

# Nickel oxide-modified composite electrode for electrochemical detection of polyhydroxyl compounds in liquid chromatographic analysis

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## Abstract

Nickel oxide-modified polymeric composite electrode was prepared for electrochemical detection in liquid chromatography (LC-ECD) or flow injection analysis of polyhydroxyl compounds. The composite electrode was prepared by incorporating semigraphitic carbon black and nickel oxide particles in polystyrene polymer matrix cross-linked with divinylbenzene. The analytical characteristics of the electrodes in LC-ECD and flow injection analysis of polyhydroxyl compounds were evaluated in terms of sensitivity, reproducibility, stability and surface renewability. The electrode revealed three–four orders of dynamic concentration ranges from sub-micromolar to  $10^{-3}$  M with high correlation coefficients from 0.9998 to 0.9999 for various polyhydroxyl compounds. The standard deviation for the surface restoration was <2%. High reproducibility, stability, and the renewability of surface are thought to be due to the catalyst particles highly dispersed through robust polymeric electrode matrix. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Electrochemical detection; Polyhydroxyl compounds; Liquid chromatographic analysis

## 1. Introduction

The analysis of polyhydroxyl compounds including carbohydrates has been a subject of great interest to analytical chemists because of their importance in physiological, clinical, medicinal, food and nutritional aspects. In contrast to the well-established separation methods of polyhydroxyl compounds using HPLC, detection methods of these analytes are not satisfactory yet. In commonly used UV-Vis detection techniques cumbersome time-consuming pre- or post-column derivatization is required to get appreciable sensitivity due to the lack of optically active functional groups

[1,2]. The sensitivity of a differential refractive index detector is relatively low and not suitable for low concentration level detection. On the other hand, electrochemical detection techniques have been concerned because of the possibility of direct detection with high sensitivity and wide dynamic concentration range of analysis [3–11]. Pulsed amperometric detection is one of the successful technique in which noble metal electrodes such as gold or platinum were used for the catalytic oxidation of carbohydrates or alditols [3,4]. However, complicated pulse schemes of cleaning, activation and detection were employed to overcome the fouling of electrode surface by oxidation products.

Simple constant potential amperometric detection of the polyhydroxyl compounds utilizing electrocatalytic properties of transition metals or their oxides has been extensively studied [6–11]. Formation of high

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valent oxyhydroxide species of transition metals such as Cu or Ni in alkaline media is known to be responsible for the catalytic oxidation of polyols [6]. The utilities of the copper and nickel metal electrodes were demonstrated with high sensitivity, but continuous anodization of the electrode surface destabilized background signal. Titanium alloy electrode containing nickel showed excellent analytical characteristics in terms of sensitivity, reproducibility, dynamic concentration range as well as long-term stability [12]. Although background drift owing to the continuous anodization of the electrode surface was suppressed, it took quite long time until the background stabilized.

Surface modified electrodes of metal oxides have been studied extensively [8,13–16]. High sensitivity resulting from catalytic activity of the metal oxides showed the possibility of the utilization of the electrodes for the detection of polyhydroxyl compounds by constant potential amperometry in alkaline media. However, the drawbacks of the surface modified electrodes are lack of the reproducibility, stability and surface renewability for practical routine analysis. Improvement of the renewability and reproducibility was possible by employing the modified carbon paste electrode technique but the stability [7,17]. A NiO-bulk modified graphite composite electrode was reported showing improved stability and renewability [11]. Nickel oxide particles were incorporated along with graphite powder in PVC composite matrix. THF was used as a solvent for slurry preparation for the casting of electrode. The electrode showed improved stability because of its robust matrix. The active surface could be restored easily by simple polishing process. However, the sensitivity of the electrode was inferior to those of the surface-modified electrodes or carbon paste electrodes modified with metal oxides. The reproducibility of surface restoration was only about 10–20% in relative standard deviation and still need improvement. A copper(I) oxide-modified graphite–polyethylene composite electrode revealing improved reproducibility, stability as well as surface renewability in the analysis of alditols and sugars was also reported [18]. However, the sensitivity was not comparable to other electrodes reported before [14,17]. Recently, we reported the preparation methods and analytical characteristics of cross-linked polystyrene-based CuO-modified composite electrodes, which revealed improved analytical characteristics in

