The Curve Fitting Analysis of D.c. and A.c. Voltammograms of a Two-step Surface-redox Reaction. The Application to the Surface-redox System of Adriamycin Adsorbed on a Pyrolytic Graphite Electrode

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A theoretical study of the two-step surface-redox reaction ($O_{ad}+n_1e \rightleftharpoons S_{ad}$, $S_{ad}+n_2e \rightleftharpoons R_{ad}$) was performed using d.c. voltammetry. A new method based on a non-linear least-squares curve fitting of cyclic d.c. and a.c. voltammograms was proposed for determining electrochemical parameters and was successfully applied to the surface-redox system of the quinone moiety of adriamycin adsorbed on a pyrolytic graphite electrode.

Recently, research regarding redox modified electrodes has been a very active field in electrochemistry and a wealth of reports have been published concerning applications to electroanalysis, electrocatalysis, and electrochromic devices (for reviews, see Refs. 1—3). In order to analyze the electrochemical behavior of surface-attached redox materials associated with biological, pharmaceutical, and chemical fields, a two-step electron-transfer surface-redox reaction mechanism seems to be very important. Numerous studies have been carried out regarding this type of electrode reaction.[4—8] However, there is little literature pertinent to the kinetic treatment of this mechanism. Kakutani and Senda have recently reported a theory of the a.c. polarization and a.c. voltammetry of the mechanism[7] and showed that a.c. voltammetry is a powerful technique for determining electrochemical kinetic parameters.[8] Alternatively, cyclic d.c. voltammetry has been widely used for the characterization of the electrochemical behavior of a modified electrode.[1—3] Analyses regarding cyclic d.c. voltammetry are, thus, very important. As far as we know, however, theoretical studies of cyclic d.c. voltammetry regarding two-step surface-redox reactions are limited to an electrochemically reversible system[4,6,7] and also to a reversible electrochemical reactions coupled with irreversible chemical reactions.[4]

In this paper, we report on a theoretical study regarding a two-step surface-redox reaction in d.c. voltammetry and also a useful method for the analysis of cyclic d.c. and a.c. voltammograms by means of non-linear least-squares curve fitting for determining the electrochemical kinetic parameters. The application of our methods to a surface-redox system of adriamycin (antitumor anthracycline antibiotics) adsorbed on a pyrolytic graphite electrode is discussed in detail.

Theoretical Treatment of D.c. Voltammetry for the Case of Two-step Surface-redox Reaction

Let us now consider a two-step surface-redox reaction:

$$\begin{aligned} O_{ad} + n_1e &\rightleftharpoons S_{ad} \quad \text{(1st step)} \\ S_{ad} + n_2e &\rightleftharpoons R_{ad} \quad \text{(2nd step)}, \end{aligned} \tag{1}$$

where all the electrochemical species (O, S, and R) are strongly adsorbed on the electrode surface. The quantities of O, S, and R due to a mass transfer from (to) the electrode surface to (from) the solution are negligibly small compared with those of the adsorbed reactants. Thus,

$$\Gamma_o + \Gamma_s + \Gamma_r = \Gamma_t = \text{const.} \tag{2}$$

Here Γ_j (j=o, s, or r) is the surface concentration of the adsorbed reactant j per unit area, and Γ_t is the total surface concentration. For the above system, theoretical studies of the reversible d.c.[4,6,7] and a.c.[7] voltammetries have already been reported. Our formulation for Scheme (1) is therefore for the d.c. voltammogram of a quasi-reversible case.

Assuming that the surface-redox reaction occurs only among the adsorbed reactants, the faradaic current, i, is given by

$$i = i(1) + i(2) \tag{3a}$$

$$i(1) = n_1FA\,\mathrm{d}\Gamma_o/\mathrm{d}t \tag{3b}$$

$$i(2) = -n_2FA\,\mathrm{d}\Gamma_r/\mathrm{d}t, \tag{3c}$$

where $i(1)$ and $i(2)$ are the faradaic currents of the first and second electron-transfer steps, respectively, and A is the electrode area. The electrode potential, E, can now be written as

$$E = E_i + vt, \tag{4}$$

E_i and v being the initial potential and the sweep rate, respectively. The current-potential-surface concentration characteristics can, thus, be expressed by the Butler-Volmer equation:[1,7,9)]

$$i(1) = n_1FAk_{\mathrm{sap}}(1)\{\Gamma_{\mathrm{s}} \exp [(1-\alpha_1)n_1F(E-E'_{\mathrm{o1}})/RT] - \Gamma_{\mathrm{o}} \exp [-\alpha_1 n_1 F(E-E'_{\mathrm{o1}})/RT]\} \quad (5a)$$

$$i(2) = n_2FAk_{\mathrm{sap}}(2)\{\Gamma_{\mathrm{r}} \exp [(1-\alpha_2)n_2F(E-E'_{\mathrm{o2}})/RT] - \Gamma_{\mathrm{s}} \exp [-\alpha_2 n_2 F(E-E'_{\mathrm{o2}})/RT]\}. \quad (5b)$$

