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Reactions between Hydroperoxides and Fe^{2+} to investigate Redox Processes in Biological Objects: A Kinetics Study.

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ABSTRACT

The kinetics of chemiluminescence induced by the Fenton reaction in organic compounds and chemiluminescence induced in organic hydroperoxides by divalent iron was calculated. The base of the simulation is 34 reactions scheme, including primary interactions of divalent iron with hydrogen peroxide (HOOH) and organic hydroperoxide (ROOH). The system of chemical kinetics equations was numerically solved by means of the program MathCad 14. The chemiluminescence light sum was investigated in terms of the concentrations of organic matter [RH], inhibitor [InH] and [ROOH]. The calculation results allow us to conclude that the variations in the chemiluminescence light sums induced in each sample by the Fenton reaction and with only divalent iron permit us to determine the susceptibility of a substratum to peroxidation by hydroxyl radicals and to evaluate the rate of free-radical processes in which the sample is involved.

Keywords: chemiluminescence, free-radical reactions, light sum, organic hydroperoxide, singlet oxygen

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INTRODUCTION

The Fenton reaction has been known for more than 100 years. In the Fenton reaction, HOOH interacts with divalent iron, forming a strong oxidant, that is, a hydroxyl radical $OH^{\bullet}$ [1]. The reaction is accompanied with radiation, which can detect by means of modern biochemiluminometers. Similar reactions of divalent iron can occur with ROOH. In this case, another kind of radical is generated, that is, $RO^{\bullet}$, which essentially has a reduced redox potential. Reactions of divalent iron with organic hydroperoxides are also accompanied with chemiluminescence. The reverse reaction, named after Haber–Weiss, is also known.

The mechanisms of these reactions are described by means of a small number of equations, which allow us to reproduce the main peculiarities of the processes. The theory of chemiluminescence in the Fenton reaction is complicated. To describe chemiluminescence quantitatively, a scheme including 20 reactions has been developed [2]. It was shown that the main luminous agent is singlet oxygen. Molecules of singlet oxygen form a dimer, the luminescence of which is registered by a photomultiplyer (PM) [2].

Peroxidation has great importance in biochemical processes. Peroxidation induced by the Fenton reaction is accompanied by chemiluminescence, but luminous product generation is only one of the peroxidation channels [3]. The hydroperoxides remain undetected if it is only chemiluminescence arising in the course of the Fenton reaction that is registered.

The Fenton reaction is used in biomedical investigations [4, 5]; chemiluminescence in Fenton reaction is widely used for chemical analysis [6, 7, 8]. Therefore, it is interesting, as the reaction between divalent iron and hydroperoxides could determine how the reaction characteristics allow us to evaluate the peroxidation ability of a sample and the rate of free-radical reactions with an object on the basis of sample analysis. The aim of this work was to investigate the peculiar reaction of divalent iron with hydrogen peroxide and organic hydroperoxide, which is accompanied by chemiluminescence, by means of kinetic calculations. Calculations (numerical solutions of differential equations system) were performed for a large number of reactions (more than 30). An analytical solution in this case was only possible under very strong simplified assumptions and not allow us to identify the general importance of the results.

MATERIALS AND METHODS

Kinetic model of the process

Fenton reaction

The Fenton reaction scheme is presented in table 1 (reactions 1–25). Reaction rate constants and the main peculiarities of the Fenton reaction were analyzed in previous work [2]. The initiation of the process is shown in reaction 1 (table 1), in which the hydroxyl radical is generated. Hydroxyl radicals can terminate in interactions with each other (reaction 5) and initiate a series of further transformations. The equilibriums are: $HO_2^{\bullet} \leftrightarrow H^+ + O_2^{\bullet-}$ ($pK_a = 4.8$) and $H_2O_2 \leftrightarrow HO_2^- + H^+$ ($pK_a = 11.5$). Hydrocarbonates, which accumulate in water during carbon dioxide dissolution from air, strongly affect the balance of active particles (reactions 18–20). Many intermediate products of the Fenton reaction are produced in exited states, which decay with UV radiation. But, this radiation is not detected with a biochemiluminometer, as its wavelength is out of the spectral range of the PM sensitivity. The luminous agent produced in the Fenton reaction is singlet oxygen [2]. The main radiation line of singlet oxygen ($\lambda = 1260$ nm) is also outside of the spectral sensitivity. However, the radiation of a singlet oxygen dimer, which is produced in reaction 24, can be detected. The transitions between the ground states of the dimer and an oxygen molecule give the line at 630 nm, whereas transitions between exited states give lines at 480, 535 and 580 nm. The production probability of this radiation is very small (reaction 24); singlet oxygen spontaneously decays (reaction 23) and molecules of singlet oxygen are deactivated with the formation of neutral products (reaction 25). But, radiation of the dimer is detected by modern biochemiluminometers, having a sensitivity of approximately 200 photons per second [2].

