The Effects of the pH and the Temperature on the Oxidation-reduction Properties of Adriamycin Adsorbed on a Mercury Electrode Surface

Kenji KANO, Tomonori KONSE, and Tanekazu KUBOTA*
Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5-chome, Gifu 502
(Received July 18, 1984)

The effects of the pH and the temperature on the oxidation-reduction properties of the quinone moiety in adriamycin adsorbed on a mercury electrode surface have been investigated by means of cyclic d.c. voltammetry. The quinone waves have been fundamentally interpreted in terms of a two-step one-electron surface-redox reaction, even in a neutral or an alkaline solution, where the reduced product is liable to cause the following chemical reaction. The pH dependences of the standard surface-redox potential of the adsorbed adriamycin are −60 mV/pH (pH 2—6), −30 mV/pH (pH 7—8), and −60 mV/pH (pH 9—12). The semiquinone formation constant is 0.15 at pH 2—6 and 5 at pH 9—12. These results can be well explained by considering the protolytic equilibria of the phenolic hydroxyl groups among the oxidized, semiquinone, and reduced forms of adriamycin, the pK_a's of which are 8.53, 6.93, and 6.83 respectively. The thermodynamic constants of the semiquinone formation reaction at pH 4.5 have been determined to be $\Delta H=-24.6$ kJ mol^{-1} and $\Delta S=-99.7$ J K^{-1} mol^{-1}.

In a previous paper,[1] we have shown that adriamycin is strongly adsorbed on a mercury electrode surface and that the electrochemical redox reaction of the quinone moiety of the adsorbed adriamycin in an acidic solution can be well explained by the theory of a two-step one-electron redox reaction.[2] The apparent charge-transfer rate constants, redox potentials, and semiquinone formation constants of the surface-redox reaction have been determined at pH 2.69 and 4.54 using cyclic d.c. and a.c. voltammetry. These electrochemical and thermodynamic properties could be useful in an elucidation of the biological action mechanism of adriamycin.

In this paper, we have studied the pH dependence of the redox potential and the semiquinone formation constant in order to obtain information about the protolytic equilibria of the reduced and semiquinone forms as well as the oxidized (or parent) form of the adsorbed adriamycin. The protolytic equilibrium and the chemical stability of the oxidized form of adriamycin in the bulk solution have also been studied spectrophotometrically. Furthermore, we have investigated the thermodynamic property of the semiquinone formation reaction.

Experimental

Chemicals. Adriamycin hydrochloride and all the other chemicals used have been described elsewhere.[1]

Electrochemical Measurements. Cyclic d.c. voltammograms were recorded with a Yanagimoto P-1000 voltammetric analyzer, equipped with a Watanabe WX-4401 X-Y recorder. In the cyclic voltammetry at a sweep rate of more than 500 mV s^{-1}, an Iwatsu DM7100-DM711D digital memory (12 bit×1024 word, 5 μs/word) was used with the above system, while the voltage ramp was applied with an NF circuit FG-121B function generator.

All the voltammetric measurements were performed under potentiostatic conditions with a three-electrode system consisting of a hanging mercury drop working electrode (HMDE), a coiled platinum counter electrode, and a saturated calomel reference electrode (SCE). A fresh mercury drop from the HMDE was exposed to the electrolysis solution for a given period of time, t_{exp}, at a constant d.c. initial potential, E_i. Then the d.c. voltage scan was started from the E_i. Buffer solutions (0.2 mol dm^{-3} sodium acetate-nitric acid for pH 2.11—6.13; 0.1 mol dm^{-3} disodium hydrogenphosphate-nitric acid for pH 6.27—7.44; 0.2 mol dm^{-3} tris-(hydroxymethyl) methanamine-nitric acid for pH 7.89—9.00; 0.1 mol dm^{-3} sodium carbonate-nitric acid for pH 9.90—10.72; 0.05 mol dm^{-3} trisodium phosphate-nitric acid for pH 11.7) were used as the base solutions. The ionic strength of the base solutions was adjusted to 0.5 mol dm^{-3} with potassium nitrate. The other details of the electrochemical measurements have been described elsewhere.[1]

Spectral Measurements. The absorption spectra were recorded at room temperature in the usual manner with a Hitachi 323 spectrophotometer. To minimize decomposition errors, 20.0 μl of the adriamycin stock solution in methanol was injected into 2.00 ml of the buffer solution in a measuring cell just before recording the spectra. The concentration of adriamycin in the cell was chosen to be less than 1×10^{-5} mol dm^{-3} in order to eliminate the effect of the self-aggregation of adriamycin.[3,4]

Results and Discussion

A. pH Effect. Figure 1 shows the cyclic d.c. voltammogram of 1.46×10^{-6} mol dm^{-3} adriamycin at pH 4.54; it was recorded after $t_{exp}=4$ min at $E_i=-0.2$ V.

