HIGHLY SENSITIVE VOLTAMMETRIC ANALYSIS BY RE-OXIDATION OF
REDUCED SUBSTANCES USING TWIN WORKING ELECTRODESSachiko SAKURA[®] and Hideo IMAIInstitute of Pharmaceutical Sciences, Fukuyama University,
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A flow cell voltammetry with twin working electrodes was applied to the trace determination of reducible organic substances in the presence of dissolved oxygen. Carbazilquinone, one of quinone drugs, which is not oxidized by itself, was chosen as a sample. The first electrode was set at the reduction potential of carbazilquinone, and the second electrode at the oxidation potential of the reductant (hydroquinone). The cathodic current at the first electrode composed of reduction currents of dissolved oxygen and the sample, while the anodic current at the second electrode depends solely on the reduced sample flowing toward the second electrode. The results of the determination based on the anodic current measurement were as follows; 1) The linear calibration plot was obtained from 0.3 nmole to 1.9 nmole of carbazilquinone. 2) The value of R.S.D. was less than 1%.

Oxygen is easily reduced to hydrogen peroxide at -0.05 V vs. SCE in acidic solution, and at $-0.05 \sim -0.2$ V in neutral or alkaline solution¹⁾, and interferes with the voltammetric determination of many reducible substances. Under normal conditions dilute aqueous solution contains 0.25 mM oxygen, and usual method of nitrogen gas bubbling can not remove trace oxygen sufficiently for highly sensitive determination of substances reducible at more negative potentials.

Hydrogen peroxide is oxidized at $+1.0$ V²⁾. The reoxidation of reduction product of oxygen is quite irreversible. Accordingly, to a sample material reversibly or quasi-reversibly reducible we can apply the following technique. The first electrode of twin working electrodes is set at the reduction potential of the sample and the second electrode at the oxidation potential of the reductant, and oxidation current at the second electrode is measured as the analytical data. This new method was applied to the determination of carbazilquinone, an anticancer alkylating drug, which showed a rather reversible electrode process.

EXPERIMENTAL

Cyclic voltammetry (CV)

A Fuso Model 311 potentiostat with a Model 321 potential scanner was used. The Working electrode was a glassy carbon electrode (GCE) (Tokai Carbon GC-20, 3 mm ϕ coated with teflon on the side). The counter electrode was a Pt wire and was referred to SCE. Temperature was controlled at $25 \pm 0.1^\circ\text{C}$ except the experiment of temperature effect. The cell was covered by a black polyvinyl sheet in order to protect the sample solution from light.

Thin-layer flow system

The voltammetric detector used was a Yanagimoto VMD-501 with a cell of twin glassy carbon electrodes closely adjacent each other in the solution passage as shown in Fig. 1. The cell volume was 2.5 μl , and the thickness of the spacer was 0.05 mm. The counter electrode was a stainless steel tubing used as the drain. The potential was referred to Ag/AgCl electrode (SSCE) ($+0.013\text{ V}$ vs. SCE). Flowing system was a Shimadzu LC-4A high performance liquid chromatograph. The delay coil (2 m $\times$ 0.1 mm I.D.) was attached to the sample injector. The eluting solution was 0.05 M McIlvaine buffer (pH 5.0).

Fig. 1 Thin-layer flow cell

W_1, W_2 : 1st and 2nd working electrode, resp.; R: reference electrode; C: counter electrode; S: spacer (W_3 was not used).

Materials

Carbazilquinone (Carboquone, CQ; 2,5-bis-(1-aziridinyl)-3-(2-carbamoyloxy-1-methoxyethyl)-6-methyl-1,4-benzoquinone, commercial name: Eskinon) was kindly offered by Sankyo Co. Ltd. (Tokyo, Japan). The other reagents were of guaranteed grade.

RESULTS

CV of CQ

The reversibility and the reduction, reoxidation potentials were investigated by CV. CQ(I) was not oxidized by itself, but the

reductant gave the reoxidation peak as shown in Fig. 2. The cathodic and anodic peak potentials were $E_p^C = -0.43 + 0.03(5 - \text{pH})\text{ V}$, and

Fig. 2 Cyclic voltammogram of 0.76 mM CQ in 0.1 M McIlvaine buffer (pH 5.0)

Scan rate: 20 mV/s; temp.: 25°C .

$E_p^a = 0.26 + 0.04(5 - \text{pH})$ V vs. SCE, respectively. The cathodic and anodic peak potentials were separated by 700 mV at pH 5, showing reoxidation occurred fairly irreversibly. Both peaks were shifted toward more negative potentials with increase of pH.

