



# Electrochemical measurement of lamotrigine using boron-doped diamond electrodes

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## ABSTRACT

In this paper we report on a study of the electrochemical behavior of lamotrigine (LTG) using boron-doped diamond (BDD) electrodes. Cyclic voltammetry of LTG in a phosphate buffer solution exhibited an oxidation signal at ca. +1.4 V (vs. Ag/AgCl). A reduction signal at –0.5 to –1.0 V (vs. Ag/AgCl) was observed only after oxidation of LTG. The oxidation signal was due to polymerization of LTG adsorbed on the BDD electrode, while the reduction signal was a consequence of a process involving the reduction of the azo group in the LTG-oxidized product. Continuous measurements of LTG aiming at *in vivo* monitoring were successfully investigated and an optimized chronoamperometry technique, in which the BDD microelectrode was clamped to the LTG oxidation potential (+1.4 V) for 2 s, then to the LTG reduction potential (–0.6 V) for 2 s, was implemented. The limits of detection ( $S/N = 3$ ) were estimated to be 29 nM and 130 nM for the oxidation and reduction of LTG, respectively. The use of this chronoamperometry technique enables a high temporal resolution of 4 s for LTG measurements. BDD microelectrodes, therefore, are the ideal electrodes for performing continuous *in vivo* measurements of LTG.

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## 1. Introduction

Lamotrigine (lamictal, LTG) is a new antiepileptic agent chemically unrelated to current antiepileptic drugs [1,2]. The anticonvulsive action of LTG by blocking Na<sup>+</sup> channel and inhibiting the release of excitatory neurotransmitters has been demonstrated. It has been reported that the Stevens-Johnson syndrome occurs in the case of overdoses [3]. This is characterized by painful blistering of the skin and the mucous membranes, and is often fatal.

To get the benefit of LTG as an anticonvulsant without side effects, it is important to measure the amount of LTG and the physiological phenomena *in situ* and *in vivo*. The conventional methods for measuring LTG are high performance liquid chromatography (HPLC) [4–6], liquid chromatography-mass spectrometry (LC-MS) [7], gas chromatography-mass spectrometry (GC-MS) [8,9], thin layer chromatography (TLC) [6,10,11], ionization-mass spectrophotometry (ESI-MS) [12], spectrofluorimetry [13], and radioimmunoassay [14]. However, the high cost of these methods is a

disadvantage, and, moreover, the measurement can take a long time, or be intermittent. Because real-time and *in-situ* measurements are required for the biological environment [15], the measurements mentioned above are unsuitable. On the other hand, electrochemical methods enable rapid and continuous measurement of LTG with low cost. There has been some research on the electrochemical detection of LTG, including cyclic voltammetry (CV) at highly oriented pyrolytic graphite (HOPG) electrodes [16] and adsorptive stripping voltammetry (AdSV) using silver nanoparticle-modified carbon screen-printed electrodes [17]. However, these methods cannot be done in real time because it takes a lot of time for LTG to be adsorbed on the electrode. While the electrochemical activity of LTG has also been reported using molecularly imprinted polymer-carbon paste electrodes [18], modified glassy carbon (GC) electrodes [19], and silver amalgam film electrodes [19], these electrodes have complex surfaces and are difficult to apply to *in vivo* measurements.

Boron-doped diamond (BDD) is an electrode material that has attracted a great deal of interest due to its outstanding electrochemical features [20–22]. First, BDD has low background current and a wide potential window. Owing to these properties, highly sensitive detection is possible [23]. Second, the sp<sup>3</sup> hybridized

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structure of BDD gives it high chemical and physical stability, making it resistant to biofouling and biocompatible with organisms [24–27]. These properties make it possible to repeatedly detect drugs *in vivo* [28,29]. Furthermore, BDD electrodes are applied not only for biosensor, but also for many fields, such as electrochemical reduction of carbon dioxide [30], water treatment [31], pH sensing [32], and so on.

In our previous study, we developed and applied a drug-sensing system [33]. This system semiquantitatively characterized the kinetics of drugs intravenously injected into a biological environment over time and simultaneously recorded changes in the electrical activity representing the function of the organ involved. Simultaneous measurements of the concentration of bumetanide and the cochlear potential, and that of the concentration of doxorubicin and local field potentials (LFPs) in the brain were developed as well as that of the concentration of LTG and LFPs in the brain. However, the electrochemical reaction, i.e., the oxidation and reduction behavior, is still not completely understood. In order to achieve *in vivo* analyses of LTG under much better conditions, it is important to investigate the electrochemical characteristics of LTG. Here, first, we used cyclic voltammetry (CV) to study the electrochemical activity of LTG using BDD electrodes. The results showed that oxidation of LTG causes a species to be adsorbed on the BDD surface and that this surface-adsorbed species could be reduced. Then, continuous measurements of LTG by chronoamperometry (CA) at an optimized potential were successfully demonstrated using BDD microelectrodes. Use of this electrode material can lead to more sensitive continuous measurements of LTG *in vivo*.

## 2. Experimental

### 2.1. Chemicals

LTG was purchased from Tokyo Chemical Industry (Japan) and used without any further purification. The solutions of 0.1 M phosphate buffer solution (PBS) pH 7.4 and a Britton-Robinson buffer (BRB) pH 2–11 were used as the electrolyte. All the solutions were prepared with pure water supplied from DIRECT-Q 3 UV (Merck Millipore Corporation) with a specific resistivity of 18.2 M $\Omega$  cm.

