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The chemistry of peroxynitrite. Reaction mechanisms and kinetics

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Contents

I.	Introduction	375
II.	Formation of peroxynitrite	375
III.	Physical and chemical properties	376
IV.	Decomposition and isomerisation	378
V.	Reactions with hydrocarbons and other compounds	381
VI.	Biochemical role of peroxynitrite	391
VII.	Conclusion	392

Abstract. Methods for the preparation of peroxynitrite and its physical and chemical properties are considered. The pathways, mechanisms and kinetics of the decomposition and best-studied reactions with hydrocarbons, dimethyl sulfoxide and other substrates are analysed. The emphasis is placed on the nature of reactive species in peroxynitrite reactions and on the reasons for its high and polyfunctional reactivity. The biochemical role of this important inorganic oxidant formed *in vivo* is outlined. The bibliography includes 268 references.

I. Introduction

Peroxynitrous acid ONOOH and peroxynitrite anion $ONOO^-$ have the same conventional name 'peroxynitrite'. [According to IUPAC recommendations,[1] they should be called hydrogen oxoperoxonitrate and oxoperoxonitrate(1 −), respectively.]

Peroxynitrite was first detected more than 100 years ago as unstable product of the interaction of nitrites with hydrogen peroxide in acid media, which possesses unusually high oxidative ability. Its formula, ONOOH, which is commonly accepted at present, was proposed in 1929.[2]

About 40 studies concerning peroxynitrite were reported before 1990. They were followed by rapidly increasing interest in this research avenue due to the discovery [3] of *in vivo* formation of $ONOO^-$ and to establishment of its important role in physiological processes,[4–11] in atmosphere chemistry [12] and in chemistry of natural water.[13] The total number of reports on the subject vigorously increases, being more than 7300 at the moment.

The main avenues of research in chemistry of peroxynitrite include (1) pathways and mechanisms of ONOOH decay over a wide range of pH values, (2) reasons for high reactivity and the mechanisms of reactions with organic compounds and biomolecules and (3) pathways of synthesis and transport of peroxynitrite in biological systems.

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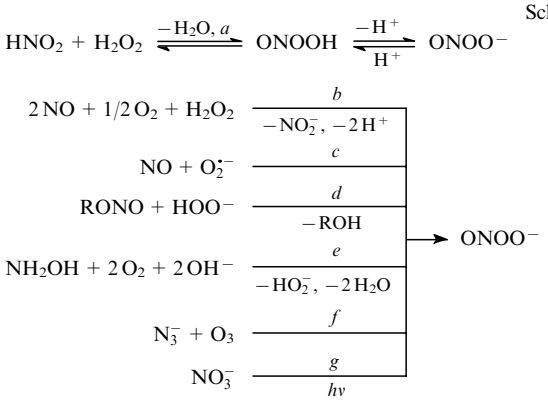
High reactivity of peroxynitrite is usually explained by generation of 'hydroxyl-like' radicals in the homolysis of ONOOH. However, in spite of numerous studies in this field, the nature of the reactive species is still highly debatable. This is due to instability of peroxynitrite in neutral and acid media, large number of possible transformation paths and to the fact that many reactions involving the compound are catalysed by metal complexes, carbon dioxide and carbonyl compounds.

The first systematic review [14] on the chemistry of peroxynitrite appeared in 1994; biochemical aspects were first considered in detail in a monograph.[15] A number of more recent interesting reviews are also available. [4, 7, 9, 11, 16–18] In this review we will dwell on the reactions of peroxynitrite formation (including *in vivo* formation), preparative synthesis, pathways and mechanisms of peroxynitrite decay and other reactions involving the compound. We will also briefly outline its role in biochemical processes. In addition to the results of studies on the interaction of peroxynitrite with sulfides, carbon dioxide, carbonyl compounds, phenols and ascorbic acid, we will present a detailed consideration of recent data on the kinetics and mechanisms of model reactions with hydrocarbons (alkanes, alkenes, alkylbenzenes) and DMSO obtained in the authors' laboratory.

II. Formation of peroxynitrite

Chemical reactions resulting in peroxynitrite are shown in Scheme 1.

Scheme 1



1. Reaction of H_2O_2 with HNO_2 (route *a*). It was established [14, 19–23] that peroxynitrous acid is rapidly formed (as

intermediate) in acidic media, but the compound is unstable (half-life is a few seconds at 0 °C).[14, 21, 24, 25] In practice, relatively stable $ONOO^-$ anions can be generated using instantaneous 'quenching'[25, 26] of the reaction mixture with an excess of alkali. Solutions thus obtained contain nitrite, hydrogen peroxide and nitrate impurities. The yield of peroxynitrite can be significantly increased using simple flow reactors (Fig. 1).[22, 23, 27] The nitrite and acidified hydrogen peroxide solutions are introduced, by means of compressed gas[27] or vacuum,[22] into a mixing cell and then, through a short pipe, into the second mixing cell where the reaction is 'quenched' with a sodium hydroxide solution. Optimisation of the reagent feed rates and the tube length allows peroxynitrite to be synthesised in high yield (> 91%) and with low impurity content.

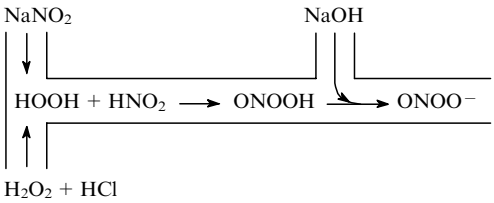


Figure 1. Scheme of peroxynitrite formation in a quenched flow reactor.[22]

Reaction *a* is reversible:[28]

$$HNO_2 + H_2O_2 \rightleftharpoons ONOOH + H_2O, \qquad (1)(-1)$$

K (25 °C) = $(1.33 \pm 0.05) \times 10^3$ litre mol^{-1}.

Formation of ONOOH in the $H^+ - HNO_2 - H_2O_2$ system involves intermediate generation of nitrosyl cation[20]

$$NO_2^- + 2H^+ \rightleftharpoons NO^+ + H_2O, \qquad (2)(-2)$$

$$NO^+ + H_2O_2 \longrightarrow ONOOH + H^+. \qquad (3)$$

The corresponding kinetic equation has the form

$$\frac{d[ONOOH]}{dt} = \frac{k_2 k_3 [H^+]^2 [NO_2^-][H_2O_2]}{k_{-2}[H_2O] + k_3[H_2O_2]},$$

where $k_2 = 5.8 \times 10^6$ litre^2 mol^{-2} s^{-1} and $k_3/k_{-2}[H_2O] =$ 2.4 litre mol^{-1} (pH 4–6, 25 °C).[20]

It is believed[23, 29] that protonated nitrous acid $H_2NO_2^+$ is a more probable nitrosating agent in this system:

$$NO_2^- + 2H^+ \rightleftharpoons H_2NO_2^+ \ (pK_a = 3.3), \qquad (4)(-4)$$

$$H_2NO_2^+ + H_2O_2 \longrightarrow ONOOH + H_2O + H^+, \qquad (5)$$

where $k_4 = 600$ litre mol^{-1} s^{-1} and $k_5/k_{-4} = 2.31$ litre mol^{-1}.

Nitrosyl chloride NOCl formed in the presence of chloride ions also reacts with hydrogen peroxide:[23]

$$H_2NO_2^+ + Cl^- \rightleftharpoons NOCl + H_2O,$$

$$NOCl + H_2O_2 \longrightarrow ONOOH + H^+ + Cl^-.$$

2. Reaction of H_2O_2 with NO in alkaline media (route *b*).[19, 30, 31] This reaction cast some doubt until it was found[31] that it proceeded only in the presence of oxygen involving formation of N_2O_3 as intermediate, which is then attacked by the hydroperoxide anion [$k = (1.0 \pm 0.6) \times 10^9$ litre mol^{-1} s^{-1}] (see Refs 19, 30, 31). The rate constant for the reaction of N_2O_3 with H_2O_2 is three orders of magnitude lower.

3. Reaction of NO with $O_2^{\cdot -}$ (route *c*). Both being of radical nature, the superoxide ion and nitrogen(II) oxide recombine at a diffusion rate [$k = (3.8 - 19) \times 10^9$ litre mol^{-1} s^{-1}, Refs 32–38] to give ^-OONO.† The reaction plays a key role *in vivo*[3, 9, 11, 16, 39] (see Section VI). It is reversible, but the equilibrium is almost completely shifted towards $ONOO^-$. Indeed, $K = (3.1 - 3.6) \times 10^{-12}$ ($k = 0.017 - 0.0023$ s^{-1} for the reverse reaction) in aqueous solutions at 25 °C.[40–42] Based on this reaction, new efficient methods of synthesis of peroxynitrite were developed, namely, the interaction of trimethylammonium superoxide with nitrogen(II) oxide in liquid ammonia[43] and a heterogeneous reaction of crystalline potassium superoxide with NO.[44] Both methods result in high yield of peroxynitrite and minimum content of nitrates and nitrites.

4. Reaction of alkyl nitrites with hydrogen peroxide (route *d*)[45, 46] proceeds in alkaline media involving hydroperoxide anion HOO^- as reagent. The reaction is of preparative value, but peroxynitrite thus obtained contains an equimolar amount of alcohol as impurity (alcohol can be oxidised with peroxynitrite). The advantage of this procedure consists in low content of nitrite, nitrate and hydrogen peroxide impurities.

5. Autooxidation of hydroxylamines and chloramines (route *e*). Alkaline oxidation of the compounds containing nitrogen in the oxidation states below +3 results in peroxynitrite in 20%–25% yield with respect to the starting substrate.[47, 48] The reaction involves formation of a triplet NO^- anion as intermediate.

$$NH_2OH + O_2 + 2OH^- \longrightarrow HO_2^- + NO^- \text{ (triplet)} + 2H_2O,$$

$$NO^- \text{ (triplet)} + O_2 \longrightarrow ONOO^-.$$

The interaction of oxygen with triplet NO^- produced in the photolysis of trioxodinitrates also leads to peroxynitrite.[49]

6. Ozonation of azides (route *f*) makes it possible to prepare peroxynitrite solutions that are of low ionic strength and free from H_2O_2 impurities.[50, 51]

7. Radiolysis and photolysis of nitrates in solutions or in the crystalline state (route *g*).[14, 52–55] These processes occur in atmosphere, natural water and on the Earth surface. Photolysis of nitrates is associated with the formation of peroxynitrite on Martian surface, which was detected by the 'Viking' Mars landers.[14] Various photolysis techniques are used for fast generation of peroxynitrite in studies of its reactivity. For instance, pulse radiolysis was used for measuring the rate constants for formation, breakdown and other reactions of peroxynitrous acid; its UV spectrum was also recorded.[55] Solid solutions of ONOOK in KNO_3 were prepared by photolysis of crystalline KNO_3 ($\lambda = 250 - 450$ nm, 2 h).[55, 56]

Preparative syntheses of peroxynitrite are mainly based on the reactions *a*, *e*, *f* and *c*. The last-named method makes it possible to obtain pure tetramethylammonium peroxynitrite that is free from nitrites, nitrates, hydrogen peroxide, hydroxylamine and azide.

III. Physical and chemical properties

The physicochemical properties of peroxynitrite are summarised in Tables 1–4. The experimental standard enthalpy of formation, $\Delta_f H^\circ$, of $ONOO^-$ anion in aqueous solutions is -42 ± 4 kJ mol^{-1}. Different values were reported for the absolute entropy (ΔS°) of $ONOO^-$ anion in solution, ranging from 63 (see Ref. 59) to 130 J K^{-1} mol^{-1} (see Refs 13 and 61). The standard free energy of formation ($\Delta_f G^\circ$) of $ONOO^-$ lies between ~70 and 42 kJ mol^{-1}. A recently reported[42] $\Delta_f G^\circ$ value of 68.5 ± 1.4 kJ mol^{-1} was derived from the experimental rate constants for the direct and reverse reactions in equilibrium

$$NO^{\cdot} + O_2^{\cdot -} \rightleftharpoons ONOO^-.$$

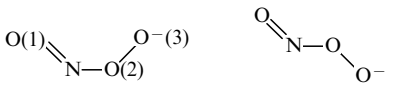
† The most reliable value of the rate constant, $(16 \pm 3) \times 10^9$ litre mol^{-1} s^{-1}, was obtained in the studies[37, 38] carried out using three different flash photolysis techniques.

Table 1. Selected thermodynamic characteristics of peroxynitrite.

End product or reaction equation	ΔH° /kJ mol^{-1}	Ref.	ΔG° /kJ mol^{-1}	Ref.	ΔS° /J K^{-1} mol^{-1}	Ref.
Formation						
$ONOO^-$ (aq.)	-42 ± 4	57, 58	42 ± 8	24	63	59
			59 ± 13	60	130 ± 29	16, 61
			67.4 ± 1.3	29	188	24
			68.5 ± 1.4	42		
ONOOH (aq.)	-59 ± 8	16, 61	21 ± 13	61	197 ± 21	16, 61
	-60 ± 4	62	29.7 ± 0.8	29		
			31 ± 2	42		
			32.2	41, 59		
ONOOH (gas)	4 ± 13	16, 61	54 ± 13	16, 61	301 ± 8	16, 59, 61
Ionisation						
ONOOH (aq.) $\rightleftharpoons$ $ONOO^-$ (aq.) + H^+	~ 0	24, 59	-36.8 ± 0.8 (see [a])	16, 61	-67 ± 21	16, 61
	17 ± 8	16, 61			-79	21
	29	21				
Isomerisation						
ONOOH $\longrightarrow$ HNO_3	-163 ± 4	16, 61	-167 ± 13	16, 61	13 ± 33	16, 61

[a] $pK_a = 6.5$.

The free energy of peroxynitrite is 150 kJ mol^{-1} higher than that of isomeric nitrate;[24, 57] nevertheless, $ONOO^-$ anion is relatively stable, that is, it lives in alkali solutions for a few weeks. The reason is the conformation of this species. According to quantum chemical calculations,[14] the order of the bond between the nitrogen atom and the peroxide oxygen atom, O(2), in peroxynitrite approaches a value of 1.5, thus providing an explanation for the existence of *cis*- and *trans*-isomers.



cis-$ONOO^-$ *trans*-$ONOO^-$

The *cis*-isomer is 8–17 kJ mol^{-1} more stable than the *trans*-isomer [70–73] due to (i) partial delocalisation of negative charge over the entire molecule and (ii) partial bonding between the O(1) and O(3) atoms (the bonding energy is comparable with the energy of a hydrogen bond). Delocalisation stabilises peroxynitrite in the *cis*-conformation and precludes either rotation of the O(3) atom about the N–O(2) bond axis or a change in the N–O–O bond angle in order to adopt the *trans*-configuration. In *cis*-$ONOO^-$, the terminal atom O(3) cannot attack the nitrogen atom to give a nitrate, in spite of high driving force of the reaction ($\Delta G = -150$ kJ mol^{-1}). Such an attack becomes possible in the *trans*-configuration owing to a decrease in the N–O–O bond angle ($\sim 117^\circ$ for the *cis*- and $\sim 112^\circ$ for the *trans*-isomer) and to elongation of the O–O bond (from ~ 1.37 Å for the *cis*- to ~ 1.40 Å for the *trans*-isomer) (results of *ab initio* calculations).

According to calculations,[70–73] the energy barrier to isomerisation of the *cis*-anion to the *trans*-isomer lies between 88 and 113 kJ mol^{-1} and the process is impossible at physiological temperatures. For peroxynitrous acid, this barrier reduces to 40–50 kJ mol^{-1}, *cis*-ONOOH being only 2–8 kJ mol^{-1} more stable than *trans*-isomer.[70, 71, 73–76]

Spectral studies confirmed that peroxynitrite in alkali solutions exists only as *cis*-isomer. In particular, the ^{15}N NMR spectra of peroxynitrite in 0.3 M NaOH exhibit [15, 71] a single peak (would both *cis*- and *trans*-isomers be present in solution, two peaks should be recorded). Small frequency shifts in the Raman spectra of ^{15}N-peroxynitrite are consistent with the presence of only *cis*-isomer.[70]

Table 2. Activation parameters of peroxynitrite isomerisation (ONOOH $\rightarrow$ HNO_3).

Parameter, units of measurement	Value	Ref.
$\Delta_{iso}H^{\neq}$ /kJ mol^{-1}	75 ± 4	24
	82	21
	86 ± 4	16
	88.7	40, 62
$\Delta_{iso}S^{\neq}$ /J K^{-1} mol^{-1}	13 ± 8	24
	31	21
	54	40, 62
$\Delta_{iso}V^{\neq}$ /cm^3 mol^{-1}	1.7 ± 1.0	16, 36
	4.8 ± 0.2	63
	6.7 ± 0.9	63
	9.7 ± 1.4	64
	10.2 ± 1.1	65
	10.7 ± 1.9	66

Table 3. Gibbs free energies of peroxynitrite homolysis reactions.

Reaction equation	ΔG° /kJ mol^{-1}	Ref.
ONOOH (aq.) $\rightarrow$	46.4	67
$NO_2^{\bullet}$ (aq.) + $HO^{\bullet}$ (aq.)	57 ± 2	40, 42
	67	16, 61
ONOOH (gas) $\rightarrow$	30	16, 61
$NO_2^{\bullet}$ (gas) + $HO^{\bullet}$ (gas)	37.7	68
$ONOO^-$ (aq.) $\rightarrow$	54.4	67
$NO^{\bullet}$ (aq.) + $O_2^{\bullet -}$ (aq.)	65.3 ± 0.2	42
	75	16, 61

Table 4. Redox potentials of peroxynitrite.

Reaction equation	E° /V (pH 0)	Ref.	E° /V (pH 7)	Ref.
ONOOH + H^+ + e^- $\rightarrow$	2.0 ± 0.1	16, 61	1.6 ± 0.1	16, 61, 69
$NO_2^{\bullet}$ + H_2O	2.12	42	1.70	59
ONOOH + 2 H^+ + 2 e^- $\rightarrow$	1.67	29	1.3 ± 0.1	16, 61, 69
HNO_2 + H_2O	1.68	42, 59	1.37	59

Infrared spectra in Ar matrix obtained at -260 and -266 °C showed [77, 78] that under these conditions the *cis*- and *trans*-isomers of peroxynitrous acid are in equilibrium, with the higher content of *cis*-isomer. Exposure of ONOOH to UV irradiation leads to an increase in the content of the *trans*-isomer, whereas the action of IR radiation causes the equilibrium to completely shift towards the *cis*-isomer.[78]

The UV spectrum of peroxynitrite anion in aqueous solutions exhibits [25] a broad absorption band with a maximum at 302 nm and an extinction coefficient, ε, of 1670 ± 50 litre mol^{-1} cm^{-1}. The UV spectrum of unstable peroxynitrous acid was obtained [55] by pulse radiolysis: $\lambda_{max} = 240$ nm and $\varepsilon = 770 \pm 50$ litre mol^{-1} cm^{-1}. The IR and Raman spectra of these species and other relevant information are also available.[70, 79, 80]

The values of the ionisation constant (pK_a) of this acid, ONOOH, measured by different methods range from 5.3 to 8.3 (Refs 3, 25, 26, 47, 54, 81–83) as a consequence of instability of the compound. More reliable values, namely, $pK_a = 6.8-6.5$ at 25 °C were obtained [4, 36] by the stopped-flow technique from the plot of the rate of ONOOH isomerisation to nitrates *vs.* pH values.

$$ONOOH \longrightarrow NO_3^- + H^+. \quad (6)$$

The reaction is described by the following kinetic equation

$$-\frac{d[PN]}{dt} = k_{eff}[PN] = \frac{k_6[PN][H^+]}{K_a + [H^+]}, \quad (7)$$

where $[PN] = [ONOO^-] + [ONOOH]$ and $k_6 = 1.2-1.3$ s^{-1} at 25 °C.[4, 24, 36, 84] The ionisation constants determined from Eqn (7) depend on the compositions and concentrations of buffer solutions, which can be illustrated using the following data for 25 °C:[36]

Buffer	Buffer concentration /mol litre^{-1}	pK_a (± 0.10)
Phosphate	0.65	7.33
	0.25	7.12
	0.05	6.71
	0.001	6.55
Ammonium	0.05	7.11
Borate	0.1	8.59

This dependence of pK_a was explained [36] by an increase in the apparent rate of isomerisation of peroxynitrous acid owing to either general acid catalysis (phosphate buffer) or the reaction of peroxynitrite with ammonium and borate components of the solutions.

The ionisation constant of ONOOD in phosphate buffer (concentration 0.1 mol litre^{-1}) at 25 °C is 3.3 times lower ($pK_a^D = 7.3$) than that of ONOOH.[85] The thermodynamic parameters of the ionisation reaction are listed in Table 1.

