

Electrochemical Oxidation of NADH at Highly Boron-Doped Diamond Electrodes

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Conductive boron-doped chemical vapor-deposited diamond thin films, already known to have superior properties for general electroanalysis, including low background current and a wide potential window, are here shown to have additional advantages with respect to electrochemical oxidation of nicotinamide adenine dinucleotide (NADH), including high resistance to deactivation and insensitivity to dissolved oxygen. Cyclic voltammetry, amperometry, and the rotating disk electrode technique were used to study the reaction in neutral pH solution. Highly reproducible cyclic voltammograms for NADH oxidation were obtained at as-deposited diamond electrodes. The response was stable over several months of storage in ambient air, in contrast to glassy carbon electrodes, which deactivated within 1 h. The diamond electrode exhibited very high sensitivity for NADH, with an amperometric detection limit of 10 nM (S/N = 7). The response remained stable, even in the very low concentration range, for several months. In addition, interference effects due to ascorbic acid were minimal when the concentrations of NADH and ascorbic acid were comparable. An NADH-mediated dehydrogenase-based ethanol biosensor incorporating an unmodified diamond electrode is demonstrated. The present results indicate that diamond is a useful electrode material for the analytical detection of NADH, making it attractive for use in sensors based on enzyme-catalyzed reactions involving NADH as a cofactor.

Polycrystalline chemical vapor-deposited (CVD) diamond films that are highly doped with boron are metallic conductors. Boron-doped diamond (BDD) exhibits several superior electrochemical properties that are significantly different from those of other carbon allotropes, e.g., glassy carbon (GC), pyrolytic graphite (PG), and highly oriented pyrolytic graphite (HOPG), which have been widely used as electrode materials for many years. BDD has received attention from electrochemists only in the past few years.^{1–17} Its attractive features include a wide electrochemical potential window in aqueous media, very low capacitance, and

extreme electrochemical stability. In addition, diamond electrodes have recently been found to show high resistance to deactivation, via fouling,¹² and insensitivity to dissolved oxygen.^{2,3} These properties make diamond a promising material for electroanalytical applications.

GC and HOPG are composed almost exclusively of sp² type carbon. The electronic and electrochemical properties depend on several factors, including surface preparation, microstructure, and the presence of carbon–oxygen functional groups.¹⁵ The surface of GC, like that of many solid electrodes, can become deactivated as a function of time when it is exposed to the laboratory atmosphere or working solution.^{18–20} The problem is more severe when biological sample solutions are used.^{21–23} Several investigations on the activation or reactivation of GC electrodes have been carried out.^{18–20} BDD electrodes appear to be relatively free from deactivation problems, as seen in several recent reports concerning their sensitivity and stability.^{5,7,13,14} For example, Jolley et al.¹³ reported a detection limit of 0.2 μM for ferrocyanide. These workers found that the electrodes were not deactivated even after several weeks of exposure to the laboratory atmosphere. In our laboratory, we have also been able to obtain well-defined voltam-

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metric responses for 0.2 μM ferrocyanide, using a BDD micro-electrode.⁷ Such electrodes exhibit a stable electrochemical response for at least two months. Another interesting feature of as-deposited BDD is that its surface is relatively nonpolar as a result of the predominant termination of the surface with hydrogen. Thus, the adsorption of polar molecules is suppressed, as demonstrated by Xu et al.¹⁵

The electrochemical oxidation of nicotinamide adenine dinucleotide (NADH) is of great interest because it is required as a cofactor in a large number of dehydrogenase-based biosensors.^{24–27} For example, NADH-dependent dehydrogenases catalyze the oxidation of compounds such as ethanol and lactic acid. Most solid electrodes, including diamond, exhibit high overvoltages for NADH oxidation. However, the most serious problem with previously used electrodes is deactivation as a result of the irreversible adsorption of oxidation products on the surface.^{21,22} Hence, pretreatment and conditioning are usually necessary to reactivate the electrode surface. Another problem inherent in the use of both unmodified and modified GC is significant background current under potentiodynamic conditions, a factor which limits the detection limit. This problem can be alleviated through the use of the steady-state polarization technique with forced convection, e.g., with a rotating disk electrode (RDE). Using this technique, Blaedel et al. were able to achieve a detection limit of 10 μM .²⁸

For GC electrodes, the use of redox mediators is a promising means of improving the electrochemical response, by reducing the overpotential. The redox mediator is usually coated on the electrode surface. For example, Wu et al.²⁵ demonstrated a drastic improvement in the electrochemical response for GC using the 1,10-phenanthroline-5,6-dione complex of Fe as a mediator, obtaining a detection limit of about 1 μM for NADH. They demonstrated an ethanol biosensor based on this modified electrode. Although the use of mediators is promising, such biosensors possess certain drawbacks, e.g., mediator leaching, lack of long-term stability, and dependence on oxygen concentration, which may limit their analytical use.^{29,30} The toxicity of the mediator is also to be taken into consideration.

