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Kinetics of Chlorpromazine Cation Radical Decomposition in Aqueous Buffers

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Abstract: The stoichiometry and kinetics of the reactions of the perchlorate salt of chlorpromazine cation radical ($CPZ^{+\cdot}$) in aqueous buffers were examined in the pH range 2–7 using electrochemistry and spectrophotometry. In phosphate and citrate media, 1 mol of radical produced 0.5 mol each of chlorpromazine and chlorpromazine sulfoxide, while in amine buffers or unbuffered solution, other products were formed. For phosphate and citrate buffers, the decay of $CPZ^{+\cdot}$ was second order in $CPZ^{+\cdot}$ first order in buffer anion concentration, inverse first order in $[H^+]$ and inverse first order in neutral chlorpromazine concentration. The kinetic data indicate the formation of a cation radical/buffer adduct which is oxidized by another molecule of $CPZ^{+\cdot}$, followed by rearrangement to the sulfoxide product. The results are inconsistent with a mechanism involving disproportionation of the radical, but rather indicate a direct reaction of cation radical with buffer components and water.

Introduction

During the last decade, there has been significant interest in the phenothiazine-based cation radicals for two fairly distinct reasons. First, the structure and reactions of the phenothiazine cation radical are similar to those of the intensely studied diphenylanthracene and thianthrene radicals. Examination of the kinetics and mechanisms of reactions of these radicals with nucleophiles has been very active.[1-6] In addition, the phenothiazine-based major tranquilizers such as chlorpromazine (CPZ) and fluphenazine are very widely used antipsychotic drugs, whose activity and metabolism are believed to involve formation of the radical cation as an intermediate.[7,8] Owing to the low stability of these radical ions in neutral aqueous environments, previous work was carried out in nonaqueous solvents, or in strong aqueous acids. It is the purpose of the present work to investigate the sulfoxidation of the CPZ cation radical under conditions which more closely approximate those in physiological fluids.

Two general mechanisms have been proposed for the reactions of nucleophiles (including water) with cation radicals, differing in the reactive form of the radical. In the first (Scheme I), the cation radical ($Ar^{+\cdot}$) is attacked directly by nucleophile (Z), then the adduct is oxidized by a second molecule of cation radical.

Scheme I

$$Ar^{+\cdot} + Z \rightleftarrows (ArZ)^{+\cdot}$$
$$(ArZ)^{+\cdot} + Ar^{+\cdot} \rightleftarrows Ar + (ArZ)^{2+}$$
$$(ArZ)^{2+} \rightarrow \text{products}$$

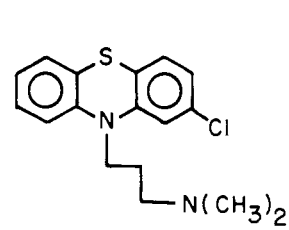
A second mechanism which must be considered involves diproportionation of the cation radical to its dication (Scheme II).

Scheme II

$$2Ar^{+\cdot} \rightleftarrows Ar + Ar^{2+}$$
$$Ar^{2+} + Z \rightleftarrows (ArZ)^{2+}$$
$$(ArZ)^{2+} \rightarrow \text{products}$$

While it is true that the overall stoichiometry of the two routes is identical, it should be emphasized that one mechanism involves direct reaction of the cation radical with nucleophile while the other involves a dication intermediate. Numerous examples of these types of reactions are available,[1-6] recent cases being the reaction of 10-phenylphenothiazine cation radical with pyridine[9] and thianthrene cation radical with water,[10] both using acetonitrile as a solvent. The details of the kinetics will not be repeated here, but in both cases, the kinetics indicate a direct attack of cation radical by the nucleophile, according to Scheme I. It is also interesting to note that the formation of thianthrene sulfoxide from thianthrene radical and water was accelerated in the presence of pyridine by the formation of a pyridine/radical reactive intermediate.[10]

The substantial pharmacological interest in chlorpromazine has prompted several studies of its oxidation and radical ion chemistry. In strong sulfuric acid CPZ (**1**) may be electrochemically oxidized via two one-electron processes to chlorpromazine sulfoxide (CPZO, **2**).[11,12] The hydrolysis of the



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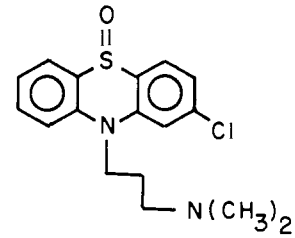

