January, 1986] © *1986 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, *59*, *265—270 (1986)*

Determination of the Equilibrium and Kinetic Parameters for the Surface-Redox Reaction of Hydroquinone Moiety of Adsorbed Adriamycin by Means of Numerical Simulation of d.c. Voltammogram

Tomonori Konse, Kenji Kano, and Tanekazu Kubota*
Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5-Chome, Gifu 502

(Received August 2, 1985)

Electrochemical equilibrium and kinetic parameters for a redox reaction caused by the hydroquinone moiety of adriamycin adsorbed on a pyrolytic graphite electrode have been determined by means of a numerical simulation of the quasi-reversible d.c. voltammograms using a theory regarding a two-step one-electron surface-redox reaction, since the electron-transfer rate constant is so small that the reversible d.c. wave can not be obtained and an analysis by a.c. voltammetry is difficult. The voltammogram was also analyzed from the viewpoint of a one-step two-electron surface-redox reaction mechanism taking into account the interaction parameters between adsorbed molecules. The analyses in terms of both mechanisms mentioned above reveal that the redox system considered here occurs through a two-step process.

The anthracycline antibiotics adriamycin and daunomycin are highly effective antitumore agents.[1] These drugs, including many new analogues and derivatives, have a dihydroxyanthraquinone structure, i.e. they contain two commonly electroactive sites: a reducible quinone moiety and an oxidizable hydroquinone moiety. The redox reaction of the quinone moiety of these drugs have been intensively investigated using various techniques, including electrochemical, in an attempt to correlate the behavior to the physiological activity of the drugs.[2–7] However, there are only a few articles concerning the redox reaction of the hydroquinone moiety. Baldwin et al.[4] have reported on a surface-redox wave of the hydroquinone moiety of adriamycin adsorbed on a carbon paste electrode. However, their analysis of the electrochemical behavior was only qualitative. One of the reasons may be related to the quasi-reversible character resulting from the small value of the electron-transfer rate constant. This character makes it difficult to estimate the equilibrium and kinetic parameters which would be obtained by an analysis of the reversible d.c. waves and a.c. voltammograms, respectively.[8,9]

In a previous paper[7] we presented a theory pertinent to the quasi-reversible d.c. voltammogram of a two-step surface-redox reaction ($O_{ad}+e\rightleftarrows S_{ad}$, $S_{ad}+e\rightleftarrows R_{ad}$), and derived a new method for determining the electrochemical equilibrium and kinetic parameters by means of a numerical simulation of d.c. voltammograms (i.e. non-linear least-squares curve fitting by the numerical calculation of current-potential curves). This new method was successfully applied to the analysis of the surface-redox reaction for the quinone moiety of adriamycin adsorbed on a basal-plane pyrolytic graphite electrode (BPGE). An excellent agreement between the equilibrium and kinetic parameters obtained from this technique and those obtained from reversible d.c. voltammograms and a.c. voltammograms indicates that the present method is very useful for determining the electrochemical parameters.

In principle, our d.c.-wave-simulation method can be employed even for surface-redox systems where the electron-transfer rate constants are so small that a direct analysis of the reversible d.c. and a.c. voltammograms is experimentally difficult. In this paper, our simulation method has been applied to the analysis of the surface-redox system of the hydroquinone moiety of adriamycin adsorbed on a BPGE. Furthermore, we have derived the theoretical equations regarding the quasi-reversible voltammetry of a one-step two-electron surface-redox mechanism ($O_{ad}+2e\rightleftarrows R_{ad}$), where the parameters describing the mutual interactions between adsorbed molecules have been taken into consideration. Then, the voltammogram was also analyzed based on the theory of a one-step mechanism. A comparison of the simulation results derived from both the mechanisms, i.e. two-step and one-step, suggests that the surface-redox system considered here obeys the above two-step mechanism.

Theoretical Treatment of Quasi-Reversible d.c. Voltammetry of Surface-Redox Reaction

At first, let us consider a one-step n-electron surface-redox reaction:

$$O_{ad} + ne \rightleftarrows R_{ad}, \tag{1}$$

under the condition that all the electrochemical species are strongly adsorbed or immobilized on the electrode surface. Then,

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

where Γ_j's (j=o, r, and t) are the surface concentrations of O_{ad}, R_{ad}, and the total. In the following, our assumption is that the interaction between the adsorbed molecules can be expressed by using Frumkin's a-parameters (a_{ij}; i, j=O or R) and, thus, the surface activity of O_{ad} and R_{ad}, a_o and a_r, is given by[15]

$$a_o = \Gamma_o \exp[-a_{OO} f\theta_t - a_{OR}(1-f)\theta_t] \tag{3}$$

$$a_r = \Gamma_r \exp[-a_{RR}(1-f)\theta_t - a_{RO} f\theta_t], \tag{4}$$

where $f=\Gamma_o/\Gamma_t$, and $\theta_t=\Gamma_t/\Gamma_m$, Γ_m being the maximum surface concentration. Theoretical studies regarding "reversible" d.c. voltammetry[8,11,12] and a.c. voltammetry[8] have been already reported for this type of redox system. Our formulation is, therefore, for "quasi-reversible" d.c. voltammetry.

