Formation of a Promazine Radical and Promazine 5-Oxide in the Reaction of Promazine with Hydrogen Peroxide: Mechanistic Insight from Kinetic and EPR Measurements

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ABSTRACT: The kinetics of the oxidation of promazine (PMZ) by hydrogen peroxide was studied in the presence of a large excess of H_2O_2 in acidic chloride media using UV–vis spectroscopy. The reaction proceeds via two consecutive steps. In the first step, oxidation leads to formation of a promazine radical. In the second step, the promazine radical is oxidized to promazine 5-oxide. Electron paramagnetic resonance spectroscopy (EPR) results provide clear evidence for the formation of an intermediate promazine radical. Linear dependences of the pseudo-first-order rate constants (k_1 and k_2) on $[H_2O_2]$ with a nonzero intercept were established for the first and the second process, respectively. The rate of the first stage of the reaction increased slightly with increasing concentration of O_2, indicating the role of the $OH^{\bullet}$ radicals on the redox process, which are transformed into the $Cl_2^{-\bullet}$ radicals. The mechanism of the overall reaction is discussed on the basis of all these kinetic measurements. © 2009 Wiley Periodicals, Inc. Int J Chem Kinet 42: 1–9, 2010

INTRODUCTION

Although considerable research has already been carried out in an attempt to understand the role of free radicals in the pathogenesis of many diseases and in normal healthy cell metabolism, the field still remains active in most disciplines in the life sciences [1,2]. Among others, reactive oxygen species are involved

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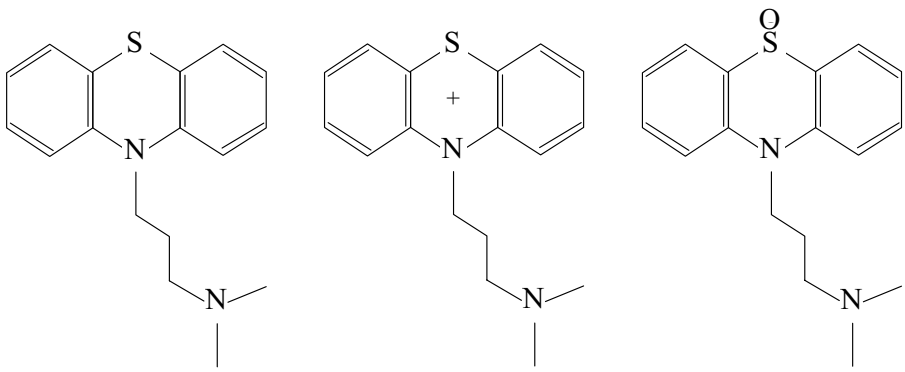
in a wide variety of physiological and pathophysiological processes [3,4]. These active oxygen species, such as the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\bullet$) can interact with cellular membrane structures and induce lipid peroxidation, which has been indicated in oxidative stress in cells [2]. However, only $OH^\bullet$ radical can directly initiates a lipoperoxidation, but H_2O_2 and $O_2^{-\bullet}$ induce this process indirectly. Hydrogen peroxide is one of the products in the dismutation of the superoxide anion radicals catalyzed by superoxide dismutases and appears as an intermediate in many reactions of organic material with dioxygen. Dioxygen is unreactive toward many organic species but can be activated by reduction to $HO_2/O_2^{-\bullet}$, H_2O_2, $OH^\bullet$, or by coordination to transition metal ions [5]. It is reasonable that hydrogen peroxide may oxidize organic substances more efficiently than oxygen. Under physiological condition in cells, hydrogen peroxide oxidizes thiols, indoles, and the imidazole, phenolic, thioester, and methionine groups in other biologically active species [6].

Promazine (Scheme 1), as have many other *N*-substituted phenothiazines, has been found to modify various neurotransmission processes [7]. In medicine, phenothiazines are also applied as antiarrhythmic drugs or photodynamic virus inactivators [8]. Their efficiency in affecting the antibiotic resistance of bacteria and tumor cells is the subject of continuous and intense investigations [9]. A quenching action of the phenothiazines on radical formation has been found to be responsible for inhibitory effects on lipid peroxidation [10,11] and characterized redox activity of this class of tricyclic compounds.