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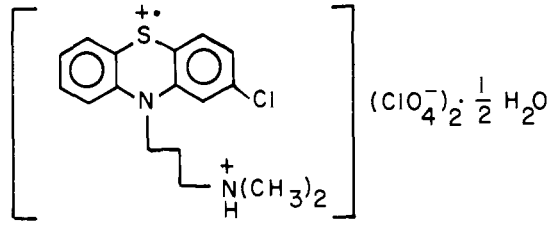
CPZ cation radical in aqueous acids and buffers yields CPZ and CPZO, the radical hydrolysis being roughly second order.[13,14] The authors proposed a disproportionation mechanism, but admitted that the kinetic picture was incomplete. The second-order plots were curved in the early part of the run, and the observed rate constant depended greatly on initial $CPZ^{+\cdot}$ concentration. A variety of ESR studies of the cation radical have been carried out, including a kinetic investigation of $CPZ^{+\cdot}$ hydrolysis in water.[8] Second-order kinetics were observed again, and a disproportionation mechanism was proposed. One is not able to conclude from past work whether the reactive form is $CPZ^{+\cdot}$ or CPZ^{2+}, nor can one explain the dependence of the rate of $CPZ^{+\cdot}$ disappearance on pH or initial $[CPZ^{+\cdot}]$.

The objective of the present work is to elucidate the mechanism of the hydrolysis of CPZ cation radical in aqueous buffers in a pH range near that of the physiological environment. The primary experimental approach involves the reaction of the perchlorate salt of $CPZ^{+\cdot}$ with water, monitored spectrophotometrically.

Experimental Section

Voltammetry. Cyclic voltammetry was performed with an operational amplifier potentiostat of conventional design. A graphite paste working electrode, platinum auxiliary electrode, and saturated calomel reference electrode (SCE) were used in a small volume (3 mL) divided cell.

Materials. Chlorpromazine hydrochloride and chlorpromazine sulfoxide were donated by Dr. A. A. Manian of the Psychopharmacology Research Branch at NIMH. Chlorpromazine cation radical perchlorate[15] (**3**) was synthesized by an electrochemical synthesis



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adapted from other chemical procedures.[16,17] CPZ HCl (200 mg) was dissolved in 3 mL of 60% perchloric acid in an electrochemical cell containing a 4-cm^2 carbon cloth electrode. The solution was electrolyzed at +0.600 V vs. SCE until the current dropped below 1% of the initial value (about 15 min). Acetone (5 mL), then 5 mL of ether were added and the solution was cooled to 0 °C. After crystallization was complete, the product was filtered and washed with cold ether, then dried in a vacuum desiccator. Anal. Calcd for $C_{17}H_{20}N_2SCl{\cdot}2ClO_4{\cdot}\frac{1}{2}H_2O$: C, 38.69; H, 4.01; N, 5.31; S, 6.07; Cl, 20.15. Found: C, 38.60; H, 4.11; N, 5.35; S, 6.05; Cl, 20.53. The melting point of the deep red solid was 194–195 °C. Coulometric reduction of the radical dissolved in 4 M H_2SO_4 required 0.98 faraday/mol, and oxidation of radical in the same medium required 1.02 faradays/mol, close to the expected one-electron values. The molar absorptivity of the radical, determined in 6 M H_2SO_4, was 12 500 M^{-1} cm^{-1} at 525 nm.

Product Analysis. A high-performance liquid chromatograph constructed from Altex components was used for product analysis. UV detection at 254 nm allowed reproducible quantitative results. The conditions for separation follow: eluent 40% MeOH, 60% H_2O, 0.025 M borate, 0.05 M $NaNO_3$; pH 9.3; flow rate 0.46 mL/min; column Du Pont Zipax strong cation exchange resin, 2 mm × 25 cm. Calibration curves were constructed from solutions containing both CPZ and CPZO, and were linear over the required range.

Kinetics. Kinetic runs were conducted by monitoring cation radical absorbance at 525 nm with a conventional spectrophotometer interfaced to a laboratory computer. The computer allowed convenient plotting of data and least-squares determination of rate constants. Except for exceedingly long runs, the reaction was carried out to at least 80% completion; the long runs (one out of five) were carried out to at least 50% completion. For experiments in unbuffered media, a flow cell allowed manual adjustment of pH to ±0.05 units. NaCl was used to adjust the ionic strength in all experiments to a value of 0.2 M. The reaction was initiated by direct dissolution of the radical perchlorate salt.

