Peroxy-radical-mediated chemiluminescence: mechanistic diversity and fundamentals for antioxidant assay

Galina F. Fedorova, Alexey V. Trofimov*, Rostislav F. Vasil'ev,* and Timur L. Veprintsev

Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, 119991 Moscow, Russian Federation
E-mail: avt_2003@mail.ru, vasilev@sky.chph.ras.ru

Dedicated to our esteemed colleague and friend Professor Waldemar Adam on the occasion of his 70th birthday

Abstract

Herein, we give a retrospective overview and analyze recent developments in the field of chemiluminescence derived from organic oxidation processes (so called oxy-chemiluminescence), most prominently, mediated by peroxy radicals. As evidenced from the presented analysis, the diversities of the reaction and the excited-state-generation mechanisms are governed mainly by a diverse chemical nature of substrates being oxidized. The notable oxy-chemiluminescence cases, which involve peroxy radicals as key reactive species, refer to oxidation of saturated and unsaturated hydrocarbons, polymers, lipids and proteins. The general feature of the considered chemiluminescence processes pertains to a formation of high-energy cyclic intermediates (tetroxides and/or dioxetanes), whose cleavage yields electronically excited products. The considered modes of the chemiluminescence enhancement encompass the energy-transfer and the electron-transfer mechanisms. Most prominent application of the discussed oxy-chemiluminescence phenomenon resides in a versatile chemiluminescent assay to monitor antioxidants (both their concentration and reactivity) in chemical and biological media.

Keywords: Chemiluminescence, excited states, oxidation, peroxy radicals, peroxides, antioxidants

Table of Contents

* To whom correspondence should be addressed

1. Introduction

The emission of light, derived either from chemical processes (chemiluminescence) or living organisms (bioluminescence), is of prime interest for both pure and applied science. Indeed, studies on chemi- and bioluminescence provide relevant insights for understanding the fundamentals of energy conversion in numerous fields of chemistry and biology and for scrutinizing the reaction mechanisms. Besides, these light-emission phenomena are of great import for numerous commercial applications, first of all, for modern analytical techniques. In this context, the diversities of light-generating processes and modes of their harnessing are impressive.[1-16] The subject matter of the present contribution encompasses salient facets of chemiluminescence derived from oxidation of organic substrates, most prominently, mediated by peroxy radicals. These oxidation reactions constitute notable chemical generators of electronically excited states. To convey a decisive role of molecular oxygen, such kind of chemiluminescent processes is sometimes called oxy-luminescence[17,18] or oxy-chemiluminescence,[19] and in the present work we follow the latter terminology. Elucidation of the excited-state generation in oxidation reactions provides an undoubted challenge for pure and applied oxidation chemistry. Herein, we discuss the relevant details of the chemiexcitation process and subsequent events (chemiluminescence emission, its enhancement and quenching by

pertinent luminophores) and give an account of the oxy-chemiluminescence kinetics with the emphasis on oxidation in the presence of antioxidants. The reason for this emphasis resides in a paramount role of antioxidants in numerous areas of biology, material science, chemical and analytical technologies. Besides, in view of interdisciplinary character of the present contribution, some related topics had to be touched on. This refers to the relevant aspects of photochemistry, photophysics, oxidation and peroxide chemistry.

2. Historical background

The discovery of oxy-chemiluminescence dates back to the end of 1950's. At that time, oxidation of organic substances in solutions was of particular interest both for pure and industrial chemistry. This process constitutes a radical-chain reaction with degenerated branching of chains.[6-8] The branching agent (peroxide) is accumulated slowly and a long (hours) induction period precedes faster chemical transformation. To shorten this period, initiators or catalysts may be introduced, but these foreign components complicate the process and form side products. Alternatively (and more efficiently), a short-duration blow of chemically active gas (*e.g.*, chlorine, ozone, nitrogen dioxide) through the solution allows to achieve the desired effect.[20,21] The advantage of the latter method is that the action of gaseous initiator may be shut off at any moment simply by stopping the blow.

Of all the gaseous initiators, ozone seemed to be the most suitable. The matter is that ozone reactions are often accompanied by chemiluminescence, which might be used to monitor the oxidation process. As a matter of fact, weak chemiluminescence was registered under the ozonolysis of isodecane at 20-90 °C.[20,21] The chemiluminescence intensity passed through the maximum; then, it immediately decreased after stopping the ozone stream and resumed quickly when the ozone flow was again switched on. Conclusion was arrived at that chemiluminescence was excited in the reaction of ozone with an intermediate which, in its turn, had been formed in the ozone reaction with isodecane.[20] This fact manifested the complex character of processes responsible for gaseous initiation.

In June 1958, one unexpected observation gave an essential impetus to the considered research.[20,21] Ozone was carefully removed from the solution, but, surprisingly, chemiluminescence did not disappear completely. This reproducible observation signified that some chemically active products of ozonolysis were responsible for the excited-state generation. As possible candidates for these chemiluminescence initiators, peroxides, hydroperoxides and ozonides were considered. For that reason, attempts were made to observe light emission from solutions of these oxygen-containing reagents. As a result, a weak chemiluminescence has been indeed found in solutions of benzoyl peroxide and hydroperoxides of some hydrocarbons (*e.g.*, tetralin, isopropylbenzene, 2,7-dimethyloctane).[20,21] It is noteworthy that photomultiplier used in these studies was sensitive to the 350-600 nm region, which corresponds to the energy interval of 80-45 kcal/mol. Chemiexcited light-emitting particles should acquire such a large amount of

energy in one elementary step, and only recombination of radicals (*i.e.*, decomposition products of starting materials) might be exothermal enough to provide this portion of energy. Once this fact was realized, it was followed by a burst of activity in this fascinating area of organic chemiluminescence.[21] In the context of the present story, it should be pointed out that high reactivity of molecular oxygen towards carbon-centered radicals causes efficient conversion of the latter into peroxy-radical species in oxygenated reaction mixtures. In subsequent sections, we consider the prominent features of the oxy-chemiluminescence derived mainly from peroxy-radical reactions.

3. Mechanistic aspects

Regardless of a mechanistic complexity, every chemiluminescent process may be conventionally divided into "chemical" and "physical" parts. The former stage relates to a formation of an excited reaction product, while the latter is a sequence of photophysical events, which ends up with a photon emission. Emitter of light is being excited either directly in the chemical process (*direct chemiluminescence*) or through "indirect" mechanisms (*indirect chemiluminescence*), most prominently by energy transfer from a primary-excited reaction product to a certain luminophore purposely added to the reaction mixture (*energy-transfer-enhanced chemiluminescence*).

3.1. Excited-state generation and direct chemiluminescence

The intensity of direct chemiluminescence (i_{DC}) is given by an obvious expression (1), in

$$i_{DC} = \Phi^*\Phi v \quad (1)$$

which Φ^* stays for the chemiexcitation yield, Φ represents the luminescence (fluorescence or phosphorescence) yield of the excited emitter and v is the reaction rate. Clearly, the Φ^* value depends heavily on the reaction mechanism and below we discuss the mechanistic diversities of oxy-chemiluminescence processes.

3.1.1. Oxy-chemiluminescence of saturated hydrocarbons

Oxidation of saturated hydrocarbons (RH) by molecular oxygen constitutes a radical-chain reaction, which involves a wealth of stages (for instance, oxidation of alkylaromatic hydrocarbons at 100 °C involves more than 40 elementary steps![19,22]). However, in most cases, merely the chain-termination is essential for the excited-state generation.[6-8,23] The nature of this step depends decisively on the radical structure (or, in other words, on the structure of the parent hydrocarbon) and herein we consider the pertinent structural peculiarities of the chemiexcitation process. In this context, the type of the oxidizable (*i.e.*, the weakest) C-H bond in a hydrocarbon molecule is of prime importance.

In alkanes, the energy of the primary carbon-hydrogen bond amounts to 98-100 kcal/mol, that of the secondary C-H bond constitutes 94-95 kcal/mol, while the tertiary one "costs" *ca.* 91 kcal/mol. The same trend remains also for alkylaromatic hydrocarbons, although, in this case the bond energies are somewhat lower.[21,24]

Hydrocarbons with a secondary C-H bond. Hydrocarbons with a secondary C-H bond as an oxidizable functionality constitute the best studied oxy-chemiluminescence case,[6-8,24-26] which merits to be considered first. Ethylbenzene, $PhCH_2CH_3$, was the first substrate, whose oxidation followed by light emission was studied in detail.[6-8,24] In this substrate, the CH_2 group furnishes the oxidizable functionality, the reactivity of methyl substituent is nominal, while the C-H bond of the aromatic ring, whose energy is as high as 103 kcal/mol, is not engaged in oxidation process at all. Scheme 1 conveys adequately the oxidation mechanism of such type of hydrocarbon (Y designates an initiator, easily decomposable peroxide or azo compound, used as source of initiating free radicals, $r^{\bullet}$).

$$Y \xrightarrow{\Delta} 2r^{\bullet}$$

$$RH + r^{\bullet} \longrightarrow R^{\bullet} + rH$$

$$R^{\bullet} + O_2 \xrightarrow{k_2} ROO^{\bullet}$$

$$ROO^{\bullet} + RH \xrightarrow{k_3} ROOH + R^{\bullet}$$

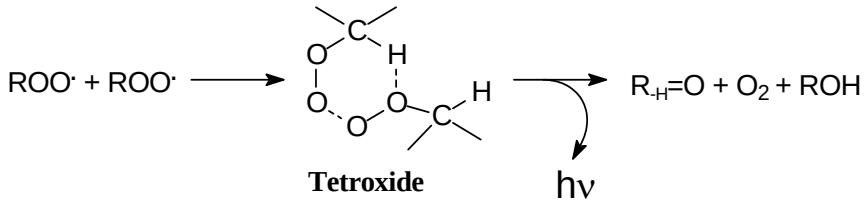
$$R^{\bullet} + R^{\bullet} \xrightarrow{k_4} \textit{Inactive products}$$

$$R^{\bullet} + ROO^{\bullet} \xrightarrow{k_5} \textit{Inactive products}$$

$$ROO^{\bullet} + ROO^{\bullet} \xrightarrow{k_6} R_{-H}{=}O + ROH + O_2 + h\nu$$

Scheme 1

For subsequent discussion, it is of mechanistic import that the peroxy radicals, $ROO^{\cdot}$, become the only chain carriers, and their self reaction thus remains the sole chain-termination reaction at oxygen concentrations, whose order of magnitude is as low as 10^{-6} M.[11,,22,24] These peroxy radicals disproportionate according to the Russell mechanism,[27] through the intermediate tetroxide[28] (Scheme 2), whose decomposition is exothermal enough (100-120 kcal/mol) to generate excited-state products, namely, ketone and singlet oxygen.[6,29] Alcohol, the third decomposition product, is formed unexcited, provided $ROO^{\cdot}$ radicals do not possess any chromophore group. Experimental evidence for the intervention of the tetroxide in the $ROO^{\cdot}$ disproportionation (Scheme 2) has been obtained from the low-temperature ESR studies.[30-32] This chain termination mechanism is general for a great variety of chemical and biological oxidation processes.[33,34]



Scheme 2

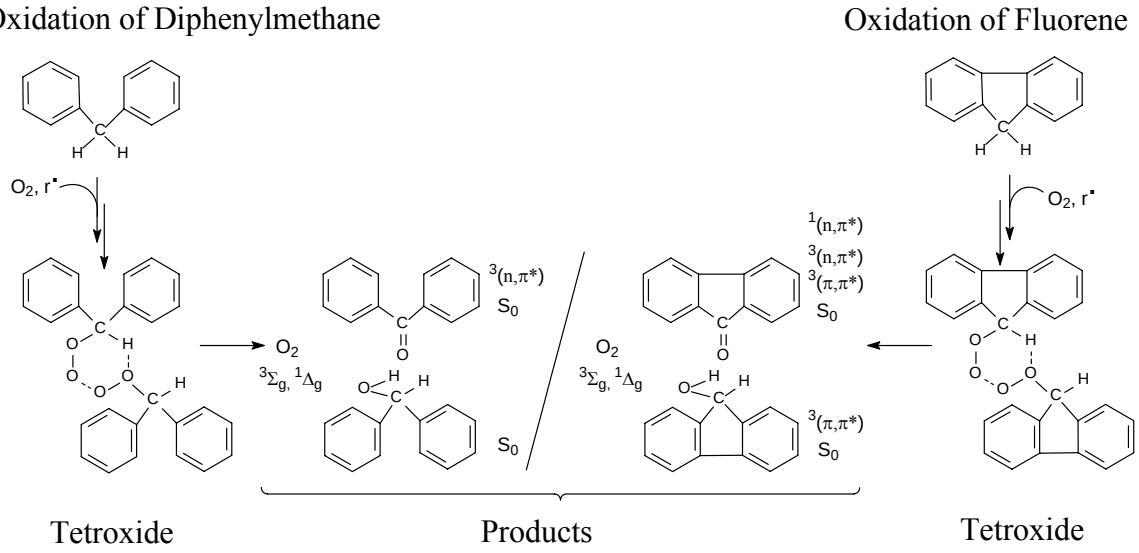
Excited ketone (R_{-H}=O*) formed in the process depicted in Scheme 2 is responsible for visible-range chemiluminescence, which is in most cases phosphorescence from the ^{3}(n,π*) state of the carbonyl group, while singlet oxygen emits infrared light from its $^1\Delta$g state. The spectroscopic manifestation for the chemically excited ketone phosphorescence rests on the oxy-chemiluminescence bands in the region of 400-500 nm with flat maxima around 420-450 nm, which are similar for various hydrocarbons being oxidized.[6] The spectral evidence for the singlet-oxygen generation in the process shown in Scheme 1 stems from the chemiluminescence emission at 1270 nm, which follows oxidation of hydrocarbons.[33-37]

The yields of triplet ketones in oxidation of hydrocarbons are normally equal to 10^{-3}-10^{-2}, whereas efficiency of the excited-singlet-state generation is several orders of magnitude lower.[8,24] The singlet-oxygen yield in the disproportionation of primary and secondary peroxy radicals averages about 0.1.[33,35-37] It is noteworthy that the quantum efficiency of the $^1O_2$ generation is independent of the peroxy-radical structure except the cases of heteroatom in proximity to the peroxyl moiety, which lowers the $^1O_2$ yield.[35-37]

The pattern of the excited-state generation considered above refers to the oxidation of structurally simple hydrocarbons (*i.e.*, with one oxidizable functionality and no extra luminophore groups). In this case, the exothermal ROO· disproportionation had no choice but to generate the ^{3}(n,π*)–excited ketone or singlet oxygen. The incentive of ensuing studies was to bring more "democracy" to the chemiexcitation process: What should happen if the alternatives to the ^{3}(n,π*) excitation were to exist? Close inspection of chemiluminescence in the diphenyl-*versus* diphenylenemethane (fluorene) oxidation shed light on this query.[21,38] What is different in chemiluminescence generated on oxidation of these akin hydrocarbons?

First, while being oxidized diphenylmethane and fluorene yield ketones, whose photophysical properties are contrasting: Contrary to benzophenone (oxidation product of diphenylmethane), 9-fluorenone (fluorene oxidation product) does not phosphoresce, but is able to fluoresce.[39-41] It is noteworthy that excitation of the ketone triplet in the ROO· disproportionation constitutes a spin-allowed process as triplet oxygen is also formed in this case, while the generation of a *singlet*-excited carbonyl product is spin-forbidden.[24] Thus, it was of mechanistic importance to find out whether oxidation of fluorene gives rise to fluorescence of fluorenone and, if so, whether singlet excitation occurs directly in the chemical process or through the intersystem crossing from the primary-excited upper triplet states.

Second, a prominent distinction between the diphenyl- and diphenylenemethane (fluorene) oxidations is that in the former case all the chromophores (carbonyl group and molecular oxygen) are formed *in the reaction*, while in the latter case there exists also a chromophore (fluorenyl moiety), which *remains unchanged* during the oxidation process. (Scheme 3). This raised the question of whether the energy released in the reaction is directed (partly) into this persistent chromophore or is forming the molecular moiety in this chemical process a necessary requisite for its chemiexcitation? In this context, a possibility of alcohol (9-fluorenol) excitation was of interest. Indeed, due to the fluorenyl group, the generation of the electronically excited fluorenol becomes *energetically* feasible; conversely, in the diphenylmethane oxidation, the electronic excitation of alcohol (diphenylcarbinol) is not possible for energy reason. Furthermore, the electronic excitation of alcohols in oxidation of hydrocarbons has never been observed before, because in all previously studied cases the alcohols did not possess the chromophore groups, whose excitation is energetically possible.



Scheme 3

Our studies have disclosed that the direct chemiluminescence constituted the *fluorescence* of fluorenone;[38] moreover, the generation of the singlet-excited carbonyl chromophore occured *directly* in the course of tetroxide cleavage (Scheme 3), which violated Wigner's rule, rather than through the intersystem crossing from the primary-excited upper triplets.[38] This was evidenced in a special experiment, in which the upper (n,π*) triplet state of fluorenone has been selectively populated by energy transfer from chemiexcited benzophenone.[38] In view of the inefficient triplet-to-singlet intersystem crossing in the fluorenone molecule, population of its ^{3}(n,π*) state did not lead to the excitation of the fluorescent state of this ketone.[38]

Violation of Wigner's rule in the ROO$^{\cdot}$ disproportionation (Scheme 3) is not the only unprecedented phenomenon disclosed in this chemiluminescence study. Also significant was the

observation of the triplet-excited alcohol (9-fluorenol).[38] Although the $^3(\pi,\pi^*)$-excited alcohol does not phosphoresce, it reveals itself through the energy transfer to the europium chelate, Eu(III) tris-thenoyltrifluoroacetonate-1,10-phenanthroline (triplet-energy acceptor), followed by the red (613 nm) luminescence of the latter.[38] (Details of the energy-transfer approach to the elucidation of the chemical excited-state generation are considered in Section 3.2.1.) It is noteworthy that for the europium chelate used in these studies as the energy acceptor triplet-triplet energy transfer from fluorenol is *exothermal* (and thereby diffusion-controlled), while transferring the triplet energy from fluorenone to the same acceptor is *endothermal* by 5 kcal/mol.[38] This substantial endothermicity prevents sensibilization of the chelate luminescence by triplet-excited fluorenone formed in the same reaction step.[38]

Thus, the reviewed studies show that, in fact, all energetically accessible excited states may be populated in the $ROO^{\cdot}$ disproportionation (Scheme 2).

Hydrocarbons with a primary C-H bond. In principle, this case retains the salient features of the oxidation and chemiexcitation mechanisms, which apply for the substrates with an oxidizable secondary C-H bond.[37,42] Indeed, the disproportionation of the primary peroxy radicals also obeys the Russell mechanism considered above for the secondary peroxy-radical chain carriers (*cf.* Scheme 2) and the decomposition of the putative intermediary tetroxide is exothermal enough for the electronic excitation of the carbonyl products and singlet oxygen.[37,42] The excitation yields observed in this case (*ca.* 0.1 for $^1O_2$ and 10^{-3} for carbonyls) are similar to those exhibited by a self reaction of the secondary peroxy radicals.[37,42] However, the overall chemiluminescence yield (a product of the chemiexcitation and the light-emission yields, $\Phi^*\Phi$; *cf.* Eq. 1) measured for a representative case of methylbenzenes turned out to be astonishingly low, namely 10^{-10}![42] Detailed scrutiny of this unprecedented observation has disclosed that such a minute quantity is accounted for by the extremely low lifetimes of the pertinent $^3(n,\pi^*)$–excited benzaldehydes, the carbonyl chemiluminescence emitters under the reaction conditions. The reason for that resides in high reactivity of the $^3(n,\pi^*)$ state of benzaldehydes and its efficient quenching by the π-system of benzene rings of the substrates and benzene as solvent.[42] It is noteworthy that the rate constants of intra- and intermolecular processes, namely triplet-singlet emission and quenching, vary in a similar way for the series toluene > *o*-xylene > *m*-xylene ≈ *p*-xylene and this correlates with the energy gap between $^3(n,\pi^*)$– and $^3(\pi,\pi^*)$–levels.[42]

Hydrocarbons with a tertiary C-H bond. Tertiary peroxy radicals furnish a peculiar case, for which the chemiluminescence mechanism depicted in Scheme 2 does not apply. Two peroxy radicals of cumene, $Ph(CH_3)_2COO^{\cdot}$, which picture the pertinent situation, cannot disproportionate through the Russell-type tetroxide (Scheme 2), because in this case such an intermediate lacks an abstractable hydrogen atom in a proximity to the peroxyl moiety.[37,43] In this case, decomposition of tetroxide may proceed through the cleavage of the O-O bonds concomitant with the formation of two oxy radicals and O_2, whose excitation is unlikely in view of endothermicity of the considered reaction path.[43]

Nevertheless, a free-radical oxidation of cumene is also accompanied by the chemiluminescence emission both in visible[19,37,43,44] and infrared[33,36,37] regions due to the excited carbonyl species and singlet oxygen respectively. The efficiency of the visible-region (carbonyl) chemiluminescence is by one or two orders of magnitude lower than that for hydrocarbons with a secondary C-H bond as oxidizable functionality.[44] The singlet-oxygen generation proceeds with the yield of ca. 0.02,[37] which is several times lower than in the cases of primary and secondary peroxy radicals. Mechanistic alternatives for this chemiluminescent process involve complex reaction schemes to account for the excited-state generation.[33,36,37,43,44] The mechanism inferred from the most recent study[43] engages not only tertiary peroxy radicals, $Ph(CH_3)_2COO^{\cdot}$, but also primary ones, $PhCH(CH_3)CH_2OO^{\cdot}$. The latter radical species are formed through the abstraction of hydrogen from the methyl group of cumene, which yields the $PhCH(CH_3)CH_2^{\cdot}$ radical with a subsequent capture of molecular oxygen. The stationary concentration of the primary-patterned radicals is expectedly low since the removal of the primary hydrogen is less probable than that of the tertiary one. But the reaction of the primary radicals with the tertiary ones is fast, since in this case the asymmetric tetroxide $PhCH(CH_3)CH_2OOOOC(CH_3)_2Ph$ is formed, which possesses the *abstractable hydrogen atom*. The latter structural feature warrants a fast irreversible decomposition of such an intermediate with the formation of excited aldehyde $PhCH(CH_3)CHO$ in the same way as in the case of secondary peroxide radicals. This mechanism has been substantiated both computationally[43] and experimentally[44] through the studies on the cumene oxidation kinetics.