The main source of background radiation is the ionized radiation in the room. Under external radiation, the same active species are produced in the sample (water) as in the Fenton reaction. The yields of these species are given in table 2 [9]. The primary active species produced under ionized radiation are transform in reactions with oxygen: $e^-_{aq} + O_2 \rightarrow O_2^{\bullet-}$, $H^{\bullet} + O_2 \rightarrow HO_2^{\bullet}$. A series of 12 differential equations,

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which include the creation and termination of active species in the Fenton reaction, was used to calculate the reaction kinetics. The active species are Fe^{2+}, Fe^{3+}, H_2O_2, HO_2^-, O_2, $HO_2^{\bullet}$, $OH^{\bullet}$, $O_2^{\bullet-}$, $O_2(a^1\Delta_g)$ (singlet oxygen), $2O_2(a^1\Delta_g)$ (dimer of singlet oxygen), HCO_3^- and $CO_3^{\bullet-}$. The yields of active species produced under ionized radiation (table 2) were added to the yields of the same species produced in reactions 1–25. For example, we write one equation (1) below, which includes rates for the generation and termination of hydroxyl radicals.

$$\frac{d[OH^{\bullet}]}{dt} = Y_b(OH^{\bullet}) + k_1[Fe^{2+}][H_2O_2] - k_2[OH^{\bullet}][H_2O_2] - k_4[OH^{*}][Fe^{2+}] - 2k_5[OH^{\bullet}]^2 - k_6[OH^{\bullet}][HO_2^{\bullet}] - k_{14}[OH^{\bullet}][HO_2^{-}] + k_{17}[H_2O_2][O_2^{\bullet-}] - k_{18}[OH^{\bullet}][HCO_3^{-}] - k_{19}[OH^{\bullet}][CO_3^{-}] - k_{21}[OH^{\bullet}][O_2^{\bullet-}] \quad (1)$$

Here, $Y_b(OH^{\bullet})$ is the yield of hydroxyl radicals under ionized radiation. To solve the system of equations, a MathCad 14 set was used. The solution was time dependence for every species concentration investigated. The initial conditions for every variabe were species concentration value before mixing all of the reagents. The rate constants for all reactions are well known; therefore, the reliability of the results was determined by the completeness of the proposed reactions scheme.

Table 1: Scheme of Fenton reaction.

No	Reaction	k, l/(mol·s), [2]
1.	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$	$k_1 = 56$
2.	$OH^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O$	$k_2 = 3 \times 10^7$
3.	$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 + O_2(a^1\Delta_g)$	$k_3 = 8.3 \times 10^5$
4.	$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^-$	$k_4 = 3 \times 10^8$
5.	$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O + ½(O_2 + O_2(a^1\Delta_g))$	$k_5 = 5.5 \times 10^9$
6.	$OH^{\bullet} + HO_2^{\bullet} \rightarrow H_2O + O_2 + O_2(a^1\Delta_g)$	$k_6 = 7.1 \times 10^9$
7.	$HO_2^{\bullet} \rightarrow H^+ + O_2^{\bullet-}$	$k_7 = 7.5 \times 10^6$ $pK_a = 4.8$
8.	$H^+ + O_2^{\bullet-} \rightarrow HO_2^{\bullet}$	$k_8 = 5.1 \times 10^{10}$
9.	$HO_2^{\bullet} + O_2^{\bullet-} \rightarrow HO_2^- + O_2$	$k_9 = 9.7 \times 10^7$
10.	$HO_2^{\bullet} + OH^- \rightarrow O_2^{\bullet-} + H_2O$	$k_{10} = 10^{10}$
11.	$O_2^{\bullet-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$	$k_{11} = 1.9 \times 10^9$
12.	$H_2O_2 \rightarrow HO_2^- + H^+$	$k_{12} = 2 \times 10^{-2}$
13.	$HO_2^- + H^+ \rightarrow H_2O_2$	$k_{13} = 10^{10}$ $pK_a = 11.5$
14.	$OH^{\bullet} + HO_2^- \rightarrow HO_2^{\bullet} + OH^-$	$k_{14} = 7.5 \times 10^9$
15.	$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3$	$k_{15} = 10^6$, pH = 12
16.	$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$	$k_{16} = 10^6$, pH = 12
17.	$H_2O_2 + O_2^{\bullet-} \rightarrow OH^- + OH^{\bullet} + O_2$	$k_{17} = 16$
18.	$HCO_3^- + OH^{\bullet} \rightarrow CO_3^{\bullet-} + H_2O$	$k_{18} = 4 \times 10^7$
19.	$CO_3^{\bullet-} + OH^{\bullet} \rightarrow CO_2 + HO_2^-$	$k_{19} = 3 \times 10^9$
20.	$CO_3^{\bullet-} + H_2O_2 \rightarrow HCO_3^- + HO_2^{\bullet}$	$k_{20} = 8 \times 10^5$
21.	$O_2^{\bullet-} + OH^{\bullet} + H^+ \rightarrow H_2O + O_2(a^1\Delta_g)$	$k_{21} = 10^{10}$
22.	$O_2^{\bullet-} + H^+ \rightarrow ½\ H_2O_2 + ½\ O_2(a^1\Delta_g)$	$k_{22} = 10^{10}$
23.	Decay $O_2(a^1\Delta_g)$	$\tau_{1/2} = 2.9 \times 10^{-4}$ c
24.	$O_2(a^1\Delta_g) + O_2(a^1\Delta_g) \rightarrow 2O_2 + \gamma$	$k_{24} = 0.1$
25.	$O_2(a^1\Delta_g) + O_2(a^1\Delta_g) \rightarrow$ products	$k_{25} = 10^{11}$

Table 2: The yield of the primary products of radiolysis in pure water at pH = 7 under radiation background 0.12 μSv/h [9].

