รายงานวิจัยฉบับสมบูรณ์

การพัฒนาระบบการไหลแบบลดขนาดที่มีความคุ้มค่าสำหรับการหาปริมาณไอออนบางชนิด

Development of Cost – effective Miniaturized Flow Systems for Determination of Some Ions

โดย นายจรูญ จักร์มุณี

กรกฎาคม 2552

สัญญาเลขที่ RMU4980047

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โดย นายจรูญ จักร์มุณี
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มหาวิทยาลัยเชียงใหม่

สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา
และสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ขอขอบคุณสำนักงานคณะกรรมการการอุดมศึกษา (สกอ.) และสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่สนับสนุนทุนเพิ่มขีดความสามารถด้านการวิจัยของอาจารย์รุ่นกลางในสถาบันอุดมศึกษาสำหรับโครงการวิจัยนี้

ขอขอบคุณภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ที่ให้การสนับสนุนการทำวิจัยอย่างดียิ่ง

ขอขอบคุณแหล่งทุนสนับสนุนอื่น ๆ ที่มีส่วนเสริมกับโครงการวิจัยนี้ ได้แก่ ทุนโครงการพัฒนากลุ่มวิจัย สกอ. (ศาสตราจารย์ ดร. เกตุ กรุดพันธ์) ทุนโครงการการสร้างกำลังคนเพื่อพัฒนาอุตสาหกรรมของฝ่ายวิชาการ (ศาสตราจารย์ ดร. เกตุ กรุดพันธ์) ทุนโครงการปริญญาเอกกาญจนาภิเษก สกว. และ ทุนโครงการพัฒนาบัณฑิตศึกษาและการวิจัยทางเคมี (PERCH-CIC) สกอ.

ขอขอบคุณอาจารย์ผู้ร่วมงาน ได้แก่ ศาสตราจารย์ ดร. เกตุ กรุดพันธ์ ผศ.ดร. ยุทธศักดิ์ วณีสอน ผศ.ดร. นภาพร ยังวิเศษ ดร. พลยุทธ์ ศุขสมิติ และผู้ร่วมงานท่านอื่น ๆ ทั้งในและต่างประเทศที่ไม่สามารถเอ่ยนามได้ทั้งหมด ซึ่งมีส่วนร่วมให้คำแนะนำ / ติชมในการทำงานวิจัยนี้ให้สำเร็จลุล่วงไปด้วยดี

ขอขอบคุณนักศึกษาทุกคนที่มีส่วนร่วมในงานวิจัย โดยโครงการนี้ได้มีส่วนสนับสนุนการเรียนและการทำวิจัยของนักศึกษาทุกระดับด้วย

สุดท้ายขอขอบคุณครอบครัวของข้าพเจ้า ที่มีความรักและความเข้าใจในการทุ่มเทเวลาในการทำงานวิจัยของข้าพเจ้ามาโดยตลอด

จรูญ จักร์มุณี

บทคัดย่อ

รหัสโครงการ: RMU4980047

ชื่อโครงการ: การพัฒนาระบบการไหลแบบลดขนาดที่มีความคุ้มค่าสำหรับการหาปริมาณไอออนบางชนิด

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ได้พัฒนาระบบการไหลแบบลดขนาดบางแบบ เช่น โฟลอินเจคชัน ซีเควนเชียลอินเจคชัน และไมโครฟลูอิดิก เป็นต้น โดยได้ออกแบบสร้างอุปกรณ์ / เครื่องมือบางส่วนของระบบขึ้นเอง โดยมุ่งเน้นให้มีราคาถูกและใช้งานได้จริง เช่น ระบบฉีดสาร เซนเนล หรือ รีแอกเตอร์ เครื่องตรวจวัด และระบบบันทึกสัญญาณ เป็นต้น ได้ใช้เครื่องมืออุปกรณ์พื้นฐานเหล่านี้ในการพัฒนาวิธีวิเคราะห์โดยระบบการไหลบางแบบเพื่อการวิเคราะห์หาปริมาณไอออนบางชนิด เช่น ระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชัน สำหรับการหาปริมาณเหล็ก แมงกานีส ไนไตรต์ ไนเตรต ฟอสเฟต และซิลิเกต ระบบซีเควนเชียลอินเจคชันสำหรับการหาปริมาณโลหะหนัก เช่น ตะกั่ว แคดเมียม สังกะสี และทองแดง โดยตรวจวัดด้วยเทคนิคแอโนดิกสตริปปิงโวลแทมเมตรี วิธีสตริปปิงโวลแทมเมตรี สำหรับการหาปริมาณตะกั่วและแคดเมียมที่ถูกชะออกมาจากภาชนะเซรามิก สารหนูในตัวอย่างทางสิ่งแวดล้อม ระบบโฟลอินเจคชันสำหรับการหาปริมาณคลอไรด์ในวัสดุสำหรับคอนกรีต การหาโปรตีนในอาหาร การวิเคราะห์ฟอสเฟตในดิน เป็นต้น เครื่องมือและวิธีการวิเคราะห์ที่พัฒนาขึ้นนี้มีความคุ้มค่าสามารถนำไปใช้งานได้จริงและเป็นองค์ความรู้พื้นฐานในการพัฒนาทางเคมีวิเคราะห์ต่อไป รวมทั้งได้ประยุกต์ในการเรียนการสอนด้วย

คำหลัก: Flow based analysis, Miniaturization, Cost effective, Ions, Metals, Nutrients

Abstract

Project Code: RMU4980047

Project Title: Development of Cost – effective Miniaturized Flow Systems for Determination of Some Ions

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Project Period: 3 years (20 July 2006 - 19 July 2009)

Some miniaturized flow systems such as flow injection, sequential injection and microfluidics has been developed. Cost effective and purposeful instruments/devices for assembling of the systems were fabricated in-house such as injection device, channel or reactor, detector and data recording system. The developed instruments/devices were used for development of some analytical methods for the determination of some ions, i.e., hydrodynamic sequential injection system for determination of iron, manganese, nitrite and nitrate, and phosphate and silicate, sequential injection anodic stripping voltammetric system for determination of lead, cadmium, copper and zinc, stripping voltammetric methods for determination of lead and cadmium leaching from ceramic wares, and arsenic in environmental samples, flow injection systems for determination of chloride in materials for concrete, proteins in food, and phosphate in soil. The developed instruments and methods are effective for real applications and provide basic knowledge for further development of analytical methods. They are worth for education as well.

Keywords: Flow based analysis, Miniaturization, Cost effective, Ions, Metals, Nutrients

สารบัญ

1. บทนำ

การวิเคราะห์ทางเคมีมีความสำคัญมากในการศึกษาวิจัยด้านต่าง ๆ รวมทั้งในการผลิตสินค้าอุตสาหกรรม การเกษตรกรรม การแพทย์และเภสัชกรรม และทางสิ่งแวดล้อม แม้ว่าจะมีเทคนิค วิธีการ และเครื่องมือวิเคราะห์ทางเคมีจำนวนมากให้เลือกใช้ แต่ในปัจจุบันการพัฒนาเครื่องมือ/เทคนิคใหม่ ๆ ทางเคมีวิเคราะห์ก็ยังมีความจำเป็น เพื่อจะให้ได้ผลการวิเคราะห์ที่มีความถูกต้อง แม่นยำ และเชื่อถือได้ มีความไววิเคราะห์ (sensitivity) และความเลือกจำเพาะ (selectivity) สูงขึ้น และในขณะเดียวกันต้องมีค่าใช้จ่ายไม่สูง วิเคราะห์ได้สะดวก รวดเร็ว มีความเป็นอัตโนมัติ และเป็นมิตรกับสิ่งแวดล้อม มีสารเคมีหรือของเสียจากกระบวนการวิเคราะห์น้อยที่สุด

แนวโน้มการพัฒนาเทคนิคทางเคมีวิเคราะห์ ที่ตอบสนองความต้องการข้างต้น แนวทางหนึ่งก็คือการพัฒนาระบบการวิเคราะห์โดยการไหลแบบลดขนาด (miniaturized flow based analytical systems) ซึ่งอาศัยการเกิดปฏิกิริยาทางเคมีภายในท่อขนาดเล็ก (เส้นผ่าศูนย์กลางน้อยกว่า 0.5 มิลลิเมตร) โดยสารเคมีที่เกี่ยวข้องในปฏิกิริยาจะถูกควบคุมให้ไหลและผสมกันในบริเวณต่าง ๆ ภายในท่อหรือ channel อย่างแม่นยำ เทคนิคนี้มีการพัฒนามาอย่างต่อเนื่อง เกิดเป็นเทคนิคย่อยต่างๆ เช่น segmented flow, flow injection (FI), sequential injection (SI), SI with bead injection, SIA-lab-on–valve, SIA-lab-at-valve (LAV), Multi-syringe flow analysis (MSFA) และ Lab-on-a-chip (LOC) หรือ micro total analysis system (μTAS) เป็นต้น โดยในปัจจุบันได้มีการพัฒนาที่พยายามลดขนาดเครื่องมือและระบบการวิเคราะห์ให้เล็กลงไปอีก และเป็นอัตโนมัติมากขึ้น ซึ่งช่วยให้มีประโยชน์เพิ่มขึ้น เช่น ทำให้วิเคราะห์ได้เร็วขึ้น ค่าใช้จ่ายต่ำลง ใช้สารเคมีและทำให้เกิดของเสียจากการวิเคราะห์น้อยลง เป็นต้น

อย่างไรก็ตาม ระบบการไหลแบบลดขนาดที่มีการพัฒนากันนั้นยังมีข้อจำกัดในการใช้งานจริงอยู่มาก การที่ต้องใช้ท่อขนาดเล็กมากทำให้เกิดการอุดตันของระบบในการวิเคราะห์ตัวอย่างจริง ที่มีองค์ประกอบซับซ้อน รวมทั้งอุปกรณ์หลายๆ อย่างยังมีขนาดใหญ่ เช่น อุปกรณ์ตรวจวัด เป็นต้น การพัฒนาเครื่องมือ/อุปกรณ์ของระบบการไหลแบบลดขนาดยังเป็นเรื่องที่มีการพัฒนากันอยู่ในต่างประเทศ ในประเทศไทยการวิจัยทางด้านนี้มักเกี่ยวข้องกับพัฒนาวิธีวิเคราะห์โดยใช้เครื่องมือที่ซื้อเข้ามา ประเทศไทยยังมีการพัฒนาเครื่องมือวิเคราะห์น้อยมาก เครื่องมือวิเคราะห์ทางเคมีที่นำมาใช้ในกิจการด้านต่างๆ ส่วนใหญ่ต้องนำเข้า จึงมีความจำเป็นอย่างยิ่งที่ต้องมีการวิจัยที่เป็นพื้นฐานเพื่อพัฒนาเครื่องมือ

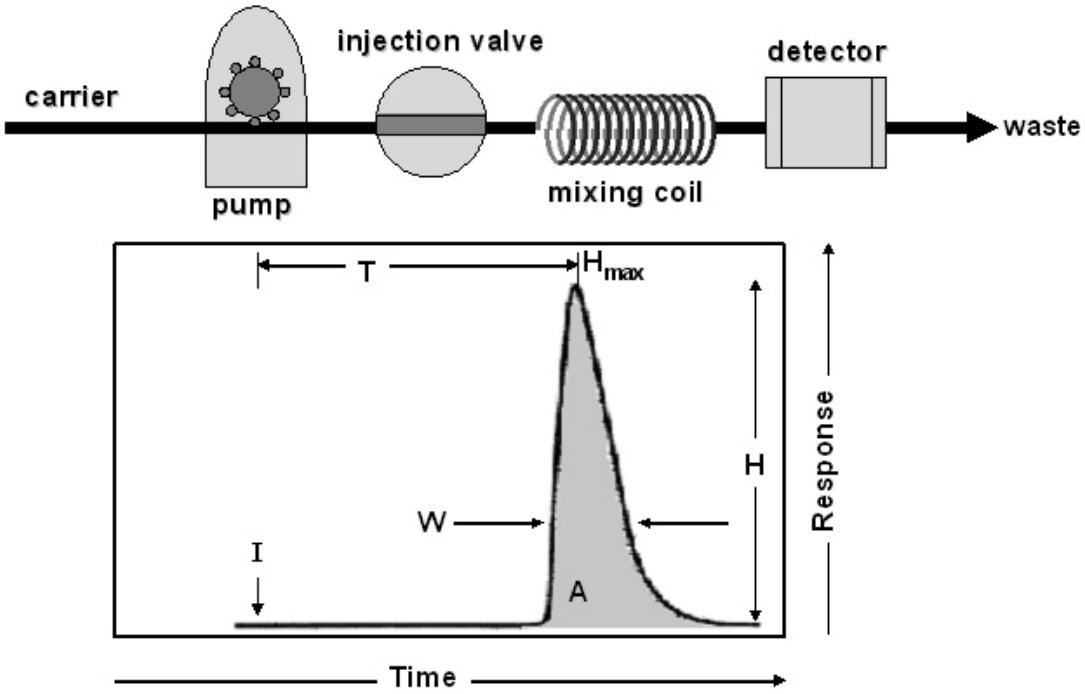
และวิธีวิเคราะห์ใหม่ ๆ ที่สามารถนำไปใช้ได้จริง ซึ่งในช่วงหลายปีที่ผ่านมาทางกลุ่มวิจัย Flow based Anaysis มหาวิทยาลัยเชียงใหม่ โดยได้รับการสนับสนุนจากแหล่งทุนต่าง ๆ โดยเฉพาะสกว. ได้มีการวิจัย/พัฒนาเครื่องมือ อุปกรณ์สำหรับระบบวิเคราะห์ทางการไหลขึ้นเอง เช่น FI, SI, SI with Lab-on-valve, bead injection (BI), SI with Lab-at-valve โดยเฉพาะได้เน้นการพัฒนาเครื่องมือที่มีความคุ้มค่า (cost effective) ซึ่งได้รับการยอมรับในระดับนานาชาติว่าเป็นงานวิจัยที่น่าสนใจ และมีประสิทธิภาพในการใช้งาน เครื่องมือและวิธีการที่มีราคาไม่สูงนักยังมีโอกาสที่จะนำไปใช้ในประเทศไทยได้ง่ายขึ้นด้วย

สำหรับในงานวิจัยนี้ จะมีการพัฒนาเครื่องมือ/อุปกรณ์ และวิธีการที่เกี่ยวข้องกับการวิเคราะห์โดยระบบการไหลที่ลดขนาดลง สำหรับการหาปริมาณไอออนบางชนิดที่มีความสำคัญทางสิ่งแวดล้อมและเกษตรกรรม เช่น พวกไอออนโลหะต่างๆ และสารอาหารของพืช เป็นต้น ซึ่งจะเป็นการพัฒนาวิทยาศาสตร์และเทคโนโลยีทางเคมีวิเคราะห์ขึ้นเองในประเทศที่สามารถนำไปประยุกต์ในการวิเคราะห์หลายๆ ด้าน และขณะเดียวกันก็ได้สิ่งใหม่ๆ ทางเคมีวิเคราะห์ที่เป็นที่ยอมรับในระดับสากลด้วย ทั้งนี้ ได้เสนอเทคนิค/แนวคิดใหม่ทางด้านเคมีวิเคราะห์ที่ใช้การไหล เช่น เทคนิคไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิส (hydrodynamic sequential injection analysis, HSIA) เทคนิคสต๊อปโฟลแบบใหม่ แลบแอทวาล์ว และอุปกรณ์นำสารตัวอย่างเข้าสู่ระบบการไหลแบบใหม่ที่ราคาถูกและมีประสิทธิภาพสูง เป็นต้น เทคนิคพื้นฐานเหล่านี้สามารถใช้พัฒนาวิธีการวิเคราะห์ทางเคมีที่มีประสิทธิภาพดี และมีความคุ้มค่าสูง สำหรับใช้วิเคราะห์สารต่าง ๆ ได้ โดยในที่นี้จะมุ่งพัฒนาวิธีสำหรับการวิเคราะห์หาปริมาณไอออนบางชนิด ที่มีความสำคัญทางด้านเกษตรกรรม และสิ่งแวดล้อม

1.1 เทคนิคโฟลอินเจคชันอะนาลิซิส

เทคนิคโฟลอินเจคชันอะนาลิซิส หรือ เอฟไอเอ เป็นเทคนิคที่ได้ถูกพัฒนามาตั้งแต่ปีค.ศ. 1975 โดยนำเสนอครั้งแรกโดย Ruzicka และ Hansen [1] เทคนิคนี้มีหลักการสำคัญคือการฉีดสารเข้าไปในกระแสการไหลของสารละลายที่ไหลอย่างอัตราคงที่ ในระหว่างที่สารละลายที่ถูกฉีดเข้าไปไหลไปยังเครื่องตรวจวัด จะเกิดการแพร่กระจาย (Dispersion) ที่มีรูปแบบแน่นอนและควบคุมได้ ทำให้เกิดการผสมของสารที่ถูกฉีดกับสารที่ไหลอยู่และเกิดปฏิกิริยาขึ้น ระยะเวลาตั้งแต่ฉีดสารจนสารไหลไปถึงจุดตรวจวัดจะมีค่าคงที่จึงทำให้ปฏิกิริยาที่เกิดขึ้นดำเนินไปเท่ากันทุกครั้งที่ฉีด ดังนั้นจึงไม่จำเป็นต้องรอให้มีการเกิดปฏิกิริยาจนถึงจุดสมดุลหรือ steady state เหมือนกับในกรณีการวิเคราะห์ใน

ระบบ batch ทั่วไป จึงช่วยลดเวลาในการวิเคราะห์ลงได้มาก นอกจากนี้ปริมาณสารที่ใช้ก็ลดลงอย่างมาก เนื่องจากการไหลที่ควบคุมได้หรือยังเป็น Laminar flow จะเกิดขึ้นในท่อขนาดเล็กมากเท่านั้น แผนผังและลักษณะของสัญญาณที่จะบันทึกได้จากระบบ FIA แสดงดังรูปที่ 1

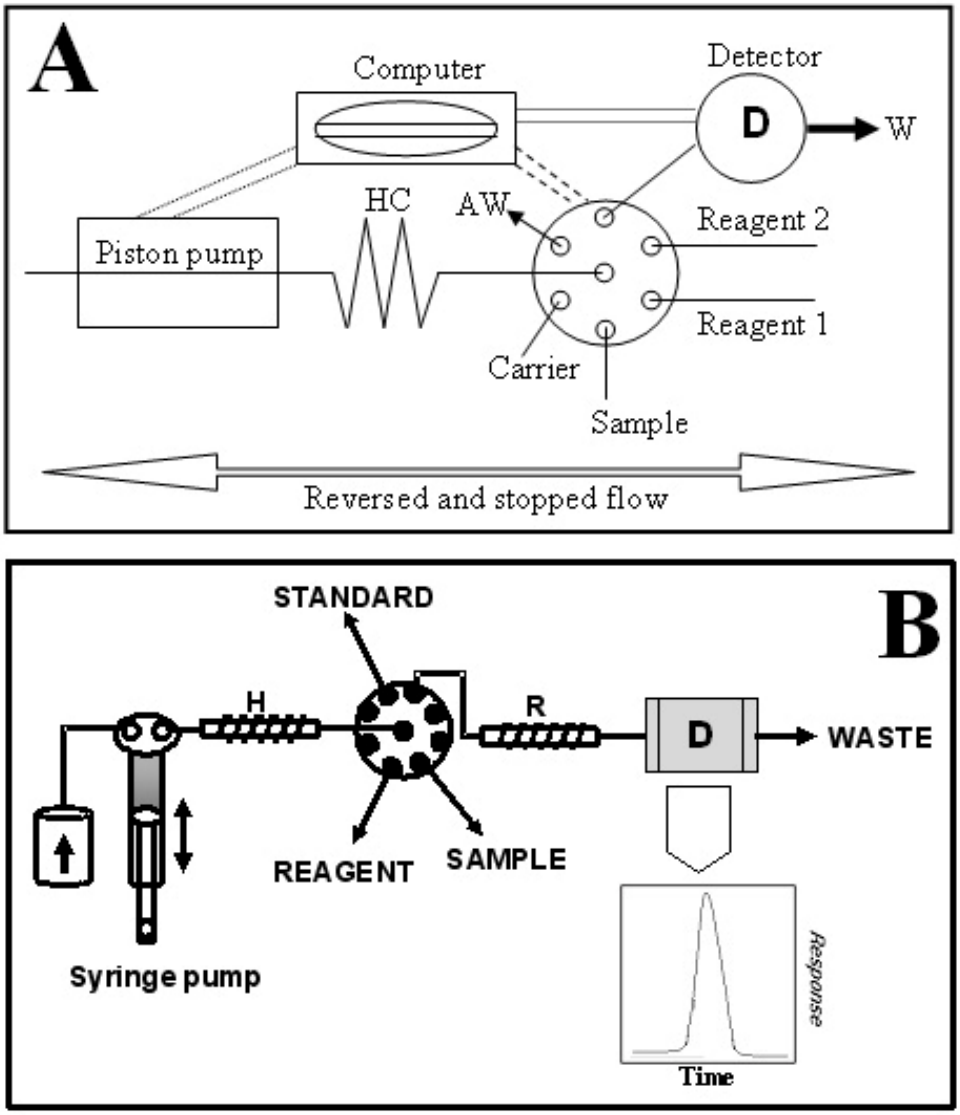


รูปที่ 1 แผนผังของระบบโฟลอินเจคชันอะนาลิซิสอย่างง่ายและสัญญาณที่จะบันทึกได้จากการฉีดสารเข้าไป 1 ครั้ง

ส่วนประกอบพื้นฐานของระบบได้แก่ ปั๊มป์ วาล์วฉีดสาร ส่วนผสมสาร หรือ reactor อุปกรณ์ตรวจวัดในระบบการไหลและเครื่องบันทึกสัญญาณ ซึ่งสามารถเลือกได้หลากหลาย ระบบเครื่องมือที่ผลิตขายในทางการค้ามีราคาในระดับ 500,000 – 1,000,000 บาท

1.2 เทคนิคซีเควนเชียลอินเจคชันอะนาลิซิส

เทคนิคซีเควนเชียลอินเจคชันอะนาลิซิส หรือ เอสไอเอ เป็นเทคนิคที่พัฒนาต่อเนื่องมาจากเอฟไอเอ โดยได้นำเสนอครั้งแรกในปี ค.ศ. 1990 โดย Ruzicka และ Marshall [2] มีหลักการพื้นฐานเหมือนเอฟไอเอ แต่มักจะใช้ปั๊มป์ channel เดียวที่มีการไหลสองทิศทางดังรูปที่ 2 โดยในขั้นแรกจะดูดสารตัวอย่างและรีเอเจนต์ผ่านทาง selection



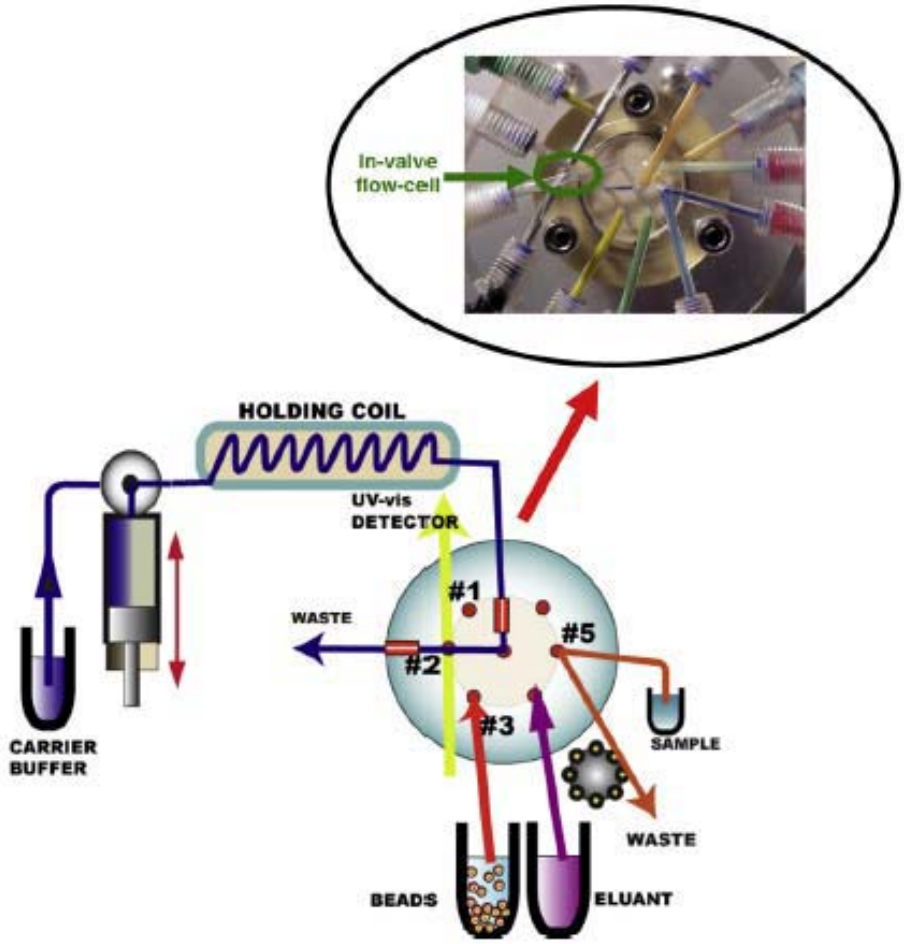
รูปที่ 2 (A) แผนผังของระบบซีเควนเชียลอินเจคชันอะนาลิซิส; HC= Holding coil, AW= auxiliary waste, W= waste. (B) ระบบซีเควนเชียลอินเจคชันอะนาลิซิสที่ใช้ syringe pump; HC= Holding coil, R= reaction coil, D=detection

valve เข้ามาพักไว้ใน holding coil ในลักษณะของ zones ที่อยู่ถัดกัน (stacked zones) จากนั้นโดยการควบคุมให้ปั๊มป์ดูด/ผลัก ทำให้เกิดการไหลกลับไปกลับมา (flow reversal) ซึ่งจะทำให้ zones ต่าง ๆ ผสมเข้าด้วยกันเนื่องจากเกิด dispersion จากนั้นจึงผลักสารไปยังเครื่องตรวจวัดเพื่อบันทึกสัญญาณต่อไป ระบบเอสไอเอ นี้สามารถตั้งโปรแกรมการไหลได้ด้วยคอมพิวเตอร์ จึงสามารถใช้อุปกรณ์ชุดเดียวในการวิเคราะห์หลาย ๆ อย่างได้เพียงแค่เปลี่ยนโปรแกรมควบคุมในคอมพิวเตอร์ และเนื่องจากสารละลายไม่ได้ไหลตลอดจึงประหยัดสารเคมีมากกว่าเอฟไอเอมาก อย่างไรก็ตามเอสไอเอ ต้องการอุปกรณ์ที่มีคุณภาพสูง เช่น ปั๊มป์ที่วัดปริมาตรได้แม่นยำและวาล์วแบบหลายช่อง ทำให้ราคา

เครื่องมือค่อนข้างสูง และเนื่องจากมีขั้นตอนต่าง ๆ เกี่ยวข้องในการวิเคราะห์มากจึงมักวิเคราะห์ได้ช้ากว่าระบบเอฟไอเอ

1.3 เทคนิคซีเควนเชียลอินเจคชันแลบออนวาล์ว

ได้มีความพยายามที่จะลดขนาดระบบวิเคราะห์โดยเทคนิคการไหลลงไปอีก โดยพัฒนาระบบเอสไอเอต่อมาเป็นระบบแลบออนวาล์ว [3] โดยนำส่วนของการทำปฏิกิริยา (reactor) และอุปกรณ์ตรวจวัดไปไว้บน selection valve เพื่อลดระยะทางและปริมาตรของสารที่ต้องใช้ในการทำปฏิกิริยาลงไปอีก ระบบ Lab-on-valve แสดงดังรูปที่ 3 ซึ่งได้มีการพัฒนาเพิ่มเติมโดยใช้รีเอเจนต์ที่เป็นของแข็ง หรือ ถูกจับไว้บนของแข็งในลักษณะของเม็ด (bead) เล็ก ๆ ซึ่งช่วยลดปริมาณสารที่ใช้และช่วยเพิ่มความไววิเคราะห์ยิ่งขึ้นไปอีก เทคนิคนี้เรียกว่า Bead injection analysis ซึ่งมักจะใช้กับระบบ SI – Lab – on -Valve นี้



รูปที่ 3 ระบบ Lab-on-valve [4]

1.4 แลบออนอะชิพ

ได้มีความสนใจลดขนาดของระบบเครื่องมือวิเคราะห์ทางเคมีมานานแล้ว เช่น Manz [5] ได้เสนอระบบ micro total analysis (μTAS) ซึ่งได้พยายามรวมขั้นตอนต่าง ๆ ทางเคมีวิเคราะห์ เช่น การเตรียมตัวอย่างการทำปฏิกิริยาการตรวจวัดเข้าไว้ด้วยกันบนอุปกรณ์ชิ้นเล็ก ๆ แทนที่จะต้องทำขั้นตอนต่าง ๆ เหล่านี้บนโต๊ะปฏิบัติการ (lab-on-bench) ของห้องปฏิบัติการ อุปกรณ์ μTAS นี้บางที่ก็เรียกว่าห้องปฏิบัติการบนชิพ หรือแลบออนอะชิพ (Lab-on-a-chip, LOC) หรือระบบไมโครฟลูอิดิก (microfluidics) เนื่องจากเกี่ยวข้องกับการไหลใน channel ขนาดเล็กมาก (ระดับ 50-100 ไมโครเมตร) ในระยะเริ่มต้นการสร้าง μTAS จะทำบนแผ่น silicon หรือแผ่นแก้วโดยใช้เทคโนโลยีของการสร้างวงจรรวม (integrated circuit, IC) ที่เรียกว่า photolithography ซึ่งมีค่าใช้จ่ายสูงมาก ต่อมามีการพัฒนาวิธีที่ง่ายและราคาถูกลง เช่น การใช้พอลิเมอร์ หรือ พลาสติกชนิดต่าง ๆ ในการสร้างชิพอย่างไรก็ตามยังต้องใช้เทคนิค photolithography และห้องปฏิบัติการไมโครอิเล็กทรอนิกส์ในการสร้างชิพต้นแบบ (template) อยู่ดี μTAS หรือ LOC ใช้สารในระดับนาโนลิตรถึงไมโครลิตรในการวิเคราะห์ จึงประหยัดได้มากโดยเฉพาะอย่างยิ่งหากต้องใช้สารเคมีราคาแพง และทำให้มีสารเหลือทิ้งจากการวิเคราะห์น้อยมากด้วย ระบบนี้ใช้ประโยชน์นาโนเทคโนโลยีได้ดี เช่น การใช้อนุภาคนาโนชนิดต่าง ๆ ในระบบเพื่อการวิเคราะห์แบบใหม่ ๆ ที่รวดเร็ว และนำไปใช้ในภาคสนามได้ อย่างไรก็ตามระบบนี้ยังมีปัญหาสำคัญคือการอุดตันของ channel ได้ง่าย และอุปกรณ์เครื่องมือที่เกี่ยวข้อง เช่น ระบบขับเคลื่อนสารละลาย เครื่องตรวจวัดยังมีขนาดใหญ่มาก

1.5 วัตถุประสงค์ของโครงการ

1. เพื่อพัฒนาเครื่องมือและอุปกรณ์สำหรับระบบการไหลแบบลดขนาด ที่จะใช้เป็น platform สำหรับพัฒนาวิธีในการหาปริมาณไอออน
2. เพื่อพัฒนาวิธีวิเคราะห์ใหม่ๆ ที่ใช้หลักการไหล สำหรับวิเคราะห์หาปริมาณไอออนบางชนิดที่มีความสำคัญทางสิ่งแวดล้อมและเกษตรกรรม
3. เพื่อประยุกต์วิธีที่พัฒนาขึ้นในการศึกษาทางสิ่งแวดล้อมและเกษตรกรรม เช่น การตรวจ/ติดตามไอออนของโลหะบางชนิด ในน้ำ/ดิน/ตะกอนท้องน้ำ การหาปริมาณไอออนบางชนิดที่มีผลต่อการเจริญเติบโตของพืช เป็นต้น

1.6 สรุปงานวิจัยในโครงการนี้

ได้นำเสนอเทคนิคไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซีส (hydrodynamic sequential injection analysis, HSIA) ซึ่งพัฒนาต่อยอดมาจากเทคนิคไฮโดรไดนามิกโฟลอินเจคชันอะนาลิซิส เพื่อลดการใช้สารเคมีลงไปอีก และใช้อุปกรณ์ราคาถูกกว่าระบบซีเควนเชียลอินเจคชันโดยทั่วไป ซึ่งได้พัฒนาเครื่องมือ/อุปกรณ์ สำหรับเทคนิค HSIA ทั้งแบบที่ง่ายและราคาถูก คือแบบ manual HSIA system ซึ่งควบคุมการทำงานของระบบทั้งหมดโดยผู้ใช้งาน และระบบ semi-automatic HSIA ที่ควบคุมการทำงานด้วย microcontroller จึงมีความแม่นยำและอัตโนมัติมากขึ้น และระบบ automatic HSIA ที่ควบคุมด้วยคอมพิวเตอร์ ระบบเครื่องมือดังกล่าวนี้ได้นำมาใช้พัฒนาวิธีวิเคราะห์ไอออนบางชนิด เช่น แมงกานีสไอออนในดิน [6] การวิเคราะห์สปีชีส์ของเหล็ก (Fe^{2+} และ Fe^{3+}) ในแหล่งน้ำธรรมชาติ [7] การหาปริมาณไนไตรต์ และไนเตรตในระดับความเข้มข้นส่วนในพันล้านส่วนในแหล่งน้ำต่าง ๆ [8] และการวิเคราะห์ไอออนของฟอสเฟตและซิลิเกตพร้อมกัน [9]

ส่วนเทคนิค SI Lab-at-valve นั้นได้นำเสนอครั้งแรกสำหรับการวิเคราะห์หาปริมาณคลอไรด์ [10] ซึ่งเป็นระบบการไหลแบบลดขนาดแบบหนึ่งที่พัฒนาต่อมาจาก Lab – on – valve [11] โดยมีประสิทธิภาพและข้อได้เปรียบคล้าย ๆ กัน แต่ Lab-at-valve ใช้เครื่องมือง่ายกว่ามาก ต่อมาได้มีการเสนอ Lab-at-valve สำหรับการวิเคราะห์ที่เกี่ยวข้องกับการสกัดด้วยตัวทำละลาย [12-13] ซึ่งช่วยให้การสกัดทำได้สะดวกและใช้ตัวทำละลายอินทรีย์น้อยลงมาก ในงานวิจัยนี้ได้พัฒนาระบบ SI Lab-at-valve สำหรับการวิเคราะห์หาปริมาณโลหะหนักบางชนิดโดยตรวจวัดด้วยเทคนิคแอโนดิกสตริปปิงโวลแทมเมตรี ซึ่งได้ออกแบบเซลล์โวลแทมเมตริกแบบใหม่ และสร้าง UV-digestion unit ขึ้นเองเพื่อช่วยในการย่อยสลายสารอินทรีย์ (dissolved organic matters) ในตัวอย่างน้ำก่อนทำการหาปริมาณโลหะ ทำให้สามารถระบุถึงสปีชีส์ของโลหะที่เป็น free form และ bound form ได้ ในส่วนของการควบคุมเครื่องมือได้พัฒนา software ขึ้นเอง ซึ่งช่วยให้การวิเคราะห์ มีความเป็นอัตโนมัติสูงขึ้น และทำให้ขั้นตอนต่าง ๆ ที่ซับซ้อนทำได้ง่าย เช่น การใช้เทคนิค monosegmented flow มาช่วยในการผสมสาร และการพัฒนาใช้ขั้วไฟฟ้า bismuth film electrode (BiFE) แทนขั้วปรอทที่มีพิษสูง สำหรับการวิเคราะห์โลหะด้วยเทคนิค ASV [14]

เทคนิคโวลแทมเมตรีสามารถวิเคราะห์โลหะได้หลายชนิดพร้อมกันในเวลาเดียวกัน และมีค่าใช้จ่ายต่ำกว่าเทคนิคทางสเปกโทรโฟโตเมตรี ในงานวิจัยนี้ได้พัฒนาวิธีวิเคราะห์

ตะกั่วและ แคดเมียม พร้อมกัน ซึ่งโลหะทั้งสองชนิดนี้จะถูกชะออกมาจากผิวของภาชนะเซรามิก โดยใช้สารสกัดตามวิธีมาตรฐานของการทดสอบความปลอดภัยของภาชนะเซรามิก วิธีที่พัฒนาขึ้นนี้มีประสิทธิภาพดีกว่าวิธีมาตรฐานที่ใช้กันอยู่ในปัจจุบัน [15] นอกจากนี้ยังได้พัฒนาเทคนิคแคโทดิกสตริปปิงโวลแทมเมตรี เพื่อวิเคราะห์หาปริมาณของ As(III) และ As(V) โดยอาศัยการเกิดปฏิกิริยารีดักชันทางไฟฟ้าของ As(III) ไปเป็นอาร์ซีน ซึ่งให้ความไววิเคราะห์และความจำเพาะที่สูง ให้ผลการวิเคราะห์สอดคล้องกับวิธีมาตรฐานที่ใช้ hydride generation AAS [16] วิธีทางโวลแทมเมตรีนี้มีศักยภาพอย่างยิ่งที่จะนำไปใช้ศึกษาการสกัดไอออนของโลหะออกมาอย่างเป็นลำดับขั้น ในที่นี้ได้ศึกษาเบื้องต้นถึงการใช้ on-line sequential extraction เพื่อศึกษาการชะธาตุพิษเหล่านี้ออกมาจากดิน ซึ่งควรทำการศึกษาพัฒนาต่อไปอีก

สำหรับเทคนิคสต๊อปโฟลอินเจคชัน (sFI) แบบใหม่นั้นได้นำเสนอในงานวิจัยก่อนหน้านี้สำหรับการวิเคราะห์คลอเรตด้วยเทคนิคแอมเพอโรเมตรี[17] ซึ่งมีข้อดีหลายประการ เช่น ช่วยเพิ่มความไววิเคราะห์ โดยที่ประหยัดสารเคมี และไม่ทำให้สารเกิดการแพร่กระจาย (dispersion) มากเกินไป ในงานวิจัยนี้ได้พัฒนาเครื่องมือสำหรับทำการวิเคราะห์โดยเทคนิคสต๊อปโฟลอินเจคชัน ให้มีความสะดวกและมีประสิทธิภาพสูงขึ้น และได้พัฒนาวิธีการสำหรับการวิเคราะห์หาปริมาณฟอสเฟตในดินและปุ๋ย [18] และการวิเคราะห์คลอเรตโดยตรวจวัดด้วยเทคนิคสเปกโทรโฟโตเมตรี เพื่อหาปริมาณคลอเรตในดินจากสวนลำไย [19]

สำหรับระบบการวิเคราะห์แบบลดขนาด ที่ใช้หลักการของโฟลอินเจคชันอะนาลิซิส ได้ทำการพัฒนาเพิ่มเติมในส่วนของอุปกรณ์ในการนำสารตัวอย่างเข้าสู่ระบบ โดยใช้โซลีนอยด์วาล์ว ซึ่งสามารถควบคุมการ load และ inject สารได้โดยใช้ไฟฟ้า ซึ่งอาจพัฒนาต่อไปเป็นระบบอัตโนมัติได้ ได้นำเสนอเทคนิคดังกล่าวในการพัฒนาวิธีวิเคราะห์หาปริมาณคลอไรด์ในตัวอย่างมวลรวม และน้ำยาผสมคอนกรีต ซึ่งต้องการควบคุมคุณภาพเกี่ยวกับปริมาณคลอไรด์ ซึ่งจะไปทำให้เกิดสนิมในเหล็กโครงสร้าง ในงานดังกล่าวนี้ได้พัฒนาขั้วไฟฟ้าเลือกจำเพาะไอออนคลอไรด์ขึ้นเองด้วย และศึกษาประสิทธิภาพของวิธีเทียบกับวิธีมาตรฐาน พบว่าวิธีที่พัฒนาขึ้นให้ผลการวิเคราะห์ที่มีแม่นยำและมีความไววิเคราะห์ดีกว่าวิธีมาตรฐาน [20] นอกจากนี้ยังไม่ใช้สารเคมีที่เป็นพิษและมีราคาแพง

ได้พัฒนาระบบโฟลอินเจคชันคอนดักโตเมตรี ที่อาศัยการแยกแก๊สโดยให้เกิดการแพร่ผ่านเยื่อบางเพื่อวิเคราะห์แอมโมเนียมไอออน ซึ่งได้ประยุกต์ในการหาปริมาณโปรตีน (โดยวิธี Kjeldahl) โดยช่วยประหยัดสารเคมีกว่าวิธีที่เคยมีรายงาน และวิเคราะห์ได้รวดเร็วกว่าวิธีไทเทรต ซึ่งเป็นวิธีมาตรฐาน [21] ในงานวิจัยนี้ได้ศึกษาประสิทธิภาพของการแพร่

ผ่านแก๊สและออกแบบสร้าง conductometric cell สำหรับการวัดความนำไฟฟ้าในระบบการไหลขึ้นเองด้วย

ได้พัฒนาระบบโฟลอินเจคชันแอมเพอโรเมตรี สำหรับการหาปริมาณฟอสเฟตในดิน [22] วิธีดังกล่าวนี้ใช้เครื่องควบคุมศักย์ไฟฟ้าขนาดเล็กและราคาไม่แพงเป็นเครื่องตรวจวัดแต่สามารถตรวจหาปริมาณฟอสเฟตในระดับความเข้มข้นต่ำระดับส่วนในพันล้านส่วนได้ โดยมีข้อดีกว่าวิธีสเปกโทรโฟโตเมตรีที่นิยมใช้กันคือไม่เกิดการรบกวนจากสารที่มีสี และทนการรบกวนจากซิลิเกตได้สูงกว่ามาก จึงได้ประยุกต์ในการหาปริมาณฟอสเฟตในดิน โดยได้พัฒนาวิธีสกัดฟอสเฟตจากดินแบบใหม่ขึ้นด้วย ซึ่งจากการศึกษาเบื้องต้นพบว่าเทคนิคดังกล่าวนี้ ยังสามารถใช้ได้ดีในการวิเคราะห์ฟอสเฟตที่ถูกชะออกมาจากตัวอย่างดิน เมื่อใช้วิธีการสกัดแบบลำดับขั้นโดยองค์ประกอบของสารละลายที่ใช้สกัดชนิดต่าง ๆ ไม่มีผลต่อระบบตรวจวัดที่พัฒนาขึ้นนี้ ซึ่งเทคนิคนี้ควรได้พัฒนาต่อไปอีกเพื่อใช้ศึกษา fraction ต่าง ๆ ของฟอสฟอรัสในดิน และดินตะกอนท้องน้ำซึ่งเป็นที่สนใจศึกษากันมาก

สำหรับระบบการวิเคราะห์แบบลดขนาดที่มีขนาดเล็กมากลงไปอีก คือ ระบบไมโครฟลูอิดิก (microfluidics) หรือ แลบออนอะชิพ (Lab-on-a-chip, LOC) ในที่นี้ ได้ศึกษาวิธีสร้างไมโครชิพด้วยวิธีที่ง่ายและราคาถูกโดยใช้ printed circuit board (PCB) เป็นต้นแบบสำหรับการลอกแบบรูปแบบ channel ที่ต้องการโดยใช้พอลิเมอร์ Polydimethylsiloxane (PDMS) และการใช้ Laser cutting ในการสร้าง PMMA chip ซึ่งจะมีค่าใช้จ่ายต่ำกว่าวิธี Lithography ที่นิยมใช้กันซึ่งต้องการ clean room และเครื่องมืออุปกรณ์ราคาแพงที่ใช้ในการผลิตวงจรรวม (integrated circuit) ได้ทดสอบการวิเคราะห์ด้วยระบบ microfluidics โดยตรวจวัดด้วยเทคนิคสเปกโทรโฟโตเมตรี

นอกจากนี้ยังได้พัฒนาระบบตรวจวัดแบบ colorimetry ขนาดเล็กที่ใช้ light emitting diode (LED) เป็นแหล่งกำเนิดแสงและ light dependent resistor (LDR) เป็นอุปกรณ์วัดแสงเพื่อให้ระบบตรวจวัดมีขนาดเล็กลงเหมาะกับการตรวจวัดบนชิพ [23] งานดังกล่าวนี้ยังต้องมีการพัฒนาเพิ่มเติมต่อไป

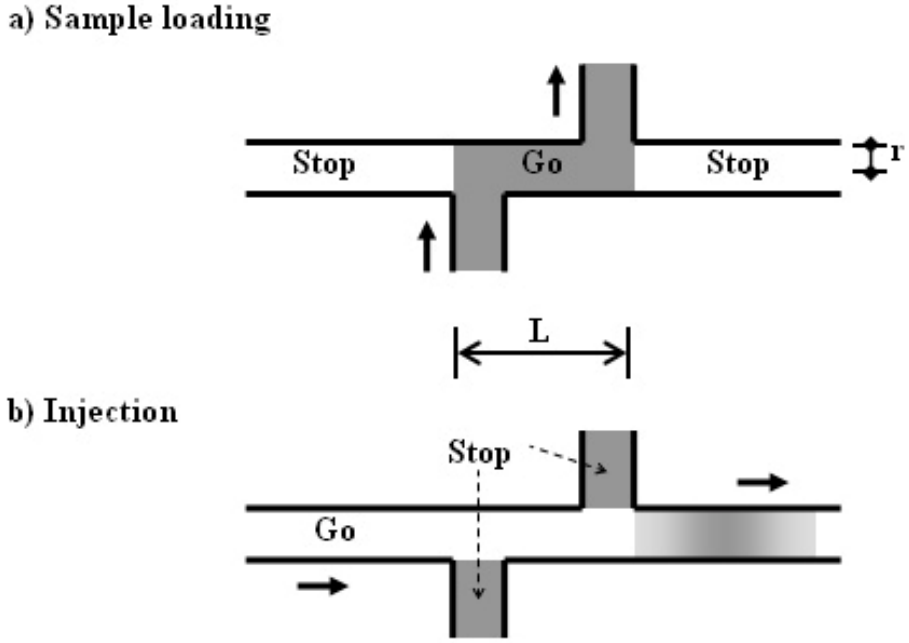
จากงานวิจัยข้างต้นนี้ นอกจากจะทำให้ได้องค์ความรู้ใหม่ที่สามารถตีพิมพ์ในวารสารชั้นนำระดับนานาชาติแล้ว ความรู้พื้นฐานที่ได้ยังสามารถพัฒนาต่อยอดต่อไปเพื่อให้ใช้งานได้จริง เครื่องมือและวิธีการวิเคราะห์ที่พัฒนาขึ้นได้ใช้ประโยชน์ในการเรียนการสอน และประยุกต์ในการนำไปใช้งานจริง ทั้งทางด้านการเกษตรและอุตสาหกรรมซึ่งเหมาะกับสภาวะสังคมเศรษฐกิจของประเทศไทยในปัจจุบัน ที่เน้นการพึ่งตนเองตามแนว

พระราชดำริเศรษฐกิจพอเพียง ซึ่งงานวิจัยในที่นี้ได้ทำในประเทศเป็นหลักเพื่อให้เกิดการพัฒนาอย่างยั่งยืน

2. การทดลอง ผลการทดลอง และวิจารณ์

2.1. การพัฒนาเทคนิคไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซีส

เทคนิคการฉีดสารแบบไฮโดรไดนามิก (hydrodynamic injection) เป็นวิธีหนึ่งในการนำสารเข้าสู่ระบบการไหลแบบเอฟไอเอ ซึ่งได้นำเสนอโดย Ruzicka และ Hansen ตั้งแต่ปีค.ศ. 1983 [24] โดยมีหลักการดังรูปที่ 2.1

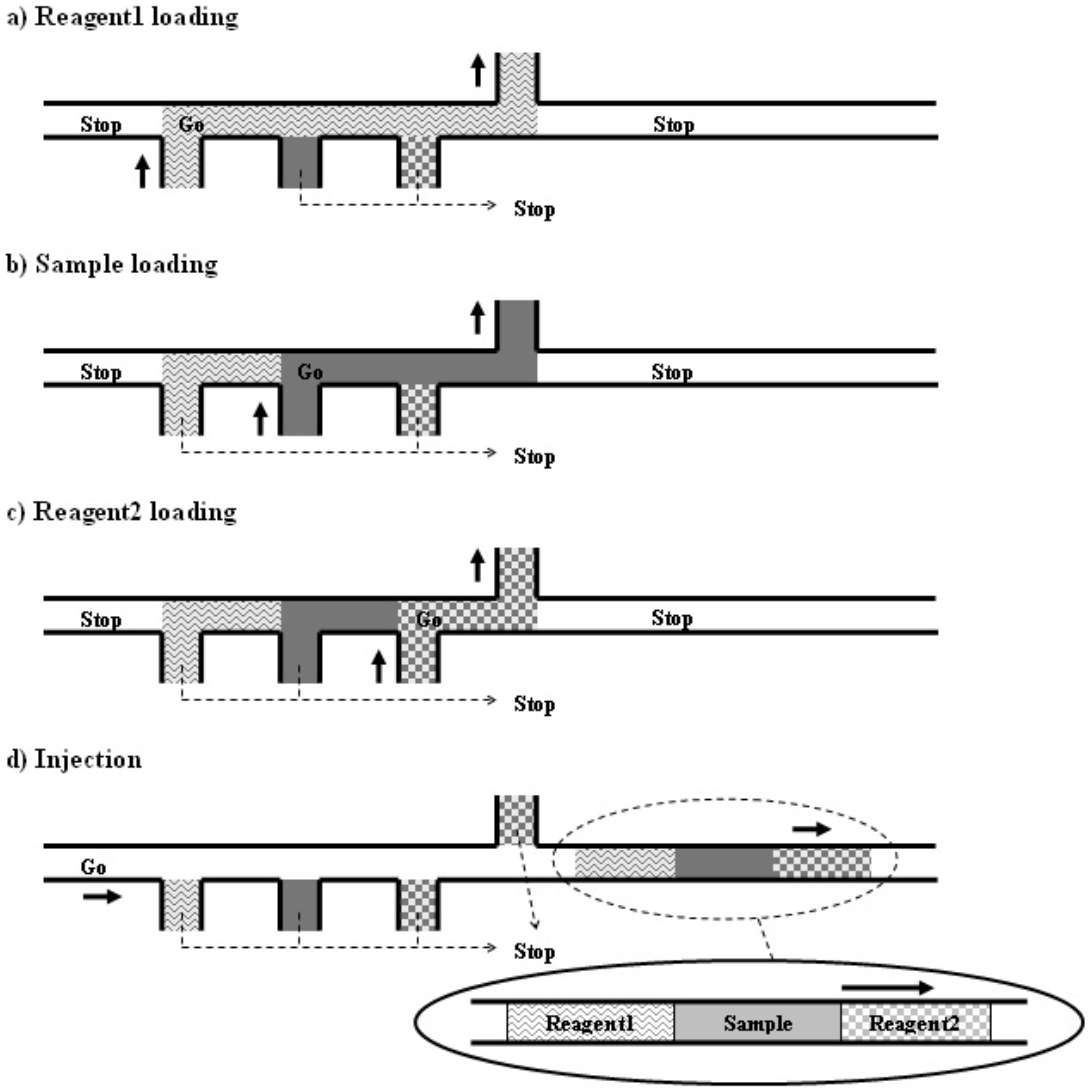


รูปที่ 2.1 หลักการฉีดสารแบบไฮโดรไดนามิก

โดยขณะทำการ load สารกระแสการไหลของสารละลายตัวพา (carrier) จะถูกหยุดไว้ ซึ่งจะทำให้มีแรงดันของของเหลวในท่อ (hydrostatic pressure) บังคับให้สารที่ฉีดเข้าไปไหลไปทางท่อที่เปิดตามทิศทางลูกศร โดยที่ไม่เกิดการผสมกับสารละลายที่หยุดไว้ในช่วงความยาวของท่อ เป็นระยะ L จะถูกเติมด้วยสารที่ฉีดเข้าไป เมื่อต้องการฉีดสารเข้าสู่ระบบก็จะหยุดไหล โดยปิดทางเข้าและทางออกที่ทำการฉีดสารและเปิดทางให้สารละลายตัวพาไหลตามเดิม sample zone ก็จะถูกผลักเข้าสู่ระบบอย่างแม่นยำ ปริมาตร

