

# An Amperometric Detector Formed of Highly Dispersed Ni Nanoparticles Embedded in a Graphite-like Carbon Film Electrode for Sugar Determination

Tianyan You,<sup>†</sup> Osamu Niwa,<sup>\*,†</sup> Zilin Chen,<sup>†</sup> Katsuyoshi Hayashi,<sup>†</sup> Masato Tomita,<sup>†,‡</sup> and Shigeru Hirono<sup>§</sup>

NTT Microsystem Integration Laboratories, 3-1 Morinosato, Wakamiya, Atsugi, Kanagawa 243-0198, Japan, and NTT AFTY Corporation, 4-16-30 Shimorenjyaku, Mitaka, Tokyo 181-0013, Japan

**We achieved improved detection limits for sugars by developing a novel thin film containing 0.8% highly dispersed Ni nanoparticles in disordered graphite-like carbon (Ni-NDC) as a detection electrode for high-performance liquid chromatography. The Ni-NDC film was prepared in one step by a simple radio frequency (rf) sputtering method at a temperature below 200 °C. We characterized the film by XPS, TEM, and AFM analysis and found that the average Ni nanoparticle size was 3 nm and that the film consisted of a mixture of Ni, NiO, Ni<sub>2</sub>O<sub>3</sub>, and Ni(OH)<sub>2</sub>. We studied the electrochemical detection of sugars using the 0.8% Ni-NDC film electrode. The film electrode had excellent electrocatalytic ability and good stability compared with a Ni-bulk electrode with regard to the electrooxidation of sugars. We employed the Ni-NDC film as an HPLC detection electrode. We achieved a good separation of four sugars (glucose, fructose, sucrose, lactose) at a relatively low constant detection potential (0.40 V vs Ag/AgCl) and a linearity of over 3 orders of magnitude. We obtained improved detection limits for the investigated sugars, namely, 20, 25, 50, and 37 nM for glucose, fructose, sucrose, and lactose, respectively. This is at least 1 order of magnitude lower than the detection limits obtained with a Ni-bulk electrode with the same measurement condition. The Ni-NDC film electrode also showed good reproducibility with a relative standard deviation of 1.75% for 40 consecutive injections of glucose in a flow system.**

The determination of carbohydrates is of considerable importance because it is widely used in various fields including those concerned with biological, environmental, clinical, and food analysis. Commercially available spectrophotometric methods such as fluorescence and UV–visible detection are commonly used for the detection of carbohydrates. However, it is difficult to

improve the sensitivity because the carbohydrates show weak optical absorbance in the UV–visible region and low refractive indexes. In addition, derivatization processing is needed for fluorescence detection. Therefore, it is essential to develop other simple and highly sensitive methods for the determination of carbohydrates.

The electrochemical detection (ECD) of carbohydrates was introduced to achieve a low detection limit at a low cost. There are two ECD techniques. One is the pulsed amperometric detection (PAD) method, which employs noble metals (Au, Pt) as working electrodes.<sup>1,2</sup> The PAD instrument is commercially available and gives us better sensitivity than that of the optical method. However, the detector needs pulse generation circuits and is relatively expensive. With PAD detection, a regeneration step using the repetitive application of a pulsed potential waveform is needed to renew the electrode surface because the surface of a noble electrode is easily fouled. The electrode surface is gradually etched during the pulse process since the pulse cycle usually contains a high-potential application step. The other technique is constant potential amperometric detection with working electrodes made of transition metals (Cu, Ni).<sup>3–8</sup> Constant potential amperometry has certain advantages over PAD in that it employs a simple instrument and provides high sensitivity with a low baseline noise.<sup>5</sup> The nickel electrode<sup>8–13</sup> has been the most widely utilized electrode for determining carbohydrates in alkaline media in the constant-potential application method. However, a

\* To whom correspondence should be addressed. Tel: 81-46-240-3517. Fax: 81-46-240-4728. E-mail: niwa@aecl.ntt.co.jp.

<sup>†</sup> NTT Microsystem Integration Laboratories.

<sup>‡</sup> Present address: Corning Technology Center, 12117, Obuchi, Osuka-cho, Ogasa-gun, Shizuoka 437-1397, Japan.

<sup>§</sup> NTT AFTY Corp.