Primary product	Radiation yield, 1/100 eV	Converted into radical	Yield, $mol(l\ s)^{-1}$
e^-_{aq}	2.8	$O_2^{\bullet-}$	$9.5\ 10^{-18}$
$H^{\bullet}$	0.5	$HO_2^{\bullet}$	$1.7\ 10^{-18}$
$OH^{\bullet}$	2.8	-	$9.5\ 10^{-18}$
H_2O_2	0.7	-	$2.4\ 10^{-18}$
H_2	0.45	-	$1.53\ 10^{-18}$

The choice of conditions for the measurement of chemiluminescence in the Fenton reaction was discussed in a previous report [2]. The reagent concentrations were taken as $[Fe^{2+}] = [H_2O_2] = 10^{-3}$ M. Therefore, all calculation were performed for this concentration of reagents. The radiation in Ref. [2] was detected by means of a BHL 07 chemiluminometer (Nizhny Novgorod, Russia), which allows the detection of radiation for 30 sec; accordingly, we calculated the chemiluminescence yield (light sum) for a time period of 30 sec. During 30 sec, 80% of divalent iron is consumed in the Fenton reaction [2]. During 90 sec, 99.9% of divalent iron is consumed, the iron is fully consumed. The trivalent iron product precipitates as ferric hydroxide $Fe(OH)_3$ and does not participate in further transformations.

Reactions with organic compounds and hydroperoxides

A scheme of the reactions arising after mixing the reactive Fenton species with a sample containing organic compound RH, inhibitor InH, hydroperoxide and, for the control reactions, only divalent iron with organic hydroperoxide ROOH is presented in table 3. The values of the reaction constants were analysed in Ref. [3, 10, 11]. Let us consider the process that is shown in scheme of table 3. Hydroxyl radicals in reaction 26 initiate the chain oxidation of organic matter RH. Chain propagation is depicted in reactions 27–29. ROOH is produced in reaction 28, whereas inert substance ROOR and singlet oxygen are produced in reaction 29. If we deal with very complex substances, for example, proteins, [RH] is the concentration of hydrocarbon fragments, which can independently to oxidize.

Table 3: Chain oxidation of organic RH and hydroperoxide ROOH with inhibitor InH.

No	Reaction	k, [3, 10, 11] l/(mol·s)
26.	$RH + OH^{\bullet} \rightarrow H_2O + R^{\bullet}$	$k_{26} = 10^8$
27.	$R^{\bullet} + O_2 + M \rightarrow ROO^{\bullet} + M$	$k_{27} = 10^4$
28.	$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$	$k_{28} = 50$
29.	$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2(a^1\Delta_g)$	$k_{29} = 10^7$
30.	$InH + OH^{\bullet} \rightarrow H_2O + In^{\bullet}$	$k_{30} = 10^9$
31.	$InH + ROO^{\bullet} \rightarrow ROOH + In^{\bullet}$	$k_{31} = 10^8$
32.	$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + OH^{-} + Fe^{3+}$	$k_{32} = 100$
33.	$RO^{\bullet} + RH \rightarrow R^{\bullet} + ROH$	$k_{33} = 50$
34.	$RO^{\bullet} + RO^{\bullet} + M \rightarrow ROOR + M$	$k_{34} = 10^6$

Chemiluminescence, caused by reaction 29 (radiation of singlet oxygen dimer), arizes after introducing the probe divalent iron and hydrogen peroxide (the reactive Fenton species). A biochemiluminometer records the reaction light sum S (value, which is proportional full number of pulses, detected during 30 s). The background for the Fenton reaction is light sum of the empty probe *S*0, which is obtained without introducing the tested sample. The ratio of S/S0 (relative light sum) is used for the analysis of the Fenton reaction results to exclude the instability of the BHL 07 device. Fenton reaction initiate peroxidation; therefore, a chemiluminescence-accompanied Fenton reaction is related with the ability of a substratum to undergo peroxidation with hydroxyl radicals, but only with the generation of radiant products.

Another product of the peroxidation of RH fragments with hydroxyl radicals is hydroperoxide (ROOH). Chemiluminescence, which is proportional to the hydroperoxide concentration, arizes after introducing divalent iron to the sample. The sum of chemiluminescence in the Fenton reaction and chemiluminescence after introducing divalent iron into the sample after the Fenton reaction should be proportional to the full oxidative ability of a sample.

