

Initial stage of lipid peroxidation with HO_2^\bullet radicals. Kinetic study

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To cite this article

I. P. Ivanova, I. M. Piskarev, S. V. Trofimova. Initial Stage of Lipid Peroxidation with HO_2^\bullet Radicals. Kinetic Study, *American Journal of Physical Chemistry*. Vol. 2, No. 2, 2013, pp. 44-51. doi: 10.11648/j.ajpc.20130202.13

Abstract: Mechanism of initial stage lipid peroxidation with HO_2^\bullet radicals was investigated. The source of radicals was electric spark discharge radiation on air, rate of radicals generation in sample was $1.2 \cdot 10^{-6} \text{ M (l s)}^{-1}$. The kinetic model of process was developed, based on the classical chain oxidation theory. Some process characteristics was calculated by means of numerical solution the system 24 differential equations of chemical kinetics. Initial lipid concentration was taken equals $2 \cdot 10^{-3} \text{ M/l}$. It was shown the unsaturated lipids concentration after 2 hours treatment to spark discharge decrease in ~ 2 times, whereas for saturated lipids decreasing is only 7%. The hydroperoxides production was calculated. The new diene conjugate appearance in treated to spark discharge sample at lipids concentration $2 \cdot 10^{-3} \text{ M l}^{-1}$ and treatment time about 10 minutes can't be detected, and just having in sample dienes are destroyed. According simulation results, diene conjugates content is increased during treatment to spark discharge radiation at large lipids concentration, having 2 double bonds, ($\sim 0.5 \text{ M l}^{-1}$). This result is confirmed in experiment with sunflower oil (69% linoleic acid). The dependence of dienes concentration in sample from properties (intensities) external irradiation is ambiguous.

Keywords: Saturated And Unsaturated Fatty Acids, Diene Conjugates, Radicals HO_2^\bullet , Simulation

1. Introduction

Today the lipid peroxidation (LP) is a well-known process associated with a chain reaction. Fundamentals of the process concerned lipids of biological membranes were formulated in the book [1]. The current status of problem is in book [2]. New researches carried out in this field, has revealed new aspects of the process.

Electric discharge plasma is widely used in practice of biomedical and biophysical researches [3]. Plasma can be hot and cold. Active factors of cold plasma are ions and radicals generated in plasma itself. Cold plasma can contact with processed object without damaging it and causing various transformations within it. Hot plasma in the case of direct contact will destroy biological object; therefore remote irradiation is used. Hot plasma of electric discharge radiates as a heated black body. The radiation frequency spectrum is continuous, spectrum is peaked at a characteristic frequency that depends only on the body's temperature. Temperature of plasma depends on electrical power of

discharge and can be easily calculated [4]. The most appropriate temperature of spark discharge is that, when maximum of radiation spectrum is in wavelength range of 180 - 260 nm, because at $\lambda < 180 \text{ nm}$ the radiation does not pass through the air, and at $\lambda > 260 \text{ nm}$ biological activity of radiation is greatly reduced. In researches [4,5] it has been established that the main reactive species created under spark discharge radiation in water are radicals HO_2^\bullet . In research [6] was calculated the yield of other active particles. OH^\bullet radicals are formed, but their yield is many orders of magnitude less, than HO_2^\bullet . HO_2^\bullet radicals are not a universal oxidant as OH^\bullet ; their oxidative capacity is much less. It is therefore interesting to examine the lipid peroxidation initiated by radicals HO_2^\bullet , which result in liquid at spark radiation.

The aim of this work is to analyze the lipid peroxidation initiated by radicals HO_2^\bullet . The focus is on the mechanism of initial stages lipid oxidation including the initiation and formation of hydroperoxides. Diene conjugates can accumulate after hydroperoxides formation of unsaturated fatty

acids having at least two double bonds. They are easily identified by the maximum in absorption spectrum at $\lambda = 232$ nm. It is well known that the concentration of conjugated dienes in biological samples characterizes the intensity of lipid peroxidation [1,7]. In this research we have calculated the concentration of diene conjugates under different conditions: the concentration of lipids, the initial concentration of conjugated dienes and the intensity of radiation. It is shown that those factors affect controversially on the change of diene conjugates concentration. Natural sunflower oil was chosen for experiment, as it contains 69% linoleic acid having 2 double bonds.

2. Experimental Methods

We have analyzed the results of lipid peroxidation studies, which were obtained in research [8]. For obtaining more results, lipids were treated with a spark generator SD-10 described in research [5] and used in research [8]. The pulse repetition rate was 10 Hz, pulse width was 100 microseconds. The radiation spectrum of SD-10 is continuous; the maximum position is $\lambda = 220$ nm. The tested samples were placed at a distance of 10 mm from discharge gap. Rate of HO_2^\bullet radicals generation was $(1.2 \pm 0.3) \cdot 10^{-6} \text{ M l}^{-1} \text{ s}^{-1}$.

