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

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Abstract

The electrochemistry of acetaminophen in phosphate buffer solution (pH 8) was studied at a boron-doped diamond (BDD) thin film electrode using 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. Comparison experiments were performed using a polished glassy carbon (GC) electrode. Acetaminophen undergoes quasi-reversible reaction at both of these two electrodes. The BDD and GC electrodes provided well-resolved cyclic voltammograms but the voltammetric signal-to-background ratios obtained from the diamond electrode were higher than those obtained from the GC electrode. The diamond electrode provided a linear dynamic range from 0.1 to 8 mM and a detection of 10 μM ($S/B \approx 3$) for voltammetric measurement. 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 ($S/N \approx 4$). Acetaminophen in syrup samples has also been investigated. The results obtained in the recovery study (24.68 ± 0.26 mg/ml) were comparable to those labeled (24 mg/ml).

Keywords: Acetaminophen; Boron-doped diamond thin film electrode; Cyclic voltammetry; Flow injection with amperometric detection

1. Introduction

Boron-doped diamond (BDD) thin film is one of the new promising materials for electrochemical applications due to its unique and extremely useful properties [1]. Electroanalysis is one field that can benefit from the attractive electrochemical properties of diamond thin film as an electrode material [2–9]. These properties including a low and stable voltammetric background current [10,11], a wide working potential window in aqueous electrolyte solutions (2.5–3 V) [12], slight adsorption of polar organic molecules and good activity toward some redox analytes without any conventional pretreatment [13].

Acetaminophen, *N*-acetyl-*p*-aminophenol or paracetamol, is a widely used antipyretic and analgesic drug. It is an attractively alternative

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drug for children and people who are sensitive to aspirin. At the recommended dosage, there are no side effects. However, overdoses cause liver and kidney damage when administrated overdose. It is suspected that a metabolite of acetaminophen is the actual hepatotoxic agent.

Several methods including conventional spectrophotometry [14–16], fluorimetry [17], high performance liquid chromatography [18,19] and capillary electrophoresis [20] have been used to determine acetaminophen in the pharmaceutical and medical applications. Electrochemical methods are also popular for this application because the cost is low, and time consuming is less. Most previous reports were performed using glassy carbon (GC) electrode, carbon paste electrode or platinum electrode to study the electrochemistry of acetaminophen [21–26]. Most of these electrodes were modified with some chemical such as nafion/ruthenium oxide in order to improve the sensitivity. To clean the surface, these electrodes must be pretreated with some chemical such as alumina slurries to provide a reproducible and stable response prior to use. BDD electrode can overcome this problem, they can be used without any complicated pretreatment even after exposure to laboratory atmosphere over a month.

In this paper, we report the electrochemical determination of acetaminophen. Cyclic voltammetry and flow injection analyses with the amperometric detection mode were used with BDD electrode.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical grade and all solutions were prepared by using deionized water.

Phosphate buffer (pH 5–8) 0.1 M were prepared from 0.1 M of potassium dihydrogen phosphate (Merck) and adjusted to pH with 0.1 M sodium hydroxide (Merck) solution. Phosphate buffer (pH 2.5) was prepared from 0.1 M of potassium dihydrogenphosphate and adjusted to pH by orthophosphoric acid (85%, Carlo Eaba).

Standard acetaminophen (Fluka) solution was freshly prepared in 0.1 M phosphate buffer prior to use.

2.2. Sample preparation

About 2 ml of paracetamol syrup was transferred to a 100 ml volumetric flask and was diluted with 0.1 M of phosphate buffer pH 8. An aliquot of this initial solution was diluted again with appropriated volume of 0.1 M of phosphate buffer pH 8 to yield a final concentration of 4.8 μg/ml. In all cases it was assumed that the actual content of the syrup corresponds to that reported by the manufacturing laboratories.

2.3. Electrode

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

The 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.

2.4. Voltammetry

Electrochemical measurement was carried out in single compartment three electrode glass cell. 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). Ohmic contact was made by placing the backside of the Si substrate on a brass plate. For comparison, a GC electrode was used. A platinum wire and a Ag/AgCl with a salt bridge were used as the auxiliary and reference electrodes, respectively. Cyclic voltammetry was performed with an Autolab Potentiostat 100 (Eco-Chemie, The Netherlands).