Assuming that a surface-redox reaction occurs exclusively for adsorbed molecules and that the current-potential-surface activity characteristics can be given by the Butler-Volmer equation,[8,13] we can write:

$$i/nFA = \mathrm{d}\Gamma_o/\mathrm{d}t \tag{5}$$

$$= k_s a_r B_r^{-1} \exp[(1-\alpha)nF(E-E_o)/RT] - k_s a_o B_o^{-1} \exp[-\alpha nF(E-E_o)/RT], \tag{6}$$

where E_o stands for the standard redox potential of the redox couple O/R in the bulk solution, B_j (j=o,r) is the adsorption coefficient of species j at E_o, and k_s is the electron-transfer rate constant at the standard surface-redox potential, E_o'. The E_o'[8,11,12] and the electrode potential, E, are expressed by:

$$E_o' = E_o + (RT/nF) \times [\ln(B_r/B_o) - (a_{OO} - a_{RR} + a_{OR} - a_{RO})\theta_t/2] \tag{7}$$

$$E = E_i + vt. \tag{8}$$

Putting α=0.5, a combination of Eqs. 2 to 8 leads to:

$$\psi = \mathrm{d}f_o/\mathrm{d}\rho$$

$$= m\{(1-f)\rho^{0.5} \exp(-G\theta_t/4) \exp(Jf\theta_t) - f\rho^{-0.5} \exp(G\theta_t/4) \exp[(J-G)f\theta_t]\}, \tag{9}$$

where $\psi=iRT/n^2F^2vA\Gamma_t$, $m=k_{sap}RT/nFv$, $k_{sap}=k_s \times (B_oB_r)^{-0.5}\exp[-(a_{RR}+a_{OR})\theta_t/2]$, $\rho=\exp[nF(E-E_o')/RT]$, $G=a_{OO}+a_{RR}-a_{OR}-a_{RO}$, and $J=a_{RR}-a_{RO}$. The current-potential characteristics for linear sweep voltammetry are, thus, obtained by solving Eq. 9. A standard fourth-order Runge-Kutta method was applied to resolve differential equation 9.[14] The initial conditions are f=0 at $\rho \to 0$ for the anodic curve and f=1 at $\rho \to \infty$ for the cathodic curve. To perform a numerical simulation of the d.c. voltammograms, a BASIC program was rewritten by modifying our program[7] to satisfy the above theoretical treatment. The parameters to be refined are generally k_{sap}, G (or $G\theta_t$), and J (or $J\theta_t$). When a reversible wave is obtained, the G value can be estimated from the peak current or the half-peak width of the "reversible" wave.[15] In such a case, the number of parameters will be diminished to two, k_{sap} and J.

On the other hand, in a previous paper[7] we have reported on a theoretical treatment of the quasi-reversible voltammetry for the two-step n-electron surface-redox reaction:

$$O_{ad} + ne \rightleftharpoons S_{ad} \quad \text{(1st step)}$$

$$S_{ad} + ne \rightleftharpoons R_{ad} \quad \text{(2nd step)}, \tag{10}$$

where a homogeneous interaction between the adsorbed molecules was assumed. The theoretical equations for this case are briefly given below for a comparison with those of the foregoing one-step mechanism. In this case, Eqs. 2, 5, and 6 must be rewritten as follows:

$$\Gamma_o + \Gamma_s + \Gamma_r = \Gamma_t = \text{const.}, \tag{11}$$

$$i = i(1) + i(2) \tag{12}$$

$$i(1)/nFA = \mathrm{d}\Gamma_o/\mathrm{d}t = k_{sap}(1)\{\Gamma_s \exp[(1-\alpha_1)nF(E-E_{o1}')/RT] - \Gamma_o \exp[-\alpha_1 nF(E-E_{o1}')/RT]\} \tag{13}$$

