Study of cell protective effects of alcohols against UV-C radiation and comparison to gamma radiation

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Abstract

A singlet oxygen production was monitored using a singlet oxygen sensor green chemical probe; reaction of chemical probe with singlet oxygen produces a fluorescent endoperoxide. Adding ethanol to the irradiated system resulted in decrease of the fluorescence signal, which indicates a decrease in concentration of singlet oxygen formed under UV-C irradiation. Thus, ethanol was likely to quench singlet oxygen in a system under study. This quenching did not occur with the use of methanol. When irradiating *E. coli* cells in the presence of ethanol and Rose Bengal for higher singlet oxygen production, there was a greater reduction in the radiation sensitivity of the cells compared to the system without Rose Bengal. Higher concentration of ethanol caused greater protection of cells; thus, it is likely that ethanol can scavenge singlet oxygen and provide a partial protection of bacteria from the effects of UV-C radiation. These results were compared with previously published data where the bacteria were irradiated by gamma radiation in presence of alcohols.

Keywords ROS · Singlet oxygen · Quenching · Alcohol · UV-C radiation · Gamma radiation · Bacteria

Introduction

It has been known for a long time that the interaction of radiation with cells creates, due to radiolysis of water, the reactive oxygen species (ROS) [1], which react with the cell structures, cause cell damage, and as a result, the death of cells. Critical structures are mainly the DNA, proteins and phospholipids that make up the cell membrane. This indirect effect of ionizing radiation is widely used in health care in the treatment of cancer, for disinfection, maintaining hygiene, sterilization of hospital instruments, etc. Indirect effects of ionizing radiation have a greater effect on cell damage compared with direct effects (a direct reaction of ionizing radiation with molecules of cell organelles), due to a greater probability of interaction of ionizing radiation with water than direct interaction with a DNA molecule [2, 3]. In the indirect effect, the largest share of damage is attributed to the hydroxyl radical [4]. To reduce these damaging effects, the OH radical scavengers are used that prevent the radical from reacting with the cell structure and thus reduce its

sensitivity to the given radiation. These include, for example, simple alcohols, potassium formate, dimethyl sulfoxide (DMSO), ascorbic acid and others. Simple alcohols have been found to reduce the radiation sensitivity of bacteria and microorganisms and to help protect cells from the effects of radiation. And moreover, radiation sensitivity is also affected by the dose and the dose rate of radiation [5, 6]. Unlike ionizing radiation, UV radiation is absorbed mainly by purine and pyrimidine bases. This absorption results in the formation of pyrimidine dimers (where T–T, C–C, or T–C bases are side by side on the same strand), which disrupts the binding at a given DNA site and thus prevents it from undergoing a standard replication process [7, 8].

ROS also includes a singlet oxygen $^1O_2$ (an excited form of oxygen with spin multiplicity 1). Its properties are used in the treatment of various forms of cancer by photodynamic therapy (PDT) [9, 10]. Photodynamic therapy is a non-invasive form of treatment of various forms of cancer, most often skin melanoma, lung cancer, brain, oral cavity, stomach, intestines, liver, bladder, prostate, and cervix, but also degenerative retinal disease, coronary artery disease or chronic periodontitis [11]. It is based on the application of photosensitive radiopharmaceuticals, such as porphyrin derivatives, which accumulate in the cancer and, when irradiated with light of the appropriate wavelength, undergo

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photochemical and photobiological processes that lead to the destruction of cancer tissue. Destruction is primarily caused by $^1O_2$, which acts as a toxic agent in biological systems.

The ground state of oxygen is triplet. The transition from triplet oxygen to singlet oxygen is a forbidden process; therefore, the transition occurs by indirect excitation through the photosensitizer. A photosensitizer is a photostable substance having a suitable absorption spectrum and, if possible, also fluorescence to detect its distribution in the organism. It is usually an organic dye, an aromatic, heterocyclic organic compound, or a coloured transition metal compound. Singlet oxygen deexcitation back to the ground triplet state occurs by phosphorescence, physical or chemical quenching [12]. During the physical quenching of singlet oxygen, the energy of the quencher molecule is transferred, which then disperses to the surroundings. In chemical quenching, the reaction of singlet oxygen with a quencher produces new oxidation products different from the input reactants [13].

