Chapter

Chemical Detection of Short-Lived Species Induced in Aqueous Media by Atmospheric Pressure Plasma

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Abstract

Non-thermal atmospheric pressure plasmas are widely used in biomedical research and clinical applications. Such plasmas generate a variety of reactive oxygen and nitrogen species upon interaction with ambient surroundings. These species further interact with a biological substrate and are responsible for the biomedical effects of plasma. Liquid water is an essential part of any biological systems. Some of the most reactive species induced by plasma in aqueous media are radicals and atoms. Hence, the presence of certain chemical components in a plasma 'cocktail' presents an important task for both understanding and further development of plasma systems with specific purposes. In this chapter, we discuss various methods of detection of the plasma-generated short-lived reactive species. We dissert various plasma-induced radicals and atoms ($\bullet OH$, $O_2\bullet^-$/$\bullet OOH$, $\bullet NO$, O), together with non-radical short-lived species (^-OONO, O_3, $^1O_2$). Electron paramagnetic resonance (EPR) is the most direct method of radical detection in water-based media. Special attention is paid to the limitations of the detection methods, with an emphasis on spin trapping used in EPR analysis.

Keywords: plasma-liquid systems, reactive species, free radicals, spin trapping, electron paramagnetic resonance

1. Introduction

Low-temperature, or 'cold' atmospheric pressure plasmas (CAPs) are gaining increasing attention in diverse fundamental and applied scientific activities [1]. The research on the industrial applications of cold plasma includes its use in plasma-assisted catalysis and thin film deposition [1–3], wastewater treatment [4, 5], photoresist removal [6], pre-treatment of polymeric solutions for the production of enhanced nano-fibres [7], etc.

Biomedical applications are the most burgeoning field of CAP research [8]. Cold plasma is used to modify or produce surfaces with high bacterial resistance, an important property in a clinical setting [8, 9]. Other applications include various sterilisation processes [10], deactivation of bacteria and viruses [11, 12], wound healing [8], and the emerging CAPs cancer treatment [8, 13]. The effects of CAPs on biological substrates are largely defined by the reactive oxygen and nitrogen species (RONS) such as •OH, $O_2^{\bullet-}$/•OOH, O_3, $^1O_2$, O, •NO, H_2O_2, $ONOO^-$, NO_2^- [14]. These species are formed either in the plasma itself, or upon its interaction with surrounding air [14, 15]. Water is an essential component of every biological system. Thus, information on both the composition of the mixture of RONS created by plasma, and their interactions with aqueous media is extremely important for tailoring-desired plasma effects [16].

Chemical modelling coupled with various analytical techniques (optical spectroscopy methods, mass spectrometry, etc.) is used to assess the composition of the gas phase plasma [1, 14, 17]. Recent works in computational chemistry have addressed the interaction of gas phase RONS with and within aqueous media [18, 19]. However, monitoring of the reactive species in liquid is paramount for benchmarking of the models. Most importantly, it provides experimental, and hence the most direct information on RONS present in the liquid.

Two main paths are used for CAP utilisation in biomedical research and applications: first, pre-treatment of a relevant medium with further application to the biological target [20, 21] and second, direct application of plasma treatment to cells in aqueous media [22], or 'dry' cells [23] (We note that generally all cells are grown in culture medium and/or washed prior to plasma exposure; thus, the 'dry' cell surface is never devoid of water, even less, so is a tissue in clinical applications [24]). In the first scenario, the effects of plasma are attributed to long-lived chemical species, which can remain in solution after plasma treatment, such as H_2O_2, NO_2^-, and NO_3^- [20, 25]. These long-lived species are usually detected using a variety of analytical techniques, of which colorimetry is commonly employed [20, 22]. The second path implies the presence of short-lived radical and atomic species: O, •NO, •OH, $O_2^{\bullet-}$, as well as non-radical chemical compounds such as, e.g., singlet oxygen $^1O_2$. Aside from creating direct oxidative stress [14], these species can regulate various cellular processes by, e.g., altering cellular uptake of metal ions [26]. These short-lived radicals were also shown to initiate radical reactions in liquid media [27].

This chapter disserts methods of detection, identification and quantification of short-lived chemical species in solutions in contact with CAPs.

