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Nickel content dependence of electrochemical behavior of carbohydrates on a titanium–nickel alloy electrode and its application to a liquid chromatography detector

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Abstract

A highly sensitive amperometric detector for the liquid chromatographic analysis of carbohydrates has been developed based on the optimization of the Ni content in a Ni–Ti alloy electrode. An optimum nickel content of 30 at. % was obtained from a signal-to-noise analysis in a flow injection experiment on glucose in 0.1 *M* NaOH. An optimum working potential of 0.5 V vs. Ag/AgCl was obtained from a hydrodynamic voltammogram. The glucose detection limit in the flow injection analysis was 11 fmol. The electrode was applied to an LC detector with a microbore column and the detection limit was 50 fmol.

Keywords: Electrochemical detection; Detection, LC; Nickel–titanium electrode; Titanium–nickel electrode; Electrodes; Carbohydrates

1. Introduction

The detection of carbohydrates is very important in various fields including food analysis and clinical testing. High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have provided an effective method for separating carbohydrates [1,2], however, very weak optical absorbance in the UV–Visible range and slight differences in refractive index make highly sensitive detection impossible. Highly sensitive detection can been achieved with HPLC or CE using the pre-column or post-column derivatized fluorescence methods [3,4] or pulsed amperometric detection [5,6]. However, amperometric detection with a fixed potential is desirable because of its simplicity and lower detection limit. Metallic electrodes, such as nickel and copper, are known to oxidize carbohydrates electrocatalytically [7–10]. The Ni–Ti (1:1) alloy electrode, in particular, offers good reproducibility and a long lifetime for carbohydrate detection [9].

Microelectrodes are of interest for several reasons including their small iR drop, fast establishment of steady-state mass transfer, and small capacitive charging currents. The Ni–Ti alloy electrode can be considered an atomically arrayed microelectrode. Titanium oxide is formed on the surface during the first anodic scan in an alkaline solution, and this

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titanium oxide surrounds many small nickel oxide dots and serves as an insulator, as shown in Fig. 1. In this structure, however, the distance between the nickel oxide dots is very small compared with the diffusion distance of the analyte. Therefore, the diffusion profiles of each dot overlap and the electrodes act as one large electrode. In an electrode with a smaller nickel content, some of the nickel atoms are isolated electrically and there would be a greater separation between the nickel dots. This results in a higher signal-to-noise ratio. In this study, we made Ni–Ti alloy electrodes containing different amounts of nickel and examined the variations in the electrochemical behavior of carbohydrates with nickel content. We then used an electrode with an optimized nickel content for the highly sensitive detection of carbohydrates by liquid chromatography. We also examined the use of a microbore column for improving the sensitivity.

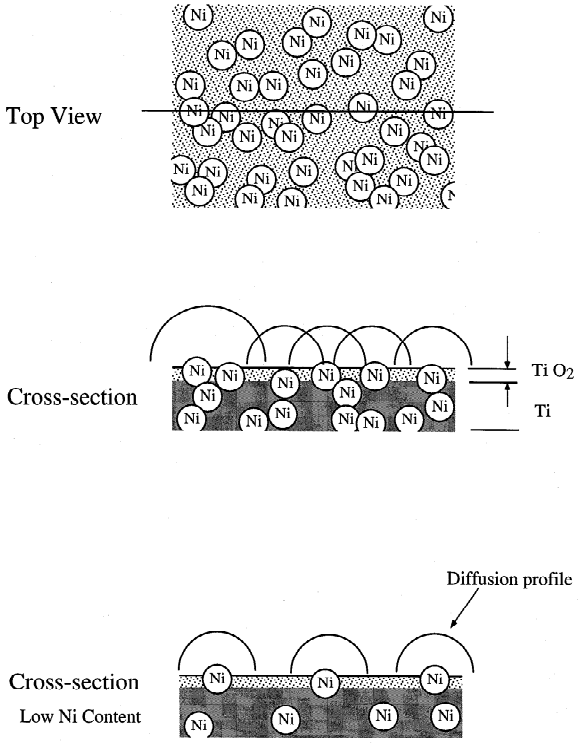

Fig. 1. Schematic diagram of diffusion profile at Ni–Ti microelectrode array.

