รายงานวิจัยฉบับสมบูรณ์

โครงการการพัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลง เพื่อการเตรียมตัวอย่างแบบออนไลน์

โดย ดร. รจนา บุระคำ และคณะ

พฤษภาคม 2550

สัญญาเลขที่ MRG4880157

รายงานวิจัยฉบับสมบูรณ์

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คณะผู้วิจัย	สังกัด
1. ดร. รจนา บุระคำ	**ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น**
2. ศ.ดร. เกตุ กรุดพันธ์	**ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่**

สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และ สำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

การพัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลง เพื่อการเตรียมตัวอย่างแบบออนไลน์

หัวหน้าโครงการ: ดร. รจนา บุระคำ
นักวิจัยที่ปรึกษา: ศาสตราจารย์ ดร. เกตุ กรุดพันธ์

โครงการนี้ได้วิจัยเกี่ยวกับการพัฒนาระบบการแยกและการวิเคราะห์ทางเคมี โดยใช้เทคนิคการไหล เพื่อประยุกต์สำหรับการเตรียมตัวอย่างและการวิเคราะห์ โดยเน้นการลดขนาดการวิเคราะห์ ปรับขั้นตอนต่างๆให้ง่ายขึ้น และพัฒนาระบบแบบใหม่โดยใช้อุปกรณ์ที่มีราคาถูก ซึ่งได้พัฒนา 2 ระบบ ได้แก่ ซีเควนเชียลอินเจคชัน-แล็ปแอทวาล์ว สำหรับการสกัดแบบออนไลน์ในระดับไมโครและ ซีเควนเชียลอินเจคชัน-โครมาโทกราฟีของเหลวสมรรถนะสูงสำหรับการเตรียมอนุพันธ์แบบออนไลน์

ระบบซีเควนเชียลอินเจคชัน-แล็ปแอทวาล์ว สำหรับการสกัดแบบออนไลน์ในระดับไมโครได้ออกแบบระบบโดยใช้กรวยแยกขนาดเล็กที่มีเครื่องตรวจวัด ติดไว้ที่พอร์ตของวาล์วเพื่อทำหน้าที่สกัดและตรวจวัดสารเป้าหมาย โดยเลือกการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในน้ำเป็นตัวอย่างในการทดสอบระบบ การวิเคราะห์อาศัยหลักการทำให้เกิดสารประกอบไอออนรวมตัวระหว่างสารลดแรงตึงผิวชนิดประจุลบกับเมทิลลีนบลู ซึ่งสามารถสกัดโดยใช้ไดคลอโรมีเทนก่อนตรวจวัดด้วยเทคนิคสเปกโทรโฟโทเมทรี ขั้นตอนการวิเคราะห์ทำได้โดยการดูดสารตัวอย่าง รีเอเจนต์ และ ไดคลอโรมีเทน เข้าสู่ระบบแล้วให้เกิดการสกัดภายในท่อเล็กๆ หลังจากนั้นสารผสมจะถูกส่งไปยังกรวยแยกที่ติดอยู่บนวาล์ว หลังจากที่ปล่อยให้ชั้นของน้ำและตัวทำละลายอินทรีย์แยกกันแล้ว จึงตรวจวัดการดูดกลืนแสงของชั้นตัวทำละลายอินทรีย์ที่มีสารตัวอย่างที่สนใจอยู่ โดยใช้สเปกโทรโฟโทมิเตอร์ผ่านทางสายใยแก้วนำแสงที่ติดอยู่บนส่วนของแล็ปแอทวาล์ว และได้ประยุกต์สำหรับการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในน้ำ โดยระบบที่พัฒนาขึ้นสามารถสกัดสารโดยใช้ตัวทำละลายอินทรีย์ในปริมาณน้อยมาก เป็นระบบออนไลน์ ช่วยลดอันตรายอันเนื่องจากไอของสารอินทรีย์ที่มีต่อผู้วิเคราะห์ได้ และสามารถประยุกต์สำหรับการวิเคราะห์สารอื่นๆได้อีกมากมาย

ระบบซีเควนเชียลอินเจคชัน-โครมาโทกราฟีของเหลวสมรรถนะสูง สำหรับการเตรียมอนุพันธ์แบบออนไลน์ ได้เลือกศึกษาการวิเคราะห์โลหะหนัก ได้แก่ Co(II) Cu(II) Ni(II) และ Fe(II)

โดยใช้ 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) เป็นรีเอเจนต์ และตรวจวัดด้วยเทคนิคสเปกโทรโฟโตเมทรี ระบบที่พัฒนาขึ้นสามารถเตรียมอนุพันธ์แบบออนไลน์และแยกสารประกอบเชิงซ้อนของโลหะทั้งสี่ชนิดได้ภายในเวลา 13 นาที และ ได้นำไปประยุกต์สำหรับการวิเคราะห์โลหะในตัวอย่างที่หลากหลาย ซึ่งข้อได้เปรียบของระบบที่พัฒนาขึ้นนี้ คือ การปรับขั้นตอนการเตรียมอนุพันธ์ให้ง่ายขึ้น โดยทำให้เป็นระบบออนไลน์ ที่ใช้ปริมาณสารตัวอย่างและรีเอเจนต์น้อยลง นอกจากนี้ระบบซีเควนเชียลอินเจคชันสามารถทำงานคู่ขนานไปกับระบบโครมาโทกราฟี จึงช่วยลดเวลาในการเตรียมตัวอย่างและการวิเคราะห์ลงได้

ABSTRACT

Development of Miniaturized Flow Separation Systems for On-Line Sample Pretreatment

Principal investigator: Dr. Rodjana Burakham

Mentor: Professor Dr. Kate Grudpan

In this project, separation and analytical systems involving flow-based techniques were developed for sample pretreatment and chemical analysis. Two developed systems, including sequential injection-lab-at-valve (SI-LAV) for on-line micro-extraction and sequential injection-high performance liquid chromatography (SI-HPLC) for on-line pre-column derivatization, emphasized miniaturization using simple and cost-effective instrumentation.

The SI-LAV system was developed for on-line micro-extraction using a small separating chamber situating spectrophotometric detector via optical fibers attached at a port of a selection valve. The extractive determination of anionic surfactant in water samples was selected as a model. The detection method is based on the formation of ion-association compound between anionic surfactant and methylene blue, which can be extracted into dichloromethane before spectrophotometric detection. Sample, reagent and dichloromethane are sequentially aspirated via the multiposition selection valve attached with an extraction coil where the extraction process is performed. The aqueous and organic phases are separated in a conical separating chamber LAV unit. The organic phase containing extracted product is then monitored spectrophotometrically. Some water samples have been analyzed for anionic surfactants by the developed procedure. The developed system involves a considerable reduction of organic solvent and can be applied for various chemical analyses.

The SI was coupled to HPLC for an on-line simple pre-column derivatization and determination of metal ions, including Co(II), Cu(II), Ni(II) and Fe(II). 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) was used as a derivatizing reagent. The detection principle is spectrophotometry. Using the proposed SI-HPLC system, on-line derivatization and separation of four metals-nitro-PAPS complexes was achieved within 13 min.

The developed method has been successfully applied for the determination of metal ions in various samples. The system offers advantages in simple pretreatment step with on-line derivatization and less consumption of sample and reagent. In addition, by parallel operation of derivatization and separation, better analysis time could be gained.

Executive Summary

ทุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ (MRG4880157)

1. ชื่อโครงการ (ภาษาไทย) การพัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลงเพื่อการเตรียมตัวอย่างแบบออนไลน์

(ภาษาอังกฤษ) Development of Miniaturized Flow Separation Systems for On-line Sample Pretreatment

2. หัวหน้าโครงการ ดร. รจนา บุระคำ
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3. สาขาวิชาที่ทำการวิจัย

เคมีวิเคราะห์ เน้นการพัฒนาเครื่องมือและระบบการวิเคราะห์แบบใหม่

5. ระยะเวลาดำเนินงาน

2 ปี (1 มิถุนายน 2548 – 31 พฤษภาคม 2550)

6. วัตถุประสงค์ของโครงการ

เพื่อพัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลง เพื่อเป็นระบบการเตรียมตัวอย่างและการวิเคราะห์แบบออนไลน์แบบใหม่ ที่สามารถทำได้ง่าย และราคาประหยัด โดยอาศัยหลักการไหล การแยกด้วยโครมาโทกราฟีและเทคนิคอื่นๆที่เกี่ยวข้อง และระบบที่พัฒนาขึ้นสามารถนำมาประยุกต์ใช้ได้กับการวิเคราะห์ตัวอย่างจริง

7. ขอบเขตของการวิจัย

(1) การพัฒนาเทคนิคซีเควนเชียลอินเจคชันร่วมกับเทคนิคการแยกทางโครมาโทกราฟีหรือเทคนิคอื่นๆที่เกี่ยวข้อง เพื่อใช้ในการเตรียมตัวอย่างแบบออนไลน์ เช่น แล็ปแอทวาล์ว แบบแนวคิดใหม่ เพื่อให้ได้ระบบการวิเคราะห์ที่สามารถวิเคราะห์ได้อย่างรวดเร็วและมีประสิทธิภาพสูง

(2) การพัฒนาระบบ miniaturized flow separation systems ที่มี on-line sample pretreatment โดยสนใจการปรับ/พัฒนาระบบให้ง่าย และใช้เวลาในการวิเคราะห์น้อยลง

8. Output

มีผลงานเป็นระบบการวิเคราะห์แบบใหม่ที่เป็น miniaturized flow separation system ที่มี on-line sample pretreatment 2 ระบบ ได้แก่ SI-LAV สำหรับการสกัดแบบออนไลน์ และ SI-HPLC สำหรับการเตรียมอนุพันธ์แบบออนไลน์และการวิเคราะห์ด้วยโครมาโทกราฟี โดยทั้งสองระบบช่วยเพิ่มความสะดวก รวดเร็วในการวิเคราะห์ และลดค่าใช้จ่ายในการวิเคราะห์ เนื่องจากใช้อุปกรณ์ที่มีราคาประหยัด และลดปริมาณการใช้สารเคมี

ผลงานบางส่วนของโครงการ ได้รับการตีพิมพ์ในวารสารวิชาการนานาชาติ 1 เรื่อง และกำลังอยู่ในระหว่างการพิจารณาอีก 1 เรื่อง มีการนำเสนอในการประชุมวิชาการระดับนานาชาติ (ทั้งแบบบรรยายและโปสเตอร์) จำนวน 3 เรื่อง และ ในการประชุมวิชาการระดับชาติจำนวน 7 เรื่อง นอกจากนี้ยังมีผลงานที่สัมพันธ์กับโครงการ ซึ่งได้รับการสนับสนุนบางส่วนจากแหล่งทุนอื่นๆ (โครงการเมธีวิจัยอาวุโส “การพัฒนาการวิเคราะห์ระดับไมโครและนาโนโดยเทคนิคที่ใช้การไหล” โดย ศาสตราจารย์ ดร. เกตุ กรุดพันธ์ และ โครงการพัฒนาบัณฑิตศึกษาและการวิจัยทางเคมี (PERCH)) ได้รับการตีพิมพ์ในวารสารวิชาการนานาชาติจำนวน 2 เรื่อง และ นำเสนอในการประชุมวิชาการทั้งระดับชาติและนานาชาติ จำนวน 8 เรื่อง

1. ความสำคัญและที่มาของปัญหาที่ทำวิจัย

การพัฒนาเทคนิคในการวิเคราะห์ที่มีประสิทธิภาพ สามารถวิเคราะห์ได้อย่างรวดเร็ว มีความไว (sensitivity) ความแม่นยำ (precision) และ ความถูกต้อง (accuracy) นับว่ามีความสำคัญต่อการวิเคราะห์ในหลายๆด้าน นอกจากนี้ ในการวิเคราะห์ตัวอย่างต่างๆ ไม่ว่าจะเป็น ตัวอย่างทางสิ่งแวดล้อม ยา อาหาร หรือตัวอย่างทางชีวภาพ นอกจากเทคนิคที่ใช้ในการวิเคราะห์ตัวอย่างแล้ว การเตรียมตัวอย่าง นับว่ามีความสำคัญอย่างยิ่ง เพราะเป็นขั้นตอนที่ใช้เวลามาก และเป็นปัจจัยหนึ่งที่ส่งผลต่อความถูกต้องของข้อมูลที่ได้จากการวิเคราะห์ เทคนิคในการเตรียมตัวอย่างจะแตกต่างกันไปแล้วแต่ลักษณะและองค์ประกอบของตัวอย่าง เช่น การสกัด (extraction) การแยกด้วยโครมาโทกราฟี (chromatography) การย่อย (digestion) เป็นต้น ซึ่งโดยปกติมักจะทำแบบ batch ต้องใช้สารตัวอย่างและรีเอเจนต์ที่เกี่ยวข้องในปริมาณมาก สิ้นเปลืองเวลาและแรงงาน การพัฒนาระบบให้มีขนาดเล็กลง (miniaturization) สำหรับการเตรียมตัวอย่างที่สามารถทำแบบออนไลน์ ใช้เวลาและสารในปริมาณน้อยและเป็นระบบอัตโนมัติ จึงน่าจะมีประโยชน์ต่อวงการเคมีวิเคราะห์เป็นอย่างมาก

การลดขนาดของระบบการวิเคราะห์ให้เล็กลง อาจพัฒนาได้โดยเกี่ยวข้องกับเทคนิคการวิเคราะห์ที่อาศัยหลักการไหล (Flow-Based Analysis) ซึ่งเป็นเทคนิคการวิเคราะห์แนวใหม่ที่มีประสิทธิภาพและเป็นที่นิยมกันอย่างแพร่หลายในปัจจุบัน เนื่องจากมีการใช้สารตัวอย่าง/รีเอเจนต์ปริมาณน้อย สามารถวิเคราะห์ได้อย่างรวดเร็ว ให้ผลการวิเคราะห์ที่มีความถูกต้อง แม่นยำ และช่วยเพิ่มความสะดวกในกรณีที่มีตัวอย่างจำนวนมาก เนื่องจากส่วนใหญ่การทำงานของระบบจะเป็นแบบอัตโนมัติ การประยุกต์ใช้เทคนิคเหล่านี้สำหรับการเตรียมตัวอย่างและการวิเคราะห์แบบออนไลน์ เช่น การสกัด การแยกด้วยโครมาโทรกราฟี และอื่นๆ จึงน่าจะช่วยเพิ่มความสะดวกในการวิเคราะห์ ทำให้สามารถลดความสิ้นเปลืองเวลา แรงงาน ค่าใช้จ่าย ตลอดจนการใช้สารเคมีและตัวอย่างในการวิเคราะห์ ซึ่งนับว่ามีประโยชน์และจำเป็นอย่างมากสำหรับการวิเคราะห์

Flow injection analysis (FIA)[1] เป็นเทคนิคการวิเคราะห์ที่ได้รับความนิยมแพร่หลายในวงการเคมีวิเคราะห์ เนื่องจากเป็นเทคนิคที่มีความสะดวก รวดเร็วและมีประสิทธิภาพ สามารถพัฒนาและประยุกต์ใช้ได้กับการวิเคราะห์ในหลายๆแขนง[2-4]

Sequential injection analysis (SIA)[5] เป็นเทคนิคที่ได้รับการพัฒนามาจาก FIA เพื่อปรับการทำงานให้เป็นระบบอัตโนมัติและมีความสามารถบางอย่างที่แตกต่างไปจากระบบ FIA เพื่อให้สามารถเลือกใช้ให้เหมาะสมกับงานแต่ละประเภท นอกจากนี้ยังเป็นเทคนิคที่ใช้สารในปริมาณน้อย

ปัจจุบัน เทคนิค SIA ยังได้รับการพัฒนาอย่างต่อเนื่องเพื่อให้เป็นระบบที่สามารถทำได้มากกว่าการวิเคราะห์สารอย่างเดียว เช่น มีการใช้ SIA ในการทำ speciation[6,7] และ preconcentration

ปัจจุบันได้มีการพัฒนาระบบการวิเคราะห์ให้มีขนาดเล็กลง เรียกว่า micro-total analysis system (μ-TAS) เพื่อลดปริมาณการใช้สารตัวอย่างและรีเอเจนต์ เช่น SIA แบบ lab-on-valve[8] และ lab-at-valve[2,9] นอกจากนี้ ยังได้มีการพัฒนาเทคนิค SIA ให้มีความสามารถในการแยกสาร เรียกว่า sequential injection chromatography (SIC)[10,11] โดยอาศัยความก้าวหน้าทางด้านโครมาโทกราฟีมาเกี่ยวข้อง ในขณะที่ SIA สามารถวิเคราะห์สารได้โดยใช้เครื่องมือที่มีความยุ่งยากซับซ้อนน้อยกว่าที่ใช้ในระบบโครมาโทกราฟี

การพัฒนาระบบการแยกโดยอาศัยหลักการไหล ที่ทำให้ขนาดเล็กลงเพื่อการเตรียมตัวอย่างแบบออนไลน์ โดยการทำงานเป็นแบบอัตโนมัติ จึงน่าจะเป็นประโยชน์ต่อการวิเคราะห์ตัวอย่างในหลายๆด้าน โดยเฉพาะในงานวิเคราะห์ที่มีจำนวนตัวอย่างมากๆ เช่น ทางด้านสิ่งแวดล้อม ยา อาหาร ฯลฯ เพื่อจะเป็นอีกทางเลือกหนึ่งของการวิเคราะห์แบบราคาประหยัด ซึ่งเหมาะกับงานวิเคราะห์ในประเทศไทย

ในโครงการวิจัยนี้ได้พัฒนาระบบการแยกโดยอาศัยหลักการไหลที่ทำให้ขนาดเล็กลง เพื่อเป็นระบบการเตรียมตัวอย่างและการวิเคราะห์แบบออนไลน์แบบใหม่ ที่สามารถทำได้ง่าย และราคาประหยัด โดยอาศัยหลักการไหล การแยกด้วยโครมาโทกราฟีและเทคนิคอื่นๆที่เกี่ยวข้อง และระบบที่พัฒนาขึ้นสามารถนำมาประยุกต์ใช้ได้กับการวิเคราะห์ตัวอย่างจริง ซึ่งจะเน้นการวิเคราะห์ตัวอย่างทางด้านสิ่งแวดล้อมและอาหาร โดยจะประกอบด้วย 2 ระบบการวิเคราะห์ คือ

1. พัฒนาระบบซีเควนเชียลอินเจคชัน-แล็ปแอทวาล์ว สำหรับการสกัดแบบออนไลน์ในระดับไมโคร โดยใช้การวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบเป็นตัวอย่างในการทดสอบระบบ
2. พัฒนาระบบการเตรียมอนุพันธ์แบบออนไลน์สำหรับการแยกและวิเคราะห์โลหะหนักด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง

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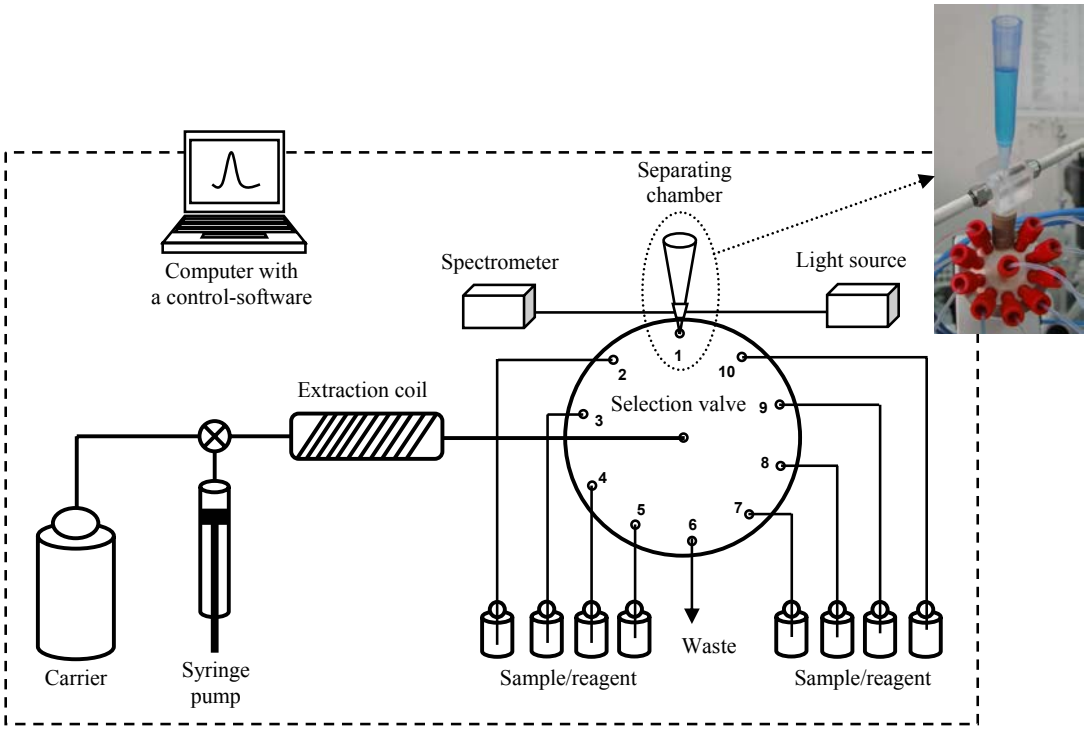
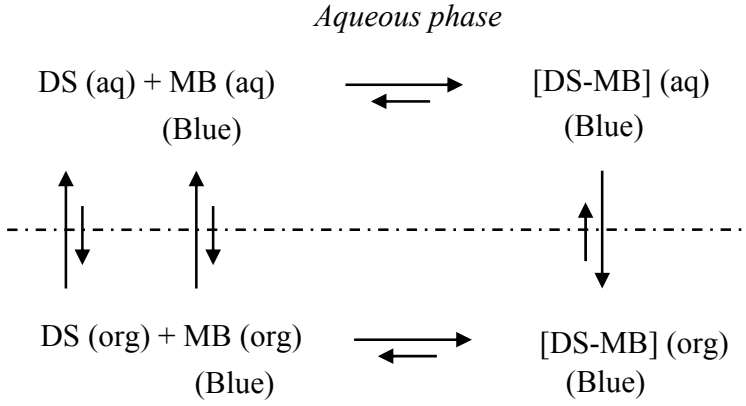
2. การพัฒนาระบบซีเควนเชียลอินเจคชัน-แล็ปแอทวาล์วสำหรับการสกัดแบบออนไลน์ในระดับไมโคร

(Development of sequential injection-lab-at-valve (SI-LAV) for on-line micro-extraction)

ระบบ SI-LAV ที่พัฒนาขึ้นสำหรับการสกัดในระดับไมโครแบบออนไลน์ แสดงดังรูปที่ 1 และได้เลือกการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในน้ำเพื่อเป็นต้นแบบในการทดสอบระบบ โดยการวิเคราะห์อาศัยหลักการทำให้เกิดสารประกอบไอออนรวมตัว (ion association compound) ระหว่างสารลดแรงตึงผิวชนิดประจุลบ (ในการทดลองนี้ใช้ sodium dodecylsulphate (SDS)) กับ methylene blue ซึ่งสามารถสกัดโดยใช้ไดคลอโรมีเทนก่อนการตรวจวัดด้วยเทคนิคสเปกโทรโฟโทเมทรี ที่ความยาวคลื่น 650 นาโนเมตร แผนภาพปฏิกิริยาแสดงดังรูปที่ 2



รูปที่ 1 ระบบ SI-LAV สำหรับการสกัดแบบออนไลน์



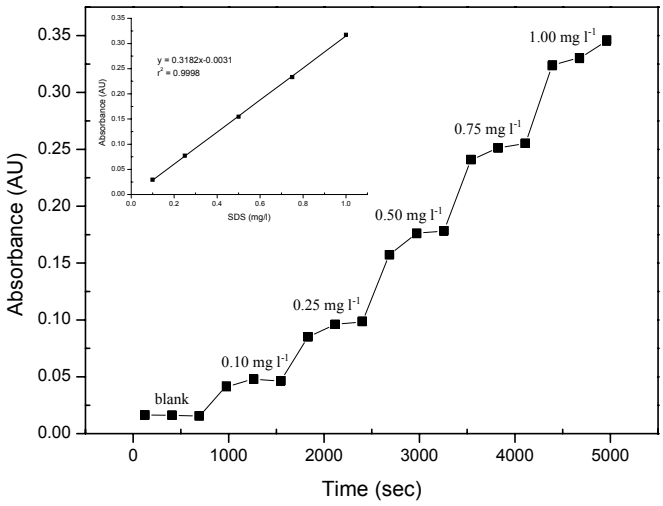
รูปที่ 2 แผนภาพปฏิกิริยาการเกิด ion association ระหว่าง dodecyl sulphate (DS) กับ methylene blue (MB) และการสกัด

ขั้นตอนการวิเคราะห์ทำได้โดย syringe pump จะดูดสารตัวอย่าง รีเอเจนต์ และ ไดคลอโรมีเทน เข้าสู่ extraction coil เป็นลำดับ สารตัวอย่างที่มีสารลดแรงตึงผิวชนิดประจุลบอยู่จะเกิด ion association compound กับ methylene blue และถูกสกัดด้วยตัวทำละลายอินทรีย์ภายใน extraction coil ด้วยการทำงานของ syringe pump หลังจากนั้นสารผสมจะถูกส่งไปที่ LAV-separating chamber และปล่อยให้เกิดการแยกระหว่างชั้นน้ำและชั้นสารอินทรีย์ ประมาณ 5 วินาที แล้วตรวจวัดชั้นสารอินทรีย์ซึ่งมีสารที่สนใจผสมอยู่ ด้วยเครื่องสเปกโทรโฟโทมิเตอร์ ผ่านทางสายใยแก้วนำแสง (optical fibers) ที่ยึดติดอยู่กับส่วนของ LAV จากนั้นจึงล้างระบบด้วยน้ำกลั่น เพื่อเตรียมพร้อมสำหรับการวิเคราะห์ตัวอย่างต่อไป

ได้ศึกษาตัวแปรที่เกี่ยวข้องในการทดลองที่มีผลต่อประสิทธิภาพในการสกัด ได้แก่ ลำดับการดูดสาร จำนวนรอบของการผสมของระบบ SIA ปริมาตรของสารตัวอย่าง รีเอเจนต์ และ ตัวทำละลายอินทรีย์ อัตราส่วนระหว่างปริมาตรของสารละลายน้ำและสารอินทรีย์ โดยที่ ลำดับของการดูดสารและจำนวนรอบของการผสมนับเป็นตัวแปรที่สำคัญมาก ซึ่งจะมีผลต่อเวลาและประสิทธิภาพที่สารละลายชั้นน้ำและชั้นสารอินทรีย์จะเกิดการสัมผัสกัน ซึ่งเป็นการเพิ่มประสิทธิภาพในการสกัด

ได้ศึกษา analytical characteristics ของระบบที่พัฒนาขึ้น พบว่า กราฟมาตรฐานมีความเป็นเส้นตรง (linear range) อยู่ในช่วง 0.10-1.00 mg l^{-1} โดยมีสมการเส้นตรงเป็น $y = 0.3182x - 0.0031$

ค่าสัมประสิทธิ์สหสัมพันธ์ (correlation coefficient, r^2) 0.9998 ตัวอย่างสัญญาณที่ได้จากการวิเคราะห์และกราฟมาตรฐาน แสดงดังรูปที่ 3 ขีดจำกัดต่ำสุดของการวิเคราะห์มีค่าเท่ากับ 0.01 mg l^{-1} ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ (relative standard deviation, RSD) ที่ได้มีค่าต่ำกว่า 6% และสามารถวิเคราะห์ได้ 5 ตัวอย่างต่อชั่วโมง



รูปที่ 3 ค่าการดูดกลืนแสงและกราฟมาตรฐานของ SDS ที่ได้จากระบบ SI-LAV

ระบบที่พัฒนาขึ้นนี้ ได้นำไปประยุกต์กับการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในตัวอย่างน้ำธรรมชาติ โดยเก็บตัวอย่างจากแหล่งน้ำธรรมชาติในเขตจังหวัดขอนแก่น ผลการวิเคราะห์ที่ได้จากวิธีที่พัฒนาขึ้นได้นำไปเปรียบเทียบกับข้อมูลที่ได้จากการวิเคราะห์ด้วยวิธีมาตรฐาน พบว่าผลที่ได้จากทั้งสองวิธีไม่มีความแตกต่างกันอย่างมีนัยสำคัญ โดยการทดสอบด้วย t-test ที่ระดับความเชื่อมั่น 95% รวมทั้งได้ศึกษาผลของ interfering ion ที่มีต่อการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบด้วยเทคนิคที่พัฒนาขึ้นด้วย (รายละเอียด reprint ในภาคผนวก ก1)

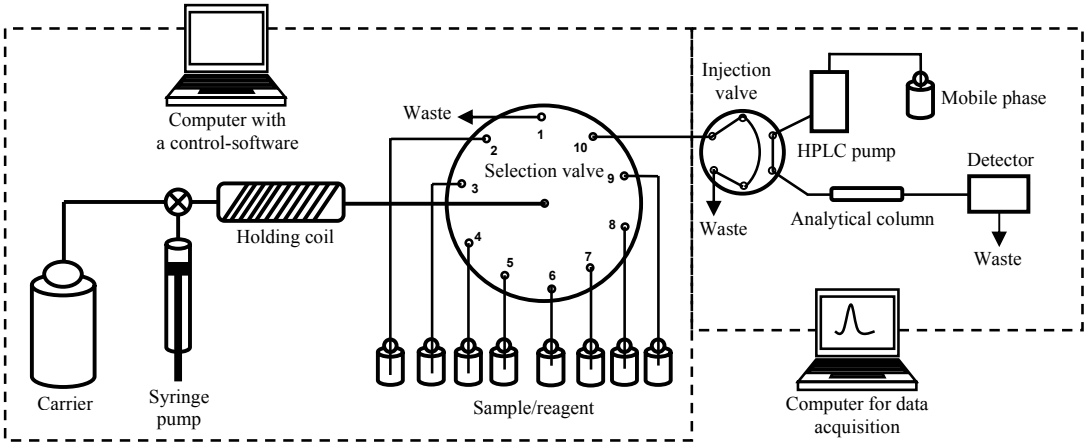
3. การพัฒนาระบบซีเควนเชียลอินเจคชัน-โครมาโทกราฟีของเหลวสมรรถนะสูงเพื่อการเตรียมอนุพันธ์แบบออนไลน์สำหรับการวิเคราะห์โลหะหนัก

(Development of sequential injection-high performance liquid chromatography for on-line derivatization and determination of heavy metals)

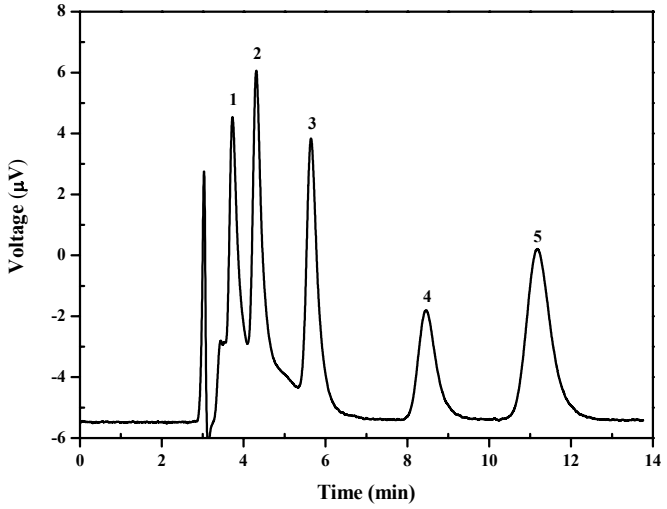
การพัฒนาระบบซีเควนเชียลอินเจคชัน เพื่อเตรียมอนุพันธ์แบบออนไลน์สำหรับการวิเคราะห์โลหะหนักด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง (high performance liquid chromatography, HPLC) โลหะที่ศึกษาได้แก่ Co(II) Fe(II) Ni(II) และ Cu(II) โดยใช้ 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) เป็นรีเอเจนต์ โดยจะทำอนุพันธ์ระหว่างโลหะหนักในตัวอย่างกับรีเอเจนต์โดยใช้ระบบ SI ก่อนที่จะ inject เข้าสู่ระบบ HPLC เพื่อแยกสารประกอบเชิงซ้อนที่เกิดขึ้น โดยใช้คอลัมน์ C_{18}-μBondapak (3.9 x 300 mm) และตรวจวัดด้วยเทคนิคสเปกโทรโฟโทเมทรีที่ความยาวคลื่น 570 นาโนเมตร

ในส่วนของการแยกสารประกอบเชิงซ้อน metal-nitro-PAPS ใช้เทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูงแบบไอออนแพร์รีเวิร์สเฟส (ion-pair reversed-phase HPLC) ได้ศึกษาองค์ประกอบของเฟสเคลื่อนที่ (mobile phase) ที่เหมาะสม ได้แก่ ความเข้มข้นและ pH ของสารละลายบัฟเฟอร์ ปริมาณของ organic modifier และ ความเข้มข้นของ ion pairing agent โดยในงานวิจัยนี้เลือกใช้ tetrabutyl ammonium bromide (TBABr) เฟสเคลื่อนที่ที่เลือกใช้ในงานวิจัยนี้ได้แก่ สารละลายผสมของ 30% อะซีโตไนไตรล์ ในบัฟเฟอร์อะซิเตต pH 5.0 ความเข้มข้น 1.0 x 10^{-2} mol l^{-1} และ TBABr เข้มข้น 3.5 x 10^{-3} mol l^{-1} ที่มีอัตราการไหล 1.0 ml min^{-1} ซึ่งสามารถแยกสารประกอบเชิงซ้อนของโลหะทั้ง 4 ชนิด และ nitro-PAPS ที่เหลือจากปฏิกิริยา ได้ภายในเวลา 13 นาที โดยมีลำดับการชะดังนี้ Cu(II)-nitro-PAPS Co(II)-nitro-PAPS excess nitro-PAPS reagent Ni(II)-nitro-PAPS และ Fe(II)-nitro-PAPS ตามลำดับ และสภาวะนี้จะใช้สำหรับการพัฒนาระบบ SI-HPLC สำหรับการเตรียมอนุพันธ์แบบออนไลน์และการวิเคราะห์สารประกอบเชิงซ้อน metal-nitro-PAPS ต่อไป โดยได้ต่อระบบ SI สำหรับทำ on-line pre-column derivatization ดังรูปที่ 4 หลังจากทำให้เกิดสารประกอบเชิงซ้อนของ metal-nitro-PAPS ภายในระบบ SI แล้วจึงส่งไปที่ injection loop ของส่วน HPLC เพื่อ inject เข้าสู่คอลัมน์ต่อไป ตัวอย่างโครมาโทแกรมที่ได้จากการระบบ SI-HPLC แสดงดังรูปที่ 5 และได้มีการศึกษา analytical characteristics ของระบบ ได้แก่ ช่วงความเป็นเส้นตรง ความแม่นยำ ขีดจำกัดต่ำสุดของการวิเคราะห์ และการประยุกต์ใช้วิธีที่พัฒนาขึ้นสำหรับการวิเคราะห์ตัวอย่างจริง โดยเลือกวิเคราะห์โลหะหนักในตัวอย่างหลายประเภท ได้แก่ นมผง

ดัดแปลงสำหรับทารก อาหารเสริม ไวน์จากโครงการหนึ่งตำบลหนึ่งผลิตภัณฑ์ และ น้ำดื่มบรรจุขวด พบว่า ผลที่ได้จากวิธีที่พัฒนาขึ้นสอดคล้องกับที่ได้จากวิธีมาตรฐาน (รายละเอียด manuscript ในภาคผนวก ก2)



รูปที่ 4 ระบบ SI-HPLC ที่พัฒนาขึ้นสำหรับการเตรียมอนุพันธ์แบบออนไลน์



รูปที่ 5 โครมาโทแกรมที่ได้จากระบบ SI-HPLC: 1, 0.100 mg l^{-1} Cu(II); 2, 0.050 mg l^{-1} Co(II); 3, excess nitro-PAPS; 4, 0.075 mg l^{-1} Ni(II); 5, 0.075 mg l^{-1} Fe(II)

Output ที่ได้จากโครงการ

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Output ที่สัมพันธ์กับโครงการ

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High-Performance Liquid Chromatography with Sequential Injection for On-line Pre-column Derivatization of Some Heavy Metals

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Abstract

High-performance liquid chromatography (HPLC) was coupled with sequential injection (SI) for simultaneous analyses of some heavy metals, including Co(II), Ni(II), Cu(II) and Fe(II). 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) was employed as a derivatizing reagent for sensitive spectrophotometric detection by on-line pre-column derivatization. The SI system offers an automated handling of sample and reagent, on-line pre-column derivatization and propulsion of derivatives to the HPLC injection loop. The metal-nitro-PAPS complexes were separated on a C_{18}-μBondapak column (3.9 x 300 mm). Using the proposed SI-HPLC system, determination of four metal ions by means of nitro-PAPS complexes was achieved within 13 min in which the parallel of derivatization and separation were processed at the same time. Linear calibration graphs were obtained in the ranges of 0.005 - 0.250 mg l^{-1} for Cu(II), 0.007 - 1.000 mg l^{-1} for Co(II), 0.005 - 0.075 mg l^{-1} for Ni(II) and 0.005 - 0.100 mg l^{-1} for Fe(II).

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The system provides means for automation with good precision and minimizing error in solution handling with the relative standard deviation (RSD) of less than 6%. The detection limits obtained were 2 μg l^{-1} for Cu(II) and Co(II), and 1 μg l^{-1} for Ni(II) and Fe(II). The method was successfully applied for the determination of metal ions in various samples, including milk powder for infant, mineral supplements, local wines and drinking waters.

Keywords: on-line pre-column derivatization, sequential injection, metal ions, HPLC, nitro-PAPS

1. Introduction

Metal ion analysis is of great importance in various fields, including food, pharmaceutical, medical and environmental sciences. Numerous analytical techniques have been reported for the determination of heavy metals. The most widely used instruments, e.g. atomic absorption [1-3] and inductively coupled plasma atomic emission spectrometers [4-5], used for metal determination suffer from both spectral and chemical interferences and involve instrumentation complexity. A common UV-visible spectrophotometry [6] is often simpler and less expensive than atomic spectroscopic methods but does not allow a multi-elemental analysis and requires derivatization process for improving the sensitivity and selectivity. High-performance liquid chromatography (HPLC) [7-10] and ion chromatography [11] have been widely recognized as one of the versatile methods for multi-element and sensitive analyses of metal ions using either pre- or post-column derivatization. In a typical post-column procedure, a chromogenic reagent is mixed with column eluent in a post-column mixer and the derivatives formed are detected. To be useful, post-

column reactions must be rapid and generate low background signals. Dead volume associated with connecting tubing, detector and mixing devices must be minimized to avoid unnecessary peak dispersion. The pre-column derivatization procedure provides that the excess of reagent is completely separated from the chelates and does not actually contribute to be the increase in baseline signals at the peak positions of the chelates. This fact indicates that the attainable sensitivity is determined only by the inherent signal to noise ratio of the detector. The potential of this approach is realized in the reversed-phase HPLC partition mode, including the ion-pair type. Ion-pair reversed-phase HPLC (IP-RPHPLC) offers both sensitivity and selectivity owning to the large degree of flexibility in the separation parameters. Several chelating agents have been successively used as a derivatizing reagent such as 4-(2-pyridylazo)resorcinol(PAR) [10], 5-(2-hydroxy-5-nitrophenylazo)thiorhodamine [12], 2,4-dihydroxybenzylidenethiorhodamine [13], 1-(4-aminobenzyl) ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate [14], 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-(3-sulfopropylamino)aniline (5-Br-PSAA) [15] and 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) [16].