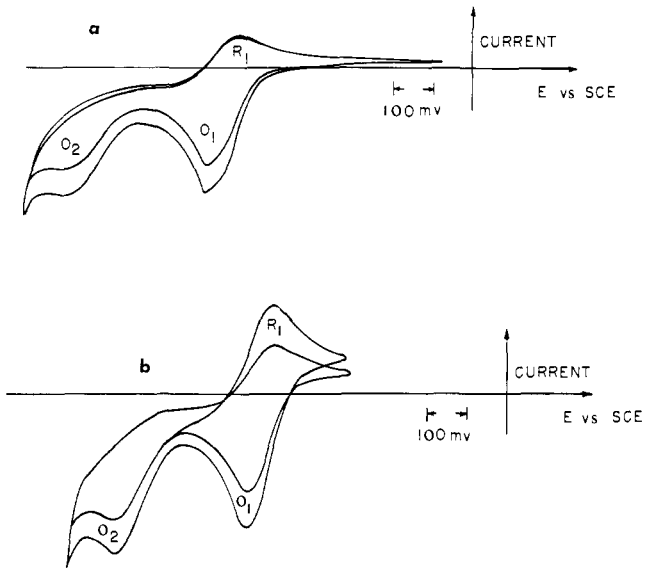


Figure 1. Cyclic voltammograms for CPZ (a) and $CPZ^{+\cdot}$ (b) in 0.01 M aqueous HCl, 0.2 M NaCl. Scan rate = 0.0185 V/s.

Results

Voltammograms. The cyclic voltammetric behavior of chlorpromazine at a carbon paste electrode in aqueous 0.01 M HCl is shown in Figure 1A. Two anodic waves (O_1, O_2), with peak potentials of +0.66 and +0.99 V vs. SCE are observed, corresponding to the oxidation of CPZ to its cation radical ($CPZ^{+\cdot}$) and subsequently to its dication (CPZ^{2+}). The abnormally sharp peak on O_1 is apparently due to adsorption of CPZ, and is observed over a wide pH range. Upon scan reversal only the cation radical is sufficiently stable to yield a cathodic wave (R_1). The dication reacts very rapidly in this medium, so that the reduction of dication is not observable even at scan rates up to 50 V/s.

Figure 1B is a comparable voltammogram of the CPZ radical perchlorate salt in 0.01 M HCl; the scan was initiated at a potential anodic of O_1. The reversible redox couple (R_1/O_1) accompanied by an oxidation wave (O_2) match those of Figure 1A. The absence of the adsorption peak on O_1 is attributable to increased solubility of the radical caused by its additional positive charge. A useful conclusion which can be drawn from these voltammograms is that the synthetic radical perchlorate salt has voltammetric behavior similar to that of the parent chlorpromazine, as expected.

Product Analysis. Quantitative analysis of the products of kinetic runs carried out in different buffers reveals that the distribution of products is highly dependent on the nature of the buffer. Figure 2 shows a set of chromatograms of solutions of $CPZ^{+\cdot}$ in different buffers, after the radical had reacted completely. Trace A was obtained with phosphate and carboxylate buffers, and shows the expected two products: the early peak is CPZO, the later CPZ. Trace B results from amine buffers such as 2-(*N*-morpholino)ethanesulfonic acid (MES) and glycine at pH 7. The extra peaks are presently unidentified, but the product distribution obviously has changed. The use of unbuffered NaCl as a medium yields trace C, qualitatively comparable to the amine buffered case. When glycine is used at low pH (2–4), the expected two products are observed (trace A); however, when the nitrogen becomes partially deprotonated at higher pH, the more complex result is observed (trace B). The results of quantitative analysis of these chromatograms

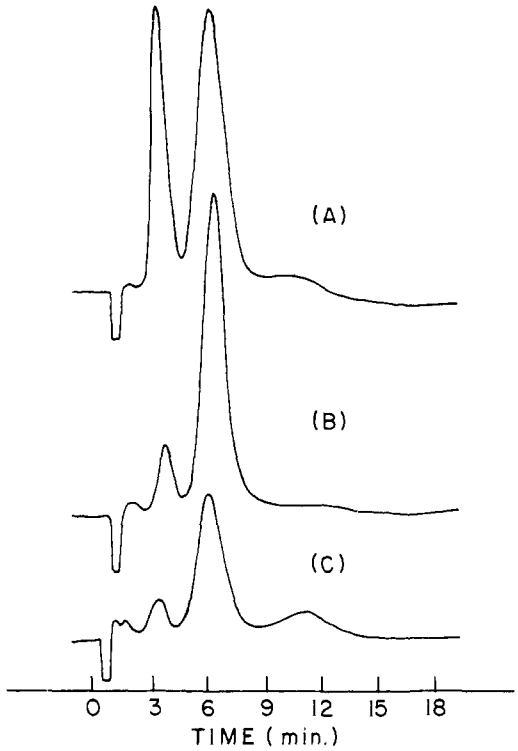


Figure 2. Liquid chromatograms of the products of $CPZ^{+\cdot}$ degradation in various media. Curve A, phosphate, pH 7; curve B, MES, pH 6.5; curve C, unbuffered, pH 7.