2. Experimental

The Ni–Ti alloy electrodes were made by the radio frequency (RF) magnetron sputtering method, because it is easy to change the Ni content in the electrode. The shielding mask covers the glass plate substrate and the electrode shape is shown in Fig. 2. The sputtering target was an Ni–Ti (1:1) plate (127 mm in diameter) partly covered with Ti chips (5×5 mm) or a Ti plate (127 mm in diameter) partly covered with Ni chips (5×5 mm) and the Ni contents were varied by changing the number of Ti or Ni chips. The nickel content was determined by inductively coupled plasma (ICP) emission spectroscopy and a linear relation was obtained for the surface Ni coverage. Table 1 shows the results of ICP analysis for the electrode we made.

We used Ni–Ti alloy rod (1 mm in diameter) with 30% Ni content which was generously donated by Tokin, Sendai, Japan. It was filed to a smaller diameter (0.5 mm) while being rotated in the chuck of an electric drill. It was then surrounded by a thermally shrinkable tube and one end of each rod was polished into a disk shape with a mirror-like finish. The finished electrode was inserted into a PTFE tube with a 1 mm inner diameter which had two small holes around the electrode position to allow eluent to flow out. It was then set up as a working electrode in an electrochemical detector cell using a cross type union (Upchurch) as shown in Fig. 3. The electrode was positioned very close to the exit of the peek tubing (0.1 mm inner diameter) connected to the separation column to minimize the dead volume. The exit end of the peek tubing was filed to give it an outer diameter of 1 mm so it could be fitted into the PTFE tubing mentioned above.

Cyclic voltammetry (CV) was performed with a BAS-100 electrochemical analyzer, and flow injection analysis (FIA) was carried out with a BAS LC-4C amperometric detector, a BAS PU-100 pump and a Reodyne Injector. The sputter deposited electrode was attached to the inlet port of a thin-layer radial flow cell with a 12 or 25 μm thick PTFE spacer [11]. The electrode potential was +0.50 V vs. Ag/AgCl. A Dionex CarboPac PA1 anion-exchange column and a modified microbore version of the Hamilton RCX-10 anion-exchange column were used for the LC separation. The microbore column

