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Electrochemical Oxidation of Chlorpromazine Hydrochloride in Aqueous Buffer

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The electrochemical oxidation mechanism of chlorpromazine hydrochloride (CPZ–HCl) was examined in aqueous buffers in the pH range from 2 to 7. Three anodic waves were observed on the cyclic voltammogram of CPZ. The peak potential of the second wave was highly dependent on the properties of the supporting electrolyte, but the first peak was independent of these properties. At a higher pH or higher concentration of electrolyte, both peak currents increased. The final oxidation product was found to be chlorpromazine sulfoxide by comparison of its ultraviolet (UV) absorption spectrum and *Rf* value of thin layer chromatography (TLC) with those of an authentic sample.

The experimental results suggest that the first wave is a catalytic wave and depends on the pH and the concentration of the supporting electrolyte, but that the second is an oxidation wave of CPZ-buffer anion adducts.

Keywords——catalytic wave; chlorpromazine; electrochemical oxidation; electrode reaction; glassy carbon electrode; voltammetry

Phenothiazines, such as chlorpromazine (CPZ), which are major tranquilizers, are easily oxidized to form cation radicals ($CPZ^{\dagger}$) as intermediates.[1–9] Several investigators have suggested that the cation radical may be an active pharmacological entity.[9–13] The stability of the cation radical, the oxidation potential of phenothiazines and the clinical doses are mutually related.[14] An understanding of the electrochemical oxidation mechanism of CPZ should therefore greatly facilitate the estimation of the clinical activity of phenothiazines.

A number of studies have been carried out on the electrolysis of CPZ in strongly acidic media[3,15] or nonaqueous media. It has been proposed that CPZ undergoes two one-electron oxidation steps in 12 N H_2SO_4. The first step is the oxidation of CPZ to $CPZ^{\dagger}$, and the second is the oxidation of $CPZ^{\dagger}$ to CPZ sulfoxide (CPZO). However, in a weakly acidic solution, the cation radical is very unstable, and few studies have been reported on the electrochemical oxidation mechanism of CPZ.

In the course of our study, it was found that the shape of the voltammogram of CPZ was affected by pH, concentration and the type of buffer. This suggests that chemical reactions such as the hydrolysis of $CPZ^{\dagger}$, *etc.* are involved in the electrochemical oxidation of CPZ. In recent studies on the hydrolysis of the CPZ cation radical in aqueous buffer solutions of pH 2—7,[16–18] it has been found that the cation radical is attacked by a weak nucleophile to form an adduct, which is oxidized by another molecule of the cation radical to produce CPZ and

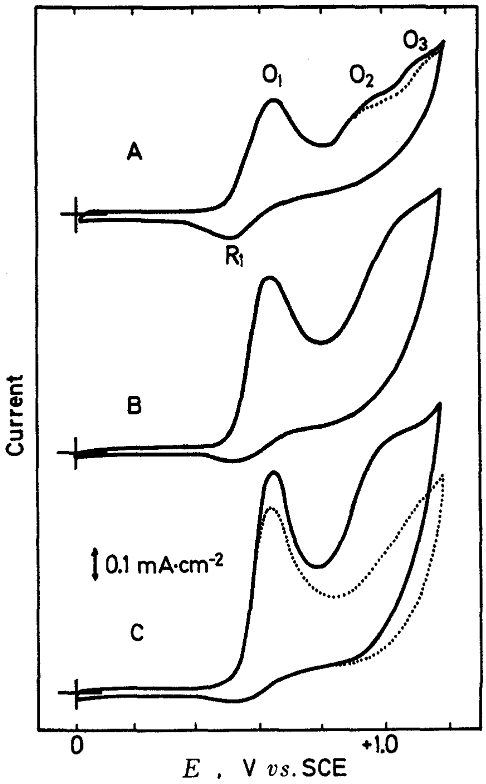

Fig. 1. Cyclic Voltammogram of 1.0×10^{-3} M CPZ in Phosphate Buffer

A, pH 3.9; B, pH 5.9; C, pH 6.5. The dotted line is the voltammogram with 1.0×10^{-3} M $CsNO_3$.

CPZO.

The objective of the present work was to examine the effect of the supporting electrolyte and pH on the oxidation peaks of CPZ, and to elucidate the electrode reaction mechanism in aqueous buffer of pH 2—7.

Experimental

Chemicals——CPZ-HCl and CPZO-HCl were kindly provided by Shionogi Co., Ltd. and were used without further purification. The CPZ cation radical was obtained according to the method of McCreery *et al.*[16] All other materials were of analytical grade. Various buffers were used as base electrolyte solutions. NaCl was used to adjust the ionic strength to 0.2.

