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ARTICLES

Photometric and Gas-Chromatographic Determination of Hydrogen Peroxide and Peroxybutanoic Acid in Oxidized Butanoic Acid

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Abstract—Procedures were developed for determining hydrogen peroxide and peroxy acids mixed with peroxide compounds of other classes in the oxidation products of butanoic acid with atmospheric oxygen and hydrogen peroxide. Conditions were found for the selective decomposition of hydrogen peroxide with catalase in the presence of an excess of the carboxylic acid deactivating the enzyme. The errors introduced by the acylation of hydrogen peroxide with the carboxylic acid in the course of sample treatment with the enzyme were eliminated by adding diphenyl sulfide or dimethyl sulfoxide, which selectively reduced the peroxy acids. The concentrations of hydrogen peroxide and the peroxy acid were found from the difference between the total concentration of the peroxide compounds before and after treating a sample with catalase and a sulfur-containing reagent by the photometric method using a reagent containing Fe^{2+} ions and *N*,*N*-dimethyl-*p*-phenylenediamine. Peroxy acids were determined by GLC from the yields of the oxidation products of diphenyl sulfide with the peroxy acid (diphenyl sulfoxide and diphenyl sulfones).

Hydrogen peroxide and (or) peroxy acids are contained in the products of the liquid-phase oxidation of many organic compounds [1–4]. In addition, these peroxide compounds enter into reaction mixtures formed in the acylation of H_2O_2 with carboxylic acids

$$H_2O_2 + RCOOH \rightleftharpoons RCOOOH + H_2O. \quad (1)$$

These peroxy acids are obtained *in situ* by reaction (1) and are later used as oxidizing agents in organic synthesis, including the Baeyer–Villiger and Prilezhaev reactions [5].

To determine H_2O_2 and peroxy acids in mixtures with peroxide compounds of other classes, methods are mainly used that are based on the ability of enzyme catalase or sulfur-containing compounds (diphenyl sulfide, dimethyl sulfoxide, etc.) to selectively decompose H_2O_2 [2, 4, 6–9] or peroxy acids [2, 4, 10–13], respectively. The observed decrease in the total concentration of peroxide compounds is detected by conventional methods, most often, by iodometry [2, 10]. It was proposed that peroxy acids be determined from the yield of a sulfur-containing reagent, sulfoxide and (or) sulfone [11–13].

H_2O_2 and peroxy acids are difficult to determine quantitatively by available methods in organic compounds oxidized by molecular oxygen or by a mixture of H_2O_2 and carboxylic acid, because the composition of the products of these reactions is rather complex. It is known that peroxide and oxygen-containing compounds can distort the results of analytical determinations of the products of liquid-phase oxidation [4]. In particular, it might be assumed that carboxylic acids can affect the results of determining H_2O_2 and peroxy acids because of the occurrence of reaction (1) and the deactivation of catalase in an acidic medium. At the same time, data on the concentrations of H_2O_2 and peroxy acids are required both to control the above processes and to study the kinetics and mechanisms of reactions involved in these processes.

The goal of this work is to develop a procedure for determining H_2O_2 and peroxy acids in oxidized butyric acid.

EXPERIMENTAL

Butanoic acid of reagent grade was purified by rectification in an argon flow. Chemically pure glacial acetic acid and chlorobenzene of reagent grade were freshly distilled. An aqueous hydrogen peroxide solution (31.2 ± 0.1%; RSD = 0.5% at $n = 8$ and $P = 0.95$), *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride of analytical grade, chemically pure ammonium iron(II) sulfate (Mohr's salt), chloroform of pharmaceutical purity, BF_3 etherate of reagent grade, and catalase from the Kamenskii Distillery (Ukraine) were used without additional purification. The molecular mass of catalase was taken equal to 250 000 [2]. The method for obtaining peroxydodecanoic acid is reported in [12]. We used a preparation that contained 99.3 ± 0.3% of the peroxy acid.

The sought parameters of kinetic equations were calculated by the least-squares method using a program written in Delphi 5.5. The set of differential equations

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was solved at each step by the fourth-order Runge–Kutta method.