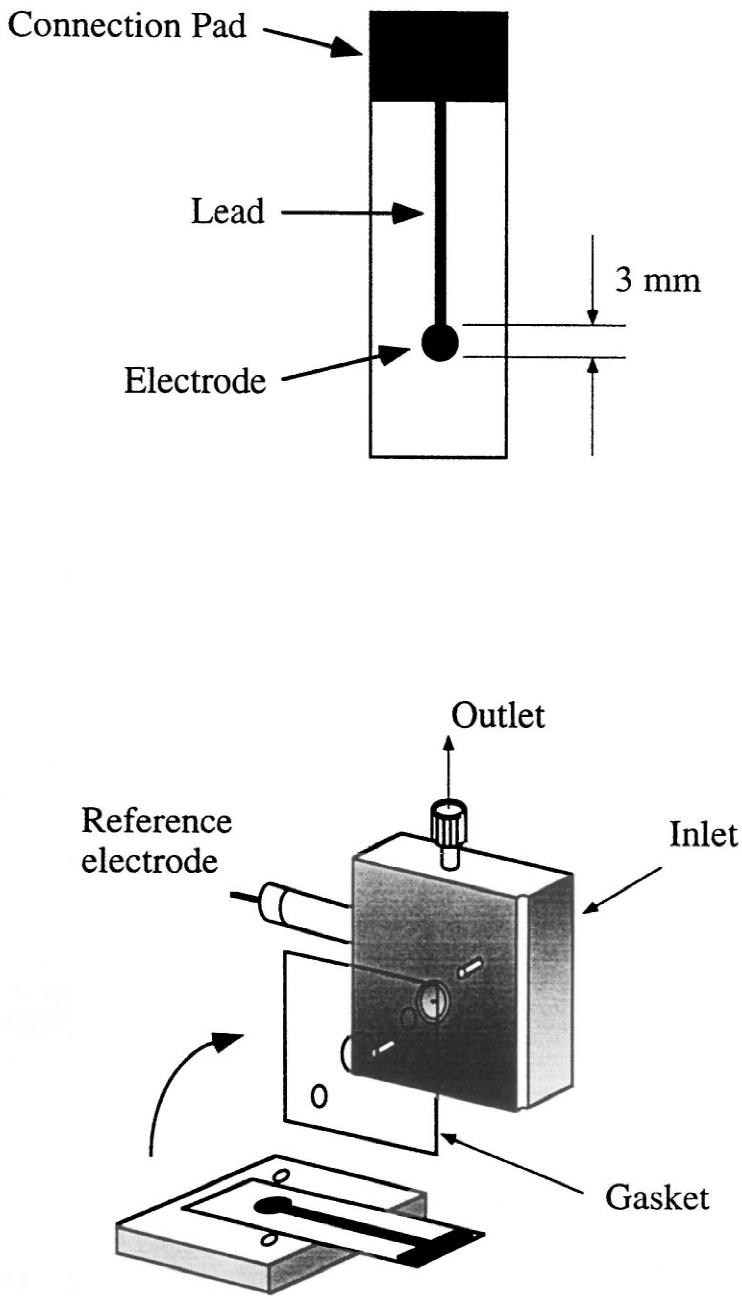

Fig. 2. Shape of sputter deposited Ni–Ti alloy thin film electrode.

was made by repacking Hamilton RCX-10 anion-exchange resin into stainless steel tubing whose inner diameter and length were 1 mm and 20 cm, respectively. The repacking procedure was as follows. Resin was withdrawn from an original column. The excess amount was slightly more than that required

Table 1
Ni content in sputter deposited electrodes and limit of detection for glucose

Target	Number of chips	Ni content (at. %)	S/N	LOD[a] ($S/N=3$)
Ni–Ti	None	53.3	16.7	45 fmol
Ni–Ti	50 (Ti chip)	40.5	51.7	15
Ni–Ti	75 (Ti chip)	31.3	66.7	11
Ti	20 (Ni chip)	17.4	63.0	12
Ti	10 (Ni chip)	12.3	30.0	25

[a] Limit of detection.

for the volume of the stainless tubing. The resin was then washed several times with packing solution (0.1 *M* NaOH) and packed at a flow-rate of 0.2 ml/min under 120 kg/cm^2 pressure.

All the carbohydrate samples were obtained from Kanto (Tokyo, Japan) and used as purchased. The carbohydrate solutions were prepared from Milli-Q system (Millipore, MA, USA) water. The required sample solutions were obtained by serial dilutions in a mobile phase solution (0.1 *M* NaOH).

3. Results and discussion

During the first CV cycle in 0.1 *M* NaOH solution, a large oxidative peak was observed corresponding to the initial formation of titanium oxide with every electrode and small reversible peaks were observed corresponding to Ni(II)/Ni(III) waves with repeated scanning. From the second scan, the oxidative peak for the formation of titanium oxide was hardly observed. This means that the surface titanium was oxidized at the first scan and this titanium oxide layer would serve as an insulator. This phenomenon is advantageous because electrical contact with the nickel dots would be realized by the unoxidized titanium metal under the thin oxidized titanium while the nickel content was low, as shown in Fig. 1.

The electrocatalytic responses in CV were observed with a 1 m*M* glucose solution. The oxidative peak was proportional to the square root of the scanning rate in an electrode with a 50% Ni content. This means that the diffusion profile to each Ni microelectrode overlapped and the process was diffusion controlled. Then the peak shape became steady-state with decreasing nickel content, resulting in a microelectrode effect. The ratio of electrocatalytic to nickel oxidation currents increased with decreasing nickel content. This means that the electrocatalytic oxidation of glucose by nickel oxide occurred effectively in the nickel microelectrode array.

