 ดวงพร โสมประยูร*(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	สำเร็จการศึกษาแล้ว
43 สุภาภรณ์ กสิวัฒน์*		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	สำเร็จการศึกษาแล้ว
44 นภาพร วรรณาพรม**(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก
45 วริศรา คชสิทธิ์*(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		-	สำเร็จการศึกษาแล้ว
46 โสภา ตนตรง(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
47 วิษณุ แจ้งใบ(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
48 วรทิพย์ ศรีเปารยะ(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
49 พรทิวา นันตาบุญ(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่	นักศึกษาปริญญาโท
50 ฐิติรัตน์ มานทิม*		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	สำเร็จการศึกษาแล้ว
51 จันทร์เพ็ญ ครุวรรณ์ *(2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	สำเร็จการศึกษาแล้ว

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล" หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน
52 ณัฐวุฒิ เชิงชั้น*(1,2,5)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกร	สำเร็จการศึกษาแล้ว
53 เบญจมาภรณ์ พรหมทอง* (2)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาโท	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี	สำเร็จการศึกษาแล้ว
54 มะลิวรรณ อมตธงไชย*(1,2,5)		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	นักศึกษาปริญญาเอก	อาจารย์	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	สำเร็จการศึกษาแล้ว
55 วีณา เสียงเพราะ		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	สำเร็จการศึกษาแล้ว
56 วนิดา วอนสวัสดิ์		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาเอก
57 สราวดี กอศรีสกุล		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	นักศึกษาปริญญาโท
58 อภิชัย แสนตลาด		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาเอก		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาเอก
59 จิราภรณ์ โฮมวงศ์		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
60 วรางคณา สุขสม		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
61 ทรงพร จึงมั่นคง		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
62 ณัฐพร บุตรวงศ์		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
63 สุธาสินี บุญเชียงมา		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
64 ปฑิตตา ราชครุฑ		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท
65 จิลาดา วิชาผง		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท		ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น	นักศึกษาปริญญาโท

* นักศึกษาสำเร็จการศึกษาแล้ว

** นักศึกษาที่ศึกษาต่อ

ตาราง 5 รายชื่อคณะผู้ร่วมวิจัยในโครงการวิจัย "การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล" หัวหน้าโครงการ: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

ชื่อ-นามสกุล	เมื่อเข้าร่วมโครงการ			ปัจจุบัน		
	ตำแหน่งวิชาการ	สังกัด	ตำแหน่งในโครงการ	ตำแหน่งวิชาการ	สังกัด	สถานภาพปัจจุบัน

1 = นักศึกษาทุน คปก.

2 = นักศึกษาทุนโครงการพัฒนาบัณฑิตศึกษาและวิจัยทางเคมี (PERCH-CIC)

4 = นักศึกษาทุนโครงการ พสวท.

หมายเหตุ

สังกัด หมายถึง ภาควิชา/คณะ/มหาวิทยาลัย ในกรณีที่อยู่หน่วยงานอื่น ให้ใส่รายละเอียดที่อยู่ต้นสังกัด

สถานภาพปัจจุบัน หมายถึง ยังอยู่ในโครงการหรือไม่ หรือหากออกจากโครงการแล้ว ขณะนี้สังกัดหน่วยงานใด หรือย้ายไปทำงานที่ใด

ภาคผนวก ก

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Some recent developments on cost-effective flow-based analysis☆

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Abstract

This paper reviews some recent developments on cost-effective flow-based analysis. They include the newly developed Lab-at-Valve (LAV), concepts in using the stopped-flow injection approach, on-line sample pretreatment systems, including bead injection–flow injection and flow injection–ion-chromatography, systems for size-based speciation, and cost-effective reagents. Applications and advantages of such techniques are discussed.

Keywords: Lab-at-Valve (LAV); Stopped-flow injection; Sample pretreatment; Bead injection; Sequential injection; Flow injection; Size-based speciation; Cost-effective reagent

1. Introduction

This paper describes some recent developments of techniques aiming for cost-effective analysis related to flow injection analysis (FIA). It is based on a presentation in the 12th International Conference on Flow Injection Analysis (12th ICFIA) including related techniques, which was dedicated to Professor Gary and Mrs. Sue Christian and also for the celebration of the 20th Anniversary of the Japanese Association for Flow Injection Analysis (JAFIA). Some of the developments were initiated and/or resulted from discussion during the previous ICFIAs. Some are from Talanta, which provides good geographical distribution in contributed papers.

Cost-effectiveness is always a consideration in performing an analysis, not only in remote places where only limited budget is available, but also in general practice [1,2]. To select a scheme for an analysis, apart from analytical characteristics of accuracy, precision, sensitivity and selectivity, and real-time analysis, the cost of analysis should also be taken into account (Fig. 1).

When considering cost of an analysis, this should include analysis time, chemical(s)/reagent(s) consumption, and instrument. The last one includes cost of the system itself, operating cost, and cost dealing with maintenance.

Flow-based analytical techniques, which offer various advantages, with the main features being high sample throughputs and much less samples/reagents consumption, and simpler instrumentation, should be suitable to serve the above considerations [2–28].

Flow injection analysis introduced in the early stage by Ruzicka and Hansen, already demonstrated well the cost-effectiveness along with high efficiency in various routine analyses in Brazil [3,4].

Sequential injection analysis (SIA) provides automation, being possible with relatively simple instrumentation, compared to other techniques [4,13,14,29–38].

Systems with cheap components in a remote place may be very valuable in research training, in investigating and demonstrating novel concepts for further development [39,40]. Examples are an overhead projector FIA set-up for demonstrating concepts [41], using an aquarium pump in various simple flow injection (FI) systems [42]: offering practical advantages in sample handling [43] for those with FI–AAS (e.g., the determination of Pb [44,45]), and for in-valve mini-column for determination of ppb-levels of ura-

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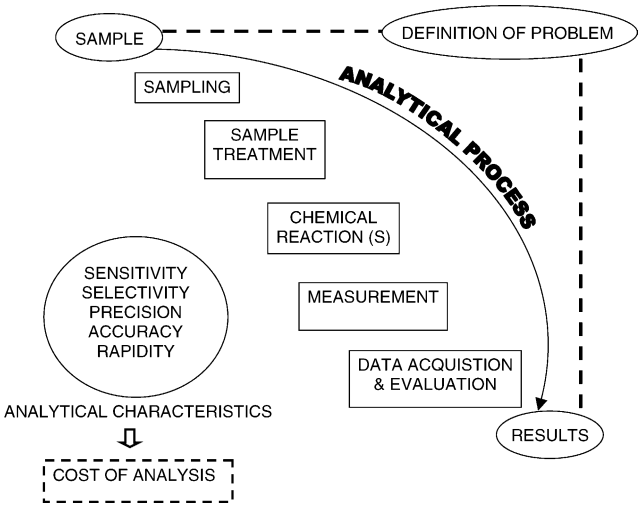

Fig. 1. Analytical process.

nium [46]. Also, such simple systems make single standard calibration possible [44–46]. By applying even simple systems, procedures become much easier, compared to the batch ones, for example, the dissolved oxygen determination by Winkler's method [47,48] and FI with on-line column system for hemoglobin typing leading to thalassemia screening [49].

Simple flow systems with some detection units offer various degrees for automation, depending on the various kinds of components of instruments involved [4,40,50–56], for example, the determination of acid concentration or acidity can be performed colorimetrically using visual detection [41], a simple peak-hold colorimeter [57], or using a very simple conductivity detector [58,59] or a computer-controlled detector [60–63]. Simple FI manifolds with radioactivity detection systems [64,65] offer basic automation using flow-based techniques. Higher degrees in automation and other advantages by employing sequential injection (SI) systems have proven to be useful to real applications for radioactivity monitoring [66–68]. Relatively simple FI and SI set-ups with dynamic surface tension detectors have proven to be very useful and fast for the economical study of some interfacial properties [69,70]. A very simple manifold with a light scattering detector using a laser pointer has been applied for nephelometric determination of sulfate [71]. Various simple FI systems for iodide determination have been reported [72–76].

2. Novel concepts for cost-effective flow-based systems

Some novel concepts have been investigated.

2.1. Sequential injection with "Lab-at-Valve" (LAV) concept

In the past decade, the field of miniaturization in chemical analysis has gained increased interest [77]. The miniaturization toward Lab-on-Chip (LOC) or micro total analysis system (μTAS) concepts includes assembly as an in integrated device, consisting of sample collection, sample pretreatment, and separation and/or chemical reaction taking place for the analytical detection, and the sensor.

Ruzicka [78] introduced a SI with "Lab-on-Valve" (LOV) system to perform analyses in micro- or nanoliter volumes by integrating sample processing, chemical reaction, and monitoring, in a conduit at a multiposition valve. A LOV system for such the above mentioned operation is a precisely fabricated monolithic structure mounted atop a conventional multiposition selection valve, by using computer-aided design (CAD). There have been various applications of LOV with various advantages [4,14,38,53,79–84], for example environmental monitoring [38,82,83], pharmaceutical applications [22], and bio- and clinical analysis [78,84].

A "Lab-at-Valve" concept has been introduced as an alternative cost-effective μTAS device. Instead of replacing a stator plate of a multiposition selection valve by a perfectly machined piece, as that of the "LOV", sample processing and detection unit(s) are attached or plugged onto port(s) of a commercial conventional multiposition selection valve without taking apart any component out of such a purchased valve.

This simpler approach has been demonstrated by SI–LAV potentiometric determination of chloride. A simple LAV flow-through electrode system, consisting of two simple laboratory-made Ag/AgCl electrodes, is plugged onto a port of a purchased multiposition selection valve (Fig. 2). Based on concentration cell behavior, chloride in a sample can be assayed [85].

SI–LAV solvent extraction has been proposed. Such a simple system was demonstrated for spectrophotometric determination of diphenhydramine hydrochloride and anionic surfactant (Fig. 3). Sample, reagents, and organic solvent are sequentially aspirated into a holding coil. By flow reversal, good extraction efficiency can be achieved. After that, the aqueous and organic phases are separated in a conical extraction chamber attached at one port of a conventional multiposition selection valve ("Lab-at-Valve" concept). The organic solution is then pushed into a flow cell of the spectrophotometer for detection of the extracted colored product.

2.2. New criteria for using a stopped-flow injection system

Stopped-flow injection analysis was proposed by Ruzicka and Hansen, for two different purposes for operating a stopped-FIA system: (1) to increase the sensitivity of measurement by increasing the residence time and thus the yield of measured species or (2) to measure a reaction rate serving a base for the analytical read-out. By stopping a flow so that the analyte and reagent(s) stay for a period at a flow-cell [86], progress of a reaction involved is continuously monitored, during the period of the stopped-flow. Various advantages can be obtained by the involvement of kinetic considerations

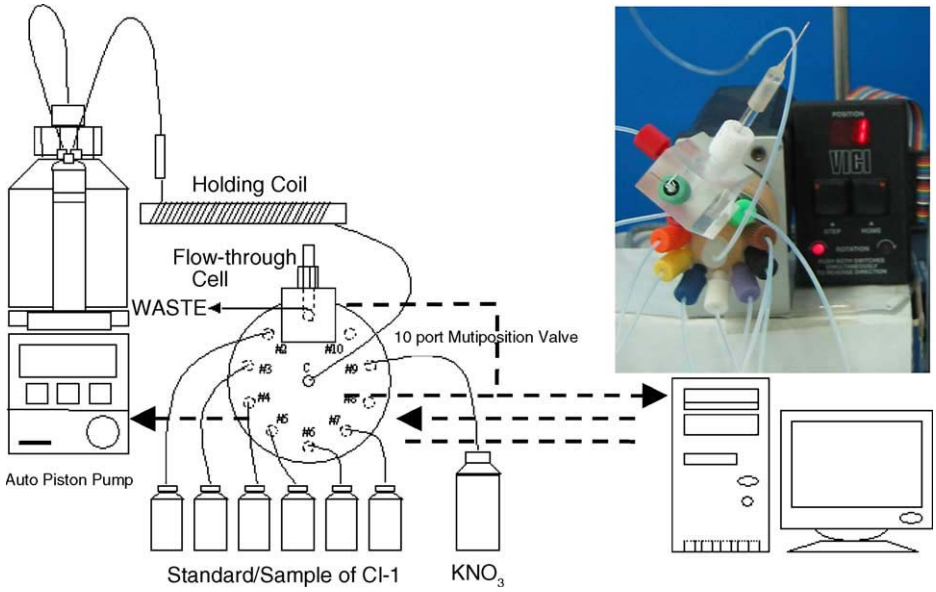

Fig. 2. SI–Lab-at-Valve (LAV) system for potentiometric determination of chloride (the inserted photo shows a close-up of the potentiometric cell (attached to the multiposition valve)).

such as kinetic information, increase in sensitivity, and kinetic separation [87].

A simple semi-automatic stopped-FI analyzer was developed (Fig. 4) [88]. One application example proposed by our group was for the simultaneous determination of phosphate and silicate by employing their differences in kinetic behaviors.

Optimization of such a stopped-FI system involves various parameters, both hardware and non-hardware, similar to the optimization in usual conventional FI systems. Hardware parameters normally are: tubing i.d., sample (loop) volume, mixing coil length and i.d., and flow through cell volume; while non-hardware ones involve: flow-rate, traveling time, stopping time, washing time, and concentration of reagents.

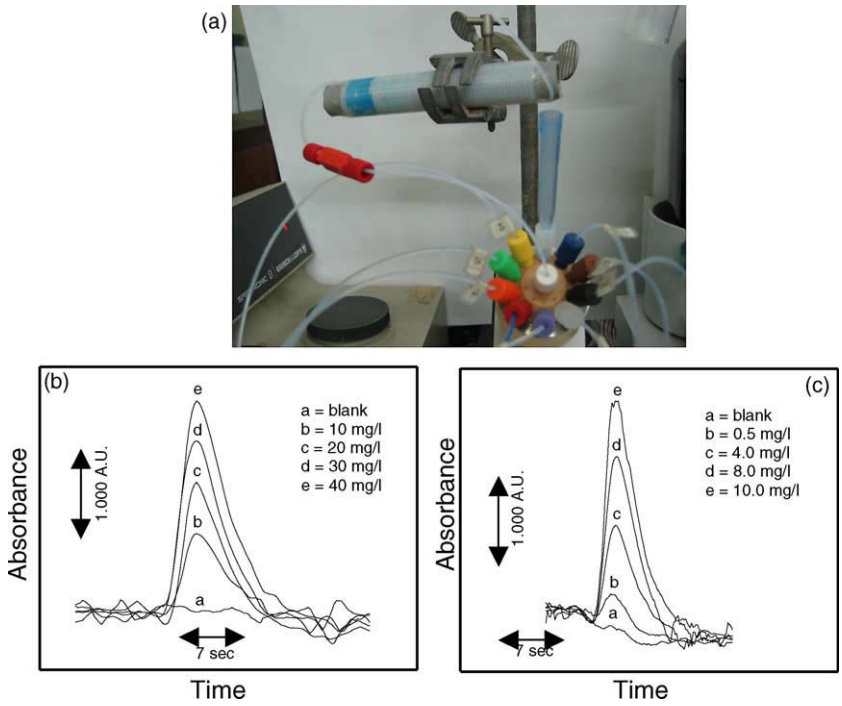

Fig. 3. SI–LAV solvent extraction spectrophotometric determination: (a) the LAV extraction system, (b) signal profiles obtained for the determination of diphenhydramine hydrochloride using bromocresol green [100], (c) SI grams for anionic surfactant determination (the methylene blue method using sodium dodecylsulfate as the standard).

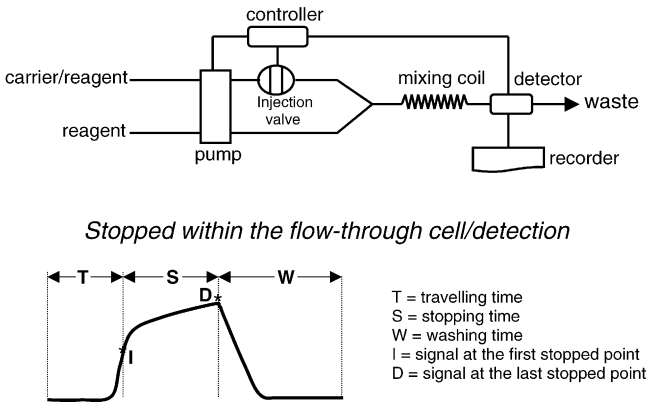

Fig. 4. Conventional stopped-FIA [88]; *T*, traveling time: a period from the injection till the flow stops; *S*, stopping time: a period of flow-stopping; *W*, washing time: a period after the flow is re-started until the next injection.

The optimization is concerned with slope in association with sensitivity (see Fig. 4).

For a conventional FI system, optimization is usually aimed for maximum sensitivity and high sample throughputs. Conventional optimization would involve the hardware parameters: tubing i.d., sample (loop) volume, mixing coil length and i.d., flow through cell volume; and the non-hardware ones: flow-rate and reagent concentration.

Now a concept for stopped-flow injection system has been investigated. By using a semi-automated stopped-flow injection analyzer being able to control pump (on–off) and switching an injection valve, after an analyte solution is injected into a carrier stream of reagent, the flow is stopped at a mixing coil for a period before allowing to flow further to the flow-through cell in a simple colorimeter as a detector. Fig. 5 illustrates the proposed concept of FIA using stopped-flow outside a detection cell. Optimization can be made by varying some parameters such as traveling time, stopping period, flow-rate, and reagent concentrations, without changing any hardware component such as injection loop (injection volume), and mixing coil length and i.d.

Ruzicka and Hansen suggested [86] to stop the flow outside the detector in order to gain reaction time and thereby generation of product to be measured. By our investigation, this would help promotion the mixing, but minimize dispersion of the product. Such a concept was demonstrated for chloride determination (Fig. 6) using mercury (II) thiocyanate and Fe(III) reagents, which were used in the previous conventional FI procedure [89]. Without changing the hardware parameters, but varying only non-hardware parameters, determination of nitrite (using well-known coupling of diazotized sulfanilamide with *N*-(1-naphtyl)ethylenediamine dihydrochloride) and determination of phosphate (using phosphomolybdovanadic acid method) can be performed. This

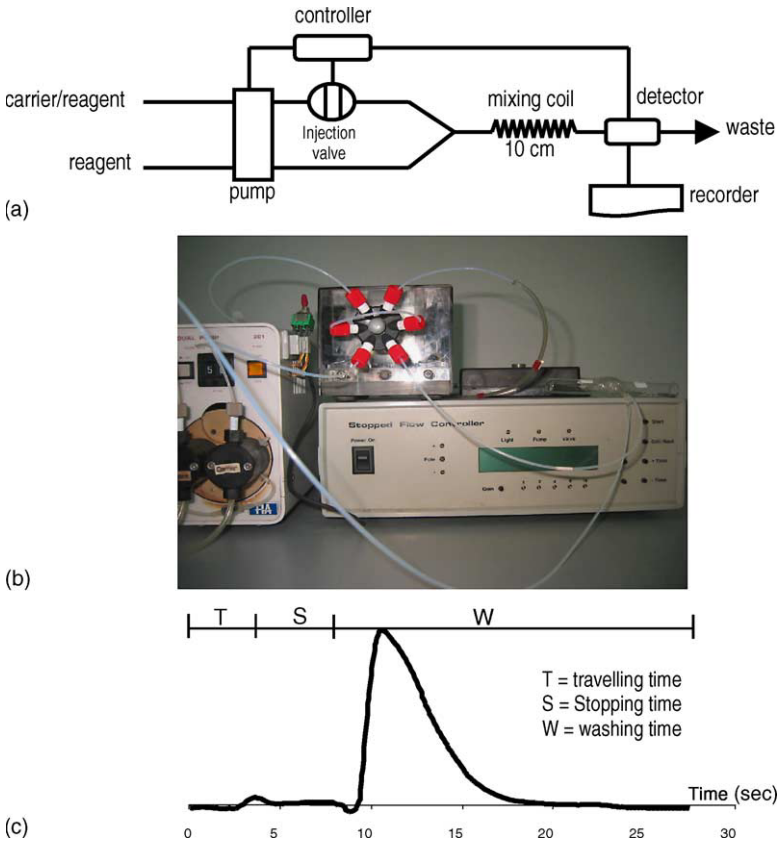

Fig. 5. A new concept stopped-FI: the flow manifold; (a) schematic diagram, (b) the picture, and (c) FI gram profile obtained, when stopping the flow at the mixing coil outside the flow through cell.

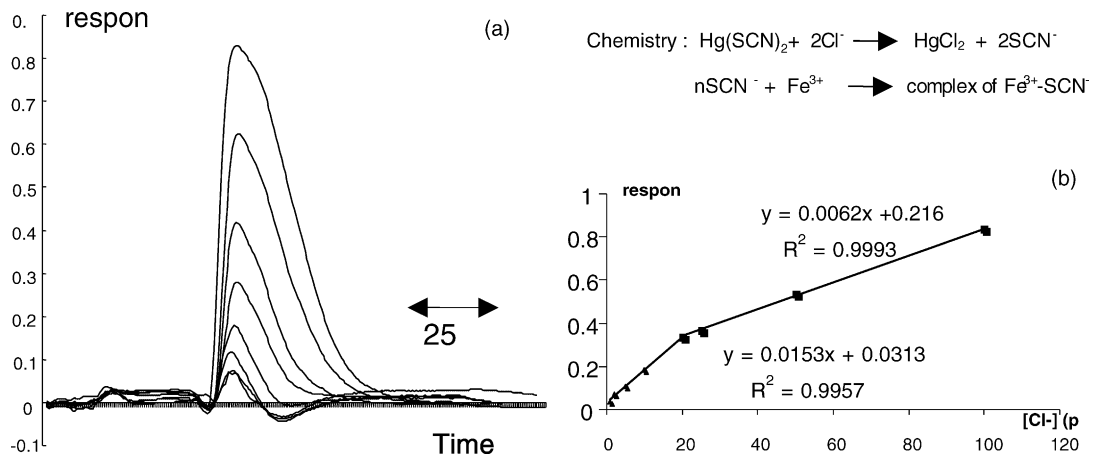

Fig. 6. The determination of chloride using the new proposed stopped-FI concept, by stopping the flow at the mixing coil (T = 1.9 s, S = 1 s, W = 15 s; a throughput of 120 injection/h): (a) FI gram profiles and (b) calibration graph (note: two breaks of the linear calibration ranges may be due to formation of different complexes for higher concentrations of chlorides).

stopped-FI approach offers various advantages, even for a determination involving fast reaction. The advantages include possibility of using only on FI analyzer with fixed components (hardware parameters) for various analytes without changing any hardware component, better sensitivity than conventional FIA, and less amounts of reagents consumption. All lead to economical aspects. This stopped-FI can be used for both slow and fast reactions.

3. On-line sample pretreatment

Sample pretreatment including preconcentration and preseparation, is an important step in analytical process (Fig. 1).

Flow-based techniques offer on-line sample pretreatment to gain advantages in less sample and reagent consumption, and possibility of automation with less sophisticate instrumentation.

Bead injection (BI) was introduced as a technique that utilizes beads as a solid surface to trap species of interest, to accommodate a chemical reaction and to transport them through the flow line or to a detection unit [33]. The renewal beads overcome the difficulty in finding suitable eluent to elute sorbed metal ions from resins, as the used beads will be discarded after each run [90].

3.1. BI with SI

This has proven to be very useful for sample preconcentration and/or preseparation for the determination of heavy metal ions in complicated matrix samples. The BI–SI can be useful to automate sample pretreatment for various types of detectors, including ET–AAS [33,79–84,91].

3.2. BI with FI

This offers an alternative for cost-effective operation for trace levels determinations, such as iron in water samples [92] and in beer samples [90]. Simple instrumentation (without a computer control) and single standard calibration are advantageous.

3.3. FI–ion-chromatograph (IC)

FI system with in-valve mini-column for sample pretreatment coupled to a simple ion-chromatograph with conductivity detector without suppressor for simultaneous determination of some cations (cadmium, lead, and zinc) in zinc ore samples, which have high amounts of matrix interference, has been proposed. The on-line sample matrix interference removals offer advantages including elimination of costly and time-consuming off-line operations, reducing IC column damage (i.e., prolong the column life-time) in addition to the simple instrumentation employed, and the normal advantages usually offered by FIA [93].

3.4. Size-based speciation

Speciation of a substance depending on its size, which has been termed as “size-based speciation”, has gained much of interest in various studies, especially in environmental aspects. Studies on combining field-flow fractionation (FFF) with analytical detection systems have been made. Some cost-effective flow-based systems have been investigated such as gravitational FFF (GrFFF)–FI chemiluminescence for Fe size-based speciation, and GrFFF–ET–AAS for size-based speciation applied to soil sample [94]. Flow FFF (FIFFF)–ICP–MS has been used for size-based speciation of some heavy ions in colloid formation of metal ions released from contaminated soil [95].

4. Cost-effective reagents

Additional advantage that flow-based systems are operated in a closed system (e.g., FIA, SIA), is that an unstable

reagent, especially one being air- or light-sensitive, can be employed.

Also, the chemicals/reagents which are not necessary to be in very high purity grade can be utilized in a flow-based technique since an analyte to be determined is to be run in the exactly the same conditions with that of the standard.

Murexide is a very common chemical in a laboratory but it is unstable in basic solution. However, it can be used in a spectrophotometric determination for Ca by a simple FI set-up [96].

$KMnO_4$, a very cheap and easily available chemical, can be used as an oxidant for an analyte with reducing species properties. Its color intensity change in the redox reaction involved can be used by employing FI [58] or SI [60] systems, whereas a batch procedure is not possible due to that it is sensitive to air and light. Pharmaceutical applications such as ascorbic acid assay in Vitamin C tablets have been reported.

Recently, aspirin, an antipyretic powder, has been proposed as a salicylate reagent, which is cheap and easily available, for the determination of Fe(III). A red Fe(III)–salicylate complex product can be monitored. An application to assay iron contents in pharmaceutical preparations has been made [97].

Solid reactants have been employed as different types of reaction columns for sulfate and sulfur dioxide [98]. Phloroglucinol has been proposed as alternative reagent, which would provide better cost-effective procedures for nitrite and nitrate determinations [99].

5. Conclusion

Sequential injection systems with Lab-at-Valve is an alternative cost-effective micro-total analysis system approach. A stopped-FI analyzer with a new concept in optimization offers the possibility for different analytes to be determined using only one fixed set-up of hardware parameters (i.e., only one instrument), while only non-hardware parameters are to be optimized. On-line sample pretreatment with flow-based techniques, such as FI–IC, BI–FI, and FFF–AAS, offers various advantages. Utilization of cost-effective reagents can be possible by using flow-based techniques, especially FI and SI.

Acknowledgements and proclaims

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Bead injection with a simple flow-injection system: an economical alternative for trace analysis

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The use of beads for sample pretreatment, such as preconcentration, isolation, and separation, and for accommodation of chemical reactions has attracted increasing interest. Beads can be used as a removable solid surface in many analytical applications. It is very practical to use beads in a flow-based system, where beads can easily be moved in and out of the system using a flowing stream of reagent(s). This is called the bead-injection (BI) technique, which was first introduced to be used with a sophisticated computer-controlled sequential injection analysis (SIA) system. In many laboratories, a relatively high-cost SIA system is not available, whereas a simple, low-cost, first-generation flow-injection (FI) analysis system is easy to assemble. The development and applications of the BI technique coupled with a lower cost first-generation FI technique is then an alternative. This simple BI–FI system can be a useful tool for pretreatment of samples that are abundant and when there is no need for micro volume control.

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1. Introduction

Sample pretreatment is often a necessary step in analysis of real samples. Depending on the matrices and the sensitivity of the detection system, the type and the degree of sample pretreatment required will be different. In the flow-injection (FI) technique, continuous flow of solutions in a closed system enables on line sample pretreatment. Even though digestion, unlike other sample pretreatment methods, normally requires heating or harsh conditions, on-line sample digestion is possible with different techniques, such as microwave assisted digestion [1,2], stopped flow digestion [3,4] and on-line destruction of samples using radiation [5], oxidizing agents [6,7] and heat [8]. In some cases (e.g., when using a detection technique that has a relatively narrow dynamic range such as atomic absorption spectroscopy (AAS)), dilution of sample is actually needed prior to passing it to the detector. Since concentrations of samples are unknown, batch-wise dilution by trial and error before performing the analysis becomes very inconvenient. On-line dilution using the FI technique, either by sample dispersion [9] or by flow manipulation [10], helps avoid some of the tedious steps encountered in batch processes. In some cases, dilution can also be useful in extending the calibration working range, such as in the FI dialysis-ion chromatographic system (FID-IC) [11]. More commonly, preconcentration and preseparation are needed for samples with trace amounts of analyte, especially when the detector does not have extremely high sensitivity. Among the many preconcentration techniques available (e.g., column [12–17], precipitation [18,19] and dialysis [20,21]), the use of a column packed with materials that can selectively adsorb or absorb the analyte is probably the most widely employed [22]. The column technique allows the use of a single standard for construction of a calibration graph by varying the loading time [16,17]. This single standard calibration technique provides various flexibilities in operation, including eliminating or reducing prearation of a set of standard solutions, use of glassware and time consumption. Isolation and separation are other processes needed for samples that comprise complex matrices that can interfere in the analysis. Conventional solvent extraction requires large amounts of solvent and produces a lot of waste, and flow-based-column solid phase extraction systems are more efficient [23,24]. Extraction of sample can be carried out in a packed column of sorbant material [25], similar to the preconcentration column.

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However, there is a drawback of the packed column in that the surfaces of the sorbant material need to be regenerated after each analysis. It is very important to get rid of memory effects to ensure accuracy and precision. Even though it is useful to reuse the same column over again and again, the washing step is normally extensive (i.e. time consuming) and may require a strong washing solution [26,27], or, in many cases, a suitable washing solution is simply not available, so a replaceable solid surface becomes interesting in many studies [28]. Microbead particles have been used in drug discovery for a long time. Now that there are many different functionalized beads commercially available together with various detection techniques, the applications of beads for preconcentration and isolation of analyte arise [29–37]. For example, the use of beads in bead-based immunoassays can be accomplished using very small diameter beads (i.e. less than 10 μm) and in different forms, such as in a small-size droplet with microelectrodes [38,39] and in a flow system, such as flow cytometry [40] and spectrophotometry [41]. Size, material of beads and modification of functional groups on the bead surface can be selected according to the applications. In systems where direct measurement of analyte is done directly on the bead surface, the properties of the bead material become very important. Beads should not interfere in the analysis in any way (e.g., beads should not scatter or absorb light in the region where analyte is detected, and they should not be decomposed or have their properties changed in the solvent).

2. Bead injection

The bead-injection (BI) technique is the combination of the use of beads with a flowing stream of solution in a FI system. Beads are utilized as solid surfaces to preconcentrate or extract the analyte or to accommodate a chemical reaction. The flowing stream of solution is used to carry beads through the system. There is no need to regenerate the bead surfaces because they are discarded after each use and are replaced by fresh ones. This helps to reduce the risk of contamination, denaturation, and system fouling, and also makes it possible to operate BI in the continuous flow system even if no suitable eluent for bead cleaning is found possible [42].

Early BI techniques were coupled with the sequential injection analysis (SIA) technique [43]. Beads were retained in a specially designed jet ring cell that let only the solution pass through. SIA was introduced in 1990 by Ruzicka and Marshall [44]. The main features of the system are that it could precisely manipulate forward and reverse flow with micro-volumes of samples and reagents, so the sample and reagent flow path could be minimized between the injector and the detector with appropriate mixing. This is possible by using a multi-port valve and a bi-directional pump with computer control. Sample and reagent zones are drawn and stacked into a holding coil. Zones are penetrated and dispersed into each other by reverse flow on the way to the detector. SIA has an increasingly wide range of applications, such as trace chemical determinations, pharmaceutical studies and food chemistry [45–49]. There are two different forms normally employed. Micro-beads may first be trapped in a flow cell or column and then physically or chemically retain the analyte of interest. Upon eluting with a suitable solvent, the solution of the analyte is detected downstream. There are examples of applications with this form of detection using ETAAS [50,51]. Another form is on-bead detection, where the change on the surface of the beads after interacting with the analyte is monitored directly using the detector located at the flow cell, or by transporting the beads into the detector. There are a number of examples of this form of detection using fiber-optic spectrofluorometry [52], fluorescence microscopy [49], ETAAS [53] and Raman spectroscopy [54].

The SIA system has also been further developed into the lab-on-valve (LOV) system that comprises specially designed micro-channels on a piece of plastic integrated as a part of the multi-channel valve [55–57]. The BI technique can be coupled to the SIA–LOV system and can be used in various applications, such as environmental studies [58], immunoassays [59], and biological studies [60]. Beads can be trapped in an adjustable volume channel on the LOV. Now, a commercially available LOV can be adjusted into two different volumes or path-lengths by moving the optical fiber [61]. Detection can be done directly on the beads with the optical fibers that are made to fit the channel or can be done downstream via the eluted sample. The main advantage of the SIA–LOV–BI system is that the micro-miniaturization of on-line sample pretreatment and detection can be done automatically with various types of detectors [62–64].

3. Bead-injection–flow-injection system

In spite of the outstanding advantages of the SIA system, some laboratories cannot afford such relatively high-cost instrumentation, so a simpler FI technique is desirable. The development of a BI–FI system can be very useful for both general and trace analysis where samples and reagents are abundant and when there is no need of micro-volume control. Assays of some contents of environmental samples, foods and beverages, of which high quantities of samples are available, can be made using a simple FI system.

The BI–FI system can be constructed using common materials that can be found in the laboratory. A simple, cost-effective configuration of the cell and simple,

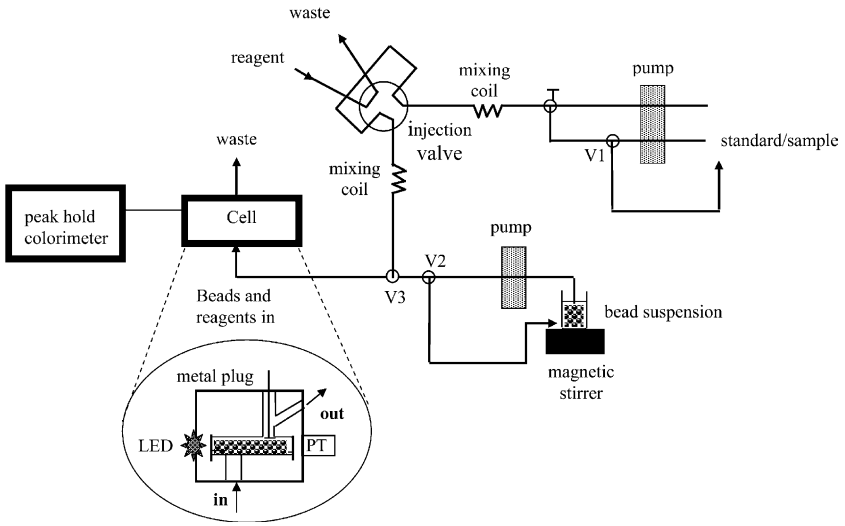

Figure 1. Bead injection with flow-injection system, adapted from Jitmanee *et al.* [65] (V1, V2 and V3 are three-way valves; and, T is a three-way connector).

unidirectional pumps can be used. The cell has the multi-purposes of bead retention, accommodation of chemical reaction (sorption for on-line preconcentration and/or separation and color development) and detection.

Our group has developed an FI–BI system [65], which is depicted in Fig. 1. The bead suspension is pumped into the cell and beads are retained while solution goes through the small space around the metal plug at the end of the cell. When a sample is passed through the cell, an analyte of interest, followed by a color reagent, is loaded onto the surface of the beads, and a color complex is formed. A suitable-wavelength LED and a phototransistor located opposite each other can monitor the intensity of the color complex directly from the surface of the beads. After each run, the used beads can be flushed out by moving the metal plug backward to open the outlet for the beads. The new analytical cycle can then be started. A time-based, single calibration can also be used with this system. There is no need for computer software control. It is also simple to operate and not expensive. The system design and the detector unit can be varied, depending on what components are available.

4. Examples of applications

To demonstrate the application of the BI–FI system, iron was first chosen as a model trace analyte to be used with the system shown in Fig. 1. Chelex-100 resin was used as solid phase and 1,10-phenanthroline was the color reagent used to form a red complex with Fe^{2+} that can be detected at 520 nm. The iron content of various types of water samples (tap, pond and drinking) were successfully determined with the proposed system. The lowest detectable concentrations of Fe^{2+} were 0.90 and 0.45 μmol/L for 3 and 5 min sample loading times, respectively. Both Fe^{2+} and Fe^{3+} were spiked for recovery studies. Fe^{3+} was reduced to Fe^{2+} with ascorbic acid before analysis. Total iron was found as 100–110% recoveries [65].

The application of the system was also extended to determine iron in a complicated sample, such as beer [42]. Even though an interference effect from tannic acid in beer was found, the results from the proposed BI–FI system agreed in all cases with the results obtained from the AOAC standard batch FAAS method and the FI–AAS within the tolerance limit of beer analysis when standard addition was performed.

The sampling rate was 10–20 analyses per hour, depending on sample loading time. The detection limit can be lowered by increasing sample loading time with, however, sacrifice of sample throughput. A precision of 4% RSD was achieved.

Another model trace element, chosen to express sensitivity and selectivity of the system, was copper. In the presence of other elements and matrices, determination of copper in swimming pool water and mineral supplement tablets was achieved with this system [66].

The FI–BI technique can be applied to analyze other ions in different samples by selecting suitable surface modified beads, color reagents and buffers [65–67].

Currently, the number of works published on FI–BI is far fewer than on SIA–BI. The main reason is possibly because SIA–BI was developed with high degree of automation and many applications were demonstrated by the developers to confirm the extended use of a commercial SIA system. However, the FI–BI system also

has potential as an alternative for versatile use in sample pretreatment and as a form of detection, similar to the SIA–BI system. The simpler, lower cost FI–BI system, having no fully automated computer control system, can be advantageous for many laboratories that do not have an SIA system. FI–BI should therefore be used more in the future, and many BI applications described above should be readily adapted to this simple system.

5. Important criteria

In the BI technique, the uniformity and the homogeneity of beads is very important for good precision. Beads should be sieved for suitable sizes and the bead suspension should be kept stirred at a fixed rate at all times of the analysis. The quantity of beads loaded into the cell or the bead loading time need to be optimized in line with the dimension of the cell. Excess beads outside the detection window will adsorb sample and reagent but cannot be detected, and that can yield false negative results, but too few beads cause space in the cell where light scattering may occur or color intensity measurement may not be effective. The concentration of the color reagent also needs to be sufficient for the range of sample concentrations. Bead- and sample-loading times for each analysis should be constant. Other important general guidelines for system design and operation involving the use of column sorption should be followed [22] (e.g., delivering sample by pumping it into the column located down stream is recommended instead of suction of the sample through the column). Tubing connecting from the pumps should be as short as possible to save reagents and to minimize the possibility of sample and reagent sorption on the tubing wall. Flow reversal should be considered to avoid tightening the column packing that may cause back pressure.

6. Conclusion

The BI technique can be used with the first-generation FI technique for successful trace-ion determination. The system is simple and economical without the need of computer technology. Contamination of samples from outside environment and from a previous run is very minute because it is a closed system, with a bead-discarding step done before a new analysis. It is suitable for the analysis of samples that are abundant and do not require micro volume control, such as environmental, food and beverage samples. The applications of the system can be extended using the suitable combination of surface-functionalized beads, buffer solutions and color reagents.

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Cost-effective flow injection spectrophotometric assay of iron content in pharmaceutical preparations using salicylate reagent☆

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Abstract

A new flow injection procedure for an assay of Fe(III) by using salicylate obtained from antipyretic powder, which is a cheap and easily available reagent, is proposed. A red complex was continuously monitored by a laboratory-made green LED colorimeter. A linear calibration was obtained in the range of 1–20 mg Fe l^{-1} with a detection limit of 0.5 mg Fe l^{-1} and R.S.D.s of 1.4–5.4% ($n = 3$, for 1–20 mg Fe l^{-1}). The new procedure was applied to assay iron contents in pharmaceutical preparations. The results were in good agreement with those of the USP standard method.

Keywords: Drugs; Flow injection; Iron; Salicylate; Aspirin

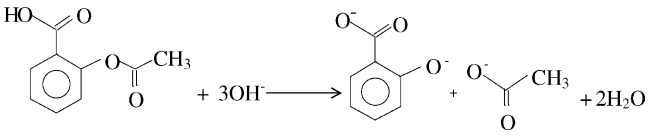
1. Introduction

Iron is a mineral essential to the human body. The average adult has 4–5 g of iron, of which 60–70% is present as heme in the circulating haemoglobin. The human body loses 0.5–1.5 mg iron per day, and sufficient amounts should be ingested (12 mg for adults, 15 mg during pregnancy and lactation and for adolescents, and 7.5–10.5 mg for children, rising to 13.5 mg in 11–14 years old group) [1]. Iron deficiencies are particularly common in premenopausal women, and even college-age women should pay particular attention to the amount of iron in their diets [2].