2.5. 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 7530) with a 20 μl injection loop, an air pump (Water) and an electrochemical detector (PG 100). The mobile phase, phosphate buffer, was regulated by an air pump (N_2 gas flow) 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.

3. Results and discussion

3.1. pH dependence study

The electrochemical parameters of the acetaminophen reaction at pH 2.5, 5, 7 and 8 at BDD electrode were studied. It was found that the oxidation potential (positive scan) decreased when the pH of analyte solution increased. This phenomenon can be explained by the previous study that acetaminophen was hydrolyzed in alkaline medium which brought more reducing compounds such as *p*-hydroxyaniline.

From this study, we found that acetaminophen in phosphate buffer of pH 8 provided the highest S/B. Therefore, we used this pH for the next experiments.

3.2. Cyclic voltammetry

Fig. 1 shows typical cyclic voltammetric i–E curves for 100 μM of acetaminophen with the corresponding background voltammogram in 0.1 M phosphate buffer (pH 8) at BDD and GC electrodes. Both of the BDD and GC electrodes exhibited well-defined peak currents, however, the voltammograms obtained with the BDD electrode provided higher S/B ratios at the same electrode area.

Table 1 shows a comparison of the voltammetric data obtained for BDD and GC electrode. Two significant points that were found in this experiment: firstly, the S/B ratios were one order of magnitude higher for the BDD than those obtained for the GC electrode. For the BDD electrode, a well-defined voltammetric peak was obtained with the S/B ratio of 3 at a concentration as low as 10 μM while the GC electrode gave a similar result at 100 μM. This was caused by the remarkably low background current of BDD electrode. The linear range obtained at the BDD electrode (0.1–8 mM) was also larger than the one at the GC electrode (0.5–5 mM).

Secondly, acetaminophen undergoes the quasi-reversible reaction (2e, 2P) at both BDD and GC electrodes. GC electrode provided $\Delta E_p \approx 30$ mV while BDD electrode exhibited $\Delta E_p \approx 60$ mV with relatively a broad and asymmetric peak. This phenomenon could be due to the slow kinetic of the reaction at BDD electrode compared with the other carbon electrodes [27]. In addition, examination of scan rate from 0.01 to 0.5 V/s showed that the oxidation peak current varied linearly with $\upsilon^{1/2}$ (scan rate)$^{1/2}$ with $r > 0.998$. The results indicate

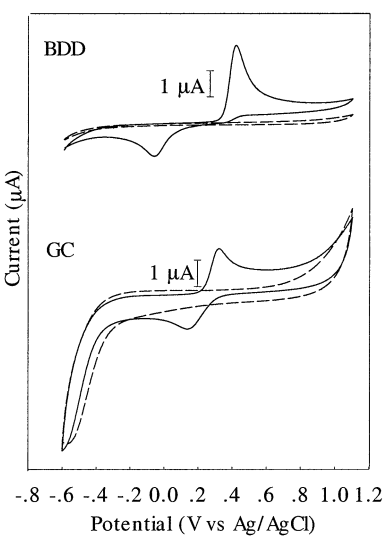

Fig. 1. Cyclic voltammograms for 100 μM acetaminophen in 0.1 M phosphate buffer (pH 8) at boron-doped thin film electrode (area 0.07 cm^2) and GC electrode (area 0.07 cm^2). The potential sweep rate was 20 mV/s (solid line). Background voltammograms (0.1 M phosphate buffer, pH 8) were also shown in this figure (dash line).

Table 1
Comparison of electrochemical parameters and S/B ratios obtained from the cyclic voltammetry for the oxidation of acetaminophen in 0.1 M phosphate buffer pH 8, for BDD and GC electrodes ($n = 3$)

Electrode	Analyte (μM)	E_p^{ox} (V)[b]	I_p^{ox} (μA)	E_p^{red} (V)[b]	I_p^{red} (μA)	S/B[a]
BDD	0.1	0.43 ± 0.01	2.6 ± 0.3	-0.10 ± 0.01	-0.9 ± 0.2	38 ± 2
	0.5	0.45 ± 0.00	9.7 ± 0.5	-0.10 ± 0.00	-4.3 ± 0.2	136 ± 4
GC	0.1	0.32 ± 0.01	1.5 ± 0.1	-0.13 ± 0.01	-0.7 ± 0.0	4 ± 1
	0.5	0.34 ± 0.00	7.5 ± 0.5	-0.05 ± 0.00	-3.4 ± 0.1	22 ± 4

[a] Calculated from I_p^{ox}/background current.
[b] Potential (V) vs. Ag/AgCl.

that the electrochemical reaction is a diffusion-controlled process.

