Electrochemical Detection of Serotonin Using Conductive Diamond Electrodes

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The electrochemical detection of serotonin with highly boron-doped diamond thin film electrodes was investigated by use of flow injection analysis (FIA) with amperometric detection. The voltammetric behavior of diamond indicated low background current and a low tendency to adsorb the oxidative products on the electrode surface, in comparison to the glassy carbon electrode. An experimental amperometric detection limit of 10 nM (0.2 pmol) was obtained for the FIA technique, demonstrating that diamond is a stable and sensitive electrode for serotonin determination.

Highly boron-doped diamond (BDD) thin-film electrodes have received much attention from electrochemists during the past few years, especially for electroanalytical applications.[1,2] In most of these studies, diamond was found to be superior compared to conventional electrodes such as glassy carbon (GC) in terms of stability and sensitivity. The attractive features of diamond include a wide electrochemical potential window in aqueous solutions, very low voltammetric background current (about one order of magnitude less than that of GC), high resistance to deactivation by fouling, extreme electrochemical stability and relative insensitivity to dissolved oxygen.[1-3] Basic studies on BDD films have revealed that the lack of oxygen-containing functional groups on the as-deposited diamond surface and very low adsorption of chemical species[3] on the inert surface of diamond are mainly responsible for the observed superiority of these films.

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a neurotransmitter that coexists with other bioamines and plays a significant role in psychiatric disorders, e.g., depression, and gastrointestinal disorders. In comparison to other methods, high-performance liquid chromatography with electrochemical detection (HPLC-ED) is considered to be one of the most highly sensitive methods for the determination of this compound.[4,5] GC is a commonly used electrode for this technique. Fast-scan voltammetry with carbon fiber electrodes is also known for serotonin determination in mast cells.[6] Although carbon electrodes are widely used, the strong adsorption of the oxidative product of serotonin causes stability problems.[6] Diamond, due to the inert nature of the surface, is a possible electrode material for the simple amperometric determination of serotonin. In the present study, we report the use of high quality, heavily boron-doped diamond electrodes for the electrochemical oxidation of serotonin by use of cyclic voltammetry and FIA with electrochemical detection (FIA-ED).

The boron-doped diamond thin films were deposited by use of a microwave plasma chemical vapor deposition (MPCVD) system (ASTeX). The details of the deposition procedure were reported earlier.[7] Diamond thin-films were deposited on Si (100) substrates. A mixture of acetone and methanol in the ratio of 9:1(v/v) was used as the carbon source. B_2O_3, the boron source, was dissolved in the acetone-methanol at a B/C atomic ratio of 10^4 ppm. The FIA system used in the present system consisted of a micro-LC pump (Bioanalytical Systems, LC-100), an injector (Rheodyne) with a 20-μL loop, a thin layer flow cell (Bioanalytical Systems), an amperometric detector (Bioanalytical Systems LC-4C) and an X-Y recorder (Graphtec, WX4000). All of the experiments were carried out in phosphate buffer (pH 7.2).

Figure 1 shows cyclic voltammograms for 10 μM 5-HT at the diamond and GC (GC-20, Tokai Carbon Co., Ltd.) electrodes. Both electrodes exhibited voltammograms with nearly identical features. However, those obtained at the diamond electrode are relatively well defined, with an oxidation peak potential at 0.42 V vs. SCE. The peak current was found to be linearly ($r = 0.993$) dependent on the square root of the scan rate. Another interesting point is that the original voltammograms for serotonin at the diamond electrode could be recovered by stirring the solution after the second cycle, in contrast to GC, where the recovery of the voltammogram was partial, even after vigorous stirring. This result indicates that the adsorption of the oxidation products is reversible on the diamond electrode, unlike the case of GC. Similar results were obtained for 5-hydroxyindoleacetic acid (5-HIAA), which is a metabolite of 5-HT and an anion at physiological pH. No electrode fouling was observed, as evident

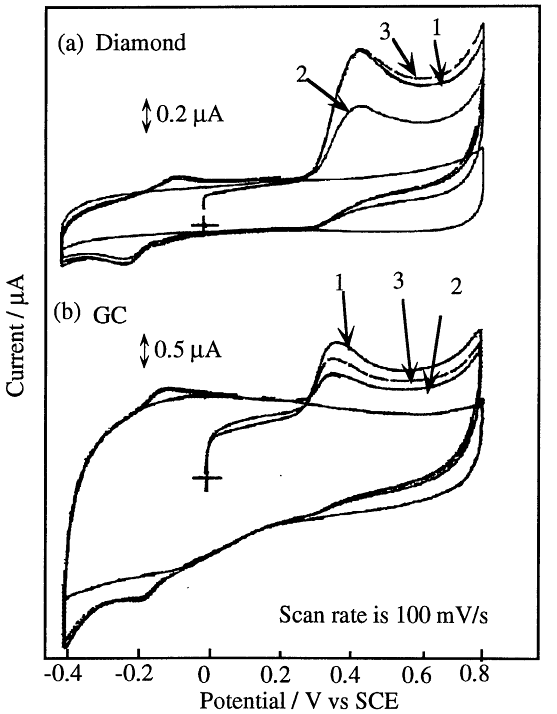

Figure 1. Cyclic voltammograms for 10 mM serotonin in 0.1 M phosphate buffer (pH 7): Background voltammograms (curves without peaks) are also shown. (a) diamond electrode (0.07 cm^2) (b) GC electrode (0.07 cm^2); (1) 1st cycle; (2) 2nd cycle; (3) after stirring.