respect of the sensitivity, reproducibility and stability as well as the surface renewability in the electrochemical detection of carbohydrates in LC analysis [11]. Improvement of the electrode characteristics was attributed from highly dispersed copper oxide particles and conducting carbon black through polymeric composite matrix. Here we report the analytical characteristics and applicability of nickel oxide-modified polymeric composite electrodes showing especially wider dynamic concentration ranges as well as sensitivity, reproducibility and stability in the analysis of the polyhydroxyl compounds compared to earlier works reported [10–12,17,18].

## 2. Experimentals

### 2.1. Reagents and instruments

All chemicals were reagent grade and used as received otherwise mentioned. All polyhydroxyl compounds were from Sigma and Aldrich. Fresh stock solutions of polyhydroxyl compounds were prepared everyday and stored in a refrigerator. They were diluted to appropriate concentrations with carbonate free sodium hydroxide solution. All solutions for LC-EC experiments were filtered with 0.45  $\mu\text{m}$  Millipore filters. BAS 100W electrochemical analyzer was used for voltammetric measurements. Pharmacia LKB HPLC pump 2150 dual head pump was used for mobile phase delivery. BAS amperometric detector LC-4C with a Rheodyne injector having 20 or 5  $\mu\text{l}$  loop and thin-layer flow cell was used for constant amperometric detection. CarboPac<sup>TM</sup> PA1 (4 mm  $\times$  250 mm) anion exchange column with CarboPac<sup>TM</sup> PA guard column from Dionex was used for separation of polyhydroxyl compounds. The surface morphology was observed using a scanning electron microscope (SEM, JSM 5200 from Jeol). Rigaku D/MAX II X-ray diffractometer was used for analysis of NiO powder.

### 2.2. Electrode preparation

Preparation procedure of the modified composite electrodes can be found elsewhere [12,19]. About 2 g of Ketjen black 600JD (semigraphitic carbon from Ackzo Chemie) was dispersed thoroughly in 200 ml of ethanol using a ultrasonicator. Pre-estimated amount

of nickel nitrate was dissolved in 300 ml of ethanol and mixed with the solution carbon black dispersed. Then the alcoholic content of the solution was adjusted to 30% approximately with deionized water. The solution was titrated with 2 M of NaOH solution slowly to form colloidal  $\text{Ni}(\text{OH})_2$  precipitates adsorbed on the carbon black surface. The mixture was filtered and washed with deionized water thoroughly, then dried at  $110^\circ\text{C}$  overnight. It was ground thoroughly with a commercial coffee bean grinder into fine powder.  $\text{Ni}(\text{OH})_2$  in the mixture was converted into NiO by heating at  $250^\circ\text{C}$  overnight in a convection oven and confirmed by XRD experiment. Finely ground carbon black/NiO mixture was added to a mixture of styrene, divinylbenzene and radical initiator AIBN (2,2'-azobisisobutyronitrile), then polymerized to form NiO polymeric composite electrode material in a sealed glass tubing at  $75^\circ\text{C}$  for 4–6 h. Typical preparative composition is 19% of NiO, 59% of styrene, 16% of divinylbenzene, 5% of carbon black and 1% of AIBN. The electrode material was fabricated into proper types of electrodes for voltammetric measurements or LC-EC experiments. The electrode was 3.0 mm in diameter. The electrodes were ground and polished by ordinary electrode surface polishing procedure.

