รายงานวิจัยฉบับสมบูรณ์

โครงการ
การพัฒนากราฟีนนาโนคอมพอสิตเพื่อสร้างระบบการตรวจวัดสารชีวโมเลกุลและวินิจฉัยโรคเบื้องต้น

โดย

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พฤษภาคม 2558

สัญญาเลขที่ TRG5680012

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สถาบันวิจัยโลหะและวัสดุ จุฬาลงกรณ์มหาวิทยาลัย

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
และ จุฬาลงกรณ์มหาวิทยาลัย

กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณ ศ. ดร. อรวรรณ ชัยลภากุล อาจารย์ที่ปรึกษาอาวุโสของโครงการ สำหรับคำแนะนำตลอดการทำวิจัยในโครงการนี้ และ Prof. Richard Vachet นางสาวนิภา พรรณ ฤาชา และ นางสาวนาฏตินันท์ พรหมเพชร ผู้มีส่วนร่วมในการดำเนินงานวิจัยจนโครงการนี้จนสำเร็จ

ท้ายที่สุดผู้วิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัยและจุฬาลงกรณ์มหาวิทยาลัยผู้สนับสนุนให้ทุนวิจัยตลอดระยะเวลา 2 ปีในการวิจัยโครงการนี้

Abstract

Project Code : TRG5680012

Project Title : Graphene Nanocomposites for Sensitive Biomolecular Detection and Medical Diagnosis

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Nanocomposites of graphene (G) and conducting polymer are developed and used as the novel electrodes for sensitive electrochemical detection of biomolecules and chemicals. Due to the high electric conductivity, large surface area, and excellent thermal stability, G is selected to modify the electrode surfaces for enhancing the electrochemical sensitivity. To prevent the agglomeration of G, the conducting polymers are employed to improve G dispersion and also enhance the nanocomposite conductivity. In this study, types of conducting polymer, ratios of G/polymer, electrode fabrication parameters, and electrochemical parameters are investigated and optimized. Electrospinning or electrospraying is selected for the electrode fabrication to further increase specific surface area and electrochemical sensitivity of nanocomposite modified electrode. The obtained results show that the presence of G-polymer nanodroplets or G-polymer nanofibers on the modified electrode surface substantially increases the electrochemical sensitivities in the detection of biomolecules and chemicals (*e.g.* cholesterol, dopamine, toxic heavy metals). Eventually, this approach is succesfully applied for the sensitive determination of such compounds in real samples and the satisfied results are achieved.

Keywords : Graphene, Conducting Polymer, Electrospinning, Electrospraying, Electrochemical detection

บทคัดย่อ

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ชื่อโครงการ : การพัฒนากราฟีนนาโนคอมพอสิตเพื่อสร้างระบบการตรวจวัดสารชีวโมเลกุลและวินิจฉัยโรคเบื้องต้น

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นาโนคอมพอสิตของแกรฟีนและพอลิเมอร์นำไฟฟ้าถูกพัฒนาขึ้นเพื่อนำไปใช้เป็นขั้วไฟฟ้าชนิดใหม่สำหรับตรวจวัดสารชีวโมเลกุลและสารเคมีที่มีความไวสูง เนื่องจากแกรฟีนมีค่าการนำไฟฟ้าสูง มีพื้นที่ผิวสูง และมีความเสถียรต่อความร้อนสูง ดังนั้นจึงเลือกแกรฟีนมาใช้ในการดัดแปรผิวหน้าขั้วไฟฟ้าเพื่อเพิ่มความไวในการตรวจวัดทางเคมีไฟฟ้า เพื่อป้องกันการรวมตัวเกาะกันเป็นกลุ่มก้อนของแกรฟีน พอลิเมอร์นำไฟฟ้าจึงถูกเลือกใช้เพื่อเพิ่มการกระจายตัวของแกรฟีนและเพิ่มค่าการนำไฟฟ้าของวัสดุนาโนคอมพอสิตที่ได้ โดยการศึกษาครั้งนี้ได้ศึกษาชนิดของพอลิเมอร์นำไฟฟ้า อัตราส่วนของแกรฟีนและพอลิเมอร์ เทคนิคและสภาวะที่เหมาะสมของการดัดแปรขั้วไฟฟ้า และ ตัวแปรที่เกี่ยวข้องกับเทคนิคการตรวจวัดทางเคมีไฟฟ้า โดยจะใช้เทคนิคอิเล็กโทรสเปรย์หรือเทคนิคอิเล็กโทรสปินในการดัดแปรขั้วไฟฟ้า เพื่อช่วยเพิ่มพื้นที่ผิวจำเพาะและความไวในการตรวจวัดสารด้วยขั้วไฟฟ้าดัดแปรดังกล่าว โดยผลการทดลองที่ได้ชี้ให้เห็นว่าการมีหยดขนาดนาโนหรือเส้นใยขนาดนาโนของแกรฟีนและพอลิเมอร์บนขั้วไฟฟ้าจะช่วยเพิ่มความไวของระบบในการตรวจวัดสารชีวโมเลกุลและสารเคมี เช่น คลอเรสเตอรอล โดพามีนและโลหะหนัก ในตอนสุดท้ายระบบขั้วไฟฟ้าดัดแปรที่ได้พัฒนาขึ้นในงานวิจัยนี้สามารถนำไปประยุกต์ใช้สำหรับการตรวจวัดสารกลุ่มที่กล่าวมาในตัวอย่างจริงได้สำเร็จและได้ผลการทดลองเป็นที่น่าพอใจ

คำหลัก : แกรฟีน พอลิเมอร์นำไฟฟ้า อิเล็กโทรสเปรย์ อิเล็กโทรสปิน การตรวจวัดทางเคมีไฟฟ้า

เนื้อหางานวิจัย

บทนำ

การพัฒนาระบบการตรวจวัดทางเคมีไฟฟ้าที่มีความไวสูงนั้นมีความสำคัญยิ่งสำหรับการประยุกต์ใช้ประโยชน์ในด้านต่างๆ เช่น การตรวจวินิจฉัยโรคเบื้องต้น การพิสูจน์หลักฐาน การควบคุมคุณภาพอาหาร และ การควบคุมคุณภาพสิ่งแวดล้อม ทั้งนี้เนื่องจากเทคนิคทางเคมีไฟฟ้ามีข้อดีเหนือเทคนิคการตรวจวิเคราะห์เทคนิคอื่นๆ หลายประการ เช่น สามารถใช้งานได้ง่าย อุปกรณ์ตรวจวัดมีขนาดเล็กทำให้สามารถพกพาได้สะดวก สามารถใช้ในการตรวจวัดได้ทั้งเชิงคุณภาพและเชิงปริมาณ ที่สำคัญคืออุปกรณ์มีราคาถูกเมื่อเทียบกับอุปกรณ์การตรวจวัดที่ใช้ในเทคนิคอื่นๆ อย่างไรก็ดีเทคนิคทางเคมีไฟฟ้าแบบดั้งเดิมนั้นเมื่อถูกออกแบบให้มีขนาดเล็กเพื่อให้สามารถพกพาได้สะดวก ขั้วไฟฟ้าใช้งาน (working electrode) จึงมักถูกจำกัดให้มีขนาดเล็ก พื้นที่ผิวในการสัมผัสสารจึงลดลง ส่งผลให้ความไวในการตรวจวัดสารด้วยเทคนิคทางเคมีไฟฟ้านั้นลดลงตามไปด้วย

ดังนั้นจึงได้มีการนำวัสดุนาโนชนิดต่างๆ ที่มีความสามารถในการนำไฟฟ้าได้ดีเข้ามาใช้ดัดแปรขั้วไฟฟ้าใช้งานเพื่อเพิ่มพื้นที่ผิวในการสัมผัสกับสารของขั้วไฟฟ้าเพราะคุณสมบัติเด่นของวัสดุนาโนคือ มีพื้นที่ผิวต่อปริมาตรที่สูง (high specific surface area) แต่ในขณะเดียวกันก็ยังคงขนาดและรูปร่างของขั้วไฟฟ้าใช้งานให้เล็กเช่นเดิมเพื่อให้สามารถพกพาไปที่ต่างๆ ได้ อนุภาคนาโนของโลหะชนิดต่างๆ เช่น อนุภาคนาโนเงิน อนุภาคนาโนทอง และอนุภาคนาโนของคาร์บอน เช่น คาร์บอนนาโนทิวป์ ฟูลเลอรีน และแกรฟีนได้ถูกนำมาใช้ในการดัดแปรขั้วไฟฟ้าใช้งานเพื่อเพิ่มพื้นที่ผิวและความไวในการตรวจวัดทางเคมีไฟฟ้า [1-2] ในงานวิจัยนี้สนใจนำแกรฟีนมาใช้ในการดัดแปรขั้วไฟฟ้าใช้งาน แกรฟีนเป็นวัสดุนาโนคาร์บอนที่มีค่าการนำไฟฟ้าสูง มีพื้นที่ผิวสูง มีความเสถียรต่อความร้อนสูง และมีราคาค่อนข้างถูกเมื่อเทียบกับวัสดุนาโนชนิดอื่น [3-4] อย่างไรก็ตามบ่อยครั้งที่แกรฟีนมักรวมตัวกันเป็นกลุ่มก้อนกลายเป็นแกรไฟต์ทำให้สูญเสียสมบัติที่สำคัญของแกรฟีนไป ดังนั้นในการใช้งานจึงนิยมเตรียมแกรฟีนในรูปของคอมพอสิต โดยในงานนี้สนใจจะเตรียมนาโนคอมพอสิตของแกรฟีนกับพอลิเมอร์นำไฟฟ้า โดยหน้าที่ที่สำคัญของพอลิเมอร์นำไฟฟ้าคือช่วยในการกระจายตัวของแกรฟีนและเพิ่มการนำไฟฟ้าของระบบ พอลิเมอร์นำไฟฟ้าที่สนใจในงานวิจัยนี้คือพอลิแอนิลีน เนื่องจากพอลิแอนิลีนมีสมบัติในการนำไฟฟ้าที่ดี สังเคราะห์ได้ง่าย มีความเสถียรสูงในสิ่งแวดล้อมและหมู่อะมิโนของพอลิแอนิลีนยังเหมาะสมสำหรับการติดกับตัวรับรู้ทางชีวภาพอีกด้วย จากสมบัติดังกล่าวจึงมีการใช้พอลิแอนิลีนในการดัดแปรขั้วไฟฟ้าสำหรับใช้ตรวจวัดทางเคมีไฟฟ้าในหลายงานวิจัย [5-11]

นอกจากการเลือกใช้วัสดุที่เหมาะสมในการดัดแปรขั้วไฟฟ้าแล้ว กระบวนการในการดัดแปรขั้วไฟฟ้าก็มีความสำคัญไม่น้อย โดยทั่วไปงานวิจัยที่ผ่านมามักใช้วัสดุที่สนใจดัดแปรขั้วไฟฟ้าโดยการหยดลงบนผิวหน้าของขั้วไฟฟ้าโดยตรงหรือใช้การเตรียมเป็นแบบฟิล์มบาง แต่ในงานวิจัยนี้สนใจที่จะศึกษาและปรับปรุงกระบวนการดังกล่าวโดยสนใจที่เทคนิคอิเล็กโทรสเปรย์หรือเทคนิคอิเล็กโทรสปิน โดยคาดว่าการดัดแปรขั้วไฟฟ้าด้วยสองกระบวนการนี้จะทำให้

เกิดหยดขนาดเล็ก และเส้นใยขนาดเล็กของนาโนคอมพอสิตของแกรฟีนและพอลิเมอร์นำไฟฟ้าบนพื้นผิวหน้าขั้วไฟฟ้า ซึ่งจะเป็นการเพิ่มพื้นที่ผิวในการสัมผัสกับสารละลาย และเพิ่มการนำไฟฟ้าของขั้วไฟฟ้า อันจะส่งผลให้เพิ่มความไวของระบบการตรวจวัดทางเคมีไฟฟ้าในที่สุด

สำหรับสารที่สนใจจะทำการตรวจวิเคราะห์ โดยระบบทางเคมีไฟฟ้าที่พัฒนาได้ในโครงการวิจัยนี้สนใจสารที่เกี่ยวข้องและมีความสำคัญกับสิ่งมีชีวิตได้แก่ คลอเรสเตอรอล ซึ่งเป็นสารบ่งชี้ทางชีวภาพที่สำคัญในการวินิจฉัยโรคหัวใจและความดันโลหิตสูง โดพามีนซึ่งเป็นสารสื่อประสาทที่สำคัญในสมองของมนุษย์และ โลหะหนักที่มีความเป็นพิษสูงต่อสิ่งมีชีวิตได้แก่ ตะกั่วและแคดเมียม เป็นต้น

วิธีการทดลอง

ขั้นตอนการทดลองโดยสรุปสำหรับการเตรียมนาโนคอมพอสิตของแกรฟีนและพอลิเมอร์นำไฟฟ้าเพื่อนำไปใช้เป็นขั้วไฟฟ้าชนิดใหม่สำหรับตรวจวัดสารชีวโมเลกุลและสารเคมีที่สนใจศึกษาในงานวิจัยนี้ประกอบไปด้วย

1. เตรียมสารละลายผสมของแกรฟีนและพอลิแอนิลีนโดยปรับสัดส่วนของแต่ละองค์ประกอบ รวมทั้งเลือกชนิดของตัวทำละลายให้เหมาะสม
2. เลือกสภาวะที่เหมาะสมในการดัดแปรขั้วไฟฟ้าด้วยเทคนิคอิเล็กโทรสเปรย์หรือเทคนิคอิเล็กโทรสปิน โดยตัวแปรสำคัญที่ทำการศึกษาได้แก่ อัตราการไหลของสารละลาย ศักย์ไฟฟ้าที่ให้กับสารละลาย ระยะห่างของปลายเข็มและฉากรับ ระยะเวลาการดัดแปร และชนิดของพอลิเมอร์ที่ใช้ช่วยในการขึ้นรูป
3. ทำการพิสูจน์ลักษณะสัณฐานวิทยาของขั้วไฟฟ้าดัดแปรที่เตรียมได้ว่ามีลักษณะเป็นหยดขนาดเล็ก หรือ เป็นเส้นใยขนาดเล็กโดยใช้กล้องจุลทรรศน์อิเล็คตรอนแบบส่องกราด (SEM) และศึกษาการกระจายตัวของแกรฟีนบนพื้นผิวขั้วไฟฟ้าโดยใช้อิเล็คตรอนแบบส่องผ่าน (TEM)
4. ทำการพิสูจน์สมบัติทางเคมีไฟฟ้าเช่น ความสามารถในการส่งผ่านอิเล็คตรอนของขั้วไฟฟ้า และ ความไวในการส่งผ่านอิเล็คตรอนบนขั้วไฟฟ้าดัดแปรที่เตรียมได้ด้วยเทคนิคไซคลิกโวลแทมเมตรี
5. ทำการเลือกเทคนิคทางเคมีไฟฟ้าที่เหมาะสมสำหรับการตรวจวัดสารแต่ละชนิดที่สนใจศึกษา เช่น amperometry, square wave voltammetry, anodic stripping voltammetry และศึกษาหาสภาวะที่เหมาะสมสำหรับสารนั้นๆ
6. ทำการตรวจวิเคราะห์สารที่สนใจด้วยเทคนิคทางเคมีไฟฟ้า หาค่าที่เกี่ยวข้องกับประสิทธิภาพการตรวจวิเคราะห์ (analytical performances) ของระบบ เช่น limit of detection (LOD), limit of quantitation (LOQ), linearity เป็นต้น

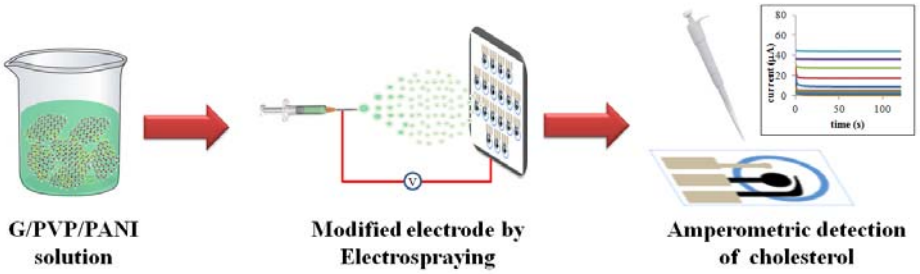
7. นำระบบที่พัฒนาได้ไปประยุกต์ใช้ในการตรวจวิเคราะห์สารในระบบที่มีตัวรบกวนและในตัวอย่างจริง รวมทั้งตรวจสอบความใช้ได้ (validation) ของระบบที่ได้ทำการพัฒนาขึ้น
8. วิเคราะห์และสรุปผลการทดลอง เขียนรายงานผลการทดลองรวมทั้งเตรียมเขียนบทความเพื่อตีพิมพ์ในวารสาร

ผลการทดลองและบทวิจารณ์

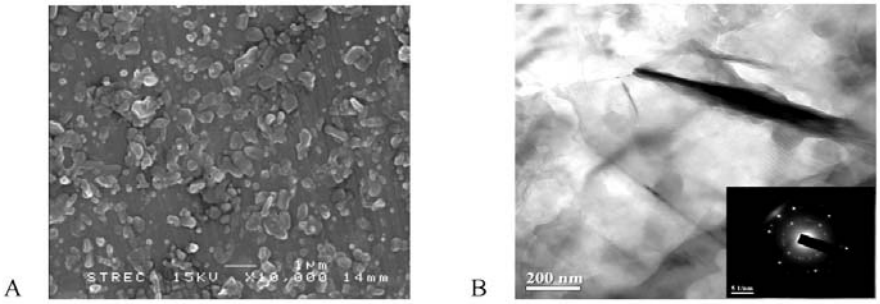
ในส่วนของผลการทดลองและวิจารณ์ผลการทดลองจะแบ่งออกเป็น 3 หัวข้อตามชนิดของสารที่สนใจจะทำการตรวจวิเคราะห์ซึ่งได้แก่ คลอเรสเตอรอล โดพามีน และ โลหะหนัก

1. การตรวจวัดคลอเรสเตอรอล (Novel Baper-based Cholesterol Biosensor Using Graphene/Polyvinylpyrrolidone/Polyaniline Nanocomposite)

งานวิจัยนี้ได้ทำการเตรียมนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีนกับพอลิไวนิลไพโรลิโดนขึ้นมาเป็นหยดขนาดนาโน (160±1.02 นาโนเมตร) โดยใช้เทคนิคอิเล็กโทรสเปรย์แล้วนำขั้วไฟฟ้ากระดาษที่ทำการดัดแปรแล้วดังกล่าวไปตรวจวัดไฮโดรเจนเปอร์ออกไซด์ซึ่งเป็นสารที่ใช้บ่งชี้ปริมาณคลอเรสเตอรอลโดยใช้เทคนิค amperometry ซึ่งภาพรวมของงานวิจัยสามารถแสดงได้ดังแผนภาพ

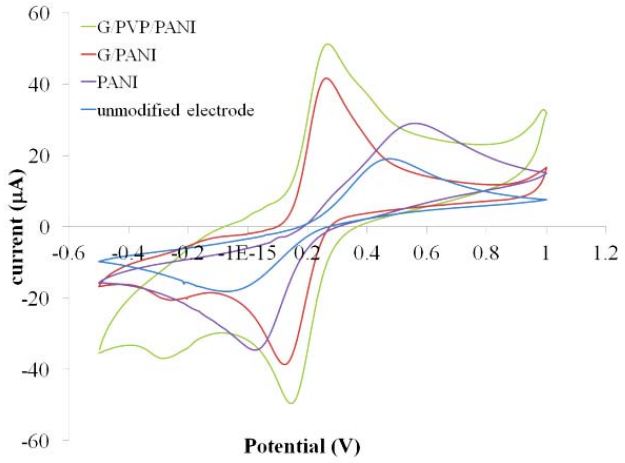


ในกระบวนการเตรียมขั้วไฟฟ้าดัดแปรในงานวิจัยนี้โดยเทคนิคอิเล็กโทรสเปรย์ได้ทำการศึกษาปัจจัยต่างๆ ในกระบวการดัดแปรที่ส่งผลต่อการนำไฟฟ้าของระบบนี้และพบว่าศักย์ไฟฟ้าที่เหมาะสมคือ 6 กิโลโวลต์ เวลาการสเปรย์ 5 นาทีและอัตราส่วนของการผสมกันของแกรฟีนและพอลิแอนิลีน 1:1 โดยลักษณะสัณฐานวิทยาของขั้วไฟฟ้าดัดแปรที่เตรียมได้มีลักษณะเป็นหยดขนาดเล็ก (160±1.02 นาโนเมตร) และยังมีลักษณะการกระจายตัวของแผ่นแกรฟีนที่บางดังรูปที่ 1-1



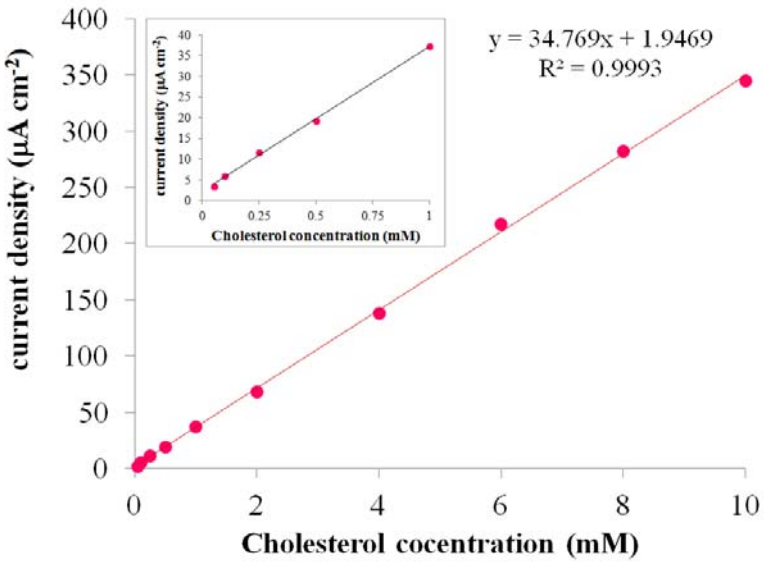
รูปที่1-1 SEM แสดงหยดขนาดเล็กของนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีนกับพอลิไวนิลไพโรลิโดน (A) และรูป TEM แสดงการกระจายตัวของแผ่นแกรฟีน (B)

เมื่อนำขั้วไฟฟ้าดัดแปรที่ได้ในงานวิจัยนี้ไปทดสอบความสามารถในการนำไฟฟ้าโดยใช้เทคนิค Cyclic voltammetry วัดสารละลายมาตรฐาน 1.0 mM $Fe(CN)_6^{3-/4-}$ และเปรียบเทียบกับขั้วไฟฟ้าที่ไม่ได้ดัดแปรพบว่าขั้วไฟฟ้าที่ดัดแปรด้วยหยดขนาดเล็กของแกรฟีนและพอลิแอนิลีนกับพอลิไวนิลไพโรลิโดนนี้มีค่าการนำไฟฟ้าที่สูงสุดโดยสังเกตได้จากความสูงของกระแสไฟฟ้าที่วัดได้ (แกน y) ดังรูปที่ 1-2



รูปที่1-2 Cyclic voltammograms ของ 1.0 mM $Fe(CN)_6^{3-/4-}$ บนขั้วไฟฟ้าต่างชนิดกัน

จากนั้นได้นำขั้วไฟฟ้าที่ดัดแปรได้ไปทำการตรวจวัดไฮโดรเจนเปอร์ออกไซด์ซึ่งเป็นสารผลิตภัณฑ์ที่ได้จากการทำปฏิกิริยาระหว่างคลอเรสเตอรอลและเอนไซม์คลอเรสเตอรอลออกซิเดส โดยใช้เทคนิค amperometry และหาค่าที่เกี่ยวข้องกับประสิทธิภาพการตรวจวิเคราะห์ (analytical performances) ของระบบ พบว่าค่า limit of detection (LOD) คือ 1 ไมโครโมล่าห์ limit of quantitation (LOQ) คือ 50 ไมโครโมล่าห์ และค่า linearity คือ 50 ไมโครโมล่าห์ to 10 มิลลิโมล่าห์ (R^2 = 0.9993) ดังรูปที่ 1-3



รูปที่1-3 Calibration curve สำหรับการตรวจวัดคลอเรสเตอรอลในช่วงความเข้มข้น 50 μM ถึง 10 mM ใน 0.1 M PBS pH 7.0.