Samples for analysis were prepared by the following two procedures:

(1) by the addition of a 31.2% hydrogen peroxide solution to butanoic acid until the concentration of H_2O_2 attained 0.02–0.03 M;

(2) by the cumyl peroxide–initiated oxidation of butanoic acid with oxygen at 90°C with an initiation rate of 3.89×10^{-8} mol L^{-1} s^{-1}.

Procedure for determining the total concentration of hydrogen peroxide and peroxybutanoic acid in butanoic acid. The solution of the reagent for determining peroxy compounds was prepared by mixing 0.1 g of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, 9 mL of a 0.02% aqueous solution of Mohr's salt, 45 mL of glacial acetic acid, and 45 mL of chloroform [14]. The reagent was prepared before use, but it can be stored in a dark vessel for 1–2 weeks. Two aliquot portions (~30 μL) of a test sample containing $(2–10) \times 10^{-7}$ mole of peroxide compounds were introduced into two 10-mL volumetric flasks. A 0.5-mL portion of a 0.005 M diphenyl sulfide (or dimethyl sulfoxide) solution in chlorobenzene and 0.5 mL of 5×10^{-6} M aqueous catalase solution were added to the first flask, and the contents were shaken and held at 20°C for 15 min. The contents of the first flask were diluted to the mark with the reagent containing *N,N*-dimethyl-*p*-phenylenediamine (the preparation procedure is given above). The absorbance of the solution was measured in 20 min at 517 nm using a Specol-21 instrument. The concentration of peroxide compounds not decomposed by catalase and diphenyl sulfide (c_{CA}) was calculated using the known molar absorption coefficient ($\varepsilon = 1.9 \times 10^4$) [14]. A 0.5-mL portion of chlorobenzene and 0.5 mL of distilled water were added to the contents of the second flask, and the mixture was diluted to the mark with the reagent solution. The total concentration of peroxide compounds (c_{total}) was determined in 20 min as described above. The total concentration of hydrogen peroxide and peroxy acids was calculated from the difference between c_{CA} and c_{total}.

Procedure for the selective determination of peroxy acids. A solution of diphenyl sulfide, 1 mL of 10-nonadecanone (internal standard) in chlorobenzene, and 1 mL of a 5×10^{-6} M catalase aqueous solution were quickly added to a 1-mL portion of the test solution immediately after sampling. The reaction mixture was shaken and allowed to stand for 20 min. The aqueous layer was separated and discarded, and the organic layer was dried with anhydrous $MgSO_4$. When test samples contained hydroxy peroxides, the products of the oxidation of butanoic acid by atmospheric air, a solution of triphenylphosphine in benzene (50% molar excess) was added and diphenyl sulfoxide and diphenyl sulfone were determined by GLC. In the determination of low concentrations of the peroxy acid in butanoic acid, a sample, after treating with catalase and triphenylphosphine, was treated with 1 mL of a 10% methanolic BF_3-etherate solution and held for 2 h at room temperature to additionally concentrate it. Excess methanol was washed out with water, and the organic layer was dried with anhydrous $MgSO_4$ and concentrated under water aspirator vacuum. GLC determinations were carried out using an LKhM-8MD chromatograph with a flame-ionization detector. Argon was used as the carrier gas, a stainless-steel column (1000 × 3 mm) was packed with 5% OV-17 silicone on N-super Chromaton; the instrument operated in the isothermal mode (220°C).

RESULTS AND DISCUSSION

The concentration of H_2O_2 in the reaction mixture can be calculated from the results of two determinations of the concentrations of peroxide compounds. The first determination is that of the total concentration of peroxide compounds in the test sample, and the second determination is the determination of the remainder of peroxides after the selective decomposition of H_2O_2 by catalase.

Selection of the conditions for hydrogen peroxide decomposition in butanoic acid. A special feature of the latter determination of the peroxide content in butanoic acid is that H_2O_2 can interact with carboxylic acid under the conditions of sample storage and catalase treatment (reaction (1)). This must lead to underestimated results. Moreover, it is necessary to optimize the conditions for H_2O_2 decomposition by catalase in the presence of butanoic acid, which, as might be assumed, can deactivate the enzyme.