We used lipids included natural sunflower oil which contain 6% palmitic acid (16:0), 18% oleic acid (18:1), 69% linoleic acid (18:2) and 6% other fatty acids. After exposure to the spark discharge radiation in order to measure the UV absorption spectra, oil was dissolved in heptane at a ratio of 1:500. Chemically pure heptane was used. Spectral measurements were made with a spectrophotometer "Fluorat-02 Panorama" (made by "Lumex" company, St. Petersburg, Russia). Analysis of oxidation kinetics (solving a system of 24 differential equations) was carried out using package MathCad 14.

3. Theoretical Approach

3.1. Oxidation Products

The final products of all organic compounds oxidation, containing elements C, H, O, are carbon dioxide and water. Compounds that make up living organisms contain other elements, the final oxidation products of which are their oxides. Intermediate products of lipid peroxidation can be malondialdehyde, Schiff bases, aldehydes and ketones. If a fatty acid contained in a lipid has at least two double bonds, during peroxidation can form diene conjugates; if acid has at least three double bonds, the dienes and trienes conjugates can be produced. Usually conceder, that concentration of conjugated dienes characterizes the intensity of ongoing chain processes [7].

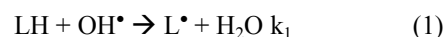
Lipids oxidation products were extracted from microorganisms after processing of lipid sample with spark discharge (SD) and then were identified in the research [8]. The exposure time to SD was not more than 60 seconds,

because during this time according to results of research [8], a 99% bactericidal effect was achieved. In all cases, there was decrease in oxidation products concentration (diene and triene conjugates, malonic dialdehyde, Schiff bases). The initial concentration of these products was determined by natural level of normal metabolic processes in cell. Reducing the concentration of products during treatment means that the rate of their accumulation is less than the rate of their consumption under interaction with active particles, generated by spark discharge.

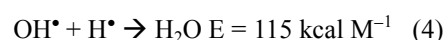
3.2. Lipid Peroxidation by Radicals OH^\bullet , HO_2^\bullet and UV-Radiation

Let us estimate the rate of accumulation the lipid peroxidation products under the spark discharge irradiation. We will use the classic free radical reaction scheme, adjusted according to recent studies [1, 2]. The oxidation reaction includes the stages of initiation, propagation, branching and termination of chain reactions. Let us consider lipid LH. In principle, oxidation can be induced by radicals OH^\bullet , HO_2^\bullet after reaction with lipid and by UV light after absorbing a quantum of radiation by a fatty acid embedded in a lipid molecule.

3.2.1. Initiation



OH^\bullet and HO_2^\bullet radicals differ in their reactivity. Constant $k_1 \sim 10^9 - 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, and $k_2 \sim 10^3 - 10^5 \text{ M}^{-1} \text{ s}^{-1}$. In the reaction of the hydrogen abstraction from the molecule LH an energy equal to the binding energy of the formed molecule is released [9].



The energy released in the reactions 4 and 5 is spent on separation of a hydrogen atom from a molecule of lipid. If this energy is greater than the binding energy of hydrogen in a molecule that interacts with radical, the abstraction of hydrogen atom is possible.

The C-H binding energy for hydrocarbons is typically in the range of 60 - 110 kcal M^{-1} [10]. In saturated fatty acids the dissociation energy of C-H bond is 91 kcal M^{-1} . In unsaturated fatty acids dissociation energy of hydrogen atom, which is located in α -position relative to the double bond is 87 kcal/mol. Thus, hydroxyl radical, for which in reaction 4 energy of 115 kcal M^{-1} is released, can oxidize any organic compound (all saturated and unsaturated fatty acids). HO_2^\bullet radical can only oxidize unsaturated fatty acids that have at least one double bond.

The photon energy at $\lambda = 260$ nm is 4.75 eV or 108 kcal M^{-1} . This energy alone is enough to carry out the reaction 3

in any lipid. However, the absorption of photons is resonant, and at wavelengths less than 260 nm, the lipid may not have a strong absorption peak. In such case, the direct initiation of oxidation by photon absorption is unlikely, and the primary initiation mechanism will be reaction 2 with radicals HO_2^\bullet , which, as shown earlier [4], are predominantly produced in the water exposed to UV radiation as secondary product.

