DIFFERENTIAL SPECTROSCOPY IN THE ASSESSMENT OF THE ORGANISM ANTIOXIDANT POTENTIAL (REVIEW)

N. M. Litvinko

UDC 543.42;577.158

Methods for determining the oxidant/antioxidant activity of free radicals, antioxidant compounds, and free-radical oxidation products in biological fluids are discussed. General approaches to the analysis of the antioxidant potential of complex natural objects using differential spectroscopy are presented.

Keywords: differential spectroscopy, antioxidant activity (capacity), phospholipolysis, oxidative stress.

Introduction. Living organisms exist in media saturated with oxygen (O_2) that can form reactive oxygen species (ROS) under physiological conditions and cause the oxidation of biological substrates. Therefore, free-radical oxidation (FRO) processes play an extremely important role in cellular metabolism [1]. ROS become rather hazardous compounds that have deleterious effects on cells upon reaching a critical point because of their reactivity. The response of the organism includes mechanisms for detoxifying them that are associated with the protective action of antioxidants. Oxidative stress, i.e., the formation of copious amounts of free radicals ($R^\bullet$) that damage cellular structural elements and disrupt exchange of compounds and energy, is observed with disbalance of cellular pro- and antioxidant systems. Oxidative stress was proven to be involved in the pathophysiology of many human diseases [2], including infection by SARS-CoV-2 [3, 4]. Radiation disease was also caused by induction of ROS through a branched chain reaction of cell membrane lipid oxidation, the products of which were toxic for cells [5]. Phospholipids of cell membranes are most predisposed to oxidation by ROS and form hydroperoxides of unsaturated fatty acid residues contained in them. The mechanism of lipid peroxidation (LPO) is well studied [6]. Lipid radicals are formed in the lipid layer of biological membranes

$$HO^\bullet + LH \rightarrow H_2O + L^\bullet .$$
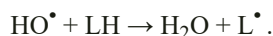
The lipid radical ($L^\bullet$) reacts with molecular O_2 dissolved in the medium to form a new free radical, i.e., a lipid peroxide radical ($LOO^\bullet$):
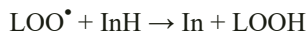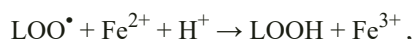
$$L^\bullet + O_2 \rightarrow LOO^\bullet ,$$
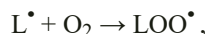
that attacks a neighboring phospholipid molecule to form a lipid hydroperoxide LOOH (Ox-PL) and a new radical $L^\bullet$:

$$LOO^\bullet + LH \rightarrow LOOH + L^\bullet .$$

The alternation of the two last reactions represents the LPO chain reaction.

The chain is broken by the reaction of the free radicals with antioxidants (InH), variable valence metal ions (e.g., Fe^{2+}), or each other:

$$LOO^\bullet + Fe^{2+} + H^+ \rightarrow LOOH + Fe^{3+} ,$$

$$LOO^\bullet + InH \rightarrow In + LOOH$$

$$LOO^\bullet + LOO^\bullet \rightarrow \text{molecular products} + \text{photon} .$$

The ROS concentration in tissues under normal conditions is insignificant (H_2O_2 10^{-8}–10^{-11} M; $HO^\bullet < 10^{-11}$ M) because they are regulated in the human body by the antioxidant system [7].

Thus, LPO processes play an important role in normal cell functioning and act as early key links of the organism response to oxidative stress [8]. Therefore, much attention is paid to the development of methods for determination of the antioxidant potential of the human body to characterize its ability for functional restoration after oxidative stress [9].

The aim of the present review was to summarize and analyze current methods for evaluating the antioxidant potential of an organism and the capabilities of differential spectroscopy for characterizing it.

Antioxidant System of Cell Protection and LPO. Disruption of the balance between FRO reactions, among which LPO makes up the main fraction, and the action of *in vivo* antioxidant systems promotes the development of pathologies such as atherosclerosis, myocardial infarct, cancer, neurodegenerative processes, etc. [10–13]. Most diseases during the acute and exacerbation periods are associated with enhanced oxidation of polyunsaturated fatty acids (PUFAs) and the development of antioxidant insufficiency manifested to different degrees and leading to pathological conditions of the body. The resulting deficit of bioantioxidants requires the corresponding intervention. Bioantioxidants are usually understood to be compounds that act as inhibitors during simulation of FRO and retain these properties upon administration to a living organism [14].

LPO is based on the reaction of ROS with unsaturated fatty acids in lipids to form peroxide radicals, i.e., mono- and dimeric, cyclic, and polymeric peroxides and lipid hydroperoxides. Divalent Fe ions and H_2O_2 or hypochlorite formed from it act as direct precursors of hydroxyl radical, which initiates the lipid chain oxidation (Fig. 1). LPO develops as chain reactions in the lipid phase of membranes or lipoproteins. However, the initial and intermediate steps of this complex reaction system occur in the aqueous cell phase. For this reason, compounds that reduce the concentration of one of these two compounds inhibit the formation of hydroxyl radical and LPO.

The SH-containing amino acid cysteine, several peptides and proteins (glutathione, albumin), ubiquinone, ascorbic and uric acids, tocopherols, carotenoids, flavonoids, etc. should be mentioned as compounds that prevent the formation of strong oxidants in the human body. LPO and antioxidant protection was found to represent a single system in dynamic equilibrium that could self-regulate. The mechanisms of antiradical protection include enzymatic and non-enzymatic processes (Fig. 1). The components of the antioxidant system (antioxidants) can be arbitrarily divided into several groups of compounds that prevent the formation of new oxygen free radicals (superoxide dismutase, glutathione peroxidase, ceruloplasmin, transferrin, ferritin); eliminate free radicals before they initiate chain reactions that damage cells (α-tocopherol, ascorbic acid, β-carotene, uric acid, bilirubin, albumin); and eliminate oxidized lipids or reduce cellular structures damaged by oxygen free radicals (phospholipase, DNA repair enzymes, methionine-sulfoxide reductase).

The system for protecting cellular structures from damage by ROS produced inside cells (endogenous ROS) and acting from the outside (exogenous ROS) is characterized by its total antioxidant capacity (AOC), i.e., an integral parameter of the antioxidant status of the organism that can be evaluated using the antioxidant activity (AOA) of its separate components (antioxidants) and the system as a whole. These characteristics are distinctly different. The AOA corresponds to the rate constant of a separate antioxidant against a given free radical. The AOC corresponds to the number of moles of a given free radical that are neutralized by the studied solution regardless of the activity of the individual antioxidants present in the mixture [1]. Therefore, the AOC expresses the antioxidant status better than the AOA for blood plasma or serum, which is a heterogeneous solution of various antioxidants.