Pre-column derivatization is normally performed off-line (batch) because of instrumentation simplicity. But off-line manipulation is proved to be laborious, time consuming, especially if a number of samples is to be processed, and may result to low precision. On the other hand, on-line derivatization offers automation, ease and high sampling rate, providing an attractive alternative [17]. Some developments have involved the coupling of flow injection (FI) [10, 18-20] or sequential injection (SI) [17, 21] with HPLC for on-line pre-column derivatization. FI involves an inexpensive hardware and a simple operational basis, while SI offers high potential for higher degree in laboratory automation with less reagent consumption.

In this work, attempts were made for coupling IP-RPHPLC with SI for on-line pre-column derivatization and determination of some heavy metals, including Co(II), Cu(II), Ni(II) and Fe(II). Nitro-PAPS was used as the derivatizing reagent for a sensitive spectrophotometric detection. Application to a variety samples was investigated.

2. Experimental

2.1 Chemicals and reagents

All of reagents used were analytical reagent grade. Deionized water was used throughout the experiments. The atomic absorption standard solutions (1000 mg l^{-1}) of copper (II) nitrate and nickel (II) nitrate were obtained from Ajax Chemicals (Australia). Stock standard solution (1000 mg l^{-1}) of cobalt (II) was prepared using cobalt (II) chloride hexahydrate in water. Stock standard solution (1000 mg l^{-1}) of iron (II) was prepared by dissolving iron (II) sulphate heptahydrate in 10^{-4} mol l^{-1} hydrochloric acid. Working standards were freshly prepared by diluting the stock solution with water to obtain appropriate concentrations. Nitro-PAPS was obtained from Dojindo laboratories (Japan). Tetrabutylammonium bromide (TBABr) was purchased from Fluka (Switzerland). Acetonitrile was of HPLC grade. All HPLC mobile phases were filtered through 0.45 μm membrane filters and sonicated prior to use.

2.2 Instrumentation

The SI-HPLC system used is schematically depicted in Fig.1(a). The SIA part consists of a 2.5 ml syringe pump (Cavro, USA), a 10-position selection valve VICI with a microelectric actuator (Valco Instruments, USA) and a holding coil (220 x 0.07

cm i.d. PTFE tubing). Instrumentation control was manipulated via the FIAlab software (FIAlab Instruments, USA). The HPLC part consists of a Waters 6000A dual pump, a Rheodyne injector with a 50 μl injection loop and a Waters 484 absorbance detector (Waters, USA) operated at wavelength of 570 nm. The SI system was connected to the HPLC injection port by means of the 37 x 0.07 cm i.d. PTFE tubing. Separations were performed on a 3.9 x 300 mm C_{18}-μBondapak column (Waters, USA). The CSW 32 software (Waters, USA) was used for data acquisition.

2.3 Procedure

Effective separation of the studied metal-nitro-PAPS complexes was achieved through the IP-RPHPLC mode using the mobile phase containing acetonitrile, buffer and ion pairing agent. Therefore, in order to ensure that there was no pairing ion reagent retained on the analytical column, the column was washed with 50% MeOH for 15 column-volumes and was then equilibrated with the mobile phase being used for 25 column-volumes before injection.

The SI-HPLC operation steps were as follows: the manifold lines were washed with water, and all of the reagents were filled into the ports of the selection valve. Then, suitable volumes of the reagents were sequentially aspirated and stacked as zones in the holding coil. By flow reversal of the syringe pump, the reaction mixture, metal-nitro-PAPS complexes in this work, was propelled to the HPLC injection loop and injected to HPLC system. A mobile phase flow rate of 1.0 ml min^{-1} was used throughout.

3. Results and discussion

3.1 IP-RPHPLC of metal-nitro-PAPS chelates

To achieve adequate separation for the studied chelates within minimum elution time, optimization of the HPLC separation was performed by off-line derivatization of metal-nitro-PAPS chelates. Nitro-PAPS chelates of Co(II), Ni(II), Cu(II) and Fe(II) were formed by mixing metal ion solutions and the nitro-PAPS solution with the concentration ratio of 1:2 and was manually injected in the HPLC system. It has been known that the composition of mobile phase plays an important role in IP-RPHPLC. The mobile phase components include concentration of buffer, pH, amount of organic modifier and concentration of ion pairing agent. The selectivity of the IP-RPHPLC was susceptible to the content of organic modifier in the mobile phase due to the variation of polarity and hydrophobicity which lead to different strengths and selectivity. The role of buffer is to control the pH of the chromatographic system, while the role of the ion pairing agent is to provide the exchange sites for the target analytes. Therefore, some degrees of variation in the retention behavior and chromatographic selectivity are expected by addition of ion pairing agent in IP-RPHPLC. The mobile phase was selected from our preliminary studies and was found to be 30% acetonitrile in 10.0 mmol l^{-1} acetate buffer pH 5.0 and 3.5 mmol l^{-1} TBABr. Separation of four metal-nitro-PAPS chelates and excess nitro-PAPS reagent was achieved within 13 min with the elution order of Cu(II)-nitro-PAPS, Co(II)-nitro-PAPS, excess nitro-PAPS reagent, Ni(II)-nitro-PAPS and Fe(II)-nitro-PAPS, respectively. The conditions were adopted for the coupling of SI to HPLC.

3.2 On-line coupling of SI to HPLC

Using the SI-HPLC system shown in Fig. 1(a), the experimental parameters affecting the sensitivity of the determination were considered. First, it must be

ensured that the most concentrated part of the reaction mixture will be injected into the separation column. Therefore, after forming derivatives by the SI system, the dispensing volumes of the syringe pump ranging from 240-380 μl were optimized and the peak areas were compared. The dispensing volume (at a constant flow rate) determined the interval time between the start of dispensing of the derivative zone by the syringe pump of the SI system and the actual injection into the HPLC system. Second, the sequence orders of aspiration together with the volumes of sample and reagent and the number of flow reversals were examined. The operational sequence in which the sample and reagent were aspirated as small segments gave a higher sensitivity, while the mixing by several flow reversals was unnecessary. The metal-nitro-PAPS derivatives could be formed immediately and the sensitivity was enhanced by mixing during the transportation to HPLC loop. However, the connection between SIA part and HPLC injection loop should be shorten to prevent further dispersion of product zone. It has been known theoretically that increase in the sample and/or reagent volumes results in increase in the sensitivity but it must be compromised between the volumes of derivative product and the volume of the HPLC injection loop. The derivative should be formed just enough for injecting into HPLC loop with adequate sensitivity and precision. In addition, the peak shape of the obtained chromatogram must be concerned. Increasing of the injection volume, the wider peak will be obtained and lead to over load the column. The selected operational sequences as well as the volumes of sample and reagent were summarized in Table 1 and Fig. 1(b). Finally, the chemical parameter, the concentration of nitro-PAPS reagent, was varied to increase the sensitivity and minimize the excess of reagent concentration. The optimum results were obtained using the nitro-PAPS concentration of 1×10^{-4} mol l^{-1}. The sensitivity determined by the peak areas of all

metal complexes remained constant and the peak of excess nitro-PAPS reagent was resolved from the derivative peaks. The typical chromatogram is represented in Fig. 2.

3.3 Analytical characteristics

Under the selected SI and HPLC conditions, quantitative features of the proposed system were evaluated by examining the linear range, precision and limit of detection (LOD). Calibration graphs were constructed by plotting the peak area against the concentration of each metal ion. The detection limits were calculated at a signal level of three times the baseline noise (S/N = 3). The equations of the obtained calibration graphs, the linear ranges and the detection limits are given in Table 2. The relative standard deviations of the peak area and retention time of each analyte were less than 6%. The effects of interferences on the SI-HPLC of metal-nitro-PAPS chelates were studied by adding the metal ions at different concentrations into a mixture of 0.060 mg l^{-1} Co(II), 0.010 mg l^{-1} Cu(II), Fe(II) and Ni(II). No interference, i.e. no signal change within ±5%, was observed from common ions with the maximum concentrations studied up to 20 mg l^{-1} of Na^{+}, Ca^{2+} and Cl^{-}; 25 mg l^{-1} of CO_3^{2-}, 30 mg l^{-1} of SO_4^{2-}, NO_2^{-} and NO_3^{-}; and 50 mg l^{-1} of Cd^{2+}, Pb^{2+} and Zn^{2+}.

3.4 Analysis of real samples

The applicability of the SI-HPLC system was investigated by determining the metal ions in various samples, including modified milk powder for infant, mineral supplements, local wines and drinking water. For milk powder and supplement samples, fine powder samples were weighted, dissolved in water and filtered before the determination. For wines and drinking waters, the samples were analyzed by the

system without any further pretreatment except filtration and dilution. Typical chromatograms of sample determination are shown in Fig.3. The results, as shown in Table 3, were in agreement with the labeled values (for milk powder and mineral supplement samples) and those obtained by AAS methods (for wines and drinking waters), evaluated by t-test at 95% confidence level. It was not surprising that some metal ions such as Co(II) and Ni(II) were not detectable in these samples and all metal ions in drinking water samples were lower than the LODs. However, the recoveries, assessed by experiment of known amounts of metal ions (0.020 mg l^{-1} each) spiked in the real samples, obtained were satisfactory (90-106%).

4. Conclusion

Coupling HPLC with SI offers on-line pre-column derivatization system for analyses of some heavy metal ions. The derivatives can be on-line formed and subsequently injected and analyzed in the HPLC system. This leads to possibility in automation. By parallel operation of derivatization and separation, advantages in less consumption of sample/reagent, as well as better analysis time could be gained. It has been demonstrated that HPLC hyphenated with SI could be an alternative for simultaneous analyses of some heavy metal ions in various samples.

Acknowledgements

We thank the Thailand Research Fund (TRF) and the Commission on Higher Education (CHE). This work was partly supported by the Postgraduate Education and Research Program in Chemistry (PERCH). Thanks are due to Professor T. Sakai for supply of the reagent (Nitro-PAPS) which was under the

Frontier Research Project-Materials for the 21st Century (Materials Development for Environment, Energy and Information).

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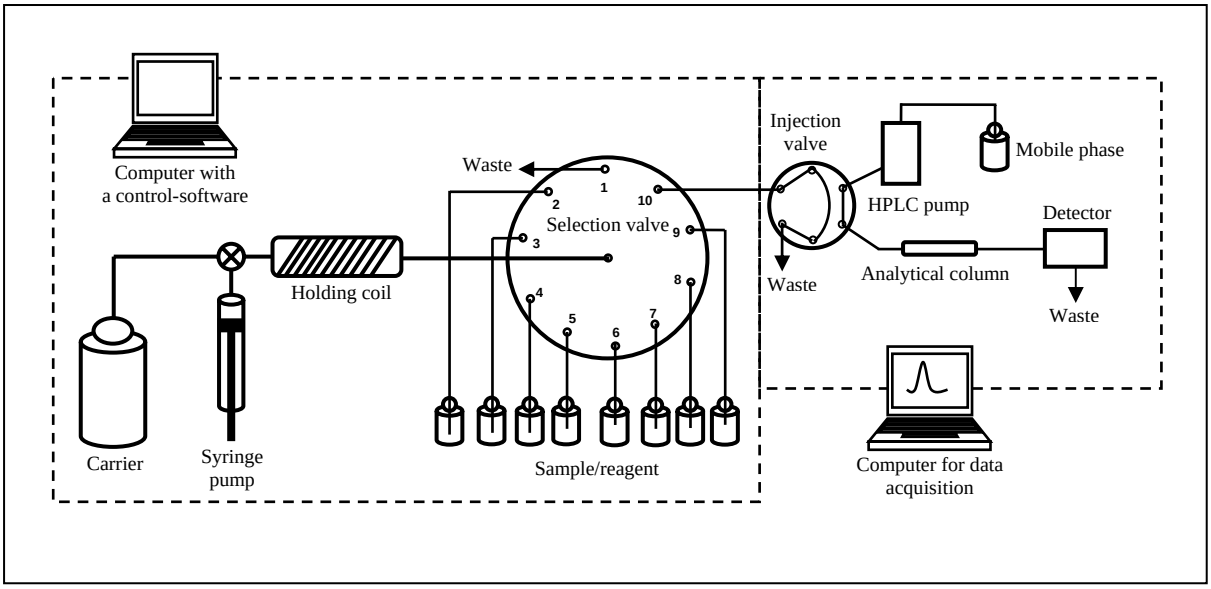
List of figure captions

Fig. 1(a) Schematic diagram of the SI-HPLC system, (b) Diagram of operational sequence of the SI-HPLC system

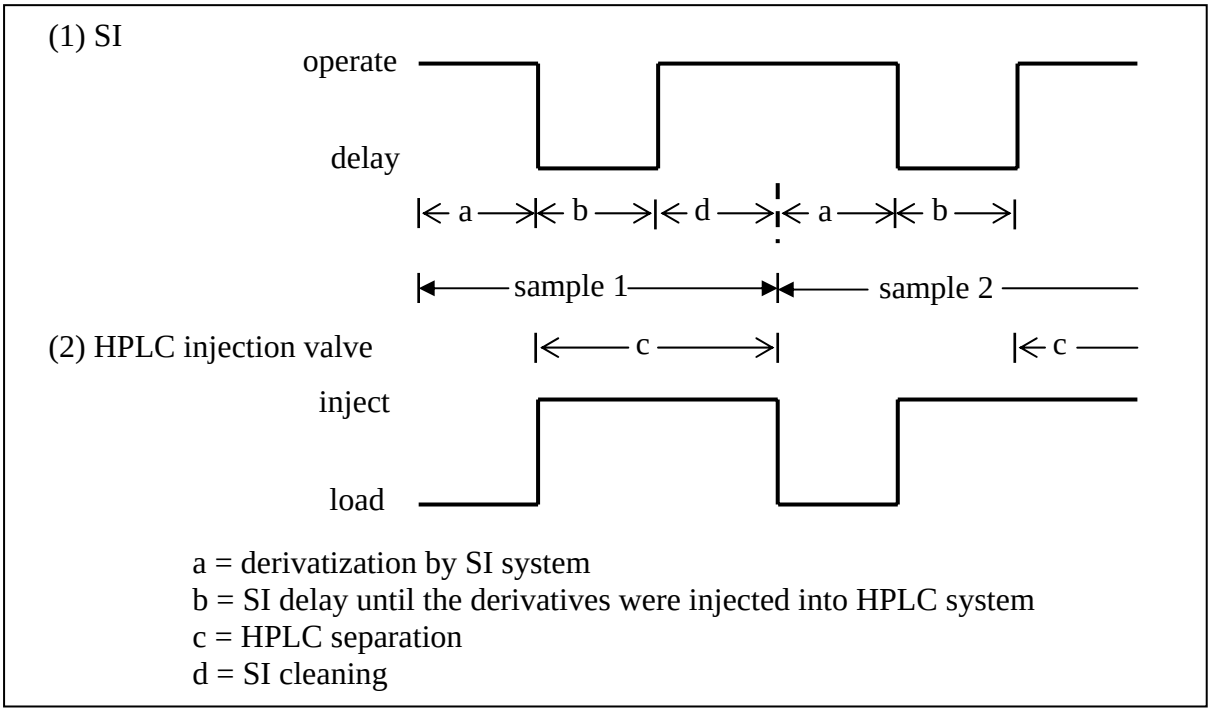
Fig.2 Typical chromatogram of metal-nitro-PAPS complexes: 1, 0.100 mg l^{-1} Cu(II); 2, 0.050 mg l^{-1} Co(II); 3, excess nitro-PAPS; 4, 0.075 mg l^{-1} Ni(II); 5, 0.075 mg l^{-1} Fe(II)

Fig. 3 Chromatograms obtained from sample determination (a) milk (b) supplement (c) wine (d) drinking water

(a)

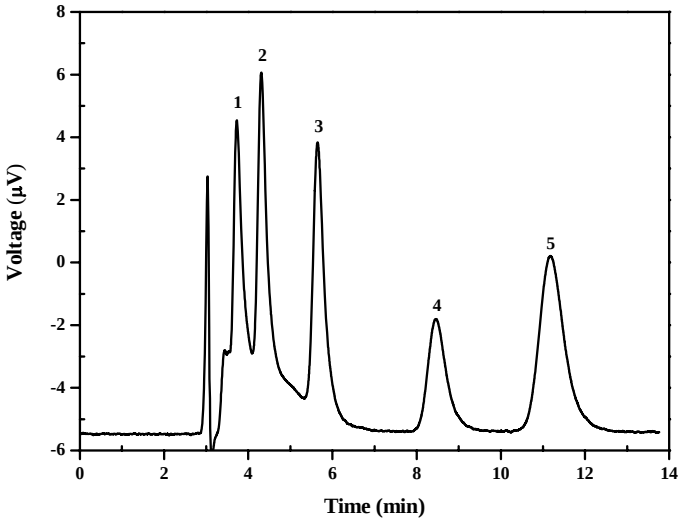


(b)



a = derivatization by SI system
b = SI delay until the derivatives were injected into HPLC system
c = HPLC separation
d = SI cleaning

Fig. 1(a) Schematic diagram of the SI-HPLC system, (b) Diagram of operational sequence of the SI-HPLC system



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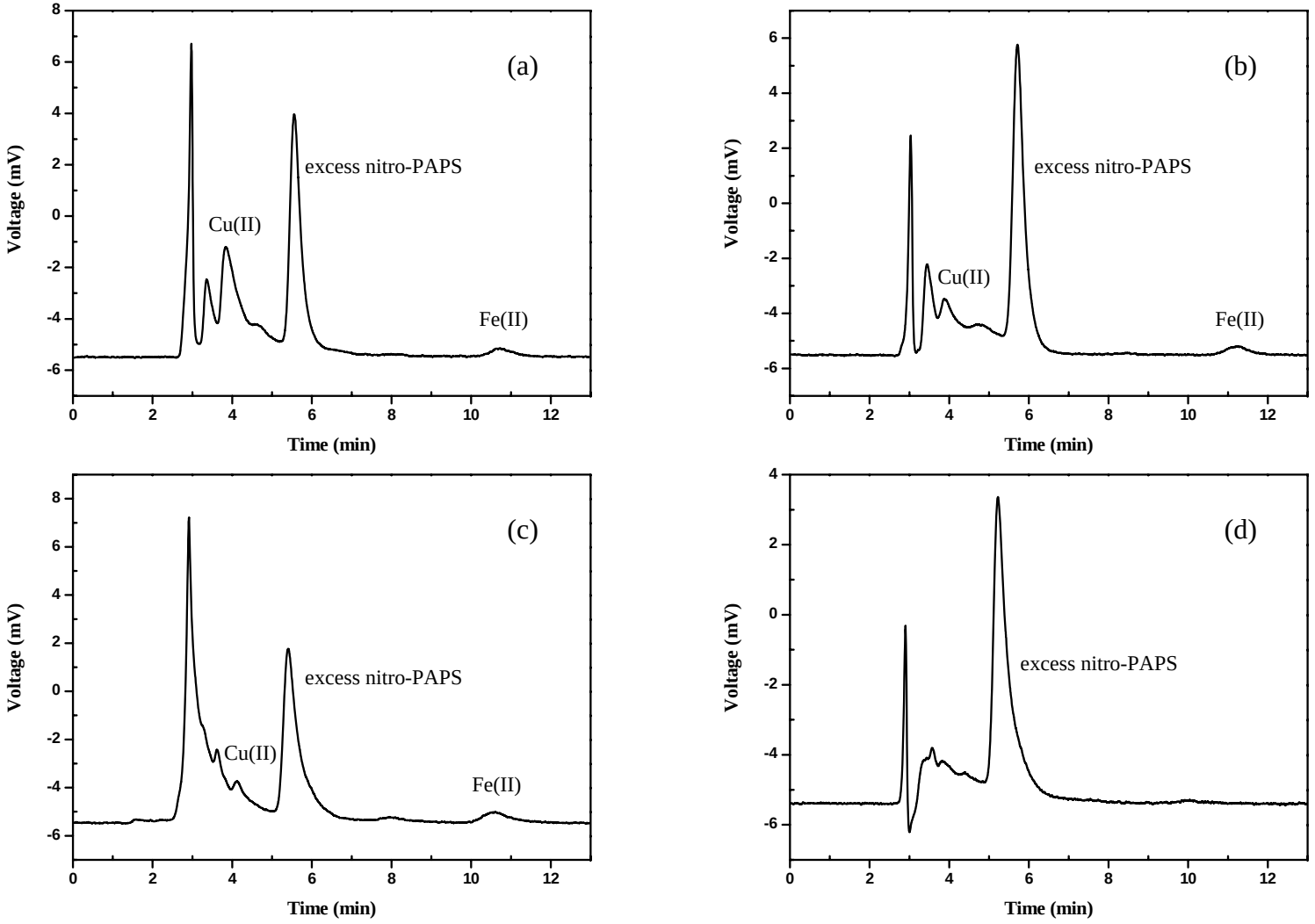