There are many methods for iron determination in pharmaceutical samples. Of these methods, USP and BP have served as the standard methods for a long time. However, both of the methods are based on a titration technique, which is tedious and involves many steps with consumption of large amounts of chemicals.

Spectrophotometric FI systems have been reported by using different color agents such as 1,10-phenanthroline [3,4], thiocyanate [5], Tiron [6] and 2,2-dipyridyl-2-pyridylhydrazone [7]. The last one was applied for the determination of iron in pharmaceutical samples.

Iron(III) was proposed to be a color agent for a batch spectrophotometric determination of acetylsalicylic acid in aspirin [8,9]. Acetylsalicylic acid in aspirin is hydrolyzed in an alkaline solution to yield salicylate dianion, as represented in Eq. (1).



(1)

Acidification, followed by addition of iron(III) ion, yields a soluble tetraaquasalicylatoiron(III) complex. The intensely purple solution exhibits a strong absorption at 520 nm [9]. In the presence of excess iron, the following complex is formed [9]:

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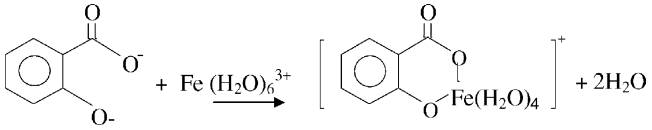

(2)

But in the presence of excess salicylic acid, as here, the iron can be present as the $Fe(III)(sal)_3$ chelate in solution. The absorption maximum was at 520 nm.

We have investigated the use of such reactions for a simple FI system to assay the iron content in pharmaceutical preparations using cheap salicylate reagent obtained from aspirin. This is a less expensive source of salicylic acid than ACS +99% grade salicylic acid.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade, unless otherwise stated. Deionised water (Milli Q, Millipore) was used throughout. A stock solution of 1000 mg l^{-1} Fe(III) was prepared by dissolving 0.8774 g of ferric sulphate in a portion of water. Concentrated sulfuric acid (0.5 ml) was added before making up to a volume of 100.00 ml. Working Fe(III) standard solutions were obtained freshly by appropriate dilutions of the intermediate 100 mg l^{-1} Fe(III), obtaining from the stock solution. A salicylate reagent solution (0.01 M) was prepared by dissolving 1.20 g aspirin drug (Tumjai, Osodsapa, commercially available in a drug store in Thailand) in 20 ml of 2 M sodium hydroxide. The solution was placed in a boiling water bath for 15 min before making to a volume of 500 ml with water.

2.2. FI manifold

A laboratory-made flow injection analysis system was employed. The system consisted of a peristaltic pump (Ismatec, Switzerland), a six port injection valve (Upchurch, USA), a home-made flow through cell, a controller and a recorder as shown in Fig. 1. The green LED and the photodiode were used as light source and sensor, respectively. The controller was a laboratory designed and assembled device with microprocessor and was used to control the operation of the pump and injection valve, and to collect and to transfer the data to a recorder.

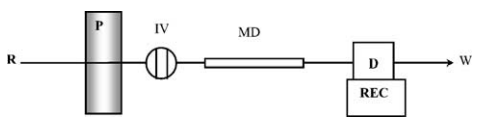

Fig. 1. FI manifold for determination of iron using salicylate: R: 0.01 M salicylate solution, P: peristaltic pump, IV: six-port injection valve, MD: mixing device, D: detector, REC: recorder, W: waste.

A series of Fe(III) standard solutions was injected into the salicylate reagent stream via an injection valve. The complex formed was continuously monitored. Calibration was made by plotting peak height versus Fe(III) concentration.

2.3. Assay of iron in pharmaceutical preparation samples

Pharmaceutical samples were taken from local drug stores. Sample preparation was adapted from AOAC 977.30 [10] as follows. Twenty tablets of a drug sample were weighed and powdered. A portion of the powder, equal in weight to the average of one tablet, was accurately weighed and transferred to a 250 ml flask. A 50 ml portion of water and 2 ml conc. hydrochloric acid were added. The mixture was boiled on a steam bath for 30 min before cooling to room temperature and was diluted with water to a volume of 100.0 ml. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask, and 0.5 ml of hydrogen peroxide was added before making to a volume of 50 ml with water. The samples were then analyzed by the proposed method.

3. Results and discussion

3.1. FI set optimization

A simple one-line manifold FIA system for iron determination using salicylate is depicted in Fig. 1. The parameters, namely, concentration, flow rate and pH of the salicylate reagent solution, mixing coil type and length were investigated. The injection volume (40 μl) and Fe(III) standard (0.5 mg Fe l^{-1}) were fixed for all studies.

Various concentrations (0.01, 0.05, 0.1 and 0.5 M) of salicylate were tried by using a flow rate of 4.0 ml min^{-1} and a mixing coil length of 100 cm. The higher the concentration of salicylate, the higher was the peak obtained. The Schlieren effect was pronounced when salicylate concentration was 0.05 M or more. Using a 200 cm mixing coil resulted in a similar observation. The mixing coil was replaced by a glass bead column (0.3 mm glass beads packed in 0.3 mm i.d. × 5 cm Tygon tube, in which both ends were plugged with 0.3 mm o.d. × 2.0 cm Tygon tubing for the connection to the system tubings) as a mixing device to promote better mixing [11]. A flow rate of 8.8 ml min^{-1} instead of 4.0 ml min^{-1} was tried, but a lower peak was obtained (0.68 cm instead of 0.75 cm).

A set of conditions was then selected: 0.01 M salicylate reagent, 40 μl injection volume, a glass bead column with the dimension described above and a flow rate of 4 ml min^{-1}.

3.2. Calibration

Using the selected conditions, a linear calibration was obtained in the range of 1.0–20.0 mg l^{-1}: peak height (cm) = 0.4618[Fe(III)(mg l^{-1})] + 0.1627, r^2 = 0.9997. Relative

Table 1
Assay of iron contents in pharmaceutical preparation samples by the proposed FI and USP method [13] (average of triplicate results)

Sample no.	Form of iron	Label (mg per tablet)	USP method		The proposed FI method			
					Modified AOAC 977.30 (HCl alone)[a]		Strong acid digestion (HNO_3 + $HClO_4$) with HCl[a]	
			$mg\,g^{-1}$	mg per tablet	$mg\,g^{-1}$	mg per tablet	$mg\,g^{-1}$	mg per tablet
1	Fumarate	200	459	221	455	219	443	213
2	Fumarate	90	67.7	90	69.8	93	72.0	96
3	Fumarate	200	446	214	436	209	481	231
4	Fumarate	200	390	197	376	190	395	200
5	Fumarate	400	424	415	405	396	400	391
6	Fumarate	200	352	194	355	196	375	208
7	Gluconate	200	509	330	296	192	330	213
8	Gluconate	150	231	161	205	157	179	138
9	Sulphate	200	322	149	438	202	444	205
10	Citrate	470.9	1094	604	688	380	909	502

[a] Sample preparation procedure

standard deviations ($n = 3$) over this range (1–20 $mg\,l^{-1}$) varied from 1.4–5.4%. A detection limit (3σ of the blank signal [12]) of 0.5 $mg\,l^{-1}$ was estimated.

3.3. Application to pharmaceutical preparations

Pharmaceutical samples available in local drug stores were taken. They were in forms of fumarate, gluconate, sulphate and citrate. The sample preparation (Modified AOAC 977.30 procedure) described in the experimental section was employed. The obtained solution was analyzed following the proposed FI procedure. The results are summarized in Table 1.

Another sample preparation procedure with very strong acid digestion was employed for comparison and to confirm that iron was converted into iron(III) and dissolved in the solution. The procedure is as follows. Twenty tablets of a drug sample were weighed and powdered. A portion equal to the weight of one tablet was transferred to a 250 ml flask. A 25 ml volume of water, 15 ml conc. nitric acid and 5 ml conc. perchloric acid were added into it. The mixture was boiled on a hot plate until nearly dry, then cooled to room temperature before adding 4 ml conc. hydrochloric acid and 10 ml water and making to a volume of 100 ml with water. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask and diluted to the mark with water. It was found that the results from the both sample preparation procedures for FI were in good correlation (Table 1), except for the citrate form (sample #10). This indicates that iron in the sample can be quantitatively converted into free Fe(III) by the sample preparation procedure using HCl and H_2O_2 in place of HNO_3 and $HClO_4$ with HCl.

The USP method was employed as a reference for validating the proposed FI method. The procedures followed the USP 24 [13] for the drug samples in the forms of fumarate, gluconate and sulphate. The sample in citrate form was also analyzed by following the USP procedure for sulphate since there is no USP method for the citrate form. It was found that the results from the proposed FI methods using both sample preparation procedures agree with that of USP method as seen from the t test ($t = 1.37$ for that of the sample preparation procedure with HCl and H_2O_2 and $t = 1.44$ for the procedure using HNO_3 and $HClO_4$ with HCl at the 95% confidence level). And there was no significant difference between the two procedures for sample preparation ($t = 0.08$ at 95% confidence level). However, although the results from the t test indicated that there were no significant differences between the methods, it can be seen from Table 1 that the milligrams per tablet obtained by the USP method for sample numbers 7–10, of which iron contents are in the forms of gluconate, sulfate and citrate, differed from that of the labels, whereas that of the proposed FI method were practically the same, except for sample number 10 (citrate form). The errors from the USP method for gluconate forms may be from zinc dust (getting through the filter paper in a step to convert Fe(III) to Fe(II) [13]), which may be contaminated in the solution and which made the values higher than labeled. For the sulphate form, the results are lower than the labeled, and this may come from the long time of the filtration step, during which some of Fe(II) may be oxidized to Fe(III). For the citrate form, using FI, both the procedures for sample preparation are not suitable and the USP procedure for sulphate is not suitable for citrate form. However, the proposed method can be well applied to the samples in which the iron contents are in the forms of fumarate, gluconate and sulphate. Further study in more detail in such cases should be made.

This study demonstrates that the proposed FI procedure is simple. Other advantages gained, in comparing to the USP method, include economics (simple FI system, cheap

and readily available reagent, simpler sample preparation), less risk from toxic and hazardous chemical wastes. The procedure offers feasibility in automation.

4. Conclusions

A new cost-effective FI spectrophotometric assay of iron in pharmaceutical preparations (in a form of fumarate, gluconate or sulphate) is proposed. Salicylate is obtained from alkaline hydrolysis of the antipyretic drug, aspirin, which is cheap and easily available as reagent. Various advantages can be benefit from the proposed FI procedure, especially in some remote places.

Acknowledgements

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Flow injection in-valve-mini-column pretreatment combined with ion chromatography for cadmium, lead and zinc determination

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Abstract

A flow injection (FI) in-valve-mini-column packed with Chelex-100 resin is proposed for on-line sample pretreatment for some metal ions, namely, Cd(II), Pb(II) and Zn(II), prior to simultaneous determination using ion chromatography (IC). A solution containing a mixture of the cations was first passed through the in-valve-mini-column, followed by on-line elution. The eluate was then flowed further to an injection valve and was injected into an ion chromatograph. Conditions of the system were optimized. A single standard calibration was possible. The recoveries of cations were found to be in the range of 95–105%. The developed method was applied to the accurate analysis of zinc ore samples.

Keywords: Sample pretreatment; In-valve-mini-column; Ion chromatography; Flow injection; Ores; Cadmium; Lead; Zinc

1. Introduction

Sample pretreatment is necessary when the analytical method cannot provide good separation and quantification due to interferences from sample matrix components [1]. Sample preparation has been a growing area in chromatography over the past several years, since samples containing interfering substances can affect chromatographic performance. These substances may mask the peak of interest or irreversibly retain on the analytical column, permanently damaging the column. To eliminate these problems, such samples need to be treated before injection [2]. A common problem in ion chromatography is poor resolution of an analyte ion from matrix ions that are present in relatively high concentrations [3–9]. Post column derivatization with a color reagent such as 4-(2-pyridylazo) resorcinol (PAR) or 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) for visible spectrophotometric detection [10–13] has been used for good sensitivity and stable baselines. One of the most important aspects of developing an IC method is the ability to recognize when undesirable chromatographic effects are derived from sample matrix interferences. Sample matrix effects can include shortened retention times, poor peak efficiency, poor resolution, poor reproducibility and irregular baseline [1].

A flow injection (FI) system can be used to introduce samples into a detector such as atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP–AES), inductively coupled plasma-mass spectroscopy (ICP–MS) and ion-selective electrode (ISE) and

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to enhance the pretreatment step. FI systems with on-line sample pretreatment are very beneficial. Automation of sample pretreatment and manipulation within the manifold have increased sample throughput and decreased the potential for sample contamination [14,15]. These include, for examples, column retention, gas diffusion, and solvent extraction [16,17]. The on-line column in a FI system could be used for sample pretreatment for complicated matrix samples prior to the determination step.

In the present work, a simple flow injection system with an in-valve-mini-column has been coupled with a simple ion chromatograph (IC) with a conductivity detector without suppressor, for on-line sample cleaning or pretreatment for the determination of Cd, Pb and Zn in a complicated matrix sample such as in a zinc ore sample, prior to their determination. An additional advantage of prolonging the life-time of the IC column is also gained.

2. Experimental

2.1. Standard solutions

Standard solutions of each cation were prepared by appropriate dilution of a stock standard solution (1000 mg l^{-1}, Merck, Germany). Ultrapure water (resistivity 18 mΩ cm^{-1}) was produced by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Buffer solutions and eluents

For the FI system, ammonium acetate buffer solution (1.0 M, pH 5.4) was prepared by mixing 57 ml of glacial acetic acid (99.8% (w/v), Merck, Germany) and 75 ml of 25% ammonia liquor (25% (w/v), Merck, Germany) and diluting to 1000 ml. Sodium citrate buffer solution pH 3 was prepared from reagent-grade chemical obtained from Merck (Germany). The eluent was 2.0 M HNO_3 (65% (w/v) HNO_3, Merck, Germany).

For the IC system, a mixture of tartaric acid and oxalic acid was employed as the eluent. The eluent concentration optimized for the best resolution was: 3 mM tartaric acid/1 mM oxalic acid.

All solutions were degassed using an ultrasonic bath before use.

2.3. FI in-valve-mini-column

Chelex-100 (sodium form, 50–100 mesh; Bio-Rad Laboratories) was packed in a mini-column, made of acrylic tubing (3 mm i.d. × 2 cm), similarly described in [16]. Teflon frits were placed at each end of the column to prevent the loss of the resin when a solution passed through the column, as shown in Fig. 1. The two ends of the column were connected to a six-port injection valve by replacing a sample loop of the valve.

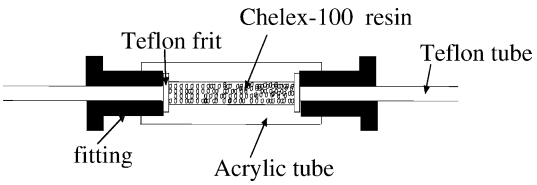

Fig. 1. Ion exchange mini-column.

2.4. Instruments

2.4.1. FI system

The FI system consisted of two peristaltic pumps (MP-3, EYELA, Tokyo Rikakikai, Tokyo, Japan and Ismatec, Glattbrugg-Zurich, Switzerland), and a Rheodyne (model 7725i) injection valve with in-valve-mini-column.

2.4.2. Ion chromatograph

The ion chromatographic system without chemical suppression (Metrohm Ltd., Switzerland) used this study consisted of a Metrohm isocratic pump, a six-port valve with 20 μl sample loop, and a Metrohm 732 conductivity detector (without suppressor). The analysis column used for the cation separation was a universal cation column (4.6 mm × 100 mm; Altech, USA). The column was packed with polybutadiene-maleic acid (PBDMA) coated on silica material. The chromatograms were recorded and handled with Metrohm software (Metrohm Ion analysis, Metrohm Ltd., Switzerland), which was also used for controlling the pump and injection valve.

2.5. Procedure

The FI–IC manifold (Fig. 2) operation was controlled by two injection valves under manual control for timing as indicated in Fig. 3. Standard/sample solution was mixed with buffer to adjust the pH for adsorption of cations on the resin, and flowed through the column with the FI-valve in the load position, with various loading times, while the unretained cations were passed to waste. After that the valve was switched to the injection position and 2.0 M HNO_3 was flowed through the mini-column to elute cations from the column. The eluate was neutralized with 2.0 M NaOH and then controlled for pH with citrate buffer. When the elapsed time was 24 s after switching the FI valve to the injection position, the zone of cations was moved into the sample loop and was injected into the IC via the IC valve.

2.5.1. Preparation of zinc ore samples

A portion (0.1 g) of a zinc ore sample was accurately weighed and digested with concenterated nitric acid (25 ml) by heating on a hot plate until it became clear (about 4 h). It was filtered and transferred into a 250 ml volumetric flask and made to the volume with 1% nitric acid.

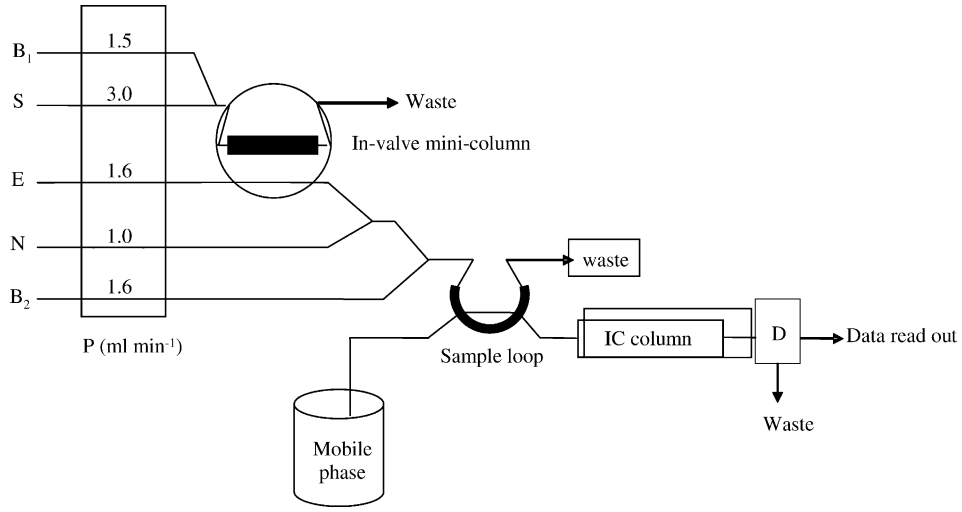

Fig. 2. Manifold of FI–IC combination; P: peristaltic pumps; B_1: ammonium acetate buffer; B_2: sodium citrate buffer; S: sample or standard solution; E: 2.0 M nitric acid; N: 2.0 M sodium hydroxide; mobile phase: mixture of tartaric acid and oxalic acid; sample loop: 20 μl loop of IC; IC column: universal cation column 100 mm × 4.6 mm; D: conductivity detector (without suppressor).

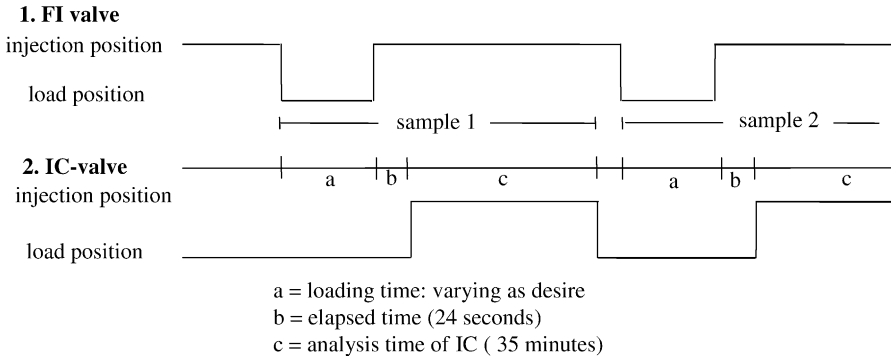

Fig. 3. Schematic diagram of timing control for operation of valves in the FI–IC system.

3. Results and discussion

3.1. Ion chromatography

The conditions, mobile phase concentration, and flow rate of mobile phase for ion chromatographic separation were optimized. Results are summarized in Table 1 and Fig. 4(a)–(d). For the flow rate studies (1.0, 1.5 and 2.0 ml min^{-1}), the lower the flow rate employed, the better sensitivity obtained, but a lower flow rate resulted in longer analysis time, as expected. Chromatograms of a blank solution and of cations obtained from conventional ion chromatography are shown in Fig. 5(a) and (b), respectively. Milli-Q water was used as a blank solution and a mixture of cations containing 3 mg l^{-1} Cu, 3 mg l^{-1} Zn, 5 mg l^{-1} Cd and 10 mg l^{-1} Pb in 20 μl was injected directly into the ion chromatograph with a mobile phase flow rate of 1.5 ml min^{-1} and an injection volume of 20 μl. In the chromatographic separation similar to a previous report [8], a mixture of 3 mM tartaric acid/1 mM oxalic acid was used as the mobile phase since this organic acid eluent can separate Cd, Cu, Pb and Zn on the PBDMA stationary phase, which is polybutadienemaleic acid coated on silica material of the

Table 1
Concentrations of tartaric acid and oxalic acid

Concentration (mM)		Retention time (min)				Total analysis time (min)	Comments
Tartaric acid	Oxalic acid	Cu	Zn	Cd	Pb		
3.0	2.0	1.1	4.4	11.8	12.9	17	Bad resolution of Cd and Pb (Fig. 4a)
1.5	2.0	1.1	5.2	15.4	15.4	20	Co-elution of Cd and Pb (Fig. 4b)
3.0	1.5	1.4	6.5	17.0	20.5	25	Good resolution of all cations (Fig. 4c)
3.0	1.0	1.9	9.4	23.0	31.3	35	Good resolution of all cations (Fig. 4d)

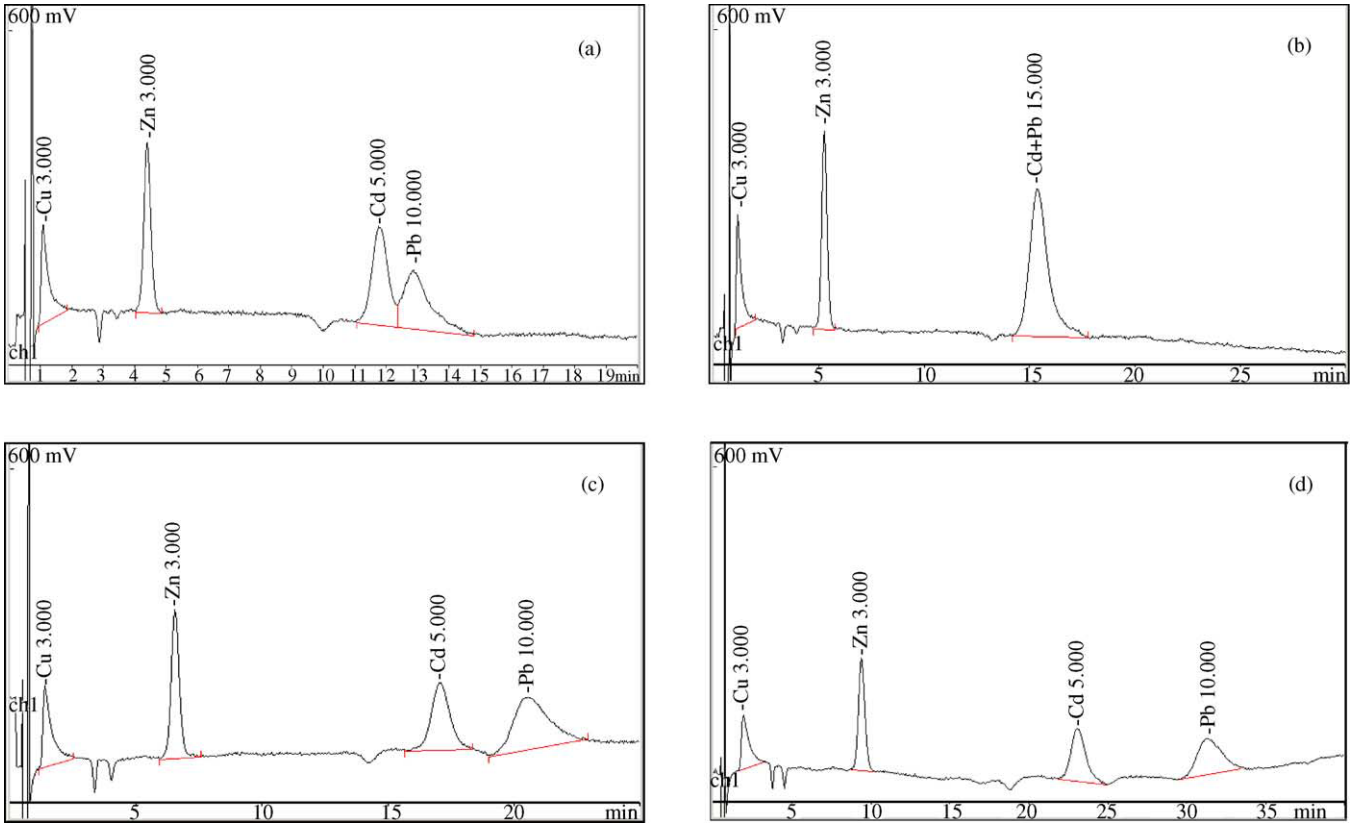

Fig. 4. Chromatograms of cations in Milli-Q water at different concentrations and ratios of tartaric acid and oxalic acid as a mobile phase: (a) 3 mM tartaric acid/2 mM oxalic acid, (b) 1.5 mM tartaric acid/2 mM oxalic acid, (c) 3 mM tartaric acid/1.5 mM oxalic acid and (d) 3 mM tartaric acid/1 mM oxalic acid. The values on the peak tops are concentrations (mg 1–7).

universal cation column. The retention time of the cations of interest in the presence of the mobile phase followed the order Cu < Zn < Cd < Pb, and the separation was achieved within 35 min at a flow rate of 1.5 ml min^{-1}.

3.2. Flow injection-ion chromatography

Using the FI–IC system and optimal conditions of the FI system in Table 2, the results in Fig. 6a show the chromatogram of a blank solution obtained on-line from the FI system to the ion chromatograph. A blank solution (without cations) was loaded on the in-valve-mini-column and followed by the on-line elution process with 2.0 M HNO_3 for FI operation. The eluate acid solution from the FI system was neutralized with 2.0 M NaOH and the pH controlled with sodium citrate buffer solution before flowing into the IC injection valve, because highly acid solution will affect the analytical column. The baseline of the blank solution in the chromatogram exhibits a large, early elution peak, which is that of a high concentration of unretained Na^+ ion. Because the conductivity of Na^+ ion is lower than that of H^+ ion, a baseline drift occurs from the change of the Na^+/H^+ ratio leading to stepwise fashion at the about 20–25th and 25–27th min.

Table 2
Proposed conditions for FI system with in-valve-mini-column

Parameters	Conditions
Buffer for adsorption process	1.0 M ammonium acetate buffer pH 5.4
Loading flow rate	3 ml min^{-1}
Eluent	2.0 M Nitric acid
Eluent flow rate	1.6 ml min^{-1}
Base for neutralized eluate	2.0 M sodium hydroxide
Buffer for eluate	Sodium citrate buffer pH 3

In Fig. 6b, the chromatogram of a mixture of cations (Cd, Cu, Pb and Zn) was obtained by using the same conditions as for the blank. From the chromatogram, the high conductivity of the unretained Na^+ ion causes a large peak and interferes with the Cu peak (t_R = 1.4 min).

Reproducibility tests were carried out for estimating the relative standard deviation (R.S.D.) of the chromatographic peak areas. This data was obtained from five repeated injections at a loading time of 40 s for a concentration of 1 μg ml^{-1} of all interested cations. Average R.S.D. values of 3.1, 3.5 and 5.1% for Zn, Cd and Pb, respectively, were obtained.

Calibration plots, with various loading times, were constructed with single standard calibration by using a plot of peak area (*y*-axis) and micrograms of cation loaded (*x*-axis). The microgram of cation was calculated by concentration (μg ml^{-1}) of a standard solution used × loading flow rate

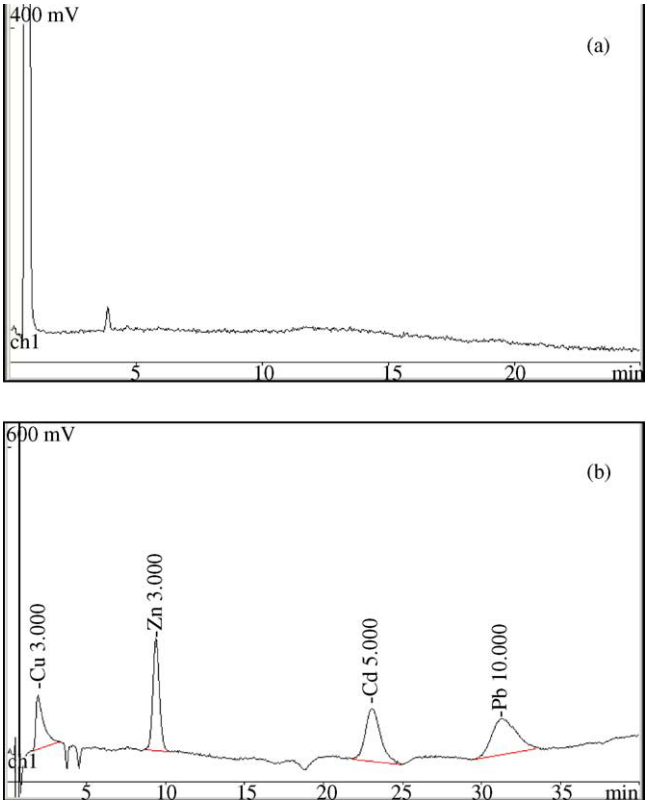

Fig. 5. Chromatograms: (a) blank solution without cations using Milli-Q water (b) cations (Cd = 5 mg l^{-1}, Pb = 10 mg l^{-1}, Zn = 3 mg l^{-1}) in Milli-Q water by direct injection into ion chromatograph without FI system. Peaks: Cd (t_R = 24.1 min), Pb (t_R = 32.5 min) and Zn (t_R = 9.8 min); column: universal cation (100 mm × 4.6 mm); eluent: 3 mM tartaric acid/1 mM oxalic acid; flow rate 1.5 ml min^{-1}; injection volume: 20 μl; detector: conductivity without suppressor.

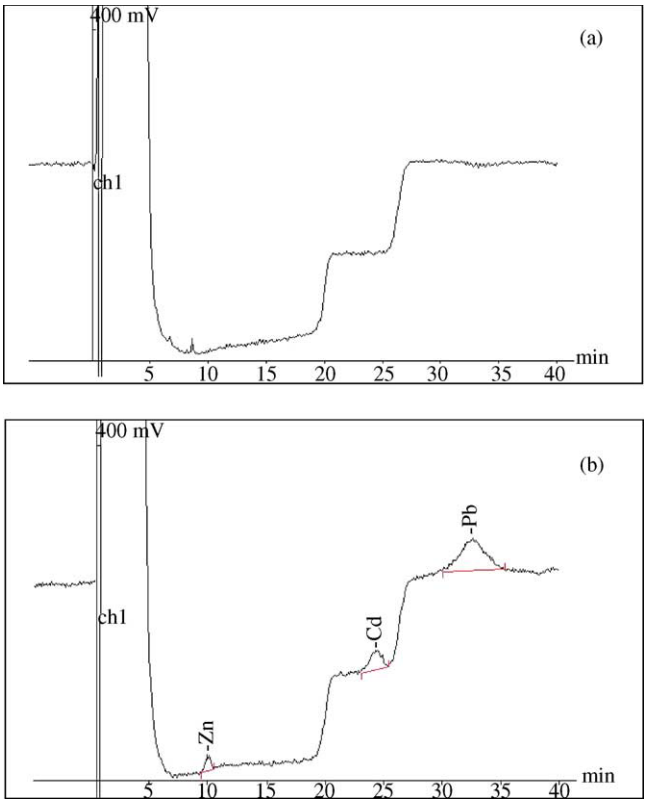

Fig. 6. Chromatograms: (a) blank solution without cations, using the FI–IC system (b) cations (Cd = 5 mg l^{-1}, Pb = 10 mg l^{-1}, Zn = 3 mg l^{-1}) by using the FI on-line sample pretreatment with loading time 1 min and loading flow rate 3 ml min^{-1} before injecting into ion chromatograph with elapsed time 24 s. Peaks: Cd (t_R = 24.3 min), Pb (t_R = 32.6 min) and Zn (t_R = 9.9 min); column: universal cation (100 mm × 4.6 mm); eluent: 3 mM tartaric acid/1 mM oxalic acid; flow rate 1.5 ml min^{-1}; injection volume: 20 μl; detector: conductivity without suppressor.

(ml min^{-1}) × loading time (min). The plots were found to be linear. For each cation, two breaks of linear calibration ranges were obtained; for Cd: $y = 105x - 99$ ($r^2 = 0.989$) (for 6−25 μg) and $y = 44x + 1035$ ($r^2 = 0.981$) (for 25–60 μg); for Pb: $y = 93x + 762$ ($r^2 = 0.983$) (for 18–54 μg) and $y = 43x + 3559$ ($r^2 = 0.992$) (for 54–180 μg) and for Zn: $y = 89x - 553$ ($r^2 = 0.986$) (for 60–200 μg) and $y = 59x + 5561$ ($r^2 = 0.992$) (for 200–600 μg). Under such conditions, detection limits were: 5, 3 and 4 μg for Cd, Pb and Zn, respectively.

The proposed method was applied to the determination of cation mixtures in zinc ore samples and ore samples with spiked cations. To determine Cd, Zn and Pb in zinc ore samples which have high matrix content, the digested sample solution was on-line pretreated using the in-valve column FI system. The pretreated sample zone was injected into the ion chromatograph. The results obtained agree with reference methods (AAS and ICP–AES), all from triplicate

Table 3
Analysis of zinc ore samples

Sample	Concentration found (%)								
	Zn			Cd			Pb		
	FI–IC[a]	ICP–AES[b]	AAS[b]	FI–IC[a]	ICP–AES[b]	AAS[b]	FI–IC[a]	ICP–AES[b]	AAS[b]
A	25.3 ± 0.6	30.4 ± 0.2	30.4 ± 0.2	0.27 ± 0.01	0.33 ± 0.02	0.32 ± 0.02	6.0 ± 0.2	5.8 ± 0.3	5.7 ± 0.3
B	49.5 ± 1.2	45.1 ± 0.5	45.1 ± 0.5	0.19 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	5.4 ± 0.2	6.3 ± 0.3	6.3 ± 0.3
C	42.4 ± 0.8	45.1 ± 0.5	45.1 ± 0.5	0.20 ± 0.01	0.20 ± 0.02	0.19 ± 0.02	4.4 ± 0.2	6.3 ± 0.3	6.3 ± 0.3
D	26.9 ± 0.4	27.8 ± 0.2	27.8 ± 0.3	0.18 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	4.6 ± 0.1	4.8 ± 0.3	4.8 ± 0.3
E	28.5 ± 0.4	27.9 ± 0.3	27.8 ± 0.3	0.18 ± 0.01	0.19 ± 0.02	0.18 ± 0.02	4.6 ± 0.2	4.8 ± 0.3	4.8 ± 0.3

Both (a) and (b) were from triplicate results: mean ± S.D.

[a] The proposed method.

[b] Analyzed by the Mineral Resources Region 3 (Chiang Mai).

determination with standard deviation as represented in Table 3. The recoveries of cations were found to be in the range of 95–105%. The proposed procedure offers simultaneous determination of Cd, Pb, and Zn. This is advantageous over the AAS, in which simultaneous determination is not possible. The proposed pretreatment using FI column reduces IC column damage. The FI–IC procedure is cheaper than the ICP–AES method, both in instrumentation and operation aspects.

4. Conclusion

In-valve column in FI system for sample pretreatment coupled to a simple IC with conductivity detector without suppressor for simultaneous determination of some cations in zinc ore samples which have high amounts of matrix interference, has been successfully applied. The on-line sample matrix interference removals are easily automated, and eliminate costly and time consuming off-line operations, and reduce IC column damage. The developed FI–IC method offers advantages over traditional analytical procedures. It allows a range of some cations to be simultaneously analyzed, and is accurate, reliable and uses readily available chemicals. The on-line sample preparation FI-column can also potentially be combined with IC using PAR/absorbance detection.

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Flow injection analysis of doxycycline or chlortetracycline in pharmaceutical formulations with pulsed amperometric detection

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Abstract

A flow injection with pulsed amperometric detection for determination of doxycycline or chlortetracycline in pharmaceutical formulations is described. Doxycycline or chlortetracycline were studied at a gold rotating disk electrode with cyclic voltammetry as a function of pH of supporting electrolyte solution. The optimized PAD waveform parameters were obtained with a flow injection system. The optimized pulsed conditions of doxycycline were 1150 mV (versus Ag/AgCl reference electrode) detection potential (E_{det}) for 220 ms (150 ms delay time and 70 ms integration time), 1500 mV (versus Ag/AgCl reference electrode) oxidation potential (E_{oxd}) for 70 ms oxidation time (t_{oxd}) and 250 mV (versus Ag/AgCl reference electrode) reduction potentail (E_{red}) for 400 ms reactivation time (t_{red}). The optimized pulsed conditions of chlortetracycline were 1050 mV (versus Ag/AgCl reference electrode) detection potential (E_{det}) for 300 ms (200 ms delay time and 100 ms integration time), 1300 mV (versus Ag/AgCl reference electrode) oxidation potential (E_{oxd}) for 70 ms oxidation time (t_{oxd}) and 250 mV (versus Ag/AgCl reference electrode) reduction potentail (E_{red}) for 400 ms reactivation time (t_{red}). The optimized PAD waveform was applied to the determination of doxycycline hydrochloride and chlortetracycline hydrochloride standard solution and in pharmaceutical formulations. The linear dynamic ranges of doxycycline hydrochloride and chlortetracycline hydrochloride were 1 μM–0.1 mM. The sensitivity of this method was found to be 23 μA/mM for doxycycline hydrochloride and 33.76 μA/mM for chlortetracycline hydrochloride. The detection limit for both compounds is 1 μM. The doxycycline hydrochloride and chlortetracycline hydrochloride content in commercially available tablet dosage forms by the proposed method was comparable to those specified by the manufacturer.

Keywords: Doxycycline; Chlortetracycline; Pulsed amperometric detection; Flow injection analysis

1. Introduction

Tetracyclines are well-known antibiotics used routinely in human and veterinary medicine for prevention and control of disease [1,2]. Chlortetracycline and doxycycline are antibiotics commonly used in food-producing animals because of their wide antibacterial spectrum, high potency and low cost. These raise the possibility that a residue of tetracyclines may remain in animal tissues intended for human consumption [3]. Moreover, they are important antibiotics widely used to control bacterial infections in human. Therefore, it is necessary to develop an assay method with high sensitivity and accuracy for monitoring the chlortetracycline and doxycycline. Many methods for determination of these compounds have been reported. These include microbiological assay [4,5]. These procedures are subject to problems such as high pH dependence, low sensitivity, low stability, as well as being time consuming. Recently, these antibiotics have been analyzed by HPLC with UV [6,7], fluorescence [8], MS [9,10] or electrochemical detection [11]. The peaks of these compounds tend to tail and exhibit low efficiency due to interaction with the residual silanol groups on silica-based packing materials. Also techniques such as spectrophotomety [12,13], chemiluminescence [14–18], spectrofluorimetry [19,20] or electrochemical methods [21,22] have been employed. However, one of the important limitations of

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method based on spectrophotometer, chemiluminescence or spectrofluorimetry is the fact that these compounds are inactive species for direct detection. Therefore, the derivatization procedure is normally required that make the methods are tedious, expensive, and long time analysis. The high sensitivity and selectivity of electrochemical detection are desired for antibiotics determination. Electrochemical techniques are alternatives, which can be cheap, fast and simple. The working electrode, mercury, is extensively used for determination tetracyclines [23,24]. This electrode has some problems such as the toxicity and limited stability of responses. In recent years, the flow injection system has received much attention and some analytical applications have been reported. A flow injection system was introduced to conventional analytical instrument to improve sample throughput and sensitivity that are the requirement to develop the assay method in pharmaceutical industry. Thus, use of the flow injection system coupled to the mercury electrode is complicated. Moreover, problems associated with easily oxidized mercury electrode have to be considered. Voltammetry and amperometry are the techniques that offer the high sensitivity. Their disadvantage is deposition of the detection product or solution impurities on the electrode surface. Therefore, pulsed amperometric detection with alternated anodic and cathodic polarization to clean and reactivate the electrode surface, has been introduced to overcome this problem. PAD offers the possibility to clean and reactivate the electrode surface effectively after measuring cycle without mechanical polishing [25–28]. 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}. All three steps of PAD require the following: (a) the oxidation of analyte during the detection step; (b) the oxidative desorption of adsorbed detection products or solution impurities at the cleaning step; (c) the cathodically dissolved of inert oxide product during reactivation step [29]. Pulsed amperometric detection has been used for the sensitive detection of numerous compounds [30–34]. It also has been successful for the determination of tetracycline in pharmaceutical formulation [35]. The goal of this work is extended the use of the PAD waveform for the determination of doxycycline or chlortetracycline in pharmaceutical formulations. In this research also employed the flow injection system with PAD to reduce time analysis and to obtain low detection limit. The present method has been proved to be simple, rapid, sensitive and suitable for automatic analysis.