- (1) Johnson, D. C.; LaCourse, W. R. *Anal. Chem.* **1990**, *62*, 589A–597A.
- (2) LaCourse, W. R.; Johnson, D. C. *Carbohydr. Res.* **1991**, *215*, 159–178.
- (3) Kafil, J. B.; Huber, C. O. *Anal. Chim. Acta* **1985**, *175*, 275–280.
- (4) Santos, L. M.; Baldwin, R. P. *Anal. Chem.* **1987**, *59*, 1766–1770.
- (5) Zadeii, J. M.; Marioli, J.; Kuwana, T. *Anal. Chem.* **1991**, *63*, 649–653.
- (6) Colón, L. A.; Dadoo, R.; Zare, R. N. *Anal. Chem.* **1993**, *65*, 476–481.
- (7) Marioli, J. M.; Kuwana, T. *Electroanalysis* **1993**, *5*, 11–15.
- (8) Luo, P.; Zhang, F.; Baldwin, R. P. *Anal. Chim. Acta* **1991**, *244*, 169–178.
- (9) Fleischmann, M.; Korinek, K.; Pletcher, D. J. *Electroanal. Chem.* **1971**, *31*, 39–49.
- (10) Schick, K. G.; Magearu, V. G.; Huber, C. O. *Clin. Chem.*, **1978**, *24*, 448–450.
- (11) Buchberger, W.; Winsauer, K.; Breitwieser, C. *Fresenius' Z. Anal. Chem.* **1983**, *315*, 518–520.
- (12) Casella, I. B.; Desimoni, E.; Cataldi, T. *Anal. Chim. Acta* **1991**, *248*, 117–125.
- (13) Fermier, A. M.; Colón, L. A. *J. High-Resolut. Chromatogr.* **1996**, *19*, 613–616.

problem with constant-potential amperometry is that there is a gradual reduction in the current response as a function of time because of the loss of activity of the electrode surface.<sup>8</sup> Therefore, several nickel-based electrodes, such as nickel alloy,<sup>14–19</sup> were prepared in order to improve the sensitivity and stability of the determination. Kuwana and co-workers reported a systematic study of Ni–Cr and Ni–Ti alloy electrodes for the detection of carbohydrates under constant-potential conditions. These alloy electrodes provided improved sensitivity and exhibited good resistance to surface fouling, which resulted in long-term stability. However, it is difficult to control the composition as well as the size and size distribution of each metal of the alloy. The direct structural observation of the alloy is also difficult because of poor separation of the phase diagrams among the metals.

Recently, some chemically modified nickel electrodes (Ni-CMEs)<sup>20–24</sup> were developed by depositing Ni particles on a traditional electrode surface (Au, carbon, or diamond). These CMEs can improve the sensitivity and reproducibility for the determination of carbohydrates. However, the problem is that it is difficult to maintain long-term stability because the electrochemical activity of CMEs gradually decreases due to the detachment and dissolution of the catalyst from the substrate.

To develop a simple method for preparing a novel electrode material for the detection of carbohydrates with high sensitivity and long-term stability, we used a radio frequency (rf) sputtering method to prepare a uniform film consisting of 0.8% highly dispersed Ni nanoparticles embedded in disordered graphite-like carbon (Ni-NDC). We characterized the film using XPS, TEM, and AFM. The electrochemical properties of the 0.8% Ni-NDC film electrode were evaluated by using it as an amperometric detector for the detection of sugars after separating them with high-performance liquid chromatography (HPLC). The Ni-NDC film electrode has a detection limit that is at least 1 order of magnitude better than previously reported Ni-based bulk electrodes due to its good electrocatalytic property and stability.

## EXPERIMENTAL SECTION

**Reagents.** Glucose, fructose, sucrose, lactose, and sodium hydroxide were obtained from Kanto Chemical Co. Ltd. (Tokyo, Japan). The water used in the experiments was purified by Milli-Q (Millipore, Bedford, MA).

**Apparatus.** The Ni-NDC film was prepared using an rf method by cosputtering Ni and carbon simultaneously. This is the same as our previously reported technique for preparing Cu-NDC film.<sup>25</sup>

X-ray photoelectron spectra were collected with an X-ray photoelectron spectroscope (XPS; XPS5700, Physical Electronics,

Inc.) using monochromatic Al K $\alpha$  radiation (1486.6 eV). We observed the film structure using a transmission electron microscope (TEM; H-9000UHR, Hitachi Ltd.) after peeling the film from the silicon substrate. An acceleration voltage of 200 kV was used for the TEM observation. We measured the conductivity of the Ni-NDC film by the conventional four-terminal method at room temperature. We undertook atomic force microscopy (AFM) in air using a Nano Scope III (Digital Instruments).

