Novel Utilization of Impedance Measurement for Electrochemical Biosensing Aiming at Elimination of Influence by Interference Materials

Susumu Kuwabata,* Hidefumi Hasegawa, and Kenji Kano[†]
Department of Materials Chemistry and Frontier Research Center, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871
[†]*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo, Kyoto 606-8502*

Application of the ac impedance technique to the electrochemical glucose sensing allowed determination of glucose concentration without influence of a serious interference material, ascorbic acid.

The electrochemical impedance measurement conducted by imposing small ac signal to an electrode is useful for elucidating resistance and capacitance components in a cell and kinetic parameters of electrode reactions.[1] Numerous theoretical equations have been derived not only for simple electrode reactions but also for electrode reactions with coupled homogeneous reactions. In the latter cases, however, relatively cumbersome treatments seem to prevent wide application to practical reaction systems. The electrochemically induced biochemical reaction using a redox enzyme belongs to such the reaction system. In fact, regarding even the electrochemical oxidation of glucose using glucose oxidase (GOx), which is widely utilized for fabrication of glucose sensors,[2] analysis of the reaction with the impedance technique was hardly made although some papers have been published concerning elucidation of kinetic parameters of specific electrodes used in glucose sensors.[3] Here, we would like to show a demonstration of the impedance measurement for a typical electrochemical oxidation of glucose, which is made using an electrolyte solution containing GOx and an electron mediator of ferrocenecarboxylic acid. As will been shown, a calibration curve for determination of glucose concentration can be drawn from the impedance plots obtained. Furthermore, the finding that we would like to emphasize in this report is that the impedance measurement can become a useful technique allowing elimination of influence by ascorbic acid, which is well known as the most serious interference material.

The electrolyte solution used in this study was 0.1 M (1 M = 1 mol dm^{-3}) KCl aqueous solution, pH of which was adjusted to 7.0 by adding phosphate buffer. A glassy carbon (GC) disk having 3 mm diameter was used as a working electrode and its surface was polished by 0.3 μm alumina. The electrochemical measurements were performed by using a one-compartment cell equipped with a reference electrode of Ag/AgCl in saturated KCl solution and a Pt foil counter electrode having ca. 5 cm^2. All measurements were conducted after bubbling the electrolyte solution with N$_2$ for 30 min at least.

Figure 1(A) shows cyclic voltammograms of a glassy carbon electrode taken at 5 mV s^{-1} in the electrolyte solution containing 0.2 mM ferrocenecarboxylic acid and 30 mM GOx. In case of the solution without glucose, a reversible redox couple due to ferrocenecarboxylic acid was obtained. Then, addition of glucose gave sigmoid voltammograms and the oxidation currents increased with the increase in glucose concentration. In this figure a
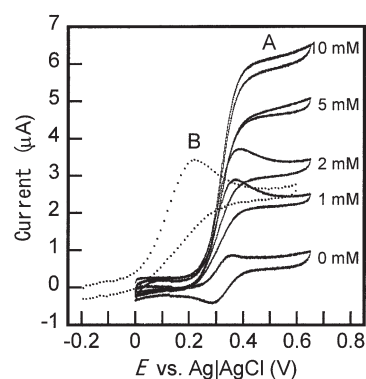
Figure 1. Cyclic voltammograms of a glassy carbon electrode taken at 5 mV s^{-1} (A) in the electrolyte solution (pH 7) containing 30 mM glucose oxidase, 0.2 mM ferrocenecarboxylic acid and glucose of given concentrations, and (B) in the electrolyte solution (pH 7) containing 0.5 mM ascorbic acid.

voltammogram (B) obtained for the electrolyte solution containing 0.5 mM ascorbic acid alone is also shown. Apparently, the oxidation of ascorbic acid occurs from more negative potential than the oxidation of glucose, indicating that ascorbic acid must be an interference material in this case.

