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Copper electrodes for stable subpicomole detection of carbohydrates in high-performance liquid chromatography

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Abstract

A new pretreatment of copper (Cu) electrodes used for catalytic oxidation of carbohydrates is presented, which involves superficial polishing of the electrochemically-oxidized Cu electrode and then air-oxidation at 100°C for 3 h. This pretreatment allows highly sensitive and stable detection of carbohydrates at 0.45 V (vs. Ag/AgCl/Sat. KCl) in anion-exchange high-performance liquid chromatography. Glucose was detected at concentration levels as low as 0.2 pmole with a dynamic range up to 10 000 pmole over a period of 1 month. The change in the electrode surface state during the pretreatment is discussed from the electrochemical point of view.

1. Introduction

Recent advances in biochemistry have stimulated a demand for highly sensitive and precise analytical methods for the determination of carbohydrates. The development of high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE) has provided effective methods for the separation of carbohydrates [1–4]. With respect to the direct and sensitive detection of carbohydrates, conventional spectrophotometric methods are not suitable due to the lack of chromophores. Thus, several electrochemical detection methods have been examined to overcome this problem. Amperometric detection with either platinum (Pt) or gold (Au) electrodes in alkaline medium is usually employed for this purpose

[4–6]. With these electrodes, however, pulsed-potential operation is required to remove adsorptive products of the electrode process and to restore the electrochemical response to carbohydrates [6].

A preferential mode of operation for electrochemical detection would be constant-potential amperometry because of its instrumental simplicity and inherent sensitivity. From this point of view, nickel (Ni)- and copper (Cu)-based electrodes have been receiving increasing attention [3,7–16]. Unfortunately, researchers in this field have not yet reached a consensus on operation, pretreatment, surface modification, sensitivity, and stability of the electrodes, in contrast with the case of Pt and Au electrodes.

In this work, we have focused our attention on Cu electrodes, because Cu electrodes have been reported to have the most favourable signal-to-noise (*S/N*) characteristics compared to other

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metal electrodes including Ni [12,15]. Although the detailed mechanism of the electrocatalytic oxidation of carbohydrates at Cu is not yet fully understood, recent studies have revealed that CuO [16] or Cu₂O [13] on the electrode surface is essential for the electrode process. With these reports in mind, we examined several ways of pretreatment and/or operation of Cu electrodes to allow sensitive and stable detection of carbohydrates based on the characterization of the electrodes by means of cyclic voltammetry and constant potential amperometry in HPLC.

2. Experimental

Cyclic voltammetry was performed on a Yanagimoto P-1100 potentiostat controlled with an NEC PC-9801 RA microcomputer with a three-electrode system consisting of a flow-through type Cu working electrode with a 1.0 mm diameter shielded in a Kel-F block, a platinum wire auxiliary electrode, and an Ag/AgCl/sat. KCl reference electrode. All measurements were done in 0.15 M NaOH under aerated conditions at 25°C. In order to obtain a fresh Cu electrode surface, the Cu electrode was thoroughly polished for ca. 6 h by hand with 0.05 μm alumina powder suspended in distilled water (Buehler, No. 3; IL, USA). The alumina powder on the electrode was removed by washing with water and then by sonication in distilled water. The electrode was immediately used for experiments as a thoroughly polished fresh Cu electrode.

HPLC experiments were carried out on a chromatographic system consisting of a Shimadzu LC-10AD chromatograph pump, a Shimadzu DGU-4A degasser, a Rheodyne 7125 sampler injector, a Shimadzu CTO-10A column oven, a Shimadzu L-ECD-6A electrochemical detector equipped with a thin-layer electrochemical cell (BAS), an Ag/AgCl/3 M NaCl reference electrode (BAS), and a Shimadzu C-R7A ChromatoPac data processor. A Dionex CarboPac PA1 (250 × 4 mm I.D.) anion-exchange column was used for the HPLC separation at

35°C. As the mobile phase, 0.15 M NaOH solution was used at a flow-rate of 0.7 ml min⁻¹.