Figure 1 displays the energy profile (as calculated by the PM3 method) for the decomposition of the considered asymmetric tetroxide, which intervenes in the cumene oxidation.[43] Along with the reaction energy profile, Figure 1 shows the *bond orders* as a function of the reaction coordinate. Inspection of this dependence provides the necessary insight into the reaction mechanism. In this context, the change in the order of the O^1O^6 bond, a precursor to the free O_2 molecule, is of prime interest. At the beginning of the final reaction stage, this bond order approaches 2, which corresponds to the double bond of $^1O_2$, *i.e.,* the system moves on the singlet potential-energy surface. However, the final product is the triplet oxygen ($^3O_2$), a biradical species with a sesquialteral bond. Hence, the intersystem crossing is inevitable to get to the lowest (triplet) surface, $R_{-H}=O(S_0) + ROH(S_0) + O_2(^3\Sigma)$. As evident from Figure 1, the decomposition of tetroxide proceeds synchronously (orders of all the bonds change simultaneously!) rather than through earlier proposed stepwise mechanism,[29] which suggests elimination of ROH from the tetroxide to yield relatively persistent biradical $^{\bullet}R_{-H}OOO^{\bullet}$, whose cleavage leads to the ketone and O_2 molecules.

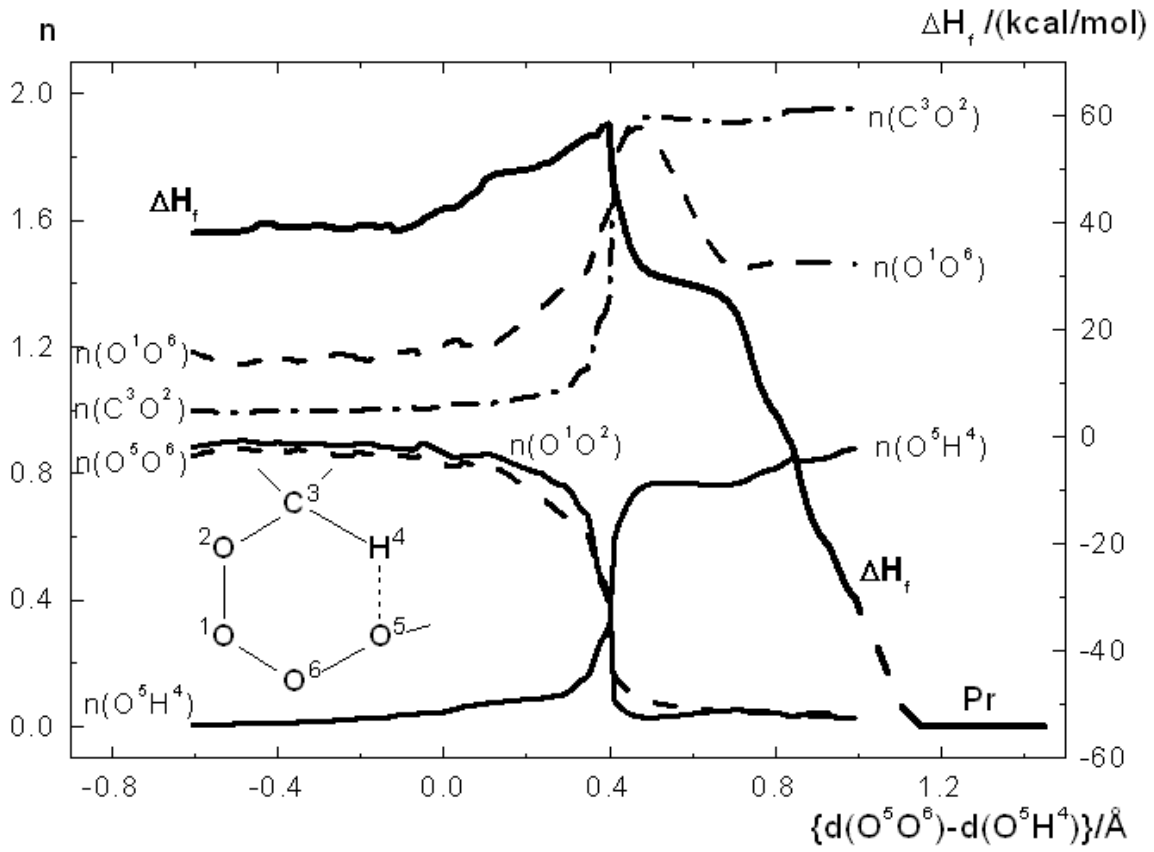


Figure 1. Dependence of energy and bond orders of the asymmetric six-membered tetroxide cycle on "reaction coordinate" (length difference of breaking the O^5O^6 and forming the O^5H^4 bonds) for the Russell-type cleavage mechanism. Calculations by the PM3 method with 3 HOMO and 3 LUMO involved in configuration interaction.

A query on the stepwise *versus* concerted mechanism of the tetroxide decomposition is general for the chemistry of cyclic peroxides. A prominent example is the cleavage of dioxetanes, which is a function of the molecular structure and exhibits concerted or stepwise features depending on substituents.[45] Very probably, the same pertains to the tetroxide, whose decomposition proceeds synchronously or stepwise also depending on structure of this intermediate. Indeed, a wide body of evidence suggests the stepwise-cleavage mechanism of the six-membered tetroxide species formed by secondary peroxy radicals,[29] while the tetroxide $PhCH(CH_3)CH_2OOOOC(CH_3)_2Ph$, a *particular case* considered herein (*cf.* Figure 1), decomposes synchronously.[43]

3.1.2. Oxy-chemiluminescence of unsaturated hydrocarbons

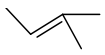
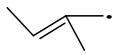
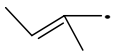
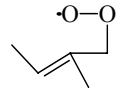
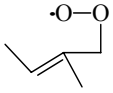
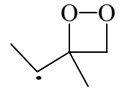
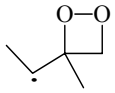
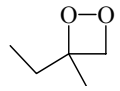
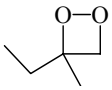
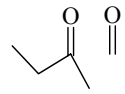
The chemiluminescence yield in oxidation of alkenes is by one to three orders of magnitude lower than that for hydrocarbons with the oxidizable secondary C-H bond and strongly varies for different compounds.[46] But the main distinction is that in this case, alongside the "classical"

chemiluminescence fraction derived from a *free-radical* process through the Russell chain-termination mechanism, a substantial *molecular* contribution is present, the latter is not quenched by antioxidants.[46] The contribution of the molecular component is also strongly varying for different alkenes.[46] Besides, its intensity changes in the course of reaction: it is rather high in the presence of O_2, but after keeping in the absence of oxygen and repeated switching on the oxygen blow through the solution it appears to be reduced. Addition of an antioxidant to the reaction mixture immediately suppresses the radical component of the overall light emission. Then, the remaining molecular component decreases according to exponential law (the rate constants are of the order of 10^{-4} s^{-1}).[46] Estimation of the emitter characteristics (lifetimes, quantum yields and radiation constants) leads to values typical for the phosphorescence of carbonyl compounds.[46] Such behavior shows that the observed molecular chemiluminescence is excited in chemical transformations of oxidation products, which are persistent enough to accumulate in the course of reaction.

Possible sources of this molecular contribution to the oxy-chemiluminescence of unsaturated hydrocarbons are dioxetane intermediates formed by a cyclization of alkylperoxy radicals.[21,47] This may be exemplary illustrated by considering the oxidation of 1,1,2-trimethylethylene, $H_3CCH=C(CH_3)_2$, as a model alkene substrate.[21] In this case, under a constant reaction-initiation rate, several types of radicals exist in their stationary concentrations. These are carbon-centered radicals, the products of addition of initiating radical $r^{\bullet}$ to the double bond, or of the H-atom abstraction by this radical from methyl groups, for instance: $H_3CCHrC^{\bullet}(CH_3)_2$, $H_3CC^{\bullet}HCr(CH_3)_2$, $H_2C^{\bullet}CH=C(CH_3)_2$, $H_3CCH=C(CH_3)C^{\bullet}H_2$. The fast addition of O_2 transforms C radicals into a number of peroxy-radical species: $H_3CCHrC(CH_3)_2OO^{\bullet}$, $H_3CC(OO^{\bullet})HCr(CH_3)_2$, $H_2C(OO^{\bullet})CH=C(CH_3)_2$, $H_3CCH=C(CH_3)CH_2OO^{\bullet}$.

As a result, various reactions of the mentioned radicals occur in solution.[21] Combination of the $ROO^{\bullet}$ radicals is exothermal, but it gives products which are not luminophoric, *i.e.*, it is not a chemiluminescent process.[21] Disproportionation of $ROO^{\bullet}$ gives the emitters of "classic" *free-radical* chemiluminescence. However, the chain =C-C-O-$O^{\bullet}$ in radicals $H_2C(OO^{\bullet})CH=C(CH_3)_2$ and $H_3CCH=C(CH_3)CH_2OO^{\bullet}$ is flexible enough (the bonds are single) for overlapping the unpaired electron with the π MO of the double bond, and so, for forming a four-membered dioxetane cycle.[21] The latter cycle stores large chemical energy, which may be easily transformed into electronic excitation upon decomposition of dioxetane. The pertinent stages of this process are illustrated in Table 1.[21]

Table 1. The sequence of reactions leading to excitation of molecular chemiluminescence

No	*Reagent*	Step / Process	Product
1		Hydrogen removal from CH_3 group of alkene by an initiating radical; formation of a C-centered radical	
2		Oxygen addition to the C-centered radical; formation of a peroxide radical of alkene	
3		Cyclization of the alkene peroxide radical; formation of a dioxetane C-centered radical	
4		Hydrogen-atom abstraction by the dioxetane C-centered radical from any substrate; formation of a dioxetane	
5		Dioxetane thermolysis giving carbonyl products; chemiexcitation	

In more detail, the steps listed in Table 1 have the following implications:

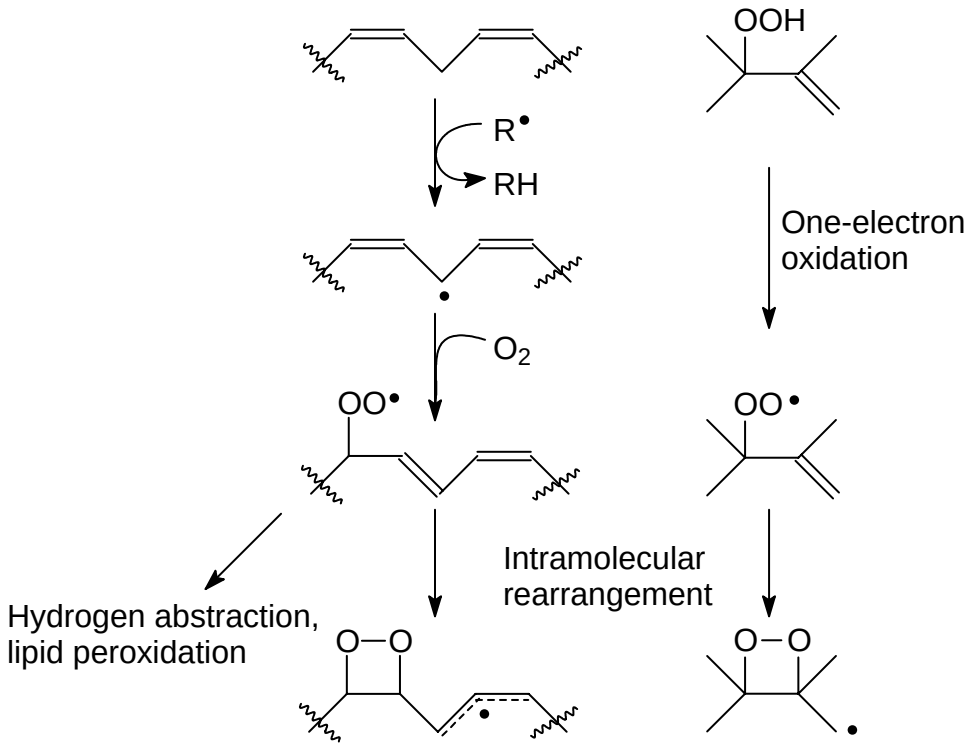
1. Entry of a hydrocarbon into a radical-chain oxidation reaction. The free valence of the C-centered radical (and, then, the $OO^{\bullet}$ group) is localized in the α position to the double bond, which is very important for further transformations;
2. Chain propagation; it readily proceeds even at low oxygen concentrations;
3. A key process for the considered mechanism. The PM3 calculations show that the heat of the peroxy-radical formation and that of dioxetane C-centered radical are equal to 5 and 6 kcal/mol respectively; consequently, reaction 3 is almost thermally neutral.[21] The barrier is equal to 20-24 kcal/mol, which provides a sufficient rate at 60 °C;[21]
4. This process is analogous in its nature to process 1;
5. Dioxetane decomposition with the formation of the T_1 and, with lower yield, the S_1 states of carbonyl products. The estimated activation energy is 27[21] (experimental value 24.5 kcal/mol[48]); the excitation yields of the T_1 and S_1 states for pertinent dioxetanes are 0.1 to 0.2 and 10^{-4} to 10^{-3}.[48]

Inspection of the literature on dioxetane thermolysis disclosed an intricate mechanism of this seemingly simple process.[4] But although full structural details of the reaction profile remain controversial, the general salient mechanistic conclusion is that thermolysis starts with the O-O bond rupture to generate a biradical intermediate, which is followed by the C-C bond cleavage to afford the final ketone products. Survey of the recent computational studies reveals that during the breakage of the O-O bond the rest of the molecular skeleton undergoes structural changes; in particular, the C-C bond is subject to essential elongation.[4] This C-C bond stretching

simultaneous with the O-O bond cleavage is consistent with the *merged* dioxetane decomposition mechanism originally proposed by Adam and Baader.[45] Thus, the excited-state generation in the dioxetane decomposition proceeds through the intervention of a biradical structure (in the present case, $^{\bullet}OCMeEtCH_2O^{\bullet}$). There exists computational evidence[21] that in the latter species the T_1 state (according to Hund's rule) is lower than the ground S_0 state, and that is why it is effectively populated providing high chemiexcitation yield of the triplet O=CMeEt or $O=CH_2$ and, then, in the presence of suitable luminophore (9,10-dibromoanthracene or europium chelate), a measurable chemiluminescence emission.[21] Of course, the luminescence is weak since chemical yields of dioxetanes are low.[21]

3.1.3. Oxy-chemiluminescence of lipids

The chemiluminescence associated with lipid peroxidation has found a widespread use in monitoring the oxidative stress.[47,49-56] The main traits of this excited-state generation are typical for oxidation of unsaturated organic substrates discussed in Section 3.1.2. Thus, both the singlet-oxygen infrared emission and the blue-green phosphorescence from triplet carbonyls have been found in oxidation of lipids.[47] The formation of these excited species has been discussed in terms of both the Russell mechanism of the alkylperoxy-radical disproportionation and the intervention of dioxetane intermediates in oxidation process (*cf.* Section 3.1.2). In polyunsaturated fatty acids (PUFA), dioxetane intermediates are suggested to be formed by cyclization of alkylperoxy radicals produced during peroxidation process (Scheme 4, *at the left*) or by one-electron oxidation of PUFA-derived hydroperoxides (Scheme 4, *at the right*).[47]



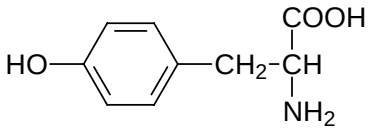
Scheme 4

To prove the mentioned dioxetane mechanism, relevant studies were pursued, in which tetramethylethene(TME)-derived hydroperoxide, 3-hydroperoxy-2,3-dimethyl-1-butene (TMEOOH), was used as a model of the PUFA hydroperoxides.[47] By using a range of one-electron oxidants and reductants and exploring a variety of experimental techniques (chemiluminescence, ESR, TLC and GC) it was convincingly shown that the peroxy radicals, derived in one-electron oxidation of TMEOOH, undergo cyclization into dioxetane intermediate species, whose cleavage results in excited acetone, a chemiluminescence emitter. Thus, in peroxidation of unsaturated substrates (including lipids) the dioxetane chemiluminescence path should be considered alongside the Russell peroxy-radical disproportionation.

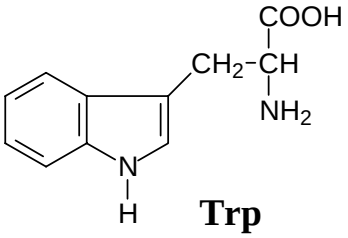
3.1.4. Oxy-chemiluminescence of amino acids and proteins

In addition to lipid peroxidation (Section 3.1.3), oxidation of proteins contributes to the chemiluminescence emission associated with the oxidative stress.[57-62] Experimental efforts to elucidate the mechanism of the excited-state generation, derived from protein systems on free-radical oxidation, involved studies on oxy-chemiluminescence of proteins, peptides and isolated amino acids.[61,62] Oxidation of these substrates was initiated by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a water-soluble source of free radicals.

While on the subject of amino-acids oxidation, the first finding which merits notice is that only tyrosine (Tyr) and tryptophan (Trp) produced marked oxy-chemiluminescence on incubation with AAPH.[61] Light emission from other amino acids did not exceed background.[61]



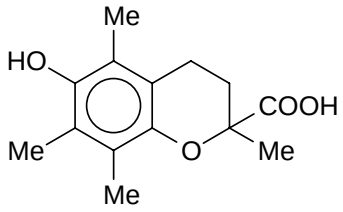
Tyr



Trp

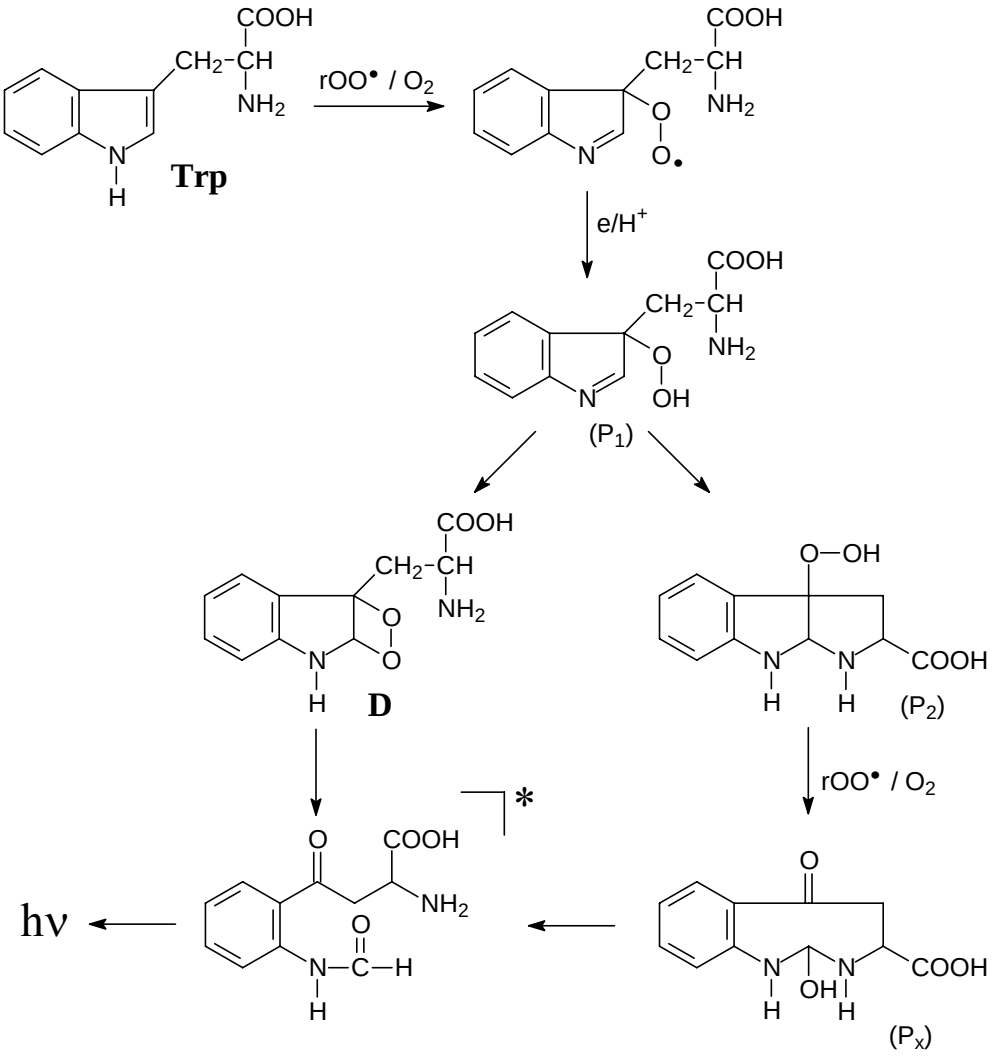
The behavior of the chemiluminescence emission arisen from free Tyr and Trp oxidation was very similar to that derived from Tyr- and Trp-containing peptides and proteins.[62] This refers to chemiluminescence time profiles and to dependencies of the light emission on reagent concentrations and oxidation rate. Besides, the effect of Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), a strong free-radical scavenger, was very similar in the case of free amino acids and in the case of proteins: In both cases, addition of this phenolic antioxidant caused an abrupt drop of essential part of the light intensity (*ca.* 70%).[61,62] The remaining chemiluminescence showed a considerably slower decay. This observation suggests that the oxy-chemiluminescence is generated in at least two pathways, involving radical-mediated and nonradical processes, the former was inhibited by Trolox, while the latter was not sensible to this inhibitor. The addition of peroxidase-like compounds, such as Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]one) results in rapid quenching of the "nonradical" light emission;[61,62]

this implies that peroxide-like and/or hydroperoxide-like compounds contribute to such chemiluminescent path both in case of protein and isolated Tyr and Trp.