Organic hydroperoxide interacts with divalent iron according to reaction 32 (table 3). The chain process (reactions 33, 27–29) with chemiluminescence can develop if organic fragments (RH) contain the probe. In the absence of organic fragments (RH) in the probe, the radicals $RO^{\bullet}$ formed in reaction 32 will terminate in reaction 34, in which the inert species ROOR is formed and chemiluminescence does not arize. New hydroperoxide is created in reaction 28, but its concentration is essentially less than the initial ROOH concentration; therefore, the new hydroperoxyde will not affect the chemiluminescence. So, the light sum, which is proportional to the hydroperoxide concentration in a sample, can be detected after introducing a divalent iron solution to the hydroperoxide sample. The background is the light sum (S_b) without divalent iron,

which is caused by ionized radiation in the room. The mechanism of luminous species production is the same in both the Fenton reaction and after divalent-iron mixing. The mechanism is determined by reactions 27 and 29.

In previous studies that are detailed in Refs. [2, 3], it was stated that the yield of chemiluminescence in the Fenton reaction reaches a maximal value at a specific sample concentration. It is attributed to the competition of chain propagation reactions 28 and 29. For a high concentration of RH, reaction 28 dominates, in which only hydroperoxide is produced. In this case, the luminescence is depressed and can stay fully out. When the concentration of RH decreases, the role of reaction 29, in which inert product ROOR and luminous species singlet oxygen are produced, will become appreciable. After maximum of chemluminescence was reached, the light sum will diminish with decreasing of [RH]. The same takes place for the reaction of divalent iron with organic hydroperoxide. Therefore, in Ref. [2, 10], it was proposed to measure the light sum for ten successive dilutions of an initial sample, starting from the maximal initial concentration. In the present work, the results of the calculations for sample concentrations of 10^0, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} received relative initial values (dilutions of 0, –1, –2, –3 and –4).

RESULTS

The role of hydroperoxide and an inhibitor in the Fenton reaction

The calculated light sum (*S*/*S*0) in the Fenton reaction, depending on the sample dilution, is shown in figure 1. The initial concentration of hypothetical compound RH is 100 M. The initial concentration [RH] = 100 M was taken on the basis that the molar concentration for organic matter could not be higher. In practice, for biological samples with molecules that have a molecular mass about 60 kDa, the concentration of molecules itself is essentially less. But, every molecule has many fragments of RH, which are susceptable to peroxidation, and the concentration may exceed 10 M. From figure 1, it is seen that, for more concentrated solutions, the maximum chemiluminescence is achieved for stronger dilutions, but the maximum values of the light sum *S*/*S*0 (if the maximum is reached) do not depend on the dilution and remain invariable.

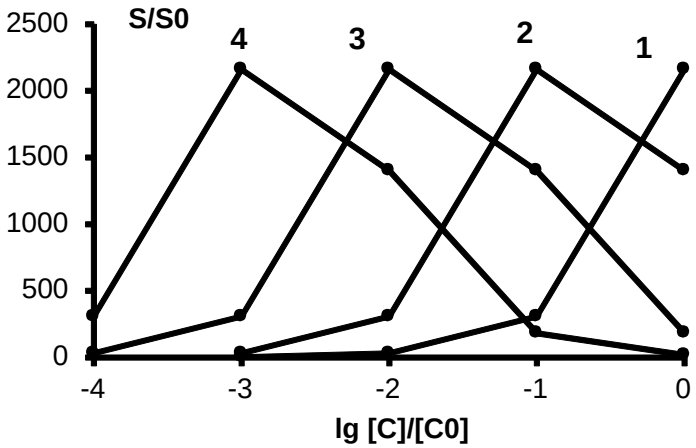

Figure 1: Light sum in the Fenton reaction (*S*/*S*0) depending on the dilution of the sample lg [*C*]/[*C*0], where *C* is the sample concentration after dilution, *C*0 is the initial sample concentration. 1 – [RH] = 0.1 M; 2 – [RH] = 1 M; 3 – [RH] = 10 M and 4 – [RH] = 100 M.

The calculated light sums in the Fenton reaction (*S*/*S*0) depend on the dilution (dilutions 0, –1, –2 and –3) for a hypothetic organic substance [RH] = 1 M and different concentrations of inhibitor and hydroperoxide, as shown in figure 2. A similar experimental dependence was observed in Refs. [2, 10]. From figure 2 it can be seen that both the inhibitor InH and hydroperoxide ROOH reduce the light sum in the Fenton reaction. We see that the location of the chemiluminescence maximum remains the same and is not dependent on [InH] and [ROOH].