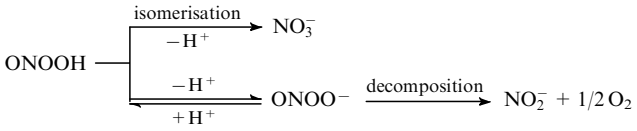
Peroxynitrite is a strong one- and especially two-electron oxidant. The E° potential of the two-electron reaction of the acid, ONOOH (see Table 4) is similar to the E° values for alkyl peroxides and peroxy acids,[59, 86] whereas the potential of one-electron reaction of ONOOH is 0.95 and ~ 0.8 V higher compared to alkyl peroxides and peroxy acids, respectively.[59]

IV. Decomposition and isomerisation

1. Decay pathways

Decay ‡ of peroxynitrite can occur as isomerisation to nitrate or as decomposition into nitrite and oxygen.

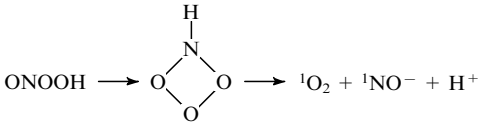
‡ According to the IUPAC recommendations [87] for the names of various processes, the decay of peroxynitrite is any process accompanied by disappearance of the compound; isomerisation is a process resulting in nitrate; and decomposition is the breakdown into nitrite and oxygen.



The former reaction is the major pathway at pH < pK_a, while the latter becomes significant at pH > 7 and peroxynitrite concentrations above 10^{-4} mol litre^{-1} (see Refs 38, 88–91). Quantitative yield of nitrate in acid solutions (pH ~ 4) was reported.[20] At pH < 7, the yield of nitrate is at least 90%, being slightly dependent on the initial concentration of peroxynitrite, pH value and temperature.[91] As the acidity of the medium decreases, the yield of nitrate also decreases, while the yield of nitrite increases and reaches a limiting value at pH ~ 10, being strongly dependent on the initial concentration of peroxynitrite.[90, 91] For instance, the yields of nitrite are 30% and 80% at pH 10 for the 5×10^{-5} and 2.5×10^{-3} M peroxynitrite solutions, respectively.[91] The formation of nitrite is accompanied by production of oxygen ($[NO_2^-] : [O_2] \approx 2$, see Refs 88–91).

In phosphate buffer, the yields of NO_3^- and NO_2^- depend on acidity (pH = 1–14) in a complex manner; the curves exhibit inflection points at pH values of about 3.1, 5.8, 6.8, 8.0 and 11.9 (Fig. 2).[90] At pH > 7, the yield of nitrite appreciably increases while the yield of nitrate correspondingly decreases, namely, $65 \pm 5\%$ for NO_2^- and $35 \pm 5\%$ for NO_3^- at pH 9–10. Contrary to earlier data,[40, 62] it was found recently [90] that at pH > 11 the yield of nitrate again increases and the nitrate becomes the major product in strong alkaline media (80% at pH 14). At all pH values, the sum of the yields of nitrite and nitrate is a constant equal to the initial concentration of peroxynitrite. The dependences of the yields of NO_2^-, NO_3^- and O_2 on the initial concentration of peroxynitrite, measured at pH 5.8, 7.4 and 8.5, were linear only at pH 5.8 (see Ref. 90).

A study [92] of the reactions of peroxynitrite with methaemoglobin and 9,10-diphenylanthracene (specific probes for the presence of singlet dioxygen, $^1O_2$) revealed the formation of nitrosylhaemoglobin and intracyclic 9,10-diphenylanthracene peroxide in high yields. Based on this, it was assumed that a possible pathway of decay of peroxynitrous acid involves its transformation to $^1O_2$ and $^1NO^-$ *via* a four-membered cyclic intermediate.



(8)

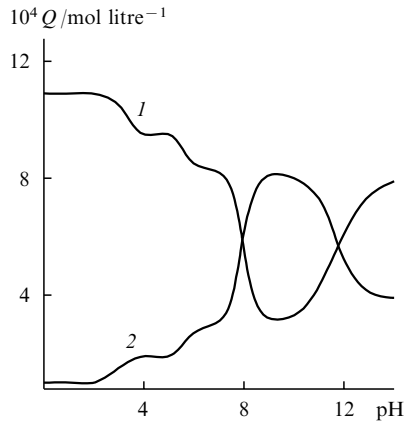
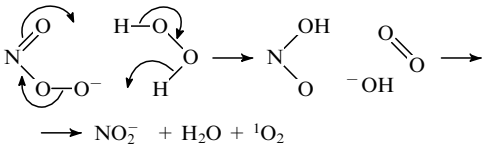


Figure 2. Yields (Q) of nitrate (*1*) and nitrite (*2*) plotted *vs.* pH value for the decay of peroxynitrite [$(1.02 \pm 0.03) \times 10^{-3}$ mol litre^{-1}] in phosphate buffer (0.05 mol litre^{-1}) at 30 °C.[90]

However, no nitrosylhaemoglobin was revealed in a more thorough study of the interaction of methaemoglobin with peroxynitrite containing no H_2O_2 and NO_2^- impurities;[93,94] nor was observed emission at 1270 nm characteristic of $^1O_2$.[94] Besides, according to quantum chemical calculations,[94] the energy barrier to formation of cyclic intermediate in reaction (8) is about five times higher than the experimental activation enthalpy of decay of peroxynitrite (415 *vs.* 86.5 kJ mol^{-1}, respectively).[85] Probably, the reason for difference between the results reported in Ref. 92 and in studies[93,94] consists in that peroxynitrite contained[92] hydrogen peroxide impurity.

The formation of $^1O_2$ in the oxidation of H_2O_2 by peroxynitrite was detected in another study.[95] Two mechanisms were proposed, namely, a concerted reaction of *cis*-$ONOO^-$ with H_2O_2 (see Ref. 95)



$$\longrightarrow NO_2^- + H_2O + {}^1O_2$$

and one-electron of oxidation hydrogen peroxide by peroxynitrous acid followed by heterolysis of peroxy nitrate that formed in the reaction[96,97]

$$ONOOH + HOOH \longrightarrow NO_2^{\bullet} + O_2^{\bullet -} + H^+ + H_2O,$$

$$NO_2^{\bullet} + O_2^{\bullet -} \longrightarrow O_2NOO^-,$$

$$O_2NOO^- \longrightarrow NO_2^- + {}^1O_2\ (\Delta G^\circ = +23\ \text{kJ mol}^{-1}). \qquad (9)$$

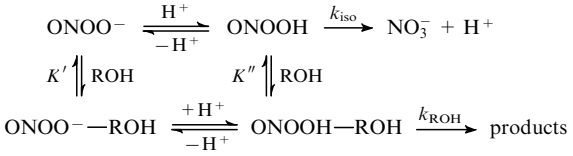
The formation of singlet oxygen in reaction (9) was reported to be thermodynamically unlikely.[94] Direct oxidation of superoxide by nitrogen dioxide

$$NO_2^{\bullet} + O_2^{\bullet -} \longrightarrow {}^1O_2 + NO_2^-\ (\Delta G^\circ = -34\ \text{kJ mol}^{-1})$$

is thermodynamically favourable and seems to be more probable.

2. Decay kinetics

Earlier studies[14,21,24–26,98] were carried out assuming that Eqn (7) is valid for the whole range of pH values at which ONOOH breaks down. However, recent data[36] showed that this holds only at low peroxynitrite concentrations and at pH < pK_a (*e.g.*, at a pH value of 7.9 and [PN] = 4.8×10^{-5} mol litre^{-1}; see Fig. 3, curve *1*). If the initial concentration of peroxynitrite is ten times higher, the decomposition of this compound is described by a simple exponent only in the initial portion and then dramatically decreases (Fig. 3, curve *2*). It was assumed[36] that at pH > 6.8 and high concentrations of peroxynitrite it decomposes involving the formation of [ONOOH···$ONOO^-$] adduct as intermediate. The decomposition rate and the character of kinetics are strongly affected by the buffer composition,[15,36] impurities (H_2O_2 [29] and NO_2^- [29,36,38,99]) and trace amounts of transition metals.[14,25,100] Recently,[101] it was established that the decomposition rate of peroxynitrous acid in phosphate buffer at pH 5.8–8.0 decreases upon addition of ethanol or mannitol. This effect was explained by the mechanism involving stabilisation of peroxynitrite owing to fast equilibrium formation of a complex with the alcohol.



The stability constants K'' are $(9.6 \pm 1.5) \times 10^{-3}$ and $(6.7 \pm 0.9) \times 10^{-3}$ and the rate constants for decomposition of the complexes k_{ROH} are 0.95 ± 0.01 and 0.83 ± 0.01 s^{-1} for ethanol and mannitol, respectively.

The plots of the apparent rate constants for decomposition (k_{eff}) on the acidity of the medium exhibit three portions (Fig. 4).[14,24] In the pH region from 10 to 7 the constant k_{eff} increases as the acidity increases and reaches a plateau at pH $\approx$ 6. Here, Eqn (7) obeys at $k_6 = 1.2$ s^{-1} and pK_a = 6.8.[24,26,36,83,85] The activation parameters for this portion are listed in Table 2. The secondary kinetic isotope effect for the decomposition of ONOOH (ONOOD), k_6^H/k_6^D, weakly depends on temperature in the range 5–55 °C and is equal to 1.6 ± 0.2 and the ionisation isotope effect, K_a^H/K_a^D, of peroxynitrous acid is 3.3.[85] At pH < 2, the decomposition rate increases with an increase in acidity. This was treated[14,21] as indication of an acid catalysed route:

$$-\frac{d[ONOOH]}{dt} = k_{H^+}[ONOOH][H^+]. \qquad (10)$$

The k_{H^+} values are listed in Table 5. The activation enthalpy and activation entropy corresponding to this route are $\Delta H^{\neq} = 61.2 \pm 0.8$ kJ mol^{-1} and $\Delta S^{\neq} = 22.2 \pm 2.9$ J K^{-1} mol^{-1}, respectively (see Refs 14 and 21).

At pH > 10, the decomposition kinetics are less understandable. Experimental k_{eff} values are much higher than those calculated from Eqn (7) for $[H^+] \ll K_a$, $k_6 = 1.2$ s^{-1} and pK_a = 6.8 and show a rather large scatter (see Fig. 4).[14] These deviations

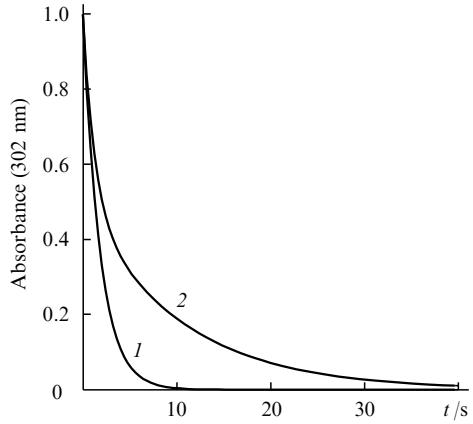


Figure 3. Kinetic curves for peroxynitrite decomposition in phosphate buffer (pH 7.9, 25 °C).[36]
[PN] = 4.8×10^{-5} (*1*) and 4.8×10^{-4} mol litre^{-1} (*2*).

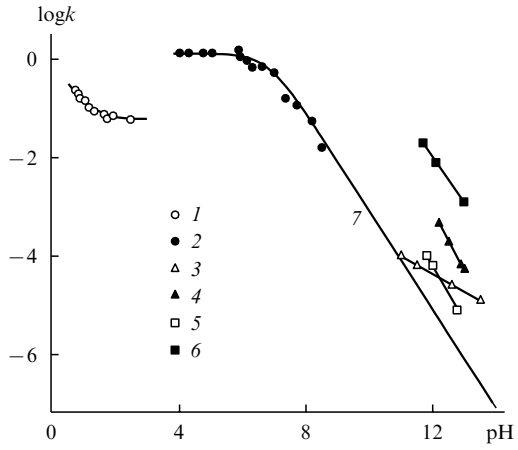


Figure 4. Rate constants for peroxynitrite decay plotted *vs.* pH values: at 1.5 °C (*1*),[21] at 25 °C (*2*),[24] (*3*),[12] (*4*),[25] (*5*)[102] and in the presence of Cu^{2+} (*6*).[17]
Line *7* corresponds to data calculated from Eqn (7) with $k_6 = 1.2$ s^{-1} and pK_a = 6.8.[24]

Table 5. Rate constants for the acid catalysed route (10) of peroxynitrite isomerisation.

T/K	k_{H^+} /litre mol^{-1} s^{-1}	Ref.
281.5	1.295 ± 0.196	14
286.0	2.450 ± 0.112	14
297.0	5.414 ± 0.312	14
308.5	15.790 ± 1.547	14
273.7	0.596 ± 0.008	14, 21
310.0	14.30 ± 0.49	14, 21

were associated [14] with a catalytic route in this range of pH values owing to the presence of transition metal (M) ions, especially copper:

$$k_{eff} = \frac{k_6[H^+]}{K_a} + k_M[M][H^+].$$

Indications of metal complex catalysis of this reaction were also reported in other studies.[25, 26, 100, 102]

3. Isomerisation mechanisms

Four routes of isomerisation of peroxynitrous acid to nitrate are assumed.[16, 24]

1. Heterolysis and interaction of nitroyl cation with water:[16, 24]

$$ONOOH \longrightarrow NO_2^+ + OH^-, \qquad (11)$$
$$NO_2^+ + OH^- \longrightarrow NO_3^- + H^+.$$

Although the change in the Gibbs free energy of reaction (11) is nearly zero,[16, 24] high activation energy (188 kJ mol^{-1}) (see Ref. 24) makes this route unlikely under normal conditions. However, it can occur in the case of acid catalysed decomposition of peroxynitrous acid at pH < 2.[16, 24] Yet another possible mechanism of acid catalysed isomerisation involves equilibrium protonation of ONOOH followed by O–O bond homolysis with the formation of two radical species, $H_2O^{\bullet +}$ and $NO_2^{\bullet}$, that recombine to give a nitrate:[14]

$$H^+ + ONOOH \rightleftharpoons ONOOH_2^+ \longrightarrow$$
$$\rightleftharpoons H_2O^{\bullet +} + NO_2^{\bullet} \longrightarrow H^+ + HNO_3.$$

2. Homolysis followed by recombination of $HO^{\bullet}$ and $NO_2^{\bullet}$ radicals:[19, 60, 62, 65, 88, 100, 103, 104]

$$ONOOH \longrightarrow NO_2^{\bullet} + HO^{\bullet} \longrightarrow NO_3^- + H^+. \qquad (12)$$

The mechanism of homolysis belongs to the most debatable issues in chemistry of peroxynitrous acid. In 1952, Halfpenny and Robinson [105] observed the formation of biaryls in the oxidation of arenes by peroxynitrous acid and assumed that ONOOH undergoes homolysis. In 1990, Beckman *et al.*[3] reported that decomposition of peroxynitrite generates a strong oxidant capable of initiating many reactions typical of OH radical. The maximum yields of $^{\bullet}OH$ in the decomposition of peroxynitrite (pH 6.0), determined using deoxyribose and DMSO as scavengers of OH radicals, are $5.1 \pm 0.1\%$ and $24 \pm 1.0\%$, respectively.[3] Different methods give for the yield of OH radicals a range of values from 0% to 40%.[40, 62, 98, 100, 103, 104, 106]

Koppenol *et al.*[16, 36, 38, 61] reported kinetic and thermodynamic arguments against the sequence of reactions (12) in aqueous solutions. Using the estimate of the free energy of homolysis of ONOOH, $(\Delta G^{\circ}_{hom})_{aq} = 67 \pm 10$ kJ mol^{-1} and the experimental rate constant for the reverse reaction

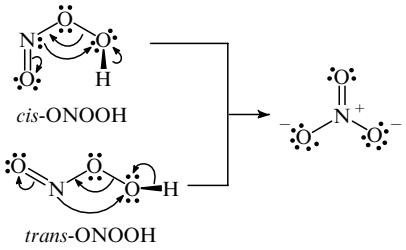
$$ONOOH \rightleftharpoons HO^{\bullet} + NO_2^{\bullet} \qquad (13)(-13)$$

($k_{-13} = 4.5 \times 10^9$ litre mol^{-1} s^{-1}), they found [61] $k_{13} = 0.6 \times 10^{-4}$ s^{-1} and, taking into account the rate constant for isomerisation (k_6), concluded that homolysis of ONOOH in aqueous solutions is unlikely. In the gas phase, the free energy of homolysis of peroxynitrous acid is much lower [$(\Delta G^{\circ}_{hom})_{gas} =$ 30 kJ mol^{-1}] and production of OH radicals is more probable.[61, 104] Besides, it was shown [16, 54] that in aqueous solutions (in contrast to the gas phase [107]) the interaction of hydroxyl radicals with nitrogen dioxide results in quantitative formation of peroxynitrous acid following reaction (–13) rather than formation of nitrate following reactions (12).

Homolysis of the O–O bond is inconsistent with two other data sets. Namely, (i) the yield of ^{15}N-nitrate in the oxidation of ^{15}N-nitrite by peroxynitrous acid is much lower than it follows from the homolytic model [38, 99] and (ii) no ascorbyl radicals were detected in the oxidation of hydroascorbate anion.[38, 108]

Sometimes, the activation volume for isomerisation ($\Delta_{iso}V^{\neq}$) (see Table 2) is used as argument *pro* (or *contra*) the homolytic mechanism. However, data obtained by different methods are significantly different (from 1.7 to 10 cm^3 mol^{-1}; see Refs 36, 63–65) and can therefore not serve as indicators of a particular mechanism.[38, 63]

3. Intramolecular rearrangement. An alternative to the homolytic mechanism of isomerisation of ONOOH involves a *cis–trans* conversion of peroxynitrite accompanied by migration of the terminal oxygen atom to the nitrogen atom and formation of nitrate.[16, 24, 70, 74, 85]



Oxygen shift in the course of *cis–trans*-isomerisation is only possible if the O–O bond is directed normal to the O=N–O plane.[16, 74] The reactive species in the oxidation reactions is thought [16, 24, 70, 74] to be the excited peroxynitrous acid. Its molecule has a *trans*-configuration with elongated O–O bond and the terminal oxygen atom located near the nitrogen atom; this is structurally similar to the transition state of the isomerisation of ONOOH to $HONO_2$. The radical-like reactivity of ONOOH is associated with the presence of two unpaired electrons in the triplet excited state.[16, 74] Quantum chemical calculations confirmed the existence of such a low-lying state.[74]

4. Homolysis in the solvent cage. A mechanism was also proposed,[4, 59, 62, 103, 104] which involves intermediate formation of a radical pair in a 'cage'. Diffusion of the radical pair out of the cage produces what is called free radicals $^{\bullet}OH$ and $NO_2^{\bullet}$ while their recombination results in nitrate.

$$ONOOH \longrightarrow [ONO^{\bullet}\cdots{}^{\bullet}OH]_{cage} \begin{cases} \xrightarrow{k_{dif}} NO_2^{\bullet} + {}^{\bullet}OH \\ \xrightarrow{k_{iso}} NO_3^- + H^+ \end{cases} \qquad (14)$$

Quantum chemical calculations [68] showed that in the gas phase the radical pair $[ONO^{\bullet}\cdots{}^{\bullet}OH]$ is 8 kJ mol^{-1} more stable than free radicals $NO_2^{\bullet} + {}^{\bullet}OH$ owing to the formation of the OH···O hydrogen bond but ~63 kJ mol^{-1} less stable than *cis*-HOONO.

The sequence of reactions (14) is similar to the homolytic mechanism if we assume that hydroxyl radicals can escape the cage and react with substrate or to the 'triplet' mechanism of intramolecular rearrangement under assumption that the radicals remain within the cage. The ratio of the rate constants for these routes, k_{iso}/k_{dif}, is about 2 (Ref. 62) and 2.6,[103] which corresponds to 30%–40% yields of the OH radicals.

Earlier,[20] it was reported that nitrate formed in the reaction retains both peroxide oxygen atoms. However, a recent study [109]

revealed that 17% of the oxygen atoms of peroxynitrite are involved in oxygen exchange with water in the course of isomerisation. These data were treated as substantiation of the intramolecular mechanism. The kinetic isotope effect of solvent (1.4 – 1.5) is also consistent with intramolecular rearrangement of ONOOH to nitrate.[16, 85] Besides, oxygen exchange between the $^{\bullet}OH$ radical in the radical pair and H_2O should be fast,[109] which excludes this structure from being considered as intermediate. Calculations of the models including a molecule water showed that homolysis (12) is the most energetically favourable mechanism and, therefore, the radicals $^{\bullet}OH$ and $NO_2^{\bullet}$ should collapse to nitrate without oxygen exchange;[110] however, this contradicts experimental data. At the same time, chemically induced nuclear polarisation (enhanced absorption of $^{15}NO_3^-$ signal) observed during the decomposition of peroxynitrous acid indicates that nitrate is formed as a result of recombination of the radical pair $[^{\bullet}OH\cdots{}^{15}NO_2^{\bullet}]$.[111]

4. Peroxynitrite decomposition mechanisms

In weak alkaline media, isomerisation of peroxynitrite is accompanied by its decomposition following the stoichiometric equation[88–91] given below

$$ONOOH + ONOO^- \longrightarrow O_2 + 2NO_2^- + H^+.$$

The mechanisms of decomposition and isomerisation have been highly debatable issues. A radical mechanism[40, 42, 62, 64, 88, 89] and a bimolecular one[36, 38, 60, 91] are possible.