In the present paper, we report on the use of BDD electrodes for the oxidation of NADH at neutral pH. Cyclic voltammetry, rotating disk voltammetry, and amperometry were used to study the reaction. Cyclic voltammograms were also obtained for a BDD microelectrode, which typically consists of one or two exposed microcrystals, to assess the effect of an absence of sp^2 carbon impurities at grain boundaries. The behavior of an ethanol biosensor based on the determination of NADH generated in the enzymatic reaction catalyzed by alcohol dehydrogenase was examined in order to demonstrate the use of unmodified BDD electrodes in biosensors. Very high sensitivity, together with excellent reproducibility and long-term stability, are shown to be attractive features.

EXPERIMENTAL SECTION

Highly doped BDD films were deposited, by use of a microwave plasma chemical vapor deposition system (ASTeX, Woburn, MA), on silicon (100) wafers, as previously reported.² Prior to deposition, the Si substrates were hand-polished with diamond powder (0.5- μm) for nucleation, following which they were rinsed with acetone. A mixture of acetone and methanol (9:1, v/v) was used as the carbon source, with dissolved B_2O_3 as the boron source, at a B/C molar ratio of 10,000 ppm (i.e., 1 mol %). High-purity (99.99%) hydrogen gas was used as the carrier gas. The hydrogen gas flow was controlled at 532 sccm with a mass flow controller. The total pressure in the reactor was 115 Torr, and the substrate temperature was estimated to be between 800 and 900 °C. The deposition of the film was carried out at a microwave power of 5 kW. A film thickness of approximately 40 μm was achieved after 10 h of deposition. The film resistivity, measured by the four-point probe method, was on the order of $10^{-3} \Omega \text{ cm}$. The Raman spectra of these films showed them to be of high quality, as evident from the strong characteristic peak at 1332 cm^{-1} . In addition, a broad peak centered at approximately 1200 cm^{-1} was observed, which is characteristic of highly boron-doped samples. Further details, including a Raman spectrum of the conductive diamond film, have been presented elsewhere.² Ohmic contact was made on the front (i.e., last-deposited) side of the film with silver paste. For the rotating disk electrode, highly conductive Si substrates were used and the ohmic contact was made from the substrate side. The GC electrode (5-mm diameter, GC-30, Tokai Carbon Co., Ltd., Japan) was pretreated by polishing with alumina (1 μm) followed by rinsing with high-purity water.

The electrochemical measurements were carried out in 0.05 M KH_2PO_4 + 0.05 M K_2HPO_4 ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH = 7.1 $\pm$ 0.05) aqueous solution in a single compartment cell. A saturated calomel electrode (SCE) was used as the reference electrode, and a platinum foil was used as the counter electrode. Cyclic voltammograms were recorded using a combined potentiostat-function generator (Toho Technical Research, PS-07) and X-Y recorder. Amperometric measurements were carried out using a potentiostat (Hokuto Denko, HA-150). All experiments were carried out at room temperature (23 $\pm$ 2 °C).

The biosensor was constructed as follows. First, the enzyme catalyst, alcohol dehydrogenase (ADH), was immobilized on a nylon disk (6-mm diameter) following a procedure described in the literature.²⁵ Prior to immobilization, the ADH powder was dissolved in 0.1 M Tris/HCl buffer (pH = 8.0) containing 50% glycerol. One hundred fifty microliters of this solution was mixed with 200 μL of BSA (10 wt %) and 200 μL of glutaraldehyde (2.5 vol %). Five microliters of this mixture was spread uniformly on the precleaned nylon disk and allowed to dry at room temperature for 30 min. The nylon net modified with alcohol dehydrogenase (ADH) was placed over the diamond electrode (0.14 cm^2), which had been mounted in a Teflon holder, leaving the front (diamond) side exposed. The nylon net was held in place by a Teflon cap with a circular hole. A schematic diagram of the biosensor is shown in Figure 6. The assembly of this sensor is similar to that previously reported by Abruña and co-workers for modified GC electrodes.²⁵

