รายงานวิจัยฉบับสมบูรณ์

การวิเคราะห์ทางเคมีไฟฟ้าของสารประกอบอินทรีย์โดยใช้ฟิล์ม
บางของโบรอน-โดปไดมอนด์ประยุกต์กับโฟลอินเจคชัน

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

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กิตติกรรมประกาศ

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ชื่อโครงการ: การวิเคราะห์ทางเคมีไฟฟ้าของสารประกอบอินทรีย์โดยใช้ฟิล์มบางของโบรอน-โดปไดมอนด์ประยุกต์กับโฟลอินเจคชัน

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ระยะเวลาที่ทำการวิจัย: 3 ปี

ในงานวิจัยนี้ได้ทำการศึกษาการวิเคราะห์ทางเคมีไฟฟ้าของ D-penicillamine captopril และ tiopronin โดยใช้ฟิล์มบางของโบรอน-โดปไดมอนด์อิเล็กโทรดด้วยเทคนิคต่างๆ เช่น ไซคลิกโวลแทมเมทรี และโฟลอินเจคชันร่วมกับแอมเพอโรเมทรี ไซคลิกโวลแทมเมทรีจะใช้ในการศึกษาปฏิกิริยาโดยเป็นฟังก์ชันกับความเข้มข้นและความเป็นกรด-ด่าง เปรียบเทียบผลการทดลองกับกลาสิคาร์บอนอิเล็กโทรด จากการทดลองพบว่าสารประกอบเหล่านี้จะเกิดปฏิกิริยาผันกลับไม่ได้กับขั้วไฟฟ้าทั้งสองชนิด โบรอน-โดปไดมอนด์อิเล็กโทรดและกลาสิคาร์บอนจะให้ไซคลิกโวลแทมโมแกรมที่ชัดเจน แต่โบรอน-โดปไดมอนด์อิเล็กโทรดจะให้ค่าสัญญาณของกระแสต่อพื้นหลังที่สูงกว่ากลาสิคาร์บอนอิเล็กโทรด นอกจากนั้นได้ทำการศึกษาโดยใช้เทคนิคแอมเพอโรเมทรีร่วมกับระบบโฟลอินชันที่มีโบรอน-โดปไดมอนด์อิเล็กโทรดเป็นขั้วไฟฟ้าทำงาน สำหรับผลที่ได้จากเทคนิคโฟลอินเจคชันให้ค่าขีดจำกัดต่ำสุดในการตรวจวัดที่ 10 นาโนโมลาร์ (S/N ≈ 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-โดปไดมอนด์, ไซคลิกโวลแทมเมทรี, โฟลอินเจคชัน, แอมเพอโรเมทรี

Abstract

Project code: RSA/21/2544

Project Tiltle: 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 amperomtric 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 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 concencentrations were obtained from 0.5 to 100 μM for captopril and 0.5 to 50 μM for D-peicillamine and tiopronin. The proposed methods were successfully applied to determine captopril, D-penicillamine, and tiopronin in some drugs fomulations. 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 สารละลายอิเล็กโทรไลท์ อัตราการสแกน เป็นต้น
2. เปรียบเทียบสมบัติทางเคมีไฟฟ้าสำหรับการวิเคราะห์ D-penicillamine, captopril และ tiopronin ตัวอย่างเช่น ค่ากระแสพื้นหลัง การเกิดการดูดซับที่ผิวหน้าขั้วไฟฟ้าระหว่างขั้วไฟฟ้าฟิล์มบางโบรอน-โดปไดมอนด์กับขั้วไฟฟ้ากลาสิคาร์บอน
3. นำขั้วไฟฟ้าฟิล์มบางโบรอน-โดปไดมอนด์มาประยุกต์ใช้เป็นอุปกรณ์ตรวจวัดในระบบโฟลอินเจคชัน 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 เพื่อปรับปรุงวิธีการวิเคราะห์ให้สามารถวิเคราะห์ได้ที่ความเข้มข้นต่ำ และเปรียบเทียบสมบัติทางเคมีไฟฟ้าของสารประกอบอินทรีย์ เมื่อใช้โบรอน-โดปไดมอนด์อิเล็กโทรดและกลาสิคาร์บอนอิเล็กโทรดโดยใช้เทคนิค Cyclic Voltammetry จากการทดลองพบว่า สารประกอบเหล่านี้สามารถเกิดปฏิกิริยาผันกลับไม่ได้กับขั้วไฟฟ้าทั้งสองชนิด แต่โบรอน-โดปไดมอนด์อิเล็กโทรดจะให้ค่าสัญญาณของกระแสต่อพื้นหลังที่สูงกว่ากลาสิคาร์บอนอิเล็กโทรด pH ที่เหมาะสมที่ให้สัญญาณในการตรวจวัดสูงสุดสำหรับ D-penicillamine, captopril และ tiopronin ได้แก่ 7, 9 และ 8 ตามลำดับ
4. หาภาวะที่เหมาะสมโดยใช้เทคนิคแอมเพอโรเมทรี ร่วมกับการตรวจวัดด้วยระบบโฟลอินเจคชันโดยใช้โบรอน-โดปไดมอนด์อิเล็กโทรดเป็นขั้วไฟฟ้าทำงาน จากผลการทดลองพบว่าศักย์ไฟฟ้าที่เหมาะสมในการตรวจวัด D-penicillamine, captopril และ tiopronin ได้แก่ 0.75, 0.8 และ 0.8 โวลต์ ตามลำดับ สำหรับผลที่ได้จากเทคนิคโฟลอินเจคชันให้ค่าขีดจำกัดต่ำสุดสำหรับสารทั้งสามชนิด ในการตรวจวัดที่ 10 นาโนโมลาร์ (S/N $\approx$ 3) จากผลการทดลอง