According to the results of researches [4, 6], the yield of OH^\bullet radicals under irradiation to UV photons is several orders of magnitude less than the HO_2^\bullet . Hydroxyl radicals can generate under other external impacts, which usually play insignificant role during the experiment with SD and UV irradiation. But their impact can reveal over a long time. Hydroxyl radicals are forming continuously when lipid sample is exposed to external background radiation (as example, cosmic radiation), and its concentration is sufficient for noticeable effects to reveal over a long time of exposure [11]. This circumstance must be taken into account in the study of various processes going on in natural environments.

3.2.2. Chain Propagation

a) Addition of oxygen resulting in formation of peroxy radicals.



The oxygen addition reaction is fast, and its activation energy is zero.

b) Formation of hydroperoxide, hydrogen abstraction.



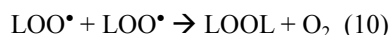
The second reaction of chain propagation with lipid LH is slow; its rate constant can be from 1 to $60 \text{ M}^{-1} \text{ s}^{-1}$ [2, 12]. This particular reaction will predominantly determine the rate of chain oxidation. At this stage, the diene and triene conjugates are produced; the detail will discuss below.

3.2.3. Chain Branching with Destruction of Hydroperoxide



Chain branching (the decay of hydroperoxides) is first-order reaction and its rate constant can be from 10^{-8} to 10^{-3} s^{-1} [12]. This reaction can occur either spontaneously or due to UV radiation exposure.

3.2.4. Chain Termination



Chain termination reaction is fast, with the rate constant from 10^6 to $10^8 \text{ M}^{-1} \text{ s}^{-1}$.

Ways of chain propagation and termination are numer-

ous, and the possibility to continue a chain under a particular scenario is determined by many factors. Complete cessation of the chain reaction at any intermediate stage, when still exist an external impact, is unlikely. The reaction rate can slow down dramatically if the intermediate products will be low active. The reaction of organic substances oxidation under continuous exposure to external factors completely ceases only when the end product will be carbon dioxide and water.

If the system still retains an organic compound, in case when radicals are generated or UV radiation exposure acts, it can take place an hydrogen abstraction. The outcome of such reaction is production of a new hydrocarbon radical, for which the oxidation chain reaction continues. The only reason for the termination of chemical reactions at any stage of oxidation is complete consumption of dissolved oxygen in the sample, and no input of new portions of it.

Therefore, the intermediate products of lipid chain oxidation, of which the most characteristic ones are the diene and triene conjugates, may appear at certain stages. The possibility of their occurrence is determined by the composition of fatty acids contained in lipids. But at deep oxidation stages all of these products will disappear.

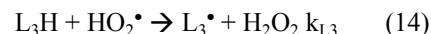
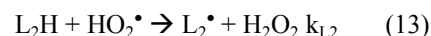
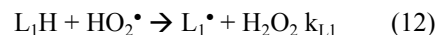
4. Calculation of Chain Oxidation of Lipids Exposed to Radiation a Spark Discharge

Lipids have a complex composition. They include fatty acids with different degrees of saturation of carbon bonds. We will distinguish LH lipids according to the degree of saturation of fatty acids: L_0H - saturated fatty acids; L_1H - fatty acid contains one double bond; L_2H - two double bonds; L_3H - three double bonds. Therefore, we will consider LH lipids as the sum total $\text{LH} = \text{L}_0\text{H} + \text{L}_1\text{H} + \text{L}_2\text{H} + \text{L}_3\text{H}$.

Let us write down specific reactions.

4.1. Initiation

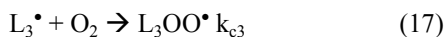
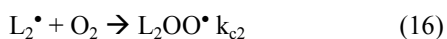
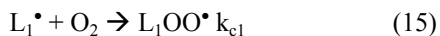
It should be emphasized that the initiation of lipid oxidation with saturated fatty acids L_0H by radicals HO_2^\bullet is impossible energetically; it begins with lipid L_1H .



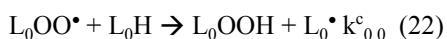
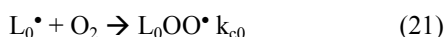
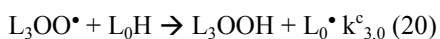
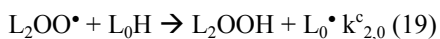
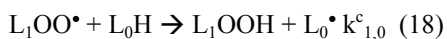
At the initiation stage, oxidation reaction may involve hydroperoxides of unsaturated lipids (not only initial lipids) accumulated earlier in the sample in course of various processes, because in these molecules hydrogen atoms in the α -position relative to double bond can remain.