The FIA and signal-to-noise (S/N) ratio for the

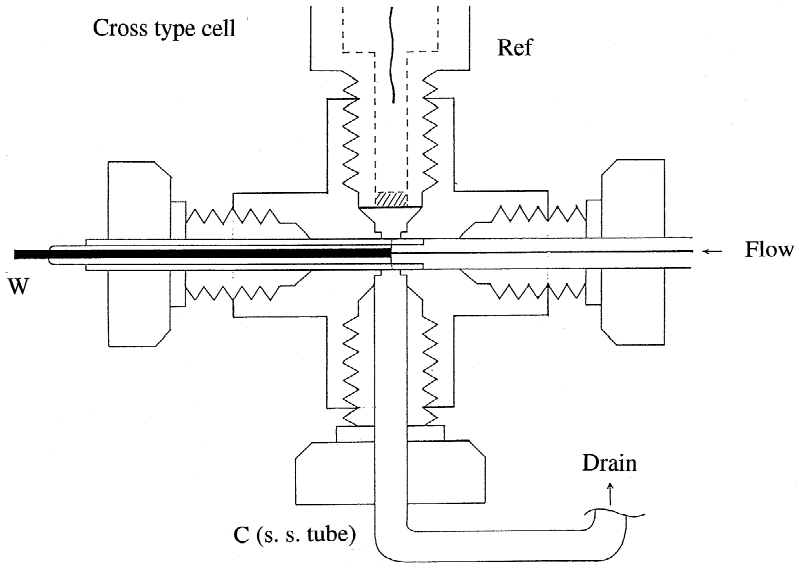

Fig. 3. Electrochemical detector cell using a cross type union.

oxidation of glucose were studied at sputter deposited different Ni content electrodes as shown in Fig. 4, and the S/N and the limit of detection was summarized in Table 1. The noise level in the FIA decreased with decreasing nickel content as did the signal current. However, the reduction in the signal current in the 50–30% nickel content region was not very large. This is because the diffusion profile of glucose to each Ni microelectrode became hemispherical rather than planar though its shape was