Singlet oxygen detection can be performed directly or indirectly. In case of the direct method, the detection is performed in real time, often by fluorescence measurement in the IR range. The disadvantage of this method is its technical complexity and low fluorescence intensity of $^1O_2$. The indirect methods rely on chemical reaction of singlet oxygen with a suitable target. The main advantage of the indirect (chemical) method is the high sensitivity of the used absorption or luminescence probes and the improved selectivity to $^1O_2$ compared to other reactive oxygen species. Commercial probes also have the advantage of their availability and easy detectability of reaction products with singlet oxygen [14]. Probes can be spectroscopic, e. g. 9,10-anthracenediyl-bis (methylene) dimalonic acid ABDMA [15] or 9,10-diphenylanthracene DPA [16], fluorescent such as SOSG (Singlet Oxygen Sensor Green) [17, 18], chemiluminescent such as 2-methyl-6-phenyl-3,7-dihydroimidazo (1,2-α) pyrazin-3-one CLA [19], etc.

Given our previous knowledge about simple alcohols as protectors of cells from the effects of ionizing radiation the aim of this paper is to monitor whether alcohols such as methanol and ethanol can quench singlet oxygen and whether they are involved in protecting cells from the effects of UV-C radiation.

Experimental

Quenching ability of alcohols

The quenching ability of alcohols was evaluated through photoluminescence measurements. Samples of physiological solution with methanol or ethanol (PENTA, Prague, Czechia) of analytical grade purity (concentrations 0; 1; 1.5 and 2 mol L^{-1}), 1×10^{-6} mol L^{-1} photosensitiser Rose

Bengal (RB) and 1.6×10^{-6} mol L^{-1} Singlet Oxygen Sensor Green (SOSG) were irradiated with UV-C radiation (a low-pressure mercury lamp Philips TUV, 11 W, 254 nm) for 0, 10, 30 and 50 min. The rate constant of singlet oxygen with methanol, ethanol, and probe SOSG can be found in Table 1. The photoluminescence emission spectra of resulting endoperoxide (SOSG-EN) were subsequently measured using FluoroMax Plus spectrofluorometer (Horiba Jobin Yvon, Kyoto, Japan) equipped with a 150-W ozone-free xenon arc-lamp, two Czerny-Turner monochromators and a photomultiplier detector; the samples were measured in plastic cuvettes. The SOSG probe was excited with a wavelength of 485 nm and its emission was measured in the range of 510–800 nm (maximum in 526 nm). The measurement was carried out by first irradiating samples containing only the probe SOSG and RB for the selected irradiation times (0, 10, 30 and 50 min). Subsequently, the samples containing SOSG, RB and alcohol were measured. The resulting data shows how the signal (maximum of emission spectra of endoperoxide) changes in the presence of the alcohol (when we consider the SOSG and RB signal to be 100 percent). Error bars are standard deviations (1) of average values for given alcohol concentrations. Standard deviation was calculated according to formula (1):

$$\text{standard deviation} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x - \bar{x}\right)^2},$$

where N is number of measurements, x is measured value (signal intensity of SOSG-EN in percent) and $\bar{x}$ is average of the measured value. For this reason, it can be noticed that for samples with no alcohol (0.0 mol/L) there are zero deviations.

Radiation sensibility of cells

The bacteria *Escherichia coli* (DBM 3125, ICT Prague, Czechia) as a prokaryotic microorganism were used in this study. The cells incubated in a standard way were transferred into the isotonic solutions containing different concentrations of scavenger, so that the cell concentration varied from 10^6 to 10^7 cells per millilitre. Ethanol was used as the protector against the effects of radiation. Its concentrations ranged

Table 1 Rate constants of reaction of singlet oxygen with methanol, ethanol, and probe SOSG