Table I. Liquid Chromatographic Analysis of Products of $CPZ^{+\cdot}$ Reactions in Various Media

Media	pH	% CPZ[a]	% CPZO[a]
Phosphate	3.5	48.0	52.5
Phosphate	7.0	50.8	52.3
Citrate	3.2	52.3	49.1
MES	6.5	63.3	16.4
Unbuffered	7.0	61.0	16.5

[a] Yields based on initial $CPZ^{+\cdot}$ concentration.

are shown in Table I and demonstrate a 50/50 yield of CPZ and CPZO in phosphate and citrate media.

The expected 1:1 CPZ:CPZO ratio was observed for phosphate, citrate, and cacodylate in the pH range 2–7, and for sulfate and glycine at low pH. These results indicate that the stoichiometry of the reaction is represented by the equation

$$2CPZ^{+\cdot} + H_2O \rightarrow CPZ + CPZO + 2H^+ \qquad (1)$$

The complications arising with the other buffers remain to be explained.

Kinetic Results. The investigation of the rate of $CPZ^{+\cdot}$ disappearance included the effects of the following variables: (1) neutral CPZ concentration, (2) buffer concentration, and (3) pH. These three effects will be discussed separately. Detailed kinetics were examined in citrate, phosphate, and low pH glycine media, since the products for the reaction in these buffers were known.

CPZ Concentration Dependence. As mentioned above, previous workers have reported that second-order plots for $CPZ^{+\cdot}$ hydrolysis were curved, and the apparent rate constant depended greatly on initial $[CPZ^{+\cdot}]$.[14] In the present work, it was determined that if the CPZ concentration is at least five times that of $CPZ^{+\cdot}$, clean second-order plots are obtained, as shown in Figure 3. The rate constant does not depend on initial $[CPZ^{+\cdot}]$ provided that the CPZ concentration is in large excess

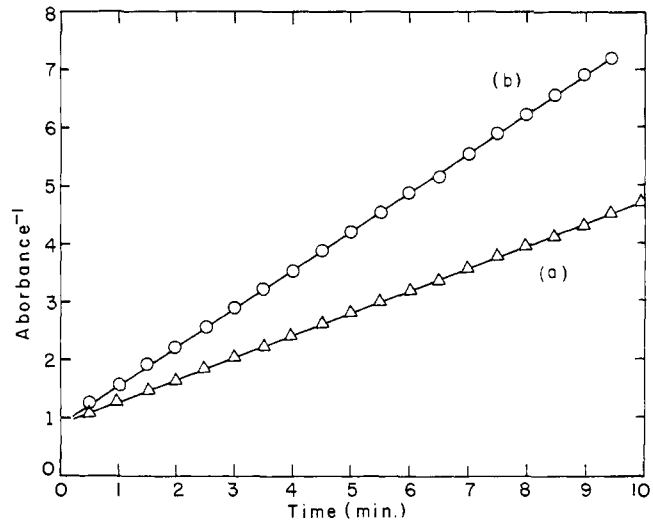


Figure 3. Second-order plots for $CPZ^{+\cdot}$ degradation in 0.05 M citrate buffer in the presence of excess CPZ. In both cases, pH 2.83, initial $[CPZ^{+\cdot}] \simeq 10^{-4}$ M. Curve a, $[CPZ] = 2.60 \times 10^{-3}$ M; curve b, $[CPZ] = 8.4 \times 10^{-4}$ M.

Table II. Second-Order Rate Constants for $CPZ^{+\cdot}$ Hydrolysis (Initial $[CPZ^{+\cdot}] \sim 10^{-4}$ M)

pH	[Citrate], M	$[CPZ] \times 10^3$, M	$k_{obsd} \times 10^{-1}$, M^{-1} s^{-1}
2.64	0.03	0.78	4.82
		1.08	4.25
		1.56	3.46
		1.96	3.14
2.64	0.05	0.75	7.84
		1.04	7.06
		1.49	5.83
		1.78	5.13
		2.60	4.13
		3.72	3.20
2.64	0.08	0.77	11.1
		1.07	9.16
		1.53	8.21
		1.84	7.17
2.45	0.05	0.77	5.42
		1.07	4.38
		1.54	3.64
		2.08	3.15
		2.85	2.45
		3.85	2.15
2.82	0.05	0.84	12.2
		1.12	10.3
		1.40	9.35
		1.82	8.25
		2.60	6.78
		3.51	5.39

of the radical. The observed rate constant decreases with increasing [CPZ] as shown in Table II. A plot of $1/k_{obsd}$ vs. [CPZ] is linear with a nonzero y intercept, indicating that eq 2 and 3 apply at constant citrate and hydrogen ion concentrations. A_1 and A_2 are as yet undetermined constants, which depend on buffer concentration and pH, as discussed below.

$$\frac{d[CPZ^{+\cdot}]}{dt} = -k_{obsd}[CPZ^{+\cdot}]^2 \qquad (2)$$

$$\frac{1}{k_{obsd}} = A_1[CPZ] + A_2 \qquad (3)$$

The curvature observed by earlier workers was apparently caused by variations in [CPZ] as the reaction proceeded.