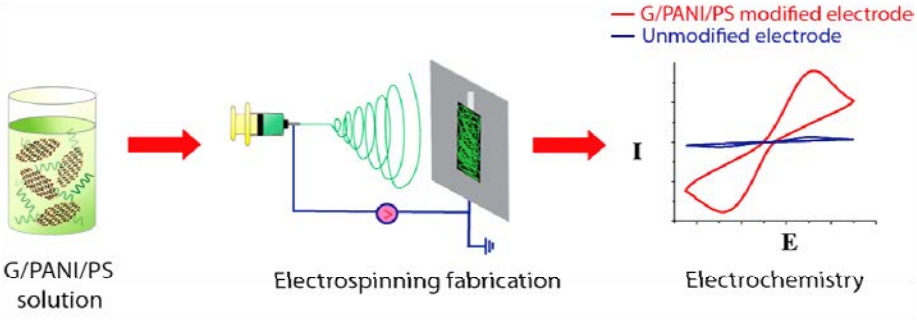
ในตอนสุดท้ายขั้วไฟฟ้าที่พัฒนาได้ในระบบนี้สามารถนำไปประยุกต์ใช้ในการตรวจวัดปริมาณคลอเรสเตอรอลในเซรั่มมนุษย์ได้สำเร็จโดยมีค่า %recovery อยู่ในช่วง 100.0-102.0% และมีค่า RSD น้อยกว่า 5% ซึ่งแสดงให้เห็นว่าระบบการตรวจวัดดังกล่าวนี้มีค่าความถูกต้องสูง ดังแสดงในตารางที่ 1-1

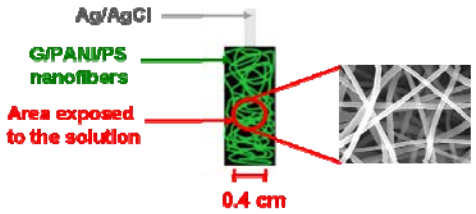
ตารางที่1-1 การหาปริมาณคลอเรสเตอรอลในเซรั่มมนุษย์

Cholesterol concentration (mM)		% Recovery	% RSD
added	found		
0.05	0.05 ± 0.04	100.0	0.8
0.10	0.10 ± 0.08	100.0	0.8
0.25	0.25 ± 0.30	100.0	1.2
1.00	1.02 ± 1.05	102.0	1.0
5.00	5.01 ± 2.84	100.2	0.6

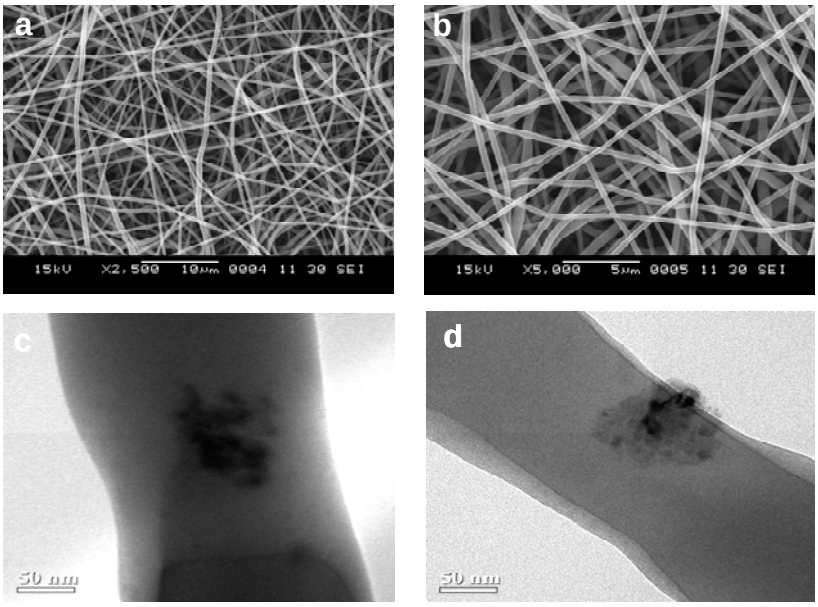
2. การตรวจวัดโดพามีน (Graphene-Loaded Nanofiber-Modified Electrodes for the Ultrasensitive Determination of Dopamine)

งานวิจัยนี้ได้ทำการเตรียมนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีนขึ้นมาเป็นเส้นใยขนาดนาโน (260±60 นาโนเมตร) โดยใช้เทคนิคอิเล็กโทรสปินแล้วนำขั้วไฟฟ้าที่ทำการดัดแปรแล้วดังกล่าวไปตรวจวัดโดพามีนโดยใช้เทคนิค square wave voltammetry ซึ่งภาพรวมของงานวิจัยสามารถแสดงได้ดังแผนภาพ



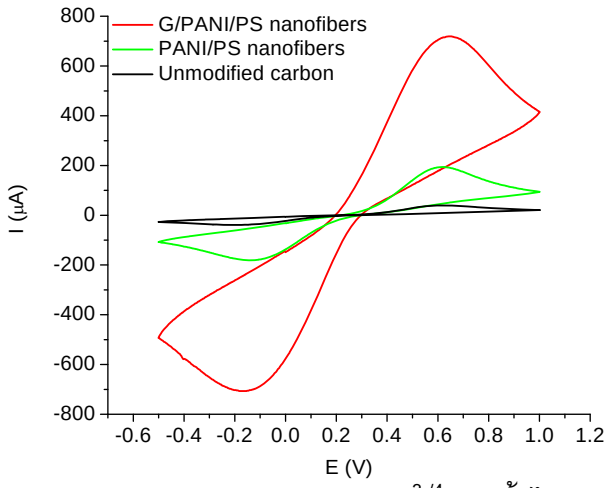


ในกระบวนการเตรียมขั้วไฟฟ้าดัดแปรในงานวิจัยนี้โดยเทคนิคอิเล็กโทรสปินได้ทำการศึกษาปัจจัยต่างๆ ในกระบวการดัดแปรที่ส่งผลต่อการนำไฟฟ้าของระบบนี้และพบว่าชนิดของตัวทำละลายที่เหมาะสมคือ dimethylformamide (DMF) ปริมาณ Graphene loading 6% และ เวลาการสปิน 15 นาที โดยลักษณะสัณฐานวิทยาของขั้วไฟฟ้าดัดแปรที่เตรียมได้มีลักษณะเป็นเส้นใยขนาดเล็ก (260±60 นาโนเมตร) มีความสม่ำเสมอสูง และยังมีลักษณะการกระจายตัวของแผ่นแกรฟีนที่บางดังรูปที่ 2-1



รูปที่2-1 SEM แสดงเส้นใยขนาดเล็กของนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีน (A,B) และรูป TEM แสดงการกระจายตัวของแผ่นแกรฟีน (C,D)

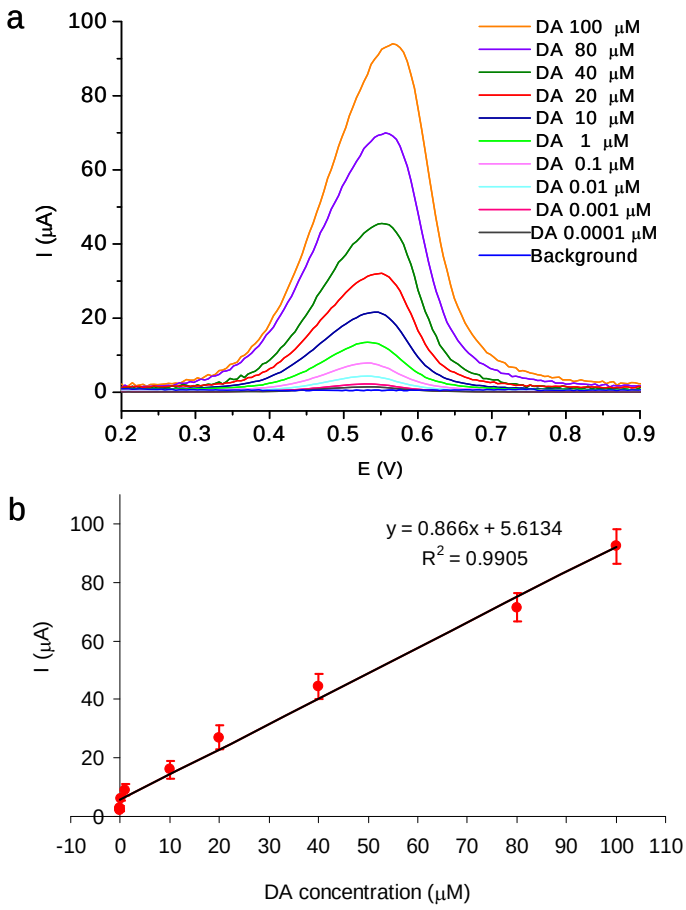
เมื่อนำขั้วไฟฟ้าดัดแปรที่ได้ในงานวิจัยนี้ไปทดสอบความสามารถในการนำไฟฟ้าโดยใช้เทคนิค Cyclic voltammetry วัดสารละลายมาตรฐาน 1.0 mM $Fe(CN)_6^{3-/4-}$ และเปรียบเทียบกับขั้วไฟฟ้าที่ไม่ได้ดัดแปรพบว่าขั้วไฟฟ้าที่ดัดแปรด้วยเส้นใยขนาดเล็กของแกรฟีนและพอลิแอนิลีนนี้มีค่าการนำไฟฟ้าที่สูงสุดโดยสังเกตได้จากความสูงของกระแสไฟฟ้าที่วัดได้ (แกน y) ดังรูปที่ 2-2



รูปที่2-2 Cyclic voltammograms ของ 1.0 mM $Fe(CN)_6^{3-/4-}$ บนขั้วไฟฟ้าต่างชนิดกัน

จากนั้นได้นำขั้วไฟฟ้าที่ดัดแปรได้ไปทำการตรวจวัดโดพามีนโดยใช้เทคนิค square wave voltammetry และหาค่าที่เกี่ยวข้องกับประสิทธิภาพการตรวจวิเคราะห์ (analytical performances) ของระบบ พบว่าค่า limit of detection (LOD) คือ 0.05 นาโนโมล่าห์ limit of

quantitation (LOQ) คือ 0.3 นาโนโมล่าห์ และค่า linearity คือ 0.0001-100 ไมโครโมล่าห์ (R^2 = 0.9905) ดังรูปที่ 2-3



รูปที่2-3 Calibration curve สำหรับการตรวจวัดโดพามีนในช่วงความเข้มข้น 0.0001-100 μM ใน 0.1 M PBS pH 7.4.

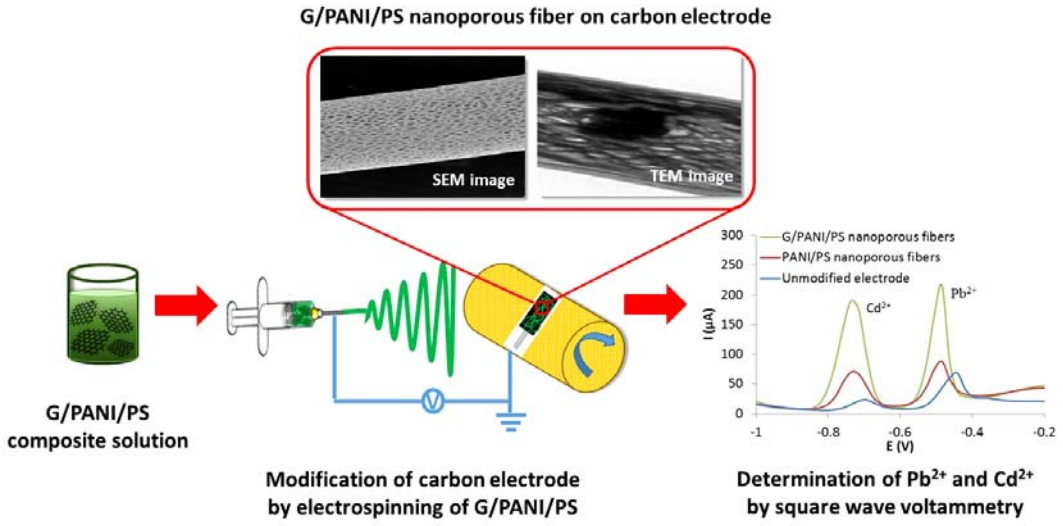
ในตอนสุดท้ายขั้วไฟฟ้าที่พัฒนาได้ในระบบนี้สามารถนำไปประยุกต์ใช้ในการตรวจวัดปริมาณโดพามีนในเซรั่มและปัสสาวะมนุษย์ได้สำเร็จโดยมีค่า %recovery อยู่ในช่วง 97.0-104.5% และมีค่า RSD น้อยกว่า 5% ซึ่งแสดงให้เห็นว่าระบบการตรวจวัดดังกล่าวนี้มีค่าความถูกต้องสูงดังแสดงในตารางที่ 2-1

ตารางที่2-1 การหาปริมาณโดพามีนในเซรั่มและปัสสาวะมนุษย์

Samples	No.	Amount of added DA (μM)	Amount of found DA (μM)	Recovery (%)	RSD (%)
Human Serum	1	1.0	0.97	97.0	2.5
	2	10.0	9.85	98.5	2.9
	3	50.0	50.90	101.8	2.6
Urine	1	1.0	1.02	102.0	2.7
	2	10.0	10.30	103.0	2.8
	3	50.0	52.25	104.5	2.9

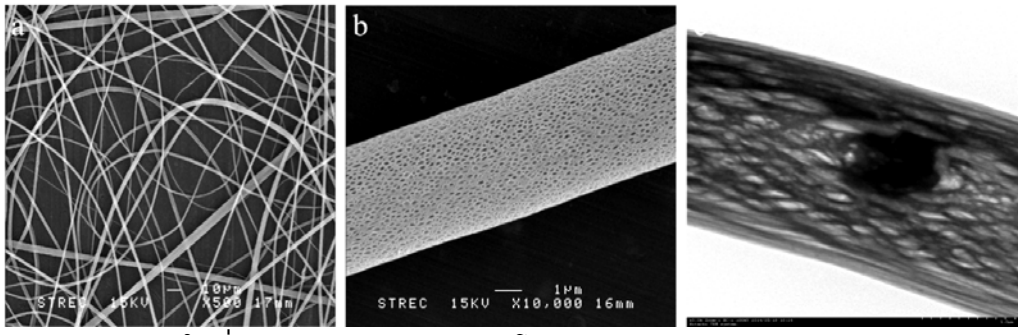
3. **การตรวจวัดโลหะหนัก ได้แก่ ตะกั่วและแคดเมียม (An Electrochemical Sensor Based on Graphene/Polyaniline/Polystyrene Nanoporous Fibers Modified Electrode for Simultaneous Determination of Lead and Cadmium)**

งานวิจัยนี้ได้ทำการเตรียมนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีนขึ้นมาเป็นเส้นใยที่มีรูพรุนขนาดนาโน โดยใช้เทคนิคอิเล็กโทรสปินแล้วนำขั้วไฟฟ้าที่ทำการดัดแปรแล้วดังกล่าวไปตรวจวัดโลหะหนัก ได้แก่ ตะกั่วและแคดเมียมโดยใช้เทคนิค anodic stripping voltammetry ซึ่งภาพรวมของงานวิจัยสามารถแสดงได้ดังแผนภาพ



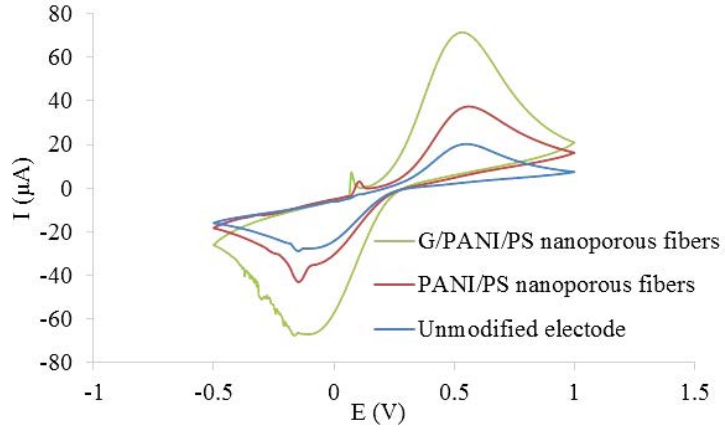
ในกระบวนการเตรียมขั้วไฟฟ้าดัดแปรในงานวิจัยนี้โดยเทคนิคอิเล็กโทรสปินได้ทำการศึกษาปัจจัยต่างๆ ในกระบวการดัดแปรที่ส่งผลต่อการนำไฟฟ้าของระบบนี้และพบว่า

ชนิดของตัวทำละลายที่เหมาะสมคือ tetrahydrofuran (THF) ปริมาณ Graphene loading 4mg/mL และ ใช้ 20% พอลิสไตรรีนเป็นพอลิเมอร์ตัวช่วยในการขึ้นรูปเส้นใยให้เป็นรูพรุน โดยลักษณะสัณฐานวิทยาของขั้วไฟฟ้าดัดแปรที่เตรียมได้มีลักษณะเป็นเส้นใยที่มีรูพรุนขนาดเล็กและยังมีแผ่นแกรฟีนอยู่ภายในเส้นใยที่มีรูพรุนดังกล่าวดังรูปที่ 3-1



รูปที่3-1 SEM แสดงเส้นใยที่มีรูพรุนขนาดเล็กของนาโนคอมพอสิตของแกรฟีนและพอลิแอนิลีน (A,B) และรูป TEM แสดงแผ่นแกรฟีนภายในเส้นใยที่มีรูพรุนขนาดเล็ก (C)

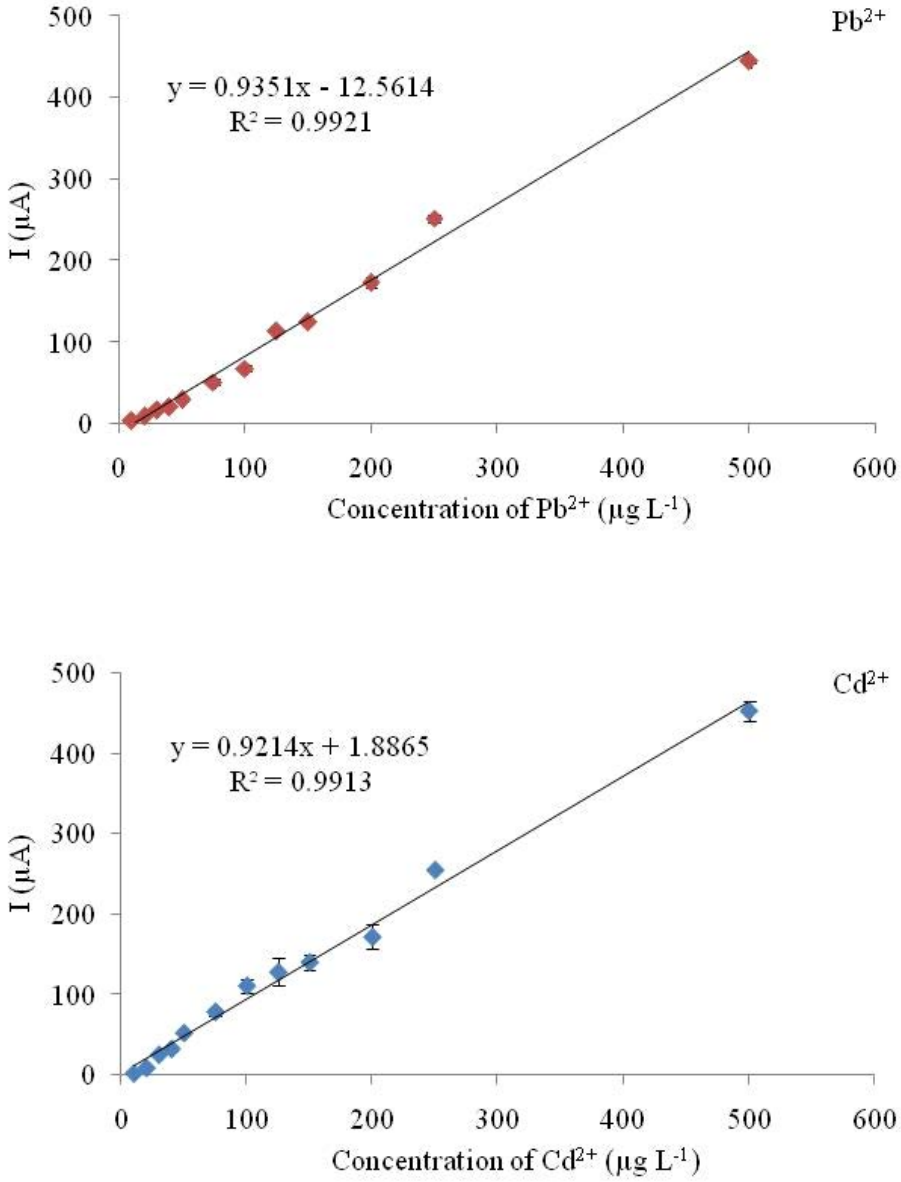
เมื่อนำขั้วไฟฟ้าดัดแปรที่ได้ในงานวิจัยนี้ไปทดสอบความสามารถในการนำไฟฟ้าโดยใช้เทคนิค Cyclic voltammetry วัดสารละลายมาตรฐาน 1.0 mM $Fe(CN)_6^{3-/4-}$ และเปรียบเทียบกับขั้วไฟฟ้าที่ไม่ได้ดัดแปรพบว่าขั้วไฟฟ้าที่ดัดแปรด้วยเส้นใยที่มีรูพรุนขนาดเล็กของแกรฟีนและพอลิแอนิลีนนี้มีค่าการนำไฟฟ้าที่สูงสุดโดยสังเกตได้จากความสูงของกระแสไฟฟ้าที่วัดได้ (แกน y) ดังรูปที่ 3-2