$$i(2)/nFA = -\mathrm{d}\Gamma_r/\mathrm{d}t = k_{sap}(2)\{\Gamma_r \exp[(1-\alpha_2)nF(E-E_{o2}')/RT - \Gamma_s \exp[-\alpha_2 nF(E-E_{o2}')/RT]\}, \tag{14}$$

where $i(j)$, $k_{sap}(j)$, and α_j are, respectively, the faradaic currents, the apparent rate constants at the formal standard surface-redox potentials E_{oj}', and the electron-transfer coefficients at the j-th steps (j=1 and 2). Here we define the semiquinone formation constant, K, as follows:

$$K = [S_{ad}]^2/[O_{ad}][R_{ad}] = \exp[nF(E_{o1}'-E_{o2}')/RT]. \tag{15}$$

The standard surface-redox potential, E_o', corresponding to the redox couples O_{ad}/R_{ad} is given by

$$E_o' = (E_{o1}'+E_{o2}')/2. \tag{16}$$

Equations 15 and 16 are introduced in Eqs. 13 and 14, and the resulting differential equations were numerically solved by combining them with Eqs. 8, 11, and 12. We can, thus, obtain the current-potential characteristics. In general, the parameters to be refined by a numerical simulation are $k_{sap}(1)$, $k_{sap}(2)$, K, α_1, and α_2. As we have already verified for the case of the surface-redox reaction of the quinone moiety of adsorbed adriamycin,[7] if the conditions $k_{sap}(1)=k_{sap}(2)$ and $\alpha_1=\alpha_2=0.5$ are experimentally satisfied, the parameters to be used are only two, $k_{sap}(=k_{sap}(1)=k_{sap}(2))$ and K. In addition, if the K values can be estimated from the reversible d.c. wave,[9] then only the k_{sap} needs to be refined.

Experimental

Adriamycin hydrochloride, which was donated from Kyowa Hakko Kogyo Co., and all other chemicals used

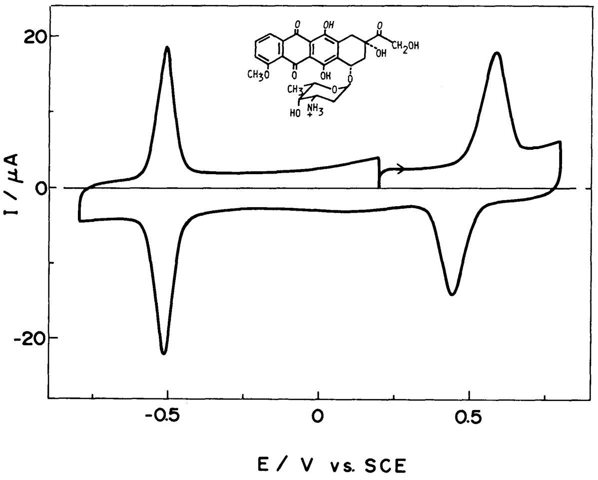

Fig. 1. Cyclic d.c. voltammograms of adriamycin adsorbed on a BPGE. Voltage scanning was done with $v=200$ mV s^{-1}. Before the measurement of cyclic voltammogram the 3.68×10^{-6} mol dm^{-3} adriamycin solution was first stirred for 1 min, then left for 1 min at $E_i=0.2$ V at 25 °C (pH=4.54, acetate buffer, $I=0.5$).

have been described elsewhere.[5–7] The electrochemical measurements and the preparation of BPGE (used as working electrodes) were, for the most part, performed as described in a previous paper.[7] The BPGE were freshly cleaved with a razor blade just before use. In the course of an analysis of the d.c. cyclic voltammograms, the base current was subtracted to determine the faradaic one. The calculation was carried out with an NEC 9801 personal computer. The computer-aided numerical simulations were done mostly performed, for the most part, as described in a previous paper.[7]

Results and Discussion

In Fig. 1 is shown a cyclic d.c. voltammogram of the adriamycin adsorbed on a BPGE in acetate buffer (pH 4.54). The voltammogram was recorded from the initial potential $E_i=0.20$ V to the switching potentials E_s's=0.80 and -0.80 V at a sweep rate $v=200$ mV s^{-1}, a saturated calomel electrode, SCE, being used as a reference electrode. One pair of the d.c. anodic and cathodic reversible waves at -0.50 V can be ascribed to the surface-redox reaction of the quinone moiety of adsorbed adriamycin.[3,5,6] Another pair of the quasi-reversible anodic and cathodic waves was observed at 0.60 and 0.45 V, respectively, and can be ascribed to a surface-redox reaction of the hydroquinone moiety of the substance.[4] The peak-separations of the anodic and cathodic waves on the two redox systems tell us that the electron-transfer rate constant of the hydroquinone moiety is considerably smaller than that of the quinone moiety. Actually, in a phase-selective a.c. voltammetry, the imaginary component pertinent to the redox system of the hydroquinone moiety was too small, even at the frequency as low as 100 Hz. Therefore, the analysis was difficult.