The radical mechanisms imply either homolysis of the ONO – OH bond at the rate-limiting step,[64, 89] resulting in the $^{\bullet}OH$ and $NO_2^{\bullet}$ radicals,

$$\begin{array}{l} ONOOH \longrightarrow {}^{\bullet}OH + NO_2^{\bullet} \\ \quad\quad\downarrow ONOO^- \qquad\quad \downarrow H_2O \\ OH^- + O_2 + NO^{\bullet} \qquad 2H^+ + 2NO_2^- \end{array} \qquad (15)$$

or homolysis of the ON – OO^- bond, resulting in nitrogen oxide and superoxide.[40, 42, 62, 64, 88]

$$\begin{array}{l} ONOO^- \rightleftharpoons NO^{\bullet} + O_2^{\bullet-} \\ \quad\quad\downarrow N_2O_3 \qquad\quad \downarrow O_2 + NO_2^- \\ ONOO^- \longrightarrow NO_2^{\bullet} + NO_2^{\bullet} + NO_2^- \end{array} \qquad (16)$$

The radical pathway is substantiated by the absence of the dependence of the relative yields of nitrite and oxygen on the initial concentration of peroxynitrite[88, 89] and by coincidence of experimental yields of NO_2^- as function of the pH value with the results of the calculations based on reactions (15).[64, 90] Production of superoxide in reactions (16) was proved using the $O_2^{\bullet-}$ scavengers, namely, tetranitromethane[41, 64, 112, 113]

$$\begin{array}{l} ONOO^- \overset{k_h}{\rightleftharpoons} O_2^{\bullet-} + NO^{\bullet} \\ \quad\quad\downarrow C(NO_2)_4 \qquad\quad \downarrow N_2O_3 \xrightarrow{2OH^-} 2NO_2^- + H_2O \\ O_2 + NO_2^{\bullet} + C(NO_2)_3^- \end{array} \qquad (17)$$

and methyl viologen ($MV^{\bullet+}$) radical[42]

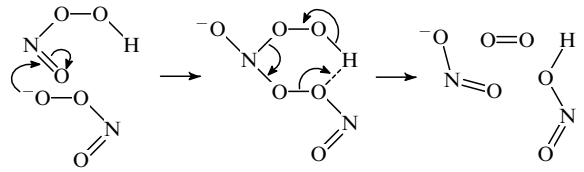
$$MV^{\bullet+} + O_2^{\bullet-} \xrightarrow[-OH^-]{+H_2O} MV^{+} + HO_2^-.$$

The rate constant for $ONOO^-$ homolysis was found to be the same for both cases ($k_h = 0.017\ s^{-1}$). The homolytic mechanism (16) is also substantiated by a decrease in the yield of $C(NO_2)_3^-$ anion in the presence of excess nitrogen oxide,[41, 113] which shifts the equilibrium in reactions (17) towards peroxynitrite.

In contrast to the results[41, 64, 113] presented above, Koppenol *et al.*[60] reported that $NO^{\bullet}$ radicals do not inhibit the formation of $C(NO_2)_3^-$ and that the decomposition of peroxynitrite ($> 10^{-4}$ mol litre^{-1}) at pH > 7 does not obey the first-order kinetics in the general case.[36] They also found that the relative yields of nitrite and oxygen[38, 91] and the apparent rate constants for decomposition[16, 36] increase as the initial peroxynitrite concentration increases. Quantum chemical estimates[114] showed that homolysis of the anion by the mechanism (16) is possible, but this is a thermodynamic 'dead end' because the $[NO^{\bullet}\cdots O_2^{\bullet-}]$ radical pair formed mostly reverts to the starting peroxynitrite. All these results are inconsistent with the homolytic mechanisms. It is believed[114] that strong solvation effects make the decomposition of $ONOO^-$ anion a more complicated process (compared to isomerisation of the acid, ONOOH) which involves the formation of an acid – anion adduct.[16, 36, 38, 91]

$$\begin{array}{l} ONOOH \overset{K_a}{\rightleftharpoons} ONOO^- + H^+ \\ \quad\quad\downarrow k_{iso} \qquad k_{-ad} \uparrow\downarrow k_{ad}, ONOOH \\ H^+ + NO_3^- \quad [ONOOH\cdots ONOO^-] \xrightarrow{k_d} O_2 + 2NO_2^- + H^+ \end{array} \qquad (18)$$

The $[ONOOH\cdots ONOO^-]$ dimer undergoes a concerted rearrangement involving a cyclic transition state.



The sequence of reactions (18) is analogous to a commonly accepted mechanism of the decomposition of peroxy acids[115] and correctly describes the dependences of the yields of nitrate and nitrite and the apparent rate constants for decomposition on the initial concentration of peroxynitrite and on the pH values at $k_{iso} = 1.15\ s^{-1}$, $pK_a = 6.8$, $k_{ad} = 1.7 \cdot 10^4$ litre mol^{-1} s^{-1}, $k_{-ad} = 0.025\ s^{-1}$ and $k_d = 0.05\ s^{-1}$ (see Ref. 38). The effective rate constant ($k_{eff} = 1.8 \times 10^4$ litre mol^{-1} s^{-1}) for peroxynitrite decomposition at pH 4.7 was obtained[116] from the slope of the initial portion of the curve of O_2 accumulation. This is similar to $k_{eff} = k_{ad}k_d/(k_{-ad} + k_d) = 1.1 \times 10^4$ litre mol^{-1} s^{-1} calculated from the results of study[38] and substantiates[116] the mechanism (18).

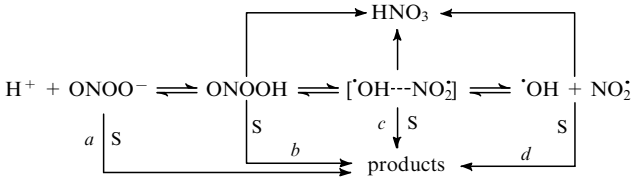
Thus, in spite of intensive research the mechanisms of isomerisation and decomposition of peroxynitrite are still to be clarified. This requires detailed studies on the kinetics of peroxynitrite decomposition and on the reactions of peroxynitrite with various model substrates.

V. Reactions with hydrocarbons and other compounds

1. Typical mechanisms

Peroxynitrite is a strong one- and two-electron oxidant (see Table 4). The interaction of peroxynitrite with the substrates (S) possessing the properties of strong reducing agents can follow four routes (Scheme 2).

Scheme 2

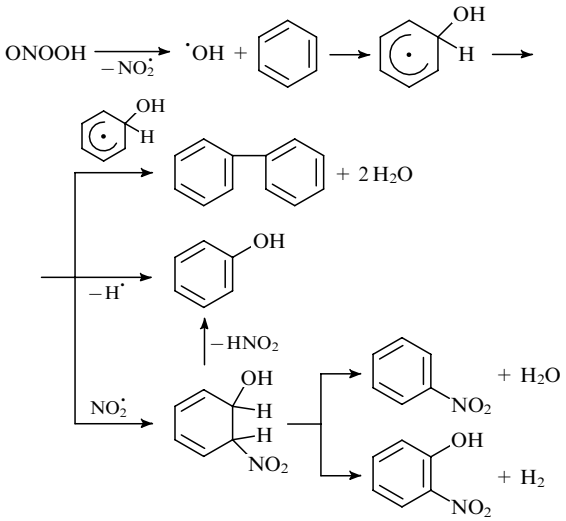


Routes *a* and *b* are first-order reactions with respect to substrate and peroxynitrite. They are characteristic of the reac-

tions involving sulfides,[117, 118] thiols,[83] Ni(II)-cyclam complex (cyclam is 1,4,8,11-tetraazacyclotetradecane),[119] Br^- and I^- anions (see Refs 105 and 119, respectively) and of nucleophilic addition of $ONOO^-$ to CO_2.[120–123] Reactions with $[Fe(CN)_6]^{4-}$ (see Ref. 119) and typical scavengers of free radicals, namely, DMSO[3, 98, 104] and 2,2′-azinobis(3-ethylbenzothiazole-6-sulfonate) (ABTS)[98] follow routes *c* and *d*, which are first-order reactions with respect to peroxynitrite and zero-order reactions with respect to substrate; they involve the formation of a hydroxyl-like reactive intermediate.[3, 68, 106, 119] In addition to free OH radical and $[HO^{\bullet}\cdots NO_2^{\bullet}]$ pair,[68] a possible candidate is the excited *trans*-ONOOH, which is similar to the radical pair.[16, 24, 70, 74, 98, 124] The oxidation of methionine, 2-keto-4-thiomethylbutanoic acid and ascorbate anion follows route *b* (or routes *b* and *d*) at high (or low) substrate concentrations.[125, 126]

Production of OH radicals in the homolysis of ONOOH was first proposed by Halfpenny and Robinson[105] who observed not only nitration and hydroxylation products, but also conversion of arenes to biaryls (Scheme 3).

Scheme 3



The rate constants for the oxidation of hydrogen peroxide and nitrite by peroxynitrous acid and hydroxyl radicals are similar.[100]

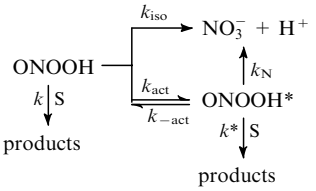
Using the stopped-flow technique, it was found[98, 119, 127, 128] that the oxidation of many compounds by peroxynitrous acid has no effect on the decomposition kinetics of ONOOH. It was assumed that the rate-limiting step of decomposition of peroxynitrous acid (but not peroxynitrite anion!) involves the formation of a reactive intermediate which then rapidly reacts with substrates in a fashion typical of OH radicals. For instance, peroxynitrous acid can initiate polymerisation of methyl acrylate,[19] hydroxylation and nitration of alkanes, alkenes[129] and arenes,[105] oxidise DMSO and deoxyribose to formaldehyde and malonic dialdehyde, respectively,[3] damage DNA and cause degradation of proteins.[56] Many of these reactions are inhibited by scavengers of OH radicals (alcohols, benzoic acid and their derivatives, uric acid) and are not catalysed by metal ions.

Hydroxylation at the aromatic ring of phenylalanine results in approximately equal yields of *ortho*-, *meta*- and *para*-derivatives. This feature is used as highly sensitive and specific method for detection of OH radicals.[130] Similar products and an identical distribution of isomers was reported for the reaction with ONOOH.[124] The yield of tyrosine and its *ortho*- and *meta*-isomers increases with the acidity of the medium (at pH 6–9) and the concentration of ONOOH, being independent of the substrate concentration. Not only hydroxylation products but also 3-nitrotyrosine and 4-nitrophenylalanine (and fluorescing bityrosine, in the reaction with tyrosine) were detected among reaction products. These facts and a decrease in the yields of reaction products in the presence of mannitol (specific scavenger of OH radicals) are consistent with route *d* (see Scheme 2).

Production of reactive radical species is accompanied by isomerisation of peroxynitrite following reaction (14). The yield of free radicals was estimated at about 32% based on the O_2 evolution in the oxidation of hydrogen peroxide or nitrite by peroxynitrous acid,[100] at about 24% based on the yield of formaldehyde in the oxidation of DMSO[3] and at 40% based on the yield of $ABTS^{\bullet+}$ radical cations in the oxidation of ABTS.[98, 106]

Both peroxynitrous acid and its anion can immediately react with various substrates (routes *a* and *b* in Scheme 2). Since many studies were carried out at neutral pH values, it is often difficult to conclude which of the two forms, ONOOH or $ONOO^-$, is more reactive. Table 6 lists the rate constants for some second-order reactions of organic and inorganic substrates with peroxynitrite. Under particular conditions, these reactions can proceed much faster than homolysis of ONOOH and be of physiological value.

A mechanism was proposed,[84, 143] which involves a direct oxidation of substrate by the acid, ONOOH, and an indirect oxidation by the activated form of the acid (ONOOH*).



In the direct oxidation (first-order reaction with respect to substrate and reagent) the yield of products with respect to peroxynitrite can be as high as 100%. In the indirect oxidation (first-order reaction with respect to peroxynitrous acid and zero-order reaction with respect to substrate) about 50%–60% of ONOOH isomerise into nitric acid (k_{iso}) and only 40%–50% is

Table 6. Rate constants for second-order reactions involving peroxynitrite in aqueous solutions.

Substrate	k /litre mol^{-1} s^{-1}	T/°C	Ref.
2-Phenyl-1,2-benzoselenazol-3[2*H*]-one [a]	2.0×10^6	25	131
Mn(III) porphyrin [b]	1.8×10^6 (see [c])	22–24	132
Fe(III) porphyrin [b]	2.2×10^6	37	133
Cistein	5.9×10^3	37	83
Methionine	9.0×10^2	25	125
	1.7×10^3	25	117
	8.6 (see [a])	25	117
4-Methylthio-2-oxo-butanoic acid	1.4×10^3	25	125
Selenomethionine	2.0×10^4	25	134
Tryptophan	1.3×10^2	25	135
	1.8×10^2	37	136
Cytochrome *c*	2.3×10^5	25	137
Dimethyl sulfide	2.1×10^3 (see [d])	25	118
	0.23 (see [e])	25	118
Dimethyl selenide	6.0 (see [e])	25	118
Alcohol dehydrogenase	$(2.6–5.6) \times 10^5$	23	138
Myeloperoxidase	2×10^7	25	139
Glutathione peroxidase [f]	8×10^6	25	16
Aconitase	2.4×10^5	25	140
Ascorbate	236	25	126
	1×10^6	25	108
Carbon dioxide [a]	3.0×10^4	25	141
	5.8×10^4	25	142
Iodide anion	2.3×10^4	27	119
Ni(II)-cyclam	3.3×10^4	27	119

[a] Reaction with peroxynitrite anion; [b] 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin; [c] at pH 7.4; [d] At pH 4.6; [e] at pH ~ 13; [f] for protein tetramer.

converted to the activated form ONOOH* (k_{act}), which then rapidly interacts with the substrate (k*) or produces nitrate (k_N). Therefore, even at very high concentration of substrate (k*[S] $\gg k_N$), the yield of oxidation products in the indirect process is less than 50%. The nature of the reagent ONOOH* is unknown. Competitive inhibition studies showed that in some cases the addition of free radical scavengers has no effect on the yields of products of indirect oxidation [144, 145] or only partial inhibition is observed,[119] which is weaker than that predicted from the known rate constants for the reactions of the scavengers with hydroxyl radicals. In those cases [3, 100, 146] where the degree of inhibition correlated to the rate constants for hydroxyl radicals, the involvement of these species in indirect oxidation of substrates was excluded [84] based on the kinetic data and product yields.

The contributions of different oxidation routes strongly depends on the pH value (Fig. 5).[3, 15, 22, 83, 127] In particular, the yields of products of DMSO oxidation following routes *c* and *d* decrease while the rate of oxidation of thiol group following route *a* increases with an increase in pH value.

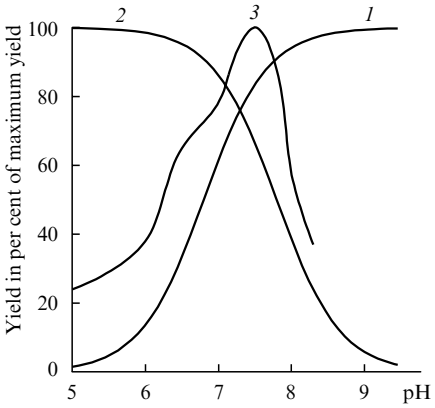


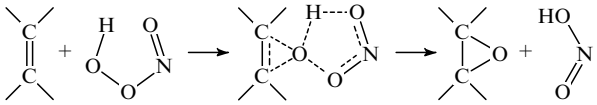
Figure 5. Oxidative reactivity of peroxynitrite plotted *vs.* pH values.[15, 22] The results are presented in per cent of the maximum yield under optimum conditions.
(*1*) Oxidation of thiol group (100% yield), (*2*) $HO^{\bullet}\cdots NO_2^{\bullet}$ (yield 25%–35%), (*3*) nitration by NO_2^+ (8% with superoxide dismutase, 100% with Fe^{3+}).

A great diversity of types of the chemical reactions of peroxynitrite is due to involvement (depending on reaction conditions) of various reactive species that are formed as a result of decomposition or activation of peroxynitrite and to unique properties of the peroxynitrite molecule that can simultaneously act as a strong one- and two-electron oxidant, an electrophile and a nucleophile.[22, 38, 69, 147]

Now we will consider specific features of the mechanisms of the best studied peroxynitrite reactions in more detail.

2. Reactions with hydrocarbons; role of $^{\bullet}$OH-like species

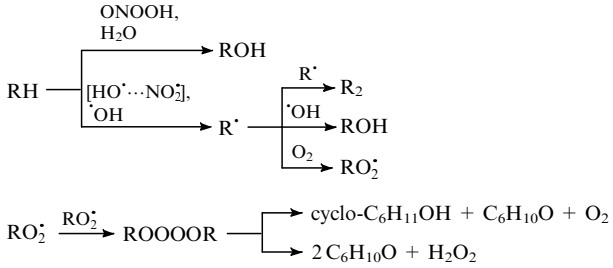
The authors of the present review have found [148–150] that the oxidation of alkanes and alkylbenzenes involves an intermediate step called 'pre-activation' of the reagent. At this step, reactive radical species, biradicals or metal complexes with a coordination vacancy are produced. The kinetics of reactions of hydrocarbons with various oxidants and radicals have been studied [148, 150–152] so thoroughly that hydrocarbons are the best substrates for establishing the nature of the reactive species. Besides, investigations on the reactions of alkanes, alkenes and arenes with peroxynitrite are important for elucidating the mechanisms of oxidation of C–H bonds of alkanes and alkenes in cell membranes and arene- and sulfur-containing fragments of proteins. Prior to our publications, only the reactions of peroxynitrite with strong reducing agents (see Scheme 2) were studied. Only two experimental investigations concerning products of the oxidation of cyclohexane and cyclohexene [129] and arenes [105] in the H_2O_2–HNO_2 system were available. According to quantum chemical calculations, the interaction of alkanes with peroxynitrous acid involves most probably production of OH radicals as intermediates [153] and the oxidation of alkenes involves *cis*-ONOOH and formation of a cyclic transition state [68, 114, 154] similarly to epoxidation of olefins by organic peroxy acids.



We pioneered in systematic studies on the kinetics of peroxynitrite reactions with hydrocarbons as reducing agents and found [155] that alkanes, alkenes and alkylbenzenes are oxidised by aqueous solutions of peroxynitrous acid at pH < 7.5. In the case of cyclohexane, a number of products are formed simultaneously; these are cyclohexanone, cyclohexanol, bicyclohexyl and trace amounts (< 1%) of nitrocyclohexane.[155–157] Oxidation of alkylbenzenes under these conditions results in a mixture of phenols, nitrophenols and nitroarenes (see Scheme 3).[105] The oxidation of cyclohexene by peroxynitrous acid in phosphate buffer (pH ~ 7) and in the H_2O_2–HNO_2 system (pH 4.3) gives a broad spectrum of products including cyclopentanecarbaldehyde, epoxycyclohexane, cyclohexanone, cyclohex-2-enol, cyclohex-2-enone, 2-hydroxycyclohexanone, 1-nitrocyclohexene and cyclopent-2-enecarboxylic acid that were identified by GC/MS.[158]

The oxidation of cyclohexane by peroxynitrite in a three-phase system 'aqueous solution–cyclohexane–gas phase' (O_2 or He) proceeds in the aqueous phase and results in cyclohexanol, cyclohexanone and bicyclohexyl.[159] In both helium and oxygen atmospheres the alcohol is formed at a constant initial rate, whereas production of ketone is preceded by a rather long induction period due to the formation of O_2 in the decomposition of ONOOH. At pH 4.7 and $[RH]_{aq} = 7 \times 10^{-3}$ mol litre^{-1}, only 1% of ONOOH is consumed for the oxidation of hydrocarbon.[116] This is due to fast decomposition of peroxynitrous acid. A decrease in the yield of cyclohexanol and an increase in the yield of cyclohexanone on going from the helium to oxygen atmosphere was explained by competition between the routes involving and not involving O_2 (Scheme 4).

Scheme 4



R = cyclo-C_6H_{11}.

Thus, only a fraction of alcohol formed in the reaction is a product of primary oxidation of cycloalkane by peroxynitrite, whereas the remaining part of alcohol and the whole amount of ketone are products of the secondary radical reactions involving oxygen.

Extremely low solubility of hydrocarbons in water and rapid decomposition of ONOOH at pH ~ 7 make the use of conventional kinetic methods impossible. The problem was solved using the kinetic distribution method (KDM)[148] and a H_2O_2–HNO_2/acetate buffer model system (pH 4.27) where peroxynitrous acid is generated *in situ*. Of particular value is the fact that the method allows one to obtain not only the rate constants but also valuable thermodynamic data on the distribution of volatile reactants between the gas phase and solution.

