



รายงานวิจัยฉบับสมบูรณ์

การวิเคราะห์ทางเคมีไฟฟ้าของสารประกอบอินทรีย์โดยใช้ฟิล์ม
บางของโบรอน-โดปไดมอนด์ประยุกต์กับโฟลอินเจคชัน

Electroanalysis of Organic Compound using a Boron-Doped
Diamond Thin-Film Applied to Flow Injection Analysis

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ขอขอบคุณ

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ชื่อโครงการ: การวิเคราะห์ทางเคมีไฟฟ้าของสารประกอบอินทรีย์โดยใช้ฟิล์มบางของโบรอน-ไดโพลมอนต์ประยุกต์กับโพลีอินเจกชัน

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ระยะเวลาที่ทำการวิจัย: 3 ปี

ในงานวิจัยนี้ได้ทำการศึกษาการวิเคราะห์ทางเคมีไฟฟ้าของ D-penicillamine captopril และ tiopronin โดยใช้ฟิล์มบางของโบรอน-ไดโพลมอนต์อิเล็กโทรดด้วยเทคนิคต่างๆ เช่น ไซคลิกโวลแทมเมทรี และโพลีอินเจกชันร่วมกับแอมเพอโรเมทรี ไซคลิกโวลแทมเมทรีจะใช้ในการศึกษาปฏิกิริยาโดยเป็นฟังก์ชันกับความเข้มข้นและความเป็นกรด-ด่าง เปรียบเทียบผลการทดลองกับกลาสีคาร์บอนอิเล็กโทรด จากการทดลองพบว่าสารประกอบเหล่านี้จะเกิดปฏิกิริยาผันกลับไม่ได้กับขั้วไฟฟ้าทั้งสองชนิด โบรอน-ไดโพลมอนต์อิเล็กโทรดและกลาสีคาร์บอนจะให้ไซคลิกโวลแทมโมแกรมที่ชัดเจน แต่โบรอน-ไดโพลมอนต์อิเล็กโทรดจะให้ค่าสัญญาณของกระแสต่อพื้นหลังที่สูงกว่ากลาสีคาร์บอนอิเล็กโทรด นอกจากนั้นได้ทำการศึกษาโดยใช้เทคนิคแอมเพอโรเมทรีร่วมกับระบบโพลีอินเจกชันที่มีโบรอน-ไดโพลมอนต์อิเล็กโทรดเป็นขั้วไฟฟ้าทำงานสำหรับผลที่ได้จากเทคนิคโพลีอินเจกชันให้ค่าขีดจำกัดต่ำสุดในการตรวจวัดที่ 10 นาโนโมลาร์ ($S/N \approx 3$) จากผลการทดลองของ captopril ให้ช่วงความเข้มข้นที่เป็นเส้นตรงจาก 0.5 ถึง 50 ไมโครโมลาร์ และสำหรับ D-peicillamine and tiopronin ให้ช่วงความเข้มข้นที่เป็นเส้นตรงจาก 0.5 ถึง 50 ไมโครโมลาร์ วิธีการที่ใช้ในงานวิจัยนี้ยังนำไปประยุกต์ใช้ในการหาปริมาณ D-penicillamine, captopril และ tiopronin ในตัวอย่างยา จากการทดลองใช้เทคนิคการเติมสารมาตรฐานได้ร้อยละของการคืนกลับอยู่ในช่วง 95.85 -109.29, 97.75 - 104.69 และ 96.5-108.3 สำหรับ D-penicillamine, captopril และ tiopronin ตามลำดับ

คำสำคัญ: D-PENICILLAMINE, CAPTOPRIL, TIOPRONIN, ฟิล์มบางของโบรอน-ไดโพลมอนต์, ไซคลิกโวลแทมเมทรี, โพลีอินเจกชัน, แอมเพอโรเมทรี

Abstract

Project code: RSA/21/2544

Project Title: Electroanalysis of Organic Compound using a Boron-Doped Diamond Thin-Film Electrode Applied to Flow Injection System

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Project period: 3 years

In this research, a boron-doped thin film (BDD) electrode was used to study the electroanalysis of D-penicillamine, captopril, and tiopronin by several techniques such as cyclic voltammetry and flow injection with amperometric detection. Cyclic voltammetry was used to study the reaction as a function of concentration of analytes and pH of analyte solutions. Comparison experiments were performed using a glassy carbon (GC) electrode. These compounds undergo irreversible reaction at both electrodes. The BDD and GC electrodes provided well-resolved cyclic voltammograms but the voltammetric signal-to-background ratios obtained from the BDD electrode were higher than those obtained from the GC electrode. In addition, the amperometric detection along with flow injection analysis with diamond electrode as the working electrode was studied. The flow injection analysis results showed the significant low detection limit of 10 nM ($S/N \approx 3$). The linear dynamic ranges of concentrations were obtained from 0.5 to 100 μM for captopril and 0.5 to 50 μM for D-penicillamine and tiopronin. The proposed methods were successfully applied to determine captopril, D-penicillamine, and tiopronin in some drugs formulations. According to the procedure based on the standard addition technique, the recoveries obtained were 95.85-109.29%, 97.75-104.69% and 96.5-108.3% for captopril, D-penicillamine, and tiopronin, respectively.

Keywords: CAPTOPRIL, D-PENICILLAMINE, TIOPRONIN, BORON-DOPED DIAMOND THIN FILM ELECTRODE, CYCLIC VOLTAMMETRY, FLOW INJECTION SYSTEM, AMPEROMETRIC DETECTION

Executive Summary

วัตถุประสงค์

1. ศึกษาภาวะที่เหมาะสมสำหรับการตรวจวิเคราะห์สารประกอบอินทรีย์ที่มีซัลเฟอร์ เป็นองค์ประกอบ ได้แก่ D-penicillamine, captopril และ tiopronin ตัวอย่างเช่น ผลของ pH สารละลายอิเล็กโทรไลต์ อัตราการสแกน เป็นต้น
2. เปรียบเทียบสมบัติทางเคมีไฟฟ้าสำหรับการวิเคราะห์ D-penicillamine, captopril และ tiopronin ตัวอย่างเช่น ค่ากระแสพื้นหลัง การเกิดการดูดซับที่ผิวหน้าขั้วไฟฟ้าระหว่างขั้วไฟฟ้าฟิล์มบางโบรอน-โดปไดมอนด์กับขั้วไฟฟ้ากลาสีคาร์บอน
3. นำขั้วไฟฟ้าฟิล์มบางโบรอน-โดปไดมอนด์มาประยุกต์ใช้เป็นอุปกรณ์ตรวจวัดในระบบโพลีอินเจคชัน เพื่อวิเคราะห์หาปริมาณ D-penicillamine, captopril และ tiopronin

การดำเนินงานวิจัย และผลงานวิจัยที่ได้รับอย่างย่อ ๆ

1. ค้นคว้าข้อมูลและเอกสารที่เกี่ยวข้อง
2. ตรวจสอบสารประกอบอินทรีย์ที่มีซัลเฟอร์ เช่น D-penicillamine, captopril และ tiopronin โดยใช้โบรอน-โดปไดมอนด์อิเล็กโทรด และเทคนิค Cyclic Voltammetry และ Amperometry
3. หาภาวะที่เหมาะสมในการวิเคราะห์ D-penicillamine captopril และ tiopronin เพื่อปรับปรุงวิธีการวิเคราะห์ให้สามารถวิเคราะห์ได้ด้วยความเข้มข้นต่ำ และเปรียบเทียบสมบัติทางเคมีไฟฟ้าของสารประกอบอินทรีย์ เมื่อใช้โบรอน-โดปไดมอนด์อิเล็กโทรดและกลาสีคาร์บอนอิเล็กโทรดโดยใช้เทคนิค Cyclic Voltammetry จากการทดลองพบว่า สารประกอบเหล่านี้สามารถเกิดปฏิกิริยาผันกลับไม่ได้กับขั้วไฟฟ้าทั้งสองชนิด แต่โบรอน-โดปไดมอนด์อิเล็กโทรดจะให้ค่าสัญญาณของกระแสต่อพื้นหลังที่สูงกว่ากลาสีคาร์บอนอิเล็กโทรด pH ที่เหมาะสมที่ให้สัญญาณในการตรวจวัดสูงสุดสำหรับ D-penicillamine, captopril และ tiopronin ได้แก่ 7, 9 และ 8 ตามลำดับ
4. หาภาวะที่เหมาะสมโดยใช้เทคนิคแอมเพโรเมทรี ร่วมกับการตรวจวัดด้วยระบบโพลีอินเจคชันโดยใช้โบรอน-โดปไดมอนด์อิเล็กโทรดเป็นขั้วไฟฟ้าทำงาน จากผลการทดลองพบว่าศักย์ไฟฟ้าที่เหมาะสมในการตรวจวัด D-penicillamine, captopril และ tiopronin ได้แก่ 0.75, 0.8 และ 0.8 โวลต์ ตามลำดับ สำหรับผลที่ได้จากเทคนิคโพลีอินเจคชันให้ค่าขีดจำกัดต่ำสุดสำหรับสารทั้งสามชนิด ในการตรวจวัดที่ 10 นาโนโมลาร์ ($S/N \approx 3$) จากผลการทดลอง

5. นำวิธีที่เหมาะสมมาใช้หาปริมาณ D-penicillamine, captopril และ tiopronin ในสารตัวอย่าง การทดลองใช้เทคนิคการเติมสารมาตรฐานพบว่า ได้ร้อยละของการคืนกลับอยู่ในช่วง 95.85 -109.29, 97.75 - 104.69 และ 96.5-108.3 สำหรับ D-penicillamine, captopril และ tiopronin ตามลำดับ จากผลการทดลองพบว่าการใช้เทคนิคแอมเพอโรเมทรีร่วมกับระบบโพลีอินเจชันให้ผลการทดลองที่ถูกต้อง มีความสามารถในการทำซ้ำ และช่วงการทำงานที่เป็นเส้นตรงที่กว้าง

Introduction

Boron-doped diamond (BDD) thin film electrode is one of the new promising materials for electrochemical applications due to its unique and extremely useful property. Electroanalysis is one field that can benefit from the attractive electrochemical properties of diamond thin film as an electrode material. Its attractive properties such as very low and stable voltammetric background current, wide potential window in aqueous electrolyte solutions (2.5-3 V), slight adsorption of polar organic molecules, high resistance to deactivation via fouling and good activity toward some redox analytes without any conventional pretreatment.

Several methods have been proposed for the determination of thiol containing drug, including high-performance liquid chromatography with various detectors such as calorimetry, fluorometry, chemiluminescence, spectrometry. One of the important limitation of these spectrophotometric techniques is the fact that sulfur containing compounds lack sufficient UV absorption so a pre or post-column derivatization procedure is normally required and this results in increased cost and complication of analysis. Electrochemical was an alternative method for the determination of sulfur containing compounds because it was cheap, simple, fast and low cost. The electrochemical detection of captopril has been reported by using graphite and selective membrane electrode as the working electrode. Nevertheless, these electrodes were generally provided low sensitivity and reproducibility because the surface of these electrodes was easy to contaminate by the fouling products

and impurities. Boron doped diamond thin film electrode can be used to eliminate this problem without any pretreatment because of the stable surface morphologies and the surface carbon atoms terminated by hydrogen. Thus, the BDD surface is relatively non-polar and suffers less adsorption of polar molecules, as reported by Xu et al. The stability is also very high.

Alternative automatic procedure based on flow injection techniques have been widely suggested, since they are enable to reduce time of analysis. Moreover, they can increase reproducible and accurate results. Flow injection with amperometric detection using boron doped diamond thin film electrode also has been reported to determine some organic compounds such as polyamine, histamine and serotonin.

In this research, we used the boron doped diamond thin film electrode to study organic thiol-containing compounds such as D-penicillamine, captopril and tiopronin by using cyclic voltammetry in comparison with glassy electrode. Hydrodynamic voltammetry and flow injection analysis with amperometric detection was also used to determine captopril in the standard chemical form and commercial available tablets.

D-Penicillamine, 3-mercapto-D-valine is a pharmaceutically important thiol compound frequently used as a medicinal agent against a number of diseases i.e. rheumatoid arthritis, cystinuria, liver disease or certain skin conditions, heavy metal poisoning and Wilson 's disease. It may also inhibit the replication of the human immunodeficiency virus, the cause of acquired immune deficiency syndrome (AIDS).

Captopril belongs to the group of anti-hypertensive drugs that affect the renin-angiotensin system and are commonly referred to as angiotensin converting enzyme (ACE) inhibitor. The chemical name of captopril is (S)-1-(3-Mercapto-2-methyl-L-oxo-propyl)-L-proline, is widely used for treatment of arterial hypertension. Recent studies suggest that it may also act as a scavenger of free radicals because of its thiol group.

Tiopronin [*N* – (2 –mercaptopropionyl) - glycine] is a drug with a thiol group that is used in clinical applications. It is effective in the treatment of cystinuria, rheumatoid arthritis as well as hepatic disorders, and as an antidote to heavy metal poisoning. Along with its needed effects, it may cause some unwanted effects such as muscle pain, yellow skin or eyes, sore throat and fever, change in taste or smell etc. Moreover this drug produces a dose - related nephrotic syndrome. Tiopronin is available only with a doctor's prescription.

2. Experimental

2.1 Chemicals and reagents

All chemicals were analytical grade and used without further purification. All solutions were prepared by using deionized water. Phosphate buffers (pH 5 - 8), 0.1 M, were prepared from 0.1 M of potassium dihydrogen phosphate (Merck) and 0.1 M disodium hydrogen phosphate (BDH). Phosphate buffer (pH 2.5) was prepared from 0.1 M of potassium dihydrogen phosphate and the pH was adjusted with orthophosphoric acid (85 %, Carlo Erba). Phosphate buffer (pH 9.0), 0.1 M, was prepared from 0.1 M of potassium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate and the pH was adjusted with 0.1 M sodium hydroxide (Merck) solution. D-penicillamine (Sigma) solutions, standard captopril (Sigma) solutions and standard tiopronin (Sigma) were freshly prepared in 0.1 M phosphate buffer prior to use.

2.2 Electrode

The commercial BDD electrodes were rinsed with ultrapure water prior to use. The glassy carbon (GC) electrode was purchased from Bioanalytical System, Inc (area 0.07 cm²). It was pretreated by sequential polishing with 1 and 0.05 micron of alumina /water slurries on felt pads, followed by rinsing with ultrapure water prior to use.

2.3 Cyclic Voltammetry

Electrochemical measurements were recorded using Autolab Potentiostat 100 (Eco-Chemie) with a standard three-electrode glass cell configuration. The BDD electrode was pressed against a smooth ground joint at the bottom of the cell isolated by an O-ring (area 0.07 cm^2) and served as the working electrode. Ohmic contact was made by placing the backside of the Si substrate on a brass plate. GC electrode was also used as a working electrode in comparison study with the BDD electrode. A platinum wire and a Ag/AgCl with a salt bridge were used as the counter and reference electrodes, respectively. Cyclic voltammetry was used to probe the electrochemical reaction. The electrochemical measurement was housed in faradaic cage to reduce electronic noise. All experiments were done at a room temperature.

2.4 Flow injection analysis with amperometric detection

The flow injection analysis system consisted of a thin layer flow cell (Bioanalytical System, Inc.), an injection port (Rheodyne 7125) with a 20- μL injection loop, a peristaltic pump (Ismatec) and an electrochemical detector (PG 100). The mobile phase, 0.1 M phosphate buffer (pH 9), was regulated by an a reagent delivery module at a flow rate of 1 ml/min. The thin layer flow cell consisted of a silicone rubber gasket as a spacer, Ag/AgCl as the reference electrode, stainless steel tube as an auxiliary electrode and an outlet of

the flow cell. The experiments were performed in a copper faradaic cage to reduce the electrical noise. A hydrodynamic voltammogram was obtained before the amperometric determination was performed. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential to obtain hydrodynamic voltammograms. The amperometric measurements were carried out at the potential giving a maximum signal-to-background (S/B) ratio in the hydrodynamic voltammograms, or in the limiting current range.

2.5 Sample preparations

A mass of powder of one capsule of penicillamine (Cuprimine 250 mg) was transferred to 100 ml volumetric flask and dissolved in 0.1 M phosphate buffer (pH 7), filtrated through a 0.45 μ M Nylon membrane syringe filter. Then, the filtrate was further diluted with 0.1 M phosphate buffer (pH 7) to obtain a final concentration of 2.5 μ g/ml.

Tablets containing captopril (Bristol-Myers Squibb) were analyzed. Five tablets were each weighed so as to obtained the mean tablet weight. An accurate weight portion of the homogenized powder corresponding to mean tablet weight was treated with 0.1 M phosphate buffer (pH 9), then dilute to the final concentration with the same solution, providing a concentration within the linear dynamic range.

Tablets containing tiopronin (Mission pharmacal) were analyzed. The drug tablet was homogenized in an agar mortar. The amount of the powdered mass analyte was

dissolved in 100 ml of 0.1 mol L^{-1} phosphate buffer (pH 8). This solution was diluted in such a way that the concentration of tiopronin in the final test solution fell within the linear dynamic range ($0.5\text{--}50 \text{ }\mu\text{mol L}^{-1}$).

3. Results and Discussion

3.1 Determination of D-Penicillamine

3.1.1 *Cyclic voltammetry*

Fig 3.1.1 shows the cyclic voltammograms for 1 mM D-penicillamine together with the corresponding background voltammogram in 0.1 M phosphate buffer (pH 7) at the BDD and GC electrodes. The background current for the GC electrode was ~ 10 times higher than that obtained for the BDD electrode. The BDD exhibits a well-defined irreversible oxidation peak at ~ 0.8 V vs Ag/AgCl, whereas the GC electrode provides an ill-defined irreversible oxidation peak at ~ 0.9 V vs Ag/AgCl.

At the BDD electrode, the cyclic voltammogram of the second cycle still exhibits a well-defined cyclic voltammogram with a slightly decreasing peak current and the current can revert to its original value after purging with a deionized water, indicating the absence of electrode fouling. In the case of GC electrode, the cyclic voltammogram in the second exhibits a featureless cyclic voltammogram and the current can only revert after polishing the electrode with the alumina slurries. This was due to electrode fouling.