5. นำวิธีที่เหมาะสมมาใช้หาปริมาณ D-penicillamine, captopril และ tiopronin ในสารตัวอย่างยา การทดลองใช้เทคนิคการเติมสารมาตรฐานพบว่า ได้ร้อยละของการคืนกลับอยู่ในช่วง 95.85 -109.29, 97.75 - 104.69 และ 96.5-108.3 สำหรับ D-penicillamine, captopril และ tiopronin ตามลำดับ จากผลการทดลองพบว่าการใช้เทคนิคแอมเพอโรเมทรีร่วมกับระบบโฟลอินเจชันให้ผลการทดลองที่ถูกต้อง 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. Elecroanalysis 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, chemiluminescene, 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 volatmmetry 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 futher 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 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) 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 measuement 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 voltamograms, 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 μM 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 μg/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 (0.5-50 µmol 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.

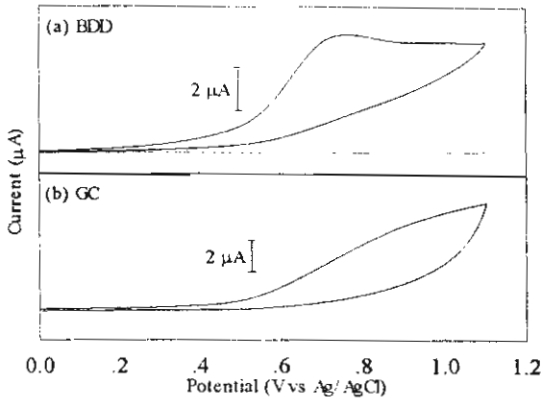

Fig. 3.1.1. Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at (a) boron-doped thin film electrode and (b) GC electrode (solid line). The potential sweep rate was 20 mV s^{-1}. Background voltammograms (0.1 M phosphate buffer, pH 7) are also shown in this figure (dotted 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 voltmmogram while the GC electrode provides an ill-defined cyclic voltmmogram. 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, $\upsilon^{0.5}$, as shown in the inset of this figure, indicating semi-infinite linear diffusion of reactant to the electrode surface.

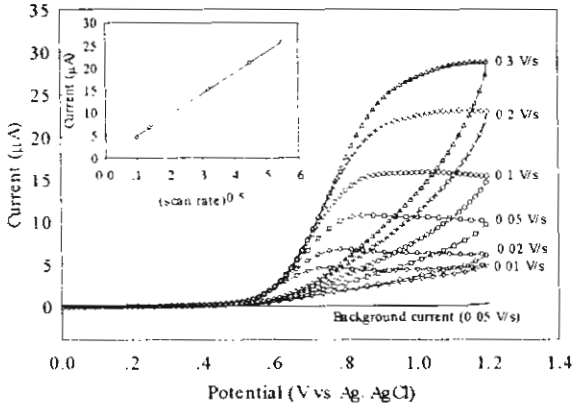

Fig. 3.1.2 Cyclic voltammograms for 1 mM D-penicillamine in 0.1 M phosphate buffer (pH 7) at boron-doped thin film electrode (the potential sweep rate was varied from 0.01 to 0.3 V s^{-1}). The curve of the relationship between current and (scan rate)$^{0.5}$ is inset.