Trolox

In the long run, protein oxy-chemiluminescence is associated almost exclusively with the oxidation of Tyr and Trp residues,[62] but the detailed mechanism of this complex excited-state generation is still far from being established. However, a wide body of evidence supports some reasonable mechanistic conjectures,[61,62] which provide a relevant insight to understand the nature of the considered light-emission processes. The salient features of the tryptophan oxy-chemiluminescence mechanism emerged in the literature (see Aspée and Lissi[61]) are shown in Scheme 5. Such reaction scheme is compatible with the majority of experimental data regarding the mechanism of the Trp oxidation, the distribution of products and the produced emitting species.[61] The key intermediate, the tryptophan hydroperoxide (P_1), has been proposed as one of the main intermediates in the hydroxyl radical-mediated oxidation of Trp.[63-65] The production of P_2 was also established in this process.[61] The formation and decomposition of the dioxetane intermediate (D), a precursor to singlet-excited emitter (*N*-formylkynurenine), has been reported for the enzymatic oxidation of indol derivatives[66,67] and for the photooxidation of Trp.[68] Besides, kynurenine and *N*-formylkynurenine were observed in studies on the AAPH-initiated oxidation of Trp[61] through their characteristic fluorescence emissions.[68,69]



Scheme 5

Certainly, Scheme 5 gives merely a provisional view on the Trp oxy-chemiluminescence mechanism, whose details require more scrutiny. Thus, one may question whether the electronically excited *N*-formylkynurenine may derive from the P_x intermediate. (The authors[61] refer to the literature, in which the P_x species has been proposed as a precursor to the ground-state *N*-formylkynurenine in thermal and photo-induced Trp oxidation.[70]) Moreover, the authors[61] of the above reaction scheme emphasized that even this complex mechanism should be considered as oversimplification, since it does not take into account other reaction pathways,[61] such as peroxy-radical addition or electron transfer[71] and participation of singlet oxygen. Singlet oxygen may be formed in Russell-type reactions and/or in the quenching of the primary-excited product.[72,73] (Plausible involvement of $^1O_2$ was evidenced, in particular, by a certain increase of the chemiluminescence intensity in the presence of D_2O[61]).

In conclusion, it is noteworthy that the characteristics of the light emission associated with oxidation of free amino acids are very similar to those observed for some cases of oxidation in membranes and organelles,[61] which suggests that the oxidation of amino acids plays sometimes a key role in biological systems.

3.1.5. Oxy-chemiluminescence of synthetic polymers

It has long been known that polymers exposed to heating under ambient-air conditions emit light,[17] which was attributed to the excited oxidation products. In addition to this light emission derived from autooxidation, incorporation of easily decomposable initiators, free-radical sources, into polymeric materials also caused generation of chemiluminescence on heating.[74,75] Thus, situation with polymers resembled observations on liquid-phase oxy-chemiluminescence. However, this similarity was rather outward. Indeed, chemiluminescence in oxidation of polymers is certainly a mechanistically more complex phenomenon. Intensive studies on polymer oxy-chemiluminescence disclosed a variety of possible chemical and physical pathways leading to the excited-state generation, which have been thoroughly reviewed.[18,,76,77]

The low oxy-chemiluminescence intensity under thermal oxidation of polymer samples was attributed to a "forbidden" character of the observed emission and its spectra indeed matched well the phosphorescence spectra of carbonyl chromophores.[18,77,78] Thus, on this point, the excited-state generation in oxidation of polymers also reminds oxy-chemiluminescence of other hydrocarbon substrates considered in the previous sections. However, the validity of the Russell mechanism in case of polymers was questioned.[79] Indeed, the latter mechanism requires at least one of the peroxy radicals, involved in the chain termination, to be primary or secondary (*cf.* Section 3.1.1), but, for instance, polypropylene, in which *tertiary* radicals predominate, nevertheless gives well-measurable oxy-chemiluminescence.[79] In this connection, it is of mechanistic import that the considered oxidation processes are accompanied by a production of alkoxy radicals that may cleave to produce, ultimately, primary and secondary peroxy radicals.[80,81] These primary and secondary radicals, in their turn, may react with tertiary peroxy-radical species through the Russell-type mechanism and thus generate chemiluminescence (similar situation applies to the oxidation of cumene, *cf.* Section 3.1.1).

Then, the suggestions were made that there may be several light-emission processes occurring in polymer[82] and that the identity of the light-emitting species changes with oxidation time.[83]

For the maximum chemiluminescence intensity and oxygen concentration, a linear relationship has been disclosed.[84] The conclusion was drawn that the chemiluminescence derives from decomposition of hydroperoxides rather than from the Russell mechanism. Indeed, recent experimental works[79,85-87] support the involvement of hydroperoxides in the light generation during oxidation of polypropylene, a model polymeric substrate.

A detailed inspection of the possible mechanisms for the polymer oxy-chemiluminescence has revealed that regardless of the mechanism in operation, *i.e.*, Russell-type peroxy-radical disproportionation or hydroperoxide cleavage, the chemiluminescence intensity should be a

function of the hydroperoxide concentration.[79] Besides, for both of these mechanistic options the concentration of the carbonyl products (=C=O) at time t, $[=C=O]_t$, should be proportional to the integral of the chemiluminescence-intensity (i) time profile, which is expressed by Eq. (2).[79]

$$[= C = O]_t \sim \int_0^t i dt \quad (2)$$

A novel technique, which allows to monitor simultaneously the chemiluminescence emission and the Fourier transform infrared (FTIR) emission spectra (FTIES), enabled to verify this expectation.[79] It turned out that in the case of the polypropylene oxidation the values of $[=C=O]_t$ acquired through the FTIES measurements did not match the $\int_0^t i dt$ values. However, the authors of the mentioned work[79] have found that the *accumulation* of carbonyls is required for the chemiluminescence emission. For instance, at 150 °C no chemiluminescence was observed for the first 15 min of the polypropylene oxidation, whereas the [=C=O] growth was well-measurable; after this induction period the chemiluminescence emission appeared and gradually increased in intensity.[79]

To rationalize these observations, two mechanisms were discussed.[79] The first one invoked energy transfer from triplet-excited carbonyls to a more efficient phosphorescer, which accumulates in the polymer during oxidation, while the second resided in the electron donor-acceptor interaction between the carbonyl species and a peroxidic compound, which is likely to be an acyl peroxide. In more detail, this hypothesis is considered in Section 3.2.2, in which the indirect chemiluminescence mechanisms are discussed.

Thus, despite a considerable progress in understanding the nature of the polymer oxy-chemiluminescence, further experimentation is required for a comprehensive description of this phenomenon.

3.2. Indirect chemiluminescence. Secondary processes and chemiluminescence enhancement

Alternatively to the immediate light emission (direct chemiluminescence), primary-excited reaction products undergo other deactivation processes, in particular, energy transfer to the molecules, capable of accepting the electronic excitation, or chemical reactions with any components of the reaction mixture ("photochemistry without light"). The former of these secondary processes leads to the excited energy acceptor, while the latter may yield either excited or ground-state final reaction product. If the luminescence (fluorescence or phosphorescence) quantum yield of a secondary-excited species is higher than that of the primary-excited reaction product, the enhancement of the chemiluminescence intensity is observed; otherwise the quenching of the overall light emission takes place.

In earlier works,[6,88] the term "*activation*" was used to designate any chemiluminescence enhancement, including energy-transfer processes. Accordingly, enhanced chemiluminescence was termed "activated chemiluminescence" and the enhancer molecule was called "activator". However, later on, the term "activation" came into use for the cases, in which the activating

agent interacts *chemically* with the substrate molecule, most prominently through the charge transfer and electron donor-acceptor mechanisms[4, 89-93] Thus, to avoid confusion in terminology, it is prudent to use exclusively the term "enhancement" instead of "activation" while talking about the physical (*i.e.*, energy-transfer) secondary processes in the chemiluminescent system.

The electronic energy transfer is of prime importance for elucidating the excited-state generation in chemical processes or simply for the light-emission enhancement to raise a sensitivity of chemiluminescence assays. For that reason, below we consider the main energy-transfer cases, which are of particular practical use in oxy-chemiluminescence studies.

3.2.1. Energy transfer in chemiluminescent systems

Energy transfer in connection with the chemiluminescence phenomenon was first reported still in 1925 by Kautsky and Neitzke,[94] and nowadays the transfer of electronic excitation to suitable luminescent acceptors is the most popular photophysical technique for counting excited singlet and triplet states generated in chemical reactions.[6,8,95] In the presence of these luminophores, both the direct light emission (i_{DC}) of the energy donor (primary excited reaction product) and sensibilized by energy transfer (ET) acceptor's emission (i_{ET}) contribute to the overall chemiluminescence intensity (i), which is expressed by Eq. 3.

$$i = i_{DC} + i_{ET} \tag{3}$$

Clearly, explicit form of Eq. 3 and other practically useful expressions derived from it depend on the energy-transfer mechanism, *i.e.*, on whether the singlet-singlet (S-S), triplet-triplet (T-T) or triplet-singlet (T-S) energy transfer operates. Herein, the following nomenclature is used throughout the kinetic derivations: "A" designates energy acceptor for the S-S energy transfer, "B" refers to the acceptor in T-T energy transfer, and "C" represents the acceptor in T-S energy transfer; the energy-transfer mediator (in most cases, naphthalene) is abbreviated by *N*.

Enhanced chemiluminescence by S-S energy transfer. If the primary-excited reaction product (emitter of the direct chemiluminescence) is singlet, the use of the S-S energy transfer to enhance the light emission is customary. In this case, 9,10-diphenylanthracece serves as the most convenient energy acceptor (A). For the enhanced chemiluminescence intensity (i_{EC}^{SS}) one easily obtains Eq. 4, where Φ^S and Φ^{fl} are the singlet

$$i_{EC}^{SS} = \frac{\Phi^s \Phi^{fl} v}{1 + k_{ET}^{SS} \tau_s^0 [A]} + \frac{\Phi^s \Phi_A^{fl} k_{ET}^{SS} \tau_s^0 [A]\, v}{1 + k_{ET}^{SS} \tau_s^0 [A]} \tag{4}$$

excitation yield and fluorescence quantum yield of the direct chemiluminescence emitter, Φ_A^{fl} is a fluorescence quantum yield of the acceptor A, τ_S^0 stays for the singlet lifetime of the energy donor molecule (*i.e.*, of the direct light emitter), and k_{ET}^{SS} is the rate constant of the S-S energy transfer from the donor to the acceptor.

The amplification factor $\kappa_A^{SS} = i_{EC}^{SS}/i_{DC}$ of the chemiluminescence intensity may be defined according to Eq. 5, in which k_{fl} is the rate constant of the fluorescence of the donor with $\Phi^{fl} = k_{fl}\tau_S^o$.

$$\kappa_A^{SS} = \frac{i_{EC}^{SS}}{i_{DC}} = \frac{1 + \frac{\Phi_A^{fl}}{k_{fl}} k_{ET}^{SS}[A]}{1 + k_{ET}^{SS}\tau_S^0[A]} \quad (5)$$

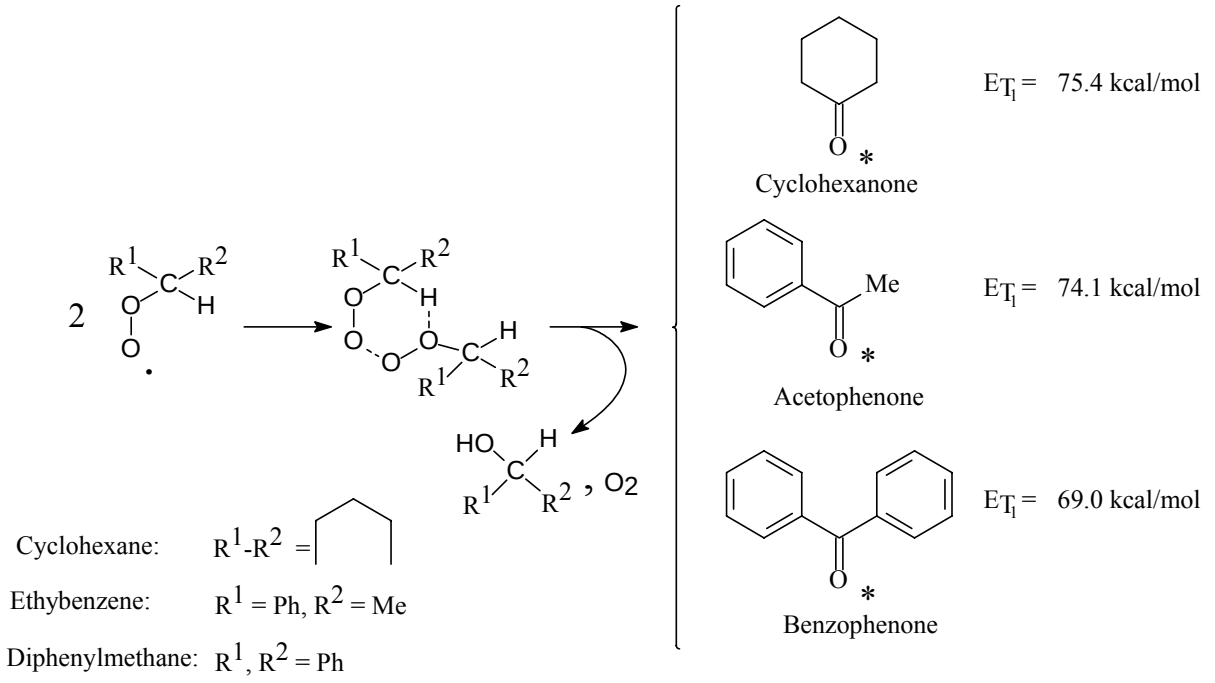
The dependence of $i_{EC}{}^{SS}$ on the concentration of A can be treated conveniently by a linear expression of Eq. 5 as shown in Eq. 6. The intercept $(\Phi_A{}^{fl}/\Phi^{fl} - 1)^{-1}$ of a double-reciprocal plot according to Eq. 6 allows the fluorescence quantum yield Φ^{fl} of the direct chemiluminescence emitter to be calculated provided the fluorescence quantum yield $\Phi_A{}^{fl}$ of A is known.

$$\left(\kappa_A^{SS} - 1\right)^{-1} = \left(\frac{\Phi_A^{fl}}{\Phi^{fl}} - 1\right)^{-1} \left(1 + \frac{1}{k_{ET}^{SS}\tau_S^0} \frac{1}{[A]}\right) \quad (6)$$

For the case of singlet-excited light emitter, Eq. 1 may be written as $i_{DC} = \Phi^S \Phi^{fl} v$. Thus, with the known reaction rate (v) and the fluorescence quantum yield (Φ^{fl}), measured by the S-S energy-transfer method according to Eq. 6, the singlet-excitation yield (Φ^S) may be easily obtained. For that, it is merely necessary to calibrate the emission intensity against any standard (for instance, luminol standard[96,97] or the Hastings-Weber scintillation "cocktail"[98]).

Alternatively, Φ^S may be obtained from the double-reciprocal plot of the enhanced-chemiluminescence intensity and concentration of A.[1,5,24] For the reliability of the results acquired through this procedure, [A] should be relatively high (for the effective quenching of the primary-excited emitter).[24] However, this is ocassionally undesirable in cases of the free-radical-mediated oxidation, in particular, because of the inhibition properties of the pertinent luminophores in these reaction systems.[24] Conversely, in the method described herein, one may use essentially smaller [A] values. That is why this method is recommended for studies on the chemiluminescence generated in the peroxy-radical reactions.

Enhanced chemiluminescence by T-T energy transfer. As outlined in Section 3.1.1, on free-radical oxidation of hydrocarbons, the generation of triplet-excited ketones is favored over their singlet excitation; thus, the disproportionation of the peroxy radicals is, in most cases, a "dark" source of the *triplet* states, which is exemplary illustrated in Scheme 6.



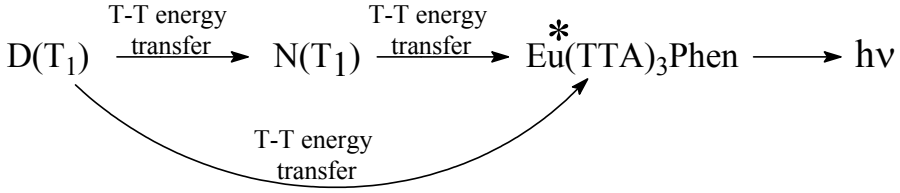
Scheme 6

In this case,[6-8] the direct chemiluminescence constitutes phosphorescence and the chemiluminescence enhancement by T-T energy transfer is described by expressions of the same form as Eqs. 4-6. To accommodate these expressions for the T-T case, the fluorescence quantum yield Φ_A^{fl} needs to be replaced by the phosphorescence quantum yield Φ_B^{ph} of acceptor B and Φ^{fl} should be substituted by the donor phosphorescence quantum yield Φ^{ph}. Analogously, the rate constant k_{ET}^{TT} of the T-T energy transfer should be used instead of k_{ET}^{SS}, the triplet donor lifetime τ_T^o should replace the singlet lifetime τ_S^o, and the singlet excitation yield Φ^S needs to be substituted by the triplet donor excitation yield Φ^T. Thus, the chemiluminescence intensity enhanced by T-T energy transfer is given by Eq. 7.

$$i_{EC}^{TT} = \frac{\Phi^T \Phi^{ph} \nu}{1 + k_{ET}^{TT} \tau_T^0 [B]} + \frac{\Phi^T \Phi_B^{ph} k_{ET}^{TT} \tau_T^0 [B] \nu}{1 + k_{ET}^{TT} \tau_T^0 [B]} \quad (7)$$

An example *par excellence* of the energy acceptor for triplet states, generated in free-radical oxidation, is furnished by the europium chelate $Eu(TTA)_3Phen$ (TTA = thenoyltrifluoroacetone, Phen = 1,10-phenanthroline).[6,8] This luminophore has been proved to be inert towards all the constituents of the chemiluminescent mixture and the pertinent free radicals (alkyl, alkoxy and peroxy ones), possesses high luminescence yield (0.04-0.2, depending on solvent and temperature[99]) and narrow emission band at 613 nm ($^5D_0 \rightarrow {}^7F_2$ of the Eu^{3+} ion).[99] Besides, the energy-accepting T_1 level of its ligands is rather low, *i.e.*, 58.4 kcal/mol, which enables to monitor the low-lying chemically excited triplet states of the reaction products.

Enhanced chemiluminescence by T-T energy transfer assisted by a long-lived mediator triplet state. The enhancement of the chemiluminescence may be insignificant when either [B] is low because of the poor solubility of B or short lifetime τ_T^o of the chemiexcited triplet. In these cases, it is instrumental to use the energy-transfer mediators, whose solubility and triplet-state lifetimes are high enough.[8] Naphthalene perfectly matches this requisite and, thus, is a suitable energy-transfer mediator.[8] Scheme 7 illustrates the



Scheme 7

naphthalene-mediated T-T energy transfer from the primary-excited triplet energy donor, $D(T_1)$, to the europium chelate, $Eu(TTA)_3Phen$. Kinetic analysis of Scheme 7 finally gives a simple expression (Eq. 8) for the additional amplification factor (κ_N^{TT}) of the

$$\kappa_N^{TT} = \frac{i_B}{i_B^0} = \frac{1 + \frac{k_{TN}k_{NB}}{k_{TB}}\tau_N^B[N]}{1 + k_{TN}\tau_T^B[N]} \quad (8)$$

chemiluminescence emission enhanced by europium chelate, $Eu(TTA)_3Phen$ (B), mediated by the T_1 state (61 kcal/mol) of naphthalene (N).[95] In this expression, i_B and i_B^0 represent the intensities of the triplet-sensitized light emission by the europium chelate (B) in the presence and in the absence of naphthalene, k_{TN}, k_{TB} and k_{NB} are the rate constants of the T-T energy transfer from the primary donor to naphthalene, from the primary donor directly to the europium chelate, and from naphthalene to the chelate; τ_T^B and τ_N^B are the lifetimes of the primary-donor and naphthalene triplet states in the presence of chelate, whose relationships to the donor and the naphthalene triplet lifetimes, τ_T^0 and τ_N^0, in the absence of B are given by the Stern-Volmer Eqs. 9 and 10.

$$\tau_T^B = \frac{\tau_T^0}{1 + k_{TB}\tau_T^0[B]} \quad (9)$$

$$\tau_N^B = \frac{\tau_N^0}{1 + k_{NB}\tau_N^0[B]} \quad (10)$$

The rate constants k_{TN}, k_{NB}, and k_{TB} in Eq. 8 represent a spin-allowed, exothermic, diffusion-controlled T-T energy transfer and all are of the same order. Therefore, from Eq. 8 it is evident that since τ_N^B is larger than τ_T^B, the chemiluminescence amplification factor becomes greater than unity ($\kappa_N^{TT} > 1$), *i.e.*, naphthalene enhances the overall light-emission intensity in the presence of the europium chelate (B).