Buffer Concentration Dependence. At constant pH and constant excess [CPZ], the reaction was first order in buffer

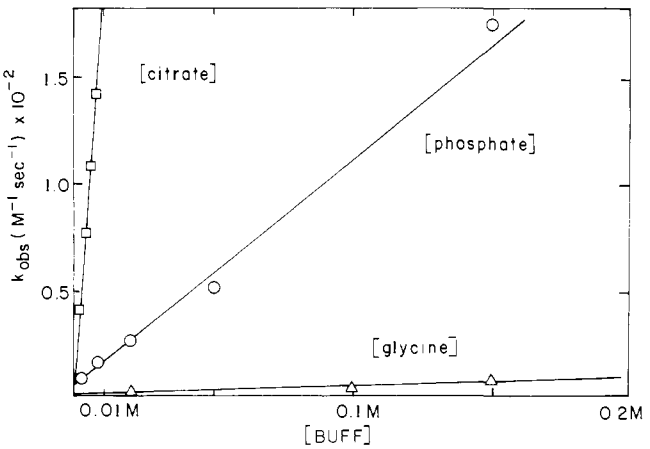


Figure 4. Second-order rate constants for $CPZ^{+\cdot}$ decay in various buffers. In all cases, pH 3.80, [CPZ] = 1.86 mM, initial [CPZ] $\simeq 10^{-4}$ M, ionic strength = 0.2 M.

Table III. Second-Order Rate Constants as a Function of Buffer Concentration (pH 3.80, [CPZ] = 1.86 mM, initial $[CPZ^{+\cdot}] \sim 10^{-4}$ M)

Buffer	Concn, M	k_{obsd}, M^{-1} s^{-1}
Phosphate	0.15	163
	0.05	47.1
	0.02	24.5
	0.08	15.0
	0.002	6.69
Glycine	0.15	8.19
	0.1	3.88
	0.02	2.14
Citrate	0.008	137
	0.006	104
	0.004	73.2
	0.002	36.8

concentration, as shown in Figure 4 and Table III. The large dependence of reaction rate on buffer indicates buffer involvement in the mechanism. The ratio of the slopes of Figure 4 for citrate, phosphate, and glycine are 300:25:1, respectively.

pH Dependence. The influence of pH on the stability of $CPZ^{+\cdot}$ is shown in Table IV. At constant CPZ and total buffer concentration, the rate of radical disappearance increases with increasing pH. Figure 5, curve a, is a plot of log k_{obsd} vs. pH for constant [CPZ] and total phosphate concentration. It is reasonable to propose that the basic form of phosphate in this pH range, $H_2PO_4^-$, is the active form catalyzing radical degradation. Since pH changes will alter $[H_2PO_4^-]$, the plot in Figure 5a should be corrected for these changes. This correction was accomplished by dividing the observed rate constant by α_1, the fraction of total phosphate in the form of $H_2PO_4^-$, obtained from pK_1 for H_3PO_4 (2.12). The corrected rate constants, which are normalized with respect to $[H_2PO_4^-]$, are plotted in Figure 5, curve b. The least-squares slope of this plot is 1.00 and indicates an inverse first order dependence of k'_{obsd} on $[H^+]$.

Discussion

From early consideration of the dependence of k_{obsd} on buffer concentration, it was inferred that the buffer anion may be accelerating proton loss from an intermediate in the rate-determining step. Two mechanisms involving rate-limiting proton transfer are Schemes III and IV, where B^- is the buffer anion.