from the reproducible behavior after stirring. It is also interesting to compare the background voltammograms for the diamond and GC electrodes (Figure 1). The background current for the GC electrode at 0.4 V (oxidation potential of serotonin) was at least five times higher than that for the diamond electrode. This indicates the possibility of obtaining high sensitivities at diamond electrodes. Furthermore, the signal/background value obtained from the voltammogram for diamond was 4.5 times greater than that for the GC electrode. Under hydrodynamic conditions, the background current for GC was found to be ~8 times higher than that for diamond electrode.

Another interesting feature is the redox behavior observed in the negative potential region of the voltammogram (pair of complementary anodic-cathodic voltammetic peaks centered at -0.15 V) (Figure 1). This nearly symmetric peak-pair (ΔE_p = 35 mV) is due to the hydroxylated form of the quinone, which is an oxidation product of serotonin.[8] This small ΔE_p value is consistent with an adsorbed species, although it is not sufficient evidence. Strong adsorption of this quinone on various carbon electrodes is known[6,8] which is undesirable for the electrochemical detection of 5-HT, as mentioned earlier. Jackson et al.[6] reported strong adsorption of the quinone product on carbon fiber electrodes. Wrona and Dryhurst[8] also observed a highly symmetric redox peak-pair and confirmed the strong adsorption of the oxidation product species on a pyrolytic graphite electrode by the observation of the linear dependence of the peak currents on the scan rate v, characteristic of a redox process involving an adsorbed species. However, the voltammogram obtained for the diamond electrode shows a peak-pair with a ΔE_p value of 75 mV, with relatively broad, asymmetric peaks. The shape of the voltammogram is characteristic of a diffusion-controlled process. Also, the peak current in this case is linearly dependent on $v^{1/2}$, which is also characteristic of a diffusion-controlled process. The ΔE_p for this pair was also found to increase with increasing v. Xu et al. have demonstrated the inert nature of the diamond surface with respect to adsorption of anthraquinone disulfonate molecules, in contrast to GC.[3]

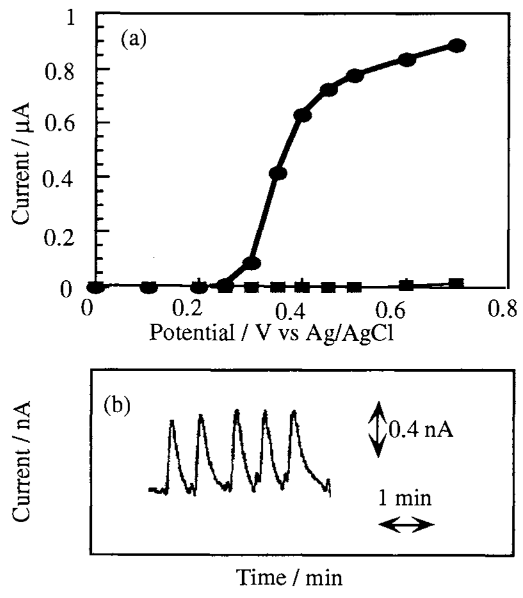

Figure 2. (a) Hydrodynamic voltammogram for 100 mM HIAA in 0.1 M phosphate buffer; (b) FIA-ED results for 10 nM 5-HT. Electrode area = 0.64 cm^2. Flow rate = 1 mL/min.

Figure 2 shows hydrodynamic voltammograms obtained at the diamond electrode for 20-µL injections of 50 µM serotonin in 0.1 M phosphate buffer, with 0.1 M phosphate buffer as the mobile phase at a flow rate of 1 mL/min. This hydrodynamic voltammogram was obtained by recording the background current after a delay of 15 min to obtain a stable response, followed by the injection of 20 µL of serotonin-containing solution. The peak current was recorded after each injection, together with the corresponding background current. It is pertinent to note that the diamond electrode produced a stable background current within 15 min after switching to the desired potential, in contrast to GC, which took about 45 min to attain a reasonably stable current. The corresponding background voltammograms are also shown. Well defined, sigmoidally shaped voltammograms, with a half-wave potential of 0.36 V vs. Ag/AgCl were obtained for 5-HT. The detection potential for amperometry was set at 0.43 V vs. Ag/AgCl in the limiting current range. Figure 2b shows the amperometric response of diamond for 20-µL injections of 10 nM (0.2 pmol) serotonin. A highly reproducible response, with a peak variability less than 3% (n = 15) is a remarkable observation. The calibration plots showed excellent linearity from 10 nM to 50 µM (r = 0.99) This value is within the range of serotonin levels encountered in real samples. Diamond exhibited high sensitivity, with a wide linear dynamic range, which could not be obtained with GC electrodes. These results indicate the superior properties of diamond and the advantage of the diamond electrode for the analysis of serotonin, particularly high stability and sensitivity with simple amperometric detection.

References and Notes

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