Fig. 3 Chromatograms obtained from sample determination (a) milk (b) supplement (c) wine (d) drinking water

Table 1 The operational sequence of the SI-HPLC system for on-line derivatization of metal-nitro-PAPS complexes

Sequence	***Port no.***	***Flow rate (μl sec^{-1})***	***Volume (μl)***	***Description***
1	3-9	50	70	Aspiration of sample into HC*
2	2	50	25	Aspiration of nitro-PAPS into HC
3	3-9	50	70	Aspiration of sample into HC
4	2	50	25	Aspiration of nitro-PAPS into HC
5	3-9	50	60	Aspiration of sample into HC
6	10	50	300	Propulsion of metal-nitro-PAPS derivatives to HPLC loop
7	10	-	-	HPLC analysis

*HC = Holding coil

Table 2 Analytical characteristics of the proposed system

Metal-nitro-PAPS	***Linear range (mg l^{-1})***	***Linear equation***	***r^2***	***LOD* ($\mu g\ l^{-1}$)***
Cu(II)	0.005-0.250	y = 1652.6x – 72.5	0.9928	2
Co(II)	0.007-0.100	y = 2479.5x – 3.2	0.9968	2
Ni(II)	0.005-0.075	y = 1607.8x – 1.5	0.9945	1
Fe(II)	0.005-0.100	y = 3018.0x – 4.9	0.9938	1

*S/N = 3

Table 3 Analysis of real samples and recovery studies

Sample	Cu(II)			Co(II)			Ni(II)			Fe(II)		
	This work* ($mg\ l^{-1}$)	Labeled ($mg\ l^{-1}$)	Recovery (%)	This work ($mg\ l^{-1}$)	Labeled ($mg\ l^{-1}$)	Recovery (%)	This work ($mg\ l^{-1}$)	Labeled ($mg\ l^{-1}$)	Recovery (%)	This work* ($mg\ l^{-1}$)	Labeled ($mg\ l^{-1}$)	Recovery (%)
Milk 1	273.7±2.1	312.0	91	-	-	99	-	-	99	5.8±0.2	7.0	97
Milk 2	314.0±3.6	340.0	95	-	-	98	-	-	96	5.5±0.1	6.0	95
Milk 3	552.5±1.8	580.0	97	-	-	99	-	-	99	7.2±0.1	7.8	99
	This work* ($mg\ tab^{-1}$)	Labeled ($mg\ tab^{-1}$)	Recovery (%)	This work ($mg\ tab^{-1}$)	Labeled ($mg\ tab^{-1}$)	Recovery (%)	This work ($mg\ tab^{-1}$)	Labeled ($mg\ tab^{-1}$)	Recovery (%)	This work* ($mg\ tab^{-1}$)	Labeled ($mg\ tab^{-1}$)	Recovery (%)
Supplement 1	1.6±0.4	2.0	93	-	-	96	-	-	98	8.7±0.1	10.0	97
Supplement 2	2.1±0.1	2.5	90	-	-	99	-	-	99	12.9±0.1	15.0	94
	This work* ($mg\ l^{-1}$)	AAS ($mg\ l^{-1}$)	Recovery (%)	This work ($mg\ l^{-1}$)	AAS ($mg\ l^{-1}$)	Recovery (%)	This work ($mg\ l^{-1}$)	AAS ($mg\ l^{-1}$)	Recovery (%)	This work* ($mg\ l^{-1}$)	AAS ($mg\ l^{-1}$)	Recovery (%)
Wine 1	1.0±0.2	1.4	102	-	-	94	-	-	105	1.5±0.1	1.8	98
Wine 2	5.2±0.6	6.8	93	-	-	97	-	-	102	1.8±0.1	2.1	93
Wine 3	-	-	95	-	-	95	-	-	98	0.8±0.2	1.0	105
Wine 4	-	-	106	-	-	101	-	-	96	0.2±0.1	0.4	101
Water 1	-	-	93	-	-	99	-	-	99	-	-	98
Water 2	-	-	90	-	-	99	-	-	99	-	-	99

- Not detected
* n = 3
Supplement 1 ingredients (tab^{-1}): Cu ($CuSO_4$) 2 mg, Fe ($FeSO_4$) 10 mg
Supplement 2 ingredients (tab^{-1}): Cu ($CuSO_4$) 2.5 mg, Fe (ferrous gluconate) 15 mg

Program Number: 875 **Day / Time:** Monday, Dec. 19, 7:20 PM - 7:40 PM

Micro - total analysis system using sequential injection analysis with lab - at - valve (SIA - LAV) for on - line micro extraction determination of anionic surfactants

R.Burakham [1]; J.Jakmunee [2]; K.Grudpan [2]

1. Chemistry, Khon Kaen University, Muang, Khon Kaen, Thailand; 2. Chemistry, Chiang Mai University, Muang, Chiang Mai, Thailand

Sequential injection analysis with lab-at-valve (SIA-LAV) was investigated for on-line micro extraction determination of anionic surfactants. Sample, reagent and organic solvent are sequentially aspirated *via* a multiposition valve attached with extraction coil in a position and a separating unit situating a fiber optic spectrophotometer. Such an integrating system serves as a micro-total analysis system. Instrumentation will be represented. Advantages and limitation of the system will be discussed. The determination of anionic surfactants is based on the methylene blue method. Optimization of the procedure and its application to water samples will be reported.

Citation: R.Burakham, J.Jakmunee, K.Grudpan. Micro - total analysis system using sequential injection analysis with lab - at - valve (SIA - LAV) for on - line micro extraction determination of anionic surfactants. Program No. 875. *2005 Abstract Viewer.* Honolulu, Hawaii: International Chemical Congress of Pacific Basin Societies

Development of On-line Sample Preparation for Chromatographic Analysis

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Research interests focus on on-line sample preparation/preconcentration for chromatographic analysis, including high performance liquid chromatography (HPLC), ion chromatography (IC) and capillary electrophoresis (CE). The on-line sample pretreatment includes flow injection pre-column derivatization, sequential injection with lab-at-valve. Applications of such development would aimed for food, environmental and pharmaceutical samples are interested. New approaches for chromatographic separation using sequential injection chromatography (SIC) will be explored.

O2

ON-LINE DERIVATIZATION AND DETERMINATION OF SOME HEAVY METALS BY COUPLING OF SEQUENTIAL INJECTION WITH ION-PAIR REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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An ion-pair reversed-phase high performance liquid chromatography (IP-RPHPLC) was proposed for simultaneous determination of some heavy metals using 2-(5-nitro-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (nitro-PAPS) as a derivatizing reagent. Sequential injection (SI) was coupled to HPLC system for a simple and efficient pre-column derivatization. Using the SI-HPLC system, separation of four metals-nitro-PAPS complexes was achieved within 12 min. Under the selected conditions, the analytical characteristics, including linear range, limit of detection, precision and accuracy, were evaluated. Advantages and limitation of the system will be discussed. The developed method was successfully applied for the determination of metal ions in various samples.

[1] N. Teshima, S. Gotoh, K. Ida, T. Sakai, Anal. Chim. Acta, 557 (2006) 387.
[2] S. Gotoh, N. Teshima, T. Sakai, K. Ida, N. Ura, Anal. Chim. Acta, 499 (2003) 91.

Sequential Injection-Lab-at-Valve for On-line Micro Extraction

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A sequential injection with lab-at-valve system was developed for on-line micro extraction determination of anionic surfactant. Sample, reagents and organic solvent were sequentially aspirated into an extraction coil connected to the center of a selection valve where extraction taking place by flow reversal. The aqueous and organic phases were separated in a LAV unit attached to one port of the valve. The LAV unit situated a fiber optic spectrophotometer to monitor absorbance change of the extract product in the organic phase.

Keywords: Sequential Injection, Lab-at-Valve, Micro-extraction, anionic surfactant

การสกัดในระดับไมโครแบบออนไลน์สำหรับการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบด้วยระบบซีเควนเชียลอินเจคชันอะนาลิซิส-แล็ปแอทวาล์ว

ON-LINE MICRO-EXTRACTION DETERMINATION OF ANIONIC SURFACTANT USING SEQUENTIAL INJECTION WITH LAB-AT-VALVE SYSTEM

รจนา บุระคำ[1*], จรูญ จักร์มุณี[2] และ เกตุ กรุดพันธ์[2*]
Rodjana Burakham[1*], Jaroon Jakmunee[2] and Kate Grudpan[2*]

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บทคัดย่อ: ได้พัฒนาระบบซีเควนเชียลอินเจคชันแบบแล็ปแอทวาล์ว สำหรับการสกัดในระดับไมโครแบบออนไลน์ เพื่อการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในน้ำ การวิเคราะห์อาศัยหลักการทำให้เกิดสารประกอบไอออนรวมตัวระหว่างสารลดแรงตึงผิวชนิดประจุลบกับเมทิล-ลีนบลูซึ่งสามารถสกัดโดยใช้ไดคลอโรมีเทนก่อนการวิเคราะห์ด้วยเทคนิคสเปกโทรโฟโทเมทรี ขั้นตอนการวิเคราะห์ทำได้โดยการดูดสารตัวอย่าง รีเอเจนต์ และ ไดคลอโรมีเทน เข้าสู่ระบบแล้วให้เกิดการสกัดภายในท่อเล็กๆ หลังจากนั้นสารละลายจะถูกส่งไปยังกรวยแยกเล็กๆ หลังจากที่ปล่อยให้ชั้นของน้ำและตัวทำละลายอินทรีย์แยกกันแล้ว จึงตรวจวัดการดูดกลืนแสงของชั้นตัวทำละลายอินทรีย์ที่มีสารตัวอย่างที่สนใจอยู่ โดยใช้สเปกโทรโฟโทมิเตอร์ผ่านทางสายใยแก้วนำแสงที่ติดอยู่บนส่วนของแล็ปแอทวาล์ว ระบบที่พัฒนาขึ้นได้ประยุกต์สำหรับการวิเคราะห์สารลดแรงตึงผิวชนิดประจุลบในน้ำ

Abstract: Sequential injection with “Lab-at-Valve (LAV)” system was developed for on-line micro-extraction determination of anionic surfactant in water samples. The detection method is based on the formation of ion-association compound between anionic surfactant and methylene blue, which can be extracted into dichloromethane before spectrophotometric detection. Sample, reagent and dichloromethane are sequentially aspirated via a multiposition selection valve attached with an extraction coil where the extraction process is performed. The aqueous and organic phases are separated in a conical separating chamber LAV unit situating a fiber optic spectrometer. The organic phase containing extracted product is then monitored spectrophotometrically. Some water samples have been analyzed for anionic surfactants by the developed procedure.

Introduction: Solvent extraction is one of the versatile techniques for sample matrix separation and/or analyte preconcentration. However, manual extraction presents a series of drawbacks such as high consumption of sample and organic solvent, low sampling frequency, loss of analyte through manipulation and contamination of atmosphere by organic vapor. Many efforts have been made to overcome these inherent drawbacks. Among them, the successful techniques are probably the on-line solvent extraction using flow systems. In this work, sequential injection analysis with a new concept called "lab-at-valve" was proposed for on-line extraction in a micro-scale.

Methodology: The SIA-LAV system consisted of a 2.50 ml syringe pumping, a 10-position selection valve, a UV-Vis light source and a spectrophotometer. The syringe pump was connected to the center of the selection valve by an extraction coil. A separating LAV unit situating a fiber optic spectrophotometer was attached at one port of the valve. Both instrumental control and data acquisition were manipulated via the FIAlab software. Sample, reagent and organic solvent were sequentially introduced into the extraction coil. An ion association formed was extracted by programming the syringe pump to aspiration and dispensing modes. The solution was then propelled into the separating chamber where the aqueous and organic phases were separated. The organic phase containing ion-associated product was detected at 650 nm.

Results, Discussion and Conclusion: The experimental parameters affected the extraction efficiency and the sensitivity of the determination were selected to be optimized. They involved operational sequence, number of flow reversal and the sample volume. It was found that the operational sequence in which the sample, reagent and organic solvent were sequentially aspirated as a segment before several cycles of flow reversal provided high sensitivity because of high degree of contact between aqueous and organic phases. Using this system, a sample and organic solvent volumes of 1000 and 300 µl, respectively, were consumed. Application to the determination of anionic surfactant in water samples was demonstrated. Good agreement between the proposed and the standard "methylene blue" methods was achieved. SI-LAV involved a considerable reduction of both reagent and sample consumption. Therefore, only small volume of organic solvent was required. The proposed integrating system serves as a micro-total analysis system.

Acknowledgements: The Thailand Research Fund and the Commission on Higher Education are gratefully acknowledged for financial support.

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(1) Kate Grudpan (2004) *Talanta*, **64**, 1084-1090.
(2) Wiboon Praditweingkum and Kate Grudpan (2000) *J. Flow Injection Anal.*, **17**, 202-205.
(3) Standard Methods for the Examination of Water and Wastewater, 7th ed., **1989**, pp. 59-63.

Keywords: sequential injection analysis, lab-at-valve, on-line extraction, anionic surfactant

Symposium on Advanced Flow-Based Chemical Analysis

Venue: Faculty of Science, Okayama University (Lecture Room 11)
Date: December 13, 2005, 14:00-18:00

Program

14:00	Opening Remarks	
14:05-14:50	K. Grudpan	Titration using flow-based techniques
14:50-15:10	R. Burakham	Sequential injection-Lab-at-Valve: An alternative system for on-line micro-extraction
15:10-15:30	L. Narong	Simultaneous flow titration for determination of iron and acid concentration
15:30-16:10	*Poster Session I**	*ODD-number papers*
16:10-16:50	Poster Session II*	EVEN-number papers
16:50-17:10	T. Takayanagi	Nonionic surfactant micelle as a pseudo-homogeneous extraction medium for the extraction of ion-associates
17:10-17:30	M. Oshima	Collection and concentration for ICP-AES and ICP-MS
17:30-18:00	S. Motomizu	Trace analysis of the atmosphere for gaseous air pollutants by flow-based analytical methods
18:00	Closing Remarks	
19:00-	Social gathering at Naritaya	

* Poster sessions will be held at 3rd floor. Free drinks are available during the poster sessions.

Posters

P01	S. Lunvongsa	Highly sensitive method for the spectrophotometric determination of copper using a catalytic reaction by flow-based technique
P02	K. Uraisin	Flow injection/spectrophotometric determination of trace amounts of bromate by using prochlorperazine
P03	A. Sabarudin	Development of novel chelating resins using chitosan as base material and their application to the adsorption/concentration of non-metallic elements
P04	Q. Li	Development of a novel flow-based method for the determination of micro amounts of formaldehyde based on Hantzsch reaction
P05	Nathawut	Gas-diffusion flow injection for kinetic determination of iodide
P06	T. Suekane	Determination of trace amounts of ammonia in air using batchwise collection/concentration method
P07	Rosi Ketrin K	Synthesis of novel chelating resin using chitosan as a base material and their application to the collection / concentration of metal ions in environmental water samples
P08	Lukman	Synthesis of novel chitosan resin and their application for metals/non-metals preconcentration
P09	M. Imamori	Preparation of a new type chelating resin based on chitosan
P10	O.Noguchi	Determination of trace elements in microsamples by air-segmented flow injection/ICP-MS
P11	D. Naito	Determination of anionic surfactants using new ion-association reagents
P12	N. Miyoshi	Determination of Vanadium by using chelating disk for collection / concentration
P13	H. Yamashita	Determination of fluorosurfactant using detection of fluoride
P14	T. Inoue	Advanced chemical analysis for EDC・HCl using flow-based analysis method
P15	F. Iwami	Study on the development of highly-sensitive chemical analysis method for trace metals
P16	Y. Joichi	Development of fully automated system for advanced trace chemical analysis
P17	T. Yamamoto	Development of on-line pretreatment method for ICP-AES
P18	M. Ishida	Highly-sensitive ion analysis by ion chromatography based on capillary electrophoresis

Sequential Injection: An On-line Sample Preparation for Liquid Chromatography and Capillary Electrophoresis

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[2] *Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200*

Research interests focus on on-line sample preparation for liquid chromatography (LC) and capillary electrophoresis (CE) using sequential injection analysis (SIA). The on-line sample preparation includes sequential injection on-line extraction-at-valve and sequential injection on-line solid phase extraction. New approaches for chromatographic separation using sequential injection chromatography (SIC) and monolithic column is also explored.

Determination of Some Heavy Metals by Sequential Injection On-line Derivatization and Ion-Pair Reversed Phase High Performance Liquid Chromatography

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An on-line derivatization system for determination of some heavy metals was developed using sequential injection (SI) and ion-pair reversed-phase high performance liquid chromatography (IP-RPHPLC). 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) was chosen as a derivatizing reagent. SI system was coupled to HPLC for a simple and efficient pre-column derivatization. Using the proposed system, separation of four metals-nitro-PAPS complexes was achieved within 12 min. Under the selected conditions, the analytical characteristics, including linear range, limit of detection, precision and accuracy, were evaluated. Advantages and limitation of the system will be discussed. The developed method was successfully applied for the determination of metal ions in various samples.

การเตรียมอนุพันธ์แบบออนไลน์สำหรับการวิเคราะห์โลหะโดยใช้เทคนิคซีเควนเชียลอินเจคชันและโครมาโทกราฟีของเหลวสมรรถนะสูง

ON-LINE DERIVATIZATION DETERMINATION OF METAL IONS USING SEQUENTIAL INJECTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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บทคัดย่อ: การใช้ระบบซีเควนเชียลอินเจคชันเพื่อเตรียมอนุพันธ์แบบออนไลน์สำหรับการวิเคราะห์โลหะด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง โลหะที่วิเคราะห์ได้แก่ โคบอลต์ เหล็ก นิกเกิล และ คอปเปอร์ โดยใช้ 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) เป็นรีเอเจนต์ และวัดด้วยเทคนิคสเปกโทรโฟโตเมทรีที่ความยาวคลื่น 570 นาโนเมตร ระบบที่นำเสนอนี้สามารถเตรียมอนุพันธ์แบบออนไลน์และแยกสารประกอบเชิงซ้อนของโลหะทั้งสี่ชนิดได้ภายใน 12 นาที และ ได้นำไปประยุกต์สำหรับการวิเคราะห์โลหะในตัวอย่างที่หลากหลาย

Abstract: Sequential injection (SI) was coupled to high performance liquid chromatography (HPLC) for an on-line simple pre-column derivatization and determination of metal ions, including Co(II), Fe(II), Ni(II) and Cu(II). 2-(5-nitro-2-pyridylazo)-5-[*N*-propyl-*N*-(3-sulfopropyl)amino]phenol (nitro-PAPS) was used as a derivatizing reagent. The detection principle is spectrophotometry at 570 nm. Using the proposed SI-HPLC system, on-line derivatization and separation of four metals-nitro-PAPS complexes was achieved within 12 min. The developed method has been successfully applied for the determination of metal ions in various samples.