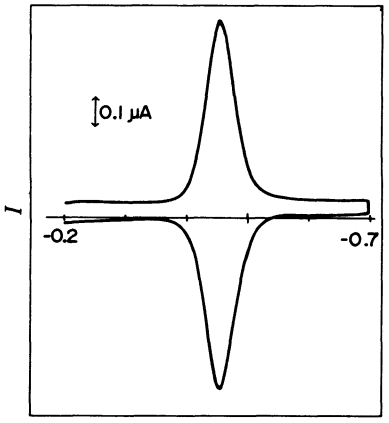

Fig. 1. Cyclic d.c. voltammogram of 1.46×10^{-6} mol dm^{-3} adriamycin at a pH 4.54 acetate buffer. $t_{exp}=4$ min at $E_i=-0.200$ V with $v=100$ mV s^{-1}.

A symmetrical pair of cathodic and anodic waves is observed. These waves are clearly to be ascribed to the surface-redox reaction of the quinone moiety in the adriamycin adsorbed on the mercury electrode.[1] When the voltammogram is recorded with an E_i value more positive than −0.3 V at pH 4.54, the peak potential, E_p^{dc}, and the half-peak width, $\Delta E_{p/2}^{dc}$, are −0.450 V and 52 mV respectively, regardless of the sweep rate, v, and the surface concentration of adriamycin, Γ.[5]

As has been described in a previous paper,[1] this wave can be interpreted in terms of a d.c. reversible two-step one-electron surface-redox reaction:[2,6]

$$\begin{aligned} O_{ad} + e &\rightleftharpoons S_{ad} \\ S_{ad} + e &\rightleftharpoons R_{ad} \end{aligned} \quad (1)$$

where O_{ad}, S_{ad}, and R_{ad} denote the oxidized (parent), semiquinone, and reduced forms of the adsorbed adriamycin respectively. In Scheme (1), the semiquinone formation constant, K, of the adsorbed adriamycin is defined by:

$$\begin{aligned} K &= [S_{ad}]^2/[O_{ad}][R_{ad}] \\ &= \exp[(F/RT)(E'_{o1} - E'_{o2})] \end{aligned} \quad (2)$$

where E'_{o1} and E'_{o2} are the standard surface-redox potentials of the O_{ad}/S_{ad} and S_{ad}/R_{ad} couples respectively. When $K<16$, the d.c. voltammogram has its maximum at the standard surface-redox potential of the O_{ad}/R_{ad} couple, E'_o, where E'_o is given by:

$$E'_o = (E'_{o1} + E'_{o2})/2 \quad (3)$$

We can estimate the K value from $\Delta E_{p/2}^{dc}$ according to:

$$\zeta^4 - K\zeta^3 + (K - 4\sqrt{K} - 6)\zeta^2 - K\zeta + 1 = 0 \quad (4)$$

where $\zeta = \exp[(F/2RT)\Delta E_{p/2}^{dc}]$, or from the peak current, i_p, *vs.* the electricity, Q, plot according to:

$$i_p = (F/RT)Qv/(2+\sqrt{K}) \quad (5)$$

The K value at pH 4.54 has been determined to be 0.138±0.043 by the use of Eq. (4) and 0.126±0.034 by the use of Eq. (5). These values agree well with each other. The E'_{o1} and E'_{o2} values can be estimated by using the K value in Eq. (2) and the E'_o value in Eq. (3). The results are given in Table 1.