The results of determining H_2O_2 in aqueous solutions of known concentrations (Table 1) showed that H_2O_2 was completely decomposed in 10 min when treated with a 2.0×10^{-6} M catalase solution. At the same time, the results of determining H_2O_2 in butanoic acid amounted to only ~35% of the results obtained in water under the same conditions (Table 1). The nature of this phenomenon is little understood. Evidently, butanoic acid either blocks active sites of catalase via the formation of hydrogen bonds or irreversibly deactivates the enzyme. It might be assumed that the dilution of butanoic acid with inert solvents (heptane, chlorobenzene) should enhance the enzyme activity, as has been observed in the determination of H_2O_2 in cyclohexanol [8].

Indeed, a study of the effect of chlorobenzene and *n*-heptane additives on the kinetics of hydrogen peroxide decomposition in butanoic acid (Fig. 1) demonstrated that the addition of heptane and especially chlorobenzene to the reaction mixture resulted in a significant acceleration of the decomposition of H_2O_2. The shape of the kinetic curve of H_2O_2 decomposition by catalase in the presence of chlorobenzene (Fig. 1)

Table 1. Effect of the conditions for treating solutions of H_2O_2 in water and in butanoic acid with catalase on the results of determining the concentrations of peroxide compounds ($n = 8$, $P = 0.95$)

Solvent H_2O_2	Taken		Catalase concentration $\times 10^6$, M	Time of the enzyme treatment, min	Found	
	$(\bar{c} \pm \delta) \times 10^3$, M	RSD, %			$(\bar{c} \pm \delta) \times 10^3$, M	RSD, %
Water	7.66 ± 0.06	1	2	10	7.63 ± 0.13	2
	7.66 ± 0.06	1	5	10	7.67 ± 0.13	2
$CH_3(CH_2)_2COOH$	19.3 ± 0.16	1	5	15	1.74 ± 0.10	7
	19.3 ± 0.16	1	5	5 + 10*	1.62 ± 0.08	6

* In 5 min after the beginning of the treatment of a sample with catalase, chlorobenzene was added to it.

Table 2. Effect of the nature of inert solvent and sulfur-containing reagent on the results of determining hydrogen peroxide in its solutions in butanoic, propanoic, and acetic acids (time of catalase treatment, 15 min, $n = 8$, $P = 0.95$)

Solvent H_2O_2	Taken		Sulfur-containing reagent	Inert solvent	Found	
	$(\bar{c} \pm \delta) \times 10^3$, M	RSD, %			$(\bar{c} \pm \delta) \times 10^3$, M	RSD, %
$CH_3(CH_2)_2COOH$	18.8 ± 0.16	1	–	C_6H_5Cl	17.9 ± 0.59	4
	17.1 ± 0.14	1	$(C_6H_5)_2S$	C_6H_5Cl	17.0 ± 0.43	3
	12.3 ± 0.10	1		C_6H_5Cl	12.4 ± 0.41	4
	19.7 ± 0.16	1	$(CH_3)_2SO$	C_6H_5Cl	19.5 ± 0.65	4
CH_3CH_2COOH	15.5 ± 0.13	1		C_6H_5Cl	5.35 ± 0.22	5
CH_3COOH	14.2 ± 0.12	1		C_6H_5Cl	2.7 ± 0.13	5