Conveniently, Eq. 8 can be rewritten in a linear form, which permits one to analyze the experimental dependence of the enhanced chemiluminescence intensity on the naphthalene concentration (Eq. 11).[95] A double-reciprocal plot of the experimental data according to Eq. 11

$$\left(\kappa_{\mathrm{N}}^{\mathrm{TT}}-1\right)^{-1}=\left(\frac{k_{\mathrm{NB}}\tau_{\mathrm{N}}^{\mathrm{B}}}{k_{\mathrm{TB}}\tau_{\mathrm{T}}^{\mathrm{B}}}-1\right)^{-1}\left(1+\frac{1}{k_{\mathrm{TN}}\tau_{\mathrm{T}}^{\mathrm{B}}}\frac{1}{[\mathrm{N}]}\right) \tag{11}$$

affords the Stern-Volmer constant $k_{\mathrm{TN}}\tau_{\mathrm{T}}{}^{\mathrm{B}}$ for the quenching of the chemiexcited triplet donor by naphthalene in the presence of B. But since [B] is normally small, $k_{\mathrm{TN}}\tau_{\mathrm{T}}{}^{\mathrm{B}}$ is approximately equal to $k_{\mathrm{TN}}\tau_{\mathrm{T}}{}^{0}$ (see Eq. 9), a true value of this Stern-Volmer constant.

Enhanced chemiluminescence by T-S energy transfer. In a context of the present story, it is noteworthy that the T-S energy transfer in liquid media owes its discovery to the studies on the enhanced oxy-chemiluminescence![6,8,88] In these works, anthracene derivatives were added to the solutions of hydrocarbons being oxidized, sources of *triplet* ketones (*cf.* Scheme 6). This caused enhancement of the light emission, however, the amplification factor poorly correlated with quantum yields of the anthracenes. But then, a fundamental feature of the observed excitation transfer has been established. The case in point is the heavy-atom effect revealed by the rate constant ($k_{\mathrm{ET}}{}^{\mathrm{TS}}$) of this process: the larger the atomic number of substituents and the number of these substituents, the higher the $k_{\mathrm{ET}}{}^{\mathrm{TS}}$ value was observed.[6,8,88] This observation has been explained in terms of the spin-orbital coupling in the acceptor (anthracene) molecule, which, according to the perturbation theory, provides the necessary "mixing" of singlet and triplet states to allow the "spin-forbidden" T-S energy transfer to proceed.[6,8,88]

Clearly, the chemiluminescence intensity enhanced by T-S energy transfer is given by Eq. 12, whose form is similar to that of Eqs. 4 and 7.

$$i_{\mathrm{EC}}^{\mathrm{TS}}=\frac{\Phi^{\mathrm{T}}\Phi^{\mathrm{ph}}v}{1+(k_{\mathrm{ET}}^{\mathrm{TT}}+k_{\mathrm{ET}}^{\mathrm{TS}})\tau_{\mathrm{T}}^{0}[\mathrm{C}]}+\frac{\Phi^{\mathrm{T}}\Phi_{\mathrm{C}}^{\mathrm{fl}}k_{\mathrm{ET}}^{\mathrm{TS}}\tau_{\mathrm{T}}^{0}[\mathrm{C}]\ v}{1+(k_{\mathrm{ET}}^{\mathrm{TT}}+k_{\mathrm{ET}}^{\mathrm{TS}})\tau_{\mathrm{T}}^{0}[\mathrm{C}]} \tag{12}$$

For a number of anthracenes, the $k_{\mathrm{ET}}{}^{\mathrm{TS}}$ value has been found[6,8,88] to be proportional to the sum of squared radial parts of the matrix elements of the spin-orbital coupling, $k_{\mathrm{ET}}{}^{\mathrm{TS}}\sim\Sigma\varsigma_{\mathrm{i}}{}^{2}$.

Later on, studies on spectroscopy of substituted anthracenes showed that the second excited triplet level, T_2, lays somewhat higher than S_1 for most of anthracene derivatives.[100-104] This implies that the T-S energy transfer to anthracenes may proceed stepwise: allowed intermolecular T-T transfer to the T_2 of acceptor, then followed by the intersystem crossing to S_1.[105] Indeed, through varying the T_1 energy of the donor (acetophenone derivatives) over the S_1-T_2 energy gap of the acceptor (9,10-dibromoanthracene) it has been shown that the $k_{\mathrm{ET}}{}^{\mathrm{TS}}$ value is controlled by the activation barrier, which matches the energy difference between T_1 of the corresponding acetophenone and T_2 of 9,10-dibromoanthracene.[106] These clear-cut data provide the evidence for the stepwise energy-transfer mechanism. However, in terms of this mechanism, the energy acceptors, whose S_1 level is higher than T_2, should violate the dependence of $k_{\mathrm{ET}}{}^{\mathrm{TS}}$ on $\Sigma\varsigma_{\mathrm{i}}{}^{2}$, which is definitively not the case.[107] Neither unsubstituted anthracene nor 9-bromoanthracene disturb this dependency, provided the donor T_1 energy is high enough, *i.e.*, if

E_{T1}(donor) > E_{S1},E_{T2}(acceptor) (in Figure 2 it corresponds to the data obtained for cyclohexanone as energy donor).[21,107] The latter fact supports the "direct" ($T_1 \rightarrow S_1$) mechanism.

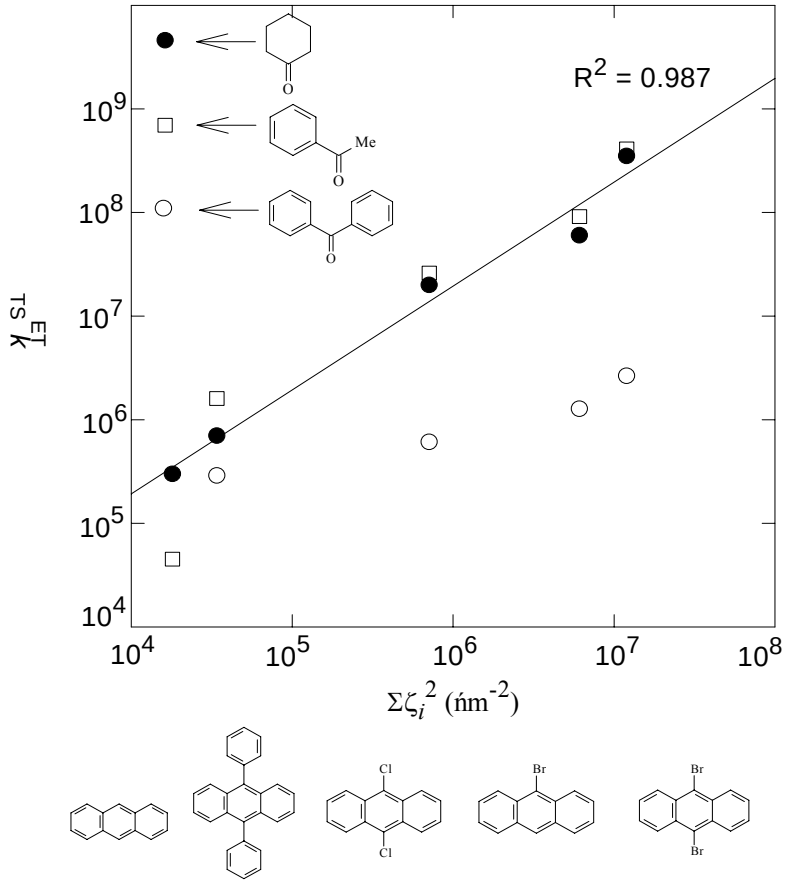


Figure 2. Dependence of the T-S energy-transfer rate constant (k_{ET}^{TS}) on the strength of spin-orbital coupling ($\Sigma\zeta_i^2$) in acceptor molecules (anthracene derivatives); chemiexcited ketones (cyclohexanone, acetophenone and benzophenone) are used as energy donors (*cf.* Scheme 6).

Thus, triplet-singlet energy transfer constitutes a mechanistic controversy. To circumvent this problem, we put forward the following concept, which reconciles conflicting mechanisms.[107] This concept resides in considering the spin-orbital coupling only in the region, in which S_1 and T_2 terms overlap. Actually, this region may be thought of as a vibronic state of "mixed" multiplicity. It is noteworthy that similar approach has been offered to rationalize some puzzling spectral features of photo- and chemiexcited acetone at low pressures.[108-110] In the latter case, a mixed-multiplicity state lies above the zero-vibrational level of S_1 and manifests itself through the short-lived luminescence band, which is seen only in the low-pressure vapors (10^{-4}-10^{-3} Torr); it provides efficient intersystem crossing to the nearest vibronic state of T_1 (with $v > 0$) due to the high density of vibrational states.

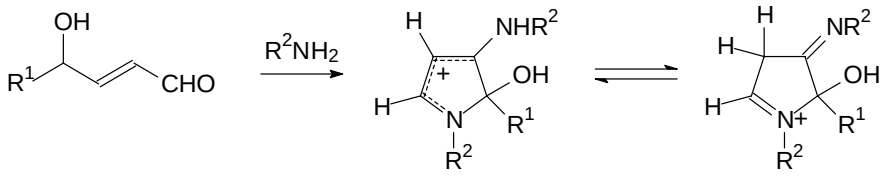
Clearly, the putative "mixed" state should be located near S_1, if $E_{S1} > E_{T2}$, but near T_2 when $E_{T2} > E_{S1}$. Hence, when the energy of the donor T_1 state exceeds *both* the S_1 and the T_2 energies of the acceptor, a distinction between the "direct" and stepwise T-S energy-transfer models makes no sense.[21,107] For that reason, acceptors with $E_{S1} > E_{T2}$ do not violate the dependence of k_{ET}^{TS} on $\Sigma\varsigma_i^2$ (Figure 2).

T-S energy transfer to 9,10-dibromoanthracene[6-8] and its water-soluble derivatives[111] is a widely used experimental tool to monitor triplet excited states in photochemical, photobiological, chemi- and bioluminescent processes.

Intricacies of the energy transfer in complex oxy-chemiluminescent systems. The above-considered energy transfer processes refer to the situation, in which the energy donor (singlet or triplet) constitutes the only primary-excited reaction product and the energy acceptors are the foreign luminophores added at will to the reaction mixture. Clearly, this "ideal" case does not always occur in chemiluminescence processes.

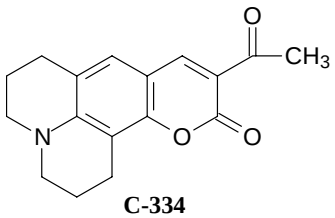
Indeed, formation of more than one electronically excited species (of the same or different multiplicities) in one chemiluminescent reaction is not a unique happenstance. Apart from the case mentioned in Section 3.1.1 (*cf.* Scheme 3, *on the right*), oxidation of methylethylketone furnishes another relevant example.[24,112] In the latter oxy-chemiluminescent reaction, the formation of both the singlet- and the triplet-excited biacetyl takes place.[24,112] Oxidation of unsaturated hydrocarbons also yields both excited singlet and reaction triplet products.[46]

Besides, sometimes extra luminophores, potential energy acceptors, may be produced in a side (nonchemiluminescent) reaction path, particularly in lipids and lipoproteins being oxidized. Thus, in lipid peroxidation 4-hydroxy-2-enals may form, giving rise to further fluorophore generation.[113,114] In this case, a major fluorophore appears to be derived from (*E*)-4-hydroxy-2-non- (or –hex)-enal [$R^1 = C_5H_{11}$ or C_2H_5] (Scheme 8).[114] These aldehydes cross-link with lysine residues in proteins. To elucidate the chemical, structural and spectral properties of such type of "endogenic" fluorophores, the model compounds have been prepared using simple amines (R^2 = Pr, Bu, CH_2CH_2OMe, *cf.* Scheme 8).[115] Typically for vinylogous amidinium cations, H(4) is exchanged by tautomeric equilibrium shown in Scheme 8. Clearly, such fluorophores, whose production *in situ* entails initial Schiff-base formation and subsequent oxidation,[114] may be involved in energy-transfer processes in bioluminescence systems.

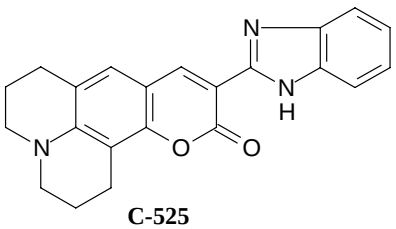


Scheme 8

Harnessing the energy transfer in oxy-chemiluminescence studies requires appropriate energy acceptors. For homogeneous hydrocarbon solutions, the substituted anthracenes and chelate complexes of lanthanides are the most suitable and efficient luminophores, which serve this purpose.[6-8] However, these luminophores are often useless in biological media, mainly because of a poor solubility and chemical reactivity towards biosubstrates. Thus, 9,10-dibromoanthracene, an efficient enhancer of the hydrocarbon oxy-chemiluminescence, only slightly enhances the chemiluminescence intensity in liposomal suspensions.[116] The water-soluble anthracene derivatives, 9,10-dibromo- and 9,10-diphenylanthracene-2-sulfonate, are more suitable fluorescence probes for chemiexcitation studies in biological media.[111] As for the lanthanide complexes, the efficient triplet-energy acceptors in hydrocarbon solutions, these chemiluminescence enhancers become unstable in biological suspensions since they dissociate in the presence of phosphates and influence kinetics of lipid peroxidation.[117,118] Some laser dyes, most prominently the quinolizin coumarins C-334 and C-525, are proved to be chemically inert and efficient light amplifiers for the oxy-chemiluminescence derived from lipid peroxidation.[118,119] In more detail, model studies[119] have shown that C-334 and C-525 can be easily integrated into the human leukemia HL-60 cells and successfully used as physical enhancers of chemiluminescence induced by the lipid-soluble free-radical azo initiator 2,2'-azobis(2,4-dimethyl valeronitrile), AMVN. These coumarins did not inhibit the AMVN-initiated peroxidation of membrane phospholipids in the HL-60 cells, and no consumption of such dyes occurred in the course of the AMVN-induced oxidative stress.[119] Redox status, evaluated by the intracellular GSH content, remained unchanged after treatment with C-334 and C-525.[119] Besides, the viability of the HL-60 cells was not affected by coumarins both in the presence and in the absence of oxidants.[119] Thus, the quinolizin coumarins constitutes a novel class of potential physical enhancers of chemiluminescence for monitoring the free-radical-mediated peroxidation in living cells.



C-334

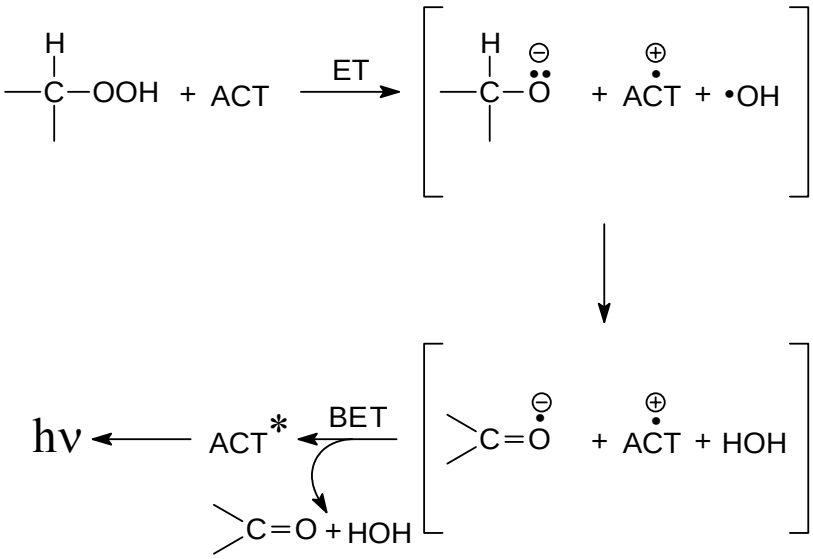


C-525

3.2.2. Alternative modes of the oxy-chemiluminescence enhancement by organic luminophores

Amplification of the chemiluminescence intensity by organic luminophores through the energy transfer, considered in the previous section, proceeds without altering the reaction mechanism and kinetics. However, this is not necessarily the case for every luminophore, which causes the enhancement of the light emission derived from oxidation processes. In this context, luminophores with low oxidation potentials are of particular interest since they are prone to the electron-[89-93] or the partial-charge-transfer[15,120] interactions with peroxide products or

intermediates giving rise to chemiluminescence emission. These important cases of the excited-state generation have been recently comprehensively reviewed.[5] The often discussed mechanism involves the forward and the backward electron-transfer steps, which are exemplary illustrated in Scheme 9 for the reaction of hydroperoxide with organic luminophore ("activator", ACT) molecule.[79,121] In this scheme, ET denotes electron transfer, while BET means electron back-transfer process. This mechanism is known as chemically initiated electron-exchange luminescence (CIEEL), which was originally proposed (with minor distinctions) for the intermolecular reactions of easily oxidizable luminophores (some anthracenes, amines, perylene, rubrene, *etc.*) with cyclic peroxides, namely diphenoylperoxide,[89,90] α-peroxy lactones[91] and appropriate dioxetanes[92] and then extended to some intramolecular cases[3,4,122-131] including the firefly bioluminescence generation.[132] Recently, the evidence for the CIEEL mechanism has been reported also for the peroxyoxalate reactions,[5,93] glowing examples of the organic chemiluminescence. It should be noted, however, that in certain systems the partial charge transfer[15,120] and the donor-acceptor interactions involving exciplex formation[133,134] may operate instead of the full-electron-transfer mechanism.



Scheme 9

It is noteworthy that the mechanism depicted in Scheme 9 has been recently discussed in a context of the polymer oxy-chemiluminescence.[79,121] It has been proposed that the peroxidic species formed in the polypropylene thermooxidation may react with the luminescent oxidation products (carbonyls or some unidentified species) to yield the chemiluminescence through the CIEEL mechanism.[79] Furthermore, doping the polypropylene with 9,10-diphenylanthracene, a readily oxidizable anthracene derivative, results in chemiluminescence emission (on polypropylene thermooxidation), whose behavior is consistent with the CIEEL mechanism, in which 9,10-diphenylanthracene plays a role of activator (ACT, see Scheme 9).[121] This was not

the case when polypropylene was doped with 9,10-dibromoanthracene,[121] whose oxidation potential (1.42 eV) is much higher than that of 9,10-diphenylanthracene (1.20 eV).[135]

In a context of electron-transfer mechanisms of the polymer oxy-chemiluminescence, it should be also mentioned that in a series of works[136-138] on autooxidation of polyamides and model amides the authors arrived at conclusion that chemiexcitation occurs in redox reactions of hydroperoxides and aldehydes formed because of oxidation.

4. Oxy-chemiluminescence as an experimental tool in studies on antioxidants

Oxy-chemiluminescence constitutes an indispensable experimental tool for modern oxidation chemistry and is most suitable in studies on antioxidants, which play a paramount role in numerous areas of biology, material science, chemical and analytical technologies.

4.1. Importance of antioxidants. General remarks

Antioxidants constitute a broad variety of compounds (such as amines, arylindandiones, phenols, tocopherols and other vitamins, *etc.*) reacting with peroxy radicals ($ROO^{\bullet}$), the chain carries in the oxidation process and thus suppressing the oxidation.[139] The natural antioxidants (*e.g.*, vitamin E) prevent oxidation of lipids and proteins in cell membranes. Synthetic antioxidants are prominent in stabilization of polymers, drugs, fats, lubricants and fuels. Antioxidant contents of materials of interest represents an important characteristics of their stability against oxidative destruction. And last but not least, antioxidants are widely used in mechanistic studies on oxidation reactions.

Of particular interest is the role of antioxidants in biological processes. Indeed, living organisms are exposed permanently to a severe oxidative stress by active oxygen species, *e.g.*, hydroxy and other oxygen-containing free radicals, singlet oxygen and superoxide anions. Nevertheless, in their normal state, the organisms are able to protect themselves against autooxidation. The biological protective system involves the enzymatic[140] and non-enzymatic defense mechanisms; the latter utilize the biochemical functions of diverse antioxidants naturally occurring in living cells.[141-144]

Although natural antioxidants are present in living organisms in fairly small quantities, their importance is difficult to overestimate. Deficiency of them reduces severely the resistance of organisms against a large variety of diseases. Indeed, a wide body of evidence suggests the oxidative nature for a broad scale of human maladies. In this context, recent attention was focused, in particular, on atherosclerosis,[145] senescence,[146,147] neurodegenerative phenomena such as stroke, Alzheimer's and Parkinson's diseases.[148-150] Thus, the chemoprotective role of antioxidants against above-mentioned diseases[148-150] and a number of inflammations, chemical toxicity and tumors[151-155] has become a subject of extensive studies.