Table 1. Electrochemical and thermodynamic parameters of adriamycin adsorbed on a mercury electrode at various pHs

pH	K	E'_o	E'_{o1}	E'_{o2}
		V *vs.* SCE		
2.11	0.10	−0.299	−0.329	−0.270
2.60	0.15	−0.333	−0.357	−0.308
2.77	0.15	−0.340	−0.365	−0.315
3.05	0.14	−0.363	−0.389	−0.337
4.54	0.13	−0.450	−0.476	−0.424
5.02	0.19	−0.489	−0.510	−0.467
6.13	0.21	−0.538	−0.558	−0.518
6.27	0.15	−0.544	−0.569	−0.519
6.59	0.44	−0.570	−0.581	−0.560
7.14	0.52	−0.586	−0.594	−0.577
7.30	0.79	−0.600	−0.603	−0.597
7.44	0.77	−0.603	−0.607	−0.600
7.89	0.95	−0.625	−0.626	−0.625
8.45	1.78	−0.646	−0.638	−0.653
9.00	2.98	−0.680	−0.666	−0.694
9.90	3.42	−0.719	−0.703	−0.735
10.59	5.16	−0.755	−0.734	−0.776
10.68	5.37	−0.758	−0.736	−0.780
11.70	5.32	−0.829	−0.807	−0.850

Figure 2A shows the d.c. cyclic voltammogram of adriamycin at pH 7.44, which was recorded with v= 100 mV s^{-1} after t_{exp}=1 min at E_i=−0.4 V. Under these conditions, the cathodic and anodic waves are not symmetrical, and the peak potentials of the cathodic and anodic waves are −0.610 V and −0.642 V respectively. Where the voltammogram is recorded with v= 1000 mV s^{-1}, however, a symmetrical pair of cathodic and anodic waves is observed. These results indicate that the reversible electrochemical redox process of the parent compound is prevented at a relatively small v, *i.e.*, for a period of a few seconds, because of a subsequent irreversible chemical reaction of the semiquinone and/or reduced form adriamycin, and that the effect of the following chemical reaction can be disregarded at a large v. As was stated in the previous paper, this kind of chemical reaction can be observed even at pH 4.54 on electrolysis for a period of several minutes, but not in the voltammetry with $v>$ 10 mV s^{-1}. This reaction may be considered as an irreversible elimination of the amino-sugar moiety, giving rise to 7-deoxyadriamycinone.[7] The chemical reaction rate of the reduced products increases with the pH. At any rate, we can estimate the E'_o and K values, and also the E'_{o1} and E'_{o2} values, from the cyclic voltammogram obtained with so large a v as to neglect the following chemical reaction.

With an increase in the pH to more than 8.5, the orange-red solution (pH<8) of the adriamycin turns blue-violet, indicating the acid dissociation of phenolic hydroxyl group in the parent molecule. Figure 3 shows the visible spectra of adriamycin at pH 6.00 and 10.72; they were recorded immediately after prep-

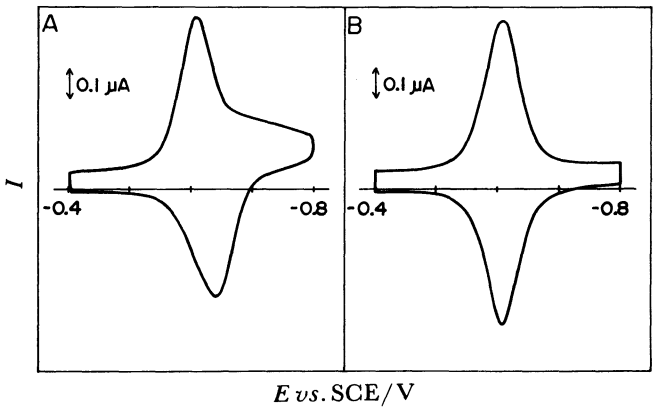

Fig. 2. Cyclic d.c. voltammograms of 4.77×10^{-6} mol dm^{-3} adriamycin at a pH 7.44 phosphate buffer with v=A) 100 mV s^{-1} and B) 1000 mV s^{-1}. t_{exp}=4 min at E_i=−0.400 V.