รูปที่3-2 Cyclic voltammograms ของ 1.0 mM $Fe(CN)_6^{3-/4-}$ บนขั้วไฟฟ้าต่างชนิดกัน

จากนั้นได้นำขั้วไฟฟ้าที่ดัดแปรได้ไปทำการตรวจวัดตะกั่วและแคดเมียมโดยใช้เทคนิค anodic stripping voltammetry และหาค่าที่เกี่ยวข้องกับประสิทธิภาพการตรวจวิเคราะห์ (analytical performances) ของระบบ พบว่าค่า limit of detection (LOD) คือ 3.30 μg L^{-1}

สำหรับPb^{2+} และ 4.43 µg L^{-1} สำหรับ Cd^{2+} และค่า linearity คือ 10-500 µg L^{-1} R^2=0.992 สำหรับPb^{2+} และR^2=0.991 สำหรับ ดังรูปที่ 3-3



รูปที่3-3 Calibration curve สำหรับการตรวจวัดตะกั่วและแคดเมียมในช่วงความเข้มข้น 10-500 µg L^{-1}

ในตอนสุดท้ายขั้วไฟฟ้าที่พัฒนาได้ในระบบนี้สามารถนำไปประยุกต์ใช้ในการตรวจวัดปริมาณตะกั่วและแคดเมียมในตัวอย่างน้ำจากแหล่งต่างๆ ได้สำเร็จโดยมีค่า %recovery อยู่ในช่วง 85-109% และ 95-103% สำหรับ Pb^{2+} และ RSD น้อยกว่า 10% แสดงให้เห็นว่าระบบการตรวจวัดดังกล่าวนี้มีค่าความถูกต้องสูง นอกจากนี้ยังได้ทำการเปรียบเทียบผลที่ได้จากระบบดังกล่าวกับเทคนิค ICP-OES และพบว่าผลการทดลองที่ได้สอดคล้องกันเป็นอย่างดีดังแสดงในตารางที่ 3-1

ตารางที่3-1 การหาปริมาณตะกั่วและแคดเมียมในตัวอย่างน้ำจากแหล่งต่าง ๆเปรียบเทียบกับเทคนิค ICP-OES

Sample	Added ($\mu g\ L^{-1}$)		Pb^{2+}		Cd^{2+}		Recovery (%)	
	Pb^{2+}	Cd^{2+}	ICP-OES ± SD($\mu g\ L^{-1}$)	Found ± SD ($\mu g\ L^{-1}$)	ICP-OES ± SD($\mu g\ L^{-1}$)	Found ± SD ($\mu g\ L^{-1}$)	Pb^{2+}	Cd^{2+}
Chao	0	0	15.05±0.62	10.30±0.47	3.18±0.07	ND	-	-
Phraya	25	25	38.60±0.63	32.94±2.64	24.86±0.05	24.92±2.40	89.93±11.02	98.68±9.61
river	50	50	61.12±0.25	53.02±2.05	46.65±0.04	51.84±4.09	85.43±4.11	103.68±8.17
	150	150	165.14±0.36	155.68±8.01	143.11±0.11	152.92±5.89	96.92±5.43	101.94±3.93
Saen	0	0	14.09±0.98	11.07±0.40	0.64±0.06	ND	-	-
Saeb	25	25	42.69±0.51	38.11±1.05	24.66±0.14	25.34±0.90	107.79±4.21	101.36±3.63
canal	50	50	62.58±2.06	65.94±3.30	45.72±0.23	47.67±3.02	109.54±6.61	95.34±6.05
	150	150	168.97±0.49	159.08±0.90	146.48±0.26	153.77±5.31	98.61±0.60	102.51±3.54

สรุปผลการทดลอง

ขั้วไฟฟ้าที่มีแกรฟีนและพอลิแอนิลีนเป็นองค์ประกอบที่ทำการดัดแปรด้วยเทคนิคอิเล็กโทรสเปรย์หรือเทคนิคอิเล็กโทรสปินในโครงการวิจัยนี้สามารถนำไปใช้สร้างระบบตรวจวัดทางเคมีไฟฟ้าที่มีความไวและประสิทธิภาพสูงสำหรับใช้ในการตรวจวัดสารที่เกี่ยวข้องและมีความสำคัญกับสิ่งมีชีวิตเช่น คลอเรสเตอรอล โดพามีนและ โลหะหนักในตัวอย่างจริงได้สำเร็จ

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1) Ruecha, N., Rangkupan, R, Rodthongkum, N., Chailapakul, O. “Novel Baper-based Cholesterol Biosensor Using Graphene/Polyvinylpyrrolidone/Polyaniline Nanocomposite” *Biosens. Bioelectron*. 2014, *52*, 13-19. *(2014 Impact Factor: 6.451)*

2) Rodthongkum, N., Ruecha, N., Rangkupan, R., Vachet, R. W., Chailapakul, O. “Graphene-Loaded Nanofiber-Modified Electrodes for the Ultrasensitive Determination of Dopamine” *Anal Chim Acta.* 2013, *804*, 84-91. *(2014 Impact Factor: 4.517)*

3) Promphet, N., Rattanarat, P., Rangkupan, R., Chailapakul, O., Rodthongkum, N. “An Electrochemical Sensor Based on Graphene/Polyaniline/Polystyrene Nanoporous Fibers Modified Electrode for Simultaneous Determination of Lead and Cadmium” *Sensor Actuat. B-Chem.* 2015, *207*, 526-534. *(2015 Impact Factor: 3.840)*

2. บทคัดย่อการนำเสนอผลงานทางวิชาการในงานประชุมวิชาการ

- ASMS 2013 Conference on Mass Spectrometry and Allied Topics ณ เมือง Minneapolis, Minnesota, USA (9-13 June 2013) เรื่อง Graphene/polyaniline nanocomposite along with MALDI-MS for sensitive detection of small biomolecules โดย Nadnudda Rodthongkum*, Nipapan Ruecha, Voravee P.Hoven, Orawon Chailapakul and Richard Vachet*

3. การนำผลงานวิจัยไปใช้ประโยชน์

- **เชิงวิชาการ:** การสร้างบัณฑิตใหม่โดยงานวิจัยบางส่วนที่ได้รับทุนจาก สกว ได้เป็นส่วนหนึ่งของวิทยานิพนธ์และดุษฎีนิพนธ์ของนิสิตซึ่งมีผู้ได้รับทุนวิจัยเป็นอาจารย์ที่ปรึกษาโดยนิสิตดังกล่าวได้สำเร็จการศึกษาในภาคการศึกษาต้น ปีการศึกษา 2558 ดังนี้

1. นางสาวนิภาพรรณ ฤาชา นิสิตระดับปริญญาเอก หลักสูตรวิทยาศาสตร์มหโมเลกุล คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
2. นางสาวนาฏตินันท์ พรหมเพชร นิสิตระดับปริญญาโท หลักสูตรปิโตรเคมีและพอลิเมอร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ภาคผนวก

ประกอบไปด้วยสำเนาบทความจำนวน 3 เรื่อง และสำเนาบทคัดย่องานประชุมวิชาการ 1 เรื่อง ที่ได้รับการสนับสนุนจาก สกว ซึ่งมีผู้ได้รับทุนวิจัยเป็นผู้แต่งชื่อแรกหรือเป็น corresponding author เรียงตามลำดับดังนี้

สำเนาบทความที่ได้รับการตีพิมพ์จำนวน 3 เรื่องดังนี้

1) Ruecha, N., Rangkupan, R, Rodthongkum, N., Chailapakul, O. “Novel Baper-based Cholesterol Biosensor Using Graphene/Polyvinylpyrrolidone/Polyaniline Nanocomposite” *Biosens. Bioelectron*. 2014, *52*, 13-19. *(2014 Impact Factor: 6.451)*

2) Rodthongkum, N., Ruecha, N., Rangkupan, R., Vachet, R. W., Chailapakul, O. “Graphene-Loaded Nanofiber-Modified Electrodes for the Ultrasensitive Determination of Dopamine” *Anal Chim Acta.* 2013, *804*, 84-91. *(2014 Impact Factor: 4.517)*

3) Promphet, N., Rattanarat, P., Rangkupan, R., Chailapakul, O., Rodthongkum, N. “An Electrochemical Sensor Based on Graphene/Polyaniline/Polystyrene Nanoporous Fibers Modified Electrode for Simultaneous Determination of Lead and Cadmium” *Sensor Actuat. B-Chem.* 2015, *207*, 526-534. *(2015 Impact Factor: 3.840)*

สำเนาบทคัดย่อจำนวน 1 เรื่องดังนี้

1) Graphene/polyaniline nanocomposite along with MALDI-MS for sensitive detection of small biomolecules

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Novel paper-based cholesterol biosensor using graphene/polyvinylpyrrolidone/polyaniline nanocomposite

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ABSTRACT

A novel nanocomposite of graphene (G), polyvinylpyrrolidone (PVP) and polyaniline (PANI) has been successfully prepared and used for the modification of paper-based biosensors via electrospraying. The droplet-like nanostructures of G/PVP/PANI-modified electrodes are obtained with an average size of 160 ± 1.02 nm. Interestingly, the presence of small amount of PVP (2 mg mL^{-1}) in the nanocomposites can substantially improve the dispersibility of G and increase the electrochemical conductivity of electrodes, leading to enhanced sensitivity of the biosensor. The well-defined cyclic voltammogram of standard ferri/ferrocyanide is achieved on a G/PVP/PANI-modified electrode with a 3-fold increase in the current signal compared to an unmodified electrode. This modified electrode also exhibits excellent electrocatalytic activity towards the oxidation of hydrogen peroxide (H_2O_2). Furthermore, cholesterol oxidase (ChOx) is attached to G/PVP/PANI-modified electrode for the amperometric determination of cholesterol. Under optimum conditions, a linear range of 50 μM to 10 mM is achieved and the limit of detection is found to be 1 μM for cholesterol. Finally, the proposed system can be applied for the determination of cholesterol in a complex biological fluid (*i.e.* human serum).

1. Introduction

The development of accurate, sensitive and low-cost biosensor is crucial for early stage screening of disease biomarkers. Recently, cellulose filter paper has become an attractive material for sensor applications due to its large surface area and low cost (Apilux et al., 2010; Dungchai et al., 2011; Songjaroen et al., 2011). Compared to other traditional substrates (*i.e.,* glass, ceramic and polymer), paper-based biosensors offers several advantages, such as low cost, high abundance, biocompatibility and disposability. Furthermore paper-based analysis only requires a small amount of samples and reagents, which make it suitable for biosensor applications (Dungchai et al., 2009). Among the detection techniques, electrochemical detection has attracted much attention due to its ease of use, portable field-based size, high specificity and rapid analysis. Moreover, both qualitative and quantitative information can be obtained simultaneously. Paper-based electrochemical biosensors have been applied to various applications, including clinical diagnosis (Dungchai et al., 2009, 2011), environmental monitoring (Apilux et al., 2010; Nie et al., 2010), and food quality control (Hossain et al., 2009). Paper-based biosensors can be fabricated by several methods, such as photolithography (Apilux et al., 2010; Dungchai et al., 2009), wax screen-printing (Dungchai et al., 2011), wax-dipping (Songjaroen et al., 2011), and wax-printing (Lu et al., 2010; Mentele et al., 2012). In this study, wax-printing is selected to create the disposable paper-based biosensors. Nowadays, an important limitation of paper-based electrochemical biosensors for the detection of low abundant biomarkers is the limited sensitivity; therefore, modification of paper based biosensor with the ultrahigh surface area material, such as metallic nanoparticles and carbon based nanostructures, is still greatly required to improve the sensor sensitivity.

In recent years, graphene (G) has received tremendous attention due to its remarkable physical, chemical, mechanical, and electrical properties. G is a single layer of carbon atoms closely

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packed into a two-dimensional honeycomb arrangement. For fabrication of G-based electronic material, it can provide excellent electrical conductivity and electron mobility. In addition, the small band gap of G is desirable for conducting electrons from target molecules in electrochemical biosensors. The ultrahigh surface area of G is also very useful for loading of bioreceptors (*i.e.,* enzyme, antibody) on its surface. It has been reported that G-based chemical sensors possess very high sensitivity because of the low electronic noise from thermal effect (Geim and Novoselov, 2007; Liu et al., 2012b; Potts et al., 2011). Compared to other carbon allotropes (*e.g.* carbon nanotubes: CNTs), G can be obtained easily by using a chemical conversion of inexpensive graphite (Geim and Novoselov, 2007).

Due to the planar sp^2-carbon of G, it has high tendency to agglomerate together via van der Waals attraction. In terms of applications, preparation of a well-dispersed G solution has become a crucial step. Recently, it has been reported that poly (vinylpyrrolidone) (PVP) can be used to stabilize the high concentration of G in a wide range of organic solvents (Wajid et al., 2012). G/PVP has been used to modify the electrodes for biosensor applications (Liu et al., 2012b; Mano and Heller, 2005). For electrode modification, the nanocomposites between G and conducting polymers have attracted more attention than the pure form of G because the composites are more compatible for electrode fabrication and biofunctionalization (Arya et al., 2011; Huang et al., 2011; Liu et al., 2012a; Qiu et al., 2012). Moreover, using conducting polymers as a matrix for G dispersion can further enhance the sensitivity of electrochemical biosensors. Various conducting polymers, including polyaniline (PANI) (Arya et al., 2011; Huang et al., 2011; Liu et al., 2012b; Qiu et al., 2012), polypyrrole (PPy) (Li et al., 2012; Lu et al., 2012), and poly(3,4-ethylenedioxythiophene) (PEDOT) (Jiang et al., 2013; Karuwan et al., 2012) have been used in biosensors. Among the conducting polymers, PANI is a promising material due to its excellent electrochemical properties, ease of synthesis and functionalization, high environmental stability, and low toxicity. Previously, it has been reported that the conducting form of PANI can be simply prepared by doping PANI with an acid, such as camphorsulfonic acid (CSA) in chloroform (Shin and Kameoka, 2012). Additionally, a number of amino groups ($-NH_2$) of PANI can be readily functionalized with biomolecules (Arya et al., 2011; Qiu et al., 2012), which make it very attractive for biosensor applications. Thus, the development of G/PVP/PANI nanocomposite modified paper-based biosensor is focused in this study.

To fabricate G/PVP/PANI nanocomposites on the paper-based biosensor, electrospraying is selected because a 3D droplet-like nanostructure can be created on the modified electrode surface. Compared to thin-film modified electrodes, G/PVP/PANI-nanodroplet-modified electrodes offer a higher specific surface area, which leads to an enhanced electrochemical sensitivity of the biosensors. Increasing the electrode surface area through this method might be very useful for further biofunctionalization, such as enzyme loading.

Biomarkers are biomolecules that can indicate a normal or pathogenic process in a biological system, including the level of exposure to environmental factors, genetic susceptibility, and an indication of response to therapy. One of the most important biomarkers for cardiovascular disease and high blood pressure is cholesterol; therefore, development of a method that can quantitatively determine the cholesterol level is very crucial. The conventional method based on spectrophotometry has been widely used for cholesterol determination (Arya et al., 2007; Dhand et al., 2007); however, this technique requires an expensive instrument and complicated sample preparation. With the advent of nanomaterials, metal nanoparticles and carbon-based nanomaterials have been applied for electrochemical biosensors to improve the electrochemical performance of cholesterol biosensors (Dey and Raj, 2010; Dhand et al., 2007; Eguilaz et al., 2011; Manjunatha et al., 2012).

Herein, a novel nanocomposite of G/PVP/PANI was prepared and used to modify the working electrode of a paper-based biosensor via electrospraying. The droplet-like structures of the G/PVP/PANI-nanocomposite-modified electrode was used for the sensitive determination of H_2O_2 and cholesterol using amperometry. The performance of this sensing system was optimized and then applied to the determination of cholesterol concentration in a complex biological fluid (*e.g.,* human serum).

2. Material and methods

2.1. Chemicals and materials

Graphene (G) nanopowders were purchased from SkySpring Nanomaterials, Inc. (Houston, TX). Cholesterol and 418 U mg^{-1} cholesterol oxidase (ChOx) from *Streptomyces* sp., sodium dodecyl sulfate (SDS), polyoxyethylene octyl phenyl ether (Triton X-100), camphor-10-sulfonic acid (CSA), polyaniline (PANI), polystyrene (Mw ~ 180,000; PS), poly (vinyl pyrrolidone) (Mw = 10,000; PVP) and trichloroacetic acid (TCA) were obtained from Sigma (St. Louis, MO). Dimethylformamide (DMF), Potassium dihydrogen phosphate (KH_2PO_4) and chloroform were purchased from Carlo Erba Reagenti-SDS (Val de Reuil, France). Disodium hydrogen phosphate (Na_2HPO_4), Potassium chloride (KCl), and Sodium chloride (NaCl) were purchased from Merck (Darmstadi, Germany). Carbon ink and silver/silver chloride ink were obtained from Gwent group (Torfaen, United Kingdom). Filter paper grade no. 1 (size, 46 × 57 cm^2) was purchased from Whatman. All chemicals were used as received without further purification. All solutions were prepared by using high-purity water from MilliQ Water System (Millipore, USA, $R \geq 18.2$ MΩ cm^{-1}). Phosphate buffered saline (PBS) was prepared by dissolving 0.144% (w/v) Na_2HPO_3, 0.024% (w/v) KH_2PO_4, 0.02% (w/v) KCl, 0.8% (w/v) NaCl in high-purity water. A stock solution of cholesterol was prepared in 5% (w/v) of Triton X-100 and high-purity water and then stored at 4 °C. A stock solution of ChOx was freshly prepared in PBS (Ruecha et al., 2011). For the determination of the cholesterol in a real biological sample, lyophilized human serum (CONSERA), obtained from Nissui Pharmaceutical, was used (Tokyo, Japan). The serum samples were precipitated using TCA prior to use.

2.2. Apparatus

All electrochemical measurements, including cyclic voltammetry and amperometry, were performed on a CHI 1232A electrochemical analyzer (CH Instruments, Inc., USA). A three electrode system was used and the working electrode was a G/PVP/PANI-modified, screen-printed carbon electrode (4 mm in diameter). An in-house electrospraying system was used for the electrode modification. A JSM-6400 field emission scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd, Japan) with an accelerating voltage of 15 kV and a JEM-2100 transmission electron microscope (Japan Electron Optics Laboratory Co., Ltd, Japan) were used for the electrode characterization.

2.3. Fabrication of paper-based biosensor

In this work, paper-based biosensor was fabricated using wax-printing method according to a previous report (Mentele et al., 2012) with slight modification. First, the patterned paper-based biosensor was designed by Adobe Illustrator and then printed onto filter paper (Whatman no. 1) using a wax printer (Xerox Color Qube 8570, Japan). Next, the printed paper-based biosensor was

placed on a hot plate at 175 °C for 40 s to melt the wax. The area covered with wax was hydrophobic, and the area without wax was hydrophilic. The block screen was designed with Adobe Illustrator software and fabricated by Chaiyaboon Co. (Bangkok, Thailand). For three electrode system of the paper-based biosensor, a working electrode (WE) and a counter electrode (CE) were screen-printed in-house using carbon ink. Silver/silver chloride (Ag/AgCl) ink was used as a reference electrode (RE) and conductive pad.

2.4. Electrospraying fabrication of G/PVP/PANI nanocomposites modified paper based biosensor

Firstly, PANI was doped with CSA to generate a conductive form of PANI and dissolved in chloroform (Shin and Kameoka, 2012). Then the stock solution of PVP was prepared by dissolving of 2 mg mL^{-1} of PVP in DMF and stirring for 10 min at room temperature. G dispersion was prepared by the adding 2 mg mL^{-1} of G into the stock solution of PVP and sonicating for 6 h at room temperature. After that, the solutions of PANI and G/PVP were mixed together, and 0.1% (v/v) PS was added into G/PVP/PANI solution. An electrospraying system consists of syringe pump, high-voltage power supply, ground collector, syringe and stainless-steel needle. During electrospraying, the CE and RE, were covered with masks to prevent the electrode modification. The G/PVP/PANI nanocomposite solution was mixed thoroughly in a syringe, and a high voltage was applied to the solution. The nanocomposite solution was electrosprayed onto the WE of a paper-based biosensor attached to a ground collector on a rotating drum. The flow rate was controlled at 1.0 mL h^{-1}, the distance between the needle tip and ground collector was fixed at 5 cm, and the applied high voltage was 6 kV.