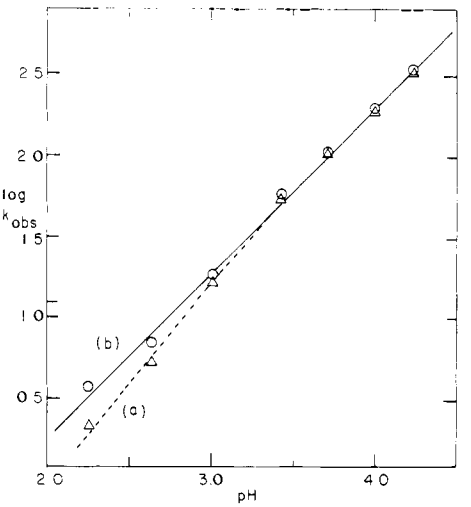


Figure 5. Dependence of k_{obsd} on pH in phosphate buffer. [CPZ] = 2.03 mM, total phosphate = 0.1 M, initial $CPZ^+ \simeq 10^{-4}$ M. Curve a, without correction for variations in $[H_2PO_4^-]$; curve b, with correction for $[H_2PO_4^-]$ variation, as described in text.

Table IV. Second-Order Rate Constants as a Function of pH (Total Phosphate = 0.1 M, [CPZ] = 2.03 mM, Initial $[CPZ^{+\cdot}] \sim 10^{-4}$ M)

pH	k_{obsd}, M^{-1} s^{-1}	α_1	k'_{obsd}, M^{-1} s^{-1}
2.24	2.16	0.572	3.78
2.63	5.39	0.707	7.03
3.00	16.5	0.884	18.7
3.41	53.6	0.953	56.3
3.70	99.0	0.974	102
3.99	177	0.986	179
4.23	316	0.992	318

Scheme III

$$2CPZ^{+\cdot} \rightleftarrows CPZ^{2+} + CPZ$$

$$CPZ^{2+} + H_2O \rightleftarrows CPZOH^+ + H^+$$

$$CPZOH^+ + B^- \xrightarrow{rds} CPZO + HB$$

Scheme IV

$$CPZ^{+\cdot} + H_2O \rightleftarrows CPZOH^{\cdot} + H^+$$

$$CPZOH^{\cdot} + CPZ^{+\cdot} \rightleftarrows CPZOH^+ + CPZ$$

$$CPZOH^+ + B^- \xrightarrow{rds} CPZO + HB$$

In both cases, the buffer acts merely as a proton acceptor reacting with protonated sulfoxide, $CPZOH^+$. This type of general base catalysis can be easily ruled out by at least three considerations. First, the rate-limiting proton transfer is between oxygen acids in the case of phosphate or citrate buffers, the donor (protonated sulfoxide) being a very strong acid. Such reactions would be diffusion limited and unlikely rate limiting unless preceded by extremely unfavorable equilibria. Second, a diffusion-limited proton transfer should not depend on the type of acceptor, clearly not the case for citrate vs. phosphate, for example. Third, for a series of carboxylate buffers (acetate, chloroacetate, dichloroacetate, citrate, glycine) no correlation whatever was found between base strength and rate of catalysis. The Brønsted catalysis law would predict such a correlation for a mechanism involving proton transfer. On these grounds, Schemes III and IV were abandoned.

Table V. $d[CPZ^+]/dt = -k_{obsd}[CPZ^+]^2$

	k_{obsd}	Abscissa intercept
	Scheme V	
Case (1)	$\frac{2k_6K_4K_5[RCO_2^-][H_2O]}{[CPZ][H^+]}$	0
Case (2)	$\frac{2k_6K_4(k_5/k_{-5})[RCO_2^-][H_2O]}{[CPZ]([H^+] + k_6/k_{-5})}$	0
Case (3)	$\frac{2k_4k_5k_6[RCO_2^-][H_2O]}{(k_{-4}k_{-5}[H^+] + k_{-4}k_6)[CPZ] + k_5k_6[H_2O][RCO_2^-]}$	$\frac{-k_5k_6[RCO_2^-][H_2O]}{k_{-4}k_{-5}[H^+] + k_{-6}k_{-4}}$
	Scheme VI	
Case (4)	$\frac{2k_9K_7K_8[RCO_2^-][H_2O]}{[CPZ][H^+]}$	0
Case (5)	$\frac{2k_9K_7(k_8/k_{-8})[RCO_2^-][H_2O]}{([CPZ] + k_9/k_{-8})[H^+]}$	$\frac{-k_9}{k_{-8}}$