aration. In Fig. 4, the absorbance for 9.70×10^{-6} mol dm^{-3} adriamycin at 483.2 nm is plotted against the pH. Considering the protolytic equilibrium of the hydroxyl group of adriamycin, the total absorbance, A, at a constant analytical concentration is given by:

$$A = A_a + (A_b - A_a)\frac{K_a(\text{bulk})}{[H^+]+K_a(\text{bulk})} \quad (6)$$

where A_a and A_b represent the absorbance of the sufficiently acidic and alkaline solutions respectively, K_a (bulk) being the acid-dissociation constant of adriamycin in the solution. Regression analysis of A *vs.* pH plot was done using Eq. (6), where the absorbances at pH 6.00 and 10.72 were used for A_a and A_b respectively. The result is pK_a (bulk)=9.33±0.06. At pH 10.0, however, the absorbance decreases with the time, and the solution becomes almost colorless after about 2 h. This reaction can be interpreted as a base-catalyzed hydrolysis of the "deprotonated" adriamycin.[8]

To minimize the effect of the decomposition at pHs higher than 8.5, we recorded the voltammogram with v=1000 mV s^{-1} immediately after injecting the adriamycin stock solution into the deaerated base solution and exposing the electrode to the solution. The solid line in Fig. 5 represents the first scan of the multicyclic voltammogram recorded at pH 9.00 after t_{exp}=1 min at E_i=−0.400 V and with v=1000 mV s^{-1}. Two cathodic waves, I_c and I_c', are observed; their peak potentials are −0.680 V for the I_c wave and −0.772 V for the I_c' wave. In the anodic scan, only a symmetrical wave, I_a, is observed. The peak potential of the I_a wave is −0.680 V, coinciding with that of the I_c wave. The broken line in Fig. 5 represents the second scan, where we see that, although the I_c' wave disappears almost completely, the decrease in the wave height of I_c and I_a is small. In addition, the wave shape of the latter (I_c and I_a) is almost symmetrical, and the peak potentials coincide with those of the first scan. As a result, the pair of waves, I_c and I_a, may be considered to be practically d.c. reversible; then, the values of E_o', E_{o1}', E_{o2}' and K can be estimated. The origin of the I_c' wave is not clear at the present stage, but it may be due to the reorientation of the adsorbed molecules. In another interpretation, the appearance of the I_c' wave may be related to a following chemical reaction, such as the dimerization of the reduced form.[6]

Table 1 summarizes the electrochemical and thermodynamic parameters determined by means of the cyclic voltammetry. The K values are 0.1—0.2 at pH<6 and 3—5 at pH>9. In other words, the semiquinone formation reaction is thermodynamically favored at higher pHs. Figure 6 shows the pH dependence of E_o', E_{o1}', and E_{o2}'. The E_o' *vs.* pH plot is composed of three linear relations, the slopes being −60 mV/pH (pH 2—6), −30 mV/pH (pH 7—8), and −60 mV/pH (pH 9—12). The first and the second inflection points at pH 6.8 and 8.5 correspond to the acid-dissociation constants of the reduced and oxidized forms of the adsorbed adriamycin respectively. These findings lead us to consider the following protolytic and electrochemical equilibria:

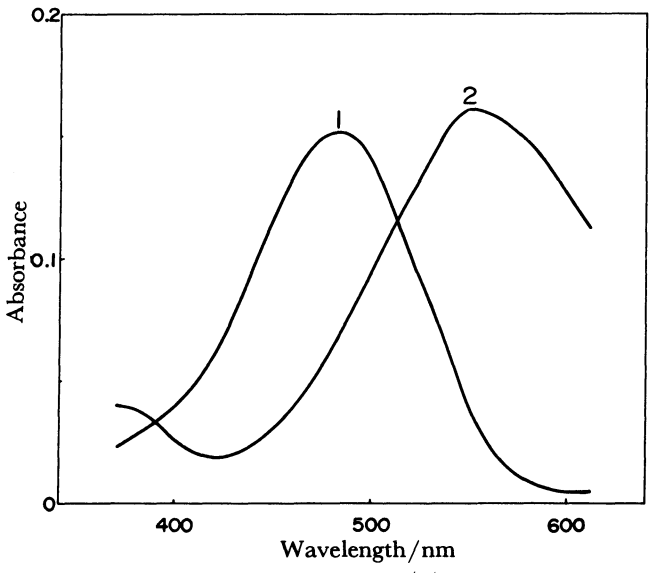

Fig. 3. Visible spectra of 9.70×10^{-6} mol dm^{-3} adriamycin at pH=1) 6.00 and 2) 10.72. The spectra were recorded immediately after preparation. (See text).