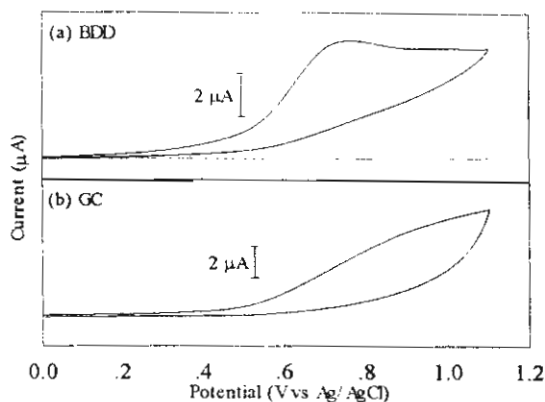


Fig. 3.1.1. Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at (a) boron-doped thin film electrode and (b) GC electrode (solid line). The potential sweep rate was 20 mV s^{-1} . Background voltammograms (0.1 M phosphate buffer, pH 7) are also shown in this figure (dotted line).

3.1.2 pH Dependence study

The effect of the buffer pH was investigated from pH 5 to 9.2. At buffer pH between pH 7 and 9.2, the BDD electrode exhibits a well-defined cyclic voltammogram while the GC electrode provides an ill-defined cyclic voltammogram. In the case of buffer pH 5, the BDD electrode provides an ill-defined cyclic voltammogram while the GC electrode provides a featureless cyclic voltammogram.

The changing of buffer pH effects the oxidation peak potential. It was found that increasing the buffer pH, decreased the oxidation peak potential, probably due to the more facile oxidation of the thiol group of D-penicillamine in the alkaline medium. In addition the buffer also affected the peak current of the cyclic voltammogram. At the BDD electrode, the maximum peak current with the maximum S/B ratio was observed at pH 7. Therefore this pH was chosen as the optimal pH.

3.1.3 Scan rate dependence study

Fig. 3.1.2 shows the cyclic voltammetric response of 1 mM D-penicillamine in 0.1 M phosphate buffer pH 7 with variation of the scan rate from 0.01 to 0.3 V/s at the BDD electrode. The oxidation current varied highly linearly ($r > 0.99$) with the square root of the scan rate, $v^{0.5}$, as shown in the inset of this figure, indicating semi-infinite linear diffusion of reactant to the electrode surface.

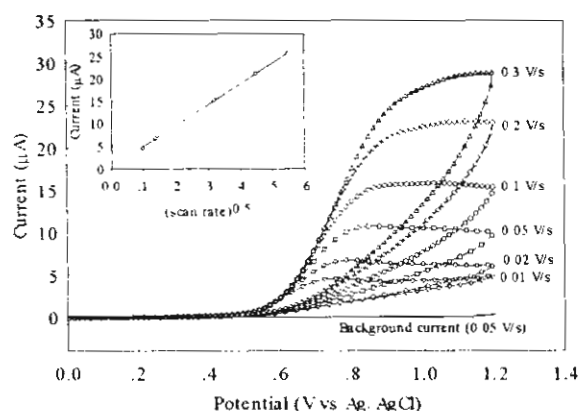


Fig. 3.1.2 Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at boron-doped thin film electrode (the potential sweep rate was varied from 0.01 to 0.3 $V s^{-1}$). The curve of the relationship between current and $(\text{scan rate})^{0.5}$ is inset.

3.1.4 Concentration dependence study

The oxidation peak current was investigated for the concentration range from 0.025 to 20 mM D-penicillamine at the BDD. Fig. 3.1.3 shows the cyclic voltammograms with the concentration of D-penicillamine varied from 0.5 mM to 10 mM. Linear regression analysis of current (μA) versus concentration (mM) profiles showed a reasonable linearity from 0.5 to 10 mM ($r > 0.99$) for the BDD electrode, as shown in the inset of this figure. The GC

electrode provides no linear regression relationship between the oxidation current and the concentration. This may be due to the electrode fouling. At a concentration as low as 0.025 mM and above, a well defined peak with the S/B ratios > 3 was obtained at the BDD electrode while the GC electrode provided this S/B value at the concentration of 0.5 mM due to its high background current.

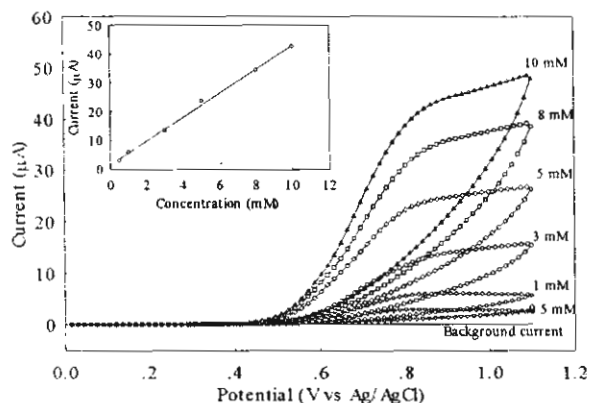


Fig. 3.1.3. Cyclic voltammograms for D-penicillamine in 0.1 M phosphate buffer (pH 7) (the concentration varied from 0.5 to 10 mM) at boron-doped thin film electrode. The potential sweep rate was 0.02 V s^{-1} . The calibration curve was also is inset.

The reproducibility of the BDD electrode was also studied. The test consisted of running the cyclic voltammogram for five aliquots (the same concentration) of three concentrations (1, 5 and 10 mM D-penicillamine). It appears that the reproducibility test is satisfied because the peak current variation value (%RSD) was 2.2 to 3.1.

3.1.5 Flow injection analysis with amperometric detection

3.1.5.1 Hydrodynamic voltammetry

Fig. 3.1.4(a) shows a hydrodynamic voltammetric i-E curve obtained at the BDD electrode for 20 μL injections of 100 μM D-penicillamine in 0.1 M phosphate buffer (pH 7),

using 0.1 M phosphate buffer (pH 7) as the carrier solution. Each datum represents the average of four injections. The absolute magnitude of background current at each potential is also shown for comparison. The hydrodynamic voltammogram for D-penicillamine did not produce a sigmoidal shape of the signal versus potential. To obtain the maxima potential point, the S/B ratio was calculated from the Fig. 3.1.4(a) at each potential. The hydrodynamic voltammetric S/B ratio vs potential curve are obtained as shown in Fig. 3.1.4(b) with the maximum S/B ratios of 0.75 V. Hence, this potential was set as the amperometric potential detection in flow injection analysis experiments.

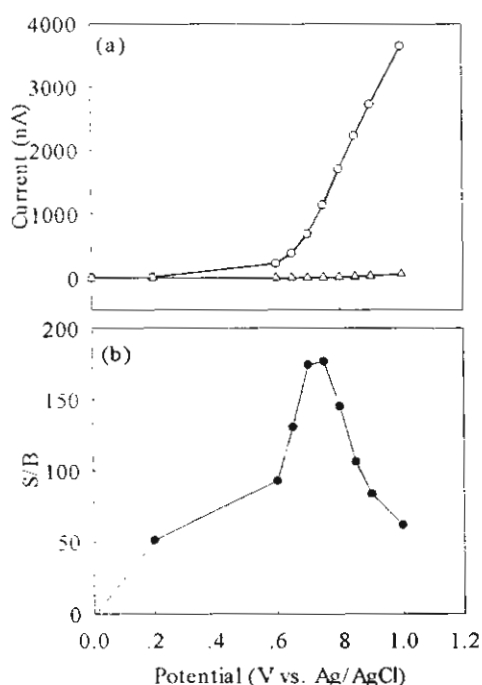


Fig. 3.1.4. (a) Hydrodynamic voltammograms of (-○-) 100 μ M of D-penicillamine in 0.1 M phosphate buffer (pH 7) and (-□-) 0.1 M phosphate buffer (pH 7, background current) with four injections of analytes, using 0.1 M phosphate buffer (pH 7) as a carrier solution. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min⁻¹.

3.1.5.2 Linear range, detection limit and reproducibility

Fig. 3.1.5 shows a series of repetitive 20 μ L injections of D-penicillamine in 0.1 M phosphate buffer pH 7 at the detection potential of 0.75 V versus Ag/AgCl. Well-defined

signals without peak tailing were obtained at all concentrations from 10 nM to 2.5 mM. The current signal increased linearly with the increasing of concentrations from 0.5 to 50 μM ($r > 0.99$) as shown in the inset of this Figure. The sensitivity of this method, which is the slope of the relation plot between the current and the concentration over the linear range, was 12.1-17.3 nA/ μM . Interestingly, the detection limit with $S/N \geq 3$ was obtained at the concentration as low as 10 nM of D-penicillamine. The reproducibility of the response was also examined. A peak variability of 1.8% was found during a course of 45 injections of 100 μL D-penicillamine, indicating the high stability of the BDD electrode.

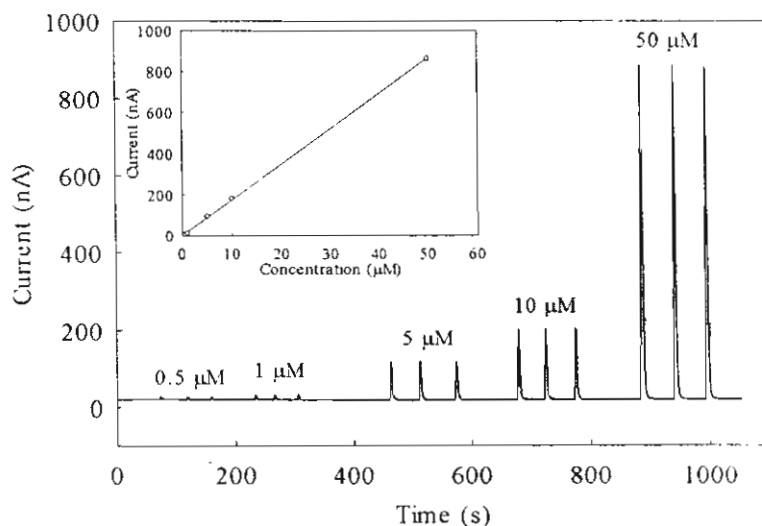


Fig. 3.1.5. Flow injection with amperometric detection results for D-penicillamine (0.5–50 μM) and calibration graph at the applied potential of 0.75 V vs. Ag/AgCl in 0.1 M phosphate buffer (pH 7) with three injections of analytes. Other conditions are the same as in Fig. 3.1.4

3.1.6 Application

3.1.6.1 Drug analysis of Penicillamine capsules

The proposed method for penicillamine was applied to the determination of penicillamine capsules. The precision of the method was obtained on the basis of intra-assay using standard addition. The intra assay study results ($n = 2$) in the same day are summarized in Table 1. There was no significant difference between the labeled contents and those obtained by the proposed method with the satisfactory recovery. Recoveries ranged from 97.75 to 104.69. Moreover, repeatability of results for the addition of D-penicillamine 0, 0.6 and 1.49 mg/ml with 10 replicates was obtained with a relative standard deviation (% RSD) of 1.5 to 2.1. The inter-assay was also study between the days ($n = 4$) and the results are summarized in Table 2. There is no significant difference in the results, indicating that the analysis of penicillamine capsules by the proposed method is reproducible.

Table 1. Recovery of D-penicillamine sample with amperometric detection on the BDD electrode applied to flow injection system of the intra assay study ($n=2$)

Amount of added ($\mu\text{g ml}^{-1}$)	Amount of found ($\mu\text{g ml}^{-1}$)	Percent of recovery (%)
0.60	0.59 ± 0.01	98.68 ± 0.93
0.90	0.94 ± 0.01	104.04 ± 0.65
1.19	1.19 ± 0.01	99.62 ± 0.39
1.49	1.48 ± 0.01	98.84 ± 0.33
250 mg per tablet	255.62 ± 0.14	102.11 ± 0.55

Table 2. Recovery of D-penicillamine sample with amperometric detection on the BDD electrode applied to flow injection system of the inter assay study ($n=4$)

Amount of added ($\mu\text{g ml}^{-1}$)	Amount of found ($\mu\text{g ml}^{-1}$)	Percent of recovery (%)
0.60	0.58 ± 0.01	98.04 ± 0.94
0.90	0.91 ± 0.03	101.30 ± 3.06
1.19	1.17 ± 0.02	99.07 ± 1.45
1.49	1.50 ± 0.03	100.43 ± 1.98
250 mg per tablet	255.55 ± 2.50	102.22 ± 0.99

3.1.6.2 *Interference study*

Talc, lactose excipients frequently added to dosage forms are talc, magnesium stearate and starch. Titanium dioxide and magnesium stearate do not dissolve in 0.1 M phosphate buffer. They were filtered out in the sample preparation step, so they did not interfere with the response. Only lactose was used in this study. It was found that the tolerance limit for the ratio between D-penicillamine and lactose concentrations was 80. This caused an error of not more than $\pm 5\%$. The separation of this interference is being studied in our laboratory.

3.2 Determination of Captopril

3.2.1 Cyclic voltammetry

Fig 3.2.1a) and 3.2.1b) show the cyclic voltammograms for 1 mM captopril together with the corresponding background voltammogram in 0.1 M phosphate buffer (pH 9) at the BDD and GC electrodes. The background current for the GC electrode was ~ 10 times higher than that obtained for the BDD electrode. The BDD exhibited a well-defined irreversible oxidation peak at ~ 0.8 V vs Ag/AgCl, whereas the GC electrode provided an ill-defined irreversible oxidation peak. No cathodic peak was observed on the reverse scan within the investigated potential range (0 V to +1.4 V) because captopril oxidation is an electrically irreversible process.

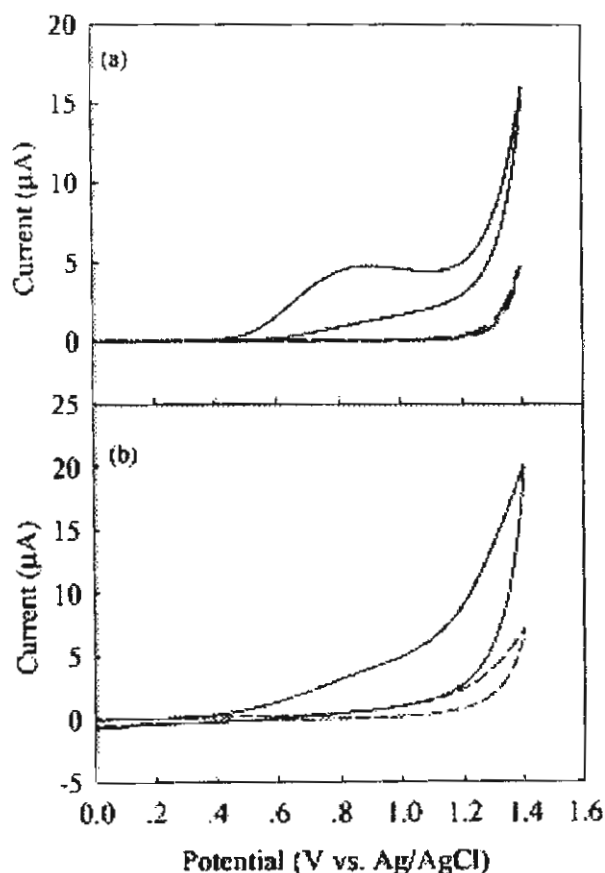


Fig. 3.2.1 Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 1.0 mM captopril in 0.1 M phosphate buffer pH 9 (solid lines) and 0.1 M phosphate buffer pH 9 (dashed lines). Sweep rate, 50 mV s^{-1} ; area of electrode, 0.07 cm^2 .

The effect of the buffer pH was investigated from pH 5 to 10. At the buffer pH between pH 7 and 9.2, the BDD electrode exhibited a well-defined cyclic voltammogram while the GC electrode provides an ill-defined cyclic voltammogram. In the case of buffer pH 5, the BDD electrode provides an ill-defined cyclic voltammogram while the GC electrode provides a featureless cyclic voltammogram.

The changing of buffer pH effects to the oxidation peak potential. At BDD electrode, it was found that increasing the buffer pH, increased of the oxidation peak potential, probably due to the facile oxidation of the thiol group of captopril structure in the alkaline medium (data were not shown). The maximum peak current with the maximum S/B ratios was observed at pH 9. Therefore this pH was chosen as the optimal pH.

3.2.2 Concentration and sweep rate dependence

The oxidation peak current was measured at the BDD electrode for the concentration range from 0.025 mM to 20mM captopril in 0.1M phosphate buffer (pH 9) at sweep rate 50 mV s^{-1} . Based on a series of cyclic voltammograms in which the concentration of captopril was varies from 0.025mM to 20mM (data shown in Fig 3.2.2), a linear regression statistical analysis of peak current (μA) versus concentration (mM) was obtained, with linearly proportional in range 0.05mM to 1mM ($r > 0.99$). The response of BDD electrode is appropriate for quantitative captopril determination even in the millimolar concentration range.

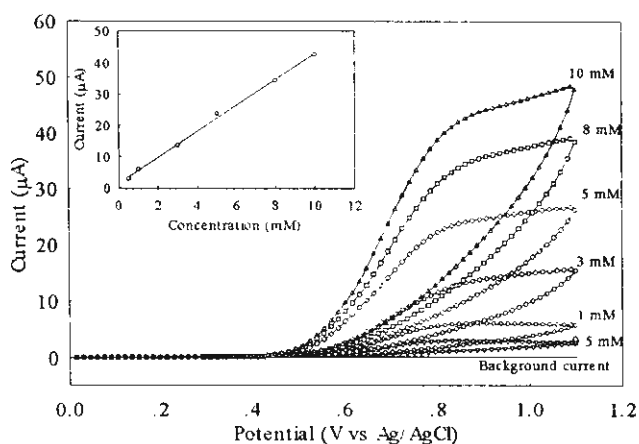


Fig. 3.2.2 Cyclic voltammogram for captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of captopril concentrations. The potential sweep rate was 50 mV s^{-1} ; area of electrode, 0.07 cm^2 . The calibration curve is shown in the inset.

We also investigated the potential sweep rate dependence, as shown in **Fig.3.2.3**

The voltammograms of 1 mM captopril were recorded during variation of the scan rates at the BDD electrode. It can be seen that peak current (μA) are linearly proportional to the square root of the sweep rate ($(\text{V s}^{-1})^{1/2}$) within the range 0.01 to 0.3 V s^{-1} . The linear regression analysis yields $r > 0.999$. From voltammograms, it was found that the peak potential shifted positively with increasing sweep rate, as expected for an irreversible process, and the linearity suggest that the reaction involves a diffusing species.