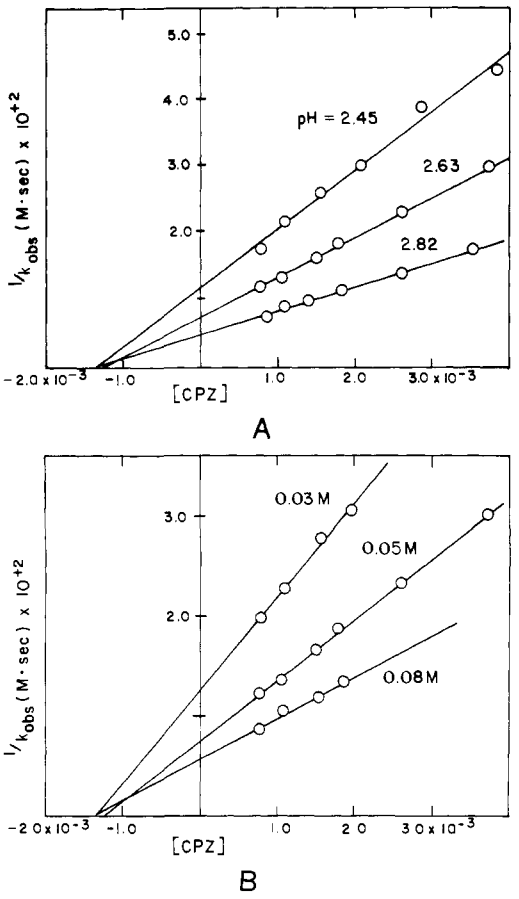


Figure 6. Plots of $1/k_{obsd}$ vs. [CPZ] for citrate buffer at various pHs (plot A) and buffer concentrations (plot B). Note that the intercept on the [CPZ] axis is independent of pH and buffer concentration. From Table VI for case 5, this intercept ($1/k_{obsd} = 0$) has the value $-k_9/k_{-8}$.

Consider Schemes V and VI, which involve nucleophilic addition of the buffer anion to the reactive CPZ species to form a covalent intermediate. RCO_2^- denotes a representative buffer constituent.

Scheme V

$$2CPZ^{+\cdot} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} CPZ + CPZ^{2+} \quad (4)$$

$$CPZ^{2+} + H_2O + RCO_2^- \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} [CPZ(RCO_2)(OH)] + H^+ \quad (5)$$

$$[CPZ(RCO_2)(OH)] \xrightarrow{k_6} CPZO + RCO_2H \quad (6)$$

Scheme VI

$$CPZ^{+\cdot} + RCO_2^- + H_2O \underset{}{\overset{K_7}{\rightleftharpoons}} [CPZ(RCO_2)(OH)]^{-\cdot} + H^+ \quad (7)$$

$$[CPZ(RCO_2)(OH)]^{-\cdot} + CPZ^{+\cdot} \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} CPZ + [CPZ(RCO_2)(OH)] \quad (8)$$

$$[CPZ(RCO_2)(OH)] \xrightarrow{k_9} CPZO + RCO_2H \quad (9)$$

Note that eq 6 and 9 are identical, both involving a loss of protonated buffer from a buffer/water/CPZ adduct. Reaction 7 is written as an equilibrium, as required by the second-order behavior of the $CPZ^{+\cdot}$ decay. Reaction 7 may be a multistep process, but the data do not justify separation of the reaction into distinct steps. Similarly, reaction 5 is written as a single step, for purposes of clarity.[18] In addition, reactions 6 and 9 may be multistep processes, but that cannot be concluded from this work.

Four experimental observations must be accounted for by any proposed mechanism: (1) second order in $[CPZ^{+\cdot}]$; (2) $1/k_{obsd}$ vs. [CPZ] plots are linear with nonzero y intercept; (3) first order in buffer anion concentration; (4) inverse first order in hydronium ion.

For such complex mechanisms, numerous rate laws are possible, depending on the relative magnitudes of the various rate constants. Of the many possibilities, the relevant examples are listed in Table V. The assumptions governing each case are as follows: case (1) Scheme V, with reactions 4 and 5 in equilibrium, reaction 6 the rate-determining step; case (2) Scheme V, reaction 4 in equilibrium, reactions 5 and 6 of comparable rate; case (3) Scheme V, reactions 4, 5, and 6 of comparable rate; case (4) Scheme VI, reactions 7 and 8 in equilibrium, reaction 9 the rate-determining step; case (5) Scheme VI, reaction 7 in equilibrium, reactions 8 and 9 of comparable rate.

Other possible mechanisms lead to a first-order decay of $CPZ^{+\cdot}$, or lack of a pH effect, or are otherwise obviously inconsistent with the observations. The rate laws in Table V were derived using steady-state approximations on CPZ^{2+}, $[CPZ(RCO_2)(OH)]^{-\cdot}$, and $[CPZ(RCO_2)(OH)]$.