We conducted the cyclic voltammetry (CV) measurements with an ALS/CHI 802 electrochemical analyzer (CH Instruments, Inc.). The CV experiments were performed with a three-electrode cell consisting of a Ni-NDC working electrode ( $d = 3$  mm), an Ag/AgCl (3 M KCl) reference electrode, and a platinum auxiliary electrode. The flow injection analysis (FIA) and HPLC experiments were performed with an HPLC Class-VP system (Shimadzu Co.), an LC-4C potentiostat (BAS, West Lafayette, IN), and a thin-layer radial flow cell (BAS). We separated the sugars using a Hamilton RCX-10 anion-exchange column (250  $\times$  4.1 mm i.d.) preceded by a CarboPac PA 100 guard column (50  $\times$  4 mm i.d.).

## RESULTS AND DISCUSSION

**Structural Characterization. XPS Analysis.** The atomic concentration and chemical state of the nickel were analyzed by XPS measurement. The binding energy for the Ni-NDC film was referenced to C 1s at 284.6 eV. The atomic concentration for Ni was 0.8% calculated from the XPS spectra of as-obtained Ni-NDC film (Figure 1A). The high-resolution XPS spectrum of the Ni 2p region is shown in Figure 1B. The Ni 2p spectrum shows shake-up satellite lines at a higher binding energy in addition to the expected Ni 2p<sub>1/2</sub> and Ni 2p<sub>3/2</sub> lines. As regards the Ni 2p<sub>3/2</sub> line, the peak around a binding energy of 853.0 eV is attributed to metallic nickel, while the peaks at a higher energy ( $\geq 855.0$  eV) are associated with nickel hydroxide (Ni(OH)<sub>2</sub>)/oxide film (NiO and Ni<sub>2</sub>O<sub>3</sub>). The peaks around binding energies of 855.0, 855.6 and 855.8, 856.3, and 856.6 eV can be assigned to the Ni 2p<sub>3/2</sub> photoelectrons from NiO, Ni<sub>2</sub>O<sub>3</sub>, NiO multiplet splitting, and Ni(OH)<sub>2</sub>, respectively. The XPS result coincides very well with that of a previous report.<sup>26</sup> We did not obtain pure metallic Ni particles but a mixture of metallic Ni, nickel hydroxide, and nickel oxide film because of the effect of a very few water molecules adsorbed on the rf chamber wall, which is similar to the effect of water on the oxidation of a nickel surface in a vacuum reported previously.<sup>27,28</sup> We suggest that the nickel formation process is as follows. During the rf sputtering, NiO, Ni(OH)<sub>2</sub>, and Ni<sub>2</sub>O<sub>3</sub> are produced due to the interaction of H<sub>2</sub>O with Ni. Because there is too little water in the reaction chamber to form any hydrogen-containing species, we obtained a mixture of Ni(0), Ni(II), and Ni(III) chemical states in the film.

**TEM Investigation.** To investigate the microstructure and the morphology of the Ni-NDC, we peeled the film from the silicon substrate for TEM measurements. Figure 2 shows typical TEM images of 0.8% Ni-NDC film. (A) shows the front view and (B) shows a cross-sectional view. The dark spots correspond to Ni nanoparticles, which are isolated from each other by the light

- (14) Marioli, J.; Luo, P. F.; Kuwana, T. *Anal. Chim. Acta* **1993**, *282*, 571–580.
- (15) Luo, P. F.; Kuwana, T. *Anal. Chem.* **1994**, *66*, 2775–2782.
- (16) Morita, M.; Niwa, O.; Tou, S.; Watanabe, N. *J. Chromatogr., A* **1999**, *837*, 17–24.
- (17) Yeo, I.-H.; Johnson, D. C. *J. Electroanal. Chem.* **2001**, *495*, 110–119.
- (18) Mora, M. I.; Marioli, J. M. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24*, 711–720.
- (19) Sinfelt, J. H. *Bimetallic Catalysts*; Wiley: New York, 1983; p 20.
- (20) Wang, E.; Liu, A. *J. Electroanal. Chem. Interfacial Electrochem.* **1991**, *319*, 217–225.
- (21) Stitz, A.; Buchberger, W. *Electroanalysis* **1994**, *6*, 251–258.
- (22) Casella, I. G.; Gatta, M. *Electroanalysis* **2001**, *13*, 549–554.
- (23) Ohnishi, K.; Einaga, Y.; Notsu, H.; Terashima, C.; Rao, T. N.; Park, S.; Fujishima, A. *Electrochem. Solid-State Lett.* **2002**, *5*, D1–D3.
- (24) Casella, I. G.; Gatta, M. *Anal. Chem.* **2000**, *72*, 2969–2975.
- (25) You, T.; Niwa, O.; Tomita, M.; Ando, H.; Suzuki, M.; Hirono, S. *Electrochem. Commun.* **2002**, *4*, 468–471.