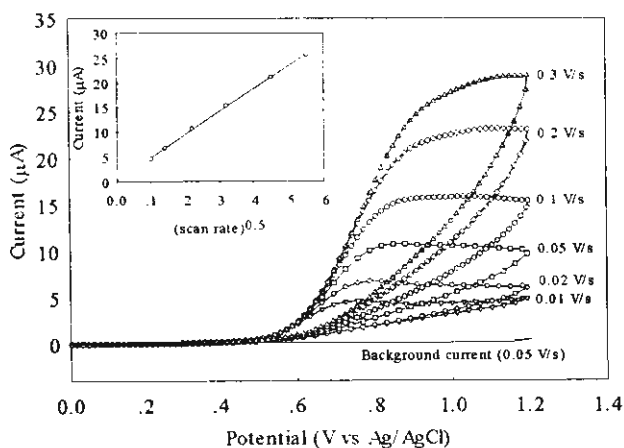


Fig. 3.2.3 Cyclic voltammograms for 1 mM captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm^2 . The calibration curve of relationship between current (μA) and $(\text{sweep rate})^{0.5}$ was also in the inset of this figure.

3.2.3 Hydrodynamic voltammetry

To obtain an optimal potential for the amperometric detection in flow injection analysis, the hydrodynamic voltammetric behavior of captopril was studied. **Fig 3.2.4** shows a hydrodynamic voltammetric i-E curve obtained at the BDD electrode for 20 μL injections of 100 μM captopril in 0.1 M phosphate buffer (pH 9), using 0.1 M phosphate buffer (pH 9) as the carrier solution. Each datum represents the average of four injections. The absolute magnitude of background current at each potential is also shown for comparison. The hydrodynamic voltammogram for captopril did not produce a sigmoid shape of the signal versus potential. The S/B ratio was calculated from the Fig. 3.2.4(a) at each potential to obtain the maxima potential point. Therefore, the hydrodynamic voltammetric S/B ratios versus potential curve are obtained as shown in Fig. 3.2.4(b) with the maximum S/B ratios of 0.9 V. Hence, this potential was set as the amperometric potential detection in flow injection analysis experiments.

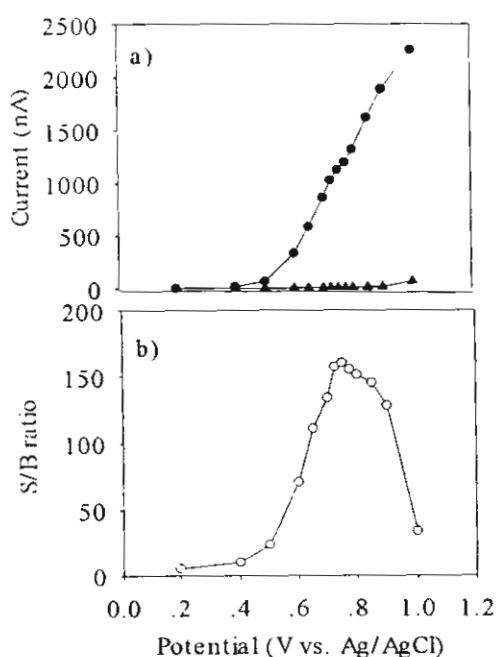


Fig. 3.2.4. (a) Hydrodynamic voltammogram of (---) 0.1 M phosphate buffer (pH 9, background current) and (—●—) 100 μM of captopril in 0.1 M phosphate buffer (pH 9) with four injections of analysis, using 0.1 M phosphate buffer (pH 9) as a carrier solution. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min^{-1} .

3.2.4 Flow injection analysis

Recently, a number of papers have appeared concerning the use of boron-doped diamond electrodes in FIA for the determination of organic compounds. In the present work, we also obtained excellent FIA results using boron-doped diamond electrodes for determination of captopril. **Fig 3.2.5** shows a series of repetitive 20- μL injections of various concentrations of captopril in 0.1 M phosphate buffer (pH 9) at detection potential 0.9 V versus Ag/AgCl. Well-defined signals were obtained at all concentrations, from 5 mM to 10 nM. The current signal decreased linearly with decreasing concentration from 100 to 0.05 μM ($r > 0.99$), as shown in the inset. In addition, a detection limit for $S/B > 3$ was determined. It can be seen that at 0.9 V, the BDD electrode could detect a captopril concentration as low as 10 nM.

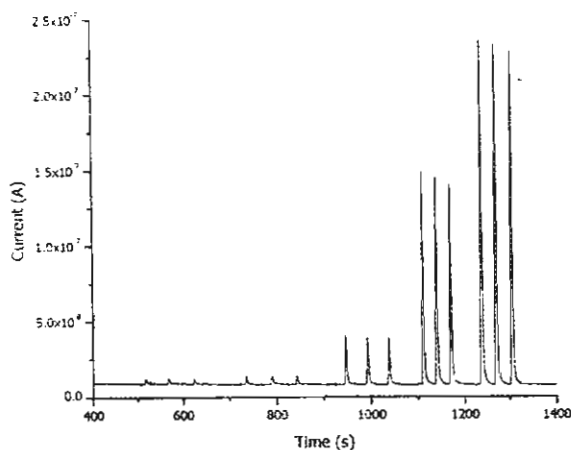


Fig.3.2.5. Flow injection analysis with amperometric detection results for a diamond electrode using 20- μL of captopril at various concentrations. The mobile phase was 0.1 M phosphate buffer (pH 9) at a flow rate of 1 ml min^{-1} . The calibration curve is shown in the inset.

3.2.5 Quantitative determination of Captopril tablets

The proposed electrode was applied to the analysis of captopril in formulation. In this work, we examined the accuracy of the proposed method for determination of captopril in pharmaceuticals by the method of the standard addition. The recoveries of the added captopril range between 95.85 and 109.29 % are also shown in **Table 1**.

Table 1. Recovery of captopril tablet samples with amperometric detection using diamond electrode applied to flow injection system ($n=2$)

Amount of added ($\mu\text{g/ml}$)	Amount of found ($\mu\text{g/ml}$)	Percent of recovery (%)
0.43	0.47 ± 0.00	109.29 ± 0.02
0.87	0.91 ± 0.14	105.62 ± 3.41
1.30	1.25 ± 0.01	95.85 ± 0.24
1.74	1.77 ± 0.06	101.93 ± 0.72
2.17	2.15 ± 0.02	98.99 ± 0.17

The precision of the method was obtained on the basis of intra-assay. Three concentrations of added solution (0.00, 0.43, 1.74 $\mu\text{g / ml}$) were chosen. Results obtained from ten injections gave 1.21-2.15 % of relative standard deviation (RSD). The RSD values for day-to-day assays of captopril were also investigated. It has been found in the same laboratory within one week that the RSD values did not exceed 2 %.

The limitation of the proposed method is the interference in the complex real samples such as plasma. Electrochemically active species, which have the potential peak close to the potential peak of captopril can produce the current response and cause the mistake. However, this interference can be separated out by HPLC technique. The determination of captopril in artificial samples using boron-doped diamond electrode as the working electrode of amperometric detection after the first separating them by high-performance liquid chromatography is under study.

3.3 Determination of Tiopronin

3.3.1 pH effect

The preliminary study of electrochemical oxidation of tiopronin using BDD and GC electrodes was performed at pH 2.5, 5.0, 7.0, 8.0, and 9.0. It was found that tiopronin gave an oxidation peak at neutral and alkali pH. The oxidation potential decreased when the pH of the analyte solution increased. This phenomenon can be explained that the thiol group of tiopronin was easily hydrolyzed in alkali medium, which brought more stable reducing form of tiopronin. We found that tiopronin in phosphate buffer at pH 8 provided the highest S/B. Therefore, we used this pH for the next experiments.

3.3.2 Cyclic voltammetry

Fig. 3.3.1a) and 3.3.1b) show cyclic voltammograms for tiopronin oxidation at BDD and GC electrodes in 2 mmol L⁻¹ tiopronin + 0.1 mol L⁻¹ phosphate buffer pH 8. The corresponding backgrounds are also shown. For the GC electrode, an anodic peak occurred at approximately + 0.8 V vs. Ag/AgCl. A rapid increase in the current at this potential was also observed in the background voltammogram due to oxygen evolution and carbon oxidation. For this reason, the voltammogram was ill-defined. In the case of the BDD electrode, a very well defined oxidation peak at approximately + 0.8 V vs. Ag/AgCl was observed. It was observed that there was very low background current. No cathodic peak was observed on the reverse scan within the investigated potential range (-0.6 to +1.4

V) for both GC and BDD electrode, because thiol oxidation was an electrochemically irreversible process.

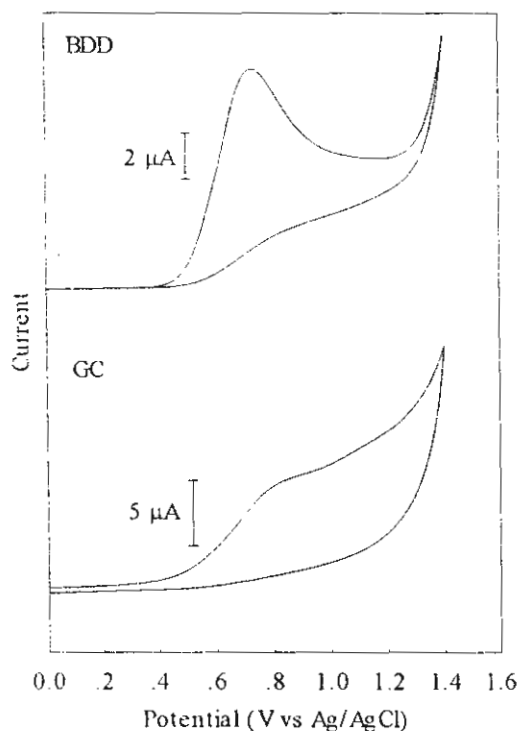


Fig. 3.3.1 Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 2.0 mmol L^{-1} tiopronin in 0.1 mol L^{-1} phosphate buffer pH 8 (solid lines) and 0.1 mol L^{-1} phosphate buffer pH 8 (dashed lines). The sweep rate was 50 mV s^{-1} ; area of electrode, 0.07 cm^2 .

Fig. 3.3.2 shows the cyclic voltammograms recorded during variation of the scan rate for the BDD electrode. It can be seen that peak currents are linearly proportional to the square root of the scan rate within the range $10\text{-}300 \text{ mVs}^{-1}$. The linear regression statistical analysis yields $R^2 = 0.9997$. The linearity suggests that the current is limited by semi-infinite linear diffusion of tiopronin in the interfacial reaction zone and that rate-limiting adsorption step and specific surface interactions can be neglected.

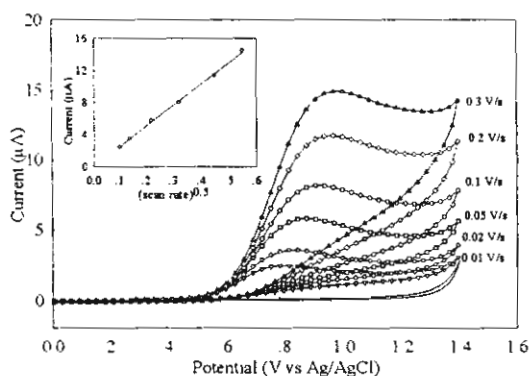


Fig. 3.3.2 Cyclic voltammograms for 1 mmol l^{-1} tiopronin in 0.1 mol L^{-1} phosphate buffer (pH 8) at BDD electrode for a series of sweep rates; area of electrode, 0.07 cm^2 . The dependence between peak current (μA) and square root of the sweep rate appears in the inset.

As shown in **Fig. 3.3.3**, at the BDD electrode the oxidation peak current is linearly proportional to the tiopronin concentration in the range of 0.05 to 10 mmol L⁻¹, while the GC electrode provided a nonlinear function of the tiopronin concentration. For the BDD electrode, a well-defined voltammetric peak was obtained with an S/B ratio of 3 at a concentration as low as 50 μ mol L⁻¹.

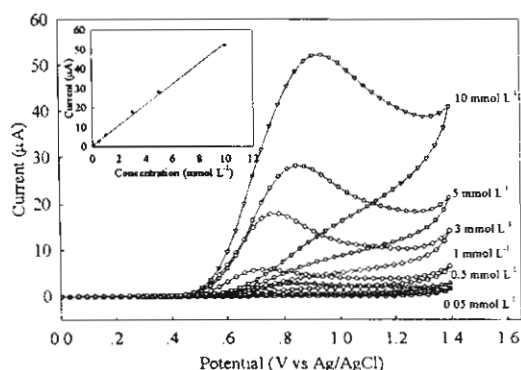


Fig. 3.3.3 Cyclic voltammograms for tiopronin in 0.1 mol L⁻¹ phosphate buffer (pH 8) at BDD electrode for a series of tiopronin concentrations. The sweep rate was 50 mV s⁻¹; area of electrode, 0.07 cm². The dependence between peak current (μ A) and concentrations at the potential of 50 mV s⁻¹ appears in the

3.3.3 Hydrodynamic voltammetry

In the FIA measurements, the first important results were obtained from hydrodynamic voltammetry. **Fig. 3.3.4** shows hydrodynamic voltammograms for 20- μ L injections of 100 μ mol L⁻¹ tiopronin + phosphate buffer pH 8, using phosphate buffer pH 8 as the carrier stream. The peak currents of tiopronin and corresponding background currents are showed in **Fig.3.2.4(a)**. Despite the well defined voltammogram of tiopronin was obtained, the hydrodynamic voltammogram is very stretching-out closer to a straight line (expanding from 0.6 to 1.1 V). The expected sigmoidal current potential curve with a defined plateau did not received. This behaviour does not outweigh the advantage of the very low residual current.

Therefore, the S/B ratios were calculated from Fig. 3.3.4(a) as a function of potential. As shown in the Fig. 3.3.4(b) the S/B ratio reaches a maximum value at 0.8 V. Hence, this potential was selected for quantitative amperometric detection in FIA experiments.

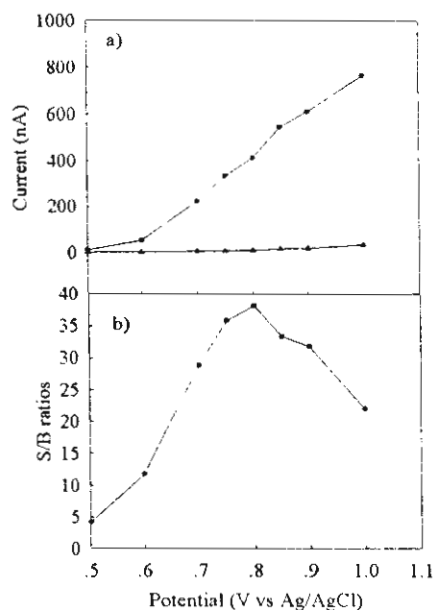


Fig. 3.3.4. (a) Hydrodynamic voltammogram of (▲) 0.1 mol l^{-1} phosphate buffer (pH 8, background current) and (●) $100 \mu\text{mol L}^{-1}$ of tiopronin in 0.1 mol L^{-1} phosphate buffer (pH 8) with averaging the peak currents from four injections (injected the analyte solution four times at each potential and then the average peak current was obtained by calculation), using 0.1 mol L^{-1} phosphate buffer (pH 8) as a carrier stream. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min^{-1} .

3.3.4 Flow injection analysis

In recent years, a number of studies have been reported concerning the development of diamond electrodes for applications as amperometric detector in flow injection analysis [10-12]. In the present paper, we obtained excellent FIA results using boron-doped diamond electrodes for the determination of tiopronin. It was found that the signal current for repetitive $20\text{-}\mu\text{L}$ injections of tiopronin at various concentrations in phosphate buffer, pH 8, at the detection potential, increased linearly with increasing

concentration from 500 nmol L^{-1} to $50 \text{ } \mu\text{mol L}^{-1}$ ($R^2 = 0.9992$). For the limit of detection, tiopronin provided a useful signal with $S/N \geq 3$ at a concentration as low as 10 nmol L^{-1} .

3.3.5 Quantitative determination of Tiopronin tablet

The BDD electrode was applied to the analysis of tiopronin in formulation. We examined the accuracy of the method for determination of tiopronin in pharmaceuticals by the method of the standard addition. Linear least squares calibration curve provide a slope of $21.07 \text{ nA } \mu\text{mol L}^{-1}$ (sensitivity) and correlation coefficient of 0.996. Relative error compared with the claimed amount was lower than 4%. The recoveries of the added tiopronin ranged between 96.5 and 108.3 % were obtained. At lower concentration, it was found some nonlinearly, with the slope gradually decreasing when the concentration grows. We expected that it was due to the inexistence of a plateau, result from hydrodynamic voltammogram, the current becomes quite potential dependent and any uncompensated IR drop in the cell can contribute to the blending. Another possible cause can be found in **Figure 3.3.2**: the anodic wave suffers displacement to more positive potential with increase concentration. This nonlinearity can be caused overestimation at low concentrations to expected values, followed by underestimation at the upper end. The tiopronin content of the drug calculated from this calibration plot ($102.8 \pm 0.03 \text{ mg per tablet } n = 2$) was found to be in satisfactory agreement with the labeled amount of 100 mg per tablet.

The precision of the method was obtained on the basis of intra-assay. Three concentrations of added solution ($0.00, 0.98, 1.63 \text{ } \mu\text{g ml}^{-1}$) were chosen. Results obtained

from ten injections were within 1.10-1.62 % of the relative standard deviation (RSD). Moreover, the RSD values between two analyses were also investigated. It was found that the RSD did not differ more than 1.5%.

3.3.6 Comparison with other methods

Table 3.3.1. summarizes data from the other methods for determination of tiopronin compared with the proposed method. It was found that using the BDD electrodes with amperometric flow injection analysis gave the similar wide linear dynamic range to other methods (two order of magnitudes). Interestingly, the proposed method provided a very low detection limit of 10 nmol L^{-1} , because the BDD electrode exhibited very low background current and noise signals. It also resulted in very high sensitivity. This outstanding performance of the BDD electrode make it is attractive for using as working electrode in FIA system for analysis of tiopronin.