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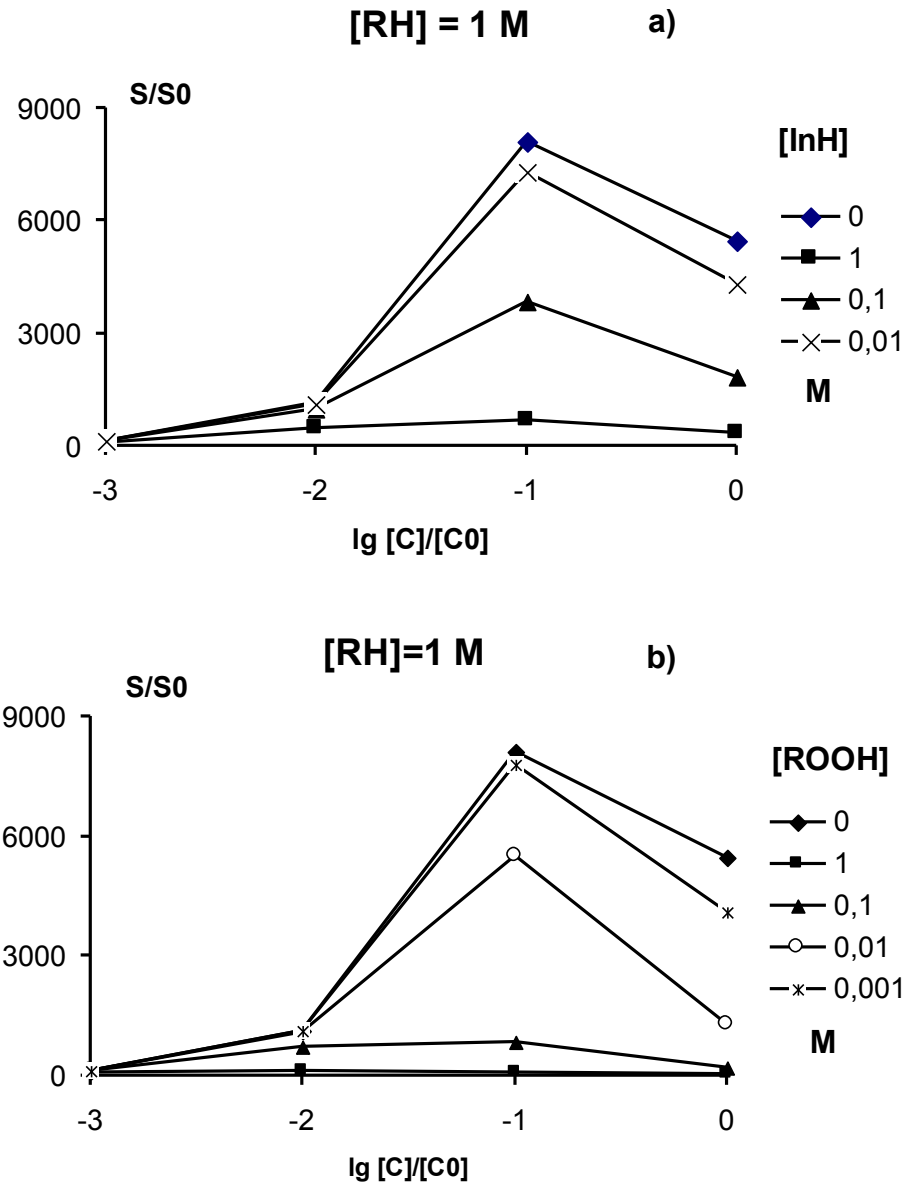

Figure 2: Light sum in the Fenton reaction (*S*/*S*0) depending on the sample dilution lg [C]/[C0], where *C* is the sample concentration after dilution and *C*0 is the initial sample concentration. The calculation was made for the case of [RH] = 1 M and [InH] = 0, 0.01, 0.1 and 1 M (a) or [ROOH] = 0, 0.001, 0.01, 0.1 and 1 M (b).

The light sum *S*/*S*0 in the Fenton reaction for organic substance [RH] = 1 M at a dilution corresponding to the maximum of chemiluminescence (dilution –1) is shown in figure 3 as a function of the concentrations of inhibitors [InH] and organic hydroperoxide [ROOH]. It can be seen that, at [InH] and [ROOH] > 0.001 M, the light sum in the Fenton reaction is diminished with increasing both [InH] and [ROOH]; the hydroperoxide concentration has a stronger influence on decreasing the light sum than the inhibitor.

The mechanism of light sum diminution was investigated next. The inhibitor absorbs radicals $OH^{\bullet}$ and $ROO^{\bullet}$ (reactions 30 and 31, table 3) and, as a result, the rate of the chain reaction is diminished. Hydroperoxide consumes the divalent iron (reaction 32, table 3) and, as a result, its concentration is decreased and the yield of hydroxyl radicals in reaction 1 (table 1) is also decreased. That is, radicals $OH^{\bullet}$ and $ROO^{\bullet}$ are terminated in reactions 30 and 31 with the inhibitor, but the divalent iron is consumed in reaction 32 with hydroperoxide. The termination of the radicals and the expenditure of divalent iron leads to a decrease in the rate of the Fenton reaction. But, the concentration of radicals $OH^{\bullet}$ and $ROO^{\bullet}$, produced in the Fenton reaction, is

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essentially less than the concentration of divalent iron (10^{-3} M). Therefore the rate of reaction 32 is more than rate of reactions 30 and 31, and the hydroperoxides have stronger effect on the decreasing Fenton reaction rate when there are equal concentrations of inhibitors and hydroperoxides. In both cases, the light sum is decreased with increasing concentrations of InH and ROOH.