สารที่ถูกนำเข้าสู่ระบบจะเท่ากับ $\pi r^2 L$ การฉีดสารแบบไฮโดรไดนามิก จะเกิดขึ้นได้ กรณีที่ท่อมีขนาดเล็กมากพอ ซึ่งการไหลยังเป็นแบบราบเรียบ (laminar) ซึ่งจะไม่ทำให้สารที่ถูกหยุดไว้ตรง 3 ทางถูกชะเข้าสู่ระบบซึ่งจะเกิดปัญหาการปนเปื้อน (carry over) ขึ้นได้

ด้วยหลักการฉีดสารแบบไฮโดรไดนามิกนี้ เราสามารถใช้นำสารตัวอย่างและรีเอเจนต์เข้าสู่ระบบการไหลได้โดยใช้อุปกรณ์/เครื่องมืออย่างง่าย ซึ่งสารละลายที่ถูกฉีดเข้าไปตามลำดับจะเกิดเป็น zones ซ้อนกันดังรูปที่ 2.2 เหมือนกับกรณีที่ใช้เทคนิคเอสไอเอ โดยปริมาตรของแต่ละ zone จะถูกกำหนดโดยความยาวและเส้นผ่าศูนย์กลางของท่อดังข้างต้น จากนั้นเมื่อทำการปิดช่องฉีดสารและให้สารละลายตัวพาไหลอีกครั้งหนึ่งสารใน zones ต่าง ๆ นี้จะถูกพาเข้าสู่ระบบเกิดการผสม เกิดปฏิกิริยา และผ่านไปยังเครื่องตรวจวัดต่อไป

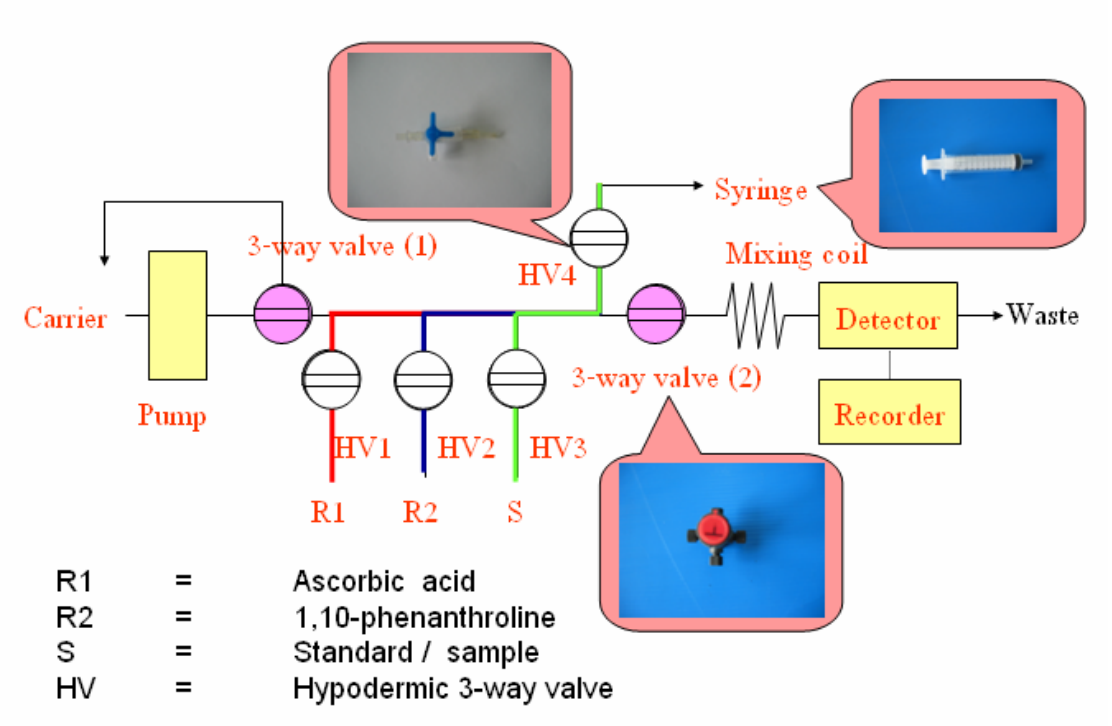


รูปที่ 2.2 หลักการของเทคนิคไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิส

เทคนิคนี้จึงถูกเรียกว่าไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิส หรือ เอช เอสไอเอ ซึ่งมีหลักการทั่วไปเหมือนกับ เอฟไอเอ และ เอสไอเอ แต่มีข้อได้เปรียบคือใช้อุปกรณ์อย่างง่าย ราคาไม่แพง สามารถทำงานได้ทั้งแบบ manual และแบบอัตโนมัติ ประหยัดสารเคมีและเกิดของเสียน้อยเหมือนระบบเอสไอเอ

2.1.1 ระบบเมนนวลไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซีส

สามารถสร้างระบบเอชเอสไอเออย่างง่าย ที่สามารถควบคุมการทำงานขั้นตอนต่าง ๆ โดยผู้ใช้งานทั้งหมด เช่น การสลับตำแหน่งของวาล์ว การดูดสารละลาย ระบบแสดงดังรูปที่ 2.3 สำหรับการวิเคราะห์หาปริมาณเหล็กในรูป Fe^{2+} และ Fe^{3+} โดยทำปฏิกิริยากับ 1,10–phenanthroline



รูปที่ 2.3 ระบบเมนนวลไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิส สำหรับการวิเคราะห์หาปริมาณเหล็ก

สามารถใช้วาล์วสามทางที่ใช้กับถุงน้ำเกลือ และกระบอกฉีดยาสำหรับดูดสารละลาย ขั้นตอนการทำงานของระบบแสดงดังตารางที่ 2.1 ในการวิเคราะห์ Fe^{2+} สารตัวอย่างจะถูกดูดเข้าไปผสมกับ 1,10–phenanthroline และถูกผลักไปยังเครื่องตรวจวัดทางสเปกโทรโฟโตเมตรี โดย Fe^{2+} จะเกิดสารเชิงซ้อนสีแดงกับ 1,10–phenanthroline สามารถวัดการ

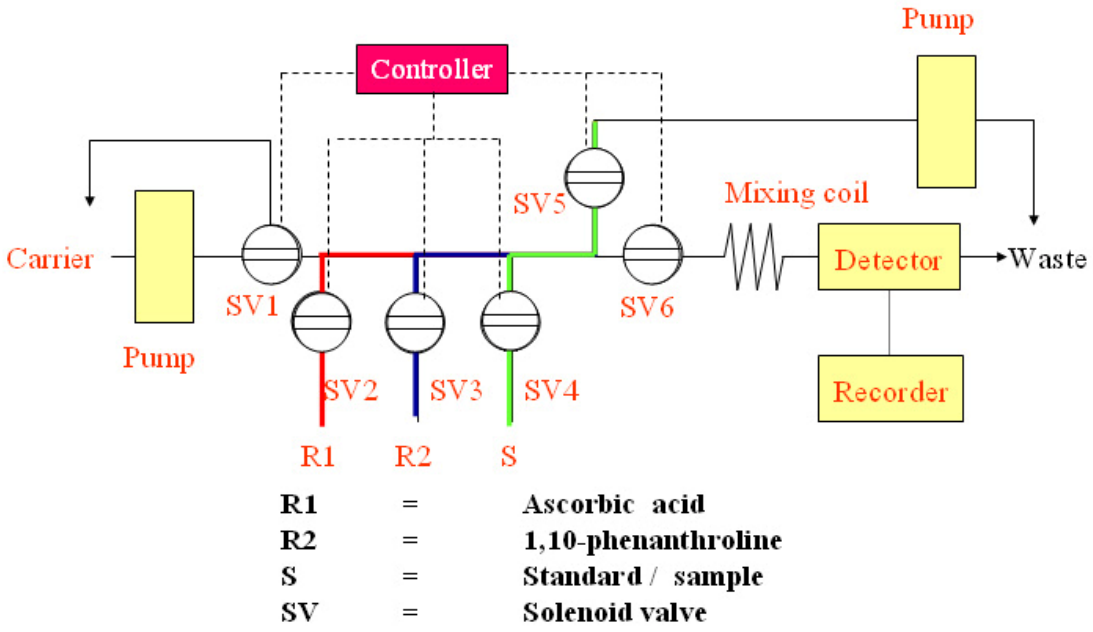
ดูดกลืนแสงได้ที่ความยาวคลื่น 510 nm ในการวิเคราะห์ Fe^{2+} + Fe^{3+} จะทำการดูด reducing agent (ascorbic acid) เข้าไปด้วยเพื่อเปลี่ยน Fe^{3+} ไปเป็น Fe^{2+} และวิเคราะห์หาปริมาณเหล็กรวม ปริมาณ Fe^{3+} จะได้จากการหักลบผลการวิเคราะห์ครั้งที่สองด้วยครั้งแรก รายละเอียดเกี่ยวกับระบบดังกล่าวโปรดดูภาคผนวก ข.1

ตารางที่ 2.1 ขั้นตอนการทำงานของระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิสสำหรับการวิเคราะห์หาปริมาณ Fe(II) และ Fe(III)

Step	Valve						Description
	TV1	TV2	HV1	HV2	HV3	HV4	
1	off	off	off	on	off	on	Draw up R2 by syringe
2	off	off	off	off	on	on	Draw up S by syringe
3	on	on	off	off	off	off	Forward the mixture to detector (for Fe(II) determination)
4	off	off	on	off	off	on	Draw up R1 by syringe
5	off	off	off	on	off	on	Draw up R2 by syringe
6	off	off	off	off	on	on	Draw up S by syringe
7	on	on	off	off	off	off	Forward the mixture to detector (for total iron determination)

2.1.2 ระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซีสแบบกึ่งอัตโนมัติ

ในระบบนี้จะใช้ไมโครคอนโทรลเลอร์มาควบคุมการสับวาล์วต่าง ๆ ตามเวลาที่กำหนดเพื่อนำสารละลายที่ต้องการเข้าสู่ระบบตามลำดับ โดยใช้ solenoid valves จำนวน 6 ตัว ดังรูปที่ 2.4



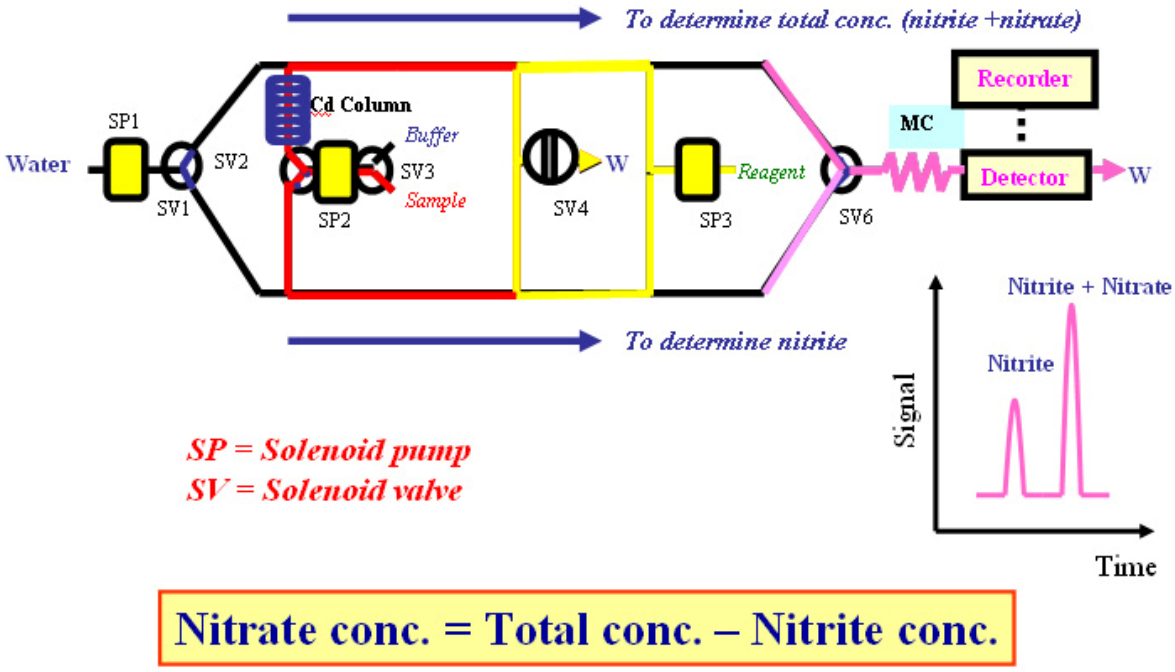
รูปที่ 2.4 ระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิสแบบกึ่งอัตโนมัติสำหรับการวิเคราะห์หาปริมาณเหล็ก

ขั้นตอนการทำงานของระบบจะเหมือนกับในระบบเมนนวล แต่จะมีความสะดวกในการใช้งานมากกว่ามาก โดยในขั้นแรกปั๊มป์จะผลักสารละลายบัฟเฟอร์ผ่านท่อของระบบไปยังจุดตรวจวัด จากนั้นจะดูดสารละลาย 1,10 – phenanthroline และสารตัวอย่างแล้วผลักไปยังเครื่องตรวจวัดเพื่อหาปริมาณ Fe^{2+} เสร็จแล้วจึงดูด ascorbic acid 1,10 – phenanthroline และสารตัวอย่างเพื่อตรวจวัดปริมาณเหล็กรวม (Fe^{2+} + Fe^{3+}) ต่อไป ระบบดังกล่าวนี้ใช้สารเคมีน้อยกว่าระบบ เอฟ ไอ เอ และสามารถวิเคราะห์หาปริมาณเหล็กได้ในระดับความเข้มข้นช่วงเดียวกับวิธีเอฟ ไอ เอ รายละเอียดเกี่ยวกับระบบนี้โปรดดูเพิ่มเติมในภาคผนวก ข.1

ระบบเอช เอส ไอ เอ แบบกึ่งอัตโนมัตินี้ได้นำมาพัฒนาวิธีวิเคราะห์หาปริมาณมังกานีสไอออนในดินด้วย โดยใช้ปฏิกิริยาการเกิดสารเชิงซ้อนระหว่าง Mn^{2+} กับ formaldoxime รายละเอียดเกี่ยวกับงานดังกล่าวนี้โปรดดูในภาคผนวก ก. 1

2.1.3 ระบบเอชเอสไอเอแบบอัตโนมัติที่ควบคุมด้วยคอมพิวเตอร์

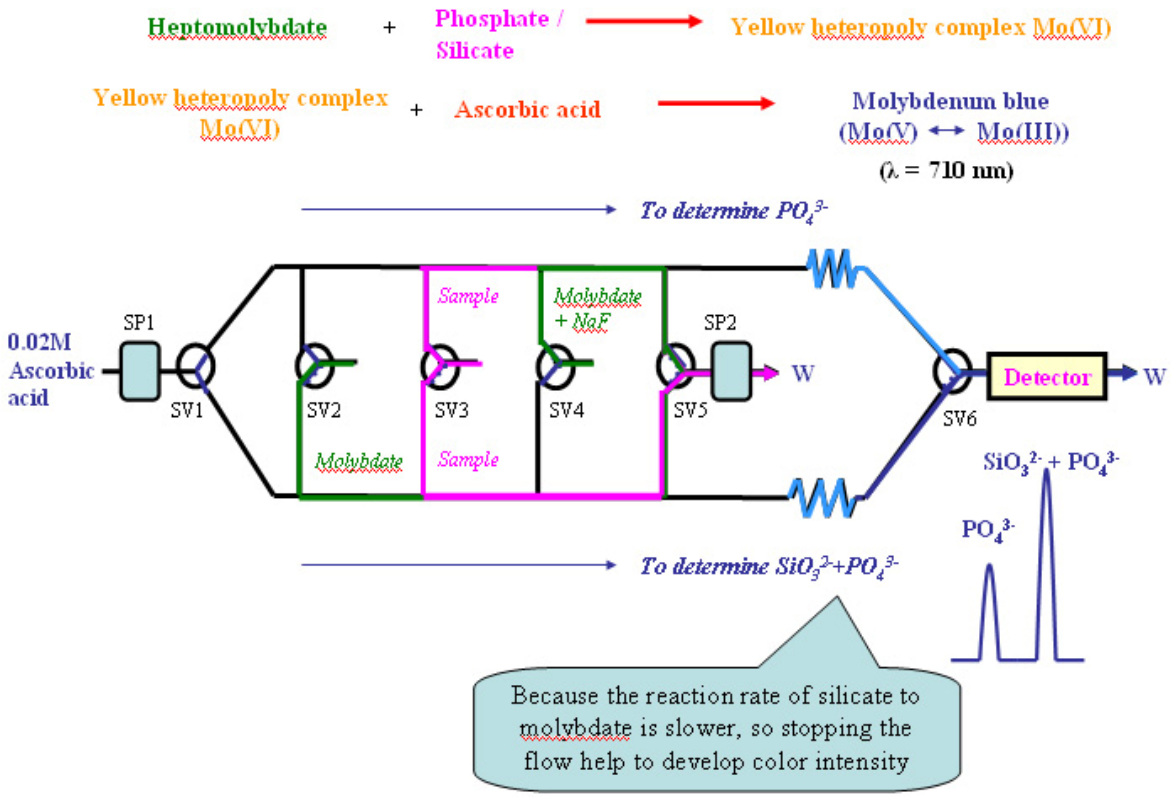
ได้พัฒนาเครื่องมือเพิ่มเติมโดยใช้ solenoid pumps และ solenoid valves และควบคุมการทำงานของระบบด้วยคอมพิวเตอร์ ซึ่งทำให้ได้ระบบเครื่องมือที่กะทัดรัดมากยิ่งขึ้น เนื่องจาก solenoid pump จะมีขนาดเล็กกว่า peristaltic pump มาก แผนผังของระบบเครื่องมือสำหรับการวิเคราะห์หาปริมาณไนไตรต์และไนเตรตแสดงดังรูปที่ 2.5



รูปที่ 2.5 ระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิสแบบอัตโนมัติสำหรับการวิเคราะห์หาปริมาณไนไตรต์และไนเตรต

สารตัวอย่างและรีเอเจนต์จะถูกปั๊มป์ผ่าน SV2 ไปทางท่อด้านล่าง ซึ่งจะใช้วิเคราะห์ไนไตรต์ ส่วนตัวอย่างที่ถูกปั๊มป์ ไปทางด้านบนจะผ่าน reduction column เพื่อเปลี่ยนไนเตรตไปเป็นไนไตรต์ก่อน จึงเป็นการวิเคราะห์หาปริมาณไนไตรต์+ไนเตรต รายละเอียดเกี่ยวกับการพัฒนาวิธีวิเคราะห์ไนไตรต์และไนเตรตนี้ โปรดดูภาคผนวก ก.2

ระบบเอช เอส ไอ เอ ที่คล้าย ๆ กันนี้ ได้นำมาพัฒนาวิธีสำหรับวิเคราะห์ฟอสเฟตและซิลิเกตในตัวอย่างน้ำ แผนผังเครื่องมือแสดงดังรูปที่ 2.6



รูปที่ 2.6 ระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิสแบบอัตโนมัติสำหรับการวิเคราะห์หาปริมาณฟอสเฟตและซิลิเกต; SP= Solenoid pump, SV=Solenoid valve, W= waste.

โดยอาศัยการเกิดปฏิกิริยาระหว่างฟอสเฟตกับโมลิบเดต และรีดิวซ์ด้วยกรดแอสคอร์บิกให้เกิดเป็นโมลิบดีนัมบูล ซึ่งสามารถตรวจวัดการดูดกลืนแสงที่ 710 nm ขั้นตอนการทำงานของระบบแสดงดังตารางที่ 2.2 โดยใน line ด้านบนจะเป็นการวิเคราะห์เฉพาะฟอสเฟต โดยใช้รีเอเจนต์ที่มี NaF เป็น masking agent สำหรับป้องกันไม่ให้ซิลิเกตเกิดปฏิกิริยา ส่วนใน line ด้านล่างจะเป็นการวิเคราะห์ฟอสเฟต + ซิลิเกต โดยจะทำการหยุดการไหลที่ mixing coil ด้วยเพื่อเพิ่มความไววิเคราะห์ รายละเอียดเกี่ยวกับวิธีดังกล่าวแสดงดังภาคผนวก ข.2

ตารางที่ 2.2 ขั้นตอนการทำงานของระบบไฮโดรไดนามิกซีเควนเชียลอินเจคชันอะนาลิซิสสำหรับการวิเคราะห์หาปริมาณฟอสเฟตและซิลิเกต

Step	Pump		Valve						Description
	SP1	SP2	SV1	SV2	SV3	SV4	SV5	SV6	
1	off	on	on	off	on	off	on	off	Draw up sample
2	off	on	on	off	off	on	on	off	Draw up reagent2
3	on	off	off	off	off	off	off	on	Forward the mixture to detector (for phosphate determination)
4	off	on	off	off	on	on	off	on	Draw up reagent1
5	off	on	off	on	off	on	off	on	Draw up sample
6	on	off	on	on	on	on	on	off	Forward the mixture for 15 seconds (for phosphate and silicate determination)
7	off	off	on	on	on	on	on	off	Stop the mixture to increase the reaction time of silicate to molybdate
8	on	off	on	on	on	on	on	off	Forward the mixture to detector (for phosphate and silicate determination)

เทคนิค เอช เอส ไอ เอ นี้ สามารถพัฒนาเป็นระบบสำหรับตรวจวิเคราะห์เพื่อเฝ้าระวัง (monitoring) ได้ ซึ่งอาจใช้ในกระบวนการผลิตของอุตสาหกรรมหรือในการเฝ้าระวังทางสิ่งแวดล้อมได้ง่ายและราคาไม่สูง จึงควรมีการพัฒนาต่อไป

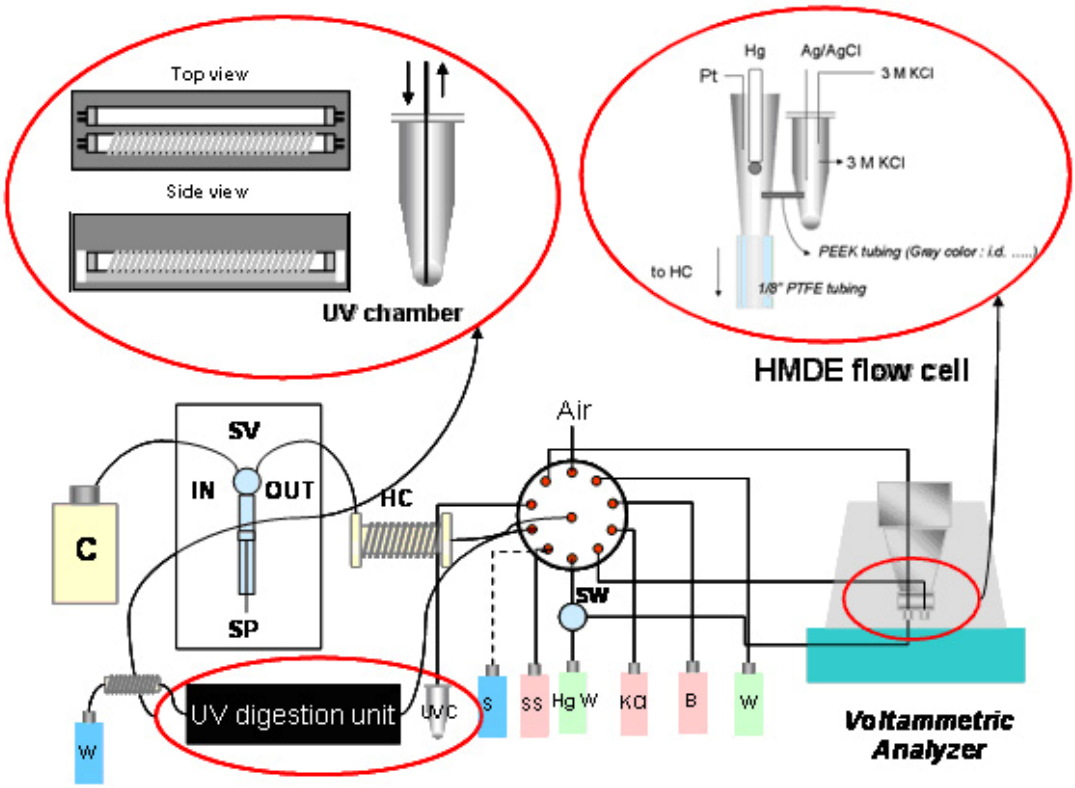
2.2 การพัฒนาซีเควนเซียลอินเจคชันแลบแอทวาล์ว

ระบบวิเคราะห์แบบ SI-Lab at valve มีลักษณะคล้ายกับ SI Lab on valve และมีลักษณะเฉพาะทางเคมีวิเคราะห์เนื่องจากตัวอย่างจะเกิด dispersion น้อย แต่ใน SI-Lab at valve ไม่จำเป็นต้องปรับเปลี่ยนชิ้นส่วนของวาล์ว ซึ่งต้องการเครื่องมือในการทำอุปกรณ์ที่มีความแม่นยำสูง เพียงแต่นำอุปกรณ์ของ Lab at valve มาต่อเข้าที่ port ของ selection valve เพื่อทำหน้าที่ตามที่ได้ออกแบบไว้ เช่น เป็นเครื่องตรวจวัด หรืออุปกรณ์ในการเตรียมตัวอย่าง เป็นต้น

ในงานวิจัยนี้ได้พัฒนาระบบ SI Lab at valve สำหรับการวิเคราะห์โลหะหนักบางชนิดด้วยเทคนิค anodic stripping voltammetry (ASV) โดยนำ voltammetric cell ที่พัฒนาขึ้นและ UV-digestion reactor มาต่อเข้าที่ port ของวาล์ว

2.2.1 การวิเคราะห์ ตะกั่ว แคดเมียม ทองแดง และสังกะสี

ระบบ SI Lab at valve สำหรับวิเคราะห์ตะกั่ว แคดเมียม ทองแดง และสังกะสี แสดงดังรูปที่ 2.7 สารละลายตัวอย่างจะถูกนำไปย่อยสลายเพื่อทำลายสารอินทรีย์ที่ UV-digestion reactor ก่อนทำการวิเคราะห์หาปริมาณโลหะโดยเทคนิค ASV ซึ่งจะได้ปริมาณโลหะโดยรวมทั้ง free form และ organic bound form ส่วนการวิเคราะห์ตัวอย่างโดยตรงโดยไม่ผ่านการย่อยสลายจะเป็นการหาปริมาณโลหะเฉพาะที่เป็น free form รายละเอียดเกี่ยวกับระบบดังกล่าวนี้ โปรดดูภาคผนวก ข.3



รูปที่ 2.7 ระบบ SI UV digestion สำหรับวิเคราะห์ตะกั่ว แคดเมียม ทองแดง และสังกะสี; C = carrier (Milli-Q water); SV = syringe valve; SP = syringe pump; HC = holding coil; SW = 3-way switching valve; UVC = UV chamber; S = sample; SS = single standard; Hg W = mercury waste; NaCl = 3 M sodium chloride; B = 3 M acetate buffer; W = waste

2.2.2 การวิเคราะห์โลหะโดยเทคนิค monosegmented flow บน Bismuth film electrode (BiFE)

เนื่องจากขั้วปรอทมีความเป็นพิษสูงแม้ว่าจะให้ศักย์ไฟฟ้าใช้งานทางด้านศักย์ลบในช่วงกว้าง จึงมีการพัฒนาขั้วไฟฟ้าชนิดใหม่ ๆ มาใช้ทดแทน ขั้วบิสมัทฟิล์มเป็นขั้วไฟฟ้าชนิดหนึ่งที่ได้รับความสนใจพัฒนากันมาก อย่างไรก็ตามการควบคุมให้ได้ผลการวิเคราะห์ที่แม่นยำ ทำซ้ำได้ดีบนขั้วบิสมัททำได้ยาก ในงานวิจัยนี้ได้ใช้เทคนิค SI- Lab at valve และ monosegmented flow เพื่อช่วยเพิ่มประสิทธิภาพในการวิเคราะห์โลหะหนักบางชนิด (แคดเมียม ตะกั่ว และสังกะสี) โดยเทคนิค ASV ทั้งนี้ได้พัฒนาเครื่องมือ SI Lab at valve และ software ที่ใช้ควบคุมขึ้นเองด้วย รายละเอียดเกี่ยวกับการพัฒนาวิธีวิเคราะห์ดังกล่าว โปรดดูภาคผนวก ก.3

2.2.3 การวิเคราะห์ แคดเมียม และตะกั่ว ในผสมภัณฑ์เซรามิก

เพื่อศึกษาวิธีการตรวจวิเคราะห์โลหะ โดยใช้ขั้วปรอทแขวนก่อนนำไปใช้ในระบบการไหล ได้พัฒนาวิธีสำหรับการหาปริมาณแคดเมียมและตะกั่วในผลิตภัณฑ์เซรามิกโดยการสกัดโลหะดังกล่าวออกมาจากผิวของผลิตภัณฑ์โดยใช้ 4 M acetic acid ตามวิธีมาตรฐาน ก่อนทำการวิเคราะห์โดยตรงในสารละลายสำหรับสกัด ด้วยเทคนิค ASV วิธีดังกล่าวนี้มีความยุ่งยากน้อยกว่าวิธีมาตรฐานที่ใช้กันในปัจจุบัน ซึ่งใช้เทคนิคอะตอมมิกแอบซอพชันสเปกโทรโฟโตเมตรี และให้ความไววิเคราะห์ที่ดีกว่า รายละเอียดโปรดดูในภาคผนวก ก.4

2.2.4 การวิเคราะห์หาปริมาณสารหนูโดยเทคนิคแคโทดิกสตริปโวลแทมเมตรี

สารหนูโดยเฉพาะสารหนูอนินทรีย์ ซึ่งมีพิษสูงจะอยู่ในรูปแอนไอออนที่สำคัญมี 2 รูปแบบ คือ อาซีไนท์(As(III)) อาซีเนต (As(V)) การทำเหมืองแร่ทำให้สารหนูถูกชะลงสู่แหล่งน้ำและปนเปื้อนเข้าสู่ห่วงโซ่อาหารได้ การวิเคราะห์สารหนูอนินทรีย์ จึงเป็นที่สนใจในงานวิจัยนี้ได้พัฒนาเทคนิคแคโทดิกสตริปปิงโวลแทมเมตรีสำหรับการหาปริมาณ As(III) และ As(V) โดยอาศัยการเกิดรีดักชันของ As(III) บนขั้วปรอทแขวนไปเป็นอาร์ซีนส่วน As(V) จะสามารถวิเคราะห์ได้หลังจากรีดิวซ์เป็น As(III) รายละเอียดเกี่ยวกับงานวิจัยนี้โปรดดู ภาคผนวก ก.5

2.2.5 การพัฒนาเทคนิค sequential extraction เพื่อศึกษาการชะโลหะจากตัวอย่างดิน

เทคนิคการสกัดเป็นลำดับขั้น (Sequential extraction) หรือ sequential leaching) ได้รับการพัฒนามาเป็นลำดับในปัจจุบันระบบ on-line extraction ได้ถูกพัฒนาขึ้น ซึ่งอาศัยระบบการไหล โดยทำการผ่านสารสกัดที่มีความแรงเพิ่มขึ้น ซึ่งสัมพันธ์กับรูปฟอร์มต่าง ๆ ของโลหะที่อยู่ในตัวอย่างดิน (หรือตัวอย่างของแข็งอื่น ๆ) และทำการตรวจวัดอย่างต่อเนื่อง ซึ่งมักใช้เทคนิคการวิเคราะห์ที่ทันสมัยเช่น ICP-MS หรือ ICP-AES ซึ่งสามารถตรวจวิเคราะห์โลหะหลายชนิดพร้อมกัน โดยมี sensitivity สูง ในงานวิจัยนี้จึงสนใจพัฒนาวิธีการสกัดแบบลำดับขั้นแบบออนไลน์ และตรวจวัดด้วยเทคนิคโวลแทมเมตรี โดยในเบื้องต้นได้พัฒนาวิธีตรวจวิเคราะห์ไอออนของโลหะโดยโวลแทมเมตรี ดังหัวข้อ 2.2.1-2.2.4 และจากการสกัดตัวอย่างดินมาตรฐาน (certified reference material) ด้วย

สารละลายตามลำดับขั้นตามมาตรฐานของ EU โดยใช้สารละลายตามลำดับดังนี้: 0.05 M $Ca(NO_3)_2$, 0.4 M CH_3COOH, 0.1 M $NH_2OH.HCl$ pH 3.6 และ 0.1 M $(NH_4)_2C_2O_4$ พบว่าสามารถตรวจวิเคราะห์ไอออนของโลหะบางชนิดได้ ดังตารางที่ 2.3 ซึ่งจะเกี่ยวข้องกับรูปฟอร์มต่างๆ ทางเคมีของโลหะในดิน การศึกษาดังกล่าวนี้ควรมีการพัฒนาต่อไปและทดลองกับตัวอย่างจำนวนมากขึ้น

ตารางที่ 2.3 ปริมาณ (mg/kg) ของ Zn, Cd, Pb and Cu ที่ถูกสกัดออกมาในสารละลายแต่ละขั้นเทียบกับปริมาณรวมของ CRM certified value

Element	Amounts of metals in fractions (mg/kg)							
	$Ca(NO_3)_2$	CH_3COOH	$NH_2OH.HCl$	$(NH_4)_2C_2O_4$	Residual	Sum	CRM value	% Recovery
Zn	27.0	62.9	40.3	49.3	106.3	285.8	350.4	81.6
Cd	1.1	15.4	4.8	N.D.	24.0	45.3	41.7	108.5
Pb	4.7	92.3	37.3	11.8	812.8	958.9	1162	82.5
Cu	N.D.	N.D.	N.D.	14.2	89.5	103.7	114	91.0

2.3 การพัฒนาเทคนิคสต๊อปโฟลอินเจคชันอะนาลิซิส

เทคนิคสต๊อปโฟลอินเจคชันอะนาลิซิส (stopped flow injection analysis, sFIA) เป็นเทคนิคที่มีการหยุดการไหลเพื่อช่วยเพิ่มเวลาให้สารตัวอย่างและรีเอเจนต์ ซึ่งผสมกันแล้วได้ทำปฏิกิริยากัน ทำให้เกิดสารผลิตผลมากขึ้นนั่นคือ สามารถตรวจวัดได้โดยมีความไววิเคราะห์สูงขึ้น เครื่องมือของระบบการวิเคราะห์ เอส เอฟ ไอ เอ นี้จะต้องสามารถตั้งเวลาตั้งแต่ฉีดสารเข้าสู่ระบบจนสารไหลไปถึงจุดที่จะหยุดการไหล (travel time) และช่วงเวลาที่หยุดไหล (stop time) ได้ ซึ่งปกติมักจะหยุดการไหลขณะสารที่ฉีดไปถึงจุดตรวจวัดเพื่อให้สามารถติดตามการดำเนินไปของปฏิกิริยาได้ สัญญาณที่บันทึกได้สามารถนำมาคำนวณอัตราการปฏิกิริยา และอาจใช้แยกแยะเพื่อหาปริมาณสารสองชนิดในเวลาเดียวกันได้ (kinetic discrimination) เทคนิคนี้นิยมใช้วิเคราะห์สารที่เกิดปฏิกิริยาได้ค่อนข้างช้า ซึ่งนอกจากจะช่วยเพิ่มความไววิเคราะห์แล้วยังช่วยประหยัดสารเคมีได้ด้วย เนื่องจากสารรีเอเจนต์ไม่ได้ไหลตลอดเวลาทำให้ใช้น้อยลง ในงานวิจัยนี้ได้พัฒนาเครื่องมือสำหรับเทคนิค เอส เอฟ ไอ เอ ขึ้นใหม่ โดยใช้ solenoid valve ที่ควบคุมด้วยไมโครคอนโทรลเลอร์ หรือ คอมพิวเตอร์ เพื่อให้ทำงานได้สะดวกมากขึ้นและได้พัฒนาวิธีวิเคราะห์ไอออนบางตัวที่เกี่ยวข้องกับการเกษตรกรรม คือ ฟอสเฟต และคลอเรต

2.3.1. การวิเคราะห์หาปริมาณฟอสเฟต

ได้ใช้เทคนิคเอส เอฟ ไอ เอ ที่มีการหยุดไหลที่จุดตรวจวัดสำหรับการหาปริมาณฟอสเฟต โดยอาศัยการเกิดปฏิกิริยากับโมลิบเดตและกรดแอสคอร์บิกเกิดเป็นโมลิบดีนัมบลู โดยอัตราการเปลี่ยนแปลงของสัญญาณที่บันทึกได้จะสัมพันธ์กับอัตราการเกิดปฏิกิริยา ซึ่งแปรผันโดยตรงกับความเข้มข้นของฟอสเฟต ซึ่งสามารถนำมาสร้างกราฟมาตรฐานเพื่อหาปริมาณฟอสเฟตในตัวอย่างดินและปุ๋ยได้ การใช้เทคนิค เอส เอฟ ไอ เอ ช่วยลดการรบกวนจาก ซิลิเกต ซึ่งเกิดปฏิกิริยาได้ช้ากว่าได้ และไม่มีผลรบกวนจากสารที่มีสีและอนุภาคคอลลอยล์ ที่มีอยู่ในสารตัวอย่าง เนื่องจากการดูดกลืนแสงหรือกระเจิงแสง เนื่องจากสารเหล่านี้จะไม่เปลี่ยนแปลงไปตามเวลา รายละเอียดเกี่ยวกับเรื่องนี้โปรดดูเพิ่มเติมในภาคผนวก ก.6

2.3.2 การวิเคราะห์หาปริมาณคลอเรต

ได้มีการใช้สารคลอเรตในการเร่งการออกดอกผลของลำไยกันมากของเกษตรกรผู้ปลูกลำไยซึ่งถือว่าเป็นพืชเศรษฐกิจในภาคเหนือ เช่น จังหวัดเชียงใหม่ ลำพูน เป็นต้น การวิเคราะห์หาปริมาณคลอเรตในดินจึงมีความจำเป็น การใช้เทคนิค เอส เอฟ ไอ เอ ช่วยเพิ่มความไววิเคราะห์ได้ จากงานวิจัยที่ผ่านมาก่อนหน้านี้ ผู้วิจัยได้เสนอเทคนิคการหยุดการไหลใน reactor ซึ่งมีการเพิ่มอุณหภูมิ เพื่อช่วยเร่งการเกิดปฏิกิริยาระหว่างคลอเรตกับไอโอไดด์ในสภาวะกรด ก่อนการตรวจวัดโดยเทคนิคแอมเพอโรเมตรี [17] แต่ยังมีข้อเสีย คือต้องมีการเพิ่มอุณหภูมิจึงจะเกิดสารผลิตผลมากพอที่จะตรวจวัดได้ ในงานวิจัยนี้ได้ใช้เทคนิค เอส เอฟ ไอ เอ ร่วมกับการตรวจวัดแบบสเปกโทรโฟโตเมตรี เพื่อวิเคราะห์คลอเรตซึ่งช่วยเพิ่มความไววิเคราะห์ได้ และไม่จำเป็นต้องใช้ตัวควบคุมอุณหภูมิอีกต่อไป รายละเอียดเกี่ยวกับเรื่องนี้โปรดดูภาคผนวก ก. 7.

เทคนิคเอส เอฟ ไอ เอ นี้มีศักยภาพมากที่จะพัฒนาต่อไปเพื่อให้ได้เครื่องมือที่กะทัดรัดมากขึ้นและสามารถนำไปใช้วิเคราะห์ในภาคสนาม หรือ ห้องปฏิบัติการ

2.4 การพัฒนาอุปกรณ์ของระบบ เอฟ ไอ เอ และวิธีการวิเคราะห์ไอออนบางชนิด

เทคนิค เอฟ ไอ เอ แบบดั้งเดิมมีความน่าสนใจที่จะพัฒนาเป็นเครื่องมือหรือระบบวิเคราะห์ทางเคมีที่มีความคุ้มค่า (cost effective) เนื่องจากใช้อุปกรณ์ที่มีความซับซ้อนน้อยและราคาถูก โดยให้ความไววิเคราะห์และความแม่นยำสูง รวมทั้งสามารถตรวจวัด

ได้รวดเร็ว มนงานวิจัยนี้จึงได้พัฒนาอุปกรณ์ของระบบเอฟ ไอ เอ ขึ้น เองบางส่วน เพื่อช่วยเพิ่มประสิทธิภาพของการวิเคราะห์และทำให้เครื่องมือมีราคาต่ำลง

2.4.1 การพัฒนาระบบบันทึกสัญญาณ

ระบบบันทึกสัญญาณของเอฟ ไอ เอ ในช่วงแรกจะใช้เครื่องบันทึกสัญญาณบนกระดาษ (chart recorder) ซึ่งมีราคาสูง และสิ้นเปลืองกระดาษบันทึก ปัจจุบันจะบันทึกสัญญาณด้วยคอมพิวเตอร์ซึ่งระบบนี้เป็นส่วนหนึ่งที่ทำให้เครื่องเอฟ ไอ เอ ทางการค้ามีราคาสูง ระบบบันทึกสัญญาณด้วยคอมพิวเตอร์ที่มีจำหน่าย เช่น บริษัท eDAQ เป็นต้น จะมีราคาประมาณ 2-3 แสนบาท โดยจะมี software สำหรับวิเคราะห์สัญญาณมาด้วย ในงานวิจัยนี้จึงได้พัฒนาระบบบันทึกสัญญาณด้วยคอมพิวเตอร์ขึ้นเอง โดยต่อยอดจากระบบที่เคยพัฒนาไว้ซึ่งใช้ Microsoft excel เป็นโปรแกรมเก็บข้อมูล [25] ได้สร้างอุปกรณ์ขยายสัญญาณอะนาลอกเป็นดิจิตอลและการเชื่อมต่อกับคอมพิวเตอร์และพัฒนาโปรแกรมบันทึกสัญญาณใหม่ โดยใช้ Visual Basic ซึ่งจะทำให้ได้โปรแกรมที่มีขนาดเล็กลงและมีประสิทธิภาพสูงขึ้นเป็นแบบ stand alone อุปกรณ์และซอฟแวร์ ที่พัฒนาขึ้นแสดงดังรูปที่ 2.8

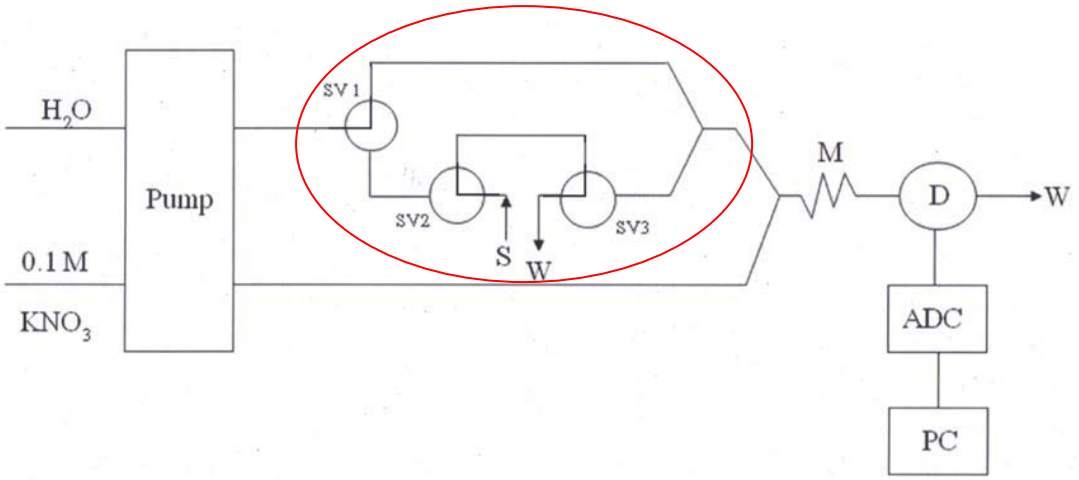
(A) (B)

รูปที่ 2.8 ระบบบันทึกสัญญาณ (A) hardware และ (B) software

2.4.2 ระบบฉีดสารแบบใหม่ที่ใช้โซลีนอยด์วาล์ว (solenoid valve)

การฉีดสารเข้าสู่ระบบ เอฟ ไอ เอ มักใช้ six port injection valve ซึ่งมีราคาค่อนข้างสูง โดยเฉพาะอย่างยิ่งถ้าเป็นระบบฉีดสารอัตโนมัติ ในงานวิจัยนี้จึงได้ออกแบบระบบฉีดสารโดยใช้โซลีนอยด์วาล์ว ซึ่งมีราคาถูกกว่าและสามารถควบคุมได้ด้วยการปิด/

เปิด การจ่ายไฟฟ้าให้แก่วาล์ว ระบบวาล์วที่ออกแบบแสดงดังรูปที่ 2.9 ซึ่งจากการทดสอบการใช้งานพบว่าให้ผลการวิเคราะห์ที่แม่นยำดี



รูปที่ 2.9 ระบบฉีดสารโดยใช้โซลีนอยด์วาล์ว

2.4.3 การพัฒนาวิธี เอฟ ไอ เอ โพเทนชิออเมตรีสำหรับการหาปริมาณคลอไรด์

โดยผู้วิจัยได้ใช้อุปกรณ์ที่พัฒนาขึ้นเอง ระบบวาล์วฉีดสารดังหัวข้อ 2.4.2 ขั้วไฟฟ้าเลือกจำเพาะไอออนคลอไรด์ potentiometric flow through cell และระบบบันทึกสัญญาณดังหัวข้อ 2.4.1 สามารถพัฒนาวิธีวิเคราะห์คลอไรด์ไอออน ซึ่งสามารถประยุกต์ในการวิเคราะห์ตัวอย่างมวลรวมและน้ำยาสำหรับผสมคอนกรีตได้ รายละเอียดเกี่ยวกับเรื่องนี้โปรดดูภาคผนวก ก.8 วิธีการวิเคราะห์นี้ให้ผลการวิเคราะห์สอดคล้องกับวิธีมาตรฐานแต่มีประสิทธิภาพดีกว่า เช่น มีความแม่นยำ และขีดจำกัดต่ำสุดในการตรวจวัดดีกว่า เป็นต้น เครื่องมือดังกล่าวนี้กำลังพัฒนาเพิ่มเติม และเผยแพร่ให้นำไปใช้ในอุตสาหกรรม

2.4.4 ระบบเอฟไอเอคอนดักโตเมตรี สำหรับการวิเคราะห์หาปริมาณแอมโมเนียมไอออน

ระบบดังกล่าวนี้อาศัยการเปลี่ยนแอมโมนียมไอออนไปเป็นแก๊สแอมโมเนีย โดยใช้เยื่อพรุนสำหรับแพร่ผ่านแก๊ส (gas diffusion membrane) ก่อนจะละลายแก๊ส ลงในน้ำและวัดการเปลี่ยนแปลงค่าความนำไฟฟ้าอย่างต่อเนื่อง ได้พัฒนาหน่วยแพร่ผ่านแก๊สและ conductometric flow cell ขึ้นเอง และประยุกต์เทคนิคดังกล่าวนี้สำหรับการวิเคราะห์หาปริมาณ Kjeldahl nitrogen หรือโปรตีนโดยรวม ซึ่งปกติต้องวิเคราะห์โดยการกลั่นและไทเทรต ซึ่งใช้สารเคมีและเสียเวลามาก ระบบที่พัฒนาขึ้นนี้ช่วยประหยัดสารเคมี และ

สามารถวิเคราะห์ได้ 30 ตัวอย่างต่อชั่วโมง รายละเอียดเกี่ยวกับเรื่องนี้ โปรดดูภาคผนวก ก.9 ระบบเครื่องมือดังกล่าวนี้กำลังพัฒนาเพิ่มเติมเพื่อให้สามารถนำไปใช้ในอุตสาหกรรมได้ และได้ใช้ในการเรียนการสอนด้วยดังภาคผนวก ค.1

2.4.5 การพัฒนา เอฟ ไอ เอ แอมเพอโรเมตรีสำหรับการวิเคราะห์กรดแอสคอร์บิก

จากงานวิจัยก่อนหน้านี้ [25] ได้พัฒนาเครื่องแอมเพอโรเมตรีขึ้นเอง และใช้สำหรับเป็นเครื่องตรวจวัดในระบบเอฟ ไอ เอ สำหรับการวิเคราะห์วิตามินซี หรือ กรดแอสคอร์บิก ในที่นี้ได้พัฒนาเพิ่มเติมในส่วนของการแยกสารโดยใช้ dialysis membrane เพื่อแยกกรดแอสคอร์บิกออกจากองค์ประกอบอื่น ๆ ในตัวอย่างและเป็นการเจือจางตัวอย่างในระบบก่อนการวิเคราะห์ โดยประยุกต์สำหรับการวิเคราะห์ยาเม็ดวิตามินซีและน้ำผลไม้ รายละเอียดโปรดดูในภาคผนวก ก.10