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.

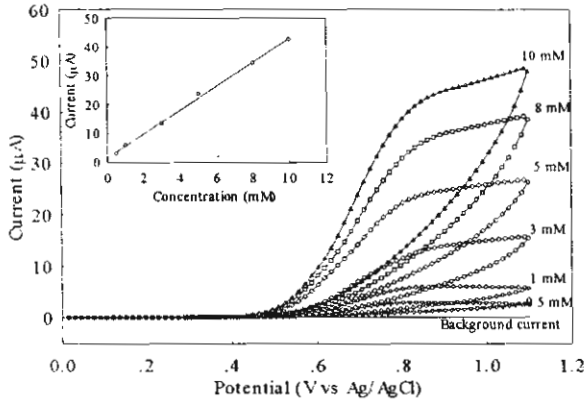

Fig. 3.1.3. Cyclic voltammograms for D-penicillamine in 0.1 M phosphate buffer (pH 7) (the concentration varied from 0.5 to 10 mM) at boron-doped thin film electrode. The potential sweep rate was 0.02 V s^{-1}. The calibration curve was also is inset.

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-penicillmine did not produce a sigmodial 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.

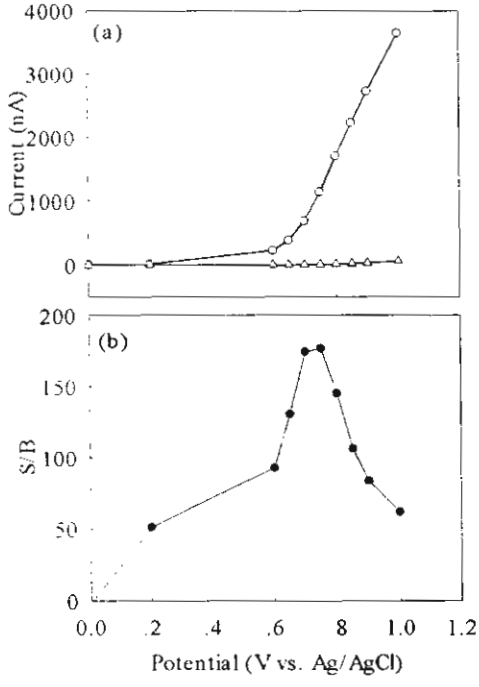

Fig. 3.1.4. (a) Hydrodynamic voltammograms of (-&z.cirf;-) 100 μM of D-penicillamine in 0.1 M phosphate buffer (pH 7) and (-□-) 0.1 M phosphate buffer (pH 7, background current) with four injections of analytes, using 0.1 M phosphate buffer (pH 7) as a carrier solution. (b) Hydrodynamic of signal-to-background 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 μL 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 ≥ 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.

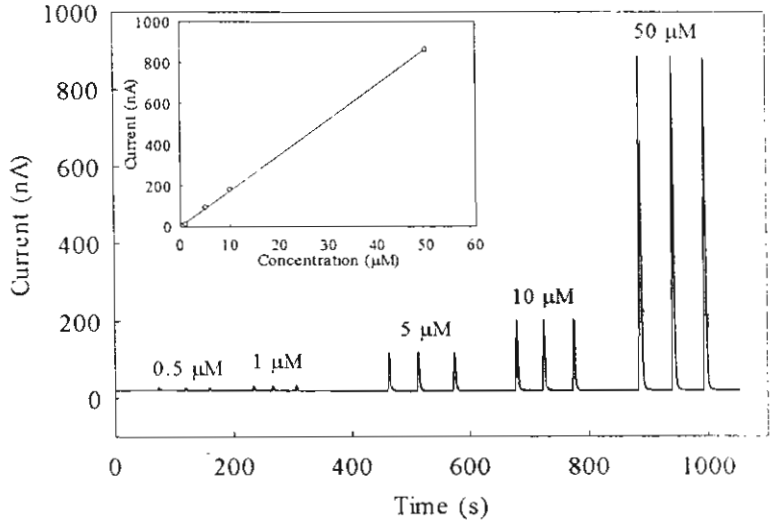

Fig. 3.1.5. Flow injection with amperometric detection results for D-penicillamine (0.5–50 μM) and calibration graph at the applied potential of 0.75 V vs. Ag/AgCl in 0.1 M phosphate buffer (pH 7) with three injections of analytes. Other conditions are the same as in Fig. 3.1.4

3.1.6 Application

3.1.6.1 Drug analysis of Penicillamine capsules

The proposed method for pencillamine 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 ($\mu g\ ml^{-1}$)	Amount of found ($\mu g\ ml^{-1}$)	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 ($\mu g\ ml^{-1}$)	Amount of found ($\mu g\ ml^{-1}$)	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 excepients frequently added to dosageforms 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 ± 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.