2. Detection of plasma-generated RONS in liquids

2.1 Hydroxyl and superoxide radicals

Upon interaction with water and oxygen moieties, CAPs generate hydroxyl radicals •OH and superoxide radical anions $O_2{\bullet}^-$. These short-lived species possess highly oxidising and cytotoxic properties, and are suggested to be one of the main causes of biomedical activity of cold plasma [14, 28].

In aqueous systems, these radicals are often detected using optical methods. These methods usually employ induction of colour or degradation of dyes (colorimetry) [29]. In this method, a coloured dye is degraded by plasma-generated species, and the loss of colour is quantified using UV-vis spectrophotometry. Some of the most commonly used dyes used to assess ROS are methylene blue and methylene red [29, 30], often used in research associated with CAPs for water treatment and pollutant removal [30, 31]. However, the main difficulty in employing this method is the non-specific degradation of such dyes. For example, decolouration of methylene blue not only occurs via reaction with •OH radicals but also with other CAP-induced species, e.g., ozone [32]. Superoxide radical anions can also be detected by degradation of dyes [33] with the same limitations.

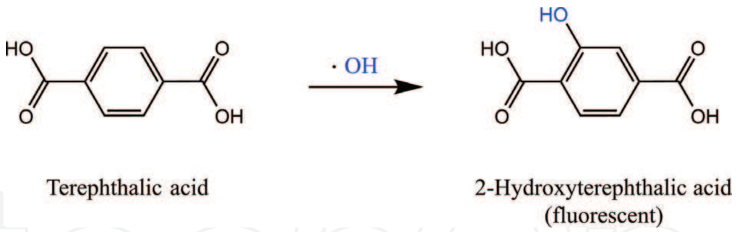
Similarly, induction/decay of fluorescent properties of chemical molecules is also used. Degradation of some fluorophores was used to detect oxygen-centred radicals on surfaces in contact with processing plasmas [34].

Terephthalic acid (TA) has been reported to detect •OH radicals in plasma-liquid systems [35–37]. The method is based on the induced fluorescence due to the formation of hydroxy-substituted TA (**Scheme 1**). This presents a simpler and more selective method of detection of the hydroxyl radicals, as demonstrated by Attri and co-workers [37]. However, possible oxidation of terephthalic acid by RONS [38] is usually ignored. Another method based on aromatic hydroxylation was recently suggested by Zhang et al. Using salicylic acid as a substrate, the authors obtained mono- and disubstituted products, which were analysed by HPLC [39].

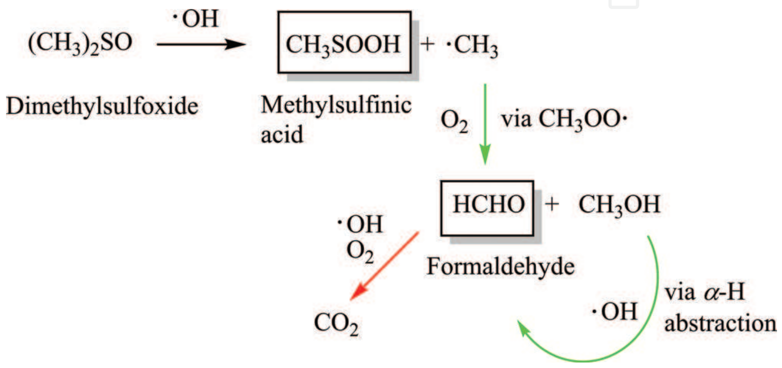
Other alternatives include different chemical probes, such as, e.g., dimethylsulphoxide, followed by quantification of the formed formaldehyde HCHO or methanesulfinic acid [35, 40] (**Scheme 2**). However, other reactions leading to both production and degradation of HCHO can occur in a plasma-liquid system, as was shown by Ma et al. [40]. The kinetics of these complex chemical processes needs to be considered for quantitative assessment of the hydroxyl radical in liquids.

Shirai et al. observed chemiluminescence at the plasma-liquid interface when alkaline solutions of luminol were exposed to CAP, presumably due to reactions with •OH and O_2•$^-$ [41]. Bekeschus et al. compared the amount of superoxide produced by different plasmas in liquid using colorimetric analysis with cytochrome C [42].