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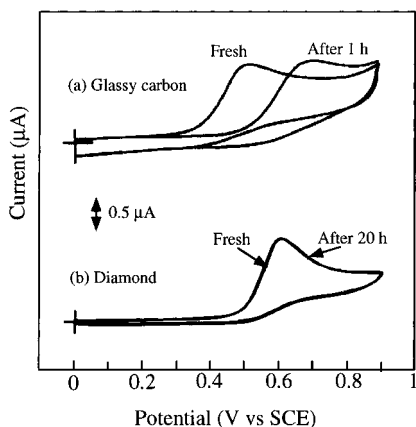


Figure 1. Cyclic voltammograms for (a) GC (0.196 cm²) and (b) BDD (0.18 cm²) electrodes in air-saturated 0.1 M phosphate buffer (pH 7.1) containing 50 μ M NADH at a potential sweep rate of 20 mV/s. The voltammograms for the diamond electrode, which were obtained before and after 1 h of exposure to the solution, followed by washing, drying and storage in the laboratory for 19 h, are superimposed.

Nicotinamide adenine dinucleotide, in the oxidized and reduced forms (NADH, NAD⁺), and alcohol dehydrogenase (ADH, EC 1.1.1.1) were obtained from the Oriental Yeast Co., Ltd. ADH was in the form of lyophilized powder containing 364 units of enzyme activity per milligram of protein. Bovine albumin (BSA), glutaraldehyde, ascorbic acid, KH₂PO₄, and K₂HPO₄ were obtained from Waco Pure Chemical Industries. High-purity ethanol was supplied by Amakasu Chemical Industries. All solutions were prepared using ultrapure water (>17 M Ω , Milli-Q).

RESULTS AND DISCUSSION

Electrochemical Oxidation of NADH. It is well-known that unmodified GC electrodes undergo rapid deactivation during the oxidation of NADH. For example, Pariente et al.²⁶ observed an anodic shift of the NADH oxidation peak by approximately 300 mV due to deactivation. Figure 1a shows cyclic voltammograms for the oxidation of NADH at a freshly polished GC electrode and at the same electrode after 1 h of contact with the solution. A positive shift (about 200 mV) in the oxidation peak was observed, demonstrating the rapid deactivation of the electrode. It should be noted that significant deactivation is also observed when the GC electrode is subjected to even a single voltammetric sweep and then washed thoroughly with distilled water, dried, and stored in air for 1 h. This shift is mainly due to strong adsorption of the NAD⁺ produced at the electrode during the oxidation of NADH.²² In contrast, the oxidation peak potential at a BDD electrode was essentially unchanged even after 1 h of exposure to the solution, followed by washing, drying, and storage in laboratory air for 19 h, as shown in Figure 1b. Moreover, the response was found to be stable for several days, even with repeated use. Prolonged use (~1 month) of the electrode resulted in a shift in the oxidation potential up to 80 mV. In this case, pretreatment with concentrated HNO₃ for 30 s was sufficient to restore the activity. Electrode fouling was essentially nonexistent. However, after each voltammetric sweep, the NAD⁺ was found to desorb somewhat slowly, so that ~2 min of recovery time was required. When the potential sweeps were carried out after a 2-min recovery, the curves were reproducible within 2–3%. The background-corrected voltammet-

ric current response at 0.58 V vs SCE was extremely linear in the concentration range examined (0–60 μ M). The peak current varied linearly with the square root of sweep rate, $v^{1/2}$, indicating semi-infinite linear diffusion of reactant to the electrode surface. This curve was also highly linear ($r = 0.999$) up to a sweep rate of 500 mV/s and passed through the origin (see Supplementary Information). It is pertinent to note that well-defined cyclic voltammograms (without background correction) were obtained even for an NADH concentration as low as 1 μ M. Voltammetric response at such low concentrations could not be obtained with GC electrodes because of large background currents. The voltammetric peak could not be distinguished from its background voltammogram. The electrochemical-oxidation response was reproducible from film to film. We have tested several films from different batches grown under identical conditions. Usually, high-quality films (confirmed by Raman) were selected for the electrochemical determination of NADH. The peak potentials were similar for all of the films: 0.58 ± 0.02 V. The current densities were also reproducible, within 4–5%.