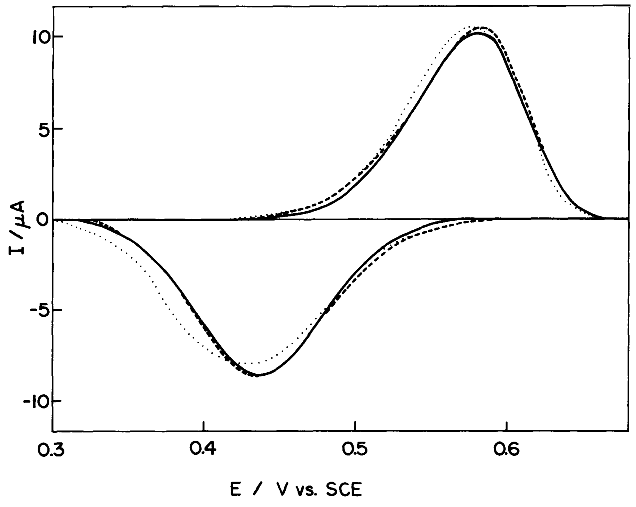

Fig. 2. Cyclic d.c. voltammograms of adriamycin adsorbed on a BPGE. Voltage scanning was done with $v=200$ mV s^{-1} under the same conditions as those in Fig. 1. The current is corrected for the base current. The broken line represents the regression curve calculated under the conditions of $k_{\mathrm{sap}}(1)=9.49$ s^{-1}, $k_{\mathrm{sap}}(2)=1.50$ s^{-1}, $K=0.06$, $\alpha_1=0.35$, and $\alpha_2=0.30$ for the case of the two-step mechanism. The dotted line shows the regression curve calculated under the conditions of $k_{\mathrm{sap}}=0.187$ s^{-1}, $G\theta_t=-3.82$, and $J\theta_t=-1.20$ for the case of one-step mechanism. $E_0'=0.466$ V, $Q=4.845$ μC, and the "geometric" area of the electrode was 0.126 cm^2.

The solid line in Fig. 2 shows the d.c. voltammogram recorded from $E_i=0.20$ V to $E_s=0.80$ V at $v=200$ mV s^{-1} under the same conditions as those in Fig. 1. In Table 1 are listed the peak potentials ($E_{p,a}$, $E_{p,c}$), peak currents ($i_{p,a}$, $i_{p,c}$), half-peak widths ($\Delta E_{p/2,a}$, $\Delta E_{p/2,c}$), peak-separations ($\Delta E_p=E_{p,a}-E_{p,c}$), and $(E_{p,a}+E_{p,c})/2$ at various v, here subscripts a and c indicate anodic and cathodic waves, respectively. Upon decreasing v, ΔE_p became smaller. However, ΔE_p did not converge to zero, even at $v=5$ mV s^{-1}, i.e. a so-called d.c. reversible wave can not be recorded. On the other hand, the shape of the anodic wave is obviously not symmetrical with that of a cathodic one at a given v. Also, the $(E_{p,a}+E_{p,c})/2$ values shift to the cathodic side with an increasing v. These circumstances are very different from those for the quinone moiety. In the following analysis of the voltammograms, E_0' was estimated to be 0.496 V by extrapolating the v vs. $(E_{p,a}+E_{p,c})/2$ relation to $v=0$ within a sweep rate of 5 to 50 mV s^{-1}.

These cyclic voltammograms were then analyzed from the viewpoint of a two-step one-electron surface-redox mechanism while assuming a homogeneous interaction between the adsorbed mole-

Table 1. Scan-Rate Dependence of the Peak Potential, Peak Current, Half-Peak Width, Peak Separation, and the Average of Peak Potential of d.c. Voltammogram of Adriamycin Adsorbed on a Pyrolytic Graphite Electrode at pH 4.54