### 3. Results and discussion

#### 3.1. Physical characteristics of the electrode

The NiO-modified composite electrodes were mostly hard and suitable to be machined. The content of carbon black should be fixed to 5–5.5 wt.% in order to maintain proper electrical conductivity of the electrode. The content of nickel oxide in the electrode matrix was limited by the mechanical strength of the electrode matrix. Maximum loading of NiO was achieved to the level of 26 wt.%. The SEM surface image of the electrode containing 12.8% of NiO is as shown in Fig. 1. The white spots represent NiO particles while the black background is believed to be carbon black and polymeric composite matrix. The particles were randomly distributed over the surface but not uniform in size. They were ranged from sub-micron to mostly several  $10\ \mu\text{m}$  in size. It was hardly to get uniform nickel oxide particles due to the coagulation of nickel hydroxide precipitates during filtering and washing step. High loading of nickel oxide resulted in large particle size mostly and was not helpful for enhancement of the catalytic activity because of small active surface area exposed. The content of nickel oxide was optimized to 19 wt.%.

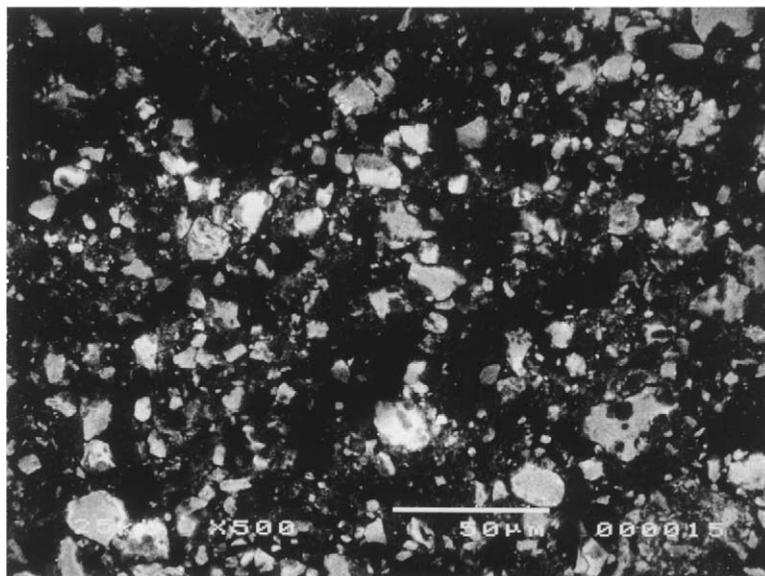


Fig. 1. Scanning electron microscopic image of the polished surface of NiO-modified composite electrode containing 12.8% of NiO.

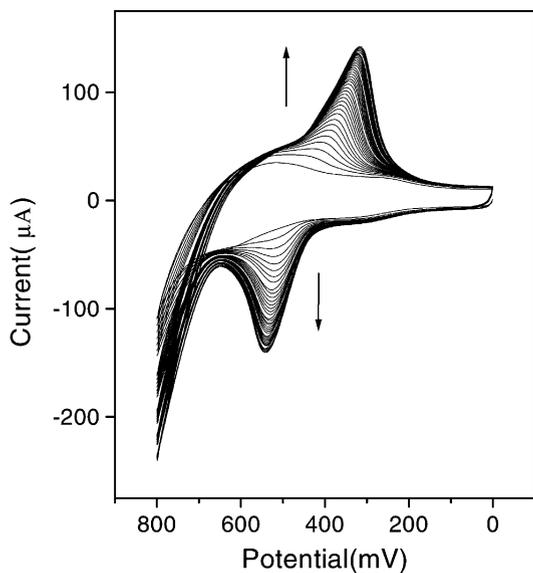


Fig. 2. Voltammetric behavior of 12.8 wt.% NiO-modified composite electrode in 0.10 M NaOH, scan rate 50 mV/s vs. Ag/AgCl.