4.2. Chain Propagation

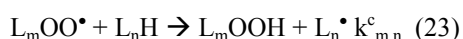
a) Formation of peroxy radicals.



b) Formation of hydroperoxides. At this stage, a radical of saturated lipid $L_0\bullet$ is formed:

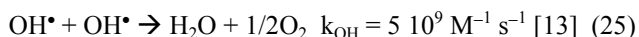
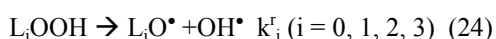


Other unsaturated lipid radicals are formed:



(m, n = 1, 2, 3)

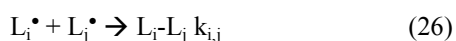
4.3. Hydroperoxide Decomposition



Our calculations in scope of describing model revealed that $OH\bullet$ radicals concentration formed as result of hydroperoxides decay was not more than $10^{-11} \text{ M l}^{-1}$. Therefore, the radicals $OH\bullet$ have not produced a significant impact on the discussed process.

4.4. Chain Termination

Reactions of recombination and chain termination are shown below. This process involves all lipids.



(i, j = 0, 1, 2, 3)

We further must take into account the absorption of $HO_2\bullet$ radicals by third molecules, other than lipids. The composition of a biological sample almost never can be uniform. Substances that absorb reactive species (radicals or photons) other than lipids will always be there. During the reaction a new compounds, which will also react with radicals, are formed. The sample cannot be completely transparent to UV radiation. Thus, an absorbing substance X will be found in the sample. The rate of reaction with the substance X can be written as $k_X[HO_2\bullet][X]$, where k_X is reaction rate constant. We have assumed that the concentration of the substance X is high and remains constant during the entire irradiation time (experiment duration). The calculation presumed that the value of the product $k_X[X]$ was given so

as to ensure the absorption of 50% radicals, generated by spark discharge in sample. Radicals, generated in sample, that have not interacted with lipids, will die in interacting with each other at the rate of $k_{HO_2}[HO_2\bullet][HO_2\bullet]$. Due to the fact that the lipids of different composition (various type of fatty aside) can interact with radicals at different rates, the $HO_2\bullet$ radicals reaction rate constants with different lipids L_1H (k_{L1}), L_2H (k_{L2}) and L_3H (k_{L3}) are given separately. As a first approximation, these constants were taken as equal. The values of reaction rate constants are listed in Table 1.

Table 1. Rate constants for lipid oxidation reactions. Values of constants were chosen according to the works [1, 2, 12]. Indexes i, j = 0, 1, 2, 3 denote the number of double bonds in fatty acids, which are the part of oxidizing lipids.

Nº	Reaction	Rate constant value *)
1.	Initiation	
	$L_1H + HO_2\bullet \rightarrow L_1\bullet + H_2O_2$	k_{L1}
	$L_2H + HO_2\bullet \rightarrow L_2\bullet + H_2O_2$	$k_{L2} \cdot 10^4 - 10^5 \text{ M}^{-1} \text{ s}^{-1}$
2.	Formation of peroxide radicals	
	$L_i + O_2 \rightarrow L_iOO\bullet$	$k_{ci} \cdot 10^2 - 10^3 \text{ M}^{-1} \text{ s}^{-1}$
	Formation of hydroperoxides	
3.	$L_iOO\bullet + L_jH \rightarrow L_iOOH + L_j\bullet$	$k_{ij}^c \cdot 1 - 60 \text{ M}^{-1} \text{ s}^{-1}$
	Decomposition of hydroperoxides	
	$L_iOOH \rightarrow L_iO\bullet + OH\bullet$	$k_i^r \cdot 10^{-8} - 10^{-3} \text{ s}^{-1}$
4.	Chain termination	
	$L_i + L_j\bullet \rightarrow L_i-L_j$	k_{ij}
	$L_iOO\bullet + L_jOO\bullet \rightarrow L_iOOL_j + O_2$	$k_{ij}^c \cdot 10^6 - 10^8 \text{ M}^{-1} \text{ s}^{-1}$
5.		
	$L_iO\bullet + L_jO\bullet \rightarrow L_iOOL_j$	k_{ij}^{cl}

*) Rate constant for reactions: k_{L1} , k_{L2} , k_{L3} – (12, 13, 14); k_{ci} – (15,16,17,21); k_{ij}^c – (18, 19, 20, 24); k_i^r – (24); k_{ij} , k_{ij}^c , k_{ij}^{cl} – (26, 27, 28).

Equation for consumption of $HO_2\bullet$ radicals can be written as:

$$[HO_2\bullet]' = k_0 - k_X[HO_2\bullet][X] - k_{HO_2}[HO_2\bullet][HO_2\bullet] - k_{HO_2,m}[HO_2\bullet][L_mH] \quad (29)$$

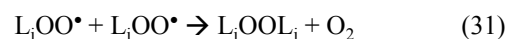
Here $k_0 = 1.2 \cdot 10^{-6} \text{ M l}^{-1} \text{ s}^{-1}$ generation rate of radicals $HO_2\bullet$, $k_{HO_2} = 8 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$ [13], $k_{HO_2,m}$ is a rate constant for reaction of $HO_2\bullet$ radicals with lipid L_mH (m = 1, 2, 3), see Table 1 (denoted as k_{L1} , k_{L2} , k_{L3}).