v		$E_{\mathrm{p,a}}$	$E_{\mathrm{p,c}}$	$i_{\mathrm{p,a}}$[a)]	$i_{\mathrm{p,c}}$[a)]	$\Delta E_{\mathrm{p/2,a}}$	$\Delta E_{\mathrm{p/2,c}}$	ΔE_{p}	$(E_{\mathrm{p,a}}+E_{\mathrm{p,c}})/2$
mV s^{-1}		V vs. SCE		μA		mV		mV	V vs. SCE
5	Obsd	0.502	0.492	0.39	−0.38	55	55	10	0.497
10	Obsd	0.509	0.488	0.78	−0.71	55	60	21	0.503
	Calcd[b)]	0.509	0.485	0.78	−0.80	56	54	24	0.497
		(0.508)	(0.489)	(0.51)	(−0.46)	(91)	(105)	(19)	(0.499)
20	Obsd	0.522	0.483	1.50	−1.34	61	66	39	0.503
	Calcd[b)]	0.518	0.478	1.43	−1.48	62	59	40	0.498
		(0.520)	(0.481)	(1.05)	(−0.88)	(88)	(109)	(39)	(0.506)
50	Obsd	0.540	0.470	3.19	−3.11	74	72	70	0.505
	Calcd[b)]	0.537	0.464	3.05	−3.13	74	70	73	0.501
		(0.541)	(0.464)	(2.63)	(−2.07)	(88)	(118)	(77)	(0.506)
100	Obsd	0.560	0.452	5.75	−5.51	81	84	108	0.506
	Calcd[b)]	0.557	0.451	5.49	−5.29	82	84	106	0.504
		(0.558)	(0.446)	(5.23)	(−4.01)	(87)	(119)	(112)	(0.502)
200	Obsd	0.580	0.436	10.20	−8.57	83	103	144	0.508
	Calcd[b)]	0.581	0.436	10.33	−8.67	87	103	145	0.509
		(0.576)	(0.428)	(10.39)	(−7.93)	(88)	(120)	(148)	(0.505)

a) The current values are normarized to $Q=4.845\ \mu\mathrm{C}$, assuming that i_{p} is proportional to Q in the narrow Q range. b) Calculated according to the theory of two-step one-electron mechanism under the conditions of $k_{\mathrm{sap}}(1)=9.49\ \mathrm{s}^{-1}$, $k_{\mathrm{sap}}(2)=1.50\ \mathrm{s}^{-1}$, $K=0.06$, $\alpha_1=0.35$, and $\alpha_2=0.30$ at $E_0'=0.496\ \mathrm{V}$ and $Q=4.845\ \mu\mathrm{C}$. The values in the parentheses denote the calculated ones according to the theory of one-step two-electron mechanism under the conditions of $k_{\mathrm{sap}}=0.187\ \mathrm{s}^{-1}$, $G\theta_{\mathrm{t}}=-3.82$, and $J\theta_{\mathrm{t}}=-1.20$.

cules.[7)] A direct estimation of K from a reversible wave[9)] is impossible in this case, and the unsymmetrical shape and the v dependence of $(E_{\mathrm{p,a}}+E_{\mathrm{p,c}})/2$ of the quasi-reversible waves indicate that $k_{\mathrm{sap}}(1)\neq k_{\mathrm{sap}}(2)$ and/or α_1, $\alpha_2\neq 0.5$.[7)] For a numerical simulation of the voltammogram in Fig. 2, the following five parameters were used: $k_{\mathrm{sap}}(1)$, $k_{\mathrm{sap}}(2)$, K, α_1, and α_2. After repeated simulation trials, the parameters finally refined were: $k_{\mathrm{sap}}(1)=9.49\pm1.06\ \mathrm{s}^{-1}$, $k_{\mathrm{sap}}(2)=1.50\pm0.07\ \mathrm{s}^{-1}$, $K=0.060\pm0.009$, $\alpha_1=0.35\pm0.02$, and $\alpha_2=0.30\pm0.02$ at $Q=4.845\ \mu\mathrm{C}$.[16)] The resultant regression curve is depicted in Fig. 2 as a broken curve. The fitting of the curves seems to be quite good. On the other hand, a numerical simulation with the three parameters, $k_{\mathrm{sap}}(1)$, $k_{\mathrm{sap}}(2)$, and K, was also undertaken while assuming $\alpha_1=\alpha_2=0.5$. However, the resulting regression curve did not reproduce very well the experimental one.[17)]

In general, attention is necessary in a least-squares analysis based on a statistical treatment. This is in case that the parameters used are sufficiently large in number compared to those of observed points, and/or the collinearity among the parameters is large. In these cases there occurs some possibility that the refined parameter values themselves, thus derived, may be physically meaningless, though the regression curve, itself, results in a good reproduction of the observed one.[18)] In order to check the validity of the parameters refined during this study, we calculated the values of $E_{\mathrm{p,a}}$, $E_{\mathrm{p,c}}$, $i_{\mathrm{p,a}}$, $i_{\mathrm{p,c}}$, $\Delta E_{\mathrm{p/2,a}}$, $\Delta E_{\mathrm{p/2,c}}$, ΔE_{p}, and $(E_{\mathrm{p,a}}+E_{\mathrm{p,c}})/2$ at various v by using the refined parameters described above, and compared them with the observed values. Table 1 gives the results. The calculated values and the observed values agreed well with each other.