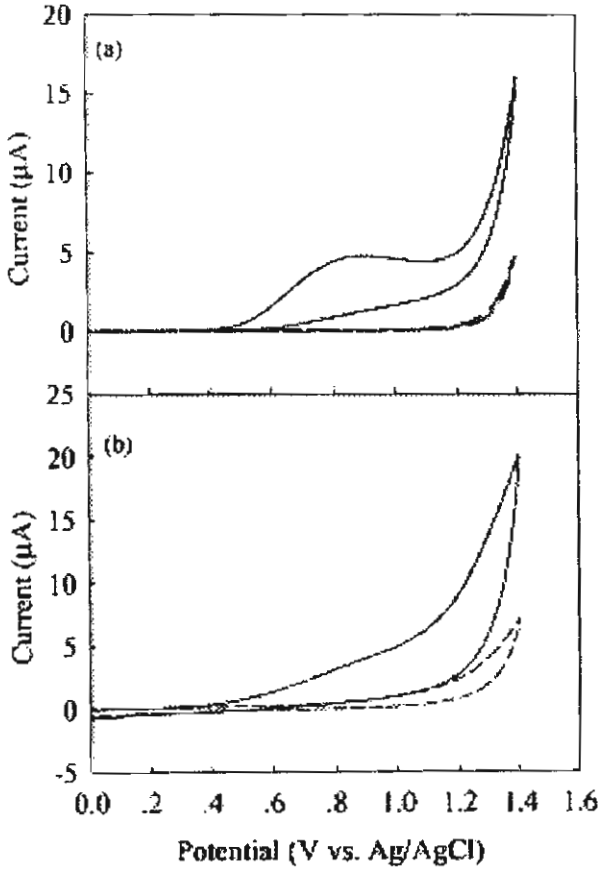

Fig. 3.2.1 Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 1.0 mM captopril in 0.1 M phosphate buffer pH 9 (solid lines) and 0.1 M phosphate buffer pH 9 (dashed lines). Sweep rate, 50 mV s^{-1}; area of electrode, 0.07 cm^2.

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 voltmmogram while the GC electrode provides an ill-defined cyclic voltmmogram. 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 eletrode, 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.

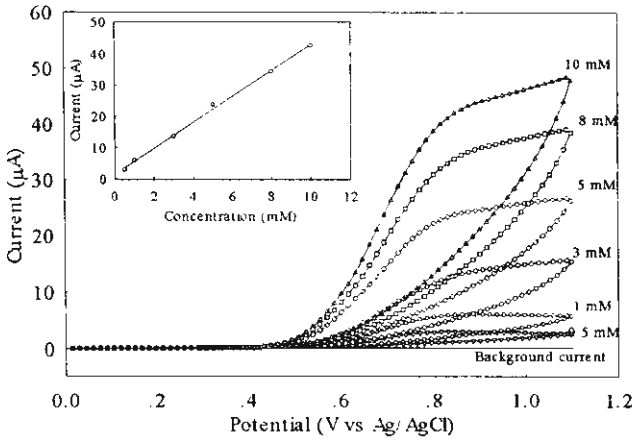

Fig. 3.2.2 Cyclic voltammogram for captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of captopril concentrations. The potential sweep rate was 50 mV s^{-1}; area of electrode, 0.07 cm^2. The calibration curve is shown in the inset.

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 $((V\ s^{-1})^{1/2})$ within the range 0.01 to 0.3 V s^{-1}. The linear regression analysis yields $r > 0.999$. From voltamograms, 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.