An additional problem with GC electrodes is the sensitivity to dissolved oxygen in the electrolyte solution, which is known to influence the linearity of the voltammetric-current response to NADH at modified GC electrodes.²⁶ As already noted, because of the high overpotential for oxygen reduction on conductive BDD electrodes,^{2,3} it was expected that there would be a negligible oxygen effect, and this was confirmed. Hence, all the experiments were carried out without deaerating the electrolyte. These results indicate that diamond may be considered to be a promising alternative to GC for NADH determination.

The long-term stability of the electrochemical behavior for NADH oxidation on the BDD electrode can be attributed to a lack of adsorption on the as-deposited surface, which, as reported by other workers,¹⁵ we find to have a very low oxygen content (≤ 3 atomic %) and to be highly hydrophobic. Thus, the surface can be assumed to be predominantly hydrogen-terminated. Adsorption of most species, particularly polar ones, is not favored. Swain and co-workers¹⁵ showed that polar molecules, such as anthraquinone-2,6-disulfonate, do not adsorb on diamond or hydrogenated GC electrodes while they adsorb strongly on GC or HOPG that contains exposed defect sites terminated by carbon–oxygen functional groups. These workers point out that the lack of polar oxygen-containing functional groups may be responsible for the lack of adsorption and the consequent resistance to fouling and, consequently, deactivation. While this is certainly true, we should also point out that, even after moderate oxidative treatment (+1.5 V vs SCE for 10 min in the same KH₂PO₄/K₂HPO₄ buffer), after which the formation of a partial monolayer of C–O functional groups would be expected, almost precisely the same short-term behavior for NADH oxidation was observed as was for the as-deposited surface. The cyclic voltammograms obtained after oxidative treatment nearly overlapped those of the untreated electrodes (see Supplementary Information). We have yet to examine the long-term behavior of such electrodes, however. An additional point of interest we find is that even heavily oxidized BDD electrodes exhibit little tendency for adsorption of such compounds as dopamine, DOPAC, and 4-methyl catechol.^{5,6}

Figure 2 shows a current–voltage curve for NADH oxidation at a BDD rotating disk electrode (RDE). The curve is well-defined,

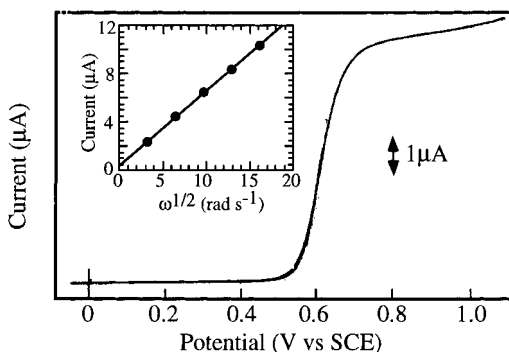


Figure 2. Voltammograms for a BDD RDE (0.135 cm²) in 0.1 M phosphate buffer (pH 7.1) containing 100 μM NADH.

with a mass transport-limited current response at potentials positive of 0.7 V vs SCE. The half-wave potential of ~0.58 V vs SCE at 100 rpm is similar to the peak potential observed in the cyclic voltammogram. A positive shift in the half-wave potential was found with increasing rotation rate, indicating partial kinetic control of the oxidation reaction. However, a plot of limiting current, i_{lim} , vs $\omega^{1/2}$ (Levich plot) is linear, indicating that the reaction is transport-limited at potentials more positive than the half-wave potential (inset of Figure 2). Using the RDE technique, we were able to obtain reproducible voltammograms during subsequent sweeps, i.e., without waiting for the products to desorb from the electrode. As noted above, Blaedel et al.²⁸ used the RDE technique with GC electrodes and steady-state polarization to minimize the background current. However, these workers found it necessary to pretreat the electrode once per day by polarizing it for 2 min at +1.35 V vs Ag/AgCl followed by 2 min at -1.35 V. Without this pretreatment, the electrochemical response was found to degrade with each day's measurements.

It is important to note that, when low-quality BDD films (as identified by Raman) were tested, they yielded voltammetric peaks at somewhat lower potentials (0.5 ± 0.03 V), which is most probably due to the presence of nondiamond sp^2 carbon present at the grain boundaries. The response of such electrodes was not stable. Thus it appears that high-quality BDD films are required for optimal behavior.