2. Experimental

2.1. Chemicals

All the chemicals were analytical grade, and the water used was deionized water. Phosphate solutions (for pH 2–4.5) were prepared from 0.1 M potassium dihydrogen phosphate (Merck) and adjusted to the desired pH using 85% phosphoric acid (J.T. Baker). For the phosphate solutions (pH 5–10) were adjusted by 0.1 M sodium hydroxide. Standard doxycycline hydrochloride and chlortetracycline hydrochloride (Sigma-Aldrich) solutions were freshly prepared in 0.1 M potassium dihydrogen phosphate solution prior to use.

Stock standards of doxycycline (0.5 mM) and chlortetracycline (0.5 mM) were prepared by accurately weighing the hydrochloride of doxycycline or chlortetracycline into volumetric flasks and dissolved with 0.1 M potassium dihydrogen phosphate solution. To prepare solutions for the standard addition method, 2.5 ml of sample (prepared as described in Section 2.2) solution was pipetted in a set of five 10 ml volumetric flasks. Then, the 0, 1.0, 2.0, 3.0 and 4.0 ml of a stock solution of standards were added in sample solutions and the volume was adjusted by 0.1 M potassium dihydrogen phosphate solution.

2.2. Sample preparation

Doxycycline hydrochloride capsules (100 mg Medochemie, USA) and chlortetracycline hydrochloride capsules (250 mg, F. E. Sillic, Thailand) were used in this study.

A mass powder of ten capsules of doxycycline hydrochloride (Medomycin, 100 mg) or chlortetracycline hydrochloride (Aureomycin, 250 mg) were transferred to each 1000 ml volumetric flask then dissolved in 0.1 M potassium dihydrogen phosphate solution (pH 2 for doxycycline and pH 2.5 for chlortetracycline). Both of sample solutions were filtrated through a 0.45 μm Nylon membrane syringe filter. The filtered solutions were further diluted with 0.1 M potassium dihydrogen phosphate solution to obtain a final concentration of 240.45 and 257.65 $\mu g\,ml^{-1}$ (0.5 mM), respectively.

2.3. Electrode

The gold rotationg 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 ultrapure 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 electrode, 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

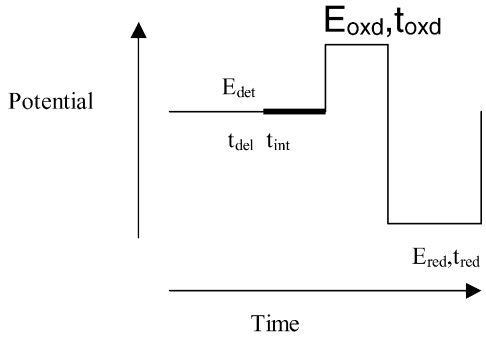

Fig. 1. Typical PAD waveform.

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 used PAD waveform to obtaine the FI-PAD response was depicted in Fig. 1.

The FI-PAD response was monitored for independent variation of all potential and time parameters. The electrode was condition 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.

3. Results and discussion

3.1. pH dependence study

In our initial experiments, the electrochemical behaviors of both doxycycline or chlortetracycline were studied at Au RDE in 0.1 M potassium dihydrogen phosphate solution of pH 2–10. It was found that the best-resolved anodic signals for oxidation of doxycycline or chlortetracycline were obtained at pH 2 and pH 2.5, respectively. Therefore, these pHs were used for the rest of the experiments.

3.2. Cyclic voltammetry

The cyclic voltammetric *(I–E)* responses are shown in Fig. 2 for the Au RDE in 0.1 M potassium dihydrogen phosphate solution with and without 1 mM doxycycline (Fig. 2a) and 1 mM chlortetracycline (Fig. 2b). The background response for the supporting electrolyte exhibits an anodic wave

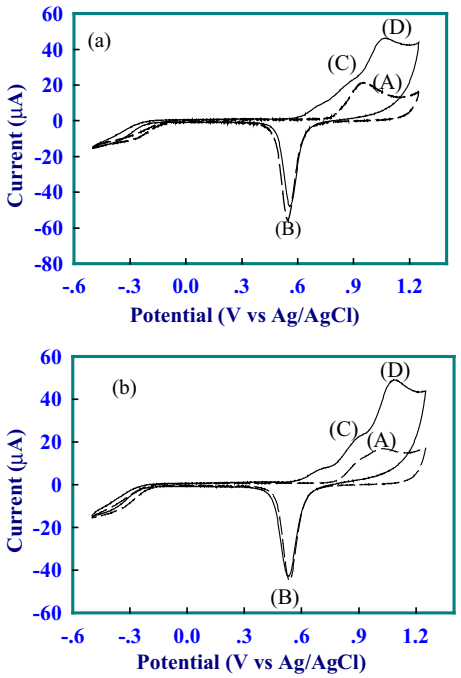

Fig. 2. Cyclic voltammetric response for (a) 1 mM doxycycline (pH 2) and (b) 1 mM chlortetracycline (pH 2.5) at the Au RDE (0.07 cm^2). Condition: 250 rpm rotation speed: 50 mV s^{-1} scan rate. The background cyclic voltammograms are also shown (dash line).

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 doxycycline or chlortetracycline, the two-step anodic signal for oxidation of them were observed on the positive scan beginning at ca. 0.6 V versus Ag/AgCl. The first and secon steps were occurred in the region 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 responses for doxycycline or chlortetracycline on the positive scan were sharply inhibited by the onset of the surface oxide formation at potential 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} to allow the charging current to decrease to a negligible value. A positive cleaning potential (E_{oxd}) that removes the oxidizable contaminant on the elec-

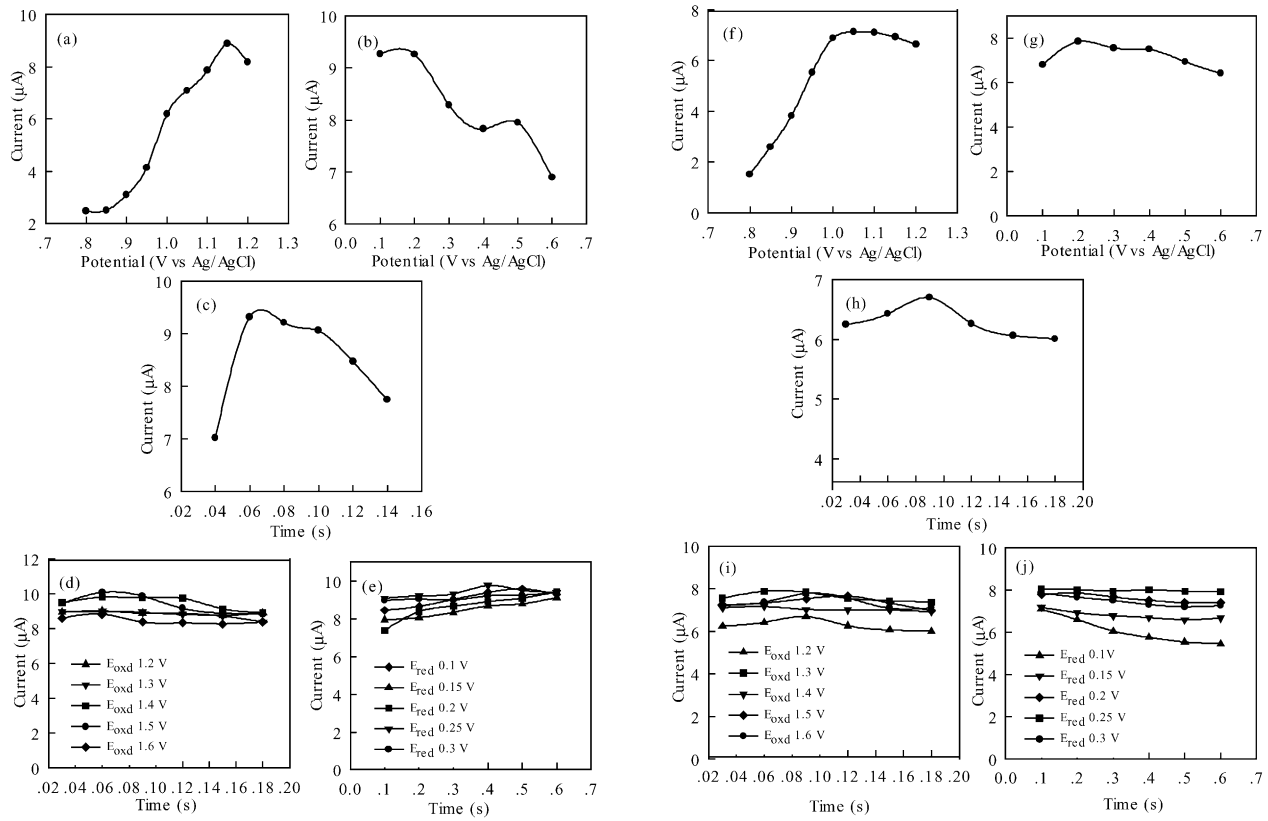

Fig. 3. FI-PAD response as a function of: (a) E_{det}; (b) t_{del}; (c) t_{int}; (d) E_{oxd} and t_{oxd}; and (e) E_{red} and t_{red}, for chlortetracycline and (f) E_{det}; (g) t_{del}; (h) t_{int}; (i) E_{oxd} and t_{oxd}; and (j) E_{red} and t_{red} for doxycycline in 0.1 M potassium dihydrogen orthophosphate solution (pH 2) at the Au RDE (0.07 cm^2).

trode 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 detection step (E_{det}, t_{int} and t_{del})

Fig. 3a and f show the FI-PAD response variations for 0.5 mM doxycycline and 0.5 mM chlortetracycline respectively 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) that the oxidation of each doxycycline or chlortetracycline occurred. The optimum detection potential for doxycycline was obtained at E_{det} = 1.15 V versus Ag/AgCl and for chlortetracycline was obtained at E_{det} = 1.05 V versus Ag/AgCl.

Fig. 3b and g show the FI-PAD response variations for 0.5 mM doxycycline and 0.5 mM chlortetracycline with t_{del} variation from 100 to 500 ms. The t_{del} optimal values of doxycycline and chlotetracycline were 150 ms and 200 ms, respectively.

Fig. 3c and h show the FI-PAD response variations for 0.5 mM doxycycline and 0.5 mM chlortetracycline with variation of t_{int} from 40 to 140 ms. The optimal values of t_{int} for doxycycline was obtained 100 ms and chlortetracycline was obtained 70 ms.

3.5. Optimization of oxidation step (E_{oxd} and t_{oxd})

A clean electrode surface is progressively fouled by the detection products during application of E_{oxd} and to avoid this problem, t_{oxd} was applied to clean the electrode surface. Fig. 3d and i show the FI-PAD response variations for 0.5 mM doxycycline and 0.5 mM chlortetracycline as a result of the variation of t_{oxd} from 30 to 180 ms at intervals of 30 ms for difference E_{oxd} values in the range +1.2 to +1.6 V versus Ag/AgCl in interval 0.1 V. The optimal values of doxycycline was obtained E_{oxd} = 1.3 V versus Ag/AgCl and t_{oxd} = 70 ms. For chlortetracycline, the E_{oxd} = 1.5 V versus Ag/AgCl and t_{oxd} = 70 ms was recommended as optimal.

3.6. Optimization of reduction step (E_{red} and t_{red})

The formation of surface oxide at the electrode surface, which reduced the electrode surface activity, occurred during the oxidation step. Therefore, it is necessary that the values of E_{red} and t_{red} are chosen to achieve complete re-

Table 1
The optimal PAD waveform parameters of doxycycline and chlortetracycline

Parameter	Doxycycline	Chlortetracycline
E_{det} (V vs. Ag/AgCl)	1.05	1.15
t_{del} (ms)	200	150
t_{int} (ms)	100	70
E_{oxd} (V vs. Ag/AgCl)	1.3	1.5
t_{oxd} (ms)	7.0	70
E_{red} (V vs. Ag/AgCl)	0.25	0.25
t_{red} (ms)	400	400

ductive dissolution of the surface oxide. Fig. 3e and j show the FI-PAD response variations for 0.5 mM doxycycline and 0.5 mM chlortetracycline with variation of t_{red} from 100 to 600 ms at intervals of 100 ms for difference E_{red} values in the range +0.1to +0.5 V versus Ag/AgCl in interval 0.1 V. For doxycycline or chlortetracycline, the optimal values of reduction step were obtained the value of $E_{red} = 0.25$ V versus Ag/AgCl and $t_{red} = 400$ ms. To conclude, the potentials and times for the optimization are shown in Table 1.

3.7. Linear range, detection limit and repeatability

From a series of repetitive 20 μl injections of doxycycline or chlortetracycline in 0.1 M potassium dihydrogen phosphate solution under the optimum pH conditions and the optimized PAD waveform parameters described above provided well-defined signals as shown in Fig. 4. The current signal increased with increase in concentration. The calibration curves for doxycycline or chlortetracycline were obtained from using the optimized PAD waveform parameters. The analytical performance results are shown in Table 2. The dynamic linear working range of both compounds is the same and over two orders of magnitude.

3.8. Drug analysis of pharmaceutical formulations

The proposed PAD methods for doxycycline or chlortetracycline were applied to the determination of doxycycline or chlortetracycline in pharmaceutical formulations by standard addition method. In order to evaluate, these proposed methods for the determination of doxycycline or chlortetracycline in drug capsules, the recovery, and within-day and between-day studies were carried out. The results are summarized in Table 3.

Table 2
Linear range, detection limit and repeatability of doxycycline hydrochloride and chlortetracyline hydrochloride

Parameter	Doxycycline	Chlortetracycline
Linear range	1 μM–0.1 mM	1 μM–0.1 mM
Sensitivity (μAmM^{-1})	23.4	33.6
Regression equation	$Y = 23.354X + 0.0272$	$Y = 33.785X + 0.037$
Correlation coefficient (R^2)	0.9992	0.9994
Detection limit	1 μM	1 μM
Repeatability (%RSD)	3.17	2.18

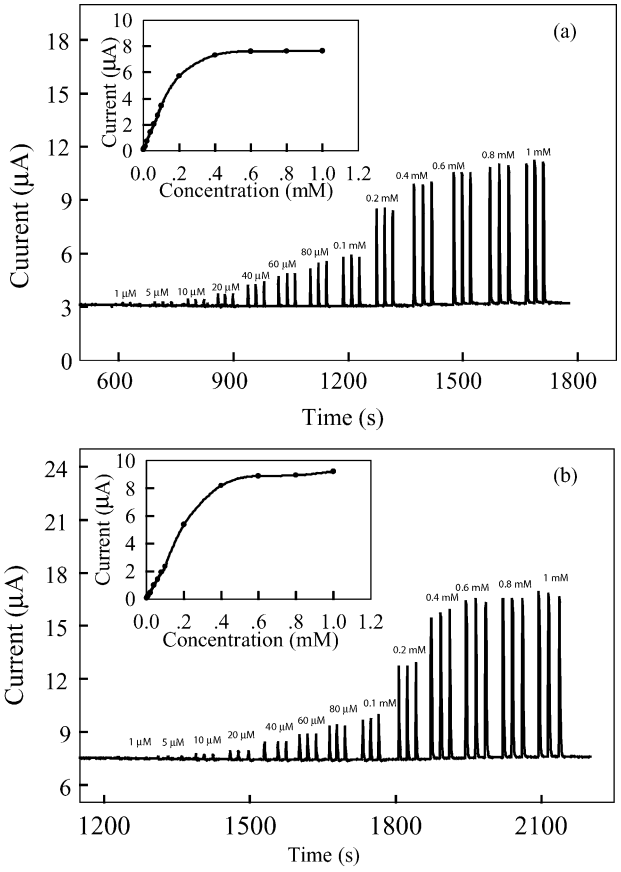

Fig. 4. FI-PAD results of (a) chlortetracycline and (b) doxycycline in 0.1 M potassium dihydrogen phosphate at gold disk electrode. The flow rate was 1 ml min^{-1}.

Table 3
Percent difference and percent recovery of doxycycline hydrochloride and chlortetracycline hydrochloride capsule samples

Parameters	Doxycycline	Chlortetracycline
% Difference	2.57 ± 1.77	7.28 ± 0.27
% Recovery of spiked standard solution	87–103	93–109

4. Conclusion

This is the first investigation of doxycycline or chlortetracycline using pulsed amperometric detection applied to a flow injection system to avoid a problem about fouling of products or interferents on the surface of a gold working electrodes. The optimized conditions, such as pH and the various potentials were investigated. The results showed that FI-PAD with optimized conditions can be used to determine doxycycline or chlortetracycline in pharmaceutical formulations. FI-PAD provided wide working concentration (0.001–0.1 mM), low detection limit (1 μM) and high re-

peatability (% RSD 3.17–2.18). The advantage of the proposed method is simple and time saving because the cleaning step occurs simultaneously during the measurement. The commercial drugs of doxycycline hydrochloride or chlortetracycline hydrochloride were also analyzed by proposed methods.

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Flow-injection determination of iodide ion in nuclear emergency tablets, using boron-doped diamond thin film electrode☆

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Abstract

The electrochemical determination of iodide was studied at boron-doped diamond thin film electrodes (BDD) using cyclic voltammetry (CV) and flow-injection (FI) analysis, with amperometric detection. Cyclic voltammetry of iodide was conducted in a phosphate buffer pH 5. Experiments were performed using glassy carbon (GC) electrode as a comparison. Well-defined oxidation waves of the quasi-reversible cyclic voltammograms were observed at both electrodes. Voltammetric signal-to-background ratios (S/B) were comparable. However, the GC electrode gives much greater in the background current as usual. The potential sweep rate dependence exhibited that the peak current of iodide oxidation at 1 mM varied linearly ($r^2 = 0.998$) with the square root of the scan rate, from 0.01 to 0.30 V s^{-1}. This result indicates that the reaction is a diffusion-controlled process with negligible adsorption on BDD surface, at this iodide concentration. Results of the flow-injection analysis show a highly reproducible amperometric response. The linear working range was observed up to 200 μM ($r^2 = 0.999$). The detection limit, as low as 0.01 μM (3σ of blank), was obtained. This method was successfully applied for quantification of iodide contents in nuclear emergency tablets.

Keywords: Boron-doped diamond thin film electrode (BDD); Iodide; Flow-injection analysis

1. Introduction

Diamond itself does not have electrical properties in terms of being conductive or semiconductive. However, with the technology of a so-called chemically vapor deposition (CVD), film of diamond with specific dopants, such as boron, can be conveniently grown on a substrate [1–3]. This diamond film offers remarkable properties of being such an electrochemical sensor.

For electrochemical measurements, thin film of boron-doped diamond on Si wafer (BDD), has benefits over other electrodes especially those sp^2-bonded carbon electrodes, e.g., carbon paste and glassy carbon [1]. Examples [1–3] of the superior properties of the BDD include (i) low and stable background current which results in improvement in signal-to-background; (ii) considerably wide working potential window in both aqueous and non-aqueous media; (iii) very low capacitance; (iv) low adsorption of polar molecules, due to the hydrogen termination during the film growth (unlike the GC surface which is usually quite polar with covered film of oxide) [4]; (v) remarkable reproducibility [5] and (vi) morphological and micro structural stability although at over potential condition.

With respect to the outstanding properties of BDD film, quite a number of applications, based on use of this new material for electrochemical quantitation, have been reported [6–19]. This synthetic type of diamond film has already been applied as amperometric detectors in flow-injection (FI) analysis [6–14,19] and in liquid chromatography [15–19]. However, there has been no report of use of BDD electrode for determination of iodide. There is only a recent report for quantitative analysis of iodide, but on diamond paste based electrodes [20].

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Presently, iodine is one of the supplements being widely publicized in our country. Campaigns have been raised against iodine deficiency disorder (IDD) in populations. Drinking water and/or table salt as well as foods are supplemented with iodide or iodate. Supplementation of iodine also comes in as multivitamins.

In the States, customers can purchase a pharmaceutical product used in the event of nuclear emergency. This product is basically potassium iodide, known as "potassium iodide tablet". The product is orally consumed to protect human thyroid from body absorption of radioactive iodine.

Since there are varieties of samples being the source of iodine, and somehow, intake of iodine should be concerned with extreme caution. Some people can be markedly sensitive to the element [21].

In the previous reports, determination of iodide can be made using several methods such as spectrometric analysis [22,23], catalytic spectrometric analysis [24,25] and by potentiometric detection [26]. Analysis of iodide using amperometric detection by other working electrodes has been well established especially for liquid chromatography [27–29]. Nevertheless, there is no report for detection on the BDD electrode.

This paper presents, for the first time, the electrochemical oxidation of iodide on thin film of boron-doped diamond. Cyclic voltammetric investigation was made for an iodide solution with comparison to the commercially known glassy carbon electrode. Amperometric determination of iodide on BDD was explored by applying the thin film for quantitative analysis in some pharmaceutical products.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade and deionized-distilled water was used throughout. A stock solution of iodide standard (0.1 M) was prepared by dissolving approximately 1.66 g (accurate weight) of potassium iodide crystals (Merck, Germany) in 100.0 ml of water. Working solutions of iodide were obtained by appropriate dilution with water. A phosphate buffer 60 mM, pH 5, was prepared by dissolving 8.9056 g of sodium dihydrogen phosphate (Fluka, Switzerland) and 0.4914 g of disodium hydrogen phosphate (Fluka) in water and making up to the mark in a 1-l volumetric flask. Phosphate buffers 60 mM, pH 4–9, were prepared similarly with pH adjustment using 1 M sodium hydroxide and 1 M hydrochloric acid solutions.

2.2. Sample preparation

Four commercial products of potassium iodide tablets, for radiation emergency, were employed in validation of the method. IOSAT^{TM} (distributed by ANBEX, USA) and Thyro-Block^{TM} (Wallace Pharmaceuticals, USA) are the samples, which contain 130 mg KI/tablet. NO-RAD^{TM} (Body Gold, USA) and RAD BLOCK KI^{TM} (distributed by USDPI, USA) both contain 65 mg KI/tablet. In the analysis, tablets were accurately weighed and dissolved in 500.0 ml of water. Suspension was removed by filtration through 0.25 μM pore of cellulose acetate membrane before analyses.

2.3. Cyclic voltammetry

Similarly to the former reports [6,11–15], an Autolab potentiostat 100 (Eco-Chemie, the Netherlands) with a single compartment three electrode glass cell, was used for all the cyclic voltammetric studies. A BDD electrode was pressed against the smooth ground joint at the bottom of the cell and was isolated by an o-ring, which resulted in 0.07 cm^2 of electrode area. Electric contact was carried out by placing the backside of electrode (Si substrate) on a brass plate. The electrochemical cell was housed in a faradaic cage to reduce electronic noise. A platinum wire and a Ag/AgCl with a salt bridge were used as the auxiliary and reference electrodes, respectively.

Cyclic voltammetric study was also carried out, in the same way, using glassy carbon as the working electrode.

2.4. Amperometric determination on BDD electrode by flow-injection

For routine analysis, we propose use of the BDD detection of iodide by flow-injection technique [30]. The system is displayed schematically in Fig. 1. An Ismatec peristaltic pump (model IS7610, Switzerland) was used for propelling the carrier stream (phosphate buffer). Arrangement of the amperometric detection with a BDD working electrode was the same as that described in previous works [6,11–15]. Again in this present work, the electrode area was utilized at 0.36 cm^2.

The final sample solutions were diluted (25 or 50 times) before injections. External calibrations with aqueous solutions of potassium iodide were adopted.

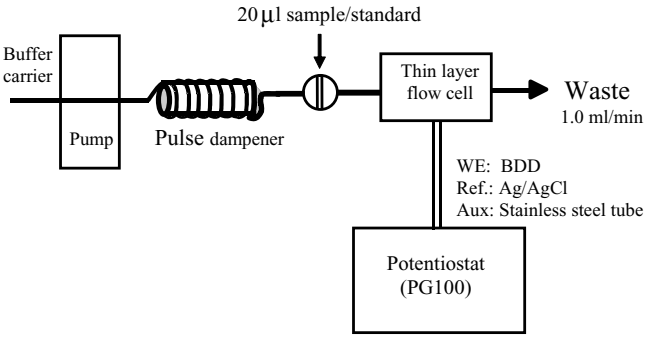

Fig. 1. The FI manifold with amperometric detection using BDD as the working (WE) electrode. Optimal condition: potential, 1.0 V; carrier, 60 mM phosphate buffer at pH 5.

2.5. Potentiometric analysis by ion selective electrode (ISE)

Accurate 10.00 ml of a sample solution was transferred into a 50 ml volumetric flask. To the flask, a 1.0 ml of 5 M $NaNO_3$ solution was added as an ionic strength adjuster. The mixture was made up to the level with water. The solution was measured for the potential developed across the employed iodide-ISE (Orion, USA) and the saturated calomel electrode (Orion). A digital Orion Ionanalyzer (model 601A) was used for the measurement.

2.6. The colorimetric method employed for comparison

Our colorimetric flow-injection method [23] which utilizes gas diffusion for selective determination of iodide was adopted for the validation. The detection is based on complex formation of I_3^-–starch. This method was previously validated against another technique.

3. Results and discussion

3.1. Cyclic voltammetry: BDD and GC electrodes

Fig. 2 shows the cyclic voltammograms that are obtained form an iodide solution together with the corresponding background voltammograms for BDD and GC electrodes. Peak-shaped oxidation responses are observed for both electrodes, but the background currents are dramatically different. The background current of the GC electrode is approximately 10 times greater than the background of BDD electrode. The BDD exhibits well-defined quasi-reversible oxidation peaks at 0.57 and 0.79 V versus Ag/AgCl, whereas the GC electrode provides the oxidation peaks at 0.48 and 0.74 V. To avoid using the high potential position, therefore, the first redox couple was considered. The potential differences between the first reduction peaks and the corresponding anodic peaks are at the order of 90 mV at GC electrode and 220 mV at BDD electrode. The peak separation suggested that the voltammetry is distorted by quasi-reversible behavior. The first anodic peak current, subtracted from background current, is 0.86 μA at BDD electrode and is 0.67 μA at GC electrode. We then expect that BDD electrode would provide a better sensitivity.

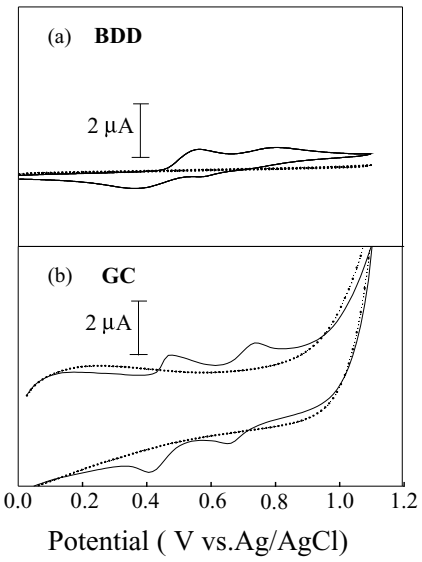

Fig. 2. Solid lines are the cyclic voltammograms that were obtained from batch experiment for 0.50 mM iodide in 60 mM phosphate buffer (pH 5). Background voltammograms (60 mM phosphate buffer, pH 5) are also shown as dotted lines for both electrodes. The scan rate was fixed at 0.02 V s^{-1}.

There have been some reports of cyclic voltammograms of iodide on platinum [31], gold and glassy carbon [32] electrodes. In these works, the authors also observed sequential oxidation of iodide, similarly to what we have seen in Fig. 2. Thus it is likely that the oxidation behaviors of iodide for both BDD and GC are the same as that reported in the systems studied by Hanson and Tobias [31] and by Qi and Hiskey [32]. These authors investigated and suggested that the oxidation sequence of iodide at the working electrodes can be represented by the following reactions:

$$3I^- \rightarrow I_3^- + 2e^- \quad (1)$$

$$2I_3^- \rightarrow 3I_2 + 2e^- \quad (2)$$

Our results also exhibit that the oxidation sequence is reversible for BDD and GC electrodes. For our work, we employed only the first oxidation peak current.

Buffer pH was also investigated from pH 4–9. The BDD and GC electrodes exhibit well-defined cyclic voltammograms of iodide in all pHs. It was observed that the oxidation peak currents slightly shift to positive potentials with increasing pH. The current background was enlarged when approaching the higher pHs. We chose pH 5 as the optimal pH for further studies on the BDD electrode.

3.2. Scan rate and concentration dependence study

Cyclic voltammograms of an iodide solution on BDD were investigated at different scan rates, the results are displayed in Fig. 3. As seen in the inset of Fig. 3, peak current varies linearly with the square root of scan rate. This indicates that the current is limited by semifinite linear diffusion of iodide to the BDD electrode.

The relationship between oxidation peak current (μA) and iodide concentration was examined from 0.50 to 10 mM. Linear calibration ($r^2 = 0.999$) was obtained with the slope of 6.07 μA mM^{-1}. These results have demonstrated that the BDD electrode is appropriate for the quantitation of iodide.

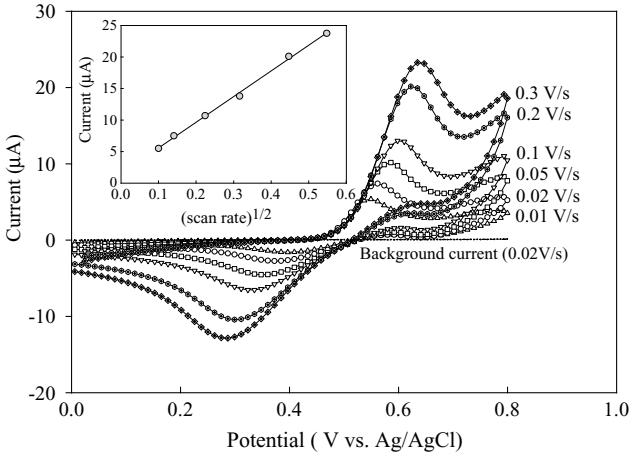

Fig. 3. Cyclic voltammograms, obtained at various potential scan rates for 1 mM iodide in 60 mM phosphate buffer (pH 5) at BDD electrode. The inserting figure shows the relationship between the oxidation current and the square root of the scan rate with regression figures of 40.74 μA $(s/V)^{1/2}$ (slope) and 0.998 (r^2).

3.3. Flow-injection analysis with amperometric detection

3.3.1. Optimal potential

For routine analysis, it is often more convenient than the potentiometric mode to use hydrodynamic injection of samples with amperometric detection. Optimization was first carried out using the FI manifold in Fig. 1 to find the most sensitive oxidation potential. The results are presented in Fig. 4. According to the results, the maxima S/B ratio was observed at 1.0 V. Therefore, this potential was chosen for amperometric detection in all FI experiments.

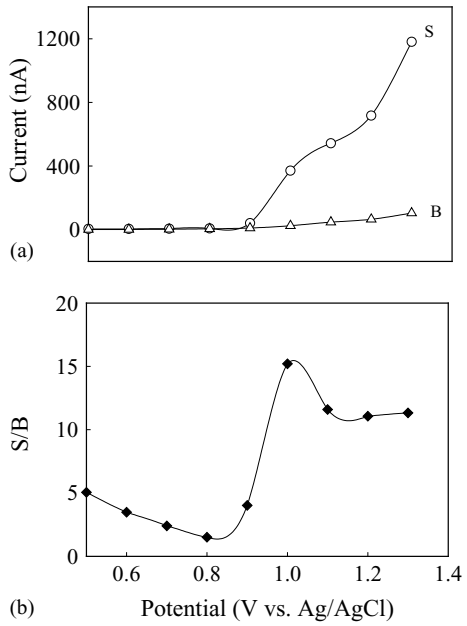

Fig. 4. Results obtained from the system of amperometric-FI (Fig. 1): (a) hydrodynamic signal of the carrier, 60 mM phosphate buffer or the background current (B) and peak current from injections ($n = 5$) of 10 μM of iodide into the carrier stream (S) and (b) plot of the signal-to-background ratio (S/B) and the applied potential.

3.3.2. Analytical features

We examined analytical performance of the manifold shown in Fig. 1. The system was operated at 1.0 V. Linear calibration was observed between 0.8 and 200 μM. The regression equation is $y = 37.41x - 2.28$ ($r^2 = 0.999$), where y and x are the area of peak current (nA s) and iodide concentration (μM), respectively. The detection limit (3σ) is as low as 0.01 μM. The system provides an impressively good precision (%R.S.D. = 2.2) for 20 μl injections ($n = 30$) of 0.1 mM iodide. Throughput of sample is 85 samples h^{-1}.

3.3.3. Performance on pharmaceutical applications

3.3.3.1. Nuclear emergency tablets. Samples of potassium iodide tablets were determined using our electrochemical method (Fig. 1). The results were compared with the values obtained from an ISE method and a colorimetric flow-injection method [23] as shown in Fig. 5. According to the ANOVA test [33], the results for IOSAT™, Thyro-Block™ and NO-RAD™ are not significantly different at 95% confidence [$F_{observed}$ are 0.1037 (IOSAT™), 0.7862 (Thyro-Block™) and 3.493 (NO-RAD™) where $F_{critical} = 4.256$]. The results of RAD BLOCK™ determined by all the method are not significantly different at 99% confidence ($F_{observed} = 6.667$, $F_{critical} = 8.022$). It can also be observed in Fig. 5 that all of our results, with three different analytical methods, agree well with the manufacture's contents.

For these samples, there is no evidence of interferents from sample matrices. Recovery studies made for all the samples have shown that the extracts' matrices do not have influence on electrochemical oxidation of iodide (86–103% recovery). For our method, the samples can be directly analysed after the following steps: water extraction, filtration, dilution and finally injection.

3.3.3.2. Multivitamin tablets. Multivitamins have always been our sample of interest. Usually these samples contain iodine at much lower levels (e.g., 150 μg I/tablet) than the raidoactive protection tablets (e.g., 49 mg I/tablet). With appropriate extraction (for example, one vitamin tablet is extracted with 100.0 ml of water, (approximately 1 mg I L^{-1})) we should be able to get reasonable signal currents from this type of sample.

Our results demonstrated that, although with C-18 clean up, the extracts still contain other electroactive species that cause positively erratic results (can be up to 500 times greater in signal current). An experiment was then carried out to study effects of foreign ions, including compounds that are likely to exist in aqueous vitamin extracts. The results are summarized in Table 1. According to these results, at least two ingredients (Vitamin B_6 and ascorbic acid) interfere the quantitative analysis of iodide.

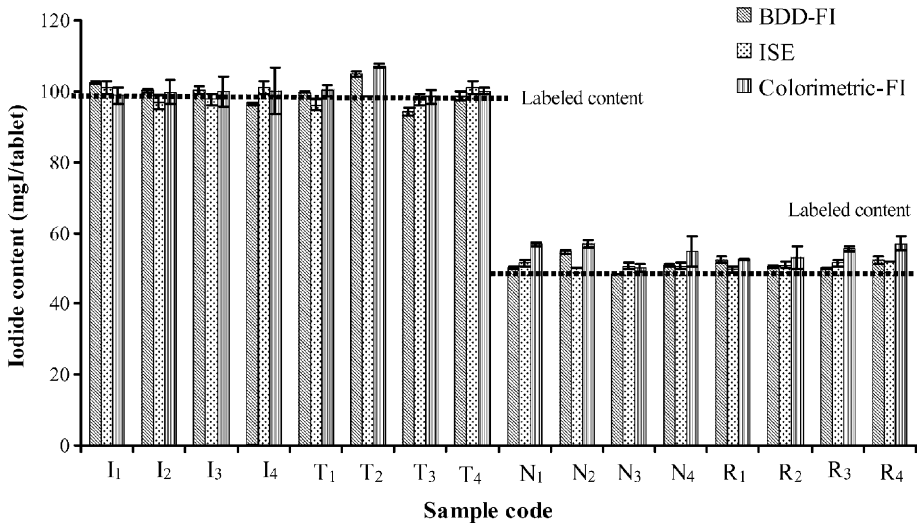

Fig. 5. Comparison of the iodide contents found in potassium iodide tablets, which were, obtained by the proposed method (BDD-FI), ISE method and colorimetric-FI method [20]. Determination by each method was carried out in triplicate for a sample. I: IOSAT, T: Thyro-Block, N: NO-RAD and R: RAD BLOCK KI.

Table 1
Effect of foreign ions on the alteration of FI signals obtained from replicate injections ($n = 5$) of standard potassium iodide 0.01 mM

Foreign species/added as	Investigated concentration ($mg\,L^{-1}$)[a]	Results[b]
1. Vitamin B_1 or aneurine hydrochloride/ $C_{12}H_{17}ClN_4OS{\cdot}HCl$	21–42	Does not interfere
2. Vitamin $B_2/C_{17}H_{20}N_4O_6$	24–48	Does not interfere
3. Vitamin B_6 or adermine hydrochloride/ $C_8H_{11}NO_3{\cdot}HCl$	30–60	Strongly interfere (e.g. 22% alteration even at $2\,mg\,L^{-1}$)
4. Vitamin C or L(+)ascorbic acid/$C_8H_8O_6$	900–1200	Strongly interfere (e.g. 19% alteration even at $1.6\,mg\,L^{-1}$)
5. Cl^-/NaCl	180–363	Does not interfere (studied up to $200\,mg\,L^{-1}$)
6. F^-/NaF	–	Does not interfere (studied up to $800\,mg\,L^{-1}$)
7. Br^-/NaBr	–	Does not interfere (studied up $200\,mg\,L^{-1}$)

[a] Likely concentrations of the foreign species, no. 1–5, in vitamin extracts.
[b] Greater than ±5% signal alteration is classified as interfering condition.

4. Conclusions

This work presents, for the first time, application of the BDD for quantitative analysis of iodide. For routine, we recommend amperometric detection in flow-injection mode. The flow-injection system is simply a single channel manifold with flow-through electrochemical cell of the working diamond electrode. With this construction, analysis of samples is very rapid (85 samples h^{-1}). Wide dynamic range (0.8–200 μM) was obtained, with good precision (R.S.D. = 2.2%).

The method is suitable for determination of iodide in nuclear emergency tablets. The BDD-FI method was proved to be valid for this type of samples. However, for multivitamins, separation prior the amperometric detection is required, since some of the ingredients have shown to interfere quite strongly. Utilization of the amperometric BDD as the detector for liquid chromatography is at present being investigated for multivitamins.

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Sequential injection with lab-at-valve (LAV) approach for potentiometric determination of chloride☆

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Abstract

Sequential injection with "Lab-at-Valve (LAV)" approach is demonstrated for potentiometric determination of chloride. The LAV flow-through electrode system consists of two Ag/AgCl electrodes: one as a reference electrode, silver chloride activated surface-silver wire soaked in a constant-concentration chloride ion solution in a small tube covered with a polymer-membrane, another as a working electrode (a similar silver chloride activated surface-silver wire) placed in a flow channel. The electrode system is attached at one port of a 10 port multiposition valve. A modified autoburette was used as a propelling device. Using SI operation via a program written in-house, based on LabVIEW®, a standard/sample is inserted, via the selection valve, in potassium nitrate as an electrolyte and water is used as a carrier. The zones are transported from the holding coil to the flow cell to monitor the difference in potential due to concentration cell behavior. The potential difference is then recorded as a peak. Peak height is proportional to logarithm of chloride concentration. The SI-LAV for chloride determination is very simple, fast, precise, accurate, automatic and economical. Applications to mineral drinking water and surface water have been made. The results agree with those of IC and titrimetric methods.

Keywords: Sequential injection; Lab-at-valve; Potentiometry; Chloride.

1. Introduction

Environmental-friendly analytical procedures are in demand for today's analytical chemistry. Green analytical chemistry concerns the use of non-toxic chemicals, with smaller amounts of reagent and hence less waste generated, and with high throughput analysis with a high degree of automation and portability. To meet these requirements, various approaches based on flow analysis have been developed to miniaturize and automate analytical systems, such as flow injection analysis (FIA) [1–3], multi-syringe FIA [4], sequential injection analysis (SIA) [5,6], SIA with lab-on-valve (LOV) [7] and micro total analysis systems (μTAS) or lab on a chip (LOC) [8].

The μTAS concept initiated by the group of the late Widmer [8] in 1990 has become an active research area today. Although, this approach provides fast analysis using minute amounts of sample and reagents, the production of the chip platform needs advanced, expensive facilities, which are not affordable by most laboratories, and the application of the technique to real samples is still limited.

Ruzicka introduced a SI-LOV concept [9] in 2000, to perform wet chemistry at microlitre levels with a relatively large bore conduits, to avoid surface contamination and clogging. The SI-LOV was designed to integrate sample processing channels with a multipurpose flow cell, and machined to perfectly fit on top of a selection valve by replacing a stator

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plate and connection port of the valve. In this way, various analytical processes, namely sampling, sample pretreatment, reaction and separation, and detection, can be incorporated onto an SIA valve. This approach is more tolerant to dirty real samples than chip based approaches.