Apparatus——Cyclic voltammetry and controlled potential electrolysis were performed with an HA-101 potentiostat (Hokuto Denko, Ltd.) and an HA-107A function generator (Hokuto Denko, Ltd.). A glassy carbon rod and a gold plate were used as the working and auxiliary electrodes, respectively. The potential was measured with reference to a saturated calomel electrode(SCE). Current-potential curves were recorded on an X–Y recorder (Yokogawa, Ltd., type 3077). All measurements were carried out at 25±1°C, and the solutions were shielded from light. Before each measurement, a sufficient amount of pure nitrogen gas was bubbled through the electrolytic solution to remove any dissolved oxygen.

Ultraviolet (UV) and visible absorption spectroscopy, and thin-layer chromatography (TLC) were used for analysis of the electrolysis products. The spectra were recorded with a Hitachi model 200-10 spectrophotometer. The products were applied to a silica gel TLC plate. Following equilibration of the plate with the vapor of the mixed solvent, benzene–dioxane–ammonia (65: 35: 5), for 10 min, the chromatogram was developed to a length of 12 cm. The spots on the chromatogram were detected under UV light and confirmed by spraying the plate with 12 N H_2SO_4.

Results and Discussion

The voltammogram of CPZ–HCl in a pH 3.9 phosphate buffer shows three oxidation waves on the first anodic scan (Fig. 1,A). The peak potentials (E_p) were +0.65 V, +0.95 V and +1.10 V *vs.* SCE. To analyze the electrolysis products, CPZ–HCl was electrolyzed at the peak potential of the first oxidation peak, +0.65 V *vs.* SCE, until the current dropped below 0.5% of the initial value. The final product gives UV absorption maxima at 240, 273, 298 and 340 nm, and *Rf* value of TLC of 0.16. By comparison of these values with those of authentic CPZO, the electrolysis product was proved to be CPZO. In this electrolysis, the solution had a red tinge. The visible absorption spectrum of this solution had a peak at 524 nm, corresponding to the spectrum of the CPZ cation radical.

At the peak potential of the second wave, +0.95 V, the final product was also CPZO, but the solution was colorless. It seems that the first peak corresponds to the oxidation of CPZ to $CPZ^{\dagger}$, and the second, to the subsequent oxidation of $CPZ^{\dagger}$. The third peak appeared due to a further oxidation of CPZO by comparison with the voltammogram of pure CPZO. The cathodic wave R_1 coupled with the first oxidation wave O_1 was observed only at low pH. The reduction waves were very small compared to the oxidation waves, indicating the decay of $CPZ^{\dagger}$ to be very rapid.

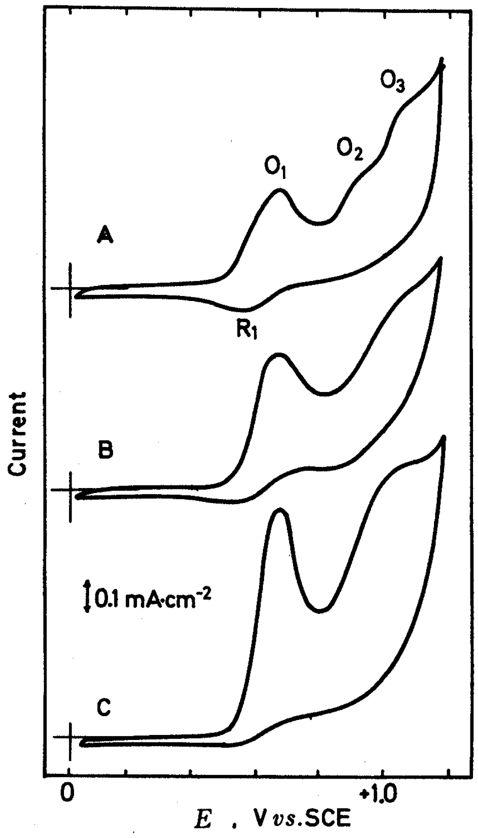

Fig. 2. Cyclic Voltammogram of 1.0× 10^{-3} M CPZ in Glycine Buffer, pH 6.1 (A), Acetate Buffer, pH 6.4 (B), and Phosphate Buffer, pH 6.5 (C)

Scan rate=0.1 V/s.