The plot of reoxidation current (i_p^a) vs. CQ concentration was linear from 0.05 mM up to 1.5 mM. The temperature dependence of i_p^a was 0 %/°C at pH 5, while that of the reduction peak current (i_p^c) was 2.0 %/°C in temperature range from 10° to 40°C as shown in Fig. 3. The value of i_p^a was not varied in the pH range from 4 to 7.4.

Chemical reaction coupled with electrolytic reduction and generation of new redox couple

Judging from the slope of E_p^c vs. pH plot, two protons were involved in the reduction of CQ. The reduction product obtained by electrolysis at controlled potential of -1.0 V vs. SCE showed the following trends: (1) A new cathodic peak appeared at -0.60 V (pH 5.0), while the anodic peak was remained unchanged at +0.26 V. (2) The wavelength of the optical absorption maximum of CQ was 328 nm, but the reduction product showed two low absorption peaks at 304 nm and 345 nm, the latter being especially low. (3) The product obtained by hydrolysis of CQ with 6 N HCl and the product of photochemical reaction of CQ showed the same trends as the reduction product. Therefore, in good conformity with Berg's reports³⁾⁴⁾ it is concluded that the reduction intermediate undergoes hydrolysis which results in the fission of ethylene imino ring, and a new redox couple with $E_p^c = -0.60$ V and $E_p^a = +0.26$ V vs. SCE at pH 5.0 is generated. The overall reaction can now be expressed as: $\text{CQ} + 2e^- + 2\text{H}^+ = (\text{CQH}_2)^*$, $(\text{CQH}_2)^* + 2\text{H}_2\text{O} = (\text{CQH}_2)_{\text{hydrolysed}}$.

Thin-layer flow cell voltammetry

Trace determination of CQ by using a thin-layer flow cell with twin electrode was carried out at the concentration level of 0.1 $\mu\text{mole/ml}$. The dependence of the reoxidation yield on the applied potential is shown in Fig. 4. When the first electrode was set at more negative potentials than -0.6 V, and the second electrode at more positive potentials than +0.5 V, the current yielded at the second electrode attained the plateau.

Figure 5 shows the relationship between sample quantity and reoxidation current at the second electrode. A linear calibration plot was obtained in the range investigated (0.3 ~ 1.9 nmole). With flow rate greater than 0.5 ml/min the yield was the same, but at slower flow rate the yield remarkably increased. Slower flow rate may result in laminar flow, which increases the yield. In the case of flow rate faster

Fig. 3 Plot of peak current versus temperature in CV

0.41 mM CQ in 0.1 M McIlvaine buffer (pH 5.0); Scan rate: 20 mV/s.

than 0.5 ml/min, the R.S.D. value ($n = 5$, within-day) was less than 1%. But, in the case of slower flow rate the R.S.D. value increased up to 3%. Under the flow rate faster than 0.5 ml/min the current efficiency of reducing electrolysis at -0.6 V was 0.5%, and the efficiency of reoxidation electrolysis was about 0.3%.

The temperature dependence of the current measured at the second electrode was $-0.8\ \%/^{\circ}\text{C}$ in the temperature range from 10 to 40°C . This negative temperature dependence seems to be a result of a spurious effect related to the flow injection device using a plunger pump. The flow stream is accompanied by inevitable ripples, which give a subtle temperature effect on laminar or eddy flow around the electrode. Thus, the eddy flow increases at higher temperature, and results in the decrease of the current yield at the second electrode.

The application of this method to a variety of samples is in progress in our laboratory.

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REFERENCES

- 1) I.M. Kolthoff, J.J. Lingane: "Polarography", Vol. 2, p. 552 (1952), (Interscience Publ., New York).
- 2) H. Imai, H. Yoshida, T. Masujima, M. Yanagitani: *Bunseki Kagaku*, **30**, 419 (1981).
- 3) A. Anhalt, H. Berg: *J. Electroanal. Chem.*, **4**, 218 (1962).
- 4) H. Berg, K. Kramarczyk: *Talanta*, **12**, 1127 (1965).

Keyword phrases

voltammetric determination of carbazilquinone; voltammetry with twin working electrodes.

Fig. 4 Dependence of reoxidation current yield on applied potential
1.1 nmole CQ in 0.05 M McIlvaine buffer (pH 5.0); 2nd electrode: $+0.5$ V.

Fig. 5 Plot of CQ quantity versus reoxidation current
1st electrode: -0.9 V
2nd electrode: $+0.5$ V
flow rate: 2 ml/min.

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