Oxygen consumption equation is written as:

$$[O_2]' = WO - k_{ci}[O_2][L_i\bullet] + k_{ij}^c[L_iOO\bullet][L_jOO\bullet] \quad (30)$$

(i, j = 0, 1, 2, 3)

where WO is the rate of oxygen diffusion into sample, preset at $10^{-6} \text{ M l}^{-1} \text{ s}^{-1}$. Oxygen is absorbed by oxidation of a lipid and is released during the chain termination reaction.



Equation of primary lipids L_jH consumption, as well accumulation and consumption of oxidation products $L_j\bullet$, $L_jOO\bullet$, L_jOOH (j = 0, 1, 2, 3) were written by the usual rules of chemical kinetics according to Table 1, which shows the reactions rate constants. Further conversion of inactive products such as L-L, LOOL were not considered.

We took into account spontaneous decay of hydroperoxides at the rate of $k'_1[\text{L}_i\text{OOH}]$. The exact value of the rate constants of reactions did not play a significant role. In living always many channels exist (as in discussed process), which led to the same final results. Decreasing reaction speed in one channel at once is compensated increasing reactions speed in another (parallel) channels owing to change the intermediate particles concentration. But result will be the same. Thus absence of strong result dependence from reaction rate constants is advantage of model.

In fact, variance of these constants within the range shown in Table 1, did not altered the final result; only concentrations of intermediate products have changed. In particular, the change in values rate constants of reactions lipids with fatty acids having different degrees of saturation with radicals HO_2^\bullet , (shown in Table 1) did not affected the conclusions set out below. Full number of independent variables (number of differential equations) was 24.

The variable are: initial lipids L_0H , L_1H , L_2H , L_3H ; lipid radicals L_0^\bullet , L_1^\bullet , L_2^\bullet , L_3^\bullet ; peroxy radicals $\text{L}_0\text{OO}^\bullet$, $\text{L}_1\text{OO}^\bullet$, $\text{L}_2\text{OO}^\bullet$, $\text{L}_3\text{OO}^\bullet$; radicals $\text{L}_0\text{O}^\bullet$, $\text{L}_1\text{O}^\bullet$, $\text{L}_2\text{O}^\bullet$, $\text{L}_3\text{O}^\bullet$; hydroperoxides L_0OOH , L_1OOH , L_2OOH , L_3OOH ; radicals HO_2^\bullet , OH^\bullet , dienes Di, oxygen O_2 . Solution the system of 24 differential equations was carried out using MathCad 14 package.

5. Results and Discussion

5.1. The Possibility of Observing Diene Conjugates

At the initial stages of peroxidation the diene and triene conjugates can occur. In UV absorption spectrum for fatty acids, which have only simple double bonds, is line at 215 nm. When conjugation occurs (after H-abstraction and formation hydroperoxides), for fatty acids with two double bonds the line 232 nm appears (diene conjugation). And for fatty acids with three double bonds line 268 nm appears (triene conjugation).

Observation the maximum at 232 nm in UV absorption spectrum, which is characteristic for conjugated dienes, is indicative to occurrence of chain oxidation reactions in lipids [7]. Conjugated diene formation is possible in the reactions with single radical HO_2^\bullet in lipids L_2H and L_3H , and conjugated triene formation is possible in reaction with two HO_2^\bullet radicals in lipids L_3H .

In the natural samples of biological probes, hydroperoxides may be found including conjugated dienes. Formation of conjugated diene in reaction with one HO_2^\bullet radical is possible for fatty acid having two double bonds. HO_2^\bullet radicals can abstract only hydrogen atom located in α -position relative to double bond. In fatty acid with two double bonds in α -position are 3 hydrogen atoms. For example, in linoleic acid 18:2 9c12c (carbon position is measured from the carbonyl end of the acid) in the α -position relative to the double bond, are hydrogen atoms 8c, 11c and 14c [14]. Only abstraction of hydrogen atom from position 11c can lead to formation of conjugated dienes and appearance