In these equations, $k_{\mathrm{sap}}(1)$ and $k_{\mathrm{sap}}(2)$ are, respectively, the apparent rate constants of the first and second steps at the formal standard redox potentials, E'_{o1} and E'_{o2}, corresponding to the redox couples $\mathrm{O_{ad}/S_{ad}}$ and $\mathrm{S_{ad}/R_{ad}}$. The transfer coefficient with regard to the (cathodic) electron-transfer of the j-th step is expressed by α_j (j=1 or 2).

Combination of Eqs. 2 to 5 may lead to current potential characteristics for linear potential sweep voltammetry. Assuming $n_1=n_2=n$ (for convenience), we define the semiquinone formation constant, K, as follows:

$$K = [\mathrm{S_{ad}}]^2/[\mathrm{O_{ad}}][\mathrm{R_{ad}}] = \exp [nF(E'_{\mathrm{o1}}-E'_{\mathrm{o2}})/RT]. \quad (6)$$

Differential equations (as dimensionless functions) are easily written as Eqs. 7a and 7b using the above equations,

$$\psi(1) = i(1)RT/n^2F^2vA\Gamma_{\mathrm{t}} = m(1)[(1-f_{\mathrm{o}}-f_{\mathrm{r}})(\rho/\sqrt{K})^{1-\alpha_1}-f_{\mathrm{o}}(\rho/\sqrt{K})^{-\alpha_1}] = \mathrm{d}f_{\mathrm{o}}/\mathrm{d}\rho \quad (7a)$$

$$\psi(2) = i(2)RT/n^2F^2vA\Gamma_{\mathrm{t}} = m(2)[f_{\mathrm{r}}(\rho\sqrt{K})^{1-\alpha_2}-(1-f_{\mathrm{o}}-f_{\mathrm{r}})(\rho\sqrt{K})^{-\alpha_2}] = -\mathrm{d}f_{\mathrm{r}}/\mathrm{d}\rho, \quad (7b)$$

the current being defined by

$$\psi = iRT/n^2F^2vA\Gamma_{\mathrm{t}} = \psi(1) + \psi(2), \quad (7c)$$

where

$$f_j = \Gamma_j/\Gamma_{\mathrm{t}} \qquad (j=\mathrm{o,\ r}) \quad (8a)$$

$$m(j) = k_{\mathrm{sap}}(j)RT/nFv \qquad (j=1,\ 2) \quad (8b)$$

$$\rho = \exp [nF(E-E'_{\mathrm{o}})/RT]. \quad (8c)$$

Here $E'_{\mathrm{o}}(=(E'_{\mathrm{o1}}+E'_{\mathrm{o2}})/2)$ is the formal standard redox potential of the redox couple $\mathrm{O_{ad}/R_{ad}}$. Other symbols have their usual meanings. Solving Eqs. 7a and 7b, we obtain values for f_{o} and f_{r}. Thus, ψ can be calculated using Eq. 7c where ψ (1) and ψ (2) are easily evaluated by introducing f_{o} and f_{r} into the original Eqs. 7a and 7b.

Two sets of the differential Eqs. 7a and 7b were solved using a standard fourth-order Runge-Kutta method.[10)] The initial conditions are $f_{\mathrm{o}}=0$ and $f_{\mathrm{r}}=1$ at $\rho\rightarrow0$ for the anodic curve, and $f_{\mathrm{o}}=1$ and $f_{\mathrm{r}}=0$ at $\rho\rightarrow\infty$ for the cathodic curve. The calculation was carried out with an NEC 8001 personal computer.[11)]

Let us also assume that the adsorbed reactants undergo homogeneous intermolecular interactions (written by Frumkin's a-parameter[12)]). Thus, E'_{o1}, E'_{o2}, $k_{\mathrm{sap}}(1)$, and $k_{\mathrm{sap}}(2)$ are now given by[7)]

$$E'_{\mathrm{o1}} = E_{\mathrm{o1}} + (RT/n_1F) \ln [B_{\mathrm{s}}(1)/B_{\mathrm{o}}(1)] \quad (9a)$$

$$E'_{\mathrm{o2}} = E_{\mathrm{o2}} + (RT/n_2F) \ln [B_{\mathrm{r}}(2)/B_{\mathrm{s}}(2)] \quad (9b)$$

$$k_{\mathrm{sap}}(1) = k_{\mathrm{s}}(1)B_{\mathrm{o}}(1)^{\alpha_1-1}B_{\mathrm{s}}(1)^{-\alpha_1} \exp (-a\theta) \quad (10a)$$

$$k_{\mathrm{sap}}(2) = k_{\mathrm{s}}(2)B_{\mathrm{s}}(2)^{\alpha_2-1}B_{\mathrm{r}}(2)^{-\alpha_2} \exp (-a\theta). \quad (10b)$$