The antioxidant protection system functions through various mechanisms. The action of exogenous ROS, which are incapable of penetrating deeply through membranes, is always mediated through stimulation of FRO in the lipid phase of cell membranes and is characterized by the degree of oxidation of unsaturated fatty acids in the phospholipids. The antioxidant potential (total AOA) is a part of cellular protective systems and is related to the reaction of antioxidants directly with the lipid phase. Another part is related to the products of ROS action in the aqueous phase. These are compounds in the aqueous phase that bind Fe ions (complexants) and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase, which eliminate H_2O_2 (Fig. 2).

Thus, the production of ROS and R$^\bullet$ in human tissues and organs is regulated by a multilevel physiological antioxidant system that includes various chemical compounds such as vitamins, pigments, hormones, and enzymes [6]. Lipid-soluble compounds that can react with radicals localized mainly in the hydrophobic membrane and lipoprotein zone are mainly used in clinical practice. Also, the first acute phase of inflammatory diseases occurs with hyperproduction of radicals localized in the aqueous phase where hydrophilic ROS scavengers are most effective [15].

Evaluation of the Degree of Development of Oxidative Stress. ROS are primarily free radicals (R$^\bullet$) from a physicochemical viewpoint. Most R$^\bullet$ are generated during FRO and undergo further transformations into more stable
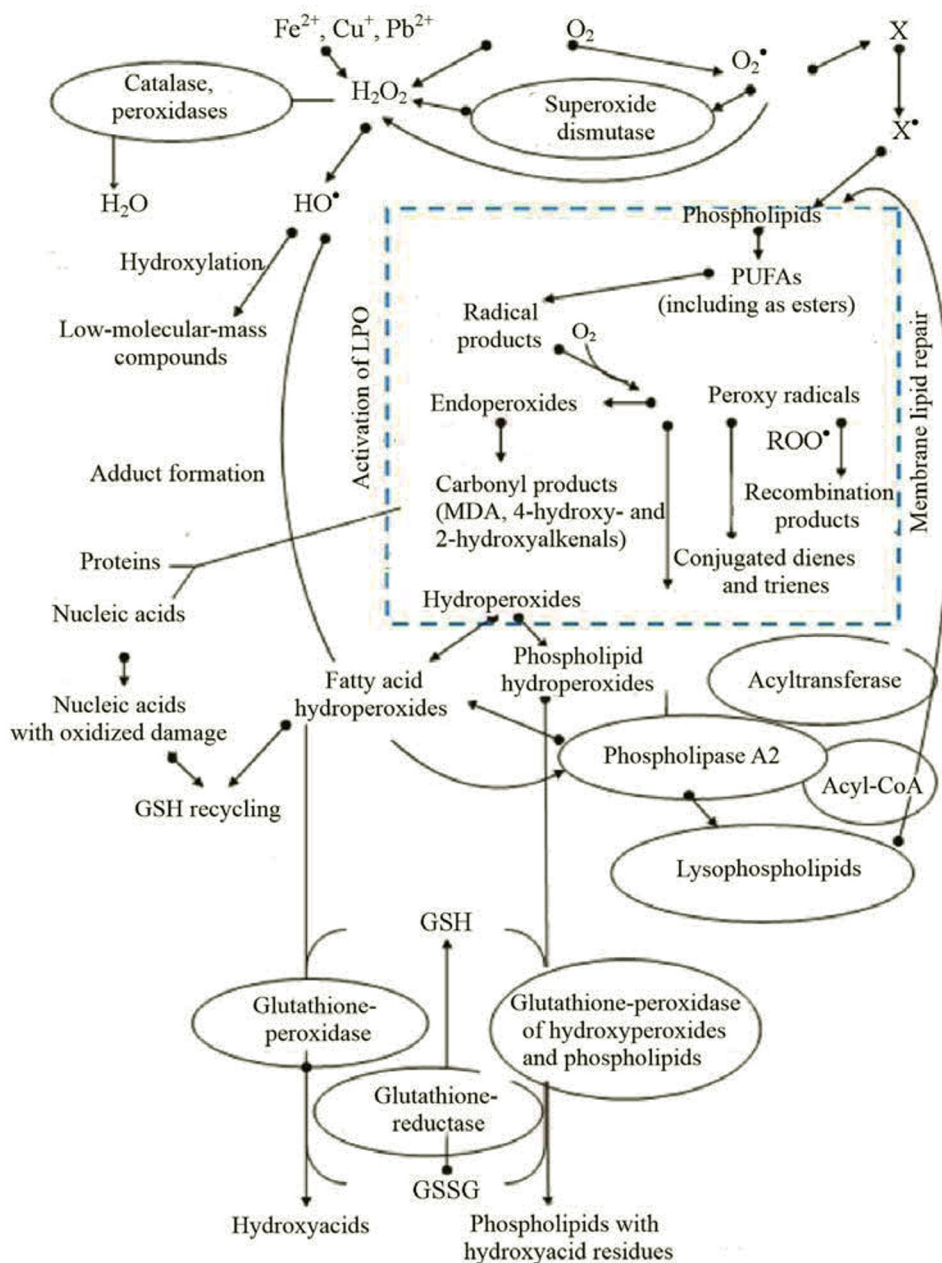
Fig. 1. Diagram of reactions of free-radical oxidation and enzyme antioxidant protection: PUFAs, polyunsaturated fatty acids; LPO, lipid peroxidation; CoA, coenzyme A; GSH, reduced glutathione; GSSH, oxidized glutathione; GST, glutathione-S-transferase; MDA, malondialdehyde; SOD, superoxide dismutase; X, any compound capable of forming radicals.

species. Active free radicals in normal cells are constantly formed in mitochondria and are used, e.g., to battle infections or to destroy toxic xenobiotics. Radiation, laser or UV irradiation, and the formation of toxic xenobiotics can change the balance between redox reactions and the antioxidant protection of cells upon forming an excess of ROS, which have a negative influence on the organism (Fig. 3). In this instance, the vitally important functions of DNA, proteins, lipids, and low-molecular-mass bioregulators are disrupted by the formation of organic hydroperoxides that modulate further FRO of these biostructures [16].