In an earlier article, we reported on the kinetics of the slow oxidation of promazine by dioxygen in solutions of aqueous copper(II) halides [12]. In this reaction, the copper(II) halides oxidized promazine to a cation radical and copper(I) was reoxidized by dioxygen to copper(II). Dioxygen dissolved in the solution was the direct oxidant in the next process leading to sulfoxidation of the cation radical. Direct interaction of O_2 with paramagnetic reductants results in an appearance of H_2O_2 as intermediate [12]. The particular emphasis of the present work was to understand the kinetics and mechanism of the nonenzymatic and noncatalyzed oxidation of promazine by hydrogen peroxide. The redox activity of hydrogen peroxide revealed the redox potential, which is equal to 0.38 V for the $H_2O_2/OH^\bullet$ couple and 1.35 V for the H_2O_2/H_2O species (pH 7) [13]. The hydroxyl radical generated in the reduction of hydrogen peroxide is known to be the most reactive oxygen species with a redox potential for the $OH^\bullet/H_2O$ couple of 2.31 V (pH 7) [13] higher than for the H_2O_2/H_2O compounds. The hydroxyl radical reacts usually at rates close to diffusion-controlled with practically all biological molecules, its half-life in cells has been estimated to be 10^{-9} s [14]. In the presence of H_2O_2 in aqueous solution, the $OH^\bullet$ radical can be transformed into the $O_2^{-\bullet}$ (HO_2) radical anion, which is less reactive toward most biological materials than the $OH^\bullet$ radical. In the present work, the $OH^\bullet$ production in the reaction was studied using the spin-trapping method of electron paramagnetic resonance spectroscopy (EPR) to establish further experimental evidence its influence on the mechanism of the reaction, although this influence is not direct. In acidic chloride solutions, the $OH^\bullet$ radical reacts with chloride ion and oxidizes to a dichloro radical anion, $Cl_2^{-\bullet}$. This radical is a very strong oxidant; its redox potentials is equal to 2.30 V ($Cl_2^{-\bullet}/2Cl^-$) [15]. The rate constants of the one-electron oxidation reactions of the $Cl_2^{-\bullet}$ radical with numerous organic and inorganic compounds were determined using pulse radiolysis and flash photolysis experiments [16].



PMZ PMZ$^{+\bullet}$ PMZO

Scheme 1

EXPERIMENTAL

Materials

Hydrogen peroxide (30%; Sigma-Aldrich, St. Louis, MO, USA), DMPO (5,5-dimethyl-1-pyrroline *N*-oxide; Aldrich, Milwaukee, WI, USA) used in spin-trapping experiments, and all other chemicals were analytical grade reagents. Water purified in a Millipore Milli-Q system was used to prepare all the solutions. The concentration of a stock solution of hydrogen peroxide was determined manganometrically. The solution was stable for 1 month when stored at 5°C. Hydrogen peroxide is unstable at higher temperatures and undergoes a dismutation reaction, which can be facilitated by Na^+, K^+ ions, as well as by traces of transition metal ions [17]. The stability of hydrogen peroxide has been analyzed in different media using manganometric titrations. The loss of H_2O_2 in the HCl solution was equal 0.4% within the standard errors ($t = 500$ min, $[H_2O_2] = 0.05$ mol dm^{-3}, $[H^+] = 0.1$ mol dm^{-3}, $I =$ 0.1 mol dm^{-3} (H^+, Cl^-), $T = 298$ K), in water it was 1%, while in an aqueous solution of NaCl this percentage was 10% ($t = 500$ min, $[H_2O_2] = 0.05$ mol dm^{-3}, $I = 0.1$ mol dm^{-3} (Na^+, Cl^-), $T = 298$ K). Promazine (Sigma-Aldrich) and its aqueous solution were stored in the dark because of their photosensitivity. Argon and oxygen gases of high purity were applied in the experiments. EDTA (disodium ethylenediaminetetraacetate dihydrate) was purchased from Sigma and was used to mask transition metal ions in the solutions.

HPLC Measurements

The final product, promazine 5-oxide, was estimated on a Shimadzu HPLC chromatograph equipped with a C18 column (Supelco; 0.46-cm i.d., 15-cm i.l., 5-μm particles), Rheodyne 7125 sample injector, Shimadzu SPD-10A (VP) UV–vis detector (250 nm), and Shimadzu LC-10AD (VP) pump. A mobile phase composition (90 vol% of acetonitrile and 10 vol% of 50 mmol dm^{-3} K_3PO_4, pH 7) was performed for chromatographic separations.

EPR Measurements

EPR spectra were recorded with Radiopan SE/X 2547 spectrometer in the X band (ca. 9.25 GHz) using a flat quartz cell. Typical parameters for acquisition of EPR spectra were as follows: central field, 330 mT; sweep width, 10 mT; microwave power, 16 mW; modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; time constant, 1 s; sweep time, 120 s; and receiver gain, (1.6–20) $\times$ 10^4. Because of long sweep time (120 s), one scan only was taken for each measurement. The spectra were scanned every 10 min during reaction time. The microwave frequency was monitored with a frequency divider. The magnetic field was measured with an automatic MNR-type magnetometer.