A simpler approach, SI with lab-at-valve (LAV) concept, has been proposed [10,11]. This is employed by attaching a device integrating sample processing and detection units on a port of a multiposition selection valve, without taking apart components of a purchased valve. This makes the LAV simpler than the LOV. The LAV unit can be built using an ordinary and less precise machine tool, to have suitable functions for chemistries of interest and with a nut that can plug in a port of the valve in the usual way. Such an integrated LAV device compiling analytical process taking place in it should be compact and economical comparing to a normal SIA system. Various advantages similar to those of the LOV should be gained by using the LAV. In this paper we demonstrate SI-LAV approach for potentiometric determination of chloride with a simple made chloride ion selective electrode (ISE), which has been introduced earlier for use in FI [1–3], but with modification to use a Ag rod instead of tubular Ag to make the electrode more easy to clean and replenish the AgCl film, and to store after use. The proposed potentiometric system is simpler than the previously reported spectrophotometric ones and does not require expensive or toxic reagents, e.g., silver nitrate [5] or mercuric thiocyanate [6,7].

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Deionized water (obtained by a system of Milli-Q, Millipore, Sweden) was used throughout. A chloride standard stock solution (0.2 M) was prepared by dissolving 5.8440 g of sodium chloride (Merck, Darmstadt, Germany) in water and making upto a volume of 500 ml in a volumetric flask. Working standard solutions of appropriate concentrations were obtained by diluting the stock solution with water. A potassium nitrate stock solution (0.10 M) was prepared by dissolving 5.05 g of potassium nitrate (Merck, Darmstadt, Germany) in 500 ml of water. Working electrolyte solutions of appropriate concentrations were prepared by diluting the stock solution with water. A ferric chloride solution (0.5 M) in 1 M hydrochloric acid was prepared by dissolving 8.11 g of $FeCl_3$ anhydrous (Merck, Darmstadt, Germany) in 100 ml of 1 M HCl.

2.2. Potentiometric flow through cell

A potentiometric flow through cell was designed to be put on a side of a selection valve by plugging into one port of the selection valve (the cell acts as the LAV unit) as shown in Fig. 1(a). The cell was made from a Perspex plastic block (see Fig. 1 (b)) by cutting and drilling to form channels for

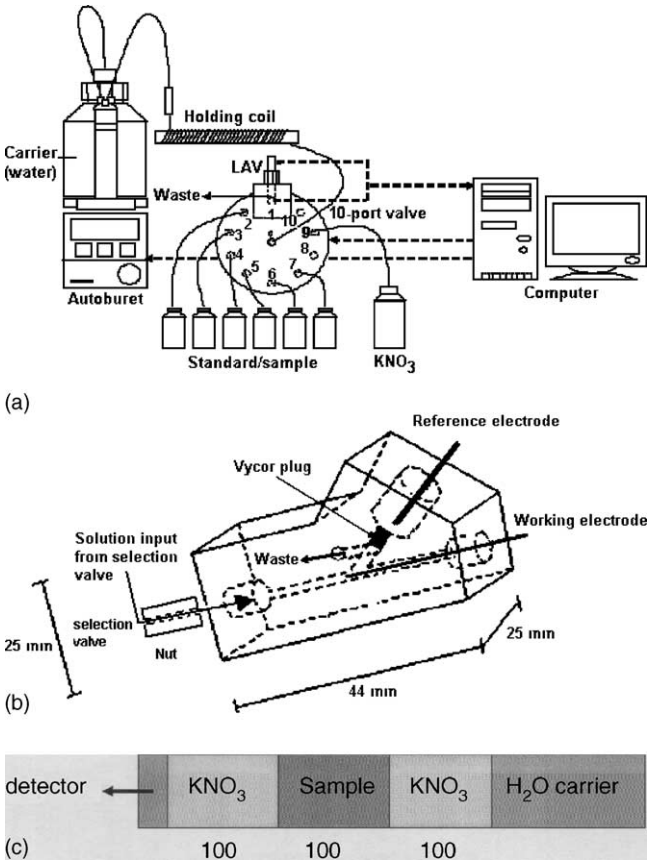

Fig. 1. (a) Schematic diagram of the SI-LAV system; (b) Potentiometric flow cell (LAV unit), the LAV plugged onto a port of the selection valve (45 mm distance from the port to the working electrode, 1.0 mm i.d. channel); and (c) Sequence of solutions aspirated into the system; the values indicating volumes of the solutions (μl).

inserting a Ag/AgCl wire working electrode (WE) and another Ag/AgCl wire reference electrode (RE), and for carrier solution inlet and outlet. The channel for RE was separated from the carrier solution channel by a small VYCOR plug (BAS, Indiana, USA), acting as a salt bridge.

2.3. SI-LAV manifold

The SI-LAV system used is schematically depicted in Fig. 1(a). It consisted of an autoburette (765 Dosimat, Metrohm Ltd., Herisau, Switzerland) equipped with a 10 ml exchange unit for the pumping system, connected to a personal computer via an RS232C interface, a ten-port multiposition valve with a microelectric actuator (C25-3180EMH, VICI, Texas, USA), and a LAV unit with two Ag/AgCl electrodes, which are connected directly or via a potentiometer (744 pH meter, Metrohm Ltd., Herisau, Switzerland) to the input terminals of a data acquisition board (AT-MIO-16X-50 equipped with SCB-68, National Instruments, Texas, USA). The autoburette was connected to the center of the selection valve via a holding coil (Tygon® tubing, 0.8 mm i.d., 4.0 mm o.d., 4.5 m long, Cole-Parmer Instrument Company, Illinois, USA) and the LAV unit was placed at

port-1 of the selection valve. Both instrumental control and data acquisition were manipulated via programs written in-house based on LabVIEW® software (Version 6; National Instruments, Texas, USA). This software provided control of the volume to be dispensed or aspirated by the autoburette, flow rate, selection of different valve positions and performance of data acquisition. The SIA-gram data were evaluated for peak height by using Microsoft® Excel 97 software (version 8.0, Microsoft, Arizona, USA).

2.4. Preparation of a Ag/AgCl electrode

A silver wire (0.5 mm in diameter) obtained from a local jewelry shop was used to prepare an Ag/AgCl electrode. The wire was polished and cleaned just before immerging it into a solution of 0.5 M ferric chloride in 1 M hydrochloric acid, to form a AgCl film on the electrode. The electrode was then washed with water and used as a working or a reference electrode by assembling each into the flow through cell described above. A VYCOR plug served as a salt bridge for the reference electrode, and the reference electrode was immersed in 1.40 mM KCl/0.01 M KNO_3 solution, unless otherwise stated.

2.5. Procedure

The solution aspiration sequence of the SI-LAV system is summarized in Table 1 and Fig. 1(c). First, the carrier (H_2O) was drawn to fill all tubes. Then, a 100 μl portion of potassium nitrate, 100 μl of standard/sample and 100 μl of potassium nitrate solution, respectively, were sequentially aspirated into a holding coil. Finally, the stacked zone was sent to the LAV unit to monitor for potential difference between WE and RE as the zone passed the WE, recording as a SIA-peak. The potential difference is based on a concentration cell, a difference in concentrations of chloride in the WE and RE half-cells. A calibration graph was plotted of the peak height obtained as a function of the logarithm value of concentration of the chloride standard. Chloride concentration in a sample was evaluated from the calibration graph. In spite of the slow operation of the autotitrator, the whole operating cycle took only about 70 s for a sample. Replacing with a more efficient pumping device would result in even a higher sample throughput.

3. Results and discussion

3.1. SI-LAV components

The SI-LAV concept was demonstrated for the determination of chloride. A LAV unit, a flow through cell for potentiometric detection with a simple home-made chloride ion selective electrode, could be fabricated small enough to put at one port of the selection valve (see Fig. 1). The upper port position was the most appropriate one to attach the flow cell to the valve because it is easier to remove any air bubble, which may be in the line out of the cell. A polymer VYCOR plug was inserted between the reference electrode and the flow channel in order to prevent leaking of chloride from the reference solution. The LAV is compact in size and has various advantages that are similarly offered by a LOV system [9]. However, the LAV can be assembled without taking apart components of a purchased valve and is easy to build to be suitable for chemistries of interest. Moreover, it can be fabricated with relatively low-cost, less precise machine tools. In this work, an autoburette was employed as an alternative inexpensive solution propelling device in place of a syringe pump. However, a solution volume of less than 100 μl cannot be aspirated due to the default setting of the autotitrator to aspirate at least 100 μl volumes each time. The WE and RE were connected directly to the signal and ground poles, respectively, of an analog input of the interfacing board. Identical signal profiles were obtained by either connecting the electrode directly to the input of the interface card or by inserting a potentiometer between them. This indicates that an impedance of the interfacing board input terminal was high enough to detect the cell potential correctly.

3.2. Optimization

Due to limitation of the autoburette used, some parameters were fixed: volume of electrolyte solution and sample, 100 μl each, flow rate for aspiration and dispensation of the solution, 15 ml min^{-1}. The effects of concentration of the electrolyte (KNO_3) solution and sodium chloride in the RE half cell on the slope of the calibration graph for chloride were investigated. It was found that the electrolyte concentration did not significantly affect to the slope, intercept and R^2 of the calibration graph, for the injection of a series of standard solutions as shown in Table 2. However, the 0.01 M KNO_3 was selected to minimize the effect of ionic species due to

Table 1
SI-LAV operating sequence

Step	Operation	SV port[a]	Volume (μl)	Flow rate (ml min^{-1})	Flow direction
1	Aspirate electrolyte solution	9	100	15	To HC
2	Aspirate standard/sample solution	2–7	100	15	To HC
3	Aspirate electrolyte solution	9	100	15	To HC
4	Dispense to LAV unit and record signal	1	2000	15	To LAV

[a] SV = Selection valve.

Table 2
Effect of potassium nitrate concentration

[KNO_3] (M)	Calibration equation (0.60–2.80 mM Cl^-)	R^2
0.005	$y = 0.053x - 0.047$	0.999
0.010	$y = 0.051x - 0.029$	0.999
0.025	$y = 0.049x - 0.038$	0.998
0.050	$y = 0.053x - 0.045$	0.999
0.100	$y = 0.051x - 0.045$	0.999

sample matrices, which may affect the total ionic strength of the solution passing through the WE. For mineral drinking water and surface water samples, this concentration of the electrolyte is sufficient for control of ionic strength of the sample.

Chloride concentration in the reference half-cell did not affect to the slope, intercept and R^2 of the calibration graph (a plot of peak height (V) (y) versus log (mM concentration of chloride standard solution) (x)). Using a reference solution containing 0.01 M KNO_3 with 0.30, 0.80, 1.40 or 2.80 mM potassium chloride, the calibration equations: $y = 0.051x - 0.030$, $R^2 = 0.998$, $y = 0.051x - 0.032$, $R^2 = 0.998$, $y = 0.051x - 0.032$, $R^2 = 0.999$ or $y = 0.051x - 0.030$, $R^2 = 0.998$, respectively were obtained. A 1.40 mM chloride solution with 0.01 M KNO_3 in the RE half cell was selected.

3.3. Analytical characteristics

Three linear calibration graph ranges, 0.10–0.80 mM ($y = 0.059x + 0.120$), $R^2 = 0.998$), 0.80–8.40 mM ($y = 0.056x - 0.105$, $R^2 = 0.998$) and 10–120 mM ($y = 0.060x - 0.128$, $R^2 = 1.000$) were obtained. Each injection consumed 200 μl of 0.01 M KNO_3 and 100 μl of standard/sample, with the whole analysis time of 70 s, which corresponds to sample throughput of about 50 h^{-1}. Relative standard deviations for 11 replicate injections of 10–40 mM chloride standards were in range of 0.7–1.3%. Higher %RSDs may be obtained for solutions containing chloride concentrations out-side of the range.

The stability of the electrode was studied by continuous injection (about 300 injections) of a series of concentrations of chloride standard solutions (0.10–0.80 mM) and some samples, for a period of time about 6 h. The slope of the calibration graphs was quite constant (0.06 ± 0.01), indicating a good stability of the electrode for at least 6 h. Although, the effects of some interferences have not been investigated here, the literature [12–14] indicates that some halides (I^- and Br^-), sulfide, cyanide and some metal ions (Fe^{3+} and Al^{3+}) have some interferences in the analysis by this kind of chloride ion selective electrode. However, these ions are usually present in natural waters at relatively low concentrations compared to chloride and do not significantly interfere, otherwise sample pretreatment is needed [12–14].

Table 3
Chloride contents in mineral drinking water (triplicate determination)

Sample	Chloride concentration (mM)	
	IC [15][a]	SI-LAV[b]
1	0.30	0.37 ± 0.01
2	0.14	0.15 ± 0.01
3	0.65	0.73 ± 0.02
4	0.37	0.35 ± 0.01
5	0.08	<0.10
6	0.06	<0.10
7	0.02	<0.10
8	0.02	<0.10
9	0.17	0.20 ± 0.01
10	2.36	2.26 ± 0.01
11	0.02	<0.10

[a] Analysis by routine laboratory of the Science and Technology Service Center, Faculty of Science, Chiang Mai University.
[b] Using the calibration equation of the range 0.10–0.80 mM Cl^-. The limit of quantitation is 0.10 mM.

Table 4
Chloride contents in surface water samples taken around a pickling industry (triplicate determination)

Sample[a]	Chloride concentration (mM)	
	Titration [16]	SI-LAV[b]
1	3.0 ± 0	2.0 ± 0.3
2	86 ± 1	86 ± 1
3	85 ± 2	86 ± 2
4	86 ± 3	88 ± 1
5	66 ± 0	69 ± 1
6	66 ± 1	68 ± 1
7	71 ± 2	71 ± 2
8	50 ± 1	45 ± 2

[a] Sample 1 = supplied pond water for the factory usage, 2–8 = surface water around the factory.
[b] Using the calibration equations of ranges of 0.80–8.40 and 10–120 mM Cl^-.

3.4. Application to water samples

The developed SI-LAV system was applied for the determination of chloride in different commercially available mineral drinking water samples from a market place in Chiang Mai, and surface water samples collected from around the vicinity of a pickling factory in Hang Dong district of Chiang Mai. Analyses by ion chromatography [15] and Mohr titration [16] were also carried out for comparison. The results (means of triplicate determinations) obtained are summarized in Tables 3 and 4. The results from the pairs of the two methods correlate well with each other, except at low concentrations of chloride. However, the t-test at 95% confidence level indicated that the results obtained from the techniques are not significantly different [17]. The chloride contents in surface water around the pickling factory were found to be significantly higher than the raw water used by the factory, indicating that salt may be introduced to the surface water via discharge water. More close investigation on this will be carried out in our laboratory.

4. Conclusion

A novel SI-LAV concept is demonstrated. Here we describe potentiometric determination of chloride in some water samples. Various advantages of the proposed LAV are similar to those of the LOV, such as economical and integrated instrumentation, small amounts of reagent consumption, rapidity, and automation in analysis. However, the LAV unit can be more easily fabricated with relatively low price materials and available instrument/machine tools, so the LAV could be a cost effective alternative. Other LAV developments such as solvent extraction and bead injection are in progress [18,19].

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A dynamic liquid–liquid interfacial pressure detector for the rapid analysis of surfactants in a flowing organic liquid

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Abstract

Design and development of a dynamic interfacial pressure detector (DIPD) is reported. The DIPD measures the differential pressure as a function of time across the liquid–liquid interface of organic liquid drops (i.e., *n*-hexane) that repeatedly grow in water at the end of a capillary tip. Using a calibration technique based on the Young–Laplace equation, the differential pressure signal is converted, in real-time, to a relative interfacial pressure. This allows the DIPD to monitor the interfacial tension of surface active species at liquid–liquid interfaces in flow-based analytical techniques, such as flow injection analysis (FIA), sequential injection analysis (SIA) and high performance liquid chromatography (HPLC). The DIPD is similar in principle to the dynamic surface tension detector (DSTD), which monitors the surface tension at the air–liquid interface. In this report, the interfacial pressure at the hexane–water interface was monitored as analytes in the hexane phase diffused to and arranged at the hexane–water interface. The DIPD was combined with FIA to analytically measure the interfacial properties of cholesterol and Brij®30 at the hexane–water interface. Results show that both cholesterol and Brij®30 exhibit a dynamic interfacial pressure signal during hexane drop growth. A calibration curve demonstrates that the relative interfacial pressure of cholesterol in hexane increases as the cholesterol concentration increases from 100 to 10,000 $\mu g\,ml^{-1}$. An example of the utility of the DIPD as a selective detector for a chromatographic separation of interface-active species is also presented in the analysis of cholesterol in egg yolk by normal-phase HPLC-DIPD.

Keywords: DIPD; DSTD; Flow-based technique; Chromatography; FIA; Liquid–Liquid interface; Cholesterol; Brij®30

1. Introduction

Surfactants are chemical species that concentrate and align preferentially at an interface due to the presence of both hydrophilic and hydrophobic moieties within their structure. Alignment at the air–liquid surface (surface tension), solid–liquid and liquid–liquid interfaces (interface tension) often results in the lowering of surface tension at the given interface. Due to their unique properties (i.e., the ability to lower surface tension, foam, and create emulsions), surfactants and other interface-active species are widely used in many industries including food and beverage, personal care products, cosmetics, metal working, mining, agriculture, paper, and leather. Furthermore, surface-active chemicals are used in large quantities for many household applications.

Several methods have been developed for the measurement of surface tension. Most of these methods can be divided into three main groups: force methods (Du Noüy ring and Wilhelmy plate), shape methods (sessile drop, pendent drop, and spinning drop), and pressure methods (small bubble

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surfactometer) [1]. When a surfactant-containing solution forms a fresh interface, a finite time interval must elapse before equilibrium surfactant concentrations can be partitioned between the interface and the bulk solution. The aforementioned methods actually measure the static surface tension since the measurement is made after the interface-active analytes attain equilibrium at the air–liquid interface. The non-equilibrium interface tension is known as the dynamic interface tension. Most surfactant-driven processes, such as foaming, emulsification, coating flows and wetting, take place under non-equilibrium conditions, and therefore, dynamic interface tension information is an imperative parameter for understanding these processes. In principle, some of the surface tension measurement methods described above can be used to measure dynamic surface tension, however, most are not appropriate for high-throughput measurements. A few of the most common dynamic surface tension techniques are maximum bubble pressure [2], oscillating jet [3] and fast forming drop [4].

In the study of surfactants, another area of interest is the interfacial tension at the liquid–liquid interface. Specifically, adsorption at liquid–liquid interfaces plays a key role in the dynamics of multiphase systems. Though the principles are similar to adsorption at the air–liquid interface, measurements at the liquid–liquid interface require unique instrumentation. Ravera et al. reviewed the use of several techniques for liquid–liquid interfacial pressure measurements and found that while in theory many of the techniques commonly employed for air–liquid measurements can also be used for liquid–liquid interface studies, in practice, however, many obstacles, such as solvent miscibility, density, and viscosity came to the forefront [5]. Thus, relative to measurements at the air–liquid interface, few instruments are available for liquid–liquid interface studies and even fewer of these measure dynamic interfacial tension. The lack of methods to measure liquid–liquid interfaces is likely due to the difficulty of designing the instrumentation.

Most of the research in the area of liquid–liquid interfacial measurements focuses on the physics of adsorption at the interface, and the development and testing of theories to understand the kinetics and thermodynamics of adsorption at the interface [4–11]. Instead, we focus on the dynamic analytical signal resulting from adsorption at the liquid–liquid interface and have developed a simple and robust instrument to detect and quantify interface-active analytes in organic samples. The technique presented here offers a mechanically simple and inexpensive means to measure the liquid–liquid interface. This instrument can also be used to qualitatively describe the adsorption kinetics of interface-active analytes and the interactions of mixtures that affect the interfacial pressure [12,13].

Another important attribute of surface and interfacial tension measurement methodologies is the ability to carry out real-time analysis on dynamic systems containing complex and interacting surfactants. Previously, the dynamic surface tension detector (DSTD) was developed as a detection system for flow-based techniques, such as high performance liquid chromatography (HPLC), flow injection analysis (FIA), and sequential injection analysis (SIA). The DSTD responds to the surface activity of analytes at the air–liquid interface and offers a mechanically simple and inexpensive means to measure the air–liquid interface of a wide variety of samples, including samples that would foam using a bubble pressure technique [12–17]. The design and construction of the DSTD has previously been discussed in detail [12–19]. The DSTD has been used for the detection and study of species, such as common surfactants, polymers and proteins at the air–liquid interface. The most recent DSTD design is based on measuring the interior pressure throughout drop growth of repeatedly forming drops, which are pneumatically detached from a capillary tip.

Herein, we present an adaptation of the DSTD to form a dynamic interfacial pressure detector (DIPD) for the detection and analysis of species that are interface-active at the liquid–liquid interface. The DIPD complements the DSTD for evaluating chemical species that are either insufficiently soluble in aqueous media, or samples with unique liquid–liquid interface properties, or samples in which the dynamic interactions at the liquid–liquid interface are vital to their performance. In the presented experiments, the DIPD was coupled with FIA and HPLC for the study of the dynamic interface properties of typical surface-active species at the hexane–water interface.

2. Theory

The DIPD measurement follows principles similar to the DSTD [12]. In the DIPD, the internal pressure of repeatedly growing drops is measured relative to the pressure of the bulk solution phase in which the drops are grown, using a pressure transducer (see Figs. 1 and 2). Pressure is measured throughout the growth of the drop and the resulting drop pressure profiles are used to calculate the relative interfacial pressure.

The time-dependent Young–Laplace equation relates to the measured drop pressure, and was applied to calculate the

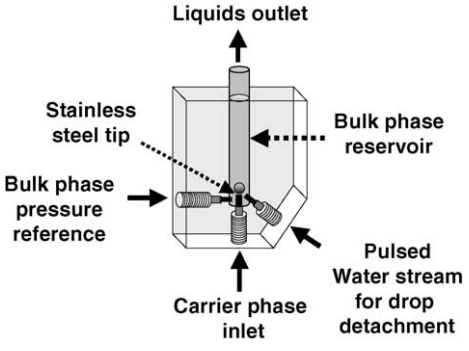

Fig. 1. A schematic diagram of the DIPD flow cell.

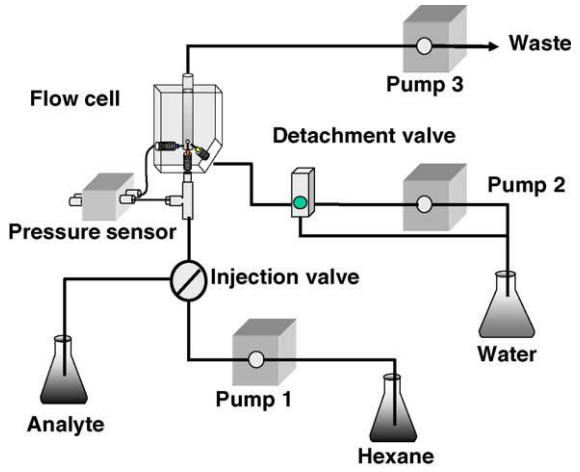

Fig. 2. A schematic diagram of FIA combined with DIPD for the liquid–liquid interfacial pressure measurement. Pump 1: piston pump at flow rate of 60 μl min^{-1} *n*-hexane, Pump 2: piston pump at flow rate of 4 ml min^{-1} water.

interfacial pressure from the pressure measurements:

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

In Eq. (1), P is the drop pressure referenced to the water bulk phase pressure, γ is the liquid–liquid interfacial tension, r is the drop radius, and P_C accounts for viscous losses in the capillary tubing. The parameters, P, γ, and r, are functions of time, t, throughout the drop growth.

Drop pressure "profiles" are the raw signals obtained for the mobile phase ($P(t)_M$) (i.e., carrier stream), standard ($P(t)_S$), and analyte ($P(t)_A$). Combined with the dynamic interfacial pressure of the standard ($\pi(t)_S$), these three signals allow the dynamic interfacial tension of the analyte ($\pi(t)_A$) to be calculated using Eq. (2):

$$\pi(t)_A = \frac{\pi(t)_S \left[P(t)_M - P(t)_A\right]}{\left[P(t)_M - P(t)_S\right]} \tag{2}$$

The dynamic interfacial tension of the analyte ($\pi(t)_A$), calculated relative to the dynamic interfacial tension of the standard ($\pi(t)_S$), contains information regarding the diffusion and arrangement of analyte at the liquid–liquid interface.

It would be ideal to calibrate the DIPD sensor using a standard solution with known interfacial pressure $\pi(t)_S$. Likewise, it is advantageous to have a standard such that $\pi(t)_S$ is constant at a given standard concentration and independent of drop time, i.e, $\pi(t)_S$ is simply π_S. Unfortunately, due to the difficulty of liquid–liquid interfacial tension measurements, the liquid–liquid interfacial pressure of a suitable standard acting at the interface of *n*-hexane and water which has been confirmed by multiple techniques or multiple research groups could not be found in the literature [4,7,8,20–23]. For this reason, we report the relative interfacial pressure values, defined by Eq. (3):

$$\pi_R(t)_A = \frac{\pi(t)_A}{\pi_S} = \frac{\left[P(t)_M - P(t)_A\right]}{\left[P(t)_M - P(t)_S\right]} \tag{3}$$

Relative interfacial pressure is simply the interfacial pressure of the analyte divided by the interfacial pressure of the standard. Since the standard is consistent, the relative interfacial pressures of analytes can be compared to understand their interfacial properties and to analyze different analytes. In this work, isopropanol in *n*-hexane and *n*-octanol in *n*-hexane were evaluated, and applied, as standard solutions to achieve a relative interfacial pressure for analytes of interest from the DIPD, using Eq. (3). We found that both isopropanol and *n*-octanol align rapidly at the water–hexane interface thus providing a π_S independent of drop time.

3. Experimental

3.1. Materials

HPLC grade *n*-hexane, *n*-octanol and 2-propanol (isopropanol) were obtained from Fisher Scientific (Fisher Scientific international Co., Fair Lawn, NJ). Cholesterol (98% purity) and Brij®30 were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI). All chemicals were used as received. Deionized (DI) water, demineralized to greater than 18 MΩ with a Millipore system (Millipore, Bedford, MA), was used as the bulk phase and in the preparation of all solutions. The water was degassed using an ultrasonic bath (Cole-Parmer Instrument Company, Vernon Hills, IL) with vacuum system for 15 min prior to use.

3.2. Dynamic interfacial pressure detector and its flow cell

A flow cell, fabricated from acrylic sheet, was designed in-house for this detector. A block of acrylic sheet was selected as the flow cell material because it is transparent, easily machined, and reasonably chemically hearty for demonstrating the detection principle. A schematic diagram of the DIPD flow cell is shown in Fig. 1. The flow cell is comprised of the following parts:

(1) Bulk phase reservoir
A 0.795 cm inside diameter (i.d.) × 2.22 cm long cylindrical hole was drilled into the block of acrylic sheet to serve as the bulk phase reservoir. In this work, water was used as the bulk phase and *n*-hexane was used as the carrier phase. To prevent *n*-hexane from adhering to the acrylic sheet flow cell wall, the bulk phase reservoir was lined with a 0.795 cm outside diameter (o.d.) × 4.40 cm long Teflon® tube (Brinkmann Instruments, Inc., Westbury, NY). This allowed the detached hexane drops to rise to the surface and be drawn to waste.

(2) Carrier inlet
The buoyant force operating on the carrier phase droplets must be sufficient to overcome the adhesive forces acting between the carrier phase and the tubing tip. The tubing tip material is chosen to minimize the adhesive forces, which consequently, minimizes droplet shape distortion. Since the DSTD uses an aqueous carrier phase, poly(etheretherketone), or PEEK™, is chosen as the tubing tip material. With the DIPD and a *n*-hexane carrier phase, however, it is more appropriate to use stainless steel as the tubing tip material. The stainless steel tip is situated inside a threaded inlet provided at the base of the flow cell. The stainless steel capillary sensing tip was made by cutting a syringe needle (Becton Drive 25 G × 11/2 in BD™, Franklin Lakes, NJ) to a length of 10 mm and polishing both ends of the tip until smooth. As can be seen in Fig. 2, a side-arm just outside the carrier inlet is connected to the pressure sensor (Validyne DP15-40-2430, Northridge, CA). With this design, most organic solvents with densities less than water and immiscible with water may be used as a carrier stream (mobile phase), such as *n*-hexane used herein.

(3) Hydraulic drop detachment
Hexane drops were hydraulically detached by degassed water flowing at a high flow rate and directed at the base of the forming hexane drop. The drop detachment stream, which flows water at 4 ml min^{-1} when briefly actuated, was regulated by an injection valve, and actuated at 2 s intervals. The hexane drop size can therefore be controlled by the detachment interval.

(4) Water pressure reference
The reference side of the pressure sensor was connected to the water reference inlet in the flow cell reservoir to measure the pressure in the bulk phase. The *n*-hexane carrier, under constant flow rate conditions, was delivered through the capillary sensing tip to form drops at the end of the tip. The drops were either detached with the hydraulic water stream or would ascend to the surface as buoyant forces overcome the forces of molecular attraction between the drop and the tip. When hydraulic detachment is employed, drops are shot with a pulsed stream of water and detached from the tip well before drops would have risen to the surface due to the buoyancy force. Hydraulic detachment is advantageous because it increases data density, improves drop time repeatability, and allows relative interfacial pressure to be calculated in real-time.

A pressure transducer continuously measured the pressure signal. The signal was conditioned with a high gain carrier demodulator (Validyne CD12, Northridge, CA). The raw pressure signal was collected at the rate of 100 Hz and instrument control was performed on a PC (1.4 GHz AMD Duron™ processor, Sunnyvale, CA) with a data acquisition board (AT-MIO-16XE-50, National Instruments, Austin, TX), using software written in-house (LabVIEW Version 6, National Instruments, Austin, TX). Data analysis was performed with MATLAB 6.0 (The MathWorks, Natick, MA).

3.3. Flow injection analysis (FIA)

A diagram of the FIA system coupled with the DIPD is shown in Fig. 2. A piston pump (Beckman, Model 114 M, Berkeley, CA) was used to introduce the carrier and sample to the flow cell at a flow rate of 60 μl min^{-1}. A second pump (Beckman, Model 114 M, Berkeley, CA) was required to control the flow of water into the flow cell chamber for hydraulic drop detachment. As the hexane drops are formed at the end of the stainless steel tip, they were detached with the pulse of water created by switching a valve in-line with the flow at a frequency of 0.5 Hz (2 s per drop). The detached hexane drops then rose to the surface and were aspirated out of the flow cell by a vacuum pump along with excess water from the hydraulic detachment process. This allowed the vacuum pump to maintain a stable bulk phase fluid level in the water reservoir.

The principles of FIA were utilized to introduce sample to the DIPD. A sample volume containing the interface-active analyte in *n*-hexane was injected into the *n*-hexane carrier using an injection valve (EC10W, Valco Instruments Co. Inc., Houston, TX) equipped with the appropriate length of PEEK™ tubing (Upchurch, Oak Harbor, WA) loop size. The sample plug then passed through PEEK™ tubing into the detector flow cell for analysis.

3.4. Normal phase high performance liquid chromatography

To demonstrate the operation of the DIPD as a selective HPLC detector, the FIA-DIPD instrument was modified to include a normal phase NP-HPLC cyano column (2.1 mm × 30 mm, Spheri-5, 5 μm, Brownlee Columns, Applied Biosystems, Inc., Foster City, CA) placed in between the DIPD flow cell and the injection valve. The *n*-hexane mobile phase was run at a flow rate of 60 μl min^{-1}. Cholesterol in egg yolk was separated by NP-HPLC and selectively detected by DIPD. To extract cholesterol from egg yolk, one egg yolk was mixed with 100 ml of *n*-hexane and the resulting biphasic mixture was shaken for two hours. Upon shaking, the cholesterol along with other organophilic compounds from the egg yolk partitioned into the *n*-hexane supernatant. Aliquots of the supernatant were injected into the NP-HPLC-DIPD system for chromatographic separation and detection.

4. Results and discussion

To test the response of the DIPD, and to ascertain the suitability of standards, a 100 μl plug of 0.5% isopropanol in hexane and a 100 μl plug of 0.1% octanol in hexane were injected separately into the water bulk phase of the DIPD

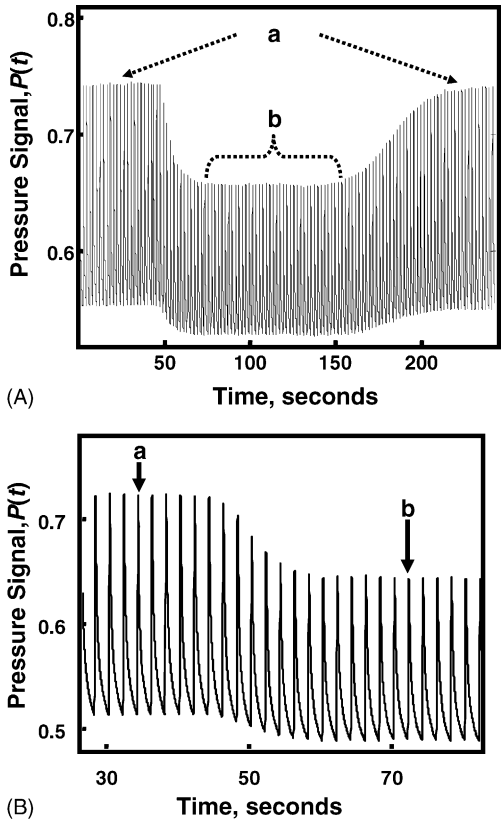

Fig. 3. (A) Raw pressure data, $P(t)$, obtained from the DIPD for a 100 μl injection of 0.1% *n*-octanol in *n*-hexane: (a) baseline signal of the *n*-hexane carrier phase; (b) steady-state concentration of 0.1% *n*-octanol in *n*-hexane. Each drop was detached from the end of the stainless steel tip at 0.5 Hz or every 2 s. (B) An enlargement of raw pressure data, $P(t)$, from Fig. 3(A). Each peak referred to the measured pressure of each drop that formed and detached at the end of the sensing tip every 2 s: (a) baseline signal of *n*-hexane; (b) steady-state concentration of 0.1% *n*-octanol in *n*-hexane.

system. Fig. 3(A) shows the raw drop pressure data, $P(t)$, of 0.1% *n*-octanol in *n*-hexane. During the first 50 s labeled "a", only *n*-hexane was present at the liquid–liquid interface. When the solution of 0.1% *n*-octanol in *n*-hexane reached the DIPD, labeled "b", the pressure signal, $P(t)$, decreased due to the lower interfacial tension (i.e., higher interfacial pressure) and achieved a steady-state signal that lasted for approximately 100 s.

In Fig. 3 (B), an enlargement of raw drop pressure data in Fig. 3 (A) is shown for clarity. The maximum of each of the sharp $P(t)$ profiles represents the maximum pressure of an individual drop. This maximum pressure, P_{max}, occurs when the liquid at the end of the sensing tip begins to form a new drop. Drops were detached from the capillary sensing tip every 2 s. Drop pressures decreased as the drop volume increased and were at their minimum values, P_{min}, just before detachment, according to Eq. (1).

Single drop pressure profiles of hexane, 0.5% isopropanol in hexane, and 0.1% octanol in hexane forming in the water bulk phase are shown in Fig. 4. These profiles were averaged from 10 raw $P(t)$ drop profiles. Comparing the pressure drop

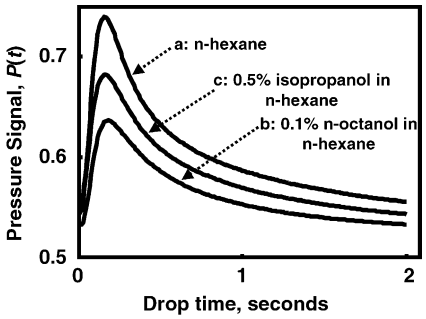

Fig. 4. Average drop pressure profiles of *n*-hexane (mobile phase carrier stream), 0.5% isopropanol in *n*-hexane, and 0.1% *n*-octanol in *n*-hexane from 10 drops under steady-state concentration conditions: (a) pure *n*-hexane drop; (b) 0.1% *n*-octanol in *n*-hexane; (c) 0.5% isopropanol in *n*-hexane.

profiles of *n*-hexane, 0.1% *n*-octanol in *n*-hexane, and 0.5% isopropanol in *n*-hexane illustrates that injecting small quantities of *n*-octanol or isopropanol into the *n*-hexane carrier dramatically lowers the interfacial tension (increases the interfacial pressure), and therefore, leads to a decrease in both P_{max} and P_{min}. The exquisite sensitivity of the DIPD is realized by noting that a volume of only 100 nl (equal to 0.1% of 100 μl) of *n*-octanol was injected into the *n*-hexane carrier, and since *n*-octanol achieved a steady-state concentration for a period of 100 s, *n*-octanol was measured with a high signal-to-noise ratio as it passed the DIPD at a rate of only 1 nl of *n*-octanol per second.

The drop pressure profiles of analytes that can be used as standards run parallel to the pure solvent drop pressure profiles from the pressure maximum to the drop detachment as shown in Fig. 4. Thus, *n*-octanol and isopropanol are good candidates as standards. Fig. 5 shows the relative interfacial pressure of 0.1% *n*-octanol and 0.5% isopropanol calculated using the drop pressure profiles shown in Fig. 4 and Eq. (3). The relative interfacial pressure of 0.1% *n*-octanol was calculated using 0.5% isopropanol as the standard and, likewise, the relative interfacial pressure of 0.5% isopropanol was calculated using 0.1% *n*-octanol as the standard. Fig. 5 shows that the relative interfacial pressure of both analytes is not

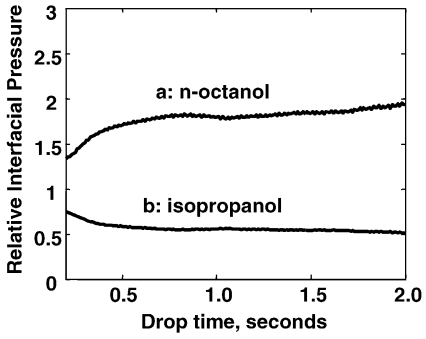

Fig. 5. Relative interfacial pressure of the analytes, after the drop pressure profile data in Fig. 4 were applied to Eq. (3): (a) the relative interfacial pressure of 0.1% *n*-octanol in *n*-hexane when 0.5% isopropanol in *n*-hexane was used as the standard; (b) the relative interfacial pressure of 0.5% isopropanol in *n*-hexane when 0.1% *n*-octanol in *n*-hexane was used as the standard.

dynamic over the measured drop growth (for drop time range of ~0.5–2 s) [16], therefore, both 0.1% *n*-octanol in *n*-hexane and 0.5% isopropanol in *n*-hexane were used as the standard solutions for sensor calibration, with 0.5% isopropanol used for calibration of the results reported herein. The calibration method defined in the Theory section (Eq. (3)) enables real-time calculation of relative interfacial pressure in a flowing system without the need of cumbersome optical measurements [16].

To demonstrate that the DIPD could be used to study the adsorption kinetics of interface-active molecules in *n*-hexane, the behavior of Brij®30 at the *n*-hexane–water interface was evaluated. The non-ionic surfactant, Brij®30 (Polyoxyethylene(4)lauryl ether), is a long chain polymer molecule that is soluble in both water and *n*-hexane. The Brij®30 hydrophobic chain is a lauryl group and the hydrophilic head group is polyoxyethylene ether. The characteristics of Brij®58 at the water/oil interface at constant drop size had been studied; although, in that work, the surfactant was dissolved in the aqueous phase [24]. The relative interfacial pressure of a 100 μl injection of 60 μg ml^{-1} Brij®30 in *n*-hexane is shown in Fig. 6(A) as a three-dimensional signal. Brij®30 shows a dynamic signal at this concentration and flow rate, with a relative interfacial pressure that changes by a factor of approximately four relative to 0.5% isopropanol in *n*-hexane as the standard, over the 2 s drop lifetime.

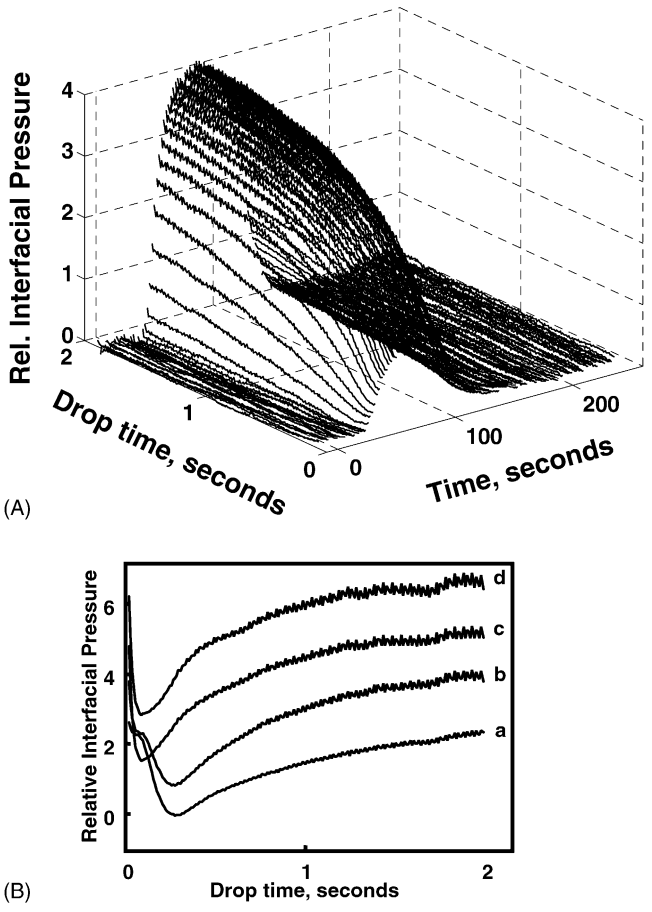

Fig. 6. (A) A three-dimensional relative interfacial pressure plot of 60 μg ml^{-1} Brij®30 in *n*-hexane using 0.5% isopropanol as standard. (B) A relative interfacial pressure plot of various concentrations of Brij®30 in *n*-hexane: (a) 20 μg ml^{-1}; (b) 40 μg ml^{-1}; (c) 60 μg ml^{-1}; and (d) 80 μg ml^{-1}.