When studying the oxidation of hydrocarbons in aqueous solutions,[148, 149] the apparent rate constants, k_λ^{RH}, for the loss of volatile substrate in the gas phase of a closed reactor are usually related to the liquid-phase rate constants k_l^{RH} as follows:

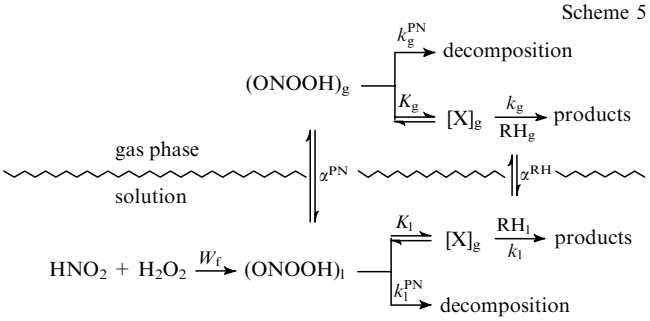
$$-\frac{1}{[RH]_g}\frac{d[RH]_g}{dt} = k_\lambda^{RH} = \frac{k_l^{RH}}{(1+\alpha^{RH}\lambda)}, \quad (19)$$

where $\lambda = V_g/V_l$ is the ratio of the volume of the gas phase to the volume of the liquid phase in the reactor and $\alpha^{RH} = [RH]_g/[RH]_l$ is the distribution coefficient of the hydrocarbon between these phases. The relative liquid-phase rate constants for substrates A and B are related to the measured quantities k_λ^A/k_λ^B as follows:

$$\frac{k_l^A}{k_l^B} = \frac{k_\lambda^A(1+\alpha^A\lambda)}{k_\lambda^B(1+\alpha^B\lambda)}. \quad (20)$$

However, the oxidation of hydrocarbons in the $H_2O_2-HNO_2$ system cannot be described by Eqn (19) in a wide range of changes in λ. The apparent rate constants are unusual bell-shaped functions of λ and the relative liquid-phase rate constants k_l^A/k_l^B for different pairs of substrates A and B change by 1.2 to 8 times for λ values ranging from 0.65 to 21.[160, 161] Calculations using Eqn (20) gave an inverted order of selectivity (alkanes > alkenes > alkylbenzenes) and inversely proportional dependence of the relative rate constants on the solubility of hydrocarbons ($1/\alpha^{RH}$). These facts have no analogies with other hydrocarbon oxidation reactions;[148, 150] they were explained [160, 161] by the following mechanism (Scheme 5):

Scheme 5



This includes the formation of peroxynitrous acid in solution at the rate $W_f = d[ONOOH]/dt$, the equilibrium distribution of the hydrocarbon (α^{RH}) and acid ($\alpha^{PN} = [ONOOH]_g/[ONOOH]_l$) between the gas and liquid phases, the decomposition of ONOOH (k_g^{PN}, k_l^{PN}), pre-activation of reagent (K_g, K_l) with production of a reactive species X (radical pair $[HO^\bullet\cdots NO_2^\bullet]$ or OH radical) and the interaction of X with the substrate RH (k_g, k_l) in both phases.

Kinetic studies of the $H_2O_2-HNO_2$ system are complicated by simultaneous occurrence of reactions in solution and in the gas phase and by rapid decomposition of ONOOH. Therefore, even at large excess of reagent the loss of hydrocarbons does not obey the first-order kinetics with respect to substrate (Fig. 6). Calculations of Scheme 5 are simplified [160, 161] at $[H^+]$ = const (buffer solutions) in two cases, namely, if $[H_2O_2]_0 \gg [NO_2^-]_0 \gg [RH]$ (system I) or $[H_2O_2]_0 = [NO_2^-]_0 \gg [RH]$ (system II).

In the case of system I the loss of substrate obeys [160] the following equation

$$\ln\frac{[RH]_g^0}{[RH]_g} = \frac{k_\lambda^{RH}}{k_\lambda^{PN}}[NO_2^-]_0\left[1-\exp\left(-k_f^I\tau\right)\right], \quad (21)$$

where $[RH]_g^0$ and $[RH]_g$ are the initial and current concentration of RH in the gas phase, respectively; k_λ^{RH} and k_λ^{PN} are the effective rate constants for the loss of substrate and decomposition of ONOOH, respectively,

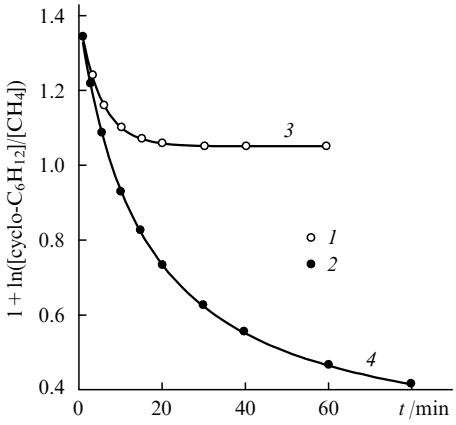


Figure 6. Kinetic curves of cyclohexane decay.[160]
Open and solid circles denote experimental data obtained at pH 4.27, 25 °C, $\lambda = 2.31$: $[H_2O_2] = 0.1$, $[NaNO_2] = 0.01$ mol litre^{-1} (*1*) and $[H_2O_2] = [NaNO_2] = 0.042$ mol litre^{-1} (*2*); lines *3* and *4* correspond to data calculated from Eqns (21) and (24), respectively. Methane was used as internal reference that is stable under the reaction conditions.

$$k_\lambda^{RH} = \frac{k_l^{RH} + k_g^{RH}\alpha^{RH}\alpha^{PN}\lambda}{(1+\alpha^{RH}\lambda)(1+\alpha^{PN}\lambda)} \quad (22)$$

($k_l^{RH} = K_l k_l$, $k_g^{RH} = K_g k_g$ are the effective rate constants for the reaction of peroxynitrite with the hydrocarbon in the liquid and gas phases, respectively),

$$k_\lambda^{PN} = \frac{k_l^{PN} + k_g^{PN}\alpha^{PN}\lambda}{1+\alpha^{PN}\lambda}. \quad (23)$$

The effective pseudo-first-order rate constant for production of ONOOH (k_f^I) is given by [20]

$$k_f^I = \frac{W_f}{[NO_2^-]} = \frac{k_2k_3[H^+]^2[H_2O_2]}{k_{-2}[H_2O] + k_3[H_2O_2]}.$$

Taking the oxidation of cyclohexane as an example, it was shown that Eqn (21) is valid in the whole range of substrate decay (see Fig. 6, curve *3*). The ratio $k_\lambda^{RH}/k_\lambda^{PN}$ is independent of the initial concentrations of H_2O_2 and $NaNO_2$ and the k_f^I values at a specified pH value depend only on $[H_2O_2]$ (Table 7). The k_2 and $k_3/k_{-2}[H_2O]$ values calculated from these data are similar to the estimates [20] obtained in basically different manner, from the kinetics of formation of ONOOH (see below).

$10^{-6}\,k_2$ /litre^2 mol^{-2} s^{-1}	$k_3/k_{-2}[H_2O]$ /litre mol^{-1}	Ref.
3.4 ± 0.6	3.6 ± 1.0	160
5.8	2.4	20

Table 7. Characteristics of cyclohexane oxidation in system I (pH 4.27, 25 °C, $\lambda = 2.31$): effect of the H_2O_2 and NO_2^- concentrations on the parameters of Eqn (21).[160]

$[H_2O_2]_0$ /mol litre^{-1}	$[NO_2^-]_0$ /mol litre^{-1}	$10^3\,k_f^I$ /s^{-1}	$k_\lambda^{RH}/k_\lambda^{PN}$ /litre mol^{-1}
0.1	0.014	3.1	30
0.1	0.010	3.2	35
0.1	0.007	3.3	38
0.1	0.005	3.0	36
0.15	0.01	4.0	34
0.2	0.01	5.2	37
0.3	0.01	6.4	33

Table 8. Dependence of the parameters of Eqn (24) on λ in system II (pH 4.27; 25 °C; $[H_2O_2]_0 = [NO_2^-]_0 = 0.042$ mol litre^{-1}).[160, 161]

λ	n-Hexane ($\alpha^{RH} = 65$)		Cyclopentane ($\alpha^{RH} = 7.7$)		Cyclohexane ($\alpha^{RH} = 8.0$)		Propene ($\alpha^{RH} = 11$)	
	$k_\lambda^{RH}/k_\lambda^{PN}$ /litre mol^{-1}	$10^2 k_f$II /litre mol^{-1} s^{-1}	$k_\lambda^{RH}/k_\lambda^{PN}$ /litre mol^{-1}	$10^2 k_f^{II}$ /litre mol^{-1} s^{-1}	$k_\lambda^{RH}/k_\lambda^{PN}$ /litre mol^{-1}	$10^2 k_f^{II}$ /litre mol^{-1} s^{-1}	$k_\lambda^{RH}/k_\lambda^{PN}$ /litre mol^{-1}	$10^2 k_f^{II}$ /litre mol^{-1} s^{-1}
0.32	24.5	2.3	–	–	–	–	–	–
0.66	25.0	2.5	18.0	2.3	26.0	2.4	98.6	2.6
1.20	24.8	2.4	21.0	2.5	27.4	2.4	108	2.3
2.31	23.6	2.6	20.8	2.2	28.0	2.6	107	2.8
5.61	21.0	2.5	18.2	2.4	26.0	2.5	91.9	2.7
8.46	–	–	16.5	2.4	24.0	2.8	81.7	2.5
12.2	14.6	2.6	14.8	2.0	21.0	2.6	72.5	2.6
21.0	15.0	2.4	–	–	–	–	–	–
	$(k_f^{II})_{aver} = (2.5 \pm 0.1) \times 10^{-2}$ /litre mol^{-1} s^{-1}		$(k_f^{II})_{aver} = (2.3 \pm 0.1) \times 10^{-2}$ /litre mol^{-1} s^{-1}		$(k_f^{II})_{aver} = (2.6 \pm 0.1) \times 10^{-2}$ /litre mol^{-1} s^{-1}		$(k_f^{II})_{aver} = (2.6 \pm 0.1) \times 10^{-2}$ /litre mol^{-1} s^{-1}	

For system II, the loss of substrate in the gas phase obeys the following equation

$$\ln\frac{[RH]_g^0}{[RH]_g} = \frac{k_f^{II} k_\lambda^{RH} [NO_2^-]_0^2 t}{k_\lambda^{PN}(1 + [NO_2^-]_0 k_f^{II} t)}, \quad (24)$$

where k_f^{II} is the effective second-order rate constant for production of ONOOH at a specified pH value.

In system I the loss of hydrocarbons in the course of reaction is at most 30%.[160, 161] On going to system II, it reaches 60% to 80% [160, 161] (see Fig. 6, curve *4*), which makes the determination of k_f and $k_\lambda^{RH}/k_\lambda^{PN}$ (Table 8) much more accurate.

The bell-shaped dependences of $k_\lambda^{RH}/k_\lambda^{PH}$ on λ (Fig. 7) were quantitatively explained in the framework of the extended KDM model (see Scheme 5).[155–157, 160, 161] From Eqns (22) and (23) it follows that the dependence of the ratio $k_\lambda^{RH}/k_\lambda^{PN}$ on λ

$$\frac{k_\lambda^{RH}}{k_\lambda^{PN}} = \frac{(k_g^{RH}\alpha^{RH}\alpha^{PN}\lambda + k_l^{RH})}{(k_g^{PN}\alpha^{PN}\lambda + k_l^{PN})(1 + \alpha^{RH}\lambda)} \quad (25)$$

includes terms proportional to λ in the numerator and terms proportional to λ and λ^2 in the denominator. At particular values of the parameters k_l^{RH}, k_g^{RH}, k_l^{PN}, k_g^{PN}, α^{RH} and α^{PN}, the plot of $k_\lambda^{RH}/k_\lambda^{PN}$ *vs.* λ passes through an extremum.

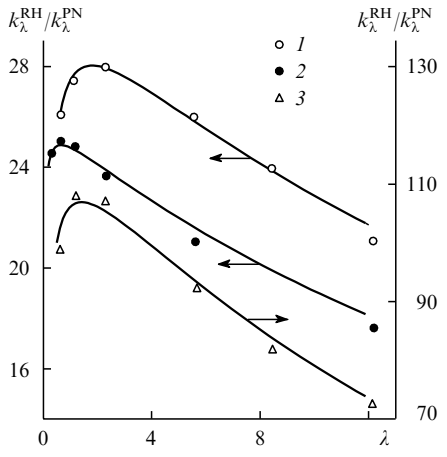


Figure 7. $k_\lambda^{RH}/k_\lambda^{PN}$ Ratios plotted *vs.* λ for the oxidation of cyclohexane (*1*), n-hexane (*2*) and propene (*3*) in the H_2O_2–$NaNO_2$/acetate buffer system (pH 4.27; 25 °C).[160]
The lines correspond to data obtained from Eqn (25) for the k_g^{PN}/k_g^{RH}, $k_g^{PN}/k_l^{PN}\alpha^{PN}$ calculated using the data from Table 9, at $(k_g^{RH}\alpha^{RH}/k_g^{PN}) \gg (k_l^{RH}/k_{rmg}^{PN}\alpha^{PN}\lambda)$.

The ratio (25) provides quite a correct description of the experimental data for alkanes, alkenes and alkylbenzenes in the whole range of changes in λ studied so far (see Fig. 7) and makes it possible to calculate [160, 161] the ratios of the rate constants k_g^{PN}/k_g^{RH} and $k_l^{PN}/k_g^{PN}\alpha^{PN}$:

k_g^{PN}/k_g^{RH} /mol litre^{-1}	$k_l^{PN}/k_g^{PN}\alpha^{PN}$
n-Hexane	
$(1.4 \pm 0.2) \times 10^{-3}$	27 ± 4
Cyclopentane	
$(1.7 \pm 0.2) \times 10^{-3}$	27 ± 3
Cyclohexane	
$(1.2 \pm 0.2) \times 10^{-3}$	26 ± 2
Propene	
$(3.3 \pm 0.5) \times 10^{-4}$	26 ± 5

Using a version of the KDM method for competitive processes, we calculated [155–157] the relative rate constants for the oxidation of a large number of hydrocarbons in solution and in the gas phase (Table 9). Because of high α values in aqueous solutions (8–110), alkanes and alkenes at $\lambda > 1$ are mainly in the gas phase where they interact with ONOOH. For arenes, the reaction rates in two phases are comparable.

The reactive species in the reactions studied are hydroxyl radicals (X = $^\bullet$OH in Scheme 5), the contribution of the radical pair being insignificant.[160, 161] The radical pair [$^\bullet$OH···$^\bullet$ONO] is 8 kJ mol^{-1} more stable than free radicals $^\bullet$OH + $NO_2^\bullet$ owing to formation of a hydrogen bond.[68] Therefore the rate constant for the reaction

$$RH + [^\bullet OH \cdots NO_2^\bullet] \longrightarrow R^\bullet + NO_2^\bullet + H_2O \quad (26)$$

cannot be higher than the rate constant for the following reaction

$$RH + {}^\bullet OH \longrightarrow R^\bullet + H_2O \quad (27)$$

with free hydroxyl radicals (10^9–10^{10} litre mol^{-1}).[148, 151, 152] For kinetic measurements, one has $[RH] < 10^{-4}$ mol litre^{-1} and $k_{26}[RH] \leqslant 10^5$–$10^6$ s^{-1}. At the same time the estimate of the rate constant for decomposition of the radical pair to $^\bullet$OH + $NO_2^\bullet$ is $k_{dif} = 10^{10}$–10^{12} s^{-1} (assuming an activation energy of 8 kJ mol^{-1} and the pre-exponent $A = 10^{12}$–10^{14}). Since $k_{26}[RH]$ is 4–7 orders of magnitude lower than k_{dif}, it was concluded that the major route of transformation of the radical pair involves its decomposition and production of free hydroxyl radicals that are the reactive species in the oxidation of hydrocarbons.

The mechanism (27) is also substantiated by (1) general correlation

$$\Delta\log k_{g(ONOOH)} = (0.98 \pm 0.01)\Delta\log k_{g(OH)}$$

Table 9. Distribution coefficients, α, and relative gas-phase rate constants, ($k_g^{RH}/k_g^{n-C_5H_{12}}$), for oxidation of hydrocarbons by $H_2O_2-HNO_2$/acetate buffer system (pH 4.27) [155–158] and by OH radicals [148, 151, 152] at 25 °C.

Hydrocarbon	α	$k_g^{RH}/k_g^{n-C_5H_{12}}$		Hydrocarbon	α	$k_g^{RH}/k_g^{n-C_5H_{12}}$	
		$H_2O_2-HNO_2$	$^{\bullet}OH$			$H_2O_2-HNO_2$	$^{\bullet}OH$
Propane	29	0.32	0.33	Isobutene	13	12	13
n-Butane	37	0.65	0.66	n-Hexene	31	8.1	9.2
n-Pentane	49	1.0	1.0	Cyclohexene	3.3	16	14
n-Hexane	65	1.3	1.6	Benzene	0.23	0.33	0.32
n-Heptane	86	1.7	2.0	Toluene	0.26	1.4	1.7
n-Octane	110	2.2	2.3	*o*-Xylene	0.18	3.2	3.8
Isobutane	48	0.58	0.64	*m*-Xylene	0.29	5.4	6.4
Cyclopentane	7.7	1.2	1.3	*p*-Xylene	0.26	3.4	4.1
Cyclohexane	8.0	1.6	1.8	Pseudocumene	0.21	8.4	11
Ethene	12	1.8	2.1	Mesitylene	0.36	13	14
Propene	11	6.1	6.6				

that is valid for gas-phase oxidation of all the substrates studied (alkanes, alkenes and alkylbenzenes) (Fig. 8);[155–158] (2) similar products of the two reactions;[155, 158, 162, 163] (3) yield ratio ([cyclohexanol]:[cyclohexanone] ≈ 2) determined [155–157] for the reaction of cyclohexane with peroxynitrous acid and characteristic of the reactions with $^{\bullet}OH$ radicals (see Ref. 162); and (4) experimental proofs of high yield of OH radicals in the decomposition of ONOOH.[40, 62, 88, 98, 103, 104]

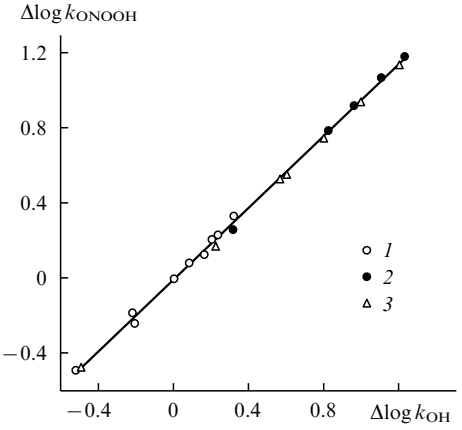


Figure 8. Generalised correlation between the relative rate constants for gas-phase oxidation of alkanes (*1*), alkenes (*2*) and alkylbenzenes (*3*) by the $H_2O_2-HNO_2$ system (see Refs 155–158) and OH radicals (see Refs 148, 151, 152).

The calculated ratios k_g^{RH}/k_g^{PN}, $k_l^{RH}/k_g^{PN}\alpha^{PN}$ and $k_l^{PN}/k_g^{PN}\alpha^{PN}$ were used for estimating the rate constant for gas-phase decomposition of peroxynitrous acid and the distribution coefficient α^{PN}. According to Scheme 5, the following reactions proceed in the two phases:

$$ONOOH \rightleftharpoons {}^{\bullet}OH + NO_2^{\bullet}, \quad (28)$$

$$ONOOH \longrightarrow HNO_3, \quad (29)$$

and also reaction (27). Based on the values of the free energy of homolysis of ONOOH, $\Delta G^{\circ}_{hom} = -RT\ln K_{28}$ (see Table 1), the equilibrium (28) in solution [$K_{28(l)} = (0.01-1)\times 10^{-12}$ mol litre^{-1}] and in the gas phase [$K_{28(g)} = (0.3-6)\times 10^{-6}$ mol litre^{-1}] is almost completely shifted to the left. With allowance for this fact the overall rate constant for the decomposition of ONOOH according to Eqns (28) and (29) in the gas phase or in solution is given by

$$k^{PN} = k_{28} + k_{29}. \quad (30)$$

Using the method of quasi-steady-state concentrations, for the effective second-order rate constant for the oxidation of RH according to Eqn (27) one gets

$$k^{RH} = \frac{k_{27}k_{28}}{k_{28}+k_{29}}. \quad (31)$$

From Eqns (30) and (31) it follows that

$$\frac{k_g^{PN}}{k_g^{RH}} = \frac{(k_{28}+k_{29})^2}{k_{27}k_{28}},$$

$$\frac{k_l^{PN}}{k_g^{PN}\alpha^{PN}} = \frac{k_l^{PN}}{(k_{28}+k_{29})_g\alpha^{PN}}. \quad (32)$$

From the known rate constants $k_l^{PN} = (k_{28}+k_{29})_{(l)} = 1.2\ s^{-1}$ (Ref. 24) and $k_{28(l)} = (0.34\pm 0.08)\ s^{-1}$ (Ref. 29) it is possible to determine the yield of OH radicals [$k_{28(l)}/k_l^{PN}$] in the decomposition of peroxynitrous acid in aqueous solutions at 25 °C, which is equal to 28%.