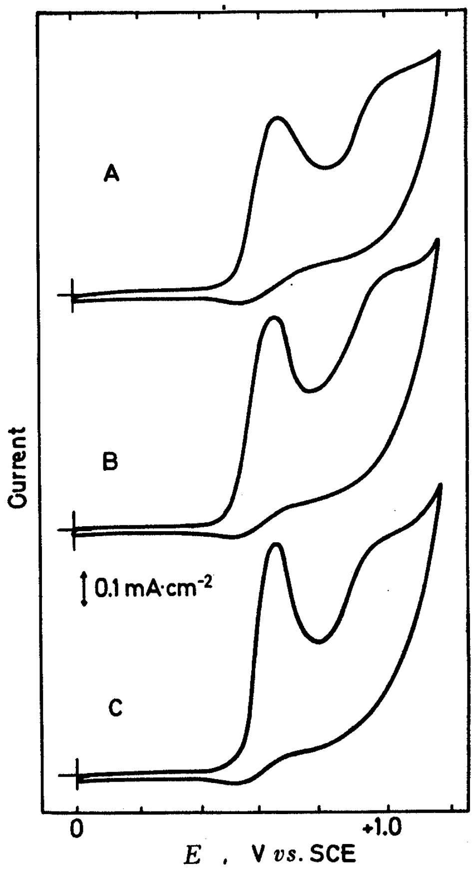

Fig. 4. Cyclic Voltammogram of 1.0× 10^{-3} M CPZ in pH 6.3 Phosphate Buffer

Scan rate=0.1 V/s. Total phosphate concentration: A, 0.04 M; B, 0.07 M; C, 0.14 M.

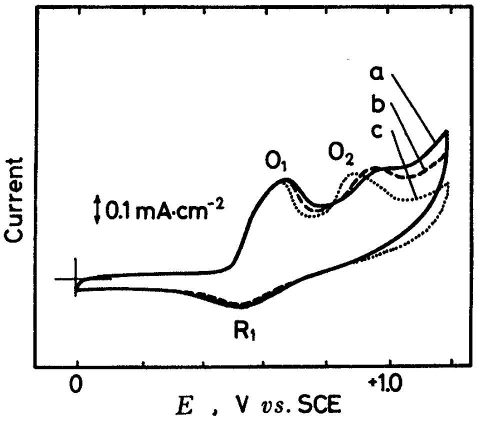

Fig. 3. Cyclic Voltammogram of 1.0× 10^{-3} M CPZ in pH 2.0 Phosphate Buffer

Scan rate=0.1 V/s. [$H_2PO_4^-$]=a, 0.06 M; b, 0.12 M; c, 0.50 M.

The peak potential (E_p) of the first oxidation wave remained unchanged irrespective of the supporting electrolyte (Fig. 2). However, the E_p of the second oxidation wave shifted to the negative potential side as the concentration of the supporting electrolyte became higher (Figs. 3 and 4). The second peak also depended on the type of supporting electrolyte (Fig. 2), suggesting that $CPZ^{\dagger}$ is associated with the supporting electrolyte and is easily oxidized in weakly acidic solution. The increase in i_p at about +1.2 V is mainly due to the overlapping of the second and third peaks, and the potential shift of the second peak to the negative side at lower concentration of $H_2PO_4^-$.

The cyclic voltammograms of CPZ–HCl at pH 4—7 are shown in Fig. 1. The peak currents of the first and second waves increase remarkably with increasing pH. It can readily be seen that the electrode reaction is accelerated at higher pH. With an increase in the supporting electrolyte concentration, both the first and the second wave heights increased, and the second wave potential shifted to the negative potential side (Fig. 4).

Plots of i_p *vs.* the square root of the scan rate (v) with the data obtained in phosphate and acetate buffers are shown in Figs. 5 and 6; straight lines are obtained in the latter but not in the former case. The results indicate that the electrode reaction in acetate buffer is primarily limited by simple diffusion, but in phosphate, particularly at higher pH, some rather complicated steps such as catalytic electrode reactions must be involved in the overall processes.