peak 232 nm. In other positions, 8c and 14c hydrogen atom abstraction will alter the structure of the molecule and don't leads to diene conjugate. If the conjugate is already formed, conjugation may be destroyed, resulting in a shift of maximum 232 nm in absorption spectrum. A similar situation occurs in acids with three double bonds, for example, α -linolenic acid 18:3 9c12c15c. Here in the α -position are 4 hydrogen atoms, and only the abstraction one of the two atoms (11c or 14c) will result in diene conjugation. If diene conjugation is already exist, for example, for an atom at the position 11c, then abstraction of the hydrogen atom from the position 14c will cause triene conjugation; the absorption peak will shift from 232 nm to 268 nm. Thus, diene conjugation for the lipids L_2H will be caused in 1/3 of initiating acts by radicals HO_2^\bullet , and for the lipids L_3H – 1/4 of initiating acts. In other cases, conjugated dienes, if they had already existed, will be destroyed, and the maximum absorption spectrum corresponding to the dienes at $\lambda = 232$ nm will decrease.

Conjugation occurs in hydroperoxides L_2OOH and L_3OOH . We have calculated the concentration of hydroperoxides formed in reactions induced by radicals HO_2^\bullet in lipids L_1H , L_2H and L_3H with concentration $[\text{L}_1\text{H}] = [\text{L}_2\text{H}] = [\text{L}_3\text{H}] = 2 \cdot 10^{-3} \text{ M l}^{-1}$ (such concentration of lipids was found in the samples used in the research [8]). In this calculation the radical generation rate was taken equal to $1.2 \cdot 10^{-6} \text{ M l}^{-1} \text{ s}^{-1}$. The rate of oxygen diffusion into the reaction zone is taken equals to $10^{-6} \text{ M l}^{-1} \text{ s}^{-1}$. The initial concentration of oxygen was taken equals to $1.5 \cdot 10^{-4} \text{ M l}^{-1}$, which is equal to its concentration in pure water at room temperature. The results of the calculation: the concentrations of lipids L_0H , L_1H , L_2H and L_3H remaining in the solution after treatment are shown in Figure 1.

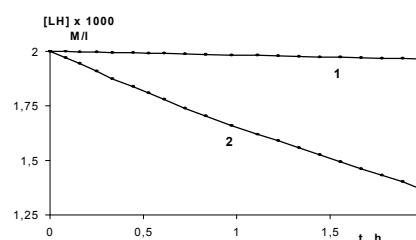


Figure 1. Calculated time dependence (t – treatment time, hours) of lipid concentration ($\lg[\text{LH}] \times 1000, \text{ M l}^{-1}$) under action of spark discharge irradiation. 1 – saturated lipids L_0H , 2 – unsaturated lipids L_1H , L_2H and L_3H . Initial lipids concentration was $[\text{L}_0\text{H}] = [\text{L}_1\text{H}] = [\text{L}_2\text{H}] = [\text{L}_3\text{H}] = 2 \cdot 10^{-3} \text{ M l}^{-1}$.

The Figure 1 shows that lipids L_0H are consumed very little. As mentioned above, the direct oxidation of saturated lipids L_0H by radicals HO_2^\bullet is impossible. They are involved into oxidation at the stage of chain propagation.

The rest lipids (unsaturated) are consumed significantly; after treatment unsaturated lipids during 2 hours their concentration decreases by almost a factor of 2. Decreasing concentration of unsaturated lipids leads to reducing speed of chain oxidation, thus to reducing speed of saturated L_0H lipids oxidation. In the case of long exposure by the source

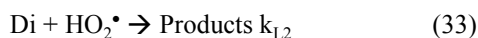
of initiation (spark discharge), the chain reaction will continue until the complete consumption of unsaturated lipids, or other products of oxidation, which could have sustained the chain reaction. Thereafter, the oxidation of saturated lipids L_0H will stop. As a result, the concentration of remaining saturated lipids after treatment will decrease by no more than 7% of their initial value and will remain further unchanged. Meantime, the unsaturated lipids concentration will decrease for two hours treatment in ~2 times (see Figure 1).

The dependence of hydroperoxides concentration, produced during the exposure time up to 2 hours is shown in Fig. 2. It is seen that concentration of hydroperoxides $[L_1OOH] = [L_2OOH] = [L_3OOH] \gg [L_0OOH]$. During the exposure time of 60 seconds used in the research [8], the concentration of hydroperoxides, which can contain conjugated dienes was $[L_2OOH] + [L_3OOH] = 2 \cdot 10^{-7} \text{ M l}^{-1}$. At this hydroperoxides concentration (if all of that were dienes) the optical density D for the band 232 nm (extinction coefficient of $2.1 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ [1]) and for the liquid layer thickness of 1 cm is $D \sim 10^{-5}$. This optical density cannot be detected, so in the research [8] the accumulation of conjugated dienes is not observed. Spontaneous decomposition of hydroperoxides [12], the possibility of which was found in case of polymers during long storage, for given time exposure (up to 2 hours) cannot occur because of its low probability.

