Reduction Kinetics of 2,3-Dimethoxy-5-methyl-1,4-Benzoquinone by Ascorbic Acid in Acid Solution

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The mechanism of the oxidation of ascorbic acid by 2,3-dimethoxy-5-methyl-1,4-benzoquinone was studied as a function of pH, ionic strength and temperature in aqueous buffer solutions, using dc polarography and UV–VIS spectrophotometry. The study was performed at pH values 3.0–6.2 at which the quinone is stable. From the experimental results, a mechanism for oxidation of the ascorbic acid which involves several steps with the participation of AH$^{\bullet}$ and QH$^{\bullet}$ radicals as intermediate species, has been proposed.

The oxidation reaction of ascorbic acid to produce dehydroascorbic acid, is of great interest in many biochemical and industrial processes. The ascorbic acid oxidation has been studied using different oxidants, *e.g.* Fe^{3+}, Cu^{2+}, Ag^{+}, Mn^{3+}, $V^{(V)}$, O_2, $Fe(CN)_6^{3-}$, $Fe(C_2O_4)_3^{3-}$, $[Fe(phenanthroline)_3]^{3+}$ and $Co(C_2O_4)_3^{3-}$,[1-14] and its kinetics have been followed by polarographic techniques, stopped flow and EPR (the latter has been used to detect the AH$^{\bullet}$ radical of this acid).

In this paper, we present, using polarographic techniques, a kinetic study of the oxidation reaction of ascorbic acid by 2,3-dimethoxy-5-methyl-1,4-benzoquinone which is a nucleus of coenzyme Q and base of structures used as anticancer drugs in several laboratory studies.[15-17] The pH range was between 3.0 and 6.2, values at which the quinone is stable.[18]

Experimental

Measurements.—Polarographic registers were made with an AMEL multipolarograph, model 471, with a three electrode system using a thermostatted cell; potentials were referred to the SCE. The capillary used when immersed in a buffer solution acetate–phosphate at pH = 7.0, at h = 55 cm and E = −1.5 V, had the following characteristics: m = 1.09 mg s^{-1} and t = 5.98 s. An ultrathermostat COLORA was used to maintain the temperature constant ±0.1 °C.

The UV and VIS spectra were registered in an Hitachi Spectrophotometer, U-3200 model. The pH values were measured using a Philips pH-meter, Model pw 9408, with a glass electrode.

Materials.—The 2,3-dimethoxy-5-methyl-1,4-benzoquinone, was a Sigma product. Ascorbic acid, and the other products used in the preparation of solutions all were Merck, 'analysis reagent grade'. Mercury was electrolytically purified and then distilled under reduced pressure three times.

Procedure.—The kinetic study was made in acetate–phosphate buffer solutions, of 0.04 mol dm^{-3} concentration both for acetic and phosphoric acid, using KOH to reach the required pH value. The ionic strength was maintained constant at 0.5 mol dm^{-3} (except in the case in which the ionic strength influence was studied) by addition of KNO_3.

The reaction was studied following the quinone reduction wave and in all the cases, we prepared two different solutions: (i) containing only the quinone, where we measured the current intensity at a zero time; and (ii) with a mixture of quinone and ascorbic acid in order to study the variation of current intensity with time.

The quinone and ascorbic acid concentrations had the same value, 2.5×10^{-4} mol dm^{-3}, except in the case in which we studied the reaction with an excess of ascorbic acid (2.5×10^{-3} to 2.0×10^{-2} mol dm^{-3}).

To determine the current intensity at a zero time, we made a full polarographic register. The measures of current intensity with the time were made, in accordance with the reaction speed, by a register of the full polarogram or by the register of the limiting current at different times of reaction. On the other hand, and in order to test that the polarographic measurements did not perturb the system under study, we used the UV–VIS spectrophotometric technique, observing that the Lambert–Beer law was obeyed. The concentration was 4.0×10^{-4} mol dm^{-3} and a wavelength of 410 nm shows an absorption maximum of quinone but not of other products.

Results

The reduction reaction of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q) by ascorbic acid (AH_2) produces 2,3-dimethoxy-5-methyl-1,4-benzohydroquinone (QH_2) and dehydroascorbic acid (A), in accordance with the following stoichiometry and global equation.

$$2Q + 2AH^- + 2H^+ \longrightarrow 2QH_2 + 2A$$

Dependence of the concentration of Q on time was followed using polarographic techniques. The corresponding values were in agreement with a kinetic process of second order when the concentrations of both reactants were equal, and with pseudo first-order kinetics with respect to the quinone when the experiments were performed with an excess of ascorbic acid (Fig. 1). Note that the dependence of the experimental rate constant, k_{exp}, on pH is not linear so that k_{exp} shows a maximum value at pH ≃ 5.1 (Fig. 2).