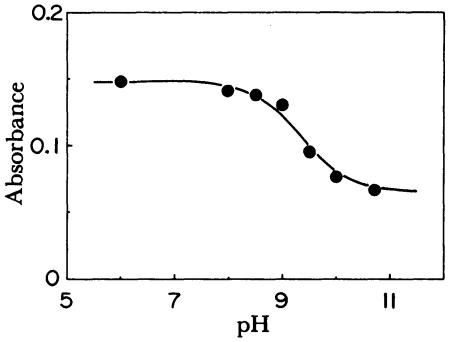

Fig. 4. pH dependence of absorbance for 9.70×10^{-6} mol dm^{-3} adriamycin at 483.2 nm. The solid line represents the regression curve calculated using the values of pK_a=9.33 for Eq. 6. (See text).

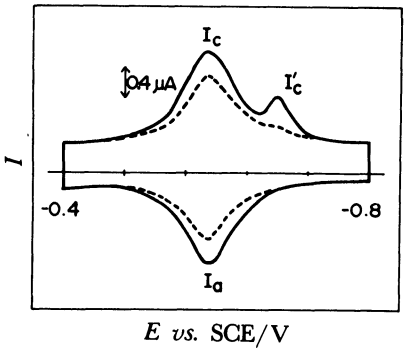

Fig. 5. Multi cyclic d.c. voltammogram of 1.38×10^{-6} mol dm^{-3} adriamycin at pH 9.00 tris buffer. t_{exp}= 1 min at E_i=−0.400 V. v=1000 mV s^{-1}. The solid and broken lines represent the first and second scans, respectively.