- (26) Kim, K. S.; Winograd, N. *Surf. Sci.* **1974**, *43*, 625–643.
- (27) Jesús, J. C. d.; Carrazza, J.; Pereira, P.; Zaera, F. *Surf. Sci.* **1996**, *369*, 217–230.
- (28) Jesús, J. C. d.; Carrazza, J.; Pereira, P.; Zaera, F. *Surf. Sci.* **1998**, *397*, 34–47.

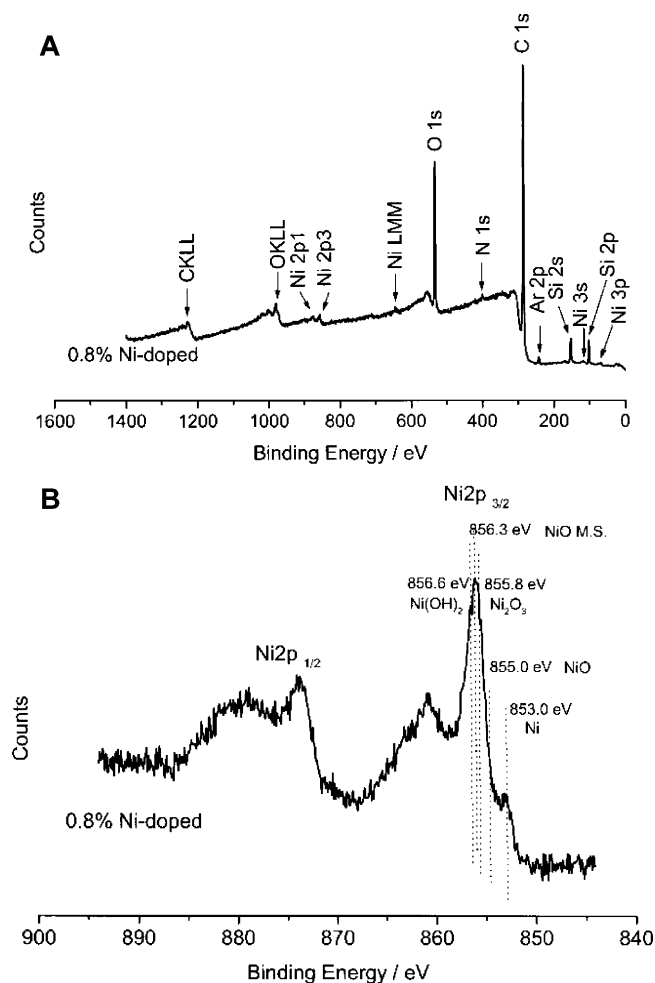


Figure 1. XPS spectra of 0.8% Ni-NDC film (A) and high-resolution XPS spectrum of Ni 2p (B).

features of the carbon matrix. The Ni particles are highly dispersed in the carbon matrix, and the film is homogeneous, unlike Ni particles on supported substrates.<sup>20–24</sup> The average size of the Ni particles is  $\sim 3$  nm. Moreover, the size distribution of the nickel particles is in a narrow range of 2.5–3.5 nm. We investigated a Ni(111) lattice with 2.034-Å spacing from both the front and cross-sectional views of the 0.8% Ni-NDC. With regard to the carbon matrix, we observed a lattice image of the carbon with a spacing of  $\sim 3.6$  Å, corresponding to graphite C (002). The spacing (3.6 Å) is wider than that of an ideal graphite crystal (3.4 Å), which indicates that the carbon matrix is not an ideal ordered graphite crystal but a disordered graphite-like structure.<sup>29–31</sup>

**Film Conductivity.** We measured the conductivity of the 0.8% Ni-NDC using the conventional four-terminal method at room temperature. The film conductivity was  $40.8 \text{ S}\cdot\text{cm}^{-1}$ , which is higher than that of rf sputtered disordered graphite-like carbon film ( $24 \text{ S}\cdot\text{cm}^{-1}$ ). This film conductivity is sufficient for the film to be used as an electrode for electrochemical detection.

(29) McCreery, R. L. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1991; Vol. 17, p 221.

(30) Hayashi, T.; Hirono, S.; Tomita, M.; Umehura, S. *Nature* **1996**, *381*, 772–774.

(31) Babonneau, D.; Cabioch, T.; Naudon, A.; Girard, J. C.; Denot, M. F. *Surf. Sci.* **1998**, *409*, 358–371.