showed that the major part of H_2O_2 was decomposed as soon as in 10 min, and then a slow decrease in the content of peroxide compounds was observed. The anamorphosis of this portion of the kinetic curve on the $\ln c$–τ coordinates was linear (Fig. 1), which points to the pseudo-first kinetic order of the decomposition of peroxide compounds. It may be suggested that the unusual shape of the curve is due to the fact that, along with H_2O_2, the reaction mixture contains the peroxy acid that slowly dissociates into H_2O_2 and carboxylic acid according to the reverse of reaction (1). Such an assumption was confirmed by the experiments on introducing sulfur-containing compounds (diphenyl sulfide or dimethyl sulfoxide) together with the enzyme into the reaction mixture. In this case, peroxide compounds were completely decomposed as soon as in 15 min, and H_2O_2 was determined quantitatively (Table 2). It is likely that butanoic acid irreversibly deactivated the enzyme, because the addition of chlorobenzene to the reaction mixture in 5 min after mixing the solution of H_2O_2 in butanoic acid with an aqueous solution of the enzyme did not enhance the degree of the decomposition of H_2O_2 with catalase (Table 2). Therefore, it may be assumed that an increase in the rate of H_2O_2 decomposition in the presence of chlorobenzene and *n*-heptane is due to the extraction of the acid with these solvents from the aqueous phase in which the decomposition of H_2O_2 occurred. The results of determining H_2O_2 in solutions of propanoic and acetic acids (Table 2) support this assumption. It is seen that the amount of determined H_2O_2 decreased in the order butanoic acid > propanoic acid > acetic acid. Such an order corresponds to the order in which the water solubility of these acids increases.

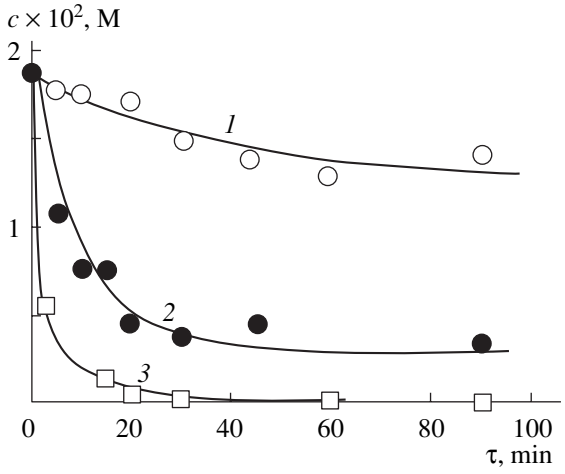


Fig. 1. Kinetic curves of H_2O_2 decomposition by a 5.0×10^{-6} M catalase aqueous solution (*1*) containing no inert solvent additive and (*2*) containing *n*-heptane and (*3*) chlorobenzene additives.

Choice of conditions for determining peroxy butanoic acid. To evaluate the errors introduced in the

determination of H_2O_2 by reaction (1), we examined the kinetics of peroxy acid accumulation on addition of H_2O_2 to butanoic acid at 20°C (Fig. 2).

The kinetics of reaction (1) can be written as follows:

$$\frac{dc_{PA}}{dt} = k_{+1}c_{CA}c_{HP} - k_{-1}c_{PA}c_{w}, \qquad (2)$$

where k_{+1} and k_{-1} are the rate constants of the forward and back reactions (1) and c_{CA}, c_{HP}, c_w, and c_{PA} are the current concentrations of carboxylic acid, H_2O_2, water, and peroxy acid, respectively. In our calculations, we set the following initial concentrations of the reagents: $c^0_{CA} = 10.87$ M; $c^0_{HP} = 0.0116$ M; $c^0_{PA} = 0.00$; and $c^0_w = 0.0388$ M. The calculated values of k_{+1} and k_{-1} (at $n = 8$, $P = 0.95$) equaled $(0.0051 \pm 0.0004) \times 10^{-4}$ (RSD = 9.6%) and $(25.5 \pm 3.1) \times 10^{-4}$ L mol^{-1} s^{-1} (RSD = 14%), respectively. Hence, the equilibrium constant of reaction (1) was $(2.0 \pm 0.4) \times 10^{-4}$ (RSD = 24%).

Thus, butanoic acid interacted with H_2O_2 by reaction (1) at a substantial rate already at 20°C. The magnitude and direction of the distortions introduced by this reaction into the results of H_2O_2 determination depend on the concentration of all products and on the closeness of the system to the equilibrium state. Therefore, it is advisable to treat samples immediately after sampling to determine the true concentrations of the peroxy acid and H_2O_2 in butanoic acid. The simultaneous treatment of samples with diphenyl sulfide and catalase made it possible to determine the total concentration of the peroxy acid and H_2O_2 from a decrease in the concentration of peroxide compounds and to determine the concentration of the peroxy acid and then the concentration of H_2O_2 from the yield of diphenyl sulfoxide and diphenyl sulfone.