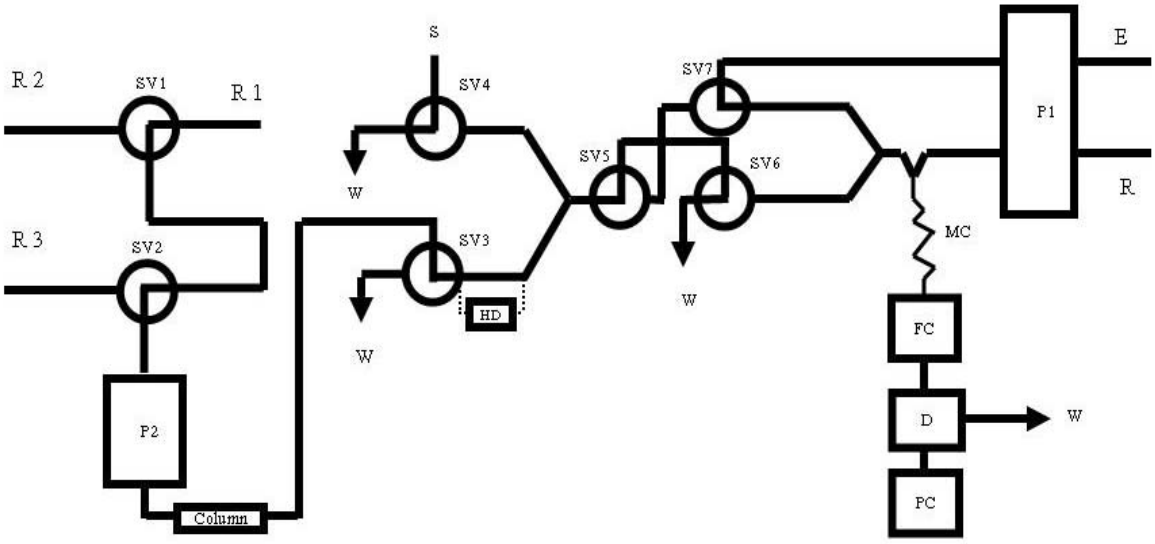
2.4.6 ระบบเอฟ ไอ เอ แอมเพอโรเมตรี สำหรับการหาปริมาณฟอสเฟต

การวิเคราะห์ฟอสเฟตด้วยวิธีทางสเปกโทรโฟโตเมตรีมีการรบกวนจากซิลิเกต และจากสารมีสีและอนุภาคคอลลอยด์ รวมทั้งจากความแตกต่างของความหนาแน่นของสารละลายซึ่งจะเกิด Schileren effect หรือ reflextive index effect ในงานวิจัยนี้ จึงได้พัฒนาวิธี เอฟ ไอ เอ แอมเพอโรเมตรี เพื่อแก้ปัญหาดังกล่าว ทำให้สามารถวิเคราะห์ฟอสเฟตในตัวอย่างดินได้สะดวกมากขึ้น รวมทั้งได้พัฒนาวิธีแบบใหม่ที่มีประสิทธิภาพกว่าเดิมในการสกัดฟอสเฟต จากตัวอย่างดินด้วย รายละเอียดโปรดดูภาคผนวก ก.11

2.4.7 การพัฒนา on-line sequential extraction สำหรับการศึกษาการชะฟอสเฟตจากดิน

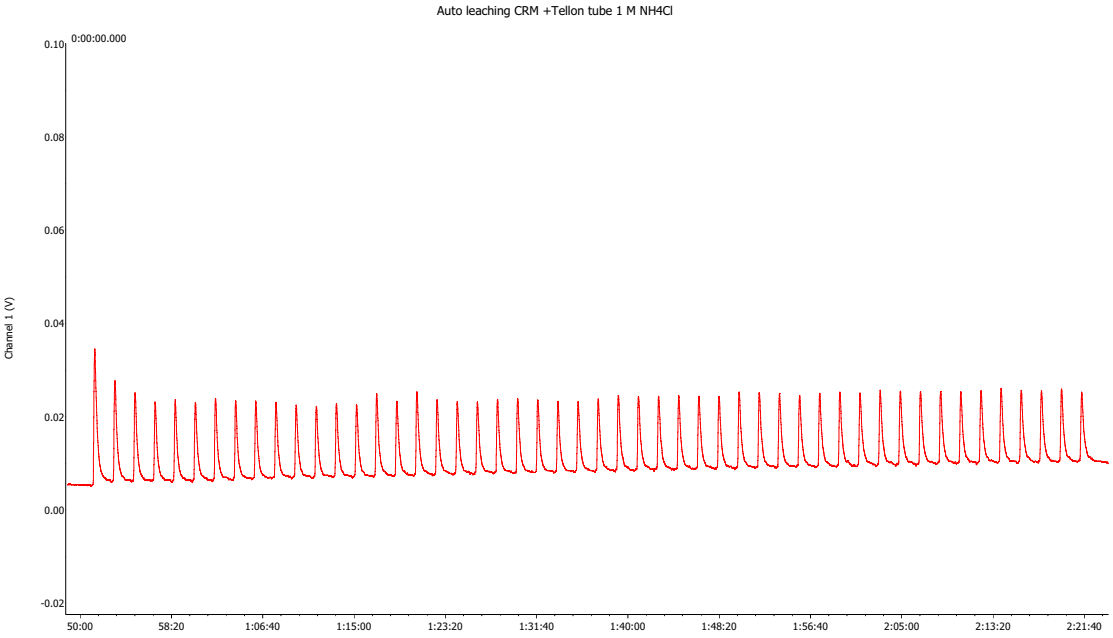
เนื่องจากระบบเอฟ ไอ เอ แอมเพอโรเมตรี สำหรับวิเคราะห์ฟอสเฟต ดังหัวข้อ 2.4.6 สามารถใช้วิเคราะห์ตัวอย่างดินได้ดีโดยทนต่อสารรบกวนต่าง ๆ ได้ดีกว่าระบบที่ตรวจวัดด้วยเทคนิคสเปกโทรโฟโตเมตรีมาก จึงได้นำมาพัฒนาต่อไปเป็นระบบการตรวจวัดสำหรับการสกัดอย่างเป็นลำดับขั้นแบบออนไลน์ เพื่อศึกษาการชะฟอสเฟตที่อยู่ในรูปฟอร์มต่าง ๆ ในตัวอย่างดิน [26] โดยออกแบบระบบดังแสดงในรูปที่ 2.10 และทำการชะฟอสเฟตจากตัวอย่างดินที่บรรจุอยู่ในคอลัมน์ โดยใช้สารละลายสำหรับการสกัดตามลำดับดังนี้ 1.0 M NH_4Cl (R1), 0.1 M NaOH (R2) และ 0.5 M HCl (R3) ซึ่งจะ

เกี่ยวข้องกับฟอสฟอรัสในรูป exchangeable, organic bound และ carbonate bound ตามลำดับ

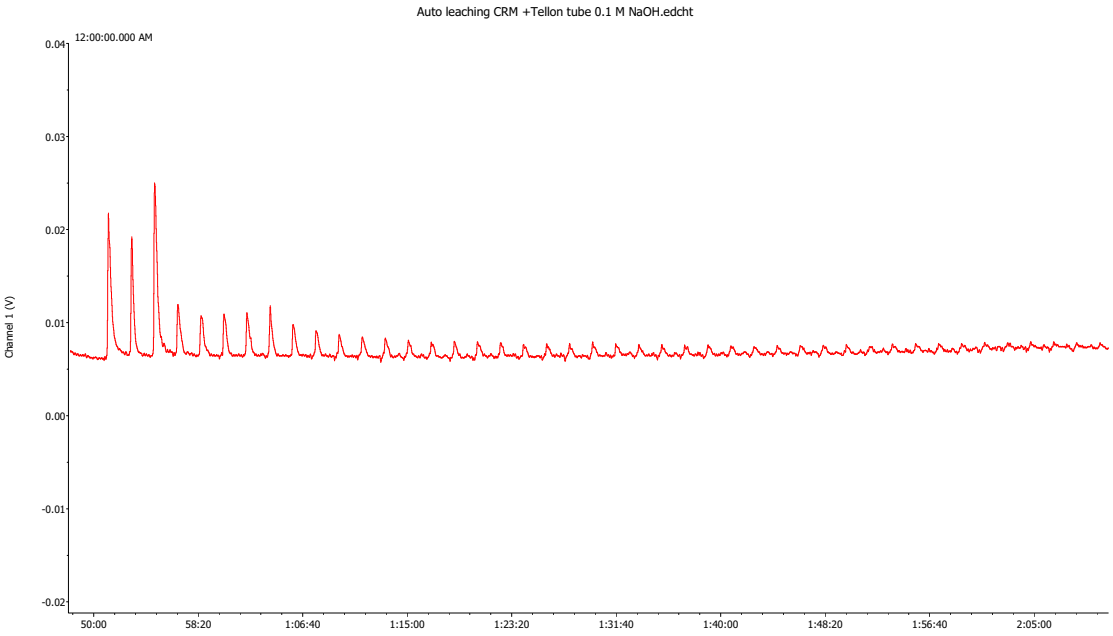


รูปที่ 2.10 ระบบ on-line sequential extraction สำหรับการศึกษาการชะฟอสเฟตจากดิน; E = 0.1 M potassium chloride, R = 0.5 % w/v acidic molybdate, SV1 – SV7 = solenoid valves, S = standard/sample, P1-P2 = peristaltic pump, I = injection valve, MC = mixing coil, HD = Holding coil, FC = electrochemical flow through cell, W = waste, D = potentiostat/amperometric detector, PC = personal computer.

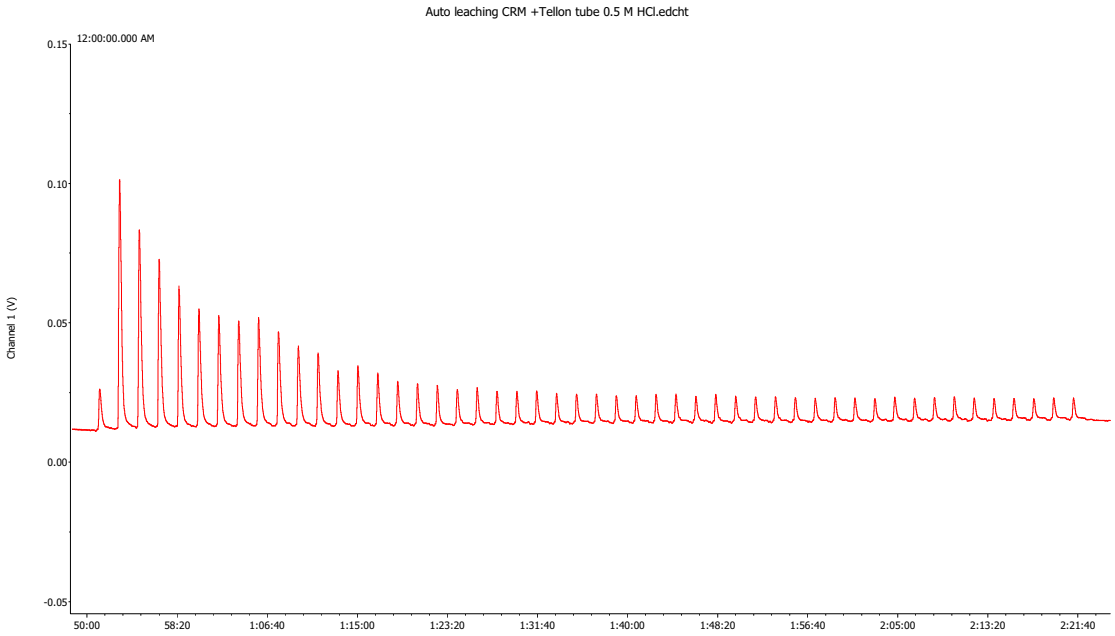
ซึ่งจะให้ extraction profiles ที่เป็น FIA peaks ดังรูป 2.11-2.13 และสามารถนำมาสร้าง sequential extraction profiles ดังแสดงในรูปที่ 2.14 และปริมาณฟอสเฟตใน fraction ต่างๆ ที่สกัดออกมาได้ แสดงดังตารางที่ 2.4



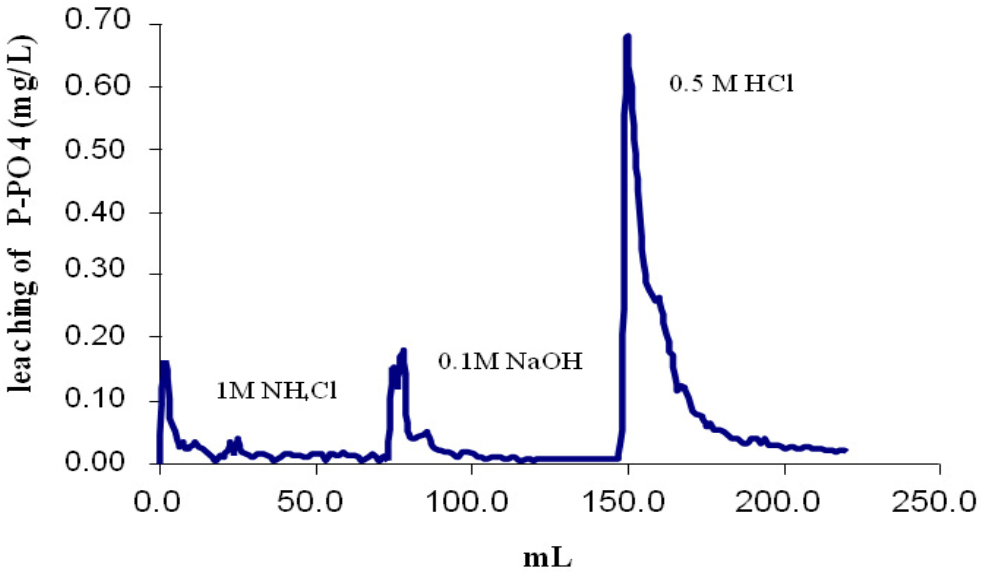
รูปที่ 2.11 Extraction profile of phosphate ในตัวอย่างดิน SRM 2711 เมื่อใช้ 1 M NH_4Cl เป็นสารสกัด



รูปที่ 2.12 Extraction profile of phosphate ในตัวอย่างดิน SRM 2711 เมื่อใช้ 0.1 M NaOH เป็นสารสกัด



รูปที่ 2.13 Extraction profile of phosphate ในตัวอย่างดิน SRM 2711 เมื่อใช้ 0.5 M HCl เป็นสารสกัด



รูปที่ 2.14 Sequential extraction profiles of phosphate in SRM 2711 by 1 M NH_4Cl, 0.1 M NaOH and 0.5 M HCl using the continuous flow system

ตารางที่ 2.4 ปริมาณฟอสเฟตที่สกัดได้ในแต่ละส่วนโดย sequential extraction method

Sample	Phosphorus ($mgKg^{-1}$ P-PO_4) from sequential extraction (mean ± S.D.)				
	NH_4Cl fraction	NaOH fraction	HCl fraction	Residue fraction	Total Phosphorus
1	273.5±3.4	241.9±9.0	744.1±22.3	113.6±1.5	1373.1±9.4
2	332.0±1.5	230.9±1.5	865.4±6.0	114.8±1.6	1543.1±2.2
3	255.1±4.8	250.7±3.6	729.2±17.0	72.6±1.8	1307.6±6.93
4	341.4±1.7	191.2±3.0	1078.1±5.6	105.9±0.6	1716.7±2.2
5	383.9±2.9	154.5±0.8	1336.8±24.0	108.8±0.4	1984.0±11.4
6	58.5±8.0	228.4±0.6	323.8±12.5	99.1±5.5	709.8±5.0
7	237.1±10.1	263.3±3.8	1002.4±5.2	115.0±0.5	1617.8±4.0
8	N.D.	58.0±1.6	37.2±3.2	104.4±0.8	199.5±1.4
9	256.6±9.1	429.3±4.1	1543.5±35.7	146.4±3.0	2375.8±15.4
10	213.4±1.8	482.3±5.9	1561.6±16.2	143.1±2.0	2400.3±6.8
11	N.D.	8.5±0.9	14.6±1.1	24.4±0.5	47.5±0.5
12	N.D.	43.9±0.2	29.2±0.3	80.7±1.1	153.8±0.5
13	N.D.	42.6±0.4	10.6±0.1	44.0±0.4	97.2±0.2
14	N.D.	16.6±0.5	33.3±0.3	134.6±1.9	184.6±0.9
15	N.D.	30.0±0.3	58.1±1.2	4.2±1.1	92.3±0.6
16	N.D.	125.85±2.75	173.88±1.46	264.7±4.5	564.4±1.9
17	N.D.	155.21±1.33	105.32±0.89	133.2±1.1	393.8±0.6
SRM *	49.9±5.6	54.91±1.31	418.48±7.84	295.0±0.7	818.2±15.5

*SRM 2711 Certified value 860 ± 70 $mgKg^{-1}$, N.D. = not detected

งานวิจัยดังกล่าวนี้กำลังอยู่ในระหว่างจัดเตรียมต้นฉบับเพื่อตีพิมพ์ และจะดำเนินการศึกษาเพิ่มเติมต่อไปอีก เนื่องจากเป็นเทคนิคใหม่ที่จะใช้ศึกษาเกี่ยวกับ mobility และ bioavailability ของฟอสเฟต ซึ่งสัมพันธ์กับรูปฟอร์มต่าง ๆ ของฟอสเฟตในดินชนิดต่าง ๆ รวมทั้งการศึกษาการจัดการดินที่ปนเปื้อนโลหะหนัก (remediation) ด้วย

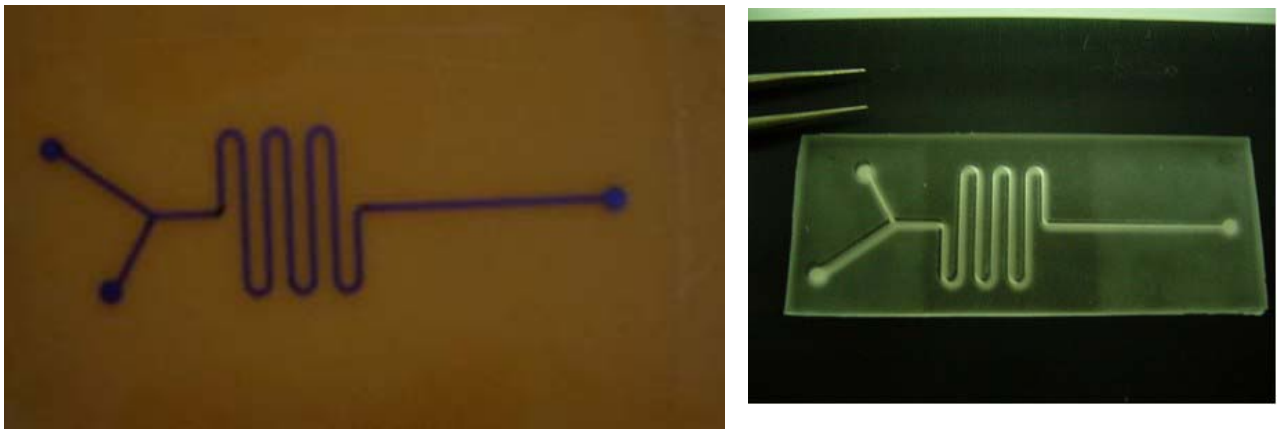
2.5 การพัฒนาเกี่ยวกับระบบวิเคราะห์แบบลดขนาดไมโครฟลูอิดิก

ปัจจุบันมีการพัฒนาไมโครฟลูอิดิกหรือแลปออนอะชิพกันมาก เนื่องจากมีการตื่นตัวทางด้านนาโนเทคโนโลยี ในงานวิจัยนี้จึงได้เริ่มพัฒนาระบบไมโครฟลูอิดิกในแนวราคาถูกหรือ มีความคุ้มค่าโดยพัฒนาวิธีการสร้างชิพโดยใช้วิธีการที่ไม่ซับซ้อน และใช้เทคโนโลยีไม่สูง รวมทั้งระบบตรวจวัดบนชิพที่มีขนาดเล็กที่ใช้คัลเลอริมิเตอร์อย่างง่าย

2.5.1 การสร้างไมโครฟลูอิดิกชิพด้วย printed circuit board

ได้ใช้วิธี photolithography ที่ใช้ในการผลิตลายวงจรอิเล็กทรอนิกส์ในการสร้างต้นแบบ (template) สำหรับการสร้าง PDMS chip โดยการลอกแบบด้วย พอลิเมอร์ polydimethylsiloxane และนำชิพที่ได้มาประกอบเข้ากับแผ่นอะคริลิกเพื่อให้เกิดเป็น channel สำหรับที่จะนำสารเข้าสู่ระบบ และต่อเข้ากับอุปกรณ์ตรวจวัดเพื่อใช้งานในการวิเคราะห์ต่อไป ลักษณะของ template และ microchip ที่สร้างขึ้นแสดงดังรูปที่ 2.15 งานดังกล่าวนี้ยังอยู่ในระหว่างการพัฒนาต่อไป

(A) (B)



รูปที่ 2.15 ไมโครฟลูอิดิกชิพ (A) template และ (B) PDMS chip

2.5.2 การสร้างไมโครฟลูอิดิกชิพโดยใช้เลเซอร์

โดยร่วมมือกับผู้ช่วยศาสตราจารย์ ดร. นภาพร ยังวิเศษ (มหาวิทยาลัยธรรมศาสตร์) ได้สร้างไมโครฟลูอิดิกชิพด้วยอีกเทคนิคหนึ่ง คือ การเซาะร่องด้วยเลเซอร์ (Laser cutting/engraving) ซึ่งสามารถออกแบบรูปแบบ channel และเขียนลงไปโดยตรงบนพอลิเมอร์ชนิด polymethylmethacrylate (PMMA) โดยตรง ได้ทดสอบการใช้งานในการ

วิเคราะห์เหล็ก [23] และเอธานอล [27] งานวิจัยดังกล่าวนี้ยังอยู่ในระหว่างการศึกษาพัฒนาเพิ่มเติม รวมทั้งการใช้คัลเลอริมิเตอร์ขนาดเล็กเป็นอุปกรณ์ตรวจวัด ดังข้อ 2.5.3

2.5.3 คัลเลอริมิเตอร์ขนาดเล็กที่ใช้ LED-LDR

เนื่องจากการตรวจวัดบนไมโครชิพยังต้องใช้เครื่องมือที่มีขนาดใหญ่และราคาแพง เช่น Laser induced fluorescence จึงได้สนใจพัฒนาเครื่องตรวจวัดขนาดเล็กที่สามารถติดตั้งเข้ากับชิพได้ โดยในเบื้องต้นได้พัฒนาเครื่องคัลเลอริมิเตอร์ที่ใช้ ไดโอดเปล่งแสงเป็นแหล่งกำเนิดแสง และความต้านทานเปลี่ยนค่าตามแสง (Light dependent resistor (LDR)) เป็นตัววัดแสงโดยได้ทดสอบการใช้งานเครื่องมือดังกล่าวกับการวิเคราะห์ในระบบระบบไมโครฟลูอิดิก ในการวิเคราะห์เหล็กดังรายละเอียดในภาคผนวก ข.4

ระบบไมโครฟลูอิดิกนี้ควรจะมีการพัฒนาต่อไปเพื่อให้สามารถใช้งานได้จริง รวมทั้งอาจพัฒนาระบบการแยกสารบนไมโครชิพด้วย เช่น การใช้เทคนิค capillary electrophoresis บนชิพ เป็นต้น

เอกสารอ้างอิง

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2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงพาณิชย์

ต้นแบบเครื่องมือที่พัฒนาขึ้นและวิธีการวิเคราะห์ที่เกี่ยวข้องอาจต่อยอดไปสู่การใช้ประโยชน์ในเชิงพาณิชย์ได้ เช่น ระบบเอฟ ไอ เอ สำหรับการหาปริมาณคลอไรด์และโปรตีน ซึ่งยังอยู่ในระหว่างปรับปรุงเพิ่มเติมและเผยแพร่แก่ผู้เกี่ยวข้อง

- เชิงวิชาการ

เครื่องมือ อุปกรณ์และวิธีการวิเคราะห์ที่พัฒนาขึ้น ได้ใช้ประโยชน์ในการเรียนปฏิบัติการเคมีวิเคราะห์เชิงไฟฟ้า ของนักศึกษาระดับบัณฑิตศึกษา ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ (ดูภาคผนวก ค)

3. อื่น ๆ

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ภาคผนวก ก

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Hydrodynamic Sequential Injection Spectrophotometric System for Determination of Manganese in Soil

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ABSTRACT A novel hydrodynamic sequential injection (HSI) spectrophotometric system for determination of manganese was developed. It is based on the complexation of Mn(II) with formaldoxime in basic solution ($pH \geq 10$) to produce product that could be monitored spectrophotometrically at 450 nm. Based on the HSI concept, both sample and reagents were aspirated through solenoid valves to fill a defined volumes conduit between 3-way connectors connected in series, forming stacked zones of solutions similar to those in normal SI. The concept was successfully demonstrated for manganese determination. A linear calibration graph over a range of 0.5 to 30 mg L^{-1} Mn(II) with a detection limit of 0.2 mg L^{-1} was obtained. Relative standard deviations for 11 replicated injections of 5 and 20 mg Mn L^{-1} were 5.6% and 2.4%, respectively. A sample throughput of 45 h^{-1} was achieved. The results from investigation of exchangeable manganese in soil samples by the developed method were found to be in good agreement with the results obtained by a batch spectrophotometric method, despite the proposed system employed simpler and more cost-effective devices/instruments, had higher degrees of automation with full microcontroller control of the operation, and consumed smaller amounts of chemicals (250 μL each of hydroxylamine, sample, and formaldoxime solutions and 2.5 mL of buffer carrier solution per operation cycle).

KEYWORDS formaldoxime, hydrodynamic sequential injection, manganese, soil, spectrophotometric

INTRODUCTION

Manganese is the one of essential micronutrients for plant growing. Manganese in soil may appear in different forms including dissolved, exchangeable, reducible, organic bound, and residual.[1] Dissolved and exchangeable manganese are an important form readily available for plant uptake.[2] Manganese deficiency in plants is shown by discoloration on lower leaves,[3] whereas the excess accumulation of manganese gives rise

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to the abnormal loss of leaves.[4] Consequently, various methods for soil analysis have been developed for quantitation of manganese.

Some official methods are based on spectrophotometric technique using periodate as a reagent and an atomic absorption spectrophotometric technique.[2] These techniques provide good accuracy and sensitivity, but consume large amounts of chemicals, take a long time, or require expensive instruments. Moreover, elevated temperature is required for the redox reaction of manganese and periodate to produce a colored product. For analysis of a large number of samples, flow-based methods that provide higher degrees of automation and better analytical features have been developed, such as flow injection (FI)[5–21] and sequential injection (SI).[22,23] FI technique, which operates in continuous flow mode, usually provides higher sample throughput, but with higher consumption of solutions. It is usually assembled requiring relatively simpler and lower cost devices. Multicommutation using a network of 3-way solenoid valves can make the FI system simpler and more versatile.[16,24] On the other hand, SI technique usually requires higher cost devices, such as high-quality syringe pump to meter precise volumes of solutions and a rotary selection valve for selection of different solutions, all under computer control. It is operated under programmable flow, thus minimizes volumes of sample, reagents, and waste. In this work, we introduce a new solution management procedure, the operation of which is similar to SI, therefore sample and reagents are sequentially aspirated to form stacked zones in a tubing while the carrier flow is halted. When the carrier flow starts to push the zones to a detector, zone penetration occurs in the same way as in SI, leading to mixing and the reaction to proceed. The introduction of solutions into tubing can be achieved by hydrodynamic flow concept[25] with use of cost-effective devices, solenoid valves and 3-way connectors, leading to the name hydrodynamic sequential injection analysis (HSIA).[26,27]

The two main detection techniques, visible spectrophotometric and atomic spectrophotometric, are usually employed in flow-based system for determination of manganese. For analysis of samples with low concentration of manganese ($\mu g\,L^{-1}$ to sub-mg L^{-1} levels) (e.g., freshwater and seawater), catalytic spectrophotometry employing oxidation reaction of periodate and various organic compounds with manganese acting as a catalyst[11–14,17] or atomic absorption/emission spectrophotometry with an on-line sorption column packing with 8-hydroxyquinoline,[21] iminodiacetate resin,[13] silica gel functionalized with 1,5-bis(di-2-pyridyl)methylene thiocarbohydrazide,[10] thiol resin,[9] or anion exchange resin[7] have been developed. Spectrophotometric detection based on oxidative conversion of Mn(II) to permanganate[5, 6, 16, 20, 22] or complex formation of Mn(II) with 4-(2-pyridylazo) resorcinol (PAR)[15] or formaldoxime[23] is widely used for manganese determination at mg L^{-1} level, such as in plant extract, wastewater effluent, and soil. The FI potentiometric method was also reported for determination of manganese in soil.[4]

In this work, we would like to propose a HSI spectrophotometric method for determination of manganese in soil. It is based on the reaction of Mn(II) and formaldoxime in basic solution to form the red-brown complex, which has a maximum absorption at 450 nm. By HSI concept, sample and reagents can be sequentially aspirated to place in a defined volumes conduit through solenoid valves and 3-way connectors under programmable control using a home-made controller unit. A linear calibration graph in range 0.5–30 mg Mn L^{-1} and detection limit of 0.2 mg Mn L^{-1} were obtained. Various advantages such as simple and cost-effective instruments used, rapid analysis with low chemical consumption, and high degrees of automation were gained.

MATERIALS AND METHODS

HSI Manifold

A schematic diagram of the proposed HSI system is illustrated in Fig. 1. The system consisted of a two-channel peristaltic pump (Alitea, Medina, WA, USA), 6 solenoid valves (Cole-Parmer, USA), a simple spectrophotometer (Spectronic 21, Bausch&Lomb, USA) equipped with a 10-mm pathlength flow-through cell (Hellma, Nuernberg, Germany) and a recorder (Linsies, NJ, USA). All tubing used PTFE tubing of inner diameter 0.5 mm, except Tygon pump tubing (Saint-Gobain Performance Plastics, NJ, USA). For automation by electronic control of the system, a home-made controller based on Basic Stamp II SX microprocessor (Parallax, USA) was employed for timing control of the operation of the solenoid valves.

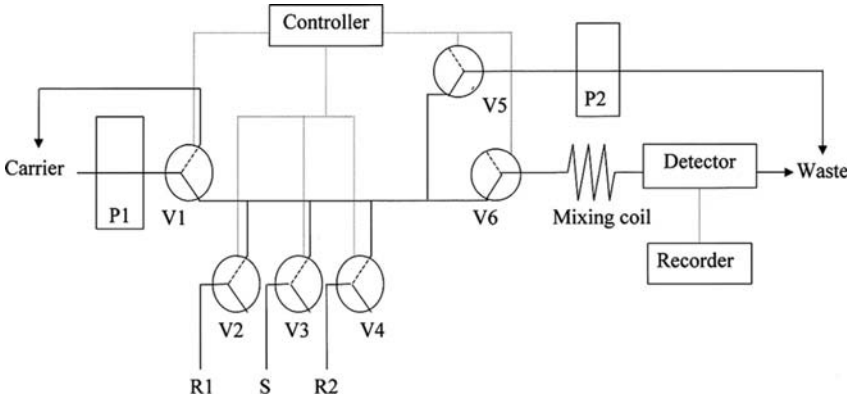

FIGURE 1 A schematic diagram of the HSI setup: V1 to V6 = solenoid valves 1 to 6; P1, P2 = peristaltic pump; R1 = hydroxylamine; R2 = formaldoxime; S = standard/sample; controller = a home-made controller unit.

Chemicals

Deionized water (Milli RX, Millipore, Bedford, MA, USA) was used throughout. All chemicals were of analytical reagent grade, unless otherwise stated. The chromogenic reagent, 0.6 mol L^{-1} formaldoxime, was prepared from the reaction of formaldehyde (M&B) (5.04 mL of 37% v/v) and hydroxylamine (Carlo Erba, Milano, Italy) (5.00 g) and adjusted the volume to 100 mL with water. A stock standard solution of 100 mg L^{-1} of Mn(II) was prepared by dissolving 0.0618 g of $MnSO_4 \cdot H_2O$ (Fluka, Buchs, Switzerland) in a portion of water. A 1.00 mL portion of concentrated nitric acid (Carlo Erba) was added before adjusting the volume to 200 mL of a volumetric flask with water. A 0.03 mol L^{-1} hydroxylamine solution was obtained by dissolving 0.21 g of the chemical in 100 mL of water.

The buffer carrier solution was prepared by dissolving 6.75 g of ammonium chloride (Carlo Erba) in 500 mL of water, and ammonia solution (BDH, Poole, Dorset, UK) was added until pH of the solution reached 10.2. Then, buffer solution was used to adjust the volume to 1000 mL with water.

Procedure

With the proposed manifold in Fig. 1, the operation sequence as shown in Table 1 was programmed to the controller. The operation sequence is briefly described as follows. First, the carrier solution was propelled by the peristaltic pump (pump channel 1) through solenoid valve 1, SV1, T-connectors, soleniod valve 5, SV5, and mixing coil to the flow cell of the detector. Then, by electronic control of the solenoid valves, the carrier stream was stopped (the carrier solution was redirected to its container) and hydroxylamine, standard/sample, and formaldoxime solutions were sequentially aspirated by pump channel 2 into the system to fill several defined volumes between the T-connectors to form stacked solution zones corresponding with the inlet port of each solution. An excess volume

TABLE 1 Operation Sequence of the HSI System (Fig. 1) for Determination of Manganese

Step	Valve	Interval(s)	Description
1	Turn off V1, V6		Stop the carrier flow
	Turn on V2, V5	5	Draw up hydroxylamine by P2
2	Turn off V2		
	Turn on V3	5	Draw up standard/sample (S) by P2
3	Turn off V3		
	Turn on V4	5	Draw up formaldoxime by P2
4	Turn off V4, V5		Stop aspiration of solution
	Turn on V1, V6	50	Carrier flow start to push the stacked zones to detector.
5	Turn off V1, V6		Return to step 1 to start next cycle

of each solution was discarded to waste. Finally, the carrier was allowed to push the stacked zones through the mixing coil to the detector while the signal profile was continuously recorded on the recorder.

Sample Preparation

Soil samples were collected at the depth of 15 cm throughout at least 3 points and mixed together. Soil extraction for exchangeable fraction of manganese was performed according to the reported procedure.[2] Briefly, a portion (10 g) of each sample was extracted with 100 mL NH_4OAc by shaking for 1 h. Then, it was filtered through a filter paper (Whatman no. 42). The filtrate was boiled to nearly dryness and continued until vapor of NH_4OAc ceased. Then, 2.00 mL HNO_3 and 1.00 mL H_2O_2 were added for digestion of organic matter, then, water added to a volume of 100 mL, the beaker was covered with watch glass and heated until dryness, 85% H_3PO_4 1.00 mL added, and the volume adjusted with deionized water to 100 mL.

RESULTS AND DISCUSSION

Effect of the Experimental Variables

In SIA, volumes of sample and reagents can be variably and accurately aspirated using a high-quality syringe pump and a selection valve. However, too large volume of the zones could reduce penetration of the sample and reagent zones leading to less reaction to occuring.[28] In HSIA, the volume of solutions was fixed by the defined volumes of the tube connected between the T-connector (about 50 μL each zone in this case), so a simple pump can be used for aspirating the solutions. Effect of the experimental variables such as carrier flow rate, mixing coil length, and concentration of reagents was studied.

Using preliminary condition (0.3 M formaldoxime, 0.06 M hydroxylamine, 100-cm mixing coil length, 0.1 M ammonia buffer pH 10 as a carrier), effect of carrier flow rate was investigated. Another peristaltic pump (P2 in Fig. 1) was employed for aspiration of the solution with constant flow rate (3 mL min^{-1}), while P1 was adjusted to give flow rate in the range 1–4 mL min^{-1}. A series of concentration of standard solution of manganese (0.5–20 mg L^{-1}) was injected and a calibration graph (a plot of peak height [mV] versus manganese concentration) was constructed in each case. Slopes of the calibration graphs of about 26 mV · L mg^{-1} were obtained for all flow rates. The flow rate of 3 mL min^{-1} was selected as it provided suitable sample throughput and was compatible with the aspiration flow rate.

The effect of mixing coil length (50, 100, 200, and 300 cm) was studied similar to that described above. Slopes of the calibration graphs obtained decreased significantly with the increase of the mixing coil length, due to higher dispersion of the product zone at longer coil. Thus, mixing coil length of 50 cm was selected as it provided higher slope and sample throughput.

Effect of chromogenic reagent, formaldoxime (concentrations 0.1, 0.3, 0.6, and 1.0 M) was investigated using the conditions previously selected. By considering slopes of the calibration graphs, it was found that formaldoxime concentration of higher than 0.3 M gives comparable sensitivities. Thus, 0.6 M formaldoxime was selected.

The reaction of Mn(II) with formaldoxime proceeds well in alkaline medium. Effect of pH of 0.1 M ammonia buffer (8, 9, 10, and 10.8) was investigated by injecting a series of concentration of manganese standard solutions (0.5–20 mg L^{-1}) with employing the above selected conditions. When pH changed from 9 to 10, a very sharp increase in sensitivity to reach maximum level (40 mV/mg L^{-1}) was observed. The pH of 10.2 was selected in order to ensure the sensitivity.

In basic solution, Mn(II) normally forms manganese hydroxide precipitate, which prevents it to react with formaldoxime. In order to avoid manganese hydroxide formation, hydroxylamine reducing agent was used. Effect of concentrations of hydroxylamine (0.01, 0.03, 0.06, and 0.10 M) was studied by carrying out the experiment similar to the above. Slopes of the calibration graphs increased with concentration of hydroxylamine and reached the plateau at 0.03 M hydroxylamine. Hydroxylamine (0.035 M) was then selected for further experiment.

It should be noted that the analog output of the Spectronic 21 spectrophotometer is linearly dependent with %T,(i.e., 1000 mV corresponded with 100 %T). Thus, a change of 40 mV should be equal to 4%T change or 0.018 absorbance units change. Therefore, the slope of a calibration graph of about 40 mV L mg^{-1} may be lower than in a batchwise

method, because in the continuous flow system the reaction may not reach equilibrium and sensitivity is controlled by extent of the reaction and degree of dispersion of the product.

Analytical Characteristics

Employing the selected conditions as studied in the previous section, a series of concentration of manganese standard solutions (0.5–30 mg L^{-1}) was injected into the HSI system. A linear calibration graph ($y = 37.6x - 13.1$, $r^2 = 0.999$) was obtained. A limit of detection (LOD)[29] of 0.2 mg L^{-1} and sample throughput of 45 h^{-1} were achieved. Relative standard deviations for 11 replicated injections of 5 and 20 mg Mn L^{-1} were 5.6% and 2.4%, respectively. The procedure consumed 250 μL each of hydroxylamine, sample, and formaldoxime solutions and 2.5 mL of buffer carrier solution per operation cycle. Analytical features of the developed method in comparison with the previously reported methods are summarized in Table 2.

Recovery of the method in the determination of exchangeable manganese in soil was evaluated by spiking manganese standard solution into the extracted solutions. Concentrations of manganese in the extracts were found in range 0.9–3.2 mg L^{-1}. Percentage recoveries in the range 91–110 were observed.

TABLE 2 **Analytical Features of the Developed Method in Comparison with the Previously Reported Methods**

Technique	Sample	Principle	Linear range (mg L^{-1})	LOD (mg L^{-1})	%RSD	Injection per hour	Ref.
SI-poten	Soil	Redox reaction of Mn(II) and hexacyanoferrate(III) leading to potential change of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ potential buffer solution	0.01–5.5	0.01	1.9 (0.33 mg L^{-1})	20	[4]
FI-spect	Mineral premixes and feedstuffs	Oxidation of Mn(II) to permanganate by periodate in slightly alkaline medium and in the presence of pyrophosphate and acetate	0–30	0.08	0.6 (10 mg L^{-1})	120	[6]
SI-spect	River water and effluent streams	Speciation of Mn(II) and Mn(VII) using 4-(2-pyridylazo) resorcinol, detected at 500 nm	0.02–0.50	0.005	0.3	30	[15]
Monosegmented flow spect	Soybean digests	Oxidation of Mn(II) by periodate in phosphoric acid medium to form permanganate anion	2.5–40.0	1.2	0.3 (17 mg L^{-1})	50	[16]
FI-spect	Natural water and effluent streams	Mn(II) was oxidized to permanganate by solid lead (IV) dioxide suspended on silica gel beads, detected at 526 nm	1.0–5.0	0.6	1.8	—	[20]
SI-spect	Tap water and effluent streams	Mn(II) was oxidized to permanganate by solid lead (IV) dioxide suspended on silica gel beads	1.0–7.0	0.6	3	50	[22]
SI-spect	Water	Formation of Mn(II)–formaldoxime complex using merging zone technique	0.5–4.0	—	6	36	[23]
HSI spect	Soil	Formation of Mn(II)–formaldoxime complex	0.5–30	0.2	2.4 (20 mg L^{-1})	45	This work

poten, potentiometry; spect, spectrophotometry.

Determination of Exchangeable Manganese in Soil Samples

Soil samples from longan orchards were analyzed for exchangeable manganese contents by the proposed method. Soil-extracted solutions were prepared as described in the "Sample Preparation" section. The solution was injected into the system, and concentration of manganese in the solution was calculated from peak height obtained using a calibration graph. Exchangeable manganese contents in soil ($\mu g\, g^{-1}$) were then calculated as summarized in Table 3. Analysis of the soil solution by the standard spectrophotometric method based on periodate reaction[2] was also carried out for comparison. Manganese contents found by using hydrodynamic injection method (x) agreed well with those found by the standard spectrophotometric method (y), indicated by the slope, intercept, and correlation coefficient (r) of the correlation graph between the two methods are close to 1, 0, and 1, respectively ($y = 1.2x - 3.0$, $r = 0.992$). According to the paired t-test at 95% confidence level,[29] there is no significant difference of the results from the two methods.

TABLE 3 Amounts of Exchangeable Manganese in Soil Samples

	Amount of exchangeable Mn in soil ($\mu g\, g^{-1}$)	
Sample number	Proposed method	Standard method[2]
1	10.7±1.0	9.5
2	8.7±1.0	8.5
3	7.7±0.8	8.5
4	16.7±2.1	12.2
5	11.7±1.0	8.5
6	13.7±2.1	8.5
7	8.7±0.8	6.9
8	68.3±1.3	79.0
9	98.1±1.3	116.8
10	30.6±2.5	36.2
11	42.0±2.1	51.2
12	26.6±1.3	30.9
13	34.5±2.5	41.6
14	17.7±1.3	19.1
15	13.7±1.3	12.2
16	11.2±1.0	14.9
17	8.7±0.8	9.5
18	8.7±0.8	8.5

CONCLUSIONS

A spectrophotometric method with a new HSI approach is introduced. The method is investigated for the determination of manganese based on the complex formation reaction of Mn(II) with formaldoxime giving a product that could be monitored for absorbance at 450 nm. The HSI provided introduction of precise and accurate volumes of sample and reagents to the flow system, even with use of a simple peristaltic pump, solenoid valves, and a simple controller. Various advantages similar to a typical SI system, such as low consumption of reagent solutions, low waste emission, and high automation, were gained. Application of the developed system to determination of exchangeable fraction of manganese in soil is demonstrated.

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ภาคผนวก ก.2

Hydrodynamic Sequential Injection with Spectrophotometric Detection for Determination of Nitrite and Nitrate in Water, ***Anal. Sci.***, 24 (2008) 1599-1603

ภาคผนวก ก.3

Sequential injection monosegmented flow voltammetric determination of cadmium and lead using a bismuth film working electrode, ***Talanta***, 79 (2009) 1118-1124

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Sequential injection monosegmented flow voltammetric determination of cadmium and lead using a bismuth film working electrode

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Bismuth film

ABSTRACT

A cost-effective sequential injection monosegmented flow analysis (SI-MSFA) with anodic stripping voltammetric (ASV) detection has been developed for determination of Cd(II) and Pb(II). The bismuth film working electrode (BiFE) was employed for accumulative preconcentration of the metals by applying a fixed potential of −1.10 V versus Ag/AgCl electrode for 90 s. The SI-MSFA provides a convenient means for preparation of a homogeneous solution zone containing sample in an acetate buffer electrolyte solution and Bi(III) solution for in situ plating of BiFE, ready for ASV measurement at a flow through thin layer electrochemical cell. Under the optimum conditions, linear calibration graphs in range of 10–100 $\mu g\,L^{-1}$ of both Cd(II) and Pb(II) were obtained with detection limits of 1.4 and 6.9 $\mu g\,L^{-1}$ of Cd(II) and Pb(II), respectively. Relative standard deviations were 2.7 and 3.1%, for 11 replicate analyses of 25 $\mu g\,L^{-1}$ Cd(II) and 25 $\mu g\,L^{-1}$ Pb(II), respectively. A sample throughput of 12 h^{-1} was achieved with low consumption of reagent and sample solutions. The system was successfully applied for analysis of water samples collected from a draining pond of zinc mining, validating by inductively coupled plasma-optical emission spectroscopy (ICP-OES) method.

1. Introduction

Heavy metals such as cadmium and lead are toxic, persistent pollutants and they can be bioaccumulated/concentrated through the food chain. Their contamination to the environment comes from different sources, e.g., soil erosion, mining and industrial activities. Therefore, the development of sufficiently sensitive, selective and reproducible analytical methods for precise and accurate determination of these metals at trace levels is essential. There are several techniques recently utilized including spectrometric, chromatographic and electroanalytical techniques. Spectrometric techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) although provide good sensitivity and selectivity, they usually involve expensive and large equipment.

Electroanalytical techniques such as stripping voltammetry on the other hand usually concern small instrument, which is relatively low cost, low power consumption and portable. The most widely used stripping voltammetric mode for determination of Cd(II) and Pb(II) is an anodic stripping voltammetry (ASV), which is conventionally performed on mercury electrode, e.g., hanging mercury drop electrode (HMDE) and mercury film electrode (MFE) [1]. Mercury electrode provides a wide cathodic potential limit for reduction of several metals and allows the formation of amalgams for accumulative preconcentration of the metals leading to very high sensitivity and reproducibility for ASV determination. However, due to toxicity of the mercury, recently mercury-free electrodes such as bismuth film electrode (BiFE) are extensively researched [2–32]. BiFE is environmentally friendly since the toxicity of bismuth and its salts is negligible. It can form "fused alloys" with heavy metals, analogously to the amalgams that mercury forms [4,11] leading to high sensitivity and reproducibility of the stripping signal and good resolution of the adjacent stripping peaks. Other attractive properties include its low background characteristics, wide alkaline pH working range and being partially insensitive to dissolved oxygen, which allows the analysis without the time-consuming de-oxygenation step [2,4–6,10,11]. Similar to the MFE, BiFE could be conveniently prepared by plating a thin bismuth film on a suitable substrate material, which can be done before (ex situ plating) [14] or at the same time (in situ plating) [8,9] with the deposition of the analyte metals. Various substrate materials could be used such as glassy carbon [2,5,6,20,25], carbon fiber [2,14], carbon paste [7,15,18], screen printed electrode [3,9,12,24,30], pencil lead [8], carbon nanotube [27], edged plane graphite [19], gold [16] and copper [17]. Other techniques for preparation of BiFE have been introduced such as sputtering of Bi film on silicon substrate to

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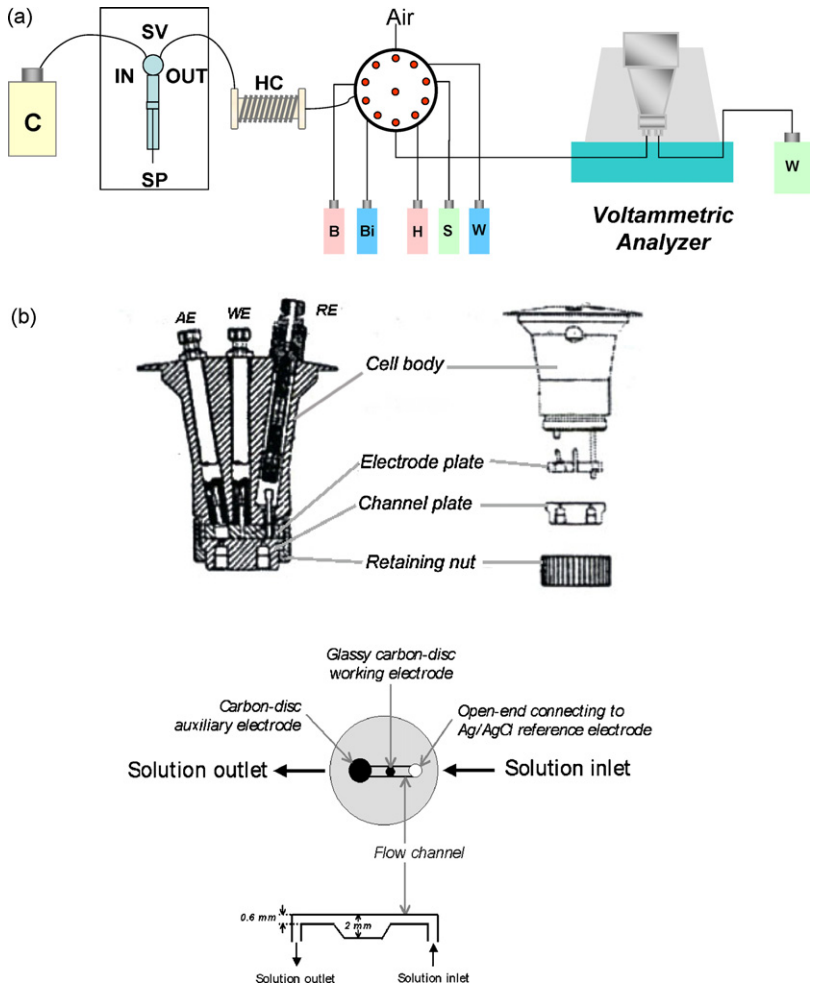

Fig. 1. The developed sequential injection anodic stripping voltammetric system. (a) Schematic diagram of the system, C: carrier (deionized water), SP: syringe pump, SV: switching valve, HC: holding coil, B: 0.2 M acetate buffer, Bi: 40 mg L^{-1} Bi(III) in 3 M acetate buffer, H: cleaning solution (0.1 M HNO_3), S: mixed metals standard/sample and W: waste. (b) A thin layer electrochemical flow-cell and the flow channel, AE: auxiliary electrode, WE: working electrode and RE: reference electrode.

produce BiFE microelectrode [22,23] and bismuth–carbon composite electrode using Bi nanoparticles [32]. BiFE is more mechanical durable than MFE which is suitable for application in flow systems [1,11]. Flow based analysis such as flow injection (FI) and sequential injection (SI) offers several advantages over batch analysis such as fast and higher degrees of automation, improvement of accuracy and precision, less risk of contamination and low consumption. Recently, several sequential injection systems have been developed for automation of ASV analysis [29–31,33–38]. However, most of them employing mercury electrodes, either HMDE [37,38] or MFE [34–36]. There is still lack of application of BiFE in flow system [11]. SI-ASV on Nafion® coated BiFE was developed for determination of Cd(II), Pb(II) and Zn(II) [29]. The hybrid FI/SI system using BiFE was also reported for ASV determination of Cd(II) and Pb(II), and AdSV determination of Co(II) and Ni(II) [30]. SI-ASV was proposed for determination of Cd(II), Pb(II) and Zn(II) employing an in situ plated bismuth film screen printed carbon electrode [31].