2.5. Electrochemical measurement

A three-electrode system fabricated on a paper-based biosensor was used throughout the experiment. To control all electroanalytical measurements, a potentiostat (CHI 1207A, CH Instruments, Austin, TX) was used. For the cyclic voltammetric measurements, the potential was scanned from −0.3 V to +0.8 V for ferri/ferrocyanide detection and +0.2 V to +1.0 V for H_2O_2 detection. Hydrodynamic voltammogram was employed to optimize the detection potential for H_2O_2 in the range of +0.2 to +0.8 V. The standard solution of H_2O_2 was dropped in the paper-based device and the current measured at a fixed time with different potentials. For the cholesterol detection, the volume of 418 U mL^{-1} ChOx enzyme was investigated between 0.1 and 1.0 μL. Prior to the detection of cholesterol, an optimum volume of ChOx enzyme was drop-cast and dried onto the surface of the G/PVP/PANI-modified electrode, and amperometry was performed at an optimum detection potential. After that, the anodic current was recorded at the steady state current for 100 s.

2.6. Preparation of Human serum

Prior to analysis, 3.0 mL of high purity water was added to the lyophilized human serum, and then the proteins in the serum were precipitated using TCA method (Rattanarat et al., 2012). For the protein precipitation, 200 μL of human serum, 50 μL of standard cholesterol at different concentration, and 250 μL of 10% (w/v) TCA were mixed together by vortexing for 5 min. The mixed solution was centrifuged at 6000 rpm (Cole-Parmer, USA) for 10 min and the supernatants were kept for further analyses. All samples were analyzed on paper-based sensors using amperometry.

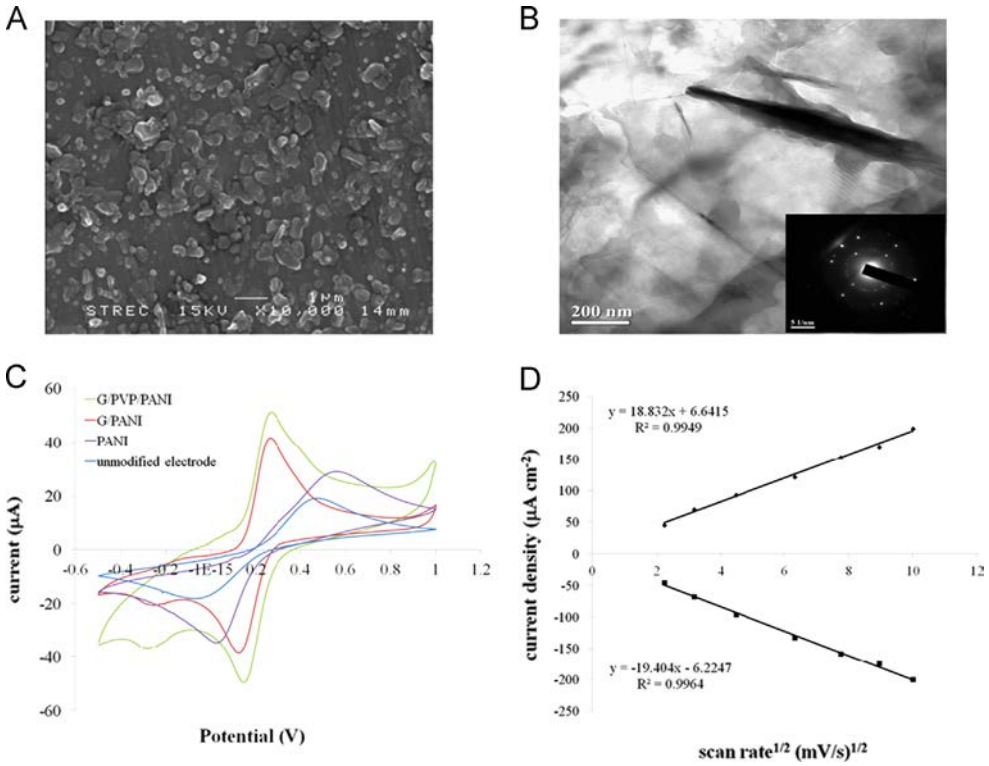


Fig. 1. Physical and electrochemical characterization of G/PVP/PANI nanocomposite-modified electrodes, (A) SEM image of G/PVP/PANI modified electrode (6 kV applied voltage), (B) TEM image of G/PVP/PANI with the electron diffraction pattern of G (inset of Fig. 1B), (C) cyclic voltammograms of 2.0 mM ferri/ferrocyanide in 0.1 M KCl measured on different working electrodes of the paper-based biosensor with a scan rate of 100 mV s^{-1} and (D) the relationship between the square root of scan rate ($\nu^{1/2}$) and peak current measured from 5 and 100 mV s^{-1}. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Characterization of G/PVP/PANI nanocomposites modified paper-based biosensor

Other than the high surface area of G, electrospraying was selected for electrode modification to further increase the surface area of the working electrode. The morphology of the G/PVP/PANI-modified electrode and unmodified electrode on a paper substrate were characterized using scanning electron microscopy (SEM) as shown in Fig. 1A and Fig. S1 (Supporting information), respectively. Interestingly, the 3D droplet-like structures of G/PVP/PANI can be uniformly generated on the modified electrode surface; the average size of each droplet was found to be 160 ± 1.02 nm, as shown in Fig. 1A. Moreover, TEM image of G/PVP/PANI indicate a good dispersion of G inside the nanocomposites without severe aggregation (Fig. 1B), and the electron diffraction pattern of G (inset of Fig. 1B) is matched very well with the previous report (Karuwan et al., 2012). TEM images of G/PVP and G/PANI were shown in Fig. S2 (Supporting information).

For electrospraying, the effect of applied voltage, spraying time, and G/PANI ratio on the electrochemical conductivity of modified electrodes were investigated and optimized. SEM images of G/PVP/PANI modified electrode and anodic current response of 2 mM ferri/ferrocyanide measured on the electrodes prepared by using different applied voltage was shown in Fig. S3 (Supporting information). In this study, 6 kV of applied voltage, 5 min of spraying time, and 1:1 of G/PANI ratio were selected for further experiments. To prepare the nanocomposite of G/polymer, other than PANI conducting media, PVP and PS were selected as graphene stabilizer and carrier polymer for electrospraying, respectively. To investigate the electron transfer process, cyclic voltammetry was performed on different electrodes including G/PVP/PANI, G/PANI, PANI and unmodified carbon electrodes using ferri/ferrocyanide as a redox probe. As shown in Fig. 1C, the anodic and cathodic peak currents of ferri/ferrocyanide show the well-defined peaks for all electrodes. The highest anodic and cathodic peak currents of ferri/ferrocyanide were observed on G/PVP/PANI modified electrode (green line) indicating the high sensitivity of the system. The peak currents gradually decrease for G/PANI, PANI and unmodified carbon electrode, respectively. Compared to PANI modified electrode (purple line), incorporation of G into the nanocomposites can increase both anodic and canodic peak currents of ferri/ferrocyanide (red line). Interestingly, the peak-to-peak potential separation (ΔEp) of ferri/ferrocyanide measured on G/PANI modified electrode ($\Delta Ep = 0.155$; red line) significantly decreases when compared to ΔEp obtained from PANI modified electrode ($\Delta Ep = 0.508$; purple line), indicating that the presence of G in the nanocomposites can accelerate the electron transfer kinetics of the system. Moreover, it can be noticed that using PVP as a stabilizer for G dispersion can further increase the anodic and cathodic peak currents in this sensing system as shown in Fig. 1C (green line *vs.* red line). Another important advantage of using PVP is decreasing of dispersion time of G from 24 h to 6 h (Wajid et al., 2012). The results of graphene dispersion in N,N-dimethylformamide (DMF) with and without PVP are shown in Fig. S4 (Supporting information). In general, without using PVP, the long dispersion time (24 h) was required for G dispersion to prevent its reaggregation to graphite form; therefore, 2 mg mL^{-1} of PVP in DMF was used as a stabilizer for G dispersion in all further experiments.

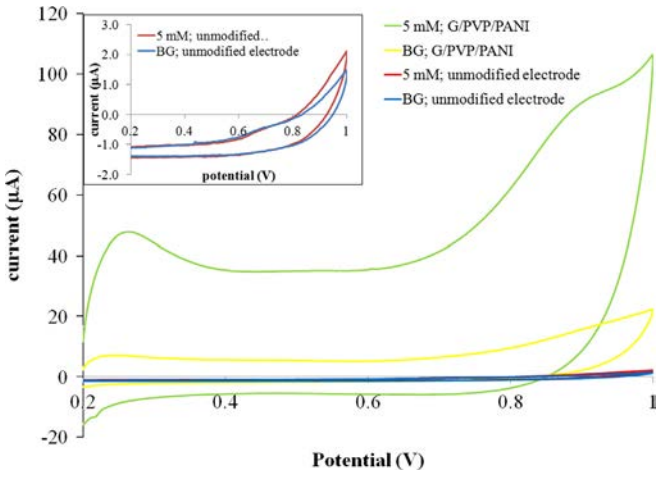


Fig. 2. Cyclic voltammograms of 5 mM hydrogen peroxide (H_2O_2) in 0.1 M PBS pH 7.0 measured on a G/PVP/PANI-modified electrode and unmodified carbon electrode at a scan rate of 100 mV s^{-1}. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Prior to the analysis of hydrogen peroxide and cholesterol, the electrochemical behavior of the G/PVP/PANI-modified paper-based biosensor was examined using standard ferri/ferrocyanide as a redox probe. 70 μL of a 2.0 mM ferri/ferrocyanide solution in 0.1 M KCl was directly dropped onto the modified electrode surface, and the cyclic voltammetric measurements were performed at different scan rates. The relationship between the square root of the scan rate ($\nu^{1/2}$) and the current density was plotted, as shown in Fig. 1D. Both anodic and cathodic peak currents of ferri/ferrocyanide are linearly proportional to the square root of the scan rate in the range from 2 to 100 mV s^{-1}. These results verify that the redox process is controlled by diffusion process.

3.2. Detection of hydrogen peroxide and cholesterol

For an electrochemical biosensor, the determination of the H_2O_2 product obtained from cholesterol oxidation can be used for the indirect quantification of cholesterol (Aravind et al., 2011; Manjunatha et al., 2012). In this study, a novel paper-based biosensor based on a G/PVP/PANI-modified screen-printed carbon electrode was used for the sensitive determination of H_2O_2 and cholesterol using cyclic voltammetry and amperometry. The cyclic voltammograms of H_2O_2, measured on the G/PVP/PANI-modified electrode and unmodified carbon electrode are illustrated in Fig. 2A. A dramatic increase (40 times) in the anodic current signal of H_2O_2 is observed (green line) when compared to an unmodified carbon electrode (red line), indicating that the modified electrode might be a promising tool for sensitive detection of cholesterol. The cyclic voltammograms of cholesterol measured on G/PVP/PANI-modified electrode was shown in Fig. S5 (Supporting information).

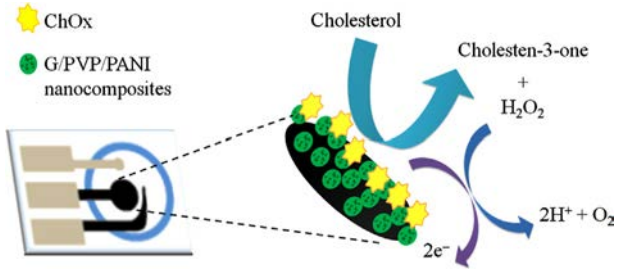
Due to the high sensitivity and wide applicability, chronoamperometry was selected for the electrochemical detection of H_2O_2 and cholesterol. Initially, the detection potential was investigated and optimized as shown in Fig. S6A and B (Supporting information). A hydrodynamic voltammogram of H_2O_2 was optimized as shown in Fig. S6A (Supporting information), the anodic current signal of H_2O_2 significantly increased as the detection potential increased (blue line); however, the background current also increased (green line). Therefore, a hydrodynamic voltammogram of signal-to-background ratios (S/B) was investigated instead of the current signal, as shown in Fig. S6B (Supporting information). The S/B ratio measured at +0.6 V shows the highest sensitivity for H_2O_2; therefore, +0.6 V was selected as the amperometric detection potential for further experiments.

For cholesterol biosensor, H_2O_2 is generated from the enzymatic reaction between cholesterol and ChOx as shown in Scheme 1. Therefore, it is important to optimize the amount of ChOx on G/PVP/PANI modified paper-based biosensor. In this study, different volumes of ChOx were directly dropped on the modified electrodes and dried out at room temperature for 10 min. The effect of enzyme

volume on the anodic current signal of 1 mM cholesterol was studied in the range of 0.1–1.0 μL and the optimum volume of enzyme was found to be 0.4 μL as shown in Fig. S7 (Supporting information).

3.3. Analytical performance of G/PVP/PANI nanocomposites modified paper-based biosensor

G/PVP/PANI-modified paper-based biosensor was used to measure cholesterol at different concentrations, and the amperometric current responses were recorded at a steady state current of 100 s to create a calibration curve for the cholesterol. As shown in Fig. 3, the calibration plot was linearly proportional to the cholesterol concentration over the range of 50 μM to 10 mM with a correlation coefficient of 0.9993. The detection sensitivity of the system, calculated by dividing the slope of linear range curve by the surface area using projection area (Parlak et al., 2013), was found to be 34.77 μA mM^{-1} cm^{-2}. The limit of detection (LOD) and limit of quantitation (LOQ) for cholesterol were 1 μM and 50 μM, respectively. LOD was calculated as the concentrations which produced the signal at 3 times of the standard deviation of a blank ($n=5$) (Dungchai et al., 2009). Previously, it has been reported by the national cholesterol education program (NCEP) that the normal level of total blood cholesterol in human is lower than 5.18 mM (200 mg dL^{-1}) (Ahmadalinezhad and Chen, 2011). This information confirms that our system can be applied for the determination of cholesterol in real biological samples. The electrochemical performance of the G/PVP/PANI-modified electrode was compared to the other modified electrodes used for the detection of cholesterol as shown in Table 1. Our electrode shows a relatively high electrochemical sensitivity, a wide linear range and a comparable LOD for cholesterol detection. Because the G/PVP/PANI-modified paper-based biosensor proposed in this study is easily prepared and inexpensive, it might be a promising tool for cholesterol detection.



Scheme 1. The enzymatic reaction between cholesterol and ChOx on G/PVP/PANI modified paper-based biosensor.

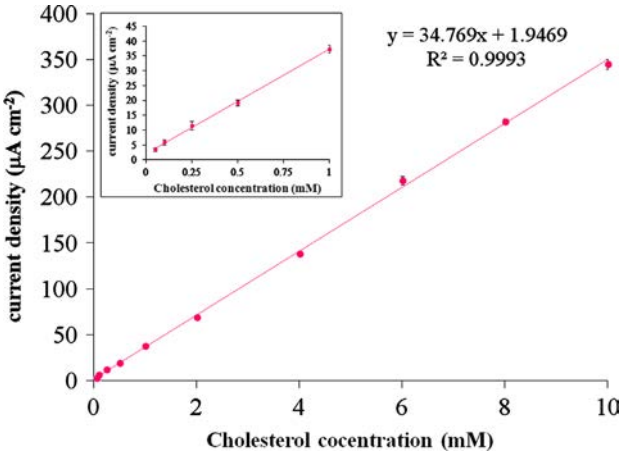


Fig. 3. The calibration graph for the detection of cholesterol over the concentration range of 50 μM to 10 mM and calibration graph between 50 μM and 1 mM (inset) in 0.1 M PBS pH 7.0.

3.4. Interference study

Previously, it has been reported that glucose and ascorbic acid are the common interfering molecules in the detection of cholesterol in complex biological fluids (*e.g.,* human serum). Therefore, selective determination of cholesterol in the presence of glucose and ascorbic acid was investigated using the highest anticipated concentrations of glucose (5.3 mM) and ascorbic acid (80 μM) in human serum (Ahmadalinezhad and Chen, 2011; Dungchai et al., 2009; Rattanarat et al., 2012). As shown in Fig. S8A (Supporting information), the amperometric result of a mixture of cholesterol and glucose shows a negligible effect on the current response (blue bar), while the ascorbic acid has an effect on the current response (green bar). To solve the problem of ascorbic acid interference, an anionic surfactant (SDS) was used to coat on the G/PVP/PANI-modified electrode. Recently, it has been reported that an electrostatic repulsion between anionic SDS and anionic AA at pH 7.4 (pKa=4.1) can prevent the interference effect from AA in the detection of target analytes (Rattanarat et al., 2012).

Table 1
Comparison of various cholesterol biosensors based on nanomaterial/polymer modified electrodes.

Modified electrode	Detection method	Sensing element	Method of enzyme immobilization	LOD (μM)	Linear range (mM)	Sensitivity (μA mM^{-1} cm^{-2})	Reference
$NiFe_2O_4$/CuO/FeO-Ch/ChOx	DPV	ChOx	Physical adsorption	0.0313	0.13–12.95	16.54	Singh et al. (2012)
Ti/NPAu/ChOx–HRP–ChE	CV	ChOx/ChEt	Entrapment	12.95	0.97–7.8	29.33	Ahmadalinezhad and Chen (2011)
AuE/dithiol/AuNPs/MUA/ ChOx	CV	ChOx	Covalent attachment	34.6	0.04–0.22	45.96	Saxena et al. (2011)
ChOx-FG/G	Amperometry	ChOx	Covalent attachment	5	0.05–0.35	–	Manjunatha et al. (2012)
AuPt–Ch–IL/GCE	Amperometry	ChOx	Cross-linking	10	0.05–6.2 and 6.2–11.2	90.7	Safavi and Farjami (2011)
AuNPs/f-G modified GCE	Amperometry	ChOx	Physical adsorption	–	0–0.135	314	Aravind et al., (2011)
CSNF–AuNPs/ChOx	Amperometry	ChOx	Physical adsorption	0.5	0.001–0.045	1.02	Gomathi et al. (2011)
ChOx/HRP/AuNPs/PDDA/ MWCNTs/GCE	Amperometry	ChOx	Physical adsorption	2.2	0.01–1.05	18.6	Eguilaz et al. (2011)
G/PVP/PANI nanocomposites	Amperometry	ChOx	Physical adsorption	1	0.05–10	34.77	Present work

Abbreviations: nPt, platinum nanoparticle; GCE, glassy carbon electrode; AuNPs, gold nanoparticles; f-G, functionalize graphene nanoplatelets; IL, ionic liquid; Ch, chitosan; HRP, horseradish peroxidase; ChEt, cholesterol esterase; CSNF, chitosan nanofibers; AuE, gold electrode; MUA, 11-mercaptoundecanoic acid; PDDA, poly-(diallyldimethylammonium chloride); MWCNTs, multi-walled carbon nanotubes.

In this study, 2 μL of SDS was dropped on the electrode surface and dried at room temperature. The optimum concentration of SDS was found to be 2 mM, which can prevent the effect of AA interference over the concentration range from 0 to 120 μM in the detection of 1 mM cholesterol (Fig. S8B, Supporting information). For G/PVP/PANI modified electrode coated with SDS, the linear relationship was found to be 0.05–10 mM, which is similar to the one obtained from the uncoated SDS electrode as shown in Fig. S9 (Supporting information).

3.5. Reproducibility and stability of the G/PVP/PANI-modified electrode

The reproducibility and stability of G/PVP/PANI nanocomposite-modified electrode were investigated by using amperometric detection of 1 mM cholesterol. The relative standard deviations (RSD) of all cholesterol concentrations in the linear range were found between 1.05% and 9.37% ($n=5$), demonstrating acceptable reproducibility for this paper-based device. The storage stability of the biosensor was studied by measuring the current responses of 1 mM using prepared paper-based biosensor. It retained 89.1% of its initial response after a storage period of 2 weeks indicating that this paper-based biosensor had good stability.

3.6. Sample analysis

To test the applicability of this system, G/PVP/PANI-modified paper-based biosensor was used for the detection of cholesterol in human serum. The blank human serum samples are the common systems for the determination of the accuracy and validation of a new diagnostic assay. Initially, different concentrations of cholesterol (0.05, 0.10, 0.25, 1.00, and 5.00 mM) were spiked into the human serum, and the proteins in the serum were precipitated by using TCA. After centrifugation, the supernatant was kept for further amperometric analysis using the G/PVP/PANI-modified paper-based biosensor. The results indicated that the current responses depended on the cholesterol concentration. An acceptable linearity with a correlation coefficient (R^2) of 0.9998 ($n=3$) was achieved. The percentages of recoveries (Table 2) were found in the range of 100.0–102.0%, and the RSD was less than 5.0%, verifying that this sensing system is highly accurate.

4. Conclusions

A novel nanocomposite based on G/PVP/PANI has been prepared and used for the modification of a paper-based cholesterol biosensor. The high conductivity and large surface area of the droplet-like nanostructures of the G/PVP/PANI-modified electrode significantly improves the electrochemical sensitivity for the detection of H_2O_2. Under optimum conditions, a high-sensitivity, wide linear range and low limit of detection for cholesterol using proposed electrode is achieved. The interferences in the detection of cholesterol can be easily eliminated by using SDS. In addition, this sensing system was successfully applied for the determination of cholesterol in human serum. This novel and sensitive paper-based biosensor might be an alternative tool for cholesterol screening in medical diagnosis due to its simplicity, low cost, disposability and portability.

Table 2
Determination of cholesterol in human serum samples ($n=3$).

Cholesterol concentration (mM)		% Recovery	% RSD
Added	Found		
0.05	0.05 ± 0.04	100.0	0.8
0.10	0.10 ± 0.08	100.0	0.8
0.25	0.25 ± 0.30	100.0	1.2
1.00	1.02 ± 1.05	102.0	1.0
5.00	5.01 ± 2.84	100.2	0.6

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.08.018.

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Graphene-loaded nanofiber-modified electrodes for the ultrasensitive determination of dopamine

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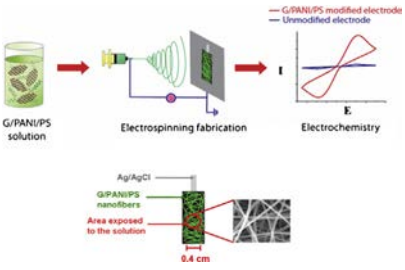
HIGHLIGHTS

- A novel electrode based on electrospun graphene/polyaniline/polystyrene nanofibers has been developed.
- The proposed system provides ultrahigh sensitivity, good selectivity and wide linearity for the determination of dopamine.
- This system was successfully applied to determine dopamine in complex biological environment with excellent reproducibility.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and highly sensitive electrochemical system based on electrospun graphene/polyaniline/polystyrene (G/PANI/PS) nanofiber-modified screen-printed carbon electrodes has been developed for dopamine (DA) determination. A dramatic increase (9 times) in the current signal for the redox reaction of a standard, ferri/ferrocyanide $[Fe(CN)_6]^{3-/4-}$ couple was found when compared to an unmodified electrode. This modified electrode also exhibited favorable electron transfer kinetics and excellent electrocatalytic activity toward the oxidation of DA. When used together with square wave voltammetry (SWV), DA can be selectively determined in the presence of the common interferents (i.e. ascorbic acid and uric acid). Under optimal conditions, a very low limit of detection (0.05 nM) and limit of quantification (0.30 nM) were achieved for DA. In addition, a wide dynamic range of 0.1 nM to 100 μM was found for this electrode system. Finally, the system can be successfully applied to determine DA in complex biological environment (e.g. human serum, urine) with excellent reproducibility.