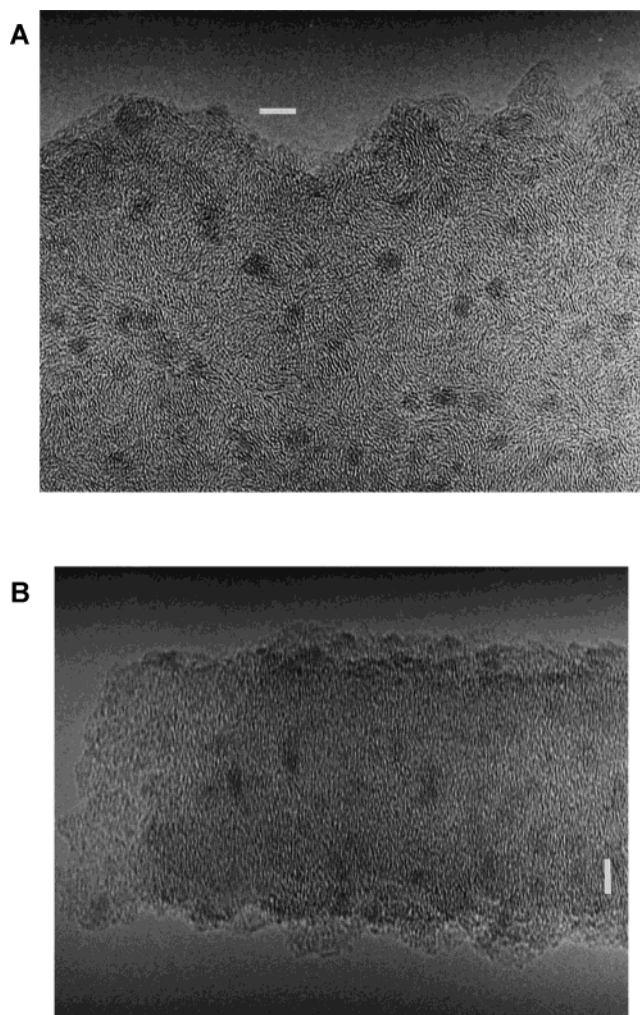


Figure 2. Typical TEM images of the front view (A) and a cross-section (B) of 0.8% Ni-NDC film. Scale bar, 5 nm.

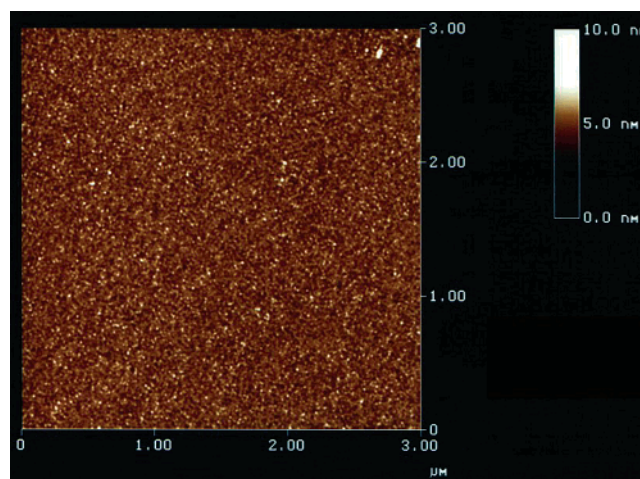


Figure 3. AFM image of 0.8% Ni-NDC film.

**AFM Image.** Figure 3 is an AFM image of the 0.8% Ni-NDC film. The film surface was very flat with an average surface roughness of  $\sim 0.39$  nm. The root-mean-squared roughness ( $R_q$ ) was  $\sim 0.50$  nm.

The structural data of the novel Ni-NDC film studied by XPS, TEM, and XPS indicate that it is a thin flat film containing a 0.8% atomic concentration of nickel nanoparticles (mixed chemical



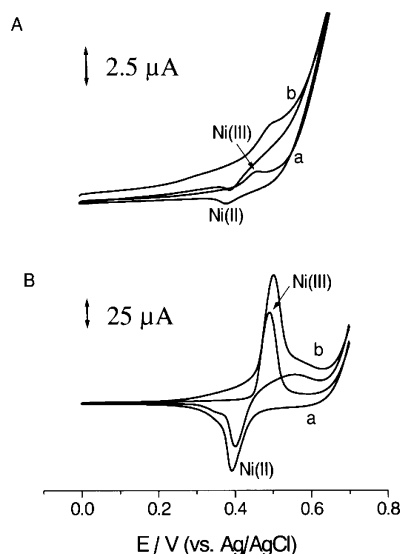


Figure 4. CVs obtained in 0.1 M NaOH solution (a) and 1 mM glucose (b) at 0.8% Ni-NDC film (A) and Ni-bulk (B) electrodes, respectively. Scan rate, 50 mV/s.

states including metallic nickel, NiO, Ni<sub>2</sub>O<sub>3</sub>, and Ni(OH)<sub>2</sub> highly dispersed and embedded homogeneously in disordered graphite-like carbon.