Kinetic Measurements

The kinetic measurements were performed on a Lambda 20 Perkin–Elmer spectrophotometer equipped with an external Julabo F25 ultrathermostat, which enabled all the solutions to be thermostatted to the desired temperature (298 $\pm$ 0.1 K). Kinetic data were acquired and processed using the UV KinLab (Perkin–Elmer) software. Kinetic runs were initiated by mixing a promazine solution with a solution containing hydrogen peroxide and hydrochloric acid. For a few runs, at pH > 1, NaCl was included to maintain constant ionic strength, $I = 0.1$ mol dm^{-3} (H^+, Na^+, Cl^-). The initial promazine concentration in reaction solutions was usually 5×10^{-5} mol dm^{-3}. The concentrations of other reagents were as follows: $[H_2O_2] =$ (5–35) $\times$ 10^{-3} mol dm^{-3}, $[H^+] = 0.02$–0.1 mol dm^{-3}. In a few experiments, kinetics was studied in the presence of transition metal ions scavenger, disodium ethylenediaminetetraacetate dihydrate ($[EDTA] = 1 \times 10^{-3}$ mol dm^{-3}). Because the rate of reaction was not affected by iron and copper chelator, the measurements were done without this species. Progress of the reaction was followed spectrophotometrically at 514 nm (a maximum for the promazine radical) and at 345 nm (a maximum for promazine 5-oxide in 0.1 mol dm^{-3} HCl where promazine radical and H_2O_2 absorbed negligibly). Usually, the kinetic measurements were done in the air atmosphere, but in a few series of experiments, the reaction was studied under either an oxygen or argon atmosphere. The spectra were scanned every 100 s up to $2t_{1/2}$. An excess of H_2O_2 over promazine was used to simplify the reactions into the pseudo-first-order processes. The pseudo-first-order rate constants were calculated from a multiexponential dependence for two consecutive one-electron processes A $\rightarrow$ B $\rightarrow$ C with fixed value of k_2 because of the long reaction time [18]. The kinetic traces at 345 nm represented changes in the concentration of the final product, and no induction time was observed. On account of this fact, a minimalization of the sum of the squares of the deviations for the absorbance dependence versus time, calculated for C, was performed from the first-order dependence. The kinetic runs were performed in a series for various reactant concentrations. The relative standard errors of the pseudo-first-order rate constants for a single kinetic trace were ca. 0.2%.

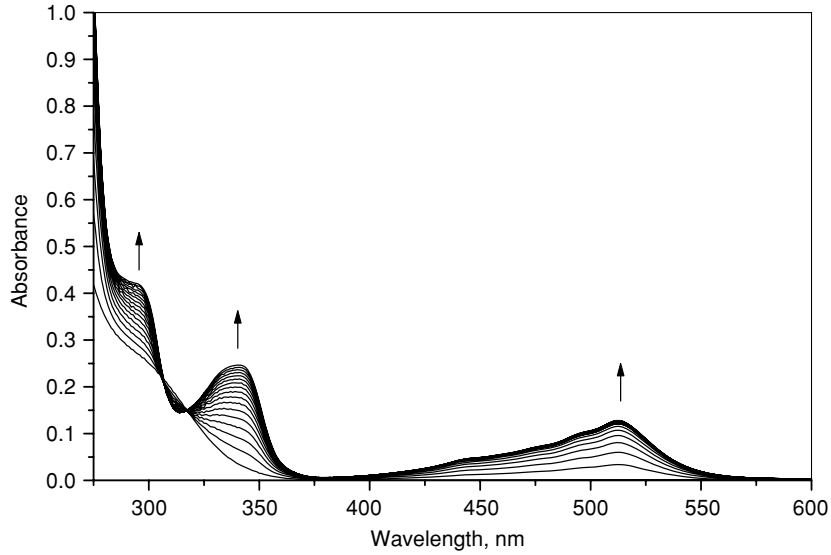


Figure 1 Spectral changes during the oxidation of promazine by hydrogen peroxide. Experimental conditions: $[H_2O_2] = 0.035$ mol dm^{-3}, [PMZ] $= 5 \times 10^{-5}$ mol dm^{-3}, $[H^+] = 0.1$ mol dm^{-3}, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), 298 K, $\Delta t = 30$ min, $t_{total} = 495$ min, 17 scans.

RESULTS AND DISCUSSION

The reaction between promazine (PMZ) and hydrogen peroxide was followed in the aqueous acidic media under an argon, air, or oxygen atmosphere ([PMZ] $= 5 \times 10^{-5}$ mol dm^{-3}, $[H_2O_2] = (5–35) \times 10^{-3}$ mol dm^{-3}, $[H^+] = 0.02–0.1$ mol dm^{-3}, $I = 0.1$ mol dm^{-3} (H^+, Na^+, Cl^-), $T = 298$ K) (Table I). The oxidation of promazine leads to the appearance of the cation radical, $PMZ^{+\bullet}$, which absorbs intensively in the visible range (Fig. 1) at 514 nm (λ_{max}, nm (ε_{max}, dm^3 mol^{-1} cm^{-1}): 513(9200)) [19] and to promazine 5-oxide detected within the UV range after the HPLC separation. The spectra of promazine 5-oxide revealed two low-field electronic transition bands at 335 and 297 nm (Fig. 1) in the ultra-violet spectral range (λ_{max}, nm (ε_{max}, dm^3 mol^{-1} cm^{-1}): 228(24,300), 269(10,300), 297(6500), 335(4700)) [20]. The reaction was monitored in the acidic chloride media, in which promazine exists in its protolytic form with a protonated nitrogen atom in the alkyloamine group in a substituent. A positive charge of this substituent is not included in Scheme 1.