The impedance measurements were performed using the same cell. The ac signal of 10 mV (peak to peak) was imposed to the electrode polarized at 0.36 V vs Ag/AgCl. It is noted that 0.36 V vs Ag/AgCl is the potential where the anodic current due to oxidation of glucose varied largely with change in the polarization potential, whereas oxidation of ascorbic acid at the GC electrode reached the diffusion controlled region. The frequencies chosen were from 10 kHz to 10 mHz. The Nyquist plots obtained for the cell containing 0–10 mM glucose are shown in Figure 2(A). The cell in the absence of glucose gave a straight line having ca. 45° slope, indicating that the reversible reaction of ferrocenecarboxylic acid is mainly controlled by the mass transfer. When glucose was added to the cell, drastic changes in the shape of plots were observed; the line tended to draw a semicircle and its diameter decreased with the
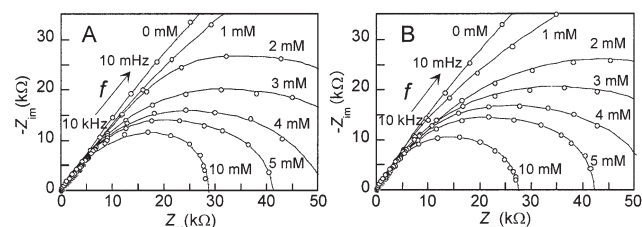

Figure 2. Nyquist plots obtained for a glassy carbon electrode polarized at 0.36 V vs Ag/AgCl in the electrolyte solution (pH 7) containing 30 mM glucose oxidase, 0.2 mM ferrocenecarboxylic acid and glucose of given concentrations in (A) the absence and (B) the presence of 0.5 mM ascorbic acid.

increase in concentration of glucose. Similar changes have been reported in literature dealing with glucose oxidation with GOx and a tetracyano-p-quinodimethane (TCNQ) electrode.[3a]

In case of the electrochemical glucose oxidation using GOx and an electron mediator like this system, enzyme reaction involving electron transfer determines the reaction rate, implying that the reaction system is kinetically sluggish in electrochemical viewpoint. Accordingly, the charge-transfer resistance (R_{ct}) and the double-layer capacitance, which should be present in parallel, give the semicircle-shape of the impedance plots as shown in Figure 2(A). In general, the R_{ct} value corresponds to dE/dI of a current-potential curve taken by a steady-state polarization measurement. In the present system, since dE/dI of the sigmoid voltammograms shown in Figure 1(A) at 0.36 V vs Ag/AgCl must reflect the R_{ct} values, the relationship between the increase in glucose concentration enlarging oxidation currents and the decrease in the diameter of the semicircle-shape of the impedance plots is qualitatively understandable. As recognized from Figure 2(A), the solution resistance is negligible as compared with the large R_{ct} values. Therefore, we estimated to the R_{ct} by extrapolating the semicircle to $f \rightarrow 0\,(Z_{re}{}^{ex})$.

The impedance analysis software in our electrochemical measurement system (Voltalab PZG-402) gave $Z_{re}{}^{ex}$ values for the impedance plots taken in the electrolyte solutions containing glucose of 2 mM and more. Then, the $1/Z_{re}{}^{ex}$ values were plotted as a function of glucose concentration and they are shown by circles in Figure 3(B). On the other hand, oxidation currents in the sigmoid voltammograms at 0.65 V vs Ag/AgCl shown in Figure 1(A) were regarded as the limiting currents (I_{lim}), and plots of I_{lim} vs glucose concentration are shown by circles in Figure 3(A). It is strongly suggested from resemblance between two profiles that dI/dE values at 0.36 V are essentially proportional to I_{lim} values.