In these equations, $B_j(1)$ and $B_j(2)$ (j=o, s, or r) are the adsorption coefficients of the species j at E_{o1} and E_{o2}, respectively. The E_{o1} and E_{o2} stand for the standard redox potentials of the redox couples O/S and S/R in the bulk solution, respectively, θ being the total surface coverage ($\Gamma_{\mathrm{t}}/\Gamma_{\mathrm{t}}^{\mathrm{max}}$; $\Gamma_{\mathrm{t}}^{\mathrm{max}}$ is the maximum value of the total surface concentration). Note that when $k_{\mathrm{sap}}(1)=k_{\mathrm{sap}}(2)=k_{\mathrm{sap}}$, the k_{sap} dependence on θ is given by

$$k_{\mathrm{sap}} = k_{\mathrm{sap}}(\theta\rightarrow0) \exp (-a\theta), \quad (10c)$$

where $k_{\mathrm{sap}}(\theta\rightarrow0)$ means k_{sap} at $\theta\rightarrow0$.

Figure 1 shows the m ($=m(1)$ $=m(2)$) dependence of the normalized anodic voltammograms at K=1 and α ($=\alpha_1=\alpha_2$)=0.5. There is a positive shift of the peak potential, $E_{\mathrm{p,a}}$, with decreasing m. Figure 2 depicts the K dependence of the normalized voltammograms under the conditions of m=0.5 and α=0.5. As in the case of a reversible system[4,7)] there is only one peak

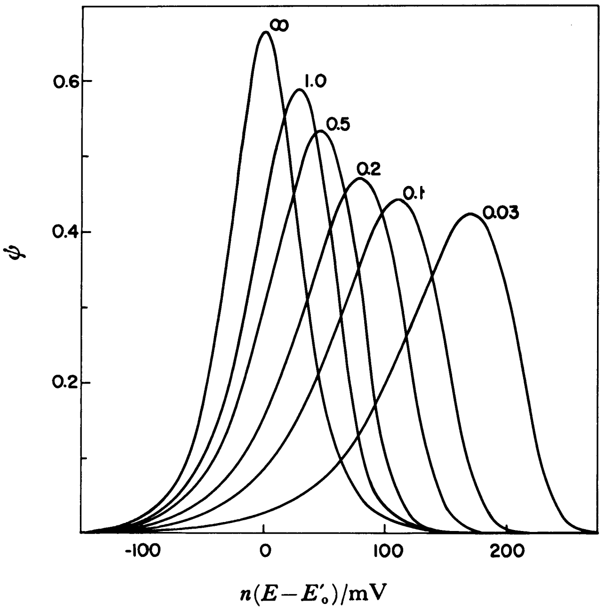


Fig. 1. The dependence of m on the normarized d.c. anodic voltammograms at K=1.0 and α=0.5. The m value is indicated on each curve.

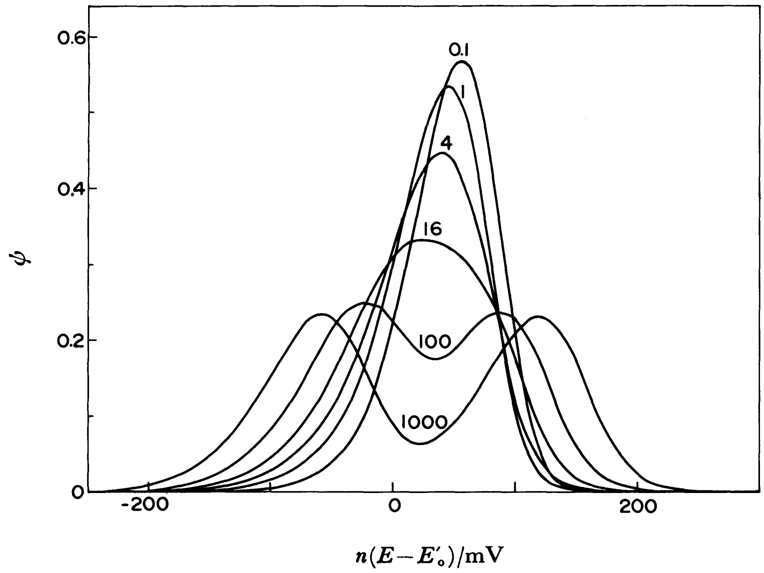


Fig. 2. The dependence of K on the normarized d.c. anodic voltammograms at $m=0.5$ and $\alpha=0.5$. The K value is indicated on each curve.