All carbohydrate samples used were obtained from Nacalai Tesque (Kyoto, Japan). Carbohydrate solutions were prepared from NANOpure water. Relatively high concentrations (ca. 0.1 M) of carbohydrates stock solutions were stored for at least 1 day in the refrigerator and diluted appropriately just before each experiment.

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 1 shows cyclic voltammograms of glucose in 0.15 M NaOH at Cu electrodes with four ways of pretreatment. A fresh Cu electrode polished thoroughly with 0.05-μm alumina powder (see Experimental) was completely insensitive to glucose (Fig. 1A). Electrochemical oxidation of the fresh Cu electrode surface at 2.0 V (vs. Ag/AgCl/Sat. KCl) in 0.15 M NaOH for 5 min resulted in a rough black appearance of the Cu electrode. The electrochemically-oxidized Cu

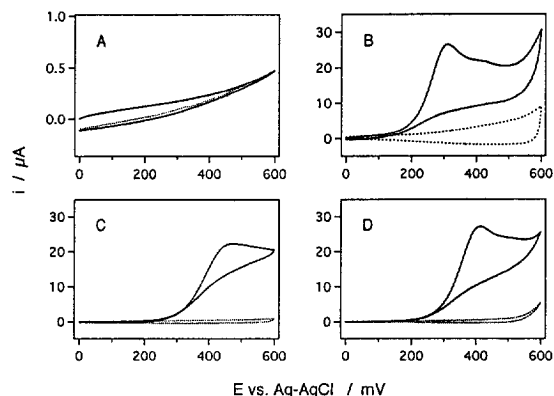


Fig. 1. Cyclic voltammograms of 5 mM glucose (solid lines) in 0.15 M NaOH at Cu electrodes under various surface conditions at a scan rate of 10 mV s⁻¹. Cu electrode pretreatment: (A) thorough polishing for ca. 6 h by hand, (B) electrochemical oxidation of fresh Cu electrode at 0.2 V for 5 min, (C) superficial polishing for ca. 5 min by hand after electrochemical oxidation, and (D) air oxidation at 100°C for 3 h in an air oven after superficial polishing. The broken lines represent the background current in the absence of glucose.

electrode exhibited a well-defined oxidative response to glucose (Fig. 1B). The black appearance can be ascribed to CuO generated during the electrochemical oxidation. This result demonstrates the significance of CuO on the electrode surface for carbohydrate oxidation, as has been previously described using copper oxide-modified electrodes [16]. At the electrochemically-oxidized Cu electrode, the background current was extremely large compared with that at the thoroughly polished Cu electrode, indicating that CuO is labile and can be oxidized to some higher Cu(III) oxide species compared with metallic Cu and that the Cu(III) oxide species serves substantially as a catalyst for carbohydrate oxidation [16].

The electrochemically-oxidized Cu electrode was, however, not suitable for the electrochemical detector of the HPLC system because of the surface roughness, which led to an increase in background current and noise in flow systems. Some superficial polishing treatment of the oxidized surface and/or much milder surface oxidation would be required. Thus, we repolished the electrochemically-oxidized Cu electrode slightly by hand for about 5 min with 0.05- μm alumina powder until the surface had a mirror-like and gold-yellow appearance and then removed the alumina powder from the electrode surface by washing and sonication as in the case of the fresh Cu surface preparation. The superficially polished Cu electrode retained the catalytic oxidation activity toward carbohydrates as shown by Fig. 1C, although the catalytic activity decreased somewhat: the anodic peak height was smaller than that at the electrochemically-oxidized electrode (Fig. 1B) and the wave shape exhibited a plateau most probably due to a kinetically controlled electrode process. Further prolonged polishing as in the case of the preparation of a fresh Cu electrode surface resulted in a complete loss of the catalytic activity, though the polishing period required to eliminate the catalytic activity depended on the history of the electrode as well as on the surface area of the Kel-F block shielding the Cu electrode. This indicates that the electrochemical oxide-formation proceeds into the more interior domain of

the electrode. There might exist a reticulated cracked structure over the electrode surface, in which the Cu oxide formation can occur, as is observed for RuO₂-TiO₂ coated titanium electrodes [17]. The Cu oxide in the cracked part would remain unchanged after superficial polishing and be responsible for the catalytic current for the carbohydrate oxidation.