### 3.2. Electrochemical characteristics of the electrodes

A cyclic voltammogram of the electrode containing 12.8% of NiO in 0.10 M NaOH solution is as shown in Fig. 2. The redox behavior of Ni(II)/Ni(III) was not clearly defined at the beginning of potential cycling. The anodic and cathodic peak currents increased gradually with increasing peak separation until steady state was reached. It is believed that NiO particles were transformed to Ni(OH)<sub>2</sub> by hydration upon the exposure of the electrode surface to solution. Then the modifier was oxidized from Ni(OH)<sub>2</sub> to NiOOH back and forth by potential cycling. Structural change of the exposed modifiers is assumed from Ni(OH)<sub>2</sub> to NiOOH and vice versa during the potential cycling. Gradual propagation of the structural change occurs toward inside of the modifier particles, and most of the modifier particles exposed at the surface become electrochemically active at steady state. It took more time to reach to steady state for the electrode having larger particle sizes of the modifier.

Fig. 3 is a cyclic voltammogram showing catalytic oxidation of sorbitol. The anodic peak current from around +600 to +700 mV versus Hg/HgO (0.10 M NaOH) reference electrode increased upon the

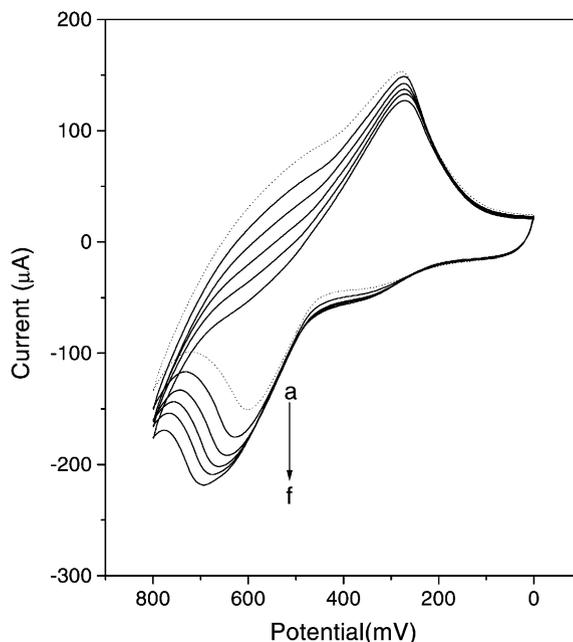


Fig. 3. Cyclic voltammograms showing catalytic oxidation of sorbitol. (a) 0.1 M NaOH blank solution, (b–f) 1.0–5.0 mM of sorbitol, 12.8 wt.% NiO-modified composite electrode, scan rate 50 mV/s vs. Ag/AgCl.

consecutive additions of sorbitol to the blank solution while the cathodic peak current decreased. It is thought that all of the active NiOOH did not participate in the oxidation of sorbitol resulting incomplete decrease of the cathodic current. Presumably it is due to the morphological limitation of the mass transfer of sorbitol inside the active NiOOH modifier. Same situation might occur in hydrodynamic voltammetry of polyols. In flow system, analytes can be oxidized catalytically only at the surface of the modifier because they do not have enough time for diffusion inside the modifier particles. Therefore, high population of the modifier particles having small uniform size distribution is desired for better performance of the electrode rather than high loading of the modifier causing large particle size distribution. Although catalytic oxidation of sorbitol seemed to be clear, the catalytic oxidation rate was sluggish yet to shift oxidation peak potential to positive direction upon consecutive additions of more sorbitol. Cyclic voltammetric behavior of the electrode for other polyhydroxyl compounds was similar to that of sorbitol.