In turn, Brown and Anson, and Kakutani et al. reported (independently) that the redox reactions of 9,10-phenanthrenequinone adsorbed on a pyrolytic graphite electrode[11)] and of flavin-adenine dinucleotide adsorbed on a mercury electrode[19)] can be reasonably explained by a one-step two-electron surface-redox system by taking account of heterogeneous interactions between the adsorbed molecules. Now, we would like to consider this reaction in terms of a one-step two-electron surface-redox system, since the K value (0.06) estimated above is very small. A numerical simulation was undertaken by using three parameters; k_{sap}, $G\theta_{\mathrm{t}}$, and $J\theta_{\mathrm{t}}$. A simulation of the voltammogram at $v=200\ \mathrm{mV\ s}^{-1}$ (Fig. 2) resulted in $k_{\mathrm{sap}}=0.187\pm0.02\ \mathrm{s}^{-1}$, $G\theta_{\mathrm{t}}=-3.82\pm0.02$, and $J\theta_{\mathrm{t}}=-1.20\pm0.15$: the regression curve is shown in Fig. 2 by a dotted curve. Also, the values of E_{p}, i_{p}, $\Delta E_{\mathrm{p/2}}$, ΔE_{p}, and $(E_{\mathrm{pa}}+E_{\mathrm{pc}})/2$ were calculated numerically using the estimated parameters (listed in parentheses in Table 1). Although the fitting of the regression curve to the observed voltammogram is good at $v=200\ \mathrm{mV\ s}^{-1}$, the discrepancy between the observed and calculated values is obvious as can be seen in

Table 1. This is especially true for i_p and $\Delta E_{p/2}$ at a decreasing v. These results indicate that this redox system is not well described in terms of the one-step surface-redox reaction, even if the heterogeneous interactions between the adsorbed species are taken into account. As a result, it may be safely said that the redox reaction obeys the two-step mechanism; then, the above five parameters, refined on the basis of the two-step mechanism, can be considered to be physically significant.

It is interesting to compare the values of the equilibrium and kinetic parameters obtained for the hydroquinone moiety of the adsorbed adriamycin with those of the quinone moiety. First of all, the E_0' value of the hydroquinone part is more positive (by 1 V) than that of the quinone part (−0.498 V at pH 4.54[7]). The oxidation and the reduction of adriamycin occur at the hydroquinone and the quinone parts, respectively; thus, the hydroquinone part in the HOMO and the quinone part in the LUMO may play an important role in the oxidation and the reduction processes, respectively. Now, the difference in the above two E_0' values can be easily understood on the basis of the well-known energy-level order. In our case, however, deprotonation and protonation are coupled with the oxidation and the reduction of the hydroquinone and quinone moieties, respectively. This is because of a redox reaction in an aqueous medium. These circumstances would make it possible to reduce the difference in the two E_0' values in comparison with those expected from the HOMO and LUMO energy difference, which is correlated directly for the case of a nonaqueous medium.[20] Secondly, the semiquinone formation reaction ($O_{ad}+R_{ad}\rightarrow 2S_{ad}$) of the hydroquinone moiety in adriamycin is thermodynamically up-hill as in the case of the quinone moiety. The estimated K value (0.06) for the hydroquinone moiety corresponds to $+7.0\,\mathrm{kJ\,mol^{-1}}$ in the free energy change of this reaction, ΔG°, which is about 2 times as large as that of the quinone moiety (K=0.211[7]). This stability difference between the semiquinone species produced from the hydroquinone and the quinone parts might be attributable to an intramolecular hydrogen bond. In case of the semiquinone formation reaction of the quinone moiety, two hydrogen bonds formed between carbonyl groups and hydroxyl groups in adriamycin are kept in the electron transfer process. On the other hand, any one of the two hydrogen bonds should be removed due to a one-electron oxidation of the hydroquinone moiety. As a result, we can easily understand that the semiquinone species derived from the quinone part is more stable than that from the hydroquinone part. Finally, let us consider the rate constant values of $k_{sap}(1)$ and $k_{sap}(2)$. These $k_{sap}(1)$ and $k_{sap}(2)$ for the hydroquinone moiety are less than one-fiftieth and one-three hundredth of those of the quinone moiety ($k_{sap}(1)=k_{sap}(2)=5.16\times10^2$ $\mathrm{s^{-1}}$ at Q=4.845 μC[7,21]), respectively. The small value of k_{sap} for the hydroquinone moiety may also, in part, be ascribed to an intramolecular hydrogen-bond breakdown and/or formation in the process of a semiquinone formation equilibrium, as has been described above. This is because the activation barrier for proceeding to the semiquinone species seems to be higher for the hydroquinone moiety than for the quinone moiety, under the assumption that the barrier height is in parallel to the above-mentioned ΔG° values (chemical non-crossing rule[22]). Here note that $k_{sap}(1)$ is 6 times as large as $k_{sap}(2)$[23,24] and also $\alpha_1=0.35$ and $\alpha_2=0.30$ for the case of the hydroquinone moiety. This unsymmetrical nature was not observed for the hydroquinone moiety in the acetate buffer (pH 4.54) containing 9.1% *N,N*-dimethylformamide (v/v) as well as the quinone moiety in the acetate buffer.[5,7] In the former case, the analysis of the voltammograms indicates that $k_{sap}(1)=k_{sap}(2)=5.16\pm0.67\ \mathrm{s^{-1}}$, $K=0.072\pm0.046$, $\alpha_1=\alpha_2=$ 0.5, and $E_0'=0.514$ V at Q=2.301 μC. This kind of solvent effect on the kinetic parameters may also suggest the significance of inter- and intramolecular hydrogen bonds in the redox reaction. Additional work will be postponed to a future study.