Table 3.3.1 Recovery of tiopronin tablet samples with amperometric detection using diamond electrode applied to flow injection system ($n=2$)

Amount of tiopronin tablet sample added ($\mu\text{g ml}^{-1}$)	Amount of tiopronin tablet sample found ($\mu\text{g ml}^{-1}$)	Percent of recovery
0.65	0.70 ± 0.02	108.3 ± 3.6
0.98	1.04 ± 0.07	105.2 ± 6.7
1.31	1.35 ± 0.04	103.2 ± 2.8
1.63	1.62 ± 0.01	99.6 ± 0.4
1.96	1.89 ± 0.002	96.5 ± 0.1

- ผลงานวิจัยที่ตีพิมพ์ในวารสารวิชาการระดับนานาชาติ

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- กิจกรรมอื่น ๆ ที่เกี่ยวข้อง

- เสนอผลงาน

1. "9th International Conference on Flow Analysis" 17-21 February, 2003, Geelong, Australia.
2. "6th International Mini-Symposium on Diamond Electrochemistry and Related Topics" March 5-6, 2003, Tokyo, Japan.
3. "Tokyo Conference 2004" (Asia Young Analytical Chemist Sessions) August 31- September 3, 2004, Chiba, Japan.
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- รางวัลที่ได้รับ

รางวัลผลงานวิจัยดี กองทุนรัชดาภิเษกสมโภช จุฬาลงกรณ์มหาวิทยาลัย ประจำปี 2546

Electrochemical analysis of D-penicillamine using a boron-doped diamond thin film electrode applied to flow injection system

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Abstract

The electroanalysis of D-penicillamine in 0.1 phosphate buffer (pH 7) was studied at a boron-doped diamond thin film (BDD) electrode using cyclic voltammetry as a function of concentration of analyte and pH of analyte solution. Comparison experiments were performing using a glassy carbon (GC) electrode. The BDD electrode exhibited a well-resolved and irreversible oxidation voltammogram, but the GC electrode provided only an ill-defined response. The BDD electrode provided a linear dynamic range from 0.5 to 10 mM and a detection limit of 25 μM ($S/B \geq 3$) in voltammetric measurement. It was also found that the peak potentials were decreased when the pH of the analyte solution was increased. In addition, penicillamine has been studied by hydrodynamic voltammetry and flow injection analysis with amperometric detection using the BDD electrode. The flow injection analysis results at the diamond electrode indicated a linear dynamic range from 0.5 to 50 μM and a detection limit of 10 nM ($S/N \approx 4$). The proposed method was applied to determine D-penicillamine in dosage form (capsules), the results obtained in the recovery study (255 ± 2.50 mg per tablet) were comparable to those labeled (250 mg per tablet).

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Keywords: D-penicillamine; Boron-doped diamond thin film electrode; Cyclic voltammetry; Flow injection system; Amperometric detection

1. Introduction

D-penicillamine, 3-mercapto-D-valine is a pharmaceutically important thiol compound frequently used as a medicinal agent against a number of diseases i.e. rheumatoid arthritis [1], cystinuria [2], liver disease or certain skin conditions, heavy metal poisoning and Wilson's disease [3]. It may also inhibit the replication of the human immuno-

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deficiency virus, the cause of acquired immune deficiency syndrome (AIDS).

Several methods have been proposed for the determination of D-penicillamine including high-performance liquid chromatography with pre or post column derivatization [4–6], calorimetry [7], fluorometry [8,9], chemiluminescence [10], capillary electrophoresis [11,12] and NMR spectrometry [13]. One of the important limitation of LC techniques is the fact that this thiol lacks sufficient UV absorption so a pre or post-column derivatization procedure is normally required [10]. Electrochemical methods are an alternative for the D-penicillamine determination because they are cheap, simple, fast and sensitive. Mercury and mercury amalgam [14–16] have been extensively used for thiol-compound determination, however, mercury has limitations due to its toxicity and the rapid deterioration of the electrode response [17]. A chemically modified electrode [18] has been also used to determine D-penicillamine but its use in flow injection analysis and liquid chromatography is limited because it needs particular mechanical and chemical stability towards the flowing solution. Recently, the boron-doped diamond thin film (BDD) electrode has emerged as a unique electrode material for several electrochemical applications, especially in electroanalysis [19,20]. It can overcome the limitations that mentioned above due to its attractive properties, such as its very low and stable voltammetric background current, its wide potential window in aqueous solution, its high resistance to deactivation via fouling, its insensitivity to dissolved oxygen, and its negligible adsorption of polar organic molecules. Flow injection with amperometric detection using the BDD electrode has been reported for the determination of some organic compounds such as polyamine [21], histamine and serotonin [22].

In this present paper, we used the BDD thin film electrode to study D-penicillamine by using cyclic voltammetry in comparison with the glassy electrode. Hydrodynamic voltammetry and flow injection analysis with amperometric detection were also used to determine D-penicillamine. The results demonstrated that diamond provided a sensitive, reproducible response for D-penicillamine oxidation.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical grade or better and all solutions were prepared by using deionized water. Phosphate buffer (0.1 M; pH 5–8) was prepared from 0.1 M potassium dihydrogen phosphate (Merck) and the pH was adjusted with 0.1 M sodium hydroxide (Merck) solution. Carbonate buffer (pH 9.2) 0.1 M was prepared by a mixing solution of 0.1 M sodium carbonate (Baker) solution and 0.1 M sodium hydrogen carbonate (Merck) solution.

D-penicillamine (Sigma) solutions were freshly prepared in 0.1 M phosphate buffer.

2.2. Electrode

The BDD electrode (obtained from Professor A. Fujishima) was grown on conductive Si(100) substrate using microwave assisted chemical vapor deposition (CVD) system. It was rinsed with ultrapure water prior to use.

The glassy carbon (GC) electrode was purchased from Bioanalytical System, Inc (area 0.07 cm²). It was pretreated by sequential polishing with 1 and 0.05 μ m of alumina/water slurries on felt pads, followed by rinsing with ultrapure water prior to use.

2.3. Cyclic voltammetry

Electrochemical measurement was carried out in a single compartment three electrode glass cell. The BDD electrode was pressed against a smooth ground joint at the bottom of the cell isolated by an O-ring (area 0.07 cm²). Ohmic contact was made by placing the backside of the Si substrate on a brass plate. The GC electrode was also used as a working electrode in comparison study with the BDD electrode. A platinum wire and a Ag/AgCl with a salt bridge were used as the counter and reference electrodes, respectively. Cyclic voltammetry was performed with an Autolab Potentiostat 100 (Eco-Chemie, The Netherlands). The electrochemical equipment was housed in a faradaic cage to reduced electronic noise.

2.4. Flow injection analysis with amperometric detection

The flow injection analysis system consisted of a thin layer flow cell (Bioanalytical System, Inc.), an injection port (Rheodyne 7125) with a 20 μ l injection loop, a reagent delivery module (Water) and an electrochemical detector (PG 100). The mobile phase, 0.1 M phosphate buffer (pH 7), was regulated by a reagent delivery module (N_2 gas flow) at a flow rate of 1 ml min⁻¹. The thin layer flow cell consisted of the Ag/AgCl reference electrode and a stainless steel tube counter electrode, which also serve as the tube for the solution outlet. A neoprene gasket was used as the spacer in the cell between the base of the cell and the BDD electrode. The analyte solution was passed through an inlet passage in the base and along a channel in the gasket contacting the electrode, then to the outlet. The BDD (1 $\times$ 3 cm) working electrode, which its size was larger than the area of the spacer was compressed between the gasket and the block of the thin layer flow cell (as shown in Fig. 1). An alligator clip was used for the electric contact with the end of BDD electrode. The experiments were performed in a copper faradaic cage to reduce electrical noise.

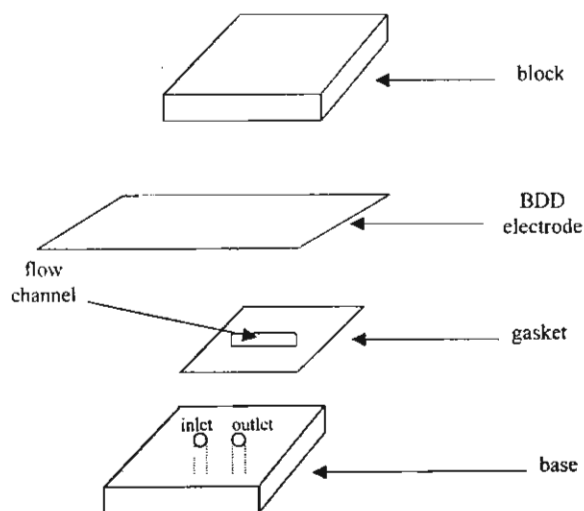


Fig. 1. Schematic diagram of the flow cell set-up.

2.5. Sample preparation

A mass of powder of one capsule of penicillamine (Cuprimine 250 mg) was transferred to 100 ml volumetric flask and dissolved in 0.1 M phosphate buffer (pH 7), filtrated through a 0.45 μ m nylon membrane syringe filter. Then, the filtrate was further diluted with 0.1 M phosphate buffer (pH 7) to obtain a final concentration of 2.5 μ g ml⁻¹.

2.6. Interference study

A stock solution of 37.3 μ g ml⁻¹ of D-penicillamine and 34.2 mg ml⁻¹ of lactose in 0.1 M phosphate buffer (pH 7) were prepared. 5 ml stock solution of D-penicillamine was pipetted into each of three 25 ml volumetric flasks, 2.5, 5 and 10 ml of lactose solution were added to obtain a concentration ratio between lactose and D-penicillamine of 20, 40 and 80, respectively. Each flask was completed to volume with 0.1 M phosphate buffer (pH 7).

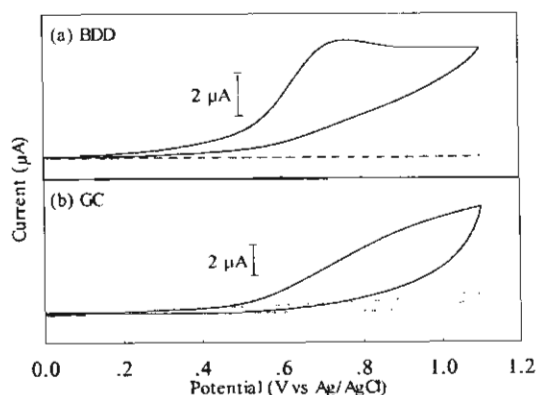


Fig. 2. Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at (a) boron-doped thin film electrode and (b) GC electrode (solid line). The potential sweep rate was 20 mV s⁻¹. Background voltammograms (0.1 M phosphate buffer, pH 7) are also shown in this figure (dotted line).

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 2 shows the cyclic voltammograms for 1 mM D-penicillamine together with the corresponding background voltammogram in 0.1 M phosphate buffer (pH 7) at the BDD and GC electrodes. The background current for the GC electrode was approximately ten times higher than that obtained for the BDD electrode. The BDD exhibits a well-defined irreversible oxidation peak at ~ 0.8 V versus Ag/AgCl, whereas the GC electrode provides an ill-defined irreversible oxidation peak at ~ 0.9 V versus Ag/AgCl.

At the BDD electrode, the cyclic voltammogram of the second cycle still exhibits a well-defined cyclic voltammogram with a slightly decreasing peak current and the current can revert to its original value after purging with a deionized water, indicating the absence of electrode fouling. In the case of GC electrode, the cyclic voltammogram in the second exhibits a featureless cyclic voltammogram and the current can only revert after polishing the electrode with the alumina slurries. This was due to electrode fouling.

3.1.1. pH dependence study

The effect of the buffer pH was investigated from pH 5 to 9.2. At buffer pH between pH 7 and 9.2, the BDD electrode exhibits a well-defined cyclic voltammogram while the GC electrode provides an ill-defined cyclic voltammogram. In the case of buffer pH 5, the BDD electrode provides an ill-defined cyclic voltammogram while the GC electrode provides a featureless cyclic voltammogram.

The changing of buffer pH effects the oxidation peak potential. It was found that increasing the buffer pH, decreased the oxidation peak potential, probably due to the more facile oxidation of the thiol group of D-penicillamine in the alkaline medium. In addition the buffer also affected the peak current of the cyclic voltammogram. At the BDD electrode, the maximum peak current with the maximum S/B ratio was observed at pH 7. Therefore, this pH was chosen as the optimal pH.

3.1.2. Scan rate dependence study

Fig. 3 shows the cyclic voltammetric response of 1 mM D-penicillamine in 0.1 M phosphate buffer pH 7 with variation of the scan rate from 0.01 to 0.3 V s^{-1} at the BDD electrode. The oxidation current varied highly linearly ($r > 0.99$) with the square root of the scan rate, $v^{0.5}$, as shown in the inset of this figure, indicating semi-infinite linear diffusion of reactant to the electrode surface.

3.1.3. Concentration dependence study

The oxidation peak current was investigated for the concentration range from 0.025 to 20 mM D-penicillamine at the BDD. Fig. 4 shows the cyclic voltammograms with the concentration of D-penicillamine varied from 0.5 to 10 mM. Linear regression analysis of current (μA) versus concentration (mM) profiles showed a reasonable linearity from 0.5 to 10 mM ($r > 0.99$) for the BDD electrode, as shown in the inset of this figure. The GC electrode provides no linear regression relationship between the oxidation current and the concentration. This may be due to the electrode fouling. At a concentration as low as 0.025 mM and above, a well defined peak with the S/B ratios > 3 was obtained at the BDD electrode while the GC electrode provided this S/B value at the concentration of 0.5 mM due to its high background current.

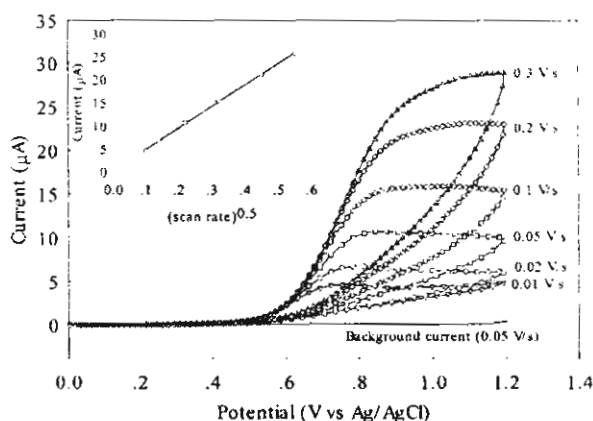


Fig. 3. Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at boron-doped thin film electrode (the potential sweep rate was varied from 0.01 to 0.3 V s^{-1}). The curve of the relationship between current and $(\text{scan rate})^{0.5}$ is inset.

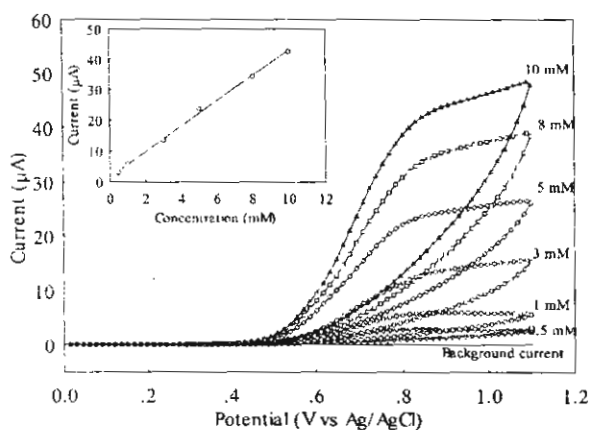


Fig. 4. Cyclic voltammograms for D-penicillamine in 0.1 M phosphate buffer (pH 7) (the concentration varied from 0.5 to 10 mM) at boron-doped thin film electrode. The potential sweep rate was 0.02 V s^{-1} . The calibration curve was also is inset.

The reproducibility of the BDD electrode was also studied. The test consisted of running the cyclic voltammogram for five aliquots (the same concentration) of three concentrations (1, 5 and 10 mM D-penicillamine). It appears that the reproducibility test is satisfied because the peak current variation value (relative standard deviation (%R.S.D.)) was 2.2–3.1.

3.2. Flow injection analysis with amperometric detection

3.2.1. Hydrodynamic voltammetry

Fig. 5(a) shows a hydrodynamic voltammetric $i-E$ curve obtained at the BDD electrode for $20 \mu\text{l}$ injections of $100 \mu\text{M}$ D-penicillamine in 0.1 M phosphate buffer (pH 7), using 0.1 M phosphate buffer (pH 7) as the carrier solution. Each datum represents the average of four injections. The absolute magnitude of background current at each potential is also shown for comparison. The hydrodynamic voltammogram for D-penicillamine did not produce a sigmoidal shape of the signal versus potential. To obtain the maxima potential point, the S/B ratio was calculated from the Fig. 5(a) at each potential. The hydrodynamic voltammetric S/B ratio versus potential curve are obtained as shown in Fig. 5(b) with the maximum S/

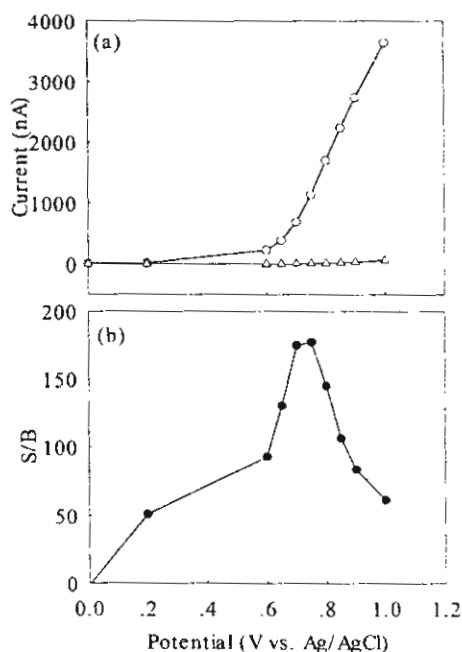


Fig. 5. (a) Hydrodynamic voltammograms of (—●—) $100 \mu\text{M}$ of D-penicillamine in 0.1 M phosphate buffer (pH 7) and (—□—) 0.1 M phosphate buffer (pH 7, background current) with four injections of analytes, using 0.1 M phosphate buffer (pH 7) as a carrier solution. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min^{-1} .

B ratios of 0.75 V versus Ag/AgCl. Hence, this potential was set as the amperometric potential detection in flow injection analysis experiments.