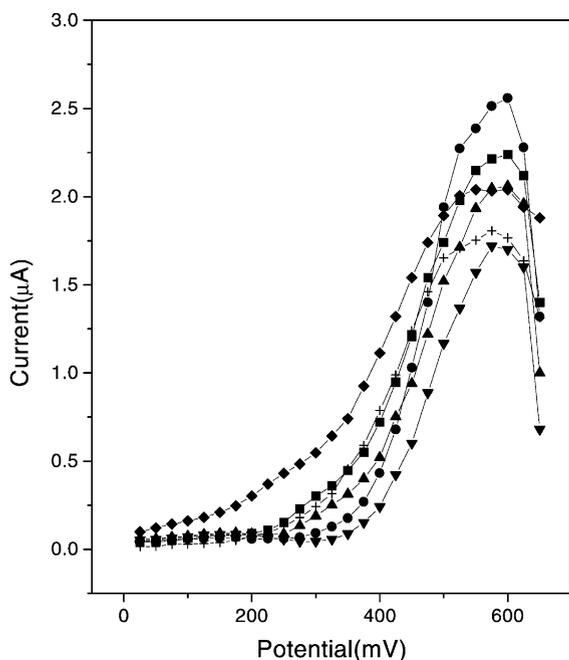


Fig. 4. Hydrodynamic voltammograms of polyhydroxyl compounds: (■) sorbitol; (●) inositol; (▲) xylitol; (▼) erythritol; (◆) glucose; (+) fructose; mobile phase, 0.10 M NaOH; flow rate, 0.5 ml/min; 20  $\mu$ l injection, without column, 19.2% NiO-modified composite electrode vs. Hg/HgO (0.10 M NaOH).

### 3.3. Analytical characteristics of the electrode

Hydrodynamic voltammetric experiments for typical polyhydroxyl compounds such as sorbitol, inositol, xylitol, erythritol, glucose, and fructose were performed to determine proper working electrode potential for LC-ECD (Fig. 4). Although there were more or less different in response, the increase of catalytic oxidation current started mostly at +250–350 mV versus Hg/HgO reference electrode and the maximum responses were observed from around +550 to +600 mV. The current decreased above +0.65 V due to the competitive background oxidation, mainly oxygen evolution, which perturbed mass transfer of polyols. So, +550 mV was selected as working electrode potential for LC-ECD.

Dynamic concentration range of the detector is one of the important factors in practical LC analysis. The response factor for each polyol was almost constant for three–five orders of concentration ranges

from sub-micromolar to  $10^{-3}$  M resulting wide dynamic ranges (Fig. 5). It was not tested for higher concentration than 1 mM due to the limitation of the current scale of the potentiostat used in this study. It is worthwhile to emphasize the improved performance of the NiO-modified composite electrode in the linear concentration ranges for various polyols. For the comparison, the linear dynamic range of the CuO-modified composite electrode prepared in our laboratory were three orders of concentration ranges of analytes [11]. The correlation coefficients of the calibration curves were ranged between 0.9998 and 0.9999. The sensitivities of the electrode were  $330 \mu\text{A}/\text{mM cm}^2$  for sorbitol and  $270 \mu\text{A}/\text{mM cm}^2$  for glucose. They are comparable to the results of other composite electrodes such as NiO-modified composite electrode based on graphite/PVC matrix ( $2.0 \mu\text{A}/\text{mM cm}^2$  for sorbitol estimated from Table 1 in [10]), Cu<sub>2</sub>O-modified graphite–polyethylene composite electrode ( $14 \mu\text{A}/\text{mM cm}^2$  for sorbitol) [18], nickel–titanium alloy electrode ( $25 \mu\text{A}/\text{mM cm}^2$  for glucose) [12], Cu<sub>2</sub>O-modified carbon paste electrode ( $215 \mu\text{A}/\text{mM cm}^2$  for glucose) [17] reported by others, and CuO-modified composite electrode prepared in our laboratory ( $365 \mu\text{A}/\text{mM cm}^2$  for glucose) [11]. Analytical characteristics of the electrodes are abbreviated in Table 1.