However, it is noteworthy that the role of antioxidants is not always benign in medical sense. For instance, the tumor tissues may contain elevated levels of antioxidants,[156] which assist the tumors to maintain a low rate of lipid peroxidation and thereby protect them from oxidative damage. Also antioxidants in the environment pollution may exert a serious harmful influence on living organisms. In this context, a pertinent example is furnished by extremely efficient volatile antioxidants evolved from aging polymeric materials into atmosphere.[157,158] (This novel phenomenon is discussed in Section 4.4.) Studies on motor and emotional activity of experimental rats revealed pronounced harmful effects imposed by these "technogenic" antioxidative species on the central nervous system of animals.[158,159]

Oxidative stress in living organism causes the increase of lipid peroxidation,[47,49-56,160-163] oxidative damage of proteins[61,62] and nucleic acids[164] and affects the cell regulatory systems through miscellaneous mechanisms.[165] All these facets result in gradual consumption of antioxidants. Therefore, the antioxidant content should correlate with a stage of pathology. Thus, antioxidant assays may serve as a convenient tool for clinical diagnostics.

Elucidation of biological function of antioxidants, which occur in living cells, is closely related and often overlaps with the studies on antioxidative effects of drugs, natural-antioxidant ingredients taken up with food and volatile free-radical scavenges acquired through the respiratory tract from the ambient air.[157-159]

As already stated above, the chemoprotective role of synthetic and natural antioxidants is of prime importance for curing the maladies of oxidative nature.[144,148-155,166] Also, the antioxidative ingredients of food and other agricultural products are often considered in terms of their protective function exerted on the living organisms, which consume them.[167-182] Another more simple but not less important role of antioxidants in food consists of preventing the food of aging and getting rancid. Interesting is that the analysis of literature on antioxidants in agricultural products discloses a particular interest in studies of antioxidant capacity of wines.[183-190]

Numerous methods have been developed to monitor lipid hydroperoxide formation and antioxidative activity,[191,192] which utilize determination of lipid-oxidized product, oxygen consumption or discoloration of β-carotene. Chemiluminescent assays for antioxidant capacity[193-222] of biological materials are widely used and essential portion of them utilize luminol reactions as the luminescence probe. The major advantages of modern chemiluminescent methods compared to routine biochemical assays are high sensitivity, rapidity and relatively low cost.

Despite numerous advantages of the luminol-based chemiluminescent assays over routine procedures for antioxidant monitoring, they are far from being perfect. The main drawbacks of the luminol-based assays are complexity of the chemiluminescence mechanism and high nonspecific sensitivity towards even trace impurities. Below, we consider the basic principles of the oxy-chemiluminescence approach, which omits these problems.

4.2. General principle of the method

The chemiluminescent assay for antioxidant monitoring is based on the competition between the self reaction of peroxy radicals (reaction step "k_6" in Scheme 1), giving rise to light emission, and scavenging the peroxy radicals by antioxidants (InH), thus inhibiting the oxidation process and thereby quenching the light emission.[6,11,19,157,158,193,222] In a most important and widespread case of the antioxidants, namely phenolic inhibitors of the free-radical oxidation, Scheme 1 should be expanded to include steps "k_7" and "k_8" shown in Scheme 10.[19] It should be emphasized that Scheme 10 furnishes a simplified case, which serves illustrative purpose and does not take into account the self reaction of the In$^{\cdot}$ species and their prooxidative ability (*i.e.*, involvement in the oxidation-chain propagation). It has been shown that in most instances these processes indeed may be neglected.[19] However, in some natural systems under certain reaction conditions the prooxidative effects should be taken into account.[223-230]

$$\mathrm{ROO^{\bullet}} + \mathrm{InH} \xrightarrow{k_7} \mathrm{ROOH} + \mathrm{In^{\bullet}}$$

$$\mathrm{ROO^{\bullet}} + \mathrm{In^{\bullet}} \xrightarrow{k_8} \textit{Inactive products}$$

Scheme 10

The mere fact that antioxidants suppress oxidation reactions and thereby quench the light emission opens a direct opportunity for the use of chemiluminescence in the antioxidant analysis. The extent of the chemiluminescence quenching and the kinetics of the chemiluminescence recovery upon gradual consumption of the antioxidant depend on the antioxidant *reactivity* towards peroxy radicals (strength of the antioxidant) and its *concentration*.[6,11,19,157,158]

The experimental procedure used in the antioxidant monitoring is very facile. Injection of a small drop of an antioxidant analyte into the probe solution causes partial or complete quenching of the chemiluminescence emission (depending on the antioxidant's strength and concentration), which is schematically illustrated in Figure 3. The chemiluminescence intensity "stolen" (quenched) by the antioxidant provides a measure of the antioxidant *concentration*.[6,11,19,157,158] On a gradual consumption of the antioxidant in the reaction mixture, the chemiluminescence intensity rises again. The kinetics of the intensity rise (at given RH and reaction initiation rate) gives an information on the antioxidant *activity* (strength).[6,11,19,157,158] Below, we consider this qualitative picture kinetically, in terms of Schemes 1 and 10.

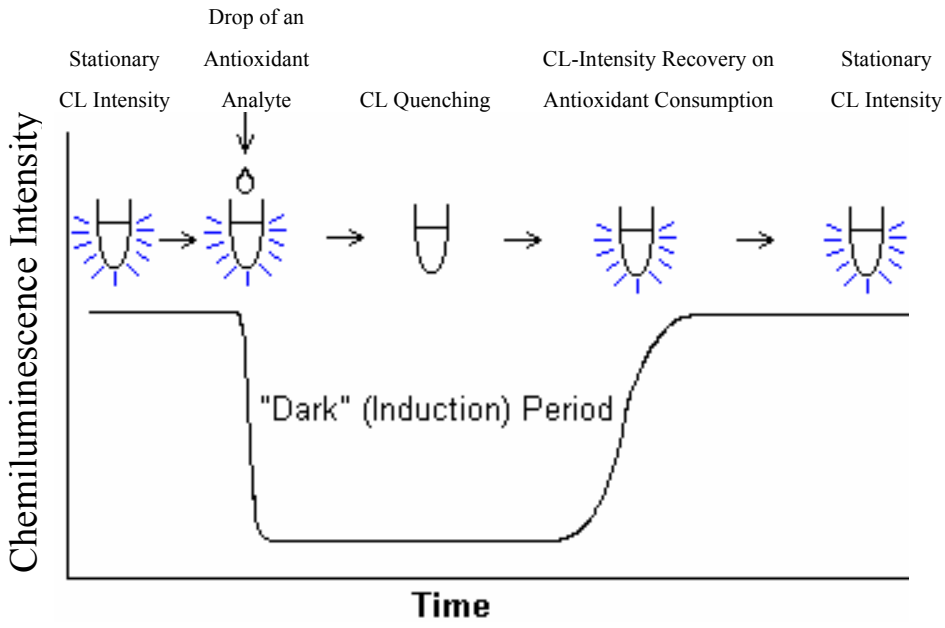


Figure 3. Typical chemiluminescence (CL) time profile for a hydrocarbon-oxidation reaction in the presence of an antioxidant.

Usually, at moderate temperatures initiated oxidation of hydrocarbons is a slow process and concentration of an initiator Y (see Scheme 1) is high enough to neglect its consumption in a course of the reaction time (t). Thus, initiation rate (v_i) of the oxidation process (Scheme 1) defined by Eq. 13 is a constant value at given [Y] and temperature. In the latter expression (Eq. 13), k_{dec} is the rate constant of the initiator (Y) decomposition to generate initiating radicals ($r^{\bullet}$ in Scheme 1) and γ_c is their probability to escape the solvent cage. The $2\gamma_c k_{dec}$ data are available for most of "standard" initiators (such as AIBN, for instance) in a number of organic solvents and in a temperature range 20 – 80 °C.[11,24,139]

$$v_i = 2\gamma_c k_{dec}[Y] \quad (13)$$

According to Schemes 1 and 10, kinetics of the oxidation process in the presence of antioxidants obeys Eqs. 14-16, from which one obtains Eq. 17. For integration of eq 17

$$d[ROO^{\bullet}]/dt = v_i - 2k_6[ROO^{\bullet}]^2 - k_7[InH][ROO^{\bullet}] - k_8[ROO^{\bullet}][In^{\bullet}], \quad (14)$$

$$d[In^{\bullet}]/dt = k_7[InH][ROO^{\bullet}] - k_8[ROO^{\bullet}][In^{\bullet}] \quad (15)$$

$$d[InH]/dt = - k_7[ROO^{\bullet}][InH] \quad (16)$$

$$d[ROO^{\bullet}] - d[In^{\bullet}] - 2d[InH] = (v_i - 2k_6[ROO^{\bullet}]^2)dt \quad (17)$$

from $t = 0$ to $t = \infty$, essential are the limited expressions for the reagent concentrations, *i.e.,* $[ROO^{\bullet}]_0$, $[ROO^{\bullet}]_\infty$, $[InH]_0$, $[InH]_\infty$, $[In^{\bullet}]_0$, $[In^{\bullet}]_\infty$. The same intensity levels before injecting antioxidant ($t = 0$) and after its complete consumption ($t = \infty$) imply that $[ROO^{\bullet}]_0 = [ROO^{\bullet}]_\infty$. Since under the stationary reaction conditions $d[ROO^{\bullet}]/dt = 0$, from Eq. 14 at $[InH] = [In^{\bullet}] = 0$ one obtains Eq. 18 for the initial and the final peroxy-radical concentrations. Clearly, the

$$[ROO^{\bullet}]_0 = [ROO^{\bullet}]_\infty = (v_i/2k_6)^{1/2} \quad (18)$$

other limited concentration are $[In^{\bullet}]_0 = [In^{\bullet}]_\infty = 0$ (no $In^{\bullet}$ radicals before the InH addition and after its complete consumption), $[InH]_\infty = 0$ (*i.e.*, InH is completely consumed), while $[InH]_0$ is the known initial concentration of the added InH. Then, it is convenient to operate with a relative chemiluminescence intensity (i_{rel}), which is the ratio of the intensity in the presence and in the absence of InH, as it is given by Eq. 19. Thus, through integration of Eq. 17 under

$$i_{rel} = i/i_0 = 2k_6[ROO^{\bullet}]^2/v_i = [ROO^{\bullet}]^2/[ROO^{\bullet}]_0^2 \quad (19)$$

aforesaid conditions one obtains Eq. 20,[19] the latter relates the antioxidant concentration

$$2[InH]_0 = v_i \int_0^\infty (1 - i_{rel})dt \quad (20)$$

added to the reaction mixture (and consumed therein) and the area above the kinetic curve (given by the integral in the right part of Eq. 20), which refers to a quenched or "stolen" (by the antioxidant) light sum.

Finally, from Eqs. 14-16 and 19, one obtains suitable relationships (Eqs. 21 and 22)[19]

$$i_{rel}^{-1/2} - i_{rel}^{1/2} = 2k_7[InH](v_i k_6)^{-1/2} \quad (21)$$

$$\ln(1 + i_{rel}^{1/2}) - \ln(1 - i_{rel}^{1/2}) - i_{rel}^{-1/2} = (k_7/(2k_6)^{1/2})v_i^{1/2}\, t + const \quad (22)$$

between the chemiluminescence intensity and the antioxidant concentration and strength. The slope of the $i_{rel}(t)$ curve at the inflection point ($i_{rel} = 0.535$) is given by Eq. 23.[19]

$$(di_{rel}/dt)_{max} = 1/T = 0.237(k_7/(2k_6)^{1/2})v_i^{1/2} \quad (23)$$

Thus, with the help of Eqs. 20-23, single experiment, *i.e.*, measuring the chemiluminescence kinetics in the presence of the antioxidant allows to acquire both the inhibitor concentration and its reactivity (k_7), provided the rate constant k_6 of the peroxy-radical self reaction is known (in fact, the k_6 data for most of model hydrocarbons are available). Yet, the absolute concentration of the peroxy radicals at any point of the kinetic curve, $[ROO^{\bullet}] = i_{rel}^{1/2}(v_i/2k_6)^{1/2}$, may be calculated if v and k_6 are known.

It should be noted that the kinetic analysis performed herein refers to a widespread situation, in which one antioxidant molecule scavenges two peroxy radicals (*i.e.*, by both the InH and the $In^{\bullet}$ species according to Scheme 10). However, this is not always the case. For more complex inhibition schemes, the key expression given by Eq. 20 should be modified through replacing the 2[InH] value by the f[InH] quantity,[19] in which effective coefficient f depends on a scavenging mechanism. Usually, $1 < f < 2$, but for some antioxidants, which contain a number of reactive groups, the f value may reach several units.[19]

Figure 4 provides an illustrative example of the inhibition effect exerted by a synthetic antioxidant, chromane C_1 (α-tocopherol analog), on the oxy-chemiluminescence process, from which kinetic behavior of the chemiluminescence intensity and concentrations of the reaction components is evident.[19]

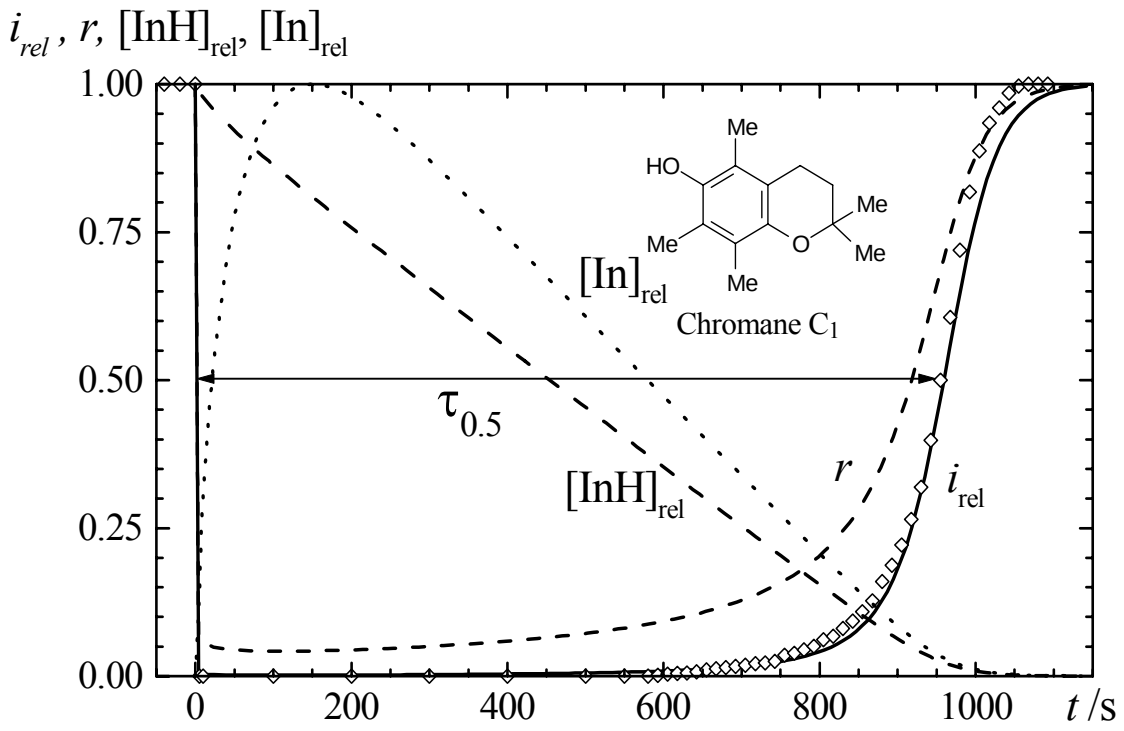


Figure 4. Kinetics of the relative chemiluminescence intensity (i_{rel}) and relative concentrations of peroxy radicals (r = $[ROO^{\bullet}]/[ROO^{\bullet}]_0$), antioxidant ($[InH]_{rel}$) and antioxidant radicals ($[In^{\bullet}]_{rel}$) for the diphenylmethane oxidation (10.2 % vol. in benzene, v_i = 2.15×10^{-9} Ms^{-1}, 60 °C). Antioxidant chromane C_1 ($[InH]_0$ = 1.01×10^{-6} M) was introduced at the moment t = 0. Symbols ◊ refer to the experimental points, lines are the computer simulation results obtained for k_6 = 1.32×10^{8} $M^{-1}s^{-1}$ and k_7 = 7.6×10^{6} $M^{-1}s^{-1}$.

If the chemiluminescence recovery on the gradual antioxidant consumption is steep, the S-shaped curve above the light-intensity curve may be approximated by a rectangle with a height Δi_{rel} and width $\tau_{0.5}$ (see Figure 5),[19] the latter refers to the time required to achieve

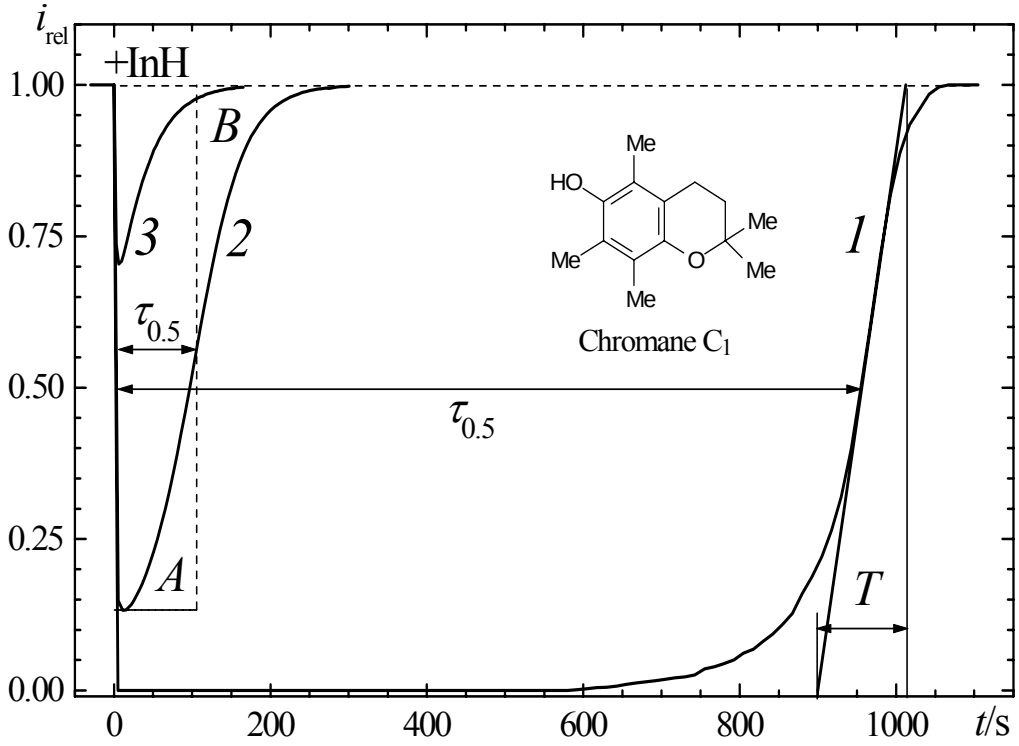


Figure 5. Kinetics of the relative chemiluminescence intensity (i_{rel}) for the diphenylmethane oxidation (10.2 % vol. in benzene, v_i =2.15×10^{-9} Ms^{-1}, 60 °C) after injection of different amounts of InH (chromane C_1): (*1*) $[InH]_0$ = 1.01x10^{-6} M, (*2*) $[InH]_0$ = 1.01x10^{-7} M and (*3*) $[InH]_0$ = 1.52x10^{-8} M.

0.5 Δi_{rel}, *i.e.*, the time of the chemiluminescence "half-recovery", which is called the induction period or effective induction period. This characteristic time, $\tau_{0.5}$, is a widely used parameter in antioxidant assays,[6,11,19,157,158] which utilize approximate relation expressed by Eq. 24 instead of Eq. 20. The accuracy of such an approximation depends on the difference

$$2[InH]_0 \approx v_i \tau_{0.5} \quad (24)$$

between the areas *A* and *B* (Figure 5, curve 2). The kinetic analysis[19] shows that the overestimation of the $[InH]_0$ value assessed with the use of Eq. 24 does not exceed 2% if $\tau_{0.5} \geq 3T$, where *T* is the inverse slope at the inflection point (see Eq. 23 and Figure 5). Alternatively, the procedure, which involves the area measurements according to Eq. 20, is applied at low concentrations of the antioxidant in analyte sample,[6,11,19,157,158] which causes incomplete quenching the chemiluminescence intensity (see curves 2 and 3 in Figure 5). It should be emphasized that this simple "area method" is suitable for the cases, in which the chemiluminescence intensity completely recovers (in other words, attains its initial level) after a consumption of the antioxidant in the reaction mixture. This requisite is not always fulfilled in

the analytical practice for several reasons: Antioxidants and/or other components present in an analyte sample may interact with the chemiluminescence emitter (*e.g.*, through energy transfer or chemical reactions with its excited state), they may cause light filtration, *etc*. Clearly, such cases require certain correction of the chemiluminescence intensity, taking into account additional quenching or light-filtering impositions, for the acquisition of the true inhibitory effects.