Table I Observed Pseudo-First-Order Rate Constants for the Reaction between Promazine (k_1) or Promazine Radical (k_2) and Hydrogen Peroxide

Atmosphere	$[H_2O_2]$ (mol dm^{-3})	$10^5\ k_1$ (s^{-1})	$10^5\ k_2$ (s^{-1})
Argon	0.0049	5.60	1.82
	0.0148	6.88	3.11
	0.0247	7.76	3.98
	0.0346	8.49	5.38
Air	0.0049	5.93	1.59
	0.0148	7.03	2.62
	0.0247	8.34	3.79
	0.0346	9.34	5.06
Oxygen	0.0049	6.51	1.62
	0.0148	8.18	2.69
	0.0247	9.97	4.25
	0.0346	11.5	5.16

[PMZ] $= 5 \times 10^{-5}$ mol dm^{-3}, $T = 298$ K, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-).

Formation of the $PMZ^{+\bullet}$ radical during oxidation of promazine by hydrogen peroxide was observed using EPR spectroscopy. Figure 2 shows the EPR spectrum of the reaction system after ca. 15 min from the initiation, and clearly indicates the presence of the $PMZ^{+\bullet}$ radical. The spectrum has unresolved hyperfine lines but its g value and overall shape is consistent with the EPR spectrum of $PMZ^{+\bullet}$, published previously [12,21]. The EPR signal of promazine radical with $g = 2.005$ could be explained as arising mainly from coupling of the unpaired electron with heterocyclic ring nitrogen nucleus ($I_N = 1$) and two equivalent β-protons ($I_H = 1/2$) of the side chain ($a_N \sim 0.7$ mT, $a_{H(\beta)} \sim 0.35$ mT), and also from weaker coupling with the four proton pairs of the lateral rings ($a_{H(\beta)} \gg a_{H(ring)}$). Owing to the large number of lines, the relatively low spin density

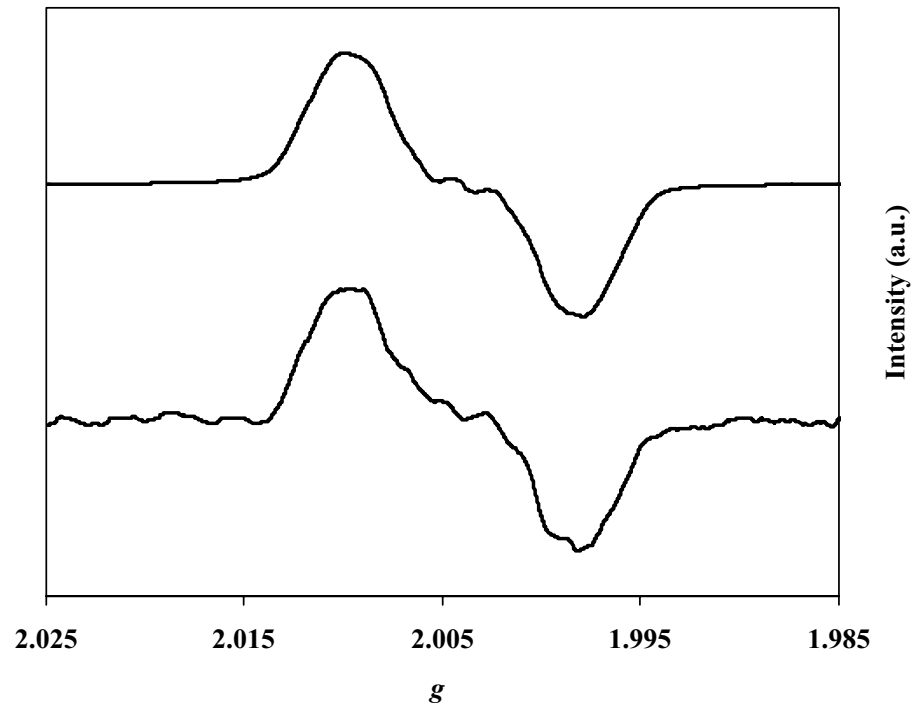


Figure 2 Simulated (top) and experimental (bottom) EPR spectra of the promazine cation radical generated via oxidation of the acidic solution of 6×10^{-3} M promazine by 5×10^{-2} M H_2O_2 under air atmosphere. Experimental conditions: [HCl] = 1×10^{-1} M, $I = 0.1$ (H^+, Cl^-), $T = 295$ K, signal after ca. 15 min, modulation amplitude, 0.02 mT, microwave frequency, 9.257 GHz; other parameters as described in the Experimental section.

of the unpaired electron at the lateral ring protons, and also incomplete averaging of the g factor and/or hyperfine anisotropy, the EPR spectrum of promazine radical cation remains poorly resolved.

The preliminary experiments showed the following interesting features of the reaction: (i) the appearance of promazine 5-oxide follows in two consecutive reactions: formation of the promazine radical and its disappearance leading to promazine 5-oxide; (ii) the highest stability of hydrogen peroxide was observed in acidic aqueous media, i.e. loss of 0.05 mol dm^{-3} H_2O_2 in acidic solution (0.1 mol dm^{-3} HCl) was less than 0.4%, in H_2O was equal to 1%, while in neutral solution of salt (0.1 mol dm^{-3} NaCl) decomposition was up to 10% during 500 min at 298 K; (iii) the oxidation of promazine in the solution at the ionic strength, $I = 1.0$ mol dm^{-3} (H^+, Na^+, Cl^-), $T = 298$ K follows two-fold faster than at $I = 0.1$ mol dm^{-3} (H^+, Cl^-) under the same conditions and temperature.

