Kinetic Current of Dehydro-reductones and α -Keto Acids

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One of the fundamental interests in the polarographic behavior of ascorbic acid (I) was that I gives a well-defined oxidation wave at a considerably positive potential, while its oxidized form, dehydroascorbic acid (II) shows no appreciable reduction wave, as far as it is polarographed with usual dilute concentrations as, for example, $10^{-3} M$.¹⁻¹⁰⁾

The characteristic reduction wave of II was first obtained by the authors in 1953 by using relatively concentrated aqueous solutions of II and the reduction product was identified to be I by means of controlled potential electrolysis^{9, 10)}. The authors interpreted the reduction wave as a kinetic current due to the dehydration reaction of the hydrated carbonyl group as it is shown in the following scheme. In the scheme;

the equilibrium between II and III should be remembered to be strongly shifted toward III. Although the hydration of other carbonyl groups in III can be considered, it may be reasonable to postulate the form III as the electro-inactive form responsible for the rate-determining step of the dehydration reaction.

This kind of phenomena of kinetic current was further found with other dehydro-reductones¹¹⁾. The following three substances were investigated.

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The kinetic current of these dehydro-reductones may be expressed by the aid of the equation derived by Koutecky and others¹²⁻¹⁶.

$$i_t = i_d \frac{0.886 \lambda \sqrt{t}}{1 + 0.886 \lambda \sqrt{t}} \quad (1)$$

$$\lambda = \sqrt{k + k'} / K_h \doteq \sqrt{k K_h} = k / \sqrt{k'} \quad (2)$$

where i_t and i_d are the kinetic limiting current and the expected diffusion current, respectively, t is the drop time, k and k' are the rate constants of the hydration and dehydration, respectively, and K_h is the hydration constant equal to k' / k . Since K_h 's for the above four substances have remained unknown, the rate constants of k and k' have been also unavailable. However, one of the characteristics of the reductones has been studied polarographically and the importance of the hydration of carbonyl group in the dehydro-reductones has been emphasized.

Discussions were also made on the relation between oxidation-reduction potential of the system investigated and the kinetic current. Of the above four substances, the lower the height of the reduction wave, the larger both the temperature coefficient of the reduction wave and the discrepancy between the half-wave potential of the oxidation wave of the reduced form and the potentiometrically obtained standard oxidation-reduction potential of the system. It may be noted that Koutecky's theoretical treatment of oxidation-reduction system for the reversible electrode reaction as for I and II did not coincide with the experimental results obtained by the authors¹⁰. The electrode reaction of these systems investigated, therefore, may be considered to be irreversible except dialuric acid-alloxan system. For the last substance, the perfect reversible character has been observed¹¹.

In order to obtain further knowledge of kinetic current of this kind, mesoxalic acid (VII) was investigated^{16, 17}. Mesoxalic acid is the acid of IV and hydration is normally expected only in the central carbonyl group.

As soon as the investigations with this acid were started, the authors encountered the very characteristic pH dependence of the limiting current. The height of the first wave and the sum of the first and the second waves had very sharp maxima in a very narrow pH range near 4, and still had kinetic character even at their maximum. Dihydroxytartaric acid (IX) was also studied in the same way as for VII, since this acid has been known as a perfectly hydrated form of the corresponding keto acid. Similar characteristics were

observed for IX as well as VII. This led the authors to reexamine the i_t -pH curve of pyruvic acid (X) obtained by Ishizaka¹⁹⁾. Although the change of the limiting current of X with pH was first studied by Müller and Baumberger on the basis of their keto-enol tautomeric equilibrium¹⁹⁾ and later by Brdička on the basis of his well-known acid recombination theory^{20, 21)}, none of them treated the change in the limiting current in pH range below 4. The actual i_t -pH curve of X obtained by Ishizaka and later checked by the authors is shown in Fig. 1.²²⁾

Fig. 1. i_t -pH curve of pyruvic acid.
 i_t : limiting current of the 1st wave.

The i_t -pH curve shows a minimum value near pH 1 and a maximum near pH 4. The effect of the hydration of carbonyl group on the limiting current of α -ketoacids has been pointed out and the i_t -pH curves have been interpreted with the following scheme.