To examine the effect of the film in greater detail, we used BDD microelectrodes for comparison. In the present work, the diamond microelectrodes were fabricated in such a way as to expose only one or two microcrystals to the solution, i.e., without significant exposure of grain boundaries. The cyclic voltammetry was characterized by sigmoidally shaped voltammograms, typical of radial-diffusion behavior. Figure 3a shows a cyclic voltammogram obtained at a diamond microelectrode in 1 mM $K_4Fe(CN)_6$ in 0.1 M KCl. A well-defined steady-state-type voltammogram was obtained, without significant hysteresis. The diameter of this electrode was calculated to be approximately 40 μm, as determined from the value of the limiting current.³¹ This value is very similar to the size of a typical single crystal on the tungsten fiber as examined using SEM.⁷ Figure 3b shows the cyclic voltammogram for NADH oxidation at the diamond microelectrode. The voltam-

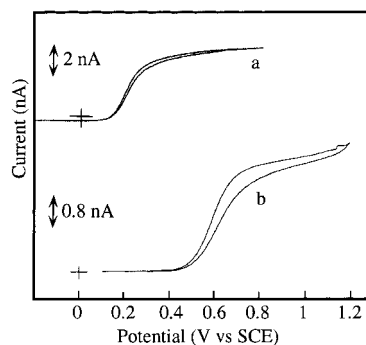


Figure 3. Cyclic voltammograms for (a) 1 mM $K_4Fe(CN)_6$ in 0.1 M KCl and (b) 1 mM NADH in 0.1 M phosphate buffer (pH 7.1) at a BDD microelectrode (radius, 20 μm).

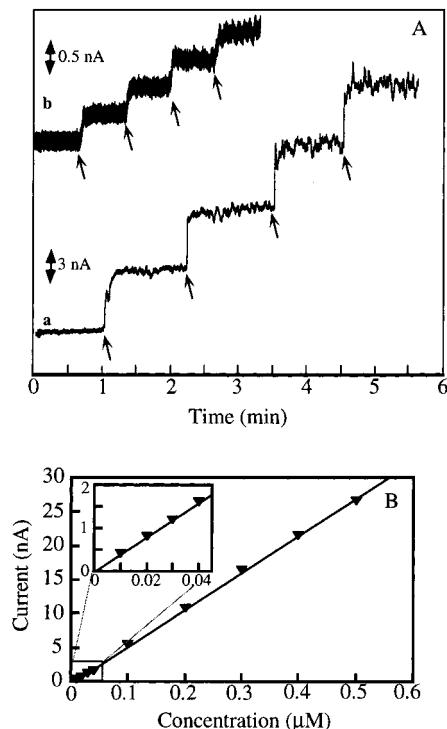


Figure 4. (A) Steady-state amperometric response of a stationary BDD electrode (0.18 cm²) to NADH additions to a stirred solution containing 0.1 M phosphate buffer (pH 7.1) at an applied potential of +0.58 V vs SCE. Each addition increased the concentration by 0.1 μM (curve a) and by 10 nM (curve b). The noise is due to the stirring. (B) Corresponding calibration plot (inset shows the calibration plot for lower concentration range).

mogram has a well-defined sigmoidal shape, and the half-wave potential (+0.6 V vs SCE) is the same as the peak potential obtained for a high-quality polycrystalline BDD macroelectrode. These results indicate that the quality of the diamond films used in this study is high and that the influence of grain boundaries is negligible.

The sensitivity of the electrode was examined with steady-state amperometry for a stationary BDD electrode in a stirred solution. Figure 4 shows the amperometric response for the BDD electrode at 0.58 V vs SCE at very low NADH concentrations. The steady-state background current in the phosphate buffer, at this potential was 12 ± 3 nA. A sudden increase in the current of ~5.5 nA was observed upon addition of the first aliquot (20 μL) of NADH solution, bringing the concentration to 0.1 μM (Figure 4A, curve

(31) The radius of the microelectrode was calculated from the equation $i_{lim} = 4nFDCr$, where i_{lim} is the limiting current, C is the concentration, D is the diffusion coefficient, r is the radius of the electrode, F is the Faraday constant, and n is the number of electrons.