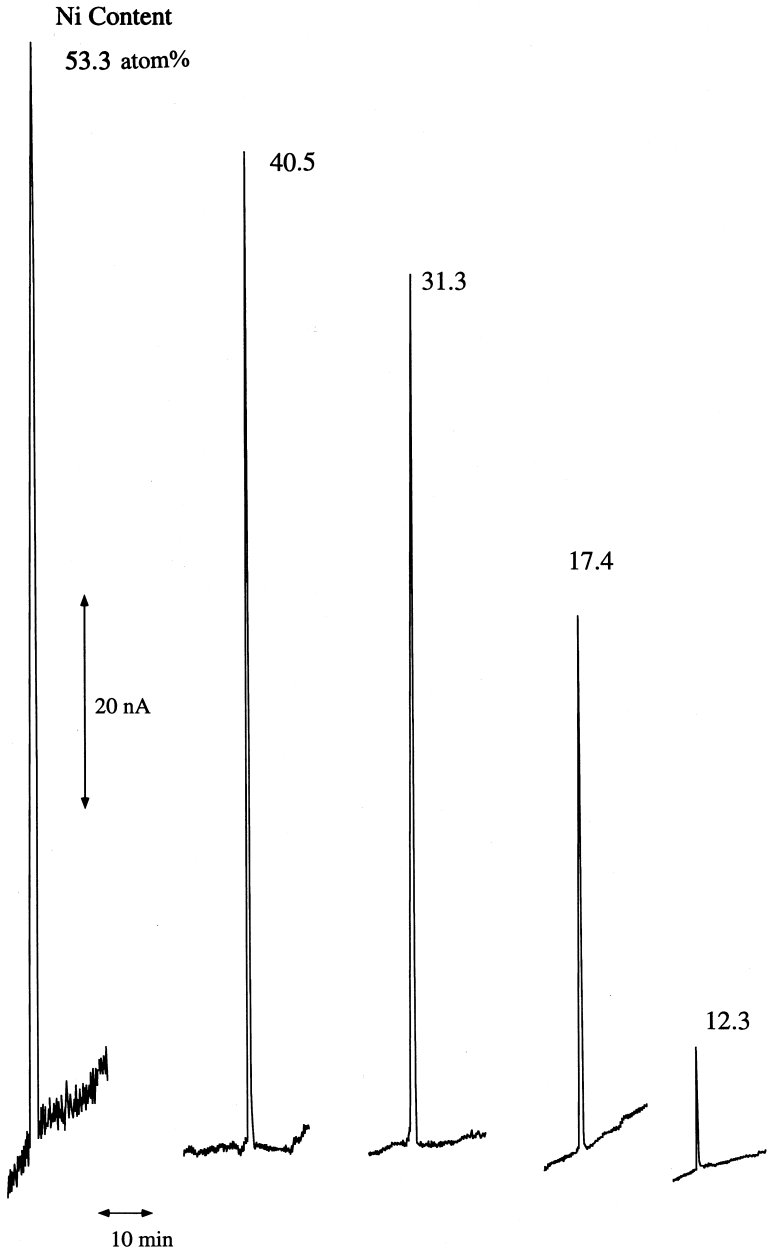

Fig. 4. Current response for flow injection analysis at different Ni content electrodes. The flow-rate was 100 μl/min, the working potential 0.5 V vs. Ag/AgCl, 2.5 pmol glucose in 0.1 *M* NaOH.

distorted by the flow of the solution. Even in such a case, the number of analyte molecules which diffuse to a unit area of electrode is much larger than that found in planar diffusion profiles at a large electrode. However, when the Ni content is very small, the Ni electrodes themselves are very few and small, resulting in higher resistivity and a change in the electron transfer properties between the electrode and the analyte. Therefore, the nickel content dependence of the S/N ratio reached its maximum at a nickel content of about 30%.

The hydrodynamic voltammogram of glucose at a Ni–Ti (30:70) sputter deposited electrode was examined, as shown in Fig. 5. The S/N ratio was analyzed at each working potential to select the best potential for LC detection. At low working potentials, the background current was small, as was the signal current, resulting in a low S/N ratio. At high potentials, the signal remained constant, while the background increased. The S/N ratio reached its maximum at a potential of 0.5 V vs. Ag/AgCl, which is approximately the same as the half-wave potential of the hydrodynamic voltammogram. The stability of the electrode was tested by repeatedly injecting 500 pmol of glucose at a working potential of 0.5 V per hour. The overall relative standard deviation was less than 2.6% ($n=30$). An 11 fmol detection limit for glucose was obtained with a Ni–Ti (30:70)electrode in an FIA experiment.

An Ni–Ti (30:70) rod electrode was used as an HPLC detector. First, a Dionex CarboPac PA1 anion-exchange column with 0.1 *M* NaOH as the mobile phase was used for the separation (Fig. 6). The applied potential was 0.5 V vs. Ag/AgCl, the flow-rate was 0.2 ml/min, the sample injection solution (1 μl) contained 50 pmol of L-fucose (fuc), D-galactosamine (galN), D-glucosamine (gluN) and D-glucose (glu). The microelectrode effect meant that only about 10 min was required for the stabilization of the background current. This is much quicker than with conventional electrodes. By calculation from each peak current, the sensitivities for these samples were