Taking into account the equilibrium constant $K_{28(g)} = (0.3-6.0)\times 10^{-6}$ mol litre^{-1} and the rate constant for the reverse reaction $k_{-28(g)} = 4\times 10^8$ litre mol^{-1} s^{-1} (Ref. 164), the rate constant for homolysis, $k_{28(g)} = K_{28(g)}k_{-28(g)}$, was estimated [160, 161] at $(0.1-2.4)\times 10^3\ s^{-1}$, which is much higher than for aqueous solutions.

If X = $^{\bullet}OH$, one has $k = k_{27} = k_{OH}$ (see Scheme 5). Using the published data for n-hexane, cyclopentane, cyclohexane and propene ($k_{OH(g)} = 3.6\times 10^9$, 2.8×10^9, 4.0×10^9 and 16×10^9 litre mol^{-1} s^{-1}, respectively),[148, 151] experimental ratios k_g^{PN}/k_g^{RH} and the value $k_{28(g)} = (0.1-2.4)\times 10^3\ s^{-1}$, the constant k_g^{PN} was estimated [160, 161] from Eqn (31) at $(0.2-1)\times 10^5\ s^{-1}$. From comparison of the k_g^{PN} and $k_{28(g)}$ values it follows that the yield of OH radicals in the gas-phase decomposition of ONOOH can range from 0.1% to 10%.

The 'gas–solution' distribution coefficient of peroxynitrous acid estimated [160] using Eqn (32) lies between the α values of H_2O_2 and O_2NOOH (calculated using the Henry law constants [165, 166] in aqueous solutions at 25 °C, $\alpha = 0.041/K_h$):

Compound	ONOOH	H_2O_2	O_2NOOH	MeCOOOH	MeOOH
$10^6\,\alpha$	0.4–2	0.5	3	50	130

The α^{PN} value corresponds to the energy of solvation of peroxynitrous acid in water $\Delta G^{\circ}_{sol} = -RT\ln\alpha^{PN} = 32.6-36.4$ kJ mol^{-1}, which is in rough agreement with the estimate $\Delta G^{\circ}_{sol} = 25\pm 12$ kJ mol^{-1} obtained from the free energies of formation of peroxynitrous acid in the gas phase and in aqueous solution (see Table 1).

3. Oxidation of dimethyl sulfoxide

Dimethyl sulfoxide is an efficient scavenger of hydroxyl radicals, being converted to methanesulfinic acid MeS(O)OH (in 91% yield) and methyl radicals that can produce formaldehyde.[167] The oxidation of DMSO by peroxynitrite in neutral media results in the same products while the reaction in alkaline solutions yields dimethyl sulfone Me_2SO_2.[98, 104] The major reaction pathways are a radical route involving $^{\bullet}OH$ at pH < 7 and bimolecular oxygen transfer from peroxynitrite to the substrate molecule in alkaline media.[168]

In addition to formaldehyde, Me_2SO_2 and methanesulfinic acid the products of DMSO oxidation by peroxynitrite include methane, methyl nitrite and methyl nitrate, which are accumulated in the gas phase of a closed reactor.[169]

Me_2SO + ONOOH (or $ONOO^-$) ⟶

- ⟶ CH_4 + MeONO + $MeONO_2$ detected in the gas phase
- ⟶ CH_2O + Me_2SO_2 + MeS(O)OH detected in solution

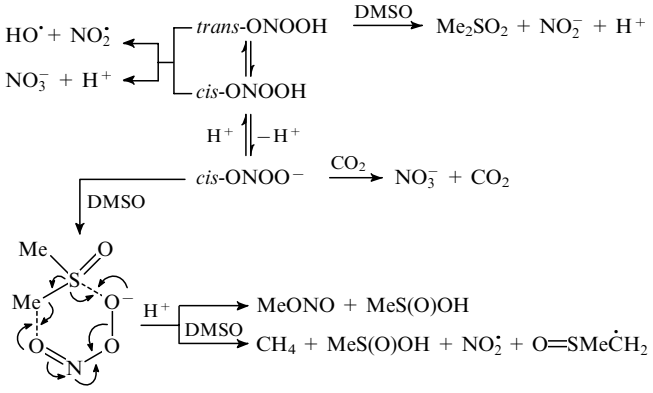
Detection of these volatile products indicates a complex mechanism of the interaction of DMSO with peroxynitrite.

The dependence of yields of CH_4, CH_2O and MeONO on acidity of the medium passes through maxima in the range of pH values 6.4–6.9 (Table 10). The major product formed under neutral conditions is methyl nitrite (~17% with respect to initial peroxynitrite). The yield of methyl nitrate rapidly increases from 1% (pH < 7) to 17% (pH ~ 10) and then abruptly decreases. The yield of Me_2SO_2 monotonically increases from 2% to 45% as the pH value increases from 6.0 to 11.5. The dependences on the pH value show that methane, formaldehyde and methyl nitrite are produced in the reaction of DMSO with the same reactive species, whereas the formation of $MeONO_2$ and Me_2SO_2 involves other reaction routes.

Under neutral conditions, the yield of CH_2O reaches a maximum value (1.8% at 0.01 M DMSO) as the substrate concentration increases (see Table 10) and then slowly decreases. The yields of CH_4, MeONO and Me_2SO_2 reach their maximum values at [DMSO] ≈ 1 mol litre^{-1} while the yield of methyl nitrate depends only slightly on the DMSO concentration. The [CH_4] : [MeONO] ratio is proportional to [DMSO], thus indicating the involvement of two DMSO molecules in production of methane. At pH 6.8, the yields of CH_4 and MeONO are linearly (that of $MeONO_2$, quadratically) related to the peroxynitrite concentration (see Table 10). Under the same conditions the nitrite anions inhibit the formation of methane and methyl nitrite but have no effect on the yield of methyl nitrate. However, the introduction of $NaNO_2$ (0.15 mol litre^{-1}) at pH 10.1 reduces the yield of $MeONO_2$ from 17% to 1%. In contrast to many other reactions involving peroxynitrite,[120–122] the formation of methane and methyl nitrite (under neutral conditions) and methyl nitrate (at pH 10.4) is inhibited by adding $NaHCO_3$. In the presence of a large excess of DMSO, the overall yield of the products (CH_4 + CH_2O + MeONO) with respect to peroxynitrite is 55 ± 10%, being higher than the upper limit (~40%)[106] for the formation of OH radicals from ONOOH. It is impossible to explain all these results by considering the reaction route involving OH radicals.

It was assumed[169] that the reaction proceeds *via* a cyclic intermediate which can decompose into MeONO and MeS(O)OH and produce methane in the reaction with the second DMSO molecule (Scheme 6). Here, the role of the reactive species is played by *cis*-$ONOO^-$ and the conversion of the intermediate to product is catalysed by the acid.

Scheme 6



trans-Peroxynitrite, which cannot form a cyclic intermediate, reacts with DMSO to give Me_2SO_2. In the presence of CO_2 and NO_2^-, competing reactions of *cis*-$ONOO^-$ with carbon dioxide proceed.[120–122] Taking into account the quadratic dependence of the yield of methyl nitrate on the peroxynitrite concentration, it

Table 10. Product (P) yields in the reaction of DMSO with peroxynitrite in phosphate buffer (36 °C, μ = 0.15) as functions of pH values and reagent concentrations (Ref. 169).[a]

pH	10^3 [ONOONa]	10^2 [Me_2SO]	10^4 [P]				
			[MeONO]	[CH_4]	[$MeONO_2$]	[CH_2O]	[Me_2SO_2]
5.66	7.8	7.2	4.1	0.72	0.1	–	–
5.95	7.8	7.2	–	–	–	1.1	1.8
6.80	2.4	7.2	3.1	0.14	–	–	–
6.80	4.7	7.2	7.9	0.38	0.5	–	–
6.80	7.8	7.2	12	0.94	1.6	–	–
6.80	14.3	7.2	20	1.4	5.0	–	–
6.95	8.6	1.8	1.1	0.043	0.26	1.6	2.0
6.95	8.6	4.0	3.5	0.28	0.30	–	–
6.95	8.6	7.2	6.3	0.86	–	1.5	5.0
6.95	8.6	30	18	2.8	0.40	–	–
6.95	8.6	120	22	9.5	–	1.4	7.0
7.10	7.8	7.2	5.8	0.4	–	–	–
7.75	7.8	7.2	1.8	0.02	4.6	0.95	3.3
10.4	7.8	7.2	0.7	0.001	13.2	< 0.02	–
11.2	7.8	7.2	0.4	0.001	5.4	–	30

[a] Concentrations of reagents and products are given in mol litre^{-1}; μ is the ionic strength.

was concluded [169] that $MeONO_2$ is formed in the interaction of DMSO with a complex between $ONOO^-$ and ONOOH.[16, 36, 38, 91]

4. Oxidation of sulfides

Sulfides are oxidised by both peroxynitrous acid and its anion to give sulfoxides and NO_2^- (Refs 117, 118, 125, 170). The yield of DMSO and nitrite in the reaction of dimethyl sulfide with $ONOO^-$ is 100% (with respect to peroxynitrite) and 80% in the interaction with ONOOH [118] The reaction of methionine (Met) with both forms of peroxynitrite is also stoichiometric but the reaction equation takes another form:[117]

$$ONOO^- (ONOOH) + Met \longrightarrow \longrightarrow \frac{2}{3}MetS{=}O + \frac{1}{3}Met + \frac{2}{3}NO_2^- + \frac{1}{3}NO_3^- (+H^+). \quad (33)$$

The reactivity of peroxynitrous acid in the reactions with sulfides is more than two orders of magnitude higher than that of its anion (see Table 6). The activation enthalpy and activation entropy of the reaction of Me_2S with ONOOH are respectively 24.7 ± 2.1 kJ mol^{-1} and $-(96 \pm 13)$ J K^{-1} mol^{-1} [*cf.* 40.6 ± 2.5 kJ mol^{-1} and $-(121 \pm 13)$ J K^{-1} mol^{-1}, respectively, for the reaction with $ONOO^-$].[118] The reaction of Me_2S with $ONOO^-$ is characterised by strong solvation effects.[118] Namely, with an increase in the concentration of the organic component (EtOH, Bu^{t}OH, MeCN, tetrahydrofuran, tetramethylenesulfone) in aqueous–organic media, the reaction rates decrease while the rate of the reaction of Me_2S with ONOOH remains unchanged.

According to quantum chemical calculations,[73] the reaction of Me_2S with *cis*-$ONOO^-$ involves transfer of an oxygen atom (two-electron oxidation) *via* an equilibrium formation of a complex $\{Me_2S\cdots{}^-OONO\}$ followed by O–O bond cleavage at the rate-limiting step:

$$ONOO^- + Me_2S \rightleftharpoons [Me_2S\cdots{}^-OONO] \longrightarrow [Me_2S\cdots{}^-O\cdots ONO]^{\neq} \longrightarrow [Me_2SO(NO_2^-)] \longrightarrow Me_2SO + NO_2^-.$$

Gas-phase interaction between the negatively charged terminal oxygen atom of peroxynitrite and the sulfur atom bearing a partial positive charge is characterised by $\Delta H^\circ = -27$ and $\Delta G^\circ = -5.0$ kJ mol^{-1}. The activation parameters, $\Delta H^{\neq}$ and $\Delta G^{\neq}$, of O–O bond homolysis in the transition state $[Me_2S\cdots{}^-O\cdots ONO]^{\neq}$ are 64 and 110 kJ mol^{-1}, respectively. The energy ΔH (ΔG) of the intermediate $[Me_2SO(NO_2^-)]$ is 215 (177) kJ mol^{-1} lower than those of the initial reagents. Dissociation of the intermediate to Me_2SO and NO_2^- is an endothermic process ($\Delta H^\circ = 72$, $\Delta G^\circ = 36$ kJ mol^{-1}) with a low activation barrier. On the whole, the reaction of Me_2S with $ONOO^-$ is exothermic, namely, $\Delta H^\circ = -143$ and $\Delta G^\circ = -141$ kJ mol^{-1}. Estimates [73] showed that solvation of $ONOO^-$ increases the reaction rate, which is consistent with the experiment.[118] The equilibrium complex between Me_2S and $(H_2O)_n(ONOO^-)$ is destabilised with respect to the reagents at $n = 1, 2$; namely, $\Delta H^\circ(\Delta G^\circ) = -19$ (13) and 0.4 (26) kJ mol^{-1}, respectively. The activation enthalpy of O–O bond homolysis decreases from 64 ($n = 0$) to 62 ($n = 1$) and 56 kJ mol^{-1} ($n = 2$).

The reaction of Me_2S with *cis*-ONOOH can proceed [73] either as a stepwise or a concerted process that is much faster than the reaction with $ONOO^-$. The stepwise route involves homolysis of the ONO–OH bond and generation of free radicals ($^{\bullet}OH$ and $^{\bullet}ONO$), formation of the intermediate $[Me_2S(OH)(NO_2)]$, which then can undergo either a barrierless endothermic transformation to radicals $[Me_2S(^{\bullet}OH)$ and $NO_2^{\bullet}]$ or an exothermic proton transfer [$\Delta H^{\neq} = 48$, $\Delta G^{\neq} = 55$ kJ mol^{-1}] to give DMSO and HONO. Parameters of the energy barrier to the concerted route involving O–O bond cleavage in the transition state $[Me_2S\cdots HOONO]^{\neq}$ were estimated as follows; $\Delta H^{\neq} = 25.5$ and $\Delta G^{\neq} = 26.4$ kJ mol^{-1}. The stepwise reaction of Me_2S with ONOOH is more probable in the gas phase, whereas both routes are efficient in solution. Since H_2O interacts with ONOOH much weaker than with $ONOO^-$, solvation effects for the reaction of Me_2S with peroxynitrous acid are less pronounced.[73, 118]

Methionine plays a key role in transfer of the unpaired electrons in peptides and proteins.[171] The oxidation of this compound can proceed as reaction (33) resulting in methionine sulfoxide and as a one-electron oxidation by activated peroxynitrous acid ONOOH* to give ethylene.[125] The second route is substantiated by nonlinear dependences of the rate constants on the concentration of methionine.[84, 125]

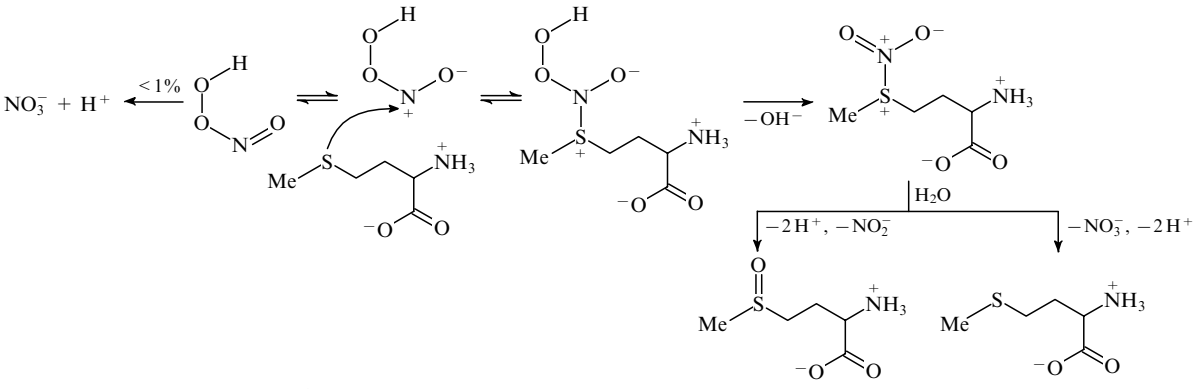
However, more recently [117] this dependence was shown to be linear and the formation of nitrate (in addition to sulfoxide and nitrite) in accordance with Eqn (33) was established; *i.e.*, methionine catalyses the isomerisation of peroxynitrite to nitrate. The relative yields of products are independent of the pH value and substrate concentration. It was concluded that the reaction obeys Scheme 7 and that nitrite (either an impurity or the species formed in the reaction) is not chemically inert. Namely, it oxidises methionine in the one-electron process to ethylene and methional ($MeSCH_2CH_2CHO$).

Using the formation of acetaldehyde as a test for one-electron oxidation, it was found [170] that only 1.5% and 1.85% of threonylmethionine are oxidised in the one-electron process under anaerobic and aerobic conditions (pH 7.4), respectively, whereas the contribution of this route estimated from the yield of ethylene is at least 8%.[125]

5. Interaction with carbon dioxide and carbonyl compounds

The reaction of peroxynitrite with bicarbonate buffer has long been known,[26] but attracted considerable attention of researchers only in 1993 when its biological importance was reported.[145] More

Scheme 7

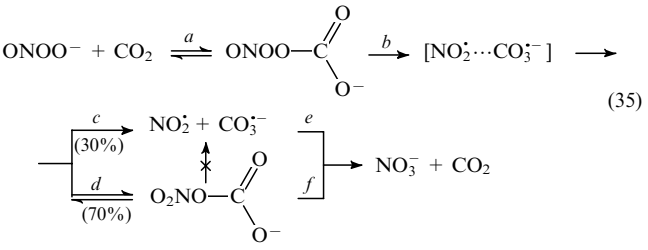


recently, it was shown [141] that $ONOO^-$ rapidly forms an unstable nitrosoperoxy carbonate with CO_2:

$$ONOO^- + CO_2 \longrightarrow ONOOCO_2^- \ (k_{34} = 3 \times 10^4 \text{ litre mol}^{-1} \text{ s}^{-1}). \quad (34)$$

The activation enthalpy and activation entropy are 45 ± 3 kJ mol^{-1} and 27 ± 2 J K^{-1} mol^{-1}, respectively. Using the stopped-flow technique, it was found [172, 173] that $ONOOCO_2^-$ is characterised by an absorption band at $\lambda_{max} = 650$ nm. At present, reaction (34) is considered as the major route of peroxynitrite decomposition in biosystems because the physiological concentration of bicarbonate $(1-3) \times 10^{-2}$ mol litre^{-1} (see Refs 96, 122, 141, 174–176) is sufficient for the process to proceed. Carbon dioxide affects many reactions involving peroxynitrite. Namely, it considerably accelerates nitration of arenes,[142, 174, 175] DNA [177] and ethyl acetoacetate,[178] catalyses one-electron oxidation of metal complexes $Fe(CN)_6^{4-}$, $Mo(CN)_8^{4-}$, $Os(bipy)_3^{2+}$, $Fe(bipy)_3^{2+}$, $Ru(bipy)^{2+}$ and I^- anion (see Ref. 121); partially inhibits the oxidation of thiols,[142] DMSO,[142] oxyhaemoglobin,[142] DNA [177] and cytochrome *c*;[142] and completely inhibits the hydroxylation of benzoate anion.[142]

In the absence of other reagents the reaction of CO_2 with peroxynitrite results in nitrate as the major product.[120, 121] It is assumed [120, 122, 123, 141, 142, 174, 176] that nitrosoperoxy carbonate formed in the reaction homolytically decomposes into a radical pair, which then transforms to nitrocarbonate ($O_2NOCO_2^-$) [18, 120, 178–182] or decomposes into radical species, $CO_3^{\bullet-}$ and $NO_2^\bullet$.



(35)

The standard Gibbs energy diagram for the sequence of reactions (35) [182] is shown in Fig. 9 and the rate constants for particular steps are listed in Table 11.

The rate of decomposition of $O_2NOCO_2^-$ to nitrate and CO_2 is 28 orders of magnitude higher than the rate of decomposition to

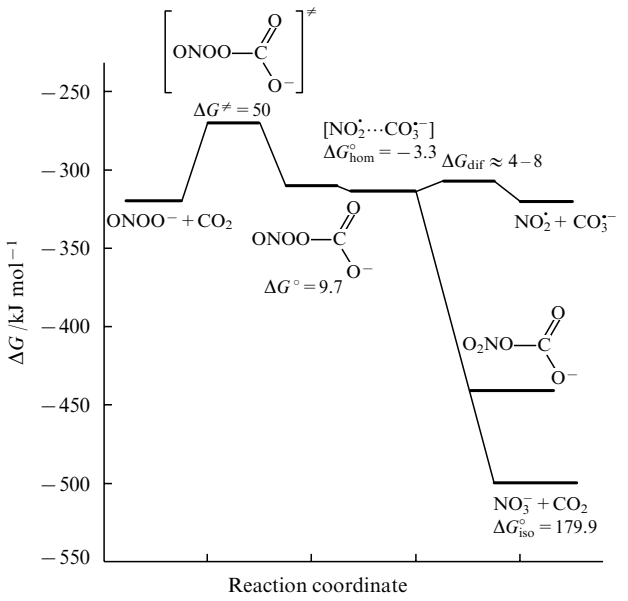


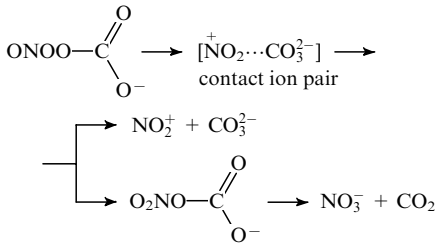
Figure 9. Standard Gibbs energy diagram of the reaction of peroxynitrite with CO_2.[182]

Table 11. Rate constants for particular steps of the reaction of peroxynitrite with CO_2 [reaction (35)] at 25 °C.