Chemical	Rate constant (L mol^{-1} s^{-1})
Methanol	3.0×10^3
Ethanol	1.7×10^3
Singlet oxygen sensor green	1.2×10^9

from 0.5 to 2.0 mol L^{-1}. The aqueous suspension of cells was irradiated with UV-C source in polypropylene tubes. The suspension was vigorously stirred during the UV irradiation. Under these conditions, it was possible to determine the nominal dose rate in Gy h^{-1} (in terms of fluency rate in W m^{-2}, the following approximate transfer relationship was found: 1 Gy min^{-1} corresponds to 0.255 W m^{-2}). The dose rate of UV-C radiation was measured by iodide–iodate actinometer and ranged from 0 to 170 Gy h^{-1}, while the dose was kept constant at 1 Gy. Immediately after irradiation, the suspension was appropriately diluted (by means of a decimal dilution method) and 0.1 mL of the suspension was plated on the complete nutrient agar (Plate count agar PCA, M 091, Himedia, Mumbai, India). The same procedure was applied to the nonirradiated samples as a control. After cultivation of bacteria for 1–2 days at 37° C, between 200 and 500 colonies were formed per dish. Each cultivation was independently repeated three times and the arithmetic mean was then taken into account. Moreover, to increase the statistical credibility of the results, two complete independent samples without the scavenger were always prepared. The non-irradiated samples containing various amount of the scavengers served as a control of the scavenger toxicity.

The σ quantity defined by the equation $\sigma = \ln S_0 / \ln S_S$ was used for description of the protection where S_0 and S_S are the fractions of surviving cells without and with the protector, respectively. The slopes k_c or k_Q of the linear dependence of the σ value on the concentration of the scavenger or on its scavenging efficiency, respectively, represent the specific protection of the cells. That means that the k_c value represents the sensitivity of protective effect on the change in the concentration of the scavenger and the k_Q value represents the sensitivity of the protective effect on the change in the scavenging rate. The scavenging efficiency Q was used for comparison different radiations (gamma and UV). Q is the product of the rate constant of the reaction of alcohol with given radical and its concentration.

Results and discussion

Rose Bengal was used as a photosensitizer to generate singlet oxygen. Figure 1 shows the emission spectrum of an aqueous system where only the SOSG probe is present and then a combination of a probe and a photosensitizer. It was observed that the luminescence signal at 526 nm, corresponding to the SOSG-endoperoxide (SOSG-EN) produced by reaction of SOSG with singlet oxygen, increased with time of irradiation. The increase of intensity of SOSG-endoperoxide signal with exposure time confirms that singlet oxygen is produced in the irradiated system. Furthermore, the increase of signal intensity of the SOSG itself can be seen with exposure time. This is because the
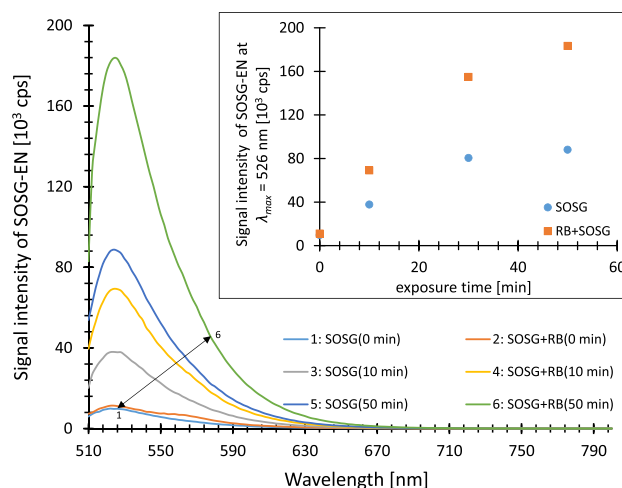

Fig. 1 SOSG-endoperoxide (SOSG-EN) emission spectrum of samples without and with Rose Bengal after different exposure times of UV-C irradiation, excitation wavelength 485 nm; inset: dependence of signal intensity of SOSG-EN at maximum wavelength on exposure time for samples without and with Rose Bengal
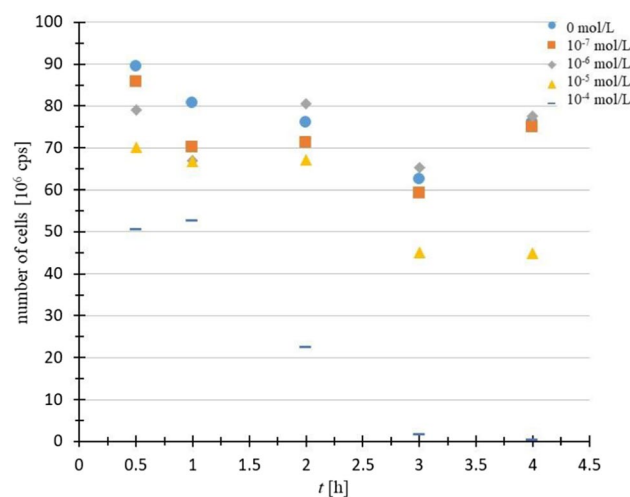

Fig. 2 Toxicity of various concentration of Rose Bengal on *E. coli* bacteria in time

SOSG itself is a photosensitizer. Therefore, in each experiment, it is necessary to irradiate the SOSG itself and monitor its increase in signal intensity over exposure time.