The SI with monosegmented flow analysis (MSFA) approach was introduced to promote good mixing of the solution zones sandwiched between two air segments, resulting from a turbulent flow in the monosegment [37,39,40]. This approach should improve efficiency in electrodeposition of metal ions on the working electrode, leading to high sensitivity and reproducibility of the analytical results. With MSFA sample dilution, single stock standard calibration and standard addition could be made in-line [37,40]. The SI-MSFA with voltammetric determination of atrazine on a HMDE was developed [37].

In this work, we developed a cost-effective SI system to perform MSFA aiming to gain benefit in convenient handling in solution preparation for in-line ASV determination of Cd(II) and Pb(II) employing an environmentally friendly BiFE as a working electrode. The BiFE was in situ plated on glassy carbon electrode and the same electrode could be repeatedly used for several times due to efficient cleaning in the flow system. The system provided sensitive and reproducible determinations of Cd(II) and Pb(II), with semi-automatic analysis and low chemical consumption. The developed system with new software offered opportunity to do complicated tasks in SIA, despite a simple script program has been used.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Deionized water (obtained from a system of Milli-Q, Millipore, Sweden) was used throughout. An acetate buffer solution (0.2 M, pH 4.6), which served as a supporting electrolyte was prepared by dissolving sodium acetate 3-hydrate (Ajax Finechem, Australia) (13.61 g) in water before adding of acetic acid (Carlo Erba, Italy) (5.7 mL) and making up to final volume of 500 mL with water. Working standard solutions of Pb(II) and Cd(II) were daily prepared by appropriate

diluting the stock standard solutions (1000 mg L^{-1} atomic absorption standard solutions, Merck, Germany) with the acetate buffer solution. A stock solution of Bi(III) (1000 mg L^{-1}) was prepared by dissolving 0.23 g of bismuth (III) nitrate 5-hydrate (Carlo Erba, Italy) in 0.5 M HNO_3 solution. A Bi(III) plating solution (40 mg L^{-1}) was daily prepared by diluting the stock solution with 3 M acetate buffer solution.

2.2. Instrumentation and apparatus

An in-house assembled sequential injection-voltammetric system is depicted in Fig. 1(a). It consisted of a syringe pump (Cavro Model XL-3000, USA), a 10-port selection valve (Valco Instrument, USA), a voltammograph (VA 757, Metrohm, Switzerland). Tygon® tubing (1.25 mm i.d., 4.5 m long) was used for assembling a holding coil. Other flow lines were made of a PTFE tubing of 0.5 mm i.d. A thin layer cross-flow cell (Metrohm, Switzerland) as shown in Fig. 1(b) was employed for voltammetric measurement. It consisted of a glassy carbon disc working electrode (WE), a carbon disc auxiliary electrode (AE) and a Ag/AgCl (3 M KCl) reference electrode (RE). An in situ plated bismuth film electrode on the WE was used in anodic stripping voltammetric analysis. The system was computerized controlled by using a home-made program written in Visual Basic 6 (Microsoft, USA). Employing this controller program, different solution sequences as shown in Fig. 2 were created for investigation of monosegmented flow for efficient mixing of various solutions.

2.3. Procedure

The operational sequences for the determination of Pb(II) and Cd(II) by the SI-voltammetric system with monosegmented flow strategy are given in Table 1. This corresponds to the solution sequence F in Fig. 2. Before running the operational sequence, the "Start-up" program sequences was firstly executed, in order to fill the HC, the electrochemical cell and the tubing connecting to the port 6 of the selection valve with 0.2 M acetate buffer solution and to fill tubings connected to other ports of selection valve with their respective solutions. Then operational sequences were started as describing as follows (sequence F, Fig. 2). First, the acetate buffer solution (800 μL) was aspirated and then delivered through port 6 to the flow cell. Then, air (100 μL) was aspirated to separate buffer solution from the following solutions. After that, Bi(III) plating solution (Bi + B) and mixed standard/sample solution (M) were alternately aspirated to form stacked zones as shown in Fig. 2(F). Then selection valve was switched to port 1 to perform flow reversal to promote mixing of the stacked zones together [39], by aspirating air (300 μL) and pushing air and 25 μL of the solution to waste (port 2). The mixing zone was then propelled through a cross-flow cell for electrodeposition of the metals by applying a potential of −1.10 V versus Ag/AgCl to the WE for a specified deposition time. The air segment at the back was taken out to waste before buffer solution was sent to the flow cell. Then, the stripping step was performed in a medium of acetate buffer electrolyte under stop flow condition. A voltammogram was recorded using the following condition: sweep mode, square wave; sweep potential, −1.10 to 0.20 V; sweep rate, 0.50 V/s. Finally, the flow cell and the electrodes were cleaned by flowing cleaning solution (0.1 M HNO_3) through the flow cell while applying a potential of 0.20 V to the WE.

3. Results and discussion

3.1. Development of SI system and software for SI operation

An SI system was assembled from commercially available OEM components in order to make the system to be cost-effective. Control of the pump and selection valve was accomplished by sending an ASCII code to the respective component via computer serial ports (COM port). An in-house developed software for control of the SI system was designed and written in Visual Basic 6.0. The control panel of the software is depicted in Fig. 3. As can be seen from the figure, the component could be controlled manually by clicking on the button on the screen or automatically by creating a program sequences or a script program. The way of writing a script program was convenient, i.e., by typing a value in a parameter box of each component and then click "Add" button to insert a script line in the Program Control box. The program script could be easily edited by highlighting on the line to be deleted or inserted and then clicking "Remove" or "Add" button, accordingly. Instruction for delay and

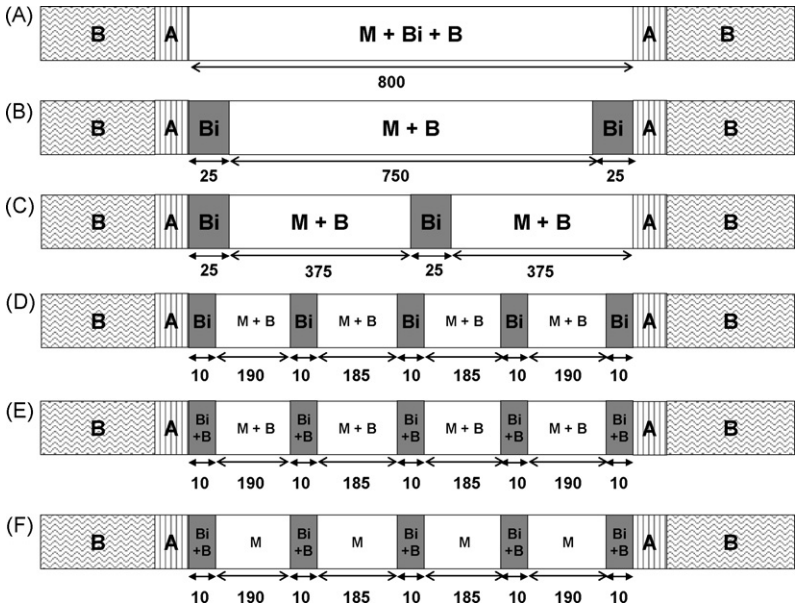

Fig. 2. Sequence of solutions in the monosegmented zone before mixing; A: air, M: mixed solution of Cd(II), Pb(II) and Zn(II) standards (0.1 mg L^{-1} each), Bi: 40 mg L^{-1} Bi (III) in nitric acid (pH 1.7), B: 0.2 M acetate buffer, Bi + B: 40 mg L^{-1} Bi (III) in 3 M acetate buffer. Volume of solution indicated under each zone is in microliter.

Table 1
Operational sequences of the SI-MSFA–ASV method for determination of Cd(II) and Pb(II).

Step	Description	Pump valve position	Selection valve position	Volume (μL)	Flow rate (μLs^{-1})	WE potential (V)
1	Load buffer solution	Out	3	800	50	
2	Deliver carrier solution to flow cell	Out	6	400	10	
3	Load air	Out	1	100	50	
4	Load bismuth plating solution	Out	4	10	10	
5	Load standard/sample	Out	9	190	10	
6	Load bismuth plating solution	Out	4	10	10	
7	Load standard/sample	Out	9	185	10	
8	Load bismuth plating solution	Out	4	10	10	
9	Load standard/sample	Out	9	185	10	
10	Load bismuth plating solution	Out	4	10	10	
11	Load standard/sample	Out	9	190	10	
12	Load bismuth plating solution	Out	4	10	10	
13	Load air	Out	1	300	50	
14	Taken air out	Out	10	325	50	
15	Deley time 5 s for clicking on start button of the voltammograph					
16	Deliver the sample zone through flow cell for deposition step	Out	6	750	10	−1.10
17	Taken air out	Out	10	150	100	
18	Push buffer to flow cell	Out	6	200	10	
19	Stripping and recording of voltammogram	Out				−1.10 to 0.20
20	Load cleaning solution	Out	7	700	50	
21	Load carrier	In		100	50	
22	Deliver zone of cleaning solution to strip bismuth film	Out	6	975	50	0.20

loop control could be inserted similarly. Additionally, the software was prepared for control of components which will be used in other applications or further development of the system to higher automation, e.g., peristaltic pump, recorder and auto-sampler.

Using the developed system, sequential injection monosegmented flow voltammetric analysis could be semi-automatically performed according to the operational sequences as described in Table 1. The script program was started to run by clicking on "Start Program" button and it can be stopped at any time by clicking "Stop Program" button. The complicated procedure could be done with higher degrees of automation employing the developed system as described in Section 2.3.

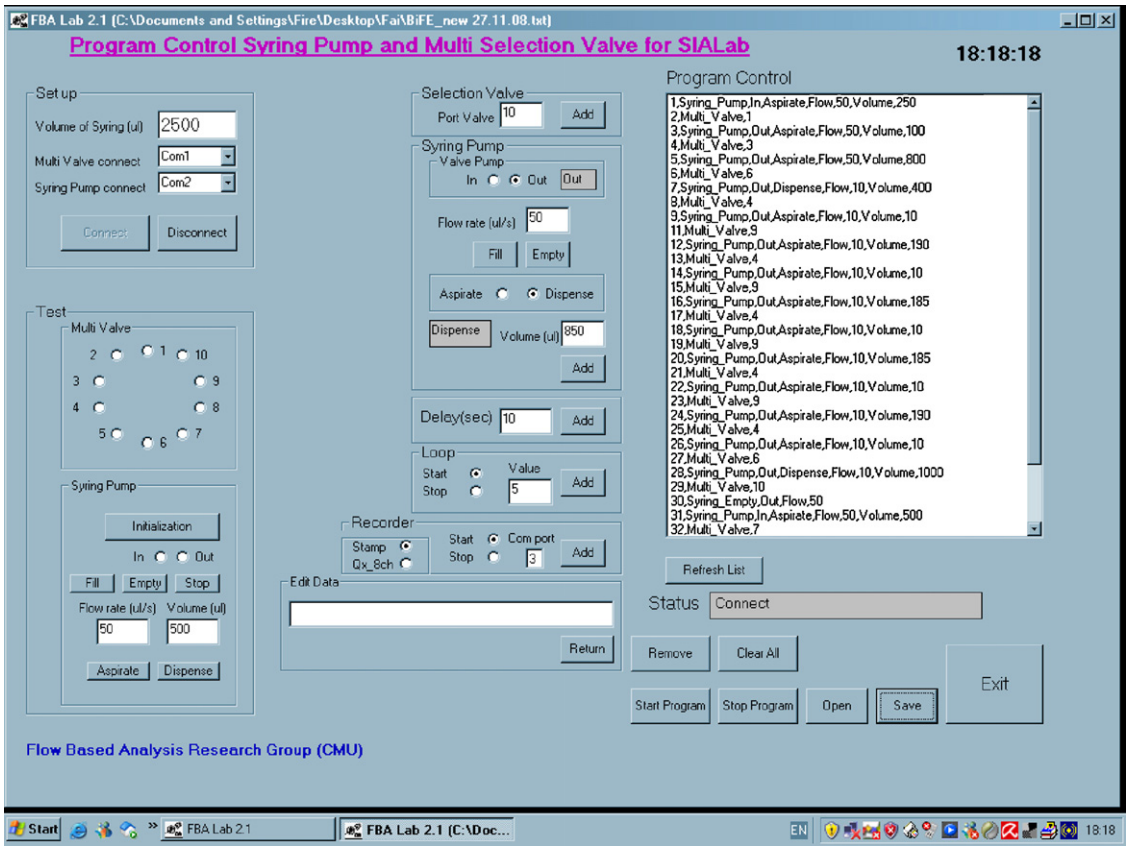

Fig. 3. The control-panel page of an in-house SI controller software (for details see text).

3.2. Optimization of experimental conditions

Conditions of the square wave anodic stripping voltammetric analysis for determination of cadmium and lead (as described in Section 2.3) was selected from the previous study [35], except bismuth film electrode (BiFE) was used instead of mercury film electrode in order to avoid the use of toxic mercury. Preparation of BiFE could be done either by ex situ plating (preplating) or in situ plating. In situ plating could be more convenient to perform by off-line spiking of the standard/sample solution with Bi(III) solution and formation of bismuth film simultaneously occurred with the analyte metals accumulation during the deposition step. In this work, a monosegmented flow analysis (MSFA) [37,39,40] approach was applied in SI systems in order to provide good mixing of Bi(III), standard/sample and acetate buffer electrolyte solutions together in a monosegmented zone. This could be carried out by using air plugs to sandwich the solution zones, which prevent dispersion of solution into a carrier stream and the turbulent flow occurring in the air segmented zone would promote mixing of the sandwiched solution zones to form a homogenized monosegmented zone. The homogeneous solution would provide good performance and reproducibility in the deposition and stripping steps of ASV analysis because the concentration gradient of the sample zone entering the electrochemical flow cell would less occur while the potential was applied to the working electrode [37]. Other segmented flow could also be used to promote mixing but complicated instrumentation and procedure may be needed, e.g., air segmentor and bubble remover devices are required. A 0.2 M acetate buffer pH 4.6 was selected as a supporting electrolyte for voltammetric analysis because this medium provided wider potential window of BiFE than the more acidic medium [5].

Preliminary investigation on the effect of Bi(III) concentration used for formation of BiFE on sensitivity of metals determination was carried out by the off-line premixing of solutions of metal ions, Bi(III) and acetate buffer. The final solution contained 0.01 and 0.1 mg L^{-1} each of Cd(II), Pb(II) and Zn(II), 0.2 M acetate buffer and different concentrations of Bi(III). The premixed solution (800 μL) was aspirated to sandwich in between air plugs and 0.2 M acetate buffer as shown in Fig. 2(A). After flow reversal, the air plug was removed and the solution zone was then pushed to the flow cell. A fixed potential of −1.10 V versus Ag/AgCl electrode was applied to the WE while the solution zone was propelled at 10 μL s^{-1} through the cell. The stripping was performed in a medium of 0.2 M acetate buffer by scanning potential from −1.10 to 0.20 V. Effect of Bi(III) concentration on peak currents of the analyte metals is illustrated in Fig. 4. It was found that peak current sharply increased with the

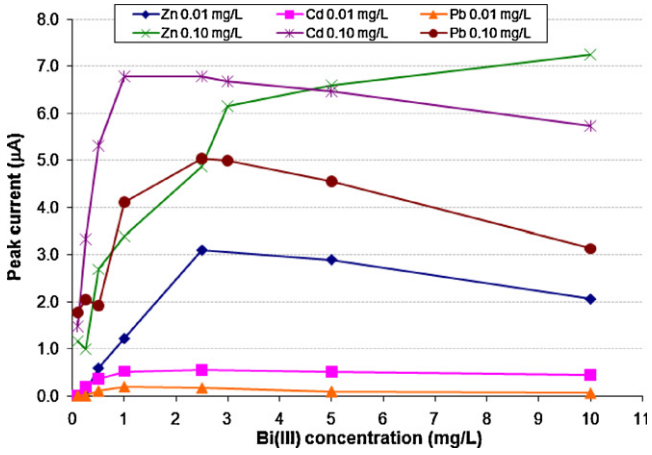

Fig. 4. Effect of concentration of Bi(III) plating solution on peak current of the analyte metals (for details see text).

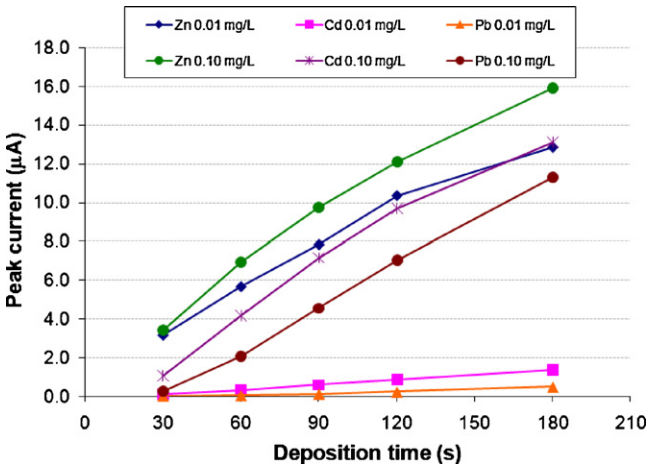

Fig. 5. Effect of deposition time on peak current of the analyte metals (for details see text).

increase of Bi(III) concentration and reached the maximum at about 2.5 mg L^{-1} Bi(III). This is the same trend with the peak current of Bi itself. Bi(III) concentration would dictate the thickness of the Bi film for deposition of the analyte metals. At high Bi(III) concentration the metals may difficultly stripped out from a thick Bi film leading to lower peak current and broader peak. The Bi(III) concentration of 2.5 mg L^{-1} was selected for further experiment. The in situ plated BiFE on GCE should provide advantages in term of simplicity, low cost and convenient operation since the same electrode could be repeatedly used after proper cleaning.

The reproducibility of peak current may depend on the cleanliness of the working electrode. The cleaning step was applied after the voltammogram was recorded by flowing a cleaning solution (0.1 M HNO_3) while the potential of WE was held at +0.20 V for 15 s. It was found that the relative standard deviations for seven consecutive determinations of 50 μg L^{-1} Cd(II) and Pb(II) were improved from 5.9 and 6.9 to 1.8 and 1.3%, respectively, for 0 s and 15 s cleaning time. Flow system helped cleaning the working electrode better than in batch method because the fresh solution was flowed through the electrode during cleaning. This would lead to repeatedly use of BiFE on GCE with better reproducibility than in batch method.

Deposition time and volume of the sample zone passing though the flow cell during deposition step were investigated by aspirating different volumes (200, 500, 800, 1100 and 1700 μL of the premixed solution), which corresponded to deposition time of 30, 60, 90, 120 and 180 s, respectively. Effect of deposition time on peak currents of 0.01 and 0.10 mg L^{-1} of each metal is depicted in Fig. 5. Roughly, peak currents linearly increased with deposition time up to 120 s. Deposition time of 90 s was chosen in order to compromise between sample throughput and sensitivity.

Effect of sequence of different solutions (standard/sample, buffer and Bi(III)) on the homogenization of the mixture zone in a monosegment was investigated by creating a monosegment of 800 μL total volume by using different sequences of solutions as depicted in Fig. 2. Once the sequence was created, the stacked zones were moved forward and backward to cause turbulent mixing of the stacked zones in the monosegment. A sequence of off-line mixed (premixed) solution as shown in Fig. 2(A) was also carried out for comparison. A plating solution (40 mg L^{-1} Bi(III)) used in sequences A–D was prepared in water, while those of sequences E and F was prepared in 3 M acetate buffer. The peak currents obtained for 0.10 mg L^{-1} each of Cd(II), Pb(II) and Zn(II) and for Bi(III) when using different sequences are shown in Fig. 6. It was found that all sequences gave comparable peak currents for all the metals.

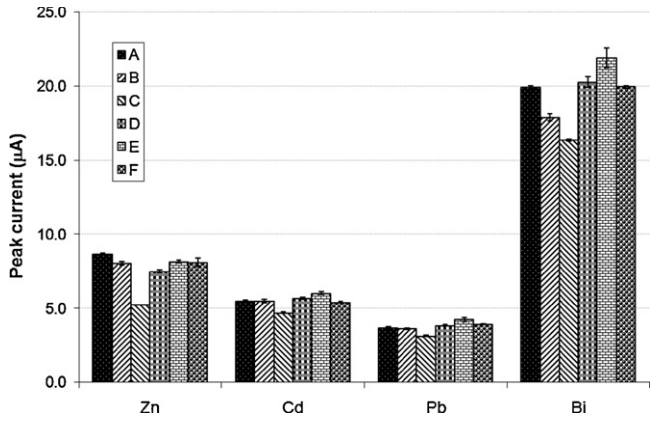

Fig. 6. Peak currents of metals obtained from different sequences A–F as shown in Fig. 5 (for details see text).

However, sequence E, which should provide better mixing and thus resulted in highest peak currents. Sequence E was modified to be sequence F by employing standard/sample solution without off-line adding of buffer, in order to simplify the sample preparation procedure. This modification resulted in a little bit lower peak currents of the metals, but with better reproducibility. The sequence F was selected for sample analysis. Thanks to the automation of the developed SIA system, this complicated procedure could be easily performed with using a simple script program.

3.3. Analytical features of the proposed system

Using SI-monosegmented flow of sequence F together with the conditions as described in Section 2.3, calibration graphs of Cd(II), Pb(II) and Zn(II) in range of 10–100 $\mu g\,L^{-1}$ of each metal were constructed by plotting peak current (μA) versus concentration of metal ions ($\mu g\,L^{-1}$). Fig. 7 shows a series of voltammograms. It could be seen that for Zn(II) a good linear calibration could not be obtained yet. Further studies should be needed for the determination of zinc. Under the selected conditions, linear calibration graphs could be obtained for Cd (II) and Pb(II) with the calibration equations, $y = 0.0551x - 0.1142$; $R^2 = 0.9999$ for Cd(II) and $y = 0.0506x - 0.5435$; $R^2 = 0.9966$ for Pb(II). The detection limits (the concentration corresponding to three times of standard deviation of blank) were obtained at 1.4 $\mu g\,L^{-1}$ for Cd(II) and 6.9 $\mu g\,L^{-1}$ for Pb(II) for deposition time of 90 s. The relative standard deviations for 11 replicate

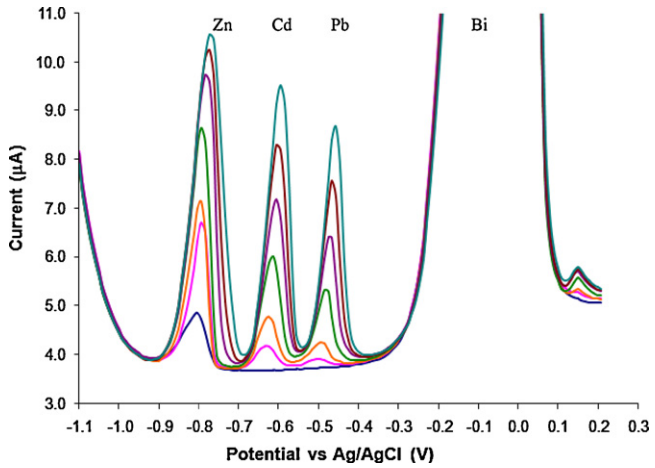

Fig. 7. Voltammograms of standard solutions containing Zn(II), Cd(II), Pb(II) obtained from SI-MSFA–ASV on BiFE; concentrations of each metal from bottom to top: 0.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 $\mu g\,L^{-1}$.

Table 2
Concentrations of Cd(II) and Pb(II) in water samples found by SI-monosegmented flow–ASV and ICP-OES methods.

Sample	Concentration of metals found ($mg\,L^{-1}$) by			
	SI-ASV		ICP-OES	
	Cd	Pb	Cd	Pb
1	0.56 ± 0.02	3.20 ± 0.02	0.51 ± 0.02	3.02 ± 0.06
2	0.78 ± 0.02	2.10 ± 0.04	0.73 ± 0.04	2.01 ± 0.03
3	0.56 ± 0.01	2.69 ± 0.02	0.52 ± 0.02	2.52 ± 0.04
4	0.23 ± 0.01	3.62 ± 0.00	0.21 ± 0.02	3.54 ± 0.05
5	0.36 ± 0.00	3.85 ± 0.00	0.34 ± 0.03	4.02 ± 0.06
6	0.44 ± 0.00	2.55 ± 0.00	0.43 ± 0.03	2.53 ± 0.04
7	0.84 ± 0.02	1.30 ± 0.02	0.82 ± 0.04	1.22 ± 0.02
8	0.57 ± 0.01	2.11 ± 0.02	0.51 ± 0.02	2.03 ± 0.05
9	0.76 ± 0.01	1.40 ± 0.03	0.82 ± 0.03	1.52 ± 0.04
10	0.34 ± 0.07	3.32 ± 0.06	0.31 ± 0.02	3.52 ± 0.05

analyses of 25 $\mu g\,L^{-1}$ Cd(II) and 25 $\mu g\,L^{-1}$ Pb(II) were 2.7 and 3.1%, respectively. The analysis time for one sample is 5 min (sample throughput of 12 h^{-1}). Each analysis cycle consumed 750 μL of sample, 50 μL of 40 $mg\,L^{-1}$ Bi(III) plating solution and 800 μL of 0.2 M acetate buffer solution.

Monosegmented flow provides completed mixing of the solution zones, thus it would open possibility to perform in-line single standard calibration and in-line standard addition procedures. Preliminary experiment was carried out for in-line single standard calibration by varying the volume of the mixed metals standard to be aspirated into the monosegmented zone. Linear calibration graphs were obtained: $y = 0.0110x - 0.0525$; $R^2 = 0.9996$ for Cd(II) and $y = 0.0701x - 0.6285$; $R^2 = 0.9994$ for Pb(II). However, more investigations and refinement for the optimum condition should be made further.

3.4. Analysis of real samples

The proposed system was employed for determination of Cd(II) and Pb(II) in surface water samples collected from a draining pond of zinc mining in northern Thailand. Such a sample was collected in a clean polyethylene bottle (1 L) with adding of HCl to acidify sample to about pH 1. No sample pretreatment was made except filtering of the sample just before the analysis and dilution of sample with water (10-fold dilution for Cd(II) and 40-fold dilution for Pb(II) determinations). Samples were also analyzed by ICP-OES at the Office of Primary Industry and Mine Region 3, Chiang Mai for comparison. The obtained results are presented in Table 2. According to *t*-test at 95% confident limit, the results obtained from both the methods were in good agreement ($t_{critical} = 2.26$, $t_{calculate} = 0.26$ and 0.05 for Cd(II) and Pb(II), respectively). The results were correlated each other well (SI = 0.9463 ICP + 0.0522, $R^2 = 0.9761$ for Cd(II) and SI = 0.9512 ICP + 0.1472, $R^2 = 0.9784$ for Pb(II)). The system was also tried for analysis of bottled mineral drinking water. Concentration of Cd(II) and Pb(II) in those samples were below detection limit of the method. By spiking 25 and 50 $\mu g\,L^{-1}$ of both metal ions into a sample, recoveries were found in range of 95–108% for Cd(II) and 100–115% for Pb(II). Application of the developed system to determination lower concentration of metal ions in water samples nearby the mining area will be further investigated.

4. Conclusion

A cost-effective sequential injection system was assembled and applied for monosegmented flow anodic stripping voltammetric determination of Cd(II) and Pb(II) employing BiFE in situ plating on a glassy carbon working electrode. The system offered non-toxic, convenient, high degrees of automation and low consumption in the analysis, with precise and accurate results for the determination

of Cd(II) and Pb(II) in contaminated water samples. The monosegmented flow help in in-line preparation of homogeneous solution mixture of sample, Bi(III) plating solution and acetate buffer supporting electrolyte solution. The system has high potential to be developed further to be automated. Further investigations for in-line dilution, in-line single standard calibration and in-line standard addition procedures employing monosegmented flow approach are in progress.

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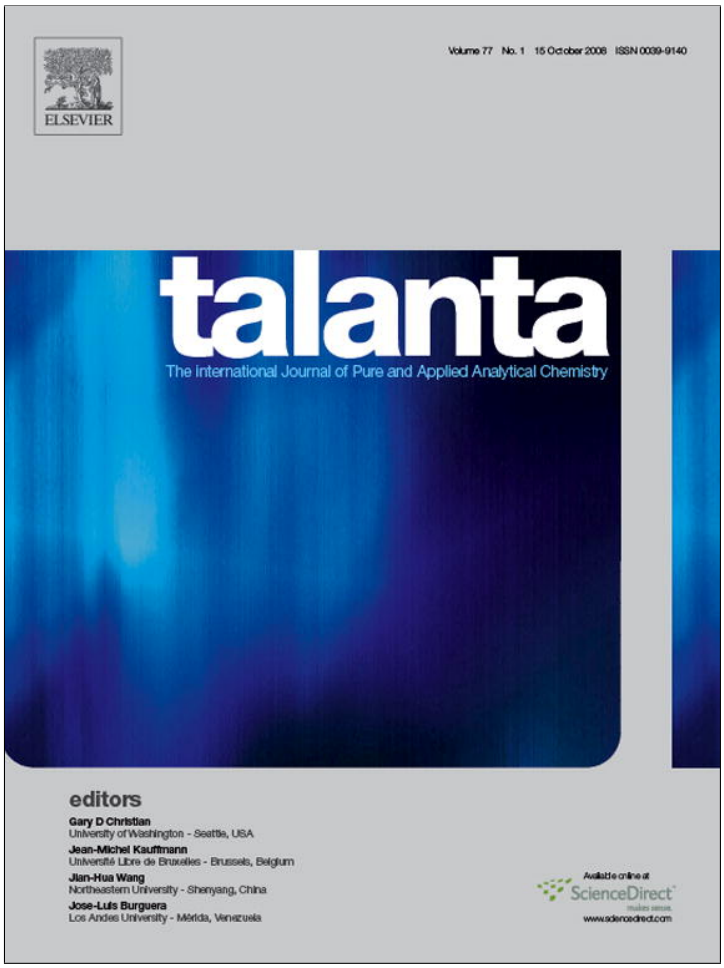
Determination of cadmium, lead, copper and zinc in the acetic acid extract of glazed ceramic surfaces by anodic stripping voltammetric method, ***Talanta***, 77 (2008) 172-175



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Determination of cadmium, lead, copper and zinc in the acetic acid extract of glazed ceramic surfaces by anodic stripping voltammetric method

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ABSTRACT

An anodic stripping voltammetric method has been developed for determination of cadmium, lead, copper and zinc in acetic acid extract of glazed ceramic surfaces. An aliquot of 4% (v/v) acetic acid solution was kept in a ceramic ware for 24 h in the dark, then 10 mL of the extracted solution was placed in a voltammetric cell. The solution was purged with oxygen free nitrogen gas for 3 min before deposition of the metals was carried out by applying a constant potential of −1.20 V versus Ag/AgCl to the hanging mercury drop electrode (HMDE) for 45 s. A square wave waveform was scanned from −1.20 to 0.15 V and a voltammogram was recorded. A standard addition procedure was used for quantification. Detection limits of 0.25, 0.07, 2.7 and 0.5 μg L^{-1} for cadmium, lead copper and zinc, respectively, were obtained. Relative standard deviations for 11 replicate determinations of 100 μg L^{-1} each of all the metals were in the range of 2.8–3.6%. Percentage recoveries obtained by spiking 50 μg L^{-1} of each metal to the sample solution were in the range of 105–113%. The method was successfully applied to ceramic wares producing in Lampang province of Thailand. It was found that the contents of cadmium, lead, copper and zinc released from the samples were in the range of <0.01–0.16, 0.02–0.45, <0.14 and 0.28–10.36 μg dm^{-2}, respectively, which are lower than the regulated values of the Thai industrial standard. The proposed method is simpler, more convenient and more sensitive than the standard method based on FAAS.

1. Introduction

Cadmium and lead are well-known toxic metals. These metals are found in glazed ceramic surfaces and are controlled by law for permissible amounts released from some ceramic products. Glaze is a thin layer of liquid, which is put on a piece of pottery and becomes hard and shiny when the pottery is heated in a hot oven. In Thailand, ceramic products are mainly produced in northern provinces, e.g., Lampang, Sukhothai and Chiang Mai. Release of cadmium and lead from ceramic ware, glass–ceramic ware and glass dinnerware intended to be used in contact with food is tested according to the Thai industrial standard (TIS 32-2546) or ISO-6486-1: 1999 [1]. The method involving extraction of the metals from the glaze by 4% (v/v) acetic acid, which is kept in the ceramic ware to be tested for 24 h in the dark, and determination of the metals in the extracted solution by flame atomic absorption spectrophotometry (FAAS). This method is tedious and time-consuming, and with relatively high detection limits. To improve detection limit and precision, sample pretreatment of the extract, e.g., evaporating up of acetic acid and adding with hydrochloric acid before FAAS determination may be performed. On-line separation/preconcentration procedures, e.g., using column packing with Pb-Spec resin [2], Muramac A-1 chelating resin [3], thioureasulfonamide resin [4] and bead injection with renewable sorption material [5] are usually employed for the determination of trace amounts of cadmium/lead by FAAS or ETAAS. Flow injection analysis with on-line preconcentration column using Pb-Spec resin and spectrophotometric detection based on formation of Pb(II)–4-(2-pyridylazo)resorcinol complex was developed for determination of lead in acetic acid leachate of glazed ceramic surfaces [6]. Detection limit of 8 μg L^{-1} and relative standard deviation for five replicate determinations of 0.8 mg L^{-1} of 0.35% were reported. However, the method is quite complicated.

On the other hand, anodic stripping voltammetry (ASV) which has an in situ preconcentration (electrodeposition) step can be applied directly for simultaneous determination of cadmium and lead at trace levels [7–13]. Metal ions were electrochemically deposited on a working electrode, e.g., a hanging mercury drop electrode (HMDE), a mercury film electrode [7–9], or a more environment friendly bismuth film electrode [10–13]. Then, the determination was done in the stripping step by scanning potential

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to positive (anodic) direction, where re-oxidation of the deposited metals was occurred at a specific potential for each metal. However, this method can detect free metal ions only and is suffered from interferences such as surface active organic substances [8]. Some modified electrodes have been developed for improving sensitivity and selectivity in the analysis of complicated samples [9–11]. Fortunately, the extract of ceramic glaze was free from organic substances, so the ASV method with a HMDE should be suitable for this sample. An electrolyte medium such as acetate buffer [7–11] is widely utilized in ASV determination of Cd and Pb. In this work, the acetic acid which has been used as an extractant for leaching of the metals from ceramic glaze surfaces, was investigated to be applied as an electrolyte in the ASV analysis.

In this paper, we developed a simple method based on anodic stripping voltammetry for simultaneous determination of cadmium, lead, copper and zinc in acetic acid leachate of glazed ceramic wares. The 4% (v/v) acetic acid extracted solution could be also used an electrolyte for voltammetric analysis, so the determination of the metals could be carried out without any sample pretreatment. A HMDE which is available in most analytical laboratories has been employed as a working electrode, making the developed method simple and convenient for quality control of ceramic wares.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Deionized water (obtained from a system of Milli-Q, Millipore, Sweden) was used throughout. Stock standard solution of lead(II) (1000 mg L^{-1}) was prepared by dissolving 0.1615 g of lead nitrate (Merck, Germany) in 0.1 M nitric acid 100 mL. Stock standard solution of cadmium(II) (1000 mg L^{-1}) was prepared by dissolving 0.1991 g of cadmium chloride (J.T. Baker, Canada) in 0.1 M hydrochloric acid 100 mL. Stock standard solution of copper(II) (1000 mg L^{-1}) was prepared by dissolving 0.3968 g of copper sulphate (Merck, Germany) in 0.1 M sulfuric acid 100 mL. Stock standard solution of zinc(II) (1000 mg L^{-1}) was prepared by dissolving 0.4443 g of zinc sulphate (Ajex Finechem, Australia) in 0.1 M sulfuric acid 100 mL. The working standard solutions were prepared daily by diluting the stock standard solution of each metal with 4% (v/v) acetic acid. The extracted solution (4% (v/v) acetic acid) was prepared by diluting 40 mL of glacial acetic acid in water and adjusting the final volume to 1000 mL. An oxygen free nitrogen (OFN) gas (99.9995%, TIG, Thailand) was used for purging the solution to remove dissolved oxygen.

2.2. Voltammetric system

A voltammetric analyzer (VA 757, Metrohm, Switzerland) including a voltammetric cell with a HMDE as a working electrode (WE), a platinum rod electrode as an auxiliary electrode (AE), and a Ag/AgCl electrode (3 M KCl) as a reference electrode (RE), was employed for anodic stripping voltammetric analysis. The voltammetric analyzer was controlled by a personal computer, using a VA Computrace version 2.0.000, SR1 software (8.757.8023, 757 VA Computrace, Metrohm).

2.3. Extraction procedure

Extraction of metals from glazed ceramic surfaces was carried out according to the standard method [1]. Briefly, the ceramic ware to be tested was cleaned to be free from grease or other matter likely to affect the test, then it was filled with 4% (v/v) acetic acid solution to produce an acid depth of >6 mm, covered the specimen and allowed to stand in the dark at $22 \pm 2\,^{\circ}C$ for 24 ± 0.5 h. Then, the extracted solution was collected in a polyethylene bottle for further voltammetric determination of the released metals. The surface area of the ceramic ware was accurately measured to 0.01 dm^{-2}.

2.4. Voltammetric analysis procedure

An aliquot of 10 mL of the extracted solution was put in a voltammetric cell and the solution was purged with OFN for 3 min. Then, a fixed potential of −1.20 V was applied to the WE for a period of 45 s, while the solution was stirred at 2000 rpm (deposition step). After that the stirring was stopped for 5 s, followed by anodically scanning of the potential from −1.20 to 0.15 V, employing a square wave waveform with amplitude of 40 mV, step potential of 10 mV, and frequency of 50 Hz (stripping step). A voltammogram was recorded. Peak potential and peak current corresponding to each metal was evaluated from the voltammogram. Standard addition procedure was carried out by adding standard solution of each metal to the sample solution, then the deposition and stripping steps were performed. Standard addition was repeated for four times. Concentration of each metal in sample was evaluated from the standard addition graph, with subtracting of concentration of metal in the blank solution. Amounts of the metal released from ceramic wares were reported as μg dm^{-2}, which were calculated from total amounts of the metal released into the extracted solution divided by contacted surface area.

3. Results and discussion

3.1. Effect of some parameters on voltammetric analysis

ASV method is based on electrochemical reduction of metal ions at WE to deposit the metals on the electrode surface with subsequent anodic striping by scanning the potential to anodic direction to allow electrooxidation of the deposited metals at a characteristic potential of each metal, as recorded as a voltammogram in this step. Conditions for deposition and stripping steps of ASV were investigated. A square wave waveform was employed in the stripping step, as it provided fast scanning and good sensitivity for the reversible redox reaction. A square wave waveform with amplitude of 40 mV, step potential of 10 mV, and frequency of 50 Hz was used. A solution of 4%(v/v) acetic acid that employing as the extracted solution was also used as an electrolyte. Standard solutions of Cd, Pb, Cu and Zn were added to the electrolyte solution and voltammetric measurement was carried out. Effect of deposition potential was investigated in the range of −0.90 to −1.30 V. It was found that the more negative potential used the higher sensitivity was obtained. Deposition potential of −1.20 V was chosen because too negative potential may lead to deposition of some interferences or evolving of hydrogen gas at the WE in the high acidic medium. Deposition time was studied for the determination of 50 μg L^{-1} of each metal. A plot of peak current versus deposition time is depicted in Fig. 1. It was found that peak currents of all the metals, except Cu, are linearly proportional to deposition time up to 2 min. At too long deposition time, the deposited metals may saturate at the HMDE so no further increase in peak current was observed. While most ASV methods used acetate buffer as an electrolyte medium, in this work acetic acid (4%, v/v) should be employed because it has been used as an extractant for leaching of metals from ceramic surfaces. Effect of concentration of acetic acid on peak current of 50 μg L^{-1} of each metal is investigated. Acetic acid in concentration range of 1–6% (v/v) did not affect either on peak potential or peak current of

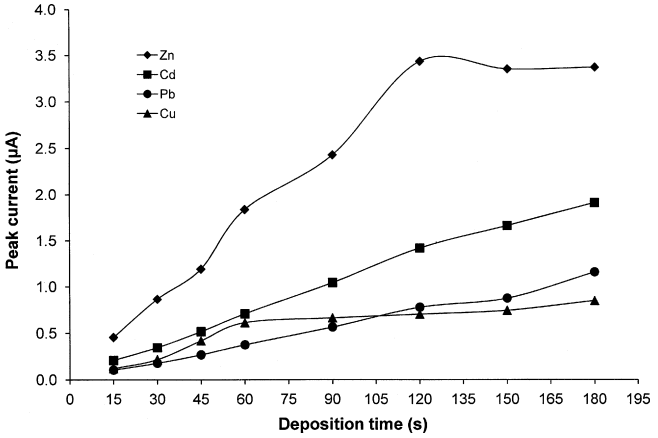

Fig. 1. Effect of deposition time on peak current. Condition: deposition potential −1.20 V, stirring rate 2000 rpm, scan rate 0.01 V s^{-1}, potential scan range −1.20 to 0.15 V.

all the metals studied. However, the higher concentration of acetic acid used, the higher zinc content in the electrolyte solution (blank) was observed. Acetic acid of 4% (v/v) was selected to be used as both the extractant and the electrolyte for ASV analysis.

3.2. Analytical characteristics

Under the selected condition: 4% (v/v) acetic acid as an electrolyte solution, deposition potential of −1.20 V, deposition time of 45 s, square wave waveform with amplitude of 40 mV, step potential of 10 mV, and frequency of 50 Hz, voltammograms were obtained as shown in Fig. 2. Linear calibration graphs in the concentration range of 0–200 μg L^{-1} of each metal were obtained as the followed calibration equations: Cd: $Y = 0.0094X + 0.0062$, $r^2 = 0.9996$, Pb: $Y = 0.0048X + 0.0065$, $r^2 = 1.0000$, Cu: $Y = 0.0033X + 0.0081$, $r^2 = 0.9990$, and Zn: $Y = 0.0159X + 1.5462$, $r^2 = 0.9999$, where Y is peak current (μA) and X is concentration (μg L^{-1}) of each metal. Detection limits calculated from three times standard deviation of blank/slope of the calibration graph [14] were 0.25, 0.07, 2.7 and 0.5 μg L^{-1} for Cd, Pb, Cu and Zn, respectively. Sensitivity and detection limit could be

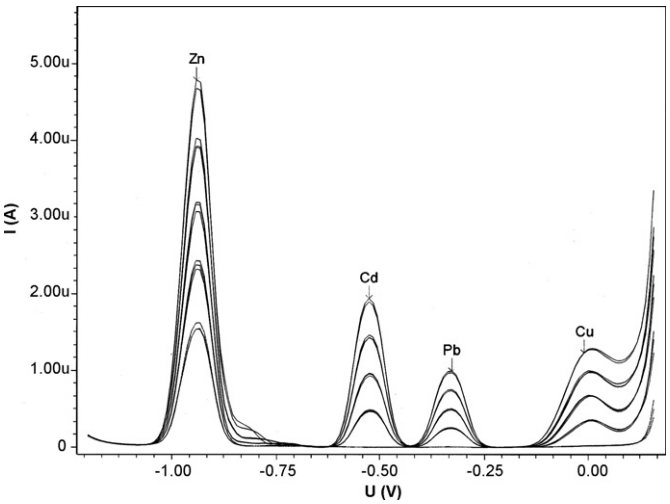

Fig. 2. Voltammograms of zinc, cadmium, lead and copper in 4% (v/v) acetic acid. Concentrations of each metal from bottom to top: 0, 50, 100, 150 and 200 μg L^{-1}. Condition: deposition potential −1.20 V, deposition time 45 s, stirring rate 2000 rpm, scan rate 0.01 V s^{-1}, potential scan range −1.20 to 0.15 V.

Table 1
Amounts of some metals released from ceramic wares

Sample	Released amounts (μg dm^{-2})*			
	Zn	Cd	Pb	Cu
1	10.36 ± 0.12	0.16 ± 0.01	0.394 ± 0.001	N.D.
2	6.31 ± 0.23	N.D.	N.D.	N.D.
3	6.00 ± 0.01	N.D.	0.447 ± 0.001	N.D.
4	0.88 ± 0.04	N.D.	0.006 ± 0.001	N.D.
5	2.84 ± 0.04	N.D.	0.272 ± 0.001	N.D.
6	0.46 ± 0.02	N.D.	0.290 ± 0.001	N.D.
7	5.65 ± 0.23	N.D.	0.109 ± 0.002	N.D.
8	6.56 ± 0.06	N.D.	0.261 ± 0.001	N.D.
9	1.18 ± 0.14	N.D.	0.299 ± 0.009	N.D.
10	1.30 ± 0.06	N.D.	0.065 ± 0.001	N.D.
11	2.89 ± 0.02	0.02 ± 0.00	0.096 ± 0.016	N.D.
12	5.25 ± 0.16	N.D.	0.064 ± 0.002	N.D.
13	4.17 ± 0.04	N.D.	0.097 ± 0.003	N.D.
14	0.64 ± 0.06	N.D.	0.056 ± 0.003	N.D.
15	2.41 ± 0.05	N.D.	0.126 ± 0.002	N.D.
16	0.28 ± 0.05	N.D.	0.076 ± 0.001	N.D.
17	2.50 ± 0.02	N.D.	0.200 ± 0.006	N.D.
18	7.24 ± 0.08	N.D.	0.431 ± 0.001	N.D.
19	3.63 ± 0.24	N.D.	0.096 ± 0.001	N.D.
20	3.53 ± 0.18	N.D.	0.127 ± 0.001	N.D.
21	1.33 ± 0.06	N.D.	0.041 ± 0.001	N.D.
22	2.29 ± 0.04	N.D.	0.316 ± 0.002	N.D.
23	0.84 ± 0.02	N.D.	0.020 ± 0.001	N.D.

*Mean of triplicated results, N.D. = not detected.

improved by using longer deposition time as described in Section 3.1. Relative standard deviations for 11 replicate determinations of 100 μg L^{-1} of each metal were 3.0, 2.8, 3.6 and 2.8% for Cd, Pb, Cu and Zn, respectively. Recoveries obtained from spiking of the metal standard solutions (50 μg L^{-1} each) into the extracted solution were found in the range of 105–113%. The analysis time is 5 min per sample by using standard addition procedure for determination of four metals, which is much faster than the standard method based on FAAS [1].

3.3. Application to real samples

The proposed method was applied to the determination of some metals extracted from the surface of ceramic wares. Standard addition method was employed for quantification in order to account for the effect of sample matrix. However, the results obtained by using calibration graph method were correlated well with those from the standard addition method (for Zn, $Y = 0.9322X - 0.8767$, $r^2 = 0.9631$). The results from standard addition method are summarized in Table 1. It was found that the contents of cadmium, lead, copper and zinc released from the samples were in the range of <0.013–0.16, 0.02–0.45, <0.14 and 0.28–10.36 μg dm^{-2}, respectively, which are lower than the permissible values of the Thai industrial standard. The developed method was convenient to be used and could be applied as an alternative method to the standard method for testing the releasing of cadmium and lead from ceramic products.

4. Conclusion

Anodic stripping voltammetric method was proposed for determination of Cd, Pb, Cu and Zn in an acetic acid extract of glazed ceramic wares. The extract can be analyzed directly by voltammetric method, where the extractant, 4% (v/v) acetic acid, also acts as an electrolyte solution. The method is simple, fast, sensitive and selective, and can simultaneously determine four metals with high accuracy using either standard addition or calibration methods. The developed method may be used in the routine determination of Cd

and Pb extracted by acetic acid from glazed ceramic wares, as an alternative to the standard method (TIS 32-2546).

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ภาคผนวก ก.5

Cathodic stripping voltammetry for speciation of inorganic arsenic in water, soil and ores samples, ***Chiang Mai J. Sci.***, (2009) in press

Chiang Mai J. Sci. 2009; 36(2) : 001-015
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Cathodic stripping voltammetric procedure for determination of some inorganic arsenic species in water, soil and ores samples

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ABSTRACT

Square wave cathodic stripping voltammetry was developed for determination of As(III) and/or As(V) in water, soil, and ore samples. The method was based on electrodeposition of As(III) on a hanging mercury drop electrode (HMDE) as a copper-arsenic intermetallic compound, which was further reduced to arsine at a higher negative potential in the stripping step. Deposition was performed in an electrolyte solution of 1 M HCl with 10 mg L^{-1} Cu(II), by applying a constant potential of -0.40 V vs Ag/AgCl to the HMDE for 60 s at a stirring rate of 2000 rpm. Stripping step was carried out by applying a square wave waveform in the potential range from -0.40 to -1.00 V. A voltammogram was obtained with peak potential at -0.78 V giving peak current linearly proportional to As(III) concentration up to 50 μg L^{-1}. The concentration of Cu(II) and deposition time were interrelated affecting the sensitivity of arsenic determination. With a fixed deposition time of 60 s, a narrow concentration range of Cu(II), 5-10 mg L^{-1} gave the highest sensitivity. Standard addition method was applied for quantification in order to account for effect of sample matrix. The detection limit of the method was 0.3 μg L^{-1} As(III) and the relative standard deviation for 5 μg L^{-1} As(III) (n =11) was 3.6%. As(V) concentration was determined after reduction to As(III) by using 80 mg L^{-1} of thiosulfate. The developed method was applied to the analysis of water, soil, ores leachate and ores digest samples. The results were in good agreement with those obtained by HG-AAS method and the certified value of soil certified reference material. Recoveries in range of 83-108% were obtained for water samples. The proposed method is simple, convenient, and low reagent consumption.

Keywords: Cathodic stripping voltammetry; CSV; Square wave; Arsenic; Water; Soil; Ores.

1. INTRODUCTION

The problems of arsenic contamination in the environment are global concerns due to its high toxicity to living organisms and its world wide distribution. Arsenic contamination in water generally comes from natural sources, through the erosion of rocks, minerals, and soils [1]. The most exposed sites of natural arsenic contamination are in Bangladesh and West Bengal, India, where arsenic levels up to 2500 $\mu g\ L^{-1}$ in ground water have been reported [2]. Others contaminated areas include the Unites States, Taiwan, Chile, Argentina, Turkey, China, Nepal, Vietnam, and Thailand. The major cases in Thailand involve arsenic contamination of soil and water at Ron Phibun District, Nakhon Sri Thammarat province due to tin mining activities during the last century [3].