4.3. Peculiarities of analyzing the natural antioxidants

It is often the case for materials of biological origin (*e.g.*, blood plasma, living tissues, plants and food products) that analyte samples contain antioxidants of essentially different strength.

To distinguish between these antioxidants, different hydrocarbons (RH) in the probe solution should be used. The matter is that different RH produce on oxidation peroxy radicals $ROO^{\bullet}$ with essentially different kinetics of their self reactions (step "k_6" in Scheme 1); the pertinent examples are given in Table 2.[19] For instance, for diphenylmethane this reaction is fast (its rate constant amounts to 1.3×10^8 $M^{-1}s^{-1}$, see Table 2), while for cumene it is very slow (rate constant is as low as 4×10^4 $M^{-1}s^{-1}$!). It is clear that the case of fast self reactions of peroxy radicals ("k_6" in Scheme 1) is suitable only for the analysis of *strong* antioxidants, since only for strong antioxidants the scavenging reaction ("k_7" in Scheme 10) may compete with the chemiluminescent channel (step "k_6"). For weak antioxidants, step "k_7" is too slow to exert any significant influence on the oxidation process and to quench the chemiluminescence emission when reaction "k_6" is fast. Consequently, to analyze *weak* antioxidants one should use hydrocarbon, for which reaction "k_6" in Scheme 1 is slow, *e.g.*, cumene (*cf.* Table 2), which meets this requirement. Thus, the analytical procedure involves two steps:

(1) With the use of hydrocarbon, for which the k_6 value is high (*e.g,* diphenylmethane), examination of the strong-antioxidant fraction is carried out.

(2) Using hydrocarbon, for which k_6 is low (cumene is the best choice), one determines the *total* antioxidant content of the analyte sample, from which the strong-antioxidant amount [determined in step (1)] is subtracted.

Another problem, which is often met in studying the natural antioxidants, stems from the existence of more than one reactive group in the antioxidant molecule. Flavonoids furnish a prominent example of such natural compounds, whose effect on oxy-chemiluminescence emission is considered below.

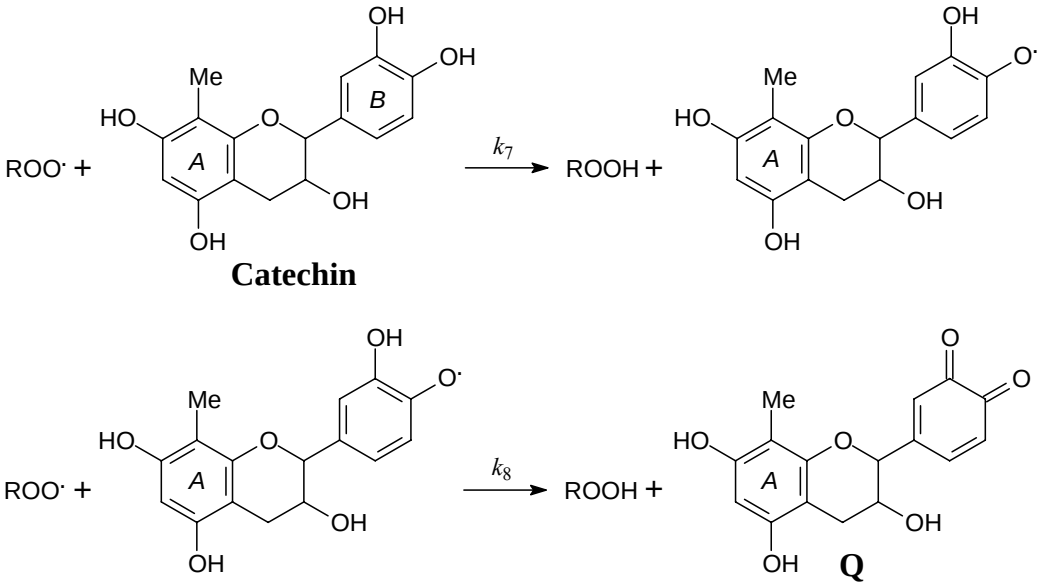
Table 2. Sample rate constants (k_6) for the self reactions of peroxy radicals[a]

Hydrocarbon being oxidized	k_6, $M^{-1}s^{-1}$
Cumene	4.0×10^4
Cyclohexane	1.6×10^6
Ethylbenzene	1.9×10^7
Diphenylmethane	1.3×10^8

[a] At 60 °C in benzene solutions

Flavonoids, natural polyphenols of plant origin, are known to be effective inhibitors of lipid peroxidation, which is commonly associated with their radical-scavenging ability.[231-236] Besides, they reveal pronounced pharmacological and vitamin-like activities explained, at least partly, by their chain-breaking ability.[237-239]

It has been found that the "classic" model of the antioxidative effect on the oxy-chemiluminescence process, whose principles are set out in Section 4.2, is not quite suitable for the case of flavonoids.[222] The first reason for that seems to be the fact that the model described in Section 4.2 is valid for antioxidants with the only active OH group, while flavonoids possess more than one OH functionality.[222] In this context, it is noteworthy that mainly catechol moiety (marked as *B*-ring in Scheme 11 for catechin molecule) of flavonoid accounts for the inhibitory effect.[222] In line with this fact, it was assumed that in such a case the phenoxyl-type radical ($In^{\bullet}$ in Scheme 10), formed through hydrogen abstraction from the antioxidant molecule (InH) by the $ROO^{\bullet}$ radical (reaction "k_7" in Scheme 10), reacts further with $ROO^{\bullet}$; this is exemplary illustrated in Scheme 11, a particular case of Scheme 10, for catechin as antioxidant.



Scheme 11

The reactivities of the OH groups in the *A*-ring and in the heterocyclic ring are expected to be much lower than that of the *B*-ring.[222] But as the parent flavonoid is consumed in the reaction with peroxy radicals and the products of type **Q** accumulate (see Scheme 11), the contribution of hydroxy groups of the *A*-ring to the overall chain-breaking process becomes essential at longer reaction times. This is particularly manifested in the kinetics of the chemiluminescence recovery (*cf.* Figure 3).[222] Clearly, this affects the data of the antioxidant reactivity (*i.e*, the k_7 values), which are normally obtained from the light-emission recovery kinetics, as it was discussed in Section 4.2.

Besides, some side reactions may be important for the behavior of the chemiluminescence emission in the presence of flavonoids.[222] It has been shown that among all possible side reactions a monomolecular transformation of the semiquinone radical (a product of hydrogen abstraction from the parent flavonoid by the $ROO^{\bullet}$ radical; see reaction "k_7" in Scheme 11) plays a decisive role.[222] However, the explicit nature of this transformation (isomerization or dissociation) remains unclear by now. It was merely suggested that the corresponding radical $PhO^{\bullet}$ (in terms of Scheme 10, $PhO^{\bullet}$ =$In^{\bullet}$), whose own activity towards $ROO^{\bullet}$ is low, undergoes conversion into more active radical $R'^{\bullet}$ (or $R'OO^{\bullet}$; see Scheme 12), and such new radical species contribute to the propagation of the oxidation chain.[222] Such an assumption enabled the computational simulation of the oxy-chemiluminescence kinetics, which successfully reproduced the experimental intensity time profiles and allowed acquisition of the true k_7 values for the flavonoid antioxidants.[222]

$$PhO^{\bullet} \longrightarrow R''^{\bullet} \xrightarrow{O_2} R'OO^{\bullet}$$

Scheme 12

Scheme 12 refers to a particular case of prooxidative effect exhibited by some natural antioxidants,[223-230] as it has been already mentioned in Section 4.2.

An essential problem, which merits to be mentioned in a context of the natural-antioxidant assay, refers to the analysis of antioxidants in lipid samples. The matter is that even strong antioxidants present in lipid analyte in high concentrations (*e.g.*, tocopherol, which retards lipid peroxidation in cell membranes) never quench the chemiluminscence emission of the probe hydrocarbon down to zero. We suggest that the remaining intensity, which is not suppressed by peroxy-radical scavengers, derives mainly from the thermolysis of dioxetane species accumulated in a lipid sample.[21,47] The plausible origin of these dioxetanes is the cyclization of alkylperoxy radicals generated in the lipid-peroxidation process, as discussed in Sections 3.1.2 and 3.13. Studies on inhibitory effects exerted by antioxidants from lipid materials are currently in progress in our laboratory.

And last but not least, the suitability of the approach presented herein for analyzing the natural antioxidants is substantiated by the possibility of examining analytes dissolved in aqueous media (most important for biology). Natural antioxidants, which constitute organic (*e.g.*, phenolic) substances, are readily soluble in organic solvents and, thereby, quickly diffuse into the probe (chemiluminescent) organic solution from aqueous samples on intensive stirring in the reaction cell, which has been demonstrated experimentally.[240] In this respect, the presented assay is well suited for determining amounts of antioxidants in natural samples. However, as for the biological significance of these radical scavengers, it should be borne in mind that results obtained *in vitro* cannot be directly extended to physiological conditions (in view of complex nature of balance between oxidant and antioxidant species in living organisms)[211,241-245] and may serve merely as a relative measure for comparing the antiradical ability of diverse natural

compounds. For that reason, some authors even use the term "antiradical" instead of "antioxidant" activity to differentiate this action from the inhibitor effect *in vivo*.[211]

4.4. Antioxidants in the ambient air

Ambient-air pollution comprises of particles, which are either natural or anthropogenic in origin, and constitutes a complex mixture of organic and inorganic compounds. The role of pollution particles in oxidative processes in living organisms is subject to extensive studies.[246-250] As reported, those particles mediate generation of reactive oxygen species, either directly through interaction with ambient oxygen or indirectly through initiation of an oxidative burst in phagocytes. As a consequence of exposure to the air pollution, the free radical formation in the lung tissues have been observed.[250]

The unprecedented result of studies performed in our laboratory[157-159] on the role of ambient air in oxidation processes resides in observation of very efficient volatile *antioxidants* in the air pollution. The probable source of volatile antioxidants, evolved into the environment, are aging polymeric materials.[157-159] The presence of antioxidants in the ambient air was manifested by an abrupt drop of the chemiluminescence intensity on bubbling the air, passed over a polymer, through a probe chemiluminescent solution of oxidizing hydrocarbon (diphenylmethane), which is exemplary illustrated in Figure 6.[157,158]

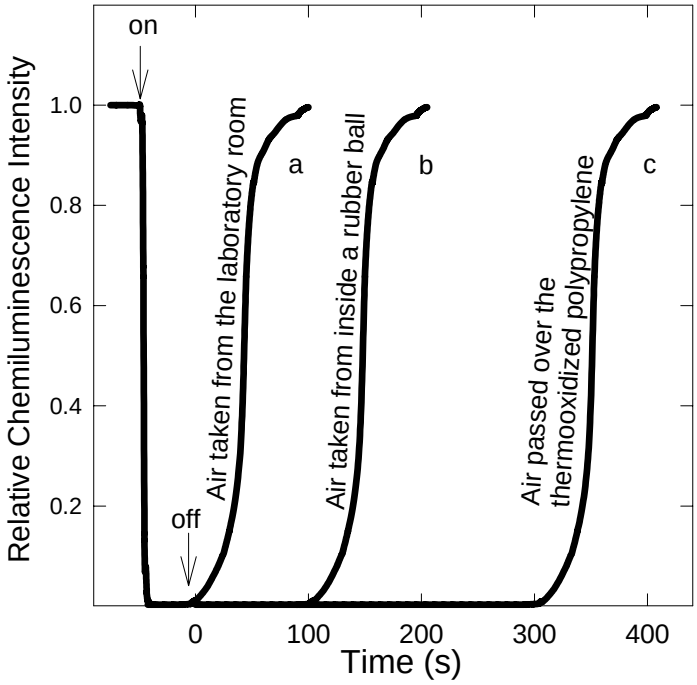


Figure 6. Effect of volatile antioxidant on chemiluminescence generated in oxidation of Ph_2CH_2 (10% in PhCl) initiated by AIBN at 60 °C (initiation rate was 1.8×10^{-11} Ms^{-1}). The antioxidant-containing air was introduced ("on") into the solution and then bubbling was ceased ("off").

Antioxidative species easily pass from the gaseous phase into the liquid organic solution, which is manifested by the drop of the oxy-chemiluminescence intensity on blowing.[157,158] When blowing was switched off, the light intensity recovered after a "dark" induction period (Figure 6), which manifests a consumption of the antioxidant. This behavior is *identical* to what is normally observed when an antioxidant analyte is initially dissolved in a liquid phase (*cf.* Figures 4 and 5). Such an observation gave the grounds to apply the same kinetic approach (with minor alterations) as developed for the liquid-phase antioxidant samples,[6,11,19] which made possible to acquire the data on reactivity of these peroxy-radical scavengers.[157,158] The measured rate constant for their reaction with the $ROO^{\bullet}$ radicals turned out to be more than 10^9 $M^{-1}s^{-1}$,[157,158] which is close to the diffusion-controlled rate constant. This means that they scavenge peroxy radicals nearly at every contact! Thus, their antioxidative effect is stronger than that of tocopherols and their synthetic analog (chromane C_1) and even galvinoxyl, a persistent phenoxyl-type free radical, which is manifested qualitatively by the steepest intensity recovery curve for volatile radical acceptors present in the ambient air (Figure 7).[157]

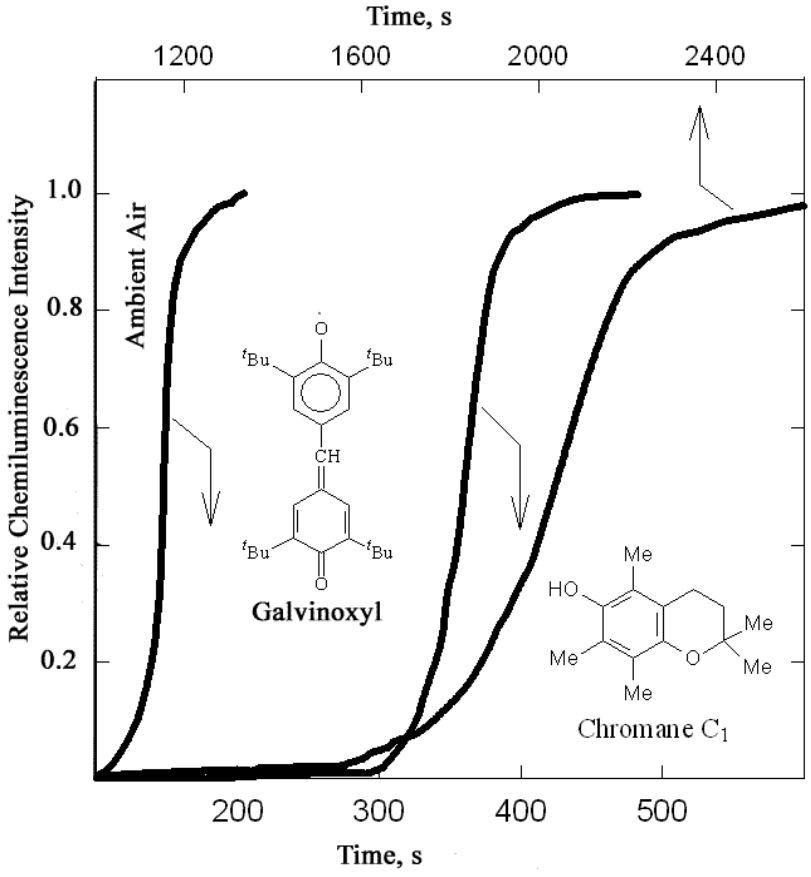


Figure 7. Kinetics of the chemiluminescence-intensity recovery upon gradual consumption of the antioxidants (galvinoxyl, chromane C_1 and volatile antioxidant from the ambient air) in the probe oxy-chemiluminescent solution (oxidation of 10% of Ph_2CH_2 in PhCl at 60 °C initiated by AIBN with the rate of 1.0×10^{-11} Ms^{-1}).

The longer the air is resident over a polymer sample and the higher its temperature, the greater the quenching of the chemiluminescence emission and the longer the induction period are observed. The induction period lengthens with increasing time *t* of air bubbling through the probe chemiluminescent solution and attains its limit at *t* of *ca.* ten seconds.[157,158] Clearly, this limit corresponds to the steady-state concentration of the antioxidant in solution. For all the polymer samples studied, the rate of antioxidant production increased with increasing oxidation of a sample, elevated sample temperature and increasing specific surface area.[157,158] Table 3 illustrates qualitatively these features.[158] Furthemore, the air taken from the rooms with plastic furniture, outdoors near the automobile roads, over rubber, resin, linoleum and other polymeric materials contained strong volatile antioxidants.[157,158] These volatile species were readily trapped by silica gel and zeolite molecular sieves and slowly returned into the gaseous phase upon heating the absorbers up to *ca.* 200 °C.[158] From these observations, it is evident that such volatile technogenic antioxidants are most likely the products of the polymer destruction.

Table 3. Chemiluminescence-quenching effect exerted by volatile antioxidants as a function of the antioxidant source[a]

Source of the Volatile Antioxidant	Light Quenching
Chemically pure unoxidazed teflon at 20 – 70 °C after storing for 3 days in a contact with air at 140 °C	No effect
Chemically pure unoxidazed polystyrene, polypropylene, polyethylene and polymethylmetacrylate at 20 – 70 °C	No effect
The same samples after contacting with air for 3 days at 140 °C	Strong effect
Black rubber at room temperature	Weak effect
The same sample after the room-temperature oxidation initiated by AIBN or by treatment with ozone or high-frequency Tesla discharge	Strong effect
Hydrocarbon and silicon caoutchoucs	No effect
The same samples after technological processing yielding black and silicon rubber	Strong effect
Automobile protector rubber powdered with extruder	Strong effect

[a] For details on probe oxy-chemiluminescent solution see caption of Figure 6.

The fact that volatile antioxidants scavenge peroxy radicals at almost every contact suggests that these scavengers are of radical nature. Contrary to a high reactivity of the volatile antioxidative species towards $ROO^{\bullet}$ radicals, their reactivity towards themselves is low.[157,158] This is not that unusual for free-radical species. Persistent free radicals exist, which may survive for many hours and even days; the carbon-centered radicals furnish prominent examples.[251] Their low self reactivity may be determined by bulky substituents, which eclipse the radical center. Such radicals have been postulated to rationalize the rates of propagation and termination of chain reactions in polymers.[252] Thus, the carbon-centered radicals seem to be prime candidates for the nature of the peroxy-radical scavengers evolved from polymer upon destruction. However, this hypothesis still needs to be substantiated experimentally.

The concentration of the technogenic antioxidants in the atmospheric air may reach 10^{-8}– 10^{-7} M,[157,158] which is high enough to cause a biological effect since living organisms are subject to a lifelong exposure to these highly reactive species. The biological role of such antioxidative pollution species, as well as the oxidative pollution particles, is harmful. As disclosed from experiments with rats, these antioxidants severely affect the excitability level of animals modifying their behavior.[158,159]

4.5. Limit of the antioxidant detection

The detection limit of the antioxidant assay refers to the lowest concentration ($[A]_{min}$) of the antioxidant analyte (A) that can be determined in the probe solution. As it has been shown before,[158] $[A]_{min}$ may be expressed by Eq. 25, in which $\Delta i_{rel} = \Delta i = 1 - i_{rel}$ and k_A is the rate

$$[A]_{min} = \Delta i_{rel}(v_i k_6)^{1/2}/k_A \quad (25)$$

constant of scavenging the peroxy radicals by the antioxidant. For the most widespread case of phenolic antioxidants considered in Sections 4.2 and 4.3 k_A refers to the k_7 value.

As evident from Eq. 25, the greater the k_A and smaller the k_6, the lower the limit of detection ($[A]_{min}$). The rate constant k_A is a characteristic of the antioxidant, whereas k_6 depends on hydrocarbon chosen for the probe chemiluminescent solution. The optimal (though quite typical) values are the following: $k_6 = 4.0 \times 10^4$ $M^{-1}s^{-1}$ for cumene as a "probe" hydrocarbon at 60 °C, $k_A = k_7 = 4.5 \times 10^6$ $M^{-1}s^{-1}$ for chromane C_1 (synthetic analog of α-tocopherol) and 10^9 $M^{-1}s^{-1}$ for the volatile antioxidants evolved from aging polymeric materials into the ambient air.[157,158] Also, as it is seen from Eq. 25, the reaction initiation rate (v) should be as low as possible to enable detection of the lowest $[A]_{min}$. In our experience, the observation of oxy-chemiluminescence is hardly practicable at v much below 10^{-12} Ms^{-1}; thus, one should take $v_i \approx 10^{-12}$ Ms^{-1} as the lowest possible value of the initiation rate.

To estimate the limit of the antioxidant detection ($[A]_{min}$) with the help of Eq. 25, one may assume that the quenched relative chemiluminescence intensity can be measured with the 10% accuracy, *i.e.*, $\Delta i = 0.10 \pm 0.01$. On substitution of $\Delta i = 0.10$ together with the k_6, k_A (k_7) and v_i values given in the above paragraph into Eq. 25, $[A]_{min}$ takes the values 4×10^{-12} M for chromane C_1 (synthetic α-tocopherol derivative) and 2×10^{-14} M (!) for the volatile "technogenic"

antioxidants occurring in the ambient air. These values clearly demonstrate high sensitivity of the discussed oxy-chemiluminescence method.