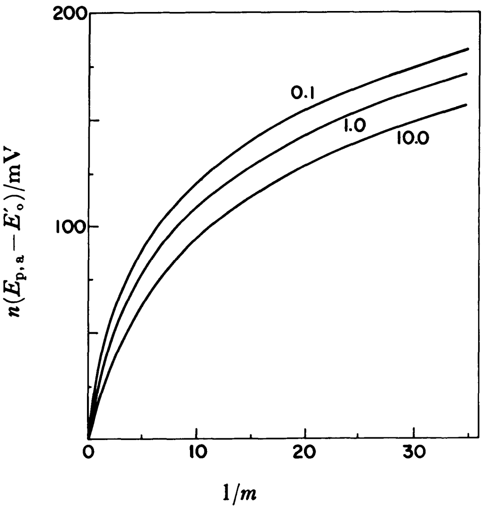


Fig. 3. Variation of $n(E_{p,a}-E'_o)$ with $1/m$ for $\alpha=0.5$ and $K=0.1$, 1.0, and 10.0. The value of K is indicated on each curve.

for $K\leq 16$, but two peaks are obtained for $K>16$. Also, Eqs. 7a to 7c indicate that the cathodic curve, having $m(1)=a$, $m(2)=b$, $K=c$, and $\alpha=d$, and the anodic curve, having $m(1)=b$, $m(2)=a$, $K=c$, and $\alpha=1-d$, are symmetrical about the C_2 axis which is vertical to the curve plane and goes through the point of $\rho=1$ ($E=E_o'$) and $\psi=0$ ($i=0$). Accordingly, when $m(1)=m(2)$ and $\alpha=0.5$ (conditions necessary to experimentally obtain cathodic and anodic voltammograms in a C_{2h} symmetry) both the waves are symmetrical about the point of $E=E_o'$ and $i=0$.

The change of $n(E_{p,a}-E'_o)$ with m is shown in Fig. 3 for various K's at $\alpha=0.5$. Using the working curves like those in Fig. 3, k_{sap} can be estimated from the observed $E_{p,a}-E_o'$ value[13], provided that n and K can be obtained by the other methods, and that $k_{sap}(1)=k_{sap}(2)=k_{sap}$ and of $\alpha_1=\alpha_2=0.5$ are kept. The latter situation can be verified by checking whether or not the average of the cathodic and anodic peak potentials, $(E_{p,a}+E_{p,c})/2$, is independent of v and Γ_t and equal to E_o',[13] and also whether the peak currents, the half-peak widths, and the electricities of the cathodic and anodic waves coincide with each other. Needless to say, the K as well as E_o' can be estimated from the reversible d.c. voltammogram.[4,7]

The above method is, however, only based on information regarding peak potentials. Furthermore, if an estimation of K is difficult, the above method can not be used. Thus, we propose here a non-linear least-squares analysis of the d.c. voltammograms for the determination of kinetic parameters.[14,15] In the following section we will discuss the results concerning the characterization of the electrochemical behavior of adriamycin adsorbed on a pyrolitic graphite electrode.

Experimental

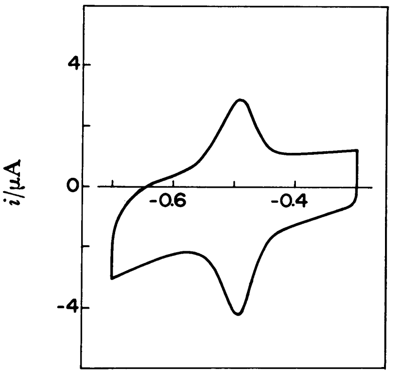
Adriamycin hydrochloride and all other chemicals used have been described elsewhere.[16] The electrochemical measurements were performed mostly as described in previous papers.[16,17] Positive feedback circuits were used for ohmic drop compensation. Electrodes, used as working electrodes; were fabricated using cylindrical rods of pyrolytic graphite carbon (Tomoe Co., Osaka), and were sealed onto glass tubing by heat-shrinkable Teflon tubing. The pyrolytic graphite electrode was cleaved with a razor blade to produce a basal-plane surface and was first polished with silicon carbide paper (No. 1000) and then with a diamond paste in order to prepare a smooth shiny surface. The electrode was ultrasonically cleaned in ethanol. The geometrical area of the electrodes were

0.126 cm^2. The freshly prepared pyrolytic graphite electrode was immersed into an electrolysis solution containing adriamycin for 1—3 min under stirring at an initial potential, E_i. After standing for 0.5—2 min, a d.c. voltage scan was started from E_i, a saturated calomel electrode (SCE) being used as a reference electrode. A sodium acetate (0.2 mol dm^{-3})-nitric acid buffer (pH 4.54, ionic strength 0.5 with potassium nitrate) was used as the base solution. Most experiments were performed in solutions containing 2—6×10^{-6} mol dm^{-3} of adriamycin.