Two mechanisms are distinguished in the theory of radical oxidation. These are linear breakage by a radical reaction inhibitor and inhibition via creation of complexes of active radicals with systems with conjugated π-bonds. Radicals were

Free-radical oxygen species

$$O_2 \text{ —— } O_2^{\bullet} \text{ ——— } H_2O_2 \text{ ——— } HO^{\bullet} \text{ —— } H_2O$$

Superoxide anion-radical — Hydrogen peroxide — Hydroxyl radical

Phospholipids (PUFAs)

Lipid phase

Intermediate products
(alkyl, alkoxyl, peroxyl radicals)

Primary products (lipid hydroperoxides, diene conjugates, etc.)

Aqueous phase

Secondary products
(malondialdehyde, triene conjugates, etc.)

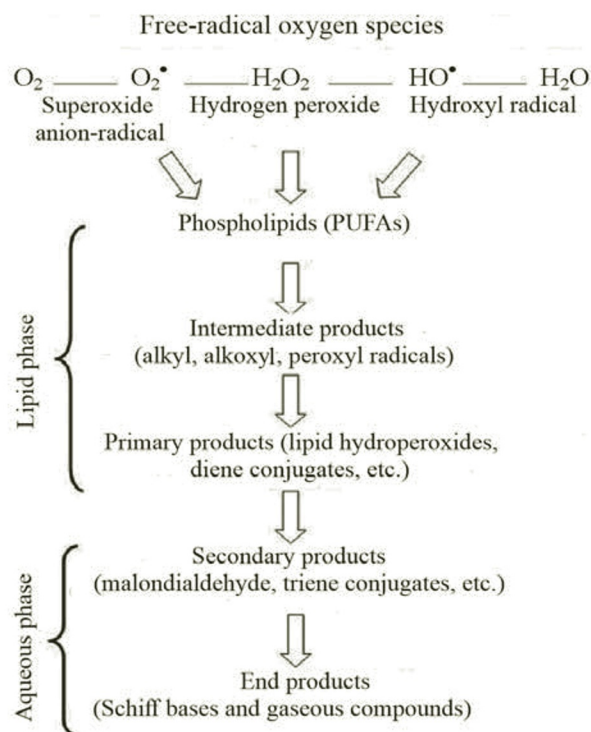End products
(Schiff bases and gaseous compounds)

Fig. 2. Distribution of ROS reaction products of phospholipids between lipid and aqueous phases.

found to undergo exchange reactions with antioxidants that resulted in the unpaired electron of the biopolymer radical ($R^{\bullet}$) transferring to the antioxidant (HIn) to form an inactive inhibitor radical ($In^{\bullet}$): $R^{\bullet} + HIn^{\bullet} \rightarrow RH + In^{\bullet}$ [14]. Sterically shielded phenols, e.g., tocopherols, were proposed to react through the first mechanism; natural biological objects, more often via the second [17].

Methods for determination of the concentration of free radicals can be combined into two main groups, i.e., direct and indirect (Table 1). Direct methods include electron paramagnetic resonance (EPR) and to a certain extent chemiluminescence (CL); indirect, determination of products from reactions involving free radicals and inhibitor analysis [18]. CL induced by the Fenton reaction allows the reaction products and not radicals to be observed. The Fenton reaction occurs practically in any substrate. The light-sum level of CL induced by hydroxyl radicals (Fenton reaction) is determined by the chain initiation, propagation, and breakage rate constants [19].

Direct determination of the concentration of free radicals in cells and tissues and suspensions and solutions of cellular organelles is complicated by their high reactivity and short lifetime. As a result, their concentration in such objects is very low and differs from the *in vivo* concentration. Therefore, the concentration of all products of FRO (primary, secondary, and tertiary) in systems simulating FRO processes is determined to evaluate the antioxidant potential.

Determination of the Antioxidant Potential of Biological Material. The AOA estimated by an individual antioxidant and the AOC defined as an integral parameter reflecting the antioxidant/oxidant status of an organism as a whole or its separate system are parameters of the degree of oxidative stress, as noted above. A method giving complete information about the state and reactions of complex systems in which ROS are observed and react with antioxidants does not currently exist. Also, a single term defining the antioxidant properties of a compound (antioxidant capacity, antioxidant activity, antioxidant power, antioxidant ability) is lacking [18]. Existing methods for determination of the AOA possess some drawbacks or others.

The systematization and analysis of methods for determination of antioxidants and the AOA of biological objects have been reviewed in the Russian literature [14, 17, 18]. Methods for studying the total AOA differ in the oxidation source, compound being oxidized, and method for measuring the oxidized compound. As a rule, methods for estimating the total AOA are based on reactions with long-lived $R^{\bullet}$ that act as prototypes of $R^{\bullet}$ formed in the living cell.
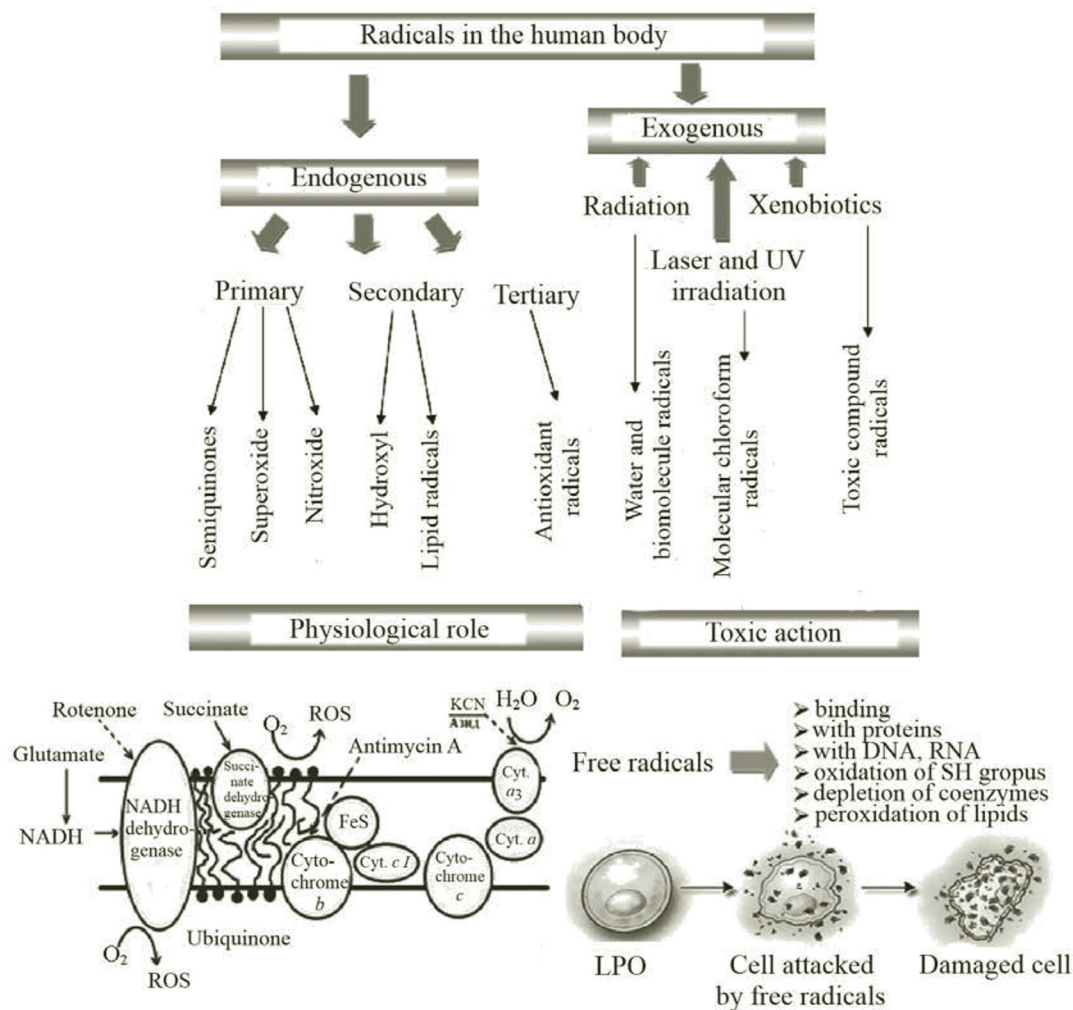
404