Stage	Rate constants	Ref.
$ONOO^- + CO_2 \rightleftharpoons$	$k_a = 3 \times 10^4$ litre mol^{-1} s^{-1}	141
$ONOOCO_2^-$ (*a*)	$k_{-a} = 1.5 \times 10^6$ s^{-1}	182
$ONOOCO_2^- \rightleftharpoons$	$k_{(b+c)} = 1.9 \times 10^9$ s^{-1}	182
$NO_2^\bullet + CO_3^{\bullet-}$ ($b + c$)	$k_{-(b+c)} = 5 \times 10^8$ litre mol^{-1} s^{-1}	182, 183
$NO_2^\bullet + CO_3^{\bullet-} \rightleftharpoons$	$k_e = 1.0 \times 10^9$ litre mol^{-1} s^{-1}	184
$NO_3^- + CO_2$ (*d*)		
$NO_2^\bullet + CO_3^{\bullet-} \rightleftharpoons$	$k_d = 5 \times 10^8$ litre mol^{-1} s^{-1}	182, 183
$O_2NOCO_2^-$ (*e*)		
	$k_{-d} = 5.6 \times 10^{-15}$ s^{-1}	182
$O_2NOCO_2^- \rightleftharpoons$	$k_f = 4 \times 10^{13}$ s^{-1}	182
$NO_3^- + CO_2$ (*f*)	$k_{-f} = 8 \times 10^3$ litre mol^{-1} s^{-1}	182

$NO_2^\bullet$ and $CO_3^{\bullet-}$ (Table 11, steps *f* and $-d$, respectively). In a number of studies [18, 59, 96, 111, 176, 185] nitrocarbonate is excluded from the sequence of reactions (35) assuming that the radical pair $[NO_2^\bullet \cdots CO_3^{\bullet-}]$ can either decompose into free radicals (30%–35%) or undergo intracage conversion to nitrate and CO_2 (65%–70%). The yield of radicals $NO_2^\bullet$ and $CO_3^{\bullet-}$ in the decomposition of nitrosoperoxy carbonate is $3 \pm 1\%$ (*cf.* $96 \pm 1\%$ for the yield of nitrate).[173]

The catalytic effect of CO_2 in the nitration of arenes by peroxynitrite was explained [120, 142, 175, 178–181] by heterolytic decomposition of nitrosoperoxy carbonate.



(36)

Although the formation of NO_2^+ cation in reaction (36) is thermodynamically allowed ($\Delta G^\circ = 16 \pm 29$ kJ mol^{-1}), the very short lifetime in aqueous solutions (1.4 ns) (see Ref. 186) makes it a poor candidate for playing the role of the reactive species.[96, 122] Nitrosoperoxy carbonate is a strong one-electron oxidant.[18]

$$ONOOCO_2^- + e^- \rightleftharpoons NO_2^\bullet + CO_3^{2-}, \ E^\circ = 1.63 \text{ V}.$$

Other possible reagents in the bicarbonate-catalysed processes included [96, 120–122, 178–181] nitrocarbonate and $NO_2^\bullet$ and $CO_3^{\bullet-}$ radicals. However, instability of the $ONOOCO_2^-$ and $O_2NOCO_2^-$ species (see the rate constants listed in Table 11) causes them to decompose much faster than to interact with the substrate.[18, 121, 174, 185, 187, 188]

The most plausible reactive species are the $NO_2^\bullet$ and $CO_3^{\bullet-}$ radicals (Refs 18, 120, 123, 141, 142, 174, 175, 178–182). The carbonate radical anion is a strong oxidant [$E^\circ_{CO_3^{\bullet-}/CO_3^{2-}} = 1.58 \pm 0.02$ V at pH 12.5 (Refs 188, 189) and $E^\circ_{CO_3^{\bullet-}/HCO_3^-} = 1.78 \pm 0.02$ V at pH 7.0 (Ref. 190)] capable of abstracting an electron from various substrates. The $NO_2^\bullet$ radical is a weaker one-electron oxidant ($E^\circ_{NO_2^\bullet/NO_2^-} = 1.04 \pm 0.02$ V).[191] A two-step mechanism was proposed for nitration of arenes (in particular, tyrosine, a compound of great biochemical value).[111, 142, 174, 192]

$$ArH + CO_3^{\bullet-} \xrightarrow[-HCO_3^-]{} Ar^\bullet \xrightarrow{NO_2^\bullet} ArNO_2$$

The rate of the reaction of tyrosine with $CO_3^{\bullet-}$ is more that 300 times higher than that of the reaction with $NO_2^\bullet$.[193, 194] In agreement with the redox potentials, direct interaction of the substrate

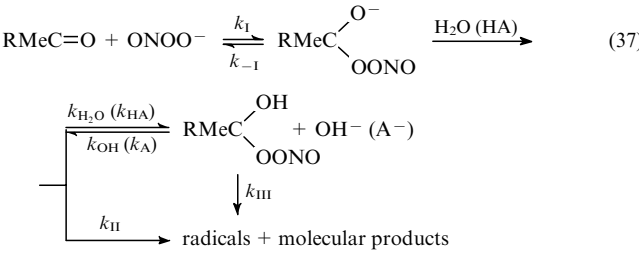
Table 12. Rate constants for different steps of reactions (37) in aqueous solutions at 22 °C.[198]

Parameter	Reagent	
	acetone	acetaldehyde
k_I /litre mol^{-1} s^{-1}	116 ± 7	$(5.8 \pm 0.4) \times 10^4$
k_{-I} /s^{-1}	$\sim 2 \times 10^9$	$\sim 3 \times 10^9$
K_I	$\sim 6 \times 10^{-8}$	$\sim 2 \times 10^{-5}$
k_{II} /s^{-1}	$\sim 3 \times 10^5$	$\sim 5 \times 10^6$
k_{III} /s^{-1}	$\sim 1 \times 10^6$	$\sim 2 \times 10^6$
k_{H_2O} /s^{-1}	$\sim 2 \times 10^7$	$\sim 6 \times 10^7$
k_{OH} /litre mol^{-1} s^{-1}	$\sim 3 \times 10^9$	$\sim 3 \times 10^9$
k_{HA} /litre mol^{-1} s^{-1} (see [a])	$\sim 3 \times 10^9$	$\sim 3 \times 10^9$
pK_a (adduct)	~ 11.8	~ 12.3

[a] HA refers to NH_4^+.

with $NO_2^\bullet$ (and in other cases) proceeds much slower than with $CO_3^{\bullet-}$ (Ref. 18).

Nucleophilic addition of $ONOO^-$ anion to carbonyl compounds[97, 185, 195–198] proceeds similarly to reaction (34). It involves rapid equilibrium between the starting reagents and tetrahedral anionic adduct, which is strongly shifted towards the former (Table 12).[185, 198]



(37)

R = H, Me.

The anionic intermediate in reactions (37) is rapidly protonated under the action of water or buffer components. The rate of the bimolecular reaction of $ONOO^-$ with RMeCO strongly depends on the pH value and buffer concentration. Rapid homolysis of weak O—O bonds in the anionic and neutral adducts is followed by production of radical pairs in the solvent cage and subsequent formation of free alkoxyl radicals and nitrogen dioxide (Scheme 8).

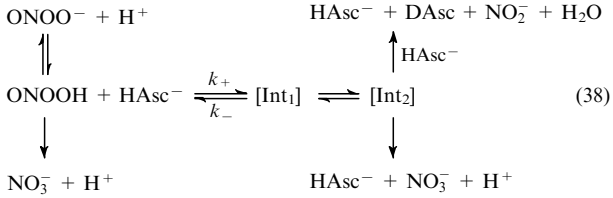
The yield of free radicals in the reactions of acetone and acetaldehyde with $ONOO^-$ is ~15% at high and low pH values.[198]

Like CO_2, aldehydes catalyse the oxidation of ABTS by peroxynitrite but, in contrast to carbon dioxide, have no effect on the rate of nitration of 4-hydroxyphenylacetate.[195] High rate constants for homolysis of peroxynitrite adducts with carbonyl compounds[198] indicate that they cannot act as reactive species, as was proposed earlier.[195]

6. Reactions with other substrates, catalysis by metal ions

Ascorbic acid has two acidic protons characterised by pK_a values of 4.04 and 11.34 (see Ref. 199). This compound is an *in vitro* and *in vivo* antioxidant of paramount importance.[200] Early studies[123, 201] on the oxidation of hydroascorbate anion ($HAsc^-$) revealed that the reaction is first-order with respect to substrate and peroxynitrous acid (k = 235 litre mol^{-1} s^{-1}). Based on the EPR spectra of ascorbyl radicals, it was concluded[201] that the reaction involves consecutive one-electron transfer.

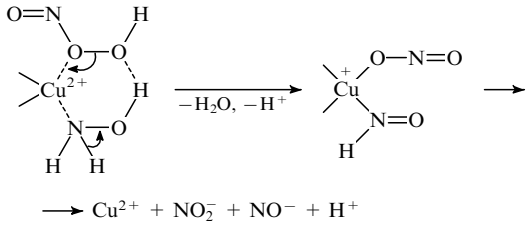
According to recent data,[38, 108] the rate constants are nearly 5000 times higher than those reported earlier (see Table 6). Kinetic, UV and EPR spectral studies and product analysis led to a mechanism that involves a rapid equilibrium formation ($k_+ = 1 \times 10^6$ litre mol^{-1} s^{-1}, $k_- = 500$ s^{-1} at 25 °C and pH 5.8; $k_+ = 1.5 \times 10^6$ litre mol^{-1} s^{-1}, $k_- = 1 \times 10^3$ s^{-1} at 37 °C) of a first intermediate (Int_1), which undergoes conversion to a second intermediate (Int_2) characterised by absorption maximum at 345 nm. At low concentration of $HAsc^-$, the second intermediate decomposes into hydroascorbate and nitrate while at [$HAsc^-$] > 4 mmol litre^{-1} it reacts with the other substrate molecule to give hydroascorbate, dehydroascorbate (DAsc) and nitrite.



(38)

Intermediates Int_1 and Int_2 are treated[108] as 1 : 1 adducts of peroxynitrous acid with hydroascorbate. According to EPR data, the yield of ascorbyl radicals is 0.24% with respect to starting peroxynitrite and accumulation of these radicals in the course of reaction is accompanied by a decrease of absorption at 345 nm. Therefore, ascorbyl radicals are not primary products. No homolysis of ONOOH into hydroxyl radicals and nitrogen dioxide was detected under the experimental conditions.

Hughes and Nicklin[202] studied the oxidation of hydroxylamine in the presence of copper(II) salts. It seems that they were the first who observed catalysis of peroxynitrite reactions by metal ions. They also postulated the formation of a complex between Cu(II) and both reagents.



It is assumed[5, 22] that nitration of phenols and aromatic acids catalysed by transition metal (Fe^{3+}, Cu^{2+}, Mn^{3+}) complexes proceeds involving reactive species similar to nitroyl cation:

$$ONOO^- + M^{n+} \longrightarrow NO_2^{\delta+}\cdots O^{\delta-}\cdots M^{n+}, \quad (39)$$

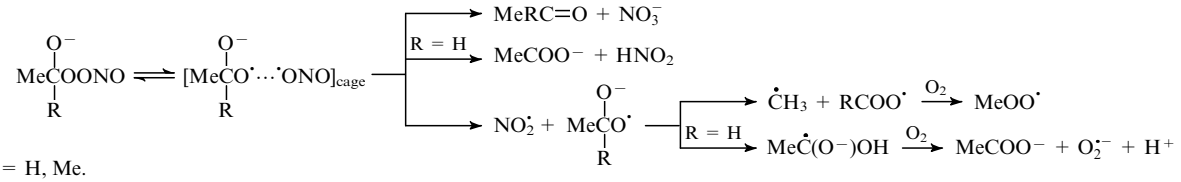
$$ArH + NO_2^{\delta+}\cdots O^{\delta-}\cdots M^{n+} \longrightarrow \quad (40)$$

$$\longrightarrow ArNO_2 + H^+ + O^-\cdots M^{(n-1)+},$$

$$O^-\cdots M^{(n-1)+} + 2H^+ \longrightarrow M^{n+} + H_2O. \quad (41)$$

Nitration of phenols catalysed by superoxide dismutase (SOD) has been best studied.[127, 203] The rate of the reaction of

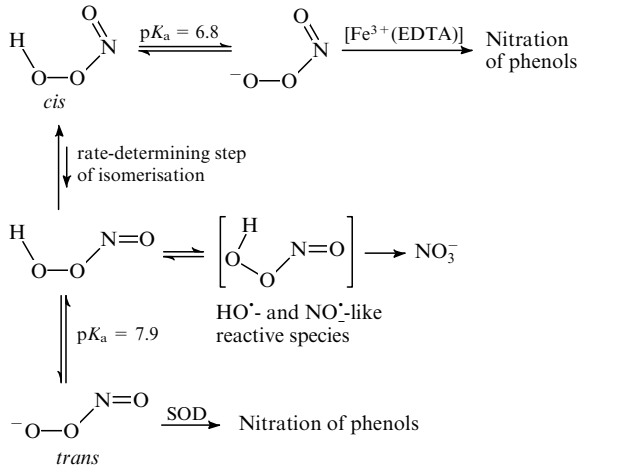
Scheme 8



R = H, Me.

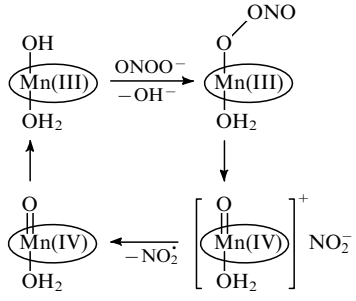
peroxynitrite with Cu,Zn-SOD is about 10^5 litre mol^{-1} s^{-1} and the maximum yield of nitrophenols ($\sim$8%) is observed at pH 7.5 (see Fig. 5). In the absence of other phenols, the tyrosine residue of SOD is transformed to nitrotyrosine [203] and Cu,Zn-SOD retains its catalytic activity. In the first stage, peroxynitrite reacts with copper; removal of copper from the SOD molecule causes nitration to cease. A complex of Fe^{3+} with EDTA is less catalytically active, however the yield of products exceeds 90% with respect to the starting peroxynitrite at pH 7.5.[127, 128] Nitration is not accompanied by production of free radicals involving 'nitroyl-like' species that are formed in reactions (39)–(41). The differences between SOD and the complex of Fe^{3+} with EDTA were explained using Scheme 9.[127]

Scheme 9



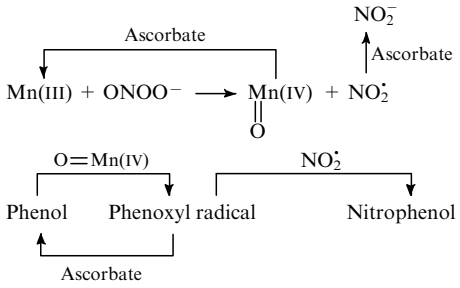
In the reaction with [Fe^{3+}(EDTA)] the role of the reactive intermediate is played by *cis*-peroxynitrite. SOD interacts only with *trans*-peroxynitrite due to specific features of the structure of Cu,Zn-superoxide dismutase. The copper ion in SOD is in a deep narrow cavity, therefore only *trans*-$ONOO^-$ can reach the active site. Positively charged amino acids near the active site facilitate the motion of peroxynitrite anion into the cavity. The rate-limiting step of this reaction is *cis*–*trans*-isomerisation.[15]

Another mechanism of catalysis is realised in the case of nitration of tyrosine in the presence of porphyrin complexes of manganese(III) and iron(III).[204–207] Metal-containing porphyrins (MPs) are rapidly oxidised by peroxynitrite (rate constants depend on the porphyrin structure and range from 2.5×10^4 to 5×10^7 litre mol^{-1} s^{-1})[204, 208–211] to relatively stable intermediates Mn(IV)=O and Fe(IV)=O from which Mn(III) and Fe(III) regenerate at the stage of catalytic decomposition of peroxynitrite.



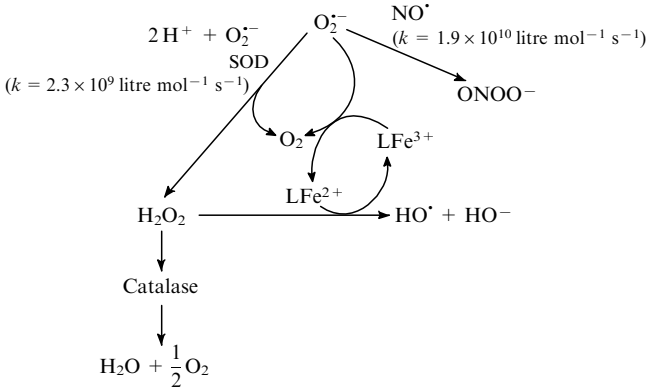
Reduction of Mn(IV)=O and Fe(IV)=O in the presence of phenols is a fast process and MPs catalyse nitration of phenols and oxidation of peroxynitrite. The Mn- and Fe-containing porphyrins cause more than five-fold (pH 7.4) and twelve-fold (pH 9.1) increase in the yield of nitration products (see Ref. 204).

It was established [204, 212] that the first stage of catalytic nitration of tyrosine and other phenols involves the oxidation of MP(3+) by peroxynitrite to MP(4+). The oxidised porphyrin rapidly reacts with phenols, thus regenerating MP(3+) and converting phenols into phenoxyl radicals that recombine with nitrogen dioxide formed in the first stage at a high rate ($k = 3\times10^9$ litre mol^{-1} s^{-1}).[194] Introduction of ascorbic acid into the system inhibits formation of nitrophenols, Mn(IV)=O and Fe(IV)=O are reduced to stable (3+) form, $NO_2^\bullet$ radical is reduced to NO_2^- and phenoxyl radical is reduced to the starting phenol.[204]



VI. Biochemical role of peroxynitrite

The possibility of *in vivo* production of peroxynitrite in fast recombination [$k = (4.3–19)\times10^9$ litre mol^{-1} s^{-1}][32–38] of nitrogen monoxide (product of enzymatic oxidation of L-arginine)[213, 214] and superoxide anion $O_2^{\bullet-}$ (conventional product of aerobic cell metabolism [215]) was first reported by Beckman *et al.*[3] Activated macrophages are assumed to be responsible for production of peroxynitrite.[216–218] If the intracellular concentration of SOD is 10 μmol litre^{-1} and the rate constant for the reaction of SOD with superoxide is 2.3×10^9 litre mol^{-1} s^{-1} (see Ref. 219), the loss rate of $O_2^{\bullet-}$ is 2×10^4 s^{-1} and the whole superoxide is transformed to oxygen and hydrogen peroxide.[16, 39] If the intracellular concentration of nitrogen monoxide is 10^{-8} mol litre^{-1}, the loss rate of superoxide in the reaction with NO is two orders of magnitude lower (2×10^2 s^{-1}) than in the reaction with SOD. No peroxynitrite is formed under these conditions. The local concentration of NO near activated macrophages is much higher (5×10^{-6}–10^{-5} mol litre^{-1}) and peroxynitrite is produced at a rate of $(2–4)\times10^4$ s^{-1}, which is twice as high as the rate of the reaction of $O_2^{\bullet-}$ with SOD.



Since the extracellular concentration of SOD is probably lower than 5×10^{-6}–10^{-5} mol litre^{-1}, it does not preclude formation of peroxynitrite near activated macrophages.[16, 39] Nitration of SOD by peroxynitrite reduces its activity and, as a consequence, increases the yield of peroxynitrite, thus switching off the signal function of NO. Peroxynitrous acid and its anion can penetrate inside cells through lipid membranes moving along anion channels or by diffusion [132, 220–222] and thus toxic effects of peroxynitrite can manifest themselves inside cells. Peroxynitrite is produced *in vivo* in the reaction of NO with superoxide; polluted air and cigarette smoke are also possible sources of this compound.[223]

Biological value of peroxynitrite is due to its high and polyfunctional reactivity with respect to various biomolecules.

The key mechanisms of biochemical action of peroxynitrite are as follows.