### 2.2. Preparation of the BDD electrodes

The BDD electrodes were prepared by growing BDD films on silicon wafers in a microwave plasma-assisted chemical vapor deposition (MPCVD) system (ASTeX Corp.). Details of the preparation are described elsewhere [20]. A mixture of acetone and trimethoxyborane with a B/C ratio of 1% was used as the carbon source. The film quality was examined using Raman spectroscopy (Renishaw System, 2000). This showed a typical spectrum for BDD with a peak for  $sp^3$  carbon ( $\sim 1333\text{ cm}^{-1}$ ) and two peaks for boron ( $500, 1200\text{ cm}^{-1}$ ) while no peak was observed at around  $1600\text{ cm}^{-1}$ , which would generally be attributed to  $sp^2$  carbon impurities. These observations indicate that the BDD is of high quality.

The BDD microelectrodes were prepared by growing thin BDD films on tungsten wires. The details of the preparation have been described previously [34]. The size of the conducting tips of the electrodes were  $<20\text{ }\mu\text{m}$  in diameter and  $100\text{--}200\text{ }\mu\text{m}$  in length.

### 2.3. Electrochemical measurement

All measurements were conducted at room temperature ( $\sim 25\text{ }^\circ\text{C}$ ) in a Faraday cage. Cyclic voltammograms (CV) and chronoamperograms (CA) were recorded using a potentiostat (ALS 852 cs, BAS Inc., Japan).

Electrochemical measurements were carried out with a three-electrode cell. An Ag/AgCl (saturated KCl) electrode was used as the reference electrode and a platinum (Pt) wire was used as the counter electrode. BDD was used as the working electrode and it was mounted on the bottom of the cell using an O-ring (JIS-standard, P-7). The geometric area of the working electrode was estimated to be  $0.363\text{ cm}^2$ . Before use, the BDD was pretreated by ultrasonication in 2-propanol for 5 min and in pure water for 10 min. Comparison was also performed using GC and Pt electrodes. Prior to use, these electrodes were polished by  $0.5\text{ }\mu\text{m}$  alumina powder for 10 min followed by ultrasonication in pure water for 10 min.

For the injection measurements, an Ag/AgCl (saturated KCl) electrode was used as the reference electrode and a BDD electrode was used as the counter electrode. A BDD microelectrode was used as the working electrode and its tip was dipped in PBS. 2, 3, 5, 10, and  $30\text{ }\mu\text{l}$  of the LTG stock solution (0.5 mM in PBS) were sequentially injected into the 5 ml PBS-filled chamber and stirred with a magnetic bar for 3 s after injection. The 4 s cycle of the applied potential protocol consisted of the sequential application of potentials at the LTG oxidation potential and the LTG reduction potential for 2 s each. The LTG oxidation potential was fixed at  $+1.4\text{ V}$ . To optimize this protocol, voltages of  $-0.6$ ,  $-0.8$ , or  $-1.0\text{ V}$  were applied for the reduction potential. Prior to each measurement, cathodic reduction at  $-2.0\text{ V}$  for 5 min (unless mentioned otherwise) was conducted to remove LTG adsorbed on the microelectrode. The current, recorded at the end of each application of the oxidation and reduction potentials was plotted as a function of the LTG concentration in PBS to construct calibration curves for LTG oxidation and reduction.

## 3. Results and discussion

### 3.1. Electrochemistry of LTG

Fig. 1a shows the CV for  $100\text{ }\mu\text{M}$  LTG in 0.1 M PBS, pH 7.4, using an as deposited BDD electrode. The potential was scanned in the negative direction at  $0.1\text{ V/s}$ . In the first scan a cathodic signal, corresponding to reduction of the LTG, was not observed, whereas the oxidation signal was observed at a potential of *ca.*  $+1.4\text{ V}$ . In the second scan the reduction signal appeared at  $-0.5$  to  $-1.0\text{ V}$  and the oxidation signal was observed at the same potential as in the first scan. The results suggest that LTG is initially oxidized, and then the oxidized LTG product is reduced.

The same measurements were conducted using GC and Pt electrodes (Fig. 1b and c). The oxidation signal and reduction signal of LTG were not clearly observed using either of these electrodes. The signal to background ratios (S/B) with the GC and Pt electrodes were apparently lower than that when the BDD electrode was used (Table 1). These results imply that the LTG oxidation signal overlaps the oxygen generation signal when using the GC and Pt electrodes due to the low overpotential for oxygen generation on these electrodes. The LTG reduction signal is also hindered by the signals due to hydrogen generation and oxygen reduction when using these electrodes. Conversely, the oxidation and reduction signals for LTG were clearly observed when using BDD electrodes owing to the high overpotential for the water electrochemical reaction on BDD. Thus, using BDD makes sensitive measurements of LTG possible.

Based on the results of the CV behavior and previous literature [35], we propose the following mechanism for the electrochemical reaction. The first electron transfer from the amino group in the LTG produces radical cations by the application of  $+1.4\text{ V}$ . The radical cations can be placed at the ortho or para positions to form resonant structures. These cations can form polymers by tail-to-tail,

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