We may now say, as a conclusion, that the present numerical simulation, based on a theory pertinent to the quasi-reversible d.c. voltammogram in terms of the two-step surface-redox mechanism, can be safely applied to estimate the electrochemical equilibrium and the kinetic parameters of the surface-redox system, of which the rate constants are so small that an observation of the d.c. reversible wave and the application of a.c. voltammetry are difficult. In addition, we believe that this technique can also be applied to surface-redox systems coupled with succeeding irreversible chemical reactions, such as redox reactions of the adsorbed adriamycin in neutral and alkaline media.[3,6] This is because the effect of the succeeding chemical reaction may be excluded by increasing the sweep rate,[6] then, the so-called quasi-reversible d.c. voltammograms are recorded.

We are grateful to Kyowa Hakko Kogyo Co. for kindly supplying the adriamycin. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 59390012) from the Ministry of Education, Science and Culture.

References

1) A. Di. Marco, F. Arcamore, and F. Zunino, "Antibiotics III-Mechanism of Action of Antimicrobial and Antitumor Agents," ed by J. W. Corcoran and F. E. Hahn, Springer, New York (1975), pp. 101—128; S. Neidle, "Topics in Antibiotic Chemistry," ed by P. G. Sammes, John

Wiley & Sons, New York (1978), Vol. 2, pp. 240—278.

2) N. R. Bachur, S. L. Gordon, and M. V. Gee, *Mol. Pharmacol.*, **13**, 901 (1977); L. W. Lown, S. K. Sim, K. C. Majumdar, and R. Y. Chang, *Biochem. Biophys. Res. Commun.*, **76**, 705 (1977); B. Kalyanaraman, E. Perez-Reyes, and R. P. Mason, *Biochim. Biophys. Acta*, **630**, 119 (1980); S. Sato, M. Iwaizumi, K. Handa, and Y. Tamura, *Gann*, **68**, 603 (1977); J. W. Lown and H.-H. Chen, *Can. J. Chem.*, **59**, 3212 (1981).

3) G. M. Rao, J. W. Lown, and J. A. Plambeck, *J. Electrochem. Soc.*, **125**, 534, 540 (1978); C. Molinier-Jumel, B. Malfoy, J. A. Reynaud, and G. Aubel-Sadron, *Biochem. Biophys. Res. Commun.*, **84**, 441 (1978); V. Malatesta, S. Penco, N. Sacchi, L. Valentine, A. Vigevani, and F. Arcamone, *Can. J. Chem.*, **62**, 2845 (1984).

4) R. P. Baldwin, D. Packeff, and T. M. Woodcock, *Anal. Chem.*, **53**, 540 (1981); E. N. Chaney, Jr. and R. P. Baldwin, *Anal. Chem.*, **54**, 2556 (1982).

5) K. Kano, T. Konse, N. Nishimura, and T. Kubota, *Bull. Chem. Soc. Jpn.*, **57**, 2383 (1984).

6) K. Kano, T. Konse, and T. Kubota, *Bull. Chem. Soc. Jpn.*, **58**, 424 (1985).