Sequential injection with lab-at-valve system for on-line micro-extraction determination of anionic surfactant

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Abstract

Sequential injection with lab-at-valve system was developed for on-line micro-extraction determination of anionic surfactant. The detection method is based on the formation of ion-association compound between anionic surfactant and methylene blue, which can be extracted into dichloromethane before spectrometric detection. Sample, reagent and dichloromethane are sequentially aspirated via a multiposition selection valve attached with an extraction coil where the extraction process is performed. The aqueous and organic phases are separated in a conical separating chamber lab-at-valve unit situating a fiber optic spectrometer. The organic phase containing extracted product is then monitored spectrophotometrically. Some water samples have been analyzed for anionic surfactant by the developed procedure.

Keywords: sequential injection, lab-at-valve, micro-extraction, anionic surfactant

Outputs

1. Burakham R, Jakmunee J, Grudpan K. Development of sequential injection-lab-at-valve (SI-LAV) micro-extraction instrumentation for the spectrophotometric determination of an anionic surfactant, Anal. Sci. 2006; 22: 137-140.

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Capillary zone electrophoresis of organic acids in beverages

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Abstract

Capillary zone electrophoresis (CZE) with photodiode array detection (PDA) was applied to simultaneously determine eleven organic acids in wine, beer, and fruit and vegetable juices after derivatisation with 2-nitrophenylhydrazine (2-NPH) in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC · HCl). The parameters affecting CZE separation included type and concentration of buffer, pH, organic additive and applied voltage. Optimum conditions at 25 °C were: 30 mmol l^{-1} borate buffer, pH 10.0, containing 100 ml l^{-1} acetonitrile, at 20 kV, sample injection at 0.5 psi for 5 s, with direct detection at 230 nm. Separation of eleven organic acids was achieved within 12 min. Linear calibration curves with good fit were obtained in the range 10.0–100.0 mg l^{-1}. Limits of detection ranged from 2.0 to 10.0 mg l^{-1}. Intra-day precision with RSD $\leqslant$ 4.0% for migration time and $\leqslant$ 5.0% for peak area, and inter-day precision with RSD $\leqslant$ 6.0% and $\leqslant$ 9.0% for migration time and peak area, were obtained. Recovery for all beverage samples was 97 ± 15%.

Keywords: Organic acids; Capillary zone electrophoresis; 2-nitrophenylhydrazine; Wine; Beer; Fruit juice; Vegetable juice

1. Introduction

Organic acids have important roles in beverages because they affect the organoleptic properties (e.g. taste, aroma and colour), stability, nutrition, acceptability and in maintaining quality (Shui & Leong, 2002; Soyer, Koca, & Karadeniz, 2003). Levels of organic acids in foods and beverages provide relevant information for monitoring the fermentation processes (Castiñeira, Peña, Herrero, & García-Martín, 2002; Esteves, Lima, Lima, & Duarte, 2004). These acids can be used for differentiation, classification, origin identification or possible adulteration of beverages. Therefore, it is important to be able to precisely determine the amounts of different acids present for quality control during beverage production, transformation, storage and distribution.

The most widely used methods for determination of organic acids are gas chromatography (GC) (Docherty & Ziemann, 2001) and high-performance liquid chromatography (HPLC) (Kotani, Miyaguchi, Tomita, Takamura, & Kusu, 2004). HPLC has been the most popular method for analysing these acids after derivatisation. Nowadays, capillary zone electrophoresis (CZE) has become an attractive alternative technique to HPLC providing high efficiency, short analysis time and low reagent and sample consumption. In CZE, detection of organic acids can be performed using either direct UV measurements (Buchberger & Winna, 1996; Cortacero-Ramírez, Segura-Carretero, Hernáinz-Bermúdez de Castro, & Fernandez-Gutierrez, 2005) or indirect UV determination (Chen, Xu, Lente, & Ip, 1996; De Villiers, Lynen, Crouch, & Sandra, 2003; Esteves et al., 2004; Wu, Lo, Lee, & Lin, 1995). However, both direct and indirect UV detection offer less selectivity and samples need purification to eliminate interfering matrices, such as sugars or phenolic compounds. Derivatisation of organic acids before separation by CZE enhances both sensitivity and selectivity (Cunha, Fernandes, & Ferreira, 2002). Derivatising reagents that have been used for analysis of organic acids are 2-nitrophenylhydrazine (2-NPH) (Miwa, 1985, 2000; Miwa & Yamamoto, 1988, 1996), phenacyl and naphthacyl bromide (Blau & Halke, 1993) and *O*-(4-nitrobenzyl)-N,*N*′-diisopropylisourea (PNBDI) (Cunha et al., 2002). 2-NPH has been

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widely used because of the simplicity and rapidity of the derivatisation procedure.

The aim of the present study is to optimise CZE for the simultaneous determination and rapid quantification of the major organic acids in wine, beer and fruit and vegetable juices. 2-NPH was used as the derivatising reagent for organic acids before separation of their acid hydrazides.

2. Materials and methods

2.1. Chemicals and standards

All chemicals were of analytical reagent grade or better. 2-NPH (Fluka, Australia) solution (100 mmol l^{-1}) was prepared by dissolving the reagent in 0.1 mol l^{-1} HCl–ethanol (1:1 v:v). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC · HCl) (Fluka, Slovakia) solution (0.25 mmol l^{-1}) was prepared by dissolving the reagent in pyridine–ethanol (3:97 v/v). Pyridine and ethanol were purchased from Carlo Erba (France). Potassium hydroxide (Merck, Germany) solution (1:10 w/v) was prepared in methanol–water (1:1 v/v). Organic acid standards used were glacial acetic acid (Carlo Erba, France); lactic acid, succinic acid, oxalic acid dihydrate, citric acid anhydrous and trans-aconitic acid (Fluka, Switzerland); suberic acid, glutaric acid and malonic acid (Acros, Belgium); malic acid (Sigma, Canada) and tartaric acid (HW, England). Stock solutions (1000 mg l^{-1}) of organic acids and various concentrations of organic acids were prepared by dissolving accurately weighed amounts in water. Sodium tetraborate (Univar-Ajax, Australia) was used for buffer preparation by dissolving accurately weighed amounts in water. Hydrochloric acid and sodium hydroxide (Carlo Erba, France) were used for adjusting buffer pH. Deionised water with resistivity of 18.2 MΩ cm from the RiO_S^{TM} Type I Simplicity 185 purification system (Millipore water, USA) was used throughout the experiments.

2.2. Instrumentation

All CZE experiments were performed using a Beckman P/ACE^{TM} MDQ v.5.0 Capillary Electrophoresis system (Beckman Instrument Inc., Fullerton, CA, USA) equipped with a photodiode array detector (190–600 nm). KARAT software was used for system control and data handling. Separations were carried out using a fused-silica capillary with total length of 40.0 cm (30.0 cm effective length) and 75 μm internal diameter (Beckman Instrument Inc., Fullerton, CA, USA).

2.3. Derivatisation procedure

Stock organic acid solutions (100 μl), 2-NPH (150 μl) and EDC · HCl (200 μl) were mixed and heated at 70 °C for 10 min. After addition of 100 μl of 100 g l^{-1} KOH, the mixture was further heated at 70 °C for 10 min and then cooled, filtered and analysed by CZE.

2.4. Capillary zone electrophoresis conditions

All new capillaries were initially conditioned with methanol for 5 min, followed by water for 2 min, 0.1 mol l^{-1} HCl for 10 min and water for 5 min. Then they were rinsed with 0.1 mol l^{-1} NaOH for 10 min, with water for 5 min and equilibrated with the running buffer for 10 min. Between each separation cycle, the capillary was automatically rinsed with 0.1 mol l^{-1} NaOH for 5 min, with water for 2 min and with running buffer for 5 min. All electrolyte solutions were filtered through a 0.45 μm membrane filter and degassed by sonication before use.

The parameters affecting the separations described below include: buffer pH (pH 5.0–11.0), type (acetate, phosphate, and borate) and concentration (20–40 mmol l^{-1}); organic modifier levels (acetonitrile, 50–200 ml l^{-1}) and separation voltage (15–30 kV). Absorbance detection at 230 nm was used throughout the experiments. The sample injection was set constant at 42 nl (hydrodynamic injection at 0.5 psi for 5 s).

2.5. Analysis of organic acids in beverages

Beverages studied include wine, beer, and vegetable and fruit juices. Samples were purchased from the local supermarkets in Khon Kaen Province in Northeastern Thailand. Wine samples are local products with various fruit origins. The samples were filtered through a 0.45 μm membrane filter and were diluted with water to the appropriate values in order to ensure that the results obtained were in the range of the calibration graph. The diluted samples were then derivatised and analysed by CZE. All analyses were performed in triplicate.

3. Results and discussion

3.1. Effect of pH

The pH of the electrolyte buffer has considerable influence on the separation because it impacts not only electroosmotic flow (EOF) but also electrophoretic mobilities of the analytes. At pH 5.0 (acetate buffer), no peaks of acid hydrazides were observed, presumably due to the predominance of non-ionised forms of acid hydrazides. Below pH 10.0 (phosphate or borate), good resolution could not be obtained. Above pH 10.0, no improvement in resolution was observed but analysis time increased (>20 min), so a borate buffer of pH 10.0 was selected as the optimum buffer for subsequent experiments.

3.2. Effect of buffer concentration

Fig. 1a shows the migration profiles of the acid hydrazides obtained for various concentrations (20–40 mmol l^{-1}) of the

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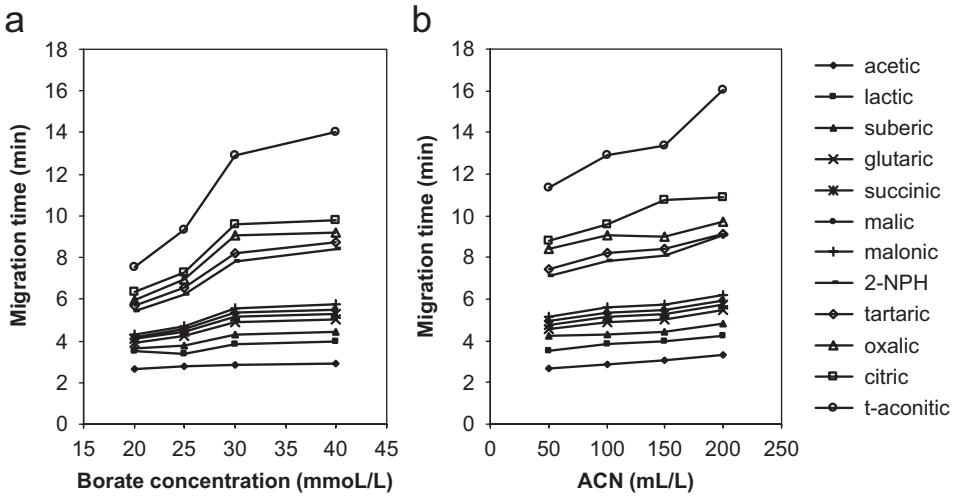

Fig. 1. Effect of (a) borate concentration and (b) acetronitrile content on migration time of acid hydrazides. Capillary electrophoretic conditions: 30 mmol l^{-1} borate and 100 ml l^{-1} ACN, voltage of 20 kV, sample injection (0.5 psi, 5 s) and detection at 230 nm.

borate buffer. With increasing borate concentration resolution increased, but the migration times also increased. In general, the electrophoretic mobility is inversely proportional to the viscosity and concentration of the running buffer (Chen, Chen, Lin, & Chang, 1999) so increasing buffer concentration results in longer migration time, associated with increased Joule heating in the capillary which results in decreasing separation efficiency. Therefore, borate buffer concentration of 30 mmol l^{-1} was chosen.

3.3. Effect of organic modifier

Organic modifiers such as methanol, acetonitrile (ACN) and iso-propanol are often added into the running buffer to improve the selectivity and alter the retention mechanism by changing the polarity of the aqueous phase (Nielsen, Nielsen, & Frisvad, 1996). An organic component with low viscosity, such as ACN, is preferred for high-speed separations. ACN is also a very weak acid as well as a very weak base and, therefore, it is a good differentiating solvent for acids (Chen et al., 1999). It was found that the addition of ACN to the running buffer extended the migration times of the acid hydrazides and provided the increase in resolution (Fig. 1b). Some acid hydrazides, such as glutaric acid, succinic acid, malic acid and malonic acid, could not be separated in the running buffer without acetonitrile. To compromise between good resolution (higher than 1.0) and short analysis time, 100 ml l^{-1} ACN was selected to be used throughout.

3.4. Effect of running voltage

The driving force behind the migration of ions in CZE is the field strength applied across the capillary, which is related to the applied voltage divided by the capillary length. In this study, voltages used were 15–30 kV operating over a 40.0 cm capillary, which gives the field strength of 375–750 V cm^{-1}. Although, the analysis time decreased with an increase in the applied voltage (data not shown), high voltage (>25 kV) resulted in high current (>300 μA) in the system. To ensure good separation and extend the lifetime of the capillary, applied voltage was compromised. Thus, the applied voltage was set to 20 kV to avoid too high current and to keep short analysis time.

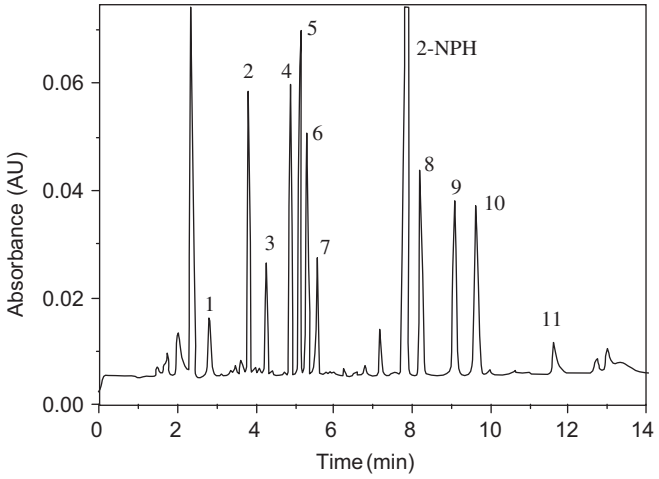

Fig. 2. Electropherogram of eleven standard organic acids (each concentration 60 mg l^{-1}); conditions: 30 mmol l^{-1} borate (pH 10.0) and 100 ml l^{-1} ACN, voltage of 20 kV, sample injection (0.5 psi, 5 s), temperature of 25 °C and detection at 230 nm; peaks: (1) acetic acid, (2) lactic acid, (3) suberic acid, (4) glutaric acid, (5) succinic acid, (6) malic acid, (7) malonic acid, (8) tartaric acid, (9) oxalic acid, (10) citric acid and (11) trans-aconitic acid.

3.5. Analytical characteristics and method validations

Fig. 2 shows a typical CZE electropherogram of eleven acid hydrazides under optimum experimental conditions, monitored at 230 nm. Grouped by their increasing negative charge-to-mass ratios, mono-carboxylic acids (acetic acid

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and lactic acid) elute first as anticipated; di-carboxylic acids (suberic acid, glutaric acid, succinic acid, malic acid, malonic acid, tartaric acid and oxalic acid) elute next, and tri-carboxylic acids (citric acid and trans-aconitic acid) elute last.

The linearity of response, limit of detection (LOD), limit of quantitation (LOQ) and precisions (i.e. intra-day and inter-day reproducibility) were investigated. Table 1 shows equation of regression lines which had good linearity in the range 10.0–100.0 mg l^{-1}, with regression coefficient (R^2) values higher than 0.999. LOD and LOQ were estimated using a signal-to-noise ratio of 3 and 10, respectively. LODs ranged from 2.0 to 10.0 mg l^{-1} whereas LOQs ranged from 10.0 to 20.0 mg l^{-1}. The reproducibility of the proposed method was studied by repeated injections of the acid hydrazide mixtures 10 times in 1 day (intra-day precision), whereas inter-day precision was tested by triplicate injections for 4 days. The results are reported in Table 1 in terms of relative standard deviation (RSD). Intra-day precision (RSD $\leqslant$ 4.0% for migration time and $\leqslant$ 5.0% for peak area) and inter-day precision (RSD $\leqslant$ 6.0% and $\leqslant$ 9.0% for migration time and peak area, respectively) were obtained. The results indicate that the proposed method has good precision for both qualitative and quantitative studies.

Recovery was studied by spiking the mixture of organic acids (each concentration of 40.0 mg l^{-1}) into individual beverage samples. The percentage recoveries of organic acids from all types of samples were in the range 81.7–112.4 as summarised in Table 1. The recoveries showed dependence on the origin of the sample and the sample matrix.

3.6. Analysis of beverage samples

The proposed method was applied to the determination of organic acids in different beverages, including wine and beer, and fruit and vegetable juices. Fig. 3 shows typical

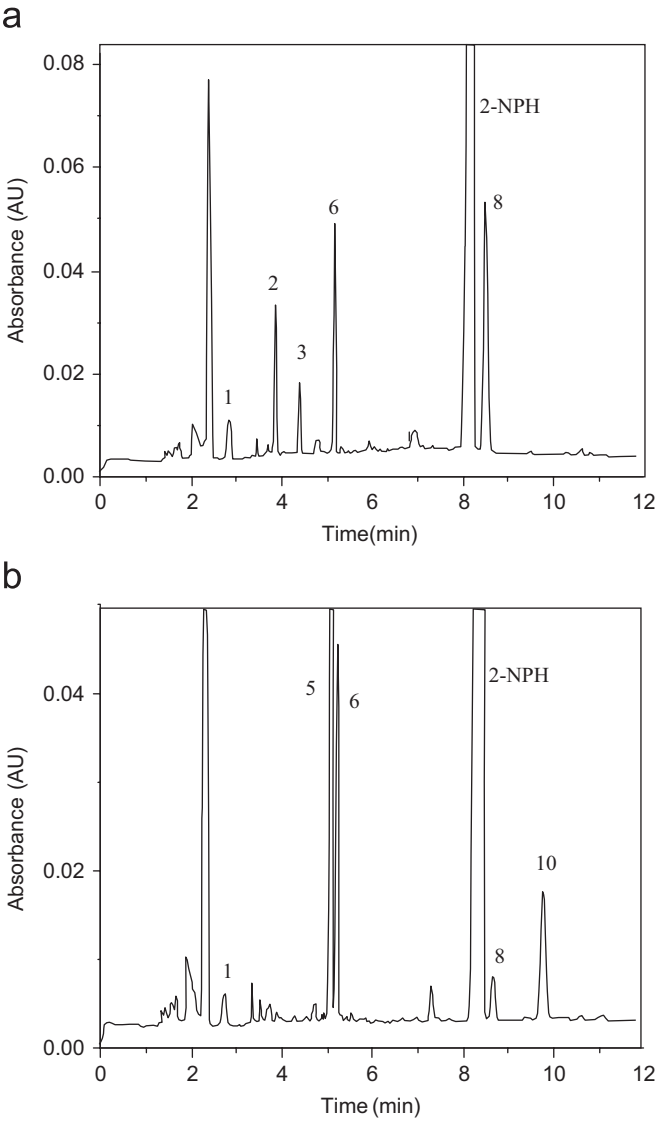

Fig. 3. Electropherograms of (a) red grape wine and (b) jambolan wine. CZE conditions and peak assignments as described in Fig. 2.