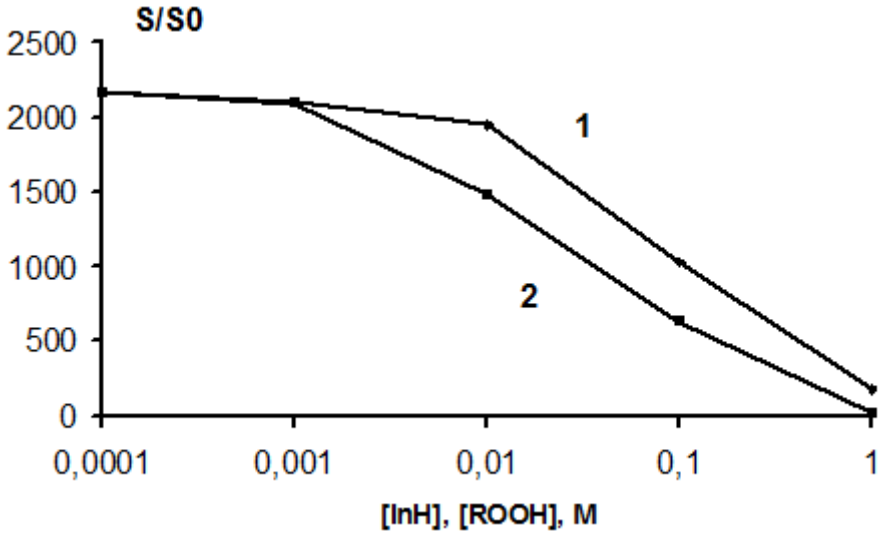

Figure 3: Calculated light sum in the Fenton reaction (*S*/*S*0) for the case of [RH] = 1 M depending on the inhibitor concentration [InH] (1) or the organic hydroperoxide concentration [ROOH] (2).

The dependence of the light sum value for the reaction with divalent iron on the hydroperoxide concentration is another important consideration. The value of the light sum *S* for a sample containing organic substance [RH] = 0.1 M as a function of hydroperoxide concentration [ROOH] after introducing a divalent iron [Fe^{2+}] = 10^{-3} M for a dilution that corresponds to a chemiluminescence maximum (dilution 0) is shown in figure 4. It can be seen that, in this case, the light sum value is increased with increasing [ROOH]. According to the calculation, the position of the chemiluminescence maximum in reaction with the divalent iron only does not depend on [ROOH] and is determined by [RH].

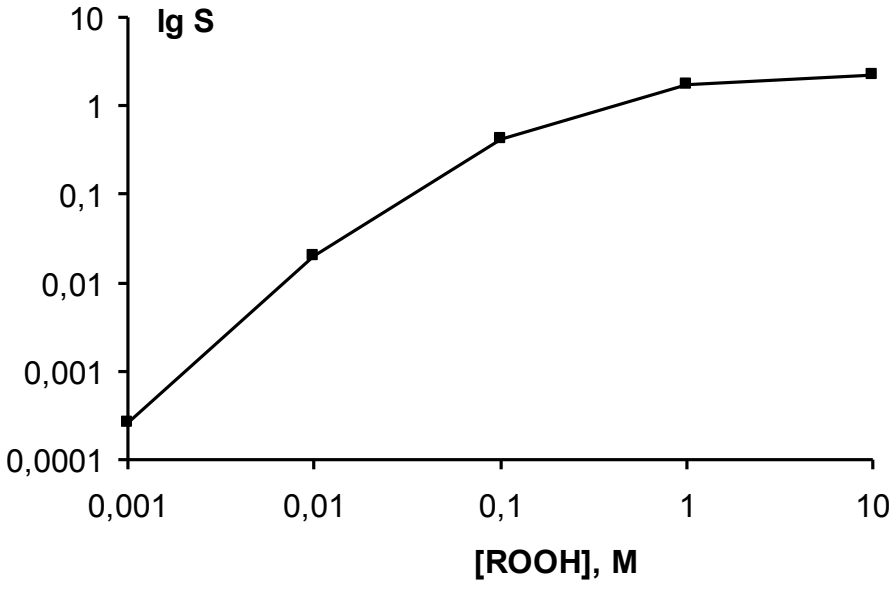

Figure 4: Calculated light sum *S* (relative units) arising in a sample of organic substance [RH] = 0.1 M, which contains hydroperoxide by introducing divalent iron [Fe^{2+}] = 10^{-3} M, depending on [ROOH]. The concentration of organic substance RH corresponds to the maximum chemiluminescence.