Fig. 3. Physiological role and toxic action of free radicals in the human body.

Individual antioxidants are determined using chromatography, e.g., TLC, high-performance TLC, gas, liquid, and high-performance liquid chromatography (HPLC) [29].

The most used major integral methods ABTS/TEAC, CUPRAC, DPPH, Folin–Ciocalteu, and FRAP have been compared [30–32]. These methods are based on electron-transfer (ET) or H-atom-transfer (HAT) processes. ET methods measure the ability of antioxidants to reduce an oxidant that changes the color of the oxidant itself or a compound specially added to the system after reacting with it. ET methods include ABTS/TEAC, CUPRAC, DPPH, Folin–Ciocalteu, and FRAP, each of which uses chromogenic redox reagents with different standard redox potentials. Most HAT methods include a competing scheme of reactions in which the antioxidant and substrate compete for peroxyl radicals thermally generated by decomposition of azo compounds [18].

The total AOA can also be established from oxygen absorption during LPO, oxidation of crocin, and CL with luminol; from oxidation of R-phycoerythrin; from the sensitivity of erythrocytes to hemolysis and the Fe-reducing activity; and from generation of lipid peroxides. The activity of antioxidant enzymes such as ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase was measured [17] and auto-oxidation of adrenalin was used [33] in several studies. Physicochemical methods for detection of AOA include flame-ionization, change of thermal conductivity, UV-visible spectroscopy, fluorescence, mass spectrometry, and electrochemical methods [32]; voltammetry [34, 35]; amperometry and chronoamperometry (biamperometry) [36], coulometry [37]; and potentiometric, titrimetric [38, 39], and polarographic methods [40]. Spectrophotometric analytical methods are most widely used to determine the AOA and AOC.

TABLE 1. Main Methods for Determination of Free-Radical Concentration

R$^\bullet$, ROS, FRO products	Detection method	Ref.
	Direct methods	
R$^\bullet$, ROS	Use of spin traps — molecules that form stable nitroxyl radicals upon reaction with unstable radicals (EPR signals are measured).	[20]
R$^\bullet$, ROS	Use of intrinsic CL activators — recombination reactions of superoxide, hydroxyl, and lipid radicals and nitric oxide, including chemical (lucigenin, luminol) reacting with certain radicals that is accompanied by emission and physical (rhodamine G dye, Eu complex with tetracycline, coumarin derivatives) not reacting with radicals but increasing the CL quantum yield by transferring electronic excitation energy from radicals, i.e., products from the reaction with activator.	[21]
	Indirect methods	
Primary and secondary products	Reaction with thiobarbituric acid (TBA) of TBA-dependent products such as conjugated dienes, particularly malondialdehyde. A red pigment (absorption maximum at 532–535 nm) is formed in the latter case.	[22] [23]
R$^\bullet$, ROS	Free radical scavengers and traps, use of superoxide dismutase enzyme in combination with catalase to remove superoxide radicals; tocopherol, for lipid radicals.	[24]
Hydroxyl radicals	Use of spectrophotometric, chromatographic and fluorescent detection of oxidation and hydroxylation products, particularly terephthalic acid oxidized by reaction with OH$^\bullet$ radicals to 2-hydroxyterephthalate that fluoresces intensely ($\lambda_{ex} = 326$ nm; $\lambda_{em} = 432$ nm).	[25, 26]
Superoxide-anion radicals	Reduction of cytochrome C during which ferricytochrome C is converted to ferrocytochrome C and spectrophotometric detection at $\lambda = 550$ nm.	[27]
Lipid hydroperoxides	Use of titration of hydroperoxides by iodide ions with amperometric detection of the formed iodine or spectrophotometric using thiocyanate to determine lipid hydroperoxides from the change of optical density at 500 nm resulting from the formation of an Fe(III) thiocyanate complex.	[28]

Spectrophotometric Methods. The antioxidant properties, e.g., of plant raw material, were characterized spectrophotometrically by determining phenolic compounds using Folin–Ciocalteu reagent [41] in models of unilamellar (single membrane) liposome oxidation by atmospheric O_2 catalyzed by Fe^{2+} followed by determination of diene products [42] and from the total content of antioxidants without separating their mixture. The results were calculated using chemometric algorithms [43].