3.2.2. Linear range, detection limit and reproducibility

Fig. 6 shows a series of repetitive $20 \mu\text{l}$ injections of D-penicillamine in 0.1 M phosphate buffer pH 7 at the detection potential of 0.75 V versus Ag/AgCl. Well-defined signals without peak tailing were obtained at all concentration from 10 nM to 2.5 mM. The current signal increased linearly with the increasing of concentrations from 0.5 to $50 \mu\text{M}$ ($r > 0.99$) as shown in the inset of this Figure. The sensitivity of this method, which is the slope of the relation plot between the current and the concentration over the linear range, was $12.1\text{--}17.3 \text{ nA } \mu\text{M}^{-1}$. Interestingly, the detection limit with $S/N > 3$ was obtained at the concentration as low as 10 nM of D-penicillamine. The reproducibility of

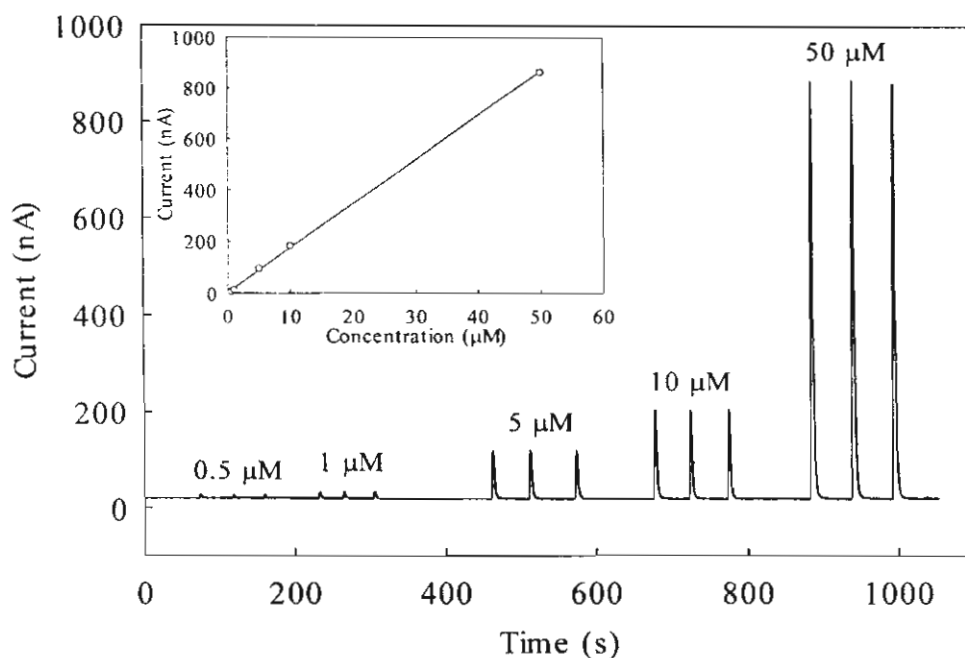


Fig. 6. Flow injection with amperometric detection results for D-penicillamine (0.5–50 μM) and calibration graph at the applied potential of 0.75 V vs. Ag/AgCl in 0.1 M phosphate buffer (pH 7) with five injections of analytes. Other conditions are the same as in Fig. 5.

the response was also examined. A peak variability of 1.8% was found during a course of 45 injections of 100 μl D-penicillamine, indicating the high stability of the BDD electrode.

3.3. Application

3.3.1. Drug analysis of penicillamine capsules

The proposed method for penicillamine was applied to the determination of penicillamine capsules. The precision of the method was obtained on the basis of intra-assay using standard addition. The intra assay study results ($n=2$) in the same day are summarized in Table 1. There was no significant difference between the labeled contents and those obtained by the proposed method with the satisfactory recovery. Recoveries ranged from 97.75 to 104.69. Moreover, repeatability of results for the addition of D-penicillamine 0, 0.6 and 1.49 $\mu\text{g ml}^{-1}$ with ten replicates was obtained with a (%R.S.D.) of 1.5–2.1. The inter-assay was also study between the days ($n=4$) and the results are summarized in Table 2. There is

Table 1

Recovery of D-penicillamine sample with amperometric detection on the BDD electrode applied to flow injection system of the intra assay study ($n=2$)

Amount of added ($\mu\text{g ml}^{-1}$)	Amount of found ($\mu\text{g ml}^{-1}$)	Percent of recovery (%)
0.60	0.59 ± 0.01	98.68 ± 0.93
0.90	0.94 ± 0.01	104.04 ± 0.65
1.19	1.19 ± 0.01	99.62 ± 0.39
1.49	1.48 ± 0.01	98.84 ± 0.33
250 mg per tablet	255.62 ± 0.14	102.11 ± 0.55

no significant difference in the results, indicating that the analysis of penicillamine capsules by the proposed method is reproducible.

3.3.2. Interference study

Excipients frequently added to dosage forms are talc, starch, magnesium stearate and lactose. Talc, starch, titanium dioxide and magnesium stearate do not dissolve in 0.1 M phosphate buffer. They were filtered out in the sample preparation step, so

Table 2

Recovery of D-penicillamine sample with amperometric detection on the BDD electrode applied to flow injection system of the inter assay study ($n=4$)

Amount of added ($\mu\text{g ml}^{-1}$)	Amount of found ($\mu\text{g ml}^{-1}$)	Percent of recovery (%)
0.60	0.58 ± 0.01	98.04 ± 0.94
0.90	0.91 ± 0.03	101.30 ± 3.06
1.19	1.17 ± 0.02	99.07 ± 1.45
1.49	1.50 ± 0.03	100.43 ± 1.98
250 mg per tablet	255.55 ± 2.50	102.22 ± 0.99

they did not interfere with the response. Only lactose was used in this study. It was found the tolerance limit for the ratio between D-penicillamine and lactose concentrations was 80. This caused an error of not more than $\pm 5\%$. The separation of this interference is being studied in our laboratory.

4. Conclusions

The BDD electrode was shown to exhibit excellent performance for the oxidative detection of D-penicillamine. Well-defined voltammograms were obtained at diamond electrode, demonstrating significant advantages over the GC electrode. Flow injection analysis with amperometric detection using the BDD electrode provided a linear range over two orders (0.5–50 μM) and a remarkably low detection limit (10 nM). Reproducible responses without pretreatment or modification of the electrode or using pulse technique were obtained. Moreover, it was found that this method could be used for detecting D-penicillamine in capsule samples with satisfactory reproducible results.

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Electrochemical determination of captopril at boron-doped diamond thin film electrode applied to a flow injection system

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Abstract

A boron-doped diamond thin film (BDD) electrode was first used to study the electroanalysis of captopril in phosphate buffer (pH = 9) by several techniques such as cyclic voltammetry, hydrodynamic voltammetry and flow injection with amperometric detection. Cyclic voltammetry was used to study the reaction as a function of concentration of analyte. The results were compared with a glassy carbon (GC) electrode at the same condition. Captopril undergoes irreversible reaction at both electrodes. However, the BDD electrode provided highly reproducible, well-resolved cyclic voltammogram and higher signal-to-background (S/B) ratio comparing with GC electrode. Linear range from 50 μM to 3 mM and detection limit of 25 μM ($S/B \approx 3$) were obtained from the BDD electrode using cyclic voltammetry. The potential sweep rate dependence of captopril oxidation (peak currents for 1 mM captopril linearly proportional to $v^{1/2}$, within the range of 0.01 to 0.3 V s^{-1}) indicates that the oxidation current is diffusion controlled with negligible adsorption on the BDD surface. The flow injection experiments of the BDD electrode at the concentration from 0.5 to 100 μM provided a linear dynamic range and very low detection limit of 10 nM were obtained ($S/N \approx 3$). In addition, the determination of captopril in the commercial available tablets by flow injection system with amperometric detection at diamond electrodes has also been investigated. The results show very good precision (1.21–2.15%).

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Keywords: Captopril; Boron-doped diamond thin film electrode; Flow injection system; Amperometric detection; Electrochemistry

1. Introduction

Boron-doped diamond (BDD) thin film electrode is one of the new promising materials for electrochemical applications due to its unique and extremely useful property [1]. Electroanalysis is one field that can benefit from the attractive electrochemical properties of diamond thin film as an electrode material [2–8]. Its attractive properties such as very low and stable voltammetric background current [6], wide potential window in aqueous electrolyte solutions (2.5–3 V) [9], slight adsorption of polar organic molecules, high resistance to deactivation via fouling and good activity toward some redox analytes without any conventional pretreatment [10].

Captopril belongs to the group of anti-hypertensive drugs that affect the renin–angiotensin system and are commonly referred to as angiotensin-converting enzyme (ACE) inhibitors. The chemical name of captopril is (S)-1-(3-mercaptopropyl)-L-proline, and it is widely used for the treatment of arterial hypertension. Recent studies suggest that it may also act as a scavenger of free radicals because of its thiol group [11–13].

Several methods have been proposed for the determination of captopril, including high-performance liquid chromatography with pre- or post-column derivatization [14–17], colorimetry [12,18], fluorometry [19–21], chemiluminescence [22–24], capillary electrophoresis [25–28], spectrometry [29–33] and amperometry [34–36]. One of the important limitation of LC techniques is the fact that captopril lacks sufficient UV absorption so a pre- or post-column derivatization procedure is normally required and these results in increased cost and complication of analysis. Electrochemical techniques are an alternative method for the captopril determination because they are simple, fast and low cost. The electrochemical detection of captopril has been reported using graphite [35,36] and selective membrane electrode as the working electrode [35]. Nevertheless, the sensitivity and reproducibility of electrodes were not high because the surface of these electrodes was easy to contaminate by the fouling products and impurities. Boron-doped diamond thin film electrode can be used to eliminate this problem without any pretreatment because of the stable surface morphologies and the surface carbon atoms terminated by hydrogen [9]. Thus, the BDD surface is relatively non-polar, very stable and suffers less adsorption of polar molecules, as reported by Xu et al. [10].

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Alternative automatic procedure based on flow injection techniques have been widely suggested, since they enable reduce time of analysis. Moreover, they can increase reproducible and accurate results. Flow injection with amperometric detection using a boron-doped diamond thin film electrode has been reported for the determination of some organic compounds such as polyamine [4], histamine and serotonin [6].

In this present paper, we report use of the boron-doped diamond thin film electrode to study captopril using cyclic voltammetry in comparison with the glassy electrode. Hydrodynamic voltammetry and flow injection analysis with amperometric detection was also used to determine captopril in the standard chemical form and commercial available tablets.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical grade and used without further purification. All solutions were prepared using deionized water. Phosphate buffers (pH 5–8), 0.1 M, were prepared from 0.1 M of potassium dihydrogen phosphate (Merck) and 0.1 M disodium hydrogen phosphate (BDH). Phosphate buffer (pH 2.5) was prepared from 0.1 M of potassium dihydrogen phosphate and the pH was adjusted with orthophosphoric acid (85%, Carlo Erba). Phosphate buffer (pH 9.0), 0.1 M, was prepared from 0.1 M of potassium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate and the pH was adjusted with 0.1 M sodium hydroxide (Merck) solution. The standard captopril (Sigma) solutions were freshly prepared in 0.1 M phosphate buffer.

2.2. Electrode

The experimental conditions and the apparatus used for the diamond film growth have been described in detail elsewhere [37]. The films were prepared by deposition of the BDD thin films on highly conductive n-Si(111) substrates by microwave plasma-assisted chemical vapor deposition. Deposition was usually carried out for 10 h to achieve a film thickness of approximately 30 μm . The nominal B/C atomic ratio in the gas phase was 100:1, and the typical boron doping level in the film was ca. 10^{21} cm^{-3} . The BDD electrodes were rinsed with ultrapure water prior to use. The glassy carbon (GC) electrode was purchased from Bioanalytical System Inc. (area 0.07 cm^2). It was pretreated by sequential polishing with 1 and 0.05 micron of alumina/water slurries on felt pads, followed by rinsing with ultrapure water prior to use.

2.3. Cyclic voltammetry

Electrochemical measurements were recorded using Autolab Potentiostat 100 (Eco-Chemie, The Netherlands)

with a standard three-electrode glass cell configuration. The BDD electrode was pressed against a smooth ground joint at the bottom of the cell isolated by an O-ring (area 0.07 cm^2) and served as the working electrode. Ohmic contact was made by placing the backside of the Si substrate on a brass plate. A GC electrode was also used as a working electrode in the comparison study with the BDD electrode. A platinum wire and a Ag/AgCl electrode with a salt bridge were used as the counter and reference electrodes, respectively. Cyclic voltammetry was used to probe the electrochemical reaction. The electrochemical measurements were housed in a Faradaic cage to reduce electronic noise. All experiments were done at a room temperature.

2.4. Flow injection analysis with amperometric detection

The flow injection analysis system consisted of a thin layer flow cell (Bioanalytical System Inc.), an injection port (Rheodyne 7125) with a 20- μl injection loop, a peristaltic pump (Ismatec) and an electrochemical detector (PG 100). The mobile phase, 0.1 M phosphate buffer (pH 9), was regulated by a reagent delivery module at a flow rate of 1 ml min^{-1} . The thin layer flow cell consisted of a silicone rubber gasket as a spacer, Ag/AgCl as the reference electrode, stainless steel tube as an auxiliary electrode and an outlet. The experiments were performed in a copper Faradaic cage to reduce electrical noise. A hydrodynamic voltammogram was obtained before the amperometric determination was performed. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential to obtain hydrodynamic voltammograms. The amperometric measurements were carried out at the potential giving a maximum signal-to-background (S/B) ratio in the hydrodynamic voltammograms, or in the limiting current range.

2.5. Sample preparations

Tablets containing captopril (Bristol-Myers Squibb) were analyzed. Five tablets were each weighed so as to obtain the mean tablet weight. An accurately weighed portion of the homogenized powder corresponding to mean tablet weight was treated with 0.1 M phosphate buffer (pH 9), then diluted to the final concentration with the same solution, providing a concentration within the linear dynamic range.

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 1(a) and (b) show the cyclic voltammograms for 1 mM captopril together with the corresponding background voltammogram in 0.1 M phosphate buffer (pH 9) at the BDD and GC electrodes. The background current for the GC

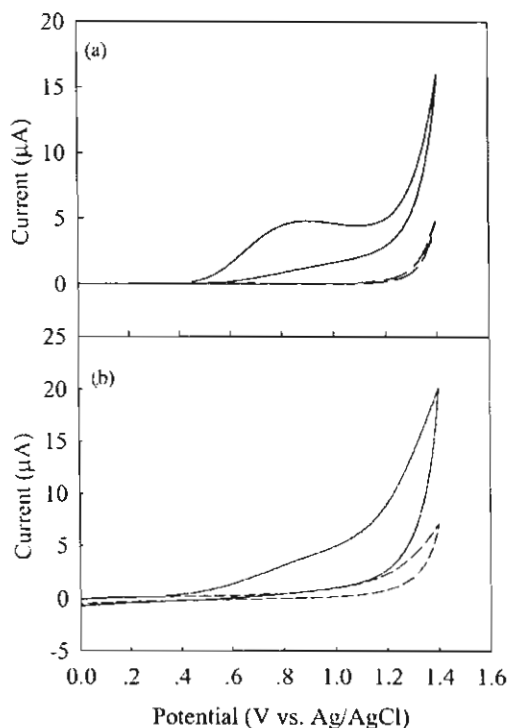


Fig. 1. Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 1.0 mM captopril in 0.1 M phosphate buffer pH 9 (solid lines) and 0.1 M phosphate buffer pH 9 (dashed lines). Sweep rate, 50 mV s⁻¹; area of electrode, 0.07 cm².

electrode was ~10 times higher than that obtained for the BDD electrode. The BDD exhibited a well-defined irreversible oxidation peak at ~0.8 V versus Ag/AgCl, whereas the GC electrode provided an ill-defined irreversible oxidation peak. No cathodic peak was observed on the reverse scan within the investigated potential range (0 to +1.4 V) because thiol oxidation is an electrochemically irreversible process.

The effect of the buffer pH was investigated from pH 2.5 to 9. At the buffer pH between pH 8 and 9, the BDD electrode exhibited a well-defined cyclic voltammogram while the GC electrode provided an ill-defined cyclic voltammogram. In the case of acidic buffer, the BDD electrode provided an ill-defined cyclic voltammogram while the GC electrode provided a featureless cyclic voltammogram.

The changing of buffer pH effects to the oxidation peak potential. At BDD electrode, it was found that increasing the buffer pH, decreased of the oxidation peak potential, probably due to the facile oxidation of the thiol group of captopril structure in the alkaline medium (data were not shown). The maximum peak current with the maximum S/B ratios was observed at pH 9. Therefore, this pH was chosen as the optimal pH.

3.2. Concentration and sweep rate dependence

The oxidation peak current was measured at the BDD electrode for the captopril concentration range from 0.025 to

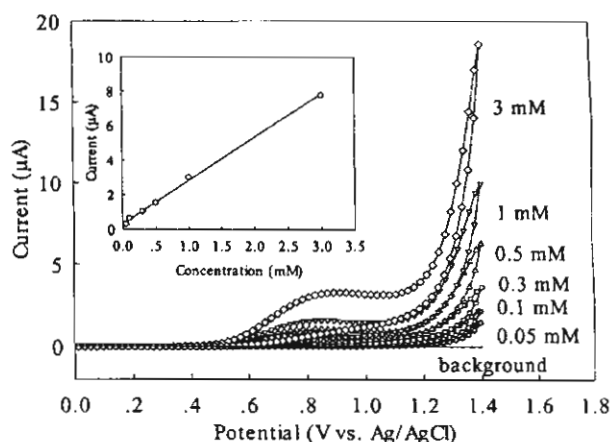


Fig. 2. Cyclic voltammogram for captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of captopril concentrations. The potential sweep rate was 50 mV s⁻¹; area of electrode, 0.07 cm². The calibration curve is shown in the inset.

20 mM in 0.1 M phosphate buffer (pH 9) at sweep rate 50 mV s⁻¹. Based on a series of cyclic voltammograms in which the concentration of captopril was varied from 0.025 to 20 mM, a linear regression statistical analysis of peak current (μA) versus concentration (mM) was obtained, linearly proportional in the range 0.05–3 mM ($r > 0.99$, data shown in Fig. 2). The response of the BDD electrode is appropriate for quantitative captopril determination even in the millimolar concentration range.

We also investigated the potential sweep rate dependence, as shown in Fig. 3. The voltammograms of 1 mM captopril

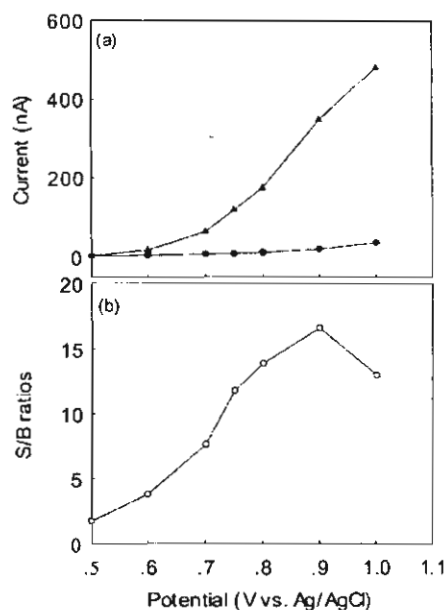


Fig. 3. Cyclic voltammograms for 1 mM captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm². The calibration curve of relationship between current (μA) and (sweep rate)^{0.5} was also in the inset of this figure.