3.3. Hydrodynamic voltammetry

Fig. 2 shows the hydrodynamic voltammetric i–E curves obtained at a BDD electrode for 20 μl injection of 100 μM of acetaminophen in 0.1 M of phosphate buffer (pH 8), using phosphate buffer (pH 8) as the carrier solution. Each datum represents the average of four injections. The magnitude of the background current at each potential is also shown for comparison. Hydrodynamic voltammetry of acetaminophen exhibited with a half peak potential at about 0.5 V versus Ag/AgCl. Therefore, the amperometric detection was set at 0.55 V versus Ag/AgCl.

3.4. Flow injection with amperometric detection

Fig. 3 shows a series of repetitive 20 μl injections of acetaminophen at a concentration of 0.5–50 μM in phosphate buffer (pH 8) using a potential of 0.55 V versus Ag/AgCl. The peaks are sharp without tailing.

Various validation parameters of the purposed method were studied as following.

- Calibration and linearity: calibration curve was obtained from three injections of five concentrations of acetaminophen (0.5, 1, 5, 10 and 50 μM). Linear regression analysis using a least square-Linear regress fit was shown in Fig. 3. It was found that slope (sensitivity) was 22.1 ± 4 μA/mM and intercept was 0.011 ± 0.001 μA. Correlation coefficient, r, is 0.999. A linear dynamic range was obtained over two orders of magnitude, from 0.5 to 50 μM.
- Limit of detection (LOD): LOD is the concentration of analyte, which provides three times of the ratio of the analyte current to noise signal ($S/N \geq 3$). LOD of this proposed method was obtained after five injections of 10 nM acetaminophen.
- Accuracy and recovery: these parameters were obtained from using real sample of acetaminophen syrup and standard addition method (adding known amounts of pure acetaminophen (six different levels). A recovery study showed average values between 94 and 103% as shown in Table 2. The concentrations of acetaminophen considered in the recovery study (24.68 ± 0.26 mg/ml) were comparable to those labeled (24 mg/ml) in the syrup samples. Relative errors compared with the claimed amount were lower than 3%.

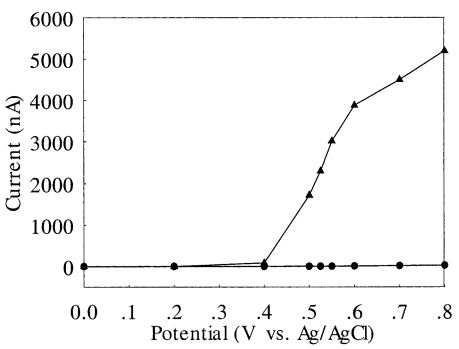

Fig. 2. Hydrodynamic voltammograms of (-▲-) 100 μM of acetaminophen in 0.1 phosphate buffer (pH 8) and (-●-) 0.1 phosphate buffer (pH 8, background current) with four injections of analytes, using 0.1 M phosphate buffer (pH 8) as a carrier solution, flow rate 1 ml/min.

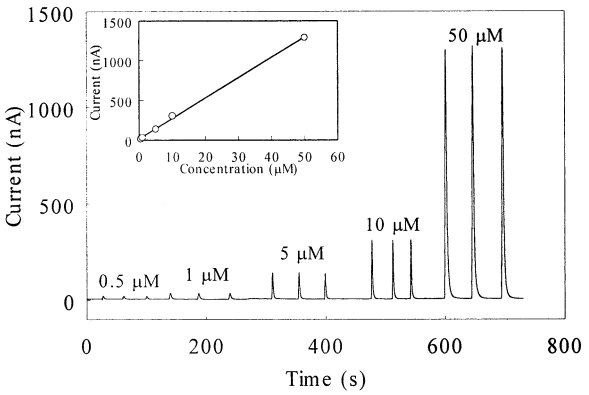

Fig. 3. Flow injection with amperometric detection results for acetaminophen (0.5–50 μM) and calibration graph at the applied potential of 0.55 V vs. Ag/AgCl in 0.1 M phosphate buffer (pH 8) with five injections of analytes. Other conditions were the same as Fig. 2.