The electrode surface was cleaned after several potential scans because of an aging effect which may have some relation to the stability of the reduced adriamycin[16,17] as well as the electrode.

Results and Discussion

Cyclic D.c. Voltammetry. Figure 4 shows the cyclic d.c. voltammogram of adriamycin adsorbed on a pyrolytic graphite electrode (recorded from $E_\mathrm{i}=-0.30$ V at $v=10$ mV s^{-1}). A symmetric pair of cathodic and anodic waves was observed at −0.5 V. This wave is ascribed to the redox reaction of the quinone moiety of the adsorbed adriamycin.[16] When the voltammograms were recorded at $v\leq 50$ m V s^{-1}, the peak heights of cathodic and anodic waves, $i_\mathrm{p,c}$ and $i_\mathrm{p,a}$, coincided with each other and were proportional to v at a given Γ_t.[18] The electricities of cathodic and anodic waves, Q_c and Q_a, also agree well with each other within the experimental error. The peak potentials ($E_\mathrm{p,c}$ and $E_\mathrm{p,a}$) and the half-peak widths ($\Delta E_\mathrm{p/2,c}$ and $\Delta E_\mathrm{p/2,a}$) are −0.498 V and 54—55 mV, respectively, for both the cathodic and anodic waves, and are independent of v and Γ_t. This electrochemical behavior should be interpreted in terms of a d.c. reversible two-step surface-redox reaction as shown in Scheme (1), where $n_1=n_2=1$.[8,16] According to the theory of a reversible two-step surface-redox reaction, we have estimated the K value to be 0.244±0.085 from the half-peak widths.[19]



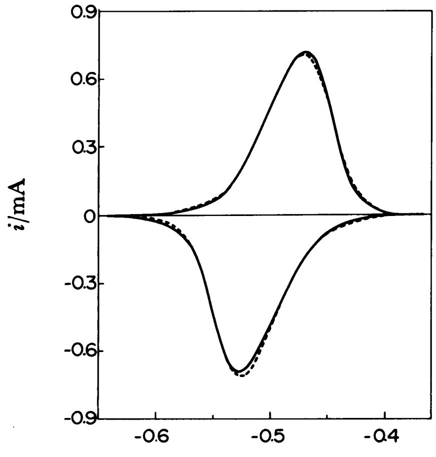
E/V *vs.* SCE

Fig. 4. Cyclic d.c. voltammogram of adriamycin adsorbed on a pyrolytic graphite electrode. Voltage scanning was done with $v=10$ mV s^{-1} after stirring for 1 min at $E_i=-0.3$ V in 5.52×10^{-6} mol dm^{-3} adriamycin solution.

Under the conditions of $v>100$ mV s^{-1}, a discrepancy occurs between $E_\mathrm{p,a}$ and $E_\mathrm{p,c}$, and the ΔE_p ($=E_\mathrm{p,a}-E_\mathrm{p,c}$) becomes larger with an increase in v. Figure 5 shows the cyclic d.c. voltammogram recorded at $v=9000$ mV s^{-1}, the current being corrected for the base. This is a typical quasi-reversible voltammogram: ΔE_p comes out 59 mV. Nevertheless, the same values between $i_\mathrm{p,a}$ and $i_\mathrm{p,c}$, and also between $\Delta E_\mathrm{p/2,a}$ and $\Delta E_\mathrm{p/2,c}$ can be within the experimental error at a given v and Γ_t. The $(E_\mathrm{p,a}+E_\mathrm{p,c})/2$ value is independent of v and Γ_t and agrees well with E_0', which is equal to $E_\mathrm{p,a}$ or $E_\mathrm{p,c}$ of the reversible waves. These facts suggest that $k_\mathrm{sap}(1)=k_\mathrm{sap}(2)=k_\mathrm{sap}$, and $\alpha_1=\alpha_2=\alpha=0.5$.