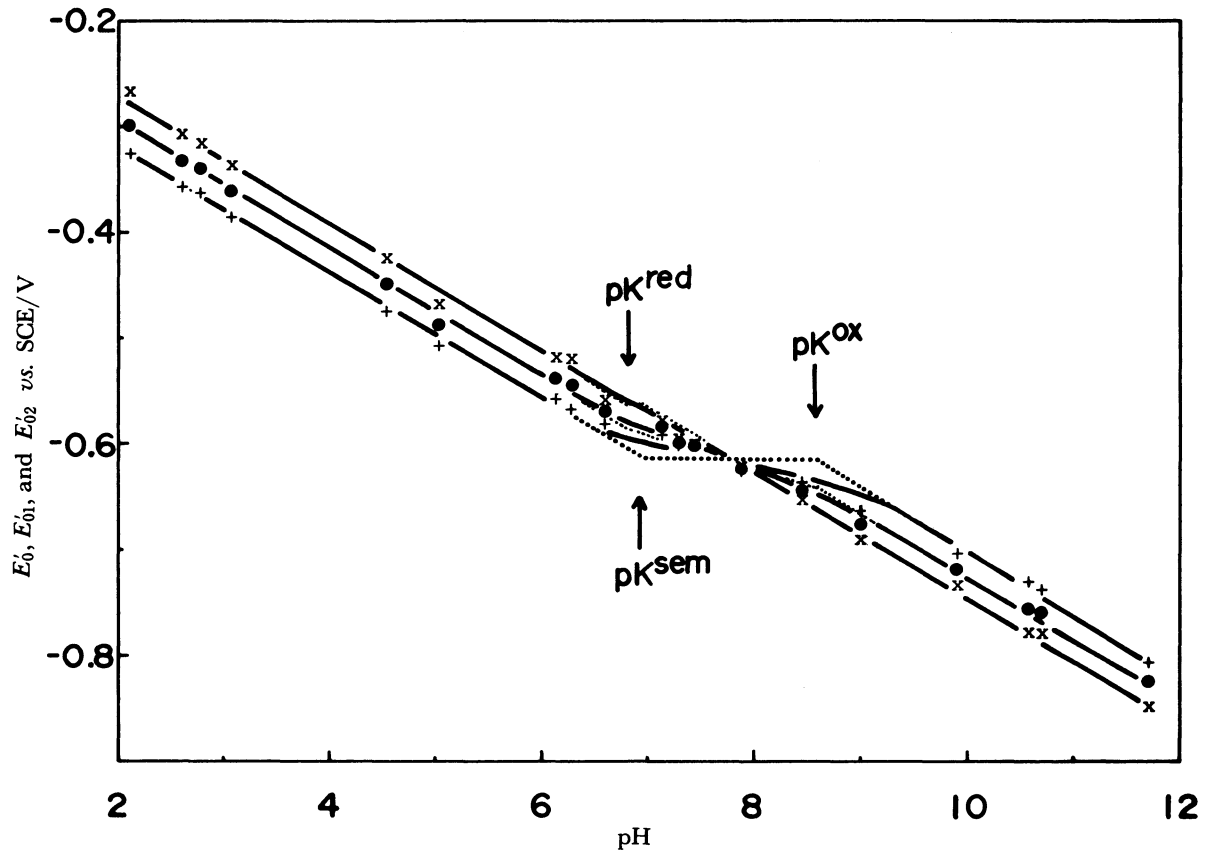

Fig. 6. Plots of E'_0 (●), E'_{01} (+), and E'_{02} (x) against pH. The solid lines represent the regression curves calculated using the values of $\bar{E}'_{01}$=−0.200 V, $\bar{E}'_{02}$= −0.153 V, pK_a^{ox}=8.53, pK_a^{sem}=6.93 and pK_a^{red}=6.83 for Eqs. 8—10.

$$\begin{array}{ccccc} (\text{Ox-H}_p)_{ad} & \overset{e,H^+}{\rightleftharpoons} & (\text{Sem-H}_{p+1})_{ad} & \overset{e,H^+}{\rightleftharpoons} & (\text{Red-H}_{p+2})_{ad} \\ H^+ \Big\| K_a^{ox} & & H^+ \Big\| K_a^{sem} & & H^+ \Big\| K_a^{red} \\ (\text{Ox-H}_{p-1})_{ad} & \overset{e,H^+}{\rightleftharpoons} & (\text{Sem-H}_p)_{ad} & \overset{e,H^+}{\rightleftharpoons} & (\text{Red-H}_{p+1})_{ad} \end{array} \quad (7)$$

where K_a^{ox}, K_a^{sem}, and K_a^{red} represent the acid-dissociation constants of the oxidized, semiquinone, and reduced forms respectively of the adriamycin adsorbed on a mercury electrode. The pH dependences of the E'_0, E'_{01}, and E'_{02} are now given by:

$$E'_0 = \frac{\bar{E}'_{01} + \bar{E}'_{02}}{2} + \frac{RT}{2F}\ln\left[\frac{[H^+]^3 + [H^+]^2 K_a^{red}}{[H^+] + K_a^{ox}}\right] \quad (8)$$

$$E'_{01} = \bar{E}'_{01} + \frac{RT}{F}\ln\left[\frac{[H^+]^2 + [H^+]K_a^{sem}}{[H^+] + K_a^{ox}}\right] \quad (9)$$

$$E'_{02} = \bar{E}'_{02} + \frac{RT}{F}\ln\left[\frac{[H^+]^2 + [H^+]K_a^{red}}{[H^+] + K_a^{sem}}\right] \quad (10)$$

where $\bar{E}'_{01}$ and $\bar{E}'_{02}$ correspond to E'_{01} and E'_{02} at pH=0 respectively. We have fitted Eqs.(8)—(10) to the three plots of E'_0, E'_{01}, and E'_{02} against the pH, using 5 parameters, $\bar{E}'_{01}$, $\bar{E}'_{02}$, pK_a^{ox}, pK_a^{sem}, and pK_a^{red}. Nonlinear least-squares analysis is performed by means of an NEC 8001 personal computer. The refined parameters are $\bar{E}'_{01}$=−0.200±0.002 V, $\bar{E}'_{02}$=−0.153±0.002 V, pK_a^{ox}= 8.53± 0.09, pK_a^{sem}=6.93±0.08 and pK_a^{red}=6.83± 0.09. In adriamycin, both the phenolic and amino groups should ionize in the pH region investigated. However, the amino group is located in the side-chain amino-sugar moiety separated from the aromatic ring by several saturated carbon atoms, so that the effect of the acid dissociation of the amino group on the redox potential should be negligible. Thus, we consider the phenolic hydroxyl group as an ionizable group in Scheme (7).[9] The present value of pK_a^{ox} (8.53) is smaller than the pK_a value of adriamycin (9.33) in the bulk solution. This disagreement is attributable to the difference between the adsorbed and dissolved states of adriamycin.