The DIPD was designed to detect interface-active analytes in a sample and give selective signals for analytes that do not quickly reach equilibrium at the interface. Due to the size of the Brij®30 molecule, as well as the concentrations studied, Brij®30 was in the process of diffusing and arranging at the interface during the DIPD measurement. Fig. 6 (B) shows the individual drop pressure profiles of Brij®30 at four concentrations. An increase in the Brij®30 concentration is accompanied by an increase in the relative interfacial pressure measured by the DIPD. The consistency in the shape of the last three-quarters of the interfacial pressure profile indicates that DIPD may be useful for analyte identification and to understand the dynamic processes at the liquid–liquid interface.

The kinetic effect in the relative interfacial pressure during the drop lifetime is similar to the phenomena observed at the air–liquid interface by the DSTD. When the relative interfacial pressure is calculated as shown in Eq. (3), subtracting the carrier phase pressure signal and dividing by the pressure signal of the standard accounts for the increase of the surface area during drop growth. Thus, the relative interfacial pressure as the drop grows is related to the concentration of the analyte at the interface. Factors that affect the shape of the drop time profile include the concentration of the analyte in solution, analyte diffusion constant, molecule arrangement at the interface, equilibrium concentration at the interface and transfer of analyte to the interface [4,7,8,20].

The DIPD and DSTD are complementary detectors in that the DSTD detects interface-active analytes that are soluble and present in aqueous samples, whereas the DIPD detects analytes that are soluble and present in organic solvents. Examples of organic-soluble analytes include petroleum products, cooking oils, hydrocarbons and high molecular weight alcohols. In this report, cholesterol was examined. Samples of cholesterol in *n*-hexane ranging in concentration from 100 to 10,000 μg ml^{-1} were evaluated with the FIA-DIPD system. A three-dimensional FIA-gram of a 100 μl injection of 1000 μg ml^{-1} cholesterol is shown in Fig. 7 as an example. The time axis shows the FIA injection profile. The drop time axis shows the relative interfacial pressure of cholesterol at the water–hexane interface using 0.5% isopropanol in *n*-hexane as the standard. The increase in relative interfacial pressure with drop time is the result of the dynamic transfer of analyte to the interface. Due to the size of the cholesterol molecule, it is suspected that much of the kinetic signal is due to the diffusion of molecules from the bulk phase (inside drop) to the interface. Fig. 7 indicates that the cholesterol response is indeed kinetically hindered possibly due to diffusion and rearrangement processes at the drop interface, and appears to then nearly reach interfacial equilibration. Future studies might involve investigation to determine whether the time required to reach a state near equilibrium can be used to

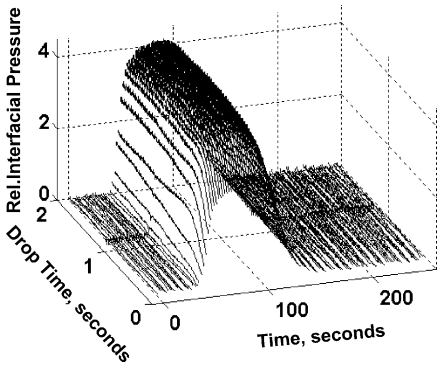

Fig. 7. A three-dimensional relative interfacial pressure plot of 1000 μg ml^{-1} cholesterol in *n*-hexane using 0.5% isopropanol as standard.

estimate the diffusion constant. Again, it is evident that the shape of an analyte interfacial pressure profile during drop growth might be useful for analyte identification.

The FIA profiles for multiple concentrations of cholesterol are given in Fig. 8(A). The interfacial pressure signals are an average of the relative interfacial pressure signals from 1.8 to 2.0 s in the drop profile (right before drop detachment, so the maximum relative interfacial pressure). The relative interfacial pressure increased with concentration of cholesterol. In Fig. 8 (B), the calibration curve of the concentration series of cholesterol from Fig. 8 (A) is shown. Fig. 8 demonstrates that the DIPD can be used to quantify the cholesterol concentration, and quantitative analysis for any surface-active analyte is possible in principle.

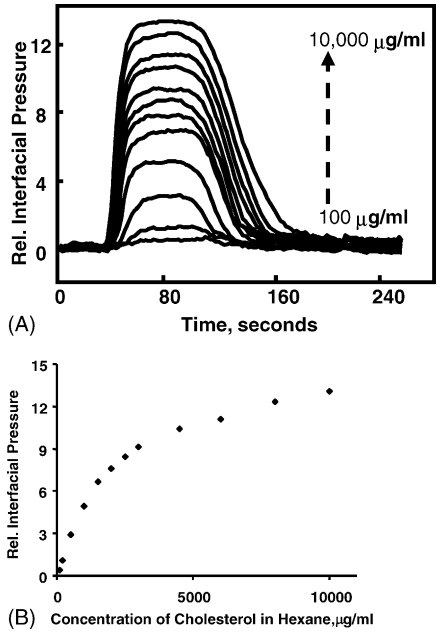

Fig. 8. (A) Overlay FIA-gram plots of relative interfacial pressure of various concentration of cholesterol over the range 100–10,000 μg ml^{-1} in *n*-hexane. The concentrations were 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 6000, 8000 and 10,000 μg ml^{-1}. (B) A calibration curve of the cholesterol data is plotted against relative interfacial pressure and concentration of cholesterol in *n*-hexane.

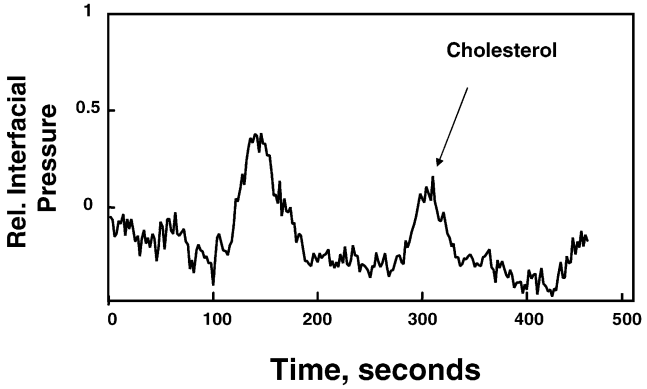

Fig. 9. Separation of cholesterol from egg yolk by NP-HPLC-DIPD. Experimental conditions are given in the text, and using 0.5% isopropanol as standard.

An example of the use of the DIPD as a selective detector for NP-HPLC was demonstrated with the extraction and normal phase liquid chromatographic separation of cholesterol from egg yolk. Because the DIPD selectively detects only interface-active compounds, only a few compounds in the sample must be separated to measure cholesterol in egg yolk. After the egg yolk was shaken with *n*-hexane, a 15 μl injection of the supernatant was injected into the *n*-hexane carrier phase of the NP-HPLC-DIPD system. The resulting chromatogram, given in Fig. 9, shows that cholesterol, with a retention time of approximately 300 s was separated from other organophilic egg yolks constituents, which eluted at approximately 150 s. The selectivity of the DIPD results in a relatively simple chromatogram, whereas use of another detector, such as absorbance or refractive index would be either not selective enough or not sensitive enough or both. The method of standard additions was used to confirm the retention time and concentration (250 μg ml^{-1}) of the cholesterol peak in the egg yolk extract, and the other organophilic egg yolk constituent remained unidentified.

5. Conclusions

An instrument known as the DIPD was designed and developed for making analytical measurements of surface-active species at the water–hexane interface. In principle, any combination of two immiscible liquids with different densities could be used with the DIPD. As a drop grows from a stainless steel capillary tip, the DIPD can be used to monitor the diffusion and arrangement of interface-active analytes at the liquid–liquid interface. The relative interfacial tension response can be used to identify and quantify surfactants and understand their behavior at liquid–liquid interfaces. The DIPD is a novel detector because it selectively measures the interfacial activity of analytes within drops eluting from a flowing stream into another immiscible liquid, and therefore, is a suitable detector for flow-based analytical techniques, such as FIA and NP-HPLC. Furthermore, the DIPD serves

as a detector for analyte quantification, as demonstrated by a calibration of cholesterol.

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Sequential injection-ELISA based system for online determination of hyaluronan

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Abstract

Sequential injection-bead-based immunoassay system has been developed. The main purpose is to make immunoassay process more automated by manipulating the precise delivery of micro-volumes of reagents and the precise timing of incubation and washing steps with a computer program that controls the bi-directional syringe pump. The manifold was designed with the aims of reducing back pressure from beads that act as solid surfaces for immobilization of the target substance, reducing dispersion and dilution of the reagent during incubation, and maximizing signal while minimizing incubation time. This was done by introducing air segment to separate the reagent zone from the carrier stream and by using a suitable sensitive detector which, in this case, was an amperometer. In this study, hyaluronan (HA) was used as a target analyte because of its clinical significance as a potential biomarker for liver, bone and cancer diseases. Amount of hyaluronan was determined using competitive enzyme linked immuno sorbent assay (ELISA) based technique where immobilized HA and HA in solution compete to bind with a fixed amount of biotinylated HA-binding proteins (b-HABPs). Upon separation of the two phases, anti-biotin conjugated with enzyme and a suitable substrate were introduced to follow the binding reaction of the immobilized HA and b-HABPs whose degree of binding is indirectly proportional to the amount of HA in solution. A calibration curve was constructed from a series of concentrations of HA standards. Lowest detectable concentration was found to be 1 ng/mL with the dynamic working range of 1–5000 ng/mL and R.S.D. of intra-assay ($n = 7$) and inter-assay ($n = 3$) of various HA concentrations were 4–10% and 9–12%, respectively. Used beads could be reused by washing with 2 M guanidine. Total analysis time for this automatic assay was about 30 min as compared to the 5–8 h used in conventional batch well ELISA. The system could be applied to assay HA in human serum.

Keywords: Sequential injection analysis; Bead ELISA; Online immunoassay; Hyaluronan

1. Introduction

Immunoassay is an analytical technique that utilizes the specific molecular recognition between antigen and antibody to selectively determine the presence of the antigen. The detection of the binding reaction between antibody and antigen can be followed using different available labels. Examples of recently used labels are radioactive isotope [1,2] chemiluminescent compounds [3,4], metals [5,6] and enzymes [7,8]. Among all the labels, the use of enzymes has gained high popularity due to their amplification properties in which they can convert substrate to product as long as the substrate is present. Assay with an enzyme label, known as enzyme linked immuno sorbent assay (ELISA), has been widely used.

Conventional immunoassay is normally carried out in a micro-well where antigen or antibody is immobilized onto the surface of the well. The rate of binding reaction between antigen and antibody depends upon the diffusion rate of antigen or antibody in the bulk solution to the immobilized ones. Conventional immunoassay, therefore, requires a long incubation

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time, mostly overnight or at least 5–8 h to ensure maximum binding. There have been attempts to reduce the analysis time of immunoassay [9–13]. Using microbeads as solid surface is one way that has been gaining popularity [14–18]. Now that there are many chemically modified beads commercially available, different formats of bead-based immunoassay have been reported [9,19–23]. The main advantages of bead-based immunoassay include increasing the surface area for immobilization of antigen or antibody and reducing the incubation time due to less diffusion distance needed between species in bulk solution and the ones immobilized on beads that are suspended in the solution. Magnetic microbeads also facilitate the phase separation during the washing steps [24,25].

The combination of beads and ELISA is very useful and has been used in many applications [9,23,24,26–28]. However, immunoassay always involves many steps of incubation and washing which are tedious and subjected to human error, mostly due to imprecise timing on each step. The small amount of each of the reagents used in the analysis process is also another difficult matter to handle by an inexperienced person. There is rarely any development on a true online immunoassay where the whole process from the first step of incubation to the last step of detection is automatic. This is due to the lack of a system that can deliver a very small volume of reagents and precisely control the incubation time. The new generation of flow injection technique called sequential injection analysis (SIA) is now available [29–33], and a true online immunoassay is possible. One very interesting work is the combination of bead injection technique with the lab-on-valve (LOV)-sequential injection analysis system for carrying out ELISA [34]. However, this work involves the sophisticated LOV device that attaches as a part of the multi-selection valve of the commercial SIA system.

The attempt of this work is to make the immunoassay process more automated and more precise than batch process by utilizing the most simple, and cost effective system as much as possible. This was done by utilizing the sequential injection analysis (SIA) system controlled by computer software to deliver micro-volumes of reagents and to precisely time the incubation and washing steps. Beads were used as solid surfaces. The system was developed with the aims of reducing back pressure in the flow line which commonly occurs when using a bead packed column, as well as to minimize the incubation time and to maximize the signal as much as possible. An air segment was introduced to separate the reagent zone from the carrier stream [35] in order to reduce the dispersion of the reagent during the incubation time when the pump was stopped. Amperometry was chosen as a detection means because of its high sensitivity and low susceptibility to the differences in non-electrochemical properties of reagents. For example, the Schlieren effect which is usually encountered when using spectrophotometric detection can be avoided when electrochemistry techniques are used.

The SIA-bead ELISA based system was developed for quantification of hyaluronan (HA) in serum. Hyaluronan is a glycoprotein, composed of gluconate acetylglucosamine disaccharide repeating units, that has been reported to be a potential biomarker for liver, cancer and bone diseases [36–43]. It can bind selectively to the proteins called HA-binding proteins (HABPs). In this study, the competitive ELISA based technique was carried out. Immobilized HA on beads and HA in solution competed to bind with a fixed amount of biotinylated HA-binding proteins (b-HABPs). Upon separation of beads from solution, anti-biotin conjugated with enzyme horseradish peroxidase (anti-b-HRP) and a suitable substrate (tetramethylbenzidine, TMB-H_2O_2) were introduced to follow the binding reaction of the immobilized HA and b-HABPs. The degree of binding is indirectly proportional to the amount of HA in solution. The demonstration of the potential use of the system with real samples was shown by performing a recovery study of the amount of spiked HA in bovine and human serum samples. The system offers an automated immunoassay process with good sensitivity and precision as compared to using a batch process, even though the incubation time was reduced to decrease the analysis time and increase sample throughput.

2. Experimental

2.1. Apparatus and reagents

2.1.1. SIA system

The system is composed of a syringe pump (CAVRO Scientific Instrument Inc., XL 3000) that can draw solution up to 2.5 mL, two 10-ports selection valves (Valco Instrument Co. Ltd., C25-3180EMH), a computer (Microsoft Window XP professional, CPU-AMD Athlon^{TM} XP 2000 + 1.67 GHz 256 MB of RAM) and SIA control software (FIAlab 3000, FIA Instrument). Holding coil is made of tygon tubing. All other tubings are PTFE tubings. The detection system was an amperometer (μAutolab Type II Eco Chemie B.V. Utrecht, The Netherlands) with its control software (Autolab Software—General Purpose Electrochemical System version 4.9). A BAS flow cell with glassy carbon working electrode, Ag/AgCl reference electrode and stainless steel auxiliary electrode was used. The applied potential was set at 100 mV for detection of the reduction of TMB product, produced from the enzyme–substrate reaction, back to TMB.

2.1.2. HA coated beads

EAH-sepharose 4B beads (Amersham Biosciences) size 45–165 μm (mean bead size 90 μm) were used as solid surfaces for immobilization of HA. These beads were washed with saline solution at the ratio of 0.5 M NaCl 250 mL: deionized water 100 mL: bead 10 mL (drained gel) and were filtered prior to use.

One of the many sources of HA is umbilical cord, which has an abundant amount of HA [44], that is also commercially available. HA was extracted from umbilical cord by mixing 0.0300 g umbilical cord (Sigma, containing approximately

30 mg HA) with 0.0010 g bovine testicular hyaluronidase (Sigma) and 10 mL of 0.15 M NaCl (BDH) in 0.1 M sodium acetate buffer solution pH 5.0 (Sigma). The mixture was shaken to let the reaction take place for 3 h before bringing it to boil for 10 min to end the reaction.

The beads and HA from previous steps were mixed and about 30 mL deionized water and 0.5751 g *N*-ethyl-*N*′-3-dimethylaminopropyl carbodiimide hydrochloride (Sigma) were added to the mixture. After mixing thoroughly, 0.1 M HCl (Sigma) was used to adjust the pH to about 4.5. This mixture was continuously shaken for 24 h at room temperature. After that, the remaining un-substituted amino groups were blocked with 1 mL glacial acetic acid and allowed to stand for 6 h. The beads were washed with 400 mL each of different solutions in the order of 1 M NaCl, 0.1 M Tris–HCl (Sigma) pH 8.1, 0.05 M formate buffer (Sigma) pH 3.1, and deionized water, respectively. In each washing step, beads were separated from washing solution by centrifugation. Finally, the beads were kept in 0.5 M sodium acetate buffer pH 5.7 at 4 °C for future use.

2.1.3. Biotinylated HABPs

HABPs was extracted from cartilage using 4 M guanidine. The extract was dialyzed against water and then was liophilized. The powder was partially digested with tripsin before dialyzation and liophilization again. Up to this step, HA that was previously bound to HABPs in cartilage is still present with HABPs. To separate out the HABPs from HA in solution, bead HA was added. HABPs can bind to HA on bead better than HA in solution. This may be because of higher amount of HA on beads and also because some portions of HA in solution were partially digested and therefore cannot bind effectively to HABPs. After that, HABPs was eluted out from bead HA using 4 M Guanidine.

Biotinylation of HA-binding proteins was performed by a standard method [45]. Briefly, HABPs which had been prepared was dissolved in 0.1 M sodium hydrogen carbonate buffer pH 8.5 and with *N*-hydroxysuccinimidobiotin (Sigma–Aldrich Chemical, 34.1 g/L in DMSO) mixed at a ratio of 3:1 (w/w) at room temperature for 1–2 h. The mixture was then introduced to a Sephadex G-25 column and was eluted with PBS pH 7.4. The excluded protein peak was collected, aliquoted and stored at −20 °C as a stock solution of the biotinylated HABPs (b-HABPs).

2.1.4. Anti-biotin conjugated with HRP

Anti-biotin HRP (Zymed) was diluted to 1:1000 ratio with PBS buffer solution pH 7.4.

2.1.5. HA standard solutions

HA standard solution (IAL®-F) has the concentration of 20 mg/2 mL which is equivalent to 10×10^6 ng/mL. Stock solution of 1 mL at the concentration of 10,000 ng/mL was made by diluting the HA standard solution with 6% BSA to match the approximate amount of protein contained in real blood. This stock solution was further diluted into a series of different concentrations; 5000, 2500, 1000, 500, 250, 125, 50, 25, 5 and 1 ng/mL.

2.1.6. Serum samples

Bovine serum (Sigma) in the powder form was dissolved in PBS to obtain a final concentration of 6% (w/v). This is to mimic the real blood in liquid form that contains approximately 6% protein.

Human serum was obtained from Chiang Mai University Hospital. Volunteers had not taken food and drink for 8 h prior to the blood drawing. These bloods were centrifuged to separate out the blood cells. Serum samples were kept at −20 °C and were used without further dilution.

2.2. Manifold design

The manifold used in this study is as shown in Fig. 1. A pipette tip (100–1000 μL blue tip, Eppendorf) with cotton wool at the tip end was used as a reservoir for beads. It was directly connected to one of the ports on the selection valve. Since one end was opened to air, there was no problem of back pressure as normally found in a packed column. Other reagents were kept in microcentrifuge tubes and light sensitive ones such as TMB were kept in a dark color tube. All tubes were closed and only a small hole was made for the tubing from selection valve to go through for solution drawing. This was to avoid air contact that might oxidize and degrade

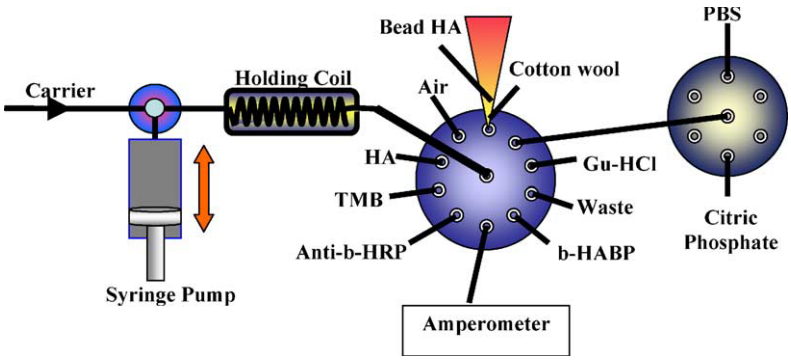

Fig. 1. SIA-bead ELISA based system for determination of HA.

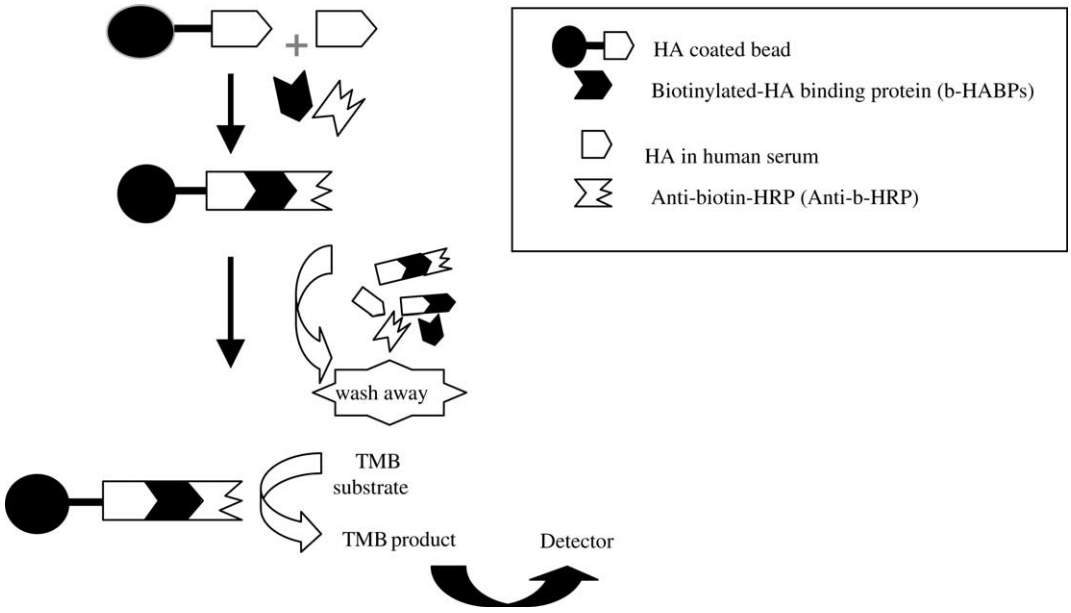

Fig. 2. Diagram illustrate bead ELISA based technique for determination of HA.

reagents. Holding coil was made of tygon tubing that can hold 2.5 mL of solution.

2.3. Operation steps

The bead-based ELISA process for determination of HA is illustrated in Fig. 2. Table 1 summarizes the operation steps. First, exactly 20 μL of HA coated bead suspension was pipetted into the pipette tip reservoir, see Fig. 1. The computer software program was started. The beads were washed once with 500 μL of PBS. Exactly 30 μL of standard HA solution or serum sample was drawn at the flow rate of 100 μL/s to mix with the bead HA in the reservoir. The mixed b-HABPs and anti-b-HRP zone was made by first drawing an air segment of 10 μL, followed by 10 μL of 1:1000 anti-b-HRP, 60 μL of 1:100 b-HABPs, and again 10 μL of anti-b-HRP. Total volume of the reagents drawn was 80 μL. The air segment helps to reduce dispersion of the zone during the reagent drawing and mixing process and the presence of anti-b-HRP on both sides helps to promote mixing. The zone was moved back and forth twice before sending 80 μL to the reservoir to mix with HA coated bead and sending the air segment to waste. These reagents were incubated with bead HA for 5 min before the unbound species were removed by washing the beads four times with PBS pH 7.4, 500 μL each time, followed by washing four times, with citric phosphate buffer pH 5.0, 500 μL each time. After that the buffer solution in the tubing leading to the detector was changed to citric phosphate buffer pH 5.0. Then TMB-H_2O_2 300 μL was drawn into the holding coil of which 250 μL was pushed into the reservoir at the flow rate of 50 μL/s to incubate with HRP that was bound to the bead HA. The rest of the TMB-H_2O_2 solution was moved to the detector to adjust the baseline. The mixing of TMB-H_2O_2 and (bead HA)-(HABPs-b)-(anti-b-HRP) was done by moving the solution in and out of the reservoir 20 times at the moving volume of 20 μL and the flow rate of 50 μL/s which resulted in a total mixing time of 200 s. The mixture was allowed to stand for another 100 s. Total incubation time was 5 min. The product of enzyme HRP and substrate TMB that

Table 1
Summarization of operation steps

Step no.	Operation	Volume (μL)
1	Pipette HA coated bead into the reservoir	20
2	Wash bead with PBS	500
3	Load HA standard solution or sample to the holding coil and then send it to mix with HA coated bead in the reservoir	30
4	Drawing air to the holding coil	10
5	Drawing anti-b-HRP to the holding coil	10
6	Drawing b-HABPs to the holding coil	60
7	Drawing anti-b-HRP to the holding coil	10
8	Mixing anti-b-HRP and b-HABPs by moving solution back and forth twice	
9	Send mixture to HA coated bead reservoir	80
10	Discard air	
11	Incubate HA solution, b-HABPs and anti-b-HRP with HA coated bead for 5 min	
12	Remove unbound species by washing beads with PBS four times followed by washing beads with citric phosphate buffer four times	500
13	Fill the detector line with citric phosphate buffer	
14	Draw TMB-H_2O_2 to the holding coil	300
15	Send some TMB-H_2O_2 to the detector to adjust baseline	50
16	Send the rest of TMB-H_2O_2 to bead reservoir and incubate for 5 min while moving solution back and forth at the end of the reservoir to promote mixing	250
17	Send the solution from the reservoir to the amperometer to detect product	
18	Change new bead reservoir to start new analysis	

occurred was detected when sending the solution to the amperometer at the flow rate of 20 μL/s. The resulting signal was the comparison of the TMB product with the TMB that had been sent previously to the detector. Used beads were collected to be washed for reuse.

2.4. Optimization

This study aims to improve the efficiency of the immunoassay technique by reducing reagent usage and time consumption as much as possible. Two main parameters studied here are the amount of b-HABPs for the given amount of HA coated beads and the incubation time.

2.5. Standard curve

The standard curve was constructed from various concentrations of standard HA solutions (1, 5, 25, 50, 100, 250, 500, 1000, 2500 and 5000 ng/mL) in 6% BSA. Lowest detectable concentration and working range were estimated from this curve.

2.6. Recovery study

To demonstrate the performance of the system on analysis of real samples, bovine serum and human serum were used. The effects of matrices were tested by spiking 50 μL of standard HA solutions of different concentrations into 950 μL of 6% bovine serum to gain the final added concentrations of HA at 5, 50 and 250 ng/mL and do the same with human serum to obtain the final added concentrations of HA at 5, 125 and 250 ng/mL. Percent recoveries were calculated from the results obtained from the standard curve as compared to the expected value from calculation.

3. Results and discussion

3.1. Optimization

Amount of b-HABPs is the most critical parameter in this immunoassay process for the selected amount of HA coated beads used in the experiment. Insufficient amount of b-HABPs may be used up by the HA in solution and therefore no signal would be produced. An excess amount of b-HABPs would accommodate all the HA present both in the solution and immobilized HA on beads, therefore no significant signal with the different levels of HA in solution would be observed. Thus, in this study the optimum amount of b-HABPs was determined by varying the concentration of a 60 μL b-HABPs from 0, 1:200, 1:100, 1:50 to 1:10 dilution in phosphate buffer while amounts of beads, anti-b-HRP and TMB-H_2O_2 were fixed at 20, 20 and 300 μL, respectively. The signals were increased with the increased b-HABPs concentration but at the concentrations higher than 1:100, there was no significant change of signal as shown in Fig. 3. This was due to the limited amount of HA on the beads. The results indicated that 60 μL of 1:100 b-HABPs was suitable to be used with 20 μL of HA coated bead.

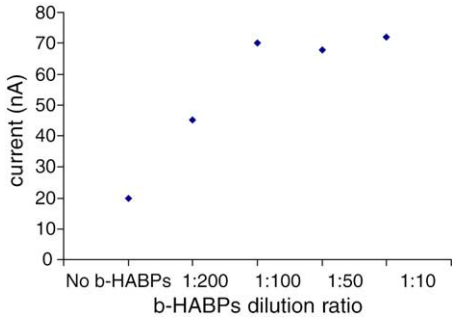

Fig. 3. Determination of optimum concentration of b-HABPs (60 μL) for the set of conditions: 20 μL HA coated bead, 20 μL anti-b-HRP and 300 μL TMB-H_2O_2.

Another important parameter that affects the sensitivity of the system is the incubation times between HA coated beads and b-HABPs and also between enzyme HRP and TMB-H_2O_2. Without stopping time for incubation, analytical signals could be observed. However, the longer the incubation time was, the better the sensitivity obtained. To compromise the analysis time and the sensitivity, the incubation time in both steps were limited to 5 min.

3.2. Standard curve and figures of merit

Standard curve is the sigmoidal (logistic) fit obtained from the computer software Origin version 7.0 as shown in Fig. 4. This standard curve is represented as $Y = [(A_1 - A_2)/(1 + (X/X_0)^P)] + A_2$ and $R^2 = 0.9963$ where Y is current in μA, X is concentration of HA in ng/mL, A_1 is initial Y value = 3.5, A_2 is final Y value = 2.5, X_0 is X value at Y equal to half of the limit A_1 and A_2 = 77.78 and P is power = 0.83. The lowest detectable concentration

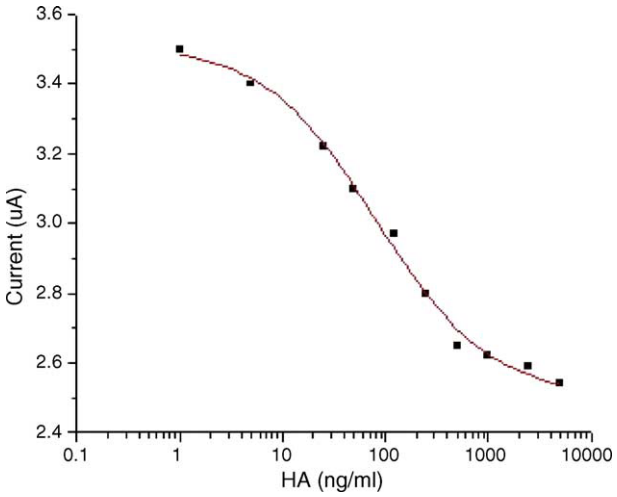

Fig. 4. Standard curve for determination of HA using the SIA system.

Table 2
Percent recovery of spiked HA in bovine serum and human serum samples

Sample	Obtained (HA, ng/mL)	Expected (HA, ng/mL)	% Recovery
Bovine serum	54	54	–
Bovine serum + 5 ng/mL standard HA	57 ± 6	59	97 ± 9
Bovine serum + 50 ng/mL standard HA	90 ± 5	104	87 ± 4
Bovine serum + 250 ng/mL standard HA	281 ± 15	304	92 ± 5
Human serum	47	47	–
Human serum + 5 ng/mL standard HA	60 ± 5	52	115 ± 9
Human serum + 125 ng/mL standard HA	188 ± 7	172	109 ± 4
Human serum + 250 ng/mL standard HA	303 ± 12	297	102 ± 4

of HA was found to be 1 ng/mL and the working range was 1–5000 ng/mL. The lowest detectable concentration was determined as the lowest distinguishable analytical signal ± S.D. with 95% confidence from the blank signal while the highest detectable concentration was determined similarly on another end of the calibration curve. This range can very well covers the HA level reported to be found in normal human at 28 ± 17 ng/mL [46] and at various elevated level in patients with liver and cancer diseases.

In this study, it was assumed that there were insignificant level of degraded HA too small to be unrecognized by HABPs. All serum samples have been kept at −20 °C and therefore HA should not be degraded during the sampling and assay process. It has also been reported that HABPs can interact with HA of the minimum 6–10 monomers and larger [47]. Small fragments of HA of 3–25 disaccharide units have been found in urine which is the last body fluid source before being discarded out of the body [48]. However, if HA macromolecules in serum were to be degraded with enzymes or other conditions, the degraded HA in serum should not be smaller than the ones found in urine and therefore the chance of having HA molecule of less than 6–10 monomers is possible but should be insignificant.

Precision of the system configured as % R.S.D. of intra-assay ($n = 7$) of various concentrations of HA in the working range was between 4 and 10% and inter-assay ($n = 3$) was between 9 and 12% comparing to % R.S.D. reported with the automated microparticle photometric agglunation assay where CV of intra-assay ($n = 10$) was 3.0–8.4% and CV of inter-assay ($n = 3$) was 4.8–8.9% [49]. The ones of batch well ELISA were found to be 6 and 21% for intra-assay ($n = 24$) and inter-assay ($n = 15$), respectively. Each analysis takes about 30 min, much less than the 5–8 h normally used with conventional batch well ELISA.

3.3. Recovery study

Table 2 shows the percent recoveries of spiked standard HA solutions in bovine and human serum samples. The recoveries were 87–115%. It is unclear at this time what matrices may cause positive error in human serum. Further study should be done to identify them.

4. Conclusion

The SIA-online bead-based immunoassay system has been developed. It can be employed to assay amount of HA in serum samples. Reuse of HA coated bead is possible by washing beads with guanidine solution to remove other reagents that have been added and leave HA attached on the bead surface. Analysis time per sample can be greatly reduced as compared to batch well ELISA without any decrease in sensitivity. Precision on drawing small amounts of reagents and timing the incubation and washing steps is improved with the use of computer control. Research has been in progress for a system performing multi-sample analysis by changing the configuration of the manifold in order to gain even better analysis time and to further develop the system into the lab-at-valve (LAV) device where integrating sample processing and detection unit are attached onto a port of a multiposition selection valve [50,51]. This would lead to an alternative cost effective μTAS for ELISA based multi-sample analysis in the future.

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Review

Review on screening and analysis techniques for hemoglobin variants and thalassemia

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Abstract

Thalassemia involves gene mutation that causes the production of an insufficient amount of normal structure globin chains while Hb variant involves gene mutation that causes the change in type or number of amino acid of the globin chain. It has been reported that some 200 million people worldwide had hemoglobinopathies of some sort. Attempts to develop effective and economical techniques for screening and analysis of thalassemia and Hb variants have become very important. In this review, we report the different techniques available, ranging from initial screening to extensive analysis, comparing advantages and disadvantages. Some indirect studies related to thalassemia indication and treatment follow-up are also included. We hope that information on these various techniques would be useful for some scientists who are working on development of a new technique or improving the existing ones.

Keywords: Hemoglobinopathies; Hemoglobin variants; Thalassemia; Screening techniques

Contents

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1. Introduction

Hemoglobin (Hb) is the molecule that carries and transports oxygen all through the body. It is composed of tetraglobin chains, two alpha and two non-alpha chains. The alpha (α) chains are encoded by the two closely related genes, alpha 1 and 2, on chromosome 16. The non-alpha chains – beta (β), gamma (γ) and delta (δ) – are encoded by a cluster of genes on chromosome 11. A fetus has a high amount of HbF ($\alpha_2\gamma_2$) as compared to other types of Hbs. A newborn has about 80% HbF and at about 1 year of age, HbF reaches a normal level. A few weeks after birth, the production of HbA ($\alpha_2\beta_2$) becomes dominant with the elevated level of HbA_2 ($\alpha_2\delta_2$) [1–3]. In normal adults, HbA is the main type of hemoglobin (96–98%) while HbA_2 and HbF are only present in 2–3% and less than 1%, respectively [4,5].

The failure in hemoglobin synthesis is a main cause of microcytosis and anemia in many population groups around the world. Hemoglobin variants (Hb variants) are characterized by the gene mutation of the globin chains that form hemoglobin (i.e., the replacement of different amino acids at a certain position). Thalassemia, which is slightly different from Hb variants, involves the gene mutation that causes production of an insufficient amount of normal structure globin chains. All types of thalassemias are considered quantitative hemoglobin disease.

Many types of hemoglobin variants have been found, depending on racial background [1–5]. Some types are not at all a problem, for example HbE heterozygous, while some types can cause severe anemia with serious clinical manifestation, for example HbS homozygous or sickel cell disease. Normally, Hb variants carriers, especially heterozygous, have no symptoms. However, combination of Hb variants and thalassemia gene on the same globin chain may result in severe symptoms. For example, combination of HbE with β-thalassemia gene becomes double heterozygote that shows symptoms similar to homozygous β-thalassemia, and combination of Hb constant spring (CS) with α-thalassemia gene will cause symptoms similar to HbH disease.

Thalassemia can be categorized into three classes – major, intermediate and minor – according to the severity of the symptoms [2,3]. The two main thalassemia syndromes (thalassemia major) are α and β thalassemias, which involve homozygous genetic defect in the α globin and β globin chain production, respectively. β-Thalassemia and sickel cell anemia have a wide distribution in tropical areas due to natural selection by malaria [6–8]. α-Thalassemia is most commonly found in Southeast Asia and Africa [1]. Related thalassemia minors or carriers are α-thal-1 (2 out of 4 globin gene deletion) and α-thal-2 (1 out of 4 globin gene deletion). Type 1 has insignificant but observable anemia while type 2 is a silent carrier without any symptoms shown [2]. Compound heterozygotes of α-thal-1 and α-thal-2 result in HbH disease. HbH is composed of β_4 chains instead of $\alpha_2\beta_2$ chains as in normal HbA. HbH is a relatively mild form of thalassemia and may go unnoticed. However, combination of HbH with HbCS, a type of hemoglobin variant of the alpha gene that has an elongated alpha chain with 31 extra amino acids, is severe and blood transfusion may be necessary. Homozygous α-thal-1 causes Hb Bart's hydrops fetalis which consists of γ_4 chains instead of $\alpha_2\gamma_2$ chains as in normal HbF. Unborn infants with Hb Bart's hydrops fetalis normally die before birth or within a short time after birth.

β-Thalassemia major is not as widely spread throughout Southeast Asia as α-thalassemia, but the β chain hemoglobinopathy HbE and HbTak are quite common. In HbE, the 26th amino acid of a normal β chain, glutamine, is replaced by lysine [1,2,4]. HbTak is another common Hb variant found in Asian population which is due to an insertion of the dinucleotide CA into codon 146 [CAC → CA(CA)C] resulting in elongation of the β chain by 11 amino acids. HbS and HbC are the main Hb variants in Africa but the

Table 1
Causes of different types of thalassemia and examples of hemoglobin variants and the areas commonly found

Thalassemia/Hb variants	Type	Cause/severity	Prevalent regions
Alpha-thalassemia	α-thal 2 trait or heterozygous α-thal 2 ($-\alpha/\alpha\alpha$ or α^+)	Only one out of four genes is deleted/No symptoms (silent carrier)	Southeast Asia, Africa
	α-thal 1 trait or heterozygous α-thal 1 ($-/\alpha\alpha$ or α^0)	Two deleted genes are on the same chromosome/observable anemia	
	homozygous α-thal 2 ($-\alpha/-\alpha$)	Two deleted genes are on different chromosomes/observable anemia	
	α-thal 1 /α-thal 2 or HbH disease ($-/-\alpha$ or α^+)	Three out of four genes are deleted and excess β-chains form β_4/significantly big liver and spleen	
	α-thal 1 /α-thal 1 or homozygous α-thal 1 or Hb Bart's hydrops fetalis ($-/-$)	There is no alpha chain production and infants have γ_4 instead of normal HbF ($\alpha_2\gamma_2$)/die before or at birth	
Hb variants related to mutation in alpha globin chain	HbCS	Alpha chain has extra 31 amino acids	Southeast Asia
	HbMahidol (HbQ)	$\alpha^{74Asp\rightarrow His}$	Southeast Asia
	HbThailand	$\alpha^{56Lys\rightarrow Thr}$	Thailand
Beta-thalassemia	β^+ thal heterozygote (β^+/β)	Less production of beta chain/insignificant symptoms	Mediterranean, Middle East, India, Southeast Asia, and also found in West and North Africa, West Asia, Italy, Greece, East and Central Europe and USA
	β^+ thal homozygote (β^+/β^+)	Less production of beta chain/need some blood transfusion	
	β^0 thal heterozygote (β^0/β)	Complete failure on beta chain production/need some blood transfusion	
	β^0 thal homozygote (β^0/β^0)	Complete failure on beta chain production/severe symptoms and need blood transfusion often	
Hb variants related to mutation in beta globin chain	HbE	$\beta^{26Glu\rightarrow Lys}$	Southeast Asia
	HbC	$\beta^{6Glu\rightarrow Lys}$	Africa, USA, Mediterranean and Caribbean
	HbS	$\beta^{6Glu\rightarrow Val}$	

most common major hemoglobinopathy is HbS homozygosity. Both HbS and HbC affect the solubility of the hemoglobin by polymerization. HbC forms crystals and makes the red blood cell rigid which causes hemolytic anemia, while deoxygenated HbS polymerizes and forms fiber structure. Similar to HbE, the glutamine at the sixth position on the β chain in HbC and HbS is substituted by lysine and valine, respectively [4,9]. Table 1 summarizes the causes of different types of commonly found Hb variants and shows the areas of their prevalent existence [8].

