Electrochemical Analysis of Acetaminophen Using a Boron-Doped Diamond Thin Film Electrode in a Flow Injection System

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The electrochemistry of acetaminophen in phosphate buffer solution (pH 8) was studied at a boron-doped diamond thin film electrode using cyclic voltammetry, hydrodynamic voltammetry, and flow injection analysis (FIA) 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 carbon electrodes. The diamond electrode and glassy carbon 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 glassy carbon electrode. The diamond electrode provided a linear dynamic range from 0.1 – 8 mM and a detection limit of 10 µM (S/B ≈ 3) in voltammetric measurement. The flow injection analysis results for the diamond electrode indicated a linear dynamic range from 500 nM - 50 µM and a detection limit of 10 nM (S/N ≈ 4).

(Received on August 9, 2001; Accepted on November 3, 2001)

Boron doped diamond thin film is one of the very new promising materials for electrochemical applications due to its unique and extremely useful properties. Electroanalysis is a field that can benefit from the attractive electrochemical properties of diamond thin film as an electrode material. These properties including a low and stable voltammetric background current, a wide working potential window in aqueous electrolyte solutions (2.5-3 V), slight adsorption of polar organic molecules and good activity toward some redox analytes without any conventional pretreatment. Acetaminophen (N-acetyl-p-aminophenol or Paracetamol) is a widely used antipyretic and analgesic drug. It is an attractive alternative drug for children and people who are sensitive to aspirin. At the recommended dosage, there are no side effects. However overdoes 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, Fluorimetry, high performance liquid chromatography and capillary electrophoresis have been used to determine acetaminophen in the pharmaceutical and medical applications. Electrochemical methods are also a general method for this application. Most previous reports were performed using glassy carbon electrode, carbon paste electrode or platinum electrode to study the electrochemistry of acetaminophen. Many 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 pretreated with some chemical such as alumina slurry to provide a reproducible and stable response prior to use. Diamond electrodes 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 analysis with the amperometric detection mode were used with boron-doped diamond thin film electrode.

Experiment

Chemicals and Reagents

All chemicals were performed with the analytical grade or better and all solutions were prepared by using deionized water. Phosphate buffer (pH 8) 0.1 M was prepared from 0.1 M of potassium dihydrogen phosphate (Merck) and adjusted to pH with 0.1 M sodium hydroxide (Merck) solution.

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

Electrodes

The boron doped diamond thin film (BDD) electrode was grown on Si(100) substrate (obtained from Prof. A. Fujishima) using microwave assisted chemical vaporization. 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 micron alumina/water slurries on felt pads, followed by rinsing with ultrapure water prior to use.

Voltammetry

Electrochemical measurement was carried out in single compartment three electrode glass cell. Diamond electrodes were 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 the brass plate. For a 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 a Potentiostat (PG-100) and Autolab software.
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).

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Analyte (mM)</th>
<th>$E_p$ (V) (*)</th>
<th>$I_p$ (µA)</th>
<th>$E_{p}^{\text{red}}$ (V) (*)</th>
<th>$I_{p}^{\text{red}}$ (µA)</th>
<th>S/B^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDD</td>
<td>0.1</td>
<td>0.43±0.01</td>
<td>2.64±0.3</td>
<td>-0.10±0.01</td>
<td>-0.94±0.2</td>
<td>38±2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.45±0.00</td>
<td>9.75±0.5</td>
<td>-0.10±0.00</td>
<td>-4.3±0.2</td>
<td>136±4</td>
</tr>
<tr>
<td>GC</td>
<td>0.1</td>
<td>0.32±0.01</td>
<td>1.54±0.6</td>
<td>-0.13±0.01</td>
<td>-0.74±0.0</td>
<td>4±1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.34±0.00</td>
<td>7.5±0.5</td>
<td>-0.05±0.00</td>
<td>-3.4±0.1</td>
<td>22±4</td>
</tr>
</tbody>
</table>

* potential (V) vs Ag/AgCl
* calculated from $I_p$/Background current

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, and an air pump (Water) and an electrochemical detector (PG 100). The mobile phase, phosphate buffer, was regulated by an air pump (N2 gas flow) at a flow rate of 1 ml/min. The thin layer flow cell consisted of a silicone rubber gasket at a spacer, Ag/AgCl as a reference electrode, stainless steel tube as an auxiliary electrode and an outlet of the flow cell. The experiments were performed in a copper Faraday cage to reduce the electrical noise.

Results and discussion

Cyclic voltammetry

Figure 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 voltammogram 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 1 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 was giving a similar result at 100 µM. This is caused by the remarkably low background current of BDD electrode. The linear range obtained at the BDD electrode (0.1 to 8 mM) was also larger than the one at the GC electrode (0.5 to 5 mM).