The photosensitizer (RB) concentration was chosen regarding the *E. coli* bacteria. The least toxicity of RB was required so that the results would not be distorted by this fact (Fig. 2). In the figure can be seen that the concentrations 1×10^{-4} and 1×10^{-5} mol L^{-1} are quite toxic for the cells in time, and it is therefore undesirable to use them for the experiments. Therefore, the photosensitizer concentration of 1×10^{-6} mol L^{-1} was chosen for further experiments.

If ethanol is added to the system prior to irradiation, the signal of SOSG-endoperoxide (SOSG-EN) is reduced. Figure 3 shows the decrease in signal in the system containing ethanol compared to the signal in the system without ethanol. The increase of signal intensity in irradiation time 0 min is probably caused by some processes as change in polarity of solution or formation of complex in the system.

Interestingly, if the system contains methanol instead of ethanol (Fig. 4), the signal does not decrease. Chemical or physical quenching is unlikely to occur. This different behaviour of the two alcohols suggests that the character of the modifier significantly affects its ability to react with various forms of ROS. This knowledge could explain previous results about different behaviour of these simple alcohols. However, the protective effect is strongly dependent on the conditions of the experiment and on many other factors, as it is a very complex and unexplored process. In previous work, we did not further analyse the cause leading to this effect. In this work, we focused on one of the possible causes of radiation damage to cells by UV radiation—the effect of singlet oxygen. It was shown that unlike methanol, ethanol can be used as a protective agent against the effect of singlet oxygen. Further systematic research would be necessary to further clarify these facts.

This knowledge was subsequently utilized for cell experiments to ascertain if it is possible to protect the cells from the effects of singlet oxygen by using simple substances such as alcohols. In Fig. 5, the protection of cells increases with increasing concentration of ethanol, when we irradiated them with UV-C radiation (nominal dose rate was 169 Gy h^{-1} and dose 1 Gy) in physiological solution with or without Rose Bengal. Therefore, the specific protections k_c are positive and have the values of 0.17 and 1.77 L mol^{-1} for
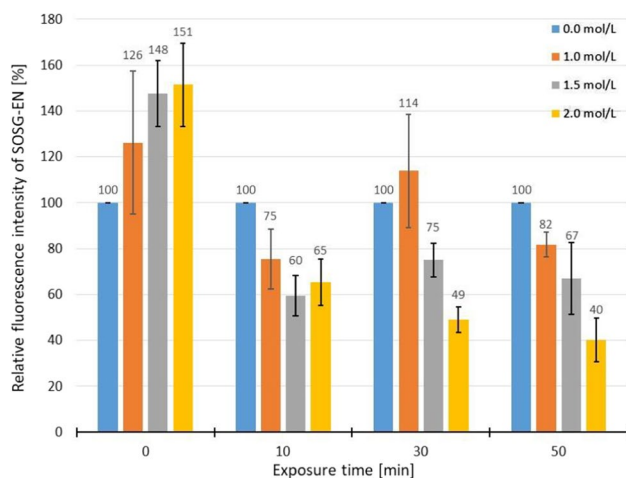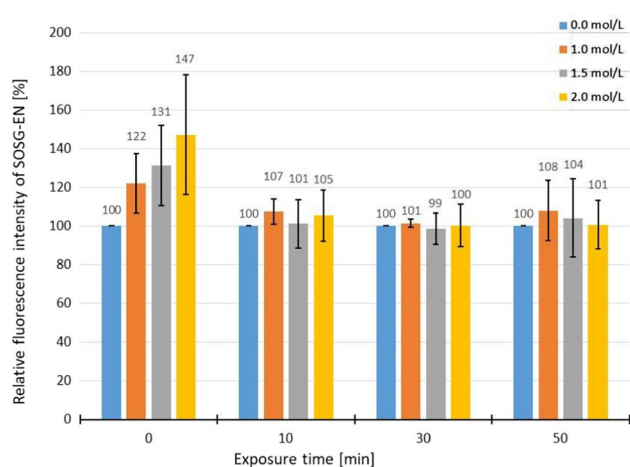

Fig. 4 Percentage quenched probe signal in the system with methanol (average values from 4 repetitions)

system without and with photosensitizer, respectively. This kind of experiment was always repeated at least three times.