Exposure to arsenic can cause a variety of adverse health effects such as dermal changes, respiratory, cardiovascular, gastrointestinal, genotoxic, mutagenic and carcinogenic effects [4]. Since 2002, U.S. EPA has lowered the maximum contaminant level (MCL) of arsenic from 50 to 10 $\mu g\ L^{-1}$, which is believed to be the improved level to protect human health [5]. World Health Organization (WHO) has also sets the same standard of 10 $\mu g\ L^{-1}$ in drinking water. Toxicity of arsenic depends on its oxidation state. In natural environment, arsenic exhibits in four oxidation states: As(V), As(III), As(0), and As(-III) which are presented as inorganic and organic forms. Recently, more than 20 arsenic species of varying levels of toxicity have been identified in environmental and biological systems [6]. Inorganic arsenic species,i.e. arsenite (As(III)) and arsenate (As(V)), predominantly found in natural water, are more toxic than organic species such as monomethyl arsenic acid (MMA) and dimethylarsonic acid (DMA) which are largely found in biological samples. Arsenobetaine (AsB) and arsenocholine (AsC) widely found in seafood are non-toxic. Generally, organisms can convert toxic inorganic arsenic species to less toxic organic species.

Arsenic determination/speciation is very important and well recognized [7]. Many reviews on arsenic detection/speciation have been published [8-10]. Various techniques have been proposed for the determination of arsenic, such as spectrophotometry which is usually applied for field test kit [11], hydride generation atomic absorption spectrometry (HG-AAS) , atomic fluorescence spectrometry (AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), X-ray spectrometry, neutron activation analysis (NAA), electrophoresis, chemiluminescence and electrochemical techniques. Currently, well established techniques which are capable to determine several inorganic and organic arsenic species are based on chromatographic separation coupled with ICP-MS, HG-AAS, or AFS detection [12-14]. However, these methods involve sophisticated instrumentation, high operating cost and cannot be applied in the field. On the other hand, electroanalytical techniques such as stripping voltammetry [9] and stripping potentiometry [10] represent less expensive and smaller size instrumentation which could be applied to on-site analysis[15]. They are usually applied for determination of inorganic arsenic species which are very important species since arsenite is 25-60 times more toxic than arsenate and several hundred times as toxic as organic arsenicals [10].

Two approaches of stripping techniques, anodic and cathodic stripping are usually employed. Although anodic stripping is able to determine at $\mu g\ L^{-1}$ to sub $\mu g\ L^{-1}$ levels of arsenic, its main drawbacks are the irreproducibility due to usage of solid electrode, hydrogen evolution during deposition step and interferences from some electrochemically

active ions such as copper, selenium, and mercury. Cathodic stripping voltammetry (CSV) [16-23] which usually uses hanging mercury drop electrode (HMDE) as a working electrode has better performance due to fresh electrode surface which can eliminate electrode memory effects. Electrochemical reactions involving in deposition/accumulation of As on HMDE and subsequently CSV determination have been proposed in previous studies [17,23]. Electroactive species, As(III), is electrochemically reduced to As(0), which is insoluble in Hg. However, its intermetallic compounds with Cu or Se can accumulate at mercury electrode and is further reduced to AsH_3 during the stripping step. Although the use of a mixture between Cu(II) and Se(IV) to co-deposit As(III) in CSV analysis has been reported on improving sensitivity [21], Cu(II) alone is commonly used [16-20,22,23]. Intermetallic compounds with different Cu: As ratios were formed, depending on the deposition potential and acid concentration. The following reactions were proposed for the formation of Cu-As intermetallic compounds in the deposition steps [17]:

$$H_3AsO_3 + 3H^+ + 3e^- \rightarrow As^0 + 3H_2O \quad (1)$$

$$Cu^{2+} + Hg + 2e^- \rightarrow Cu(Hg) \quad (2)$$

$$As^0 + 3CuCl_3^{2-} + 3e^- \rightarrow Cu_3As + 9Cl^- \quad (3)$$

Then, in the stripping step by scanning potential of the working electrode to negative direction (cathodic scanning), the electrochemical reduction of arsenic to arsine (AsH_3) would occur:

$$Cu_3As + 3H^+ + Hg + 3e^- \leftrightarrow 3Cu(Hg) + AsH_3 \quad (4)$$

During this period, a voltammogram was recorded and peak current at peak potential of about -0.8 V(vs. Ag/AgCl) was directly proportional to As(III) concentration [17,18]. The other arsenic species were determined after being converted to As(III). As(V) could be reduced to As(III) by various types of reducing agents, such as potassium iodide and ascorbic acid, sodium thiosulfate, sodium sulfite, sodium bisulfite, aqueous sulfur dioxide, hydroxylamine hydrochloride, L-cysteine and hydrazine. Table 1 summarizes the CSV methods for arsenic determination/ speciation.

In this work, a simple square wave cathodic stripping voltammetric procedure was developed for the determination of As(III) and As(V) under the same condition at ppb levels using, 1 M HCl with 10 mgL^{-1} Cu(II) as a medium. It was found that lower concentration of Cu(II) provided higher sensitivity for arsenic determination than the one which have been previously reported. As(V) was determined after reduction to As(III) by using 80 mg L^{-1} thiosulfate. The higher concentration of thiosulfate as suggested in the literatures [18,22] (650 mg L^{-1}) produced colloid of sulfur which affects the sensitivity and the reproducibility for As determination. A standard addition method was used in order to accounting for matrix interferences. The proposed procedure was applied for determination of these inorganic arsenic species in water, soil, and ores samples. The method was validated using soil CRM and the results were compared with those obtained from HG-AAS.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used were of analytical reagent grade and ultrapure water (Milli-Q, Millipore) was used for preparing all solutions. As(III) stock standard solution (1000 mg L^{-1}) was prepared by dissolving 0.1320 g of arsenic trioxide(Merck) with 1 mL of 25%

w/v NaOH (Lab Scan), then immediately acidified with 2 mL of conc. HCl (Carlo Erba) and adjusting to volume of 100 mL in a volumetric flask with ultrapure water. As(V) stock standard solution (1000 mgL^{-1}) was obtained by dissolving 0.4164 g of sodium arsenate(Riedel-De Han) and adjusting volume with water in a 100 mL volummetric flask. The stock solutions were daily diluted to the concentration of 10 and 1 mgL^{-1} As. By dissolving 6.7065 g of cupric chloride dihydrate (BDH) in ultrapure water, a Cu(II) solution (10 g L^{-1}) at the volume of 100 mL was obtained. Thiosulfate solution (10 g L^{-1}, 100 mL) was prepared by dissolving 2.2134 g sodium thiosulfate heptahydrate (Fluka) in cold boiled water. Oxygen free nitrogen gas (Ultra high-purity grade, 99.9995%, Thai Industry Gas, Thailand) was used for purging solution before the analysis to remove oxygen and gaseous compounds.

2.2 Instrumentation

Cathodic stripping voltammetry was performed by using a Metrohm 757 VA Computrace Voltammograph (Metrohm, Switzerland) equipped with a voltammetric cell, a multi-mode mercury electrode, a nitrogen purge tube, and a motor-driven PTFE stirring rod. The instrument was controlled by a computer using VA Computrace software version 2.0.000 (Metrohm, Switzerland). A hanging mercury drop electrode (HMDE) working electrode, a Pt auxiliary electrode, and a Ag/AgCl/3M KCl double junction reference electrode were used.

2.3 Square wave CSV procedure

A Milli-Q water or sample solution (23.00 mL) was placed into a voltammetric cell and 2.00 mL of conc. HCl was added to obtain 1 M HCl in solution. Cu(II) solution was added to obtain 10 mg L^{-1} Cu(II) final concentration. In the case of As(V) determination, thiosulfate solution was added into the vessel to yield the final concentration of 80 mg L^{-1} thiosulfate. The same conditions of square wave CSV procedure was employed for both As(III) and As(V) determination by setting the operating parameters as follows: initial purging time: 300 s (sufficient period for complete reduction of As(V) to As(III) by thiosulfate), stirring rate: 2000 rpm, deposition potential: -0.40 V, deposition time: 60 s, equilibration time:10 s, end potential: -1.00 V, voltage step: 2 mV, amplitude: 20 mV, frequency: 140 Hz and sweep rate: 277.8 mV. After the potential scanning, a voltammogram was recorded and peak current (μA) at the peak potential of -0.78 V was measured. Standard addition method was employed for the determination of As(III) and the total As (As(III) plus As(V)) and As(V) concentration was evaluated by subtracting total As with the As(III) concentrations.

2.4 Sample collection and preparation

Surface and well water samples from Nakhon Sri Thammarat and Lampang provinces were collected in 1000 mL polyethylene bottle. An aliquot (2 mL) of conc. HCl was added to preserve the sample. Each sample was filtered through a Whatman No. 42 filter paper immediately before the analysis.

Soil samples were collected from a gold mine in Pichit province. Samples were dried and ground to the particle size less than 180 μm after sieving. A portion (0.15 g) of the ground sample was accurately weighed and added with 10 mL of 1:1 v/v HCl, then heated until near dryness. The deigested residue was then diluted, filtered and adjusted to 100 mL with a 1% hydrochloric acid. This treatment was adequately considered to determine readily available arsenic in soil samples [24]. If total arsenic, including that

Table 1. Comparison of cathodic stripping voltammetric methods for determination/speciation of arsenic in different types of samples.

Technique	Arsenic species	Sample	Detection condition*	Reductant for As(V) reduction	Linear range (μgL^{-1})**	Detection limit (μgL^{-1})	Precision (%RSD)	Ref.
DPCSV	As(V)	sediment and water	HMDE, E_d -0.55 V, t_d 60 s,10 mV/s, ΔE 50 mV, unstir solution, 2M $NaClO_4$+0.5M mannitol+0.3M NaCl+2mM $CuSO_4$ (250 mgL^{-1} Cu(II)) pH 1.7	0.5 M D-mannitol	10-100; 0-10 (t_d 140 s)	4.4 (t_d 1 min)	5% (n=3)	16
SWCSV	As(III)	river and sea waters	HMDE, E_d -0.4 V, t_d 1-10 min, E_{step} 2 mV, ΔE 25 mV, f 70 Hz, 2M HCl, 0.8 mM $CuCl_2$ (100 mgL^{-1} Cu(II)), 0.04 mM hydrazine sulfate	-	0-42 (E_p -0.8V)	0.005 (t_d 10 min)	8% (n=11, 0.083 μgL^{-1}, t_d 1 min)	17
SWCSV	As(III) , As(V)	spring and mineral waters	As(III): HMDE, E_d -0.39 V, t_d 40 s,E_{step} 2 mV, ΔE 40 mV, f 300 Hz, stir 2000 rpm, 1M HCl, 45 mgL^{-1} Cu(II); As(V): HMDE, E_d -0.40 V, t_d 180 s,E_{step} 2 mV, ΔE 40 mV, f 300 Hz, stir 2000 rpm,1M HCl, 400 mgL^{-1} Cu(II)	3.2 mM (650 mgL^{-1}) thiosulfate	0-20 (E_p -0.82V)	As(III) 0.2 (t_d 40 s); As(V) 2.0 (t_d 180 s)	6% (n=13, 8 μgL^{-1})	18
SWCSV	As(III) , As(V)	thermal, spring and sea waters	HMDE, E_d -0.40 V, E_f -1.1V, t_d 40 s, E_{step} 2 mV, ΔE 160 mV, f 100 Hz, stir 300 rpm, 0.45M HBr, 50 mgL^{-1} Cu(II), 10 $mgmL^{-1}$ hydrazine sulfate	3 mM sodium dithionite	0.05-0.5 ; 0-15 (E_p -0.8V)	As(III) 0.01, As(V) 0.02 (t_d 5 min)	5% (n=7, 1 μgL^{-1}, t_d 40 s)	19
CSV	As(III)	carrot, beet, irrigation water	HMDE, E_d -0.5 V, E_f -1.1V, t_d 6 min, E_{step} 5 mV, ΔE 25 mV, f 250 Hz, stir 400 rpm, 1M HCl, 150 mgL^{-1} Cu(II) + ADDTP ligand	-	0.8-12.5 (E_p -0.77 V to -0.82V)	0.5 (t_d 1 min)	5% (n=3)	20
DPCSV	As(III) ,As(V), MMA, DMA	tap, ground, sediment pore and stream waters	HMDE, E_d -0.44 V, t_d 1 min, 25 mV/s, ΔE 50 mV, stir 1000 rpm, 1M HCl, 4.6 mgL^{-1} Cu(II) + 7.4 μgL^{-1} Se(IV)	20 mM L-cysteine + 0.03 M HCl 70°C, 6 min/org. As: UV +peroxydisulfate	2.5-190 (E_p -0.68V)	0.3 (t_d 1 min)	5% (n=3)	21
DPCSV	As(III) , As(V)	natural water	copper amalgam drop electrode (Cu 211 $mgmL^{-1}$), E_d -0.38 V,E_f -0.86V, td 6 min, Estep 4 mV, ΔE 50 mV, stir 640 rpm, 2M HCl	3.2 mM (650 mgL^{-1}) thiosulfate	0.04-1.25 (E_p -0.68V, t_d 1 min)	0.02 (t_d 4 min)	2.5% (n=7, 0.42 μgL^{-1})	22
SWCSV	As(III), As(V)	water, soil, ore	HMDE, E_d -0.40 V, t_d 1 min, E_{step} 2 mV, ΔE 20 mV, f 140 Hz, stir 2000 rpm, 1M HCl, 10 mgL^{-1} Cu(II)	80 mg L^{-1} thiosulfate	0.3-50 (E_p -0.78V, t_d 1 min)	0.3 (t_d 1 min)	3.6% (n=11, 5 μgL^{-1}, t_d 1 min)	This work

* E_d = deposition potential, E_f = final potential, t_d = deposition time, E_{step} = step potential, ΔE = amplitude, f = frequency, rpm = round per min ** E_p = peak potential.

bound to silica, is required, a more rigorous digestion by heating with a mixture of concentrated strong acids such as hydrofluoric acid should be employed.

Ore samples were collected from lignite mines in Lampang province. Ore samples were ground to the particle size less than 180 μm after sieving. An accurately weighed of a portion (1 g) of the sample was soaked in a 100 mL pH 3 solution (adjusted by using acetic acid) for a period of 7 days. The leachate solution was obtained by filtering and adjusting to a volume of 100 mL. Ore samples (0.25 g) were digested with 10 mL hydrochloric-sulfuric acid (1:1 v/v) in a sealed Teflon vessel using a microwave oven (Perkin Elmer, USA). After cooling, samples were transferred to volumetric flask and diluted to 100 mL with a 1% hydrochloric acid.

3. RESULTS AND DISCUSSION

3.1 Optimization for As(III) determination

The principle of CSV for arsenic determination involved the reduction of electroactive As(III) in the presence of an excess of copper and/or selenium to accumulate on HMDE as intermetallic compounds which were further stripped in the cathodic scan, as previously described. As presented in Table 1, several electrolytes were proposed for CSV method for the determination of As(III) though slight improvement in sensitivity was observed. Hydrochloric acid is widely employed as a reaction medium because Cl^- can stabilize the electrogenerated Cu(I) which is required for the formation of the Cu-As intermetallic compounds (equation (3)). HBr [19] or $NaClO_4$ with NaCl [16] can also be used as a reaction medium. HCl was selected to be the supporting electrolyte in this work due to its availability and lower cost in high purity chemical grade. Cu(II) was chosen instead of Se(IV) based on the analogous reasons. It has been reported that a higher HCl concentration would result in a higher sensitivity, but baseline deterioration was found [17]. In our observation, a higher HCl concentration resulted in a higher variation in peak current of 10 μg L^{-1} As(III), i.e., relative standard deviations (n=11) of peak current when using 1 M and 2 M HCl were 3.0 and 10.9%, respectively. Therefore, the 1 M HCl was selected as a supporting electrolyte for further optimization.

The effect of deposition potential was re-investigated. Similar findings to the previous report were observed [17]. At a higher negative potential than -0.40 V, peak currents decreased due to the reduction of As to AsH_3. The deposition potential of -0.40 V was then selected for further investigation.

As it had been observed that the sensitivity for As(III) determination depended on the deposition time and Cu(II) concentration in a complex manner. This may be due to the formation of the Cu-As intermetallic compounds of different ratios [17]. For higher concentration of Cu(II), the maximum sensitivity was obtained at a shorter deposition time. Effect of Cu(II) concentration on the sensitivity of As (III) determination was investigated, at a fixed deposition time of 60 s, by considering calibration graphs (0-20 μg L^{-1} As(III)) in electrolyte solutions containing 1 M HCl with different concentrations of Cu(II). Figure 1 illustrates a plot of the slope of the calibration graph versus Cu(II) concentration. It can be seen that Cu(II) concentration is a critical parameter affecting the sensitivity (as indicated by the slope of the calibration graph) for As(III) determination. An electrolyte solution containing 5 mg L^{-1} Cu(II) provided the highest sensitivity. This should be as a result of the in-situ deposition of Cu(II) on HMDE to form intermetallic compound with As as described above, thus a suitable minimum concentration of Cu(II) was needed. However, higher concentration

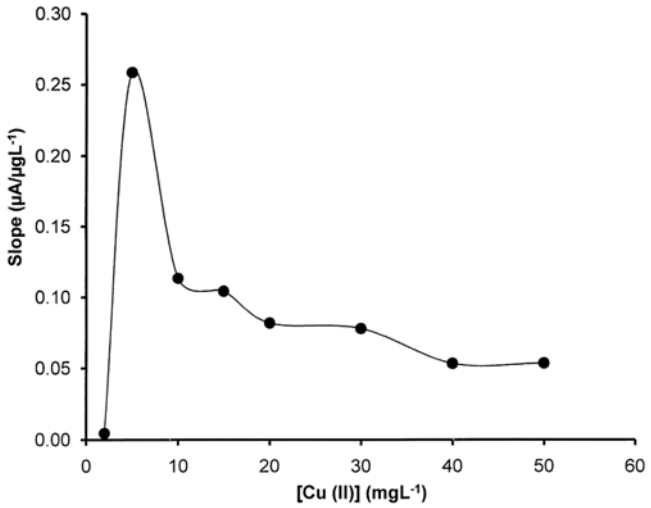

Figure 1. Effect of concentration of Cu(II) on the sensitivity of As(III) determination. (see text for conditions used).

of Cu(II) caused a decrease in sensitivity. This may be because of the formation of the intermetallic compounds of different As:Cu ratios.

By using an electrolyte solution containing 1 M HCl and 5 mg L^{-1} Cu(II), voltammograms of As(III) at the concentration range of 5-50 µg L^{-1} were recorded as shown in Figure 2. A linear calibration graph (peak current versus concentration) (y = 0.59x -2.65;

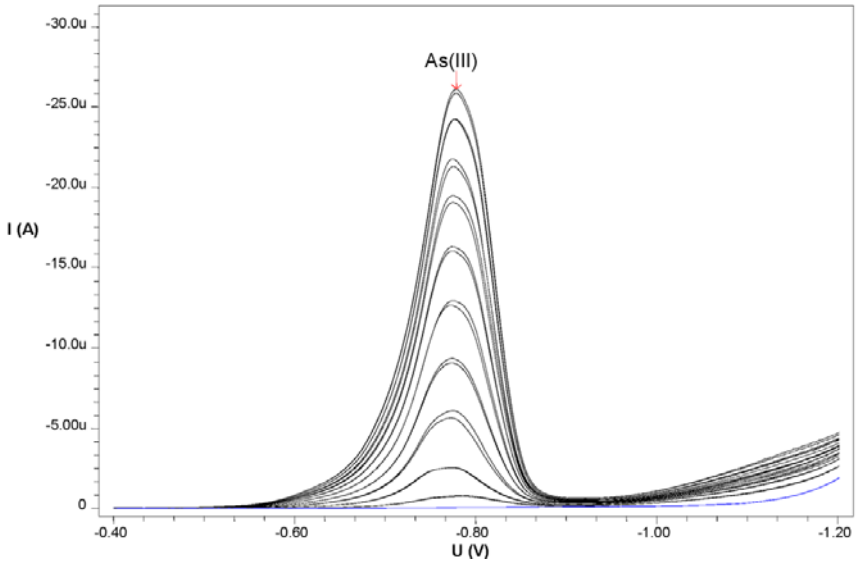

Figure 2. Cathodic stripping voltammograms of As(III) in concentration range of 5-50 µg L^{-1} (from bottom to top: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg L^{-1} of As(III)). (see text for conditions used).

R^2 = 0.9935) was obtained. It was demonstrated that in the presence of Cu(II) at different concentrations led to the variation in sensitivity for the determination of As(III). Similar effects would be observed if any species, that can form complex or react with Cu(II) to alter the concentration of Cu(II) in the electrolyte solution, was present in the analyzed solution. The standard addition method should be applied for the CSV determination of As(III). This was investigated by spiking water sample with 5 mgL^{-1} of As (III) to be used as a model sample. The experiments were performed in electrolyte solutions with different concentrations of Cu(II) (5,10,15 and 20 mgL^{-1} Cu(II), as presented in Figure 3. The results indicated that only 5 and 10 mg L^{-1} of Cu(II) yielded acceptable results (for 5.0 and 4.8 mg L^{-1} As(II) found having no error and 4 % error, respectively). The more Cu(II) concentration in the electrolyte solution, the more errors were observed (about 50% and 65% errors for 15 and 20 mgL^{-1} Cu(II) as shown in Figure 3). This indicated that the sensitivity by CSV for the determination of As(III) depended on the ratio of As(III): Cu(II) in a complicate manner.

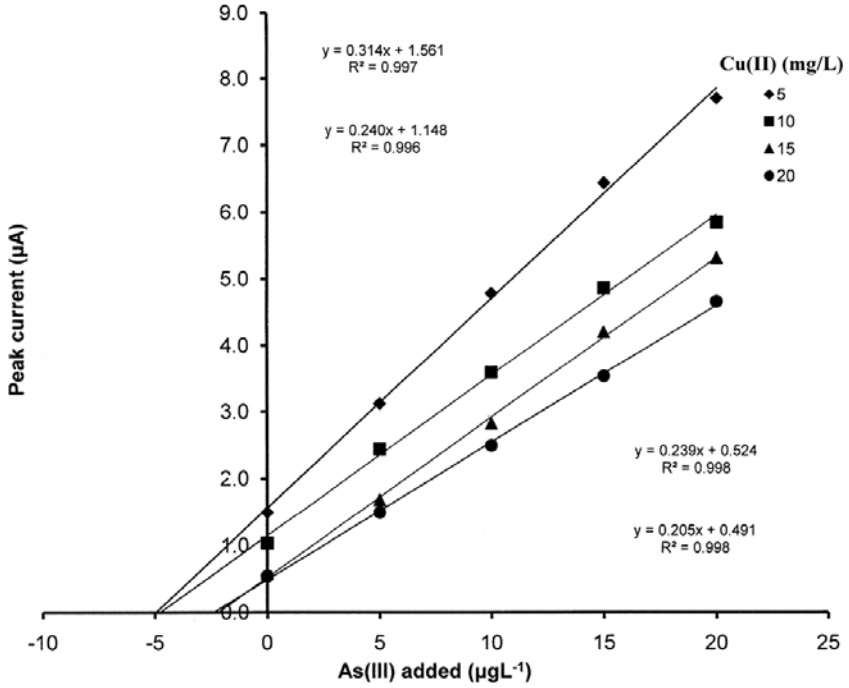

Figure 3. Standard addition graphs using different concentrations of Cu(II) for the determination of As(III) in the model water sample containing 5 µg L^{-1} As(III). (see text for conditions used).

The effect of concentration (0-100 mg L^{-1}) of Cu(II) was re-investigated for the analysis of different types of samples spiking with 30 µg L^{-1} of As(III), in order to obtain a suitable Cu(II) concentration for the analysis of real samples. The results are depicted in Figure 4. It was found that similar trends as shown in the Figure 1 were observed, but the

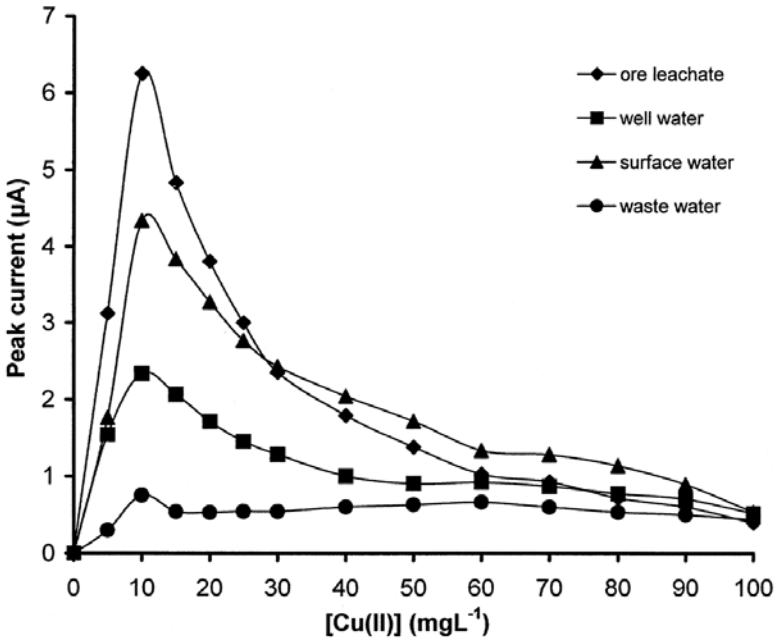

Figure 4. Effect of concentration of Cu(II) on the peak current of As(III) determination in different types of samples. (see text for conditions used).

highest sensitivity was obtained with using of 10 mg L^{-1} Cu(II). Therefore, it was chosen for the standard addition method.

The effect of stirring rate was studied in range of 400-2500 rpm of a stirring speed. It was found that sensitivity increased with increasing of stirring rate. The stirring speed of 2000 rpm was chosen as higher variation in peak current was observed at a higher speed.

3.2 Reduction of As(V) to As(III)

Different types of reducing agents have been used for reduction of As(V) to As(III) with different advantages and drawbacks. Several reductants are not stable, require high working temperature condition and some reductants (e.g. sulfite and hydrogen sulfite) may interfere with the As determination step, so the excess amounts of reductants have to be eliminated before the CSV step. Sodium thiosulfate was reported as a more convenient and reliable reductant which allowed rapid and complete reduction of As(V) to As(III) at room temperature without any interfering of excess amount of thiosulfate in the following As determination step [18]. Thiosulfate was chosen in this work as it is stable, readily available and easy to use. Effect of thiosulfate concentration was studied for reduction of 20 μg L^{-1} As(III) with reduction time of 300 s. Percentage reduction was obtained by comparing the peak current of the identical concentration of As(III) and that of As(V) with adding of the reducing agent. It was found that thiosulfate concentration higher than 80 mg L^{-1} should completely reduce As(V) to As(III) as shown in Figure 5. However, thiosulfate decomposed to sulfite and sulfur in acidic medium, which sulfite is dehydrated to sulfur dioxide, while the white colloid suspension of sulfur was observed at

concentration of thiosulfate ≥ 90 mg L^{-1}. This colloid may affect the deposition of As on the HMDE, hence leading to the decrease in peak current of As and low reproducibility of the results. Thiosulfate at the concentration of 80 mg L^{-1} which was much lower concentration than those had been previously suggested in the literatures [18, 22] (650 mgL^{-1}), was selected as a reducing agent. By using thiosulfate at the concentration of 80 mg L^{-1}, the effect of the reduction time was studied as shown in Fig. 6. The reduction time of 300 s was selected as the complete reduction of As(V) to As(III) was accomplished. The reduction could be done during nitrogen gas purging period before voltammetric measurement, therefore no additional time was required for the analysis.

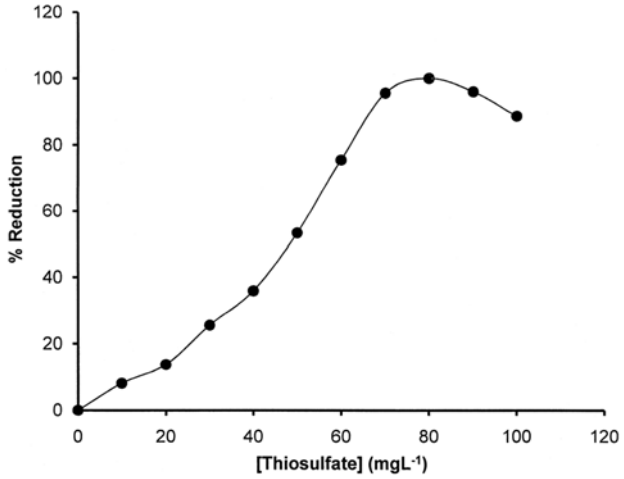

Figure 5. Effect of concentration of thiosulfate as reducing agent on reduction efficiency of As(V) to As(III) using the reducing time of 300 s. (see text for conditions used).

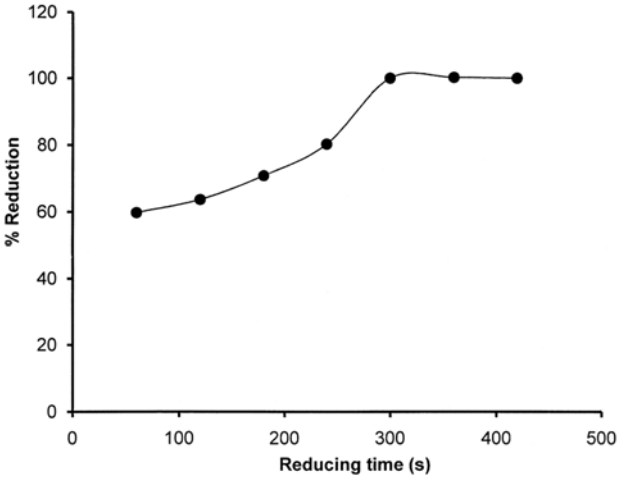

Figure 6. Effect of reducing time on reduction efficiency of As(V) to As(III) by thiosulfate at the concentration of 80 mg L^{-1}. (see text for conditions used).

3.3 Analytical features

Under the optimized conditions as described in the section 2.3, a linear response in the concentration range up to 50 mg L^{-1} with a detection limit (calculated from 3 times of the standard deviation of blank/ slope of the calibration graph) of 0.3 mgL^{-1} was obtained, which was suitable for application to various types of environmental samples. Relative standard deviation for 5 μg L^{-1} As(III) (n =11) was 3.6%. The effect of sample matrix can be suppressed by using standard addition method. The proposed procedure is more convenient than the previously reported CSV methods, using the same condition for determination of As(III) and total As, after reduction of As(V) to As(III) by using thiosulfate. The analytical performances of the proposed method were compared with those of the previously reported methods as shown in Table 1.

3.4 Interferences

Interferences for the determination of As by CSV method can be classified into, firstly, metal ions that can be reduced and accumulated at the mercury electrode, which some metals can also form intermetallic compounds with As or Cu, secondly, surface active compounds that can adsorb on electrode surface, and lastly, compounds which can form complex or precipitate with As or Cu. Effects of these interferences have been investigated in literatures [16-23]. Most common ions found in natural water have been reported and these ions did not interfere [18] for the determination of As. Some interferences have been studied in this work by spiking the potential interfering ions into the solution containing 10 μg L^{-1} (0.12 μM) As(III). It was found that Fe^{3+}, Cd^{2+}, Zn^{2+}, NO_2^- and NO_3^- did not interfere up to the concentration of 100 folds of As(III), whereas Sn^{2+} and Bi^{3+} at the concentration of 100 folds of As(III) caused reducing in As peak current almost half. With adding sulfide concentration at 100 folds of As, As peak disappeared at the potential of -0.78 V and a peak at more positive potential was observed (at -0.48 V). A low concentration of sulfide can be eliminated by increasing the acidity of the medium in order to transform S^{2-} to gaseous H_2S, which can be removed during purging step. Se(IV) caused the shift of the As peak to more positive potential (-0.68 V). This might be as a result of the $Se_xAs_yCu_z$ intermetallic compound formation [18].

3.5 Analysis of water, soil, ore leachate and ore digested samples

The proposed procedure was applied to the analysis of water, soil, ores leachate and ore digest, in comparison with hydride generation atomic absorption spectrophotometry (HG-AAS). Samples were prepared as described in section 2.4. The results obtained are summarized in Tables 2-4. As(III) concentration found was below the detection limit of the method in most samples. According to the reduction procedure, the total As (the combining concentration of As(III) and As(V)) was determined, and concentration of As(V) in each sample could be obtained by subtraction of the total As by the As(III) concentrations. According to paired t-test at 95% confidence level [25], the results obtained by the proposed method were not significantly different from those by HG-AAS. CRM of soil (CM PCM-6) was also analyzed. The result obtained was agreed well with the certified value.

Recoveries were studied by spiking 4 μg L^{-1} of As(III) and/or 5 μg L^{-1} As(V) into water samples. Percentage recoveries of 87-104 % and 83-108 % were obtained for the determination of As(III) and As(V), respectively.

Both As(III) and As(V) in μg L^{-1} levels were found in surface and well water samples

Table 2. Concentration of arsenic in water samples determined by SWCSV and HG-AAS

Sample	Concentration of arsenic found (μg L^{-1})*			
	SWCSV		HG-AAS	
	As (III)	As (V)	As (III)	As (V)
Surface water**				
1	N.D.	N.D.	N.D.	N.D.
2	N.D.	N.D.	N.D.	N.D.
3	N.D.	N.D.	N.D.	N.D.
4	N.D.	N.D.	N.D.	N.D.
5	N.D.	22.9 ± 0.0	N.D.	23.2 ±0.2
6	N.D.	1.88 ± 0.02	N.D.	1.9 ±0.1
7	N.D.	N.D.	N.D.	N.D.
Well water***				
1	0.72 ± 0.09	1.57 ± 0.16	-	-
2	N.D.	2.01 ± 0.06	-	-
3	N.D.	N.D.	-	-
4	N.D.	N.D.	-	-
5	0.72 ± 0.09	1.57 ± 0.16	-	-
Surface water***	1.10 ± 0.03	6.87 ± 0.20	-	-
Tap water	N.D.	N.D.	-	-

* mean of triplicate results, N.D. = not detected, - = not analysis

** samples from Lignite mines, Lampang province

*** samples from Ron Phibun, Nakhon Sri Thammarat province

Table 3. Concentration of arsenic in soil and certified reference material of soil, determined by SWCSV.

Sample	Total As found (mg kg^{-1})*
1	1.88 ± 0.03
2	56.7 ± 2.6
3	3.1 ± 0.1
4	33.4 ± 2.4
5	2.54 ± 0.04
6	340 ± 10
7	117 ± 3
CRM : PCM – 6**	154 8

* mean triplicate results

** Certified value 162.0 ± 14.2 mg kg^{-1}

Table 4. Concentration of arsenic in ore digest and ore leachate samples, determined by SWCSV and HG-AAS.

Sample	Concentration of arsenic found (mg kg^{-1} or $\mu g L^{-1}$)*			
	SWCSV		HG-AAS	
	As (III)	As (V)	As (III)	As (V)
Ores digest				
1	N.D.	30.6 ± 1.3	N.D.	31.5 ± 0.8
2	N.D.	N.D.	N.D.	N.D.
3	N.D.	N.D.	N.D.	N.D.
4	N.D.	N.D.	N.D.	N.D.
Ores leachate				
1	N.D.	N.D.	N.D.	N.D.
2	N.D.	3.2 ± 0.2	N.D.	2.9 ± 0.2
3	N.D.	1.26 ± 0.02	N.D.	1.3 ± 0.2
4	N.D.	8.4 ± 1.0	N.D.	8.6 ± 0.2
5	N.D.	N.D.	N.D.	N.D.
6	N.D.	N.D.	N.D.	N.D.
7	N.D.	1.56 ± 0.03	N.D.	1.7 ± 0.2
8	N.D.	N.D.	N.D.	N.D.
9	N.D.	N.D.	N.D.	N.D.
10	N.D.	N.D.	N.D.	N.D.
11	N.D.	N.D.	N.D.	N.D.
12	N.D.	5.3 ± 0.2	N.D.	5.7 ± 0.3

* mean of triplicate results, mg kg^{-1} for ores digest or $\mu g\ L^{-1}$ for ores leachate, N.D. = not detected.

from arsenic contaminated site, Ron Phibun, Nakhon Sri Thammarat province, and in soil and ore samples from mining areas of the North of Thailand (Pichit and Lampang provinces). More investigations as well as arsenic remediation study should be of interest in a wider area and in various types of samples, e.g., sediment, soil, and plant [3]. The proposed method should be useful as an analytical tool for such investigation.

4. CONCLUSION

In this work, cathodic stripping voltammetry has been developed for determination of inorganic arsenic (As(III) and As(V)) in different types of sample matrices. It was found that Cu(II) which has been proposed as a metal involve in intermetallic compound formation [17,18] to increase the solubility of As in mercury electrode strongly affected on the sensitivity of As determination. A narrow concentration range (5-10 mg L^{-1}) of Cu(II) was found to provide accurate results for As determination by employing standard addition method. The developed CSV method in the present work for speciation of the inorganic As species involves the condition for reduction of As(V) to As(III) using thiosulfate solution. Thiosulfate at the concentration of 80 mg L^{-1} could completely

reduce As(V) to As(III) at room temperature within 5 min, while the higher concentration of thiosulfate reduced peak current of As due to the formation of colloid/precipitate of sulfur in the solution.. The proposed method was sensitive, simple, and convenient, using the same set of voltammetric conditions and chemical parameters (electrolyte solution containing 1 M HCl and 10 mg L^{-1} Cu(II)) for CSV determination of both As(III) and As(V)). The method was successfully applied for determination of the inorganic arsenic species in water, soil, ore leachate and ore digest samples.

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ภาคผนวก ก.6

Stopped-flow injection method for determination of phosphate in soils and fertilisers, ***Mj. Int. J. Sci. Tech.***, 2(01) (2008) 172-181

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Full Paper

Stopped-flow injection method for determination of phosphate in soils and fertilisers

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Abstract: A stopped-flow injection system for the determination of phosphate has been developed. It involves the phosphate-molybdate-ascorbic acid reactions in the molybdenum blue method. The system is controlled by a semi-automatic stopped-FI analyser with a light emitting diode (LED)-colorimeter for monitoring the absorbance change relating to the concentration of a reaction product formed during the stopping period while the injected zone of a standard or sample is being in the flow cell. The slope of the FIAgram obtained is linearly proportional to the reaction rate, which depends on the phosphate concentration. Effects of concentration of reagents, viz. sodium molybdate, ascorbic acid and nitric acid, on the slope of the FIAgram were studied. The suitable concentration is 0.02 M, 0.25 %w/v and 0.15 M, respectively. A linear calibration graph in the range of 0.3-6.0 mg P L^{-1} was employed for the determination of phosphate in soil and fertiliser samples. The results obtained agree well with those from a standard spectrophotometric method.

Keywords: phosphate, stopped-flow injection, molybdenum blue, fertiliser, soil

Introduction

Phosphorus is an element which is widely distributed in nature. It is never found in a free or uncombined state because of its great affinity for oxygen [1]. The common species of phosphorus are phosphate (PO_4^{3-}), phosphorus trioxide (P_2O_3) or phosphorus oxide (P_4O_6), phosphorus tetraoxide

(P_2O_4 or PO_2), and phosphorus pentoxide (P_2O_5 or P_4O_{10}). A condensed phosphate is formed by the combination of oxides of phosphorus and water [2].

In agriculture, phosphorus is one of the essential elements for plants. It is absorbed by plants preferably in the form of a phosphate ion (HPO_4^{2-} or $H_2PO_4^-$). Under acidic conditions the latter is the dominant ion of the soil system. Plants need this nutrient for cell division, transformation of starch to sugar, seed germination, fruiting and flowering [3]. Thus, knowing the amounts of phosphate in soils or fertilisers is useful for monitoring the amounts of phosphorus for plants. Various methods for determination of phosphate have been reported such as ion chromatographic [4], batch [5], and flow injection (FI) [6-10] methods. Ion chromatography can quantitate different anions simultaneously at low concentration levels, but it requires an expensive and complicated instrument. Batch and FI spectrophotometric methods are used popularly for determination of phosphate by employing different chemical reactions such as molybdenum blue formation [10], complexation of orthophosphate with alizarin red sulphonate [11], malachite green ion association [12], and molybdate-crystal violet-phosphate reaction [13]. Both of the methods can provide simplicity and rapidness of analysis, but they suffer from interferences and low sensitivity. In order to improve sensitivity and selectivity of analysis, a stopped FI method is proposed in this research for determination of phosphate employing molydenum blue reaction.

The stopped FI method can increase sensitivity of the measurement by increasing the residence (reaction) time, the elapsed time after sample and reagent are mixed together prior to detection of the reaction product. By stopping the flow the residence time can be prolonged without increasing the length of the reaction coil, thus avoiding an increase of dispersion [14]. The absorbance signal due to the phosphomolybdenum blue product is continuously recorded during the stopping period. Improvement of selectivity is another advantage, since the response of an analyte increases with time whereas the background signal remains unchanged during the stopped-flow period. Therefore, by using the slope of the signal profile during the stopping period for analysis, interferences from coloured and colloidal substances present in the sample, which is a serious problem in the batch or normal FI method, can be eliminated.

Materials and Methods

Chemicals

Deionised water (Milli RX, Millipore) was used throughout. All reagents were of analytical grade, unless otherwise stated. Potassium dihydrogen phosphate (Merck) was used to prepare a stock standard solution of 1000 mg P L^{-1}, by dissolving 0.2197 g of the chemical in water, making up to a volume of 500 mL in a volumetric flask. Acidic molybdate reagent was prepared by dissolving sodium molybdate dihydrate (Fisher Scientific) (2.4195 g) in water. Then 5.4 mL of nitric acid was added before making up to a volume of 500 mL with water. Ascorbic acid (0.25 %w/v) was prepared freshly by dissolving 1.25 g of ascorbic acid in 500 mL water.

Sample preparation

Soil samples were collected from different areas in Chiang Mai. Each sample was collected from at least 15 points by digging at the depth of 6 inches (15 cm) then combining together. The sample was

air- dried and ground to less than 300 μm particle diameter. A portion of 10 g of each sample was extracted with 25 mL of 0.8 M acetate buffer (pH 4.8) by shaking for 30 min and the mixture filtered through a filter paper and the final volume of the filtrate adjusted to 50 mL with water prior to analysis.

Fertiliser samples were obtained from local suppliers. An appropriate amount of the sample was dissolved with water prior to determination of available phosphate. The solution was filtered before analysis.

Stopped FI setup

The stopped FI system used is illustrated in Figure 1. A lab-built semi-automatic stopped-FI analyser as reported previously [10] was employed. It consists of a peristaltic pump (MP-3, Eyela, Japan), a 6-port injection valve (Upchurch, USA), a home-made colorimetric detector, a recorder (Philip, The Netherland) and a microcontroller for timing control of the pump and valve. The colorimeter had a light emitting diode (LED) as the light source and a photodiode as the light sensor. All tubings used were PTFE tubing with inner diameter of 0.5 mm, except for Tygon pump tubing (Saint-Gobain Performance Plastics, USA).

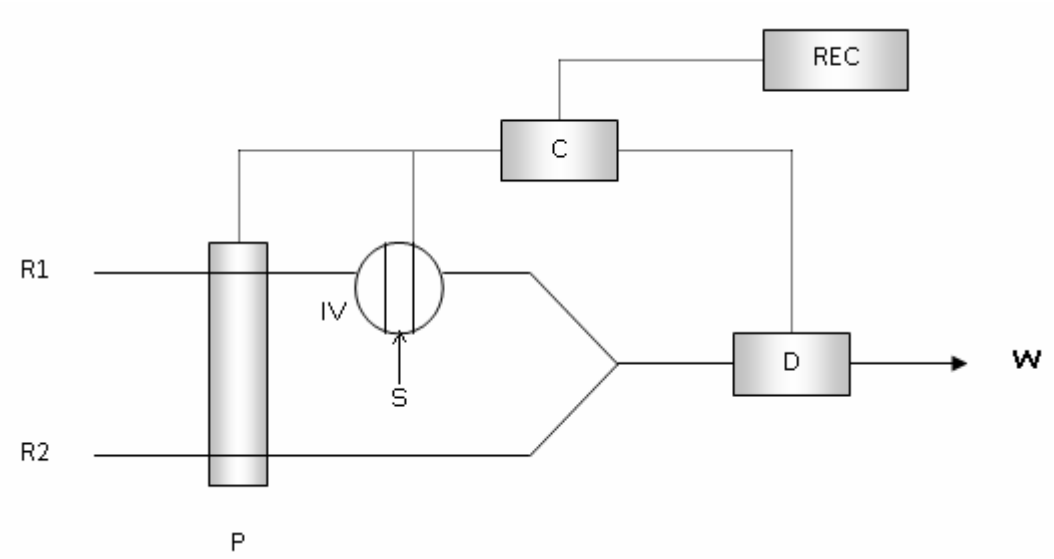

Figure 1. Stopped FI manifold for determination of phosphate: R1 = 0.02 M sodium molybdate in 0.15 M nitric acid, R2 = 0.25%w/v ascorbic acid, S = sample, P = Peristaltic pump, C = controller, IV = six-port injection valve, D = colorimetric detector, REC = recorder, W = waste

Procedure

A standard or sample (55 μl) was injected into a stream of 0.02 M sodium molybdate, which was then merged with a stream of 0.25%w/v ascorbic acid and flowed further to a colorimetric flow cell (see Figure 1). The flow rate of each stream was 2.0 mL min^{-1}. After injection, the injected zone was travelled for 3 s (travelling time) before being stopped for a period of 10 s (stopping time) in the flow cell at the detection point of the detector by stopping the pump. During this period, the colour intensity of the phosphomolybdenum blue product increased continuously, and the absorbance at about 630 nm was recorded as a stopped FI profile (FIgram). Then the flow was started again to

propel a bolus of the solution mixture out of the flow cell for a period of 8 s (washing time). The slope of stopped-FIgram was used for phosphate determination by plotting slope versus phosphate concentration. A linear calibration graph was obtained, which could be utilised for the determination of phosphate in a sample. The whole cycle took about 40 s, with a consumption of 0.4 mL of each reagent.

Results and Discussion

The stopped-flow injection system for the determination of phosphate involves the phosphate-molybdate-ascorbic acid reaction to form a molybdenum blue product as shown [15].

$$PO_4^{3-} + \text{Acidic molybdate} \rightarrow \text{Heteropoly acid}$$

$$\text{Heteropoly acid} + \text{Ascorbic acid} \rightarrow \text{Molybdenum blue}$$

Ascorbic acid is selected as a reducing agent because it is efficient and more friendly to the environment. Tin (II) chloride can also be used, but it produces a heavy metal waste. With sodium sulfite as a reducing agent, the bubble of sulfur dioxide is evolved in the flow system, which may cause many problems.

In this work, a semi-automatic stopped FI-analyser was employed (see Figure 1). Via the controller, presetting values can be defined for travelling time (T), the period for sample to flow from the injection point to the detection point prior to the stopping of the flow and the monitoring of the reaction product. Also defined is stopping time (S), the period during which the flow is stopped, and washing time (W), the period during which the system is washed by restarting the flow (after stopping). Each operation cycle comprises these three periods. The analyser is on standby (the flow is halted) after each operation cycle is finished, ready for the user to start the next cycle. Due to the non-continuous flow, the stopped FI method consumes a smaller amount of reagent than the normal FI one.

During the stopping period, the change of absorbance due to the reaction product was recorded versus time, so that the kinetics of the reaction could be continuously monitored. Using the reagents mentioned above, the reaction of the phosphate was relatively fast compared to the silicate, and the kinetic data could be used for a discriminative determination of both of the species [10]. In this work, a stopping period of 10 s was selected in order to avoid interference from the silicate, which might be present in the soil extract at a high concentration. The stopped-FI signal profiles of standard solutions containing different concentrations of phosphate are illustrated in Figure 2. It can be noticed that the slope of the signal profile is linearly proportional to the phosphate concentration.

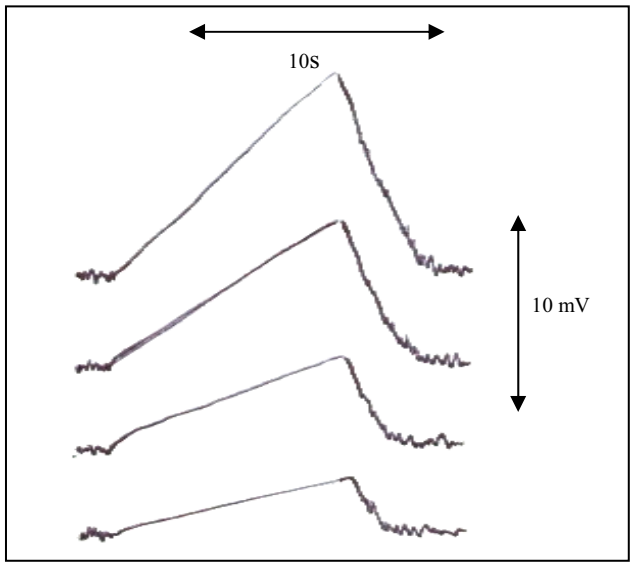

Figure 2. Stopped-FI profiles obtained with different concentrations of phosphate (0.3, 0.5, 1.0 and 2.0 mg P L^{-1}, respectively)

Effect of reagent concentration

1. Sodium molybdate

The sodium molybdate concentration was varied from 0.005 to 0.04 M while ascorbic acid and nitric acid concentration was fixed at 0.5% (w/v) and 0.08 M, respectively. A series of standard phosphate solutions was injected and calibration graphs (plot of slope of signal versus phosphate concentration) were constructed. A plot of slope of the calibration graph (sensitivity) versus concentration of sodium molybdate is illustrated in Figure 3, indicating that 0.02 M sodium molybdate should be selected.