1. Introduction

Dopamine (DA) is one of the most important natural catecholamine neurotransmitters in the mammalian brain, playing a significant role in the function of the central nervous, renal and hormonal systems. Abnormal levels of DA may result in neurological disorders, such as Parkinson's disease and Schizophrenia

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[1–4]. The development of simple and accurate methods for the sensitive determination of DA in biological fluids is crucial to conveniently trace and diagnose diseases. Various analytical techniques including capillary electrophoresis (CE), high performance liquid chromatography (HPLC), flow injection analysis and spectrophotometry have been used to determine DA [5–8]; however, these existing techniques require time-consuming sample preparation and expensive instrumental equipment. Due to its electrochemical activity, the electroanalysis of DA has attracted tremendous interest [9–12]. Nonetheless, an important challenge when measuring DA under physiological condition is the presence of very low levels of DA, which usually lead to false-negative results. Furthermore, the fouling of the electrode surface by the DA oxidation products can reduce the performance of conventional electrodes and lead to a low detection sensitivity [13]. Thus, the development of new electrochemical systems for the sensitive determination of DA is still greatly required for medical diagnoses.

To enhance the detection sensitivity of electrochemical systems, a variety of carbon nanostructures, such as carbon nanotubes (CNTs), carbon nanofibers (CNFs) and carbon nanodots (CNDs) have been used for electrode surface modifications [14–17]. Recently, graphene (G), a two-dimensional sheet of carbon atoms bonded through sp^2 hybridization, has been considered as a "rising-star" carbon material. G has been intensively researched because of its remarkable properties, such as large surface area, high electrical and thermal conductivities, high mechanical strength and potentially low manufacturing cost [18–20]. The attachment of G with electrode surfaces and bioreceptors is possible through a plethora of nanocomposites [21]. Compared to other carbon allotropes, G has a higher surface area, more excellent electrical conductivity and electron mobility at room temperature [22,23]. The ultra-high surface area of G can provide more active sites to expose to the analytes, leading to enhanced electrochemical sensitivity without compromising temporal responses. Thin film electrodes based on G and its nanocomposites have been used for the electrochemical detection of various biomolecules [24–26].

Conducting polymers, such as polyaniline (PANI), polypyrrole (PPY), poly(3,4-ethylenedioxythiophene) (PEDOT) and their composites, have also been widely used in electrode surface modifications [27–29]. Of these conducting polymers, PANI has attracted much attention due to its unique and controllable chemical and electrical properties, easy synthesis, biocompatibility, high electrochemical and environmental stabilities [30]. The redox reactions and electrocatalytic property of PANI have been reported previously [31,32]. PANI can be used as a redox system for transporting electrical charges and immobilized matrices for bioreceptors in biosensors. The advantages of PANI in electrochemical biosensor are indicated by impressive signal amplification and elimination of electrode fouling [30]. Recently, it has been reported that doping PANI with carbon based nanomaterials (*e.g.* CNTs and G) can remarkably enhance both the electrocatalytic activity and mechanical strength, leading to improved performance of these nanocomposite electrodes [26,33].

Electrospinning fabrication has emerged as a versatile and cost-effective method for producing large specific surface area and high porosity nanofibers with three dimensional (3D) structures. These properties are very attractive for sensing applications [34,35]. For electrochemistry, an important advantage of electrospun nanofiber-modified electrodes compared to thin film coated electrodes is the surface area. Electrospun nanofibers were reported to possess surface area per unit volumes up to two orders of magnitude higher than those of continuous thin films [35]. This relatively huge surface area can potentially provide ultra-high sensitivity and a fast response time to electrochemical detection. However, because electrospun conducting nanofibers are usually produced from a blended solution between conducting and non-conducting polymers, these nanofibers can be handicapped in terms of their electrical properties compared to conducting nanofibers prepared via other methods, such as electrodeposition or electropolymerization. For electrochemical applications, an effective strategy to improve the electrical properties of electrospun conducting nanofibers is incorporation of conducting nanomaterials (*e.g.* gold nanoparticles (AuNPs), CNTs and G) into the nanofibers [33,36,37].

In this study, a novel electrode system was constructed and then applied for the sensitive determination of DA by combining the advantages of G, PANI and electrospinning fabrication. The electrospun G/PANI/PS nanofiber-modified electrodes were characterized and then used for the selective determination of DA in the presence of ascorbic acid (AA) and uric acid (UA) via square wave voltammetry (SWV). The analytical performance of the proposed system was evaluated, and the optimal condition was used to determine DA in complex biological matrix (*e.g.* human serum, urine). In addition, the performance of this proposed system was compared to the previously reported systems. This novel system has great promise to be extended for electrochemical based biosensor applications in the future.

2. Experimental

2.1. Materials

2.1.1. Materials and reagents

Graphene nanopowders (99.5+ %C) with average thickness of 11–15 nm were purchased from SkySpring Nanomaterials. Inc. (Houston, TX, USA). The electrical conductivities of graphene nanopowders are 10^7 S m^{-1} (parallel to surface) and 10^2 S m^{-1} (perpendicular to surface). The thermal conductivities of graphene nanopowders are 3000 W m^{-1} K (parallel to surface) and 6 W m^{-1} K (perpendicular to surface). Transmission electron microscopy (TEM) image and X-ray photoelectron spectroscopy (XPS) spectrum of G nanopowders used in this work are shown in Figs. S1 and S2 in the Supporting Information. Polyaniline emeraldine base (M_W = 65,000), (+)-camphor-10-sulfonic acid (CSA), polystyrene (M_W = 180,000), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), trichloroacetic acid (TCA), phosphate buffered saline (PBS, pH 7.4) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Dopamine and ascorbic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Potassium chloride (KCl) was purchased from RFCL, Ltd. (New Delhi, India). Chloroform, dichloromethane, N,N-dimethylformamide (DMF) were obtained from Carlo Erba Reagents (Milano, Italy). All aqueous solutions were prepared in MilliQ water (12.8 MΩ cm) and used throughout the experiments. All other reagents were of analytical grade.

2.2. Instrumentation

2.2.1. Electrospinning of the G/PANI/PS nanofibers onto the screen-printed carbon electrodes

The G/PANI/PS nanofibers were aligned on a screen-printed carbon electrode attached to a rotating drum. All of the carbon electrodes were attached at the same position. An electric potential difference of 15 kV was applied between the collector and a syringe tip, and the distance between the collector and tip was 10 cm. The flow rate of the G/PANI/PS composite solution was kept steady at 0.5 mL h^{-1} using a syringe pump. The optimal collection time was found to be 15 min.

Since the diameter and length of nanofibers produced from electrospinning are random, the collecting time and other factors affecting the nanofiber morphology (*e.g.* solvent, humidity, and temperature) were controlled to ensure the reproducibility of our

approach. After electrospinning, the weight and morphology of nanofibers were determined by using 5-digit analytical balance, SEM and TEM to confirm that the morphology of nanofibers is reproducible prior to use in electrochemistry. For each condition of electrospinning, the experiment was repeated at least five times and the relative standard deviation was determined.

2.2.2. Morphology characterization

The morphology of G/PANI/PS nanofibers was characterized using a JSM-6400 field emission scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd., Japan) and a JEM-2100 transmission electron microscope (Japan Electron Optics Laboratory Co., Ltd., Japan).

2.2.3. Electroanalytical measurements

All electroanalytical measurements were performed on a μAUTOLAB type III potentiostat (Metrohm Siam Company Ltd.) controlled with the General Purpose Electrochemical System (GPES) software. A three-electrode system with an in-house electrochemical cell was employed. The configuration of an in-house electrochemical cell is shown in Fig. S3 in the Supporting Information. The G/PANI/PS nanofiber-modified screen-printed carbon electrodes were used as the working electrode (WE), and the area of the electrode exposed to the solution was limited by using the same electrochemical cell (Fig. S3 right). A Pt wire and Ag/AgCl were used as the counter electrode (CE) and reference electrode (RE), respectively. For all electrochemical measurement, the analyte solution volume was limited to 3 mL in a well (diameter 2.5 cm and height 2.0 cm) as shown in Fig. S3 (right). The electrochemical measurements were performed via cyclic voltammetry (CV) and square-wave voltammetry (SWV). In general, the CV measurements were performed over a potential range from −0.5 to +1.0 V at a scan rate of 100 mV s^{-1}. The SWV measurements were performed over a potential range from +0.2 V to +1.8 V with a pulse amplitude of 15 mV, square wave frequency of 30 Hz and step height of 5 mV. All measurements were conducted at the room temperature.

The G/PANI/PS nanofiber-modified screen-printed carbon electrodes were electrochemically characterized by cyclic voltammetry using a standard solution of $[Fe(CN)_6]^{3-/4-}$ as previously reported [36,38] prior to DA determination.

2.3. Procedures

2.3.1. Preparation of the screen-printed carbon electrodes

The screen-printed carbon electrodes were prepared using an in-house screen-printing technique. The commercial carbon ink (Adheson Electrodag PF-407C) consisting of very finely divided carbon particles dispersed in thermoplastic resin was purchased from Henkel (Bridgewater, NJ, USA). The screen-printed carbon electrode patterns were designed in Adobe Illustrator. First, the silver/silver chloride ink was screened onto a polyvinyl chloride (PVC) substrate to form the conductive layer to connect with electrochemical detection part. Then, the carbon ink was screened onto the same PVC substrate as the second layer (0.8 cm × 2.0 cm) to obtain the screen-printed carbon electrodes. The preparation of an in-house screen-printed carbon electrode is shown in Fig. S4 in the Supporting Information. These electrodes were dried in an oven at 55 °C for 1 h and stored in a desiccator at the room temperature until used.

2.3.2. Preparation of the solution

The G/PANI/PS composite solution was prepared via the following procedure. G nanopowders (4.0 mg) were dispersed in 1 mL of N,N-dimethylformamide using an ultrasonicator for 24 h at room temperature. PANI emeraldine base (0.40 g) was doped with CSA (0.52 g) and then dissolved in 15 mL of chloroform. The obtained PANI solution was stirred at 1000 rpm for 12 h at room temperature and filtered through a filter paper (Whatman No. 1) to remove any particulate matter. A 20% (w/v) PS solution was prepared in dichloromethane/N,N-dimethylformamide (1:1). Finally, 1000 μL of 20% (w/v) PS was mixed with different volumes of the G solutions ranging from 0, 60, 120, 180 to 240 μL and different volumes of the PANI solution from 1000, 940, 880, 820 to 760 μL to obtain the total volume of 2000 μL. Nanocomposite solutions with 0, 3, 6, 9 and 12% (v/v) of G in PANI/PS were obtained and mixed by vortexing for 2 min at room temperature prior to electrospinning.

2.3.3. Preparation of human serum and urine

Lyophilized human serum was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) and normal human urine was obtained from Innovative Research (Novi, MI, USA). According to the product information, the serum and urine provide a complex biological environment system, but they do not contain DA. For the determination of DA, the known DA concentrations were added to the serum and urine and then determined by using standard addition method as the previous report [12].

Prior to use, 3.0 mL of high purity water was added to the lyophilized human serum and urine. Then the proteins in serum and urine were precipitated by using TCA precipitation method [39]. For protein precipitation, 400 μL of human serum or urine, 100 μL of DA at different concentrations and 500 μL of 10% (w/v) TCA were mixed together by vortexing for 5 min at room temperature. The mixed solutions were centrifuged at 1100 RCF for 10 min and the supernatants were kept for further analyses.

3. Results and discussion

3.1. Optimization of the electrode modification

Prior to electrospinning fabrication, a homogeneous nanocomposite solution containing G, PANI and PS was freshly prepared. In this study, G was used to increase the surface area and electrochemical sensitivity of the system. PANI (emeraldine salt) was used as a conducting matrix to generate the conducting nanofibers and improve the dispersibility of G in the nanocomposite solution. PS was utilized as a carrier polymer to assist the formation of electrospun conducting nanofibers of PANI since it is very difficult to electrospin PANI into nanofibers with uniform morphology and size. Then, the conducting nanofibers of G/PANI/PS were fabricated on screen-printed carbon electrodes via electrospinning. Several factors affecting the performance of these modified electrodes, such as the type of organic solvent used for G dispersion, type of non-conductive polymer, percentage of G loading (%G) and collection time were investigated and optimized. In this study, N,N-dimethylformamide (DMF) and polystyrene (PS) were selected as the organic solvents for the G dispersion and non-conductive polymer, respectively.

Among all the parameters, the percentage of G loading (%G) and collection time were the two main factors that exhibit a profound influence upon the electrochemical response. The effects of the %G loading (v/v) on the electrochemical sensitivity of the G/PANI/PS modified electrodes was investigated by cyclic voltammetry using the commonly used standard $[Fe(CN)_6]^{3-/4-}$ as the redox probe. As shown in Fig. 1 and Fig. S5, the anodic peak currents increased rapidly upon increasing the %G loading from 0 to 6%, which verifies that incorporating G into the PANI/PS nanofibers significantly improves their electrical conductivities. However, the anodic peak currents tend to decrease once the %G loadings are increased above 6%, which suggests a hindered mass transfer process. This decrease in current was probably caused by the agglomeration of G within the nanofibers (Fig. S6), which results in a decreased

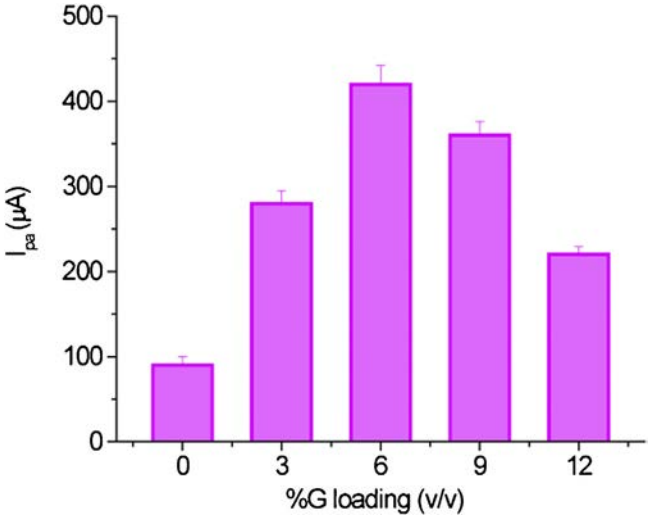

Fig. 1. Anodic peak current (i_{pa}) obtained from the cyclic voltammetry of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl using G/PANI/PS nanofiber-modified electrodes with different %G loadings. The error bars correspond to the standard deviations obtained from 5 measurements.

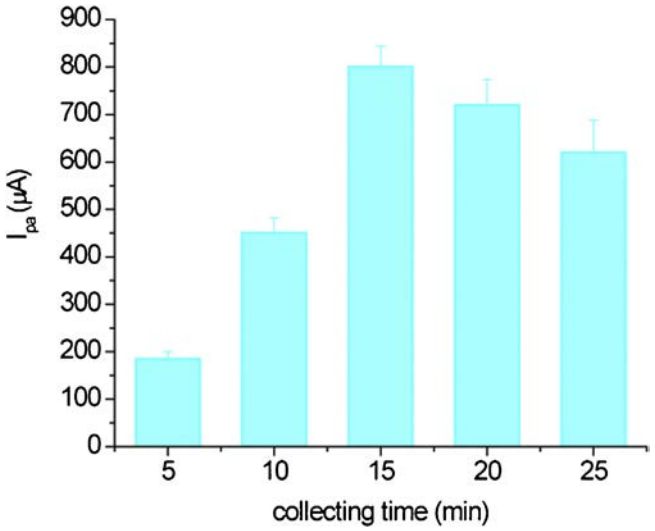

Fig. 2. Anodic peak current (i_{pa}) obtained from the cyclic voltammetry of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl using G/PANI/PS nanofiber modified electrodes at different collecting time. The error bars correspond to the standard deviations obtained from 5 measurements.

electrochemical response. Therefore, a 6% G loading was chosen for subsequent studies.

To optimize the collection time of the electrospinning fabrication, the G/PANI/PS nanocomposite solutions were collected on different screen-printed carbon electrodes for times ranging from 5 to 25 min. The electrochemical sensitivity of each modified electrode was then investigated via cyclic voltammetry using standard $[Fe(CN)_6]^{3-/4-}$. The anodic peak currents obtained using different collection time for each of the modified electrodes indicated that the highest electrochemical response was achieved at 15 min collection time (Fig. 2 and Fig. S7). The anodic peak currents decreased slightly for longer collection times. This decrease in current was probably caused by nanofiber overlap on the electrode surface, which decreased the active surface area exposed to the analytes. Moreover, increasing the collection time enhances the total amount of non-conductive polymer in the nanofibers. Although the ratio between the conductive and nonconductive polymers in the nanofibers was held constant, it is possible that the nanofibers lose their electrical properties once the total amount of non-conductive polymer reaches a certain limit. Therefore, 15 min was selected as the optimal collection time for further experiments.

Since the electrochemical activity of each compound is different, we need to confirm that 6% G loading (v/v) and 15 min collection time of electrospinning are the optimal conditions for DA determination. Therefore, the effect of %G loading and collection time on the current response of DA were investigated on G/PANI/PS modified electrode by SWV. The results of DA determination (Figs. S8 and S9) showed a similar trend as seen with $[Fe(CN)_6]^{3-/4-}$ (Figs. 1 and 2); thus, these conditions were selected for DA determination in subsequent studies.

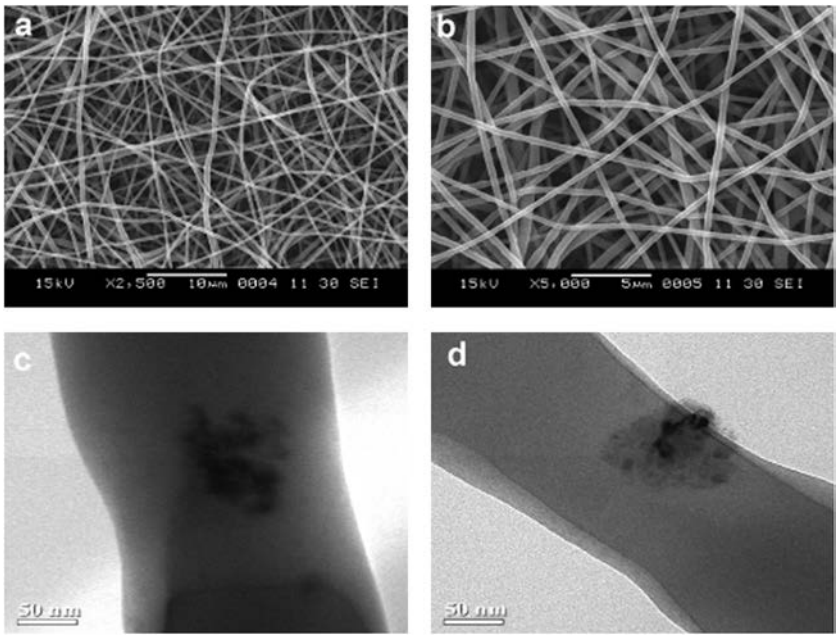

Fig. 3. SEM images of the G/PANI/PS nanofibers with 2500× magnification (a) and 5000× magnification (b), and TEM images of random G distributions in the G/PANI/PS nanofibers (c and d).

3.2. Characterization of the G/PANI/PS nanofibers

The morphology of the electrospun G/PANI/PS nanofibers was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Under optimal electrospinning conditions, well-defined G/PANI/PS nanofibers with an average diameter of 260 ± 60 nm were obtained as shown in Fig. 3a and b. The SEM images indicate a relatively uniform size distribution and continuous alignment for the G/PANI/PS nanofibers. Moreover, the TEM images (Fig. 3c and d) confirmed that the G was randomly dispersed inside the nanofibers without any severe aggregation. Even though some of the incorporated G was not fully aligned in the middle axis of the nanofibers (Fig. 3d), they were still surrounded by a conducting matrix of PANI in the nanofibers. The similar morphology was also obtained for electrospun PANI/PS nanofibers as shown in Fig. S10.

Cyclic voltammetry (CV) is an effective and convenient tool for monitoring the electron transfer process in the modified electrodes [36,40]. In this study, the electrochemical characteristics of the newly developed G/PANI/PS nanofiber-modified screen-printed carbon electrodes were investigated by CV using a standard $[Fe(CN)_6]^{3-/4-}$ redox couple and compared to the results obtained from PANI/PS nanofiber-modified electrodes and unmodified screen-printed carbon electrodes (Fig. 4). The G/PANI/PS nanofiber-modified electrodes yielded the highest current response, which was approximately 9 times greater than the unmodified carbon electrode and 4 times higher than the PANI/PS nanofiber-modified electrode. These results verify that the G/PANI/PS nanofibers can facilitate the electron transfer process and thus significantly enhance the electrochemical sensitivity of the modified electrode. In this study, the dramatic increase of current response was focused and then applied for ultrasensitive determination of DA.

Moreover, the cyclic voltammograms of the G/PANI/PS nanofiber-modified electrodes showed well-defined anodic and cathodic peaks with peak potential values (E_{pa} and E_{pc}) virtually identical to the unmodified carbon electrode. As shown in Fig. 4, the peak potential difference values (ΔE_p) obtained from all electrodes exceed 60 mV, which is probably caused by: (1) IR drop due to non-ideal electrode configuration and (2) the nonconductive property of resin used as a binder in the commercial carbon ink.