For the oxidation of promazine to the cation radical, the pseudo-first-order rate constants, k_1, were found to be linearly dependent on [H_2O_2] and were higher in the presence of oxygen than under the argon atmosphere. The change of atmosphere affected the slopes, but the statistically insignificant, nonzero intercepts in the plots of k_1 versus [H_2O_2] are illustrated in Fig. 3. The observed rate constants were found to be independent of the acid concentration within the 0.02–0.1 mol dm^{-3} range (data not shown).

Plots of k_2 versus [H_2O_2] for the second stage—the oxidation of the cation radical ($PMZ^{+\bullet}$) to promazine 5-oxide—are presented in Fig. 4. The pseudo-first-order rate constants were found to be linearly dependent on the H_2O_2 concentration. The rate constants did not differ substantially with increasing [O_2] and the differences in the k_2 values did not affect the intercepts and the slopes under the oxygen atmosphere.

These kinetic data can be rationalized by the following reaction scheme:

$$PMZ + H_2O_2 \xrightarrow{H^+} PMZ^{+\bullet} + OH^\bullet + H_2O \quad \text{(rate determining)} \tag{1}$$

$$PMZ^{+\bullet} + H_2O_2 \xrightarrow{H^+} PMZ^{2+} + OH^\bullet + H_2O \quad \text{(rate determining)} \tag{2}$$

$$PMZ^{2+} + H_2O \longrightarrow PMZO + 2H^+ \tag{3}$$

$$OH^\bullet + Cl^- \rightleftharpoons ClOH^{-\bullet} \quad 4.3 \times 10^9 mol^{-1} dm^3 s^{-1}\ [22] \tag{4}$$

$$ClOH^{-\bullet} \xrightarrow{H^+} Cl^\bullet + H_2O \quad 2.1 \times 10^{10} mol^{-1} dm^3 s^{-1}\ [22] \tag{5}$$

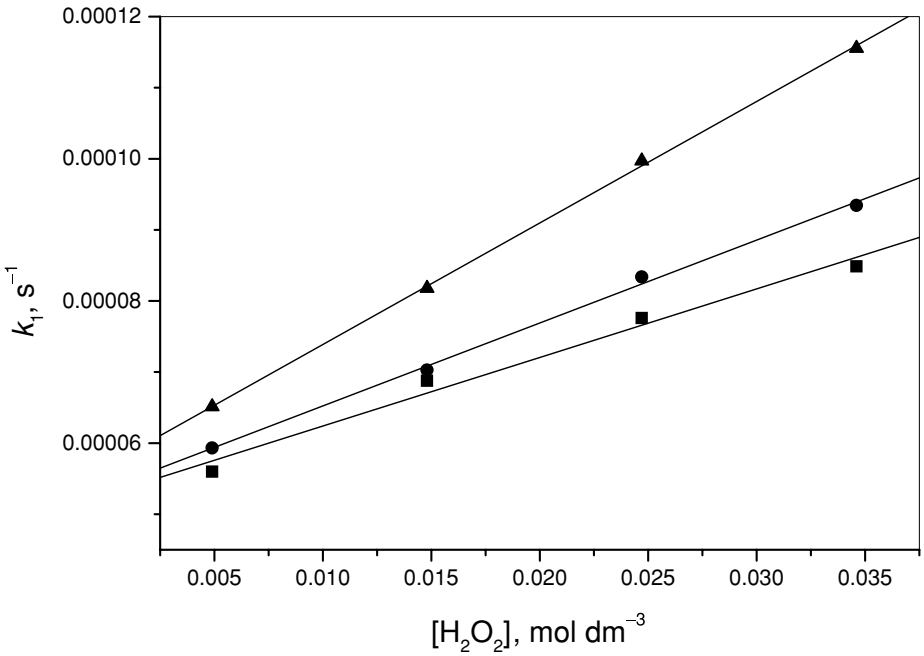


Figure 3 Plots of k_1 versus $[H_2O_2]$ for the oxidation of promazine with hydrogen peroxide. Experimental conditions: $[PMZ] = 5 \times 10^{-5}$ mol dm^{-3}, $T = 298$ K, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), (■) argon, (●) air, (▲) oxygen atmosphere.

$$Cl^{\bullet} + Cl^- \rightleftharpoons Cl_2^{-\bullet}$$
$$8.0 \times 10^9 mol^{-1}dm^3s^{-1}\ [23] \qquad (6)$$

$$PMZ + Cl_2^{-\bullet} \longrightarrow PMZ^{+\bullet} + 2Cl^-$$
$$5 \times 10^9 mol^{-1}dm^3s^{-1}\ [24] \qquad (7)$$

$$PMZ^{+\bullet} + O_2 + H^+ \longrightarrow PMZ^{2+} + HO_2^{\bullet} \quad \text{(very slow process)} \qquad (8)$$

$$2PMZ^{+\bullet} \longrightarrow PMZ + PMZ^{2+} \quad \text{(very slow process)} \qquad (9)$$