7) K. Kano, T. Konse, and T. Kubota, *Bull. Chem. Soc. Jpn.*, **58**, 1879 (1985).

8) T. Kakutani and M. Senda, *Bull. Chem. Soc. Jpn.*, **52**, 3236 (1979).

9) T. Kakutani and M. Senda, *Bull. Chem. Soc. Jpn.*, **53**, 1942 (1980).

10) B. B. Damaskin, O. A. Petri, and V. V. Batrakov, "Adsorption of Organic Compounds on Electrodes", Plenum Press, New York (1971).

11) A. P. Brown and F. C. Anson, *Anal. Chem.*, **49**, 1589 (1977).

12) E. Laviron, *J. Electroanal. Chem.*, **52**, 395 (1974); D. F. Smith, K. Millman, K. Kuo, and R. W. Murray, *J. Electroanal. Chem.*, **95**, 217 (1979).

13) E. Laviron, *J. Electroanal. Chem.*, **101**, 19 (1979).

14) H. Margenau and G. M. Mupphy, "The Mathematics of Physics and Chemistry," the 2nd Japanese, ed by T. Sato and M. Kunimune, Kyoritsu Press, Tokyo (1961), p. 534.

15) See Eqs. 25—28 of Ref. 8.

16) In simulation of voltammogram in Fig. 2, the observed currents were measured at every 10 mV from 0.490 V to 0.630 V for the anodic wave (15 points) and from 0.550 V to 0.360 V for the cathodic wave (20 points). The observed current was normarized to an average value of Q pertinent to the anodic and cathodic waves, which were determined by measuring the area under the waves.

17) The regression curve shows that $E_{p,a}=0.576$ V, $E_{p,c}=0.428$ V, $i_{p,a}=9.00$ μA, $i_{p,c}=-8.50$ μA, $\Delta E_{p/2,a}=98.1$ mV, and $\Delta E_{p/2,c}=104.8$ mV. The discrepancy between the observed and calculated values is observed especially for $E_{p,c}$, $i_{p,a}$, and $\Delta E_{p/2,a}$ (see Table 1).

18) T. Nakagawa and Y. Oyanagi, "Analysis of Experimental Data by Least-Squares Method—Program SALS," (in Japanese), Applied Mathematics Series, Vol. 7, Tokyo Univ. Press, Tokyo (1982), Chap. 7; T. Kubota, J. Hanamura, K. Kano, and B. Uno, *Chem. Pharm. Bull.*, **33**, 1488 (1985) and references cited therein.

19) T. Kakutani, I. Katasho, and M. Senda, *Bull. Chem. Soc. Jpn.*, **56**, 1761 (1983). They concluded that the surface-redox reaction of flavin-adenine dinucleotide is brought about by way of the two-step one-electron system at $\theta_t<0.83$ ($\Gamma_t=5\times10^{-11}$ mol cm^{-2}) and of the one-step two-electron system at $\theta_t=0.83-1$.

20) H. Miyazaki, T. Kubota, and M. Yamakawa, *Bull. Chem. Soc. Jpn.*, **45**, 780 (1972); T. Kubota, H. Miyazaki, K. Ezumi, and M. Yamakawa, *Bull. Chem. Soc. Jpn.*, **47**, 491 (1974); T. Kubota, H. Miyazaki, M. Yamakawa, K. Ezumi, and Y. Yamamoto, *Bull. Chem. Soc. Jpn.*, **52**, 1588 (1979).

21) The k_{sap} of the quinone moiety depends on the total surface coverage θ_t.(7) This k_{sap} value was estimated by interpolating the $\log k_{sap}$ vs. θ_t plot (Fig. 6 in Ref. 7) to $\theta_t=0.277$ ($Q=4.845$ μC).

22) T. Nagata, "Introduction to Quantum Biology," (in Japanese), Tokyo Univ. Press, Tokyo (1975), p. 24.

23) Armstrong et al.(23) reported that the rate constant of the first step one-electron transfer would be smaller than that of the second step one-electron transfer for the oxidized flavodoxin. In connection with their report we may say that our analytical method of voltammograms gives clearly the electron-transfer rate constants of the first and second steps separately, though the application is limited to only the redox reaction of surface-attached species. Our method then seems to be very useful.

24) F. A. Armstrong, H. A. O. Hill, B. N. Oliver, and N. J. Walton, *J. Am. Chem. Soc.*, **106**, 921 (1984). See also: S. G. Meyhew, V. Massey, *Biochim. Biophys. Acta*, **315** 181 (1973).