Determination of sample characteristics during reactions with divalent iron

The next set of experiments are for the evaluation of biological sample properties, in which the main measured value is the chemiluminescence light sum that appears in the Fenton reaction and during the reaction with divalent iron, which are proposed on the basis of calculations.

1) A divalent iron solution is added to a sample. In this step, the reaction of iron with organic hydroperoxides, already accumulating in the sample, is evaluated and we can obtain light sum $S1$. To obtain the background level, we measure the luminous light sum S_b of the same sample under radiation in the room before adding the iron. The chemiluminescence light sum $S1 - S_b$ is proportional to the concentration of organic hydroperoxides in the sample: $S1 - S_b \sim [ROOH]_0$.

2) Divalent iron and hydrogen peroxide solutions are added to a sample (the Fenton reaction). In this step, the chemiluminescence of the sample with hydroxyl radicals generated in the Fenton reaction is measured. We obtain the yield of luminous products, light sum $S2$. Furthermore, the chemiluminescence of an empty probe (only resolvent, divalent iron and hydrogen peroxide) in the Fenton reaction is measured and we obtain light sum $S0$. The light sum of chemiluminescence $S2 - S0$ is proportional to the yield of the Fenton reaction of luminous species, that is, singlet oxygen: $S2 - S0 \sim [O_2(a^1\Delta_g)]$. In addition to singlet oxygen, organic hydroperoxides (ROOH) are produced. The concentration of hydroxyl radicals N_f, from which the singlet oxygen and hydroperoxide are produced, equals the divalent iron concentration that is consumed in reaction 1 (table 1): $N_f = [Fe^{2+}] = 10^{-3}$ M.

3) A solution of divalent iron is added to sample 2, 90 sec after the start of step 2. In step 3, we evaluate the reaction of iron with the sum of the accumulating sample of organic hydroperoxides and hydroperoxides, arising from the Fenton reaction, and we obtain light sum $S3$. The divalent iron added to the sample (step 2) during the Fenton reaction is fully consumed [2]. The background in this experiment is light sum S_b, which was obtained earlier. The light sum of chemiluminescence is proportional to the concentration of organic hydroperoxides in the initial sample $[ROOH]_0$ and that produced in the Fenton reaction $[ROOH]_f$: $S3 - S_b \sim [ROOH]_0 + [ROOH]_f$. The concentration of hydroperoxides produced in the Fenton reaction ($[ROOH]_f$) under the action of N_f hydroxyl radicals, will be proportional to $S3 - S1 \sim [ROOH]_f$.

As shown earlier, the mechanism of luminescence production is the same for all steps, that is, the luminous product is a dimer of singlet oxygen; therefore, light sums $S1$, S_b, $S2$, $S0$ and $S3$ could be expressed with the same relative units, and the light sum values can be compared directly. All light sums must be measured at dilutions from 0 to –4 and, after experimental analysis, we use the middle value of the light sums for all dilutions, as the maximum chemiluminescence position was previously not known, the light sum value does not depend on [RH] and the light sum is determined by [InH] and [ROOH]. In all cases $[Fe^{2+}] = [H_2O_2] = 10^{-3}$ M.

DISCUSSION

Evaluation of substratum susceptibility to peroxidation

It is essential to know for organic samples and living organisms, the susceptibility of a substratum (sample) to peroxidation. Let us evaluate the possibility of reactions, initiated by divalent iron discussed above, for the estimation of substratum susceptibility to peroxidation with hydroxyl radicals. Peroxidation with hydroxyl radicals is realized in the Fenton reaction.

As shown above, chemiluminescence arizes in samples after introducing divalent iron into the reaction if hydroperoxides are there in the organic probe. The value of light sum $S1$ that was detected in this case is determined to be the concentration of organic hydroperoxides. If $S1 > S_b$, it means that, in the sample, there is organic hydroperoxide. The value of $S1 - S_b$ is proportional to hydroperoxide concentration $[ROOH]_0$. We also obtained the value of light sum $S2$ in the Fenton reaction with the same sample probe. If $S2 > S0$, it means that, in the Fenton reaction, the chain process is developed; $S2 - S0$ is proportional to the number of chain reaction acts (steps) producing luminous agents in the Fenton reaction. We received the value of light sum $S3$ by adding an additional portion of divalent iron into the sample after the Fenton reaction. Introducing

the divalent iron 90 sec after the start of the Fenton reaction ensures that the Fenton reaction itself is fully stopped, and the light sum can only be determined by the concentration of organic hydroperoxide at the moment the iron is introduced. The value of $S3 - S_b$ is proportional to the concentration of organic hydroperoxides [ROOH] in the sample after the Fenton reaction. The concentration of hydroperoxides [ROOH] after the Fenton reaction equals the sum of the hydroperoxide concentration in the initial sample $[ROOH]_0$ plus the concentration of hydroperoxide produced in the Fenton reaction $[ROOH]_f$ under the action of hydroxyl radicals N_f: $[ROOH] = [ROOH]_0 + [ROOH]_f$. We thus obtain the equation:

$$S3 - S_b \sim [ROOH] = [ROOH]_0 + [ROOH]_f \qquad (2)$$

Hence, the number of hydroperoxides produced under the action of hydroxyl radicals, with concentration N_f, is proportional to the light sum:

$$[ROOH]_f \sim S3 - S1 \qquad (3)$$

Full susceptibility of the substratum to peroxidation with hydroxyl radicals, which is determined by the sum of the luminous product yield and the concentration of hydroperoxides produced in the Fenton reaction, is proportional to the light sum:

$$[O_2(a^1\Delta_g)] + [ROOH]_f \sim S2 - S0 + S3 - S_b \qquad (4)$$

Comparing the obtained value of the light sum $S2 - S0 + S3 - S_b$ (4) for the tested probe with the analogous value for a substance, which is possible to obtain as a reference, allows us to receive the relative susceptibility of a substratum to peroxidation with hydroxyl radicals.

Evaluation of the intensity of the free-radical processes

The level (intensity) of free-radical processes in object determines the hydroperoxide concentration, which is accumulated in an object at the moment of sampling. To determine the intensity of free-radical processes in objects before sampling, with a hydroperoxide concentration as a trace of that process, we determine light sums $S1$, $S2$ and $S3$. We obtained value $S1$ after introducing divalent iron ions into sample. Light sum $S2$ is obtained during the Fenton reaction. We obtained the value of light sum $S3$ for the sample after divalent ions are introduced into the sample after Fenton reaction. As stated above, the mechanism of chemiluminescence in all cases is the same, that is, the radiation of the singlet oxygen dimer is detected, singlet oxygen is produced in reaction 24 (table 1) and it is possible to immediately compare light sums $S1$, $S2$, $S3$ and S_b.

The light sum of initial sample $S1$ is a trace of free-radical processes, which take place in the object before sampling. The comparison of light sums $S1$ and $S3$ allows us to determine a number of acts (steps) in free-radical processes in the object before sampling, as it is known that it increases light sums $S3 - S1$, owing to interactions with sample of N_f hydroxyl radicals, which are generated in the Fenton reaction. The value of N_f is well known. At concentration $[Fe^{2+}] = 10^{-3}$ M, this value equals $N_f = 10^{-3}$ M, as the number of hydroxyl radicals generated in reaction 1 (table 1) equals the number of Fe^{2+} ions. For the concentration in hydrocarbon fragments [RH] ~ $1 \div 10^{-3}$ M, almost all hydroxyl radicals are consumed in the reaction with RH, as the concentration of secondary products in the Fenton reaction is essentially less than the RH concentration [1]. The number of hydroxyl radicals N_f give rize to the light sum $N_f \rightarrow \Delta S = S3 - S1$. In the object before sampling, reactions initiated by radicals take place. Let us denote N_{OH} as the number of radicals in an object, the action of which is equivalent to the number of hydroxyl radicals. As a result of free-radical processes in the object, the radicals N_{OH} lead to the appearance of hydroperoxides, the chemiluminescence of which with divalent iron gives light sum $S1 - S_b$. Light sum $S1 - S_b$ appears after the action of N_{OH} radicals in the object: $N_{OH} \rightarrow S1 - S_b$. Light sum $S3 - S1$ appears after the action of $N_f = 10^{-3}$ M radicals in a Fenton reaction. We can thus obtain:

$$N_{OH} = N_f \cdot \frac{S1 - S_b}{S3 - S1} \qquad (5)$$

So, the measurement of chemiluminescence arising from the introduction of divalent iron in a sample (if the sample contains organic hydroperoxides) and chemiluminescence of a sample in the Fenton reaction allow the determination of the susceptibility of a substratum to peroxidation in the presence of hydroxyl radicals as well as the intensity of free-radical processes in the object.

CONCLUSION

- The light sum of chemiluminescence in the Fenton reaction and in a reaction with only divalent iron, depending on the sample dilution, has a maximal value at a certain dilution.
- The dilution for which there is a maximal chemiluminescence value is determined by the initial concentration of the sample RH content.
- The value of the light sum at the maximum does not depend on the initial concentration of sample RH and its dilution, but it is determined by the proportion of inhibitor [InH]/[RH] and hydroperoxide [ROOH]/[RH] in the sample.
- The light sum in the Fenton reaction is diminished with increasing inhibitor and hydroperoxide concentration.
- The light sum upon introducing hydroperoxide and only the divalent iron into solution is increased with increasing hydroperoxide concentration.
- The sum of yields for luminous products and hydroperoxides produced in the Fenton reaction is proportional to the susceptibility of a substratum to peroxidation in the presence of hydroxyl radicals. It is proportional to light sum: $S2 - S0 + S3 - S_b$.
- The results of chemiluminescence measurements allow us to determine the intensity of the free-radical processes in an object before sampling. This intensity is expressed by an equivalent number of hydroxyl radicals, the reactions of which led to the accumulation of hydroperoxides in the initial probe: $$N_{OH} = 10^{-3}\frac{S1 - S_b}{S3 - S1}$$

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