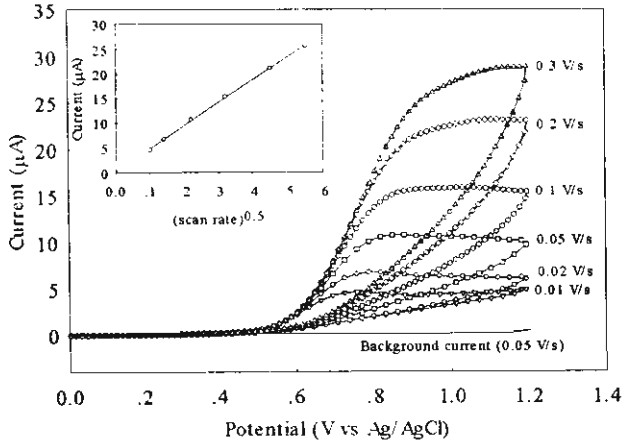

Fig. 3.2.3 Cyclic voltammograms for 1 mM captopril in 0.1 M phosphate buffer (pH 9) at boron-doped diamond electrode for a series of potential sweep rates; area of electrode, 0.07 cm^2. The calibration curve of relationship between current (μA) and $(\text{sweep rate})^{0.5}$ was also in the inset of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

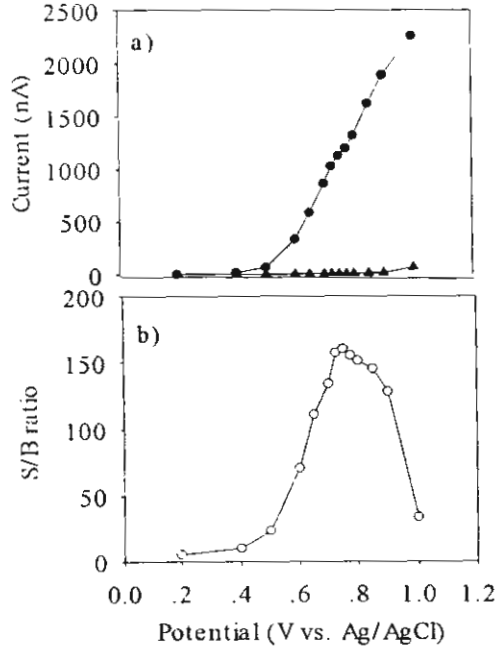

Fig. 3.2.4. (a) Hydrodynamic voltammogram of (-•-) 0.1 M phosphate buffer (pH 9, background current) and (-▼-) 100 μM of captopril in 0.1 M phosphate buffer (pH 9) with four injections of analysis, using 0.1 M phosphate buffer (pH 9) as a carrier solution. (b) Hydrodynamic of signal-to-background ratio. 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-dope 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 additional, 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.

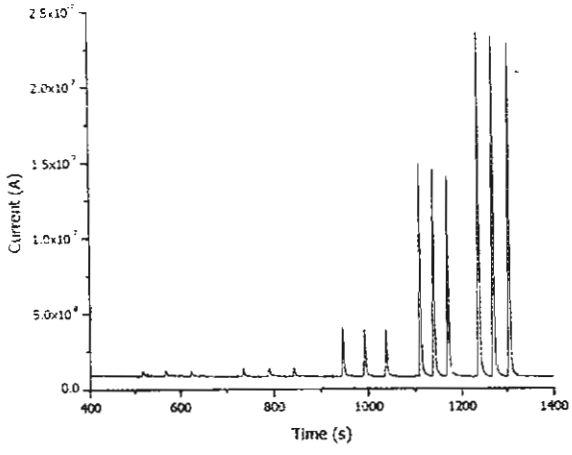

Fig.3.2.5. Flow injection analysis with amperometric detection results for a diamond electrode using 20-μl of captopril at various concentrations. The mobile phase was 0.1 M phosphate buffer (pH 9) at a flow rate of 1 ml min^{-1}. The calibration curve is shown in the inset.

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 (μg/ml)	Amount of found (μg/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 μg / 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.