In 1995, it was reported that nearly 200 million people worldwide had thalassemia or Hb variants of some type [1]. Intermarriage causes various combinations of globin chain defects. Even though carriers of some types of hemoglobin defection do not have any health effect, if in combination with thalassemia trait, then there would be a 25% chance that their children could have thalassemia symptoms [10].

Thalassemia patients in severe cases normally have iron overloading of tissues and malfunctioning liver and spleen due to increased iron collection from blood transfusion treatment [11,12]. The symptoms (i.e., abnormal facial features, big tummy and pale) and the treatment processes (i.e., regular blood transfusion) usually have an affect mentally, physically and economically on the patients and their society [13,14]. The lack of education and knowledge about the disease, along with the neglect of couples to have family planning, causes this genetic disease to spread widely. Therefore, in many countries, couples are now encouraged to have their health checked before or at the very beginning of pregnancy [15,16]. Attempts to develop effective and economic techniques for thalassemia screening and analysis have become very important, especially in the countries that have populations with high percentage of thalassemia trait, high birth rate and low funding [17,18].

There are many techniques that have been used to screen and diagnose for hemoglobin variants and thalassemia, mostly done in combinations. In this article, the authors attempt to report the different techniques available, ranging from initial screening to extensive analysis, including a few indirect studies. Screening techniques are the group of techniques that can initially indicate a defect in hemoglobin synthesis. Positive results from these tests need confirmation by a more extensive analysis technique. Negative results normally help in cutting down the number of subjects that need to be further diagnosed by a more advanced and complicated testing. Extensive analysis techniques can give more precise information about types of Hb variants or types of thalassemia. They normally involve higher technologies and instrumentation, and therefore are more expensive than screening techniques. In some cases, it is necessary to perform a more extensive test instead of a combination of many cheaper techniques, such as in prenatal diagnosis where sampling procedures are difficult and the amount of sample is limited. The flow chart shown in Fig. 1 summarizes the techniques for diagnosis of hemoglobinopathies that are commonly used in most laboratories. Different laboratories may have differ-

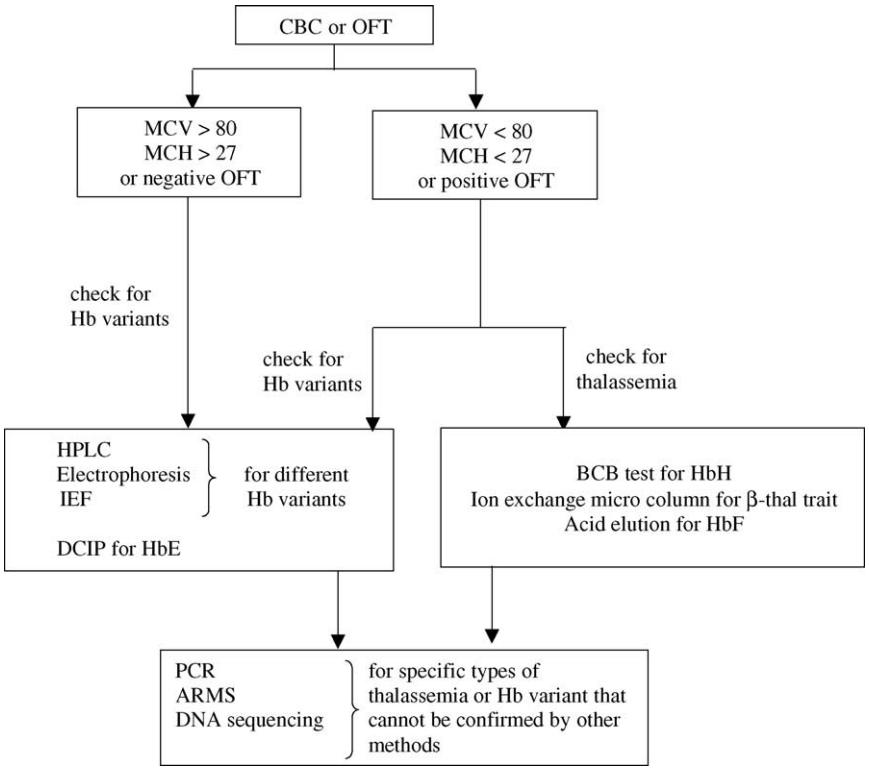

Fig. 1. Flow chart summarizes the normal process of thalassemia and Hb variants diagnosis (see text).

ent choices of analysis techniques, depending on availability of instrumentation and funding. In the developing countries where economic restrictions do not allow for the use of the relatively more expensive technologies, the development of low cost analysis techniques will always be needed. It is hoped that this will be useful information for those who are interested in learning about thalassemia and Hb variants diagnosis techniques for general knowledge, for the development of a new technique or for the improvement of the existing ones.

2. Initial screening techniques for thalassemia

Initial screening techniques are defined as techniques that are simple and relatively low cost which can indicate the possibility of having thalassemia. These techniques should involve the least sample pretreatment and be rapid, and may not need special instrumentation. This would lead to low cost and high sample throughput analysis. They provide a "yes/no" type answer. Positive samples need further confirmatory test while negative samples can be eliminated from further complicated and expensive testing. The complete blood count (CBC) or the alternative osmotic fragility test (OFT) can be used to screen for thalassemia. The negative result eliminates the possibility of having thalassemia, but does not completely exclude the possibility of having Hb variants. Therefore, if necessary, Hb variants testing is needed. Positive results reveal the possibility of having either thalassemia or Hb variants. These screening techniques cannot provide the information on the exact type of hemoglobinopathies, but can help in cutting down the number of samples from unnecessary complicated and expensive testing.

2.1. Complete blood count (CBC)

Complete blood count, a screening test involving the measurement of important characteristics of the blood, has been used as part of the diagnosis process for many diseases, including blood disorders, heart disease, kidney problems and nutritional status [1,19]. The main features of the blood normally tested in the CBC are the total white blood cell count (WBC), red blood cell count (RBC), hematocrit (Hct), hemoglobin (Hb), red cell distribution width (RDW), peripheral blood smear and other important erythrocyte indices (EI), namely mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) [19–22]. Among these parameters, MCV and MCH are the most important ones that can indicate the existence of thalassemia trait, i.e., when MCV < 80 and MCH < 27.

Table 2 summarizes the tests performed in the CBC, the calculation needed for each and the approximate normal cut-off level. However, due to the similar low red blood cell count between the patients with thalassemia (iron overloading) and the ones with iron deficiency, it has been suggested that in the geographic regions where iron deficiency rate is high, the cutoffs for thalassemia interpretation should be adjusted to more suitable values by using a receiver operator char-

Table 2
Summary of tests done for CBC and the interpretation of results for thalassemia and hemoglobinopathies indication

Test	Activity or calculation	Normal level
WBC[a]	Number of white blood cells in cubic millimeter (microliter) of blood	4500–10000 cells μl^{-1}
RBC	Number of red blood cells in cubic millimeter (microliter) of blood	Male 4.7–6.1, female 4.2–5.4 million cells μl^{-1}
Hct	MCV × RBC	Male 40.7–50.3%, female 36.1–44.3% (varies with altitude)
Hb	Spectrophotometric measurement at 540 nm of the cyamethemoglobin (Hb bound with cyanide)	Male 13.8–17.2 g dl^{-1}, female 12.1–15.1 g dl^{-1}; using 64,500 g/mole Hb
MCV	10 (Hct/RBC)	80–95 fl
Mentzer index	MCV/RBC	Less than 13 favors thalassemia over iron deficiency
MCH	(10 Hb)/RBC	27–31 (>30) pg per cell
MCHC	(100 Hb)/Hct	28–33%
Peripheral smear	Visual inspection for shape and number of red blood cells by staining with colored chemicals	
Erythrocyte sedimentation rate[a]	The rate that RBCs settled in the tube within 1 h (mm h^{-1})	Varies with age, gender and pregnancy; male age/2, (female age + 10)/2
Platelet count[a]	–	100000–300000 cells μl^{-1}
Fibrinogen[a]	–	200–400 mg dl^{-1}

[a] Parameters that are normally tested in CBC, but are not directly related to determination of thalassemia and Hb variants.

acteristic (ROC) curve [23]. The ROC curve is the plot of the true-positive results (Y) against the false-positive results (X) for the various sets of results used for constructing the decision threshold. It is used to statistically determine an optimal cutoff point for the medical tests [24–27], which in this case should better differentiate thalassemic microcytosis from non-thalassemic ones (i.e., iron deficiency patients). Other tests such as erythrocyte sedimentation rate, platelet count, and fibrinogen may also be done along with the CBC [28,29].

Many laboratories use an automated CBC machine which can provide many blood parameters in one run. However, the high cost limits its use in many hospitals around the world. Osmotic fragility test, an alternative screening test for thalassemia, is therefore performed instead.

2.2. Osmotic fragility test (OFT)

The main purpose of this technique is to diagnose the hereditary spherocytosis and it is also useful for screening of thalassemia. This simple test utilizes osmosis, the movement of water from lower to higher salt concentration region, to test for the osmotic resistance of the red blood cell [30]. A single hypotonic saline solution can be prepared from dilution of a Tyrode's solution, which is composed of NaCl, KCl, $CaCl_2{\cdot}6H_2O$, $MgCl_2{\cdot}6H_2O$, $NaHCO_3$, NaH_2PO_4, glucose and distilled water [31]. Whole blood is thoroughly mixed with this solution. In a hypotonic condition, the concentration of salt on the outside of a cell is lower than that on the inside, resulting in net water movement into cells. Normal red blood cells are broken within 1–2 min and the mixture then turns clear and reddish. Abnormal red blood cells have deviated osmotic resistances as compared to normal red cells. Spherocytes and erythrocytes with various membrane defects may show decreased osmotic resistance. However, red blood cells of thalassemia have higher osmotic resistance and thus have slower rupture rate, therefore the mixture remains turbid even after 1–2 h [32]. This technique can be carried out in one test tube and it is also called one tube method. Different laboratories may be using slightly different recipes for preparation of hypotonic salt solution, but all are normally based on the same concept of kinetic osmotic fragility. The OFT is a quick preliminary and very economic test before performing further studies of the blood cells.

3. Conventional confirmatory tests for thalassemia and Hb variants

These are useful tests to confirm the existence of certain Hb variants or abnormal level of some Hb types. Confirmatory tests for Hb variants include deoxyhemoglobin solubility test (DST) for detection of HbS and dichlorophenol indophenol precipitation test (DCIP) for detection of HbE. HbH disease which relates to α-thalassemia can be detected by DCIP and brilliant cresyl blue test (BCB). Alkaline resistant hemoglobin test (ART) and acid elution stain (AES) are used for detection of abnormal levels of HbF, which can help identify some types of thalassemia. The ion exchange microcolumn technique is used to quantify the amount of HbA_2 and HbF to identify β-thal trait, E-trait and EE homozygotes. These conventional techniques are relatively low cost and do not require complicated instrumentation. However, some of these techniques may need a highly experienced operator to translate the results. Therefore, availability of more modern instrumentation that can provide more precise information with less requirement of an experienced operator and less usage of toxic chemicals diminishes the use of some of these conventional techniques such as DST and ART.

3.1. Deoxyhemoglobin solubility test

This is a simple test for HbS based on its insolubility in a potassium phosphate saponin buffer solution (composed of K_2HPO_4, KH_2PO_4, saponin and distilled water). Turbidity would be observed within 5 min if the whole blood containing HbS were mixed with sodium hyposulfite and saponin buffer. This test can discriminate samples with HbS from samples with almost all other hemoglobins except Hb Bart's and some rare sickle Hb such as C-Georgetown and S-Travis. Therefore, if a positive test result is shown (i.e., high enough turbidity that newsprint cannot be seen through the test mixture when placed behind the tube), then a follow-up test by electrophoresis is recommended. A false-negative result may be from a high anemic condition [33–35].

3.2. Hemoglobin precipitation test

Some hemoglobin variants such as HbH (β_4 with α-thalassemia) and Hb Köln ($\beta^{98Val\rightarrow Met}$) are classified as unstable hemoglobins which can be precipitated by heating or adding a chemical such as isopropanol or dichlorophenol indophenol [2,36]. The heat stability test can be carried out at either medium temperature (50 °C) for 1–2 h or at high temperature (68 °C) with chemical reaction aids for 1 min. Although taking longer time, the medium temperature stability test is very simple. The clear supernatant of erythrocyte hemolysate in Tris buffer medium, obtained after removing plasma, hemolyzing with distilled water and removing stroma, is placed in the 50 °C water bath for 1 h. Normal hemolysates remain completely clear, while unstable hemoglobins cause flocculation of various turbidities. The test can be done much faster by using chemicals, i.e., KCN and $K_3Fe(CN)_6$, to form hemolysate cyanmethemoglobin. In a phosphate buffer medium, this hemolysate cyanmethemoglobin is agitated rapidly in the 68 °C hot water bath. After 1 min, normal hemolysate may show slight cloudiness and therefore this high temperature method, even though very fast, may need high experience in interpretation in order to avoid a false-positive reading [19,37].

Another way to demonstrate the instability of Hb is with isopropanol precipitation. Packed erythrocytes, cold deionized water and CCl_4 (1:1:1.5 ratio) are placed in a closed tube and vortexed for a few minutes, followed by centrifugation. The clear supernatant is then mixed with isopropanol–Tris buffer at a control temperature of about 37 °C. Unstable hemoglobins cause more turbidity over time, while normal hemoglobins remain clear for at least 30 min. The isopropanol test is reported to have some limitations on the subjects that contain ≥5% HbF, and those that are inappropriately preserved (i.e., unrefrigerated or too old samples) may give false-positive results. Adding anticoagulating reagent can help reduce the false reading but it is suggested that the samples with high HbF should be tested by heat stability, as it is not interfered by HbF [19,38,39].

Similarly, the dichlorophenol indophenol (DCIP) precipitation test is also used widely to screen for HbE and HbH. DCIP can oxidize HbE and HbH faster than any other type of hemoglobin, and therefore it can be used to screen for HbE and HbH. Interpretation of results can be difficult since it involves observing the cloudiness in a deep blue color of DPIC solution. However, a reducing agent may be added to overcome this problem. For example, in the AOAC standard titration method for ascorbic acid, the color of an oxidant DCIP is changed from dark blue to light blue on the way to the end point pink [40]. Therefore, if a small amount of ascorbic acid were added to the DCIP thalassemia test, then the observation could be made more accurately under the light blue condition.

Hemoglobin precipitation tests can be used to screen for some hemoglobin variants but they may not be able to speciate the types of hemoglobins (i.e., HbE and HbH show similar results). Further tests are needed to pinpoint the exact type.

3.3. Brilliant cresyl blue test or new methylene blue test

Both tests are based on the same procedures but with different reagents. They are specifically performed for HbH diagnosis, which cannot be indicated using other techniques such as affinity column and electrophoresis. Polymerase chain reaction can be used to diagnose for HbH, but the cost is higher than these simple color tests. HbH is unstable and it precipitates in the red cells, giving the appearance of many small golf balls inside the cells that can be observed when staining the blood film with brilliant cresyl blue ($C_{17}H_2OClN_3O$) or new methylene blue ($C_{18}H_{22}ClN_3S{:}SClZnCl_2$) [41,42]. The incubation time of blood and the reagents (brilliant cresyl blue in sodium citrate media) takes about 1 h in a controlled temperature setting of about 37 °C [43]. This test is very useful to confirm for α-thalassemia involving HbH inclusion body. However, the technique yields low sensitivity for α-thal trait and therefore it should only be used as a confirmatory test, but not for screening of α-thalassemia.

3.4. Alkaline resistant hemoglobin test

This is a test for abnormal level of fetal hemoglobin (HbF). Normally hemoglobins are denatured at alkaline pH such as in NaOH solution and they can be precipitated readily with saturated ammonium sulfate ($(NH_4)_2SO_4$) solution. However, HbF is not denatured as easily and remains soluble. Differences in alkaline resistance of the normal Hb and fetal Hb allow for rapid testing for the amount of HbF in blood. The procedure consists of a few experimental and calculation steps as reported before [19,44,45]. A suspended mixture of Hb-cyanide–ferricyanide (or cyanmethemoglobin) is prepared by adding packed red cells, obtained from centrifugation of whole blood in isotonic saline solution, into a cyanide–ferricyanide solution (KCN and $K_3Fe(CN)_6$ in distilled water). Then NaOH is added and the solution is mixed

for a few minutes before adding the saturated $(NH_4)_2SO_4$ solution. Coagulated protein can be removed by filtering the mixture until a clear filtrate is obtained. The percent of alkaline resistant hemoglobin is calculated based on the absorbance of the filtrate (Df) and the absorbance of the 1:10 dilution of the original cyanmethemoglobin without NaOH and $(NH_4)_2SO_4$ added (Db) at 540 nm, using the following equation: (100 Df)/(10 Db). In a normal person more than 1 year old, the percentage of HbF should be expressed as being less than 1–2% by using this method. Higher levels of HbF will be suspected of having a hemoglobin disorder of some kind. Although the method was found to mistakenly yield lower results for a subject with HbF higher than 30% of total hemoglobin, such as in umbilical cord blood of newborns, this method was sufficiently sensitive and reproducible for measuring 1–10% HbF, providing that final cyanmethemoglobin concentration is higher than 480 mg/100 ml [46]. In the cases where high amount of HbF is present, an alternative method such as immunological determination of HbF, e.g., by the gel precipitation or immuno-diffusion, involving the use of monoclonal antibody against HbF, may be used to avoid incorrect results obtained from the alkaline resistant hemoglobin test [8,47,48].

3.5. Acid elution stain (modified Kleihauer–Betke test)

This is a simple test for HbF and Hb Bart's. After smearing a blood sample on the slide and letting it dry, the slide is immersed in an 80% alcohol solution (ethyl, methyl or propyl alcohol) for 2–3 min. After that, the slide is immersed in a staining solution of Amido Black 10B ($C_{22}H_{14}N_6O_9S_2Na_2$) prepared in alcohol with pH adjusted to 2.0. After 3 min, the slide is washed under running water for 1 min. In the acidic condition, HbA, HbA_2, HbE, and HbH will be eluted out of the blood cells, leaving the cells empty (ghost cells) and showing no color. HbF and Hb Bart's can tolerate acid and are stained by the Amido Black 10B, showing dark blue color of the cells which can be observed under the microscope.

There are a few precautions that need to be taken when working with this technique. If the slide is left dry for too long, HbA will not be eluted out. The concentration of alcohol is also important because alcohol higher than 85% will cause HbA to stay in the cell, while alcohol lower than 65% will cause vacuolization of HbF. In addition, if the pH of the solution is higher than 2.5, HbA will not be eluted. All these cases will show false results [49,50].

The drawbacks of this technique are time consuming and subjected to human error. Another possible way of detection of HbF is flow cytometry which is more precise as described later.

3.6. Ion exchange micro-column

In the regions where economic restriction does not allow for the use of a relatively higher cost instrument such as HPLC, a cheaper method such as this ion exchange micro-column along with other inexpensive tests can be used in combination to diagnose the type of thalassemia. This technique is based on ion exchange chromatography as a simplified version of high performance liquid chromatography. The use of diethylaminoethyl DEAE anion exchanger, packed in a relatively cheap and small syringe, and Tris–HCl mobile phase can be adapted to separate HbA_2 and HbF effectively. The relative amounts of these Hbs can be estimated by calculating the peak areas of the absorbance, measured at 415 nm, of fractions eluted from the column. It has been shown that the results obtained from the batchwise micro-column are in agreement with those from HPLC, though the method lacks automation and yields lower precision [51–55]. However, the result from ion exchange micro-column technique is acceptably accurate and precise and can be used to confirm some types of thalassemias such as β-thalassemia trait. In addition, with its simplicity and low cost, some laboratories perform this technique together with the OF tests as regular screening techniques, especially where thalassemia cases related to abnormal ratio of HbA_2 and HbF is commonly found such as in Thailand.

It has been estimated that the cost for chemicals and materials per test of the micro-column technique is approximately five times less than that of HPLC. Even though the total analysis time per run is longer than automated HPLC (4 h versus 20 min), many ion exchange micro-columns can be set up and run at the same time. Therefore, the total analysis time of, e.g., 50 tests using multiple micro-columns at one time is less than performing 50 continuous HPLC runs (16 h using HPLC and 4 h using ion exchange micro-columns). In addition, an attempt to reduce the analysis time per run and to make the micro-column technique more automated has been carried out. A flow injection analysis system was joined together with a much smaller ion exchange column to improve the analysis time for hemoglobin typing as compared to the batch process [56]. More work needs to be done, but the preliminary results have suggested that the flow based and reduced volume ion exchange column system has the potential to improve the analysis time per run and to greatly reduce the amount of blood sample needed for the analysis.

4. Instrumental techniques for determination of thalassemia and Hb variants

These techniques involve modern technologies of complicated instrumentation. They can be automated and are usually faster and more reliable but more expensive than the conventional techniques. Even though these techniques can provide detailed information and can help in diagnosis of many types of Hb variants, there are a few exceptional Hb variant cases that cannot be identified with these techniques, and more extensive confirmatory tests are needed. Most instrumental techniques can perform qualitative and quantitative analysis, but with limited ways to accurately quantitate the signals, such as in gel electrophoresis, these techniques have been

used mainly for diagnosis of Hb variants rather than for detection of abnormal level of Hbs in thalassemia diagnosis, as shown in the flow chart in Fig. 1.

4.1. High performance liquid chromatography (HPLC)

In high performance liquid chromatography, particle size of the stationary phase packed in the column is quite small (about 2–5 μm). High pressure is required to force the mobile phase to continuously flow through the column. As the sample solution flows with the liquid mobile phase through the stationary phase, the components of the sample will migrate according to the non-covalent interactions of the compounds with the stationary phase. The degree of interactions determines the degree of migration and separation of the components (i.e., the component with a stronger interaction with the mobile phase than with the stationary phase will have a shorter retention time and thus will be eluted from the column first and vice versa) [57,58].

HPLC has become a very important tool for thalassemia and Hb variants diagnosis because of its ability to accurately and rapidly qualitate and quantitate different types of Hbs. However, in most laboratories, HPLC has been used for diagnosis of Hb variants rather than for quantification of normal Hb or thalassemia diagnosis, except for the case of prenatal analysis. The HPLC technique requires a very small amount of blood samples (μl), therefore, it is very suitable for prenatal diagnosis of thalassemia [59–63] where sample may be limited and difficult to obtain. There are many reports showing the agreement of results obtained from HPLC and those obtained from other techniques such as the globin synthesis technique, isoelectrofocusing, carboxymethylcellulose chromatography and DNA sequencing [59–64].

Anion exchange resin DEAE and gradient Tris–HCl buffer solution, pH 8.5–6.0, is a widely used stationary–mobile phase system for HbA_2 and HbF quantification to effectively diagnose β-thalassemia and Hb Bart's hydrop fatalis that occur frequently in Southeast Asia [62,65]. The system can also separate other Hb variants such as HbS, HbC and HbJ [66,67]. Cation exchangers, such as CM-cellulose (CMC) and silica supported with carboxylic acid residues with bis-Tris–KCN developer, can also be used for the same purpose [60,68,69]. The system based on carboxymethylated poly(vinyl alcohol) resin and sodium phosphate buffer solution as a stationary–mobile phase has been developed for separation and quantification of St-HbA1c, which is a marker of blood glucose regulation in diabetic patients [70].

The ratio of different globin chains (e.g., β:γ for β-thalassemia diagnosis) can also be determined with HPLC using a reverse phase C18 column and shows similar results to those obtained from CMC which is normally employed for this purpose [59]. Determination of Hb types using HPLC has gained high popularity over globin chains determination using CMC because of HPLC's relatively easier and faster analysis which is a result of its having fewer sample preparation steps and an automated data analysis system [61]. HPLC has an overall performance better than electrophoresis.

4.2. Electrophoresis

Electrophoresis is one of the widely used techniques for analyzing hemoglobin variants based on the movement of different Hb or different globin chains, containing different charges, in the electric field. At an alkaline pH, Hb is negatively charged and will move toward the anode (positively charged) terminal. Electrophoresis of total Hb is different from electrophoresis of separated globin chains. To perform electrophoresis of globin chains, a few steps need to be done in order to obtain free globin chains. First, heme is removed from hemoglobin by treating with mercaptoethanol. Then the four globin chains are split apart without denaturing them using 8 M urea. A cellulose acetate membrane is mainly used in alkaline pH electrophoresis. Normal operating voltage is about 250 mV and the approximate run time is about 90 min. After that, the membrane needs to be stained, de-stained and air dried before separation of globin chains can be observed. The main limitation of electrophoresis at alkaline pH is the inability to differentiate HbA_2, HbC, HbO and HbE from one another, nor can HbD, HbG and Hb Lepore be differentiated from HbS [33,71,72]. Therefore, it is normally used to screen for some types of Hb variants. The confirmatory test can be done using electrophoresis in acidic media.

At a lower pH of about 6.0, a better separation of different hemoglobins is obtained. Those Hbs that co-migrate in alkaline pH electrophoresis can be separated in acidic media. Nevertheless, the main technique for Hb quantification by densitometric scanning of the gel is still somewhat difficult and unreliable [33] and therefore electrophoresis technique has been used mainly for detection of Hb variants rather than measuring level of Hb in thalassemia diagnosis. It is highly specific in the detection of certain Hb disorders such as sickle cell disease. Even though the electrophoresis in acidic media is quite a powerful technique in separation of many types of Hbs, please keep in mind that not all Hb variants can be separated by electrophoresis in acidic media. For example, Hb Okayama cannot be separated using electrophoresis, but can be done so in HPLC [73].

Capillary electrophoresis is the new format of electrophoresis where separation takes place in a small fused silica capillary. It is rapid, easily automated and consumes low amounts of reagents, as compared to conventional gel electrophoresis. It also offers much higher throughput as compared to HPLC [74]. However, some researchers found that CE has higher instrumentation cost and is less accurate as compared to automated HPLC [75].

4.3. Isoelectric focusing (IEF)

This technique is based on the electrophoresis technique but with a higher degree of separation. Different Hbs migrate in a pH gradient to the point where their net charges are zero.

The order of migration is the same as in alkaline electrophoresis but the narrower bands obtained from this method (IEF) allow for the resolution of HbC, HbE, HbO, HbS, HbD and HbG [76,77].

Two different formats of IEF, thin layer gel and capillary, have been reported [78–81]. Cossu et al. [82] applied the immobilized pH gradient method (IPG) with a thin layer gel that has a pH range of 6.7–7.6 to differentiate heterozygous from homozygous β-thalassemia in newborns. The group suggested the use of umbilical cord blood because it contains only HbF, HbA and acetylated HbF (HbFac) and the ratio of HbA:HbFac or HbF:HbA is used instead of the conventional β:α ratio in the IEF of globin chains.

Capillary IEF showed very promising performance both in qualitative and quantitative aspects. A single IEF run can replace the main tests that normally have to be carried out in combination for qualitative and quantitative analysis of Hbs, for instance, alkaline and acid electrophoresis for major Hb variants, ion exchange chromatography for HbA_2 quantification and alkaline resistant test for HbF [78,81]. It has been proven to have a comparable performance to chromatography or radioactive globin chain methods [79] and can be used for analysis of hemoglobin variants in adult and newborn [83].

4.4. Flow cytometry

Even though acid elution stain test seems to be simple, it is rather time consuming and subject to human error. The more precise and sensitive quantification of HbF can be done using the instrumental based flow cytometric technique [84,85]. The interested component of the cell is bound to a fluorescence label. Light scattering can identify the cell population of interest. Fluorescence intensity is measured to quantify the component of interest. The discovery of monoclonal antibody production has extended the use of flow cytometry. Antibody against HbF tagged with fluorescent dye can be used to specifically determine the amount of HbF. It has been demonstrated that detection of both a fetal cell surface antigen and HbF using two different monoclonal antibodies and two colored dyes is a precise way to identify the fetal cells [86]. The technique called gradient centrifugation has been proved to enrich the fetal cells from the adult blood and can extend the sensitivity of the flow cytometric analysis of HbF [87].

5. Extensive analysis techniques for thalassemia and Hb variants

These are advanced techniques used to detect thalassemia and Hb variants. They are complicated and expensive and therefore are used in the cases for which there are no other ways to accurately confirm or identify the types of thalassemia or Hb variants. They involve DNA technology that can provide in-depth detailed information of gene mutation.

5.1. Polymerase chain reaction (PCR) with different formats of gel electrophoresis

Polymerase chain reaction is a technique that allows a small amount of DNA to be amplified in vitro. The process is composed of cycles of the three following steps: perform heat denaturing to separate the DNA sequence target into two strands, anneal each strand to the specific primers and then extend the polymerase chain from the primer termini [10]. Once there are enough of the DNA target sequences produced, further analysis can be performed. Gel electrophoresis is commonly done following the PCR to separate different DNA fragments. Many additional methods can be coupled with gel electrophoresis and PCR to obtain better information such as those described briefly here.

Direct DNA sequencing of PCR products is quite a straightforward method to indicate the mutation site [88,89].

The restriction fragment length polymorphism (RFLP) technique can differentiate between different DNA sequences based on the length of fragments yielded by a particular enzyme restriction and can indicate the mutation point of a gene in thalassemia patients [90,91].

The amplification refractory mutation system (ARMS-PCR), also known as allele specific PCR, is another technique that has been introduced to be used for thalassemia diagnosis. This technique utilizes two PCR reactions: one contains a primer specific for the normal allele and the other contains one for the mutant allele. Gel electrophoresis is then employed to separate specific DNA bands. Diagnosis of genotyping is based on whether there is amplification in one or both reactions (i.e., the band in normal reaction only indicates normal allele, the band in mutant reaction only indicates mutant allele, and bands in both reactions indicate a heterozygote) [92–94]. ARMS-PCR is more accurate as compared to RFLP.

Single stranded conformation polymorphism (SSCP) is the technique that was developed based on the fact that the mobility in gel electrophoresis of single strands of DNA drastically depends on nucleotide sequence. Single stranded DNA is produced by adding one primer at a concentration higher than another primer in the PCR step. After the primer with lower amount is used up, the reaction will continue producing only the product of the excess primer. The mobilities of single strands are then compared [95]. Single stranded DNA may also be produced by denaturing double stranded DNA, as in the technique called denaturing gradient gel electrophoresis (DGGE). The DGGE technique utilizes the gradient of low to high pH to denature different gene fragments and retard their mobilities in gel. A mobility shift can be detected even with a slight difference in the base pair sequence [92,96,97].

5.2. DNA technology: DNA probe/DNA microchip

Analysis of nucleic acids has led to the understanding of the gene expression that controls Hbs formation. This information is more detailed as compared to information obtained

from protein analysis that normally only suggests type and amount of different Hbs production. The advance of DNA studies and fabrication technology together has led to the development of methods for diagnosis using a DNA microchip. Normally the segment of a gene of interest first has to be amplified by PCR to obtain a sufficient amount prior to hybridization with allele specific oligonucleotide probes that are immobilized on the solid phase or chip [98–100]. The bound target gene can be detected using either labels such as fluorescent substances [101,102] or electronic transducers such as piezoelectronic and ion sensitive field effect transistors (ISFETs) [103]. The attempt to pinpoint the DNA sequences that become over-expressed in a patient has become important because it can lead to the cure or prevention of the disease. In this case the precision and accuracy of detection requires highly sophisticated devices. Therefore, development of a highly sensitive and accurate device or method of detection is currently an important research trend.

One example of devices that has been used commonly is a cytometer. Cytometry is a laser based technique that allows for analysis of physical properties and fluorescence intensity of an individual cell in a heterogeneous environment. The image can differentiate different types of cells or DNA sequences that are labeled with different colors by comparing the ratios of fluorescence of different targets. With the aid of a computer, detection and visualization of many different probes can be done simultaneously [104,105].

6. Indirect studies related to thalassemia indication and treatment follow-up

These studies are not intended to be used for thalassemia diagnosis. However, the relevance of the variable of interest and the existence of thalassemia may help open up a new way to economically test for thalassemia or treatment follow-up.

6.1. Ferritin

Ferritin is the iron storage protein and its level in serum directly relates to the amount of iron stored in the body, which is important for red blood cell production. Normal ranges of ferritin are 12–300 and 12–150 ng ml^{-1} for male and female, respectively [106]. The technique commonly used to quantify ferritin is immunoassay [107,108]. A significantly high level of ferritin is found in patients with iron overload and this may help differentiate thalassemia patients from those with iron deficiency, both of which will have a low red blood cell count [109]. However, please note that iron deficiency does not exclude thalassemia disorder. In addition, any inflammatory disorder can cause a high level of ferritin. Therefore, long term monitoring of ferritin would be necessary, if its level is chosen to be observed, to gain any additional information for thalassemia diagnosis or treatment follow-up [110].

6.2. Skin tissue Fe concentration

Thalassemia patients have an increased amount of iron storage that has been reported to cause an increased risk of cardiovascular disease due to arterial stiffness [111–113]. Evaluating the level of iron in the body over time is one way to obtain additional information for thalassemia diagnosis and to evaluate the efficiency of the treatment. The X-ray spectrometric techniques (diagnostic X-ray and X-ray fluorescence spectrometry) have been employed to rapidly and non-invasively quantify the amount of iron overload in the skin of thalassemia patients [114,115]. Since the amount of iron found in the outer body skin correlates to the amount of iron overloaded in the liver, heart and spleen, the results from the skin can be used as markers for iron-overload organs. However, as mentioned previously, it is necessary to keep in mind that iron deficiency does not exclude thalassemia.

6.3. Magnetic behavior of erythrocytes

A study in physics based on observation of the change in magnetic behavior of Hb at different states (i.e., normal, oxidized and reduced states) has been conducted [116]. The reduced form of Hb induces paramagnetism while the oxidized form of Hb shows diamagnetic behavior. It was found that normal state Hb from β-thalassemia minors has lower diamagnetic response as compared to that of Hb from iron deficiency patients. This may indicate a low oxygen intake of β-thalassemia minor blood.

6.4. Nuclear magnetic resonance spectroscopy

The nuclear magnetic resonance technique is based on the magnetic properties of some nuclei that when placed in the magnetic field, would take up radio frequency energy that matches the magnetic field strength and later re-emit that energy [117]. The phenomenon is known as nuclear magnetic resonance (NMR) because it involves the nucleus in a magnetic field that has its strength in resonance with the applied radio frequency. Originally NMR spectroscopy was used for the study of composition of chemical compounds. Later, the technique was developed into the imaging technology, magnetic resonance imaging (MRI), that became a major breakthrough in medical fields because it can reveal the image of the parts of the body and seems to be the most sensitive means at present. Contrast medium such as gadoteric acid may be introduced to obtain better results [118].

NMR has been widely applied to study body iron overload [119–128]. NMR spectroscopy has been employed mainly for study of iron level in the fraction of tissue in vitro, while NMR imaging has been used mainly for determination of iron in vivo. So far, there has been no report on health hazards directly related to NMR and therefore the NMR technique is considered a safe and non-invasive way to study body iron content.

7. Conclusion

There are many different techniques available for thalassemia diagnosis, but used alone they may not be able to satisfactorily answer every question. Therefore, it is quite common to utilize more than one technique to ensure the diagnostic result. Fig. 1 summarizes techniques commonly used for diagnosis of Hb variants and thalassemia in most laboratories. If MCV, MCH or OFT screening test reveals a normal result, the possibility of having thalassemia can be eliminated, but analysis of Hb variants should be done. If an abnormal result is obtained from the screening test, there is a possibility of having either Hb variants or thalassemia case. If Hb variants tests do not show any abnormal results, thalassemia tests should still be performed. Choices of techniques depend mainly on budget and equipment available. For example, some countries with limited budget such as Thailand, Indonesia and The Philippines utilize OFT rather than MCV and MCH (from automated CBC machine) for thalassemia screening. It should be pointed out that even in laboratories equipped with high technology and many years of experience, quite a few false diagnoses were reported, which resulted in the births of thalassemia children or the abortions of unaffected fetuses [93]. It is very important to take precaution in every step of the diagnostic procedures to ensure the most accurate diagnosis. For example, be sure to use a fresh blood sample, always perform sufficient sample cleaning, and always run duplicate tests with suitable controls. Developments in chemical analysis methodologies are still very useful to this field.

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Size-based speciation of iron in clay mineral particles by gravitational field-flow fractionation with electrothermal atomic absorption spectrometry

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Abstract

Gravitational field-flow fractionation (FFF) coupled to UV and ETAAS detectors has been tested for micron-size particles in the range of 5–20 μm using three Fe-rich clay samples. The iron content estimated after aqua regia extraction was about 20–40 mg kg^{-1}. The ETAAS analysis was performed both off-line from collected fractions and in an online continuous sampling mode using a specially designed flow through vial placed in the autosampler of the ETAAS. Comparison of the direct injection method with total analysis after aqua regia digestion shows that slurry injection of the dilute samples in the gravitational field-flow fractionation (GrFFF) effluent is quite efficient in these samples. In the majority of cases, more than 90% recovery was obtained for the slurry injection method. Fe mass-based particle size distributions and Fe concentration versus particle diameter plots can be generated using certain assumptions. This provides detailed information on size-based speciation of particulate samples. Generally, the Fe concentrations in the particles decreased slightly with an increase in particle size as is often found for soil and sediment samples.

Keywords: Clay mineral particles; Size-based iron speciation; Micron-size particles; Gravitational field-flow fractionation; Electrothermal atomic absorption spectrometry

1. Introduction

The size of particles influences many properties of solid materials. An important example is the transport and fate of particles in aquatic environments [1] through soil profiles [2] and as atmospheric aerosols [3]. Since many elements and compounds are associated with such particulate material, the study of size-based speciation is very relevant in environmental science. One approach for such work is to use conventional separation methods, such as filtration and settling (gravitational or centrifugal), to prepare subsamples with specified size ranges and to analyze these for the components of interest [4–6]. However, filtration suffers from many artefacts, and settling methods are usually quite time consuming [5,6].

For several decades, various groups have been developing methods involving field-flow fractionation (FFF) for separation purposes [7]. FFF has the advantage of generating a continuous distribution of polydisperse samples which can be either collected as discrete subsamples for analysis [8–10], or in certain cases, the FFF eluent can be fed directly into an analytical device [11]. The data generated by such experiments are often in the form of an analyte-based size distribution [8–11].

Most studies on size-based speciation of environmental samples to date have involved the use of sedimentation FFF (SdFFF) [8,12–15] or flow FFF (FlFFF) [16–22] to characterize submicron colloids. These have been coupled

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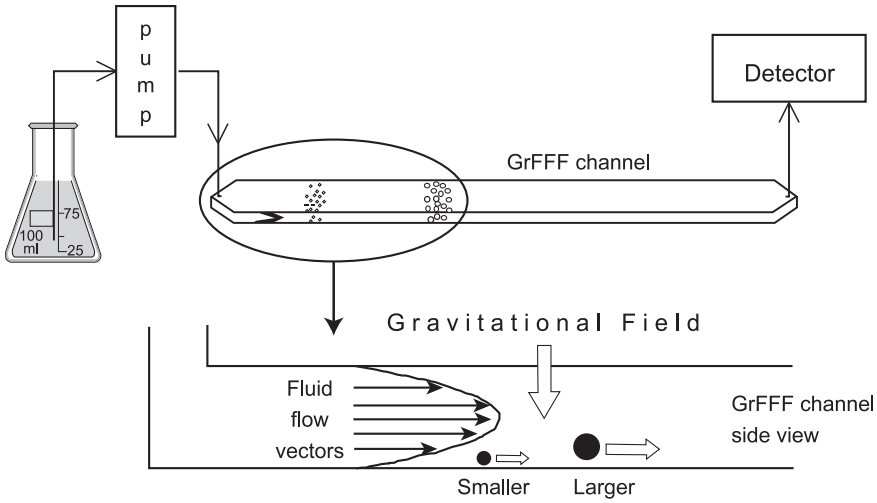

Fig. 1. GrFFF instrument and steric/hyperlayer separation mechanism for micron-size of particles.

both off-line and online with inductively coupled plasma mass spectrometry (ICP-MS) [12–22], and electrothermal atomic absorption spectrometry (ETAAS) is employed as tool [8–11] for element speciation. FFF-ICP-MS has been currently reviewed for some selected applications by Barnes and Siripinyanond [23]. One of the limitations for elemental analysis of particulate slurries is that the efficiency decreases for particles greater than about 5–10 μm. This could be due to trapping of particles in the nebulizer or incomplete atomization/ionization for these larger particles. Recently, we have demonstrated that gravitational FFF (GrFFF) can be combined with ETAAS to achieve size-based speciation data for micron-size silica particles up to 10 μm in diameter [9]. This was done off-line by collecting subfractions for Fe analysis by ETAAS. The digestion efficiency of ETAAS with slurry samples proved to be good at least up to 10-μm diameter particles.