**Electrochemical Properties.** To evaluate the 0.8% Ni-NDC film electrode as an amperometric detector for carbohydrate detection, it was studied with regard to the electrooxidation of mono- and disugars as target compounds compared with the Ni-bulk electrode.

**Cyclic Voltammograms.** Figure 4 shows cyclic voltammograms obtained in 0.1 M NaOH solution (a) and in the presence of 1 mM glucose (b) at the 0.8% Ni-NDC film (A) and Ni-bulk (B) electrodes. The anodic and cathodic peaks in 0.1 N NaOH alkaline solution (curve a) are assigned to the Ni(II)/Ni(III) redox couple, which is thought to catalyze the oxidation of small organic molecules at the Ni electrodes.<sup>9,32–34</sup> The peak current of glucose (corrected for the background current of 0.1 M NaOH) is 3.5 and 32  $\mu$ A at the 0.8% Ni-NDC film and bulk electrodes, respectively. Given that the concentration of nickel particles in the Ni-NDC film electrode is only 0.8%, the current density at the Ni-NDC film electrode is calculated to be over 10 times that at the Ni-bulk electrode. Electrode fouling was not observed during 100 cycles of voltammetric measurements.

We studied the dependence of the scan rate on the peak current response of glucose at the 0.8% Ni-NDC film electrode in the 10–400 mV/s range. The peak current is proportional to the square root of the scan rate, indicating that sugar oxidation is a diffusion-limited process, which coincides with previous reports.<sup>32–34</sup> The mechanism of the sugar electrooxidation process at the 0.8% Ni-NDC film electrode is not by direct electron transfer from the sugars to the anode but by a surface catalytic reaction between the sugars and high-valence species of Ni, which is the same as that at a Ni-bulk electrode with a thin layer of nickel hydroxide.<sup>9,32–34</sup> The Ni-NDC film electrode worked as a Ni nanoarray

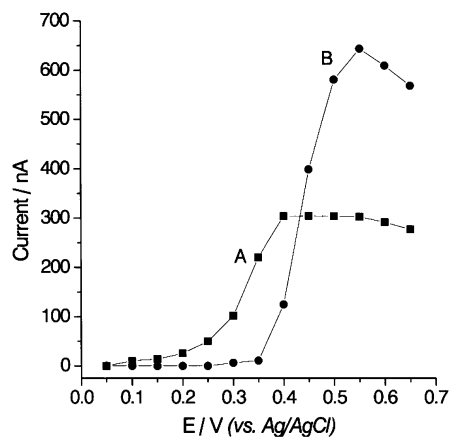


Figure 5. Hydrodynamic voltammograms of glucose at 0.8% Ni-NDC film (A) and Ni-bulk (B) electrodes.

electrode with regard to sugar oxidation because Ni nanoparticles were highly dispersed in the carbon matrix. Although the atomic concentration of Ni in the film was as low as 0.8%, it exhibited a high electrocatalytic ability for the oxidation of sugar, and therefore, we still observed diffusion-limited current at the Ni-NDC film electrode.

We investigated the 0.8% Ni-NDC film electrode for the electrooxidation of sugars by means of FIA and HPLC measurements to evaluate its application as an amperometric detector in a flow system.

**Hydrodynamic Voltammograms (HDVs).** Figure 5 shows the HDVs of glucose at 0.8% Ni-NDC film (A) and Ni-bulk (B) electrodes. The oxidation current of glucose at the Ni-bulk electrode was very low when the detection potential is lower than 0.40 V. Above 0.4 V, the current rapidly increased until it reached its highest peak value at 0.55 V. In contrast, the 0.8% Ni-NDC film electrode exhibited different HDV behavior during the glucose measurement. The current response at the film electrode increased gradually when the applied potential was lower than 0.25 V. It then increased quickly and reached its plateau above 0.40 V. The response at the 0.8% Ni-NDC film electrode decreased thereafter, but much more slowly than that at the Ni-bulk electrode. Compared with the Ni-bulk electrode, the 0.8% Ni-NDC exhibited a high oxidation current for the detection of glucose at a comparatively low applied potential of 0.40 V. This is because the electrocatalytic ability of the Ni-NDC film electrode is better as regards oxidizing glucose than the bulk Ni electrode at the lower applied potential. A Ni-NDC film electrode contains a mixture of Ni chemical states (0, II, III). This is unlike the Ni-bulk electrode, which needs a high potential for Ni(0) to be oxidized to Ni(III). We can expect enhanced sensitivity at the Ni-NDC film electrode because we can obtain a high current response for glucose oxidation and a low background current at a low detection potential.