1. Interaction with sulfide and thiol groups [24, 83, 117, 118, 224, 225]

$$ONOO^- + R{-}SH \xrightarrow[-NO_2^-]{} R{-}S{-}OH \xrightarrow{R{-}SH} \longrightarrow R{-}S{-}S{-}R + OH^-$$

mediates the activity of many enzymes and receptors.[226–230] Toxic effects of peroxynitrite are often associated with its ability to oxidise the methionine residue in the active site of α-1-protease (elastase inhibitor), which causes inactivation of enzyme.[231]

2. Protonation under physiological conditions and subsequent homolysis to hydroxyl radicals and NO_2 produces oxidative stress and apoptosis; this is related to neurotoxicity and molecular mechanisms of attack on immunocompetent cells.[218, 226, 232–235]

3. Oxidation of the alkyl and alkene C–H bonds in polyunsaturated fatty acids causes oxidative damage of lipid membranes.[132, 220, 221, 236, 237]

4. Metal-ion (Cu^{2+}, Zn^{2+}, Fe^{3+}, Mn^{3+}) catalysed nitration of phenols and aromatic amino acids. Many DNAs [56, 177, 238, 239] and enzymes are nitrated and oxidised, *e.g.*, SOD [15, 218, 234, 240–242] (its activity reduces; the concentrations of superoxide and peroxynitrite increase, thus producing oxidative stress); actine and neurofilaments [226, 243] (cytoskeleton and myofibrillae are damaged); cytochrome *c*, cytochrome P450 and mitochondrial ATPase [137, 212, 243–246] (inhibition of oxidative phosphorylation).

Peroxynitrite in organisms is a carrier of the bound form of nitrogen monoxide, a key transmitter in central and peripheral nervous system, and can induce damage to neurons in the case of strokes and neurodegenerative disease.[15, 216, 247, 248] The effect of peroxynitrite, a possible terminal neurotoxic agent, is responsible for neurotoxic action of NO.[249] For instance, amyotrophic lateral sclerosis can be due to nitration and oxidation of proteins, which damage the nuclei of motor neurons in spinal cord.[5, 250]

Oxidation of low density lipoproteins (LDLPs) in arterial walls is thought to be the key step leading to atherosclerosis. Peroxynitrite initiates the oxidation of lipids [132, 220, 237, 251] and oxidises LDLPs [6, 251–258] to a potentially active atherogenic form, which results in accumulation of cholesterol esters in cells. Besides, peroxynitrite rapidly oxidises α-tocoferol, which precludes the oxidation of LDLPs.[253] Nitration of tyrosine at *ortho*-position is the major route of peroxynitrite attack on protein molecules, which manifests itself in increased content of *o*-nitrotyrosine in patients with atherosclerosis.[6, 251, 257, 258]

Diffusion of peroxynitrite across cell membranes inhibits mytochondrial electron transfer [259] and damages cation channels.[260] High cytotoxicity of peroxynitrite to *Trypanosoma cruzi* [261] and bactericidal activity against *Escherichia coli* [262] are due to deactivation of thiol-containing enzymes and inhibition of cell respiration.[229]

Since peroxynitrite is produced *in vivo* and can damage cells, an enzyme system for deactivating or utilising it should exist. It was shown that myeloperoxidase (MPO), horse raddish peroxidase and lactoperoxidase rapidly react with peroxynitrite. For instance, the reaction with MPO results in compound MPO-II.[139]

$$MPO + ONOOH \longrightarrow [MPO\text{-}I\cdots NO_2^-] \longrightarrow [MPO\text{-}II] + NO_2^{\bullet} \quad (k = 3.2\times10^6 \text{ litre mol}^{-1}\text{ s}^{-1}). \quad (42)$$

The character of the dependence of the rate for reaction (42) on the pH value indicates that the enzyme reacts only with peroxynitrous acid. Thus, MPO can reduce the concentration of peroxynitrite by converting it into important bactericidal agent $NO_2^{\bullet}$ and protect SOD from the action of peroxynitrite, thus increasing the lifetime of NO generated in neutrophiles.

Another possible way of peroxynitrite utilisation is the reaction with hydroascorbate anion. The concentration of $HAsc^-$ is $\sim10^{-3}$ mol litre^{-1} in lungs and neutrophiles and 10^{-2} mol litre^{-1} in neurons, ranges from 3×10^{-5} to 1.5×10^{-4} mol litre^{-1} in plasma and is 7×10^{-3} mol litre^{-1} in leucocites and 1.3×10^{-3} mol litre^{-1} in liver.[263–266] Therefore, with allowance for the rate constant for reaction (38), ascorbate can be treated as efficient intracellular scavenger of peroxynitrite.

Increasing interest in chemistry of ONOOH and nitrogen oxides is due to very important role played by these compounds in physiological processes (respiration, regulation of blood pressure and sexual function, growth of malignant cells, *etc.*). Both positive role (bactericidal and antimicrobial effects) and negative role of peroxynitrite are considered.[4–11, 226, 247, 251, 258, 262, 267]

VII. Conclusion

The chemistry of peroxynitrite emerged more than 100 years ago. However, systematic, comprehensive investigations of the kinetics and mechanisms of reactions involving this compound began only in the last decade. Complexity of the studies of peroxynitrite reactions is due to its instability at physiological pH values, diversity of transformation pathways and to the influence of impurities (nitrite, hydrogen peroxide, metal complexes, CO_2) on the rates and mechanisms of reactions. Recently, application of methods for studying fast reactions and the kinetic distribution method made it possible to achieve some advances in studies of the kinetics and mechanisms of peroxynitrite decomposition and isomerisation, its reactions with hydrocarbons and DMSO, catalytic action of CO_2 and metal complexes. Considerable literature is devoted to biochemical aspects of the action of peroxynitrite.

At the same time a number of key problems concerning the mechanisms of influence of peroxynitrite are still to be solved. Pathways of isomerisation and decomposition of the compound belong to the most debatable issue. Recently, the homolytic mechanism of isomerisation involving intermediate formation of $^{\bullet}OH$ and $NO_2^{\bullet}$ radicals, which was accepted in earlier studies, has cast some doubt.[38, 104] Experimental and theoretical arguments *pro et contra* the homolytic mechanism in different types of reactions are available. Undoubtedly, this is due to polyfunctional reactivity of peroxynitrite, which underlies high chemical and biochemical activity of the compound.

An interesting classification of the mechanisms of peroxynitrite reactions with a broad spectrum of organic substrates was reported [147] by researchers from Japan. It includes five reaction classes. These are (1) electron transfer with involvement of the reactive species NO^+, $NO_2^{\bullet}$ and $^{\bullet}OH$ (nitration, hydroxylation and dimerisation of phenols, oxidation of phenols to quinones, dealkylation of *N*,*N*-dialkyl-*p*-toluidine); (2) *O*-electrophilic substitution involving ONOOH (formation of *N*-oxides in the oxidation of amines, oxidative cleavage of C=C double bond and oxidation of alcohols to ketones); (3) *N*-electrophilic substitution (formation of triazoles from aromatic 1,2-diamines and *S*-nitrosation); the reactive species are ONOOH and NO^+, (4) *O*-nucleophilic substitution involving $ONOO^-$ and HOO^- (epoxidation of quinones and oxidation of aldehydes); and (5) radical mechanisms involving $NO_2^{\bullet}$ and $^{\bullet}OH$ (oxidation of lipids and alcohols, hydroxylation of activated aromatic compounds).

Analysis of the results presented in the present review and obtained in our kinetic studies of the reactions of peroxynitrite with hydrocarbons and DMSO (see Sections V.2 and V.3) suggest the following.

1. High oxidation potential and high electrophilic and nucleophilic activity allows peroxynitrite to react with a broad spectrum of substrates.

2. The variety of pathways and mechanisms of peroxynitrite reactions and the nature of the active centers in the peroxynitrite molecule are determined by not only the reaction conditions (pH, presence of transition metal impurities, CO_2, hydrogen peroxide, nitrite) but also the properties of substrates:

(a) with highly reactive substrates, whose reactions with peroxynitrite proceed faster than the latter decomposes, either peroxynitrous acid or its anion can act as reactive species;

(b) reactions with substrates of low reactivity proceed by radical mechanisms involving $^{\bullet}$OH-like reactive species (hydroxyl radical, radical pair or the excited form of peroxynitrous acid), which was proved taking the oxidation of inert alkane molecules under mild conditions as examples.

It is the reactivity of substrates that can reconcile the research group headed by Koppenol [16, 38, 61, 108] (they assume a bimolecular interaction of peroxynitrite with many substrates) with the groups headed by Goldstein [40, 62, 65] and Hurst [64, 88] who prefers the mechanisms involving $^{\bullet}$OH-like reactive species.

3. Volatile substances, *e.g.*, low-molecular-mass hydrocarbons, can react with peroxynitrous acid (but not its anion!) not only in solution, but also in the gas phase.

4. The ability of ONOOH to distribute between different phases (aqueous, gas phase and lipophilic phase) and generate OH radicals in each phase is of great importance for understanding the mechanisms of peroxynitrous acid initiated oxidation of lipid fragments in cells and diffusion of peroxynitrite across cell membranes.

High chemical and biological activity of peroxynitrite requires further systematic studies on the kinetics and mechanisms of reactions, including the reactions with biomolecules and their models under physiological conditions.

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While the manuscript was in preparation, a new review [268] has appeared. This indicates incessant interest of researchers in the subject in question.

References

1. G J Leigh (Ed.) *IUPAC. Nomenclature of Inorganic Chemistry. Recommendations* (Oxford: Blackwell, 1990)
2. K Gleu, E Roell *Z. Anorg. Allg. Chem.* **179** 233 (1929)
3. J S Beckman, T W Beckman, G Chen, P A Marshal, B A Freeman *Proc. Natl. Acad. Sci. U.S.A.* **87** 1620 (1990)
4. W A Pryor, G L Squadrito *Am. J. Physiol.* **268** L699 (1995)
5. J S Beckman, M Carson, C D Smith, W H Koppenol *Nature (London)* **364** 584 (1993)
6. N Hogg, V M Darley-Usmar, A Graham, S Moncada *Biochem. Soc. Trans.* **21** 358 (1993)
7. J S Beckman, W H Koppenol *Am. J. Physiol.* **271** C1424 (1996)
8. I Y Haddad, H Ischiropoulos, B A Holm, J S Beckman, J R Baker, S Matalon *Am. J. Physiol.* **265** L555 (1994)
9. J K Hurst, S V Lymar *Acc. Chem. Res.* **32** 520 (1999)
10. D Stuehr, S Pou, G M Rosen *J. Biol. Chem.* **276** 14533 (2001)
11. R Radi, G Peluffo, M N Alvarez, M Naviliat, A Cayota *Free Radical Biol. Med.* **30** 463 (2001)
12. H M Papée, G L Petriconi *Nature (London)* **204** 142 (1964)
13. O C Zafiriou, in *Chemical Oceanography* Vol. 8 (2nd Ed.) (New York: Academic Press, 1983) p. 339
14. J O Edwards, R C Plumb, in *Progress in Inorganic Chemistry* Vol. 41 (Ed. K D Karlin) (New York: Wiley, 1994) p. 599
15. J S Beckman *Nitric Oxide. Principles and Actions* (Ed. J Lancaster) (San Diego, CA: Academic Press, 1996)
16. W H Koppenol, in *Interrelation Between Free Radicals and Metal Ions in Life Processes* (Eds A Singel, H Singel) (New York, Basel: Marcel Dekker, 1999) p. 597
17. D A Wink, J B Mitchell *Free Radical Biol. Med.* **25** 434 (1998)
18. O Augusto, M G Bonini, A M Amanso, E Linares, C C X Santos, S L De Menezes *Free Radical Biol. Med.* **32** 841 (2002)
19. E Halfpenny, P L Robinson *J. Chem. Soc.* 928 (1952)
20. M Anbar, H Taube *J. Am. Chem. Soc.* **76** 6243 (1954)
21. D J Benton, P Moore *J. Chem. Soc. A* 3179 (1970)
22. J S Beckman, G Chen, H Ischiropoulos, J P Crow *Methods Enzymol.* **233** 229 (1994)
23. A Saha, S Goldstein, D Cabelli, G Czapski *Free Radical Biol. Med.* **24** 653 (1998)
24. W H Koppenol, J J Moreno, W A Pryor, H Ischiropoulos, J S Beckman *Chem. Res. Toxicol.* **5** 834 (1992)
25. M N Hughes, H G Nicklin *J. Chem. Soc. A* 450 (1968)
26. W G Keith, R E Powell *J. Chem. Soc. A* 90 (1969)
27. J W Reed, H H Ho, W L Jolly *J. Am. Chem. Soc.* **96** 1248 (1974)
28. G Merényi, J Lind, G Czapski, S Goldstein *Inorg. Chem.* **42** 3796 (2003)
29. S Goldstein, G Czapski *Inorg. Chem.* **35** 7735 (1996)
30. G L Petriconi, H M Papée *Can. J. Chem.* **44** 977 (1966)
31. S Goldstein, G Czapski *Inorg. Chem.* **35** 5935 (1996)
32. M Saran, C Michel, W Bors *Free Radical Res. Commun.* **10** 221 (1990)
33. R E Huie, S Padmaja *Free Radical Res. Commun.* **18** 195 (1993)
34. S Goldstein, G Czapski *Free Radical Biol. Med.* **19** 505 (1995)
35. K Kobayashi, M Miki, S Tagawa *J. Chem. Soc., Dalton Trans.* 2885 (1995)
36. R Kissner, T Nauser, P Bugnon, P G Lye, W H Koppenol *Chem. Res. Toxicol.* **10** 1285 (1997)
37. T Nauser, W H Koppenol *J. Phys. Chem. A* **106** 4084 (2002)
38. R Kissner, T Nauser, C Kurz, W H Koppenol *IUBMB Life* **55** 567 (2003)
39. W H Koppenol *Free Radical Biol. Med.* **25** 385 (1998)
40. G Merényi, J Lind, S Goldstein, G Czapski *Chem. Res. Toxicol.* **11** 712 (1998)
41. G Merényi, J Lind *Chem. Res. Toxicol.* **11** 243 (1998)
42. S V Lymar, G A Poskrebyshev *J. Phys. Chem. A* **107** 7991 (2003)
43. D S Bohle, P A Glassbrenner, B Hanset *Methods Enzymol.* **269** 302 (1996)
44. W H Koppenol, R Kissner, J S Beckman *Methods Enzymol.* **269** 296 (1996)
45. J R Leis, M E Pena, A Rios *J. Chem. Soc., Chem. Commun.* 1293 (1993)
46. R M Uppu, W A Prior *Methods Enzymol.* **269** 322 (1996)
47. G Yagil, M Anbar *J. Inorg. Nucl. Chem.* **26** 453 (1964)
48. M N Hughes, H G Nicklin *J. Chem. Soc. A* 164 (1971)
49. C E Donald, M N Hughes, J M Tompson, F T Bonner *Inorg. Chem.* **25** 2676 (1986)
50. W A Pryor, R Cueto, X Jin, W H Koppenol, M Ngu-Schwemlein, G L Squadrito, P L Uppu, R M Uppu *Free Radical Biol. Med.* **18** 75 (1995)
51. R M Uppu, G L Squadrito, R Cueto, W A Pryor *Methods Enzymol.* **269** 311 (1996)
52. F Barat, B Hickel, J Sutton *J. Chem. Soc. D* 125 (1969)
53. R C Plumb, J O Edwards *J. Phys. Chem.* **96** 3245 (1992)
54. M Gratzel, A Henglein, S Taniguchi *Ber. Bunsen-Ges. Phys. Chem.* **94** 292 (1970)
55. T Løgager, K Sehested *J. Phys. Chem.* **97** 6664 (1993)
56. P A King, V E Anderson, J O Edwards, G Gustafson, R C Plumb *J. Am. Chem. Soc.* **114** 5430 (1992)
57. J D Ray *J. Inorg. Nucl. Chem.* **24** 1159 (1962)
58. M Manuszak, W H Koppenol *Thermochim. Acta* **273** 11 (1996)
59. G Merényi, J Lind *Chem. Res. Toxicol.* **10** 1216 (1997)
60. T Nauser, M Merkofer, R Kissner, W H Koppenol *Chem. Res. Toxicol.* **14** 348 (2001)
61. W H Koppenol, R Kissner *Chem. Res. Toxicol.* **11** 87 (1998)
62. G Merényi, J Lind, S Goldstein, G Czapski *J. Phys. Chem. A* **103** 5685 (1999)
63. R Kissner, C Thomas, M S A Hamsa, R van Eldik, W H Koppenol *J. Phys. Chem. A* **107** 11261 (2003)
64. S V Lymar, R F Khairutdinov, J K Hurst *Inorg. Chem.* **42** 5259 (2003)
65. S Goldstein, D Meyerstein, R van Eldik, G Czapski *J. Phys. Chem. A* **103** 6587 (1999)
66. S Goldstein, D Meyerstein, R van Eldik, G Czapski *J. Phys. Chem. A* **101** 7114 (1997)
67. S Pfeiffer, B Mayer, R Yanoschek *J. Mol. Struct. (THEOCHEM)* **623** 95 (2003)
68. K N Houk, K R Condroski, W A Pryor *J. Am. Chem. Soc.* **118** 13002 (1996)
69. W H Koppenol *Redox Rep.* **6** 339 (2001)