Table 1
Linear equations, regression coefficients, LOD, LOQ, precision and recovery range of the studied organic acids

Acid	Linear equation	R^2	LOD (mg l^{-1})	LOQ (mg l^{-1})	Precision, RSD (%)				Recovery range[a] (%)
					Intra-day ($n = 10$)		Inter-day ($n = 4 \times 3$)		
					t_M	Peak area	t_M	Peak area	
Acetic	$y = 641.12x + 17918$	0.9995	5.0	10.0	2.34	4.64	2.32	6.64	83.2–110.9
Lactic	$y = 206.02x + 3223.6$	0.9998	5.0	10.0	1.44	3.58	2.04	6.55	85.0–109.9
Suberic	$y = 364.67x + 441.4$	0.9999	5.0	12.0	1.48	4.04	2.00	5.14	82.7–111.9
Glutaric	$y = 905.13x + 1413.4$	0.9991	2.0	10.0	1.67	4.38	2.27	8.44	83.4–112.4
Succinic	$y = 1126.80x + 10787$	0.9998	2.0	10.0	1.74	2.29	2.34	5.18	82.6–104.6
Malic	$y = 808.42x + 1450.6$	0.9992	3.0	10.0	1.86	3.98	2.45	6.99	89.4–111.9
Malonic	$y = 459.50x + 82.64$	0.9992	5.0	12.0	1.86	3.94	2.67	8.49	84.6–106.2
Tartaric	$y = 1122.60x + 1078$	0.9992	3.0	10.0	2.26	4.90	3.17	5.44	84.7–111.1
Oxalic	$y = 1186.9x + 773.69$	0.9998	4.0	10.0	3.15	2.66	5.25	8.60	81.7–105.4
Citric	$y = 523.61x + 896.33$	0.9994	3.0	10.0	2.46	2.95	3.22	5.00	84.8–102.3
t-Aconitic	$y = 151.46x + 4310.3$	0.9996	10.0	20.0	2.87	4.71	5.54	6.92	83.9–112.0

[a] $n = 3$ for replicate injections in all types of beverage samples.

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Table 2
The contents of organic acids in wine and beer samples

Sample	Content of acid ($g l^{-1}$)								
	Acetic	Lactic	Suberic	Glutaric	Succinic	Malic	Malonic	Tartaric	Citric
Grape red wine	0.54	4.11	0.99	n.d.	n.d.	1.44	n.d.	2.34	n.d.
Grape white wine	n.d.	n.d.	0.26	n.d.	0.56	4.41	0.44	2.38	13.49
Strawberry white wine	0.36	0.52	0.30	0.12	0.75	6.48	n.d.	5.09	1.66
Organic fruit wine	0.95	n.d.	n.d.	n.d.	2.10	1.39	n.d.	5.17	12.28
Black galingale wine	1.16	n.d.	n.d.	n.d.	1.68	0.74	n.d.	3.86	7.34
Jambolan wine	0.48	n.d.	n.d.	n.d.	5.73	3.58	n.d.	0.42	4.69
Roselle wine	1.23	n.d.	n.d.	n.d.	2.52	0.51	n.d.	n.d.	12.44
Mangosteen wine	0.76	n.d.	n.d.	n.d.	3.58	1.58	n.d.	0.16	2.58
Star gooseberry wine	4.54	n.d.	n.d.	n.d.	3.25	11.01	n.d.	n.d.	n.d.
Beer 1	0.05	0.45	n.d.	0.05	0.06	0.21	0.24	n.d.	0.41
Beer 2	0.06	0.32	n.d.	0.05	0.08	0.28	0.12	n.d.	0.65
Wines									
Esteves et al. (2004)	0.06–0.46	0.53–2.46	n.r.	n.r.	0.24–0.71	0.49–2.38	n.r.	0.82–2.75	n.r.
De Villiers et al. (2003)	0.40–1.08	0.61–4.03	n.r.	n.r.	0.41–1.59	0.3–3.05	n.r.	0.92–2.67	n.d.–0.32
Beers									
Pérez-Ruiz et al. (2004)	n.r.	0.55–0.63	n.r.	n.r.	n.r.	0.41–0.72	n.r.	n.d.–0.22	0.73–0.20

n.d.: not detected, n.r.: not reported.

Table 3
The contents of organic acids in fruit and vegetable juice samples

Sample	Content of acid ($g l^{-1}$)					
	Acetic	Succinic	Malic	Malonic	Tartaric	Citric
Red grape 1	n.d.	n.d.	0.83	n.d.	0.50	1.79
Red grape 2	n.d.	n.d.	10.30	n.d.	7.58	2.49
Tangerine orange	n.d.	n.d.	0.62	n.d.	n.d.	5.39
Apple	n.d.	n.d.	2.96	n.d.	n.d.	1.77
Mixed berry	n.d.	n.d.	0.73	n.d.	0.16	7.55
Longan	n.d.	n.d.	0.05	0.03	n.d.	0.09
Grapefruit flavoured	n.d.	n.d.	0.37	n.d.	n.d.	6.36
Muscat flavoured	n.d.	n.d.	0.65	n.d.	0.07	5.23
Cherry flavoured	n.d.	n.d.	0.76	n.d.	0.06	3.84
Mixed fruit and vegetable	n.d.	n.d.	0.89	0.20	n.d.	8.95
Broccoli	n.d.	n.d.	2.46	n.d.	0.45	9.90
Purple carrot	n.d.	0.06	5.50	0.19	n.d.	7.47
Tomato	0.14	n.d.	7.66	n.d.	n.d.	13.23
Apple juice						
Miwa (2000)	n.r.	n.r.	0.20–7.20	n.r.	n.r.	0.30–8.50
Cunha et al. (2002)	n.r.	n.r.	0.20–4.90	n.r.	n.r.	0.10–3.70
Chinnici et al. (2005)	n.r.	0.10–0.84	1.37–5.61	n.r.	n.r.	3.3–1.09
Orange juice						
Cunha et al. (2002)	n.r.	n.d.–0.20	n.d.–4.30	n.r.	n.d.	1.50–12.50
Grape juice						
Soyer et al. (2003)	n.r.	n.r.	1.40–3.50	n.r.	4.10–8.00	0.03–0.20

n.d.: not detected, n.r.: not reported.

electropherograms obtained from the samples. Organic acids in the samples were identified by comparing their migration times (t_M) with authentic compounds. As noted in Table 2, five acids (i.e. acetic acid, succinic acid, malic acid, tartaric acid, and citric acid) were almost always present in wines, and there was considerable variation in levels (e.g., 0.51–11.01 $g l^{-1}$ for malic acid). Tartaric acid was detected in seven wine samples, but neither of the two beer samples. Some organic acids such as lactic acid, suberic acid, glutaric acid and malonic acid were found at low levels. In juice samples (Table 3), malic acid and citric acid were consistently present, but they varied considerably

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in samples (e.g., 0.05–10.30 g l^{-1} for malic acid). The acid contents obtained by the proposed method are comparable to those reported in literatures. These results demonstrate that the present method is suitable for determining organic acids in various types of beverage samples.

4. Conclusion

CZE has been shown to be a powerful method for rapid and simultaneous analysis of organic acids in wine and beer, and in fruit and vegetable juices. The separation of eleven organic acids as their acid hydrazides was achieved within 12 min. The derivatisation with 2-NPH was simple and provided sensitive spectrometric detection. The main organic acids found in the beverages consisted of malic acid and citric acid, whereas the other organic acids were found at low levels. Oxalic acid and trans-aconitic acid were not found in any sample. This method is well suited for determining organic acids in beverages.

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Field-amplified sample injection and in-capillary derivatization for capillary electrophoretic analysis of metal ions in local wines

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Field-Amplified Sample Injection and In-Capillary Derivatization for Capillary Electrophoretic Analysis of Metal Ions in Local Wines

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Abstract

In-capillary derivatization and field-amplified sample injection (FASI) coupled to capillary zone electrophoresis (CZE) was evaluated for the analysis of metals (Co(II), Cu(II), Ni(II), and Fe(II)) using 2-(5-Nitro-2-Pyridylazo)-5-(N-Propyl-N-Sulfopropylamino)Phenol (Nitro-PAPS) as the derivatizing agent. For FASI, the optimum conditions were water as sample solvent, 1 s hydrodynamic injection (0.1 psi) of a water plug, 5 s of electrokinetic introduction (10 kV) of the sample. The in-capillary derivatization was successfully achieved with zone-passing strategy in order tandem injection of Nitro-PAPS reagent (0.5 psi, 7 s), a small water plug (0.1 psi, 1 s), and metal ion introduction (10 kV, 5 s). The solution of 45 mmol L^{-1} borate pH 9.7 and $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS containing 20% acetonitrile was used as the running buffer. Limit of detection obtained by the proposed method was lower than those from pre-capillary derivatization about 3 – 28 times. The recovery of the method was comparable to pre-capillary derivatization method. In-capillary derivatization-FASI-CZE was applied to analysis of metals in wine samples. The results were compared with those obtained by CZE with pre-capillary derivatization method and atomic absorption spectrometry (AAS).

Key words: Capillary zone electrophoresis; Field-amplified sample injection; In-capillary derivatization; Metal ions; Nitro-PAPS; Wines

1 Introduction

The analysis of heavy metals in a range of substances is of considerable interest due to potential human toxicities. There is increasing demand for simple and reliable methods for the simultaneous detection of several metal ions with low detection limits and low sample/reagent consumption. Atomic absorption spectrometry (AAS) is the most widely used method for metal analysis but simultaneous multi-element analysis is not possible. Inductively coupled plasma atomic emission spectrometry (ICP-AES) is capable of multi-element analysis, but the instrumentation is relatively expensive. The simultaneous determination of metal ions with conventional high performance liquid chromatography (HPLC) and ion chromatography (IC) have several disadvantages, such as long analysis times and consumption of large volumes of reagents. Recently, capillary electrophoresis (CE) has been shown to be a powerful separation technique for the analysis of metals in a wide variety of sample matrices [1–8] because of the significant advantages of resolution, speed, simplicity and lower sample/reagent consumption. The electrophoretic determination of metals can be easily achieved by indirect detection through the addition of appropriate species to the background electrolyte to produce UV-absorbing species [9, 10], but the number of appropriate compounds for this environment is limited. The pyridylazo reagent, 2-(5-Nitro-2-Pyridylazo)-5-(N-Propyl-N-Sulfopropylamino) Phenol (Nitro-PAPS) is a synthesized chromogenic reagent which forms water-soluble chelates with giving high molar absorptivities of ca. $10^4 – 10^5$ L mol^{-1} cm^{-1} [11–13]. Nitro-PAPS has been used as a chromogenic reagent in various analytical techniques for the determination of various metal ions. Flow-injection spectrophotometry for the determination of Fe(II) in purified salts [13] and the trace analysis of V(V) in river water [14] using Nitro-PAPS have been demonstrated. The HPLC-based determination of Cu(II), Co(II), Ni(II) and Fe(II) in rain and river waters using Nitro-PAPS has been reported [11]. The use of Nitro-PAPS chelates incorporating pre-capillary derivatization in CE for the determination of trace metal impurities in nickel and iron salts has been described [15].

In CE, derivatization has been accomplished by either pre-capillary [16, 17], post-capillary [18] and in-capillary [1, 2, 19–21] methods. Pre-capillary derivatization requires large amounts of derivatization ligand, whereas post-capillary derivatization requires significant post-capillary hardware modification, and loss of efficiency during separation and peak broadening has been noted [18, 22, 23]. The increased

popularity of in-capillary derivatization takes advantage of the fact that there is minimal sample dilution compared to pre-capillary and post-capillary derivatization. In-capillary derivatization can be performed by three modes: zone-passing, at-inlet and throughout-capillary [24–26]. Among these strategies, zone-passing is appropriate for the Nitro-PAPS derivatization since the formation of the metal complex occurs within seconds.

In order to increase the sensitivity of detection in CE, the on-line preconcentration methods have been developed with different strategies, such as large volume sample stacking [27], sweeping [28], dynamic pH junction-sweeping [29], and field-amplified sample injection (FASI) [8, 30–35]. FASI is very popular since it is quite simple, requiring only the electrokinetic injection of the sample after the introduction of a small plug of high-resistivity solvent (mainly water). In FASI, samples are normally prepared in a low conductivity buffer and injected electrokinetically into a capillary containing a high-conductivity running buffer.

In this work, the feasibility of combining FASI with in-capillary derivatization of metal ions (Co(II), Cu(II), Ni(II), and Fe(II)) with Nitro-PAPS before their analysis by capillary zone electrophoresis (CZE) is demonstrated. The combination of electrokinetic injection and water or organic solvent field amplified stacking is investigated. Studies of effects of diluents (aqueous versus organic) on the stacking by the electroinjection under FASI is performed. Finally, in-capillary derivatization-FASI-CZE is applied to the analysis of these metal ions in local wine samples.

2 Materials and methods

2.1 Chemicals and reagents

All the reagents used in this work were of analytical reagent grade. Nitro-PAPS was purchased from Dojindo (Japan). A $1x10^{-3}$ mol L^{-1} stock solution of Nitro-PAPS was prepared in deionized water. The 1000 mg L^{-1} stock atomic absorption standard solutions of metal ions including Co(II), Cu(II), Ni(II) and Fe(II), were purchased from Ajax Finechem (Australia). The working solutions of each metal were daily prepared in deionized water except for Fe(II) was prepared in acidic medium of HCl (1% v/v). Sodium tetraborate was purchased from Univar-Ajak (Australia). Water for preparation of samples and buffer solution was the deionized water obtained from a RiO_s^{TM} Type I Simplicity 185 (Millipore-Waters, U.S.A.) with the resistivity of 18.2 MΩ.cm.

2.2 Instrumentation

Experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument (Fullerton, CA, U.S.A.) equipped with a photodiode array detection system. The 32 Karat software version 5.0 (Beckman) was used for instrument control, data acquisition and data analyses. Electrophoretic separations were carried out using uncoated fused-silica capillaries (Beckman) with a total length of 60.2 cm (50.0 cm effective length) and 75 μm I.D. On-capillary detections were performed at either selected wavelengths (200, 214, 250 and 570 nm) or in the scanning mode (190 – 600 nm). All CZE experiments were thermostated at 25 °C.

A Perkin-Elmer Instruments AAnalyst 100 atomic absorption spectrometer (U.S.A.) equipped with Perkin-Elmer single element hollow cathode lamps and an air-acetylene flame with air and acetylene flow rates of 10 and 3 mL min^{-1} was used for the determination of metals. The wavelengths (nm) selected for the determination of the analytes were as follows: Co 240.7, Cu 324.8, Ni 232.0, and Fe 248.3.

2.3 Pre-capillary derivatization (as a reference method)

The working solutions of metal ions were mixed with Nitro-PAPS solution ($1x10^{-3}$ mol L^{-1}) in the mole ratio of ligand:metal of 2:1 at pH 6.0 at room temperature. The sample solution was introduced into the electrophoretic system by hydrodynamic injection at 0.5 psi (1 psi = 6895 Pa) for 5 s before separation. The absorbance was detected at 250 nm.

2.4 In-capillary derivatization and FASI

In-capillary derivatization of metals and Nitro-PAPS was performed by zone-passing option in the tandem mode. Briefly, the capillary was first filled with the carrier electrolyte and then a plug of Nitro-PAPS solution was introduced at 0.5 psi for 7 s. After that, FASI was performed by introduction of a small water plug (0.5 psi, 1 s) and then the sample was introduced into the capillary with electrokinetic injection at 10 kV for 5 s. Subsequently, the separation voltage of 20 kV was applied through the capillary and the on-line reaction occurred inside the capillary when the metals mixed with the Nitro-PAPS. The complexing reaction occurs during the separation to

obtain the corresponding metal-Nitro-PAPS chelates and subjected to spectrometric detection.

2.5 Electrophoretic separation

Prior to use, all new capillaries were initially pretreated with the following cycles: methanol, 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaOH for 10 min each and deionized water for 5 min between each rinses and finally equilibrated with the buffer solution for 10 min before applying voltage of 10 kV across the capillary for 5 min. To maintain reproducible migration times, the capillary was pre-conditioned with the running buffer for 3 min before each run. After completing the separation session, the capillary was flushed with 0.1 mol L^{-1} NaOH for 5 min and then washed with deionized water for 3 min. The running buffer used throughout the separation was 45 mmol L^{-1} borate buffer pH 9.7 and $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS containing 20 % acetonitrile. All running buffers were filtered through a 0.45 μm membrane filter (Nylon, Chrom Tech) and degassed by sonication before use.

2.6 Wine Samples

The wine samples studied included a variety of homemade red and white wines from a variety different fruits. The samples were purchased from the local supermarkets in Khon Kaen Province in Northeastern Thailand. The wines were filtered through a 0.45 μm membrane filter and were appropriately diluted about 10 times with water before derivatization and analysis. All analyses were performed in triplicate.

The AAS determinations of metals in wines were performed on standard calibration curves. The calibration curves were constructed in range 0 – 5 mg L^{-1} with obtained correlation coefficients higher than 0.999 for all studied metals. To obtain the results in line with the calibration curves, wine samples were appropriately diluted with water before directly analyses.

3 Results and discussion

3.1 Spectrometric properties

Although the metal ion-Nitro-PAPS complexes are normally detected at visible wavelengths, higher sensitivity and good signal-to-noise ratios were achieved

with detection at 250 nm. Using Job's method, the ligand-to-metal ratio of 2:1 was found for the Co(II), Ni(II) and Fe(II) complexes, and 1:1 for the Cu(II) complex.

3.2 In-capillary derivatization and FASI

In-capillary derivatization can be achieved by successive introductions of a sample solution and the reagent solution followed by application of the specified voltages. Reaction occurs while both zones are overlapping each other in the capillary. In the present study, in-capillary derivatization of metal ions and Nitro-PAPS reagent was performed based on the zone-passing strategy. The Nitro-PAPS reagent was first introduced into the capillary by the hydrodynamic injection mode, followed by hydrodynamic injection of a small water plug and then the metal ions were injected using the electrokinetic mode. All solutions were successively introduced at the anodic end of the capillary. After the specified voltage was applied, the metal ions as positively charged species zones migrated faster than the Nitro-PAPS reagent zone toward the cathode, whereas the negatively charged Nitro-PAPS species migrated to the anode. Thus, the metal ions moved into the reagent zone and reacted to form the metal-Nitro-PAPS chelates. The mixing scheme is illustrated in **Figure 1**. The parameters affecting FASI such as injection voltage and sample matrix were investigated and optimized. Preliminary studies involving metal ions dissolved in different solvents such as 10% acetonitrile, water, and 0.4 mol L^{-1}sodium chloride showed that metal ions prepared in water gave the highest peaks for all the chelates. Although metal ions in acetonitrile provided good peak shape, the migration times of the analytes were shifted to longer times. Thus, aqueous solutions of metal ions were used for further studies.

The effect of injection voltage was studied by introducing metal ions dissolved in water at different applied voltages (7 – 15 kV) with a constant time interval (5 s). The peak areas increased up to 10 kV and remained nearly constant thereafter, possibly due to the competing migration directions and rates that affect local concentrations. Thus, an injection voltage of 10 kV for 5 s was chosen. **Figure 2** summarizes the results corresponding to each derivatization strategy; R, S, and W refer to the reagent, sample, and water, respectively. Among the various zone-passing introduction modes, the R-S-R configuration gave the lowest responses, except for Cu(II). The R-S tandem mode gave higher sensitivities for all metal ions, compared to RSR. If the R-S tandem mode was carried out by introduction of a small water plug

(at 0.1 psi for 1 s) before electrokinetic metal introduction, RW(1)S, increasing responses were observed. While a time of 2 s, designated RW(2)S, produced an increase for Co(II), the responses for other metals decreased significantly, possibly due to dilution in the reaction zone. The optimum condition is, therefore, represented by RW(1)S.