**Flow Injection Analysis.** When conducting FIA experiments at the 0.8% Ni-NDC film electrode, we waited 0.5 h for the baseline of the film electrode to stabilize under a constant applied potential of 0.40 V. No baseline drifts occurred during the detection process. The response at the Ni-NDC film electrode decreased to 90% of its initial value and became stable after 2 h during the first day's use, whereas it decreased to 70% of its initial value at the Ni-bulk electrode. Figure 6 shows the FIA response of 100  $\mu$ M glucose

(32) MacArthur, D. M. *J. Electrochem. Soc.* **1970**, *117*, 422–426.

(33) Visscher, W.; Barendrecht, E. *J. Electroanal. Chem.* **1983**, *154*, 69–80.

(34) Hahn, F.; Beden, B.; Croissant, M. J.; Lamy, C. *Electrochim. Acta* **1986**, *31*, 335–342.

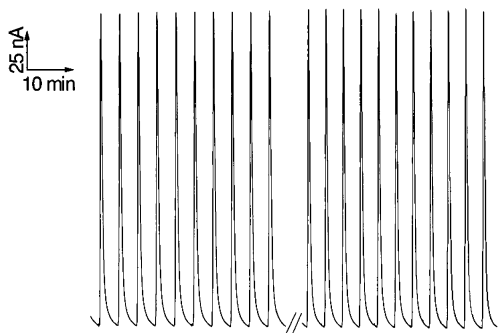


Figure 6. Reproducibility of 100  $\mu\text{M}$  glucose at 0.8% Ni-NDC film electrode. Conditions: 30- $\mu\text{L}$  injection; run buffer, 0.01 M NaOH; flow rate, 0.1 mL/min; detection potential, 0.40 V vs Ag/AgCl.

(30- $\mu\text{L}$  injection) at the 0.8% Ni-NDC film electrode. No significant loss was observed in the peak current signal with consecutive glucose injections ( $n = 40$ ). The relative standard deviation (RSD) was 1.75% ( $n = 40$ ), compared with an RSD of 9.7% obtained at the Ni-bulk electrode under the same condition. This indicates that the 0.8% Ni-NDC film electrode exhibits a better reproducibility than a Ni-bulk electrode because the film electrode surface is not easily passivated. The day-to-day stability was investigated for one month by estimating the precision with six injections of glucose each day. The RSD was less than 6.7%.

**HPLC Determination of Sugars.** We compared the performance of a Ni-NDC film electrode with that of a Ni-bulk electrode when they were used in combination with HPLC as the detection electrodes for sugars. Figure 7A shows a chromatogram of 10  $\mu\text{M}$  each of glucose, fructose, sucrose, and lactose detected at the 0.8% Ni-NDC film electrode, following separation by a Hamilton RCX-10 anion-exchange column (250  $\times$  4 mm i.d.) with a CarboPac PA 100 guard column (50  $\times$  4 mm i.d.) and a 0.03 M NaOH solution as the mobile phase at a 1.0 mL/min flow rate. The applied potential was 0.40 V. Good chromatographic separations of the four sugars were obtained. In contrast, the response of the sugars at the Ni-bulk electrode was very low under the same conditions (Figure 7B). The 0.8% Ni-NDC exhibits high sensitivity for the determination of sugars at the very low detection potential of 0.40 V. Figure 7C shows a chromatogram of a solution of 0.05  $\mu\text{M}$  mixed sugars. The detection limits (LODs) ( $S/N = 3$ ) calculated from Figure 7C are 20, 25, 50, and 37 nM (or 0.2, 0.25, 0.5, and 0.37 pmol) for glucose, fructose, sucrose, and lactose, respectively. These values are lower than those obtained at a Ni-bulk electrode (4 pmol for glucose)<sup>8</sup> and Ni alloy (between 0.5 and 1 pmol)<sup>15</sup> and Ni-based CME (between 3.3 and 5.5 pmol)<sup>22</sup> electrodes under optimized detection conditions (0.55 or 0.6 V as detection potential). The results including linear range, LODs, and precision are summarized in Table 1. The linear range for each sugar was over 3 orders of magnitude. The reproducibility of 10 cycles of HPLC detection showed a variation of less than 5.0% for each sugar at the 0.8% Ni-NDC film electrode.