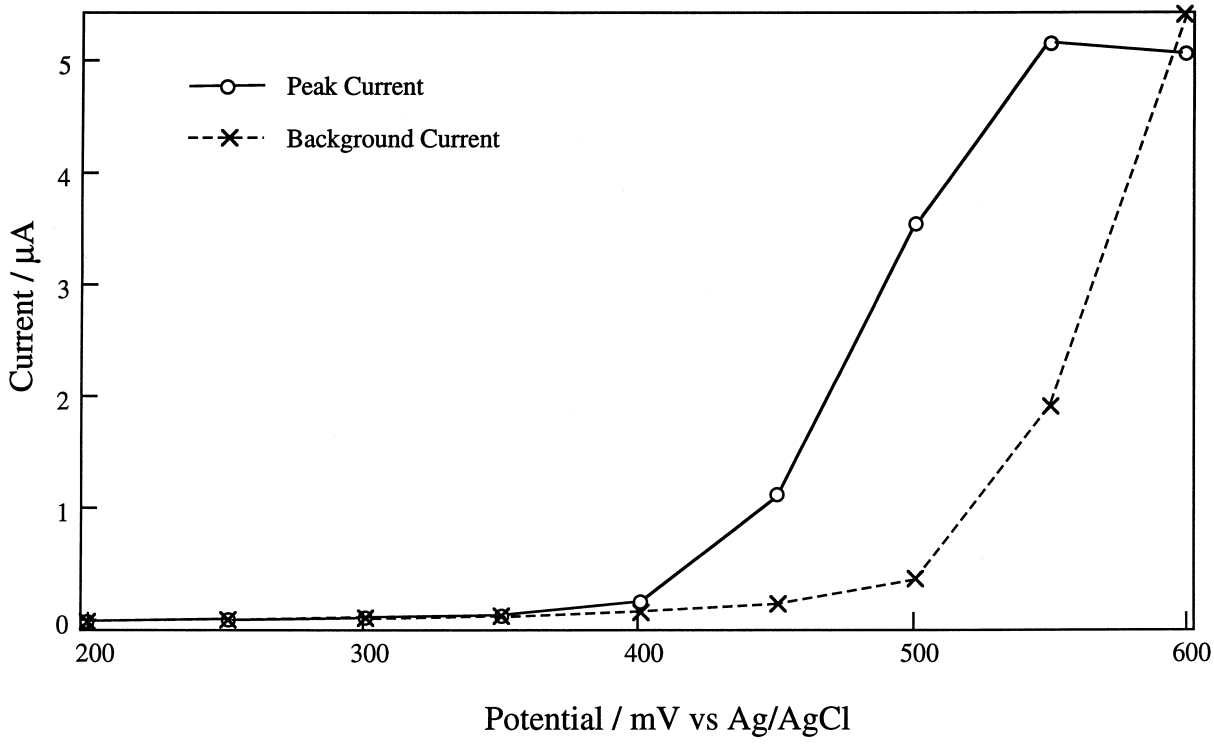

Fig. 5. Hydrodynamic voltammogram of glucose at a Ni–Ti (30:70) sputter deposited electrode using a radial flow cell with a 12 μm spacer. The flow-rate was 15 μl/min, 500 pmol glucose per injection.

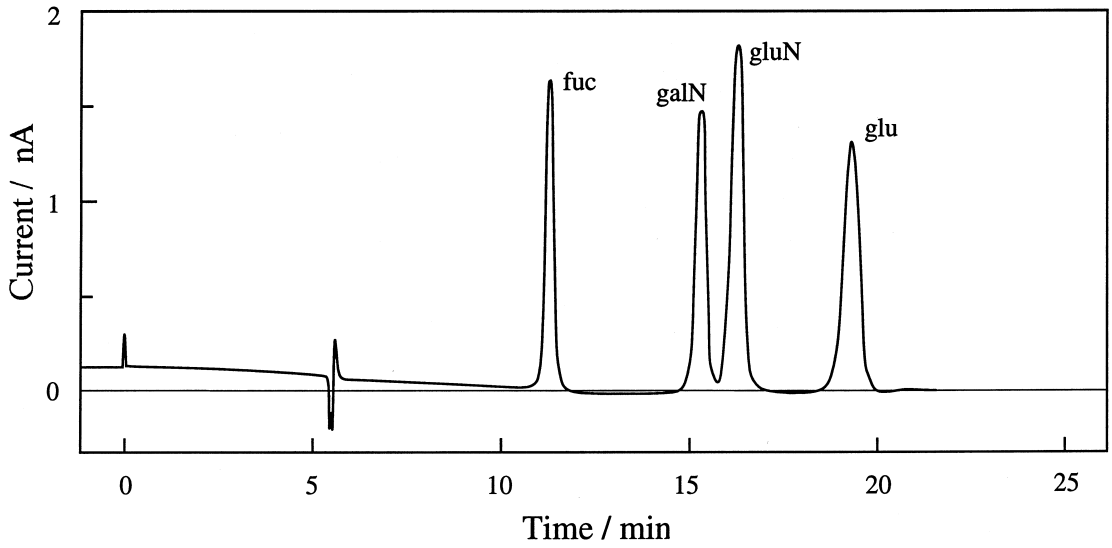

Fig. 6. Chromatogram of fucose, galactosamine, glucosamine and glucose at a Ni–Ti (30:70) electrode. Stationary phase: Dionex CarboPac PA1 column. Mobile phase: 0.1 *M* NaOH at a flow-rate of 0.2 ml/min. A 50 pmol concentrationin each injection.

32 pA/pmol for fuc, 29 pA/pmol for galN, 38 pA/pmol for gluN and 28 pA/pmol for glu.