GrFFF is carried out by applying a gravitational field perpendicular to the laminar flow direction in a thin GrFFF separation channel (see Fig. 1). One significant advantage of GrFFF is that the channel can be constructed quite easily and cheaply, and the system can be assembled using widely available components for HPLC or flow injection systems. However, the weak gravitational field restricts the size range that can be fractionated to about 2–50 μm in diameter. Nonetheless, this silt-size range is of importance for sediment transport in rivers and atmospheric aerosols.

As an extension of our work to develop analytical methods for size-based element speciation, we present for the first time data for online GrFFF–ETAAS of micron-size clay particles. This was achieved by continuously feeding the eluent from the GrFFF into a special flow through sampler set in one position of the autosampler. The stream was sampled at regular time intervals, and the Fe content was obtained by slurry introduction into the ETAAS. A major advantage of this method is that samples are withdrawn from a flowing suspension, thus avoiding the problem of settling of sample particles in a vial. This same approach may be useful in other applications where slurry injection is used.

The reason for our focus on Fe, in this and previous publications, is that hydrous Fe oxide coatings are thought to exist on aquatic and soil particles and are generally believed to be involved in the uptake of contaminants such as toxic metals [3] and phosphate [2]. In this paper, we continue with this emphasis, but instead of using pure silica with a coating of synthetic goethite [9], we study the Fe speciation of a series of natural iron-rich silt-size clay minerals.

2. Experimental section

2.1. Instrumentation

2.1.1. GrFFF

The components of the GrFFF instrument have been described in previous work [9,24]. The channel dimensions were 0.095×300×20 mm for the channel thickness, tip-to-tip length and breadth, respectively. The geometric void volume was 0.57 mL. Although the GrFFF channel was only 95 μm thick, no clogging problems were experienced with the dilute (2 mg mL^{-1}) <45-μm diameter sieved samples used in this work. A Milton Roy Constametric III pump (Pennsylvania, USA) was used to deliver the carrier, 10^{-4} M sodium hydroxide solution. Particle suspensions were injected into the carrier stream with a hypodermic syringe (40 μL) through a homemade injection port [24]. The concentration of particles in the FFF eluent was monitored by a UV detector (Model UV8, BAS, USA) at an operating wavelength of 254 nm. The Pocket Sampler (Dick Smith, Australia) with the software supplied was used as the interfacing device for digitizing the GrFFF-UV detection signal.

2.1.2. ETAAS

The ETAAS instrument was a Perkin–Elmer Model 5100 (Norwalk, CT, USA) 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 fast-heating program for the graphite furnace is summarized in Table 1. The peak area across the 3-s heating segment (step 2) of the program was recorded. This program enables more analysis data to be collected across the GrFFF fractogram. The total analysis time for each replicate was 86 s (including autosampler operating time and cooling step). By using this temperature program, there was no difference for the Fe atomic peak profiles from the slurry, and solution analysis was observed.

2.1.3. GrFFF–ETAAS

Off-line and online determination of iron by GrFFF with ETAAS detection can be carried out by employing the instrument set-ups, as shown in Fig. 2. Off-line GrFFF with ETAAS analysis was done by collecting fractions (ISCO, Model RETRIEVER 500 fraction collector, Lincoln, USA) of the eluent after passing through the GrFFF separation device, as shown in Fig. 2(a). Analysis of the iron content in each fraction was obtained through slurry introduction into an electrothermal atomic absorption spectrometer (ETAAS). For online GrFFF–ETAAS analysis, the eluent from FFF unit was continuously fed into the bottom of a specially designed flow through sampler vial (see Fig. 2(b)) which was placed in the autosampler of the ETAAS. Sample suspensions (10–20 μL of 2.01–2.07 mg mL^{-1} depending on the sample) were introduced into the GrFFF, and the eluted particle suspension was continuously introduced through the bottom of the sampler vial. The overflow was directed to waste (Fig. 2(b)). The autosampler arm was set always to sample from this same position so that it introduced discrete samples of the eluent into the ETAAS. A photograph of the online GrFFF–ETAAS instrumentation is shown in Fig. 2(c).

2.2. Chemicals and samples

2.2.1. Chromatographic silica particles (5 and 10 μm)

Chromatographic silica was obtained from used HPLC columns (CN packing from PARTISPHERE RTF Columns, Whatman, UK). Particle suspensions of about 2 mg mL^{-1} in water were prepared. The 5-μm particles were spherical, and the 10-μm particles had more irregular shapes. These were used as particle size standards.

Table 1
Fast heating graphite furnace temperature program

Step	Furnace temperature(°C)	Ramp time (s)	Hold time (s)	Internal Ar gas flow (mL min^{-1})
1	140	1	15	300
2[a]	2400	3	10	0
3	2600	1	5	300

[a] Peak area measurement.

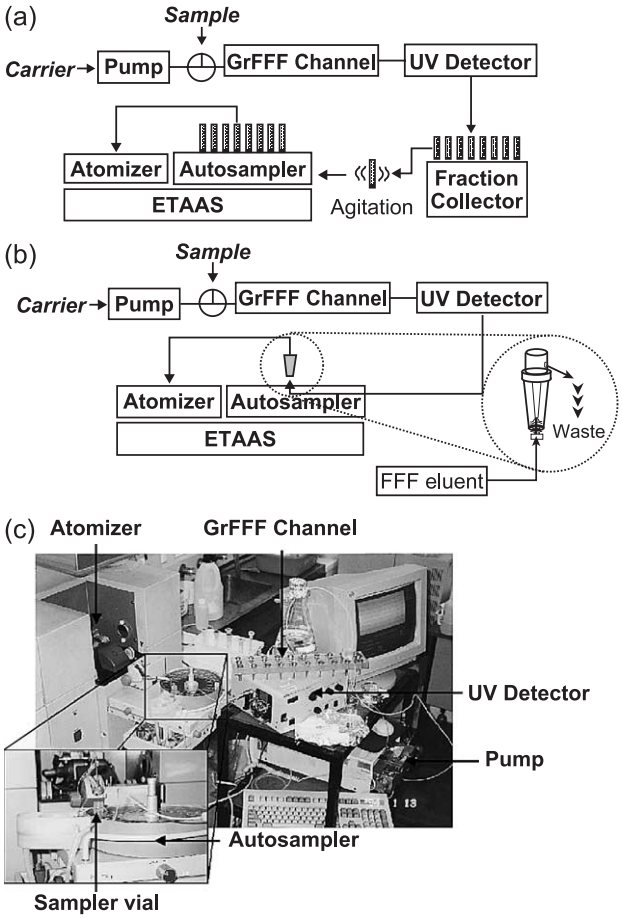

Fig. 2. Instrumental set-up for GrFFF coupled with ETAAS. (a) Off-line GrFFF–ETAAS, (b) online GrFFF–ETAAS showing the flow through sampler vial and (c) photo of the online GrFFF–ETAAS instrumentation.

2.2.2. Clay samples

All clay samples were of natural origin and were obtained from different provinces in Thailand. The clays are referred to as red clay (Kanchanaburi province), ball clay 1 and ball clay 2 (sampled from different sites in Payao province). They were dry sieved through a 45-μm sieve, then dried further at 60 °C for 12 h in an oven and stored in a desiccator until required. Each sample was suspended in water (2 mg mL^{-1}).

2.2.3. Reagents

All reagents were prepared using water obtained from a Milli-Q system (Millipore, Milford, MA, USA). Carrier of sodium hydroxide (10^{-4} M) was prepared using a reagent from Aka Chemicals (Sweden). A standard iron solution (1000 mg L^{-1}) was SpectrosoL® grade from BDH. The desired standard concentrations (10, 20, 40, 60 and 80 μg L^{-1}) were diluted appropriately from the stock standard and used to obtain the calibration line: Y=0.0049×, r^2=0.9947.

3. Results and discussion

3.1. Mass- and Fe-based fractograms obtained by GrFFF with ETAAS

Fractograms of the three clays generated with off-line and online GrFFF–ETAAS analysis for iron are given in Fig. 3. It was found that the UV and Fe profiles obtained by off-line and online operations from all samples agreed reasonably well with each other. The largest discrepancy was between the off-line and online UV fractograms for ball clay 1, which might have been caused by specific errors of the runs. In all subsequent plots, the online fractogram data were used for the calculations. The fact that the sample peak was not resolved from the void peak indicated the presence of large particles of about 20 μm or more.

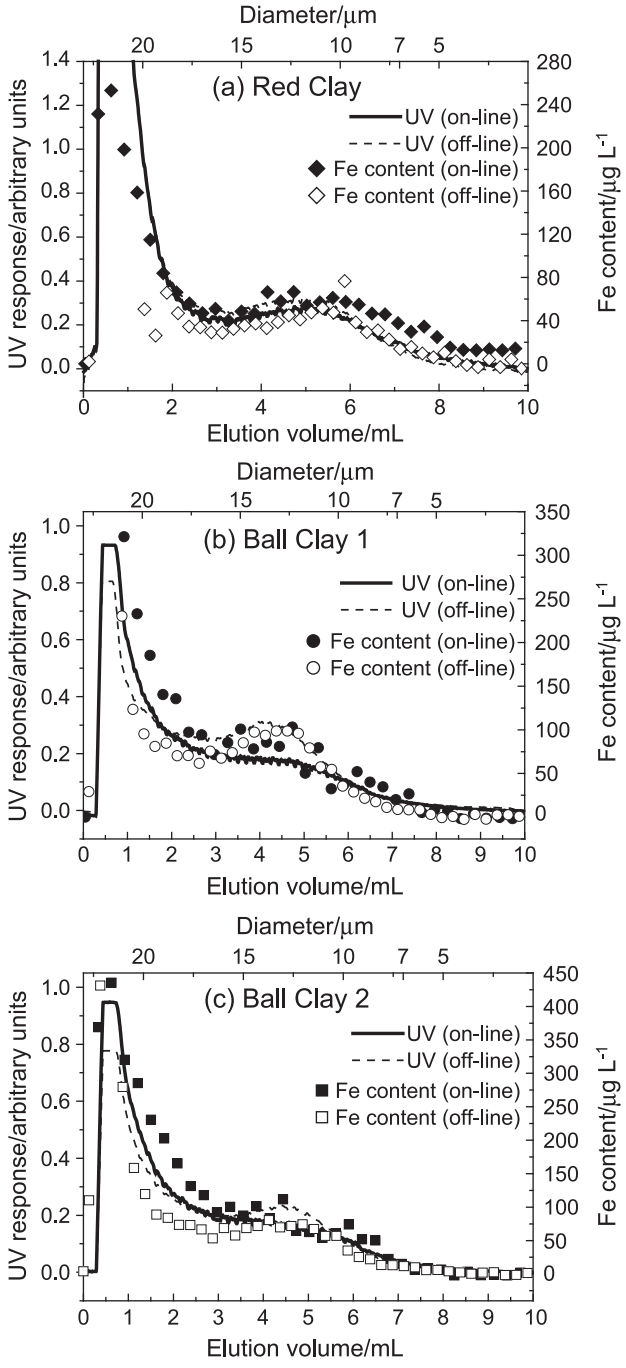

Fig. 3. UV and Fe-based fractograms of clay samples obtained by off-line/online GrFFF–ETAAS (a) red clay, (b) ball clay 1 and (c) ball clay 2.

3.2. Conversion from elution time to diameter

Due to the complications in the steric/hyperlayer model, the conversion of the elution time or volume to diameter must be done empirically via calibration with suitable standard particles. By assuming that the standard particles have the same density as the clay particles, the conversion of the diameter scale can be made employing the empirical formula [25],

$$\log t_r = -S_d \log d + \log t_{r1} \quad (1)$$

where t_r is measured retention time, d is the diameter of the particles, S_d the size selectivity, and t_{r1} is a constant equal to the extrapolated value of t_r corresponding to particles of unit diameter.

For the purposes of illustrating the general approach, a calibration line was obtained using only two chromatographic silica samples (5 and 10 μm) as particle size standards. Although it is not adequate for accurate size determination, the errors are not likely to be excessive as many previous studies have found that the calibration graph is linear [7,26]. Fig. 4 shows fractograms of the 5- and 10-μm silica particles and the plot of log t_r versus log d. This plot was used to generate the equation for converting the x-axis to a diameter scale as described above. In this case, the calculated selectivity using the data for the 5- and 10-μm silica was found to be 0.92, and t_{r1} was 98.

It should be noted that there may be a systematic error in the calibration performed because the 5-μm silica standard particles are spherical, but the 10-μm silica standard and clay particles are more platey in shape. It is known that platey particles experience higher lift forces than spheres of the same volume and hence elute at shorter times [27].

A rough estimate of the magnitude of the error introduced by shape can be deduced as follows. A survey of the literature shows that the selectivity for sedimentation FFF (including the gravitational version) usually falls between 0.7 and 0.8. The gradient for our data in Fig. 4(b) is higher (0.92) because t_r for the 10-μm nonspherical particles is smaller than would be expected for a spherical particle of the same volume. If we use a gradient of 0.75 and the single 5-μm spherical particle calibration point, we can estimate that, for a 10-μm spherical sample particle, we would obtain a diameter of

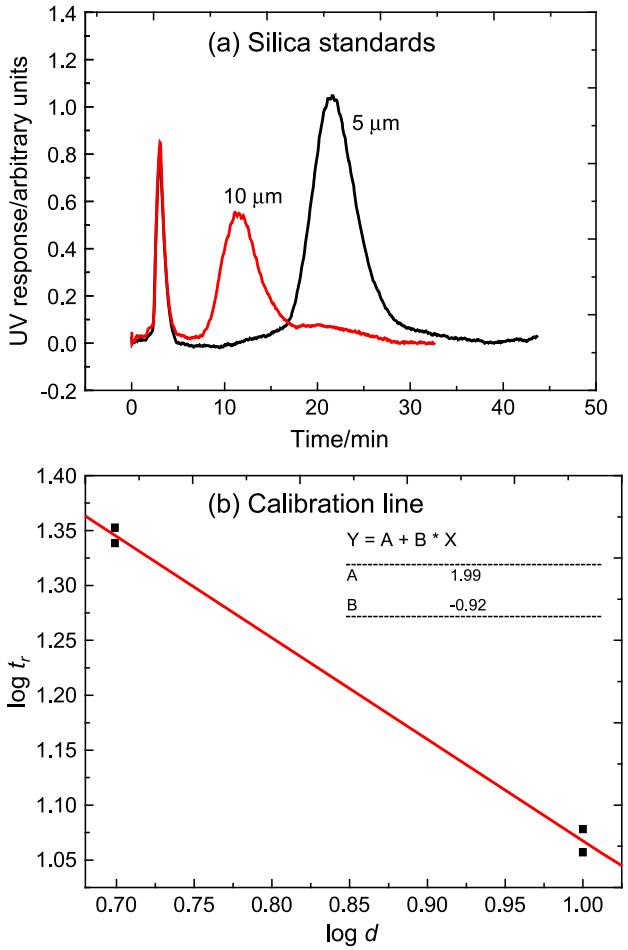

Fig. 4. (a) GrFFF Fractograms of 5- and 10-µm silica particles and (b) plot of log t_r versus log d.

8.8 µm or thus incurring an error of 12%. This simple calculation illustrates the magnitude of the shape error to be expected but should not be taken as the error in our results. Indeed, the situation here is even more complicated as the 5 µm standard was spherical, the 10 µm standard appeared to be somewhat irregular in shape, and the ball clay samples were very platey.

The density of the silica particles may also differ from the clays although this is likely to be less than 20%. Ideally, the calibration standards and samples should have the same shape and density. The calibration error encountered here are of no importance in this paper which is intended just to outline the concept of GrFFF–ETAAS approach for size-based speciation.

3.3. Conversion from UV detector signal to eluted mass

For submicron particles, the UV detector response is usually assumed to be directly proportional to the mass concentration of particles ($\mathrm{d}m^{\mathrm{c}}_{\mathrm{pi}}/\mathrm{d}V_{\mathrm{i}}$). However, according to the approach of Reschiglian et al. [28,29] for micron-size particles, the general relationship for the suspension concentration for large particles (10-fold bigger than the incident wavelength), which comes from Mie theory, can be expressed as

$$\frac{\mathrm{dm}^{\mathrm{c}}_{\mathrm{pi}}}{\mathrm{dV_i}} \propto UV_i d_i \tag{2}$$

where UV_i; is the UV detector response at point i along the FFF elution profile; $m^{\mathrm{c}}_{\mathrm{pi}}$ is the mass of sample eluted up to elution volume V_i, and d_i is the particle diameter eluting at V_i. It should be noted that the superscript c in these quantities signifies that it is the cumulative amount eluted up to point i on the fractogram.

3.4. Mass- and Fe-based particle size distributions of clay samples

The appropriate y-axis for a particle size distribution ($\mathrm{d}m^{\mathrm{c}}_{\mathrm{pi}}/\mathrm{d}d_i$) is given by [7]

$$\frac{\mathrm{dm}^{\mathrm{c}}_{\mathrm{pi}}}{\mathrm{dd_i}} = \frac{\mathrm{dm}^{\mathrm{c}}_{\mathrm{p}}}{\mathrm{dV_i}}\left|\frac{\delta V_i}{\delta d_i}\right| \propto \mathrm{UV_i d_i}\left|\frac{\delta \mathrm{V_i}}{\delta \mathrm{d_i}}\right| \tag{3}$$

where δd_i is the increment in d_i corresponding to increment δV_i in V at point i along the fractogram. The mass-based size distribution is thus a plot of $\mathrm{d}m^{\mathrm{c}}_{\mathrm{pi}}/\mathrm{d}d_i$ versus d_i.

When the GrFFF was connected to the ETAAS system, the Fe content can be evaluated. The mass concentration of the Fe present in the eluent ($\mathrm{d}m^{\mathrm{c}}_{\mathrm{Fe}_i}/\mathrm{d}V_i$) is used to plot the Fe fractogram. This is then converted to an Fe-based particle size distribution using the equation,

$$\frac{\mathrm{dm}^{\mathrm{c}}_{\mathrm{Fe_i}}}{\mathrm{dd_i}} = \frac{\mathrm{dm}^{\mathrm{c}}_{\mathrm{Fe_i}}}{\mathrm{dV_i}}\left|\frac{\delta V_i}{\delta d_i}\right| \tag{4}$$

where $m^{\mathrm{c}}_{\mathrm{Fe}_i}$ represents the cumulative mass of Fe eluted up to digitized point i on the fractogram. The Fe-based particle size distribution is obtained by plotting $d^{\mathrm{c}}_{\mathrm{Fe}_i}/\mathrm{d}d_i$ against particle diameter d_i.

Fig. 5 shows the particle mass and Fe distributions of the three clay samples. It was found that the samples contained particles in a broad size range starting from about 2 µm and extending beyond 20 µm. However, in these GrFFF runs, the larger particles (>20 µm) are eluted with the void peak where there is no resolution of particle size. Again, we caution that extrapolation beyond the 10-µm upper limit of the calibration standards is not recommended, but in this illustration of the method, it is tolerated because of the expected linear calibration graph.

In all samples, the size distributions decrease rapidly in the range from 4 to 2 µm. This truncation of the distributions is expected in these runs since no stop flow relaxation step was used. Stop flow relaxation is sometimes required in order that the particles travel across the channel to the accumulation wall under the influence gravity. Thus, some of the particles <2 µm would be eluted in the void peak as their relaxation time is >35 s compared to the mean residence time of the carrier in the channel of 171 s [7]. The

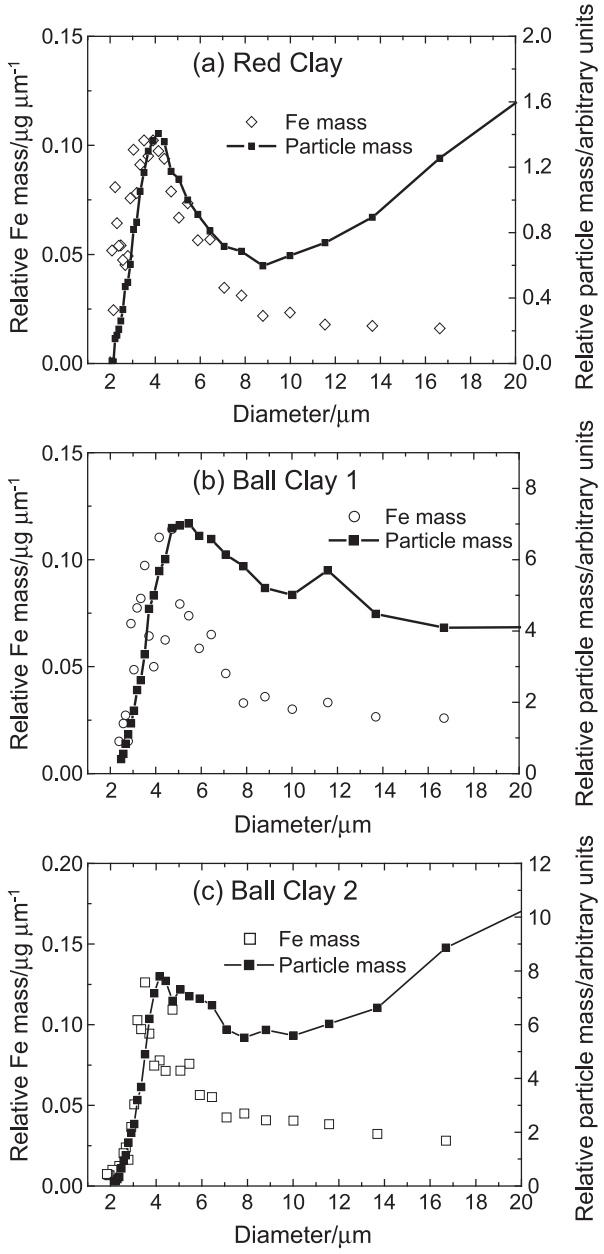

Fig. 5. Mass- and Fe-based particle size distributions (a) red clay, (b) ball clay 1 and (c) ball clay 2.

relaxation time is estimated as the time for the smallest particle of interest to settle across the channel width, w. The relaxation time (t_{relax}) is calculated by transposing the Stokes settling velocity equation to give $t_{relax}=18\eta w/g\Delta\rho d^2$, where η is the carrier liquid viscosity, w is the channel thickness, d is the particle diameter, g is the gravitational acceleration and $\Delta\rho$ is the density difference between the particle and the carrier liquid. However, optical microscope observation of these samples revealed that only small amounts of material were present with diameter <2 μm. The lack of sample relaxation will cause some spreading of the smaller particles but should be insignificant for particles greater than about 4 μm which have relaxation times <9 s.

3.5. Fe content distributions

The Fe concentration in the particles is given by

$$\frac{dm^c_{Fe_i}}{dm^c_{p_i}} = \frac{dm^c_{Fe_i}/dV_i}{dm^c_{p_i}/dV_i} \propto \frac{dm^c_{Fe_i}}{dV_i}\frac{1}{UV_id_i} \quad (5)$$

The Fe concentration distributions were obtained by plotting $\frac{dm^c_{Fe_i}/dV_i}{UV_id_i}$ i.e $\frac{[Fe_i]}{UVd}$ against particle diameter.

The distribution of the Fe concentration in the particles is plotted in Fig. 6. The data are only plotted between 5 and

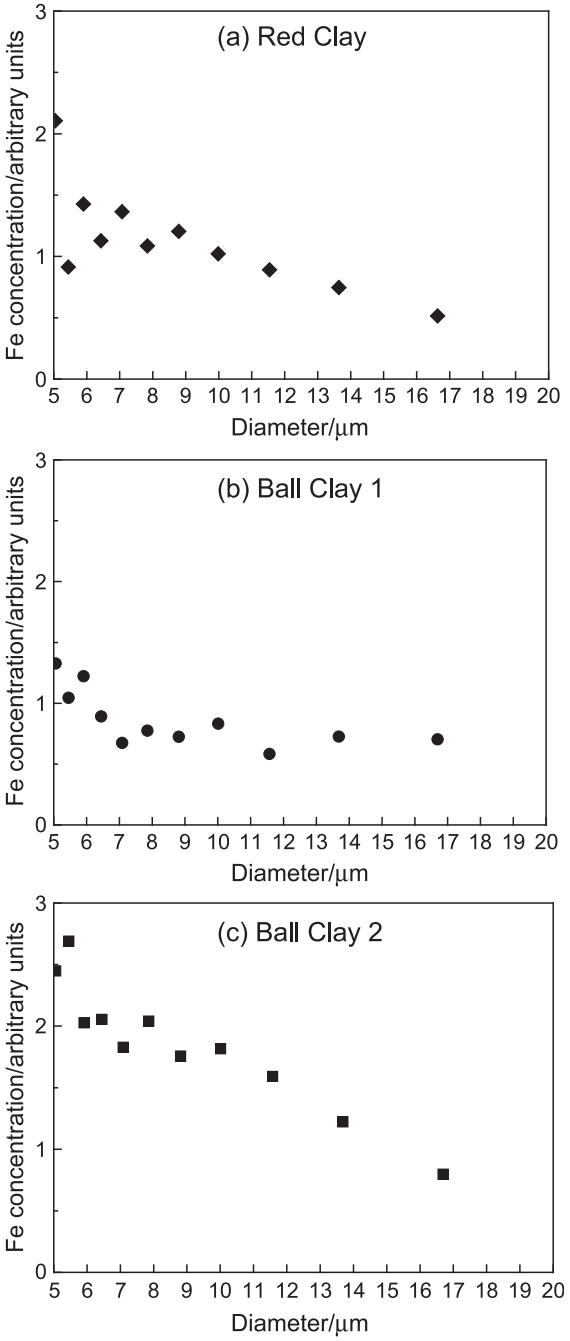

Fig. 6. Distribution of Fe concentrations in the particles as a function of diameter (a) red clay, (b) ball clay 1 and (c) ball clay 2.

Table 2
Total Fe concentrations in the three clay samples

Samples	Fe content (mg g^{-1})		
	Digested	Online	Off-line
Red clay	41.7	32.6	38.6
Ball clay 1	19.9	18.2	18.3
Ball clay 2	21.7	23.9	21.7

20 μm. For >20-μm particles, no resolution from the void peak is obtained at the run conditions employed. The data below 5 μm are not shown as both the UV and Fe concentration values are small, which can result in a large swing in the graph due to errors in setting the correct baselines in the fractograms.

The Fe content increases in the smaller particles, particularly for ball clay 2. This is commonly found for soil and sediment samples [1,11,14]. This may indicate that a significant proportion of the Fe is present as a surface coating on the particles, or that there is an increase in the proportion of Fe containing particles in the smaller size range.

3.6. Efficiency of Fe analysis by GrFFF–ETAAS

An evaluation was carried out on the effectiveness of the slurry ETAAS method for Fe analysis after GrFFF separation. Summation of the iron amounts in each fraction across the entire Fe-based fractogram (including the void peak) provides an estimate of the total mass of Fe injected into the GrFFF with the samples. This was done by integration of the area under the Fe-based fractograms obtained by both off-line and online operations (Fig. 3).

The results were compared with the total iron contents of the original samples analyzed by flame atomic absorption spectrometry after being digested with aqua regia. This comparison is presented in Table 2. It can be seen that the total Fe contents obtained by the GrFFF–ETAAS methods (both off-line and online slurry injections) agreed reasonably well with the digestion analysis. Thus, it would appear that the slurry ETAAS analysis for Fe does not suffer from the same loss of efficiency. This agrees with some previous studies [30].

Using the mean value of all three methods for a given sample, the percentage deviations of each method from this mean can be calculated, as shown in Table 3. It was found that the percent deviations from the mean for the digestion, online and off-line methods were 3–11%, 3–13% and 3%, respectively.

Assuming that the digestion method gave the most accurate estimate of the total Fe content of the sample, the percentage deviations from this value for the slurry methods were found to be 8–22% for the online method and 0–8% for the off-line method. The mean deviation (discounting the sign) was 9%, and only one value was greater than 10%. By far, the most significant outlier was the online red clay result (22% deviation). One contributing factor may be the fact that only 10 μL of sample was injected compared to 20 μL for the ball clays.

It was found that for ball clay 2, the slurry results were higher than those obtained from the digestion analysis. This suggested that there could be other errors in the analysis in addition to inefficient slurry atomization. Thus, it is reasonable to conclude from the results in Tables 2 and 3 that the slurry efficiency is quite good. However, optimization of the slurry method is required to improve the overall accuracy of the Fe analysis.

4. Conclusion

GrFFF–ETAAS was demonstrated to be an effective method for investigating the Fe size-based speciation of micron-size particles. Direct injection of the dilute suspensions of GrFFF eluent into the graphite furnace was shown to be quite efficient. The average deviation of the Fe contents from the values determined after aqua regia digestion was 9%.

The method was illustrated using some Fe-rich clay samples. The Fe concentration in the particles increased slightly with decreased particle size, perhaps indicating that significant amounts of the Fe exists as surface hydroxy oxide coatings. An alternative explanation is an increase in the proportion of Fe-rich minerals in the smaller particles.

GrFFF is the most cost effective of the FFF suite of separation techniques and could be constructed in the simplest workshops. However, it is only applicable over a size range of about 2–50 μm. The ETAAS can be operated

Table 3
Percentage deviations of the total mass of Fe in the three clay samples obtained by various ETAAS methods from either the mean Fe content value or the Fe content of the total digested sample

Sample	%Deviation of Fe content from the mean value[a]			%Deviation of Fe content from the digested value[b]	
	Digested	Online	Off-line	Online	Off-line
Red clay	11	13	3	22	7
Ball clay 1	6	3	3	8	8
Ball clay 2	3	6	3	10	0

The mean value was for the three methods.
"methodi" refers to either the digest, online or off-line method for ETAAS analysis.
[a] %Deviation of Fe content the from mean value=(Total $Fe_{(methodi)}$−Total $Fe_{(mean)}$×100/Total $Fe_{(mean)}$).
[b] %Deviation of Fe content from digest value=(Total $Fe_{(methodi)}$−Total $Fe_{(digest)}$×100/Total $Fe_{(digest)}$).

online using a specially designed sampler vial which was used in the autosampler. GrFFF–ETAAS has been demonstrated for Fe speciation here but should also be useful for a range of other elements.

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Novel approach for mono-segmented flow micro-titration with sequential injection using a lab-on-valve system: a model study for the assay of acidity in fruit juices

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A new concept for micro-titration using a "lab-on-valve" (LOV) system with sequential injection of mono-segmented flow is proposed. The performance of the system was demonstrated by the assay of acidity in fruit juices which is based on acid–base neutralization. A standard/sample solution containing citric acid, indicator, sodium hydroxide, were sandwiched between air segments and were aspirated in microliter volumes through a selection valve into a holding coil. The acid, indicator, and base were mixed by flow reversal. After removing air segments, the solution was pushed to the detector for monitoring of the change in absorbance of the indicator color, which depended on the concentration of the remaining base. With LOV, microliter volumes of the solution can be detected without dispersion of the color zone. A calibration graph (plot of absorbance *vs.* acidity value) in the range of 0.2–1.2% (w/v) as citric acid was established. Sample throughput of 30 sample h^{-1} and good reproducibility (RSD = 1.2%, n = 11 for 0.6% acidity) were achieved. The procedure has been applied to determine acidity in fruit juices.

Introduction

Titration is an analytical technique for measuring the concentration of the analyte based on the reaction of known stoichiometric ratio between analyte and standard reagent of known concentration (titrant). Apart from visual detection, there are various ways of end point estimation including electrochemical ones which are more precise and accurate[1–3] but these applications are only limited to analytes that have electrochemical properties. All batch titrations normally involve quite high volume of reagents consumption and manually operations which are subjected to personal error.

The development of the flow injection technique has opened a new way for titration.[4–8] However, there has been a debate whether the technique of "flow injection titration" really fits the definition of titration.[9,10] This is because it is based on different principles compared to normal titration. When the sample plug, for example an acid, is injected into a carrier stream of titrant, for example a base, the dispersed zones at the two interfaces will become neutralized. The distance between the two boundaries increases with the increase of sample concentration, resulting in increasing of peak width of the FIA gram.[11,12] The calibration graph can be constructed from the peak widths (or peak area) of the standard solution of various concentrations. Then, the concentration of sample can be estimated from this calibration graph without the need for knowledge on stoichiometric ratio of sample and titrant. Alternatively, the mixing chamber can be placed in line to mix sample and titrant. The end point can be observed from the titration curve obtained after suitable sequential addition of the titrant, similar to normal batch titration where there's no need for a calibration graph.[10,13–16]

Despite the debate over this "flow injection titration" technique and whether it is really a titration, this technique has been applied to determine various analytes and parameters.[17–26] The flow injection technique offers many advantages over batch titration. The continuous flow of reagents at constant flow rate makes it possible to record the signal at constant time at the early stage of titration rather than titration until reaching the end point. This system helps to shorten the analysis time and increase sampling rate. The system can be fully automated and therefore personal error can be reduced. As a closed system, it also helps to prevent sample contamination from the outside environment and *vice versa* it helps to prevent the operator from direct contact with the chemicals. Unstable reagents can be used in the flow injection technique because they will not be exposed to air and light as much as in the batch procedure. The development of third generation FIA or sequential injection analysis (SIA) offers a higher degree of automation using computer software control and a more feasible system for many applications using bi-directional pump with micro volume manipulation.[27–30]

A mono-segmented flow is a system where air segments are used to sandwich the sample–reagent plug to eliminate dispersion between the sample–reagent zone and the carrier stream. Thus, longer resident time and better sensitivity with low reagent consumption can be achieved.[31–35]

We propose the new concept of mono-segmented flow micro-titration using a sequential injection analysis-lab-on-valve (SIA-LOV) system. The LOV is a newly developed device that is integrated as part of the selection valve of the SIA system. Since the first report by Ruzicka,[36] the applications of

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the LOV has been extended.[37–44] Reagents can be drawn, reacted and detected right on the valve.

The performance of the proposed mono-segmented flow with the SIA-LOV system was demonstrated by acid–base titration. The acid–base balance can be very important even at the cell level.[45] Studies of some substances of limited amount will also benefit from micro-volume titration. For example, determination of enzyme activity (*e.g.* pectinmethylesterase) may involve a reaction that can produce or consume an acid or base (*e.g.* when reacting with pectin, the methyl group on pectin will be changed to a carboxylic group that can be detected with an acid–base indicator)[46] and it should be possible to employ the proposed system for this purpose. Therefore, determination of acidity at small volumes has important potential. In this work, the determination of citric acid in fruit juices at the micro volume level was chosen as a model study because of the simplicity of the process and wide availability of samples and reagents.

Experimental

Apparatus

The sequential injection with the lab-on-valve system (FIAlab 3000, FIAlab, USA[47]) was composed of a syringe pump (with 2500 μl syringe), a peristaltic pump, a 6 port selection valve with lab-on-valve (LOV-SIA, FIAlab, USA) on the top, a fibre optic spectrophotometer (USB2000 UV/VIS, Ocean Optic, USA) with a tungsten halogen light source (LS-1, Ocean Optic, USA) and a computer. The first optical fibre guided light from the light source to the LOV, while the second one carried light from the LOV to the spectrophotometer. The two optical fibres were placed facing each other on a channel of LOV, forming a flow cell of 1 cm path length. All connections were made with 0.8 mm id PTFE tubing. The system was controlled by a computer with FIAlab software for windows (FIAlab, USA). Fig. 1 illustrates various components of the manifold used in this system.

Reagents and sample

Deionized water (Milli Q, Millipore) was used in all the experiments. All reagents were of analytical grade, unless otherwise stated. Citric acid (Carlo Erba) was used to prepare standard solutions of various acidities. A stock standard solution of 10.0% (w/v) citric acid was prepared by dissolving 27.3420 g of citric acid in 250 ml of 10% (w/v) sucrose solution. The working solutions were prepared by diluting the stock solution with 10% (w/v) sucrose solution. Sodium hydroxide solution was prepared by dissolving 2.00 g of sodium hydroxide (Lab-scan) in 250.00 ml deionized water and then diluting to 0.10 M. It was standardized with 0.10 M potassium hydrogen phthalate (Univar) if the exact concentration was required.

Indicator solution (0.1% (w/v)) was prepared by dissolving 0.10 g of Indigo carmine (BDH) in 100.00 ml deionized water. Other concentrations were prepared in the same manner.

Fruit juice samples were collected from local convenience stores. They all contain real fruit juices, 25–40%. They were filtered through Whatman #1 filter paper before being analysed. The clear sample solutions were kept in the refrigerator and were used within one week.

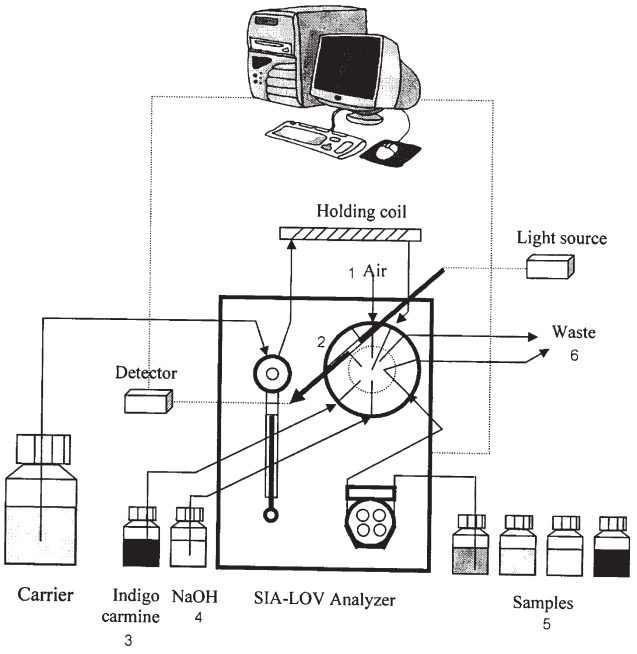

Fig. 1 A schematic diagram of the SIA-LOV manifold using a FIALab 3000 system for the mono-segmented flow titration system.

Procedure

An operation cycle of the system shown in Fig. 1 was controlled by a computer program (FIAlab 5.0). First, the carrier (H_2O) was drawn passing all the lines to clean them and to remove air from the system. The sample or standard citric acid solutions and reagents were drawn to fill up their lines. Next, 100 μl of water were aspirated into the multi-purpose flow cell at the flow rate of 0.5 ml min^{-1}. The reference absorbance of the carrier solution at this condition was detected and memorized. Then, air (15 μl), 0.0–1.2% (w/v) standard solution or samples (20 μl), 0.1% (w/v) indigo carmine solution (6 μl), 0.12 M sodium hydroxide solution (40 μl) and finally, another air segment (200 μl) were drawn in that order into the holding coil at the flow rate of 1 ml min^{-1} through port 1, 5, 3, 4 and 1 respectively. The mono-segment reagent zone was formed as shown in Fig. 2. After mixing the reagents in the mono-segment zone by reversing the flow once, the last air segment was removed through port 6. The solution zone was propelled at the flow rate of 1 ml min^{-1} through the multi-purpose flow cell *via* port 2. The change in absorbance of the indicator was detected at 608.9 nm. The first air segment was also discarded. Finally, the system was cleaned by

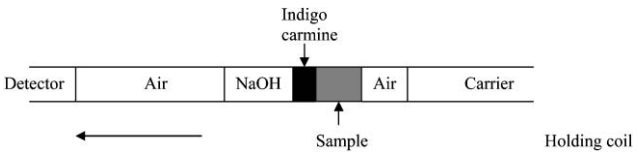

Fig. 2 The sequential drawing of regents and air to form a mono-segment.

pumping water through the flow cell. A calibration graph was constructed by plotting the peak heights (maximum absorbance) obtained *vs*. acidity values (as % w/v of citric acid) using a Microsoft® Excel program. In all experiments, triplicate measurements were done.

Standard AOAC method

All samples were analysed by the AOAC official method 942.15 for acidity (titratable) of fruit products[48] and the results were compared with those obtained from the proposed SIA-LOV mono-segmented titration. For the colorless or slightly colored juices, a 25 ml juice was diluted to 250 ml with recently boiled water and about 0.8 ml of phenolphthalein was added. Then it was titrated with 0.1 M NaOH until the indicator turned pink. For highly colored juices, more dilution of samples was needed. First, juices were diluted the same way as in the case of colorless samples. Then the solution was titrated with 0.1 M NaOH to just before the end point and after that about 0.8 ml of phenolphthalein was added. Next, a 2 or 3 ml precisely measured volume of solution was further diluted to 20 ml with water. This extra dilution helps to dilute the color of fruit juices so that the end point was easily seen. In the case where the end point is not reached after a long titration, this dilute portion should be poured back into the original solution and more titrant should be added until the end point can be observed.