A useful diagnostic tool for discerning which case applies to the $CPZ^{+\cdot}$ hydrolysis is provided by a plot of $1/k_{obsd}$ vs. [CPZ], shown in Figure 6 for citrate media at various buffer and hydronium ion concentrations. Table V includes theoretical expressions for the abscissa intercept ($1/k_{obsd} = 0$) for such

plots, as predicted from the various rate laws. Cases 1, 2, and 4 can easily be ruled out, because they predict that the $1/k_{obsd}$ vs. [CPZ] plot should intercept the origin for all buffer and hydronium ion concentrations. Case 3 predicts an abscissa intercept which depends upon $[RCO_2^-]$ and $[H^+]$. Indeed, a factor of 2 change in $[RCO_2^-]$ should shift the abscissa intercept by a factor of 2, clearly not the case for the experimental data in Figure 6. It can be shown that the y intercept for case 3 is $1/2k_4$, which is itself independent of pH and $[RCO_2^-]$. Thus, case 3 can be ruled out, leaving only case 5. Case 5 correctly predicts an abscissa intercept which is independent of $[RCO_2^-]$ and $[H^+]$, as verified in Figure 6.

Thus one concludes that the mechanism for the hydrolysis of $CPZ^{+\cdot}$ in aqueous buffers is represented by the equations

$$CPZ^{+\cdot} + RCO_2^- + H_2O \overset{K_7}{\rightleftharpoons} [CPZ(RCO_2)(OH)]^{-\cdot} + H^+ \quad (10)$$

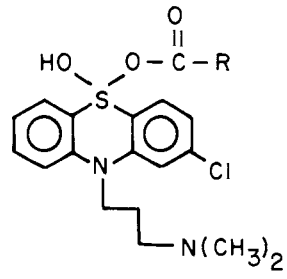
$$[CPZ(RCO_2)OH]^{-\cdot} + CPZ^{+\cdot} \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} CPZ + [CPZ(RCO_2)(OH)] \quad (11)$$

$$[CPZ(RCO_2)(OH)] \xrightarrow{k_9} CPZO + RCO_2H \quad (12)$$

While individual rate constants cannot be determined from these data, the expressions k_9/k_{-8} and K_7k_8 may be calculated from the intercept and slopes of Figure 6. In citrate media at 0.2 M ionic strength, $k_9/k_{-8} = (1.37 \pm 0.06) \times 10^{-3}$ M, and $K_7k_8 = 0.049$ M^{-2} s^{-1}. In phosphate at the same ionic strength, $k_9/k_{-8} = 1.8 \times 10^{-3}$ M and $K_7k_8 = 3.1 \times 10^{-3}$ M^{-2} s^{-1}. Comparable behavior was observed in less detail for carboxylate and other nonamine buffers. The products and mechanism of the reaction in amine buffers and unbuffered media are presently under investigation.

The direct involvement of buffer in the $CPZ^{+\cdot}$ degradation was unexpected but not completely without precedent. In the reaction of thianthrene cation radical with water in acetonitrile to form its sulfoxide, pyridine catalyzes the reaction by initially attacking the sulfur atom.[10] The thianthrene radical also reacts with the phosphate group of ATP, again in nonaqueous solvents.[19] pyridine forms a 10-phenylphenothiazine adduct with a three-coordinate sulfur center in acetonitrile, during the formation of 10-phenylphenothiazine sulfoxide.[9] Structural data are not presently available for the oxidized radical buffer adduct, but a reasonable structure for $[CPZ(RCO_2)OH]$ is shown below (**4**). Given this structure, the last step in the reaction would be a loss of RCO_2H to form CPZO.

It is apparent from Figure 4 that the rate of reaction without buffer is extremely slow, an observation confirmed by unbuffered experiments. Much of the disparity in previous work on the stability of $CPZ^{+\cdot}$ arises from the use of different buffers and lack of excess [CPZ]. The buffer effect described here indicates not only that radical stability in the body depends on the physiological environment, but also that the radical may form short-lived covalent bonds with common nucleophiles such as carboxyl and phosphate groups. The possibility that these nucleophiles may be part of the nerve membrane which CPZ affects is a notion worth consideration in the future.



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(15) Note that in the weakly acidic solutions used here, the radical will be a dication due to protonation of the terminal nitrogen. For simplicity, the cation radical will be abbreviated by $CPZ^{+\cdot}$.
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(18) Rate laws for a stepwise reaction 5 may be derived easily but are very complex. In all cases, the $1/k_{obsd}$ vs. CPZ plot has the form $1/k_{obsd} = \alpha[CPZ] + \frac{1}{2}k_4$ where α depends on pH and $[RCO_2^-]$. Thus a plot of $1/k_{obsd}$ vs. [CPZ] should have a y intercept which is independent of pH and $[RCO_2^-]$, a case which is not observed in Figure 6. The results of Figure 6 indicate that proton loss must occur before oxidation of the adduct, so the disproportionation mechanisms, with a stepwise reaction 5 or not, do not apply.
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