The variation of the experimental rate constant values with pH can be explained if we consider the ascorbate monoanion, AH^-, as a reactive species whose concentration increases as the pH-values become larger. Thus, and since the pK values of ascorbic acid are pK_1 = 4.17, pK_2 = 11.5, this implies that k_{exp} increases with pH and must reach a maximum value at pH ≃ 5.1.

In turn, the fact that k_{exp} decreases for pH values larger than 5.1 is explained by taking into account that protons are also a reactive species that are involved in the reaction mechanism. Thus, for 5.1 < pH < 6.2 the diminution of k_{exp} is due to the fact that the effect of increase of AH^- concentration is compensated by the decrease of the concentration of protons in the reaction media. We must also consider that at larger values of pH, the AH^- concentration decreases to provide the A^{2-} dianion.

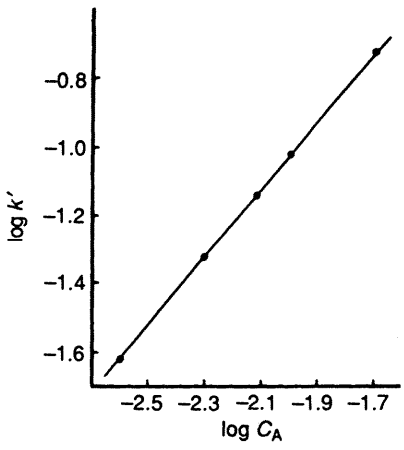

Fig. 1 Variation of the pseudo-first-order constant logarithm (log k'), with the concentration logarithm of the ascorbic acid; pH = 5.1, T = 25 °C

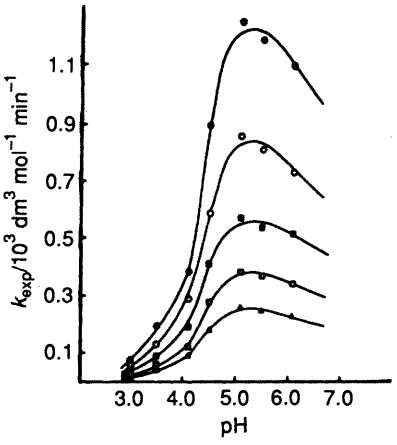

Fig. 2 Dependence of the experimental rate constant (k_{exp}) on pH; ▲, 15 °C; □, 20 °C; ■, 25 °C; ○, 30 °C; ●, 35 °C

From the study of the influence of the ionic strength of the media, μ, on the rate constant, we found that the slope of the plot log k_{exp} *vs.* $\sqrt{\mu}$ was $\simeq 0$ (Fig. 3). This confirms that one of the reactants in the determinant step of the rate has a zero charge, in our case, the Q species. Thus, k_{exp} also decreases at low values of pH (see Fig. 1), and this is accounted for by considering that Q is another of the reactive species that at very low pH values is protonated in the inactive form QH^+ (the pK_Q value of the quinone used is 6.4).

In order to test the experimental results obtained by polarography, we also used another technique, UV–VIS spectrophotometry. The measurements were carried out at 25 °C and at concentrations similar to those previously used in polarography (4.0×10^{-4} mol dm^{-3}). Under these conditions the Lambert–Beer law was accomplished. The wavelength selected was 410 nm, where 2,3-dimethoxy-5-methyl-1,4-benzoquinone shows an absorption maximum and the products obtained do not absorb. The k_{exp} values obtained are, within the experimental error, equal to those found by polarography.

The dependence of k_{exp} with temperature was also studied. Thus, the values of the activation parameters have been determined and they are given in Table 1. Note that $\Delta G^{\ddagger}$ is almost independent of pH. The most negative values of $\Delta S^{\ddagger}$ are obtained at pH-values where the ascorbic acid is mainly in the form AH_2. At these pH-values, the interaction of the solvent molecules with the activated complex is stronger than on the individual molecules of the reactives. This pattern for the $\Delta S^{\ddagger}$-values is reversed at pH > 5.1.