In order to enhance the catalytic activity of the superficially polished Cu electrode, the electrode was oxidized mildly in an air-oven at 100°C for 3 h. The surface appearance of the resultant Cu electrode remained mirror-like smooth but had changed to shiny brown. The latter suggests the formation of a mixture of Cu₂O and CuO. This treatment enhanced the electrocatalytic response to glucose, as shown in Fig. 1D. Comparing the cyclic voltammogram with that at the electrochemically-oxidized electrode (Fig. 1B), the anodic peak height was comparable and a similar diffusion-tail was observed, although the peak appeared at 0.4 V as compared to at 0.3 V. Thus we can conclude that the catalytic oxidation at the air-oxidized Cu electrode is fast enough to attain a diffusion controlled process at potentials more positive than 0.4 V under the present conditions. Interestingly, the background current was much smaller than that at the electrochemically oxidized electrode (Fig. 1B) and comparable with that at the superficially polished electrode (Fig. 1C). This electrochemical behavior was reproducible and the preparation of the fresh Cu electrode surface was not necessary to exhibit such voltammetric response to glucose. That is to say, the catalytic activity of the air-oxidized electrode decreased by superficial repolishing and the activity was restored by air-oxidation.

3.2. Constant-potential amperometric detection in HPLC

Amperometric response of the air-oxidized Cu electrode was examined in HPLC at a constant-potential mode. When the pretreated Cu electrode was equipped with a flow cell, the background current gradually decreased to ca. 30 nA within 1–2 h at 0.45 V (vs. Ag/AgCl/3 M NaCl).

The preconditioning time to reach a substantially stable background current is much shorter than for a fresh metallic Cu electrode, for which it took 1 or 2 days [15]. This implies a relatively high kinetic barrier for the oxidation of fresh metallic Cu, compared with partially oxidized Cu such as Cu_2O . Once preconditioning has been performed, the background current quickly stabilized within 30 min from the second time of the closed-circuit.

The hydrodynamic voltammogram was sigmoidal with a plateau at potentials higher than +0.45 V. Such ideal hydrodynamic voltammogram was not observed at Pt and Au electrodes at a constant-potential mode, where the catalytic activity was suppressed by adsorption or fouling of the products (and/or analytes) on the electrode surface. Compared with other common metallic electrodes, Cu-based electrodes have a relatively higher activity to complete the catalytic oxidation of carbohydrates into formate [16]. This would be one of the reasons that the adsorptive inhibition effect encountered in Au and Pt electrodes is scarcely observed and thus implies that Cu-based electrodes are suitable for constant-amperometric detection of carbohydrates. In the following, the electrode potential was set at 0.45 V.

The amperometric peak height was ca. 60 nA for 100 pmole glucose. The response and the background current decrease to 50 nA and 15 nA, respectively, after a 2-week period. During this period, the appearance of the electrode surface had changed to black but it remained relatively smooth compared to the electrochemically-oxidized Cu surface. The black layer is due to gradual CuO build-up in the constant-potential mode at 0.45 V, most probably during the first precondition process. The smooth surface would be responsible for the high sensitivity.

Fig. 2 illustrates an anion-exchange chromatogram of a laboratory-prepared mixture containing seven different carbohydrates. A low detection limit of 0.2 pmole (or 0.1 pmole under optimum conditions) at an S/N ratio of 3 was accomplished for glucose. This is the highest sensitivity found for the direct detection of glucose in HPLC. The linear dynamic response