Major advantages of the NiO-modified composite electrode are improved reproducibility of the response and surface renewability. Since the electrode contains the modifier through the electrode matrix uniformly the surface can be renewed easily by simple polishing procedure whenever the electrode become contaminated or deactivated. For the test of the surface renewability the electrode surface was renewed for 10 trials, then the reponse of the electrode was measured at +550 mV by injecting 20  $\mu$ l of 0.1 mM sorbitol 10 times for each trial. The relative standard deviation of the averaged signals was 1.6%, which was comparable to 10–20% for the NiO-modified composite electrode based on the graphite/PVC matrix [10]. The reproducibility of the electrode was also estimated by pooling the data obtained during surface renewability test. The relative standard deviation of the pooled data ( $n = 100$ ) was 0.83% only. Since long-term stability of the detector in HPLC analysis is essential, the stability of the electrode response was also tested. Average response for every 10 injections of 20  $\mu$ l of

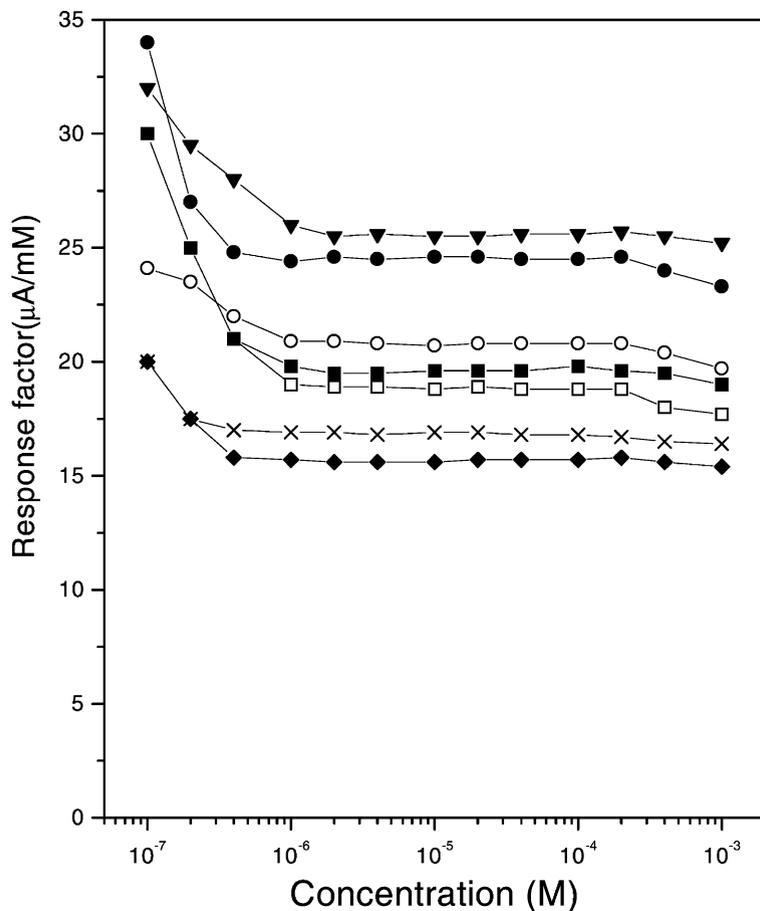


Fig. 5. Plots of response factors ( $\mu\text{A}/\text{mM}$ ) vs. concentration of polyhydroxyl compounds: (●) sorbitol; (○) xylitol; (■) adonitol; (□) mannitol; (▼) inositol; (×) erythritol; (◆) arabitol; 19.2 wt.% NiO-modified composite electrode,  $E_{\text{app}} = 550 \text{ mV}$  vs. Hg/HgO (0.10M NaOH), mobile phase, 0.10M NaOH; flow rate, 0.5 ml/min; 20  $\mu\text{l}$  injection, without column.