As indicated above, incorporating only 6% (v/v) of G into the PANI/PS nanofibers increased the electrical conductivity by a factor of 4, indicating that the incorporation of a small amount of G is promising for producing sensitive electrochemical biosensors. Likewise, the SWV responses of DA (0.1 mM) on PANI/PS and G/PANI/PS modified electrodes shown in Fig. S11 in the Supporting Information demonstrate that the presence of G in the nanofibers can significantly increase the current response of DA leading to enhanced detection sensitivity. Furthermore, the peak potential of DA measured on G/PANI/PS modified electrode (0.55 V) is lower than the peak potential of DA on PANI/PS modified electrode (0.70 V). The lower peak potential value is important for selective determination of DA in the present of common interferences (*i.e.* ascorbic acid and uric acid) because the interference peaks usually overlap with DA at higher detection potential. Therefore, in this study, G plays an important role for increasing both sensitivity and selectivity in the detection of DA.

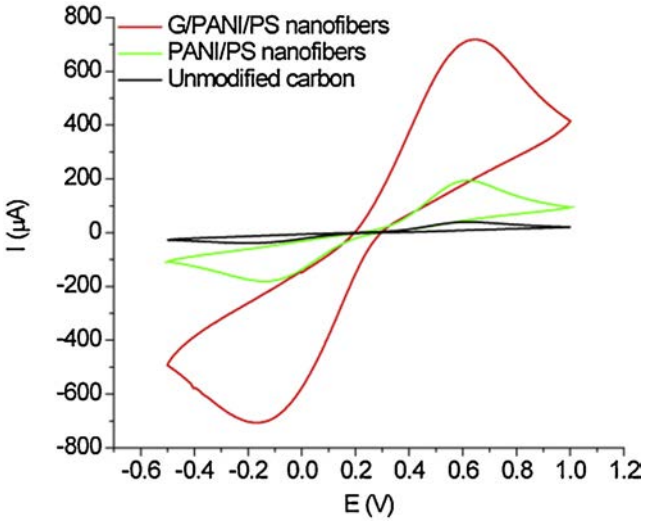

Fig. 4. Cyclic voltammograms using 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl with the unmodified screen-printed carbon electrode (black), PANI/PS nanofibers modified carbon electrode (green) and G/PANI/PS nanofibers modified carbon electrode with a 6% G loading (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

The improved performance of the G/PANI/PS nanofiber-modified electrodes is presumably due to the high specific surface area of these materials. To estimate this surface area, we weighed the electrodes before and after modification. Using certain assumptions, as shown in the Supporting Information (Table S1), we then estimated a specific surface area value of 13.78 $m^2\ g^{-1}$, which falls in a normal range of specific surface area of nanofibers prepared by electrospinning [41].

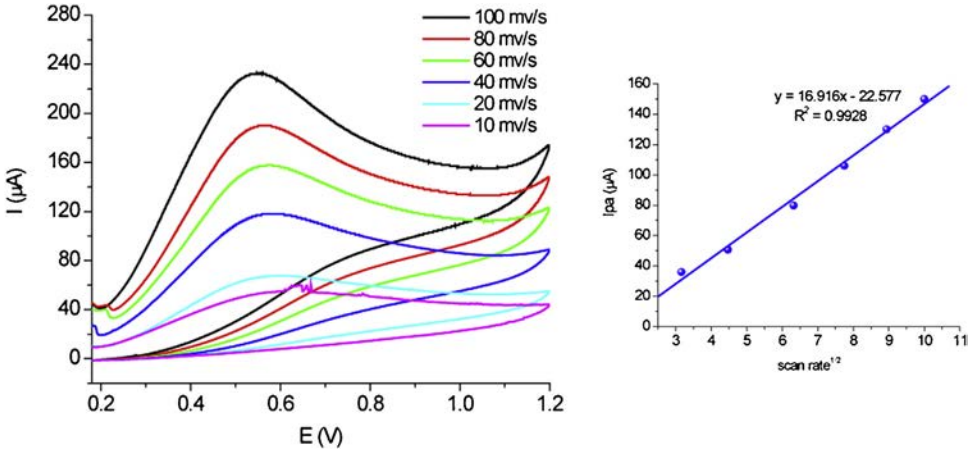

Fig. 5. Cyclic voltammograms of 1.0 mM DA in 0.1 M PBS (pH 7.4) at scan rates of 10, 20, 40, 60, 80 and 100 mV s^{-1} on the G/PANI/PS nanofiber-modified electrodes and the anodic peak current as a function of square root of scan rate (inset) measured on the G/PANI nanofiber-modified electrodes.

3.3. The performance of the G/PANI/PS nanofibers modified electrodes

To assess their suitability as a novel platform for sensitive determination of DA, the electrochemical behavior of the electrospun G/PANI/PS nanofiber-modified screen-printed carbon electrodes was examined in more detail. As shown in a previous report, the redox reaction of DA is irreversible [40]; therefore, only the anodic peak current of 1 mM DA was examined at different voltage scan rates (Fig. 5). The resulting data conformed to the Randles–Sevcik equation as shown below.

$$I = kn^{3/2}FAD^{1/2}\nu^{1/2} \quad (1)$$

where $k = 2.69 \times 10^5$ (C mol^{-1} v$^{-1/2}$), n is the number of transferred electrons, F is Faraday's constant (96,485 C mol^{-1}), A is the electrode surface area (cm^2), D is the diffusion coefficient of $[Fe(CN)_6]^{3-/4-}$, which is 6.70×10^{-7} cm^2 s^{-1}, and ν is the scan rate (mV s^{-1}).

As expected, the anodic peak currents increase as a function of scan rate (Fig. 5). The linearity of the anodic peak currents versus the square root of the scan rate over a range of 10–100 mV s^{-1} with a correlation coefficient (R^2) of 0.9928 verified that the oxidation process of DA on G/PANI/PS nanofiber modified screen printed carbon electrode is diffusion controlled.

3.4. Selective determination of DA using square wave voltammetry

The high electrochemical response of the G/PANI/PS nanofiber-modified electrodes makes it very attractive for the sensitive detection of biomolecules. To test their capabilities in this regard, the modified electrodes were used to determine DA via SWV. A major challenge encountered with DA determination is interference from AA because the oxidation potential of DA and AA are very close in value, which leads to the overlap of their voltammetric responses. Moreover, the oxidation product of DA can catalyze the oxidation of AA, which results in electrode fouling and both poor reproducibility and selectivity [42]. As shown in Fig. 6a, SWV voltammograms of the individual solution of 40 μM DA (black) and 40 μM AA (red) in 0.1 M PBS (pH 7.4) that were analyzed on an unmodified screen-printed carbon electrode overlapped completely; therefore, it was impossible to selectively detect DA in the presence of AA on an unmodified electrode. Because the highest AA concentration in normal human serum is 80 μM [43,44], the ability to selectively determine DA using the G/PANI/PS nanofiber-modified electrode at the highest anticipated concentration of AA was studied. As shown in Fig. 6b, the individual voltammograms for 40 μM DA (black) and 40 μM AA (red) in 0.1 M PBS (pH 7.4) analyzed on the G/PANI/PS nanofiber-modified electrode reveals that the anodic peak current of AA (red line) is separated completely from the anodic peak current of DA (black line) with a ΔE_p of 0.65 V.

Furthermore, the SWV voltammograms of a mixture of the two compounds containing the highest anticipated AA concentration (80 μM) in serum (green line) demonstrated the well separated anodic peaks of these two compounds. Another important interference in the detection of DA in biological fluids is uric acid (UA) [12,43,44], therefore, selective determination of DA at the highest anticipated concentration of UA (400 μM) and AA (80 μM) on the modified electrode was also studied as shown in Fig. S12 in the Supporting Information. The results verify that G/PANI/PS nanofiber-modified electrodes can be used to selectively determine DA in the presence of both AA and UA. A possible reason to explain the high selectivity of G/PANI/PS nanofiber-modified electrode for DA determination is a favorable interaction between aromatic structure of PANI, PS or sp^2-carbon of G and the aromatic structure of DA.

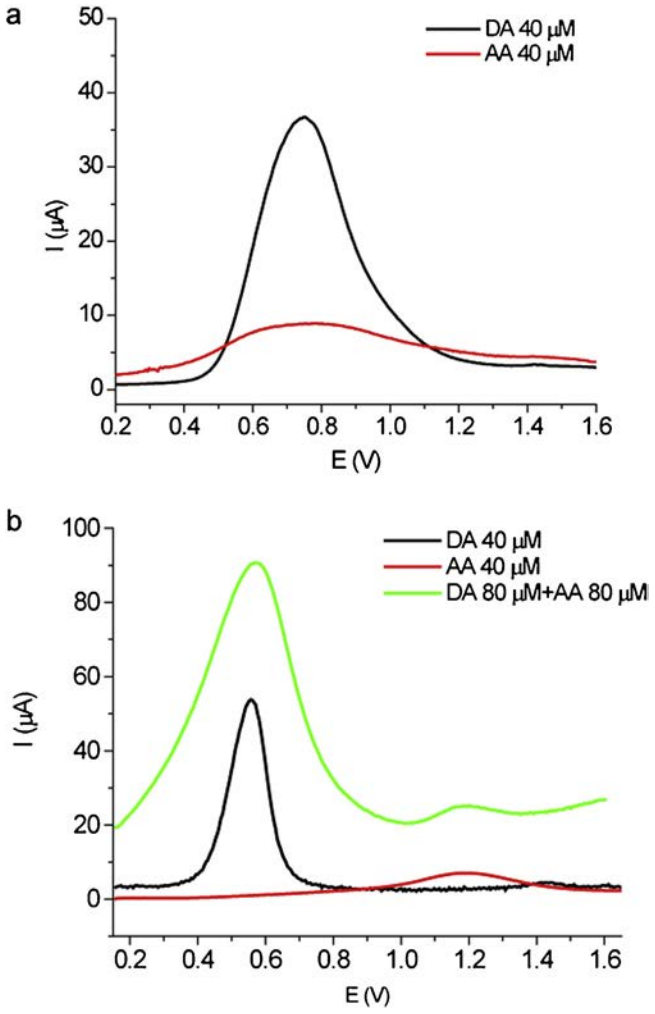

Fig. 6. Square wave voltammograms of 40 μM of DA in 0.1 M PBS (pH 7.4) (black) and 40 μM of AA in 0.1 M PBS (pH 7.4) (red) analyzed on an unmodified screen-printed carbon electrode (a), and the square wave voltammogram of 40 μM of DA in 0.1 M PBS (pH 7.4) (black) and 40 μM of AA in 0.1 M PBS (pH 7.4) (red) and a 80 μM mixture of both DA and AA in 0.1 M PBS (pH 7.4) (green) analyzed on a G/PANI/PS nanofiber-modified screen-printed carbon electrode. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

To investigate the analytical performance of this system, the relationship between the DA concentration and current response was studied by SWV. As shown in Fig. 7, the current response increased as a function of the DA concentration, and the linear range was found to be 0.0001–100 μM with a correlation coefficient (R^2) of 0.9905. The limit of detection (LOD) and limit of quantification (LOQ) for the system evaluated using signal-to-noise ratios of three (S/N = 3) and ten (S/N = 10) were found to be 0.05 nM and 0.30 nM, respectively.

A literature comparison of these results to DA determinations using different modified electrodes is shown in Table 1. Considering previous work, we find that the electrospun G/PANI/PS nanofiber-modified electrodes provide lower detection limits and a comparable linear range for DA determination. Although the aptamer/G-PANI composite film modified glassy carbon electrode [26] provides a lower LOD, the aptamer-based biosensor is much more expensive and complicated than our proposed system. These comparisons show the promise of G/PANI/PS nanofiber-modified electrodes for the sensitive determination of DA in complex biological fluids.

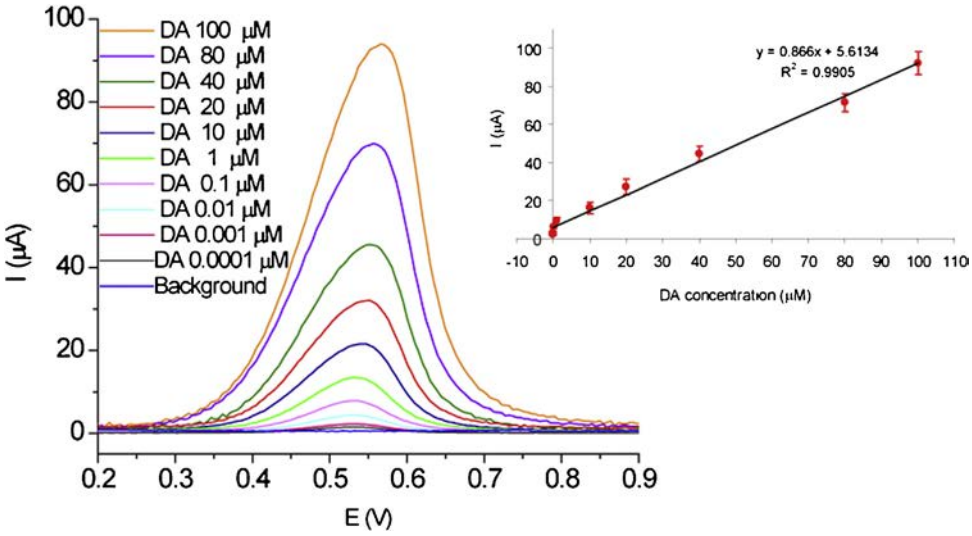

Fig. 7. Square wave voltammograms of DA in the concentration range of 0.0001–100 μM in 0.1 M PBS (pH 7.4) and a linear plot of the DA concentration versus the current response (inset) measured on the G/PANI/PS nanofiber-modified screen-printed carbon electrode.

Table 1
Comparison of the proposed electrode to other modified electrodes for DA determination.

Modified electrode	Linear range (μM)	Detection limit (μM)	Reference
MWCNTs modified graphite electrode	0.5–10	0.1	[45]
CNTs modified pyrolytic graphite electrode	0.5–10	0.1	[46]
CNTs-ionic liquid gel modified glassy carbon electrode	1–10	0.1	[47]
SDS-MWCNTs modified glassy carbon electrode	20–200	3.75	[48]
CNFs modified carbon paste electrode	0.04–5.6	0.04	[2]
Pd/CNFs modified carbon paste electrode	0.5–160	0.20	[49]
G modified glassy carbon electrode	4–100	2.64	[50]
G-PVP modified glassy carbon electrode	0.0005–1130	0.0002	[42]
Stacked G nanofiber/ionic liquids/chitosan modified glassy carbon electrode	0.05–240	0.04	[25]
PANI/Au nanocomposite-modified boron-doped diamond electrode	0.15–500	0.03	[40]
3,4,9,10-Perylene tetracarboxylic acid functionalized G/MWCNTs/ionic liquid modified glassy carbon electrode	0.03–3820	0.0012	[24]
Phenylethynyl ferrocene/G nanocomposite modified glassy carbon electrode	0.05–200	0.02	[51]
Ferrocene thiolate stabilized Fe_3O_4@Au nanoparticles with G sheet modified glassy carbon electrode	0.5–50	0.1	[52]
Aptamer/G-PANI composite film modified glassy carbon electrode	0.000007–0.09	0.000002	[26]
G/PANI/PS nanofibers modified screen-printed carbon electrode	0.0001–100	0.00005	This work

Abbreviations: MWCNTs, multi-walled carbon nanotubes; CNT, carbon nanotubes; SDS, sodium dodecyl sulphate; CNF, carbon nanofibers; Pd, palladium; G, graphene; PVP, polyvinylpyrrolidone; PANI, polyaniline; Au, gold; Fe_3O_4, iron oxide; PS, polystyrene.

3.5. Reproducibility and stability of the modified electrode

The reproducibility and stability of the electrospun G/PANI/PS nanofiber-modified electrodes were investigated by measuring the SWV response to 1 μM of DA in PBS solution (pH 7.4) multiple times. The relative standard deviation (RSD) of the oxidation peak currents by ten successive measurements was 3.21%, indicating excellent detection reproducibility. Furthermore, the fabrication reproducibility, which was determined from five different modified electrodes prepared under the same conditions, had an RSD of 3.85% revealing a reliable and reproducible electrode preparation procedure. The storage stability of the modified electrodes was also evaluated. When the electrodes were stored at 4 °C for 10 days, the current responses remained above 88.5% of their initial values. These results verify the good reproducibility and stability of the modified electrodes described in this study.

3.6. Sample analysis

To evaluate the applicability of the proposed system in complex biological matrices, the DA concentration in human serum and human urine was determined using a standard additions method. Three human serum and three urine containing spiked DA were analyzed on six independently prepared electrodes. Each sample was detected three times and the detection value was the average of these three results. The analytical results are summarized in Table 2. The recovery ranged from 97.0 to 104.5% and the RSD ($n = 3$) was below 3.0%, which indicates this system is highly accurate. These results further verified the promising applications of the G/PANI/PS nanofiber-modified electrodes for the direct determination of DA in complex biological fluids.

Table 2
The determination of DA in human serum and urine samples ($n = 3$).

Samples	No.	Amount of added DA (μM)	Amount of found DA (μM)	Recovery (%)	RSD (%)
Human serum	1	1.0	0.97	97.0	2.5
	2	10.0	9.85	98.5	2.9
	3	50.0	50.90	101.8	2.6
Urine	1	1.0	1.02	102.0	2.7
	2	10.0	10.30	103.0	2.8
	3	50.0	52.25	104.5	2.9

4. Conclusions

A novel system for the ultrasensitive determination of DA based on G/PANI/PS nanofiber-modified electrodes has been successfully developed. The modified electrodes exhibited excellent sensitivity, good selectivity and a wide linear range for DA determination. This high sensitivity was attributed to the fact that electrospun G/PANI/PS nanofibers can enhance the electrode surface area and improve the analytes ability to reach the electrode surface. In addition, this system can be applied for the determination of DA in human serum and human urine with excellent reproducibility. Further functionalization of the amino group ($-NH_2$) of PANI on the G/PANI/PS nanofibers with specific bio-receptors might expand the performance of this promising platform for biosensor applications.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.09.057.

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An electrochemical sensor based on graphene/polyaniline/polystyrene nanoporous fibers modified electrode for simultaneous determination of lead and cadmium

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ABSTRACT

The development of graphene/polyaniline/polystyrene (G/PANI/PS) nanoporous fiber modified screen-printed carbon electrode (SPCE) using electrospinning fabrication for simultaneous determination of lead (Pb^{2+}) and cadmium (Cd^{2+}) was achieved. Initially, the important factors controlling the electrospun fiber morphology and electrochemical sensitivity (e.g. type of solvent, amount of G loading) were investigated and optimized. Then, the electrospun G/PANI/PS nanoporous fibers were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Cyclic voltammetric (CV) measurements using a standard ferri/ferrocyanide $[Fe(CN)_6]^{3-/4-}$ redox couple were performed for electrochemical characterization of the modified electrode. Due to the increase of specific surface area of the electrospun G/PANI/PS nanoporous fibers, the electrochemical sensitivity of modified SPCE was enhanced by a factor of three compared to an unmodified SPCE. In terms of application, square-wave anodic stripping voltammetry (SWASV) was employed for the simultaneous determination of Pb^{2+} and Cd^{2+} in the presence of bismuth (Bi^{3+}) on G/PANI/PS nanoporous fiber-modified SPCE. Under optimal conditions, a linear relationship between anodic current and metal ion concentration was found in a range of 10–500 $\mu g\,L^{-1}$ with the detection limit (S/N = 3) of 3.30 $\mu g\,L^{-1}$ and 4.43 $\mu g\,L^{-1}$ for Pb^{2+} and Cd^{2+}, respectively. In addition, the effects of common cation and anion interferences commonly found in environmental water were studied, and the satisfied results were obtained. Interestingly, by simple washing step, this described electrode can be reused for more than ten replicates with high reproducibility. Finally, this new electrode system was successfully applied for the simultaneous determination of Pb^{2+} and Cd^{2+} in real river water samples, and the results correlated well with conventional inductively coupled plasma optical emission spectroscopy (ICP-OES).

1. Introduction

Electrospinning is a well-known fiber fabrication technique that has gained significant interest in recent years owing to its versatility in various fields. Applications include biosensor, metal sensor, enzyme immobilization, tissue engineering, filtration, controlled release and wound dressing [1–4]. This technique can be used to produce continuous fibers of polymers with size from tens nanometers to micrometer range scale [5]. In the process, a surface of a polymer liquid, either solution or melt, is charged by high voltage electric fields. Due to Coulombic repulsion among surface charges at sufficient electrical field strength, the liquid surface deformed and self-ejected from the spinneret to form a continuous stream of charged polymer jet. Subsequent elongation via electrically-driven begin instability and solidification of the jet lead to a formation of ultrafine or nanofibers [6]. The electrospun fibers possess several advantages, such as large specific surface area, high porosity, and high mechanical properties [7]. Moreover, the morphology of electrospun fibers is readily controlled by adjusting the electrospinning parameters, such as polymer concentration, type of solvent, applied

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voltage, flow rate, and distance between tip and ground collector [8]. Electrospun fibers with different sizes and shapes have been produced, such as ribbon, core shell, hollow, smooth and porous fibers [7,9,10]. Among these morphologies, fibers with nanoporous surface have received considerable attention for sensor application development due to its high specific surface.

Electrochemical sensors are versatile tools for the determination of various analytes in different fields, such as food inspection, clinical diagnosis as well as environmental monitoring because they are simple, rapid, portable and inexpensive [11,12]. Nonetheless, an important problem for electrochemical sensors is the limited surface area of the working electrode since the electrode is usually designed to be small to provide the portability for on-site monitoring and compatibility with trace amounts of sample. Tiny electrodes significantly decrease the sensitivity of electrochemical detection. Therefore, electrospinning is selected for electrode surface modification because electrospun nanoporous fibers can increase the surface area per unit volume of working electrodes, leading to enhanced electrochemical sensitivity [13–18]. Electrospun fibers have been used for electrode modification in electrochemical sensors to increase the electrochemical sensitivity and the percentage of enzyme loading. Furthermore, the electrode stability and life time are significantly improved compared to an unmodified electrode [14,15].