Results and discussion

Preliminary study

The concept of this novel approach for mono-segmented flow micro-titration involves a fixed amount of NaOH used as a titrant to titrate citric acid in samples. Sample, titrant and indicator were aspirated into the system and formed a stack zone that was separated from the carrier by air segments. After the titrant and a suitable volume of acidic sample in a mono-segment were well mixed, the remaining amount of NaOH, which had not been used in the reaction at the time when the detection took place, would define pH of the final solution. For different concentrations of acid samples, different amounts of NaOH were left leading to various pHs of the final solutions which could be followed by the change in color of a suitable acid–base indicator. The change can be observed in a short time and there is no need to reach the equivalent point.

Fig. 3 illustrates the pH change with volume of titrant obtained from calculation of the excess amount of NaOH left after completion of neutralization with citric acid. This was done to consider the preliminary condition to be used in the experiment. The change in color of the indicator was observed at the region shown at the beginning of the curve. According to the results obtained from the standard batch titration method, acidities found in fruit juice samples were in the range of 0.2–1.1% (w/v) expressed as citric acid, therefore the citric acid concentration in this range was selected for the calculation. 0.12 M NaOH (40 μl) was chosen to ensure a complete protolysis of citric acid which would occur at pH higher than 8.5. A volume ratio of NaOH : acid sample solution of 2 : 1 was selected because it gave a good discrimination among

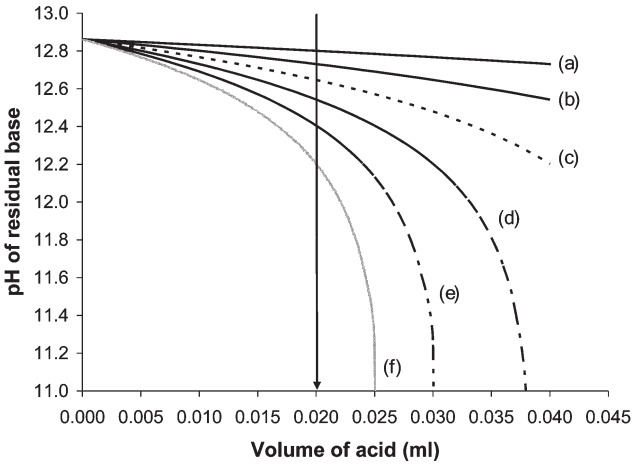

Fig. 3 Calculated pH changes in the titration between NaOH *versus* citric acid. The concentration of NaOH is 0.100 M and concentrations of citric acid are (a) 0.2, (b) 0.4, (c) 0.6, (d) 0.8, (e) 1.0, and (f) 1.2% (w/v). The arrow marks the pH values that were observed in the experiments when using different acid concentrations.

various concentrations of acidity. In this work, indigo carmine was selected because its color intensity is linearly proportional to NaOH concentration in the pH range of 11.4–13.0[49] which is the range of pH change in this particular titration. It is yellow in basic solution and blue in acidic solution. The decrease in the blue acidic form was selected to be monitored because the blue color was less interfered by colored substances in the fruit juice samples. Maximum absorption wavelengths of the indigo carmine indicator are at 400 nm (yellow) and 609 nm (blue).

The combined benefits of the mono-segmented flow technique and those of the SIA-LOV system have led this proposed system to have high sensitivity and sample throughput with less reagents and time consumption. Concentration of sample can be determined without any complicated calculations.

Optimization

Flow rate for aspiration of air segment and other reagents. The effect on flow rate for drawing air segment was investigated. The flow rate for aspiration of the air segment may not be the most obvious parameter to be optimized but it is very important. Since unsuitable flow rate for air led to small bubbles and the experiment could not be carried out, we studied this parameter at the beginning to ensure the proper handling of the air segment in the system for further experiments.

A flow rate of 1 ml min^{-1} was selected as it provided a steady air segment and short analysis time. With higher flow rate, the air segment was broken into a number of small air bubbles which may be difficult to be completely removed from the system.

For aspiration of other reagents, the flow rate of 1 ml min^{-1} was also chosen to compromise the sensitivity and sample throughput.

Concentration of an indicator. Calibration graphs were constructed using different concentrations of indigo carmine which varied from 0.05, 0.10, 0.20, to 0.40% (w/v). It was found that the slopes and R^2 values of the calibration graphs

decreased when the concentration of indigo carmine was increased. Therefore, the 0.05% (w/v) indigo carmine indicator was selected for further studies because it gave a greater slope and R^2 values.

Volumes of acid, base, indicator and air. Volumes of acid sample, base and indicator were chosen to find a compromise condition according to reagent consumption and sensitivity. The amounts chosen were 20, 40 and 6 μl for sample, base and indicator, respectively.

The air segment of 15 μl effectively separated the sample–reagent mono-segment from the carrier. However, at the beginning of the zone, 200 μl air was used just to save the carrier.

Number of flow reversals. It was found that one reversal flow was enough to mix the sample and reagents in the mono-segmented zone. The calibration signal profiles, obtained in the range of 0.0–1.2% (w/v) citric acid, are shown in Fig. 4.

Sample matrix. Fruit juices normally contain various organic matters and the most common one is sugar which is either added or is naturally present in juices. Sugar severely interfered with the determination of citric acid at low concentration. This might be due to the light refraction of high density and high viscosity sugar solution. The interference from sugar in samples could be overcome by adding sugar into the standard solutions to adjust the density and viscosity of the standard solutions to be similar to those of sample solutions as much as possible. An improved optical cell might be useful in reducing this problem.

Table 1 Summary of selected operation condition for determination of 0.0–1.2% (w/v) citric acid

Parameter	Condition
Reagent solution	40 μl of 0.12 M sodium hydroxide 6 μl of 0.05% (w/v) indigo carmine indicator
Sample volume	20 μl
Volume of air	15 μl for the end of zone 200 μl for the beginning of zone
Blank solution	10% (w/v) of sugar solution
Flow rate	0.5 ml min^{-1} for aspiration 1.0 ml min^{-1} for dispensation to detector
Volume of detection	80 μl
Wavelength	608.9 nm

Normally, the amounts of sugar in samples are approximately 10% (w/v) as indicated on the product labels. Therefore, the series of standard citric acid solutions was prepared in 10% (w/v) sugar solution to compensate for any effect of sugar in real samples.

Selected conditions. The selected conditions for the SIA-LOV determination of acidity in the range of 0.0–1.2% (w/v) as citric acid are summarized in Table 1.

Analytical characteristics

The experiment was carried out as described previously. The corresponding calibration graph, $Y = 0.4347X + 0.3414$ (where Y is absorbance and X is concentration of citric acid) with $R^2 = 0.9980$, was obtained from the signal profiles, as shown in Fig. 4.

A solution containing 0.6% (w/v) citric acid and 10% (w/v) sugar was used to study precision of the proposed method. The analysis of 11 replicates was done under the selected condition. The relative standard deviation (RSD) was found to be 1.2%. This indicates that the proposed system has good precision. Sample throughput was found to be 30 samples h^{-1}.

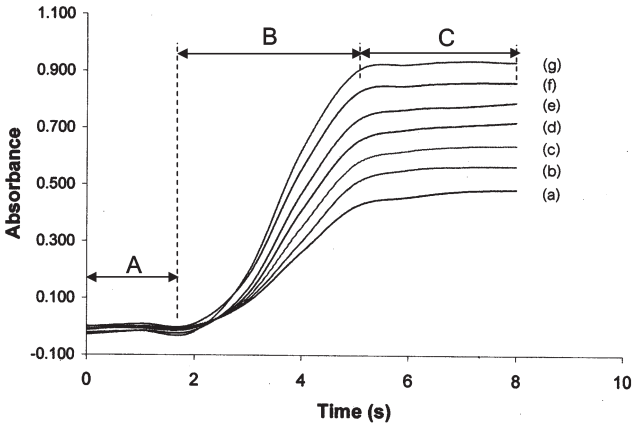

Fig. 4 Signal profiles of the standard solution. (a) 0.0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.0 and (g) 1.2% acidity. Part A is absorbance due to carrier (water). Part B is absorbance due to the interface region between water and mixing zone (air segment has been prior discarded). Part C is absorbance due to the final pH of the mixing zone which depends on amount of remaining NaOH.

Table 2 Comparison of % (w/v) acidity found by the mono-segmented flow SIA-LOV system and the standard AOAC titration method

Sample no.	Type of fruit juice	% (v/v) real fruit juice (as labeled)	Citric acid found (% (w/v))[a]	
			Standard AOAC method	SIA-LOV
1	Lychee	30	0.30 ± 0.01	0.33 ± 0.01
2	Lime	25	0.37 ± 0.02	0.39 ± 0.03
3	Lychee and pineapple	40	0.41 ± 0.01	0.44 ± 0.03
4	Pineapple	25	0.42 ± 0.01	0.43 ± 0.01
5	Lime	15	0.44 ± 0.01	0.47 ± 0.01
6	Apple and pineapple	40	0.45 ± 0.01	0.49 ± 0.02
7	Orange	25	0.45 ± 0.01	0.48 ± 0.04
8	Pineapple	25	0.50 ± 0.01	0.53 ± 0.02
9	Apple	25	0.52 ± 0.01	0.58 ± 0.02
10	Pineapple	100	0.91 ± 0.03	0.94 ± 0.01
11	Pineapple	100	1.02 ± 0.03	1.08 ± 0.02

[a] Mean of triplicate results.

Analysis of fruit juice samples

The accuracy of the proposed method was determined by comparing results obtained from SIA-LOV with those obtained from a standard AOAC titration method. Comparative analyses of the same samples were carried out on the same day and the results are shown in Table 2.

From Table 2, the acidity found in fruit juice samples (expressed as citric acid concentration) using the SIA-LOV method agrees well with those found by the standard AOAC titration method. The correlation of the two methods are linear: $Y = 1.03X + 0.02$ with $R^2 = 0.9965$ where the Y axis represents the results from the LOV mono-segmented micro-titration method and the X axis represents the results from the AOAC standard titration method. The slope and intercept of the correlation graphs of the fruit juices are close to 1 and 0, respectively. This indicated that both methods correlate well.

The results imply that if the matrices of sample are known and standard solution can be prepared in matrices matching those in sample solution, the proposed method would be very useful for routine analysis. Alternatively, the standard addition technique could be applied to the analysis of samples with unknown matrices.

Conclusion

A new concept of mono-segmented flow micro-titration with SI-LOV was proposed and demonstrated by acid–base titration for determination of acidity of fruit juices. The analysis by this system helps to reduce amounts of sample, reagents and time consumption. Micro-volume of reagents used make it possible to be applied to rare samples. This system provides good reproducibility and it is convenient for in-line dilution of sample. The proposed system should be able to be adapted to study other acids or other substances involving reactions that produce or consume acid or base. It can be coupled to other types of detection.

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Evaluation of on-line preconcentration and flow-injection amperometry for phosphate determination in fresh and marine waters

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Abstract

Dissolved reactive phosphorus (DRP) was determined as orthophosphate (PO_4-P) in fresh and saline water samples by flow-injection (FI) amperometry, without and with in-valve column preconcentration. Detection is based on reduction of the product formed from the reaction of DRP with acidic molybdate at a glassy carbon working electrode (GCE) at 220 mV versus the Ag/AgCl reference electrode. A 0.1 M potassium chloride solution was used as both supporting electrolyte and eluent in the preconcentration system. For the FI configuration without preconcentration, a detection limit of 3.4 $\mu g\,P\,l^{-1}$ and sample throughput of 70 samples h^{-1} were achieved. The relative standard deviations for 50 and 500 $\mu g\,P\,l^{-1}$ orthophosphate standards were 5.2 and 5.9%, respectively. By incorporating an ion exchange preconcentration column, a detection limit of 0.18 $\mu g\,P\,l^{-1}$ was obtained for a 2-min preconcentration time (R.S.D.s for 0.1 and 1 $\mu g\,P\,l^{-1}$ standards were 22 and 1.0%, respectively). Potential interference from silicate, sulfide, organic phosphates and sodium chloride were investigated. Both the systems were applied to the analysis of certified reference materials and water samples.

Keywords: Phosphate; Flow-injection amperometry; Preconcentration; Bio-Rad AG-X8; DRP; Schlieren effect

1. Introduction

Phosphorus is an essential plant and animal nutrient. However, due to human activities, inadvertent addition of phosphates to watersheds has caused eutrophication, which is commonly manifested by algal bloom formation [1]. Analytical methods for determination of phosphorus species, especially readily bioavailable dissolved reactive phosphorus (DRP) are essential for investigating the sources, cycling and fate of phosphorus (P) in aquatic ecosystems.

Spectrophotometric methods for determination of DRP are commonly based on the reduction of the phosphomolybdate complex (formed from reaction between reactive phosphate and acidic molybdate) to form the intensely coloured "molybdenum blue" product. Both batch [2] and flow-based [3,4] procedures have been reported. Although the spectrophotometric method provides good sensitivity, it suffers both from interferences, e.g. silicate, turbidity [3] and refractive index (Schlieren) effects in estuarine and marine samples, which can cause large errors in quantitation [5]. Electroanalytical methods are more tolerant of these interferences, and do not suffer from the Schlieren effect. Voltammetric or amperometric determination of phosphate has been performed using a range of different electrochemical reactions including, (1) reduction of 12-molybdophosphate to molybdenum blue [6–12], (2) oxidation of molybdenum blue, which was electrochemically prereduced and adsorbed on the electrode [13,14], (3) reduction of molybdovanadophosphate [15] and (4) oxidation of $FePO_4$ after reductive accumulation of Fe(II) in the presence of phosphate [16]. Flow-injection has also been applied for electrochemical determination of

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phosphate in order to improve performance of the technique. Some electrochemical methods involving the use of enzymes for determination of DRP have also been reported [17,18]. An FI amperometric system incorporating inorganic pyrophosphatase and nucleoside phosphorylase–xanthine oxidase reactors was used for simultaneous determination of phosphate and pyrophosphate [17]. An FI amperometric system with a nucleoside phosphorylase–xanthine oxidase column was also used for determination of phosphate with a detection limit of 1.25 μM [18]. The analytical characteristics of these electroanalytical techniques are summarized in Table 1.

The three major objectives of this work, based on the reduction of 12-molybdophosphate on a glassy carbon working electrode (GCE), were to evaluate amperometric detection of phosphate in fresh and marine waters, investigate the use of an in-valve preconcentration column and develop a simple manifold suitable for application in field instruments.

2. Experimental

2.1. Instrumentation

The FIA manifolds used in this study are illustrated in Fig. 1. A laboratory-made FIA workstation, consisting of two peristaltic pumps (Ismatec CA5E, Switzerland), an

Table 1
Summary of electroanalytical techniques and their analytical characteristics for determination of phosphate

Technique	Electrochemical reactions	Working electrode	Linear range ($\mu g\,P\,l^{-1}$)	Detection limit ($\mu g\,P\,l^{-1}$)	Reference
Batch injection analysis—amperometry	Reduction of molybdophosphate complex	Carbon paste	30–600	10	[6]
Batch/FI—differential pulse voltammetry (DPV)	Reduction of 12-molybdophosphate in acetone–water mixture	–	–	–	[7]
Voltammetry/amperometry	Reduction of molybdophosphate complexes	Gold microdisk	30–30000	–	[8]
Voltammetry	Reduction of 12-molybdophosphate in a capillary fill device	Screen-printed carbon	150–60000	150	[9]
FI-voltammetry	Reduction of 12-molybdophosphate in a reverse FI system	Glassy carbon	150–15000	–	[10]
Batch/FI—DPV	Reduction of 12-molybdophosphate	–	–	–	[11]
FI-voltammetry	Reduction of 12-molybdophosphate	Glassy carbon	30–15000	–	[12]
Adsorptive stripping voltammetry (AdSV)	Reoxidised of the preadsorbed hetoropoly blue	Glassy carbon	0.3–30	0.3	[14]
Voltammetry (DPASV)	ASV of β-heteropolymolybdates stabilized by acetone	Glassy carbon	–	–	[13]
Cyclic voltammetry	Reduction of 12-molybdophosphate	Carbon paste	400–25000	40	[22]
SIA-amperometry	Detection of molybdophosphate	Glassy carbon	100–3000	100	[23]
Voltammetry	Reduction of molybdovanadophosphate	Hanging Hg drop	0.06–300 mg $P\,l^{-1}$	0.15	[24]
FI-voltammetry	Reduction of molybdovanadophosphate	Glassy carbon	–	30	[15]
Cathodic stripping voltammetry (CSV)	Reduction of Fe(III) phosphate	Glassy carbon	–	100	[16]
FI-amperometry	Reduction of H_2O_2 produced from enzyme reaction (pyrophosphatase, nucleoside phosphorylase, xanthine oxidase)	–	–	–	[17]
FI-amperometry	Reduction of H_2O_2 produced from enzyme reaction (nucleoside phosphorylase, xanthine oxidase)	Platinum	–	40	[18]
FI-amperometry	Reduction of 12-molybdophosphate	Platinum	– 1550	0.6	[25]
FI-amperometry	Reduction of 12-molybdophosphate	Glassy carbon	50–1000	3.4	This work
FI-amperometry, with preconcentration	Reduction of 12-molybdophosphate	Glassy carbon	0.1–10	0.18	This work

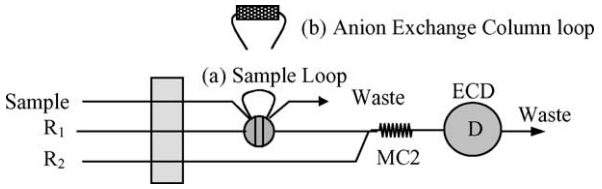

Fig. 1. FIA manifolds for the direct determination of orthophosphate: 60 cm mixing coil (MC2), $R_1 = 0.1\ mol\ l^{-1}$ potassium chloride, $R_2 = 0.5\%$ ammonium molybdate in 2.5% (v/v) sulfuric acid, (a) 50 μl sample loop; (b) in-valve anion exchange column for preconcentration.

injection valve (Rheodyne 5041, USA) actuated by pressure from a compressed air-gas cylinder, and an electrochemical detector (Princeton Applied Research model 400, USA) was employed. The amperometric signal (μA) was converted to voltage (mV) within the detector, where 1 V full scale corresponds to 100 μA. PTFE tubing (0.5 mm i.d.) was used for mixing coils, and all manifold lines. The FCS computer program (A-Chem Technologies, Australia) was used to control the system and collect the data from the detector. The thin-layer flow-through cell (5 mm × 15.6 mm × 0.09 mm) consisted of a GCE (3 mm diameter, Princeton Applied Research, MP1305), a Ag/AgCl reference electrode and stainless steel cell body auxiliary electrode. The GCE was polished daily and rinsed with deionized water.

For the manifold in Fig. 1, standard/sample was injected into a stream of potassium chloride before merging with the acidic molybdate reagent. This manifold was also modified by replacing the sample loop with an ion exchange minicolumn to preconcentrate the analyte before injecting into the system. The potassium chloride served both as an electrolyte to promote electrical conduction and decrease the difference in electrical conductivity between sample and acidic molybdate and as the eluant to elute the phosphate from the ion-exchange column. The volume of standard/sample loaded onto the column was defined by the loading flow-rate and time.

2.2. Reagents

All solutions were prepared from analytical grade reagents and high purity, deionized water (Continental). The 500 ml of acidic molybdate solution (R_2 in Fig. 1) was prepared from 2.5 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (BDH) and 12.5 ml of concentrated H_2SO_4 (BDH). A 0.1 M potassium chloride solution was prepared from oven-dried KCl (BDH). Standard phosphate solutions in the concentration range of 10–1000 $\mu g\ P\ l^{-1}$ were prepared daily from an intermediate solution (5 $mg\ P\ l^{-1}$), diluted from a 100 $mg\ P\ l^{-1}$ stock solution (0.2197 g oven-dried KH_2PO_4 (BDH) in 500 ml water). A 200 $mg\ l^{-1}$ sulfide stock solution was prepared from $Na_2S{\cdot}9H_2O$ (Sigma–Aldrich).

The anion-exchange mini-column was prepared by placing Bio-Rad AG1-X8 resin (Bio-Rad, USA, 200-400 mesh, chloride form) in a perspex column (5 mm × 1.5 mm i.d.), and plugging both ends with nylon mesh. The following certified reference materials were purchased from the Queensland Health Scientific Services, Australia, natural waters NLLNCT-Round 7: bottle 1, bottle 3, bottle 5 and bottle 7.

2.3. FI procedure

Potassium chloride and molybdate flow-rates in the FI system shown in Fig. 1 were 2.0 and 0.9 ml min^{-1}, respectively. The potential applied to the GCE was fixed at 220 mV versus the Ag/AgCl reference electrode. The sensitivity range of the detector was 0.2 and 0.1 μA for the preconcentration system. Orthophosphate standard solutions (10–1000 $\mu g\ P\ l^{-1}$) were measured in triplicate. Potential interferences including sodium chloride, sulfide, silicate and some organic phosphates were studied. The developed methods were validated using four certified reference materials. Eight water samples, which were collected from the water overlying the sediment in laboratory bioreactors were also analysed.

3. Results and discussion

3.1. Manifold without preconcentration

DRP concentrations, measured as orthophosphate (PO_4-P), were determined using the manifold depicted in Fig. 1a). The detection limit of this FI system, calculated using the criterion of 3× the standard deviation of the blank signal [19] was 3.4 $\mu g\ P\ l^{-1}$. Linear calibration was obtained over the concentration range 50–1000 $\mu g\ P\ l^{-1}$; the equation was: $y = 0.7834x - 2.4996$, $R^2 = 0.9995$. There was no curvature evident in the calibration curve, suggesting that even higher orthophosphate concentrations could be determined, if wastewater analysis was required. Attempts to simplify the manifold by using a single manifold line with injection of the sample into the acidic molybdate reagent resulted in a high negative blank peak due to the electrical conductivity difference between the reagent and sample streams. The detection limit in this case was 22 $\mu g\ P\ l^{-1}$.

3.1.1. Signal reproducibility

The stability of the FI system was examined by measuring 50 replicate injections of 50 and 500 $\mu g\ P\ l^{-1}$ phosphate standards. Fig. 2 shows that at a lower concentration of phosphate (50 $\mu g\ P\ l^{-1}$), the peak-height response was very reproducible. The mean and R.S.D. of peak heights were 21.3 mV and 1.3%, respectively. More variability was observed with the higher phosphate concentration, where the mean and R.S.D. of peak heights were 182.6 mV and 9.5%, respectively. The poor reproducibility of these results at higher phosphate concentrations suggests that the reduced molybdenum blue complex may have precipitated in the flow cell.

3.1.2. Interference study

Several potentially interfering species have been studied. Quintana et al. [6], as well as many others, have reported

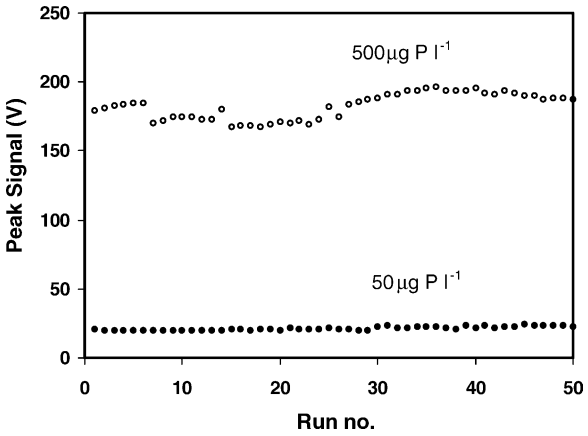

Fig. 2. Reproducibility of orthophosphate standard peak heights (50 and 500 μg P l^{-1}). Fifty replicate injections using the FI system 1a.

that silicate can cause major problems with phosphate determinations [2,6,15]. It was found here with silicate solutions of concentrations lower than 50 mg l^{-1}, no peak signal was obtained. Given that silicate is usually less than 20 mg l^{-1} in natural waters, silicate presents no significant interference for the determination of phosphate by this method.

Sulfide is another interferent in spectrophotometric phosphate determination [20]. Sulfide can be reduced at 220 mV (versus Ag/AgCl) at the working electrode and gives a negative peak. It was found that 5 mg l^{-1} of sulfide affected the peak-height responses of phosphate (50–100 μg P l^{-1}), but no interference was detected for 2 mg l^{-1} sulfide.

Potential interference by five organic phosphate species: adenosine-5-monophosphate, 2-aminoethyl phosphonic acid, glycerophosphate, phenyl phosphate and phytic acid, was also investigated. It was found that 100 μg P l^{-1} of each organic phosphate did not produce FIA peaks using the same conditions and manifolds investigated here for the determination of DRP. In natural waters, total organic phosphate concentrations do not normally exceed 50 μg P l^{-1}, although this may not be the case in sediment pore waters.

It was found that sodium chloride concentrations up to 4% (40 mg l^{-1}) did not affect peak height responses of phosphate over the range 50–1000 μg P l^{-1}. This method is therefore directly applicable to sea water samples without the need for salinity compensation. This represents a major advantage of this approach compared with the spectrophotometric FI determination of phosphate where adjustments are required to overcome the Schlieren (RI) effect [5].

3.1.3. Method validation and applications

Four reference materials: fresh waters, NLLNCT-Round 7: bottle 1 and bottle 3, and sea waters, NLLNCT-Round 7: bottle 5 and bottle 7, were analysed. The results shown in Table 2, indicate good agreement between expected and measured P concentrations, in both fresh and marine waters.

The method was also applied to eight saline water samples collected from the Gippsland lakes in eastern Victoria. The results, shown in Table 3, agreed well with the FI-spectrophotometric method (paired *t*-test, $t_7 = -0.10$, $p = 0.923$).

Table 2
Analysis of the certified reference materials (CRM)

Water type	CRM NLLNCT-round 7	Certified value (μg P l^{-1})	Found[a] (μg P l^{-1})
Fresh water	Bottle 1	27.0 ± 0.8	29.4 ± 1.4
	Bottle 3	97.9 ± 1.1	86.8 ± 1.1
Sea water	Bottle 5	27.7 ± 1.2	23.1 ± 0.3
	Bottle 7	11.8 ± 0.9	13.6 ± 1.7

[a] Mean of triplicate injections.

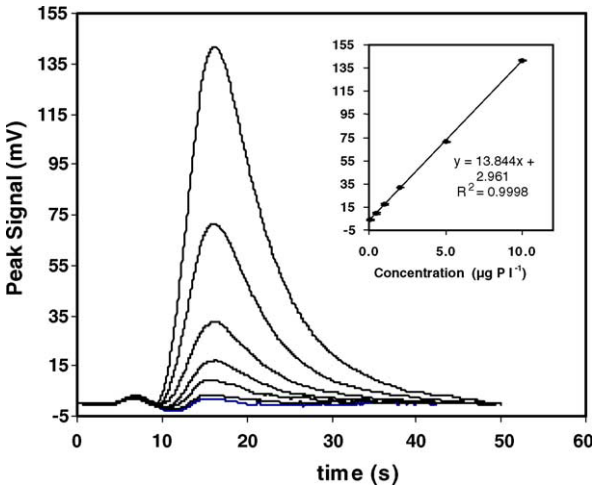

Fig. 3. FI graph and calibration graph of standard orthophosphate concentrations of 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg P l^{-1}: using the FI system 1b and preconcentration time = 2 min, detector range = 0.1 μA.

3.2. The FI system with preconcentration

The anion exchange minicolumn replaced the sample loop in the manifold shown in Fig. 1(b) and was used for preconcentration and subsequent determination of orthophosphate concentrations below 10 μg P l^{-1}. The calibration graph (Fig. 3) was linear between 0.1 and 10 μg P l^{-1}. Using a 2 min preconcentration time, at a loading flow-rate of 0.9 ml min^{-1}, the detection limit was 0.18 μg P l^{-1}. An

Table 3
Analysis of phosphate concentrations in sea water samples

Sample	Found (μg P l^{-1})	
	Electroanalytical procedure	Conventional spectrophotometric FIA
BB11	17	39
BB12	146	138
BB13	319	337
BB14	408	389
BB15	393	380
BB16	417	419
BB17	417	418
BB18	435	436

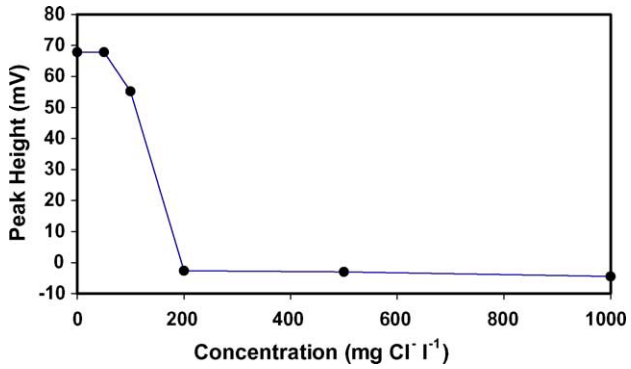

Fig. 4. The effect of chloride on peak signal response of 5 μg P l^{-1}. Preconcentration time: 2 min.

enrichment factor of 18, determined as the ratio of sensitivities with and without the preconcentration, was achieved.

Potential competition for ion-exchange sites by chloride ions present in the sample was investigated by analysing a series of 5 μg P l^{-1} standards containing chloride at a range of concentrations (0–1000 mg l^{-1}). Results indicate that chloride ion concentrations higher than 50 mg l^{-1} reduce the peak height response of 5 μg P l^{-1} (Fig. 4). At 200 mg l^{-1} of Cl^-, the phosphorus peak height response was indistinguishable from the blank signal. Similar interferences might be expected with other anions, especially divalently charged species such as sulfate with higher affinity for ion exchange sites on the column.

The preconcentration method was then applied to tap water samples. Recoveries for spiked orthophosphate standards were in the range of 92–126% (Table 4).

The viability of a single standard calibration using the preconcentration system was investigated using 5 and 10 μg P l^{-1} phosphate standards. By varying the preconcentration time (or loading time) for a sample, a linear relationship between μg P and peak height was obtained. The μg P was calculated by flow-rate (ml min^{-1}) × loading time (min) × standard solution concentration (μg P l^{-1}) × 10^{-3}. The data points using the 5 μg P l^{-1} solution coincided with the 10 μg P l^{-1} data (equation $y = 7345x + 7.47$, $R^2 = 0.996$), hence demonstrating the validity of the single standard calibration method.

Table 4
Spike recovery of phosphate by adding phosphate to tap water samples, using the preconcentration manifold (FI system 1b)

Sample	Concentration (μg P l^{-1})		% Recovery[a]
	Added	Found	
A	–	0.74[a]	–
	3.00[b]	3.50[c]	92
B	–	1.05[a]	–
	5.00[b]	6.83[c]	116
C	–	0.66[a]	–
	1.00[b]	1.92[c]	125
D	–	0.50[a]	–
	0.50[b]	1.09[c]	118

These data were calculated from the calibration equation: $y = 12.989x - 0.4932$, $R^2 = 0.999$.

[a] %Recovery = $[(c - a)/b] \times 100$.

4. Conclusion

An FI method using electrochemical detection has been developed for sensitive and rapid determination of orthophosphate. The efficacy of this method is demonstrated by a linear range (50–1000 μg P l^{-1}), covering orthophosphate concentrations commonly found in natural waters, with a detection limit of 3.4 μg P l^{-1} and sample throughput of 70 samples h^{-1}. The method is applicable to both fresh and saline samples and totally avoids the refractive index problems typically found with spectrophotometric methods. Analysis of oligotrophic freshwater samples is facilitated using the preconcentration system, where a detection-limit of 0.18 μg P l^{-1} was achieved using a 2 min preconcentration time. This method was only applicable to water samples with low chloride concentrations (<50 mg l^{-1}). It was demonstrated that a convenient, single standard calibration could be used with this preconcentration system. The analytical performance of these methods compares extremely favorably with the other studies listed in Table 1. The successful application of this simple, two line manifold to DRP determination in fresh and marine waters indicates great potential for further development into an instrument capable of field use, where artifacts engendered by sample preservation and storage can be avoided [21].

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Determination of linear alkylbenzene sulfonates in water samples by liquid chromatography–UV detection and confirmation by liquid chromatography–mass spectrometry

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed for the separation and determination of individual (C_{10}–C_{13}) linear alkylbenzene sulfonates (LAS). New sets of conditions have been established for routine analysis of individual chemical forms of four LAS surfactants, i.e. C_{10}–C_{13} LAS. Under a condition set using a mobile phase containing 1.5 mM ammonium acetate in methanol/water 80:20 (v/v) mixture, detection limits obtained were in the range 1.5 ppb (for C_{10} LAS) to 11.5 ppb (for C_{13} LAS). This 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

1. Introduction

Linear alkylbenzene sulfonates (LAS), synthetic anionic surfactants, have been used in household laundry and dish-washing 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. Although the method cannot differentiate individual anionic surfactant, it is normally expressed as methylene blue-active substances (MBAS) concentrations down to 25 ppb [10]. 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

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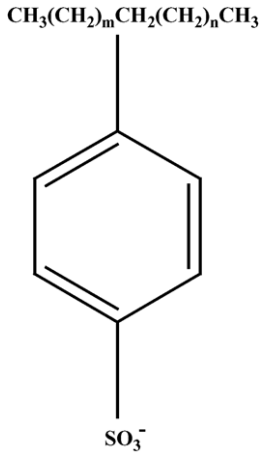

Fig. 1. General chemical structure of LAS.

(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 10–13 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 atoms [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 analytical 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 × 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 V, 20 psi, $10\,l\,min^{-1}$, 350 °C and 3500 V, 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 × 4.6 mm dimension and 5 μm diameter packing material. 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

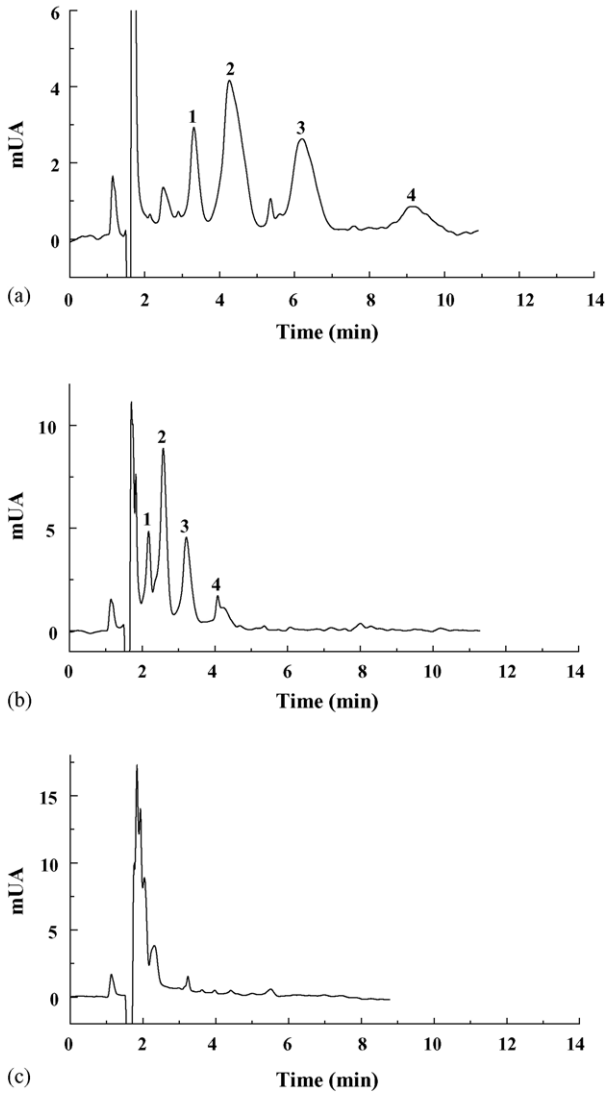

Fig. 2. Chromatograms of mixture of four LAS compounds obtained using various mobile-phase compositions of MeOH–H_2O: (a) 70:30; (b) 75:25; (c) 80:20. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

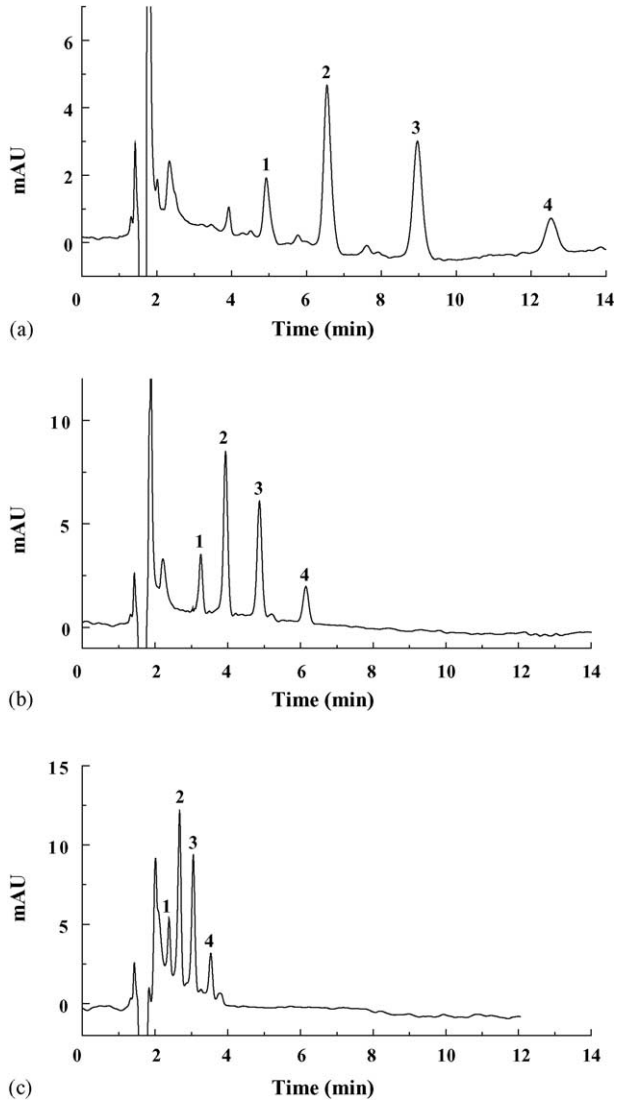

Fig. 3. Chromatograms of mixture of four LAS compounds obtained using various mobile-phase compositions in the presence of 3.5 mM NaCl and MeOH–H_2O: (a) 75:25; (b) 80:20; (c) 85:15. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

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.

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) along 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

Linearity, accuracy and detection limit for individual LAS compounds were studied (Table 1). The mobile phase

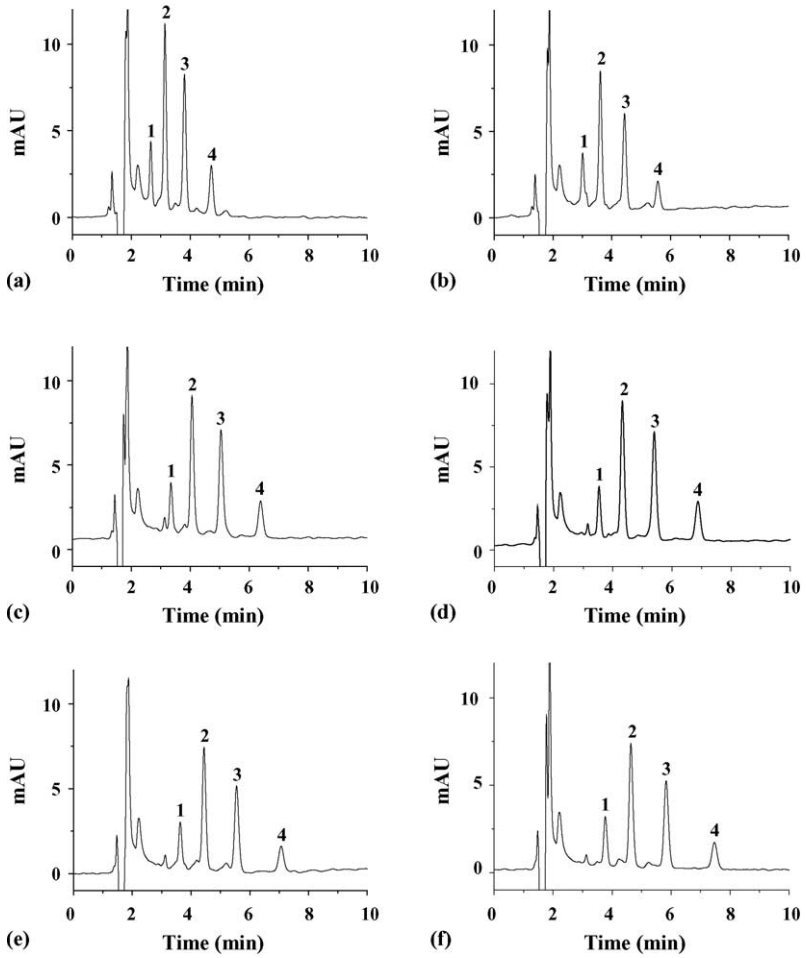

Fig. 4. Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium chloride: (a) 1 mM; (b) 2 mM; (c) 4 mM; (d) 6 mM; (e) 8 mM; (f) 10 mM. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.