Table 1

Analytical characteristics of the NiO-modified composite electrode<sup>a</sup> in LC-ECD analysis of polyhydroxyl compounds

Compound	$I (\mu\text{A}) = a + bC (\text{mM})^b$			Linear range <sup>c</sup> ( $\mu\text{M}$ )	Detection limit <sup>d</sup> (mM)
	$a \pm t_{95} s_a$	$b \pm t_{95} s_b$	$r$		
Sorbitol	$0.053 \pm 0.076$	$23.4 \pm 0.2$	0.9999	0.2–1000	0.044
Inositol	$0.022 \pm 0.034$	$25.2 \pm 0.1$	0.9999	0.4–1000	0.042
Xylitol	$0.055 \pm 0.079$	$19.8 \pm 0.2$	0.9999	0.4–1000	0.052
Erythritol	$0.013 \pm 0.015$	$16.4 \pm 0.1$	0.9999	0.2–1000	0.064
Mannitol	$0.013 \pm 0.033$	$17.7 \pm 0.1$	0.9999	1–1000	0.057
Arabitol	$0.011 \pm 0.027$	$15.4 \pm 0.1$	0.9999	0.4–1000	0.069
Adonitol	$0.033 \pm 0.052$	$19.0 \pm 0.2$	0.9999	0.4–1000	0.055
Glucose	$-0.005 \pm 0.008$	$19.2 \pm 0.2$	0.9998	0.1–1000	0.056
Fructose	$-0.004 \pm 0.026$	$16.4 \pm 0.1$	0.9998	0.2–1000	0.065

<sup>a</sup> 19.2 wt.% NiO;  $E_{\text{app}} = 550 \text{ mV}$ ; flow rate, 0.5 ml/min; 20  $\mu\text{l}$  injection.

<sup>b</sup> The  $t$  was taken at 95% of the confidence level ( $n = 12$ ).

<sup>c</sup> The linear range was estimated by the criteria of  $\pm 10\%$  of average response factor.

<sup>d</sup> The detection limit was estimated for  $S/N = 3$  from the signal of 2  $\mu\text{M}$  of each analyte.