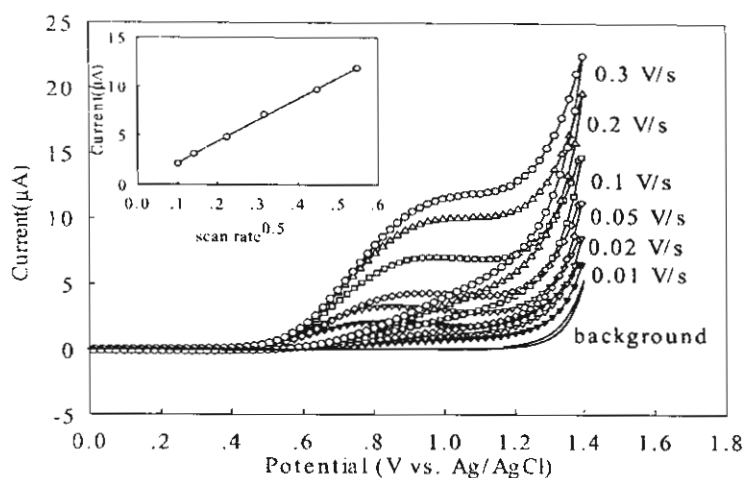


Fig. 4. (a) Hydrodynamic voltammogram of (—●—) 0.1 M phosphate buffer (pH 9, background current) and (—▼—) 100 μM of captopril in 0.1 M phosphate buffer (pH 9) with four injections of analysis, using 0.1 M phosphate buffer (pH 9) as a carrier solution. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min^{-1} .

were recorded during variation of the scan rates at the BDD electrode. It can be seen that peak current (μA) is linearly proportional to the square root of the sweep rate (V s^{-1}) within the range $0.01\text{--}0.3 \text{ V s}^{-1}$. The linear regression analysis yields $r > 0.999$. From the voltammogram, it was found that the peak potential shifted positively with increasing sweep rate, as expected for an irreversible process, and the linearity suggest that the reaction involves a diffusing species.

3.3. Hydrodynamic voltammetry

To obtain the optimal potential for amperometric detection in flow injection analysis, the hydrodynamic voltammetric behavior of captopril was studied. Fig. 4 shows a hydrodynamic voltammetric $i\text{--}E$ curve obtained at the BDD electrode for $20\text{-}\mu\text{l}$ injections of $100 \mu\text{M}$ captopril in 0.1 M phosphate buffer (pH 9), using 0.1 M phosphate buffer (pH 9) as the carrier solution. Each datum represents the average of four injections. The absolute magnitude of the background current at each potential is also shown for comparison. The hydrodynamic voltammogram for captopril did not produce a sigmoid shape of the signal versus potential. The S/B ratio was calculated from the Fig. 4(a) at each potential to obtain the maxima potential point. Therefore, the hydrodynamic voltammetric S/B ratios versus potential curve is shown in Fig. 4(b) with the maximum S/B ratio at 0.9 V . Hence, this potential was set as the amperometric potential detection in flow injection analysis experiments.

3.4. Flow injection analysis

Recently, a number of papers have appeared concerning the use of boron-doped diamond electrodes in FIA for the determination of organic compounds. In the present work, we also obtained excellent FIA results using boron-doped

diamond electrodes for the determination of captopril. Fig. 5 shows a series of repetitive $20\text{-}\mu\text{l}$ injections of various concentrations of captopril in 0.1 M phosphate buffer (pH 9) at detection potential 0.9 V versus Ag/AgCl. Well-defined signals were obtained at all concentrations, from 5 nM to 10 nM . The current signal decreased linearly with decreasing concentration from 100 to $0.5 \mu\text{M}$ ($r > 0.99$), as shown in the inset. In addition, a detection limit for $S/N \approx 3$ was determined. It can be seen that at 0.9 V , the BDD electrode could detect a captopril concentration as low as 10 nM .

3.5. Quantitative determination of captopril tablets

The proposed electrode was applied to the analysis of captopril in formulation. In this work, we examined the accuracy of the proposed method for determination of captopril in pharmaceuticals by the method of the standard addition (results shown in Fig. 6). The captopril content of the drug calculated from two replicate measurements ($12.88 \pm 0.01 \text{ mg per tablet}$) was found to be in satisfactory agreement with the declared amount of $12.5 \text{ mg per tablet}$. The recoveries of the added captopril range between 95.85 and 109.29% are also shown in Table 1.

Table 1
Recovery of captopril tablet samples with amperometric detection using diamond electrode applied to flow injection system ($n = 2$)

Amount of added ($\mu\text{g/ml}$)	Amount of found ($\mu\text{g/ml}$)	Percent of recovery (%)
0.43	0.47 ± 0.00	109.29 ± 0.02
0.87	0.91 ± 0.14	105.62 ± 3.41
1.30	1.25 ± 0.01	95.85 ± 0.24
1.74	1.77 ± 0.06	101.93 ± 0.72
2.17	2.15 ± 0.02	98.99 ± 0.17

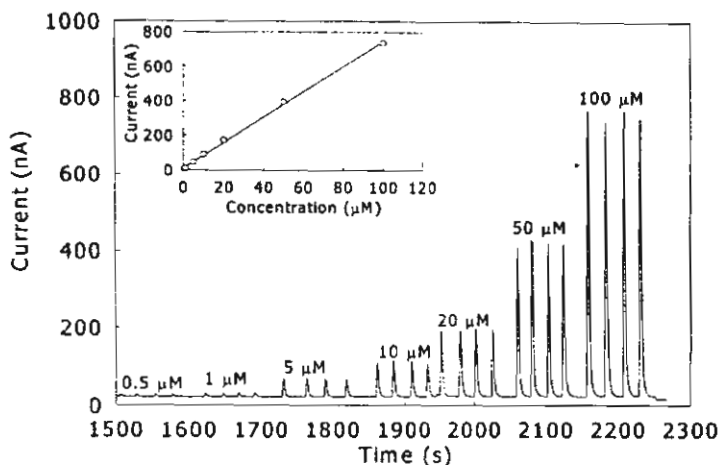


Fig. 5. Flow injection analysis with amperometric detection results for a diamond electrode using 20-μl of captopril at various concentrations. The mobile phase was 0.1 M phosphate buffer (pH 9) at a flow rate of 1 ml min⁻¹. The calibration curve is shown in the inset.

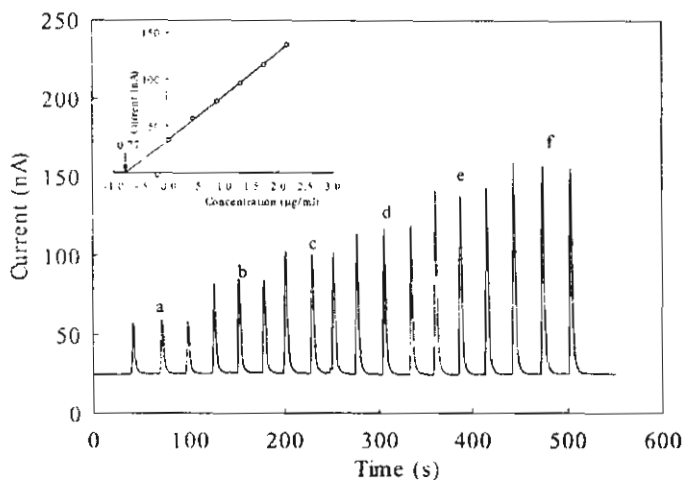


Fig. 6. Flow injection analysis results for determination of captopril in commercially available tablets. Using the standard addition for the added captopril: (a) 0.00 μg/ml, (b) 0.43 μg/ml, (c) 0.87 μg/ml, (d) 1.30 μg/ml, (e) 1.74 μg/ml, (f) 2.17 μg/ml.

The precision of the method was obtained on the basis of intra-assay. Three concentrations of added solution (0.00, 0.43, and 1.74 μg ml⁻¹) were chosen. Results obtained from 10 injections were within 1.21–2.15% of the relative standard deviation (R.S.D.). The R.S.D. values for day-to-day assays of captopril were also investigated. It has been found in the same laboratory within 1 week that the R.S.D. values did not exceed 2%.

The limitation of the proposed method is the interference in the complex real samples such as plasma. Electrochemically active species, which have a potential peak close to the potential peak of captopril can produce the current response and cause the mistake. This interference can be separated out by HPLC technique. The determination of captopril in an artificial samples using a boron-doped diamond electrode as the working electrode for amperometric detection after the separation by HPLC is under study.

4. Conclusions

Captopril can be detected directly with high sensitivity in alkaline medium using boron-doped diamond electrodes operating in an amperometric detection mode for FIA. Well-defined sweep rate dependent cyclic voltammograms were obtained. The pH dependence of captopril oxidation shows that, at the diamond electrode, a well-defined response can be obtained in an alkaline medium, while a response can not be obtained at a GC electrode in either acidic or basic media. FIA results show that captopril can be detected amperometrically with high sensitivity and reproducibility. The detection limit of 10 nM (0.2 pmol) was obtained with a response variability less than 3% (*n* = 10). A linear dynamic range from 0.5 to 100 μM was achieved. Its advantages over chromatographic methods include simplicity, cost effectiveness and rapidity of the analysis. Application of the proposed

method for determination of captopril in commercially available tablet dosage forms indicates that this method is precise, accurate and very sensitive.

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Electrochemical oxidation of tiopronin at diamond film electrodes and its determination by amperometric flow injection analysis

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Abstract

The electrooxidation of tiopronin at diamond film electrodes was investigated using cyclic voltammetry. Comparison experiments were performed with glassy carbon electrodes. At the diamond film electrode, it was found that tiopronin provided highly reproducible and well-defined cyclic voltammograms, with peak potential at a potential of +0.8 V versus Ag/AgCl. Conversely, tiopronin gave ill-defined cyclic voltammograms at the glassy carbon electrode. The linearity at diamond film electrode was observed over the range of 0.05–10 mmol l⁻¹ and the detection limit of 50 μmol l⁻¹ (S/B ≥ 3). In addition, the amperometric hydrodynamic voltammetry along with flow injection analysis with diamond electrode as the working electrode was studied. A detection potential of +0.8 V was chosen, at which tiopronin exhibited the highest signal-to-background ratios. The flow injection analysis results showed the significant low detection limit of 10 nmol l⁻¹ (S/N ≈ 3) and a linear dynamic range of 0.5–50 μmol l⁻¹. The method was applied to the determination of tiopronin in a pharmaceutical formulation. According to the procedure based on the standard addition technique, the recoveries obtained were 96.5–108.3% and the RSD values were 1.10–1.62% (*n* = 10).

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Keywords: Tiopronin; Boron-doped diamond thin film electrode; Flow injection system; Amperometric detection; Electrochemical oxidation

1. Introduction

The electrochemical use of synthetic conductive diamond thin film electrodes is being increasingly reported [1–8]. Diamond is one of nature's best insulators, but when doped with boron, the material can possess either semiconducting or semimetallic electronic properties depending on the doping level [9]. Therefore, boron-doped diamond (BDD) thin film electrode is one of the new promising materials for electroanalytical measurements due to its attractive electrochemical properties over other elec-

trodes. Its attractive features include low and stable voltammetric background currents, a wide working potential window in aqueous electrolyte solution, low adsorption of polar molecules from aqueous solution, long-term response stability, and good activity without any conventional pretreatment [10–13]. These material properties are the impetus for our interest in studying and developing diamond electrodes for electrochemical applications.

Tiopronin [*N*-(2-mercaptopropionyl)-glycine] is a drug with a free thiol group that is used in clinical applications. It is effective in the treatment of cystinuria, rheumatoid arthritis as well as hepatic disorders, and as an antidote to heavy metal poisoning [14,15,19]. Along with its needed effects, it may cause some unwanted effects such as muscle pain, yellow skin or

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eyes, sore throat and fever, change in taste or smell, etc. Moreover this drug produces a dose-related nephrotic syndrome. Tiopronin is available only with a doctor's prescription [17,18,20].

Various methods have been proposed for the determination of tiopronin. Most of these involve conventional spectrophotometry based on the formation of complexes with metal ions [19,28], fluorimetry [14], chemiluminescence [15,21–23], liquid chromatography [24,25], and gas chromatography [26,27]. These techniques require expensive apparatus and reagents as well as skilled operators. They are also time-consuming. Tedious pre- and post-column derivatization is often required for liquid chromatography. A sensitive and rapid method for the analysis is still needed.

Electrochemical methods are popular for many applications because the procedures are simple and fast, and the cost is low. Nevertheless, the determination of thiols has provided a substantial challenge to the electroanalytical community as the direct oxidation of thiol functionality is generally hampered by poor voltammetric behaviour at solid electrodes. Numerous strategies have been developed to alleviate some of the problems that affect both the selectivity and sensitivity of analytical procedures. Using mercury electrodes can often provide significant enhancements in sensitivity. However, the method based on mercury has a limit due to its possible toxicity and the rapid deterioration of the electrode response [29]. Boron-doped diamond thin film electrodes can be used to eliminate these problems. Thus, the BDD electrode is an interesting material for studying the electrochemical oxidation of tiopronin.

Boron-doped diamond thin film electrodes have been successfully used in amperometric detection schemes coupled with automatic system based on flow injection analysis (FIA) for several analytes, since they can reduce time of analysis. Moreover, they can provide reproducible and accurate results [30–32]. In this present work, we report the use of BDD electrodes to study the electrochemical oxidation of tiopronin using cyclic voltammetry. Focus is placed on comparing the results with glassy carbon (GC) electrodes. In addition, the performance of the BDD electrode for the detection of this analyte was examined by hydrodynamic voltammetry and FIA with amperometric detection for determination of tio-

pronin in standard chemical form and commercially available tablets.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade or better and were used without further purification. All solutions and subsequent dilutions were prepared in deionized water. Phosphate buffers (pH 5–8), 0.1 mol l^{-1} , were prepared from 0.1 mol l^{-1} potassium dihydrogen phosphate (Merck) and 0.1 mol l^{-1} disodium hydrogen phosphate (Fluka). Phosphate buffer (pH 2.5) was prepared from 0.1 mol l^{-1} potassium dihydrogen phosphate and the pH was adjusted with orthophosphoric acid (85%, Carlo Erba). Phosphate buffer (pH 9), 0.1 mol l^{-1} , was prepared from 0.1 mol l^{-1} potassium dihydrogen phosphate and the pH was adjusted with 0.1 mol l^{-1} sodium hydroxide. The standard tiopronin (Sigma) solutions were freshly prepared with the same buffer prior to use.

2.2. Electrodes

The experimental conditions and the apparatus used for the diamond film growth have been described in detail elsewhere [33]. The films were prepared by deposition of the BDD thin films on highly conductive n-Si (1 1 1) substrates by microwave plasma-assisted chemical vapour deposition. Deposition was usually carried out for 10 h to achieve a film thickness of approximately $30 \mu\text{m}$. The nominal B/C atomic ratio in the gas phase was 1:100, and the typical boron-doping level in the film was ca. 10^{21} cm^{-3} . The BDD electrodes were rinsed with ultra-pure water prior to use. The GC electrode was purchased from Bioanalytical System, Inc. (area 0.07 cm^2). Pretreatment involved sequential polishing with 1 and 0.05μ of alumina/water slurries on felt pads, followed by rinsing with ultra-pure water prior to use.

2.3. Cyclic voltammetry

Electrochemical measurements were recorded using an Autolab Potentiostat 100 (Metrohm, Switzerland) with a standard three-electrode configuration. The

planar working BDD electrode was pressed against a smooth ground joint at the bottom of the cell, isolated by an O-ring. The geometric area of the working electrode is 0.07 cm^2 . A platinum wire served as the auxiliary electrode and Ag/AgCl was used as the reference. Placing the backside of the Si substrate on a brass plate made ohmic contact. A GC electrode (0.07 cm^2 , Bioanalytical System, Inc.) was also used as a working electrode in the comparison study with the BDD electrode. Cyclic voltammetry was used to probe the electrochemical reaction. The electrochemical measurements were housed in a Faradaic cage to reduce electronic noise. All experiments were done at room temperature.

2.4. Flow injection analysis with amperometric detection

The FIA system consisted of a thin layer flow cell (Bioanalytical System, Inc.), an injection port (Rheodyne 7725) with a $20\text{-}\mu\text{l}$ injection loop, a peristaltic pump (Ismatec), and an electrochemical detector (PG100). The carrier stream, 0.1 mol l^{-1} phosphate buffer (pH 8), was regulated by a reagent delivery module at a flow rate of 1 ml min^{-1} . A pulse dampener was used in series to reduce the pulsation introduced by the alternation of the roller of the peristaltic pump. The thin layer flow cell consisted of a silicone gasket as a spacer, Ag/AgCl as the reference electrode, stainless steel tube as an auxiliary electrode and outlet. The experiments were performed in a copper Faradaic cage to reduce electrical noise. A hydrodynamic voltammogram was obtained before the amperometric determination was performed. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential to obtain hydrodynamic voltammograms. The amperometric measurements were carried out at the potential giving a maximum signal-to-background (S/B) ratio in the hydrodynamic voltammograms.

2.5. Sample preparations

Tablets containing tiopronin (Mission pharmaceutical) were analyzed. The drug tablet was homogenized in an agar mortar. The amount of the powdered mass analyte was dissolved in 100 ml of 0.1 mol l^{-1} phos-

phate buffer (pH 8). This solution was diluted in such a way that the concentration of tiopronin in the final test solution was within the linear dynamic range ($0.5\text{--}50\text{ }\mu\text{mol l}^{-1}$).

3. Results and discussion

3.1. pH effect

The preliminary study of electrochemical oxidation of tiopronin using BDD and GC electrodes was performed at pH 2.5, 5.0, 7.0, 8.0, and 9.0. It was found that tiopronin gave an oxidation peak at neutral and alkali pH. The oxidation potential decreased when the pH of the analyte solution increased. This phenomenon can be explained by the fact that the thiol group of tiopronin was easily hydrolyzed in alkaline medium to produce the more stable reduced form. We found that tiopronin in phosphate buffer at pH 8 provided the highest S/B. Therefore, we used this pH for the next experiments.