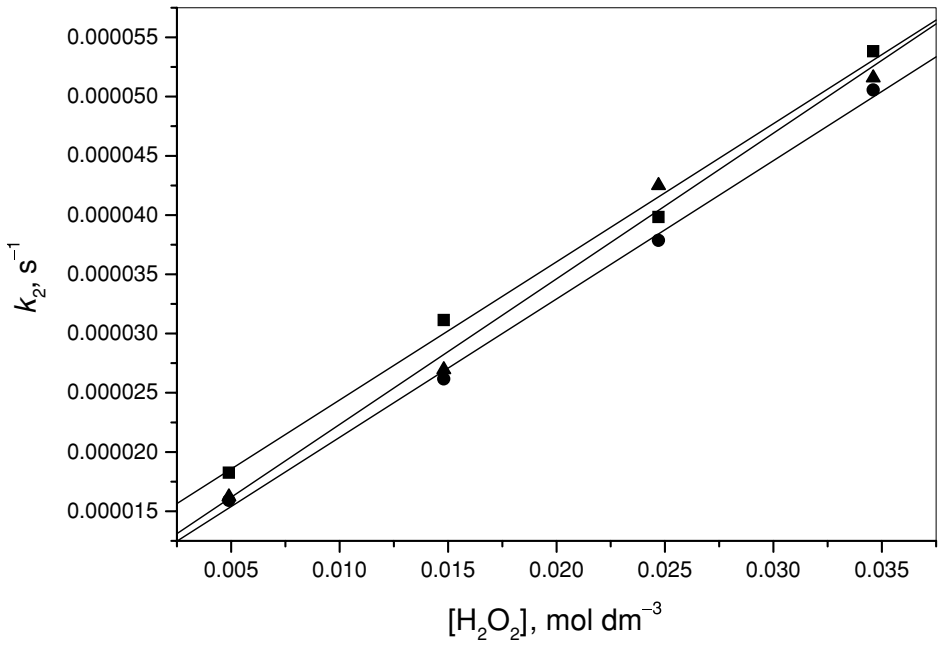


Figure 4 Plots of k_2 versus $[H_2O_2]$ for the oxidation of promazine radical with hydrogen peroxide. Experimental conditions: $[PMZ] = 5 \times 10^{-5}$ mol dm^{-3}, $T = 298$ K, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), (■) argon, (●) air, (▲) oxygen atmosphere.