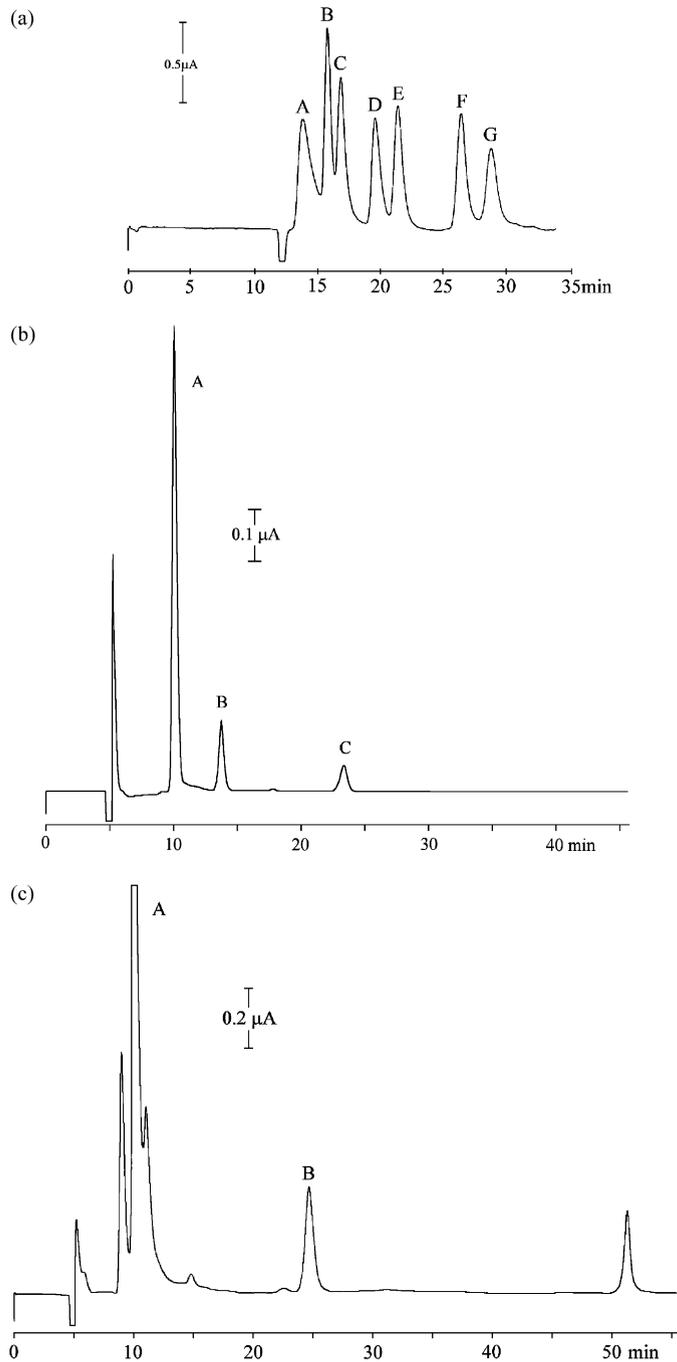


Fig. 6. Liquid chromatograms of polyhydroxyl compound mixtures. (a) A synthetic mixture of (A) 0.05 mM ethanol, (B) 0.10 mM inositol, (C) 0.10 mM sorbitol, (D) 0.10 mM xylitol, (E) 0.10 mM erythritol, (F) 0.10 mM glucose, (G) 0.10 mM fructose. (b) Enzymatic hydrolysis product of barley starch, (A) glucose, (B) endogenous peak from  $\alpha$ -amylase, (C) maltose. (c) Acid hydrolysis product of barley, (A) glucose, (B) maltose. 0.30 M NaOH, CarboPac<sup>TM</sup> PA1 (4 mm  $\times$  250 mm) anion exchange column with CarboPac<sup>TM</sup> PA guard column, flow rate 0.1 ml/min for the synthetic mixture and 0.25  $\mu\text{l}/\text{min}$  for the barley starch samples, 20  $\mu\text{l}/\text{injection}$ .

0.10 mM sorbitol with an hour interval was monitored for 50 h. Although there were some fluctuations, the electrode was not much deactivated during experiment. After 50 h, the average response reached to 96% of the initial value. This result is very similar to that of CuO-modified composite electrode prepared in our laboratory (2.4% decrease in 50 h) [11]. It is comparable to those of Ru<sub>2</sub>O-modified carbon paste electrode (8% decrease in 48 h) [7] and NiO-modified graphite/PVC composite electrode (5% within 72 h) [10]. The improved stability, reproducibility of the electrode response and surface renewability are due to the uniform distribution of the modifier particles held strongly by physically stable cross-linked polymeric binder through the electrode matrix. These characteristics of the electrode afford practical applicability of the NiO-modified composite electrode in long-term HPLC analysis.

A synthetic mixture of four polyhydroxyl compounds and two carbohydrates (Fig. 6a), and hydrolysis products of barley starch (Fig. 6b and c) were separated using an anion exchange column, then detected with the NiO-modified composite electrode to demonstrate practical applicability of the electrode in LC-ECD analysis. The barley starch was either enzymatically hydrolyzed with  $\alpha$ -amylase in pH 4.8 buffer at 40 °C for 12 h or acid-hydrolyzed with 2 M HCl at 90 °C for 30 min. The mixtures were successfully separated using mobile phase of 0.30 M NaOH and detected.

In conclusion, we have developed NiO-modified composite electrodes having improved analytical characteristics in LC-ECD analysis of polyhydroxyl compounds and carbohydrates. The electrode showed wide dynamic concentration range of three–five order of magnitude with high stability and reproducibility. These improved characteristics of the electrodes were mainly due to physical and chemical stability of catalytic modifier. The modifier particles, NiO

were randomly distributed with small particle size and strongly bound by cross-linked polymeric matrix through the electrode matrix. And they were exposed to the surface upon polishing with high repeatability and showed high catalytic activity. The results obtained in this work ensure the practical applicability of the NiO-modified composite electrode in LC-ECD analysis of polyhydroxyl compounds and carbohydrates. Further utilization of metal oxide-modified composite electrodes in detection of other organic compound is under investigation.

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