Based on the above consideration, we analyzed the voltammogram shown in Fig. 5 by means of a non-linear least-squares analysis using two parameters, k_sap and K, at $E_0'=-0.498$ V, $Q=5.94$ μC[20] ($\Gamma_\mathrm{t}=2.44\times 10^{-10}$ mol cm^{-2}),[18] and $\alpha=0.5$.[21] The refined parameters are $k_\mathrm{sap}=503\pm19$ s^{-1} and $K=0.229\pm0.023$. The resultant regression curve is depicted in Fig. 5 as a broken curve. Table 1 gives the regression results of the voltammograms at various Γ_t and v. We can see that although k_sap decreases with an increase in Γ_t, K is almost independent of Γ_t and stays within 0.211±0.099. This is in good agreement with the K estimated from the reversible voltammograms. This indicates the effectiveness of the non-linear least-squares curve fitting analysis of d.c. voltammograms.

In addition, a one-parameter curve fitting was also



E/V *vs.* SCE

Fig. 5. Cyclic d.c. voltammogram of adriamycin adsorbed on a pyrolytic graphite electrode. Voltage was scanned with $v=9000$ mV s^{-1} after stirring for 1 min at $E_i=-0.3$ V in 1.84×10^{-6} mol dm^{-3} adriamycin solution. The current is corrected for the base current. The broken line represents the regression curve calculated under the conditions of $k_\mathrm{sap}=503$ s^{-1}, $K=0.229$, $\alpha=0.5$, $E_0'=-0.498$ V *vs.* SCE, and $Q=5.94$ μC.

TABLE 1. k_{sap} AND K VALUES OF ADRIAMYCIN ADSORBED ON A PYROLYTIC GRAPHITE ELECTRODE ESTIMATED BY MEANS OF CYCLIC D.C. VOLTAMMETRY

Γ_{t} / 10^{-10} mol cm^{-2}	v / V s^{-1}	k_{sap} / s^{-1}	K
1.72	4.0	532	0.104
2.44	9.0	503	0.229
2.72	8.0	519	0.062
3.14	4.0	320	0.280
3.33	9.0	358	0.187
4.20	4.0	239	0.290
6.30	4.0	167	0.325

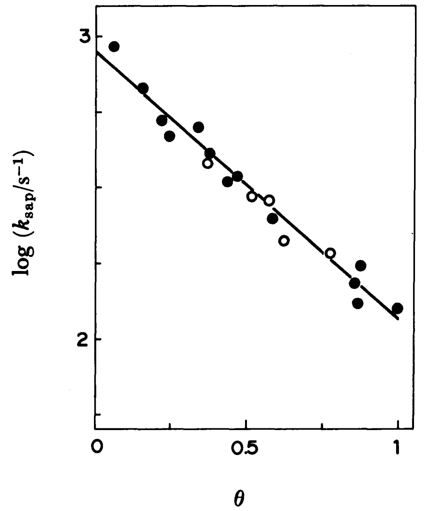


Fig. 6. Surface coverage dependence of the log k_{sap} values estimated by one-parameter curve fitting (K= 0.211) of cyclic d.c. (●) and of a.c. (○) voltammograms. The solid line represents the regression result; *i.e.* $\log k_{\mathrm{sap}}[=\log k_{\mathrm{sap}}(\theta\rightarrow 0)-a\theta/2.303]=2.94-0.882\theta$ ($r=0.983$).

carried out using a constant K value of 0.211 (the average value obtained by two-parameter fitting). A linear log k_{sap} *vs.* θ (surface coverage) plot for the refined k_{sap} is depicted in Fig. 6 (closed circles); the linearity seems to be quite good. These results prove the adequacy of Eq. 10c for the adsorbed adriamycin; *viz.* the interaction among the adsorbed reactants is homogeneous, and the reactants obey Frumkin's adsorption isotherm. The negative log k_{sap} *vs.* θ plot indicates an attractive interaction.

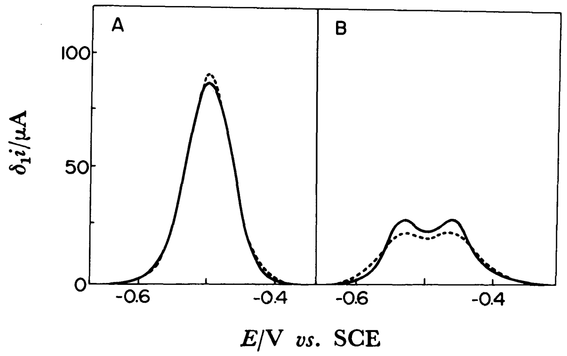


Fig. 7. (A) Real component and (B) imaginary component of a.c. voltammograms of adriamycin adsorbed on a pyrolytic graphite electrode. D.c. voltage sweep was done with $v=20$ mV s^{-1} after stirring 3 min at $E_i=-0.3$ V in 1.84×10^{-6} mol dm^{-3} adriamycin solution. Amplitude of the superimposed a.c. voltage was 10 mV peak-to-peak and the a.c. frequency was 100 Hz. The current is corrected for the base current. The broken lines represent the regression curves calculated under the conditions of $k_{\mathrm{sap}}=194$ s^{-1}, $K=0.199$, $\alpha=0.5$, $E_0'=-0.498$ V *vs.* SCE, and $Q=13.37$ μC.

