CHEMISTRY LETTERS, pp. 849-852, 1991.

On-Line Elimination of Electroactive Interferents for Flow-Type Electrochemical Biosensor System[1)]

Yusuke OKAWA,* Hiroyuki KOBAYASHI, and Takashi OHNO
Department of Image Science, Faculty of Engineering,
Chiba University, 1-33 Yayoi-cho, Chiba 260

A flow-through electrolytic cell (electrochemical filter; ECF) was newly designed for the elimination of electroactive interferents with electrochemical biosensors. The ECF has been combined with a glucose oxidase monolayer electrode in a flow injection analysis (FIA) setup. The addition of ferricyanide to the carrier buffer drastically increased the elimination efficiency for L-ascorbic acid and the biosensor output. Glucose content in soft drinks has been determined without any pretreatment.

A biosensor-FIA system features the continuous, rapid, and easy determination of biological molecules. We have applied to an FIA detector an amperometric glucose sensor and obtained an excellent performance for glucose standard solutions.[2)] For serum analysis, however, a nonspecific current response caused by the direct electrolysis of electroactive species in the serum severely overlapped the output. This is a general disadvantage of solid electrode type amperometric biosensors, and in order to avoid this interference, we used a chromatographic column.[2)] Several investigators have tried to decrease such nonspecific responses by use of permselective membranes.[3-5)] Another simple and attractive way for eliminating the electroactive interferents is on-line electrolysis in an electrolytic cell placed in front of the detector.[6)] This method may retain high analysis frequency and easy operation, which are the primary merits of FIA. The electrolysis can be easily controlled by controlling the applied potential, and the amount of the interferents can be evaluated coulometrically. In the present communication, we describe a new design of such a flow-through electrolytic cell, named "electrochemical filter" (ECF), and preliminary results for the analysis of commercial soft drinks.

Figure 1 illustrates the design of the ECF. A carbon felt[7)] (Mitsui Engineering and Shipbuilding Co. Ltd., apparent size, 2 x 2 x 70 mm^3) was

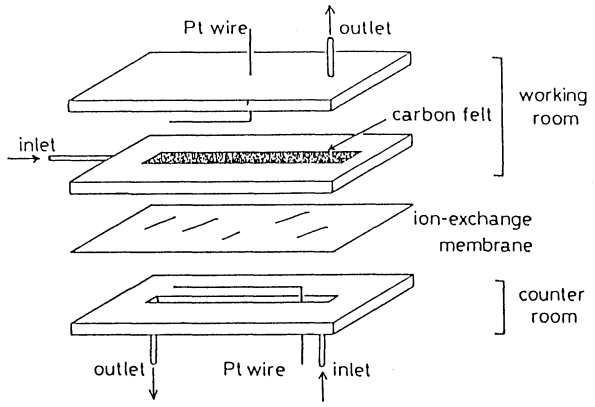

Fig. 1. Design of ECF.

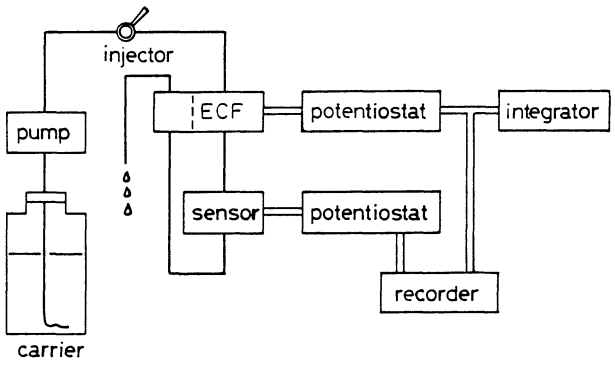

Fig. 2. Schematic diagram of the ECF/biosensor FIA setup.

used as a working electrode (WE) featuring high surface area relative to sample size, and a platinum wire as a counter electrode (CE). The WE and CE rooms were separated with a Nafion membrane. The potential of the WE was controlled with a potentiostat against the CE. The ECF current was integrated for the coulometric analysis. A glucose sensor was prepared as reported previously and placed in a flow-through electrochemical cell.[2] The sensor detects glucose by monitoring the enzymatic generation of hydrogen peroxide (or the reduced mediator) through the direct electrooxidation on the base tin oxide electrode at +0.75 V vs. Ag/AgCl. Figure 2 shows the schematic diagram of the ECF/biosensor FIA system. The flow rate of a carrier (0.067 M phosphate buffer, pH 6.4, 1 M = 1 mol·dm^{-3}) and the injection volume of sample was 1.0 mL·min^{-1} and 10 μL, respectively, throughout. All the operations were carried out at room temperature.