With the advent of nanotechnology, another strategy for increasing electrode surface area in electrochemical sensor is the use of nanostructured materials. Various materials in nanoscopic-scale such as metallic nanoparticles, carbon based nanomaterials (i.e. fullerene, carbon nanotube, graphene) have been used for working electrode surface modification [19,20]. Nowadays, graphene (G), a two-dimensional single atom thick of carbon material, has become a material of interest for electrode surface modification due to its outstanding electrical conductivity, high mechanical strength and large surface area. However, pure G agglomerates easily to form graphite [21,22]. In order to prevent the restacking of G, conducting polymers are used to improve G distribution on the electrode surface. Several conducting polymers including polyaniline (PANI) [13,23–30], polypyrrole (PPy) [31–33], poly (3, 4-ethylenedioxythiophene) (PEDOT) [34–36] have been used to prepare well-dispersed G on electrode surfaces for electrochemical detection. Comparing to other conducting polymers, PANI has been studied extensively as an attractive polymer because of its high conductivity, good environment stability, and easily controllable properties [23,30]. Numerous researchers have reported alternative sensing approaches based on electrode surface modification using carbon nanomaterial-conducting polymer nanocomposite. The nanocomposite of PANI and carbon based material has been used to produce high conductivity electrodes via electrospinning fabrication [27]. Recently, our group has developed a new electrode system based on electrospun G/PANI/PS nanofiber [13]. The modified electrode exhibits ultra-high sensitivity, good selectivity and wide linear range for dopamine detection. From previous reports, it is clear that G and PANI nanocomposite electrodes have a tendency to significantly improve the electrochemical sensitivity and analytical performance of electrochemical sensors [13,25,26,28,37]. Together with the sponge-like morphology of the electrospun fibers, a novel electrochemical sensor is created in this study.

The contamination of heavy metals (*e.g.* Pb^{2+}, Cd^{2+}) in environmental areas has become a serious concern due to their wide applications in industrial processes (e.g. electroplating, batteries, paint). Particularly, lead (Pb^{2+}) and cadmium (Cd^{2+}) are toxic heavy metals due to non-biodegradability. Furthermore, the accumulation of these metals in the human body can cause serious disorders to human organs (e.g. kidney, liver, central nervous system, bone) [38]. Several analytical techniques have been used for the determination of Pb^{2+} and Cd^{2+} in environment, such as atomic absorption spectrometry (AAS), inductively couple plasma optical mass spectrometry (ICP-MS), and X-ray fluorescence spectroscopy (XRF). However, these techniques require expensive instrumentation, specialized operator, and long analysis time [20]. In contrast, electrochemical related techniques offer several advantages such as fast analysis, low cost, portability and high sensitivity [39]. Anodic stripping voltammetry (ASV) has been established as a highly sensitive electrochemical method for trace metal ion analysis due to the incorporation of two procedures including a deposition step and a measurement step [40]. In the deposition step, the potential is held at a low value to accumulate the metal of interest onto the electrode surface. Conventionally, mercury electrodes have been used for ASV to obtain high sensitivity; however, mercury is highly toxic. Instead of using hazardous mercury-modified electrodes, bismuth (Bi) film electrodes have been introduced as an environmental friendly modifier because they offers high sensitivity, well-defined peak shapes and highly reproducible stripping signal [12,24,41–44].

Herein, we introduce electrospun G/PANI/PS nanoporous fiber modified SPCE for ASV in the simultaneous determination of Pb^{2+} and Cd^{2+}. The electrode preparation and electrochemical characteristic of electrospun G/PANI/PS nanoporous fiber modified SPCEs are investigated and optimized. This modified electrode exhibits outstanding analytical performance with good electrochemical sensitivity compared to previous reports. Moreover, the electrode performance is evaluated by river water samples spiked with these heavy metal ions. Overall, the determination of Pb^{2+} and Cd^{2+} by using this approach offers several advantages including low cost, simple preparation, high selectivity, good sensitivity and reusability. This approach might be an alternative tool for heavy metal detection in environmental monitoring.

2. Material and methods

2.1. Reagents and materials

Graphene (G) nanopowders were purchased from SkySpring Nanomaterials Inc (Houston, TX, USA). Polyaniline emeraldine base (Mw of 65,000), (+)-camphor-10-sulfonic acid (CSA), polystyrene (Mw of 180,000), potassium ferricyanide ($K_3[Fe(CN_6)]$) and potassium ferrocyanide ($K_4[Fe(CN_6)]$) were obtained from Sigma–Aldrich (St. Louis, Mo, USA). Potassium chloride (KCl) was purchased from PFCL, Ltd. (New Delhi, India). Chloroform ($CHCl_3$), N, N-dimethylformamide (DMF), tetrahydrofuran (THF) and hydrochloric acid (HCl) were obtained from Carlo Erba reagent (Milano, Italy). Bi^{3+}, Cd^{2+} and Pb^{2+} solutions were diluted from their respective 1000 mg L^{-1} standard solutions purchased from VWR International Ltd. (Poole, England). All aqueous solutions were prepared in Mili-Q water (12.8 MΩ cm). All chemicals were used as received without further purification.

2.2. Electrode fabrication and electrospinning modification of SPCE

A carbon electrode was fabricated on a polyvinyl chloride (PVC) substrate using screen-printing technique [45]. Briefly, the electrode pattern was designed by Adobe Illustrator CS5 (Adobe system, Inc.), and an ink-blocking stencil was fabricated by Chaiyaboon Co. (Bangkok, Thailand). Initially, the silver/silver chloride ink was printed on PVC substrate as a conductive pad. Then, the carbon ink was printed on top of patterned silver layer to be used as a working electrode area. The ink-coated PVC substrate was then

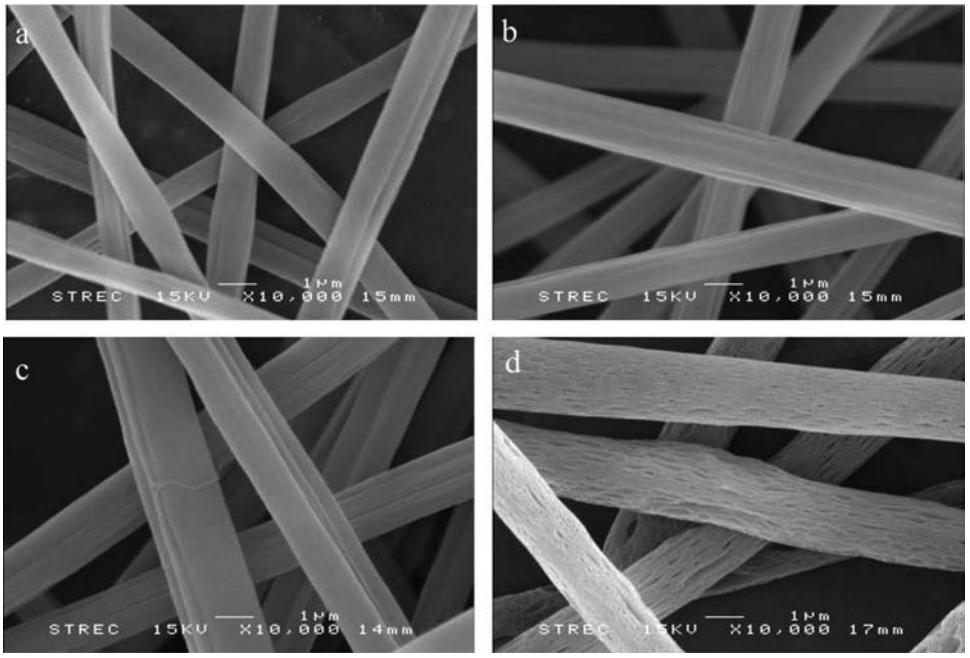

Fig. 1. SEM images of electrospun fiber obtained from different solvent systems including (a) 25/75% THF/DMF, (b) 50/50% THF/DMF, (c) 75/25% THF/DMF, and (d) 100% THF.

dried in an oven at 55 °C for 1 h after each screen-printing step to remove residual solvent.

For SPCE surface modification, G/PANI/PS nanoporous fibers were fabricated on the electrode by using an in-house electrospinning system. The solution of G, PANI, and PS were prepared separately as follows. G nanopowders in a range of 0–10 mg were dispersed in DMF in an ultrasonicator for 24 h. For the PANI solution, PANI emeraldine base 0.60 g and CSA (doping agent) 0.77 g were dissolved in 15 mL of chloroform. After that, the PANI mixture was stirred at 1000 rpm for 6 h and filtered to obtain a clear PANI solution. A 23% w/v PS solution was prepared in various ratios of THF: DMF including 25:75, 50:50, 75:25, and 100:0. Next, 1000 μL of PS (23% w/v) and 150 μL of a G: PANI solution (1:1 ratio) were mixed thoroughly. The G/PANI/PS mixture was loaded into a syringe. The G/PANI/PS nanocomposite solution flow rate was kept constant at 0.5 mL h^{-1} by a syringe pump (New Era Pumps, NE300, USA). The electric field between the syringe tip and rotating drum collector attached with SPCE was established with an applied voltage of 10.5 kV using a high voltage DC module (Gamma High Voltage, model UC5-30P/CM/VM (3), Florida) connected to a DC regulated power supply. An optimal tip-to collector distance was maintained at 15 cm. Finally, the nanoporous fiber was produced and directly collected on the SPCE surface.

2.3. Physical and electrochemical characterization

For physical characterization, the morphology of G/PANI/PS nanoporous fibers on SPCE surface was investigated using a JSM-6400 field emission scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd., Japan) and a transmission electron microscope (Hitachi/s-4800). The surface area analysis was performed using Brunauer–Emmett–Teller (BET) technique (Quantachrome/Autosorb-1, Thermo Finnigan/Sortomatic 1990).

All electrochemical measurements were performed on a μAUTOLAB type III potentiostat (Metrohm Siam Company Ltd.) controlled with General Purpose Electrochemical System (GPES) software. A three electrode system was used and consisted of an auxiliary Pt wire electrode, a reference Ag/AgCl electrode and the G/PANI/PS electrospun fibers modified SPCE as a working electrode. An in-house electrochemical set up used in the previous report was employed for all experiments [13]. The electrochemical characterization of the electrospun fibers was performed by cyclic voltammetry (CV) using 1 mM ferri/ferrocyanide in 0.5 M KCl with scanning potential in the range of −0.5 to +1.0 V at a scan rate of 100 mV s^{-1}. The square-wave anodic stripping voltammetry (SWASV) was employed under optimal conditions and involved a deposition potential of −1.2 V, a deposition time of 180 s, a frequency of 100 Hz, a potential amplitude of 40 mV, and a step potential of 21 mV. All standard metal solutions were prepared in HCl supporting electrolyte at a pH of 1.0 in situ Bi^{3+} 900 μg L^{-1} for the determination of Pb^{2+} and Cd^{2+} [46].

2.4. Preparation of river water samples

The river water samples were collected from different sources (i.e. Chao Phraya river, Saen Saeb canal). Firstly, known amounts of Cd^{2+} and Pb^{2+} were spiked into a portion of the water samples, and the samples were digested by using a nitric acid digestion method as shown in the previous report [47]. Then, all river water samples were acidified with 3% nitric acid and heated to boil on a hot plate. Two aliquots of milli Q water were added to the samples. Between each addition of milli Q water, the sample solution was allowed to evaporate by heating to eliminate the residue of nitric acid. Lastly, the pH and final volume of digested solution was then adjusted with supporting electrolyte of 0.1 M HCl and then stored in a freezer. The recovery and precision of the acid digestion method were evaluated by using standard addition method [12,47] for the analysis of water samples following spiking with either metal ions at final concentrations of 25, 50 and 150 μg L^{-1}. For method validation, the results obtained from the proposed method were compared with those obtained from ICP-OES method.

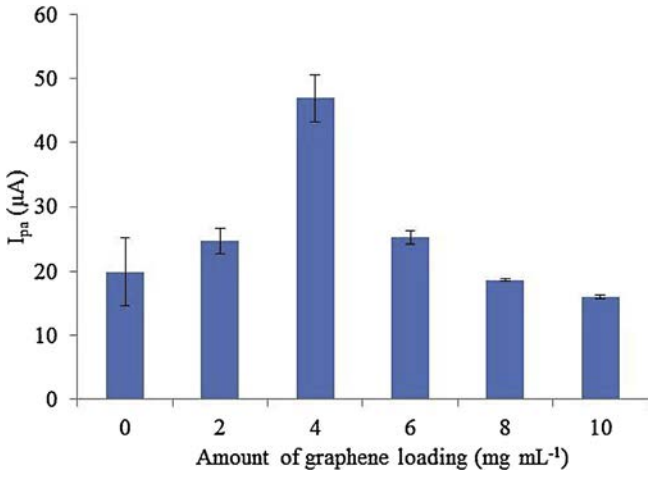

Fig. 2. Anodic peak current (I_{pa}) obtained from the cyclic voltammetric measurement of 1.0 mM $[Fe(CN)_6]^{4-/3-}$ standard redox couple in 0.5 M KCl measured on a G/PANI/PS nanoporous fiber modified SPCE with different amounts of G loaded. The error bars correspond to the standard deviation obtained from five measurements ($n = 5$).

3. Results and discussion

3.1. Optimization of the electrode composition and electrode modification process

The three components of the electrospun nanoporous fiber fabricated on the SPCE surface include G, PANI, and PS. G was used to enhance the electrical conductivity and surface area of the nanoporous fiber. PANI and PS were used as a conducting media and a carrier polymer for electrospinning fabrication, respectively. For SPCE modification by electrospinning, two important factors controlling the morphology of the electrospun G/PANI/PS nanoporous fiber and electrochemical sensitivity of the modified electrode are the type of organic solvent and amount of G loading. Initially, 20% w/v of PS was selected as an optimal concentration of the carrier polymer because this concentration continuously produces uniform electrospun fibers on SPCE. The type of organic solvent and amount of G loading were investigated and optimized. As shown in Fig. 1, the morphologies of G/PANI/PS fibers produced by using different solvent system were observed by SEM. For solvent contain DMF, the fiber surface appeared to be fairly smooth with longitudinal ridged along fiber axis and no porous structure observed. The formation of small ridges of G/PANI/PS fibers increases when the percentage of THF increases from 25% to 100% (Fig. 1a–d). This suggested that some part of fibers contained cavity underneath fiber skin which collapsed under atmospheric pressure similar to the core–shell formation of ribbon electrospun fiber. For 100% THF solvent system, numerous nanopores were observed on electrospun G/PANI/PS fibers surface (Fig. 1d). The nanopore formation could be contributed to different in evaporation rate of THF (with boiling point of 66 °C and DMF (with boiling point of 153 °C) and moisture effect, which led to microphase separation on fiber skin prior to solidification [48,49]. The porous surface area of electrospun fiber was expected to increase the specific surface area of modified SPCE, which in turn could improve electrochemical sensitivity of this sensor; thus, 100% THF (Fig. 1d) was chosen for further studies.

The amount of G loading was then investigated and optimized. The electrochemical performance of electrospun G/PANI/PS nanoporous fiber modified SPCE containing G in a range of 0–10 mg mL^{-1} was tested by cyclic voltammetric detection of standard 1.0 mM $[Fe(CN)_6]^{4-/3-}$ in 0.5 M KCl. As shown in Fig. 2, the anodic peak current significantly increases when the amount of G increases from 0 to 4 mg mL^{-1}. Nonetheless, the anodic peak current decreases when the amount of G greater than 4 mg mL^{-1}. This is probably cause by the self-agglomeration of G inside the nanoporous fibers, leading to decreased surface area and electrochemical conductivity. Therefore, 4 mg mL^{-1} of G loading was selected for further experiments.

3.2. Characterization of G/PANI/PS nanoporous fiber modified SPCE

After the composition of the G/PANI/PS nanoporous fiber was optimized, the morphology of nanoporous fibers was then characterized by scanning electron microscopy (SEM) and transmission microscopy (TEM). As shown in Fig. 3a and b, the nanoporous fibers with an average diameter of 2.44 ± 0.53 μm were created on the modified SPCE. These nanoporous secondary structures, with an apparent size in the range of 100–200 nm, were uniformly distributed throughout the fibers. As a result, the surface area of the fibers as determined by BET technique was found to be 12.23 $m^2\ g^{-1}$, which is relatively high for electrospun fiber of this size range. Additionally, a TEM image (Fig. 3c) of the nanoporous fibers verifies that G is randomly distributed inside the fiber, with no severe agglomeration of G within the nanoporous fiber. The TEM images also revealed many bright regions within the fiber matrix. Since the brightness of the TEM image is related to electron density of atom or the specimen thickness (or matrix body in this case), we speculated that these bright regions are voids or open cavities inside the matrix. While we do not know whether these voids are close cell or open cell in nature, nor do we know about the interconnectivity between these voids and surface pores, the surface area result indicated low inter-connectivity between these cavities and the surface pores.

3.3. Electrochemical characterization

As shown in Fig. 4a, cyclic voltammetry (CV) was performed using 1.0 mM $[Fe(CN)_6]^{4-/3-}$ standard redox couple for electrochemical characterization of the unmodified SPCE, PANI/PS nanoporous modified SPCE and G/PANI/PS nanoporous fiber modified SPCE. The peak potential values (E_{pa} and E_{pc}) of the G/PANI/PS nanoporous fiber modified SPCE (green) matched well with the unmodified SPCE (blue). Interestingly, both anodic and cathodic current responses (I_{pa} and I_{pc}) that were measured on the G/PANI/PS nanoporous fiber modified SPCE (green) increased approximately 3-fold and 2-fold compared to the unmodified SPCE (blue) and PANI/PS nanoporous fiber modified SPCE (red), respectively. The significant current response increases indicate that the presence of the G/PANI/PS nanoporous fibers enhance the electrochemical sensitivity of the unmodified SPCE. These results suggest that our modified electrode is potentially useful for development of a sensitive electrochemical sensor.

To study the mass transfer process of the modified electrode, the relationship between peak current and scan rate in a range of 10–100 mV s^{-1} was investigated on the G/PANI/PS nanoporous fiber modified SPCE as demonstrated in Fig. 4b. The results show that both anodic and cathodic current responses are directly propotional to square root of the scan rate ($\nu^{1/2}$), assuming it follows the Randle–Sevick Eq. (1).

$$I = (2.69 \times 10^5) n^{3/2} A C D^{1/2} \nu^{1/2} \quad (1)$$

where the constant n is the number of transferred electrons, A is the electrode surface area (cm^2), C is the concentration of the electroactive species (mol cm^{-3}), D is the diffusion coefficient of $[Fe(CN)_6]^{3-/4-}$ equal to 6.70×10^{-7} $cm^2\ s^{-1}$, and ν is scan rate ($V\ s^{-1}$).

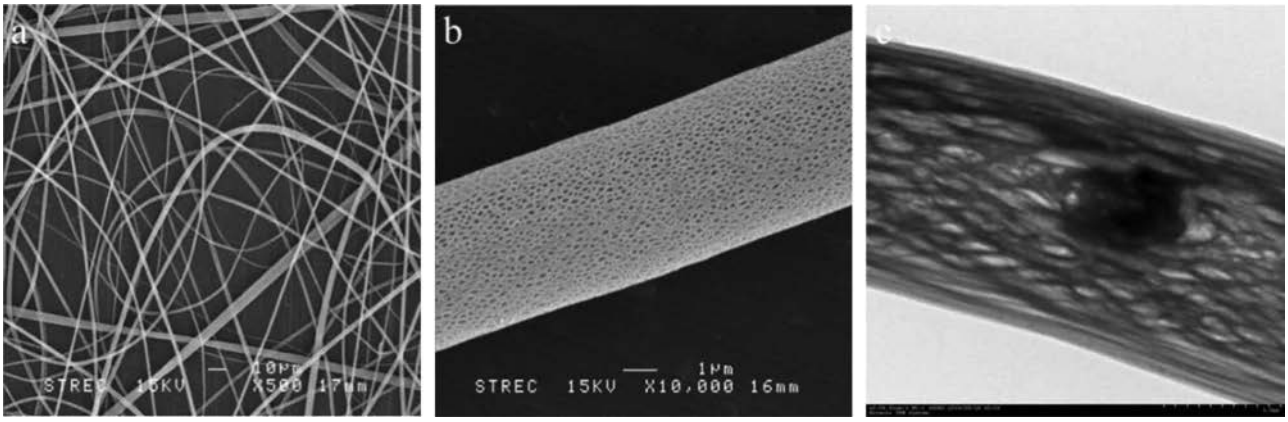

Fig. 3. SEM images of the G/PANI/PS nanoporous fibers (a) low magnification, (b) high magnification and (c) TEM image of random distribution of G in the G/PANI/PS nanoporous fiber.

As shown in Fig. 4b (inset), the currents increase linearly with the square root of scan rate in a range of 10–100 mV s^{-1}. The linearity of peak currents versus the square root of the scan rate with correlation coefficients (R^2) of 0.995 and 0.992 are obtained for both anodic and cathodic peak currents, indicating that a diffusion-controlled mass transfer process occurs on G/PANI/PS nanoporous fiber modified SPCE.