70. J H M Tsai, J G Harrison, J C Martin, T P Hamilton, M van der Woerd, M J Jablonsky, J S Beckman *J. Am. Chem. Soc.* **116** 4115 (1994)
71. H H M Tsai, T P Hamilton, J H M Tsai, M van der Woerd, J G Harrison, M J Jablonsky, J S Beckman, W H Koppenol *J. Phys. Chem.* **100** 15087 (1996)
72. Y Zhao, K M Houk, L Olson *J. Phys. Chem. A* **108** 5864 (2004)
73. D G Musaev, Y V Geletii, C L Hill *J. Phys. Chem. A* **107** 5862 (2003)
74. W H Koppenol, L Klasinc *Int. J. Quantum Chem. Quantum Biol. Symp.* **20** 1 (1993)
75. M P McGrath, M M Francl, F S Rowland, W J Hehre *J. Phys. Chem.* **92** 5352 (1988)
76. B M Cheng, J M Lee, Y P Lee *J. Phys. Chem.* **95** 2814 (1991)
77. W J Lo, Y P Lee, J H M Tsai, H H M Tsai, T P Hamilton, J G Harrison, J S Beckman *J. Chem. Phys.* **103** 4026 (1995)
78. B Liang, L Andrews *J. Am. Chem. Soc.* **123** 9848 (2001)
79. W J Chen, W J Lo, B M Cheng, Y P Lee *J. Chem. Phys.* **97** 7167 (1992)
80. M C R Symons *J. Inorg. Biochem.* **78** 299 (2000)
81. U Shuali, M Ottolenghi, J Rabani, Z Yelin *J. Phys. Chem.* **73** 3445 (1969)
82. F Barat, L Gillies, B Hickel, J Sutton *J. Chem. Soc. A* 1982 (1970)
83. R Radi, J S Beckman, K N Bush, B A Freeman *J. Biol. Chem.* **266** 4244 (1991)
84. S Goldstein, S Squadrito, W A Pryor, G Czapski *Free Radical Biol. Med.* **21** 965 (1996)
85. S Padmaja, R Kissner, P L Bounds, W H Koppenol *Helv. Chim. Acta* **81** 1201 (1998)
86. G Meréni, J Lind, L Engman *J. Chem. Soc., Perkin Trans. 2* 2551 (1994)
87. A D McNaught, A Wilkinson *Compendium of Chemical Terminology. IUPAC Recommendation* (Oxford: Blackwell, 1997)
88. J W Coddington, J K Hurst, S V Lymar *J. Am. Chem. Soc.* **121** 2438 (1999)
89. S Pfeiffer, A C F Gorren, K Schmidt, E R Werner, B Hansert, D S Bohle, B Mayer *J. Biol. Chem.* **272** 3465 (1997)
90. M Kirsch, H-G Korth, A Wensing, R Sustmann, H de Groot *Arch. Biochem. Biophys.* **418** 133 (2003)
91. R Kissner, W H Koppenol *J. Am. Chem. Soc.* **124** 234 (2002)
92. A U Khan, D Kovacic, A Kolbanovskiy, M Desai, K Frenkel, N E Geacintov *Proc. Natl. Acad. Sci. U.S.A.* **97** 2984 (2000)
93. G Merényi, J Lind, G Czapski, S Goldstein *Proc. Natl. Acad. Sci. U.S.A.* **97** 8216 (2000)
94. G R Martinez, P Di Mascio, M G Bonini, O Augusto, K Briviba, H Sies, P Maurer, U Röthlisberger, S Herold, W H Koppenol *Proc. Natl. Acad. Sci. U.S.A.* **97** 10307 (2000)
95. P Di Mascio, E J N Behara, M H G Medeiros, K Briviba, H Sies *FEBS Lett.* **355** 287 (1994)
96. S Goldstein, G Czapski *J. Am. Chem. Soc.* **120** 3458 (1998)
97. G R Hodges, K Ingold *J. Am. Chem. Soc.* **121** 10695 (1999)
98. J P Crow, C Spruell, J Chen, C Gunn, H Ischiropoulos, M Tsai, C D Smith, R Radi, W H Koppenol, J S Beckman *Free Radical Biol. Med.* **16** 331 (1994)
99. P Maurer, C F Thomas, R Kissner, H Rüegger, O Greter, U Röthlisberger, W H Koppenol *J. Phys. Chem. A* **107** 1763 (2003)
100. L R Mahoney *J. Am. Chem. Soc.* **92** 5262 (1970)
101. B Alvarez, G Ferrer-Sueta, R Radi *Free Radical Biol. Med.* **24** 1331 (1998)
102. M N Hughes, H G Nicklin, A C Sakrule *J. Chem. Soc. A* 3722 (1971)
103. O V Gerasimov, S V Lymar *Inorg. Chem.* **38** 4317 (1999)
104. C E Richeson, P Mulder, V W Bowry, K U Ingold *J. Am. Chem. Soc.* **120** 7211 (1998)
105. E Halfpenny, P L Robinson *J. Chem. Soc. A* 939 (1952)
106. G Yang, T E G Candy, M Boaro, H E Wilkin, P Jones, N B Nazhat, R A Saadalla-Nazhat, D R Blaket *Free Radical Biol. Med.* **12** 327 (1992)
107. J B Burkholder, P D Hammer, C J Howard *J. Phys. Chem.* **91** 2136 (1987)
108. C Kurz, R Kissner, T Nauser, D Perrin, W H Koppenol *Free Radical Biol. Med.* **35** 1529 (2003)
109. D S Bohle, B Hansert *Nitric Oxide* **1** 502 (1997)
110. B S Jursic, L Klasinc, S Pecur, W A Pryor *Nitric Oxide* **1** 494 (1997)
111. M Lehnig *Arch. Biochem. Biophys.* **368** 303 (1999)
112. S V Lymar, J K Hurst *Chem. Res. Toxicol.* **11** 714 (1998)
113. S Goldstein, G Czapski, J Lind, G Merényi *Chem. Res. Toxicol.* **14** 627 (2001)
114. L P Olson, M D Bartberger, K N Houk *J. Am. Chem. Soc.* **125** 3999 (2003)
115. J F Goodman, P Robson *Trans. Faraday Soc.* **59** 2871 (1963)
116. V P Tret'yakov, L A Min'ko, E S Rudakov *Teor. Eksp. Khim.* **40** 298 (2004) [a]
117. D Perrin, W H Koppenol *Arch. Biochem. Biophys.* **377** 266 (2000)
118. Y V Geletii, D G Musaev, L Khavrutskii, C L Hill *J. Phys. Chem. A* **108** 289 (2004)
119. S Goldstein, G Czapski *Inorg. Chem.* **34** 4041 (1995)
120. R M Uppu, G L Squadrito, W A Pryor *Arch. Biochem. Biophys.* **327** 335 (1996)
121. S V Lymar, J K Hurst *Inorg. Chem.* **37** 294 (1998)
122. S Goldstein, G Czapski *Inorg. Chem.* **36** 5113 (1997)
123. S V Lymar, J K Hurst *Chem. Res. Toxicol.* **9** 845 (1996)
124. A van der Vliet, C A O'Neill, B Halliwell, C E Cross, H Kaur *FEBS Lett.* **339** 89 (1994)
125. W A Pryor, X Jin, G L Squadrito *Proc. Natl. Acad. Sci. U.S.A.* **91** 11173 (1994)
126. G L Squadrito, X Jin, W A Pryor *Arch. Biochem. Biophys.* **322** 53 (1995)
127. J S Beckman, H Ischiropoulos, L Zhu, M van der Woerd, C D Smith, J Chen, J Harrison, J C Martin, M Tsai *Arch. Biochem. Biophys.* **298** 438 (1992)
128. M S Ramezanian, S Padmaja, W H Koppenol *Chem. Res. Toxicol.* **9** 232 (1996)
129. T Inoe, N Sonoda, S Tsutsumi *Bull. Chem. Soc. Jpn.* **36** 1549 (1963)
130. H Kaur, I Fagerheim, M Grootveld, A Puppo, B Halliwell *Anal. Biochem.* **172** 360 (1988)
131. M Masumoto, R Kissner, W H Koppenol H Sies *FEBS Lett.* **398** 179 (1996)
132. S S Marla, J Lee, J T Groves *Proc. Natl. Acad. Sci. U.S.A.* **94** 14243 (1997)
133. M K Stern, M P Jensen, K Kramer *J. Am. Chem. Soc.* **118** 8735 (1996)
134. S Padmaja, G L Squadrito, J N Lemercier, R Cueto, W A Pryor *Free Radical Biol. Med.* **21** 317 (1996)
135. S Padmaja, M S Ramezanian, P L Bounds, W H Koppenol *Redox Rep.* **2** 173 (1996)
136. B Alvarez, H Rubbo, M Kirk, S Barnes, B A Freman, R Radi *Chem. Res. Toxicol.* **9** 390 (1996)
137. L Thomsonn, M Trujillo, L Telleri, R Radi *Arch. Biochem. Biophys.* **319** 491 (1995)
138. J P Crow, J S Beckman, J M McCord *Biochemistry* **34** 3544 (1995)
139. R Floris, S R Piersma, G Yang, P Jones, R Werver *J. Biochem.* **215** 767 (1993)
140. L Castro, M Rodrigues, R Radi *J. Biol. Chem.* **269** 29409 (1994)
141. S V Lymar, J K Hurst *J. Am. Chem. Soc.* **117** 8867 (1995)
142. A Denicola, B A Freeman, M Trujillo, R Radi *Arch. Biochem. Biophys.* **333** 49 (1996)
143. S Goldstein, G Czapski *Nitric Oxide* **1** 417 (1997)
144. N W Kooy, J A Royall, H Ischiropoulos, J S Beckman *Free Radical Biol. Med.* **16** 149 (1994)
145. R Radi, T P Cosgrove, J S Beckman, B A Freeman *Biochem. J.* **290** 51 (1993)
146. B Alvarez, A Denicola, R Radi *Chem. Res. Toxicol.* **8** 859 (1995)
147. N Nonoyama, H Oshima, C Shoda, H Suzuki *Bull. Chem. Soc. Jpn.* **74** 2385 (2001)
148. E S Rudakov *Reaktsii Alkanov s Okislitelyami, Metallokompleksami i Radikalami v Rastvorakh* (Reactions of Alkanes with Oxidants, Metal Complexes and Radicals in Solutions) (Kiev: Naukova Dumka, 1985)

149. E S Rudakov, V L Lobachev *Izv. Sib. Otd. Akad. Nauk, Ser. Khim. nauk* (3) 25 (1987)
150. V V Goncharuk, G L Kamalov, G A Kovtun, E S Rudakov, V K Yatsimirskii *Kataliz. Mekhanizmy Gomogennogo i Geterogennogo Kataliza, Klasternye Podkhody* (Catalysis. Mechanisms of Homogeneous and Heterogeneous Catalysis, Cluster Approaches) (Kiev: Naukova Dumka, 2002)
151. R Atkinson *J. Phys. Chem. Ref. Data* **26** 215 (1997)
152. R A Perry, R Atkinson, J Pitts *J. Phys. Chem.* **81** 296 (1977)
153. G V Shustov, R Spinney, A Rauk *J. Am. Chem. Soc.* **122** 1191 (2000)
154. R D Bach, M N Glukhovtsev, C Canepa *J. Am. Chem. Soc.* **120** 775 (1998)
155. E S Rudakov, V L Lobachev, Yu V Geletii, G G A Balavoine *Zh. Org. Khim.* **32** 522 (1996) [b]
156. V L Lobachev, E S Rudakov *Kinet. Katal.* **41** 208 (2000) [c]
157. E S Rudakov, V L Lobachev, Yu V Geletii *Chem. Res. Toxicol.* **14** 1232 (2001)
158. V L Lobachev, S L Kluger, G P Zimtseva, Yu V Geletii, E S Rudakov *Teor. Eksp. Khim.* **39** 36 (2003) [a]
159. V P Tret'yakov, L A Min'ko, E S Rudakov, T A Alekhina *Teor. Eksp. Khim.* **39** 32 (2003) [a]
160. V L Lobachev, E S Rudakov *Kinet. Katal.* **46** 370 (2005) [c]
161. V L Lobachev, S L Kluger, E S Rudakov *Teor. Eksp. Khim.* **40** 23 (2004) [a]
162. H Zegota, M N Ichuchman, C von Sontage *J. Phys. Chem.* **88** 1589 (1984)
163. A A Fokin, P R Schreinen *Chem. Rev.* **105** 1551 (2002)
164. D M Matheu, W H Green *Int. J. Chem. Kinet.* **32** 245 (2000)
165. D W O'Sullivan, M Lee, B C Noone, B G Heikes *J. Phys. Chem.* **100** 3241 (1996)
166. J-M Regimbal, M Mozurkewich *J. Phys. Chem. A* **101** 8822 (1997)
167. D Veltwisch, E Janata, K-D Asmus *J. Chem. Soc., Perkin Trans. 2* 146 (1980)
168. G G A Balavoine, Yu V Geletii, in *Proceedings of the 3rd International Meeting on Biology of Nitric Oxide, Cologne, Germany, 1993* p. 175
169. V L Lobachev, G P Zimtseva, E S Rudakov, Yu V Geletii *Teor. Eksp. Khim.* **36** 41 (2000) [a]
170. J L Jensen, B L Miller, X P Zhang, G L Hug, C Schöneich *J. Am. Chem. Soc.* **119** 4749 (1997)
171. W A Prütz, J Butler, E J Land, A J Swallow *Int. J. Radiat. Biol.* **55** 539 (1989)
172. R Meli, T Nauser, W H Koppenol *Helv. Chim. Acta* **82** 722 (1999)
173. R Meli, T Nauser, W H Koppenol *J. Biol. Inorg. Chem.* **7** 31 (2002)
174. S V Lymar, Q Jiang, J K Hurst *Biochemistry* **35** 7855 (1996)
175. A Gow, D Duran, S R Thom, H Ischiropoulos *Arch. Biochem. Biophys.* **333** 42 (1996)
176. S Goldstein, G Czapski *J. Am. Chem. Soc.* **121** 2444 (1999)
177. V Yermilov, Y Yoshie, J Rubio, H Ohshima *FEBS Lett.* **399** 67 (1996)
178. R M Uppu, W A Pryor *Biochem. Biophys. Res. Commun.* **229** 764 (1996)
179. W A Pryor, J-N Lemercier, H Zhang, R M Uppu *Free Radical Biol. Med.* **23** 331 (1997)
180. H Zhang, G L Squadrito, R M Uppu, J-N Lemercier, R Cueto, W A Pryor *Arch. Biochem. Biophys.* **339** 183 (1997)
181. J-N Lemercier, S Padmaja, R Cueto, G L Squadrito, R M Uppu, W A Pryor *Arch. Biochem. Biophys.* **345** 160 (1997)
182. G L Squadrito, W H Pryor *Chem. Res. Toxicol.* **15** 885 (2002)
183. Z B Alfassi, T Dhanasekaram, R E Huie, P Neta *Radiat. Phys. Chem.* **56** 475 (1999)
184. J Lilie, R J Hanrahan, A Henglein *Radiat. Phys. Chem.* **11** 225 (1978)
185. S Goldstein, J Lind, G Merényi *J. Chem. Soc. J., Dalton Trans.* 808 (2002)
186. R B Moodie, K Schofield, P G Taylor *J. Chem. Soc., Perkin Trans. 2* 133 (1979)
187. S Goldstein, G Czapski, J Lind, G Mereni *Chem. Res. Toxicol.* **14** 1273 (2001)
188. R E Huie, C L Clifton, P Neta *Radiat. Phys. Chem.* **38** 477 (1991)
189. S V Lymar, H A Schwarz, G Czapski *Radiat. Phys. Chem.* **59** 387 (2000)
190. M G Bonini, O Augusto *J. Biol. Chem.* **276** 9749 (2001)
191. D M Stanbury *Inorg. Chem.* **33** 69 (1989)
192. H Zhang, G L Squadrito, W A Pryor *Nitric Oxide* **1** 301 (1997)
193. S-N Chen, M Z Hoffman *Radiat. Res.* **56** 40 (1973)
194. W A Prutz, H Monig, J Butler, E J Land *Arch. Biochem. Biophys.* **243** 125 (1985)
195. R M Uppu, G M Winston, W A Pryor *Chem. Res. Toxicol.* **10** 1331 (1997)
196. L S Nakao, D Ouchi, O Augusto *Chem. Res. Toxicol.* **12** 1010 (1999)
197. D Yang, Y-C Tang, J Chen, X-C Wang, M D Bartberger, K N Houk, L Olson *J. Am. Chem. Soc.* **121** 11976 (1999)
198. G Merényi, J Lind, S Goldstein *J. Am. Chem. Soc.* **124** 40 (2002)
199. M H Williams, J K Yandell *Aust. J. Chem.* **35** 1133 (1982)
200. M Levine *New Engl. J. Med.* **314** 892 (1986)
201. D Bartlett, D F Church, P L Bounds, W H Koppenol *Free Radical Biol. Med.* **18** 85 (1995)
202. M N Hughes, H G Nicklin *J. Chem. Soc. A* 925 (1970)
203. H Ischiropoulos, L Zhu, J Chen, M Tsai, J C Martin, C D Smith, J S Beckman *Arch. Biochem. Biophys.* **298** 431 (1992)
204. J P Crow *Arch. Biochem. Biophys.* **371** 41 (1999)
205. G G A Balavoine, Yu V Geletii, D Bejan *Nitric Oxide* **1** 507 (1997)
206. J T Groves, S S Marla *J. Am. Chem. Soc.* **117** 9578 (1995)
207. G Ferrer-Sueta, L Ruiz-Ramirez, R Radi *Chem. Res. Toxicol.* **10** 1338 (1997)
208. J Lee, J A Hunt, J T Groves *J. Am. Chem. Soc.* **120** 7493 (1998)
209. J P Crow *Free Radical Biol. Med.* **28** 1487 (2000)
210. M P Jensen, D P Riley *Inorg. Chem.* **41** 4788 (2002)
211. G Ferrer-Sueta, D V Vitturi, I Batinić-Haberle, I Fridovich, S Goldstein, G Czapski, R Radi *J. Biol. Chem.* **278** 27432 (2003)
212. A Daiber, M Bachschmid, J S Beckman, T Munzel, V Ullrich *Biochem. Biophys. Res. Commun.* **317** 873 (2004)
213. S Moncada, R M J Palmer, E A Higgs *Pharmacol. Rev.* **43** 109 (1991)
214. M A Marletta *J. Biol. Chem.* **268** 12231 (1993)
215. B Chance, H Sies, A Boveris *Physiol. Rev.* **59** 527 (1979)
216. H Ischiropoulos, L Zhu, J S Beckman *Arch. Biochem. Biophys.* **298** 446 (1992)
217. T J Evans, L D K Buttery, A Carpenter, D R Springall, J M Polak, J Cohen *Proc. Natl. Acad. Sci. U.S.A.* **93** 9553 (1996)
218. R Radi, A Cassina, R Hodara, C Quijano, L Castro *Free Radical Biol. Med.* **33** 1451 (2002)
219. E M Fiedlen, P B Roberts, R C Bray, D J Lowe, G N Mautner, G Rotilio, L Calambrese *Biochem. J.* **139** 49 (1974)
220. A Denicola, J M Souza, R Radi *Proc. Natl. Acad. Sci. U.S.A.* **95** 3566 (1998)
221. R Radi *Chem. Res. Toxicol.* **11** 720 (1998)
222. N Romero, A Denicola, J M Souza, R Radi *Arch. Biochem. Biophys.* **368** 23 (1999)
223. W A Pryor, K Stone *Ann. N.Y. Acad. Sci.* **686** 12 (1993)
224. C Quijano, B Alvarez, R M Gatti, O Augusto, R Radi *Biochem. J.* **322** 167 (1997)
225. G Scorza, M Minetti *Biochem. J.* **329** 183 (1998)
226. C Ducrocq, B Blanchard, B Pignatelli, H Ohshima *Cell. Mol. Life Sci.* **55** 1068 (1999)
227. E A Konorev, N Hogg, B Kalyanaraman *FEBS Lett.* **427** 171 (1998)
228. M Whiteman, B Halliwell *FEBS Lett.* **414** 497 (1997)
229. H Rubbo, A Denicola, R Radi *Arch. Biochem. Biophys.* **308** 96 (1994)
230. J P E E Pasquet, M H Zou, V Ulrich *Biochimie* **78** 785 (1996)
231. J J Moreno, W A Pryor *Chem. Res. Toxicol.* **5** 452 (1992)
232. K T Lin, J Y Xue, F F Sun *Biochem. Biophys. Res. Commun.* **115** 230 (1997)
233. A G Estvez, N Spear, S M Mannuel *J. Neurosci.* **18** 923 (1998)
234. B N Lyu *Usp. Sovr. Biol.* **121** 488 (2001)
235. E Battinelli, J Loscalzo *Blood* **95** 3451 (2000)

236. N Hogg, B Kalyanaraman *Biochim. Biophys. Acta* **1411** 378 (1999)
237. R F Khairutdinov, J W Coddington, J K Hurst *Biochemistry* **39** 14238 (2000)
238. C Szabó, H Ohshima *Nitric Oxide* **1** 373 (1997)
239. V Yermilov, J Rubo, H Ohshima *FEBS Lett.* **376** 207 (1995)
240. J N Keller, M S Kindy, F M Holstberg, D K S Clair, H C Yen, A Germeyer, S M Steiner, A J Bruce-Keller, J B Hutchins, M P Mattson *J. Neurosci.* **18** 687 (1998)
241. F Yamakura, H Taka, T Fujimura, K Murayama *J. Biol. Chem.* **273** 14085 (1998)
242. C Quijano, D Hernandez-Saavedra, L Castro, J M McCord, B A Freeman, R Radi *J. Biol. Chem.* **276** 11631 (2001)
243. J P Crow, Y Z Ye, M Strong, M Kirk, S Barnes, J S Beckman *J. Neurochem.* **69** 1945 (1997)
244. A M Cassina, R Hodara, J M Souza, L Thomson, L Castro, H Ischiropoulos, B A Freeman, R Radi *J. Biol. Chem.* **275** 21409 (2000)
245. E S Roberts, H Lin, J R Crowley, J L Vuletich, Y Osawa, P F Hollenberg *Chem. Res. Toxicol.* **11** 1067 (1998)
246. A M Cassina, R Radi *Arch. Biochem. Biophys.* **328** 309 (1996)
247. E Culotta, D E Koshland Jr *Science* **258** 1862 (1992)
248. C J Lowenstein, J L Dinerman, S H Snyder *Ann. Intern. Med.* **120** 227 (1994)
249. S A Lipton, Y-B Choi, Z-H Pan, S Z Lei, H-S V Chen, N J Sucher, J Loscalzo, D J Singel, J S Stamler *Nature (London)* **364** 626 (1993)
250. C W Olanow *Trends Neurosci.* **16** 439 (1993)
251. R Radi, J S Beckman, K N Bush, B A Freeman *Arch. Biochem. Biophys.* **288** 481 (1991)
252. V M Darley-Usmar, N Hogg, V J O'Leary, M T Wilson, S Moncada *Free Radical Res. Commun.* **17** 9 (1992)
253. N Hogg, V M Darley-Usmar, M T Wilson, S Moncada *FEBS Lett.* **326** 199 (1993)
254. W D Stanbro *J. Theor. Biol.* **205** 465 (2000)
255. W D Stanbro *J. Theor. Biol.* **205** 473 (2000)
256. S R Thomas, M J Davies, R Stocker *Chem. Res. Toxicol.* **11** 484 (1998)
257. C R White, T A Brock, L Y Chang, J Crapo, P Brisco, D Ku, W A Bradley, S H Gianturco, J Gore, B A Freeman, M T Tarpey *Proc. Natl. Acad. Sci. U.S.A.* **91** 1044 (1994)
258. J S Beckman, Y Z Ye, P G Anderson, J Chen, M A Accavitti, M M Tarpey, C R White *Biol. Chem. Hoppe-Seyler* **375** 81 (1994)
259. R Radi, M Rodriguez, L Castro, R Tellery *Arch. Biochem. Biophys.* **308** 89 (1994)
260. M L Bauer, J S Beckmann, R J Bridges, C M Fuller, S Matalon *Biochim. Biophys. Acta* **1104** 87 (1992)
261. A Denicola, H Rubbo, R Radi *Arch. Biochem. Biophys.* **304** 279 (1993)
262. L Zhu, C Gunn, J S Beckman *Arch. Biochem. Biophys.* **298** 452 (1992)
263. P Washko, D Rotrosen, M Levine *Am. J. Clin. Nutr.* **54** 1221S (1991)
264. M E Rice, I Russo-Menna *Neuroscience* **82** 1213 (1998)
265. H R Griffiths, J Lunec *Environ. Toxicol. Pharmacol.* **10** 173 (2001)
266. X Li, C E Cobb, K E Hill, R F Burk, J M May *Arch. Biochem. Biophys.* **387** 143 (2001)
267. A R Butler, D L H Williams *Chem. Soc. Rev.* 233 (1993)
268. S Goldstein, J Lind, G Merényi *Chem. Rev.* **105** 2457 (2005)

[a] — *Theor. Exp. Chem. (Engl. Transl.)*
[b] — *Russ. J. Org. Chem. (Engl. Transl.)*
[c] — *Kinet. Catal. (Engl. Transl.)*