3.3 The optimization of CZE conditions

In order to achieve complete separation of metal-Nitro-PAPS chelates, the crucial parameters affecting the separation were: buffer pH, concentration of buffers, organic modifier, and separation voltage. Preliminary experiments with three common buffers, acetate (near pH 5.0), phosphate (near pH 7.0), and borate (near pH 9.0) buffers showed that acetate and phosphate did not lead to complete separation due to overlap of peaks from some metals and Nitro-PAPS, whereas borate buffer provided successful separation. Borate buffer was then investigated at the pH ranges of 9.0 – 10.5. Co(II), Cu(II) and excess Nitro-PAPS were well separated, but by increasing of pH, increased the resolution of Ni(II)- and Fe(II)-Nitro-PAPS could be achieved. At pH values higher than pH 10.5, excess Nitro-PAPS and Cu(II)-Nitro-PAPS peaks co-eluted. The best separation was obtained at pH 9.7.

The effect of borate concentration was then evaluated ranging from 35 to 55 mmol L^{-1}. Increasing the borate concentration resulted in decreased mobility and better resolution. However, high current in the capillary system was observed at high concentrations, so 45 mmol L^{-1} was chosen as the optimum value.

The type and concentration of organic modifier are other parameters that can improve resolution and selectivity in separations [17, 20]. For the present study, acetonitrile was selected and varied over the range of 10 – 25 % (v/v). The chelates of metals were completely separated with the running buffer containing 20% acetonitrile, so this was selected for subsequent runs.

In this study, voltages over the range of 15 – 22 kV showed little effect on separation. However, a poor baseline was observed when the separation was performed at the highest applied voltages, so the optimum of 20 kV was used throughout.

In all the studies, Nitro-PAPS (1.0×10^{-5} mol L^{-1}) was added into the running buffer in order to prevent the dissociation of metal-Nitro-PAPS chelates during the

separation and provided good baselines [36]. No effects of Nitro-PAPS concentration changes were observed other than some baseline drift at higher values.

In summary, the optimum running buffer was 45 mmol L^{-1} borate pH 9.7 and 20% acetonitrile containing $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS. The separation voltage was 20 kV and on-capillary detection was carried out at 250 nm. Under the optimum CZE conditions, the four metal ions was completely separated within 20 min (**Figure 3**) with the migration order of Co(II)-, unreacted Nitro-PAPS, Cu(II)-, Ni(II)- and Fe(II)-Nitro-PAPS, respectively. The peak of excess Nitro-PAPS (migration time 15 min) was close to the Cu(II) peak (16.0 min).

3.4 Analytical validations

To validate the quantitative aspects of the proposed method using FASI combined with in-capillary derivatization method, quality and quantity parameters using the optimized CZE method were evaluated and the results are shown in **Table 1**. Calibration curves based on the peak area at concentrations between 0.05 mg L^{-1} and 5.00 mg L^{-1} exhibited good linearity was obtained with R^2 values higher than 0.995. The reproducibility of the method was reported in terms of relative standard deviation (RSD) using six repeat determinations for intra-day precision and four replicate injections for inter-day comparisons. The RSD obtained from both of intra-day and inter-day precisions were lower than 0.5 % and 1.2 %, respectively, for migration time whereas 3.0 % and 4.5 % for peak area. Limit of detection (LOD) was estimated based on signal-to-noise ratio (S/N) of 3:1 for both pre-capillary and in-capillary methods. The results are shown in **Table 2**. Co(II)-Nitro-PAPS has larger molar absorptivity (ε) than the other studied metal ions about 1 order of magnitude (i.e. 10^5 L mol^{-1} cm^{-1} for Co(II)-Nitro-PAPS whereas the other metal ions have ε about 10^4 L mol^{-1} cm^{-1}). Thus, larger signal of Co(II)-Nitro-PAPS as well as its low LOD is obtained. Similar result was observed [11]. The in-capillary method with the electrokinetic injection (10kV, 5 s) gives lower concentration detection than pre-capillary approach by a factor of 3 – 28. Whereas the in-capillary method with the electrokinetic injection produced lower LODs in the range of 5 – 40 times when compared to pressure injection (0.5 psi, 5 s). The results obtained by the proposed method can be used for the analysis of metals in sub-ppm level as lower or similar to those obtained by others [9, 10, 19]. To ensure that the present method can be used

for the analysis of metals in real samples, the recovery was studied by spiking the metal ion standard solutions at 2.0 mg L^{-1} each into all samples and analyzed in triplicate. As shown in **Table 2**, the recovery obtained from in-capillary method was comparable to the pre-capillary method. The recovery ranges showed dependence on the origin of the sample and the sample matrix (see below).

3.5 Application

The analysis of 12 wine samples was performed following the procedure described in Section 2.6. **Figure 4** shows the typical electropherograms obtained by the proposed method of Emblic fruit wine and white grape wine samples, respectively. Identification of peaks was carried out by comparing the migration time (t_M) of known metal ions in a standard containing only a single metal ion with those in a metal mixture and by spiking with known metals and looking for increases in peak height. The contents of metals present in all studied wines are listed in **Table 3**. Only Cu(II) and Fe(II) were found in the studied wine samples whereas Co(II) and Ni(II) were not detected. Cu(II) was found in Emblic fruit and Black Galingale wines with a high level about 20 mg L^{-1} for the first sample. The levels of copper in various beverages (e.g. wines, tea, fruit juices, etc.) from some other countries have been reviewed [37]. In wines, the copper concentration was found to be less than 2 mg L^{-1}. Although copper was classified as an essential metal, excessive levels in wines are potentially toxic. The maximum acceptable level of copper in wine as established by the Office International de la Vigne et du Vin (OIV) is 1 mg L^{-1} [38]. Iron is usually present in musts and wines at a concentration range varying from 0.5 up to 20 mg L^{-1} [39]. The highest permissible concentration of iron in table wines set by the official national legislation has been determined to be 10 mg L^{-1}. Iron contents higher than 8 – 10 mg L^{-1} may result in turbidity and color changes [40]. In the present study, Fe(II) was found at different amounts in all wine samples. The highest value (~ 51 mg L^{-1}) of Fe(II) was found in Emblic fruit wine which also has the highest content of Cu(II). The Longan wine contained Fe(II) at a level of 34 mg L^{-1} and Fe(II) in Roselle wine at 19 mg L^{-1}. Fe(II) was also detected in other wine samples at levels of 16 mg L^{-1} or lower. The presence of copper and iron at these levels suggest that all wines may be risk of cloudiness and lack of stability. To establish the reliability of the proposed method for analysis of metals in wine samples, the results obtained using the CZE method with in-capillary derivatization and FASI method have been

compared to those using pre-capillary derivatization method and AAS (**Table 3**). The statistical significant as determined by the *t*-test at 95% confidence level showed no significant difference in the results obtained by the three methods. Comparisons presented here suggest that rapid and reliable results can be obtained in a highly automated process to establish the quality of wines. Iron contamination in homemade preparations is of particular interest.

4 Conclusion

The method and results described here demonstrate that FASI in combination with in-capillary derivatization is a useful technique for CZE determination of cobalt(II), copper(II), nickel(II), and iron(II) using Nitro-PAPS as the complexing agent. FASI of metal ions and in-capillary mixing with Nitro-PAPS were successfully obtained with zone-passing strategies using tandem injection order of Nitro-PAPS, a small water plug, and metal ions. Under the optimum condition, the separation of four metals can be achieved within 20 min. Limit of detections obtained from in-capillary derivatization with electrokinetic injection were 3 – 28 folds higher than those obtained from the pre-capillary method. The successful application of this method for the determination of metals in wines has been demonstrated. Only copper and iron were found at high levels in wine samples that and these may influence on color and stability of the homemade wines. In-capillary derivatization and FASI were superior in terms of simplicity and ease of automation when compared to pre-capillary derivatization. Moreover, small quantities (~ nL level) of reagent and sample were required. Thus, the method can be considered to be an alternative to other more complicated methods for analysis of metals in wines.

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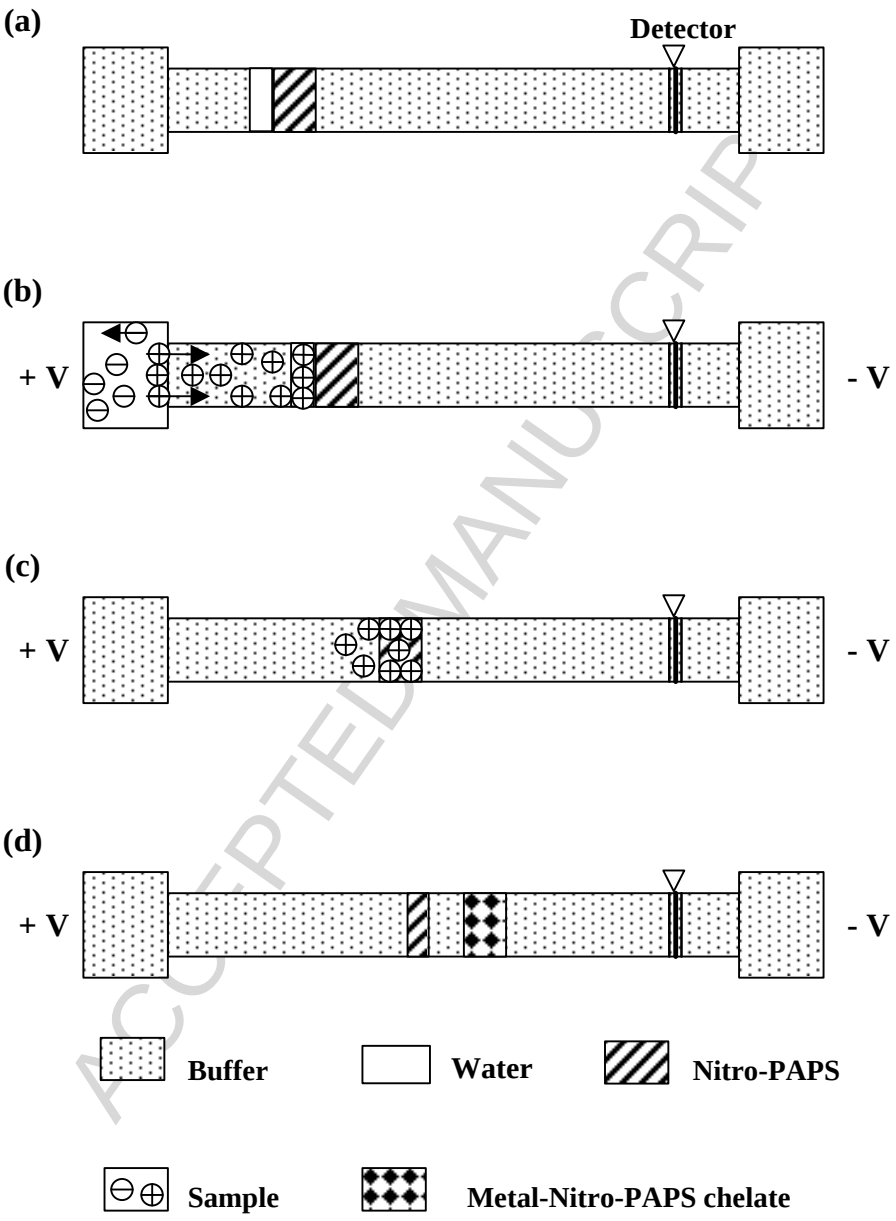
Figure Captions

Figure 1 The schematic reaction of metal ions and Nitro-PAPS based on in-capillary derivatization. (a) Hydrodynamic introduction of buffer, Nitro-PAPS and water plug, (b) electrokinetic injection of the sample, (c) mixing and reaction of metals with Nitro-PAPS, and (d) separation of the metal-Nitro-PAPS chelates.

Figure 2 Comparison of introduction order for in-capillary derivatization based on zone-passing. Conditions: metal ion standards = 3.0 mg L^{-1} each, reagent solution=$1.0x10^{-3}$ mol L^{-1}, reagent injection time=7 s at 0.5 psi; sample introduction=10kV for 5 s; water injection time=1 s at 0.1 psi; running buffer=45 mmol L^{-1} borate pH 9.7 and $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS containing 20% acetonitrile, potential=20 kV. Segment assignment: S, Sample; R, reagent; W, water.

Figure 3 Electropherogram of metal ion standards (3.0 mg L^{-1} each) obtained from in-capillary derivatization and FASI under the optimum condition as followed: 45 mmol L^{-1} borate pH 9.7 and $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS containing 20% acetonitrile, applied voltage of 20 kV, temperature of 25 °C and on-capillary detection at 250 nm.

Figure 4 Electropherograms of wine samples under the optimum conditions: (a) Emblic fruit wine and (b) white grape wine. Remarkable with asterisks (*) are unknown peaks.



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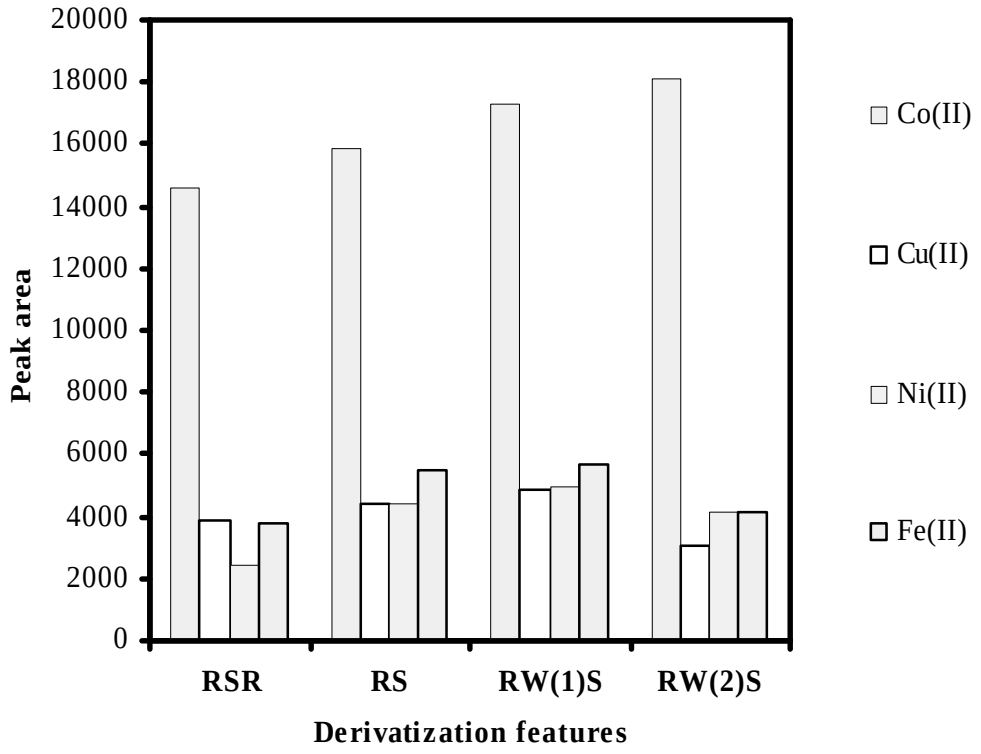
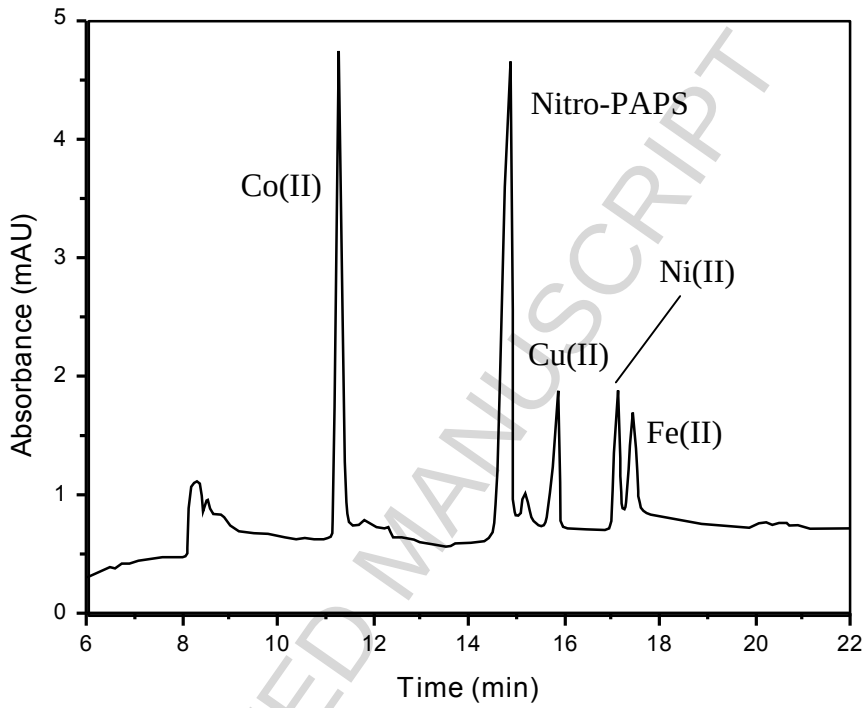


Figure 2 Comparison of introduction order for in-capillary derivatization based on zone-passing. Conditions: metal ion standards = 3.0 mg L^{-1} each, reagent solution=$1.0x10^{-3}$ mol L^{-1}, reagent injection time=7 s at 0.5 psi; sample introduction=10kV for 5 s; water injection time=1 s at 0.1 psi; running buffer=45 mmol L^{-1} borate pH 9.7 and $1.0x10^{-5}$ mol L^{-1} Nitro-PAPS containing 20% acetonitrile, potential=20 kV. Segment assignment: S, Sample; R, reagent; W, water.



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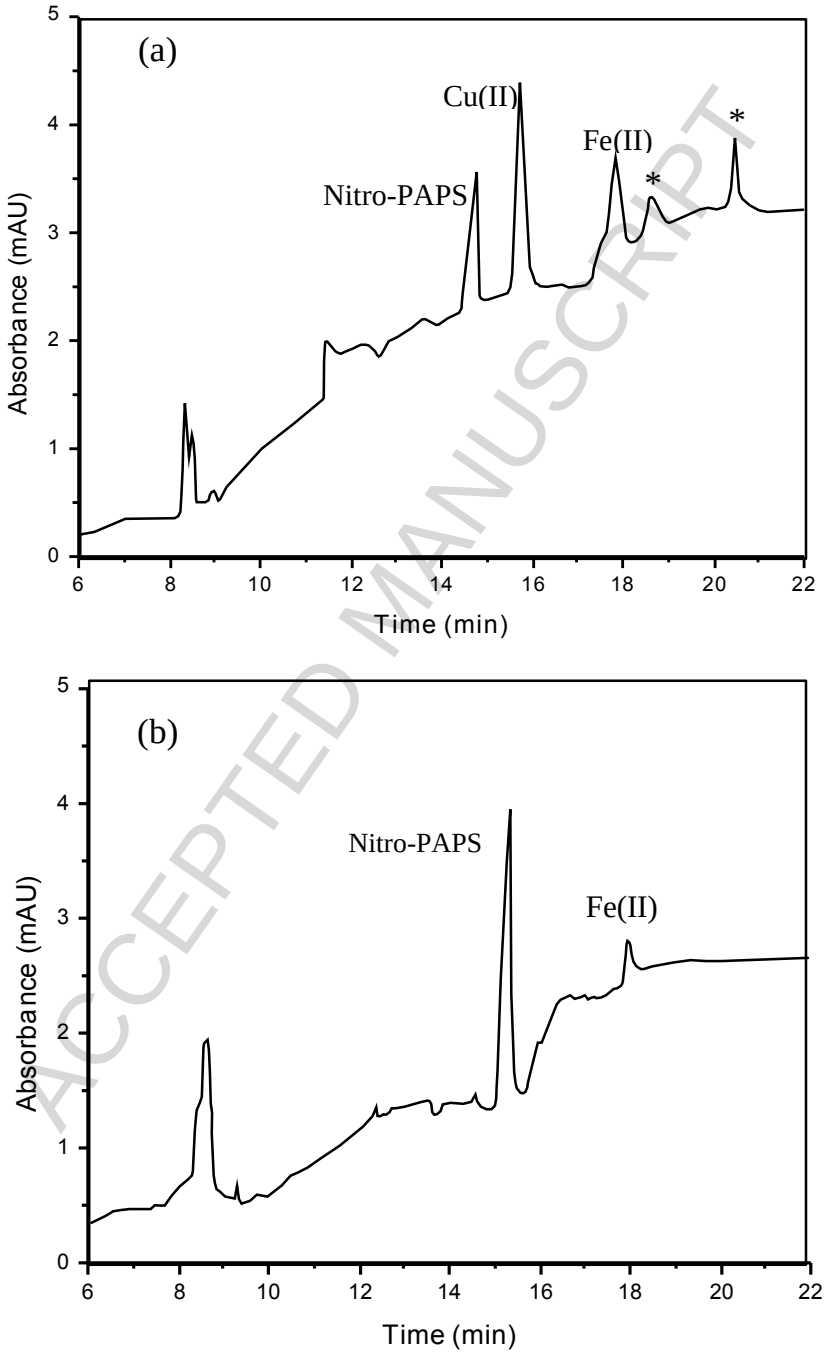

Figure 4 Electropherograms of wine samples under the optimum conditions: (a) Emblic fruit wine and (b) white grape wine. Remarkable with asterisks (*) are unknown peaks.