3.2. Cyclic voltammetry

Fig. 1(a) and (b) shows cyclic voltammograms for tiopronin oxidation at BDD and GC electrodes in 2 mmol l^{-1} tiopronin + 0.1 mol l^{-1} phosphate buffer pH 8. The corresponding backgrounds are also shown. For the GC electrode, an anodic peak occurred at approximately $+0.8\text{ V}$ versus Ag/AgCl. A rapid increase in the current at this potential was also observed in the background voltammogram due to oxygen evolution and carbon oxidation. For this reason, the voltammogram was ill-defined. In the case of the BDD electrode, a very well-defined oxidation peak at approximately $+0.8\text{ V}$ versus Ag/AgCl was observed. It was observed that there was very low background current. No cathodic peak was observed on the reverse sweep within the investigated potential range (-0.6 to $+1.4\text{ V}$) for both GC and BDD electrode, because thiol oxidation was an electrochemically irreversible process.

Fig. 2 shows the cyclic voltammograms recorded during variation of the sweep rate for the BDD electrode. It can be seen that peak currents are linearly proportional to the square root of the sweep rate within the range $10\text{--}300\text{ mV s}^{-1}$. The linear regression statistical analysis yields $R^2 = 0.9997$. The linearity suggests

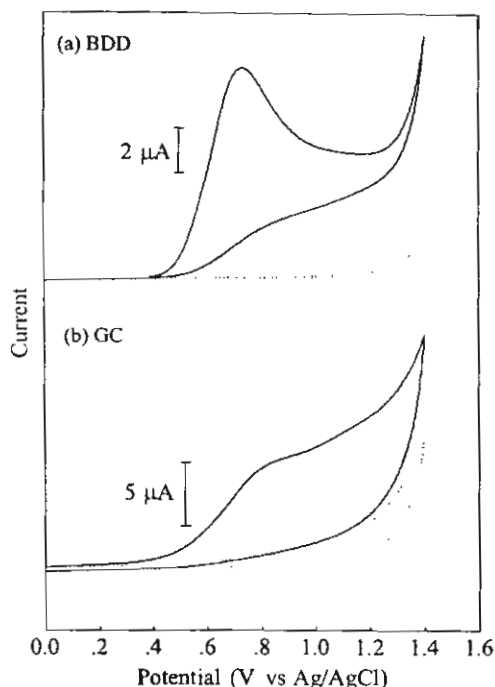


Fig. 1. Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 2.0 mmol l^{-1} tiopronin in 0.1 mol l^{-1} phosphate buffer pH 8 (solid lines) and 0.1 mol l^{-1} phosphate buffer pH 8 (dashed lines). The sweep rate was 50 mV s^{-1} ; area of electrode, 0.07 cm^2 .

that the current is limited by semi-infinite linear diffusion of tiopronin in the interfacial reaction zone and that rate-limiting adsorption step and specific surface interactions can be neglected.

As shown in Fig. 3, at the BDD electrode the oxidation peak current is linearly proportional to the tiopronin concentration in the range of $0.05\text{--}10 \text{ mmol l}^{-1}$, while the GC electrode provided a non-linear function of the tiopronin concentration. For the BDD electrode, a well-defined voltammetric peak was obtained with an S/B ratio of 3 at a concentration as low as 50 µmol l^{-1} .

3.3. Hydrodynamic voltammetry

In the FIA measurements, the first important results were obtained from hydrodynamic voltammetry. Fig. 4 shows hydrodynamic voltammograms for 20-µl injections of 100 µmol l^{-1} tiopronin + phosphate buffer pH 8, using phosphate buffer pH 8 as the carrier stream. The peak currents of tiopronin and corresponding background currents showed in Fig. 4a. Despite the well-defined voltammogram of tiopronin was obtained, the hydrodynamic voltammogram (Fig. 4a) is very stretched out closer to a straight line (extending from 0.6 to 1.1 V). The expected sigmoidal

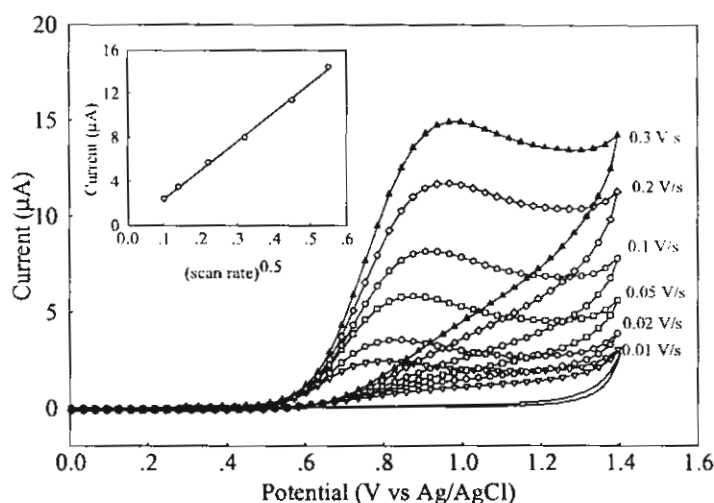


Fig. 2. Cyclic voltammograms for 1 mmol l^{-1} tiopronin in 0.1 mol l^{-1} phosphate buffer (pH 8) at BDD electrode for a series of sweep rates; area of electrode, 0.07 cm^2 . The dependence between peak current (μA) and square root of the sweep rate appears in the inset.

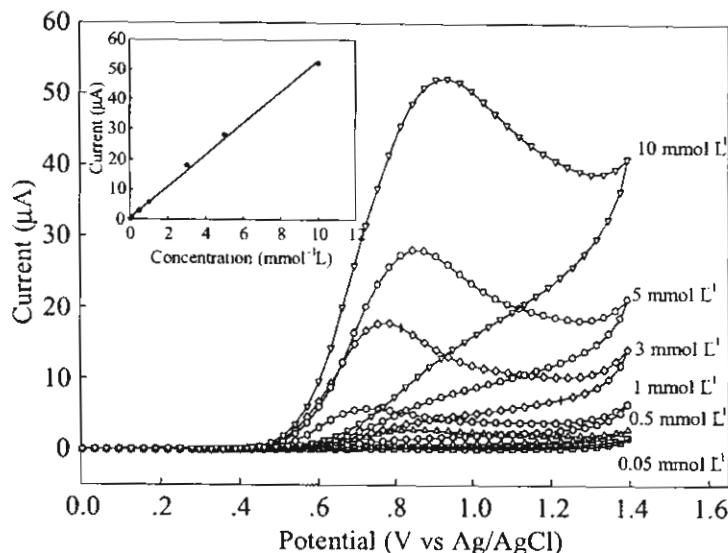


Fig. 3. Cyclic voltammograms for tiopronin in 0.1 mol l^{-1} phosphate buffer (pH 8) at BDD electrode for a series of tiopronin concentrations. The sweep rate was 50 mV s^{-1} ; area of electrode, 0.07 cm^2 . The dependence between peak current (μA) and concentrations at the potential of 50 mV s^{-1} appears in the inset.

current potential curve with a defined plateau did not receive. This behaviour does not outweigh the advantage of the very low residual current. Therefore, the S/B ratios were calculated from Fig. 4a as a function of potential. As shown in Fig. 4b the S/B ratio reaches a maximum value at 0.8 V. Hence, this potential was selected for quantitative amperometric detection in FIA experiments.

3.4. Flow injection analysis

In recent years, a number of studies have been reported concerning the development of diamond electrodes for applications as amperometric detector in FIA [10–12]. In the present paper, we obtained excellent FIA results using BDD electrodes for the determination of tiopronin. It was found that the signal current for repetitive $20\text{-}\mu\text{l}$ injections of tiopronin at various concentrations in phosphate buffer, pH 8, at the detection potential, increased linearly with increasing concentration from 500 nmol l^{-1} to $50 \text{ }\mu\text{mol l}^{-1}$ ($R^2 = 0.9992$). For the limit of detection, tiopronin provided a useful signal with $S/N \geq 3$ at a concentration as low as 10 nmol l^{-1} .

3.5. Quantitative determination of tiopronin tablet

The BDD electrode was applied to the analysis of tiopronin in formulation. We examined the accuracy of the method for determination of tiopronin in pharmaceuticals by the method of the standard addition. Linear least square calibration curve provides a slope of $21.07 \text{ nA}/\mu\text{mol l}^{-1}$ (sensitivity) and correlation coefficient of 0.996. Relative error compared with the claimed amount was lower than 4%. The recoveries of the added tiopronin ranged between 96.5 and 108.3% were obtained as shown in Table 1. At lower concentration, it was non-linear, with the slope gradually decreasing when the concentration grows. We expected that it was due to the inexistence of a plateau, result from hydrodynamic voltammogram, the current becomes quite potential-dependent and any uncompensated IR drop in the cell can contribute to the blending. Another possible cause can be found in Fig. 2: the anodic wave suffers displacement to more positive potential with increase concentration. This non-linearity can be caused overestimation at low concentrations to expected values, followed by underestimation at the upper end. The tiopronin content of the drug calculated

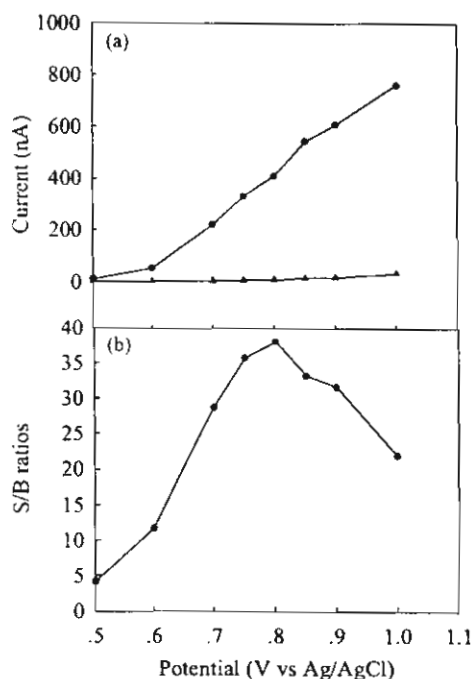


Fig. 4. (a) Hydrodynamic voltammogram of ($\blacktriangle$) 0.1 mol l^{-1} phosphate buffer (pH 8, background current) and ($\bullet$) $100 \mu\text{mol l}^{-1}$ of tiopronin in 0.1 mol l^{-1} phosphate buffer (pH 8) with averaging the peak currents from four injections (injected the analyte solution four times at each potential and then the average peak current was obtained by calculation), using 0.1 mol l^{-1} phosphate buffer (pH 8) as a carrier stream. (b) Hydrodynamic of signal-to-background ratio. The flow rate was 1 ml min^{-1} .

from this calibration plot ($102.8 \pm 0.03 \text{ mg per tablet}$, $n = 2$) was found to be in satisfactory agreement with the labelled amount of $100 \text{ mg per tablet}$.

The precision of the method was obtained on the basis of intra-assay. Three concentrations of added solution (0.00 , 0.98 , $1.63 \mu\text{g ml}^{-1}$) were chosen. Results

Table 1

Recovery of tiopronin tablet samples with amperometric detection using diamond electrode applied to flow injection system ($n = 2$)

Amount of tiopronin tablet sample added ($\mu\text{g ml}^{-1}$)	Amount of tiopronin tablet sample found ($\mu\text{g ml}^{-1}$)	Percent of recovery
0.65	0.70 ± 0.02	108.3 ± 3.6
0.98	1.04 ± 0.07	105.2 ± 6.7
1.31	1.35 ± 0.04	103.2 ± 2.8
1.63	1.62 ± 0.01	99.6 ± 0.4
1.96	1.89 ± 0.002	96.5 ± 0.1

obtained from 10 injections were within 1.10 – 1.62% of the relative standard deviation (RSD). Moreover, the RSD values between two analyses were also investigated. It was found that the RSD did not differ more than 1.5% .

Further efforts will study the use of amperometric techniques coupled with HPLC system for the determination of tiopronin in complex real samples to improve the selectivity, sensitivity, and detection limit.

3.6. Comparison with other methods

Table 2 summarizes data from the other methods for determination of tiopronin compared with the proposed method. It was found that using the BDD electrodes with amperometric FIA gave the similar wide linear dynamic range to other methods (two order of magnitudes). Interestingly, the proposed method provided a very low detection limit of 10 nmol l^{-1} , because the BDD electrode exhibited very low background current and noise signals. It also resulted in very high sensitivity. This outstanding performance of the BDD electrode make it attractive for using as working electrode in FIA system for analysis of tiopronin.

Table 2
Comparison of data for tiopronin determination

Method	Linear dynamic range ($\mu\text{mol l}^{-1}$)	Detection limit ($\mu\text{mol l}^{-1}$)	Precision (percent RSD)	Reference
Flow injection fluorometric	0.8–20	0.098	1.0 ($1 \mu\text{mol l}^{-1}$)	[14]
Flow injection chemiluminescent	0.1–70	0.036	2.6 ($3 \mu\text{mol l}^{-1}$)	[15]
Liquid chromatography with electrochemical detection	5–20	0.710	—	[16]
Chemiluminescent HPLC coupled with flow injection	1–100	0.80	4.8 ($50 \mu\text{mol l}^{-1}$)	[21]
Diamond thin film electrode applied to flow injection analysis	0.5–50	0.010	1.1 ($17.8 \mu\text{mol l}^{-1}$)	^a

(—) No report.

^a This proposed method.

4. Conclusion

This is the first use of BDD thin film electrodes for the electroanalysis of tiopronin. It was found that BDD electrodes exhibited excellent performance for the oxidative detection of tiopronin. Well-defined voltammograms were obtained at the BDD electrode, which exhibited high sensitivity, and demonstrated significant advantages over the GC electrode, because of its superior electrochemical properties. In addition, the use of the BDD electrode is a simple analytical procedure, because no chemical modification is required. Cleaning of the electrode with time is also not necessary due to the long-term stability of the BDD electrode response. The outstanding capabilities of the BDD electrode were demonstrated by coupling with FIA. The results indicate that tiopronin can be detected amperometrically without derivatization or the use of a pulse waveform. The advantage of the BDD electrode is that it is able to achieve a highly stable and sensitive response with simple amperometric detection. Application of the proposed method for determination of tiopronin in commercially available tablet forms shows that this method is precise, accurate, and very sensitive.

Acknowledgements

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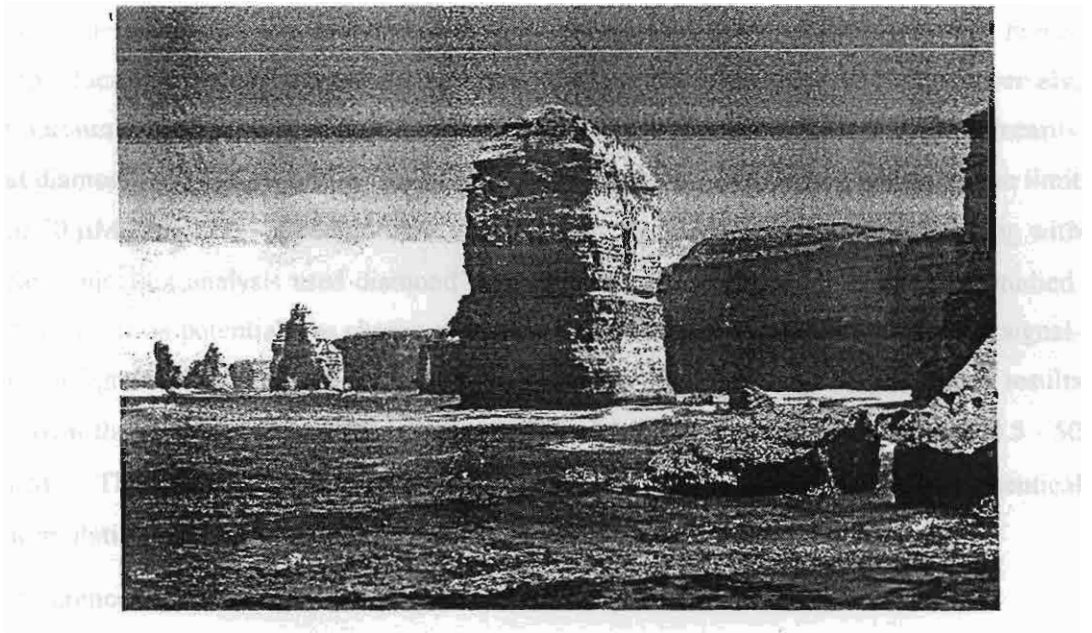
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FLOW ANALYSIS



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BOOK OF ABSTRACTS

C2

**ELECTROCHEMICAL OXIDATION OF TIOPRONIN AT DIAMOND FILM
ELECTRODES AND ITS DETERMINATION BY AMPEROMETRIC FLOW
INJECTION ANALYSIS**

W. Siangproh, N. Wangfuengkanagul and O. Chailapakul*

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The electrooxidation of tiopronin at diamond film electrodes was investigated using cyclic voltammetry (1,2). The comparison experiments were performed with glassy carbon electrode. At diamond film electrode, it was found that tiopronin provided highly reproducible and well-defined cyclic voltammogram at potential of +0.8 V. Conversely, tiopronin gave ill-defined cyclic voltammogram at glassy carbon electrode. The linearity at diamond film electrode was observed over the range of 0.05 -10 mM and detection limit of 50 μ M ($S/B \geq 3$). In addition, the amperometric hydrodynamic voltammetry along with flow injection analysis used diamond film electrode as a working electrode was studied. The detection potential was chosen at +0.8 V which tiopronin exhibited the highest signal-to-background ratios under the optimum condition. The flow injection analysis results shown the significant low detection limit of 10 nM ($S/N \approx 3$) and a linear range of 0.5 - 50 μ M. The method was applied to determination of tiopronin in a pharmaceutical formulation and the results will be discussed.

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Announcement

6 th International Mini-Symposium on Diamond Electrochemistry and Related Topics, March 5-6, 2003, Tokyo, Japan

Scope of the Symposium

The diamond electrochemistry mini-symposia are held every year starting from 1997 with the aim of covering the latest developments in this field. The present symposium will include mainly basic electrochemistry, biosensors and photoelectrochemistry of diamond electrodes.

These topics will be discussed in oral presentations, in short oral presentations and in poster session. A book of abstracts will be distributed at the symposium.

Symposium Venue

The symposium will take place on **March 5-6, 2003**, at the **Sanjo Kaikan**. The University of Tokyo. The conference party is evening on March 5 at Matsumoto-rou restaurant (in the University campus).

Registration

The registration fee is free. However, preliminary registration is essential. If you are interested and wish to attend the Mini-symposium, send an e-mail to Prof. Einaga (Keio Univ.) **now** (by **January 10, 2003**) at the address given below.