The high sensitivity of the 0.8% Ni-NDC film electrode for the determination of sugars results from the Ni nanoarray structure in the carbon matrix. Two main factors contribute to the good stability. One is that Ni nanoparticles are difficult to detach from the film because they are embedded in the carbon matrix. Another factor could be the disordered graphite-like carbon conductive matrix in this film that may absorb impurities and thus play an

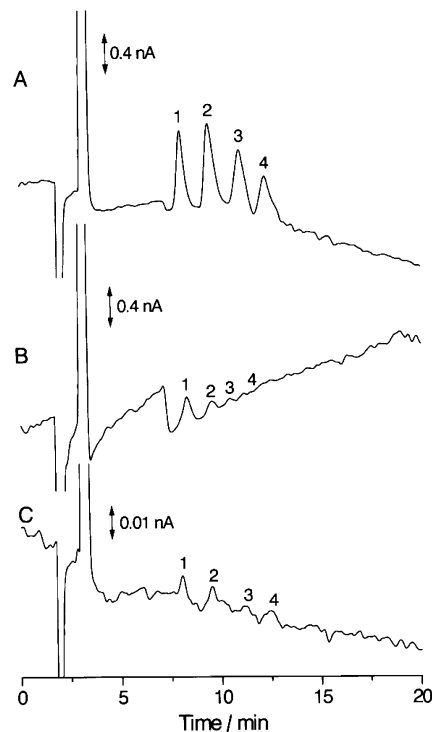


Figure 7. Liquid chromatogram separation of mixture of glucose (1), fructose (2), sucrose (3), and lactose (4) at 0.8% Ni-NDC (A, C) and Ni-bulk (B) electrodes carried out with 0.03 M NaOH as mobile phase. Separation column, Hamilton RCX-10 anion-exchange column (250  $\times$  4 mm i.d.) plus CarboPac PA 100 guard column (50  $\times$  4 mm i.d.); injection volume, 10  $\mu\text{L}$ ; concentration of sugars, 10  $\mu\text{M}$  (A, B), 0.05  $\mu\text{M}$  (C); flow rate, 1.0 mL/min; applied potential, 0.40 V vs Ag/AgCl.

Table 1. Quantitative Result for Sugars at a 0.8% Ni-NDC Film Electrode<sup>a</sup>

| compound | LOD <sup>b</sup> (nM) | Lin      | $r^2$ <sup>c</sup> |
|----------|-----------------------|----------|--------------------|
| glucose  | 20                    | 0.05–500 | 0.9999             |
| fructose | 25                    | 0.05–500 | 0.9999             |
| sucrose  | 50                    | 0.10–250 | 0.9997             |
| lactose  | 37                    | 0.08–250 | 0.9998             |

<sup>a</sup> The conditions are the same as those in Figure 7. <sup>b</sup> The LOD value was calculated based on  $S/N = 3$ . <sup>c</sup> The correlation coefficient ( $r^2$ ) was calculated in a linear range from 12 experimental data points.

important role in preventing surface fouling. A similar phenomenon whereby the carbon matrix contributed to the prevention of electrode surface passivation has also been reported.<sup>35</sup> The Ni-NDC film electrode has the advantages not only of high sensitivity and good stability but also that it can be formed in one step by the rf sputtering method at low temperature. It is unnecessary to modify the electrode substrates with Ni particles, and this improves the reproducibility of electrode formation. Therefore, with regard to sugar oxidation, the Ni-NDC film electrode has certain advantages over other Ni-based electrodes; namely, it is simple to prepare, is highly sensitive, and has good stability.

## CONCLUSION

A novel thin flat film, consisting of a 0.8% mixture of nickel state nanoparticles highly dispersed and homogeneously embed-

(35) Pocard, N. L.; Alsmeyer, D. C.; McCreery, R. L.; Neenan, T. X.; Callstrom, M. R. *J. Mater. Chem.* **1992**, *2*, 71–784.

ded in disordered graphite-like carbon (Ni-NDC), was successfully prepared by a simple rf sputtering method. We characterized the structure of the 0.8% Ni-NDC film and investigated its electrochemical behavior. An electrode made of this film was used to determine sugars at a low applied detection potential of 0.40 V with high sensitivity. Low detection limits of 20, 25, 50, and 37 nM were obtained for glucose, fructose, sucrose, and lactose, respectively. The film was proved to be a potential electrode

material for sugar detection by HPLC with high sensitivity and good reproducibility. Further experiments will be conducted to evaluate its potential, when combined with capillary electrophoresis separation, for determining other carbohydrates.

Received for review February 28, 2003. Accepted August 11, 2003.

AC034204K