The Dionex column had a 4 mm inside diameter, therefore the elution volume and band-broadening was rather large, resulting in reduced sensitivity. A column with a 1 mm or sub-millimeter diameter offers the advantage of low solvent consumption, a smaller sample volume requirement and smaller volume of eluting solute bands. Compared with a 3.2 mm I.D. column, a 1 mm I.D. column provides a nine times lower detection limit (by amount) [12]. To retain this advantage of the microbore column in the post column detection port, electrochemical detection is an attractive means of attaining small

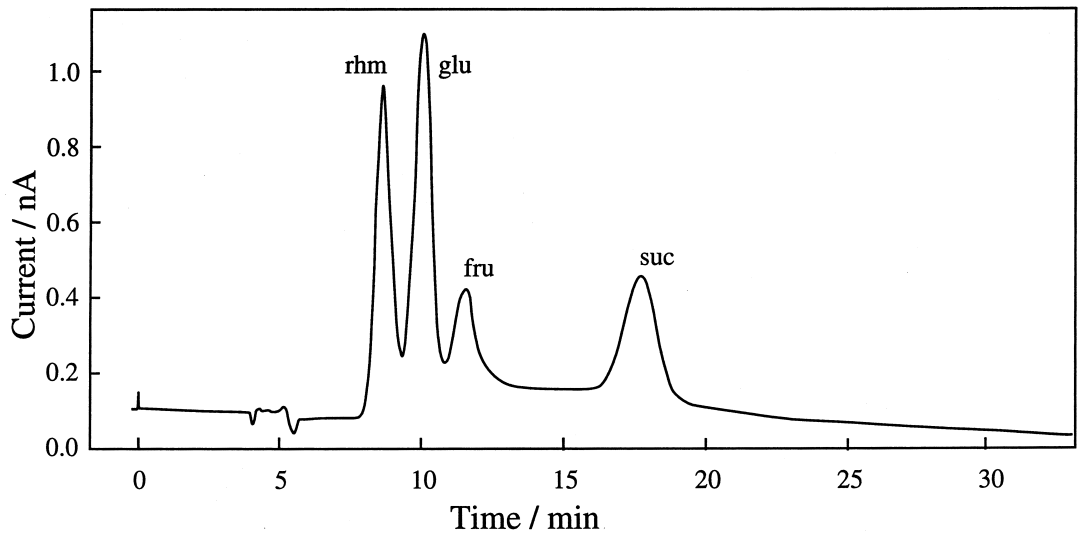

Fig. 7. Chromatogram of rhamnose (0.5 pmol), glucose (0.5 pmol), fructose (0.2 pmol) and sucrose (2 pmol) at the Ni–Ti (30:70) electrode. Stationary phase: Hamilton ion-exchange column. Mobile phase: 0.1 *M* NaOH at a flow-rate of 30 μl/min.

detector cell volumes, because electrochemical reactions require only a surface, rather than a volume, and electrodes are easily miniaturized.

Fig. 7 shows a chromatogram of rhamnose (0.5 pmol), glucose (0.5 pmol), fructose (0.2 pmol) and sucrose (2 pmol) at the Ni–Ti (30:70) electrode. The separation column was 20 cm×1 mm I.D. and was made by repacking Hamilton RCX-10 anion-exchange resin. The mobile phase was 0.1 *M* NaOH at a flow-rate of 30 μl/min. Compared with the Dionex column, the peak current of each sugar showed almost the same level even though the injected amount was 100 times smaller than the previous experiment. The sensitivities for these samples were 1.8 nA/pmol for rhamnose, 2.0 nA/pmol for glucose, 1.2 nA/pmol for fructose and 0.16 nA/pmol for sucrose. The detection limit was calculated to be below 50 fmol ($S/N=3$) for glucose. These high sensitivities result from the reduction in the separation column diameter and higher electrochemical reaction. The 1 mm microbore column is 16 times higher sensitivity than that of the 4 mm diameter column. The conversion efficiency of the analytes on the electrode by electrocatalytic reaction in the 4 mm I.D. column was less than 5%. By contrast, that of the 1 mm I.D. column reached more than 70%, owing to the smaller dead volume and slower flow-rate. Because of repacking procedure, the peak symmetry and column efficiencies in Fig. 7 reduced compared to those of Fig. 6. Those parameters and the detection limit will be improved by optimizing the microbore column packing.

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