Differential spectroscopy has been used to estimate the antioxidant potential. An original approach for determination of the antioxidant potential that was based on development of the differential spectrum of hemoproteins myoglobin (Mb) and hemoglobin (Hb) during formation of its low-spin hexacoordinated form hemichrome was proposed [44–55]. This simply reproduced method was based on the spectral changed of Mb and Hb near the Soret band through the action of phospholipolysis products or UV-irradiated phospholipids and their derivatives [44, 45]. Auto-oxidation of Hb is known to be a spontaneous process in which either O_2 bound to an Fe atom (Fe^{2+}) of a heme group spontaneously dissociates as a superoxide radical or free O_2 in the solution reacts with a heme group to form a transition state, i.e., aquadeoxyheme, leaving the resulting Fe in the trivalent state (Fe^{3+}). The heme Fe atom after this reaction is axially coordinated on one side by a histidine (proximal or F_8 histidine) and, on the other, by H_2O or OH^- depending on the pH. If the heme pocket is opened from the folded position, distal histidine His58 (E_7) of this heme-containing protein (or other close-lying basic amino acids on the distal side of the heme ring) can coordinate to the Fe^{3+} atom. The resulting bihistidyl adduct is called hemichrome [56]. The conversion of Hb into hemichrome is dose-dependent and is detected from the amplitude change of the differential visible spectrum.

Differential spectroscopy and the activity of phospholipase A_2 in estimating the AOA. The amplitude between the maximum (λ_{423}) and minimum (λ_{405}) in difference absorption spectra of Mb and Hb (ΔD) was observed to increase during hydrolysis of phospholipids by phospholipase A_2 (PLA$_2$, 3.1.1.4; phospholipolysis) in their presence through the action of one of the reaction products, i.e., a fatty acid. Spectral changes due to the action of the fatty acid cleaved during phospholipolysis were shown to be directly proportional to its concentration in a model system during conversion of Hb into hemichrome [57]. Differences in the contents of the two Hb forms were associated with a proportional change of the difference spectrum that allowed the PLA$_2$ activity to be measured from the shift in the region of the Hb Soret band and were indicative of changes during phospholipid peroxidation. The changes of Mb and Hb spectral properties by native phosphatidylcholine (PC) or lysophosphatidylcholine (LPC) were insignificant, in contrast with the free fatty acid.

PLA$_2$ is very sensitive to a change of membrane physicochemical properties under the influence of various factors, including oxidation. Exogenous ROS that cannot penetrate membranes always have only mediated effects on cells via stimulation of LPO in the plasmic membrane. Therefore, protection from damage by exogenous ROS is targeted primarily at utilizing fatty acids and lipid hydroperoxides as LPO products that stimulate FRO through chain reactions. PLA$_2$ plays the main role in this protection because phospholipids with peroxy-oxidized fatty-acid residues are more preferred as substrates for this enzyme than normal unoxidized phospholipids [58]. This allows the use of difference spectroscopy to determine the PLA$_2$ activity during LPO [46, 47]. A feature of the approach was that phospholipase activity was not used to characterize the degree of LPO and to estimate the antioxidant potential of biological fluids although it is one of the enzyme antioxidants [48].

The system oxidized-phospholipids–PLA$_2$–hemoproteins was found to be convenient for testing the AOA of biological fluids [49]. For this, oxidized phospholipids (Ox-PL) and their derivatives were used as the primary target for ROS. PLA$_2$ activity against Ox-PL and unoxidized phospholipids was measured from spectral changes of Mb [50] and Hb [51, 52].

Any method for determination of total AOA of an individual FRO inhibitor, their mixture, or biological fluids is known to be based on the use of a model system that includes at least two components, i.e., a mechanism for generating a certain type of free-radical reaction and a system for detecting it. Introduction into such a model system of a free-radical scavenger or compounds affecting the concentration or state of catalytic ions leads to a concentration decrease of the free radicals or catalysts that is reflected in the parameters of the detecting system.

The physical action of UV radiation on phospholipid micelles was chosen as the factor causing LPO in several studies [44–46, 52] to exclude additional reagents such as aggressive H_2O_2 from the system. The primary LPO products (hydroperoxides etc.) damaged by ROS were selectively eliminated by PLA$_2$. Therefore, the degree of phospholipid oxidation was judged from the activation of this enzyme and was detected using a hemoprotein method. The change of enzyme activity was monitored in kinetic mode by recording spectral changes of Hb near the Soret band caused by phospholipolysis products
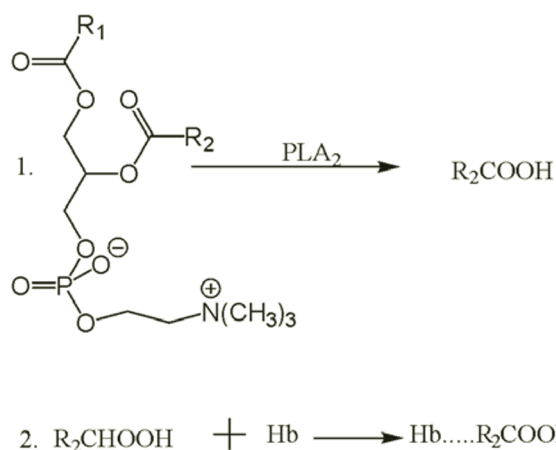
A kinetic curve was obtained by recording spectra every 0.5 min. The slope of this curve reflected product ingrowth per unit time ($\Delta P/\Delta t$) and characterized the initial rate of the phospholipase reaction. The proposed method showed that PLA$_2$ activity was 1.5–2 times greater for oxidized phospholipids regardless of the structural organization of the lipid–water surface formed as bilayer liposomes from phospholipid and mixed micelles of phospholipid with detergent [52]. This approach gave adequate results for the determination of total AOA of blood serum, tea, and juices upon using biological fluids
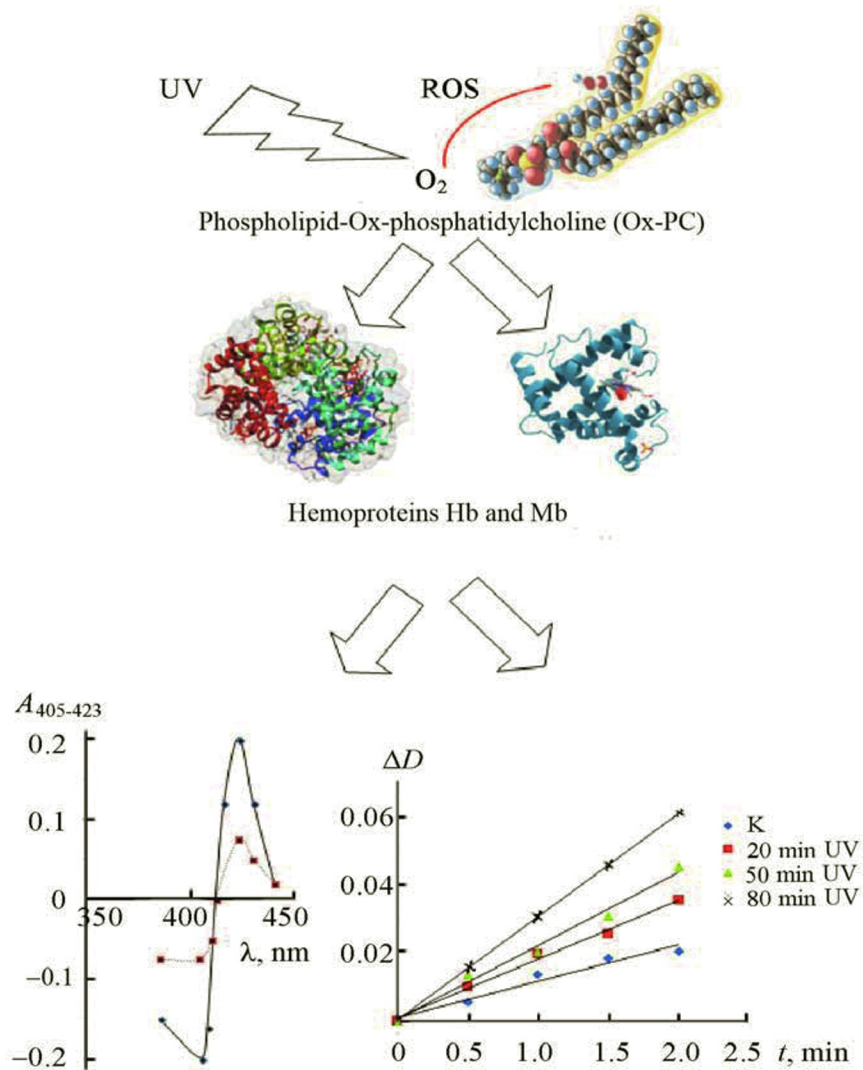
Fig. 4. Reaction of Mb and Hb with UV irradiated (solid line) and unirradiated (dashed line) phosphatidylcholine (Ox-PC) and its kinetics; the steric structure of Mb was constructed using PDB 1MBO; Hb (3odq).