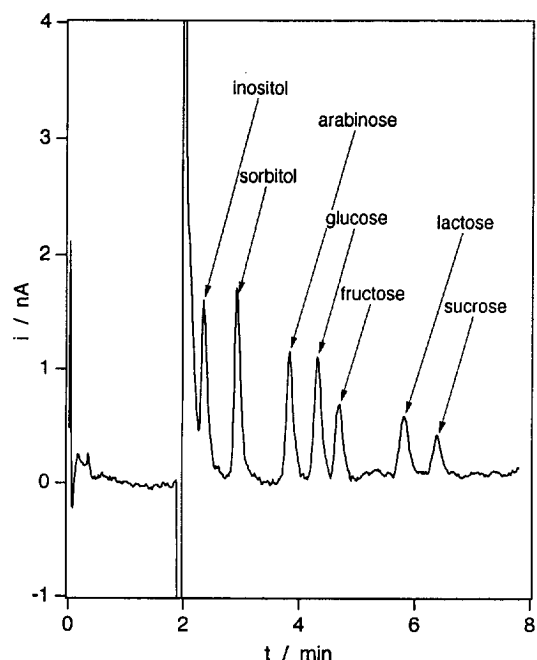


Fig. 2. Anion-exchange chromatogram of a sample mixture of carbohydrates (2 pmole each) detected with an air-oxidized Cu electrode at 0.45 V. See text for other details.

was obtained at least for 0.2 to 10 000 pmole, in which the logarithmic correlation gave a slope of 0.98 with a correlation of 0.999. The within-day reproducibility for five repeated manual injections was 0.5–2%. The long-term stability was examined monitoring the response for 41 days. The daily average response to 100 pmole glucose and the background current are plotted in Fig. 3.

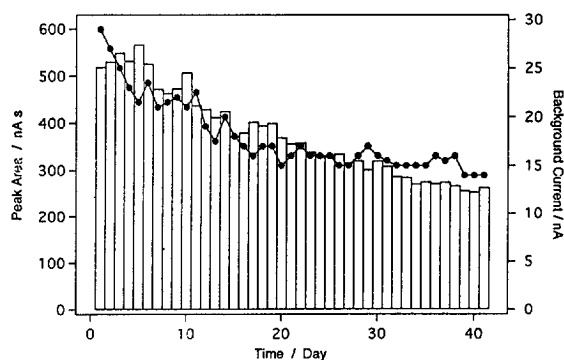


Fig. 3. Daily response (average peak area) of 100 pmole glucose (left hand y-axis) and the background current (right hand y-axis) at an air-oxidized Cu electrode.

A gradual decrease in glucose response was observed together with that of the background current. Thus, the detection limit was almost constant. Because of the high within-day reproducibility, the electrode can be considered to have an acceptable long-term stability. After long-period usage over 1 or 2 months, an increase in the background noise was occasionally observed. This might be ascribed to excess build-up of the surface oxide layer, resulting in an increase in surface roughness. However, the electrode can be easily reactivated by the superficial polishing and subsequent air-oxidation. Preliminary thorough polishing to produce a fresh Cu electrode surface is not necessary.

In conclusion, we have described an effective pretreatment of the Cu electrode surface, i.e. superficial polishing of the electrochemically-oxidized black Cu electrode surface and subsequent air-oxidation in an air-oven at ca. 100°C for 3 h. This pretreatment allows enhanced sensitive and stable response toward carbohydrates in a constant-potential amperometric mode. The period required for superficial repolishing can be reduced by employing a small size Kel-F block for the electrochemical flow cell. With respect to sensitivity and stability, Cu electrodes with appropriate pretreatments are superior to Ni electrodes, which are considered to be possible electrodes in the constant-potential mode. One exception reported so far is a Ni–Ti alloy electrode [9], which provides sensitivity down to 0.5 pmole and high stability comparable with the present Cu electrode. The sensitivity of the present Cu electrode is still superior to that of the Ni–Ti alloy electrode and, in addition, Cu material is cheaper and easily available.

The important point in the pretreatment of Cu electrodes is to form a smooth CuO layer. In this sense, an alternative might be some mild electrochemical oxidation. Indeed, a Cu-oxide layer with high catalytic activity can be constructed from a fresh metallic Cu electrode at 0.45 V, although it takes 1 or 2 days [15]. A positive shift of the electrolysis potential would reduce the time required for the oxide layer formation, but

the potentials may not exceed 0.6–0.7 V, beyond which the electrochemical oxidation of Cu becomes very fast, resulting in rough oxide layer formation. Therefore, the air oxidation (combined with electrochemical preconditioning at 0.45 V in the flow system) seems to be best for the electrode activation.

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