As a typical substance giving the nonspecific response to the biosensor, L-ascorbic acid (AA) was studied. The sensor responses to 1 mM AA and 10 mM D-glucose solutions were shown in Fig. 3. The flow system was the same as in Fig. 2 but no potential was applied to the ECF. The output to 1 mM AA is severely higher than that to 10 mM glucose. This demonstrates that the elimination of the interference is needed for accurate analysis.

When a potential (+0.8 V vs. CE) was applied to the ECF, an anodic current to the injection of AA was observed, and the nonspecific biosensor response decreased correspondingly. The elimination of AA is, however, incomplete, i.e. about 1% of the injected AA was detected by the biosensor, when 1 mM AA was injected. The magnitude of the elimination efficiency is not acceptable for accurate determination of glucose at the mM level. Application of higher potential to the ECF slightly improved the elimination efficiency. In order to improve the efficiency 0.1 M potassium ferricyanide (hexacyanoferrate(III)) was added to the carrier;[8] ferricyanide ions mediate the electrooxidation of AA and suppress the polarization at the CE, thus resulting in the effective electrolysis of AA. Figure 4 shows

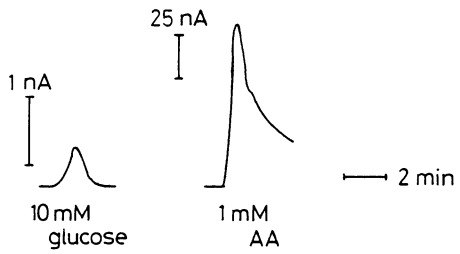

Fig. 3. Typical responses of the biosensor to 10 mM glucose and 1 mM AA. Carrier, phosphate buffer.

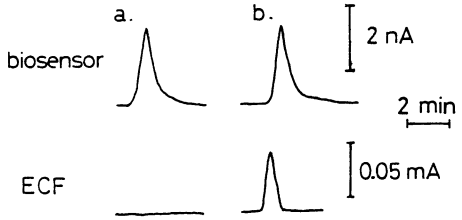

Fig. 4. Responses of the ECF/biosensor system to 10 mM glucose (a) and 10 mM glucose + 1 mM AA (b). Carrier, phosphate buffer containing 0.1 M $K_3Fe(CN)_6$.

the responses to 10 mM glucose and 10 mM glucose + 1 mM AA. The output currents of the biosensor show good agreement between in the presence and absence of AA. AA below about 1 mM was eliminated in the ECF to the negligible level for the determination of glucose at the mM level or higher. Other electroactive interferents which can reduce ferricyanide may be effectively eliminated in this system.

The calibration curve for the ECF/biosensor system for D-glucose is shown in Fig. 5. The addition of ferricyanide to the carrier also improved the sensitivity to glucose by the factor of ca. 4.5. Ferricyanide is reduced to ferrocyanide during the glucose oxidase reaction,[9] and ferrocyanide is detected on a tin oxide electrode much more easily than the peroxide, thus resulting in the improved sensitivity.[10] However, a ferricyanide solution generally contains ferrocyanide naturally formed (by light, for instance), causing high and unstable baseline of the biosensor. Then use of the ECF gives a further merit; initially contained ferrocyanide is completely oxidized in the ECF and the baseline of the biosensor is thus lowered and stable.