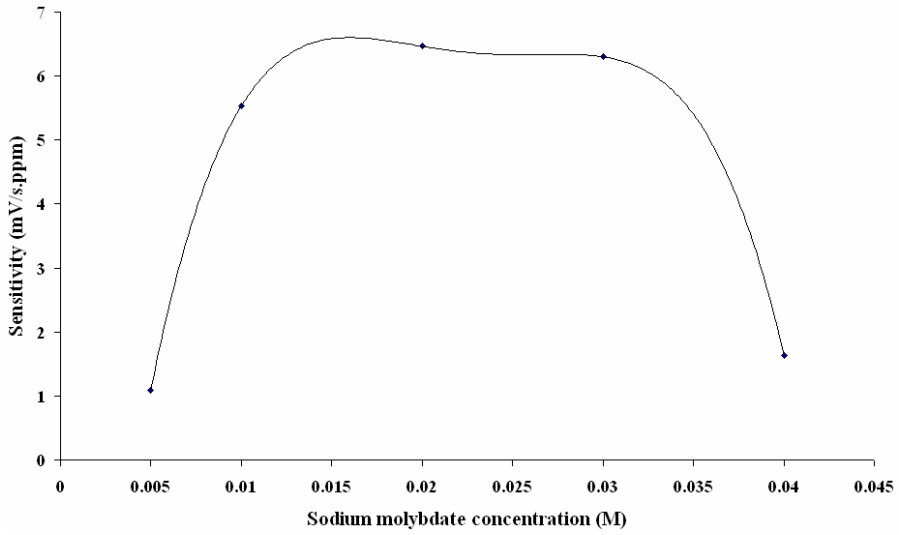

Figure 3. Effect of sodium molybdate concentration

2. Ascorbic acid

Ascorbic acid concentrations in the range of 0.05 to 2%w/v were tried while the sodium molybdate and nitric acid concentration was fixed at 0.02 and 0.08 M, respectively. The effect of ascorbic acid concentration on the slope of the calibration graph is illustrated in Figure 4. Although 1 and 2% w/v ascorbic acid provided higher sensitivity than 0.05, 0.25 and 0.5% w/v, both of these concentrations gave a high noise signal that caused difficulty in measuring the slope of stopped-FIAgrams. Thus, ascorbic acid of 0.25% w/v was chosen because it provided higher sensitivity than 0.05 and 0.5% w/v.

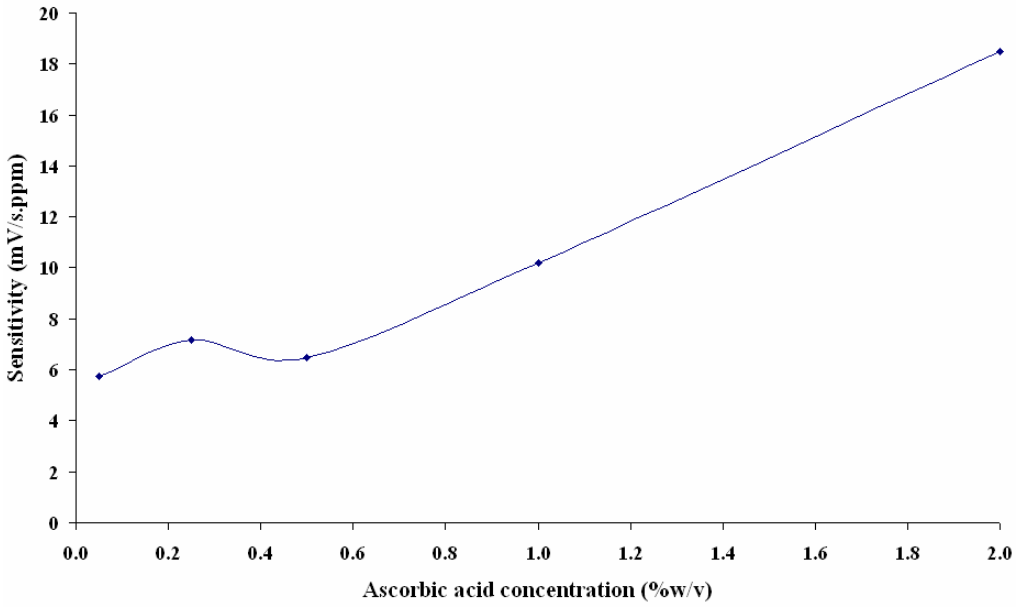

Figure 4. Effect of ascorbic acid concentration

3. Nitric acid

The effect of nitric acid concentration (0.05 to 0.4 M) was investigated while the sodium molybdate and ascorbic acid concentration was fixed at 0.02 M and 0.25% w/v, respectively. A sharp increase in sensitivity was observed when nitric acid concentration was increased up to 0.15 M, but at concentration higher than 0.15 M the sensitivity declined, as shown in Figure 5. In addition, when sulfuric acid of different concentrations was used, a similar trend was also observed, indicating that the reaction seemed to progress well in a narrow range of acid concentration.

Calibration graph and precision

Using the above selected conditions, a linear calibration graph in the range of 0.3 - 6 mg P L^{-1} ($y=10.091x + 5.5403$, $R^2=0.9987$) is obtained. The detection limit calculated from the calibration data is found to be 0.02 mg P L^{-1}. A relative standard deviation obtained for 10 replicated injections of 2 mg P L^{-1} is 2.6%. The method has a sample throughput of 90 h^{-1}, with a consumption of 0.4 mL of each reagent.

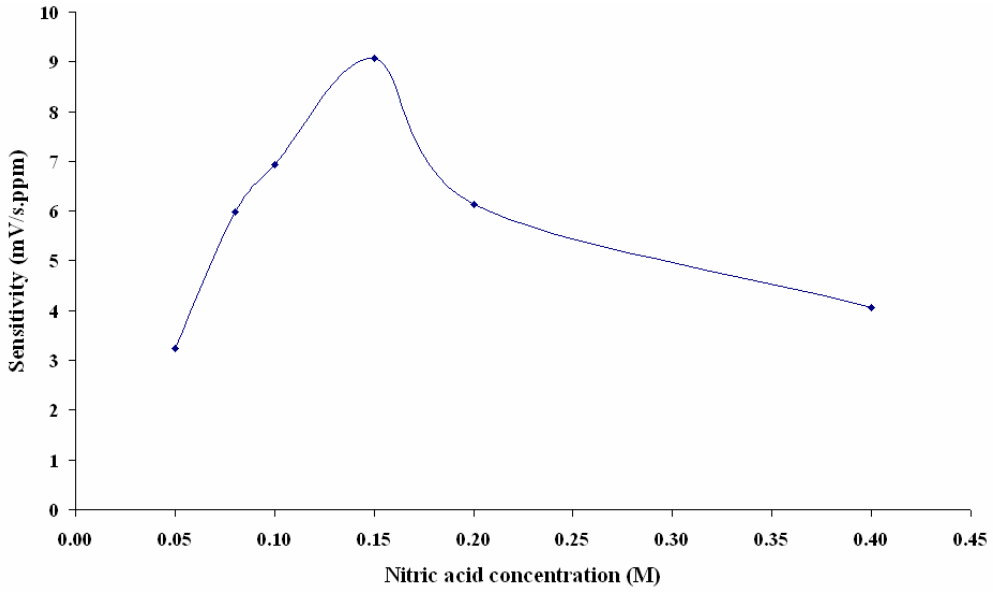

Figure 5. Effect of nitric acid concentration

Application to real samples

The developed method was applied to soil samples. The procedure for collecting and extracting soil has been described in the materials and methods section. The accuracy of the proposed method was determined by comparing results obtained with those from a batch spectrophotometric method [16]. Comparative analyses of the same samples were carried out on the same day and the results are summarised in Table 1. The phosphate contents obtained from the stopped-FI method (x) agree well with those obtained by the batch method (y), which is indicated by the value of the slope, the intercept and R^2 of the correlation graph between the two methods being closed to 1, 0 and 1, respectively ($y=1.0064x+0.0357$, $R^2=0.9997$). According to the t-test at 95 % confidence [17], these methods correlate to each other well.

The proposed method was also applied to the determination of soluble phosphate in fertilisers. The results are shown in Table 2. Again, according to the t-test at 95 % confidence, the two methods correlate well to each other.

The determination of phosphate in soil and fertiliser samples by the stopped-FI method seems to have more advantages than the batch method. The former method can reduce the effect of interferences from such species as silicate and arsenate, because their reaction rates in the molybdenum blue reaction are slower than that of the phosphate. By using the slope of FIAgram instead of peak height, the effect due to coloured and colloidal species usually found in these samples is also decreased. In addition, the stopped-FI technique is simple, inexpensive, rapid, and has a good sensitivity. Also, very small amounts of reagents and sample are consumed.

Table 1. Content of available phosphate in soil samples found by stopped-FI and batch method

Sample number	Physical appearance	[P] in soil sample (µg/g) [a]		% Difference [b]
		sFI method	Batch method	
1	Friable, wet	11.2 ± 0.3	11.2 ± 0.0	0.0
2	Friable, wet	11.1 ± 0.2	11.0 ± 0.2	0.9
3	Friable, wet	13.3 ± 0.3	13.5 ± 0.2	-1.5
4	Friable, wet	24.0 ± 1.2	24.1 ± 0.0	-0.4
5	Friable, wet	15.2 ± 0.7	15.0 ± 0.2	1.3
6	Laterite	7.4 ± 0.2	7.4 ± 0.1	-0.8
7	Friable, wet	12.8 ± 0.6	12.6 ± 0.1	1.6
8	Friable, wet	11.9 ± 0.5	11.8 ± 0.0	0.8
9	Black soil	120 ± 2	119 ± 1	0.8
10	Sand	42.0 ± 0.5	42.5 ± 0.0	-1.2
11	Friable, sand	12.4 ± 0.5	12.6 ± 0.0	-1.6
12	Friable, wet	5.7 ± 0.2	5.7 ± 0.1	0.0
13	Hard, dry	23.4 ± 1.2	23.6 ± 0.2	-0.8
14	Friable, wet	11.0 ± 0.5	10.9 ± 0.1	0.9
15	Friable, wet	5.1 ± 0.3	5.0 ± 0.1	2.0
16	Hard, dry	24.0 ± 2.4	24.2 ± 0.3	-0.8
17	Friable, wet	43.2 ± 0.6	43.1 ± 0.0	0.2
18	Friable, wet	14.5 ± 0.0	14.3 ± 0.0	1.4
19	Mud	3.0 ± 0.1	3.0 ± 0.0	0.0
20	Mud	5.0 ± 0.0	5.0 ± 0.1	0.5

[a] mean of triplicate results

[b] % difference = [(FIA value – batch value)/batch value] x 100

Table 2. Content of soluble phosphate in fertiliser samples found by stopped-FI and batch method

Sample number	$[P_2O_5]$ in fertiliser sample (mg/g)		% Difference[a]
	sFI method	Batch method	
1	85 ± 6	83 ± 2	3
2	69 ± 4	62± 1	10
3	50 ± 1	36± 1	37
4	396 ± 5	321 ± 0	23
5	63 ± 2	53 ± 2	19
6	76 ± 4	67 ± 2	13
7	98 ± 5	90 ± 2	8
8	136 ± 5	125 ± 2	9
9	0.7 ± 0.0	1.0 ± 0.0	-26
10	0.8 ± 0.0	0.7± 0.0	13

[a] % difference = [(FIA value – batch value)/batch value] x 100

Conclusions

The stopped FI method based on the phosphate-molybdate-ascorbic acid reaction (the molybdenum blue method) has been developed for the determination of phosphate in soil and fertiliser samples. The effect of concentration of reagents was investigated. The suitable concentration for sodium molybdate, ascorbic acid and nitric acid, is 0.02 M, 0.25 %w/v and 0.15 M, respectively. A linear calibration graph (plot of slope of the stopped-FIgram versus phosphate concentration) in the range of 0.3-6.0 mg P L^{-1} was employed for the determination of phosphate in samples. The developed method provides various advantages, including high sensitivity and selectivity, and a simple, fast and cheap analysis.

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ภาคผนวก ก.7

Stopped-flow injection spectrophotometric method for determination of chlorate in soil, ***Mj. Int. J. Sci. Tech.***, 2(01) (2008) 383-390

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Full Paper

Stopped-flow injection spectrophotometric method for determination of chlorate in soil

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Abstract: A stopped-flow injection (FI) spectrophotometric procedure based on iodometric reaction for the determination of chlorate has been developed. Standard/sample was injected into a stream of potassium iodide solution and then merged with a stream of hydrochloric acid solution to produce triiodide. By stopping the flow while the sample zone is being in a mixing coil, a slow reaction of chlorate with iodide in acidic medium was promoted to proceed with minimal dispersion of the triiodide product zone. When the flow started again, a concentrated product zone was pushed into a flow cell and a signal profile due to light absorption of the product was recorded. Employing a lab-built semi-automatic stopped-FI analyser, the analysis can be performed with higher degree of automation and low chemical consumption. Linear calibration graph in the range of 5-50 mg ClO_3^- L^{-1} was obtained, with detection limit of 1.4 mg ClO_3^- L^{-1}. Relative standard deviation of 2.2% (30 mg ClO_3^- L^{-1}, n=10) and sample throughput of about 20 h^{-1} were achieved. The system was applied to soil samples and validated by batch spectrophotometric and standard titrimetric methods.

Keywords: chlorate, stopped-flow injection, iodometry, soil

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Introduction

Chlorate is utilised in the bleaching process in the pulp and paper industry [1]. In addition, chlorate has been employed in agriculture as a herbicide and as a defoliant especially between the years 1930 and 1950. Recently, especially in Thailand, chlorate compounds, e.g. potassium chlorate, have been popularly used for promoting flowering and fruiting of longan. The plant can absorb chlorate through both leaf and root [2]. However, there have been some reports on the effect of chlorate causing damage to plants. The intake of chlorate in high amounts caused falling of leaves and death in plants [2]. Chlorate may compete with nitrate as a substrate for the enzyme nitrate reductase [3-5], which can reduce chlorate to chlorite which is toxic to plants [2,6].

From the above instance, information on chlorate content in soil is therefore useful for controlling the effect of chlorate on plants and the environment. Various methods for determination of chlorate have been reported such as ion chromatography [4], infrared spectrophotometry [7], batch spectrophotometry [8], and flow injection (FI) [9-10]. Ion chromatography and infrared spectrophotometry can quantitate chlorate at low concentration levels, but they require relatively high operating costs and complicated instruments. Batch and FI spectrophotometric methods have gained interest. They are based on different chemical reactions such as iodometric reactions [11], complexation of chlorate with rhenium-α-furildioxime [12], and decolourisation of indigo carmine by chlorate [8]. Although those procedures may provide simplicity and rapidness of analysis, they suffer from interferences and low sensitivities. Stopped-FI procedure can increase sensitivity by increasing the reaction time in a stopping period, thereby promoting more product. It also reduces the main interference due to oxygen in air by allowing the reaction to occur in a closed tube. The stopped-flow injection method with amperometric detection system has been developed for determination of chlorate in soil [13]. However, it involves a complicated system employing a water bath of 55 ^{o}C to accelerate the reaction.

In this work, we propose a stopped-FI spectrophotometric procedure for the determination of chlorate utilising iodometric reactions which were reported for batchwise analysis [14]. The sample is injected into a stream of potassium iodide and merged with a stream of acidic solution. Then, the sample-reagent mixture zone is stopped in a mixing coil to promote the slow reaction of chlorate with iodide in acidic medium to produce triiodide while no dispersion of the product zone occurs during the stopping period. When the flow starts again, the product zone is pushed into a flow cell giving rise to a highly sensitive signal to be recorded as a peak. Since the reaction proceeds in a closed system the interfering effect of oxygen in the air which is usually observed in a batch method is minimised. The peak height obtained is proportional to the chlorate concentration. A calibration graph in the range of 5-50 mg ClO_3^- L^{-1} is achieved with a detection limit of 1.4 mg ClO_3^- L^{-1}. Stopped FI also reduces the consumption of reagent and consequently minimises waste. The whole analysis cycle takes about 160 s, with a consumption of 2.3 mL of each reagent.

Materials and Methods

Chemicals

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Deionised water (Milli RX, Millipore) was used throughout. All reagents were of analytical reagent grade unless otherwise stated. Potassium chlorate (99.5 %w/w, Merck) was used to prepare a stock standard solution of 1000 mg L^{-1} ClO_3^-, by dissolving 0.1474 g of the chemical in water, making up to 100 mL in a volumetric flask. Potassium iodide (iodate free, Carlo Erba) (4.1711 g) was dissolved in 250 mL of water to obtain a 0.1 M iodide solution. Hydrochloric acid (7 M) was prepared by diluting 150 mL of conc. hydrochloric acid (Carlo Erba) with water to the final volume of 250 mL.

Sample preparation

Soil samples were collected from the longan plantation field in Chiang Mai, northern Thailand. A soil sample was taken from 15 points around the rim of the longan tree, at the depth of 15 cm. The sample was dried and ground before a portion of 100 g was taken for further treatment.

A portion (20 g) of each sample was extracted with water (20 mL) by shaking for 1 h. The mixture was then filtered through a filter paper (Whatman, No. 42), rinsed with water and the filtrate adjusted to a volume of 25.00 mL with water prior to analysis.

Stopped FI setup

The stopped FI system used is illustrated in Figure 1. A lab-built semi-automatic stopped-FI analyser as reported previously was employed. It consisted of a peristaltic pump (MP-3, Eyela, Japan), a 6-port injection valve (Upchurch, USA) and a microcontroller (Basic stamp II SX) for timing control of the pump and the valves. A detector was a simple spectrophotometer (Spectronic 21, Spectronic Instrument, USA), equipped with a 10 mm path-length flow-through cell (Hellma, Germany). All tubing used was of PTFE tubing of 0.5 mm inner diameter, except Tygon pump tubing (Saint-Gobain Performance Plastics, USA).

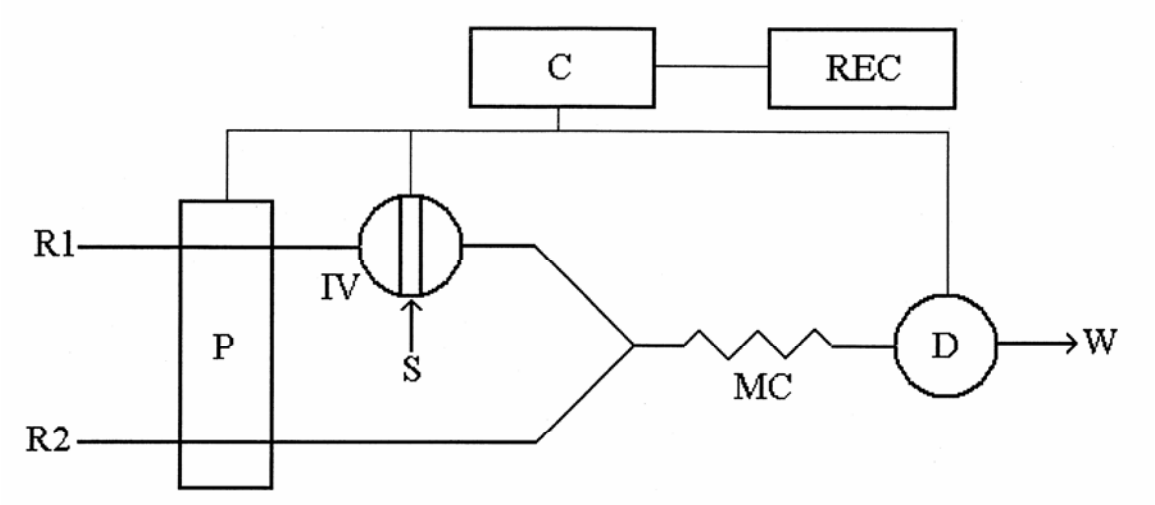

Figure 1. Stopped-FIA manifold for determination of chlorate: R1 = 0.1 M potassium iodide, R2 = 7 M hydrochloric acid, P = peristaltic pump, C = controller, IV = six-port injection valve, MC = mixing coil, D = detector, REC = recorder, S = standard/sample, W = waste

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Procedure

A standard or sample (55 µl) was injected into a stream of 0.1 M KI, before merging with a stream of 7 M HCl and flowing to a mixing coil (see Figure 1). This design prevents the contact of concentrated acid with the injection valve, which may cause corrosion of the valve. The flow rate of each stream was 2.0 mL min^{-1}. The operation cycle and peak profiles are illustrated in Figure 2. After injection for 9.5 s, the sample zone was halted for 90 s in the mixing coil by stopping the pump via the control unit of the stopped flow analyser. Then the flow was restarted again to push the zone through the detecting flow cell where absorbance at 400 nm was continuously recorded. The last step would take 60 s. A calibration graph was a plot of peak height versus chlorate concentration. Concentration of chlorate in an unknown sample was then evaluated from the calibration graph.

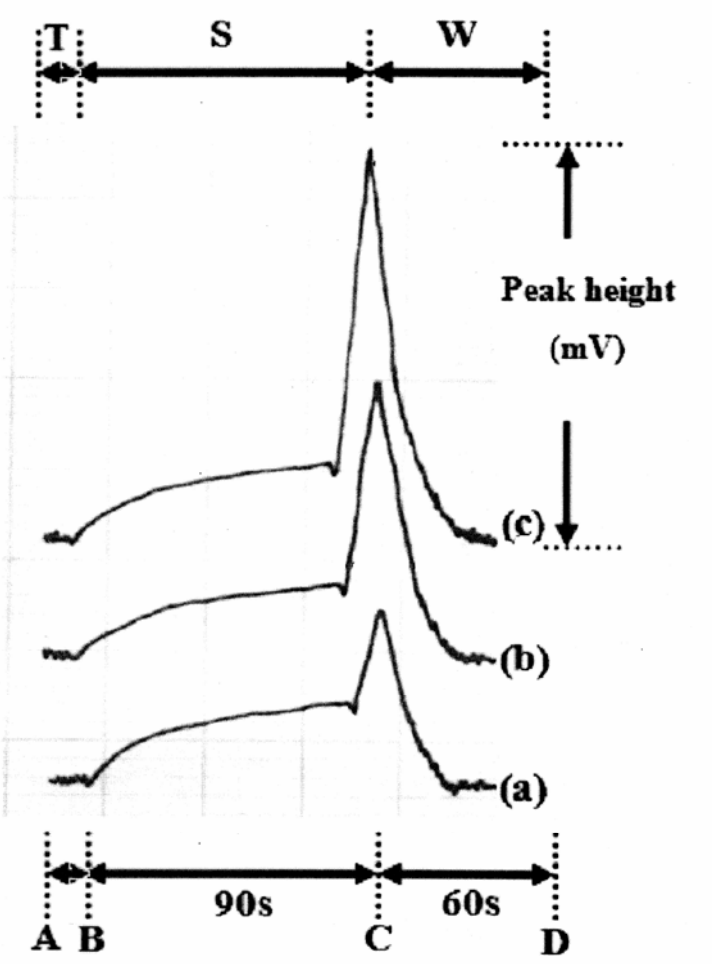

Figure 2. Stopped-FI signal profiles obtained for chlorate concentration of (a) 5 (b) 10 and (c) 20 mg ClO_3^- L^- (T = travelling time: a period from the injection to the stopping point, S = stopping period: a period during which the flow is halted, W = washing time: a period from the restarting of the flow to the end of the analysis, A = sample injecting point, B = point of stopping the flow, C = point of restarting the flow, D = end point of operation cycle)

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Results and Discussion

The stopped-FI method for determination of chlorate involves iodometric chemical reactions as follows [14]:

$$ClO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + Cl^- + 3H_2O \quad (1)$$

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

The triiodide from this reaction can be detected spectrophotometrically at 400 nm. Hydrochloric acid is appropriate for this reaction. An oxy acid such as nitric acid and sulphuric acid is to be avoided in this reaction because it can be oxidised by chlorate.

The first reaction is slow, depending on concentration of ClO_3^-, I^- and H^+. By increasing the concentration of either H^+ or I^- the reaction can be accelerated. Use of elevated temperature also helps [13,15]. However, at high temperature and/or high concentration of H^+, iodide is quantitatively oxidised by oxygen, so the determination cannot be easily carried out in a batch method. Employing a FI manifold, the reaction product can be enhanced during the stopping period designed to extend the reaction time in a closed system of the mixing coil before spectrophotometric measurement is taken [13,16]. This novel approach is different from the conventional stopped-FI in that the reaction zone is halted in the flow cell so that a change in absorbance can be monitored [17-18]. A calibration graph was plotted as peak height versus concentration of chlorate. Various parameters affecting the procedure were then studied.

Effect of stopping time

With concentration of potassium iodide and hydrochloric acid being kept constant at 0.3 and 3 M respectively, stopping time was varied: 15, 30, 60 and 90 s. A series of standard chlorate solutions (100-500 mg ClO_3^- L^{-1}) was injected. It was found that higher sensitivity was obtained the longer the stopping time. The stopping time of 90 s was chosen due to the limitation of the instrument for which a maximum stopping time of 99 s can be set and this period provided enough sensitivity for chlorate determination in soil sample.

Effect of iodide and hydrochloric acid concentration

Effects of concentration of potassium iodide and hydrochloric acid solution were studied in the ranges of 0.1-0.6 M and 5-7 M respectively. With the stopping time of 90 s, a series of standard chlorate solutions (5-50 mg ClO_3^- L^{-1}) were injected in order to construct a calibration graph for each condition. The slopes of the calibration graphs are shown in Figure 3. A higher slope of the calibration graph (higher sensitivity) was obtained with using of higher concentrations of either iodide or hydrochloric acid, except at 7 M hydrochloric acid wherein a higher concentration of potassium iodide led to a lower slope of the calibration graph. This may be due to the oxidation of the iodide ion by air or iodate impurity in potassium iodide chemical, which is more pronounced at high content of acid [13] causing high blank signal and low slope. However, the reaction was minimised at lower iodide

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concentration (0.1 M) because of inadequate quantity of iodide and/or iodate ion for this reaction. Potassium iodide and hydrochloric acid at 0.1 M and 7 M respectively were selected for further study.

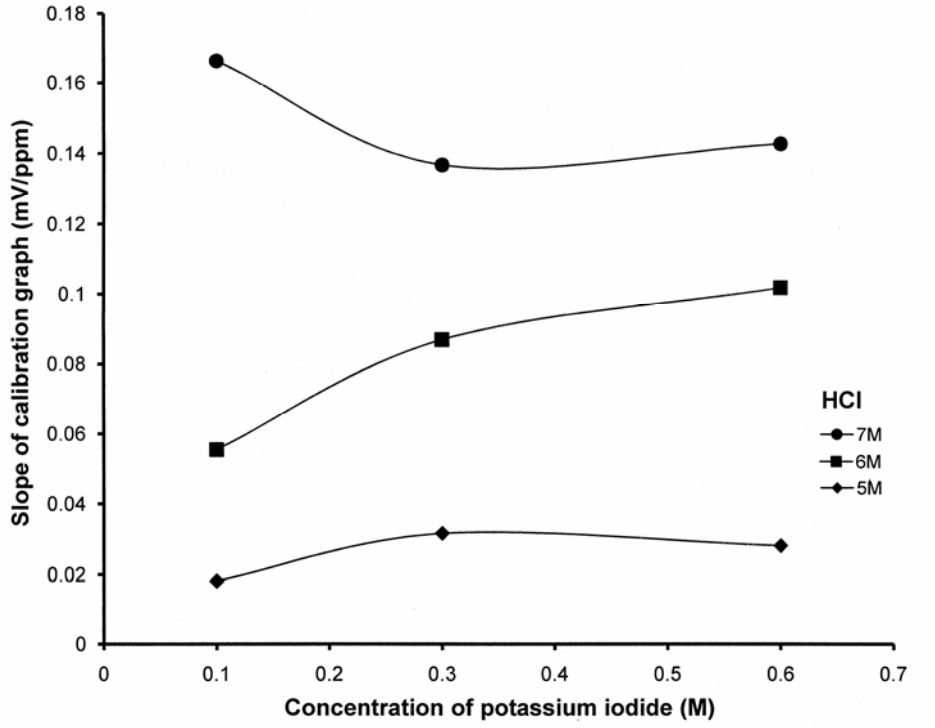

Figure 3. Effect of potassium iodide concentration on sensitivity (slope of the calibration graph of chlorate in concentration range of 5-50 mg ClO_3^- L^{-1}) at different concentrations (5, 6 and 7 M) of hydrochloric acid

Analytical characteristics

A linear calibration graph in the range of 5-50 mg ClO_3^- L^{-1} (y=0.130x + 0.012, R^2=0.996) was obtained. The detection limit calculated from the calibration data was found to be 1.4 mg ClO_3^- L^{-1}. The relative standard deviation was 2.2% for 10 replicated injections of 30 mg ClO_3^- L^{-1}. With the stopped-FI, the consumption of reagent and consequently the production of waste could be reduced. Each analysis cycle consumed about 2.3 mL each of 0.1 M KI and 7 M HCl solutions.

Analysis of soil samples

Soil samples were analysed for chlorate content by the proposed method. They were also analysed by a batch spectrophotometric method [8] and the titrimetric standard method [14]. The results are summarised in Table 1. Chlorate content found by stopped-FI method (x) agrees well with that found by the standard titrimetric method (y), as indicated by the slope, intercept and R^2 of the correlation graph of the two methods being closed to 1, 0 and 1 respectively (y=0.95x + 0.08, R^2=0.997). According to the t-test at 95% confidence level [19], there is no significant difference of the results from the three methods.

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Table 1. Chlorate content in soil samples obtained by stopped-FI, standard titrimetric and batch spectrophotometric method

Sample number	Chlorate content found (μg g^{-1}) by		
	Stopped-FI*	Titrimetry [14]*	Batch spectrophotometry[8]
1	194 ± 5	189 ± 1	196
2	258 ± 2	245 ± 1	226
3	107 ± 4	103 ± 7	101
4	36.5 ± 0.4	40 ± 2	33.7
5	49.5 ± 0.1	50 ± 1	47.8
6	69 ± 1	62 ± 2	75.7
7	167 ± 2	155 ± 2	142
8	57.8 ± 0.9	46 ± 2	28.9
9	37.8 ± 0.3	36 ± 2	32.3
10	101 ± 4	102 ± 1	91.2
11	127 ± 4	122 ± 1	91.7
12	9.8 ± 0.4	9 ± 1	10.8
13	50.4 ± 0.5	46 ± 1	43.1
14	106 ± 1	96 ± 1	119
15	214 ± 4	201 ± 2	163
16	30.0 ± 0.6	27 ± 1	22.7
17	10.6 ± 0.2	9 ± 1	6.4
18	12.6 ± 0.2	11 ± 0	6.2

*mean of triplicate results

Conclusions

A novel stopped-FI method is proposed, in which there is a stopping of the flow to hold an injected zone of standard/sample in a mixing coil for promoting the reaction to take place without dispersion of the resulting product. It is applied to a slow reaction of chlorate with acidic iodide for the determination of chlorate. The procedure provides a suitable sensitivity for analysis of soil from longan plantation fields where chlorate is used to promote fruiting of the longan trees. With a simple instrument set-up employing a lab-built semi-automatic stopped-FI analyser, the determination can be done with high degree of automation and low reagent consumption.

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ภาคผนวก ก.8

Determination of chloride in admixtures and aggregates for cement by a simple flow injection potentiometry, ***Talanta***, 76 (2008) 365-368

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Determination of chloride in admixtures and aggregates for cement by a simple flow injection potentiometric system

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ABSTRACT

A simple flow injection system using three 3-way solenoid valves as an electric control injection valve and with a simple home-made chloride ion selective electrode based on Ag/AgCl wire as a sensor for determination of water soluble chloride in admixtures and aggregates for cement has been developed. A liquid sample or an extract was injected into a water carrier stream which was then merged with 0.1 M KNO_3 stream and flowed through a flow cell where the solution will be in contact with the sensor, producing a potential change recorded as a peak. A calibration graph in range of 10–100 mg L^{-1} was obtained with a detection limit of 2 mg L^{-1}. Relative standard deviations for 7 replicates injecting of 20, 60 and 90 mg L^{-1} chloride solutions were 1.0, 1.2 and 0.6%, respectively. Sample throughput of 60 h^{-1} was achieved with the consumption of 1 mL each of electrolyte solution and water carrier. The developed method was validated by the British Standard methods.

1. Introduction

Reinforced concrete is a concrete with incorporating of reinforcement bars (rebars) to strengthen a material that would otherwise be brittle. Typically, concrete has high resistance to compressive stresses (about 4000 psi), but not to tension, while steel has high strength in tension. The reinforced concrete structures can support 300–500 times their combined weight [1]. The alkaline chemical environment of concrete can protect steel from corrosion. However, chloride (Cl^-) content in concrete plays important role on quality of reinforced concrete, therefore, Cl^- induces depassivation of the steel rebars and initiation of the corrosion process leading to degradation of the structure. Chloride in concrete comes from cement, aggregate materials and water used for creating concrete, or by diffusion of Cl^- from outside of the structure through pore water in concrete. Determination of Cl^- in materials for concrete is necessary. Aggregate materials could be analyzed according to the British Standard (BS 812: Part 117: 1988), by mean of back titration of silver nitrate used in precipitation of Cl^- by using thiocyanate as titrant [2]. Referring to BS EN 480-10:1997, Cl^- in admixtures for concrete, mortar and grout was determined by potentiometric titration with precipitation as silver chloride, monitoring of end point by using ion selective electrode (ISE) [3]. However, these methods are tedious, slow and consume large amounts of reagents, not suitable for analysis a large number of samples. New method which is faster, less chemical consumption and has higher degrees of automation would be needed. Several flow based methods have been reported for determination of Cl^- using different detection principles [4–17]. Spectrophotometric detection based on reaction of Cl^- with mercuric thiocyanate to release SCN^- to form complex with Fe^{3+} has been widely used [4–8]. Turbidity measurement due to formation of AgCl [9,10] or absorbance measurement of chloranilate released from silver chloranilate solid phase reactor [11] was also proposed to avoid using of toxic mercury compound. By new flow techniques, e.g., sequential injection (SI) [6], FI with reagent immobilized on solid phase [7], and multisyringe flow injection (MSFI) [8], the amounts of toxic mercury reagent could be reduced several orders. Potentiometric detection was also popularly used in flow system for Cl^- determination, which provided the same sensitivity as the spectrophotometric one [12–17]. A simple home-made Cl^- ISE as in wire [17] or tubular [14,15] format, or a commercial Cl^- ISE [12] has been employed in different flow techniques.

In this paper, we developed a simple FI system using three 3-way solenoid valves as an electronic control injection valve and with a simple home-made Cl^- ISE based on Ag/AgCl wire as a sensor for determination of water soluble Cl^- in admixtures and aggregates for cement. The Cl^- ISE could be prepared by oxidation of a jewelry grade Ag wire electrochemically or chemically and could be used for at least 3 months by storage in 3 M KCl. A linear calibration graph plotting between peak height (mV) versus logarithms of

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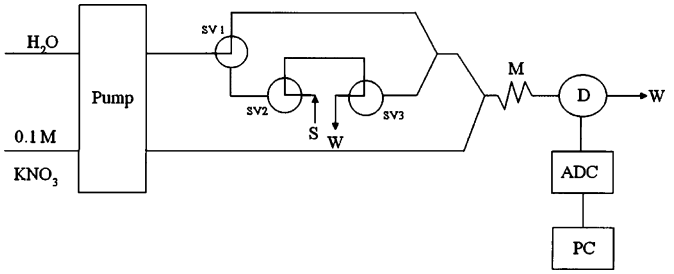

Fig. 1. FI manifold of flow injection potentiometric detection of chloride; SV1-3 = solenoid valves, S = standard/sample, W = waste, M = mixing coil, D = detector, ADC = analog to digital converter unit, and PC = personal computer (injection valve is in loading position).

Cl^- concentration in range of 10–100 mg L^{-1} was obtained with a detection limit of 2 mg L^{-1}. Relative standard deviation in range of 0.6–1.2% was observed. Sample throughput of 60 h^{-1} was achieved with the consumption of 1 mL each of 0.1 M KNO_3 electrolyte solution and water carrier.

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 (1000 mg L^{-1}) was prepared by dissolving 0.1648 g of sodium chloride (Merck, Darmstadt, Germany) in water and making up to a volume of 100 mL in a volumetric flask. 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. 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 and Cl^- ISE

A potentiometric flow through cell was fabricated from a Perspex plastic block, similar to the previous reported [17], by cutting and drilling to form channels for inserting a Ag/AgCl wire working electrode (WE) and a Ag/AgCl reference electrode (RE) (3 M KCl) (MW-2030, BAS, Indiana, USA), and for solution inlet and outlet.

A silver wire (0.5 mm in diameter) obtained from a local jewelry shop was used for preparation of a 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 electrode by assembling into the flow through cell described above. The electrode was placed in 3 M KCl solution after used and can be last long more than 3 months.

2.3. FI manifold

The FI system used is schematically depicted in Fig. 1. It consisted of a peristaltic pump (Ismatec, Switzerland), a 3-way connector and three 3-way solenoid valves (Biochemvalve, USA) connecting to be an injection valve (see Fig. 1), a flow through cell with Ag/AgCl electrodes connecting to a home-made potentiometer, an analog to digital converter unit for data recording with relevant software (PowerChrom280, eDAQ, Australia) and a computer. Data acquisition was controlled by eDAQ chart software and eDAQ FIA extension software was used for data analysis. Sample loading or injection was performed manually by switching electrical switch for applying power to the solenoid valves. All tubings used were of PTFE tubing of inner diameter of 0.5 mm, except Tygon pump tubing (Saint-Gobain Performance Plastics, USA).

2.4. Procedures

The water carrier and 0.1 M KNO_3 solution were propelled with constant flow rate of 1 mL min^{-1} each, to attain a stable baseline recording on the computer. While the injection valve was at loading position (see Fig. 1), a standard/sample was filled in a sample loop (100 μL), then by turn on the switch to change all three solenoid valves to another position (injection valve turn to injecting position), the solution in the sample loop was injected into the system and flowed to the flow cell to monitor for potential difference between WE and RE as the zone passed the WE, recording as a peak. A calibration graph was a plot of the peak height obtained as a function of the logarithm value of concentration of the Cl^- standard. Chloride concentration in a sample was evaluated from the calibration graph.

Aggregate sample preparation was carried out following the standard method [2], briefly, soak 2 kg of aggregates (particle size <20 mm) in 2 kg of deionized water for 24 h and take the supernatant for analysis.

Admixture sample was prepared following the standard method [3], briefly, accurately weigh 10 g of a liquid admixture sample and dilute with deionized water to obtain Cl^- concentration within about the middle range of the calibration graph (dilution about 2–5 times).

3. Results and discussion

3.1. Preparation of Ag/AgCl wire Cl^- ISE and condition for FI system

The Ag/AgCl wire could be prepared by oxidation of a Ag wire electrochemically or chemically using ferric chloride in HCl. Effect of oxidation time on sensitivity of the electrode was investigated. The produced Ag/AgCl electrodes were tested for theirs performance by using FIA system described in Section 2.3. After preliminary studies (described below), some parameters were fixed, i.e., concentration of KNO_3 electrolyte solution of 0.1 M, flow rate of water carrier and the electrolyte solution of 1 mL min^{-1} each line, injection volume of 100 μL and mixing coil of 50 cm. A series of Cl^- standard solutions was injected into the systems and a calibration graph (a plot of peak height (Y) versus logarithm of Cl^- concentration (X)) was obtained for each electrode. By electrooxidation of Ag wire in a 0.1 M HCl for 0.5–3 h to produce AgCl coated Ag wire, the resulted electrode gave closely response to Cl^- ion in concentration range of 3.6–3550 mg L^{-1}, with slope of 49.8–55.4 mV/decade. The electrode was tested for its durability by placing it in a 3 M KCl solution, which was vigorously stirred using magnetic stirrer and the potential of the electrode was recorded versus a Ag/AgCl reference electrode for a period of 17 h. It was found that the potential of the electrode was only slightly change during this period.

Despite the Cl^- ISE prepared by this method worked well, the voltage supplier is needed for electrooxidation of Ag, so the preparation of the electrode by chemical oxidation [17] was tried. The clean Ag wire was dipped into a solution containing 0.5 M $FeCl_3$ and 1.0 M HCl for 1–24 h. The electrode was tested for determination of Cl^- in range of 10–100 mg L^{-1}, which was expected to be found in samples. It was found that the comparable slopes of calibration graphs (58.1–60.3 mV/decade) were obtained with good linearities ($r^2 > 0.999$), indicating that the dipping time does not affect on sensitivity of the electrode. Dipping time of ≥24 h was

Table 1
Analytical features of the developed FI potentiometric method versus some reported methods

Method	Principle	Sample	Linear range (mg L^{-1})	LOD (mg L^{-1})	R.S.D. (%)	h^{-1}	Ref.
FI spect	Cl^--$Hg(SCN)_2$-Fe^{3+}	Water	0–50	3	2.5	37	[6]
FI spect	$Hg(SCN)_2$ impregnated on epoxy resin bead	Water	2.0–7.8	0.5	2.2	100	[7]
MSFI spect	Cl^--$Hg(SCN)_2$-Fe^{3+}, use small amounts of reagent	Water	1–40	0.2	0.8	130	[8]
SI turbidity	Forming of AgCl precipitate	Ground, surface, wastewater	2–400	2	3.7	57	[9]
FI spect	Release of chloranilate from silver chloranilate solid phase reactor	Water	0.5–100	0.3	1.1	80	[11]
SI poten	On-line dialysis, tubular Ag/AgCl electrode	Electroplating bath	3550–35500	–	–	40	[14]
FI poten	Ag/AgCl electrode with on-line dialysis	Milk and coconut water	4–1000	0.4	1.2	90	[16]
SI-LAV poten	Ag/AgCl electrode	Water	3.6–28.4, 28–298	3.6	0.7–1.3	50	[17]
FI poten	Ag/AgCl wire	Cement aggregates and admixtures	10–100	2	0.6–1.2	60	This work

Spect = spectrophotometry, poten = potentiometry, FI = flow injection, SI = sequential injection, MSFI = multisyringe flow injection, and LAV = lab-at-valve.

selected for electrode preparation to ensure a good sensitivity and long time stability of the electrode.

Effect of purity of Ag wire was investigated by using a high purity Ag wire (diam. 0.25 mm, >99.99%, Sigma–Aldrich) to prepare the Ag/AgCl electrode. It was found that the sensitivity obtained from this electrode is comparable to that of preparing from Ag wire from jewelry shop, usually called Sterling silver which has purity of 92.5% Ag (slopes of calibration graphs were 58.9 and 58.7 mV/decade, respectively).

Effect of electrode contacting area was investigated by dipping Ag wire at different depth (0.5–2.0 cm) for 24 h and the resulted ISE were tested using FIA system as described above. It was found that the length of Ag/AgCl electrode did not affect on sensitivity of Cl^- determination (electrode slopes in range of 58.2–59.9 was observed). However, a broader peak was observed for the longer electrode, thus an electrode length of 1.5 cm which gave a calibration graph of $Y = 59.9X - 24.7$, $r^2 = 0.9996$) was selected for further experiment. By keeping the electrode in a solution of 3 M KCl after used, stability of the electrode was good for longer than 3 months, with slope of calibration graph changing from ~59 to ~53 mV/decade.

Carrier and KNO_3 electrolyte solutions flow rate in range of 1–4 mL min^{-1} and length of mixing coil in range of 20–80 cm were studied, by injecting of 50 mg L^{-1} Cl^- into the system. It was found that only slightly different in peak height was observed for all flow rate and mixing coil length studied. A flow rate of 1 mL min^{-1} and mixing coil length of 50 cm were selected. Effect of pH of 0.1 M KNO_3 solution was investigated and found that similar peak heights were obtained in pH range of 1–8 and shorter peaks were observed at pH higher than 10. This has been explained that Ag_2O was formed on the electrode surface at high pH, lead to diminishing of electrode sensitivity [18]. A KNO_3 solution without adjusting pH was selected. According to the theory, the potential obtained by ISE depends on temperature, however, all experiments were carried out in an air condition room of about 25 ± 1 °C, thus no significant variation due to temperature change was observed.

Table 2
Chloride contents in cement aggregates and admixtures found by the FI potentiometric and standard methods

Sample	Chloride content (%, w/w)	
	FI–potentiometry[a]	Standard method[b]
Admixtures		
1	0.0109 ± 0.0003	0.01
2	0.0113 ± 0.0003	0.01
3	0.0076 ± 0.0002	0.01
4	0.0141 ± 0.0003	0.01
5	0.082 ± 0.003	0.08
6	0.093 ± 0.002	0.09
Aggregates		
1	0.0010 ± 0.0001	<0.01
2	0.0080 ± 0.0001	<0.01
3	0.0010 ± 0.0001	<0.01
4	0.0013 ± 0.0001	<0.01
5	0.0014 ± 0.0001	<0.01
6	0.0068 ± 0.0003	0.01
7	0.0069 ± 0.0004	0.01

[a] Mean of triplicate results.
[b] BS EN 480-10: 1997 [3] was applied for admixture samples, BS 812: Part 117: 1988 Method A [2] was applied for aggregates samples.

3.2. Analytical characteristics

Under the selected condition as described above although the linear range may be extended to higher concentration up to at least 1000 mg L^{-1} Cl^-, a linear calibration graph in the concentration range of 10–100 mg L^{-1} Cl^- ($Y = 58.8X - 18.9$, $r^2 = 0.9996$) was constructed. By using low concentration range, the appropriate dilution of sample solution was made, thus gave advantages in terms of minimizing interference effect and matching of viscosity of the sample to the carrier solution. A detection limit (3 times the standard deviation of the blank/slope of analytical curve) of 2 mg L^{-1} Cl^- was achieved. Relative standard deviations for 7 replicates injecting of 20, 60 and 90 mg L^{-1} chloride solutions were 1.0, 1.2 and 0.6% respectively. Recoveries were found to be 99.7–102.6% for spiking of Cl^- to the sample at concentrations of 20–90 mg L^{-1}. Performance of the developed method which used a home-made Cl^- ISE was compared with other methods as shown in Table 1. The developed method provided comparable performance to the reported methods but it is simpler, cheaper, consumed smaller amounts of non-toxic and inexpensive chemicals (1 mL each of water and 0.1 M KNO_3 per injection).

According to literatures [18,19], some halides (I^- and Br^-), sulfide, cyanide and some metal ions (Fe^{3+} and Al^{3+}) are the interferences in Cl^- determination by this kind of ISE in acidic medium, but not the other common ions, e.g., NO_3^-, NO_2^-, CO_3^{2-}, SO_4^{2-} and PO_4^{3-}. Effects of NO_2^-, CO_3^{2-}, SO_4^{2-} and PO_4^{3-} on the proposed method where performed in neutral medium were studied by spiking each ion into 50 mg L^{-1} Cl^- solution. It was found that NO_2^-, CO_3^{2-}, SO_4^{2-} and PO_4^{3-} at least up to 100 mg L^{-1} did not interfere (causing a change in peak height of less than 5%). Effects of I^-, Br^-, S^{2-}, Fe^{3+} and Al^{3+} have also been investigated. It was found that I^-, Br^- and S^{2-} seriously interfere, but Fe^{3+} and Al^{3+} up to 500 mg L^{-1} caused a change in peak height of less than 5%. By injecting a series of I^-, Br^- or S^{2-} standard solutions (10–100 mg L^{-1}), calibration graphs of $Y = 70.8X - 25.7$ ($r^2 = 0.998$), $Y = 70.2X + 14.5$ ($r^2 = 0.998$)

or $Y = 96.5X - 16.1$ ($r^2 = 0.989$), respectively, were obtained. However, these ions are usually present in the sample at relatively low concentrations compared to Cl^- and do not significantly interfere.

3.3. Application to real samples

The system was applied to cement aggregate and admixture samples. The samples were prepared as described in Section 2.4. The contents of Cl^- in the samples were calculated using a calibration graph ($Y = 57.6X - 23.3$, $r^2 = 0.9994$) and were found in range of 8–113 mg L^{-1}, which were converted to % (w/w) of Cl^- in samples as summarized in Table 2. The analysis of aggregate samples by titrimetric standard method [2] and admixture samples by potentiometric titration standard method [3] was also carried out for comparison. The standard methods are applied for determination of total halides, except F^-, reported as Cl^- content. The Cl^- contents obtained from the proposed method were in good agreement with those from the standard methods, evaluating by *t*-test at 95% confidence level [20]. It should be noted that the proposed method has lower detection limit (0.0002%, w/w), so it can determine lower level of Cl^- in samples with good precision. It is also faster and consumed smaller amounts of reagents. The permission level of Cl^- in concrete admixtures by Thai Industrial Standard (TIS 733–2530) is 0.20 ± 0.01%, while British Standard is 0.10%. The application of the method could be extended to analysis of water used in preparing of cement.

4. Conclusion

A simple Cl^- ISE and FI potentiometric method have been developed for the determination of water soluble Cl^- content in aggregates and admixtures for concrete. In comparison to the standard methods, the developed method provided agreeable results, moreover it is lower cost, lower reagent consumption, faster and has lower detection limit. The system gave linear range of 10–100 mg L^{-1} Cl^-, detection limit of 2 mg L^{-1} Cl^-, relative standard deviation of 0.6–1.2% and sample throughput of 60 h^{-1}, which are comparable to the previous flow based systems, but with simpler in instrumentation and no use of toxic or expensive chemicals.

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Flow injection conductometric system with gas diffusion separation for the determination of Kjeldahl nitrogen in milk and chicken meat

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ABSTRACT

A simple flow injection (FI) conductometric system with gas diffusion separation was developed for the determination of Kjeldahl nitrogen (or proteins) in milk and chicken meat. The sample was digested according to the Kjeldahl standard method and the digest was diluted and directly injected into the donor stream consisting of 4 M NaOH. In alkaline medium, ammonium was converted to ammonia, which diffused through the PTFE membrane to dissolve in an acceptor stream (water). Dissociation of ammonia caused a change in conductance of the acceptor solution, which was linearly proportional to the concentration of ammonium originally present in the injected solution. A conductometric flow through cell and an amplifier circuit was fabricated, which helped improve sensitivity of the conductometric detection system. With using a plumbing Teflon tape as a gas diffusion membrane and without thermostating control of the system, a linear calibration graph in range of 10–100 mg L^{-1} N-NH_4 was obtained, with detection limit of 1 mg L^{-1} and good precision (relative standard deviation of 0.3% for 11 replicate injections of 50 mg L^{-1} N-NH_4). The developed method was validated by the standard Kjeldahl distillation/titration method for the analysis of milk and chicken meat samples. The proposed system had sample throughput of 35 h^{-1} and consumed much smaller amounts of chemical than the standard method (275 mg vs 17.5 g of NaOH per analysis, respectively).

1. Introduction

Proteins are essential for growth and survival of human and animals. Proteins in food are commonly found in peanuts, meat, poultry and seafood. Standard methods for determination of total proteins are based on Kjeldahl method [1] and Dumas method [2]. Both the methods involved the quantitative determination of total nitrogen contents in sample and then the protein contents were calculated using different multiplying factors suitable for different kind of samples [3]. The factors are needed in order to account for different amino acid sequences of different proteins.

The Kjeldahl method consists of the digestion by heating a substance with sulfuric acid to decompose the organic nitrogen to ammonium sulfate, and the distillation of the digest after being alkalized for titrimetric determination of the released ammonia nitrogen. Potassium sulfate is added in the digestion step in order to increase the boiling point of the medium. Some catalysts (mercuric oxide and copper sulfate) were added to speed up the decomposition. The digested

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solution is distilled with sodium hydroxide, which converts the ammonium to ammonia. The ammonia is trapped in an acidic solution, e.g., 4% (w/v) boric acid, and was determined by titration. Although the method is accurate, reproducible, and has been used for standardization of other methods, its drawbacks such as the need to use concentrated sulfuric acid at high temperature and the relatively long testing time (an hour or more), compare unfavorably with the Dumas method.