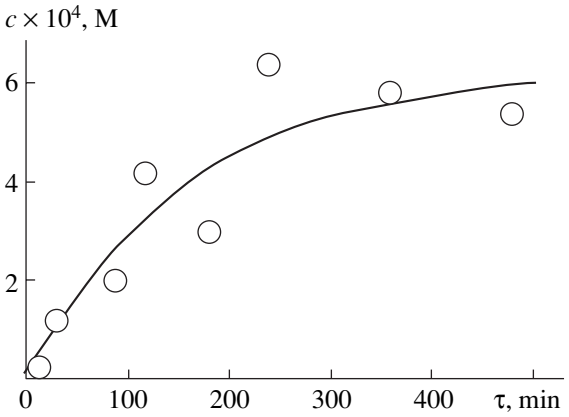


Fig. 2. Kinetic curve of peroxybutanoic acid accumulation in the reaction of 10.87 M of butanoic acid with a 0.01155 M H_2O_2 solution at 20°C.

The results of determining peroxydodecanoic acid, which is taken as a model peroxy acid, in its butanoic acid solutions of known concentrations are presented in Table 3 and indicative of the accuracy of the used procedure.

Determination of hydrogen peroxide and peroxybutanoic acid. The concentrations of H_2O_2 and peroxybutanoic acid were determined in butanoic acid obtained by initiated oxidation at 90°C by the developed procedures (Table 4). It is seen that the results of determining peroxybutanoic acid in butanoic acid obtained by initiated oxidation are independent of the degree of oxidation and higher than we might expect based on the current concentrations of butanoic acid, water, and H_2O_2 and on the equilibrium constant for reaction (1) obtained above. The overestimated results of determining the peroxy acid are due to the distorting effect of the initiator of the oxidation reaction, cumyl peroxide. It was found that the partial interaction of the

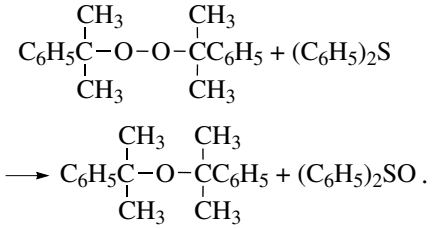
Table 3. Determination of peroxydodecanoic acid in its solution in butanoic acid ($n = 8$, $P = 0.95$)

Taken $(\bar{c} \pm \delta) \times 10^4$, M	Found	
	$(\bar{c} \pm \delta) \times 10^4$, M	RSD, %
0.270 ± 0.001	0.268 ± 0.02	9
1.512 ± 0.001	1.497 ± 0.07	6
5.641 ± 0.005	5.851 ± 0.24	5

Table 4. Determination of consumed oxygen (c_{oxygen}), the total concentration of hydroxyperoxides (c_{hp}), hydrogen peroxide (c_{HP}), and peroxybutanoic acid (c_{PA}) in oxidized butanoic acid ($n = 8$, $P = 0.95$)

Reaction time, min	$(\bar{c}_{oxygen} \pm \delta) \times 10^3$, M	RSD, %	$(\bar{c}_{hp} \pm \delta) \times 10^4$, M	RSD, %	$(\bar{c}_{HP} \pm \delta) \times 10^4$, M	RSD, %	$(\bar{c}_{PA} \pm \delta) \times 10^4$, M	RSD, %
150	1.24 ± 0.04	4	3.35 ± 0.11	4	1.50 ± 0.02	5	0.45 ± 0.02	5
210	2.24 ± 0.07	4	4.08 ± 0.17	5	1.86 ± 0.09	6	0.81 ± 0.03	5

latter with diphenyl sulfide in the course of GLC determination resulted in diphenyl sulfone:



(3)

Unfortunately, we failed to eliminate this interfering effect. At the same time, the approach implemented in this work can also be used in determining H_2O_2 and peroxy acids in the autooxidation products of carbonyl-containing compounds and in the reaction products of H_2O_2 with the corresponding acids.

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