The system was able to detect glucose down to 1 mM. The dynamic range in higher glucose concentration range is surprisingly wider than those of generally reported systems, as shown in Fig. 5, and the output current did not saturate even for a 1 M-sample. This is caused by the dilution of sample in the void volume of the ECF and enables direct determination of a sample containing high concentration of glucose, such as serum from a diabetic, fresh juice, and soft drinks, although the calibration curve is of course needed because of the non-

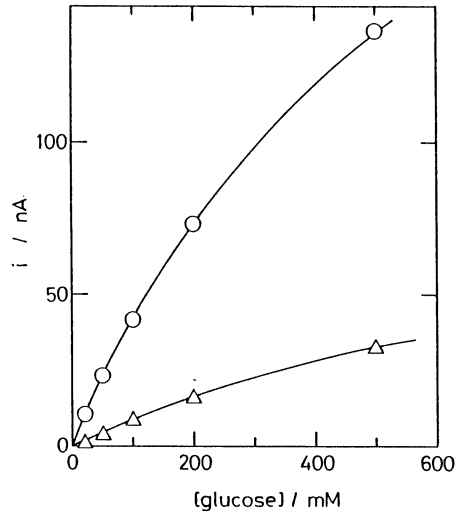

Fig. 5. Calibration curves of the ECF/biosensor system for glucose. Carrier, phosphate buffer (△) and phosphate buffer containing 0.1 M $K_3Fe(CN)_6$ (○).

linearity of the response.

Taking into account the above-stated advantageous characteristics of the system, we have tried the determination of glucose in commercial soft drinks without any pretreatment. Many of soft drinks contain high concentration of glucose (and other sugars) as a sweetening, and AA as an acidulant. The analysis was carried out by only injecting a non-treated sample to the system. For comparison, a conventional enzyme-based colorimetric method (the glucose oxidase-peroxidase-aminoantipyrine method) was also applied to the diluted and then aerially oxidized sample. The analytical values based on the two methods showed good agreement, as summarized in Table 1. From the ECF current, the approximate AA content of all the samples except for B were estimated to be of 1 mM-level. When the ECF was not used, the determined glucose concentrations were unbelievably high. The present results clearly ensure the applicability and potentiality of the present ECF/biosensor system.

Table 1. Determination of glucose in soft drinks ($g \cdot cm^{-3}$)

Sample	This method	Colorimetry
A	0.23	0.26
B	0.43	0.48
C	0.47	0.46
D	0.35	0.35
E	0.27	0.23

We are grateful to Prof. Emeritus S. Mizusawa for his valuable suggestions. We also thank Mr. M. Takabatake and Mr. O. Hamamoto (Mitsui Engineering & Shipbuilding Co., Ltd.) for the generous donation of the carbon felt. This work is partly supported by a Grant-in-Aid from the Ministry of Education, Science and Culture (No. 02750561).

References

1) Electrochemical Filter. Part I.
2) Y. Okawa, H. Tsuzuki, S. Yoshida, and T. Watanabe, Anal. Sci., 5, 507 (1989).
3) D. J. Harrison, R. F. B. Turner, and H. P. Baltes, Anal. Chem., 60, 2202 (1988).
4) S. V. Sasso, R. J. Pierce, R. Walla, and A. M. Yacynych, Anal. Chem., 62, 1111 (1990).
5) S. Yamauchi, M. Yaoita, A. Katayama, A. Sakurai, Y. Yoshida, and Y. Ikariyama, Proc. 11th Chem. Sensor Symp., 1990, 117.
6) T. Yao and Y. Kobayashi, Bunseki Kagaku, 32, 253 (1983).
7) S. Uchiyama, M. Ono, S. Suzuki, and O. Hamamoto, Anal. Chem., 60, 1835 (1988).
8) S. Uchiyama, T. Yamaguchi, O. Hamamoto, and S. Suzuki, Bunseki Kagaku, 38, 286 (1989).
9) P. Schläfer, W. Mindt, and Ph. Racine, Clin. Chim. Acta, 57, 283 (1974).
10) Y. Okawa, T. Tatsuma, T. Watanabe, H. Kobayashi, T. Ohno, and S. Mizusawa, Proc. 9th Chem. Sensor Symp., 1989, 29.

(Received January 23, 1991)