- Precision: precision of the method was obtained on the basis of intra-assay using standard addition. Three concentrations of added solution (0.15, 0.45 and 0.60 μg/ml) were chosen. Results obtained from ten injections gave 1.8–2.3% of relative standard deviation (R.S.D.).
- Stability of electrode: the BDD electrode with amperometric detection of acetaminophen was very stable, even with continuous use for 1 month. The flow amperograms (The Figure was not shown) obtained from repetitive twenty injection of 100 μM acetaminophen on the BDD electrode after 1 month of continuous use provided the peak variation of only about 2.2%. The results indicated that BDD electrode was very stable with the high reproducibility. This unique response stability was also reported by Swain [1,10].
- Selectivity: the limitation of the proposed method is the interference of electrochemically active species which have the potential peak (E_p) close to the potential peak of acetaminophen (0.55 V). However, these interferes can be separate out by HPLC technique. Various interferences such as caffeine, ascorbic acid etc. and the tolerance are under investigation by this proposed method. In addition, the determination of acetaminophen in mixed artificial sample using BDD as the working electrode of amperometric detector after first separating them by high-performance liquid chromatography is under study.

Table 2
Recovery of acetaminophen sample with amperometric detection on the BDD electrode applied to flow injection system ($n = 2$)

Amount added (μg/ml)	Amount found (μg/ml)	Percent of recovery (%)
0.30	0.28 ± 0.01	93.66 ± 1.34
0.36	0.36 ± 0.00	99.35 ± 0.12
0.45	0.44 ± 0.01	97.52 ± 2.36
0.54	0.55 ± 0.01	102.03 ± 0.13
0.60	0.58 ± 0.00	96.10 ± 0.74
0.91	0.93 ± 0.01	102.71 ± 1.26

3.5. Comparison with any other methods

Table 3 summarizes the electroanalytical methods for acetaminophen from this study compared with other methods. It can be seen that using the BDD electrode with flow injection with amperometric detection provides a significant low detection limit (especially when compared with the results obtained by Danet [28] using flow injection analysis), high sensitivity and reproducible responses without pretreatment or modification of the electrode because there is no fouling at the BDD electrode and the background current is also very low. Moreover, the use of the BDD electrode applied to flow injection with amperometric detection is simple, rapid and has high sample throughput.

4. Conclusions

The aim of the study was to propose the use of novel electrode for the quantitative determination of acetaminophen. BDD electrode exhibits attractive properties for the determination of acetaminophen such as, (1) low background current; (2) slow kinetics and only slight adsorption of acetaminophen onto the surface of the electrode. Flow injection with amperometric detection using the BDD electrode also enhances the sensitivity

Table 3
Comparison of electroanalytical data for acetaminophen determination

Electrode	Method	Linear (dynamic) range (μM)	LOD (μM)	References
Carbon fiber microelectrode	Micellar liquid chromatography with wall jet cell	0.13–460, (0.02-70 ppm), ($r = 0.997$)	0.13, (0.02 ppm), $S/N = 2$	[26]
Chemically modified electrode	Square wave voltammetry	5–250, ($r = 0.999$)	1.2 (3σ)	[22]
Pt-microelectrode	Flow injection with biamperometric Detection	Up to 410	5.10	[28]
BDD electrode	Cyclic voltammetry	100–8000, ($r = 0.997 \pm 0.002$), $n = 2$	10 ($S/B \approx 3$), $n = 2$	[a]
	Amperometry applied to flow injection	0.5–50, ($r = 0.999 \pm 0.001$), $n = 2$	0.01 ($S/N \approx 4$), $n = 2$	[a]

[a] This proposed method.

and improves the detection limit (as low as 10 nM) with a reproducible response and without pretreatment and modification of electrode or using pulse technique.

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