5. Concluding remarks

The analysis presented herein disclose a rich diversity of mechanistic aspects giving an account of the excited-state generation and light emission in the peroxy-radical-mediated processes. The novel results, which refer to the elucidation of structural effects in the chemiexcitation process and oxy-chemiluminescence studies with polymeric, biological and antioxidant materials, qualify this interdisciplinary realm as evolving and growing field of knowledge. The incentive of our contribution is not only to entertain the readership, but to stimulate the chemical community to explore the oxy-chemiluminescence as an experimental tool to monitor oxidative and antioxidative processes in chemical and biological media.

6. Acknowledgements

The generous financial support by the Russian Foundation for Basic Research (grant № 05-03-32730) and the Division of Chemistry and Material Science of the Russian Academy of Sciences (Research Program № 1) is gratefully appreciated. Also we are indebted to Dr. Yu.B. Tsaplev for the valuable inputs and relevant suggestions.

7. References

1. Adam, W.; Cilento, G. Eds. *Chemical and Biological Generation of Excited States*; Academic Press: New York, 1982.
2. Adam, W.; Cilento, G. *Free Radic. Biol. Med.* **1995**, *19*, 103.
3. Adam, W.; Reinhardt, D.; Saha-Möller, C.R. *Analyst*, **1996**, *121*, 1527.
4. Adam, W.; Trofimov, A.V. In: *The Chemistry of Peroxides*; Rappoport, Z. Ed.; Patai Series; Wiley: Chichester, 2006; Vol. 2, Part 2, pp 1171-1209.
5. Baader, W. J.; Stevani, C. V.; Bastos, E. L. In *The Chemistry of Peroxides*; Rappoport, Z. Ed.; Patai Series; Wiley: Chichester, 2006; Vol. 2, Part 2, pp 1211-1278.
6. Vasil'ev, R. F. *Prog. React. Kinet.* **1967**, *4*, 305.
7. Vasil'ev, R. F. *Russ. Chem. Rev.* **1970**, *39*, 529.
8. Belyakov, V. A.; Vasil'ev, R. F. *Photochem. Photobiol.* **1970**, *11*, 179.
9. Sharipov, G. L.; Kazakov, V. P.; Tolstikov, G. A. *Chemistry and Chemiluminescence of 1,2-Dioxetanes* (in Russian); Vasil'ev, R. F. Ed.; Nauka: Moscow, 1990. *Chem. Abstr.* **1990**, *114*, 143390.

10. Bulgakov, R. G.; Kazakov, V. P.; Tolstikov, G. A. *J. Organomet. Chem.* **1990**, *387*, 11.
11. Shlyapintokh, V. Ya.; Karpukhin, O. N.; Postnikov, L. M.; Zakharov, I. V.; Vichutinskii, A. A.; Tsepalov, V. F. *Chemiluminescence Techniques in Chemical Reactions*; Consultants Bureau: New York, 1968.
12. McCapra, F. *Prog. Org. Chem.* **1973**, *8*, 231.
13. Gundermann, K. D.; McCapra, F. *Chemiluminescence in Organic Chemistry*; Springer-Verlag: Berlin, 1987.
14. Campbell, A. K. *Chemiluminescence: Principles and Applications in Biology and Medicine*; VCH, Ellis Horwood Ltd.: New York, 1988.
15. Wilson, T. *Photochem. Photobiol.* **1995**, *62*, 601.
16. Wilson, T.; Hastings, J.W. *Annu. Rev. Cell. Dev. Biol.* **1998**, *14*, 197.
17. Ashby, G. E.; *J. Polym. Sci.* **1961**, *1*, 99.
18. Jacobson, K.; Eriksson, P.; Reitberger, T.; Stenberg, B. *Adv. Polym. Sci.* **2004**, *169*, 151.
19. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *Kinetics and Catalysis* **2004**, *45*, 329.
20. Shliapintokh, V. Ja.; Vasil'ev, R. F.; Karpukhine, O. N.; Postnikov, L. M.; Kibalko, L. A. *J. Chim. Phys.* **1960**, 1113.
21. Vasil'ev, R. F. In *Chemical and Biological Kinetics. New Horizons* (in Russian)*;* Burlakova, E. B.; Shilov, A. E.; Varfolomeev, S. D.; Zaikov, G. E. Eds.; Chemistry: Moscow, 2005; Vol. 1, pp 453-493; English Translation: Brill, VSP: Leiden, The Netherlands, 2005.
22. Emanuel, N. M.; Denisov, E. T.; Maizus, Z. K. *Liquid-Phase Oxidation of Hydrocarbons*; Plenum Press: New York, 1967.
23. Vasil'ev, R. F. *Nature* **1962**, *194*, 1276.
24. Belyakov, V. A. Ph.D. thesis, ICP USSR Acad. Sci., 1970.
25. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *High Energy Chem.* **1978**, *12*, 208.
26. Mendenhall, G. D.; Sheng, X. C.; Wilson, T. *J. Am. Chem. Soc.* **1991**, *113*, 8976.
27. Russell, G. A. *J. Am. Chem. Soc.* **1957**, *79*, 3871.
28. Bartlett, P. D.; Guaraldi, G. *J. Am. Chem. Soc.* **1967**, *89*, 4799.
29. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *Bull. Acad. Sci. USSR Phys. Ser.* **1978**, *42*, 138.
30. Bennett, J. E.; Summers, R. *J. Chem. Soc., Faraday Trans. II.* **1973**, *69*, 1043.
31. Bennett, J. E.; Brown, D. M.; Mile, B. *J. Chem. Soc., Chem. Comm.* **1969**, 504.
32. Adamic, K.; Howard, J. A.; Ingold, K. U. *Canad. J. Chem.* **1969**, *47*, 3803.
33. Kanofsky, J. R. *J. Org. Chem.* **1986**, *51*, 3386.
34. Miyamoto, S.; Martinez, G. R.; Medeiros, M. H. G.; Di Mascio, P. *J. Am. Chem. Soc.* **2003**, *125*, 6172.
35. Niu, Q. J.; Mendenhall, G. D. *J. Am. Chem. Soc.* **1990**, *112*, 1656.
36. Niu, Q. J.; Mendenhall, G. D. *J. Am. Chem. Soc.* **1992**, *114*, 165.
37. Adam, W.; Kazakov, D. V.; Kazakov, V. P. *Chem. Rev.* **2005**, *105*, 3371.
38. Belyakov, V. A.; Vasil'ev, R. F.; Trofimov, A. V. *Bull. Acad. Sci. USSR Phys. Ser.* **1990**, *54*, 93.

39. Andrews, A.; Deroulede, A.; Linshitz, H. *J. Phys. Chem.* **1978**, *82*, 2304.
40. Bicźok, L.; Bérces, T. *J. Phys. Chem.* **1988**, *92*, 3842.
41. Kobayashi, T.; Nagakura, S. *Chem. Phys. Lett.* **1976**, *43*, 429.
42. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *Sov. J. Chem. Phys.* **1990**, *6*, 1213.
43. Vasil'ev, R. F.; Fedorova, G. F. *Kinetics and Catalysis* **2004**, *45*, 655.
44. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G.F. *Dokl. AN SSSR* **1978**, *239*, 344.
45. Adam, W.; Baader, W. J. *J. Am. Chem. Soc.* **1985**, *107*, 410.
46. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *Russ. Chem. Bull.* **1983**, *32*, 2429.
47. Timmins, G. S.; Dos Santos, R. E.; Whitwood, A. C.; Catalani, L. H.; Di Mascio, P.; Gilbert, B. C.; Bechara, E. J. H. *Chem. Res. Toxicol.* **1997**, *10*, 1090.
48. Adam, W.; Beinhauer, A.; Hauer, H. In *CRC Handbook of Organic Photochistry*; Scaiano, J. C. Ed.; CRC Press: Boca Raton, 1989, pp 271-327.
49. Boveris, A.; Cadenas, E; Chance, B. *Fed. Proc.* **1981**, *40*, 195.
50. Lissi, E. A.; Caceres, T.; Videla, L. A. *Free Radic. Biol. Med.* **1988**, *4*, 93.
51. Cadenas, E. *Annu. Rev. Biochem.* **1989**, *58*, 79.
52. Prat, A. G.; Turrens, J. F. *Free Radic. Biol. Med.* **1990**, *8*, 319.
53. Sharov, V. S.; Driomina, E. S.; Vladimirov, Yu.A. *J. Biolumin. Chemilumin.* **1996**, *11*, 91.
54. Cash, G. A.; George, G. A.; Bartly, J. P. *J. Sci. Food. Agric.* **1988**, *43*, 277.
55. Wright, J. R.; Rumbaugh, H. D.; Colby, H. D.; Miles, P. R. *Arch. Biochem. Biophys.* **1979**, *192*, 344.
56. Tilbury, R. N. *Cell. Mol. Life Sci.* **1992**, *48*, 1030.
57. Albertini, R.; Abuja, P. M. *Free Rad. Res.* **1998**, *29*, 75.
58. Pollet, E.; Martinez, J. A.; Metha, B.; Watts, B. P.; Turrens, J. F. *Arch. Biochem. Biophys.* **1998**, *349*, 74.
59. Watts, B. P.; Barnard, M.; Turrens, J. F. *Arch. Biochem. Biophys.* **1995**, *317*, 324.
60. Barnard, M.; Gurdian, S.; Diep, D.; Ladd, M.; Turrens, J. F. *Arch. Biochem. Biophys.* **1993**, *300*, 651.
61. Aspée, A.; Lissi, E. A. *Luminescence* **2000**, *15*, 273.
62. Aspée, A.; Lissi, E. A. *J. Protein Chem.* **2001**, *20*, 479.
63. Simat, T. J.; Steinhart, H. *J. Agric. Food Chem.* **1998**, *46*, 490.
64. Nakagawa, M.; Kato, S.; Kataoka, S.; Hino, T. *J. Am. Chem. Soc.* **1979**, *101*, 3136.
65. Gebicki, S.; Gebicki, J. M. *Biochem. J.* **1993**, *289*, 743.
66. Escobar, J. A.; Vásquez-Vivar, J.; Cilento, G. *Photochem. Photobiol.* **1992**, *55*, 895.
67. Pires de Melo, M.; Escobar, J. A.; Metodiewa, D.; Dunford, H. B.; Cilento, G. *Arch. Biochem. Biophys.* **1992**, *296*, 34.
68. Slawinski, J.; Elbanowski, M.; Slawinska, D. *Photochem. Photobiol.* **1980**, *32*, 253.
69. Friedman, M.; Cuq, J. L. *J. Agric. Food Chem.* **1988**, *36*, 1079.
70. Saito, I.; Imuta, M.; Nakada, A.; Matsugo, S.; Matsura, T. *Photochem. Photobiol.* **1978**, *28*, 531.

71. Parcker, J. E.; Mahood, J. S.; Wilson, R. L. Wolfenden, B. S. *Int. J. Radiat. Biol.* **1981**, *39*, 135.
72. Boveris, A.; Cadenas, E.; Reiter, R.; Filipkowski, M.; Nakase, Y. *Prod. Natl. Acad. Sci. USA* **1980**, *77*, 347.
73. Roldán, J. A.; Pinus, C. R.; Turrens, J. F.; Boveris, A. *Gut* **1989**, *30*; 184.
74. Phillips, D.; Anissimov, V.; Karpukhin, O.; Shlyapintokh, V. *Nature* **1967**, *215*, 1163.
75. Phillips, D.; Anissimov, V.; Karpukhin, O.; Shlyapintokh, V. *Photochem. Photobiol.* **1969**, *9*, 183.
76. Matisová-Rychlá, L.; Rychlý, J. In *Polymer Durability: Degradation, Stabilization and Lifetime Prediction*; Clough, R. G.; Billingham, N. C.; Gillen K. T. Eds.; *Adv. Chem. Ser.* 249; ACS: Washington, DC, 1996; pp 175-193.
77. Mendenhall, G. D. *Angew. Chem. Int. Ed.* **2003**, *29*, 362.
78. Matisová-Rychlá, L.; Rychlý, J.; Vavrekova, M. *Eur. Polym. J.* **1978**, *14*, 1033.
79. Blakey, I.; George, G. A. *Macromolecules* **2001**, *34*, 1873.
80. Niki, E.; Decker, C.; Mayo F. R. *J. Polym. Sci. Plym. Chem.* **1973**, *11*, 2813.
81. Mayo F. R. *Macromolecules* **1978**, *11*, 942.
82. Billingham, N. C.; George, G. A. *J. Polym. Sci., Part B: Polym. Phys.* **1990***, 28*, 257.
83. Lacey, D.; Dudler, V. *Polym. Degrad. Stab.* **1996**, 51, 109.
84. Achimsky, L.; Audouin, L.; Verdu, J.; Rychlá, L.; Rychlý, J. *Eur. Polym. J.* **1999**, *35*, 557.
85. Rychlý, J.; Matisová-Rychlá, L.; Jurcák, D. *Polym. Degrad. Stab.* **2000**, *68*, 239.
86. Matisová-Rychlá, L., Rychlý, J. *Polym. Degrad. Stab.* **2000**, *67*, 515.
87. Rychlý, J.; Matisová-Rychlá, L; Tiemblo P.; Gomez-Elvira, J. *Polym. Degrad. Stab.* **2001**, *71*, 253.
88. Vasil'ev, R. F. *Nature* **1963**, *200*, 773.
89. Koo, J.-Y.; Schuster, G. B. *J. Am. Chem. Soc.* **1978**, *100*, 4496.
90. Schuster G. B. *Acc. Chem. Res.* **1979**, *12*, 366.
91. Adam, W.; Cueto, O. *J. Am. Chem. Soc.* **1979**, *101*, 6511.
92. Adam, W.; Zinner, K.; Krebs, A.; Schmalstieg, H. *Tetrahedron Lett.***1981**, *22*, 4567.
93. Stevani, C. V.; Silva, S. M.; Baader, W. J. *Eur. J. Org. Chem.* **2000**, 4037.
94. Kautsky, H.; Neitzke, O. *Z. Phys.***1925**, *31*, 60.
95. Trofimov, A. V.; Vasil'ev, R. F.; Mielke, K.; Adam, W. *Photochem. Photobiol.* **1995**, *62*, 35.
96. Lee, J.; Seliger, H. H. *Photochem. Photobiol.* **1965**, *4*, 1015.
97. Lee, J.; Seliger, H. H. *Photochem. Photobiol.* **1972**, *15*, 227.
98. Hastings, J. W.; Weber, G. *J. Opt. Soc. Am.* **1963**, *53*, 1410.
99. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *Russ. Chem. Bull.* **1996**, *45*, 1596.
100. Kellogg, R. E. *J. Chem. Phys.* **1966**, *44*, 411.
101. Gillispie, G. D.; Lim, E. C. *Chem. Phys. Lett.* **1979**, *63*, 3.
102. Dreeskamp, H.; Pabst, J. *Chem. Phys. Lett.* **1979**, *61*, 262.

103. Fukumura, H.; Kikuchi, K.; Koike, K.; Kokubun, H. *J. Photochem. Photobiol. A: Chem.* **1988**, *42*, 283.
104. Hamanoue, K.; Nakayama, T.; Ikenaga, K.; Ibuki, K. *J. Photochem. Photobiol. A: Chem.* **1993**, *74*, 147.
105. Schmidt, R.; Bauer, H.-D.; Kelm H. *J. Photochem.* **1978**, *8*, 217.
106. Catalani, L. H.; Wilson, T. *J. Am. Chem. Soc.* **1987**, *109*, 7458.
107. Belyakov, V. A.; Vasil'ev, R. F.; Trofimov, A. V. *Chem. Phys. Repts.* **1995**, *13*, 1785.
108. Greenblatt, G. D.; Ruhman, S.; Haas, Y. *Chem. Phys. Lett.* **1984**, *112*, 200.
109. Haas, Y.; Ruhman, S.; Greenblatt, G. D.; Anner, O. *J. Am. Chem. Soc.* **1985**, *107*, 5068.
110. Zuckerman, H.; Schmitz, B.; Haas, Y.; *Chem. Phys. Lett.* **1988**, *151*, 323.
111. Catalani, L. H.; Wilson, T.; Bechara, E. J. H. *Photochem. Photobiol.* **1987**, *45*, 273.
112. Belyakov, V. A.; Vasil'ev, R. F. *Photochem. Photobiol.* **1967**, *6*, 35.
113. Shimasaki, H.; Maeba, R.; Ueta, N. In *Lipofuscin and Ceroid Pigments*; Porta, E. A. Ed.; Plenum Press, New York, 1990, pp 323–331.
114. Da Silva, E. L.; Piskula, M. K.; Yamamoto, N.; Moon, J. H.; Terao, J. *FEBS Lett.* **1998**, *430*, 405.
115. Xu, G.; Liu, Y.; Sayre, L. M. *J. Org. Chem.* **1999**, *64*, 5732.
116. Sharov, V. S.; Kazamanov, V. A.; Vladimirov, Yu. A. *Free Radic. Biol. Med.* **1989**, *7*, 237.
117. Vladimirov, Yu. A.; Sharov, V. S.; Suslova, T. B. *Photobiochem. Photobiophys.* **1981**, *2*, 279.
118. Vladimirov, Yu. A.; Sharov, V. S.; Driomina, E. S.; Reznitchenko, A. V.; Gashev, S. B. *Free Radic. Biol. Med.* **1995**, *18*, 739-745.
119. Vladimirov, Yu. A.; Arroyo, A.; Taylor, J. M.; Tyrina, Yu. Y.; Matsura, T.; Tyrin, V. A.; Kagan, V. E. *Arch. Biochem. Biophys.* **2000**, *384*, 154-162.
120. Catalani, L. H.; Wilson, T. *J. Am. Chem. Soc.* **1989**, *111*, 2633.
121. Blakey, I.; George, G. A.; Billingham, N. C. *Macromolecules* **2001**, *34*, 9130.
122. Schaap, A. P.; Chen, T.-S.; Handley, R. S.; DeSilva, R.; Giri, B. P. *Tetrahedron Lett*. **1987**, *28*, 1155.
123. Schaap, A. P.; Handley, R. S.; Giri, B. P. *Tetrahedron Lett.* **1987**, *28*, 935.
124. Bronstein, I.; Edwards, B.; Voyta, J. C. *J. Biolumin. Chemilumin.* **1988**, *2*, 186.
125. Edwards, B.; Sparks, A.; Voyta, J. C.; Bronstein, I. *J. Biolumin. Chemilumin.* **1990**, *5*, 1.
126. Trofimov, A. V.; Mielke, K.; Vasil'ev, R. F.; Adam, W. *Photochem. Photobiol.* **1996**, *63*, 463.
127. Adam, W.; Bronstein, I.; Edwards, B.; Engel, T.; Reinhardt, D.; Schneider, F. W.; Trofimov, A. V.; Vasil'ev, R. F. *J. Am. Chem. Soc.* **1996**, *118*, 10400.
128. Adam, W.; Bronstein, I.; Trofimov, A. V. *J. Phys. Chem. A* **1998,** *102*, 5406.
129. Adam, W.; Matsumoto, M.; Trofimov, A. V. *J. Org. Chem.* **2000,** *65,* 2078.
130. Watanabe, N.; Kobayashi, H.; Azami, M.; Matsumoto, M. *Tetrahedron* **1999**, *55*, 6831.
131. Matsumoto, M.; Ito, Y.; Matsubara, J.; Sakuma, T.; Mizoguchi, Y.; Watanabe, N. *Tetrahedron Lett.***2001**, *42*, 2352.