The voltammograms and impedance measurements were also taken with use of the electrolyte solution containing 0.5 mM ascorbic acid as an interference material. The Nyquist plots
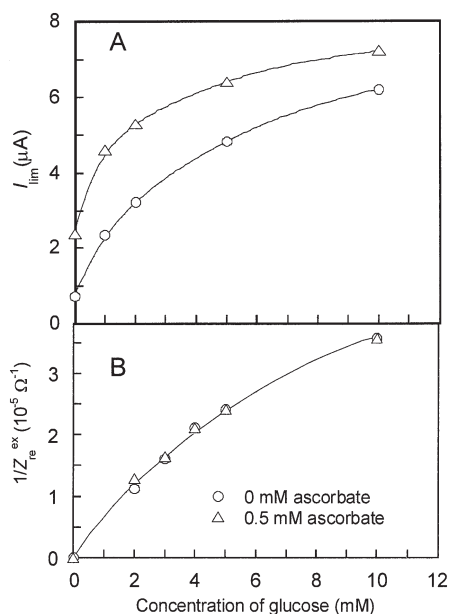
obtained for glucose concentration from 0 to 10 mM are shown in Figure 2(B). It can be recognized by comparing Figure 2(A) and (B) that the presence of ascorbic acid did not influence the impedance measurements. This was more conspicuous by drawing plots of I_{lim} and $1/Z_{re}{}^{ex}$ as a function of glucose concentration, as shown by triangle marks in Figure 3(A) and (B). The plots of I_{lim} vs glucose concentration exhibited apparent superimpose of the oxidation currents due to ascorbic acid on those due to glucose oxidation. However, the plots of $1/Z_{re}{}^{ex}$ were not influenced by ascorbic acid. As mentioned above, the impedance measurements were conducted by polarizing the electrode at 0.36 V where ascorbic acid oxidation reached the diffusion-controlled region. In the diffusion-controlled region, since dI/dE is null, definite $Z_{re}{}^{ex}$ value should not be given. As a matter of fact, the Nyquist plots for the electrolyte solution containing 0.5 mM ascorbic acid alone gave a straight line. It is, therefore, certain that the plots exhibiting semicircle shapes shown in Figure 2(B) reflect the glucose oxidation alone. Then, the $Z_{re}{}^{ex}$ values obtained form the plots are determined by only glucose concentration, resulting in avoidance of interference due to ascorbic acid.

It is already well known that electrochemical measurements conducted by imposing small signals on polarization potential are useful for acquiring information of each species separately from mixed electrochemically active species. The ac voltammetry and the differential pulse voltammetry are typical methods. Therefore, determination of glucose concentration with evasion of interference materials can be made by those electrochemical measurements. However, considering that the amperometric measurement adopted in almost all commercially available biosensors can be done by simple circuits without any potential sweeper and recorder, it is desired to make smaller improvement of the circuits to gain larger benefit. Of course, the circuits of the apparatus allowing impedance measurements while changing frequencies are very complicated. However, the R_{ct} value can basically be determined by ac impedance measurement with a fixed frequency. Development of such the biosensing method using a simple impedance meter is our present subject.

Figure 3. Plots of (A) I_{lim} obtained at $E = 0.65$ V vs Ag/AgCl and (B) $1/Z_{re}{}^{ex}$ obtained from the Nyquist plots as a function of concentration of glucose in the electrolyte solution in the absence and the presence of ascorbic acid.

References
1   M. Sluyters-Rehbach and J. H. Sluyters, *J. Electroanal. Chem.*, **26**, 237 (1970); M. Sluyters-Rehbach and J. H. Sluyters, in "Electroanalytical Chemistry," ed. by A. J. Bard, Marcel Dekker, New York (1970), Vol. 4, pp 1–128; A. J. Bard and L. R. Faulkner, "Electrochemical Methods," 2nd ed., John Wiley & Sons, Inc. (2001), pp 368–416.
2   A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott, and A. P. F. Turner, *Anal. Chem.*, **56**, 667 (1984); Y. Degani and A. Heller, *J. Electroanal. Chem.*, **91**, 1285 (1987); N. C. Foulds and C. R. Lowe, *Anal. Chem.*, **60**, 2473 (1988); R. Maidan and A. Heller, *J. Am. Chem. Soc.*, **113**, 9003 (1991); P. N. Bartlett and J. M. Cooper, *J. Electroanal. Chem.*, **362**, 1 (1993); S. Kuwabata and C. R. Martin, *Anal. Chem.*, **66**, 2757 (1994); S. Kuwabata, T. Okamoto, Y. Kajiya, and H. Yoneyama, *Anal. Chem.*, **67**, 1684 (1995); F. Battaglini, E. J. Calvo, C. Danilowicz, and A. Wolosiuk, *Anal. Chem.*, **71**, 1062 (1999); O. A. Raitman, E. Katz, A. F. Bueckmann, and I. Willner, *J. Am. Chem. Soc.*, **124**, 6487 (2002).
3   a) C. C. Jung and A. H. Hall, *Anal. Chem.*, **67**, 2393 (1995). b) E. J. Calvo, R. Etchenique, C. Danilowicz, and L. Diaz, *Anal. Chem.*, **68**, 4186 (1996). c) C. F. Chen and C. M. Wang, *J. Electroanal. Chem.*, **466**, 82 (1999). d) F. Patolsky, M. Zayats, E. Katz, and I. Willner, *Anal. Chem.*, **71**, 3171 (1999).