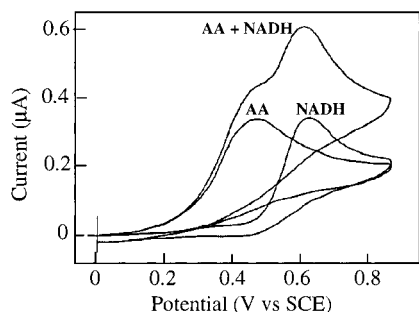


Figure 5. Cyclic voltammograms for a BDD (0.18 cm²) diamond electrode in the phosphate buffer containing (a) 60 μM NADH, (b) 60 μM ascorbic acid, and (c) 60 μM NADH + 60 μM ascorbic acid.

a). Furthermore, we were able to detect NADH down to a concentration of 10 nM (Figure 4A, curve b). The statistical detection limit for the amperometric method was also found to be 10 nM, with an S/N ratio of 7.4 ($n = 7$), which is at least 2 orders of magnitude lower than those previously reported for modified GC electrodes.²⁶ A linear calibration curve was obtained in the concentration range examined, 10–500 nM ($r > 0.999$), as shown in Figure 4B. It is pertinent to note that, even after 3 months of exposure to ambient air, a typical electrode exhibited a stable amperometric response for the very low concentration range. The decrease in the response for the detection of 10 nM NADH after three months of exposure was approximately 15–20%. These results provide evidence for the excellent performance of the BDD electrode in terms of sensitivity and stability for NADH oxidation.

There are several possible explanations for the low background currents commonly observed for conductive diamond electrodes. These include (a) negligible porosity, (b) lack of electroactive carbon–oxygen surface functional groups,¹³ except in the case of electrochemically or chemically oxidized surfaces, (c) a high degree of resistance to electrochemical and chemical oxidative attack,¹³ which effectively prevents the surface from acquiring porosity, and (d) a depressed density of electronic states at the Fermi level, compared with that in a metal,^{2,13} but a greater such density than that for highly oriented pyrolytic graphite (HOPG).³²

Interference of Ascorbic Acid. The interference of ascorbic acid (AA) is an important problem for the electrochemical determination of biological substrates such as dopamine and NADH. To overcome this problem, the electrode can be covered with a membrane such as Nafion, which electrostatically excludes anions such as ascorbate. However, such membranes usually cause a reduction in the sensitivity. Furthermore, at neutral pH, NADH is negatively charged, and thus it is also excluded by the membrane.²⁴ Another method would be to make use of ascorbate oxidase, an enzyme capable of selectively oxidizing ascorbate in the presence of molecular oxygen. Immobilization of this enzyme on a membrane held over the electrode could prevent the diffusion of ascorbate to the electrode surface. Pariente et al. showed that such an approach is effective for an NADH-based ethanol sensor with a modified GC electrode.²⁶

We have examined the detection of NADH in the presence of AA on a bare, as-deposited diamond electrode. Figure 5 shows

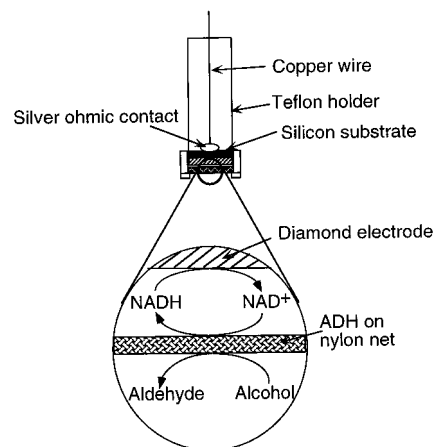


Figure 6. Schematic diagram of the diamond/ADH biosensor assembly, together with an expanded view, showing the processes involved in the determination of ethanol. In the present study, alcohol dehydrogenase (ADH) was immobilized on nylon mesh disks (50 × 50 μm openings), with a loading of ~33 activity units.

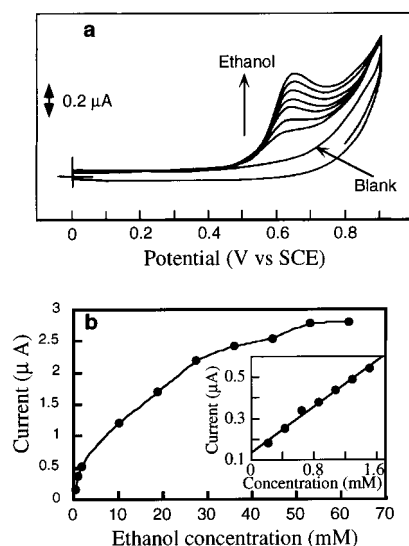


Figure 7. (a) Cyclic voltammograms for a diamond (0.14 cm²)/ADH (33 units) biosensor for various ethanol concentrations (increasing in steps of 0.215 mM up to 1.505 mM) in 0.1 M phosphate buffer (pH 7.1) containing 5 mM NAD⁺. (b) Corresponding calibration curve for a wide concentration range (inset, linear portion of the curve for a lower concentration range).