The Dumas method involved combustion of a sample of known mass in a high temperature (900 °C) chamber in the presence of oxygen. This leads to the release of carbon dioxide, water and nitrogen. The gases are passed over special columns that absorb the carbon dioxide and water, and then nitrogen was separated from residual carbon dioxide and water by another column before being measured by a thermal conductivity detector using helium as a carrier gas. The method is fast (taking a few minutes per measurement) and does not use toxic chemicals or catalysts. However, its instrument and operation costs are high. Both the Kjeldahl and Dumas methods do not give a measure of true protein, as they register non-protein nitrogen in addition. The Dumas method usually gives higher protein contents than the Kjeldahl method [4,5].

Flow injection (FI) technique has been applied to automate the distillation/determination step of the Kjeldahl method [6–9]. The FI methods help shorten analysis time, reduce chemical consumption, and provide more reproducible results with the easy to use, relatively low-cost automated instrumentation. The FI methods are based on separation of ammonium from the Kjeldahl digest by using gas diffusion membrane and then determination of ammonium by different detection techniques, e.g., UV–vis spectrophotometry [8], potentiometry [9], conductometry [6] and bulk acoustic wave-impedance sensor [7]. Although the conductometric detection is not selective, its high sensitivity, wide linear response to the analyte concentration and relatively simple instrumentation are attractive. By employing a gas diffusion membrane, selectivity can be extremely improved. However, gas diffusion efficiency is usually low (<40%), so high temperature with thermostat control is required to improve sensitivity and reproducibility [6,7,10]. Non-linear gas diffusion characteristic is observed [6], which results in a non-linear calibration graph to be used for quantification of ammonium. Potentiometric and bulk acoustic wave-impedance sensor also provides a non-linear response.

In this work, a simple FI conductometric (FIC) system with gas diffusion separation was developed for the determination of Kjeldahl nitrogen (or proteins). A conductometric flow through cell and an amplifier circuit were fabricated, which helped improve sensitivity of the conductometric detection system. Although a simple and low-cost plumbing Teflon tape was used as a gas diffusion membrane in a planar gas diffusion unit and without either using of thermostat bath or optimization for good gas diffusion efficiency, the system could provide a linear calibration graph in range of 10–100 mg L^{-1} N-NH_4, with detection limit of 1 mg L^{-1}, which was appropriate for determination of Kjeldahl nitrogen in food samples. A good precision (0.3% R.S.D. for 11 replicate injections of 50 mg L^{-1} N-NH_4) was achieved. Moreover, the system provided sample throughput of 35 h^{-1} and consumed very small amounts of NaOH (275 mg per analysis), which is much smaller than the standard Kjeldahl method. The proposed method was applied to the determination of Kjeldahl nitrogen in milk and chicken meat samples.

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. Stock standard solution of ammonium (1000 mg L^{-1} N-NH_4) was prepared by dissolving 0.3821 g of ammonium chloride (Merck, Germany) and adjusting the volume to 100 mL with water. Working standard solutions were daily prepared by diluting the stock solution with water. Sodium hydroxide solution (4 M) was prepared by dissolving 16.00 g NaOH (Merck, Germany) in 100 mL of water. The solutions used for the Kjeldahl technique were prepared according to the reference method [1].

2.2. Instrument

FI conductometric system (Fig. 1a) consisted of a peristaltic pump (Model ISM 935, Ismatec, Switzerland) with pump tubing, an injection valve (Upchurch, USA), a home-made gas diffusion unit (GDU) [11], a home-made flow through conductometric cell as depicted in Fig. 1(b), a conductometer (Model 712, Metrohm, Switzerland), a lab-built amplifying and data collecting unit, and a personal computer. The planar GDU was fabricated by drilling a groove of 1 mm wide × 30 cm long × 0.5 mm deep on each of 2 Perspex plastic blocks, inserting a PTFE membrane (a commercial PTFE (Teflon) tape for household and plumbing purposes) between the Perspex blocks to form two channels opposite to each other. The conductometric cell was built by drilling a Perspex block to form a channel of 1.5 mm i.d. for solution to flow in and out, and for inserting of two Cu wires (1 mm o.d.) to be used as the electrodes, as shown in Fig. 1(b). The amplifying and data collecting unit was built employing a Basic Stamp 2SX microcontroller (Parallex, USA), as similar to the previously reported one [12], excepting the new software based on Visual Basic 6.0 (Microsoft, USA) was used instead of Microsoft excel. The unit accepted the analog input signal in the range of 0–5 V. PTFE tubing of 0.5 mm i.d. was used to assemble the FI system, including a mixing coil (C1) and back pressure coils (C2 and C3).

A block digester instrument (Model DK6, VELP Scientifica, Italy) was used for Kjeldahl digestion and a steam distillation unit (Model UDK132, VELP Scientifica, Italy) was employed for ammonia distillation in the Kjeldahl standard method. Dumas method was performed by using an automated system (FP-528 Protein/Nitrogen Determinator, LECO, USA).

2.3. FI conductometric procedure

Using the FIC system as shown in Fig. 1(a), a standard/sample solution was injected via an injection valve into the stream of 4 M NaOH donor stream. Ammonia gas was produced during the injected zone flowed through a mixing coil (C1) to the GDU, where the gas diffused though a PTFE membrane to dissolve in

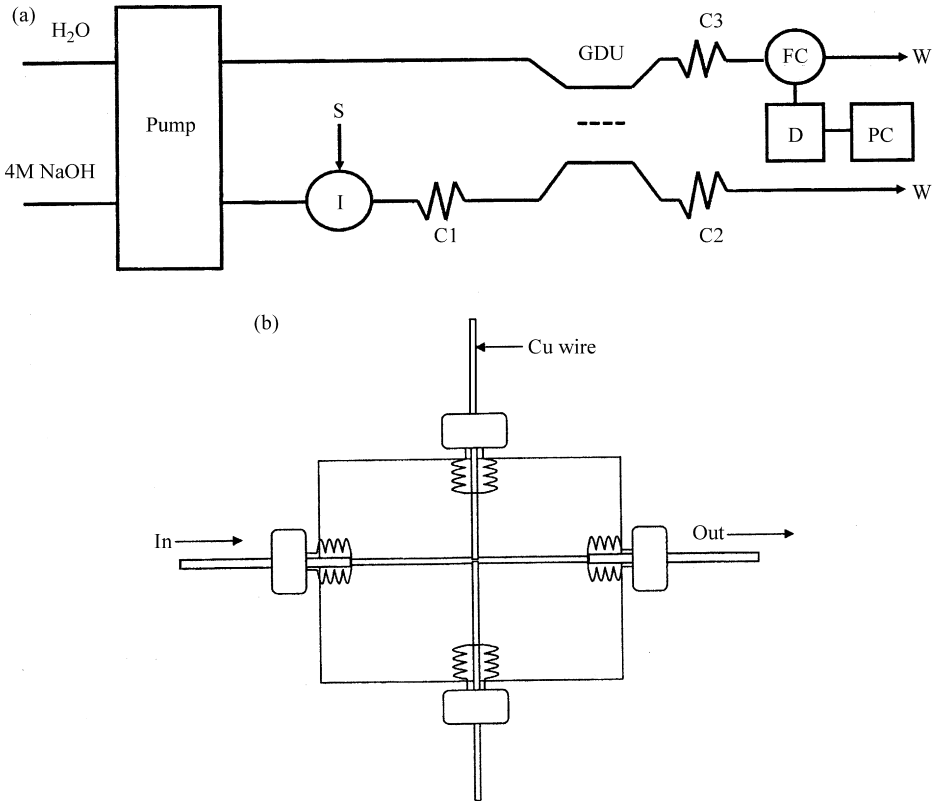

Fig. 1 – FI conductometric system for determination of Kjeldahl nitrogen, (a) FIC manifold and (b) conductometric flow through cell; Pump = peristaltic pump (1.0 mL min^{-1} each line), S = standard/sample, I = injection valve (75 μL sample loop), C1, C2, C3 = coiled tubes (all 50 cm long), GDU = gas diffusion unit, FC = conductometric flow through cell, W = waste, D = conductometer, PC = personal computer.

an acceptor stream (water). Both the NaOH and water streams had the same flow rate of 1.0 mL min^{-1} each in order to maintain an identical pressure on both sides of the membrane. The ammonium and hydroxide ions produced on the dissolution of ammonia gas increased conductance of the acceptor stream, which could be detected and recorded as a peak when the zone passed through the flow cell. Peak height was linearly proportional to concentration of ammonium in the injected solution. A linear calibration graph was constructed for examining of ammonium concentration in the samples.

2.4. *Kjeldahl digestion and determination procedures*

Sample digestion was carried out according to the reference procedure [1]. Briefly, an aliquot of 5.00 mL of milk sample was digested with 7.00 mL of concentrated H_2SO_4 in the presence of 7.00 g of K_2SO_4, 0.10 g of $CuSO_4$ and 5.00 mL of 35% (v/v) H_2O_2, in a block digester at 420 °C for 30 min or until the clear solution was obtained. The digest was allowed to cool down before taking to analysis by the proposed FIC method or standard Kjeldahl titrimetric method. Thus, two digested solutions for each sample were employed.

For FIC method, the digest was added with 80 mL water and filtered through a Whatman No. 40 filter paper into a 250 mL volumetric flask, then adjusting the volume to the mark with water. A multi-steps dilution may be applied to reduce the volume of water used. The solution was injected into the FIC system in triplicate and the peak heights obtained were used to examine for ammonium concentration and protein content of the sample.

For the standard titrimetric determination, the digest was added with 50 mL water and the digestion tube was assembled to the distillation unit, where a 50 mL of 35% (w/v) NaOH was added and steam distillation was performed with the distilled inserted in a 25 mL of 4% (w/v) boric acid solution. Continue the distillation until 100 mL of the distillate was obtained. A few drops of an indicator solution was added to the distillate before titrating with a standardized 0.2 M HCl solution for ammonium content.

3. Results and discussion

3.1. *Optimization of the FIC system*

The FIC system was developed aiming for a simple automation system to be used as an alternative to the distillation/titrimetric determination system of the Kjeldahl standard method. Kjeldahl digestion converts protein nitrogen (and other nitrogen) to ammonium. However, apart from ammonium the Kjeldahl digest contains high concentration of acid and ions of various salts, which are sensed by conductometric detector, a non-selective detection system. A gas diffusion membrane was employed to selectively separate ammonium

(as ammonia gas) from other ions. A previously reported planar GDU [11] was used in this work because it is simple and easy to fabricate from low-cost materials. Sensitivity of the developed FIC system depended on gas diffusion efficiency and detector sensitivity. Thickness and porosity of the membrane should affect gas diffusion efficiency. Expensive membranes of known thickness and porosity are available but in this work we aim for simple and low-cost system, so the plumbing tape was use as a membrane. Several PTFE (Teflon) tapes which are widely available for household and plumbing uses were tried. They are variations in the thickness and smoothness of the tapes and may be with different in loading materials and degrees of polymer crystallinity, which results in different degrees of gas permeation. Porosity and accurate thickness of this kind of membranes are difficult to measure and with the aim for simple system, we did not investigate on these issues. It was found that all of the PTFE tapes gave reproducible FI profiles but with variation in peak heights (±25%). The one which was thin and had smooth surface (manufactured by Indy Hand Tools Co., Ltd., Thailand) was selected as it provided the best sensitivity. The membrane could be used for at least 150 injections of sample/standard solutions, without drastic changes in sensitivity and reproducibility.

Different parameters affected the degree of gas diffusion through the membrane, such as membrane thickness and porosity, configuration of GDU, flow rate of a donor and an acceptor streams, volume of the injected solution, pressure difference between the donor and the acceptor channels and temperature. A higher ratio of surface area to volume of GDU provided small improvement in diffusion efficiency [13]. However, longer path length GDU may result in higher dispersion and lower sample throughput. Hence, we used GDU of 30 cm path length without further optimization. An equal flow rate of both the streams was chosen in order to maintain nearly identical pressure on both sides of the membrane, so avoiding membrane deformation or breakage. It has been reported in literatures [6,10] that the lower the flow rate the higher diffusion efficiency was obtained, e.g., 80% increment in sensitivity was achieved by reducing flow rate from 3.2 to 0.9 mL min^{-1}. A flow rate of 1.0 mL min^{-1} was selected in order to compromise between sensitivity and sample throughput.

Effect of injection volume was investigated. Injection volumes of 75 and 100 μL provided nearly equal peak heights of 10 mg L^{-1} $N\text{-}NH_4$, but with significantly broader peak for the larger volume, so the injection volume of 75 μL was selected.

A mixing coil C3 of 50 cm long was placed before the flow cell in order to help maintain a constant pressure in the acceptor line, leading to a stable baseline and more reproducible signal to be obtained from the conductometric detector. The pressure difference between the two sides of the membrane could be slightly altered by using back pressure coils on both the streams. The higher pressure on the donor side would promote diffusion to the acceptor stream. However, too high pressure difference might lead to membrane deformation and breakage. The length of back pressure coil of the donor side (C2) was varied (0–100 cm) while the mixing coil C3 acted also as an acceptor back pressure coil of fixed length. It was found that the longer the C2 length the higher sensitivity was obtained. The coil length of 50 cm was selected because the longer coil caused breakage of the membrane occasionally.

A thermostat bath is usually needed to obtain reproducible signal from the gas diffusion systems [6,7,10]. Temperature change affected both the diffusion efficiency and the detector response (e.g., in case of potentiometric and conductometric detectors). By raising the temperature, the higher diffusion degree was achieved, but with a non-linear calibration graph obtained [6,7]. In the proposed FIC system, a linear calibration graph and reproducible signal were obtained without usage of a thermostat bath, excepting the experiment was performed in an air conditioning room of about 25 ± 2 °C. Thus, the effect of temperature was not studied and the FIC system without thermostat control was used.

Another approach to increase sensitivity of the determination, improvement of detector performance was investigated. With the conductometric cell shown in Fig. 1(b), the sensitivity could be increased by carefully adjusting the gap between the electrodes, which the shorter the gap the higher the sensitivity was obtained. Sensitivity could be further improved by using amplification circuit of the data acquisition unit. However, the higher amplification may lead to lower signal to noise ratio. Amplifier gain of 40-fold was selected as it gave the signal in a suitable range for the data acquisition unit with acceptable S/N. Further improvement in sensitivity could be achieved by adjusting the analog output range of the detector to be suitable with the amplifier gain. The analog output range of 3 μS V^{-1} was set in this work as it gave enough sensitivity for the determination of ammonium in the concentration range of 10–100 mg L^{-1}, suitable for analysis of food samples.

Kjeldahl digest contained high concentration of sulfuric acid that would react with NaOH in the donor stream, affecting the production of ammonia gas. Effect of H_2SO_4 was investigated in range of 0–2 M by adding the acid into 10 mg L^{-1} $N\text{-}NH_4$ standard solution. The solutions were injected into the donor stream containing different concentrations of NaOH (2, 4 and 6 M). The results as shown in Fig. 2 indicated that the higher NaOH concentration provide the more tolerance to acid concentration in the sample solution. NaOH of 4 M was selected as it could tolerate to H_2SO_4 up to about 1 M and it provided

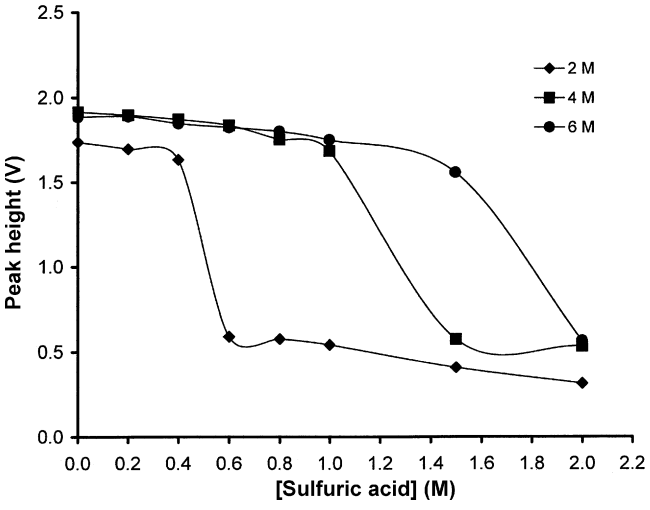

Fig. 2 – Effect of concentration of sulfuric acid on peak height of 10 mg L^{-1} $N\text{-}NH_4$, with different concentrations of NaOH solutions (2, 4 and 6 M) as the donor stream.

Table 1 – Analytical features of different methods for determination of nitrogen content

Method	Detection principle	Sample	Calibration range ($mg L^{-1}$)	Detection limit ($mg L^{-1}$)	Precision (%R.S.D.)	Sample throughput (h^{-1})	Remarks	Ref.
Kjeldahl method	Distillation of ammonia and titrimetric determination	Kjeldahl digest of milk	Absolute method	14	0.5, 2.0	9	Standard method, 17.5 g NaOH/sample	[1,4]
Dumas method	Determination of nitrogen gas from combustion of sample	Milk	–	–	1.5	12	Standard method, Automated system	[4]
FI–GD conductometry	Gas diffusion and conductometric detection	Kjeldahl digest of vegetable and animal feed	14–112	–	1.0	100	Non-linear calibration, Temperature control 25 °C 60 mg NaOH/sample	[6]
FI–GD–BAW-impedance sensor	Gas diffusion and bulk acoustic wave-impedance sensor	Kjeldahl digest of amino acids and blood products	0.07–56	0.01	1.0	45–50	Non-linear calibration, Temperature control 25 °C 40 mg NaOH and 1.3 mL of 0.01 M boric acid/sample	[7]
FI–GD colorimetry	Gas diffusion and colorimetric detection of ammonia using acid-base indicator	Kjeldahl digest of animal feed	40-400	–	0.8	–	Non-linear calibration, Partial least square (PLS) analysis method, Determination of ammonium and acid	[8]
FI–GD potentiometry	Gas diffusion and detection of NH_4^+ by PVC tubular membrane ammonium ion selective electrode	Kjeldahl digest of milk and dairy products	11–210	7	0.5	100	Logarithmic response of ISE vs ammonium concentration 110 mg NaOH and 3.5 mL of 0.01 M Tris buffer/sample	[9]
Ion chromatography	Ion chromatographic determination of ammonium	Kjeldahl digest of animal feeds	0.015–25	0.015	0.6	4	1000-fold dilution of sample	[14]
FI–GD conductometry	Gas diffusion and conductometric detection	Kjeldahl digest of milk and chicken meat	10–100	1	0.3	35	Linear calibration, 275 mg NaOH/sample, No thermostat control	This work

higher sensitivity than 2 M NaOH. The H_2SO_4 concentration in the diluted Kjeldahl digest was about 0.5 M, so the solution could be directly injected into the system.

3.2. Analytical characteristics

The FIC manifold as shown in Fig. 1(a) was employed with a set of the selected conditions: 4 M NaOH as a donor stream, water as an acceptor stream, flow rate of each stream of 1.0 mL min^{-1}, the length of all mixing coil and back pressure coils of 50 cm each, sample volume of 75 μL, amplifier gain 40×, and detector analog range of 3 μS V^{-1}. Under the above selected conditions, a linear calibration graph ($y = 0.038x + 0.6816$, $r^2 = 0.9945$) for the concentration range of 10–100 mg L^{-1} N-NH_4 was obtained with a detection limit (3 S.D.) of 1 mg L^{-1} N-NH_4, which was a suitable concentration range for the determination of Kjeldahl nitrogen in food sample. With higher amplifier gain, a lower linear calibration range (1.0–10.0 mg L^{-1} N-NH_4) was obtained with a detection limit of 0.2 mg L^{-1} N-NH_4, which might be appropriate for other kinds of sample. Relative standard deviation was 0.3%, for 11 replicate injections of 50 mg L^{-1} N-NH_4. The relative standard deviation of overall analytical process (digestion and determination) was evaluated by 7 replicate analyses of the sample and was found to be 1.2%, while that of the standard Kjeldahl titration was 2.3%. Sample throughput of 35 h^{-1} was achieved with the consumption of 1.7 mL each of water and 4 M NaOH (275 mg NaOH) per analysis, which is much lower than the amounts used by the standard method (17.5 g analysis^{-1}). Furthermore, the NaOH solution could be recycled after purging out the ammonia gas from the solution. No sample treatment was needed, except the dilution.

In the alkaline donor solution, acidic gases did not interfere as they were converted to ions and could not pass though a gas diffusion membrane. Volatile amines [10,13] should be potential interferences in GD system but they would not expect to present in the Kjeldahl digest. Interferences from solution turbidity and/or metallic species are negligible, because the digest was diluted several folds and the injected volume was only 75 μL. No precipitate was observed in the donor line and a stable baseline signal was obtained for at least 2 h.

3.3. Comparison of the developed method with existing methods

Analytical features of different methods for determination of nitrogen content in food are presented in Table 1. The widely used standard methods are Kjeldahl method based on digestion/distillation/titrimetry and Dumas method based on combustion of sample/determination of nitrogen gas. The Kjeldahl method is used for standardize other methods, despite it is slow, tedious and has high consumption. Comparison to the standard methods, the developed method is simpler in instrumentation, lower consumption and has higher sample throughput. Although the Dumas method is fast and does not use toxic chemicals or catalysts, its instrument and operation costs are high.

Comparing to the previous developed FI systems for Kjeldahl nitrogen determination, the proposed system provided wider linear calibration graph than the spectrophotometric one and with no interference from carbon dioxide. A high sensitivity could be obtained from the linear calibration graph of the proposed system, which is better than the logarithmic response of the potentiometric detection [9] or a non-linear calibration graph of the other systems [6–8]. Thus, chemometric treatment of the analytical data was not required. The developed method is simpler and more convenient than ion chromatographic method [14] and the FI methods based on spectrophotometric [8], potentiometric [9] and bulk acoustic wave-impedance sensor [7] detection systems and with no need for thermostat control of the system. A sample throughput of 35 h^{-1} achieved was quite low for FIA systems but it is better than the previously reported multi-commuted flow injection system for spectrophotometric determination of ammonium [13].

Table 2 – Kjeldahl nitrogen contents in milk samples determined by FIC method and Kjeldahl standard method [1]

Sample	Nitrogen content (%, w/w)		% Different
	FIC method[a]	Kjeldahl method	
1[b]	0.414 ± 0.001	0.43	−3.4
2[b]	0.431 ± 0.001	0.44	−1.9
3[b]	0.450 ± 0.002	0.47	−3.6
4[b]	0.464 ± 0.002	0.48	−2.8
5[b]	0.464 ± 0.002	0.48	−2.8
6[b]	0.481 ± 0.002	0.49	−2.7
7[b]	0.433 ± 0.002	0.44	−2.1
8[b]	0.441 ± 0.001	0.45	−1.4
9[b]	0.487 ± 0.001	0.51	−5.3
10[b]	0.428 ± 0.003	0.44	−2.6
11[b]	0.464 ± 0.001	0.49	−5.2
12[b]	0.322 ± 0.001	0.32	−0.6
13[b]	0.397 ± 0.001	0.42	−4.8
14[b]	0.449 ± 0.002	0.47	−5.3
15[b]	0.517 ± 0.002	0.51	1.2
16[b]	0.526 ± 0.003	0.52	1.0
17[b]	0.401 ± 0.002	0.41	−1.2
18[b]	0.508 ± 0.001	0.51	0.2
19[b]	0.500 ± 0.001	0.50	−0.8
20[c]	0.404 ± 0.002	0.40	−0.0
21[c]	0.403 ± 0.001	0.41	−1.0
22[c]	0.408 ± 0.001	0.41	0.2
23[c]	0.401 ± 0.001	0.41	−1.5
24[c]	0.389 ± 0.001	0.41	−4.4

[a] Mean ± S.D. of triplicate results.
[b] Cow milk.
[c] Soy milk.

3.4. Application to Kjeldahl digests of milk and chicken meat

The developed system was applied to the determination of protein contents in various cow and soy milks and instant chicken meat. The digests were prepared as described in Section 2.3, before injecting into the FIC system. Concentrations of the Kjeldahl nitrogen were calculated from the linear calibration equation constructing under the same condition as the samples. The Kjeldahl nitrogen contents in milk and chicken meat samples are summarized in Tables 2 and 3, respec-

Table 3 – Nitrogen contents in chicken meat samples determined by FIC method, Kjeldahl standard method [1] and Dumas standard method [2]

Sample	Nitrogen content (%, w/w)			% Different	
	FIC method[a] (A)	Kjeldahl method (B)	Dumas method (C)	A vs B	A vs C
1	4.62 ± 0.02	4.85	5.05	−4.9	−8.5
2	4.67 ± 0.01	4.74	5.14	−1.4	−9.1
3	4.78 ± 0.01	4.89	5.00	−2.4	−4.4
4	4.61 ± 0.01	4.60	5.07	0.2	−9.1
5	4.71 ± 0.01	4.65	5.06	1.4	−6.9
6	4.66 ± 0.03	4.65	5.05	0.2	−7.7
7	4.70 ± 0.01	4.78	5.03	−1.6	−6.6

[a] Mean ± S.D. of triplicate results.

tively. The results obtained by the proposed procedure were agreed well with those from the standard Kjeldahl distillation/titration procedure [1], with percentage different of less than 5%. According to the paired *t*-test at 95% confidence level [15], the results from both the methods were not significantly different ($t_{tabulated} = 2.000$ and $t_{calculated} = 0.998$ ($n = 31$)). Nitrogen contents in chicken meat obtained from Dumas method were slightly higher than those obtained from both the proposed method and the standard Kjeldahl method. This fact is agreed with that previously reported in the literatures [4,5]. Protein contents in samples could be calculated from the Kjeldahl nitrogen contents by using different multiplying factors for different types of samples, i.e., 6.38 for cow milk, 5.71 for soy milk, and 6.25 for chicken meat [3].

4. Conclusion

A simple FI conductometric system with gas diffusion was developed for the determination of Kjeldahl nitrogen (or proteins) in milk and chicken meat samples. The sensitivity of conductometric detection system was improved by using a special design flow through cell and amplifier circuit. The system employing a plumbing Teflon tape as a diffusion membrane and without thermostat bath could be used to provide a linear calibration graph in range of 10–100 mg L^{-1} N-NH_4, with good precision (0.3% R.S.D. for 11 replicate injections of 50 mg L^{-1} N-NH_4). The proposed system provided sample throughput of 35 h^{-1} and consumed very small amounts of NaOH, so it is interesting to be used as an alternative method for determination of proteins.

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Contributed Paper

Flow Injection Amperometric Method with Dialysis Sample Pretreatment for Determination of Ascorbic Acid

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ABSTRACT

A flow injection amperometric method with dialysis sample pretreatment for determination of ascorbic acid (vitamin C) has been developed. Standard/sample solution (50 μL) was injected into a donor stream (deionized water) via an injection valve. The sample zone was passed through a dialysis unit to separate the analyte from the matrices. The dialysate containing ascorbic acid, in the acceptor stream of 0.1 M phosphate buffer pH 5.6, was flowed to the amperometric detector for current measurement. Ascorbic acid was electrochemically oxidized at glassy carbon electrode giving an anodic peak current proportional to ascorbic acid concentration. A linear calibration graph in range of 50 - 800 mg/L ascorbic acid was obtained. The relative standard deviation of 10 replicate injections was 1.5 % for 50 mg/L of ascorbic acid. A sample throughput of 20 h^{-1} was achieved. The proposed method was applied to vitamin C tablets and fruit juice samples collected from drug stores, supermarkets and local suppliers in Chiang Mai. The results obtained for vitamin C samples are agreed well with the labeled values, and comparable with those determined by voltammetric method. The proposed method is rapid, low reagent consumption and does not suffer from colored and colloidal substances presented in the sample.

Keywords: flow injection, amperometry, dialysis, ascorbic acid, vitamin C, fruit juice.

1. INTRODUCTION

Ascorbic acid (vitamin C), a water soluble vitamin is an important micronutrient and plays many physiological roles in the body, including as a free radical scavenger, which may help to prevent free radical induced diseases such as cancer and Parkinson's diseases[1]. It has also been used for the prevention and treatment of the common cold, mental illness, infertility, cancer and AIDS[2]. Fruits and vegetables are the principal source of ascorbic acid. Ascorbic acid is also added into food and beverage products and pharmaceutical preparations as a supplementary source of vitamin C in human

diets. However, ascorbic acid is not stable in the presence of air, heat, light and some metal ions. It is necessary for industry to have rapid and sensitive methods for routine and reliable determination of ascorbic acid. Although method based on visual end point indophenols titration has been approved by the Association of Official Analytical Chemist (AOAC) for the determination of ascorbic acid in food products, it has several drawbacks such as it is tedious and time consuming procedure with high chemical consumption, and colored species in sample may interfere a lot on end point detection. Various instrumental methods have been proposed including spectrophotometry[3], chemiluminescence[4], enzymatic analysis[5], high performance liquid chromatography[6], electrophoresis[7] and electrochemistry[8-9].

Many flow injection (FI) methods have been reviewed for the determination of ascorbic acid in pharmaceutical preparations, food products, and biological samples using different detection systems [10]. Most of the methods employed the reducing property of ascorbic acid. Spectrophotometry in visible region is the technique more frequently used. It involved redox reaction with ascorbic acid in which a colored compound is formed or decomposed in a reaction. For example, the method using chloramine T in the presence of starch-KI solution[11], the titration with 2,6-dichlorophenolindophenol[12], the formation of Fe(II)-phenanthroline[13], Fe(II)-ferrocine[14] or Cu(I)-bathocuproine [15] complexes previous metal ions reduction by ascorbic acid have been reported. Decolorized methods such as by reduction of cerium(IV)[16], reduction of triiodide [17], reduction of Co(III)-EDTA complex [18] or photochemical reduction of methylene blue[19], and a simple fading of permanganate color by ascorbic acid[20] were proposed. Colored substances presented in sample may seriously interfere in these methods. On the other hand, electrochemical technique did not suffer from this kind of interference. The technique is based on direct electrochemical oxidation of ascorbic acid on a bare glassy carbon (GCE) or platinum (Pt) electrode or other modified electrodes. Electrooxidation on bare GCE or Pt electrode requires higher potential than the modified electrode, which may lead to electrode fouling, poor reproducibility and low selectivity, especially in batch procedure[21]. Modified electrodes have been proposed to avoid these problems [21,22], but complicated process in preparation and limited stability of the electrode were found.

In this work, FI amperometric system using a bare GCE as a working electrode was developed for determination of ascorbic acid. With injecting small volume of sample and incorporating of on-line dialysis unit, reproduce signal with improving in selectivity and without electrode fouling was obtained. The dialysis unit also provided on-line dilution of sample, which percentage dialysis of 0.3% was achieved for cellulose acetate membrane with molecular weight cut off of 12,000 Da. Although a lab-built amperometer with a home-made data acquisition device was employed, a good sensitivity and linearity calibration graph was obtained in range of 50-800 mg/L ascorbic acid. The proposed method was applied for pharmaceutical and fruit juice samples.

2. MATERIALS AND METHODS

2.1 Chemicals and Samples

All solutions were prepared with analytical grade chemicals and with deionized water. Standard solution of ascorbic acid (1000 mg/L) was prepared daily by dissolving 0.1000 g of ascorbic acid (UNILAB) in water and adjusting to 100 mL in a volumetric flask. Supporting electrolyte (0.1 M phosphate

buffer of pH 6) was prepared by dissolving 14.71 g of sodium dihydrogenphosphate dihydrate(Fluka) and 1.00 g of disodium hydrogenphosphate dihydrate(BDH) in water and making up volume to 500 mL.

Pharmaceutical tablet samples were obtained from local pharmaceutical stores. Twenty tablets of the sample were weighed and an average weight of a tablet was calculated before being ground into fine powder. Then, 0.6-4.0 g portions were accurately weighed, dissolved in water to get appropriate concentrations of ascorbic acid in solutions.

Fruit juice samples were collected from supermarkets and local suppliers. Then, the samples were filtered with cotton wool before analyses.

2.2 FI Manifold and Procedure

Schematic diagram of the instrumental set-up of FI amperometric system is shown in Figure 1. It consisted of a peristaltic pump (Ismatec, Switzerland), a six port injection valve (Upchurch, USA), a home-made dialysis unit[23] and a lab-built amperometer with a flow through electrochemical cell (BAS, USA) and an in-house developed data recording unit[24] or a commercial data acquisition system(eDAQ, Australia). The planar dialysis unit was similar to the previous report[23], but with a dialysis membrane (cellulose acetate membrane of MW cut off 12,000 Da) instead of a Teflon membrane. A GCE (3 mm diameter), stanless steel and Ag/AgCl electrodes were used as working, auxiliary and reference electrodes, respectively. Standard or sample (50 μL) was injected into a water carrier stream and flowed to dialysis unit, where ascorbic acid dialysed to an acceptor stream, 0.1 M phosphate buffer pH 5.6. Flow rates of both the donor and acceptor streams are 1.5 mL/min. Ascorbic acid was diluted and separated from other matrices in the samples. A zone of ascorbic acid solution in the acceptor stream flowed further to the flow through electrochemical cell, where a constant potential (0.8 V) was applied to a GCE. Electrooxidation of ascorbic acid produced anodic peak current which was converted to voltage and recorded as FI peak. A calibration graph is a plot of peak height versus ascorbic acid concentration. Concentration of ascorbic acid in sample was calculated using an equation obtained from a linear calibration graph.

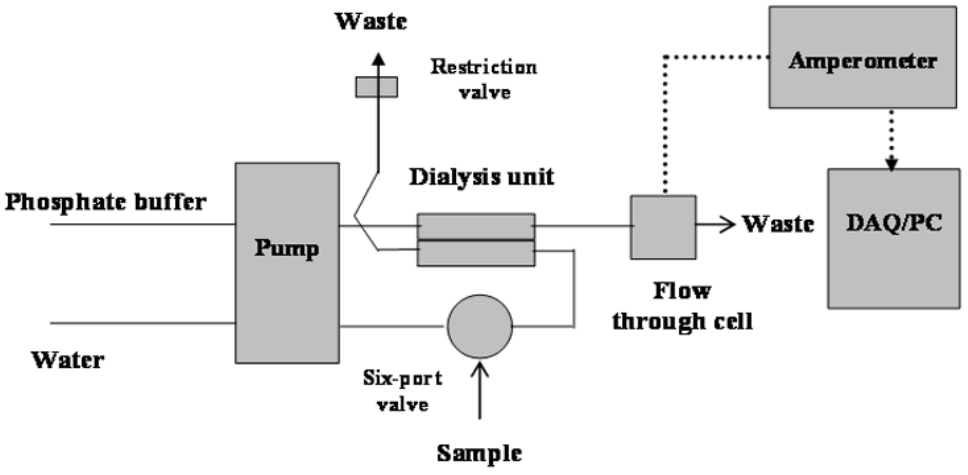

Figure 1. Schematic diagram of the instrumental set-up of FI amperometric system with on-line dialysis.

3. RESULTS AND DISCUSSION

3.1 Optimization of the FI Amperometric Detection System

Using a single line FI – system without dialysis unit, 50 µL of the ascorbic acid standard solution was introduced into phosphate buffer carrier stream via a six-port valve. The injected solution flowed through the flow cell whereas ascorbic acid was oxidized to dehydroascorbic acid at a working electrode. Preliminary conditions, 0.1 M phosphate buffer solution of pH 6.0 as a carrier solution, flow rate of 1.0 mL/min were employed. Effects of various parameters were investigated as followed.

3.1.1 Type of Working Electrode

Two types of working electrode, platinum disc (2 mm diameter) and glassy carbon disc (3 mm diameter) electrodes were investigated, with a fixed potential of 0.1 V applying to the electrode. A standard solution of 100 mg/L ascorbic acid was injected in 11 replicates into the system. The peak heights of 19.2±0.6 mV and 20.5±0.6 mV were obtained for Pt and GC electrodes, respectively. Despite both of the electrodes could be used, the GCE was selected for further experiment.

3.1.2 Applied Potential

The applied potential was varied in the range of 0.1-1.4 V vs. Ag/AgCl reference electrode. A 40 mg/L ascorbic acid standard solution was injected into the system. It was found that peak current obtained increased sharply with applied potential in the range of 0.1-0.8 V and reached maximum at 1.0 V (Figure 2). However, baseline drift was observed for applied potential higher than 0.8 V and higher potential may lead to oxidation of some interfering substances, so applied potential of 0.8 V was selected.

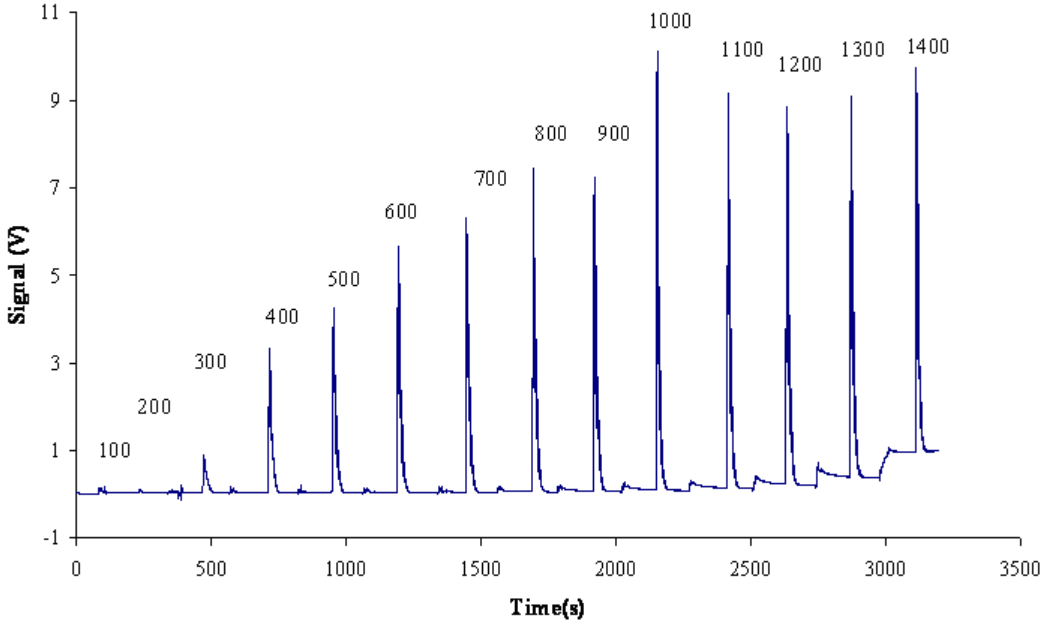

Figure 2. FIgram obtained from injecting of 40 mg/L ascorbic acid with different applied potentials (mV) to the GCE.

3.1.3 Concentration and pH of Phosphate Buffer

Concentration of phosphate buffer in the range of 0.005 – 0.5 M was investigated, while pH of buffer was fixed at 6.0. Calibration graphs in concentration range of 20 – 100 mg/L ascorbic acid were constructed by plotting peak height obtained versus ascorbic acid concentration. Slopes, intercepts and r^2 of the calibration graphs are shown in Table 1. Phosphate buffer concentration of 0.1 M was chosen because it provided high sensitivity with using smaller amounts of reagent than 0.5 M.

The pH of phosphate buffer was varied from 3.0 -11.0, while phosphate buffer concentration was fixed at 0.1 M. Standard solutions of ascorbic acid in concentration range of 20 – 100 mg/L were injected. With increasing of the pH, slope of the calibration graphs slightly decreased as shown in Table 2. Therefore, buffer of pH 5.6 was chosen because this solution gave high sensitivity and was easy to prepare.

3.1.4 Flow Rate

Effect of flow rate of phosphate buffer carrier solution (0.5 – 4.8 mL/min) was investigated by injecting various concentrations of ascorbic acid standard solution (20 -100 mg/L) and constructing a calibration graph for each flow rate. Slope of the calibration graphs are plotted versus flow rate of the phosphate buffer, as illustrated in Figure 3. It was found that the higher flow rate of the carrier stream, the higher sensitivity was

Table 1. Effect of concentration of phosphate buffer on the determination of ascorbic acid by FI amperometric method.

Concentration of phosphate buffer (M)	Calibration data		
	Slope (mV.L/mg)	Y – intercept (mV)	r^2
0.005	1.0	164.9	0.9888
0.05	16.6	130.9	0.9866
0.10	21.0	11.5	0.9995
0.50	25.8	-81.3	0.9973

Table 2. Effect of pH of phosphate buffer.

pH of phosphate buffer	Calibration data		
	Slope (mV.L/mg)	Y – intercept (mV)	r^2
3.0	24.7	-32.9	0.9976
5.6*	21.9	-48.5	0.9968
7.3	23.0	-41.6	0.9964
11.1	18.4	-81.3	0.9937

*Not adjusting pH

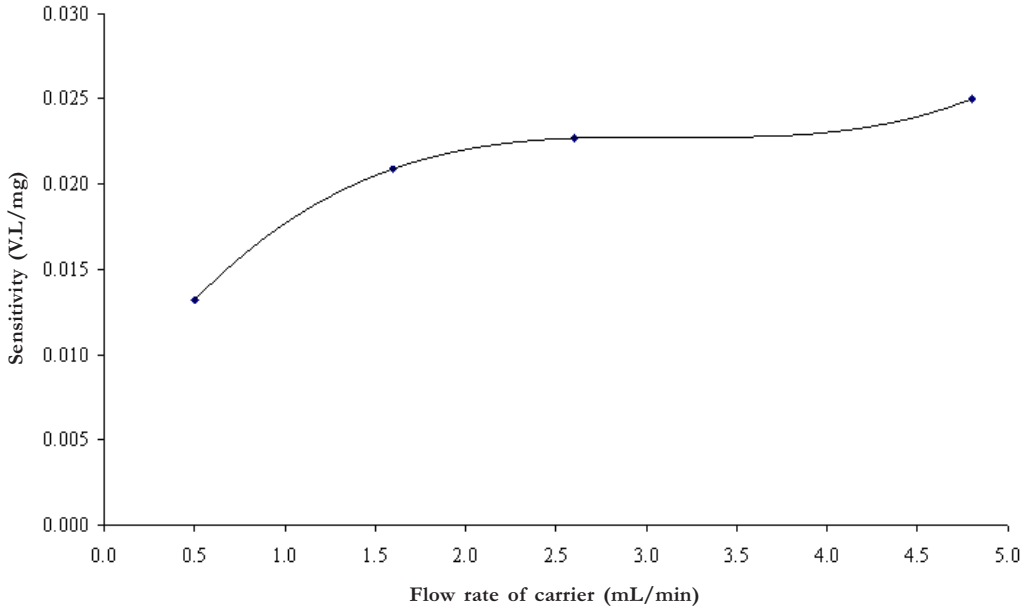

Figure 3. Effect of flow rate of carrier stream on sensitivity of ascorbic acid determination.

obtained. The flow rate of 1.5 mL/min was chosen because it provided enough sensitivity and sample throughput (20 injections per hour) with lower reagent consumption.

3.2 FI Amperometric System with Dialysis Unit

Dialysis unit was incorporated to the FI system as shown in Figure 1, in order to separate ascorbic acid from other matrices in samples. A cellulose acetate membrane of molecular weight cut off of 12,000 Da was utilized as a dialysis membrane. Employing the selected conditions obtained in section 3.1, ascorbic acid solutions in concentration range of 50 – 500 mg/L were injected. A linear calibration graph ($y=6x10^{-5}x$ -0.0006, r^2 = 0.9915) was obtained. Due to low dialysis efficiency (about 0.3%), a decrease in sensitivity was observed. However, it is still useful for determination of ascorbic acid in pharmaceutical and fruit juice samples, which high concentration of ascorbic acid is concerned. By on-line dialysis, dilution factor of about 300 (calculated from the ratio of the slope of calibration graphs from the systems without and with dialysis unit) could be reproducibly achieved.

3.3 Analytical Characteristics

Using the recommended conditions as described in section 2.2, a linear calibration graph in the range of 50 – 800 mg/L ascorbic acid was obtained ($y = 5x10^{-5}x$-0.0023 , r^2 = 0.9919). Detection limit calculated from the calibration data[26] was found to be 45 mg/L. The relative standard deviation of 10 replicate injections was 1.5 % for 50 mg/L of ascorbic acid, indicated a good precision of the method. Each injection consumed about 5 mL of reagent and 150 μL of sample.

3.4 Application of the Developed Method to Real Samples

3.4.1 Vitamin C Tablet Samples

The vitamin C samples from local drugstores were analyzed for ascorbic acid by the proposed method. All samples were

Table 3. Ascorbic acid contents in vitamin C tablet samples.

Sample	Label (mg/tablet)	Ascorbic acid found (mg/tablet)				% Different[c]
		FI – amperometric method [a]	% label	Voltammetric method [b]	%label	
1. Bio C 1000	1000	1021±6	102	1143±23	114	-11
2. Nat C	1000	998±15	100	1018±15	102	-2.0
3. Berocca	500	487±3	97	539±24	107	-9.6
4. C-500 nopparat	500	491±13	98	588±24	117	-16
5. Mag - C 500	500	525±8	105	615±15	123	-15
6. Flavettes	250	245±23	98	316±13	126	-22
7. Vitacimin	100	97±1	97	117±2	117	-17
8. Vit C 50 Frx	50	49±1	98	58±1	116	-16

[a] triplicate results

[b] duplicate results

[c] % different = [(FI value – Voltammetric value) x100] / Voltammetric value

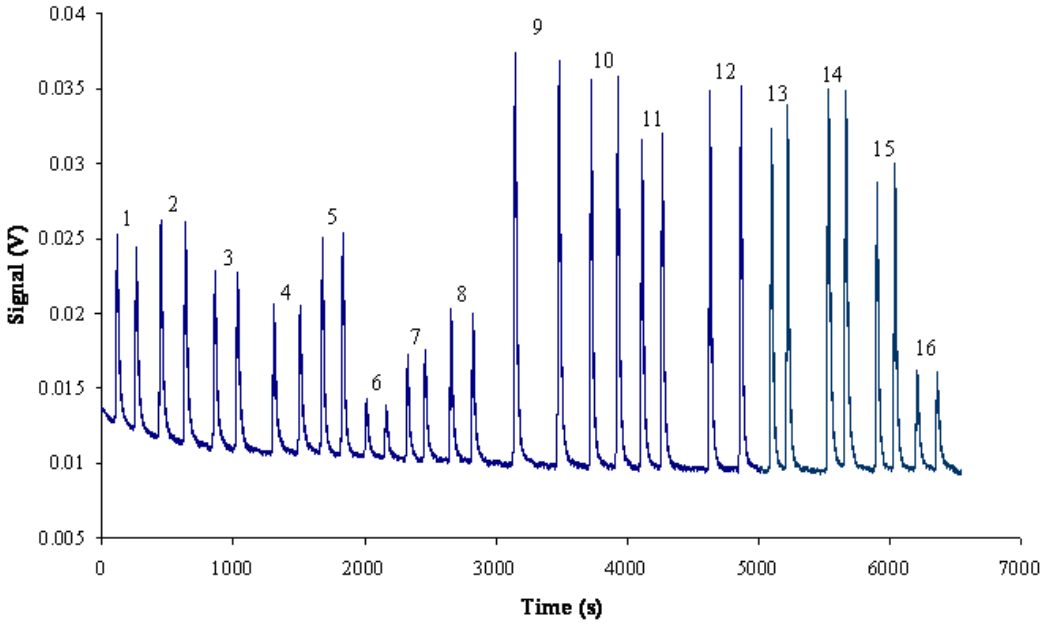

Figure 4. FIgram obtained from injecting of various fruit juice samples.

Table 4. Ascorbic acid contents in fruit juice samples

Sample No.	Type of sample	Label (mg/L)	Ascorbic acid found (mg/L)	
			FI – amperometric method	%label
1	Orange juice	-	292±6	-
2	Orange juice	-	337±4	-
3	Orange juice	480	280±2	58
4	Guava juice	-	244±1	-
5	Apple juice	-	337±7	-
6	Orange mixed carrot juice	-	118±4	-
7	Blueberry mixed apple juice	180	188±2	104
8	Orange mixed banana juice	180	247±3	137
9	Tangerine juice	330	587±4	178
10	Sai Nam Phueng orange juice	-	563±4	-
11	Shogun orange juice	420	488±3	116
12	Guava juice	360	554±4	153
13	Kiwi and grape juice	480	515±18	107
14	Pineapple juice	-	549±4	-
15	Broccoli and fruit juice	-	440±16	-
16	Mixed vegetable and mixed fruit juice	-	175±1	-

prepared as described in section 2.1. Concentration of ascorbic acid in the sample solution was calculated from peak height obtained using the calibration graph. Accuracy of the proposed method was determined by comparing results from FI-amperometric method with those obtained from voltammetric method [25] and the labeled values. The voltammetric method was used because it does not interfere by the intense color of sample. The results are summarized in Table 3. It was found that the results obtained from voltammetric method had a more positive bias than those obtained by FI-amperometric method. Regression equation: Ascorbic acid content by voltammetric method = 1.05 (Ascorbic acid content by FI method) + 34.6; r^2 = 0.9909, indicated a good correlation between the two methods (the slope is about 1.0). The positive bias obtained from voltammetric method may be due to interference of other ingredients such as multivitamins. This problem is avoided by using FI-amperometric system with dialysis sample pretreatment. According to t-test[26] at 95% confidence level, the results obtained from the proposed method agreed well with the labeled values. Moreover, the

developed method consumed small amounts of reagent and sample per analysis, and is faster than voltammetric method.

3.4.2 Fruit Juice Samples

Preliminary investigation on application of the developed system to the determination of ascorbic acid in fruit juice samples was carried out. Each sample was injected in duplicate and FIgram was obtained as shown in Figure 4. Ascorbic acid contents determined by FI-amperometric method and the labeled one are summarized in Table 4. In most of the samples, ascorbic acid concentrations found are higher than the labeled values. More investigations including comparison of the developed method with other methods (e.g., voltammetric and chromatographic methods) will be performed. It should be noted that the proposed method should be more appropriate than spectrophotometric and titrimetric ones for fruit juice analysis since the amperometric detection does not suffer from colored and colloidal interferences and Schileren's effect.

4. CONCLUSION

A new FI amperometric system with on-line dialysis sample pretreatment has been developed. The proposed system has many advantages such as low chemical consumption, high sample throughput, no interference from colored substances and colloids, good sensitivity and precision, and a simple bare GCE could be employed. Application to pharmaceutical and fruit juice samples was demonstrated. The method may be useful for research and quality control of food and pharmaceutical products which a large number of samples is involved.