as traps for products of free-radical reactions in the lipid phase that were formed upon UV irradiation of the phospholipid [53–55].

Differential spectroscopy and UV irradiated phospholipids for estimation of the AOC. Photo-oxidation of lipids is a two-step, two-quantum process. In the first step, lipids are oxidized by UV radiation through a free-radical mechanism to form hydroperoxides. In the second step, the peroxides are cleaved upon absorption of a second quantum of UV radiation to form stable products, primarily aldehydes. In cases where peroxidation, including after UV irradiation, reaches the secondary products (aldehydes), C9-aldehyde (acid) and 2-azeloyl derivatives are formed from PC, which can contain up to 61% oleic acid in the second position depending on the source. UV irradiated phospholipids were first used to determine the degree of their peroxidation using difference spectrophotometry [54, 60] because these products could convert Hb into hemichrome [59]. In fact, UV irradiation of PC for a long time led to secondary oxidation that eventually promoted the conversion of Hb into hemichrome (Fig. 4). The correlation of changes in the LPO index of a UV-irradiated PC film with the amplitude changes of Hb differential spectra in the presence of micelles of UV-irradiated PC-detergent and the accumulation of LPO secondary products (malondialdehyde) confirmed this [61].

Unirradiated PC was shown not to cause spectral changes of Hb [57]. The Hb differential spectrum was recorded in the presence of PC already 15 min after its irradiation. The amplitude of the Hb difference spectrum increased in proportion

to the PC irradiation time. The intensity of the difference spectrum exceeded the control values by 12–15 times after 80 min [61]. One of the most common standards, i.e., the water-soluble tocopherol (vitamin E) analog Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), was used for quantitative evaluation of the antioxidant protection. Irradiation of PC combined with Trolox showed a dose-dependent reduction of the initiating effect of the UV radiation on the spectral response of the hemoproteins. This allowed the use of calibration curves for quantitative evaluation in Trolox units of the AOC of blood serum, which acted as a trap for free-radical reaction products in the lipid phase that formed during UV irradiation of the phospholipid. Biological fluids combined with a lipid phase formed by PC or unsaturated fatty acids significantly reduced the intensity of Hb and Mb difference spectra upon UV irradiation because of their antioxidant potential. This was a measure of the action of Ox-PC or Ox-PUFA on hemoprotein and acted as an LPO indicator. This phenomenon provided a basis for new quantitative determination methods for total AOC because of the protective action of blood serum and other biological fluids.

Conclusions. The variety of compounds of various chemical natures with oxidant and antioxidant properties, the multicomponent composition of biological objects, and the rapid change of their composition after taking a sample make the problem of evaluating oxidative stress and the antioxidant potential far from trivial. The situation is complicated by the lack of unified terms and comparable units for expressing the concentration and antioxidant properties of compounds or compound complexes. As a rule, relative units expressed in grams of ascorbic acid, Trolox, rutin, etc. are used as units of measurement.

Two main methodological approaches to estimating the oxidant/antioxidant status of an organism are known. The first relates to the determination of the content and/or activity of individual antioxidants, including those of high-molecular-mass (enzyme systems of glutathione, superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, phospholipase, decarboxylating malate dehydrogenase, redox-sensitive transcription factors, etc.) and low-molecular-mass (glutathione, uric and ascorbic acids, tocopherols, polyphenols, carotenoids, retinol, etc.). The second is based on an evaluation of the integral antioxidant capacity considering the total antioxidant potential regardless of the individual antioxidants. Considering the multitude of *in vivo* redox reactions, the many varied antioxidants, the different mechanisms of action, and the possibility for synergism of their action, the second approach is considered more informative and; therefore, preferred.

Spectrometry should be identified among physicochemical methods for determination of the AOA and AOC. Photometric detection is a widely employed method for estimating the antioxidant potential and is the most convenient and accessible in our opinion. The use of *in vitro* oxidized-phospholipids–PLA$_2$–hemoprotein original systems based on phospholipolysis and oxidized phospholipids and hemoproteins as a simple model of phospholipid peroxidation as the primary target of ROS through the action of UV radiation using differential spectroscopy can reliably determine the AOA and AOC. Use in both systems of the known antioxidant Trolox as a standard for determination of the AOA and AOC of blood can quantitatively characterize the *in vitro* prooxidant and antioxidant potential of biological fluids.

A rapid method using difference spectroscopy and biological fluids as free-radical traps [44, 45] has clear advantages because it does not require lengthy tests on laboratory animals and does not use toxic reagents and expensive detection techniques. This creates favorable conditions for adequate characterization of the antioxidant potential among the broad assortment of methods discussed in this review.