cyclic voltammograms for the individual compounds, i.e., NADH and AA, and that for an equimolar mixture of the two compounds. The peak corresponding to AA oxidation appears as a shoulder in the voltammogram for the mixture. The peak current at the approximate potential for NADH oxidation (+0.58 V) has increased as a result of the simple addition of the AA oxidation current. It is important to note that both reactions involve two electrons.³³ This assumption makes it possible to calibrate both NADH and AA in the mixture. The calibration plots obtained for both compounds, individually and in the binary mixture, are linear. The plots for the mixtures lie close to those for the pure single-component solutions. The calibration plots for AA in the presence and in the absence of NADH were first obtained at a potential

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(+0.4 V vs SCE) where the current due to NADH oxidation was small. The plots for NADH were obtained at 0.58 V, where the contribution of NADH oxidation was the largest. These results indicated that the NADH oxidation current can be corrected for AA interference when both compounds are present at comparable concentrations. Such corrections for AA interference have previously been reported for modified GC electrodes.²⁵

Ethanol Biosensor Response. To demonstrate the operation of the diamond electrode in a dehydrogenase-based sensor, the assembly depicted in Figure 6 was constructed. The assembly of the sensor is similar to that previously reported by Pariente et al. for modified GC electrodes.²⁴ The enzyme catalyst, alcohol dehydrogenase (ADH), was immobilized on a nylon mesh disk, as already mentioned. In an ethanol biosensor assembly, the immobilized ADH oxidizes ethanol to acetaldehyde in the presence of NAD⁺. The cofactor, NAD⁺, is converted to its reduced form (NADH) by accepting the electrons generated in the enzymatic reaction. Oxidation of the NADH at the electrode generates an electrical signal the magnitude of which is a measure of the ethanol present in the electrolyte solution.

Figure 7a shows the cyclic voltammetric response for a diamond/ADH biosensor in a solution containing phosphate buffer and 5 mM NAD⁺, both in the absence and in the presence of ethanol. In the absence of ethanol, no peak was observed at the oxidation potential of NADH. Upon addition of aliquots of ethanol (each addition increases the concentration by 0.215 mM), a monotonic increase in the anodic peak current at the potential corresponding to oxidation of NADH was observed up to 1.5 mM. Figure 7b shows the voltammetric-current response over a wide concentration range. In the low concentration range, the response was found to be linear (inset of Figure 7b). At higher concentrations, the slope of this curve decreases, most likely as a result of control by the enzymatic reaction. Similar behavior has been reported previously for modified GC electrodes.²⁵ The biosensor response was also tested at various NAD⁺ concentrations in order to obtain the concentration at which maximum response can be obtained. An increase in the biosensor response was observed with increasing NAD⁺ concentration, reaching a maximum at about 5.5 mM. The decrease in the response above 6 mM may be due to an inhibitory effect, as observed previously.²⁴

CONCLUSIONS

The oxidation of NADH in neutral aqueous media has been investigated using high-quality, conductive, boron-doped diamond thin-film electrodes. The BDD electrode exhibited excellent performance for NADH oxidation in terms of stability and high resistance to deactivation, providing significant advantages compared to GC. A well-defined voltammogram with a sigmoidal shape was obtained using rotating-disk voltammetry. The resulting linear Levich plot indicated mass-transport-limited behavior. The high quality of the planar-film electrode was confirmed by comparison with a microelectrode, for which the amount of grain boundary exposed is thought to be minimal. The film behavior was similar to that of the microelectrode. Using the film electrode, the amperometric detection limit was found to be ~10 nM at an S/N ratio of 7 ($n = 7$). The electrode response was found to be stable for long periods of storage (up to 3 months), indicating the possibility of its applicability for NADH-based sensors. In addition, interference effects due to ascorbic acid are shown to be correctable when the concentrations of NADH and AA are comparable. An ethanol biosensor was shown to operate successfully, demonstrating the feasibility of the use of BDD electrodes in NADH-based biosensors.

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SUPPORTING INFORMATION AVAILABLE

A plot of peak current vs the square root of sweep rate for the oxidation of NADH and cyclic voltammograms for the BDD electrode before and after anodic oxidation treatment. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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