In the catalytic electrode reaction given in equations (1) and (2), the oxidation peak current, i, is represented by equation (3), where R is the reduced form of the drug, O is the oxidized form and Z is a reducing agent. C_R is the concentration of R.[19] If a catalytic process takes part in the electrode reaction in the phosphate buffer, the peak current should be proportional to the square root of the rate constant (k_f) of the chemical reaction of the oxidation product.

$$\mathrm{R} \rightleftharpoons \mathrm{O} + ne^- \qquad (1)$$

$$\mathrm{O} + \mathrm{Z} \xrightarrow{k_f} \mathrm{R} \qquad (2)$$

$$i = nFAC_R\sqrt{Dk_f} \qquad (3)$$

Chart 1

$$\mathrm{CPZ}^{\dot{+}} + \mathrm{H_2PO_4^-} \underset{}{\overset{k_1}{\rightleftharpoons}} [\mathrm{CPZ(HPO_4)}]^{\dot{-}} + \mathrm{H^+} \qquad (4)$$

$$\mathrm{CPZ}^{\dot{+}} + [\mathrm{CPZ(HPO_4)}]^{\dot{-}} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [\mathrm{CPZ(HPO_4)}] + \mathrm{CPZ} \qquad (5)$$

$$[\mathrm{CPZ(HPO_4)}] + \mathrm{H_2O} \xrightarrow{k_3} \mathrm{CPZO} + \mathrm{H_2PO_4^-} + \mathrm{H^+} \qquad (6)$$

$$k_{\mathrm{obs}} = \frac{2k_1(k_2/k_{-2})k_3[\mathrm{H_2O}][\mathrm{H_2PO_4^-}]}{([\mathrm{CPZ}] + k_3[\mathrm{H_2O}]/k_{-2})\,[\mathrm{H^+}]} \qquad (7)$$

Chart 2

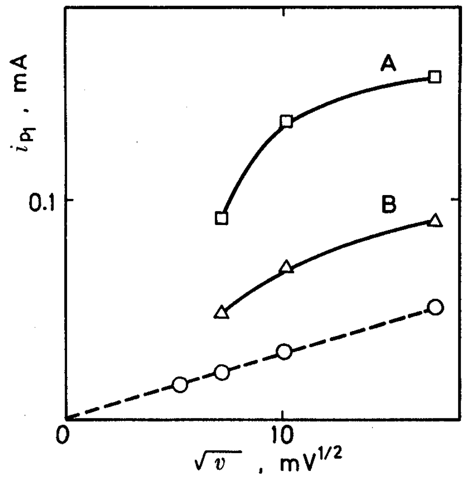

Fig. 5. Peak Current of First Wave *vs.* Square Root of Scan Rate in Phosphate Buffer

A, pH 6.8; B, pH 4.5. The dashed line was measured in 8.4 N H_2SO_4.

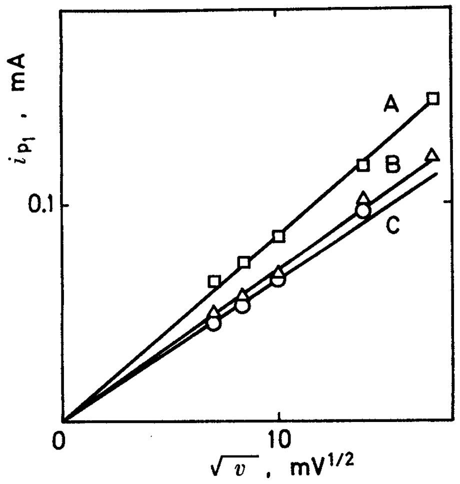

Fig. 6. Peak Current of First Wave *vs.* Square Root of Scan Rate in Acetate Buffer

A, pH 6.4; B, pH 4.7; C, pH 3.6.

The degradation rate constant (k_{obs}) of CPZ^{+} in bulk solution and the reaction mechanism as proposed by McCreery *et al.*,[14] are shown in Chart 2. Provided that the relations given in equations (3) and (7) hold, log i_p is proportional to pH, and i_p is proportional to the square root of the buffer concentration. These relations of course exist for the first peak of CPZ in the phosphate buffer, as shown in Figs. 7A and 8A. The straight lines suggest that the first peak is catalytic in nature. In contrast, i_p is independent of both pH and the buffer concentration in acetate buffer (Figs. 7B and 8B). This seems to be due to the slow reaction rate of the hydrolysis of $CPZ^{+}$and reproduction of CPZ in the acetate buffer, as pointed out by McCreery *et al.*[18]

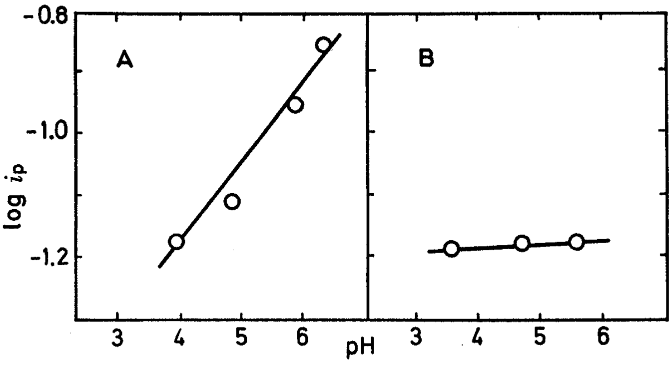

Fig. 7. Logarithm of Peak Current of O_1 *vs.* pH

Ion strength=0.2. [CPZ]=1.0×10^{-3} M. A, in phosphate buffer; B, in acetate buffer.