Let us consider how the concentration of diene conjugates is related with the concentration of unsaturated lipid L_2H , the fatty acid of which has two double bonds, and with the intensity of external exposure, under which the dienes could have accumulated. Dienes Di can form during the reaction of hydroperoxides formation:



As shown above, 1/3 (one-third) of produced hydroperoxides may contain dienes Di . $[Di] = 1/3 [L_2OOH]$. Then a hydroperoxide again can react with the radical resulting in diene conjugation destruction.



The rate of dienes formation is:

$$[Di]' = 1/3 k_{2,2} [L_2OO\cdot][L_2H] - 2/3 k_{1,2}[Di] [HO_2\cdot] \quad (34)$$

Suppose there is a steady state in which $[LOO\cdot]$ does not change much and $[Di]' = 0$.

Then we will obtain:

$$[Di] = \frac{k_{2,2}[LOO\cdot][L_2H]}{2k_{1,2}[HO_2\cdot]} \quad (35)$$

From this expression it follows that the steady-state concentration of dienes increases with rising concentration of lipid L_2H and with decreasing the intensity of exposure (radicals $HO_2\cdot$ generation rate). This means that if we take a lipid sample with already accumulated concentration of

dienes and expose it by the spark radiation, then there exist a critical concentration of dienes $Di(\text{critical})$. If initial diene concentration $[Di]_0 > [Di(\text{critical})]$, the value $[Di]$ will be diminish, dienes will be consumed, and if $[Di]_0 < [Di(\text{critical})]$, the dienes will be accumulated.

This situation is illustrated in Figure 3, which shows the kinetics of changes in concentration of dienes at a concentration of linoleic acid $[L_2H] = 2 \cdot 10^{-3} \text{ M l}^{-1}$ exposed by spark radiation. It is seen that for initial concentration of dienes $[Di]_0 = 10^{-4} \text{ M l}^{-1}$ (Fig. 3a), their concentration during the exposure will be reduced; at $[Di]_0 = 10^{-5} \text{ M l}^{-1}$ (Fig. 3b) the concentration of dienes first decreases until it has attained a significant amount of hydroperoxide. When the concentration of hydroperoxides will reach approximately value of 10^{-5} M l^{-1} (see Fig. 2), the concentration of dienes increases. At $[Di]_0 = 10^{-6} \text{ M l}^{-1}$ (Fig. 3c) the diene concentration increases.

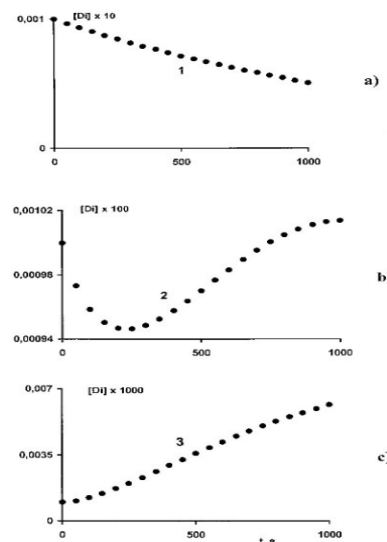


Figure 3. Calculated time dependence (t – treatment time, seconds) of diene conjugates concentration $[Di]$ (M l^{-1}) under action of spark discharge at various initial diene conjugates concentrations $[Di]_0$ during treatment of linolenic acid with concentration $[L_2H] = 2 \cdot 10^{-3} \text{ M l}^{-1}$. a) $[Di]_0 = 10^{-4}$, b) $[Di]_0 = 10^{-5}$, c) $[Di]_0 = 10^{-6} \text{ M l}^{-1}$.

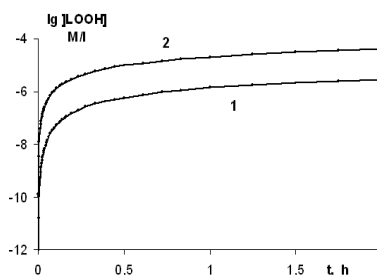


Figure 2. Calculated time dependence (t – treatment time, hours) of hydroperoxide concentration $\lg [LOOH]$, M l^{-1} under action of spark discharge irradiation. 1 – saturated lipids L_0OOH ($\lg [L_0OOH]$), 2 – unsaturated lipids L_1OOH , L_2OOH u L_3OOH ($\lg [L_mOOH]$, M l^{-1} , $m = 1, 2, 3$). Initial concentration lipids was $2 \cdot 10^{-3} \text{ M l}^{-1}$.