Promazine undergoes the oxidation reaction with hydrogen peroxide according to reaction (1), in which the hydroxyl and promazine radicals are produced. The promazine radical as an intermediate product is transformed finally into the promazine dication (2), which hydrolyzes with the diffusion-controlled rate to promazine 5-oxide (3). The PMZO is a final product of the overall reaction with H_2O_2. It is known that the hydroxyl radical reacts unselectively with organic species, and hydroxo derivatives or other characteristic products are produced in the reaction with this radical. As is seen in the reaction scheme, the hydroxyl radical is scavenged by chloride ion (4, 5), which is transformed into the dichloro radical anion, $Cl_2^{-\bullet}$ (6). Under the conditions used in the kinetic experiments, when $[H^+, Cl^-] = 0.1$ mol dm^{-3}, $OH^\bullet$ almost never reacts with promazine. The second-order rate constant for the reaction of $OH^\bullet$ with chlorpromazine is equal to 1.5×10^{10} mol^{-1} dm^3 s^{-1} [25] and is similar to the rate constant for the reaction of $Cl_2^{-\bullet}$ with this phenothiazine derivative. Thus, the reactions of the hydroxyl radical with PMZ and $PMZ^{+\bullet}$ are suppressed and the dichloro radical anion reacts with PMZ in reaction (7). A very slow oxidation of $PMZ^{+\bullet}$ by dioxygen produces the superoxo radical (8). Preliminary experiments showed that the $HO_2^\bullet$ radical in acidic media did not oxidize PMZ under the concentration conditions used in these experiments, when the $HO_2^\bullet$ radical is present in a very low steady-state concentration. In the acidic media, the $HO_2^\bullet$ radical ($pK_{a\,HO_2} = 4.88$) [26] disproportionates and recombines with other radicals in the solution ($E^\circ = 0.152$ V (O_2/HO_2), pH 0, $p_{O_2} = 1$ atm) [26]. This suggestion was supported by spin-trapping experiments in EPR ($[DMPO] = 0.1$ mol dm^{-3}, $[H_2O_2] = 0.05$ mol dm^{-3}, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), $T = 295$ K, signal after ca. 23 min, modulation amplitude, 0.1 mT, microwave frequency, 9.256 GHz), in which a signal of a DMPO·$OOH^\bullet$ adduct was detected directly only in the solution of H_2O_2. The rate of the DMPO·$OOH^\bullet$ adduct formation is slow at neutral pH, however at acidic pH increases and then the second-order rate constant is equal to 27 mol^{-1} dm^3 s^{-1} [27,28] at pH 6.2 and 10^3 mol^{-1} dm^3 s^{-1} at pH 5.0 [27,29]. The high reactivity of $O_2^{-\bullet}$ to DMPO in acidic pH was proposed to be due to the protonation of $O_2^{-\bullet}$ to form the hydroperoxyl radical, $HO_2^\bullet$, and the fact that $HO_2^\bullet$ is a stronger oxidizer than $O_2^{-\bullet}$ ($E^\circ = 1.06$ and 0.94 V at pH 0, respectively) [30]. Although the rate of the DMPO·$OOH^\bullet$ adduct formation is not fast, this species was observed in the H_2O_2 solution in the presence of chloride ions. The rate constant of the $OH^\bullet$ radical addition to DMPO, $(2.7 - 4.3) \times 10^9$ mol^{-1} dm^3 s^{-1} [31], is on the same level of magnitude as the rate constant of the $OH^\bullet$ radical reaction with Cl^-, 4.3×10^9 mol^{-1} dm^3 s^{-1} [22]. For that reason, the concentration of DMPO was high, ca. 0.1 mol dm^{-3}, and their reaction with $OH^\bullet$ can proceed. A signal of a DMPO·$OH^\bullet$ adduct was also detected in the same solution of H_2O_2 ($[DMPO] = 0.1$ mol dm^{-3}, $[H_2O_2] = 0.05$ mol dm^{-3}, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), $T = 295$ K, signal after ca. 23 min, modulation amplitude, 0.1 mT, microwave frequency, 9.256 GHz). The adduct intensity increased during experiments (at the same time a signal of DMPO·$OOH^\bullet$ adduct completely disappeared). EPR spectrum of DMPO-H_2O_2 acidic mixture is shown in Fig. 5, where 1:2:2:1 quartet characteristic of DMPO-$OH^\bullet$ adduct is clearly seen [32]. Another weak radical 1:1:1 triplet signal with $a_N \sim 1.4$ mT is also seen. It comes probably from the further oxidation or/and degradation of DMPO-$OH^\bullet$ adduct. In the mixture of promazine, H_2O_2, and DMPO, however, a very intensive signal characteristic for the $PMZ^{+\bullet}$ radical was observed, and the signal of DMPO·$OH^\bullet$ disappeared on the timescale of the experiments ($t_{1/2} =$ 23 min) [33]. It was mentioned earlier that the loss of H_2O_2 was less than 0.4% in 0.1 mol dm^{-3} HCl and did not affect the kinetics of the oxidation reaction by H_2O_2, but the reactive oxygen species may influence the mechanism of reaction. The rate of the H_2O_2 decomposition is 1×10^{-6} mol^{-1} dm^3 s^{-1} ($[H_2O_2] =$ 0.1 mol dm^{-3}, pH 10, $T = 308$ K) [17] and is negligible on the timescale of this experiment.

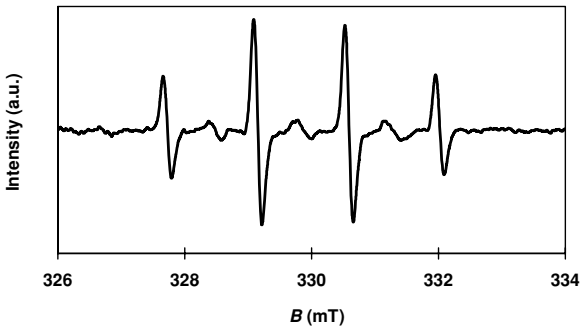


Figure 5 Experimental EPR spectrum of radicals generated via oxidation of the acidic solution of 0.1 M DMPO by 5×10^{-2} M H_2O_2 under air atmosphere. Experimental conditions: $[HCl] = 1 \times 10^{-1}$ M, $I = 0.1$ (H^+, Cl^-), $T = 295$ K, signal after ca. 23 min, modulation amplitude, 0.1 mT, microwave frequency, 9.256 GHz; other parameters as described in the Experimental section.

The cation radical of promazine is very stable in the solution [34] and very slowly disproportionates (9). The second-order rate constant for their disproportionation in 0.1 mol dm^{-3} H_2SO_4 calculated from the dependence of log k on $[H_2SO_4]$ [34] is equal to 4.55 mol^{-1} dm^3 s^{-1}. This means that the rate of this reaction, 1×10^{-10} mol dm^{-3} s^{-1}, is lower than the rate

Table II Linear Regression Data for the k_1 Dependences on $[H_2O_2]$ (Fig. 3) for the Reaction between Promazine and Hydrogen Peroxide

Atmosphere	k_1^{I} (slope) (mol^{-1} dm^3 s^{-1})	k_{-1}^{I} (intercept) (s^{-1})
Argon	$(0.96 \pm 0.08) \times 10^{-3}$	$(5.27 \pm 0.20) \times 10^{-5}$
Air	$(1.17 \pm 0.04) \times 10^{-3}$	$(5.35 \pm 0.09) \times 10^{-5}$
Oxygen	$(1.71 \pm 0.06) \times 10^{-3}$	$(5.68 \pm 0.16) \times 10^{-5}$