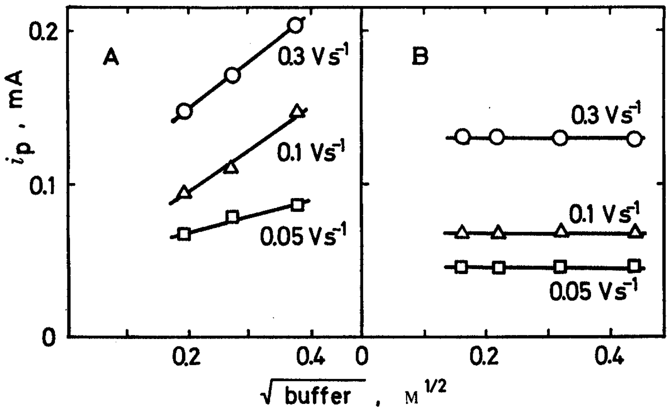

Fig. 8. Peak Current of O_1 *vs.* Square Root of Buffer Concentration

pH=4.7. Ion strength=0.2. [CPZ]=1.0×10^{-3} M. A, in phospate buffer; B, in acetate buffer.

The influence of the presence of Cs^{+} on the cyclic voltammogram of CPZ was examined to confirm the formation of CPZ^{+}-buffer anionic associates. No appreciable change was observed in the voltammogram at lower pH, but the oxidation peaks were suppressed at higher pH as shown in Fig. 1. In particular, the effect on the second peak was marked. This suggests that Cs^{+} reacts with certain anionic species, probably to form CPZ-supporting electrolyte adducts, as a result of which the reproduction of CPZ may be suppressed. This suppression of the second peak, as well as the effects of the kind of supporting electrolyte and its concentration on the E_p of the second peak described above, suggest that the second peak may be attributed to the oxidation of the adducts.

first peak

$$CPZ \underset{+e}{\overset{-e}{\rightleftarrows}} CPZ^{+} \qquad (8)$$

$$CPZ^{\dot{+}} + H_2PO_4^- \rightleftarrows [CPZ(HPO_4)]^{\dot{-}} + H^+ \qquad (9)$$

$$[CPZ(HPO_4)]^{\dot{-}} + CPZ^{\dot{+}} \rightleftarrows [CPZ(HPO_4)] + CPZ \qquad (10)$$

$$[CPZ(HPO_4)] + H_2O \longrightarrow CPZO + H_2PO_4^- + H^+ \qquad (11)$$

second peak

$$CPZ^{\dot{+}} + H_2PO_4^- \rightleftarrows [CPZ(HPO_4)]^{\dot{-}} + H^+ \qquad (12)$$

$$[CPZ(HPO_4)]^{\dot{-}} \xrightarrow{-e} [CPZ(HPO_4)] \qquad (13)$$

$$[CPZ(HPO_4)] + H_2O \longrightarrow CPZO + H_2PO_4^- + H^+ \qquad (14)$$

Chart 3

In conclusion, we propose the oxidation mechanism shown in chart 3 on the basis of all the findings presented above. CPZ is oxidized to the cation radical, which associates with the supporting electrolyte to become a CPZ-electrolyte adduct (equations (8) and (9)). The adduct is oxidized by another radical to produce CPZ(HPO_4) and CPZ (equation (10)). The former is then hydrolyzed to CPZO and the latter latter is reoxidized. At higher pH or higher concentration of the supporting electrolyte, reproduction of CPZ proceeds rapidly, resulting in the enhancement of the first peak current. The second peak may be due to the oxidation of the CPZ-supporting electrolyte adduct.

The oxidation potential of the phenothiazine cation radical in electrochemical oxidation can be correlated to clinical activity.[14] In human beings, the interaction between CPZ and a dopamine receptor seems to play an important role in drug activity.[20-22] The value of the oxidation potential of the CPZ cation radical is influenced by pH and the supporting electrolyte, and thus the situation in biofluids surrounding the receptor sites may affect the clinical activity of CPZ. If the electrode surface can be regarded as a very simple model of receptors, the details of the electrode reaction mechanism of CPZ may be worthy of further study to cast light on the nature of the drug-receptor interaction.

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