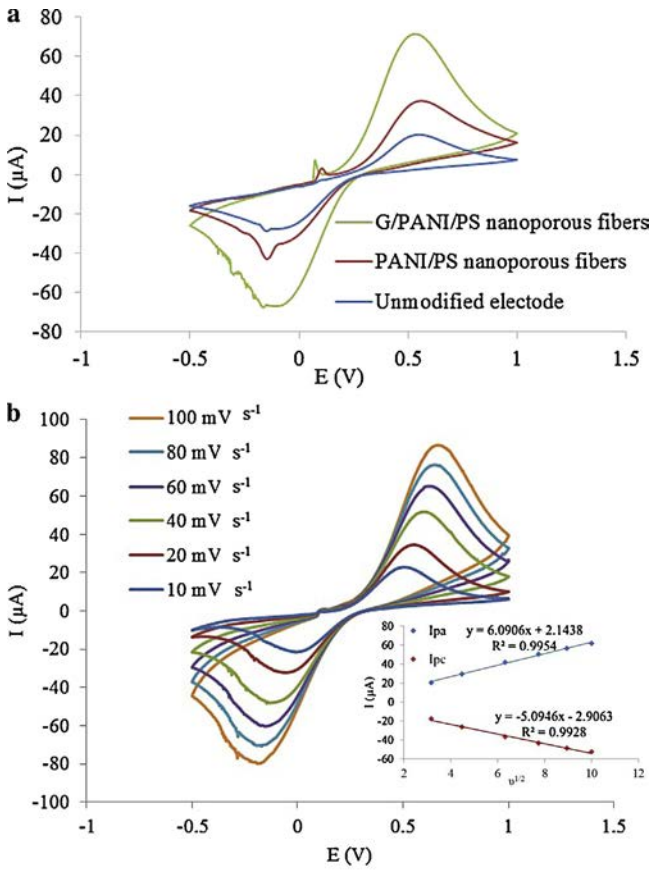

Fig. 4. Cyclic voltammograms of 1.0 mM $[Fe(CN)_6]^{4-/3-}$ in 0.5 M KCl with scan rate of 100 mV s^{-1} measured on an unmodified SPCE (blue), PANI/PS nanoporous fiber modified SPCE (red), G/PANI/PS nanoporous fiber modified SPCE (green) (a) and measured on G/PANI/PS nanoporous fiber modified SPCE at scan rate of 10, 20, 40, 60, 80 and 100 mV s^{-1} (b) and the anodic and cathodic peak currents as a function of the square root scan rate ($\nu^{1/2}$) (inset). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.4. Optimization of anodic striping voltammetry

With the high electrochemical sensitivity of G/PANI/PS nanoporous fiber modified SPCE, it was then applied for the simultaneous determination of Pb^{2+} and Cd^{2+} using anodic stripping voltammetry (ASV). Initially, several parameters related to the anodic stripping voltammetric response were optimized, including the type of supporting electrolyte, concentration of Bi^{3+}, deposition potential, deposition time, frequency, potential amplitude and step potential. To obtain the highest anodic current response and a well-defined stripping peak of Pb^{2+} and Cd^{2+}, hydrochloric acid (HCl) was selected as a supporting electrolyte and a frequency of 100 Hz, a potential amplitude of 40 mV, and a step potential of 21 mV were also used for all ASV experiments. Moreover, the parameters playing an important role in the stripping response of Pb^{2+} and Cd^{2+}, such as the concentration of Bi^{3+} and deposition potential and time were investigated. Bi^{3+} was selected to improve ASV performance of the G/PANI/PS nanoporous fiber modified SPCE because it can form a "fusible alloy" with heavy metals (e.g. Pb^{2+} and Cd^{2+}) at the electrode surface during preconcentration, and the ASV measurement can be performed without deoxygenation and without the use of more toxic mercury modified electrodes [42,43,50]. The stripping performance of the Bi/G/PANI/PS modified SPCE was studied with respect to the dependence of the electrode response for 200 μg L^{-1} of both Cd^{2+} and Pb^{2+} on the concentration of Bi^{3+} (100–1700 μg L^{-1}) in the measurement solution. In Fig. 5a, the mean anodic peak current for Cd^{2+} increases when the Bi^{3+} concentration increases from 100 to 900 μg L^{-1} while the mean anodic peak current of Pb^{2+} slightly increases and reaches a maximum value at 900 μg L^{-1}. At very high concentrations of the Bi^{3+} solution, the stripping peak responses of both Cd^{2+} and Pb^{2+} decrease probably because of the mass transfer limitation of Cd^{2+} and Pb^{2+} diffusing out from the Bi-thick film during the stripping step. Therefore, an optimum concentration of 900 μg L^{-1} of Bi^{3+} was chosen for the subsequent measurement.

The influence of the deposition potential or accumulation potential was optimized from −1.5 to −1.0 V (Fig. 5b) with a SW frequency of 100 Hz and a step potential of 21 mV. The current responses of the anodic stripping voltammograms for both Pb^{2+} and Cd^{2+} increase from −1.0 to −1.2 V. At deposition potentials more negative than −1.2 V, the current response decreases because of hydrogen evolution. The highest peak current of both Pb^{2+} and Cd^{2+} were obtained at −1.2 V (Fig. 5b); therefore, −1.2 V was chosen as an optimal deposition potential.

The effect of the deposition time on the G/PANI/PS nanoporous fiber modified SPCE's current response for Pb^{2+} and Cd^{2+} was studied in a range from 60 to 300 s. In Fig. 5c, the peak currents of

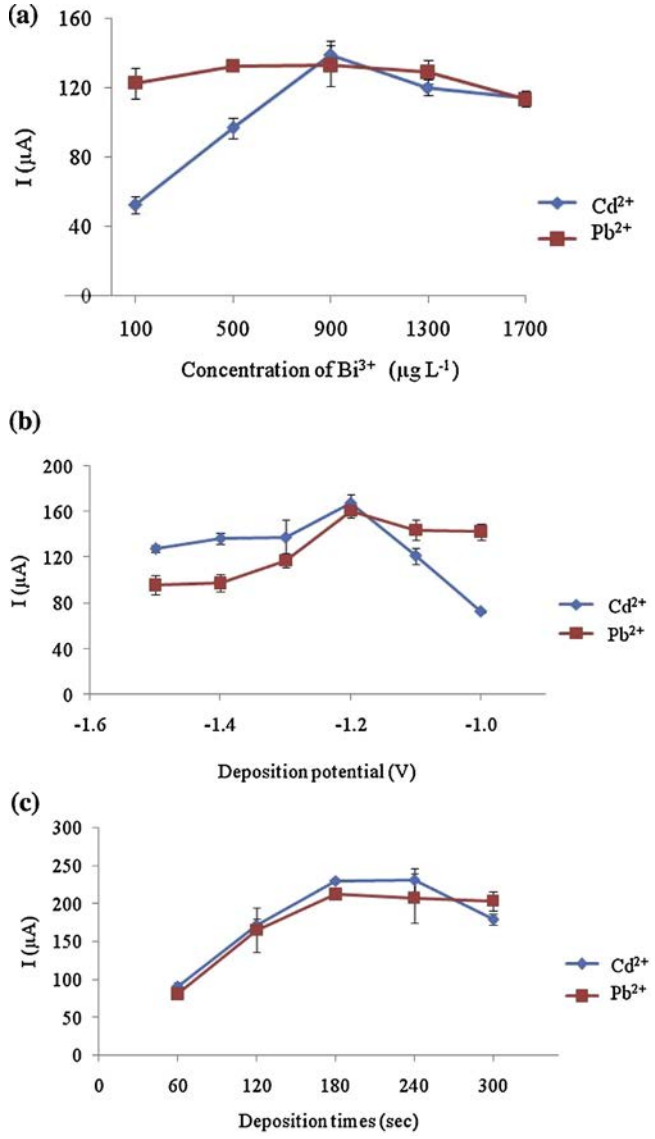

Fig. 5. Effect of Bi^{3+} concentration (a); measurement parameters: deposition potential of −1.2 V, deposition time of 120 s, frequency of 100 Hz, potential amplitude of 40 mV, and step potential of 21 mV, effect of deposition potential (b); measurement parameters: deposition time of 120 s, frequency of 100 Hz, potential amplitude of 40 mV, and step potential of 21 mV, and effect of deposition time (c); measurement parameters: deposition potential of −1.2 V, frequency of 100 Hz, potential amplitude of 40 mV, and step potential of 21 mV (c) on the stripping peak of 200 μg L^{-1} Pb^{2+} (red line) and Cd^{2+} (blue line) in 0.1 M HCl (pH 1.0). The error bars correspond to the standard deviation obtained from 5 measurements (n = 5). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

both Pb^{2+} and Cd^{2+} rapidly increase from 60 to 180 s and slightly decrease at a deposition time of 300 s. The level off and drop of peak current at higher deposition time is probably due to the electrode fouling from the excess amount of deposition metals on the electrode surface. Thus, 180 s of deposition time was chosen for further experiment.

Overall, the optimized conditions include HCl as a supporting electrolyte at a pH of 1.0, a Bi^{3+} concentration of 900 μg L^{-1}, a deposition potential of −1.2 V, a deposition time of 180 s, a frequency of 100 Hz, a potential amplitude of 40 mV, and a step potential of 21 mV. All of these parameters were used for further ASV experiments to provide the highest electrochemical sensitivity in the simultaneous determination of Pb^{2+} and Cd^{2+}.

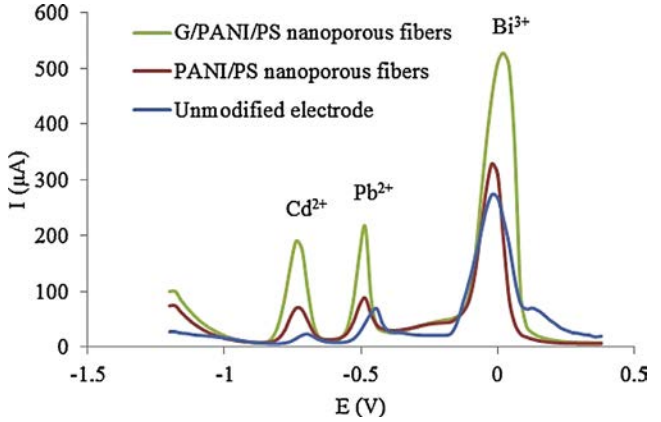

Fig. 6. Anodic stripping voltammograms of 200 μg L^{-1} Pb^{2+} and Cd^{2+} in the presence of 900 μg L^{-1} Bi^{3+} in 0.1 M HCl (pH 1.0). ASV measurement parameters: deposition potential of −1.2 V, deposition time of 180 s, frequency of 100 Hz, potential amplitude of 40 mV, and step potential of 21 mV measured on unmodified SPCE (blue), PANI/PS fiber modified SPCE (red) and G/PANI/PS nanoporous fiber modified SPCE (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.5. Analytical performance of G/PANI/PS nanoporous fiber modified SPCE

The voltammograms of 200 μg L^{-1} of Pb^{2+} and Cd^{2+} measured on the unmodified SPCE (blue), PANI/PS nanoporous fiber modified SPCE (red), and G/PANI/PS nanoporous fiber modified SPCE (green) under the optimized conditions are shown in Fig. 6. By using Bi^{3+} modification, the anodic peak currents of both metal ions exhibit well-defined peak shapes. For Cd^{2+} detection, the ASV responses measured on the G/PANI/PS nanoporous fiber modified SPCE (green) is higher than the one measured on the PANI/PS nanoporous fiber modified SPCE (red) and unmodified SPCE (blue) by 3-fold and 8-fold, respectively. For Pb^{2+} detection, the highest anodic peak current is observed on the G/PANI/PS nanoporous fiber modified SPCE (green), which is greater than the anodic peak current measured on the PANI/PS nanoporous fiber modified SPCE (red) and the unmodified SPCE (blue) by 2-fold and 5-fold, respectively. Similar to the cyclic voltammetric results for the $[Fe(CN)_6]^{4-/3-}$ standard redox couple shown in Fig. 4a, these data verify that the high surface area and great electrochemical conductivity of the G/PANI/PS nanoporous fiber modified SPCE lead to enhanced electrochemical sensitivity.

The analytical performance of the G/PANI/PS nanoporous fiber modified SPCE in the determination of Pb^{2+} and Cd^{2+} using the optimized conditions was evaluated. As shown in Fig. 7, the linear range was found in a range of 10–500 μg L^{-1} with a correlation coefficient (R^2) of 0.992 and 0.991 for Pb^{2+} and Cd^{2+}, respectively. The limits of detection (LOD), calculated by LOD = $3S_b/m$, where S_b is a standard deviation of the blank (estimated by eight replicate determination of the blank signals), m is a slope of calibration graph [38] were found to be 3.30 μg L^{-1} for Pb^{2+} and 4.43 μg L^{-1} for Cd^{2+}. According to the maximum allowable limits of Pb^{2+} and Cd^{2+} set by the National Environment Board, 50 μg L^{-1} for Pb^{2+}, 50 μg L^{-1} for Cd^{2+} (when water hardness as $CaCO_3$ is more than 100 mg L^{-1}) and 5 μg L^{-1} for Cd^{2+} (when water hardness as $CaCO_3$ is not more than 100 mg L^{-1}) in river water remain acceptable [51]. Thus, our method is still effective for the determination of both Pb^{2+} and Cd^{2+} in real river water.

Table 1 shows the analytical performances for determining Pb^{2+} and Cd^{2+} using our system as compared to the literatures. It verifies that our electrode system provides a comparable detection limit and wide linear range for the simultaneous determination

Table 1
Comparison of our proposed electrode to other modified electrodes in the determination of Pb^{2+} and Cd^{2+}.

Electrode	Detection limit ($\mu g\,L^{-1}$)		Linear range ($\mu g\,L^{-1}$)		Ref.
	Pb^{2+}	Cd^{2+}	Pb^{2+}	Cd^{2+}	
MWCNTs/synthesis Schiff base/CPE	0.25	0.74	0.4–1100	1–1200	[38]
Bi film/crown ether/Nafion/SPCE	0.11	0.27	0.5–60	0.5–60	[43]
TiO_2/ZrO_2 composite/CPE	0.48	0.77	1–200	1–200	[52]
ERGNO film/SPCE	0.80	0.50	1–60	1–60	[53]
Bi-CNT/SPCE	1.3	0.7	2–100	2–100	[54]
P(DPA-co-2ABN)/GC	165	255	260–58,730	1260–907,800	[55]
Diacetyldioxime/CPE	2.07	4.48	20.7–3105	28–2800	[56]
PANI/GC	20.7	14.56	0–414	0–224	[29]
G/PANI/PS nanoporous fiber/SPCE	3.30	4.43	10–500	10–500	This work

MWCNTs: multi-walled carbon nanotubes; CPE: carbon paste electrode; SPCE: screen-printed carbon electrode; TiO_2: titanium dioxide; ZrO_2: zirconium dioxide; ERGNO: electrochemically reduced graphene oxide; Bi: bismuth; GC: glassy carbon electrode; CNT: carbon nanotube; P(DPA-co-2ABN): Poly(diphenylamine-co-2-aminobenzonitrile).

of Pb^{2+} and Cd^{2+}. Interestingly, the G/PANI/PS nanoporous fiber modified electrode can be reused more than 10 times, and the relative standard deviation (RSD) was found to be 4.67% for Pb^{2+} and 3.52% for Cd^{2+} while maintaining good analytical performances.

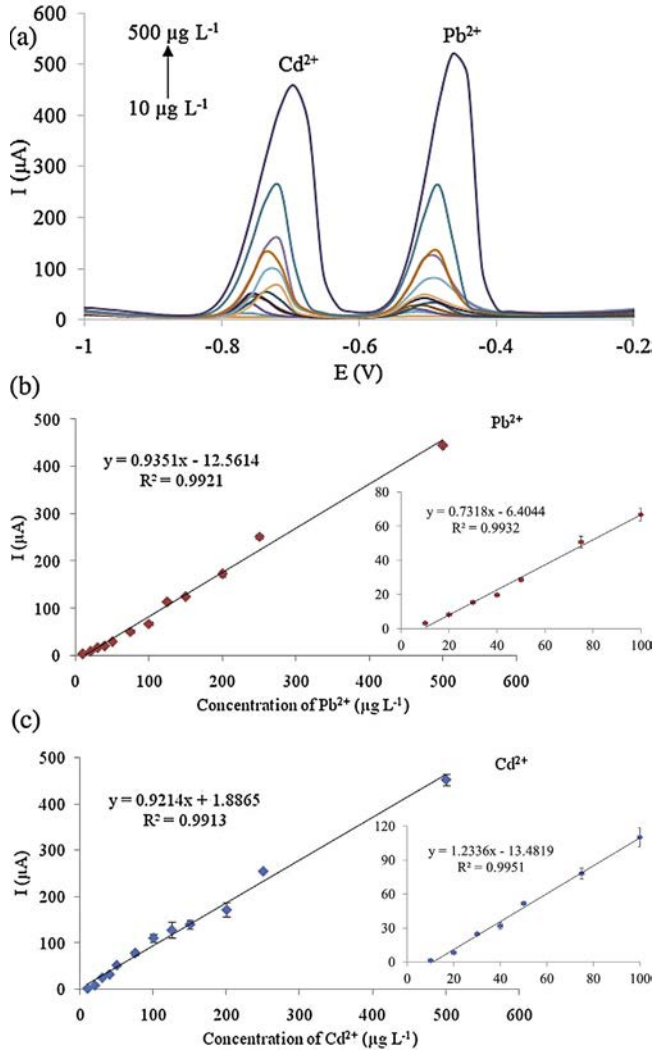

Fig. 7. Anodic stripping voltammograms of Pb^{2+} and Cd^{2+} in the concentration range of 10–500 $\mu g\,L^{-1}$ (a), the linear plot of Pb^{2+} concentration versus the current response (inset: linear plot of the concentration of 10–100 $\mu g\,L^{-1}$) (b) and the linear plot of Cd^{2+} concentration versus the current response (inset: linear plot of the concentration of 10–100 $\mu g\,L^{-1}$) (c). The error bars correspond to the standard deviation obtained from five measurements ($n = 5$).

3.6. Interference study

Since water samples usually contain other metal ions that could interfere with our system, the tolerance ratio for metal interferences was also evaluated. The tolerance ratio is defined as the peak response change ±5.0% from the Pb^{2+} and Cd^{2+} anodic peaks in the presence of foreign metals ions. The results show that a 500-fold mass ratio of Na^+, K^+, Mg^{2+}, Fe^{3+}, Co^{2+}, Cl^- and SO_4^{2-}, a 250-fold mass ratio of Ba^{2+}, Ca^{2+} and NO_3^{2-}, a 20-fold mass ratio of Ni^{2+}, and a 1-fold mass ratio of Cu^{2+} and Zn^{2+} were found as the tolerance ratios for the detection Pb^{2+} and Cd^{2+} at 50 $\mu g\,L^{-1}$. It can be seen that most of the metal ion interferences studied do not affect the determination of Pb^{2+} and Cd^{2+}. Nevertheless, Cu^{2+} and Zn^{2+} possibly influence the accuracy and sensitivity of our sensing system. Thus, water samples containing high levels of Cu^{2+} and Zn^{2+} need to be pretreated, masked or diluted to a level that is lower than their tolerance ratios [44].

3.7. Real sample analysis

To validate and evaluate the efficiency of our system, the G/PANI/PS nanoporous fiber modified SPCE was used to measure Pb^{2+} and Cd^{2+} in river water samples obtained from different sources. Fig. 8 shows the representative anodic stripping voltammograms of spiked Pb^{2+} and Cd^{2+} concentration at 0, 25, 50, 150 $\mu g\,L^{-1}$ in the Cho Phraya River. As shown in Table 2, the results obtained from our method were compared to those obtained from the conventional ICP-OES method. The % recoveries were found in the range of 85–109% and 95–103% for Pb^{2+} and Cd^{2+}, respectively. Nonetheless, both samples were in good agreement with the values obtained from ICP-OES. Obviously, these results demonstrate that

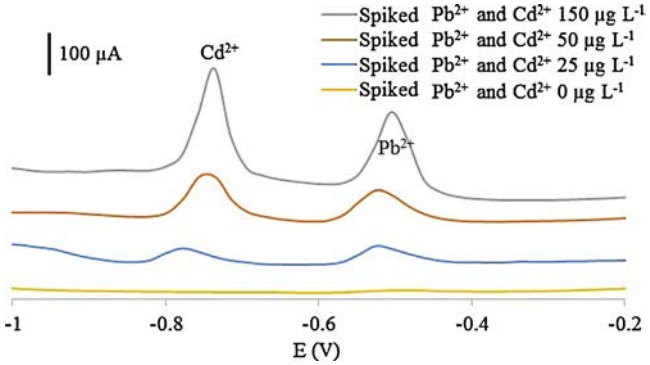

Fig. 8. Representative anodic stripping voltammograms of spiked Pb^{2+} and Cd^{2+} concentration at 0, 25, 50, 150 $\mu g\,L^{-1}$ in environmental water sample corrected from Cho Phraya River. ASV measurement parameters: deposition potential of −1.2 V, deposition time of 180 s, frequency of 100 Hz, potential amplitude of 40 mV, and step potential of 21 mV.

Table 2
Determination of Pb^{2+} and Cd^{2+} in river water samples using the proposed electrodes along with ASV compared with ICP-OES method ($n = 5$).

Sample	Added ($\mu g\,L^{-1}$)		Pb^{2+}		Cd^{2+}		Recovery (%)	
	Pb^{2+}	Cd^{2+}	ICP-OES ± SD($\mu g\,L^{-1}$)	Found ± SD ($\mu g\,L^{-1}$)	ICP-OES ± SD($\mu g\,L^{-1}$)	Found ± SD ($\mu g\,L^{-1}$)	Pb^{2+}	Cd^{2+}
Chao Phraya river	0	0	15.05 ± 0.62	10.30 ± 0.47	3.18 ± 0.07	ND	–	–
	25	25	38.60 ± 0.63	32.94 ± 2.64	24.86 ± 0.05	24.92 ± 2.40	89.93 ± 11.02	98.68 ± 9.61
	50	50	61.12 ± 0.25	53.02 ± 2.05	46.65 ± 0.04	51.84 ± 4.09	85.43 ± 4.11	103.68 ± 8.17
	150	150	165.14 ± 0.36	155.68 ± 8.01	143.11 ± 0.11	152.92 ± 5.89	96.92 ± 5.43	101.94 ± 3.93
Saen Saeb canal	0	0	14.09 ± 0.98	11.07 ± 0.40	0.64 ± 0.06	ND	–	–
	25	25	42.69 ± 0.51	38.11 ± 1.05	24.66 ± 0.14	25.34 ± 0.90	107.79 ± 4.21	101.36 ± 3.63
	50	50	62.58 ± 2.06	65.94 ± 3.30	45.72 ± 0.23	47.67 ± 3.02	109.54 ± 6.61	95.34 ± 6.05
	150	150	168.97 ± 0.49	159.08 ± 0.90	146.48 ± 0.26	153.77 ± 5.31	98.61 ± 0.60	102.51 ± 3.54

the G/PANI/PS nanoporous fiber modified SPCE developed in this study can be applied for simultaneous determination of Pb^{2+} and Cd^{2+} in river water samples with the satisfactory results.

4. Conclusions

A G/PANI/PS nanoporous fiber modified SPCE fabricated by electrospinning was prepared and applied for the simultaneous determination of Pb^{2+} and Cd^{2+} by using anodic stripping voltammetry (ASV). The high surface area and electrical conductivity of the G/PANI/PS nanoporous fiber significantly improve the electrochemical sensitivity in the determination of Pb^{2+} and Cd^{2+}. By using the modified electrode along with ASV, a linear range of 10–500 $\mu g\,L^{-1}$ was obtained for both Pb^{2+} and Cd^{2+}. The limits of detection (LOD) were found to be 3.30 $\mu g\,L^{-1}$ for Pb^{2+} and 4.43 $\mu g\,L^{-1}$ for Cd^{2+}, respectively. This modified electrode could be used as an alternative tool for environmental monitoring of Pb^{2+} and Cd^{2+} in real river water samples.

Acknowledgements

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