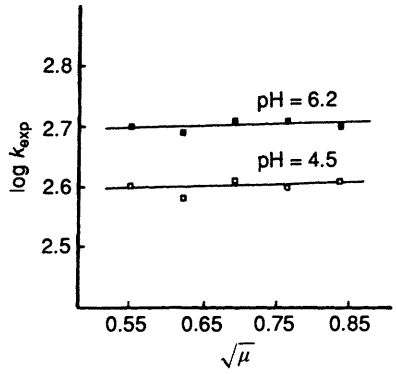

Fig. 3 Dependence of the experimental rate constant (k_{exp}) on the square root of the ionic force ($\sqrt{\mu}$) at pH 4.5 and 6.2

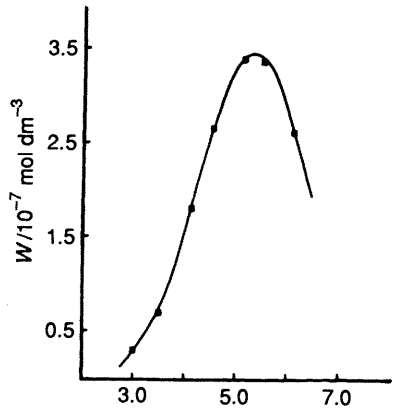

Fig. 4 Dependence of W on pH, from eqn. (7)

The experimental profiles k_{exp}/pH show a much less symmetrical maximum than the profiles theoretical (Fig. 4). This fact can be explained by assuming that when pH becomes larger there is a new step involved: the reaction of Q with OH^-, that increases the values of k_{exp}. This step is due to the nucleophilic attack of hydroxide ion on a quinonoid double bond giving the hydroxy substituted hydroquinone, which may then be oxidized by unreacted quinone to give hydroxyquinone (QOH) and hydroquinone (QH_2).[19–21]

The global reaction may be expressed by the following scheme.

$$2Q + OH^- + H_2O \longrightarrow QH_2 + QOH$$

The hydroxyquinone is an unstable compound in solution.[19]

The reaction of the quinone with hydroxide ion makes only a minor contribution to the reaction rate in the pH-range examined. It has not been included in the reaction mechanism proposed below.

In accordance with the experimental results, the following reaction mechanism is proposed for acid medium.

$$Q + AH^- + H^+ \xrightarrow{k_1} QH^{\bullet} + AH^{\bullet} \quad \text{(I) (slow)}$$

$$QH^{\bullet} + AH^- + H^+ \xrightarrow{k_2} QH_2 + AH^{\bullet} \quad \text{(II) (fast)}$$

$$AH^{\bullet} + Q \xrightarrow{k_3} A + QH^{\bullet} \quad \text{(III) (fast)}$$

$$AH^{\bullet} + QH^{\bullet} \xrightarrow{k_4} QH_2 + A \quad \text{(IV) (fast)}$$

$$2Q + 2AH^- + 2H^+ \longrightarrow 2QH_2 + 2A$$

In step I, the ascorbate AH^- ion is oxidized to give the $AH^{\bullet}$ radical. The existence of this intermediate radical has been

Table 1 Kinetic and thermodynamic data[a]

pH	E_a/Kcal mol^{-1}	A/dm^3 mol^{-1} s^{-1}	$\Delta S^{\ddagger}$/cal mol K^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta G^{\ddagger}$/kcal mol^{-1}
3.0	32.72	9.22×10^{11}	−5.8	32.14	33.87
3.5	32.06	1.43×10^{12}	−4.9	31.47	32.93
4.1	32.38	3.73×10^{12}	−3.0	31.79	32.68
4.5	32.00	6.48×10^{12}	−1.9	31.41	30.98
5.1	32.25	1.07×10^{12}	−0.9	31.66	31.93
5.5	32.21	9.86×10^{12}	−1.1	31.62	31.94
6.2	32.16	8.75×10^{12}	−1.3	31.57	32.55

[a] Reaction: ascorbic acid (A) and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q); $[A]_0 = [Q]_0 = 2.5 \times 10^{-4}$ mol dm^{-3}, $T = 25$ °C. 1 kcal mol^{-1} = 4.184 kJ mol^{-1}.

Table 2 Variation k with pH[a]

pH	$k = k_1 + \left(\frac{k_1k_2k_3}{k_4}\right)^{\ddagger} / 10^9$ dm^6 mol^{-2} min^{-1}
3.0	1.75
3.5	1.62
4.1	1.30
4.5	1.93
5.1	2.11
5.5	1.97
6.2	2.32

[a] Reaction between ascorbic acid (A) and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q). $[A]_0 = [Q]_0 = 2.5 \times 10^{-4}$ mol dm^{-3}, $T = 25$ °C.

detected by EPR transforming the quinone to the QH$^{\bullet}$ radical, and proposed in different references.[24–29] We also assume that, before the formation of this radical, the quinone takes an electron from the AH^- ascorbate anion to give the $Q^{\bullet -}$ anion, which suffers a fast protonation to give the radical QH$^{\bullet}$.[22,23,30–33] This result is supported by the fact that in the absence of protons in the medium, it is only possible to detect the $Q^{\bullet -}$ radical anion.[34,35]