Secondly, acetaminophen undergoes the quasi-reversible reaction (2e2P) at both carbon electrodes. GC provided $\Delta E_P = 30$ mV while BDD electrode exhibited $\Delta E_P = 60$ mV with relatively a broad and asymmetric peak. This phenomenon could be due to the slow kinetics of the BDD electrode compared with the other carbon electrodes. In addition, examination of scan rate from 0.01 to 0.3 V/s showed that the oxidation peak current varied linearly with $u^{1/2}$ or (scan rate)$^{1/2}$ with $r > 0.998$. The results indicated that the electrochemical reaction is a diffusion-controlled process.

Hydrodynamic voltammetry

Figure 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 exhibits with a half peak potential at about 0.5 V vs. Ag/AgCl. Therefore, the amperometric detection was set at 0.55 V vs. Ag/AgCl.

Flow injection with amperometric detection

Figure 3 shows a series of repetitive 20 µl injections of acetaminophen at a concentration of 10 nM (Figure 3a) and 10 µM (Figure 3b) in phosphate buffer (pH 8) using a potential of 0.55 V versus Ag/AgCl. The peaks are sharp without tailing. For the repetitive injection of 100 µM acetaminophen (n = 20), the results were reproducible with the peak variation about of only about 2.2 % indicating the high stability of BDD electrode.

![Figure 1 Cyclic voltammograms for 100 µM acetaminophen in 0.1 M phosphate buffer (pH 8) at BDD electrode (area 0.07 cm²) and GC electrode (area 0.07 cm²). The potential sweep rate was 20 mV s⁻¹ (Solid lines). Background voltammogram 0.1 M phosphate buffer, pH 8) was also shown in the figure (dashed line).](image-url)

![Figure 2 Hydrodynamic voltammograms for 100 µM acetaminophen in 0.1 M phosphate buffer (pH 8) at BDD electrode (area 0.07 cm²) and GC electrode (area 0.07 cm²). The potential sweep rate was 20 mV s⁻¹ (Solid line).](image-url)

![Figure 3 Flow injection with amperometric detection](image-url)
A linear dynamic range was obtained over two orders of magnitude, from 0.5 to 50 μM (r = 0.999) as shown in Figure 4. Interestingly, an injected concentration as low as 10 nM acetaminophen still provided a useful signal (detection limit) with S/N ≥ 3.

Figure 2 Hydrodynamic voltammogram of (A) 100 μM of acetaminophen in (--) 0.1 phosphate buffer (pH 8, background current) with 4 injections of analyte, using 0.1 M phosphate buffer (pH 8) as carrier solution, flow rate 1 mL min⁻¹.

Figure 4 The calibration plot of acetaminophen in 0.1 M phosphate buffer (pH 8). Other conditions were the same as for Figure 3.

Comparison with any other methods
Table 2 summarizes the analytical methods for acetaminophen from this study compared with other methods. From the table, it can be seen that using the BDD electrode with flow injection and amperometric detection provides a significant low detection limit, high sensitivity and reproducible responses without pretreatment or modification of the electrode because there is no fouling of 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.

In conclusion, 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 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.

Acknowledgements
This research was supported by Chulalongkorn University, The Thailand research fund and Student Grant of ASIANALYSIS IV. Special thanks are extended to Prof. A. Fujishima (Tokyo University) for the BDD electrodes used in this research. The author also thank to Dr. R. Bates (Chulabhorn Research Institute) for the kindly help and invaluable suggestion.

Figure 3 FIA-ED result for a) 10 nM and b) 10 μM concentrations of acetaminophen at the applied potential 0.55 V vs. Ag/AgCl in 0.1 M phosphate buffer (pH 8) with 5 injections of analyte. Other conditions were the same as for Figure 2.
<table>
<thead>
<tr>
<th>Electrode</th>
<th>Method</th>
<th>Linear (dynamic) range (µM)</th>
<th>Limit of detection (µM)</th>
<th>Sensitivity (nA/µM)</th>
<th>%RSD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon fiber microelectrode</td>
<td>Micellar liquid chromatography with wall jet cell</td>
<td>0.13 - 460 (r = 0.997) (0.02 - 70 ppm)</td>
<td>0.13 (S/N = 2) (0.02 ppm)</td>
<td>3 x 10^-4</td>
<td>3.1</td>
<td>26</td>
</tr>
<tr>
<td>Chemically modified electrode</td>
<td>Square wave voltammetry</td>
<td>5 - 250 (r = 0.999)</td>
<td>1.2 (3σ)</td>
<td>-</td>
<td>2.2</td>
<td>17</td>
</tr>
<tr>
<td>BDD electrode</td>
<td>Cyclovoltammetry</td>
<td>100 - 8000 (r = 0.996±0.002)</td>
<td>10 (S/B = 3)</td>
<td>10.17±0.68</td>
<td>2.7</td>
<td>this study</td>
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<td></td>
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<td>22.08±3.62</td>
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<td>n = 2</td>
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References