132. Koo, J.-Y.; Schmidt, S. P.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 30.
133. Weller, A.; Zachariasse, K. *Chem. Phys. Lett.* **1971**, *10*, 197.
134. Weller, A.; Zachariasse, K. *Chem. Phys. Lett.* **1971**, *10*, 424.
135. Smith, J. P.; Schrock, A. K.; Schuster, G. B. *J. Am. Chem. Soc.* **1982**, *104*, 1041.
136. Lánská, B.; Matisová-Rychlá, L.; Rychlý, J. *Polym. Deg. Stab.* **1998**, *61*, 119.
137. Lánská, B.; Matisová-Rychlá, L.; Brozek, J.; Rychlý, J. *Polym. Deg. Stab.* **1999**, *66*, 433.
138. Lánská, B.; Doskocilova, D.; Matisová-Rychlá, L.; Puffr, R; Rychlý, J. *Polym. Deg. Stab.* **1999**, *63*, 469-480.
139. Denisov, E. T.; Afanas'ev, I. B.; Denisova, T. T.; Trepalin, S. V.; Drozdova, T. I. *Oxidation and Antioxidants in Organic Chemistry and Biology;* CRC Press: Boca Raton, FL, 2005.
140. Simic, M. G.; Karel, M. Eds.; *Autooxidation in Food and Biological Systems*; Plenum: New York, 1980.
141. Ingold, K. U. *Chem. Rev.*, **1961**, *61*, 563.
142. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194.
143. Burlakova, E. B.; Khrapova, N. G. *Russ. Chem. Rev.* **1985**, *54*, 907.
144. Burlakova, E. B.; Varfolomeev, S. D. Eds. *Chemical and Biological Kinetics. New Horizons, Vol. 2: Biological Kinetics;* Brill, VSP: Leiden, The Netherlands, 2005.
145. Yla-Herttuala, S.; Palinski, W.; Rosenfeld, M. E.; Steinberg, D.; Witztum, J. L. *Eur. Heart. J.* **1990**, *11*, 88.
146. Melov, S.; Ravenscroft, J.; Malik, S.; Gill, M. S. Walker, D. W.; Clayton, P. E.; Wallace, D. C.; Malfroy, B.; Doctorow, S. R.; Lithgow, G. J. *Science* **2000**, *289*, 1567.
147. Von Zglinicki, T.; Pilger, R.; Sitte, N. *Free Radical. Biol. Med.* **2000**, *28*, 64.
148. Braginskaya, F. I.; Zorina, O. M.; Pal'mina, N. P.; Gaintseva, V. D.; Burlakova, E. B.; Selezneva, N. D.; Kolykhalov, I. V.; Gavrilova, S. I. *Neuroscience and Behavioral Physiol.* **2001**, *31*, 457.
149. Becker, D. A.; Ley, J. J.; Echegoyen, L.; Alvarado, R. *J. Am. Chem. Soc.*, **2002**, *124*, 4678.
150. Becker, D. A. *Cell. Mol. Life Sci.* **1999**, *56*, 626.
151. Wattenberg, L. W. *Cancer Res.* **1985**, *45*, 1.
152. Yang, C. S.; Landau, J. M.; Huang, M. T.; Newmark, H. L. *Annu. Rev. Nutr.* **2001**, *21*, 381. Garewal, H. S. Ed. *Antioxidants and Disease Prevention (Modern Nutrition Series)*; CRC Press: New York, 1997.
153. Ahmad, S. Ed. *Oxidative Stress and Antioxidant Defenses in Biology*; Chapman & Hall: New York, 1995.
154. Ma, Q.; Kinneer, K. *J. Biol. Chem.* **2002**, *227*, 2477.
155. Appenroth, D.; Karge, E.; Kiessling, G.; Wechter, W. J.; Winnefeld, K.; Fleck, C. *Toxicol. Lett.* **2001**, *122*, 255.
156. Ahmed, S. M.; Slater T. F. In *Recent Advances in Lipid Peroxidation and Tissue Injury*; Slater T. F.; Garner, A. Eds. Brunel University Printing Services: Uxbridge, UK, 1981; pp 177-194.

157. Belyakov, V. A.; Vasil'ev, R. F.; Fedorova, G. F. *J. Photochem. Photobiol. A: Chem.* **1993**, *72*, 73.
158. Belyakov, V. A.; Vasil'ev, R. F.; Trofimov, A. V.; Fedorova, G. F. In *Free Radicals in Biology and Environment (NATO Adv. Sci. Inst. Series*, *Series 3: High Technology)*; Minisci, F. Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands; 1997, Vol. 27, pp. 233-250.
159. Belyakov, V. A.; Burlakova, E. B.; Vasil'ev, R. F.; Arkhipova, G. V.; Fedotova, I. B.; Chernyavskaya, L. I. *Dokl. Biochemistry* **1994**, *336*, 45.
160. Mead, J. F. In *Free Radicals in Biology*; Pryor, W. A. Ed.; Academic Press: New York, 1976; Vol. 1, pp 51-68.
161. Bors, W.; Saran, M.; Tait, D. Eds. *Oxygen Radicals in Chemistry and Biology*; De Gruyter: Berlin, 1984.
162. Mehlhorn, R. J., Cole, G. *Adv. Free Radic. Biol. Med.*, **1985**, *1*, 165.
163. Ames, B. N. *Science* **1983**, *221*, 1256.
164. Cadet, J.; Di Mascio, P. In *The Chemistry of Peroxides*; Rappoport, Z. Ed.; Patai Series; Wiley: Chichester, 2006; Vol. 2, Part 2, pp 915-999.
165. Skulachev, V. P. *Biochemistry (Moscow)* **2001**, *66*, 1153.
166. Ricardo, M. A. G.; Andreo, M. A.; Cavalheiro, A. J.; Gamboa, I. C.; Bolzani, V. S.; Silva, D. H. S. *Arkivoc* **2004** (viii), 127.
167. Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. *Nature* **2000**, *405*, 903.
168. Roginsky, V.; Barsukova, T. *J. Med. Food* **2001**, *4*, 219.
169. Sellés, A. J. N.; Castro, H. T. V.; Agüero-Agüero, J.; González-González, J.; Naddeo, F.; De Simone, F.; Rastrelli, L. *J. Agric. Food Chem.* **2002**, *50*, 762.
170. Urizzi, P.; Monje, M.-C.; Souchard, J. P.; Abella, A.; Chalas, J.; Lindenbaumm, A.; Vergnes, L.; Labidalle, S.; Nepveu, F. *J. Chim. Phys. PCB* **1999**, *96*, 110.
171. Richelle, M.; Tavazzi, I.; Offord, E. *J. Agric. Food Chem.* **2001**, *49*, 3438.
172. Cano Lario, A.; Acosta Echeverria, M.; Banon Arnao, M. *Alimentaria (Madrid)* **1998**, *295*, 73.
173. Pietta, P.; Simonetti, P.; Mauri, P. *J. Agric. Food Chem.* **1998**, *46*, 4487.
174. Miyagi, Y.; Miwa, K.; Inoue, H. *Am. J. Cardiol.* **1997**, *80*, 1627.
175. Koleva, I. Linssen, J. P. H.; Van Beek, T. A.; Evstatieva, L. N.; Kortenska, V. Hanjieva, N. *J. Sci. Food Agric.* **2003**, *83*, 809.
176. Lee, J. H.; Renita, M.; Fioritto, R. J.; Martin, S. K. St.; Schwartz, S. J.; Vodovotz, Y. *J. Agric. Food Chem.* **2004**, *52*, 2647.
177. Suja, K. P.; Jayalekshmy, A.; Arumughan, C. *J. Agric. Food Chem.* **2004**, *52*, 912.
178. Castro, I. A.; Berlanga, S. M. B.; Marques, U. L.; Motizuki, M.; Sawada, T. C. H. *Food Res. Int.* **2005**, *38*, 861.
179. Prior, R.; Wu, X.; Schaich, K. *J. Agric. Food Chem.* **2005**, *53*, 4290.
180. Roginsky, V.; Lissi, E. A. *Food Chem.* **2005**, *92*, 235.

181. Castro, I. A.; Rogero, M. M.; Junqueira, R. M.; Carrapeiro, M. M.; *Int. J. Food Sci. Nutr.* **2006**, *57*, 75.
182. Castro, I. A.; Rogero, M. M.; Junqueira, R. M.; Carrapeiro, M. M.; *Int. J. Food Sci. Technol.* **2006**, *41*, 59.
183. Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. *J. Agric. Food Chem.* **1994**, *42*, 64.
184. Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. *J. Agric. Food Chem.* **1995**, *43*, 890.
185. Teissedre, P.-L.; Waterhouse, A. L.; Frankel, E. N. *J. Int. Sci. Vigne Vin* **1995**, *29*, 205.
186. Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. *J. Agric. Food Chem.* **1998**, *46*, 25.
187. Costin, J. W.; Barnett, N. W.; Lewis, S. W.; McGillivery, D. J. *Anal. Chim. Acta* **2003**, *499*, 47.
188. Roginsky, V.; De Beer, D.; Harbertson, J. F.; Kilmartin, P. A.; Barsukova, T.; Adams, D. O. *J. Sci. Food Agric.* **2006**, *86*, 834.
189. Burns, J.; Gardner, P. T.; O'Neil, J.; Crawford, S.; Morecroft, I.; McPhail, D. B.; Lister, C.; Matthews, D.; MacLean, M. R.; Lean, M. E. J.; Duthie, G. G.; Crozier, A. *J. Agric. Food Chem.* **2000**, *48*, 220.
190. Pellegrini, N.; Simonetti, P.; Gardana, C.; Brenna, O.; Brighenti, F.; Pietta, P. *J. Agric. Food Chem.* **2002**, *48*, 732.
191. Budnikov, G. K.; Ziyatdinova, G. K. *J. Anal. Chem.* **2005**, *60*, 600.
192. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
193. Burlakova, E. B.; Kukhtina, E. N.; Ol'khovskaya, I. P.; Sinkina, E. B. *Biophysics (Moscow)* **1979**, *24*, 965.
194. Whitehead, T. P.; Thorpe, G. H. G.; Maxwell, S. R. J. *Anal. Chim. Acta*, **1992**, *266*, 265.
195. Hirayama, O.; Takagi, M.; Hukumoto, K.; Katoh, S. *Anal. Biochem.* **1997**, *247*, 237.
196. Smith, R.; Vantman, D.; Ponce, J.; Escobar, J.; Lissi, E. *Human Reproduction* **1996**, *11*, 1655.
197. Pascual, C.; Reinhart, K. *Luminescence* **1999**, *14*, 83.
198. Popov, I.; Lewin, G. *J. Biochem. Biophys. Methods* **1996**, *31*, 1
199. Tawa, R.; Sakurai, H. *Analytical Lett.* **1997**, *30*, 2811.
200. Desmarchelier, C.; Barros, S.; Repetto, M.; Latorre, L. R..; Kato, M.; Coussio, J. Ciccia, G. *Planta Medica* **1997**, *63*, 561.
201. Toyo'oka, T.; Ogawa, A.; Arai, H.; Tanizawa, H. *Biomedical Chromatography* **1999**, *13*, 101.
202. Oosthuizen, M. J.; Greyling, D. *Redox Rep.* **1999**, *4*, 277.
203. Palaron, W. S.; Bergantin, J. Jr.; Sevilla, F. III *Anal. Lett.* **2000**, *33*, 1797.
204. Parejo, I.; Codina, C.; Petrakis, C.; Kefalas, P. *J. Pharmacol. Toxicol.* **2001**, *44*, 507.
205. Crestanello, J. A.; Lingle, D. M.; Kamelgard, J.; Millili, J.; Whitman, G. J. *J. Surgical Res.* **1996**, *65*, 53.
206. Park, D. K.; Song, J. H. *Korean Biochem. J.* **1994**, *27*, 473.

207. Gómez-Taylor Corominas, B.; Antón Fos, G. M.; García Mateo, J. V.; Lahuerta Zamora, L.; Martínez Calatayud, J. *Talanta* **2003**, *60*, 623.
208. Georgetti, S. R.; Casagrande, R.; Di Mambro, V. M.; Azzolini, A. E. C. S.; Fonseca, M. J. V. *AAPS Pharm. Sci.* **2003**, *5*, 1.
209. Papadopoulos, K.; Triantis, T.; Yannakopoulou, E.; Nikokavoura, A.; Dimotikali, D. *Anal. Chim. Acta* **2003**, *494*, 41.
210. Toyo'oka, T.; Kashiwazaki, T.; Kato, M. *Talanta* **2003**, *60*, 467.
211. Bastos, E. L.; Romoff, P.; Eckert, C. R.; Baader, W. J. *J. Agric. Food Chem.* **2003**, *51*, 7481.
212. Latté, K. P.; Kolodziej, H. *J. Agric. Food Chem.* **2004***, 52*, 4899.
213. Parejo, I.; Viladomat, F.; Bastida, J.; Schmeda-Hirschmann, G.; Burillo, J.; Codina, C. *J. Agric. Food Chem.* **2004***, 52*, 1890.
214. Tian, B.; Wu, Y.; Sheng, D.; Zheng, Z.; Gao, G.; Hua, Y. *Luminescence* **2004**, *19*, 78.
215. Wenli, Y.; Yaping, Z. *Biochim. Biophys. Acta* **2005**, *1725*, 30.
216. Marquele, F. D.; Di Mambro, V. M.; Georgetti, S. R.; Casagrande, R.; Valim, Y. M.; Fonseca, M. J. *J. Pharm. Biomed. Anal.* **2005**, *39*, 455.
217. Bancířová M.; Šnyrychová, I. In: *Bioluminescence and Chemiluminescence. Progress and Perspectives*; Tsuji, A.; Matsumoto, M.; Maeda, M.; Kricka, L. J.; Stanley, P. E. Eds. World Scientific: Singapore, 2005; pp 279-282.
218. Popov, I.; Lewin, G. *Luminescence* **2005**, *20*, 321.
219. Triantis, T. ; Stelakis, A.; Dimotikali, D.; Papadopoulos, K. *Anal. Chim. Acta* **2005**, *536*, 101.
220. Nälsén, C. Ph.D. thesis, Uppsala University, 2006.
221. Pavlova, E. L.; Savov, V. M. *Biochemistry (Moscow)* **2006**, *71*, 861.
222. Belyakov, V. A.; Roginsky, V. A.; Bors, W. *J. Chem. Soc. Perkin Trans. 2* **1995**, 2319.
223. Takahashi, M.; Yoshikawa, Y.; Niki, E. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1885.
224. Bowry, V. W.; Stocker, R. *J. Am. Chem. Soc.* **1993**, *115*, 6029.
225. Schwartz, J. L. *J. Nutr.* **1996**, *126*, 1221S.
226. Mukai, K.; Sawada, K.; Kohno, Y.; Terao J. *Lipids* **1993**, *28*, 747.
227. Siraki, A. G.; O'Brien, P. J. *Toxicology* **2002**, *177*, 81.
228. Sakihama, Y. Cohen, M. F.; Grace, S. C.; Yamasaki, H. *Toxicology* **2002**, *177*, 67.
229. Zinchuk, V. V.; Dorokhina, L. V.; Maltsev, A. N. *J. Therm. Biol.* **2002**, *27*, 345.
230. Cotelle, N. *Curr. Top. Med. Chem.* **2001**, *1*, 569-590.
231. Hudson, B. J. F.; Lewis, J. I. *Food Chem.* **1983**, *10*, 47.
232. Torel, J.; Cillard, J.; Cillard, P. *Phytochemistry* **1986**, *25*, 383.
233. Affany, A.; Salvayre, R.; Dousté-Blazy, L. *Fundam. Clin. Pharmacol.* **1987**, *1*, 451.
234. Ratty, A. K.; Das, N. P. *Biochem. Med. Metab. Biol.* **1988**, *39*, 69.
235. Mora, A.; Paya, M.; Rios, J. L.; Alcaraz, M. J. *Biochem. Pharmacol.* **1990**, *40*, 793.
236. Chen, Y.; Zheng, R.; Jia, Z.; Ju, Y. *Free Radical Biol. Med.* **1990**, *9*, 19.
237. Larson, R. A. *Phytochemistry* **1988**, *27*, 969.

238. Teel, R. W.; Castonguay, A. *Cancer Lett.* **1992**, *66*, 107.
239. Morel, I.; Lescoat, G.; Cogrel, P.; Sergent, O.; Paseloup, N.; Brissot, P.; Cillard, P.; Cillard, J. *Biochem. Pharmacol.* **1993**, *45*, 13.
240. Belyakov, V. A.; Fedorova, G. F.; Naumov, V. V.; Trofimov, A. V.; Vasil'ev, R. F. In *Bioluminescence and Chemiluminescence. Progress and Perspectives*; Tsuji, A.; Matsumoto, M.; Maeda, M.; Kricka, L. J.; Stanley, P. E. Eds. World Scientific: Singapore, 2005; pp 283-286.
241. Ghiselli, A.; Serafini, M.; Ferro-Luzzi, A. *Free Radical. Biol. Med.* **1994**, *16*, 135.
242. Halliwell, B.; Gutteridge, J. M. C. *Free Radical. Biol. Med.* **1995**, *18*, 125.
243. Prior, R. L.; Cao, G. *Free Radical. Biol. Med.* **1999**, *27*, 1173.
244. Arnao, M. B. *Trends Food Sci. Technol.* **2000**, *11*, 419.
245. Ghiselli, A.; Serafini, M.; Natella, F.; Scaccini, C. *Free Radical. Biol. Med.* **2000**, *29*, 1106.
246. Prahalad, A. K.; Inmon, J; Dailey, L. A.; Madden, M. C.; Ghio, A. J.; Gallagher, J. E. *Chem. Res. Toxicol.* **2001**, *14*, 879.
247. Prahalad, A. K.; Ghio, A. J.; Lehmann, J. R.; Winsett, D. W.; Tepper, J. S.; Park, P.; Gilmour, M. I.; Dreher, K. L.; Costa, D. L. *Inhal. Toxicol.* **1996**, *8*, 457.
248. Ghio, A. J.; Stonehuer, J.; Pritchard, R. J.; Piantadosi, C. A.; Quigley, D. R.; .; Dreher, K. L.; Costa, D. L. *Inhal. Toxicol.* **1996**, *8*, 479.
249. Prahalad, A. K.; Inmon, J.; Ghio, A. J.; Gallagher, J. E. *Chem. Res. Toxicol.* **2000**, *13*, 1011.
250. Kadiiska, M. B.; Mason, R. P.; Dreher, K. L.; Costa, D. L.; Ghio, A. J. *Chem. Res. Toxicol.* **1997**, *10*, 1104.
251. Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1976**, *9*, 13.
252. Emanuel, N. M.; Buchachenko, A. L. *Chemical Physics of Degradation and Stabilization of Polymers*; VNU: Amsterdam, 1988.

Authors' biographical data

Galina F. Fedorova, born in 1938 in Leningrad (now St. Petersburg), Russia, graduated from the St. Petersburg (Leningrad) State University of Information Technologies, Mechanics and Optics (1962, M.Sc. in spectroscopy and optical engineering) and received Ph.D. degree (1979,

with Prof. R.F. Vasil'ev and Dr. V.A. Belyakov) in physics and mathematics from the Russian Academy of Sciences (Moscow, Russia). During her further academic work, Dr. Fedorova was affiliated to the Institutes of Chemical/Biochemical Physics, Russian Academy of Sciences. Currently, she has a position of Senior Scientist at the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences. Her main research interests refer to organic photochemistry, photophysics and chemiluminescence (most prominently, in oxidation reactions), chemical kinetics and studying the antioxidants (including bioantioxidants). The sample accounts on her research may be found in *Spectroscopy Lett.* **1978**, *11*, 549-561; *NATO Adv. Sci. Inst. Series*, *Series 3: High Technology* **1997**, *27*, 233-250 and *Kinetics and Catalysis* **2004**, *45*, 329-336.

Alexey (*Alexei*) V. Trofimov, born in 1965 in Moscow, Russia, received his M.Sc. (1988) and Ph.D. (1991, with Prof. R.F. Vasil'ev and Dr. V.A. Belyakov) degrees in physics and mathematics from the Moscow Institute of Physics and Technology (State University) and D.Sc. ("Habilitation") degree in chemistry (2005) from the Russian Academy of Sciences. During his academic career, he held positions at the Institutes of Chemical/Biochemical Physics, Russian Academy of Sciences (Moscow, Russia), as Senior Scientist and at the Institute of Organic Chemistry, University of Würzburg (Germany), as postdoctoral fellow and Senior Research Associate with Prof. W. Adam. Currently, he holds a position of Leading Scientist (Research Professor) and runs the Chemiluminescence Group at the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences. His research interests comprise chemical kinetics, organic photochemistry and photophysics, chemiluminescence, energy and electron transfer, oxidation processes and high-pressure chemistry in supercritical fluids. The pertinent summaries and reviews on his recent research are given partly in *Acc. Chem. Res.* **2003**, *36*, 571-579 and in *The Chemistry of Peroxides*; Rappoport, Z. Ed.; Patai Series; Wiley: Chichester, 2006; Vol. 2, Part 2, pp. 1171-1209. The research activity of Dr. Trofimov was marked by various awards and fellowships, including the *Academia Europaea* Prize in chemistry received in 1999.

Rostislav F. Vasil'ev, born in 1928 in Leningrad (now St. Petersburg), Russia, graduated from the Leningrad (St. Petersburg) State University (1952, M.Sc. in physics), received degrees of Ph.D. in chemistry (1955 with Profs. A.N. Terenin and N.M. Emanuel) from the Russian Academy of Sciences and D.Sc. ("Habilitation") in physics and mathematics (1964) from the Leningrad (St. Petersburg) State University. Over years, he was a leader of the Chemiluminescence Group at the Institutes of Chemical/Biochemical Physics, Russian Academy of Sciences (Moscow, Russia). Presently, he is Full Professor in physical chemistry and holds positions at the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, as Head of the research group and at the Council for Luminescence, Russian Academy of Sciences, as Chairman of the Chemiluminescence Section. Prof. Vasil'ev is well known for his pioneering works on oxy-chemiluminescence (*e.g.*, see *Nature* **1962**, *194*, 1276) and triplet-singlet energy transfer in liquid solutions (*Nature* **1962**, *196*, 668 and *Nature* **1963**, *200*, 773). His current research interests encompass diverse aspects of chemical kinetics, excited-state chemistry, photophysics and organic chemiluminescence (most prominently, oxy-chemiluminescence, "photostorage" chemiluminescence and chemical models of bioluminescence processes). The recent research activities of Prof. Vasil'ev are partly summarized in *NATO Adv. Sci. Inst. Series*, *Series 3: High Technology* **1997**, *27*, 233-250; *High Energy Chem*. **2002**, *36*, 170-178 and *Russ. Chem. Rev.* **2006**, accepted).

Timur L. Veprintsev, born in 1979 in Moscow, Russia, graduated from the M.V.Lomonosov Moscow State Academy of the Fine Chemical Technology (2001, M.Sc. in chemical technology). His current Ph.D. project at the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, deals with elaboration of chemiluminescent techniques for studying the oxidative and antioxidative processes in chemical and biological media.