Namely, the first wave of α -keto acid is due to the electro-reduction of the unhydrated and undissociated acid (a) which can be regenerated via both electro-inactive forms (b) and (c) by chemical reactions. The greater degree of hydration in the undissociated form than in the dissociated form ($K_h^o > K_h^-$), can be expected from the inductive effect of carboxyl group to carbonyl group. The increase of the limiting current with decreasing pH in the neighborhood of pH 0 can be understood by considering catalytic effect of hydrogen ion on the rate of dehydration. It must be noted that the same scheme has been independently suggested by Kůta²³⁾ for the polarographic behavior of glyoxylic acid, the simplest α -keto acid, and later by Becker and Strehlow^{24, 25)} for α -keto acids, but the latter authors investigated the acids in a limited pH range.

However, in the course of further investigations with many different α -keto acids, the authors found another factor in determining the limiting current of α -keto acids. Some of the α -keto acids studied distinctly showed the contribution of enol form²²⁾, though the i_t -pH curves had quite a similar shape to that of X (Fig. 1) in all the instances studied. The α -keto acids studied by the authors can therefore be classified as follows.

1) *Hydration of carbonyl group possible but enolization impossible*; they are trimethylpy-

Trimethylpyruvic acid
(XI)

Phenylglyoxylic acid
(XII)

ruvic acid (XI), phenylglyoxylic acid (XII), VII and IX. Dihydroxytartaric acid (IX) is not α -keto acid, but the polarographic behavior is quite similar. The form $\text{COOH-CO-C(OH)}_2\text{COOH}$ was considered for the electro-active form responsible for the limiting current, comparing the half-wave potential with those of other α -keto acids.

2) *Both hydration and enolization apparent*; they are phenylpyruvic acid (XIII) and

oxalacetic acid (XIV). The evidence of the contribution of enol form has been mostly obtained with UV-absorption spectra. For the former, kinetic studies were carried out by following the change of the optical density at $287 \text{ m}\mu$ (due to enol form) and polarographic limiting current (due to keto form). Consistent results were obtained for kinetic studies by both polarography and spectrophotometry. It may be interesting to note that both XIII and XIV are enol forms in their crystalline states as (XV) and (XVI). They attained equilibrium between keto and enol forms in solutions.

3) *Both hydration and enolization possible, but indistinguishable*; they are X and α -ketoglutaric acid (XVII). For these two acids, the existence of enol form can be expected from their configuration, but no distinct evidence has been obtained. However, the different behavior in UV-spectra of these acids from that of XI may imply a certain possibility of enol contribution in their polarographic limiting current.

For the latter two of the classified cases, the consideration, therefore, should be made with the following scheme (C).

General theoretical treatment of i_t -pH curve of α -keto acids has not yet succeeded, since the equilibrium between electro-active and electro-inactive species is not strongly shifted towards electro-inactive form in most α -keto acids and still the limiting current exhibits considerable kinetic character. However, some simply specified cases have been treated and discussed^{26, 27}.

Trimethylpyruvic acid (XI) has no possibility of enolization. Further, by spectrophotometric studies the hydration constant (K_h) was obtained and even the rate constant of dehydration was available under limited conditions²⁶. The rate of dehydration reaction of hydrated XI seems slow, compared with other α -keto acids (probably due to the steric

hindrance of methyl groups), so that the limiting current in the pH range where the current increases with pH may be simply expressed as proportional to the concentration of unhydrated species in bulk solution. Neglecting the hydrated anion form (on the basis of the spectrophotometric studies), the scheme for XI may be simplified as

where AH, AH_{aq} and A⁻ are XI, the hydrated form and the anion of XI, respectively, k_d and k_r are the rate constants of dissociation and recombination of AH, respectively, and K_h^0 is the hydration constant. The limiting current is then expressed as

$$i_l = \frac{i_a[\text{H}^+]}{K_a + [\text{H}^+]} \left(\frac{1}{K_h^0 + 1} + \frac{0.886 K_a [k_r t / K_a (K_h^0 + 1)]^{1/2}}{1 + 0.886 [\text{H}^+] [k_r t / K_a (K_h^0 + 1)]^{1/2}} \right) \quad (3)$$

where K_a is the apparent dissociation constant and equal to $[\text{A}^-] / ([\text{AH}] + [\text{AH}_{\text{aq}}])$, so that the dissociation constant for free acid K_a^0 is

$$K_a^0 = k_d / k_r = [\text{A}^-] [\text{H}^+] / [\text{AH}] = K_a (K_h^0 + 1) \quad (4)$$