To our present knowledge, the determination of the acid-dissociation constant of the reduced and semiquinone forms of adriamycin is the first such case, though the values correspond not to the bulk state, but to the adsorbed state. The potentiometric titration may give information about the standard redox potential and the semiquinone formation constant of the bulk species.[10,11] This method, however, can not be applied to an unstable species such as adriamycin in a neutral or an alkaline solution.

On the other hand, the accelation of the chemical reaction rate following the electrochemical reduction in a neutral or an alkaline solution may be attributable to the instability of the deprotonated form of the reduced adriamycin and/or semiquinone intermediate. Here it is noteworthy to consider that, if the reduced (or

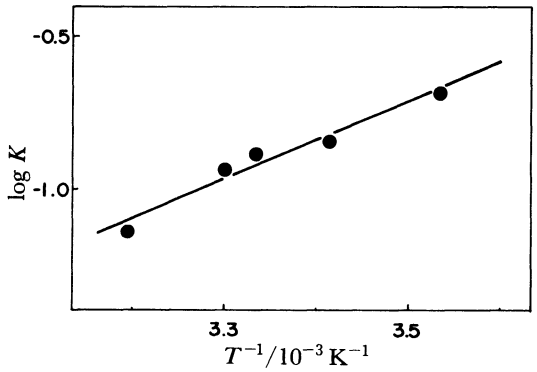

Fig. 7. Temperature dependence of the semiquinone formation constant of the adsorbed adriamycin in an acetate buffer (pH 4.54 at 25 °C).

semiquinone) form is generated in biological media (pH≈7.4), this species easily undergoes the elimination of the amino-sugar moiety to give 7-deoxyadriamycinone. This coincides with the fact that 7-deoxyaglycon is an important metabolite.[12,13]

B. Temperature Effect. We have further studied the temperature effect on the cyclic voltammogram of the adriamycin adsorbed on the HMDE in an acetate buffer (pH 4.54 at 25 °C). In the temperature range from 10 °C to 40 °C, the d.c. voltammogram is reversible and resembles that shown in Fig. 1. The peak potential shifts to the negative side with the increase in the temperature, the slope being $-0.9\ mV\,K^{-1}$. The half-peak width decreases with the increase in the temperature; in other words, the semiquinone formation constant, K, decreases with the temperature. Figure 7 shows the temperature dependence of the K value as determined by the use of Eq. 4.[14,15] The value of log K increases linearly with the reciprocal of the absolute temperature. The linear plot gives the enthalpy $\Delta H=-24.6\ kJ\,mol^{-1}$ and the entropy $\Delta S=-99.7\ J\,K^{-1}\,mol^{-1}$[16,17] for the semiquinone-radical-formation reaction ($O_{ad}+R_{ad}\rightarrow 2S_{ad}$). These values indicate that, because of the large negative entropy, the semiquinone formation reaction is not thermodynamically favored above 0 °C in an acidic solution.

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8) The time-course analysis of the absorbance at 478 nm (pH 8.00—9.50) or 588 nm (pH 10.00—10.72) indicates that the decomposition is a (pseudo) first-order reaction. The evaluated apparent rate constants are ≈0 (pH 8, 9), 4.03×10^{-3} (pH 9.5), 1.14×10^{-2} (pH 10.0), and $1.59\times10^{-2}\,s^{-1}$ (pH 10.72).

9) The protolytic equilibrium involving the amino qroup affects the equilibrium involving the phenolic hydroxyl group, since overlapping protolytic equilibria are possible in adriamycin. Therefore, the pK_a's estimated in this work correspond to the apparent pK_a's.

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14) Under the experimental conditions employed here, the adsorption of adriamycin on the HMDE surface is not in the equilibrium state, but is diffusion-controlled.[5] Therefore, Γ increases with an elevating temperature because of the increasing diffusion coefficient.[15] On the other hand, the K and E_0' values are independent of Γ,[1] so that, in this study, the effect(s) of the temperature (and also of the pH as well as the concentration and species of the supporting electrolytes) on the adsorption state is not taken explicitly into consideration.

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16) In this discussion, the change in the pH with the temperature is not taken into account; it is calculated to be 4.51 to 4.57 for the temperature change from 10 °C to 40 °C in the acetate buffer.[17] In this pH region, the K value is practically independent of the pH. Therefore, this correction for the pH change should not affect the estimated thermodynamic constants.

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