REFERENCES

1. A. Ghiselli, M. Serafini, F. Natella, and C. Scaccini, *Free Radical Biol. Med.*, **29**, 1106–1114 (2000).
2. C. E. Thomas and B. Kalyanaraman, *Oxygen Radicals and the Disease Process*, Harwood Academic Publishers (1998).
3. M. M. Rogero, M. C. de Leao, T. M. Santana, M. V. de M. B. Pimentel, G. C. G. Carlini, T. F. F. da Silveira, R. C. Goncalves, and I. A. Castro, *Free Radical Biol. Med.*, **156**, 190–199 (2020); doi: 10.1016/j.freeradbiomed.2020.07.005.
4. E. Barberis, S. Timo, E. Amede, V. V. Vanella, Ch. Puricelli, G. Cappellano, D. Raineri, M. G. Cittone, E. Rizzi, A. R. Pedrinelli, V. Vassia, F. G. Casciaro, S. Priora, I. Nerici, A. Galbiati, E. Hayden, M. Falasca, R. Vaschetto, P. P. Sainaghi, U. Dianzani, R. Rolla, A. Chiocchett, G. Baldanzi, E. Marengo, and M. Manfredi, *Int. J. Mol. Sci.*, **21**, 8623–8648 (2020).
5. B. N. Tarusov, *Principles of the Biological Action of Radioactive Emissions* [in Russian], Medgiz, Moscow (1954).
6. B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, 4th edn., Oxford University Press, New York (2007).
7. A. M. Pisoschi and A. Pop, *J. Med. Chem.*, **97**, 55–74 (2015).

8. V. Bochkov, B. Gesslbauer, Ch. Mauerhofer, M. Philippova, P. Erne, and O. V. Oskolkova, *Free Radical Biol. Med.*, **111**, 6–24 (2017).

9. I. V. Babenkova, E. A. Buravlev, K. V. Buravleva, and Yu. O. Teselkin, *Evraziisk. Soyuz Uchenykh*, No. 13, *Ser. Biol. Nauki*, No. 4, 1–7 (2015).

10. J. A. Berliner, N. Leitinger, and S. Tsimikas, *J. Lipid Res.*, **50**, S207–S212 (2009); doi: 10.1194/jlr.R800074-JLR200.

11. L. Butler, Y. Perone, J. Dehairs, L. E. Lupien, V. de Laat, A. Talebi, M. Loda, W. B. Kinlaw, and J. V. Swinnen, *Adv. Drug. Deliv. Rev.*, **159**, 245–293 (2020), published online July 23, 2020; doi: 10.1016/j.addr.2020.07.013.

12. E. Gianazza, M. Brioschi, A. Martinez Fernandez, and C. Banfi, *Redox Biol.*, **23**, (2019); doi: 10.1016/j.redox.2019.101119.

13. T. S. Anthonymuthu, E. M. Kenny, A. M. Lamade, V. E. Kagan, and H. Bayir, *Free Radical Biol. Med.*, **124**, 493–503 (2018); doi: 10.1016/j.freeradbiomed.2018.06.031.

14. E. B. Burlakova, *Ross. Khim. Zh. (Ross. Khim. O'va im. D. I. Mendeleeva)*, **LI**, No. 1, 3–12 (2007).

15. V. S. Kamyshnikov (Ed.), *Handbook of Clinical Laboratory Diagnostics* [in Russian], Vol. 2, Minsk, Belarus (2000).

16. Yu. A. Vladimirov, O. A. Azizova, and A. I. Deev, *Itogi Nauki Tekh., Ser. Biofiz.*, **52**, 1–250 (1992).

17. O. V. Trineeva, *Razrab. Regist. Lek Sredstv*, **21**, No. 4, 180–197 (2017).

18. M. Ya. Khodos, Ya. E. Kazakov, M. B. Vidrevich, and Kh. Z. Brainina, *Vestn. Ural. Med. Akad. Nauki*, **14**, No. 3, 262–274 (2017); doi: 10.22138/2500-0918-2017-14-3-262-274.

19. I. M. Piskarev and I. P. Ivanova, *Sovrem. Tekhnol. Med.*, **8**, No. 3, 16–26 (2016); doi: 10/17691/stm2016/8/3/02.

20. L. Valgimigli, G. F. Pedulli, and M. Paolini, *Free Radical Biol. Med.*, **31**, 708–716 (2001).

21. Yu. A. Vladimirov and E. V. Proskurina, *Usp. Biol. Khim.*, **49**, 341–388 (2009).

22. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, A. V. Kozlov, A. N. Osipov, and D. I. Roshchupkin, *Itogi Nauki Tekh., Ser. Biofiz.*, **29**, 1–251 (1991).

23. F. Karaas and M. Karatepe, *Anal. Biochem.*, **311**, No. 1, 76–79 (2002).

24. Yu. A. Vladimirov, *Vestn. Ross. Akad. Med. Nauk*, **7**, 43–51 (1998).

25. E. Peralta, G. Roa, J. A. Hernandez-Servin, R. Romero, P. Balderas, and R. Natividad, *Electrochim. Acta*, **129**, 137–141 (2014).

26. G. Cao, H. M. Alessio, and R. G. Cutler, *Free Radical Biol. Med.*, **14**, No. 1, 303–311 (1993).

27. M. M. Tarpey and I. Fridovich, *Circ. Res.*, **89**, 224–236 (2001).

28. V. I. Dontsov, V. N. Krut'ko, B. M. Mrikaev, and S. V. Ukhanov, *Tr. Inst. Sist. Anal. Ross. Akad. Nauk*, **19**, 50–69 (2006).

29. W. Jesionek, B. Majer-Dziedzic, and I. M. Choma, *J. Liq. Chromatogr. Relat. Technol.*, **38**, 1147–1153 (2015).

30. R. Apak, K. Guclu, B. Demirata, M. Ozyurek, S. Esin Celik, B. Bektasoglu, K I. Berker, and D. Ozyurt, *Molecules*, **12**, 1496–1547 (2007).

31. K. Pyrzynska and A. Pekal, *Anal. Methods*, **5**, No. 17 (2013); doi: 10.1039/C3AY40367J.

32. A. M. Pisoschi, A. Pop, C. Cimpeanu, and G. Predoi, *Oxid. Med. Cell. Longevity*, Article ID 9130976 (2016); https://www.hindawi.com/journals/omcl/2016/9130976/.