$T = 298$ K, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), standard errors.

of the oxidation of PMZ to $PMZ^{+\bullet}$ (1), 5.75×10^{-10} mol dm^{-3} s^{-1}, and the rate of oxidation of $PMZ^{+\bullet}$ to PMZO (2), 2.5×10^{-10} mol dm^{-3} s^{-1}, for 10% of conversion degree ($[H_2O_2] = 0.0346$ mol dm^{-3}, $[PMZ] = 5 \times 10^{-5}$ mol dm^{-3}, $[H^+] = 0.1$ mol dm^{-3}, $I = 0.1$ (H^+, Cl^-). The concentration of $PMZ^{+\bullet}$ at the time of overall reaction is not higher than 30% of total promazine concentration. Under such circumstances, the rate of the oxidation reaction of PMZ to $PMZ^{+\bullet}$ (1) and the $PMZ^{+\bullet}$ disproportionation are similar. However, including the disproportionation reaction in the scheme of two one-electron consecutive processes has not improved kinetic results.

The differential equations are too complicated to be solved. No analytical solutions have been found for the proposed reaction scheme. Moreover, if all the reactions were included, too many variables would have to be determined simultaneously in the numeric calculations.

The linear regression data for the dependences of the observed rate constants k_1 and k_2 on $[H_2O_2]$ are presented in Tables II and III. Thus, those for the first and second stages of the reaction can be expressed by the following equations:

$$k_1 = k_{-1}^{I} + k_1^{I}[H_2O_2] \quad (10)$$

$$k_2 = k_{-2}^{II} + k_2^{II}[H_2O_2] \quad (11)$$

in which the first-order (k_{-1}^{I}) and second-order rate constants (k_1^{I}) are dependent on the concentrations of reactive oxygen forms, which are transformed into $Cl_2^{-\bullet}$ radicals, according to reaction scheme (1)–(9), and the first-order (k_{-2}^{II}) and second-order rate constants (k_2^{II}) for the second stage are almost unaffected by the reactive oxygen species and oxygen atmosphere. In previously published data [12], copper(II) in the oxidation reaction of promazine by dioxygen accelerated the first stage of the redox process into the submicrosecond scale and the very slow oxidation of $PMZ^{+\bullet}$ by dioxygen was the rate-limiting step. The product of the second-order rate constant for the copper(II) halide mediated oxidation of $PMZ^{+\bullet}$ by dioxygen, calculated from the activation parameters and the O_2 concentration, $k_{O_2}[O_2] = 3 \times 10^{-5}$ s^{-1} ($I = 1.0$ mol dm^{-3}, $[O_2] = 1 \times 10^{-3}$ mol dm^{-3}, $T = 298$ K) [12] is higher than the first-order rate constant, k_{-2}^{II}, for the second stage of the reaction with H_2O_2; moreover, this last value remains unchanged in different atmospheres. This means that the very slow reaction of $PMZ^{+\bullet}$ with O_2 is ineffective as a reaction path in the spontaneous oxidation using H_2O_2.

Table III Linear Regression Data for the k_2 Dependences on $[H_2O_2]$ (Fig. 4) for the Reaction between Promazine Radical and Hydrogen Peroxide

Atmosphere	k_2^{II} (slope) (mol^{-1} dm^3 s^{-1})	k_{-2}^{II} (intercept) (s^{-1})
Argon	$(1.17 \pm 0.07) \times 10^{-3}$	$(1.27 \pm 0.16) \times 10^{-5}$
Air	$(1.17 \pm 0.04) \times 10^{-3}$	$(0.96 \pm 0.09) \times 10^{-5}$
Oxygen	$(1.23 \pm 0.08) \times 10^{-3}$	$(1.00 \pm 0.19) \times 10^{-5}$

$T = 298$ K, pH 1, $I = 0.1$ mol dm^{-3} (H^+, Cl^-), standard errors.

CONCLUSIONS

The results of the present study indicate that the degradation of promazine using hydrogen peroxide proceeds via two consecutive reactions, which occur with very similar rates and appear to be influenced by reactive oxygen species, i.e. the $OH^\bullet$ radicals, which are transformed into the $Cl_2^{-\bullet}$ radical anions. However, the direct proof of its participation in the reaction was not obtained. Under physiological condition, the redox potential for $H_2O_2/OH^\bullet$ species, 0.38 V (pH 7), protects many organic compounds against oxidation by H_2O_2 [13]. In acidic solution, this redox potential is equal to 0.8 V (pH 0) [13] and enables production of the $OH^\bullet$ radical in the reaction. The very high stability of the promazine radical and *N*-substituted phenothiazine radicals evoked the very characteristic reaction step involving the oxidative degradation of the promazine, PMZ → $PMZ^{+\bullet}$ → PMZO, as well as the other *N*-substituted phenothiazine drugs and the kinetics of this process.

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