Yasuaki Einaga

**Department of Chemistry, Faculty of Science and Technology,
Keio University**

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Abstract

Abstracts (Maximum 2 pages) also should be sent to Prof. Einaga (Keio University) **by February 1, 2003**. Attached files by e-mail (einaga@chem.keio.ac.jp) are also available.

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Electroanalysis of Captopril, Tiopronin and Tetracycline Using the Boron-Doped Diamond Thin-Films

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Abstract

The electrochemical comparison between the boron-doped diamond electrode (BDD) and the glassy carbon (GC) electrode was studied. Organic compounds with different functional groups such as captopril, tiopronin and tetracycline were employed by cyclic voltammetric technique.

The electrooxidation of captopril and tiopronin at diamond film electrodes was investigated using cyclic voltammetry. The comparison experiments were performed with glassy carbon electrode. Captopril undergoes irreversible reaction at both electrodes. However, the BDD electrode provided well-resolved cyclic voltammogram and higher signal-to-background (S/B) ratio comparing to GC electrode. At diamond film electrode, it was found that tiopronin provided highly reproducible and well-defined cyclic voltammogram at potential of +0.8 V. Conversely, tiopronin gave ill-defined cyclic voltammogram at glassy carbon electrode. The Linearity of captopril was from 50 μM – 5 mM and detection limit was 10 μM ($\text{S/B} \approx 3$). For tiopronin was observed over the range of 0.05 -10 mM and detection limit of 50 μM ($\text{S/B} \geq 3$). In addition, the amperometric hydrodynamic voltammetry along with flow injection analysis used as deposited diamond film electrode as a working electrode was studied. The method was applied to determination of tiopronin and captopril in a commercial available tablet. The results show very good precision and accuracy.

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ELECTROCHEMICAL ANALYSIS OF SULFUR-CONTAINING COMPOUNDS AT BORON-DOPED DIAMOND ELECTRODE

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Abstract:

The use of boron-doped diamond electrode to detect several types of sulfur-containing compounds, including thiol drugs and specific important sulfur-containing compounds, such as homocysteine, cysteine, tiopronin, and captopril was reported. Using cyclic voltammetry, it was found that BDD electrode exhibited high sensitivity, and demonstrated significant advantages over the GC electrode, because of its superior electrochemical properties. The oxidation of these compounds at the BDD electrode exhibited well-defined irreversible cyclic voltammograms. The concentration dependence has been studied and has indicated the promise of using BDD electrodes for quantitative determination. It was also shown that there was no adsorption on the surface of the BDD electrode for low concentrations. This advantage should be valuable for the electroanalysis of sulfur-containing compounds without pretreatment of the electrode. In addition, the amperometric detection applied to flow injection system or HPLC system with BDD electrode as the working electrode was studied. The results showed the significant low detection limit, wide linear range, and good reproducibility. The application of their methods also studied in the real samples, such as pharmaceutical formulations. The results were satisfactory.

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บทคัดย่อ ABSTRACTS

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THE SCIENCE SOCIETY OF THAILAND UNDER THE PATRONAGE
OF HIS MAJESTY THE KING



คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น
FACULTY OF SCIENCE,
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SC-263P: THE ELECTROANALYSIS OF METHIMAZOLE AT BORON-DOPED DIAMOND THIN FILM ELECTRODES APPLIED TO FLOW INJECTION SYSTEM

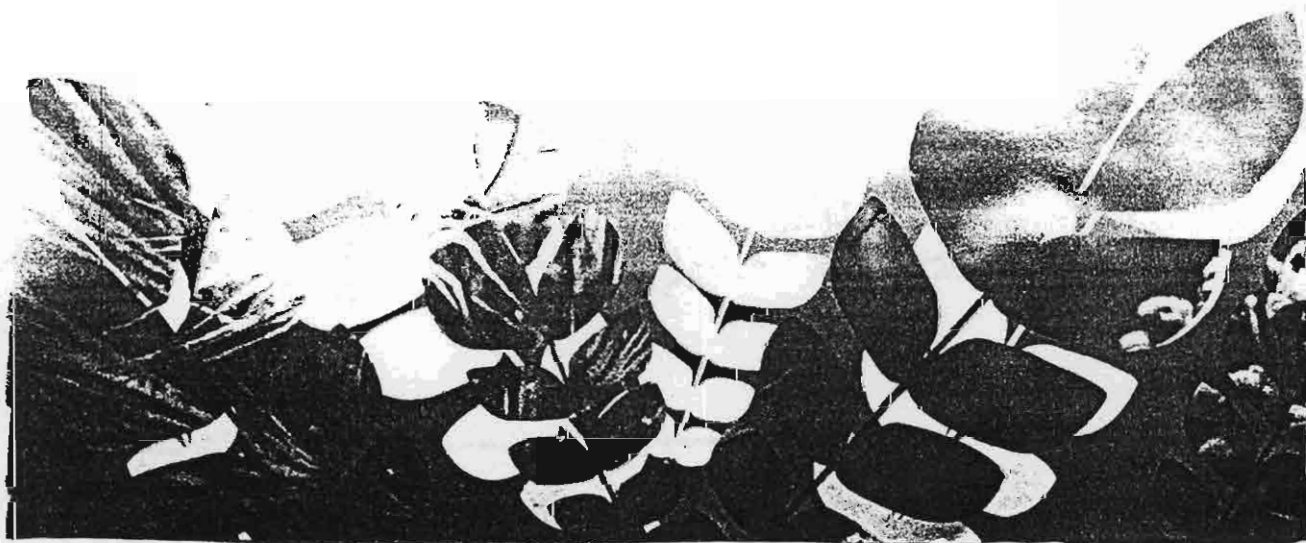
Weena Siangproh and Orawon Chailapakul

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Abstract: In this research, electroanalysis of methimazole was studied in phosphate buffer boron-doped diamond thin film (BDD) electrodes by several techniques such as cyclic voltammetry, hydronamic voltammetry and flow injection with amperometric detection. Cyclic voltammetry was used to study the reaction as a function of concentration of analyte. The results were compared with a glassy carbon (GC) electrode at the same condition. Methimazole underwent irreversible reaction at both electrodes. However, the BDD electrode provided highly reproducible, well-defined cyclic voltammogram and higher signal to background (S/B) ratio comparing with GC electrode. The detection limit of 25 μM was obtained from the BDD electrode using cyclic voltammetry. The potential sweep rate dependence of methimazole oxidation (peak currents for 1 mM methimazole linearly proportional to $V^{1/2}$, within the range of 0.01 to 0.3 V s^{-1}) indicated that the oxidation involved a diffusing species, with negligible adsorption on BDD surface at this concentration. In addition, methimazole has been studied by hydrodynamic voltammetry and flow analysis injection with BDD electrode. From the system, boron-doped diamond exhibited a highly reproducible amperometric response, with a peak variation less than 2% at concentration of 1 μM ($n=2$). An extremely low detection limit (10 nM) was obtained at 0.75 V vs. Ag/AgCl.

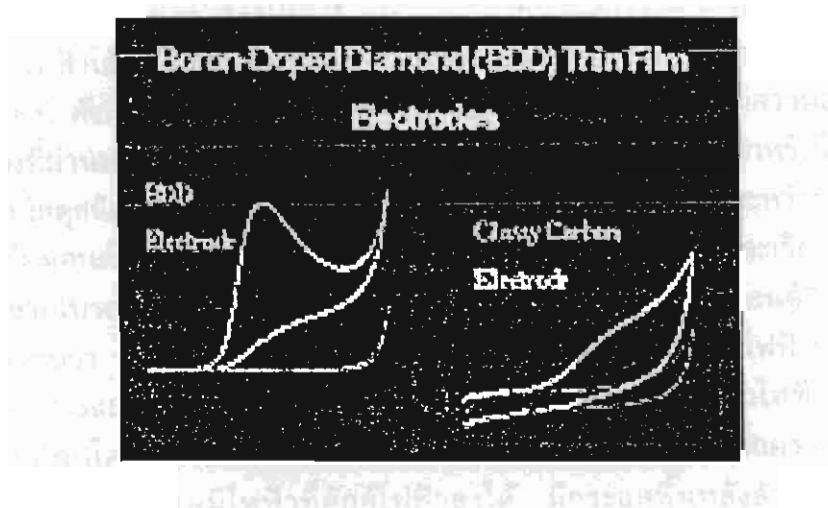


ร่างข้อเสนองานวิจัย
จุฬาลงกรณ์มหาวิทยาลัย ปี ๒๕๔๖



รางวัลผลงานวิจัย
กองทุนรัชดาภิเษกสมโภช จุฬาลงกรณ์มหาวิทยาลัย
ประจำปี ๒๕๔๖

างวลผลงานวิจัยดี



ผลงานวิจัยเรื่อง การประยุกต์ใช้ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์สำหรับการหาปริมาณสารประกอบอินทรีย์

(The Application of Boron-Doped Diamond Thin Film Electrodes for the Determination of Organic Compounds)

ดย ผู้ช่วยศาสตราจารย์ ดร. อรรณ ชัยลภากุล

แหล่งทุนที่ได้รับ กองทุนรัชดาภิเษกสมโภช กองทุนสนับสนุนการวิจัย (สกว.)
โครงการถ่ายทอดเทคโนโลยีไทย-ญี่ปุ่น

การวิเคราะห์ทางเคมีไฟฟ้าเป็นวิธีที่มีประโยชน์อย่างกว้างขวางในการวิเคราะห์สารประเภทต่างๆ ทั้งในการควบคุมคุณภาพและมาตรฐานยา การวิเคราะห์ทางคลินิก การวิเคราะห์ทางอุตสาหกรรม การวิเคราะห์สารจากสิ่งแวดล้อม และการศึกษาวิจัยทางวิทยาศาสตร์ในสาขาอื่นๆ สิ่งที่สำคัญของการวิเคราะห์ทางเคมีไฟฟ้าคือ ขั้วไฟฟ้าใช้งานหรืออิเล็กโทรด ซึ่งทำหน้าที่เป็นตัวติดตามหรือตรวจวัดสารที่สนใจวิเคราะห์ ดังนั้นการเลือกใช้วัสดุที่นำมาทำเป็นขั้วไฟฟ้าจึงเป็นสิ่งที่มีความสำคัญยิ่งในช่วงที่ผ่านมาพบว่า ผู้วิจัยกลุ่มหนึ่งได้ทำการพัฒนาวัสดุชนิดใหม่สำหรับใช้เป็นขั้วไฟฟ้า วัสดุชนิดนี้คือ โบรอนโดปไดมอนด์ เนื่องจากการเติมโบรอนในระหว่างการสร้างฟิล์มไดมอนด์ ทำให้วัสดุชนิดนี้มีพฤติกรรมเป็นขั้วไฟฟ้าตัวนำได้ และเรียกขั้วไฟฟ้าที่ทำจากโบรอนโดปไดมอนด์ว่า “ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์” จากการศึกษพบว่า ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์มีสมบัติทางเคมีไฟฟ้าที่น่าสนใจ ซึ่งแตกต่างและเด่นกว่าขั้วไฟฟ้าชนิดอื่นๆ หลายประการ ได้แก่ ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์ให้ช่วงศักย์ไฟฟ้าการใช้งานที่กว้างทำให้สามารถตรวจวัดสารที่เกิดปฏิกิริยาทางเคมีไฟฟ้าที่ศักย์ไฟฟ้าสูงได้ มีกระแสพื้นหลังสำหรับเทคนิคไซคลิกโวลแทมเมทรีที่ต่ำและเสถียร โดยมีค่ากระแสพื้นหลังที่ต่ำกว่าขั้วไฟฟ้ากลาสคาร์บอนประมาณ ๑๐ เท่า ทำให้การตรวจวัดมีสภาพไวที่สูง สมบัติที่สำคัญมากอีกประการหนึ่งคือ ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์ เป็นขั้วไฟฟ้าที่มีเสถียรภาพสูง เนื่องจากไม่เกิดการดูดซับของสารประกอบอินทรีย์บนผิวหน้าขั้วไฟฟ้า นอกจากนี้ยังพบว่าขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์ไม่ไวต่อการศึกษาของออกซิเจนที่ละลายในสารละลายทั้งในกรณีที่สารละลายมีความเป็นกรดหรือด่าง ดังนั้นการใช้ขั้วไฟฟ้าฟิล์มบางโบรอนโดปไดมอนด์จึงไม่จำเป็นต้องทำการไล่ออกซิเจนออกจากสารละลายก่อนทำการวิเคราะห์ ทำให้ประหยัดเวลาในการทดลอง

ในงานวิจัยนี้ได้เลือกทำการวิเคราะห์สารประกอบอินทรีย์ชนิดต่างๆ เช่น สารประกอบอินทรีย์ที่ใช้เป็นยารักษาโรค สารประกอบจำพวกกรดอะมิโน และสารประกอบอินทรีย์จำพวกอื่นๆ โดยสารประกอบอินทรีย์เหล่านี้ล้วนเป็นสารที่มีความสำคัญทางคลินิกและทางด้านสิ่งแวดล้อม

จากการสืบค้นข้อมูลพบว่า การวิเคราะห์สารประกอบอินทรีย์เหล่านี้ สามารถทำได้หลายวิธี แต่เกือบทุกเทคนิคที่นำใช้จำเป็นต้องเปลี่ยนสารประกอบอินทรีย์ให้อยู่ในรูปของอนุพันธ์ที่สามารถตรวจวัดได้ด้วยตัวตรวจวัดที่เลือกใช้ ทำให้เป็นวิธีที่มีความยุ่งยากซับซ้อน ใช้เวลาในการวิเคราะห์นาน และมีค่าใช้จ่ายสูง เทคนิคทางเคมีไฟฟ้าเป็นเทคนิคที่มีวิธีการใช้งานง่าย ราคาถูก มีความไวสูง จึงเป็นอีกเทคนิคหนึ่งที่น่าสนใจสำหรับการวิเคราะห์สารประกอบอินทรีย์ชนิดต่างๆ งานวิจัยนี้ได้ทำการศึกษาสมบัติทางเคมีไฟฟ้าของสารประกอบอินทรีย์ที่เลือกมาโดยใช้ขั้วไฟฟ้าฟิล์มบางโบรอนโดปโดมอนด์ และทำการเปรียบเทียบผลการทดลองกับขั้วไฟฟ้ากลาสสึคาร์บอนด้วยเทคนิคไซคลิกโวลแทมเมทรี นอกจากนี้ได้นำระบบโพลีอินเจกชันมาประยุกต์ใช้ร่วมกับเทคนิคทางเคมีไฟฟ้า ทำให้การวิเคราะห์มีความถูกต้องและความไวสูงขึ้น และยังช่วยลดเวลาในการวิเคราะห์ ดังนั้นงานวิจัยนี้จึงนำขั้วไฟฟ้าฟิล์มบางโบรอนโดปโดมอนด์และระบบการวิเคราะห์โพลีอินเจกชันมาใช้สำหรับวิเคราะห์ปริมาณของสารประกอบอินทรีย์ด้วยเทคนิคแอมเปอร์โรเมทรี

จากผลการทดลอง สามารถสรุปได้ว่าขั้วไฟฟ้าฟิล์มบางโบรอนโดปโดมอนด์เป็นขั้วไฟฟ้าชนิดใหม่ที่สามารถนำมาใช้สำหรับงานด้านเคมีวิเคราะห์ ทำให้หาปริมาณสารประกอบอินทรีย์ ได้ทั้งในด้านคุณภาพวิเคราะห์และปริมาณวิเคราะห์ และการใช้ขั้วไฟฟ้าชนิดนี้ยังสามารถแก้ปัญหาต่างๆ ที่พบในขั้วไฟฟ้าชนิดอื่นๆ กล่าวคือ เป็นขั้วไฟฟ้าที่ไม่เป็นพิษ ไม่เกิดการดูดซับของสารที่ผิวหน้าขั้วไฟฟ้า มีเสถียรภาพสูงทำให้ข้อมูลเชื่อถือได้ และการที่ขั้วไฟฟ้าชนิดนี้มีค่ากระแสพื้นหลังที่ต่ำทำให้ได้ผลการตรวจวัดที่มีความไวสูง และเมื่อนำขั้วไฟฟ้าฟิล์มบางโบรอนโดปโดมอนด์มาประยุกต์ใช้ร่วมกับระบบโพลีอินเจกชันและวิธีแอมเปอร์โรเมทรีเพื่อวิเคราะห์หาปริมาณสารพบว่า สามารถวิเคราะห์สารประกอบอินทรีย์ได้โดยตรง โดยไม่ต้องเปลี่ยนสารให้อยู่ในรูปของอนุพันธ์ก่อนตรวจวัดหรือเลือกใช้เทคนิคที่มีความซับซ้อน เช่น ฟัลส์แอมเปอร์โรเมทรี ทำให้วิธีวิเคราะห์ที่เสนอนี้มีความง่าย ประหยัดค่าใช้จ่าย และใช้ระยะเวลาในการทดลองสั้น ดังนั้นจึงสามารถกล่าวได้ว่าขั้วไฟฟ้าฟิล์มบางโบรอนโดปโดมอนด์เป็นวัสดุที่มีสมบัติดีมากและเหมาะสมที่จะนำมาใช้สำหรับงานวิเคราะห์ทางเคมีไฟฟ้า

สิ่งที่ดีเด่นในผลงานวิจัย

งานวิจัยนี้ประกอบด้วยองค์ความรู้ใหม่ ซึ่งได้แก่การนำขั้วไฟฟ้าชนิดใหม่มาใช้สำหรับการตรวจวิเคราะห์สารและสามารถตีพิมพ์ในวารสารนานาชาติได้ดังต่อไปนี้

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จากผลงานที่ได้ตีพิมพ์แสดงให้เห็นว่างานวิจัยนี้เป็นงานวิจัยใหม่ที่ยังไม่มีการศึกษามาก่อน สารที่เลือกมาทำการวิเคราะห์ล้วนแต่มีความสำคัญต่อสิ่งมีชีวิต นอกจากนี้วิธีการวิเคราะห์ที่เสนอในงานวิจัยนี้ยังเป็นวิธีที่ง่าย ประหยัด เมื่อเปรียบเทียบกับงานวิจัยในอดีตที่ผ่านมา วิธีการดังกล่าวยังสามารถนำไปประยุกต์ใช้กับสารตัวอย่างจริงได้ในกรณีที่สารตัวอย่างนั้นไม่ใช่สารที่มีความซับซ้อน ซึ่งจัดเป็นการประยุกต์ใช้ขั้นพื้นฐานที่ประสบความสำเร็จ

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