Thus, at the same initial concentration of the lipid $[L_2H]$ and constant irradiation rate, dienes can be consumed, their concentrations can oscillate or increase. It depends on the

conditions under which the particular lipid sample had been placed earlier. Diene concentration is in a complicated way connected with irradiation conditions. In researches [8, 15] it was experimentally found out that after irradiation of lipid samples by discharge, previously accumulated lipid oxidation products are destroyed. In other work the diene concentration increases [1].

Figure 4 shows the calculated dependence for concentration of conjugated dienes being treated by a hypothetical source HO_2^\bullet radicals with different rates of radical generation. At radical generation rate of $10^{-7} \text{ M l}^{-1} \text{ s}^{-1}$ diene concentration decreases. With a decrease in generation radicals rate by 10 times the concentration of dienes first decreases slightly and then increases sharply. The calculations showed that at low intensities of external impact, which is characteristic for natural processes, concentration of dienes will grow as long as the source lipid L_2H has not been consumed. Kinetic cases found by means of calculation are confirmed experimentally for different samples (Table 2). Here, the optical density of 232 nm band after exposure of sunflower oil (69% linoleic acid) to SD increases (case of high unsaturated lipid concentration 0.5 M l^{-1}), but for lipids extracted from microorganisms decreases [8] (case of low unsaturated lipid concentration, as full lipid concentration was $2 \cdot 10^{-3} \text{ M l}^{-1}$). Complete analysis is not possible, because only relative measurements of diene concentration were conducted.

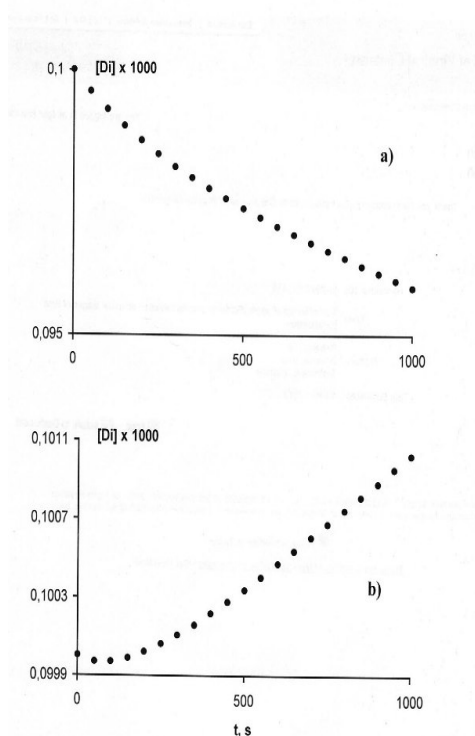


Figure 4. Calculated time dependence (t – treatment time, seconds) of diene conjugates concentration (initial concentration $[\text{Di}]_0 = 10^{-4} \text{ M l}^{-1}$) under action of hypothetical HO_2^\bullet radicals source with rate of radicals generation: a) $10^{-7} \text{ M l}^{-1} \text{ s}^{-1}$; b) $10^{-8} \text{ M l}^{-1} \text{ s}^{-1}$. Lipid concentration $[\text{L}_2\text{H}] = 2 \cdot 10^{-3} \text{ M l}^{-1}$.

Table 2. Variations of optical density (arbitrary units) for bond 232 nm in various lipid samples after spark discharge treatment (experiment).

Time treatment, minutes	Sunflower oil	S. aureus [8]	E. coli [8]
Before treatment	0.33 ± 0.05	0.18 ± 0.004	0.012 ± 0.008
1	–	0.031 ± 0.009	0.0022 ± 0.0004
30	0.41 ± 0.05	–	–
60	0.45 ± 0.05	–	–

6. Conclusion

Radicals HO_2^\bullet can oxidize only unsaturated lipids, fatty acids of which have not more 1 double bond. Lipids with saturated fatty acids start to oxidize on the stage chain propagation. When unsaturated fatty acid will consume, the oxidation of saturated fatty acids by HO_2^\bullet radicals fully stops.

The formation of new diene conjugates in experiments under spark discharge radiation for biological samples, having lipid concentration $\sim 2 \cdot 10^{-3} \text{ M l}^{-1}$, is impossible to found in view of small diene yield. It don't means the absence of lipid peroxidation. Evidence for lipid peroxidation process is decreasing diene conjugate concentration, which was accumulated in sample at natural conditions before experiment with spark discharge treatment.

The increasing of diene concentration in course of treatment to spark discharge was found in samples with linoleic acid concentration $\sim 0.5 \text{ M l}^{-1}$.

The diene concentration begin appreciable increase with diminution external irradiation rate. Accumulation of diene conjugates must be usual for biological objects in natural condition at low irradiation rate.

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