TABLE 2. k_{sap} AND K VALUES OF ADRIAMYCIN ADSORBED ON A PYROLYTIC GRAPHITE ELECTRODE ESTIMATED BY MEANS OF A.C. VOLTAMMETRY

Γ_{t} / 10^{-10} mol cm^{-2}	f / Hz	k_{sap} / s^{-1}	K
2.63	100	415	0.135
3.69	100	238	0.217
4.12	200	311	0.137
4.48	100	225	0.156
5.50	100	194	0.199

A.c. Voltammetry. Figure 7 shows the real and imaginary components of the a.c. voltammogram of the adsorbed adriamycin, recorded at 100 Hz (a.c. frequency, f). The imaginary component is less than the real component and has two peaks. The peak potential of the real component and the valley potential between the two imaginary component peaks coincide with each other and also with the E_0' estimated from d.c. voltammetry. This result makes it possible to set $k_{\mathrm{sap}}(1)=k_{\mathrm{sap}}(2)=k_{\mathrm{sap}}$ and $\alpha=0.5$, and agrees well with the situation derived from the d.c. voltammetry (previously described). The theory of a.c. voltammetry[7)] tells us that the a.c. frequency dependence of the current ratio of the real and imaginary components at the E_0' and a constant Γ_{t} leads to information regarding kinetic parameters. In case of solid electrodes, however, it is a quite difficult matter to always obtain a constant Γ_{t}. This renders the experiment regarding the frequency dependence of a.c. voltammograms at a given Γ_{t} difficult. Here, we have analyzed the a.c. voltammogram by a direct non-linear least-squares analysis[15)] according to the theory of a.c. voltammetry.[7,22)] Using the same procedure as that regarding an analysis of the d.c. voltammograms, we have used two parameters, k_{sap} and K, for the simulation. The analysis of the voltammograms (Fig. 7) results in $k_{\mathrm{sap}}=194\pm 12$ s^{-1} and $K=0.199\pm 0.028$, at $E_0'=-0.498$ V, $Q=13.37$ μC[20)] ($\Gamma_{\mathrm{t}}=5.50\times 10^{-10}$ mol cm^{-2}),[18)] and $\alpha=0.5$.[23)] The broken lines in Fig. 7 represent regression curves. The

TABLE 3. ELECTROCHEMICAL DATA OF ADRIAMYCIN ADSORBED ON A PYROLYTIC GRAPHITE ELECTRODE AND ON A MERCURY ELECTRODE AT pH 4.54

Electrode	E'_0 / V *vs.* SCE	Γ_t^{max} / 10^{-10} mol cm^{-2}	$k_{sap}(\theta \to 0)$ / 10^3 s^{-1}	K	a
Pyrolytic Graphite[a)]	−0.498	7.21	0.870	0.211	2.03
Mercury[b)]	−0.450	1.10	19.8	0.132	1.66

a) This work. b) Ref. 16.

refined parameters, thus obtained, are shown in Table 2. It was found that an increasing Γ_t becomes a decreasing k_{sap}; however, K is almost independent of Γ_t. The average value of K (0.169±0.037) agrees with that estimated by d.c. voltammetry. We have also simulated a.c. voltammograms with only one parameter, k_{sap}, by setting K=0.211. In Fig. 6, a linear graph of log k_{sap} *vs.* θ (open circles) for the refined k_{sap} is given. The agreement between the k_{sap} values obtained by d.c. and a.c. voltammetries is quite good. The results now verify that the curve fitting analysis of d.c. and a.c. voltammograms is a useful technique for determining electrochemical kinetic parameters.

Table 3 summerizes the electrochemical data regarding the adriamycin adsorbed on a pyrolytic graphite electrode as well as that regarding a hanging mercury drop electrode (HMDE), reported in a previous paper.[16)] The E'_0 value at a pyrolytic graphite electrode is more negative by 48 mV than at an HMDE. This difference can be attributed to a disagreement in the adsorption energies on the electrodes, as predicted by Eqs. 9a and 9b. Nevertheless, the K's obtained for the two electrodes are almost the same, and less than unity, indicating the semiquinone formation reaction to be unfavorable thermodynamically at pH 4.54.[17)] Note that the Γ_t^{max} value at a graphite electrode is almost 7 times as large as that at an HMDE, the former being determined by using the geometrical surface area. The "effective" surface area of the pyrolytic graphite electrode, however, is postulated to be no more than tenfold the geometrically calculated surface area. This assumption causes us to believe that the adsorption state of adriamycin on a pyrolytic graphite electrode is essentially the same as that on an HMDE; that is, the aromatic ring of the adsorbed adriamycin must be oriented parallel to the basal-plane of a pyrolytic graphite electrode. The agreement of the values of the Frumkin's α-parameter derived from the two electrodes may support the above consideration. In turn, it was also found that the $k_{sap}(\theta \to 0)$ value, *i.e.* extrapolated k_{sap} value at $\theta \to 0$, evaluated using a pyrolytic graphite electrode is markedly small compared with that at an HMDE. Similar results were observed for flavin-adenine dinucleotide adsorbed on a graphite electrode[5b,24)] and on an HMDE.[8c)]