From the dependence of the specific radiation protection on the radiation dose rate (Fig. 6), it can be seen that there is a higher specific protection of the cells against the effects of UV-C radiation in the presence of Rose Bengal than in the case without the RB in the dose rate range 0–170 Gy h^{-1}. The regression slope for system with Rose Bengal ($y = 2x + 4.33$) is twice bigger than system without Rose Bengal ($y = 0.13x$). This growing dependence suggests that the higher the dose rate, the greater the sensitivity of the protection σ to changes in the scavenger efficiency. From this it can be deduced that with increasing density of singlet oxygen and hydroxyl radicals respectively (with increasing dose rate) the k_Q value increases in both kinds of radiation (Fig. 6A, B). It is evident from the k_Q values (Fig. 6A,
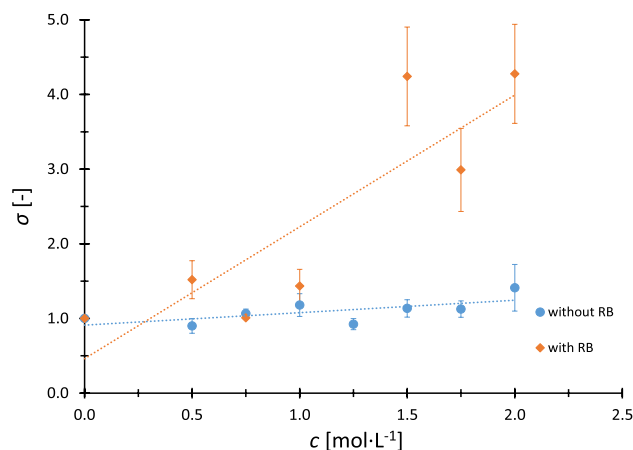

Fig. 3 Relative signal intensity of SOSG-EN in different exposure time in system with ethanol (average values from 2 repetitions)