From this mechanism the rate at which Q disappears is given by eqn. (1).

$$-\frac{d[Q]}{dt} = k_1[Q][AH^-][H^+] + k_3[AH^{\bullet}][Q] \quad (1)$$

The concentrations of the ascorbate ion AH^- and of the quinone Q are obtained by considering the following pre-equilibria.

$$AH_2 \overset{K_1}{\rightleftharpoons} AH^- + H^+$$

$$AH^- \overset{K_2}{\rightleftharpoons} A^{2-} + H^+$$

$$QH^+ \overset{K_Q}{\rightleftharpoons} Q + H^+$$

In turn, [AH$^{\bullet}$] is obtained by applying the steady state approximation to the QH$^{\bullet}$ and AH$^{\bullet}$ radicals. Thus, we have:

$$\left.\begin{aligned} [AH^-] &= \frac{K_1C_T^A}{[H^+] + K_1 + \frac{K_1K_2}{[H^+]}} \\ (Q) &= \frac{C_T^QK_Q}{K_Q + [H^+]} \\ [AH^{\bullet}] &= \left(\frac{k_1k_2}{k_3k_4}\right)^{\ddagger}[AH^-][H^+] \end{aligned}\right\} \quad (2)$$

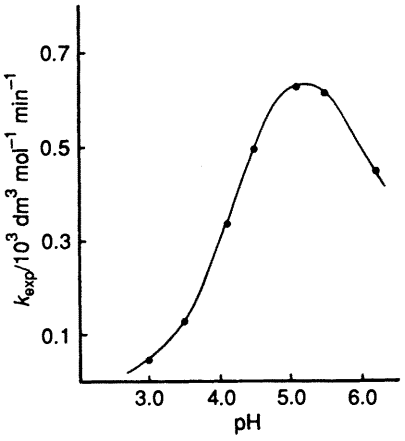

Fig. 5 k_{exp}-values determined from eqn. 4 on pH, at constant temperature

Where C_T^A and C_T^Q are, respectively, the total concentrations ascorbic acid and quinone. By taking into account this equation, the rate at which the quinone disappears is given by the following expression:

$$-\frac{d[Q]}{dt} = \left\{k_1 + \left(\frac{k_1k_2k_3}{k_4}\right)^{\ddagger}\right\} \left\{\frac{K_QC_T^Q}{K_Q + [H^+]} \times \frac{C_T^AK_1}{[H^+] + K_1 + \frac{K_1K_2}{[H^+]}}[H^+]\right\} \quad (3)$$

Finally, the experimental rate constant is given by the expression:

$$k_{exp} = k\left\{\frac{K_QK_1}{K_Q + [H^+]} \times \frac{[H^+]}{[H^+] + K_1 + \frac{K_1K_2}{[H^+]}}\right\} \quad (4)$$

where

$$k = k_1 + \left\{\frac{k_1 \cdot k_2 \cdot k_3}{k_4}\right\}^{\ddagger} \quad (5)$$

Eqn. (4) shows a maximum when plotted as a function of pH, and this is in agreement with the experimental results. Eqn. (4) allows us to obtain k at different pH-values from the experimental rate constants k_{exp}, and this is shown in Table 2. Note that k remains practically constant for all pH-values and this justifies the proposed mechanism for the studied reaction (k_1, k_2, k_3, k_4 are the true rate constants for the four steps involved in the mechanism, and hence its values are also constant). The average value of k is 1.85×10^9 (dm^6 mol^{-2} min^{-1}). By using this value in eqn. (4) the k_{exp}-values at different values of the pH have been determined, and they are shown in Fig. 5. Values of k_{exp} in this figure are in agreement with

the values of the rate constants obtained experimentally (Fig. 2).

From this mechanism it follows that the rate determining step of the reaction is the first one, in which are involved the species AH^-, Q and the H^+. In the following steps some radicals participate and therefore, these steps must be faster.

Eqn. (4) can be rewritten as eqn. (6).

$$k_{exp} = kW \tag{6}$$

where

$$W = \frac{K_Q K_1}{K_Q + [H^+]} \times \frac{[H^+]}{[H^+] + K_1 + \frac{K_1 K_2}{[H^+]}} \tag{7}$$

Since we do not know the theoretical values of the true rate constant k_1, k_2, k_3, k_4, the k value is not known either. Hence, we have plotted W *vs.* pH (Fig. 4), and we have found a maximum at pH $\simeq$ 5.1 which is in accordance with the maximum found experimentally (see Fig. 2).

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