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Determination of available phosphorus in soils by using a new extraction procedure and a flow injection amperometric system

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ABSTRACT

A new extraction procedure based on an off-line extraction column was proposed for extracting of available phosphorus from soils. The column was fabricated from a plastic syringe fitted at the bottom with a cotton wool and a piece of filter paper to support a soil sample. An aliquot (50 mL) of extracting solution (0.05 M HCl + 0.0125 M H_2SO_4) was used to extract the sample under gravity flow and the eluate was collected in a polyethylene bottle. The extract was then analyzed for phosphorus contents by a simple flow injection amperometric system, employing a set of three-way solenoid valves as an injection valve. The method is based on the electrochemical reduction of 12-molybdophosphate which is produced on-line by the reaction of orthophosphate with acidic molybdate and the electrical current produced was directly proportional to the concentration of phosphate in range of 0.1–10.0 mg L^{-1} PO_4-P, with a detection limit of 0.02 mg L^{-1}. Relative standard for 11 replicate injections of 5 mg L^{-1} PO_4-P was 0.5%. A sample through put of 35 h^{-1} was achieved, with consumption of 14 mg KCl, 10 mg ammonium molybdate and 0.05 mL H_2SO_4 per analysis. The detection system does not suffer from the interferences that are encountered in the photometric method such as colored substances, colloids, metal ions, silicate and refractive index effect (Schlieren effect). The results obtained by the column extraction procedure were well correlated with those obtained by the steady-state extraction procedure, but showed slightly higher extraction efficiency.

1. Introduction

Phosphorus is a major nutrient for plants and is typically present in soils for agricultural purposes in the concentration range of 0.03–0.22%. However, the major fractions of P in soil are in the insoluble forms, which plants cannot utilize. Therefore, it is the plant-available P, the amount of P extracted by the chemical extractant, which is correlated well with the amount of P taken up by the plant that is more important than the total amount of P in the soil [1]. Orthophosphate is the dominant form of available P useful for plant grown. Release of available P from soil depends on several factors such as pH, humidity and type and amount of soil minerals. In agricultural practices, P is usually obtained from the applied fertilizer. However, in soils which contain high levels of iron and aluminum, P in fertilizer may be absorbed by soils, thus only small fractions (3–25%) would be available for plants. On the other hand, application of excessive amounts of fertilizer may cause impact to the environment due to leaching of the nutrient to the water body.

The determination of available P in soil is necessary for agricultural practices and research in this field. The test values obtained are useful for the producers to best manage for the application of fertilizer and make decisions concerning the profitability of their operations while managing for impacts such as erosion, nutrient runoff and water quality [2]. Several chemicals have been used as extractants for available P. The suitable extractant would give the best correlation between the amounts of P extracted and P taken up by the particular plant, which depends on several factors, such as type and amount of soil minerals, and pH of soil. There are some commonly used extraction protocol for available P such as Bray No. 1 (0.025 M HCl + 0.03 M NH_4F), Bray No. 2 (0.10 M HCl + 0.03 M NH_4F), North Carolina (0.05 M HCl + 0.0125 M H_2SO_4), Troug (0.001 M H_2SO_4 + $(NH_4)_2SO_4$ (pH 3.0)), Citric acid (1.0% citric acid), Egner (0.01 M calcium lactate + 0.02 M HCl), Morgan (0.54 M HOAc + 0.7 M NaOAc (pH 4.8)) and Olsen (0.50 M $NaHCO_3$ (pH 8.5)) [3,4]. The procedure for soil extraction is based on shaking known amounts of soil with an accurate volume of the extractant for extended periods of time to reach a steady-state condition between the solid and liquid phases. The extract is then filtered and analyzed for P content by spectrophotometric methods, employing either molybdenum blue or vanadomolybdate reactions. A stirred flow extraction chamber [5] and an extraction micro-column [6] have been proposed for on-line fractionation of P from soil and sediment under non-steady-state condition. In these procedures, a fresh extracting solution was continuously propelled though the

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sample bed, resulting in better extraction efficiency to be obtained. However, on-line extraction procedure requires a pump and a micro-column for each soil sample and concerning a long extraction time, so it is not suitable for extraction of available P involving a large number of soil samples. Such a protocol is usually applied for generating the extraction profiles for kinetic study of the leaching process [6]. Thus, we proposed here a batchwise gravity feed column extraction procedure, which on the other hand utilized a simple syringe as extraction vessel for each sample and despite it takes long extraction time, extraction of several samples at the same time could be carried out in order to increase sample throughput.

The spectrophotometric detection based on molybdenum blue method either in batch or flow systems is commonly used for determination of P. The flow system provided higher degrees of automation and reduced consumption of the chemicals. A multi-syringe flow injection analysis was proposed for determination of available P in soils [7]. All injection analysis was developed for determination of P in soil and sediment extracts [8]. However, the spectrophotometric detection method suffers potential interference from colored and colloidal substances and silicate. In flow analysis, the effect of refractive index or the Schlieren effect can also be a problem [6,8]. These interferences may present in the soil extract at high extents. We have developed the FI amperometric systems, which have higher tolerant to these interferences [9], thus they should be appropriate for soil analysis.

In this work, a new extraction procedure based on off-line column extraction was developed for extracting available P from soil. The collected extract was analyzed for P concentration by using a FI amperometric method which was based on the electrochemical reduction of the on-line formed 12-molybdophosphate, producing an electrical current, which was directly proportional to P concentration. The column extraction was simpler, more convenient to use and provided higher extraction efficiency than the standard extraction procedure based on batchwise shaking. The amperometric detection provided higher selectivity than the spectrophotometric one, as it did not suffer from colored substances, particulates and refractive index effect (Schlieren effect), and could tolerate to silicate at high concentration (up to 1000 mg L^{-1}). The system could be applied to soil extract without the requirement of masking agent and with low consumption of chemicals (2 mL each of KCl and reagent solutions per analysis).

2. Experimental

2.1. Chemicals and materials

All chemicals used were of analytical reagent grade. Deionized water (obtained by a system of Milli-Q, Millipore, Sweden) was used throughout. Acidic molybdate solution (0.5%, w/v in 2.5%, v/v sulfuric acid) was prepared by dissolving 1.25 g of ammonium molybdate (Ajax Finechem, Australia) in water and 6.50 mL of concentrated sulfuric acid (Merck, Germany) was added before adjusting the final volume to 250 mL. A 0.1 M potassium chloride was prepared by dissolving 1.86 g KCl (Merck, Germany) with water and adjusting volume to 250 mL. Orthophosphate stock solution (1000 mg L^{-1} PO_4-P) was prepared by dissolving 0.4394 g KH_2PO_4 (Merck, Germany) with water and adjusting volume to the mark of a 100-mL volumetric flask. Working standard solutions in the concentration range of 0.1–10 mg L^{-1} PO_4-P were daily prepared from an intermediate solution (10 mg L^{-1} PO_4-P), diluted from the stock solution. A solution for extraction of phosphate from soil (0.05 M HCl + 0.0125 M H_2SO_4) was prepared by diluting 2.10 mL conc. HCl and 0.35 mL conc. H_2SO_4 in water and adjusting the volume to 500 mL.

Electrode polishing kit (model MF-1000, BAS, USA) was used for cleaning of the working electrode.

2.2. FI manifold

The FI system used is schematically depicted in Fig. 1(a). It consisted of a peristaltic pump (Ismatec, Switzerland) with pump tubing, an injection device assembled from a set of three-way solenoid valves (Biochemvalve, USA) [10], a flow through electrochemical cell (cross-flow cell, Model MF-1093, BAS, USA) assembling with a 3-mm diameter glassy carbon working electrode (GCE), a stainless steel auxiliary electrode and a Ag/AgCl reference electrodes (3 M KCl), and a compact potentiostat (Palmsens Vs 3.6, Netherlands) with connecting to a personal computer. The three-way solenoid valves were assembled to be used as an injection valve, as shown in Fig. 1. Actuation of the valves for loading and injecting positions was performed by manually switching the electrical power supplied to the valves. PTFE tube of inner diameter of 0.5 mm was used for assembling the system.

2.3. Sample collection and extraction procedures

Soil samples were collected from longan orchards and vegetable growing field in Chiang Mai, northern of Thailand. A soil sample was taken from 15 points at a depth of 7 cm and combined together. The sample was dried in air and ground to a particle sizes of less than 100 mesh.

The standard extraction procedure was carried out by accurately weighing 1.00 g of soil, putting in a 100-mL Erlenmeyer flask, adding 15.00 mL of the extracting solution and shaking for 30 min. The extract was filtered through a Whatman No. 5 filter paper into a polyethylene bottle.

Column extraction was carried out batchwise employing a plastic syringe fitted at the bottom with cotton wool and a

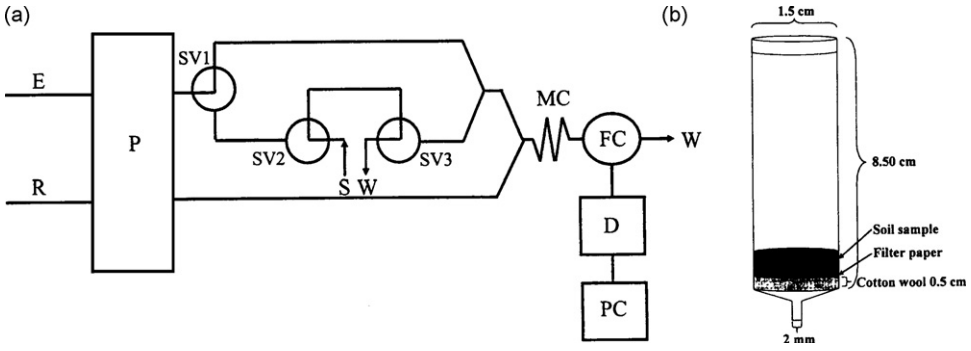

Fig. 1. (a) FI manifold of flow injection amperometric system for determination of phosphate; (E = 0.1 M KCl, R = 0.5%, w/v molybdate in 2.5%, v/v sulfuric acid, P = peristaltic pump, S = standard/sample, SV1–SV3 = three-way solenoid valves, MC = mixing coil, FC = electrochemical flow through cell, W = waste, D = potentiostat/amperometric detector, PC = personal computer) and (b) an extraction column for off-line leaching of soil samples (adapted from a 10-mL plastic syringe).

piece of filter paper to support the sample (Fig. 1(b)). A portion (1.0000 g) of ground soil was accurately weighed and put in the column. An extracting solution (50.00 mL) was carefully poured into the column and the leachate was collected in a polyethylene bottle.

Extraction was carried out at room temperature of about $25 \pm 2\,^{\circ}C$.

2.4. FI amperometric determination procedure

A KCl electrolyte solution and an acidic molybdate solution were pumped at 1.0 mL min^{-1} each. A standard/sample solution (75 μL) was injected into the stream of 0.1 M KCl and then merged with the stream of the acidic molydate solution which then flowed through a mixing coil (50 cm in length) to the amperometric flow cell. The 12-molybdophosphate complex produced on-line was then electrochemically reduced at the glassy carbon working electrode producing an electrical current, that was directly proportional to the concentration of phosphate (PO_4-P). The current was converted to voltage and continuously recorded as a FI peak. A calibration graph was a plot of peak height obtained vs. P concentration. Concentration of P in sample was then evaluated from the calibration graph.

3. Results and discussion

3.1. FI amperometric determination of orthophosphate

The FI amperometric method for orthophosphate determination is based on the electrochemically reduction of the 12-molybdophosphate which formed on-line by the reaction of orthophosphate and molybdate in an acidic medium [9]. The reduction current is linearly proportional to orthophosphate concentration. The FI amperometric system for the determination of orthophosphate in the soil extract was modified from the previously reported system [9] by replacing the six port injection valve with the injection valve assembled from three solenoid valves (SV1–SV3) and a three-way connector, as shown in Fig. 1(a). At the loading position (as shown in Fig. 1(a)), a standard/sample was loaded into a sample loop and then by manually switching current, the solenoid valves were actuated to another position to allow the carrier solution (0.1 M KCl) to inject sample into the reagent solution (0.5%, w/v ammonium molybdate in 2.5%, v/v H_2SO_4). The reaction product, 12-molybdophosphate was reduced at the GCE, at a constant applied potential of 0.20 V vs. Ag/AgCl, producing a FI peak that was recorded by a portable amperometer. Fig. 2 illustrates the FI profiles of phosphate standard solution (1.0–10.0 mg L^{-1} PO_4-P) and some samples. Linear calibration graphs for phosphate in the concentration ranges of 0.1–1.0 ($y = 0.2455x + 0.0014$, $r^2 = 0.9996$) and 1.0–10.0 ($y = 0.2111x + 0.1389$, $r^2 = 0.9966$) mg L^{-1} PO_4-P were obtained. The detection limit calculated from three times standard deviation of blank/slope of the calibration graph was found to be 0.02 mg L^{-1} PO_4-P.

Relative standard deviations for 11 replicate injections of 5 mg L^{-1} PO_4-P were 0.5%. A sample throughput of 35 h^{-1} was achieved, with the low consumption of reagents (about 2 mL each of electrolyte and reagent solutions, corresponding to 14 mg KCl, 10 mg ammonium molybdate and 0.05 mL sulfuric acid per analysis).

As reported previously for the determination of P in water samples [9,10], the FI amperometric method has higher selectivity than those based on spectrophotometric detection. The method can tolerate silicate up to 1000 mg L^{-1}, so no masking agent was needed for soil analysis. The interferences from some metals such as Fe and Al at high concentration can be avoided by passing the sample solution through a cation exchange resin column. However, no interference was observed for the studied soil samples, so such a treatment was not necessary. The developed system did not suffer from colored substances, colloids and Schlieren effect.

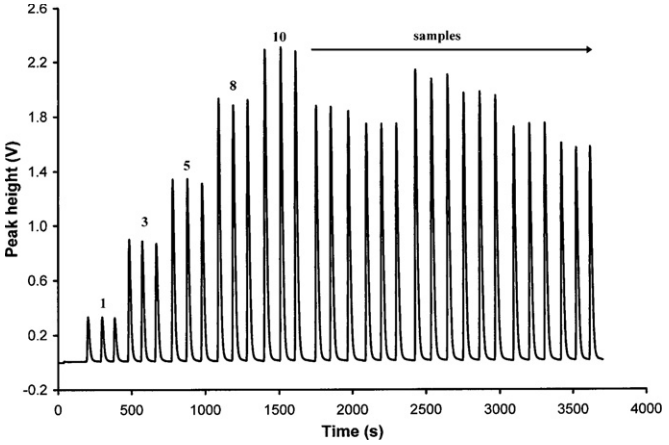

Fig. 2. FI profiles for standard phosphate solutions and samples, determined by FI amperometric system in Fig. 1(a); condition: applied potential 0.20 V, $E = 0.1$ M KCl, $R = 0.5\%$, w/v molybdate in 2.5%, v/v sulfuric facid, and flow rate of each line 1.0 mL min^{-1}. Values indicate concentration of phosphate in mg L^{-1} PO_4-P.

3.2. Leaching of available P from soil by extraction column

The standard procedure for extraction of available phosphorus from soil is based on shaking of the suspension of soil in the extractant for a period of time to reach equilibrium (45 min) and filtering of the extract. Different extracting solutions can be utilized, which may give slightly different available P contents dependent on the type of soils. The proposed extraction procedure was based on column extraction and the North Carolina solution (0.05 M HCl + 0.0125 M H_2SO_4) was selected as the extracting solution. A simple 10 mL plastic syringe was adopted to use as a column, by taking out the plunger and placing a cotton wool and a piece of a Whatman No. 40 filter paper fitted to the syringe for supporting a soil sample as shown in Fig. 1(b). An accurate amount of soil sample (about 1.00 g) was put in the syringe and an accurate volume of extracting solution was added carefully to fill the remainder volume of the syringe. The extraction gravity feed profiles were investigated by collecting the eluate fractions (15.00 mL each) to be determined for phosphate concentration. It was found that the similar profiles were obtained for different soil samples and more than 99% of the phosphate could be extracted within fraction number 3, corresponding to extraction volume of 45.00 mL, as shown in Fig. 3. The volume of extracting solution of 50.00 mL was selected for further extraction of soil samples. Although the extraction time of about 50 min was needed, several extractions could be performed in parallel at the same time, leading to high sample throughput. The relative standard deviation was 6.0%, for six replicate extractions of the same soil sample, which was comparable to that of the standard extraction procedure (6.8%). Although the mini-column could be incorporated to the FI amperometric system in order to perform on-line extraction/determination, the low sample throughput was resulted due to the long extraction time involved. Thus the on-line extraction was not suitable for determination of available P in soil, but it might be appropriate for study on sequential extraction of P associated with different phases of soil employing various extracting solutions [6].

It should be noted that some extracted solutions obtained had intense color due to the organic matter present in the soils, which

Table 1
Available P contents in soil samples, extracted by the proposed and the standard procedures, and determined by the FI amperometric and standard spectrophotometric [11] methods.

Sample	Available phosphorus content (mg kg^{-1} as PO_4-P)*			
	Column extraction		Standard extraction	
	FI amperometric	Spectrophotometric	FI amperometric	Spectrophotometric
1	1173 ± 4	1152 ± 5	1083 ± 1	1095 ± 7
2	1349 ± 14	1368 ± 3	1235 ± 10	1244 ± 2
3	1085 ± 1	1119 ± 1	960 ± 2	995 ± 2
4	1718 ± 8	1725 ± 9	1535 ± 7	1505 ± 6
5	1810 ± 1	1729 ± 1	1567 ± 4	1533 ± 1
6	473 ± 6	467 ± 1	421 ± 4	422 ± 2
7	1387 ± 8	1352 ± 2	1217 ± 5	1182 ± 2
8	73.9 ± 1.3	60.4 ± 0.1	58.6 ± 0.9	51.5 ± 0.1
9	2160 ± 5	2040 ± 1	1859 ± 4	1749 ± 1
10	1951 ± 1	2060 ± 7	1527 ± 6	1697 ± 1
11	3.7 ± 0.2	4.3 ± 0.1	2.3 ± 0.1	3.2 ± 0.2
12	35.4 ± 0.3	30.8 ± 0.3	28.2 ± 0.1	24.4 ± 0.3
13	45.2 ± 0.2	44.6 ± 0.4	31.0 ± 0.1	31.4 ± 0.2
14	12.9 ± 0.1	13.6 ± 0.1	7.1 ± 0.1	7.6 ± 0.1
15	38.5 ± 1.0	34.9 ± 0.2	24.0 ± 0.2	23.1 ± 0.2
16	65.8 ± 1.0	59.2 ± 0.5	56.6 ± 2.1	52.2 ± 0.3
17	22.4 ± 0.6	26.0 ± 0.2	29.7 ± 0.4	31.9 ± 0.1

* Mean of triplicate results.

may interfere in the spectrophotometric determination, but not in the FI amperometric system.

3.3. Determination of available P in soil samples

The developed method was applied to the determination of available P in different soil samples, collected from Longan orchards and vegetable field in Chiang Mai province of Thailand. Soil extraction was performed using the developed procedure and a standard extraction procedure for comparison. The extracts were determined for available P contents by the proposed FI amperometric method and standard spectrophotometric method [11] based on molybdenum blue reaction using ascorbic acid as a reducing agent and potassium antimonyl tartrate acting simultaneously as a catalyst and masking agent. According to *t*-test at 95% confidence level, the results obtained from both methods were in good agreement ($t_{critical} = 2.021$, $t_{calculated} = 0.025$, $n = 17$) [12], as presented in Table 1. It should be noted that the FI amperometric method consumed much smaller amounts of chemicals and was simpler, faster, more convenient to use and provided better tolerance to the interferences

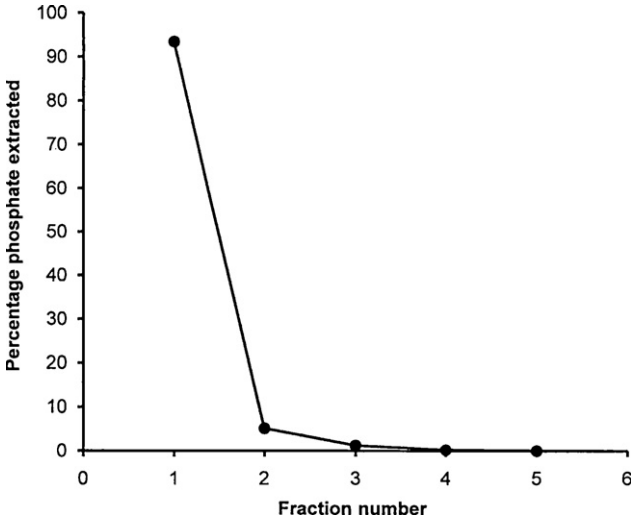

Fig. 3. Percentage extraction of phosphate from soil in different fractions of the extracting solution.

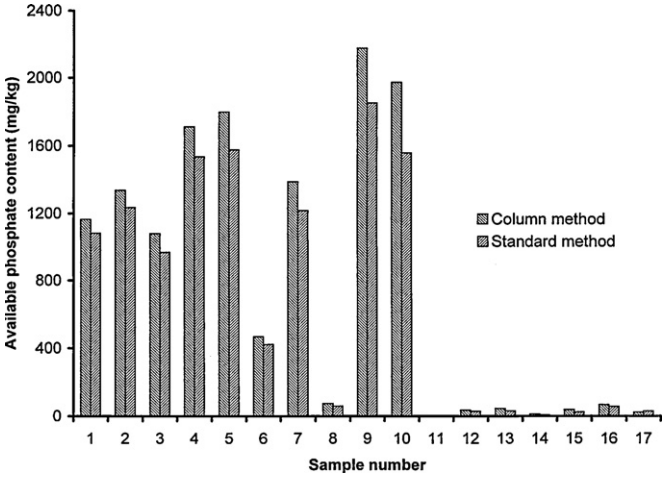

Fig. 4. Available P contents extracted by using the column extraction and standard extraction procedures.

presented in soil (e.g., colored substances, colloids and silicate) than the standard spectrophotometric method.

It was found that the phosphate contents found in the extracts from both the extraction procedures were in good correlation. However, the proposed extraction procedure provided better extraction efficiency than the standard method, as shown in Fig. 4. This may due to the advantage of dynamic extraction that the sample was in contact with the fresh extracting solution, which flowed through the sample bed in the extraction column, so no re-adsorption of the extracted P was occurred. Moreover, the proposed extraction procedure was simpler, and more convenient to use than the standard method. The extraction of several samples could be performed at the same time.

4. Conclusion

A flow injection amperometric method and a new extraction procedure were proposed for the determination of available phosphorus in soil samples. Although the extraction was done by using a simple column made from a plastic syringe, a better extraction efficiency than the standard extraction procedure could be accom-

plished, which was more convenient to perform. The proposed extraction procedure may be a new alternative way to perform extraction of available P in routine analysis of soil. The FI amperometric method provided the precise and accurate results, with using of simple and cost-effective components to assemble the system, and consumed of smaller amount of chemicals. The amperometric detection also provided significant advantages over the spectrophotometric detection of the standard method, e.g., it can tolerate to silicate up to 1000 mg L^{-1} and does not suffer interference from turbidity and colored substances present in soil samples. The system could be developed to higher degrees of automation by control of the injection valve via a computer.

Acknowledgements

The Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), the Commission on Higher Education (CHE) and the Thailand Research Fund (TRF) are acknowledged for financial supports.

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ภาคผนวก ข

บทคัดย่อผลงานบางส่วนที่นำเสนอในการประชุมวิชาการ

Speciation of Fe(II) and Fe(III) by Hydrodynamic Sequential Injection (HSI) System

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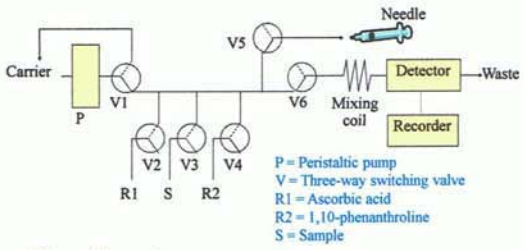
Introduction

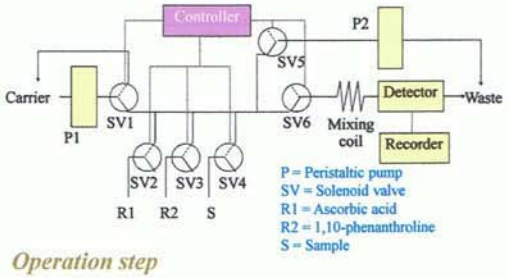
Hydrodynamic injection was first proposed by Ruzicka and Hansen (1983) as an alternative injection procedure in FIA. By this idea, an exact volume of sample was inserted with hydrodynamic pressure into well-defined conduit without injection devices. In this work, we propose a new concept, named, hydrodynamic sequential injection (HSI), which both reagents and sample were injected sequentially into a flow conduit based on hydrodynamic injection principle. The manual and semi-automated HSI system with simple and low cost instrumentation are exploited. This proposed system was demonstrated for the speciation of Fe(II) and Fe(III) based on 1,10-phenanthroline method and applied to water and soil analysis.

Manifold and Results

A manual HSI system
(applied to water sample analysis)



A semi-automated HSI system
(applied to soil sample analysis)



Operation step

Step	Valve						Description
	V1	V2	V3	V4	V5	V6	
1	off	off	off	on	off	on	Draw up R2 by syringe
2	off	off	off	off	on	on	Draw up S by syringe
3	on	on	off	off	off	off	Forward the mixture to detector (for Fe(II) determination)
4	off	off	on	off	off	on	Draw up R1 by syringe
5	off	off	off	on	off	on	Draw up R2 by syringe
6	off	off	off	off	on	on	Draw up S by syringe
7	on	on	off	off	off	off	Forward the mixture to detector (for total iron determination)

Operation step

Step	Valve						Description
	SV1	SV2	SV3	SV4	SV5	SV6	
1	on	off	on	off	off	on	Draw up R2 by P2
2	on	off	off	on	on	on	Draw up S by P2
3	off	off	off	off	off	off	Forward the mixture to detector (for Fe(II) determination)
4	on	on	off	off	on	on	Draw up R1 by P2
5	on	off	on	off	on	on	Draw up R2 by P2
6	on	off	off	on	on	on	Draw up S by P2
7	off	off	off	off	off	off	Forward the mixture to detector (for total iron determination)

Features of HSI system

Parameters	Fe(II)	Fe(III)
Linear range	0.5 - 20 μg ml^{-1}	0.5 - 20 μg ml^{-1}
Limit of detection (LOD)	66 μg l^{-1}	70 μg l^{-1}
Precision (n=11, %RSD)	3.0 (5 μg l^{-1})	2.0 (5 μg l^{-1})
Sample throughput (h^{-1})	15	

Features of HSI system

Parameters	Fe(II)	Fe(III)
Linear range	0.5 - 20 μg ml^{-1}	0.5 - 20 μg ml^{-1}
Limit of detection (LOD)	110 μg l^{-1}	95 μg l^{-1}
Precision (n=11, %RSD)	2.4 (5 μg l^{-1})	1.2 (5 μg l^{-1})
Sample throughput (h^{-1})	25	

Conclusion

The hydrodynamic sequential injection (HSI) systems was developed. Despite simple and low cost devices were employed, the systems show good performance. This HSI concept should be a cost effective alternative approach with respect to other flow based systems for application to environmental analysis..

Acknowledgement

We appreciate the support from Chiang Mai University, the Postgraduate Education and Research Program in Chemistry: the Center for Innovation in Chemistry (PERCH-CIC) and the Thailand Research Fund (TRF) for the scholarships to S. Somnam.

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Introduction

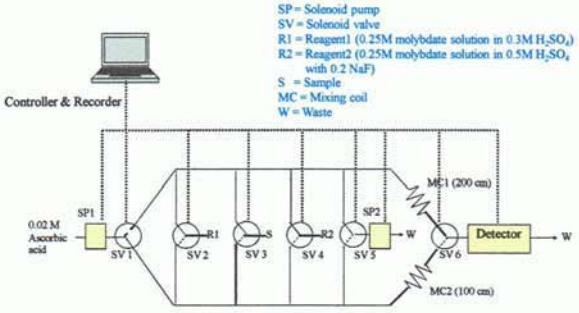
Hydrodynamic injection was first proposed by Ruzicka and Hansen [1] as an alternative injection procedure in FIA. By this idea, an exact volume of sample was inserted with hydrodynamic pressure into well-defined conduit without injection devices. In this work, we propose a new concept, named, hydrodynamic sequential injection (HSI), which both reagents and sample were injected sequentially into a flow conduit based on hydrodynamic injection principle. The fully automated HSI system with simple and low cost instrumentation are exploited. This proposed system was demonstrated for the determination of phosphate and silicate based on molybdenum blue method and applied to waste and fish pond water analysis.

Objectives

- To develop the fully automated HSI system for the determination of phosphate and silicate
- To apply the developed system to real sample

Manifold and Operation

A fully automated HSI system with a computer control



Operation step

Step	Pump		Valve						Description
	SP1	SP2	SV1	SV2	SV3	SV4	SV5	SV6	
1	off	on	on	off	on	off	on	off	Draw up sample
2	off	on	on	off	off	on	on	off	Draw up reagent2
3	on	off	off	off	off	off	off	on	Forward the mixture (for phosphate determination)
4	off	on	off	off	on	on	off	on	Draw up reagent1
5	off	on	off	on	off	on	off	on	Draw up sample
6	on	off	on	on	on	on	on	off	Forward the mixture (for phosphate plus silicate determination) for 15 seconds
7	off	off	on	on	on	on	on	off	Stop the mixture to increase the reaction time of silicate to molybdate
8	on	off	on	on	on	on	on	off	Forward the mixture to detector (for phosphate plus silicate determination)

Results and Discussion

Selected conditions

Parameters	Phosphate	Total concentration
Reagent concentration (M)		
- molybdate solution	0.25	0.25
- H_2SO_4	0.50	0.30
- NaF	0.20	-
- ascorbic acid	0.020	0.020
Stopping time (s)	-	15

Features of HSI system

Parameters	Phosphate	Total concentration
Linear range	Up to 10 mg l^{-1}	Up to 12 mg l^{-1}
Limit of detection (LOD)	80 µg l^{-1}	90 µg l^{-1}
Precision (n=11, %RSD)	1.8 (0.8 mg P l^{-1})	0.9 (0.8 mg P l^{-1} + 4.0 mg Si l^{-1})
Sample throughput (h^{-1})	20	

Application to Real Samples

Sample	Phosphate (mg l^{-1})		Silicate (mg l^{-1})	
	Proposed method	Standard method	Proposed method	Standard method
Waste 1	5.0	5.4	3.1	2.8
Waste 2	1.3	1.0	4.8	5.0
Waste 3	0.9	0.7	6.0	6.2
Waste 4	*ND.*	*ND.*	5.6	5.9
Waste 5	1.9	1.7	6.1	6.4
Fish pond 1	*ND.*	*ND.*	0.8	0.7
Fish pond 2	5.3	5.5	7.4	7.8
Fish pond 3	*ND.*	*ND.*	1.5	1.4

Comparisons between two methods (for both phosphate and silicate determination) were done by t-test at 95% confidence level. It was found that the calculated t-values were less than the tabulated t-values. So, there are no significant differences between the two methods at this level.

Conclusion

The fully automated hydrodynamic sequential injection (HSI) systems was developed. Despite simple and low cost devices were employed, the systems show good performance. This HSI concept should be a cost effective alternative approach with respect to other flow based systems for application to environmental analysis..

Reference

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We appreciate the support from Chiang Mai University (Thailand) and Okayama University (Japan), the Postgraduate Education and Research Program in Chemistry: the Center for Innovation in Chemistry (PERCH-CIC), the Thailand Research Fund (TRF) and the JASSO short-term student exchange promotion program for the scholarships to S. Somnam.

On-line UV Digestion and Anodic Stripping Voltammetric System for Determination of Zn Cd Pb and Cu in Water Samples

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INTRODUCTION

Some trace metals such as Zn, Cd, Pb and Cu are known to occur organically complexed in natural water sample. The organic complexation causes these trace metals to be partially non-reactive (non-labile) during voltammetric analysis unless the dissolved organic matter (DOM) is destroyed [1].

Wet digestion is a commonly used method for the destruction of DOM prior to metal analysis. However, the high level of oxidants added by this method often introduce contamination to the samples.

UV digestion is a method that is often used for destruction of DOM prior to voltammetric trace metal analysis [2]. It is clean method, as it does not generally require added oxidants. In addition, it is effective and can easily be incorporated in an automated trace metal analyzer. Therefore, it is a more preferable method for destruction of DOM prior to voltammetric trace metal determination.

OBJECTIVES

- To develop an on-line UV digestion and anodic stripping voltammetric system for determination of Zn(II), Cd(II), Pb(II) and Cu(II)
- To apply the developed system for determination of Zn(II), Cd(II), Pb(II) and Cu(II) in model water samples

EXPERIMENTAL

The sequential injection -anodic stripping voltammetric (SI-ASV) system consisted of a syringe pump (SP), a selection valve (SV) and a voltammograph equipped with a modified flow through electrochemical cell (working electrode : HMDE, reference electrode : Ag/AgCl, auxiliary electrode : Pt wire). A home-made UV digestion unit employing a small size and low wattage (6 W) UV lamps has been fabricated. The flow reactor was made from PTFE tubing coiled around the UV lamp and was connected to the SI-ASV system. Optimum conditions as shown in Table 1 were employed.

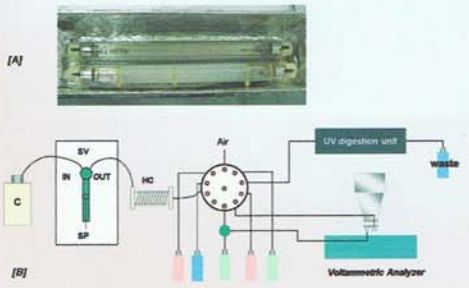


Figure 1 A home-made UV digestion unit (A) and sequential injection UV digestion voltammetric system (B)

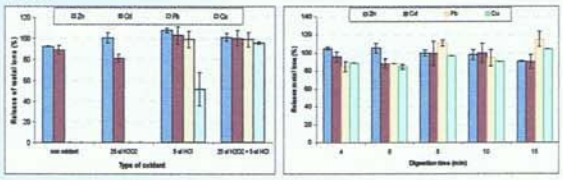
Table 1 Optimum conditions for SI-UV-ASV system

Parameters	Value
Deposition potential (V)	-1.1
Deposition time (s)	180
Sweep mode	Square wave
Sweep potential (V)	-1.1 - 0.2
Type and volume of oxidant	25 µl H_2O_2 + 5 µl conc.HCl (added in 5 ml sample solution)
Sample digestion volume	1500 µl
Digestion time	10 min

RESULTS AND DISCUSSION

1. Digestion efficiency

The parameters affecting digestion efficiency, i.e., type, concentration and volume of oxidant, and digestion time were investigated. The release of metal ions from organic complexes with strong organic ligand (EDTA) at mole ration of 1 : 10 metal : EDTA were used as test solution. It was found that the developed system provided good digestion efficiency of 91-101% when using 25 µl H_2O_2 + 5 µl conc.HCl as oxidants and UV digestion time of 10 minutes.



[A] [B]

Figure 2 Effect of oxidant type [A] and digestion time [B] on digestion efficiency of the proposed on-line UV digestion combined with SI-ASV system

2. Digestion performance

Some organic matters i.e. EDTA (strong organic ligand), humic acid or HA (dissolved organic matter), TritonX-100 (surfactant) were added to the metal solution as model water samples. Performance of the proposed system was tested. The wet acid digestion method of aqueous samples [4] for total metal analysis (USEPA 3010a) was used as standard digestion method for comparison. According to t-test at 99.8% confident limit, the results obtained from both methods were in good agreement ($t_{critical}$ = 4.025, $t_{calculate}$ = 1.387, 3.862, 0.477 and 3.365 for Zn(II), Cd(II) Pb(II) and Cu(II),respectively).

Table 2 Comparative results of the release metal ions in model water samples using UV-digestion method and wet digestion method

No.	Organic matter concentration (mg/l)			Release of metal ions (%)							
				Zn		Cd		Pb		Cu	
	EDTA	HA	Triton X	UV digestion	Wet digestion	UV digestion	Wet digestion	UV digestion	Wet digestion	UV digestion	Wet digestion
1	5	-	-	81.1 ± 2.3	113.8 ± 5.9	104.4 ± 0.9	110.3 ± 2.5	104.7 ± 4.2	97.4 ± 12.7	97.3 ± 5.7	114.7 ± 6.9
2	10	-	-	77.8 ± 2.0	94.0 ± 3.8	104.9 ± 1.1	112.8 ± 2.1	103.1 ± 1.4	101.0 ± 12.7	98.1 ± 9.7	109.9 ± 2.8
3	25	-	-	86.3 ± 1.5	89.2 ± 13.6	100.6 ± 2.5	107.8 ± 6.6	94.0 ± 1.5	108.6 ± 2.0	101.9 ± 1.3	106.6 ± 4.2
4	-	5	-	90.1 ± 4.2	115.0 ± 14.7	102.7 ± 3.6	103.8 ± 14.8	97.7 ± 4.5	91.2 ± 13.6	101.7 ± 6.7	115.6 ± 3.5
5	-	10	-	100.4 ± 2.2	77.5 ± 6.5	100.1 ± 5.6	113.2 ± 0.8	93.7 ± 1.2	108.6 ± 6.2	99.9 ± 12.9	108.8 ± 4.8
6	-	25	-	100.9 ± 2.9	115.4 ± 4.2	102.4 ± 5.5	99.6 ± 6.6	96.3 ± 4.0	98.0 ± 4.1	104.6 ± 4.0	106.5 ± 7.2
7	-	-	5	95.2 ± 2.9	76.3 ± 6.9	103.0 ± 4.0	119.4 ± 1.1	104.2 ± 2.3	99.5 ± 3.2	106.3 ± 15.5	96.5 ± 8.9
8	-	-	10	85.8 ± 1.6	118.8 ± 3.6	115.5 ± 7.9	113.9 ± 3.3	105.5 ± 1.7	101.2 ± 1.2	104.1 ± 4.9	111.9 ± 16.3
9	-	-	25	99.1 ± 4.2	91.2 ± 11.7	100.5 ± 5.5	110.0 ± 3.8	99.7 ± 1.0	94.8 ± 10.7	100.2 ± 9.1	123.9 ± 9.2
10	5	5	5	88.3 ± 3.5	85.0 ± 12.5	102.0 ± 4.7	113.0 ± 11.8	100.6 ± 7.2	107.6 ± 5.4	107.6 ± 5.9	117.9 ± 17.4
11	10	10	10	83.4 ± 6.4	92.9 ± 8.8	106.0 ± 0.3	109.9 ± 3.8	93.6 ± 3.3	112.5 ± 2.0	106.2 ± 4.0	116.5 ± 4.9
12	25	25	25	85.6 ± 0.9	93.2 ± 1.7	104.6 ± 2.0	114.2 ± 2.7	104.8 ± 4.3	93.7 ± 1.2	109.2 ± 0.7	107.9 ± 8.3

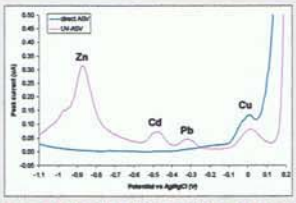


Figure 3 Voltammograms of 100 µg/l Zn, 50 µg/l Cd, 50 µg/l Pb and 100 µg/l Cu in model water samples No.11 [EDTA, HA, TritonX 10 mg/l each] before and after UV digestion

CONCLUSIONS

- The proposed on-line UV digestion system was successfully used for simultaneous determination of zinc, cadmium, lead and copper using only small volume of sample and reagent solutions and with high degrees of automation.
- The UV digestion efficiency results of model water samples are in good agreement with the results obtained from wet digestion method.

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- The Royal Golden Jubilee (RGJ) Ph.D. Program
- The Commission on Higher Education (CHE)
- The Center of Excellence for Innovation in Chemistry : Postgraduate Education and Research Program in Chemistry (PERCH-CIC)
- The Thailand Research Fund (TRF)

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A SIMPLE AND COST EFFECTIVE MICROFLUIDIC SYSTEM WITH LIGHT DEPENDENT RESISTOR FOR IRON(III) ANALYSIS

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Abstract

In this research, a simple and cost effective microfluidic device with light dependent resistor (LDR) as light sensor has been designed and investigated for monitoring the color change in channel of the microchip. The system consisting of black acrylic chip with the channel network, 300 μm i.d. x 50 μm depth, was fabricated by laser technology and covered by transparent molded-PDMS, and fixed with a low cost optical sensor based on LDR and light emitting diode (LED) light source. This low cost sensor has been applied to monitor the color change of $Fe(SCN)^{2+}$ in this microfluidic system and it was found that the linearity in range of 0.0005-0.005 mol L^{-1} of Fe(III) with R^2 of 0.9981. The most advantage of this system is to minimize analysis cost and miniaturize total analysis system.

Keywords; microfluidics, lab on chip, sensor

Introduction

The microfluidic devices are often described as miniature versions of their macro-scale counterparts. In this research, we are interested to design chip and light detection unit that the most significant benefit would be scaling down the size and reduction of manufacturing cost by using PMMA-PDMS chip and Light.

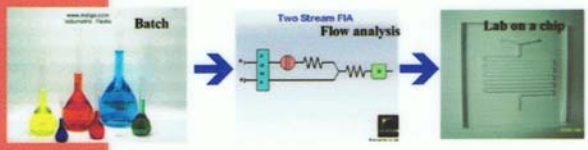
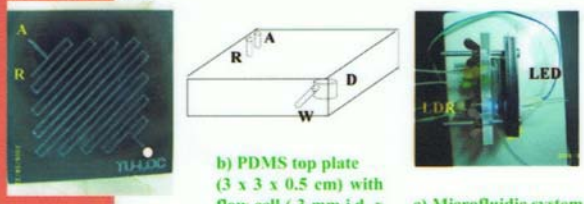


Fig. 1 The Generation of analytical system's scaling down

Methodology

This black acrylic chip was fabricated by laser technique with channel 300 μm i.d. x 50 μm depth and covered by molded-PDMS.



a) Chip platform

b) PDMS top plate (3 x 3 x 0.5 cm) with flow cell (3 mm i.d. x 1 mm height)

c) Microfluidic system

Fig. 2 Microfluidic system with LDR light sensing and LED light source. A: analyte, R: Reagent , D: flow cell (3mm id x 1 mm height) and W: waste out

LDR; Light dependent resistor act as light sensor. This system is applied to monitor red complex of the reaction of Fe(III) and SCN^-

$$Fe^{3+} + SCN^- \rightarrow [Fe(SCN)]^{2+}$$

Results and Discussion

Calibration graph

This microfluidic system was successful to monitor signal and the calibration graph was linear in range of 0.0005-0.005 mol L^{-1} of Fe(III) as shown in Fig. 3.

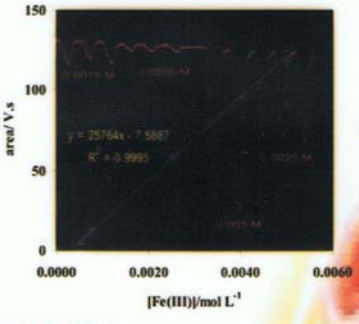


Fig. 3 Calibration graph

Conclusion

This LDR in a microflow system was successful to apply as optical sensor in a chip with a low manufacturing cost and miniturize size. However, in the next work, the sensitivity have to be improved.

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ภาคผนวก ค

การประยุกต์งานวิจัยสำหรับการเรียนการสอน

การทดลองที่ 2

การหาปริมาณแอมโมเนียมไอออนโดยเทคนิคโฟลอินเจคชันคอนดักโตเมตรี

วัตถุประสงค์

1. เพื่อศึกษาเทคนิคโฟลอินเจคชันอะนาลิซิสที่มีการตรวจวัดด้วยการวัดค่าความนำไฟฟ้า
2. เพื่อศึกษาวิธีเพิ่มความจำเพาะในการวิเคราะห์สารด้วย gas diffusion unit

หลักการ

การวัดค่าความนำไฟฟ้าของสารละลายโดยตรง ไม่สามารถใช้หาปริมาณสารได้เพราะไม่มีความจำเพาะ (selectivity) เนื่องจากค่าความนำไฟฟ้าที่วัดได้เป็นผลรวมของความนำไฟฟ้าของไอออนทั้งหมดในสารละลาย จากค่าความนำไฟฟ้า(conductivity) ที่วัดได้จึงมักใช้บอกภาพรวมของปริมาณไอออนในสารละลาย ซึ่งอาจใช้ประโยชน์ในการบอกถึงความบริสุทธิ์ของน้ำ ส่วนการหาปริมาณสารโดยเทคนิคนี้จึงต้องใช้เทคนิคต่าง ๆ มาช่วยเพิ่มความจำเพาะในการวิเคราะห์ เช่น การแยกไอออนออกจากกันด้วยคอลัมน์ที่บรรจุสารแลกเปลี่ยนไอออน การแยกสารโดยใช้เยื่อเลือกผ่าน เป็นต้น

ในการทดลองนี้จะได้ศึกษาเทคนิคโฟลอินเจคชันอะนาลิซิสที่มี gas diffusion unit ช่วยเพิ่มความจำเพาะ ในการวิเคราะห์โดยช่วยแยกแอมโมเนียออกมาจากสารละลายตัวให้ (donor stream) และมาละลายลงในสารละลายตัวรับ (acceptor stream) ก่อนจะนำไปวัดค่าความนำไฟฟ้าที่เครื่องตรวจวัด [1-3] ดังรูปที่ 1

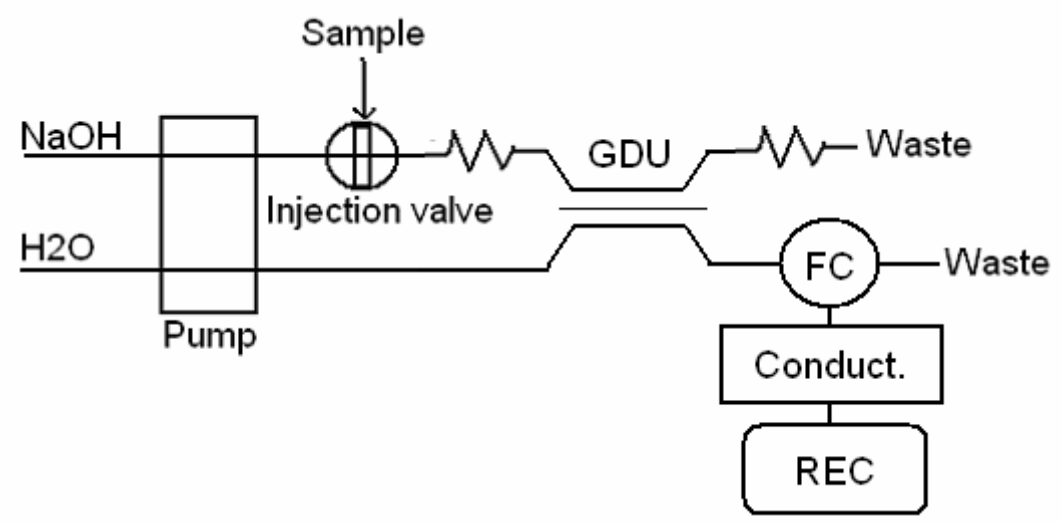
เมื่อฉีดสารมาตรฐานหรือตัวอย่างลงในสารละลายตัวให้ (donor stream) (1 M NaOH) NH_4^+ จะถูกเปลี่ยนเป็นแก๊สแอมโมเนีย ดังสมการที่ 2.1

$$OH^- + NH_4^+ = NH_{3\,(g)} + H_2O \quad \text{- - - - - (2.1)}$$

ซึ่งแก๊สแอมโมเนียจะสามารถแพร่ผ่านเยื่อเทปลอนไปยังสารละลายตัวรับ(acceptor stream) และเกิดปฏิกิริยาดังสมการ

$$NH_3 + H_2O = NH_4^+ + OH^- \quad \text{- - - - - (2.2)}$$

ซึ่งจะทำให้การนำไฟฟ้าของสารละลายตัวรับ (acceptor stream) เพิ่มขึ้นโดยแปรผันตรงกับปริมาณ NH_3 ที่แพร่ผ่านเข้ามา สามารถบันทึกเป็นพีกบนเครื่องบันทึก (recorder) ความสูงพีกที่ได้แปรผันโดยตรงกับความเข้มข้นของแอมโมเนียมไอออนในสารละลายตัวอย่างที่ฉีดเข้าสู่ระบบ ซึ่งสามารถสร้างกราฟมาตรฐานเพื่อใช้หาปริมาณแอมโมเนียมไอออนในตัวอย่างได้



รูปที่ 1 Flow injection conductometric system; GDU= gas diffusion unit, FC = Flow through cell, Conduct. = conductometric detector, and REC = Recorder.

เครื่องมือและอุปกรณ์

1. peristaltic pump
2. injection valve
3. gas diffusion unit
4. conductometric flow through cell
5. conductometer
6. เครื่องบันทึก (recorder)

สารเคมี

1. แอมโมเนียมคลอไรด์ (NH_4Cl) stock solution 1000 ppm 100 mL
2. โซเดียมไฮดรอกไซด์ (0.5 M) 250 mL
3. ตัวอย่างยาแก้ไอ
4. ตัวอย่างน้ำ

วิธีการทดลอง

1. ติดตั้งระบบโฟลอินเจคชันอะนาลิซิสดังรูปที่ 1
2. ปรับสภาวะต่าง ๆ ของการทดลองดังนี้
   2.1 อัตราการไหลของ donor stream 1 mL/min
   2.2 อัตราการไหลของ acceptor stream 1 mL/min
   2.3 Injection volume 100 μL
   2.4 Recorder full scale 0.5 V
3. บันทึกสัญญาณ baseline จนได้สัญญาณที่เรียบ ทำการฉีดสารสารละลายมาตรฐานความเข้มข้น 200 ppm NH_4^+ ปรับ recorder full scale ให้ได้ความสูงพีกที่เหมาะสม (เกือบเต็มหน้ากระดาษบันทึก)
4. ฉีดสารละลายมาตรฐานความเข้มข้น 0, 20, 40, 60, 80, 100, 150, 200 ppm NH_4^+ บันทึก FIAgram สร้างกราฟมาตรฐานโดยเขียนระหว่างความสูงพีก (mV) กับความเข้มข้นของ NH_4^+
5. ฉีดสารละลายตัวอย่างแล้วหาปริมาณ NH_4^+ จากกราฟมาตรฐาน

เอกสารอ้างอิง

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คำถาม

1. Gas diffusion unit ช่วยเพิ่มความจำเพาะในการหาปริมาณแอมโมเนียมโดยการตรวจวัดค่าความนำไฟฟ้าได้อย่างไร
2. ท่านคิดว่าระบบที่ใช้ในการทดลองนี้จะมีการรบกวนจากสารใดบ้าง