Fig. 5 Dependence of the protection σ on the concentration of ethanol for *E. coli* bacteria irradiated with UV-C radiation, with or without Rose Bengal in the system
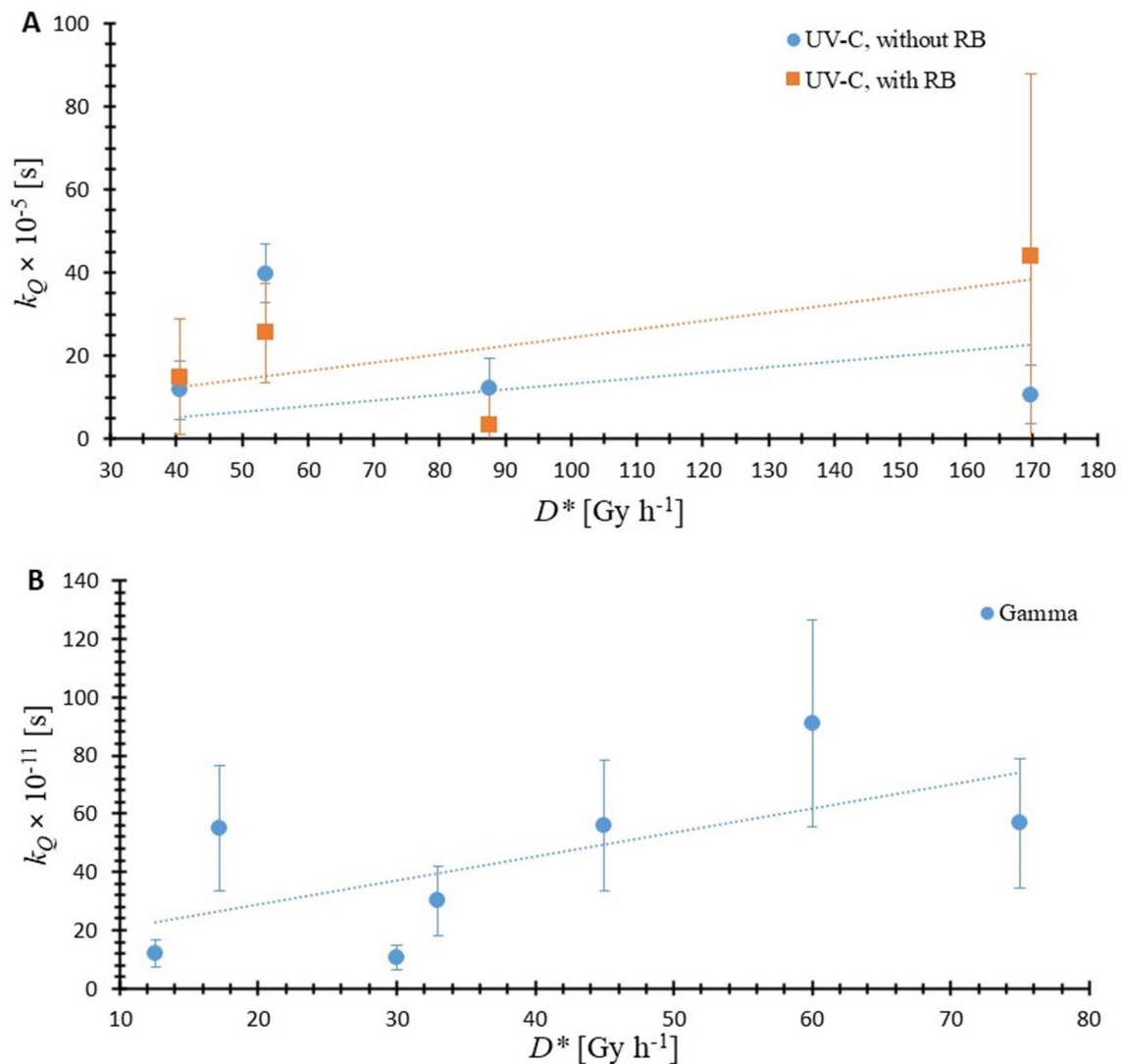
Fig. 6 Dependence of the specific protection k_Q on the dose rate D* of UV-C radiation (**A**) with and without photosensitizer Rose Bengal (RB) compared to gamma radiation [20] (**B**) for bacteria *E. coli* and ethanol as a scavenger

B) that the sensitivity of the protective effect on the reaction rate of the ethanol with singlet oxygen is by six odder higher than with the OH radicals. The values k_Q are slopes of dependences of the protection σ and the scavenging efficiency Q, where is used rate constant of the reaction of ethanol with singlet oxygen in the case of UV radiation (Fig. 6A) and the rate constant of the reaction of ethanol with hydroxyl radicals in the case of gamma radiation (Fig. 6B).

The protection process is a complex process. Apparently, therefore, a simple relationship between the quantitatively expressed protection and the rate constant of a particular reaction cannot be expected in general [5, 6]. Alcohols can react with various other substrates present in the system. In addition, singlet oxygen can be scavenged in this complex system by other reductants than the alcohol of interest. Another factor that has a non-negligible effect on the

resulting protective effect is the superposition of the protective effect of alcohol and its toxic effect. This can also be reflected in the different final effect of the two alcohols. In further research, it will be appropriate to focus on the singlet oxygen yield in our systems, because otherwise this determination is indicative.

Conclusions

Alcohols were shown to slightly protect cells from the effects of UV (254 nm) radiation in comparison with gamma radiation. Ethanol, unlike the methanol, quenches the signal of the fluorescent probe used and thus seems to react with the singlet oxygen in the system. The radiation specific

protection of EtOH is greater in a system when the photo-sensitizer Bengal Rose was used.

The possible interpretation of the observed difference between k_Q for scavenging OH radicals or singlet oxygen is that the extent of protection, depending on the rate of the mentioned reactions, is greater for singlet oxygen than for OH radicals.

Declarations

Conflicts of interest There are no conflicts to declare.

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