33. E. I. Ryabinina, E. E. Zotova, E. N. Vetrova, N. I. Ponomareva, and T. N. Ilyushina, *Khim. Rastit. Syr'ya*, No. 3, 117–121 (2011).

34. Kh. Ghanbari and N. Hajheidari, *Anal. Biochem.*, **473**, 53–62 (2015).

35. R. Q. Ferreira and L. A. Avaca, *Electroanalysis*, **20**, No. 12, 1323–1329 (2008).

36. Kh. Z. Brainina, D. P. Varzakova, and E. L. Gerasimova, *Zh. Anal. Khim.*, **67**, No. 4, 409–415 (2012).

37. G. K. Ziyatdinova, H. C. Budnikov, V. I. Pogorel'tzev, and T. S. Ganeev, *Talanta*, **68**, No. 3, 800–805 (2006).

38. A. Agarwal, S. Roychoudhury, R. Sharma, S. Gupta, A. Majzoub, and E. Sabanegh, *Reprod. BioMed. Online*, **34**, No. 1, 48–57 (2016).

39. I. F. Abdullin, E. N. Turova, G. K. Ziyatdinova, and G. K. Budnikov, *Zh. Anal. Khim.*, **57**, No. 4, 418–421 (2002).

40. B. D. Atanasova, R. J. Simpson, I. S. Mudway, A. C. Y. Li, K. N. Tzatchev, and T. J. Peters, *Bulg. Chem. Commun.*, **35**, No. 3, 167–173 (2003).

41. V. N. Sorokopudov, N. A. Luchina, and O. A. Mostovoi, *Nauchn. Vedomosti BelGU, Ser. Med. Farm.*, **99**, No. 4, issue 13/2, 196–198 (2011).

42. V. V. Khasanov, G. L. Ryzhova, and E. V. Mal'tseva, *Khim. Rastit. Syr'ya*, No. 3, 63–75 (2004).

43. T. G. Tsyupko, I. S. Petrakova, N. S. Brilenok, N. A. Nikolaeva, D. A. Chuprykina, Z. A. Temerdashev, and V. I. Vershinin, *Anal. Kontrol*, **15**, No. 3, 287–298 (2011).

44. N. M. Litvinko, L. A. Skorostetskaya, and D. O. Gerlovskii, BY Patent No. 19,670, Dec. 30, 2015, *Method for Determination of Total Antioxidant Capacity of Biological Fluid Using the Lipid Phase.*

45. N. M. Litvinko, L. A. Skorostetskaya, and D. O. Gerlovskii, BY Patent No. 19,669, Dec. 30, 2015, *Composition and Method for Determination of Total Antioxidant Activity of Blood Serum.*

46. N. M. Litvinko, L. A. Skorostetskaya, and D. O. Gerlovskii, *Dokl. Nats. Akad. Nauk Belarusi*, **61**, No. 4, 60–68 (2017).

47. N. M. Litvinko, *Vestsi Nats. Akad. Navuk Belarusi, Ser. Khim. Navuk*, **56**, No. 4, 115–128 (2020).

48. N. M. Litvinko, L. A. Skorostetskaya, D. O. Gerlovskii, and Yu. Sh. Ermakovich, *Science — Innovative Development of Society* [in Russian], Belaruskaya Navuka, Minsk (2020), pp. 391–403.

49. V. S. Kamyshnikov, N. N. Yakovlev-Malykh, N. M. Litvinko, O. V. Sviridov, L. V. Dubovskaya, T. M. Yuraga, and T. D. Borisenko, *Lab. Diagn. Vost. Evropa*, **9**, Nos. 1–2, 98–115 (2020).

50. N. M. Litvinko, L. A. Skorostetskaya, D. O. Gerlovskii, Yu. Sh. Ermakovich, and G. S. Evdokimova, *Dokl. Nats. Akad. Nauk Belarusi*, **63**, No. 1, 44–54 (2019); doi: 10.29235/1561-8323-2019-63-1-44-54.

51. N. M. Litvinko, L. A. Skorostetskaya, T. G. Gudko, M. M. Timokhova, V. S. Kamyshnikov, E. I. Vizhinis, and V. A. Vorobei, *Dokl. Nats. Akad. Nauk Belarusi*, **60**, No. 4, 82–88 (2016).

52. N. M. Litvinko, L. A. Skorostetskaya, and D. O. Gerlovsky, *Chem. Phys. Lipids*, **211**, 44–51 (2018); doi: 10.1016/j.chemphyslip.2017.10.010.

53. N. M. Litvinko, L. A. Skorostetskaya, and D. O. Gerlovsky, *Curr. Res. Bioorg. Org. Chem.*, **3**, 19 (2019); doi: 10.29011/2639-4685-02.

54. L. A. Skoorstetskaya, N. I. Pavlyuchenko, Yu. Sh. Ermakovich, S. P. Konopel′ko, and N. M. Litvinko, in: *Proc. II Int. Conf. "Free Radicals in Chemistry and Life"* [in Russian], October 19–20, 2017, Bel. Gos. Univ., Minsk (2017), pp. 124–126.

55. M. S. Osipchik, E. A. Remeeva, N. I. Pavlyuchenko, L. A. Skorostetskaya, and N. M. Litvinko, in: *Proc. III Int. Conf. "Free Radicals in Chemistry and Life"* [in Russian], October 10–11, 2019, Bel. Gos. Univ., Minsk (2019), pp. 70–71.

56. A. N. Schechter, *Blood*, **112**, 3927–3938 (2008).

57. N. M. Litvinko, G. M. Andreyuk, and M. A. Kisel, *FASEB J.*, **11**, No. 9 (Suppl.) (Abstracts of *17th Int. Congress Biochem. Mol. Biol.*, 2623, CA 1306 (1997).

58. L. R. McLean, K. A. Hagaman, and W. S. Davidson, *Lipids*, **28**, No. 6, 505–509 (1993).

59. G. M. Andreyuk and M. A. Kisel′, *Bioorg. Khim.*, **23**, No. 4, 290–293 (1997).

60. N. M. Litvinko, Yu. Sh. Yermakovich, L. A. Skorostetskaya, and D. O. Gerlovsky, *Proc. BIT's 7th Annual Conf. AnalytiX-2019*, April 12–15, 2019, Singapore (2018), p. 59.

61. Y. Yermakovich, L. A. Skorostetskaya, and N. M. Litvinko, *Thesis Int. Conf. "Chemistry, Structure and Functional of Biomolecules,"* May 22–25, 2018, Inst. Bioorg. Chem. NAS Belarus, Minsk (2018), pp. 185–186.