As a conclusion we may say that the thermodynamic and kinetic informations concerning a two-step surface-redox reaction system can be successfully evaluated by means of a direct non-linear least-squares analysis of d.c. as well as a.c. voltammograms. Particularly, note that in these analyses we do not need (necessarily) information regarding K values, since K can be treated as one of the parameters. The viewpoint that a cyclic d.c. voltammetry has been widely used for the study of the surface-redox reaction would make it noteworthy that the kinetic information can be obtained from the theoretical analysis of the d.c. voltammetry.

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11) Our "basic" computer program is distributed upon request.

12) The value of the *a*-parameter is positive for attraction and negative for repulsion.

13) Strictly speaking, $E_{p,a}$ and $E_{p,c}$ correspond to the peak potentials of the anodic and/or cathodic waves for $K \leq 16$, and also to the valley potentials between two peaks of anodic and/or cathodic waves for $K > 16$.

14) The "basic" program pertinent to the non-linear least-squares analysis was rewritten from that found in the literature[15] with some modification, and combined with a program for the numerical solution of the d.c. voltammogram. Our program is distributed upon request.

15) Y. Tanaka and Y. Yamaoka, "Microcomputer Guide for Chemist," (in Japanese) 2nd ed by Nankodo Press, Tokyo (1983), pp. 114—119. In this program, the criterion for convergence of parameters is $1-[SS(n)/SS(n-1)] \leq 0.001$, where $SS(n)$ is the residual sum of squares for the n-th iteration.

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18) The value of Γ_t was estimated from the electricity, Q, using the relation, $Q=(n_1+n_2)FA\Gamma_t$, where $n_1=n_2=1$ for the redox of the quinone moiety and A is the "geometric" surface area of a pyrolytic graphite electrode.

19) Equation 40 in Ref. 7 was used for the estimation of K, where $\alpha+\beta=1$ and $n=1$.

20) The Q value is the average of Q_a and Q_c of the d.c. voltammogram.

21) In curve-fitting analysis of the voltammogram in Fig. 5, the observed currents were measured at every 10 mV from −0.600 V to −0.380 V from the anodic wave (23 points) and from −0.410 V to −0.650 V for the cathodic wave (25 points). The initial conditions for numerical solution were set as $f_o=0$ and $f_r=1$ at −0.650 V for the anodic wave and $f_o=1$ and $f_r=0$ at −0.360 V for the cathodic wave. The initial parameters were roughly estimated as follows: K was evaluated from the $\Delta E_{p/2}$ of the reversible d.c. wave[19] and k_{sap} was estimated from the ΔE_p by using the working curves like those in Fig. 3 (see text). In a simulation of these voltammograms, the parameters set under the above conditions usually converged at an iteration of no more than 5 cycles.

22) See Eqs. 34a and 34b in Ref. 7 for the analytical solutions of the real and imaginary components of the a.c. voltammograms.

23) In a simulation of the voltammograms in Fig. 7, the observed currents of the real and imaginary components were measured at every 10 mV from −0.360 V to −0.620V (27 points for each component). The initial parameters were estimated as follows: K was the value (0.211) obtained by d.c. voltammetry (see text), and k_{sap} was estimated (to be 234 s^{-1}) using the ratio of the real component current to the imaginary one at $E=E_0'$, according to Eq. 37 in Ref. 7 under the assumpsion of $K=0.211$. In a simulation of these voltammograms, the parameters converged at an iteration of no more than 5 cycles.

24) The authors of Ref. 5b have treated this system as a "one-step" surface-redox reaction and reported the standard electron-transfer rate constant to be about 1 s^{-1}. A reanalysis of the cyclic d.c. voltammograms of Fig. 1 in Ref. 5b by the curve-fitting method proposed in this paper has revealed that $k_{sap}=1.8\ s^{-1}$ and $K=7.6$, under the assumption that $k_{sap}(1)=k_{sap}(2)=k_{sap}$ and $\alpha_1=\alpha_2=0.5$.