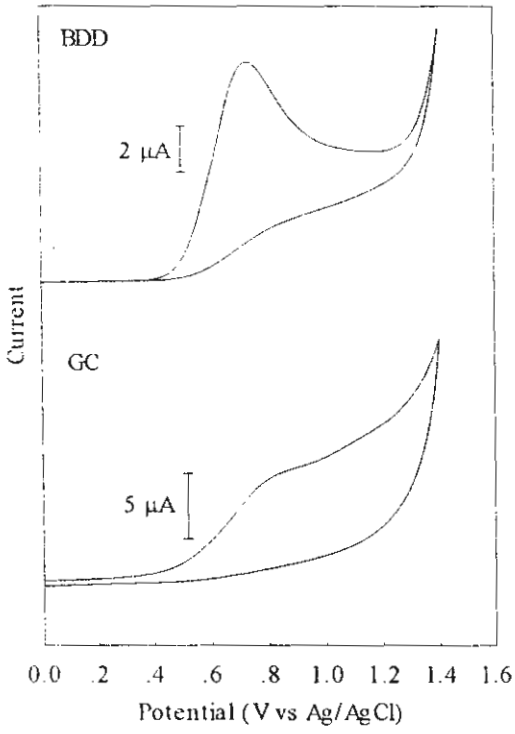

Fig. 3.3.1 Cyclic voltammograms for (a) BDD and (b) GC vs. Ag/AgCl in 2.0 mmol L^{-1} tiopronin in 0.1 mol L^{-1} phosphate buffer pH 8 (solid lines) and 0.1 mol L^{-1} phosphate buffer pH 8 (dashed lines). The sweep rate was 50 mV s^{-1}; area of electrode, 0.07 cm^2.

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 10-300 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.

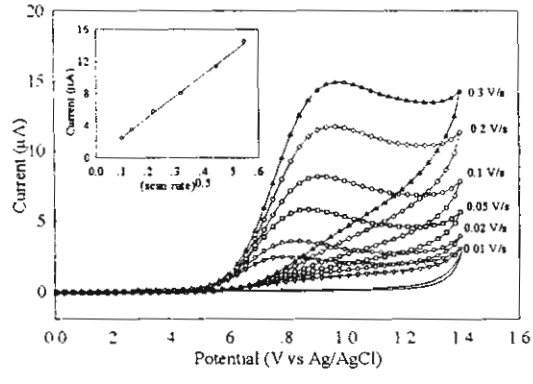

Fig. 3.3.2 Cyclic voltammograms for 1 mmol l^{-1} tiopronin in 0.1 mol L^{-1} phosphate buffer (pH 8) at BDD electrode for a series of sweep rates; area of electrode, 0.07 cm^2. The dependence between peak current (μA) and square root of the sweep rate appears in the inset.

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 10mmol 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 μmol L^{-1}.

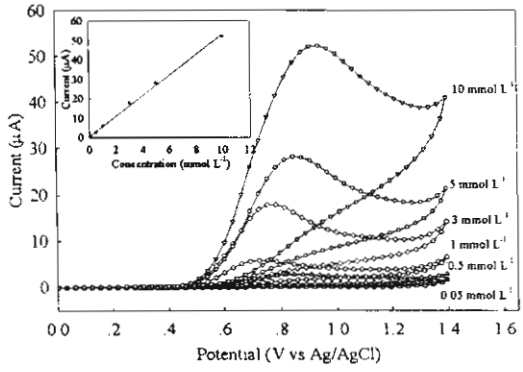

Fig. 3.3.3 Cyclic voltammograms for tiopronin in 0.1 mol L^{-1} phosphate buffer (pH 8) at BDD electrode for a series of tiopronin concentrations. The sweep rate was 50 mV s^{-1}; area of electrode, 0.07 cm^2. The dependence between peak current (μA) and concentrations at the potential of 50 mV s^{-1} 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 voltammmograms 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 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 (expending 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.

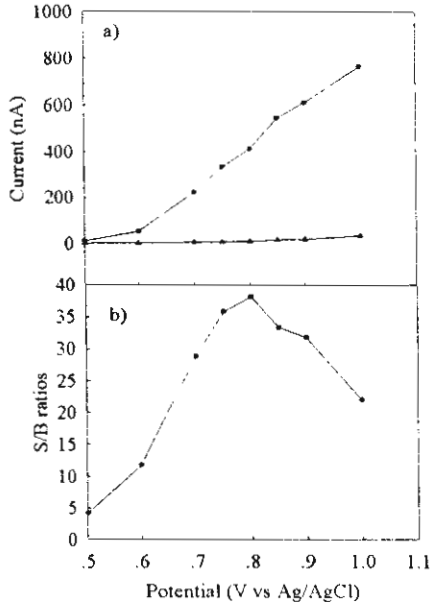

Fig. 3.3.4. (a) Hydrodynamic voltammogram of (▲) 0.1 mol l^{-1} phosphate buffer (pH 8, background current) and (•) 100 μmol L^{-1} of tiopronin in 0.1 mol L^{-1} phosphate buffer (pH 8) with averaging the peak currents from four injections (injected the analyte solution four times at each potential 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-µL 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 μmol L^{-1} (R^2 = 0.9992). For the limit of detection, tiopronin provided a useful signal with S/N ≥ 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 nA/μmol 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 ±0.03 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 μg 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 ($\mu g\ ml^{-1}$)	Amount of tiopronin tablet sample found ($\mu g\ ml^{-1}$)	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