Table 1 The analytical characteristics of the proposed method

complex	linear equation, y = ax + b		linearity (mg L^{-1})	R^2	intra-day precision, RSD(%), n = 6		inter-day precision, RSD(%), n = 4x3	
	a	b			t_M	peak area	t_M	peak area
Co(II)-Nitro-PAPS	27032.0	-87.0	0.05 – 3.00	0.9982	0.28	2.00	0.35	4.00
Cu(II)-Nitro-PAPS	1476.7	472.5	0.10 – 3.00	0.9989	0.38	2.43	0.51	4.20
Ni(II)-Nitro-PAPS	1769.1	537.4	0.05 – 5.00	0.9953	0.42	2.75	0.67	3.85
Fe(II)-Nitro-PAPS	4384.0	-524.6	0.20 – 5.00	0.9996	0.25	2.55	0.32	4.36

Table 2 The limit of detection (LOD) and recovery for in-capillary and pre-capillary methods

complex	LOD (mg L^{-1})			LOD factor		Recovery[d] (%), n = 3	
	pre-capillary[a]	in-capillary					
		electrokinetic injection[b]	pressure injection[c]	pre-capillary/ electrokinetic	pressure / electrokinetic	pre-capillary	in-capillary
Co(II)-Nitro-PAPS	0.20	0.025	0.30	8.0	12.0	80 – 102	80 – 95
Cu(II)-Nitro-PAPS	0.20	0.080	0.50	2.5	6.2	83 – 104	85 – 102
Ni(II)-Nitro-PAPS	0.70	0.025	1.00	28.0	40.0	80 – 103	82 – 97
Fe(II)-Nitro-PAPS	0.70	0.100	0.50	7.0	5.0	82 – 94	87 – 103

[a] injection of complexes at 0.5 psi for 5 s

[b] metal ion injection at 10 kV for 5

[c] metal ion injection at 0.5 psi for 5 s

[d] reported as mean values for the twelve studied wine samples

Table 3 The content of metals in wine samples (mean, n=3)

wine	copper(II), mg L^{-1}			iron(II), mg L^{-1}		
	in-capillary	pre-capillary	AAS	in-capillary	pre-capillary	AAS, total iron
Emblic fruit	19.53	19.96	20.36	50.89	51.37	51.80
Mangosteen	n.d.	n.d.	n.d.	7.78	7.36	7.54
Jambolan	n.d.	n.d.	n.d.	11.17	11.95	13.84
Star gooseberry	n.d.	n.d.	n.d.	12.29	12.59	11.26
Organic fruit	n.d.	n.d.	n.d.	10.03	10.28	11.30
Black galingale	5.37	5.52	5.46	15.25	15.73	15.78
Roselle	n.d.	n.d.	n.d.	19.09	20.69	19.60
Longan	n.d.	n.d.	n.d.	34.49	35.38	39.40
Bel fruit	n.d.	n.d.	n.d.	14.18	15.67	13.30
Strawberry	n.d.	n.d.	n.d.	15.86	16.71	14.52
White grape	n.d.	n.d.	n.d.	15.26	15.78	14.34
Red grape	n.d.	n.d.	n.d.	13.03	13.89	12.90

n.d.: not detected

Program Number: 677 **Day / Time:** Sunday, Dec. 18, 8:00 PM - 10:00 PM

Simultaneous determination of Co (II) , Cu (II) , Ni (II) , Fe (II) and V (V) as their nitro - PAPS chelates by flow injection coupled to high performance liquid chromatography

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A presented method involves the on-line complexation of metal ions with 2-(5-nitro-2-pyridylazo)-5-(N- *n*-propyl-N-sulfopropyl)-aminophenol (Nitro-PAPS) and subsequent simultaneous separation by ion-pair reversed phase high performance liquid chromatography. The lab made flow injection system was used for an on-line complexation and was coupled with HPLC *via* switching valve. The separation was performed on a μ-Bondapak C18 column and visible detection at 570 nm. Mobile phase compositions such as percentage of organic modifier, concentration of buffer and concentration of ion pairing agent were optimized using the sequential simplex method. The optimum mobile phase was obtained after 19 experiments. Using the optimum condition, baseline separation of five metal-(nitro-PAPS) chelates was achieved within 17 min. Detection limits ranged from 1.0 to 55.0 ng mL^{-1} for Co(II) and V(V), respectively. Application of the proposed method to the analysis of water and wine samples is demonstrated.

Citation: S.Srijaranai, W.Pimrote, C.Kukusamude, T.Sakai, R.Burakham. Simultaneous determination of Co (II) , Cu (II) , Ni (II) , Fe (II) and V (V) as their nitro - PAPS chelates by flow injection coupled to high performance liquid chromatography. Program No. 677. *2005 Abstract Viewer.* Honolulu, Hawaii: International Chemical Congress of Pacific Basin Societies

Program Number: 676 **Day / Time:** Sunday, Dec. 18, 8:00 PM - 10:00 PM

Capillary zone electrophoresis of carboxylic acids

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The simultaneous separation and determination of carboxylic acids was performed by capillary zone electrophoresis (CZE) with UV detection of their 2-nitrophenylhydrazine derivatives. Optimum conditions for the derivatization and capillary electrophoresis separation of 12 carboxylic acids were investigated. Twelve studied carboxylic acids, including acetic, succinic, glycolic, suberic, glutaric, citric, tartaric, lactic, sorbic, suberic, malonic and *trans*-aconitic acids, were separated in 10 minutes. Advantages of such a system will be discussed. The proposed method has been successfully applied to the determination of carboxylic acids in real samples.

Citation: A.Santalad, S.Srijaranai, P.Teerapornchaisit, R.Burakham. Capillary zone electrophoresis of carboxylic acids. Program No. 676. *2005 Abstract Viewer.* Honolulu, Hawaii: International Chemical Congress of Pacific Basin Societies

Some Development of Chromatographic Separation for Foods and Pharmaceutical Applications

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The research group has been active in explorting some novel packing materials for chiral chromatographic separation. The applications for such development are aimed for enantiomeric drugs. Some other investigations involve phenolic acids and antioxidants in foods, sugar, amino acids and carboxylic acids inraw sugar and cane juice for sugar cane industry.

O57

EXPLOITING FLOW ANALYSIS WITH LAB-AT-VALVE (LAV) APPROACH

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A "Lab-at-Valve (LAV)" system has been proposed as an alternative micro-total analysis system (μTAS). A LAV system is designed to be an integrated unit to accommodate an analytical process including sampling, chemical reaction for detection and detector. The LAV device is attached to a position of a selection valve of a SI system. Manipulation of SI-LAV is made via a software control. LAV is similar to Lab-on-Valve (LOV) which is a device attached on top of a selection valve after the top piece of the purchased selection valve is taken out.
Discussion on similarity and difference of LAV and LOV will be made. LAV instrumentation will be briefed. Some LAV applications will be demonstrated. They include potentiometric determination of chloride, on-line micro extraction spectrophotometric determination of surfactant and pharmaceutical preparations. LAV-bead injection for some biochemical compounds such as hyaluanan and chrondroitin sulfate.

Analysis of Carboxylic Acids using Capillary Zone Electrophoresis with Pre-Capillary Derivatization

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The capillary zone electrophoresis (CZE) with photo diode array detection was developed for analysis of carboxylic acids. The carboxylic acids were derivatized with 2-Nitrophenylhydrazine (2-NPH) in the presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl). The conditions for separation of carboxylic derivatives were investigated including pH, type and concentration of buffers, organic modifier and applied voltage. The optimum conditions were 30 mM borate buffer (pH 10.0) containing of 10 % acetonitrile, voltage of 20 kV, temperature of 25 °C, hydrodynamic injection at 0.5 psi for 5 sec and detection at 230 nm. The electrophoretic separation of 11 carboxylic acids, performed on fused-silica capillary 75 μm I.D. x 40 cm, was achieved within 10 min. The present method was successfully applied for analysis of carboxylic acids in beverage.

Keywords: Capillary Zone Electrophoresis, 2-Nitrophenylhydrazine, Carboxylic Acids, Beverage

การวิเคราะห์กรดคาร์บอกซิลิกด้วยเทคนิคแคพพิลลารีโซนอิเล็กโทรฟอริซีส

ANALYSIS OF CARBOXYLIC ACIDS USING CAPILLARY ZONE ELECTROPHORESIS

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บทคัดย่อ : งานวิจัยนี้นำเสนอเทคนิคแคพพิลลารีโซนอิเล็กโทรฟอริซีส (CZE) ร่วมกับการตรวจวัดด้วยโฟโตไดโอด แอเรย์ (PDA) สำหรับการวิเคราะห์กรดคาร์บอกซิลิก หลังจากการเตรียมอนุพันธ์กับ 2-Nitrophenylhydrazine (2-NPH) และ N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC.HCl) ศึกษาปัจจัยที่มีผลต่อการแยกด้วยเทคนิค CZE ได้แก่ พีเอช (pH) ชนิดและความเข้มข้นของสารละลายบัฟเฟอร์ สารอินทรีย์ และความต่างศักย์ที่ใช้ในการแยก พบว่า สภาวะที่เหมาะสมสำหรับการแยกกรดคาร์บอกซิลิกทั้ง 11 ชนิด คือ สารละลายบัฟเฟอร์บอเรต เข้มข้น 30 มิลลิโมลต่อลิตร (พีเอช 10.0) ที่มีอะซิโตไนไตรล์ 10 เปอร์เซ็นต์ ศักย์ไฟฟ้าในการแยก 20 กิโลโวลต์ และอุณหภูมิ 25 องศาเซลเซียส ฉีดสารตัวอย่างด้วยความดัน 0.5 psi นาน 5 วินาที และตรวจวัดที่ความยาวคลื่น 230 นาโนเมตร ท่อแคพพิลลารีที่ใช้มีขนาดเส้นผ่านศูนย์กลางภายใน 75 ไมโครเมตร ความยาว 40 เซนติเมตร สามารถแยกกรดคาร์บอกซิลิกทั้ง 11 ชนิด ภายในเวลา 10 นาที นำวิธีนี้ไปใช้วิเคราะห์กรดคาร์บอกซิลิกในตัวอย่างเครื่องดื่ม

Abstract: The capillary zone electrophoresis (CZE) with photo diode array detection (PDA) is developed for the analysis carboxylic acids. The method is based on derivatization with 2-Nitrophenylhydrazine (2-NPH) in the presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC.HCl). The parameters affecting CZE separation were studied, including, pH, types and concentration of buffer, organic additive and applied voltage. The optimum conditions were 30 mM borate buffer (pH 10.0) containing 10 % acetonitrile, voltage of 20 kV, temperature of 25 ºC, hydrodynamic injection at 0.5 psi for 5 sec and detection at 230 nm. The separation of 11 carboxylic acids, using a 75 µm i.d. x 40 cm fused-silica capillary tube, was achieved within 10 min. The present method was successfully applied for analysis of carboxylic acids in beverages.

Introduction: Carboxylic acids are widely distributed in foods and beverages with different contents that affect on their flavor, stability, nutrition, acceptability and keeping quality of products. Ion chromatography (IC), gas chromatography (GC) and high performance liquid chromatography (HPLC) have been recognized as powerful techniques for analyses of carboxylic acids. Recently, capillary electrophoresis (CE) becomes an alternative for the separation of various substances. This work presents the

development of carboxylic acid determination using CZE and photo diode array detection after derivatization with 2-Nitrophenylhydrazine.

Methodology: The studied carboxylic acids including, acetic acid, lactic acid, suberic acid, glutaric acid, succinic acid, malic acid, malonic acid, tartaric acid, oxalic acid, citric acid and trans-aconitic acid. The derivatization was performed by reacting of carboxylic acids with 2-NPH in the presence of EDC.HCl and was heated at 70 ºC for 10 min. The acid hydrazides were cooled and filtered before the introduction into CE system. The experimental parameters affecting the separation of carboxylic acids were investigated. Those parameters were types and concentration of buffer, pH, organic additive, separation voltage and the detection wavelength. A fused-silica of 75 µm i.d. x 40 cm was used.

Results, Discussion and Conclusion: The optimum conditions were found to be 30 mM borate buffer (pH 10.0) containing of 10 % acetonitrile, hydrodynamic injection at 0.5 psi for 5 sec, applied voltage of 20 kV and detection wavelength at 230 nm. The separation of 11 carboxylic acids was completed within 10 min. The calibration graphs were linear in the range of 10.00-100.00 mg l^{-1} with the correlation coefficient greater than 0.99. The limits of detection (LOD) were in the range of 2.00-10.00 mg l^{-1}. The applicability of the method was investigated by analyzing carboxylic acids in beverage samples. The recoveries of 82-116% were obtained.

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Keywords: Carboxylic acids, 2-Nitrophenylhydrazine, Capillary zone electrophoresis, Beverage

Analysis of Amino Acids in Beverages by Capillary Zone Electrophoresis and Pre-Capillary Derivatisation

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A simple and rapid method for the simultaneous analysis of amino acids has been demonstrated. Amino acids were derivatised based on pre-capillary derivatisation with 1,2-naphthoquinone-4-sulfonate (NQS) in basic medium and mild conditions. Their derivatives were analysed by capillary zone electrophoresis (CZE). The crucial parameters affecting CZE separation were investigated including buffer (pH, type and concentration), organic modifier and separation voltage. The separation of seven amino acids was achieved within 17 min. The present method was successfully applied to the analysis of amino acids in beverage samples.

การเตรียมอนุพันธ์ในแคพพิลลารีและเทคนิคแคพพิลลารีโซนอิเล็กโทรฟอริซิสสำหรับการวิเคราะห์โลหะโดยใช้ Nitro-PAPS เป็นรีเอเจนต์

IN-CAPILLARY DERIVATISATION AND CAPILLARY ZONE ELECTROPHORESIS FOR ANALYSIS OF METALS USING NITRO-PAPS AS DERIVATISING REAGENT

อภิชัย แสนตลาด, ศุภลักษณ์ ศรีจารนัย และ รจนา บุระคำ

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บทคัดย่อ: ศึกษาการเตรียมอนุพันธ์ในแคพพิลลารีร่วมกับเทคนิคแคพพิลลารีโซนอิเล็กโทรฟอริซิสสำหรับการวิเคราะห์โลหะ 4 ชนิด คือ โคบอลต์(II) คอปเปอร์(II) นิกเกิล(II) และเหล็ก(II) โดยใช้ Nitro-PAPS เป็นรีเอเจนต์ ซึ่งปฏิกิริยาจะเกิดขึ้นจากการผสมกันระหว่างโลหะกับรีเอเจนต์ภายในแคพพิลลารีเมื่อมีการให้ความต่างศักย์เข้าไปในระบบ ศึกษาวิธีสำหรับเตรียมอนุพันธ์แบบในแคพพิลลารี ประกอบด้วย รูปแบบการเตรียมอนุพันธ์ และการฉีดสารตัวอย่างและรีเอเจนต์เข้าสู่แคพพิลลารี นอกจากนี้ยังศึกษาพารามิเตอร์ที่มีผลต่อการแยกในระบบอิเล็กโทรฟอริซิส คือ พีเอชและความเข้มข้นของสารละลายบัฟเฟอร์ ตัวทำละลายอินทรีย์ และความต่างศักย์ เปรียบเทียบผลที่ได้ระหว่างการเตรียมอนุพันธ์แบบในแคพพิลลารี และก่อนแคพพิลลารี ซึ่งถือว่าการเตรียมอนุพันธ์แบบในแคพพิลลารีเป็นวิธีที่น่าสนใจและเป็นอีกทางเลือกหนึ่งสำหรับการวิเคราะห์โลหะ

Abstract: The feasibility of in-capillary derivatisation (ICD) and capillary zone electrophoresis (CZE) were investigated for the determination of four metals ions (Co(II), Cu(II), Ni(II) and Fe(II)). 2-(5-Nitro-2-pyridylazo)-5-[*N*-n-propyl-*N*–(3-sulfopropyl)amino]phenol (Nitro-PAPS) was used as derivatising reagent. The derivatisation was carried out inside the capillary. The strategies for in-capillary derivatisation include modes of derivatisation and types of reagent/sample introduction. The crucial electrophoretic parameters, including buffer pH, buffer concentration, organic modifier and applied voltage were optimised to provide good resolution. The analytical features obtained from in-capillary and pre-capillary derivatisations were compared. The scheme of in-capillary derivatisation is attractive and serves as alternative technique for metal analysis.

Introduction: The analysis of trace heavy metals is interesting task because of their toxicity. The favourable analytical techniques for simultaneous metal analysis are high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) based on derivatisation pre-, on- and post-column derivatisation. Using CE, on-column (or in-capillary) derivatisation gives more advantage features comparing to pre-column, such as the small reaction chamber in capillary which means the minimum dilution and less reagent consumption. In the present work, CE with in-capillary derivatisation was proposed for analyses of some heavy metals. Nitro-PAPS was chosen as the derivatising reagent.

Methodology: The different strategies of in-capillary derivatisation were investigated for developing reaction of metals and Nitro-PAPS. The segment of Nitro-PAPS solution ($1x10^{-3}$ M) was introduced into capillary by hydrodynamic injection at 0.5 psi for appropriated time. A mixture of metal ions was subsequently introduced by electrokinetic injection (10 kV, 5 s). Then, the voltage was applied between both ends of the capillary at 10 kV for 1 min to develop the reaction inside the capillary before the separation. The crucial parameters affecting CZE separation were optimised including buffer pH, buffer concentration, organic additive and applied voltage. A fused silica of 75 µm I.D. x 60.2 cm was used. .

Results, Discussion and Conclusion: The derivatisation was successfully performed by zone passing technique. The Nitro-PAPS segment was introduced into capillary at the end of anode by hydrodynamic mode before introducing of metal ions by electrokinetic injection. After the application of voltage, Nitro-PAPS as neutral migrated to the cathode by electroosmotic flow (EOF) whereas the metal species migrated with the higher velocities than reagent. Thus, the metals mixed with the reagent inside the capillary to form their derivatives. The separation conditions were 45 mmol L^{-1} borate (pH 9.7) containing 20 % acetonitrile, applied voltage of 20 kV and on-capillary detection at 250 nm. Under the optimum condition, four metal-Nitro-PAPS complexes were completely separated within 20 min. The order of migration was Co(II), Cu(II), Ni(II) and Fe(II), respectively.

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