The equation (3) was checked with the spectrophotometrically measured value of K_a and K_h^0 , and with polarographic data. Data were consistent except for pH range below 0.5 where the catalytic effect of hydrogen ion on dehydration reaction should be taken into consideration.

Another interesting instance treated by the authors is glyoxylic acid (XVIII)²⁷. K_ut_a

studied polarographic behavior of this acid in detail²⁷ and pointed out the kinetic character of the limiting current in all the pH range studied. Becker and Strehlow treated the change of the limiting current only in a narrow pH range where the current of the first wave decreases with pH due to the decrease of the rate of recombination of the acid^{24, 25}.

Using the same scheme as B with necessary rate constants, let us consider with the following scheme.

The rates of acid dissociation and recombination are assumed to be fast enough, compared with those of hydration and dehydration, so that the latter are the rate-determining step. Since the limiting current of XVIII is very small and the equilibrium of hydration seems to be such that the substance exists predominantly as the hydrated form even in the dissociated state, the equation (1) may be applied to the present problem of the limiting current of XVIII, each constant being regarded as that of the over-all reaction.

Now, remembering that the rates of the hydration and dehydration are proportional to the equilibrated bulk concentrations of the corresponding species, and using other constants given in scheme D', the rate constants of the over-all reaction k and k' may be expressed as

$$k = k_1 \frac{[\text{H}^+]}{K_a^h + [\text{H}^+]} + k_2 \frac{K_a^h}{K_a^h + [\text{H}^+]} \quad (5)$$

$$k' = k_1' \frac{[\text{H}^+]}{K_a^o + [\text{H}^+]} + k_2' \frac{K_a^o}{K_a^o + [\text{H}^+]} \quad (6)$$

consequently

$$\lambda = \frac{(k_1[\text{H}^+] + k_2 K_a^h)(K_a^o + [\text{H}^+])^{1/2}}{(K_a^h + [\text{H}^+])(k_1'[\text{H}^+] + k_2' K_a^o)^{1/2}} \quad (7)$$

Using equation (1) with (7), i_t -pH curve of XVIII can be expressed, as far as the acid dissociation and recombination are fast enough, i.e. in the range where only the first wave is observed. The increase of the limiting current at pH near 0 is due to the catalytic effect of H^+ . For that, k_1 should be considered to be $k_1 = k_1^o + k_1^h [\text{H}^+]$, where k_1^o and k_1^h are uncatalyzed rate constant and catalytic constant of H^+ , respectively. However, even in the pH range where two waves appear, this equation may be valid, if i_t is regarded as the expression for the sum of the both limiting currents. The above expressions (5), (6) and (7) have been obtained by the authors, starting with simple reaction layer concept, as it is often used for kinetic current, but the same results could be obtained from Senda's⁽²⁸⁾ more rigorous mathematical treatment for scheme B, by simplifying the results with the same assumption. It may be interesting to note that Los and Gaspar studied kinetic current of 2-ketogulonic acid and explained i_t -pH curve with the reaction layer concept⁽²⁹⁻³¹⁾, but their treatment involves some erroneous assumption of back-ward reaction, which becomes apparent when compared with the present results.

In addition few comments will be made from the practical point of view for the determination of dehydro-reductones and α -keto acids. Since the polarographic limiting currents of these substances have more or less kinetic character, the application of limiting current for the determination is not recommendable. To make this feasible, addition of some amine compounds can be used. The use of the *o*-phenylenediamine for the determination of dehydro-reductones was successfully applied.⁽³²⁾

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