Furthermore, the information on the availability of radical species in liquids exposed to CAPs is often obtained using electron paramagnetic resonance (EPR) spectroscopy. EPR is the most direct method of radical detection in liquids [43, 44]. The method is based on the detection of paramagnetic species, e.g., free radicals with an unpaired electron. In EPR, several methods of radical detection are used [45]. Free radicals such as hydroxyl and superoxide are very short-lived and cannot be detected directly. Hence, spin probes and spin traps are employed. An example



Scheme 1.
Detection of the OH radical using induced fluorescence via the reaction with terephthalic acid.



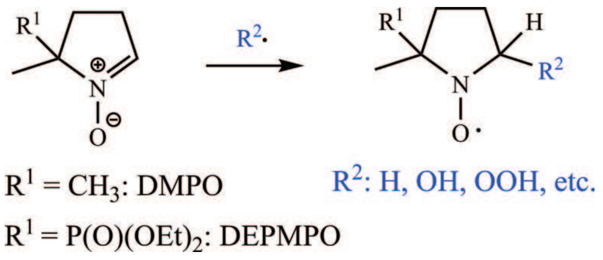
Scheme 2.
Reactions of dimethylsulphoxide used in detection of the •OH radical. Production and loss pathways of HCHO are indicated with green and red arrows, respectively.

of a spin probe is 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH). CMH is a cyclic hydroxylamine, which reacts with superoxide radicals to form a nitroxide radical detected by EPR: =N—OH → =N—O• [46]. However, this method is mostly used in biological systems rather than plasma-liquid systems. The selectivity is not explicitly known, and possible difficulties may arise from interferences by other plasma-induced ROS.

The second method is the formation of radical adducts in reactions of spin traps (organic molecules, usually nitrones) with free radicals (**Scheme 3**). The formed spin adducts are organic nitroxides with longer half-lives compared to the analysed radicals, and thus detectable by EPR [47, 48]. In the past decade, the spin trapping of CAP-induced radicals in liquids has gained vast attention. Numerous groups have performed detection of •OH and $O_2^{\bullet -}$/•OOH radicals in aqueous media by EPR. Tani et al., Takamatsu et al. and Uchiyama et al. performed the detection of radical species in plasma-treated liquids by using various spin traps [49–51]. Tresp et al. assessed the concentrations of the •OH and $O_2^{\bullet -}$ radicals by monitoring the radicals adducts of 5-tert-butoxycarbonyl-5-methyl-1-pyrroline *N*-oxide (BMPO) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) spin traps in liquid samples [52]. We have previously performed detection of hydroxyl and superoxide radicals, as well as hydrogen atoms, in CAP-treated water with DMPO, 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (DEPMPO) and *N*-benzylidene-*tert*-butylamine *N*-oxide (PBN) spin traps [15, 22, 44]. We note that according to their pKa values, superoxide radical exists in its anion form $O_2^{\bullet -}$ in a physiological solution (pH 7–7.6), but the radical adducts are protonated forms —OOH [53].

The advantage of spin trapping over other methods is that the non-selectivity of a spin trap is not a drawback. Indeed, the characteristic features of the EPR signals are different for different radical adducts due to the hyperfine coupling: interaction with magnetic moments of nearby nuclei with non-zero spin numbers (^{14}N, ^{1}H, ^{13}C, ^{17}O, etc.). The hyperfine values of an adduct therefore depend on the structure of a specific adduct: DMPO-OH and DEPMPO-OH have different chemical structures and thus different features of their EPR spectra (see, e.g., [15, 44]). Also, if a spin trap (e.g., DEPMPO) forms adducts with several radicals such as •OH and $O_2^{\bullet -}$, the amounts of both adducts can be obtained from the same EPR spectrum [45]. This feature enables studying the source of the radicals produced by CAPs by using isotopically labelled water ($H_2{}^{17}O$, $^{2}H_2O$) to distinguish between the radicals formed from the gas phase water and the liquid water [15, 44, 54]. More recently, the use of PBN and DMPO spin traps helped identify the nature of the radicals generated by CAPs from organic solvents (chloroform and *N,N*-dimethylformamide) [7].

However, despite being a very versatile and the most direct method of radical detection in liquids, spin trapping and EPR analysis have limitations, which need to be considered in experimental settings. Before proceeding to the detection of other short-lived species, we first address in the following section the factors limiting the applicability of spin trapping and EPR analysis in CAP-liquid systems.



Scheme 3.
Formation of nitroxide radical adducts in reactions with CAP-induced radicals by DMPO and DEPMPO spin traps.

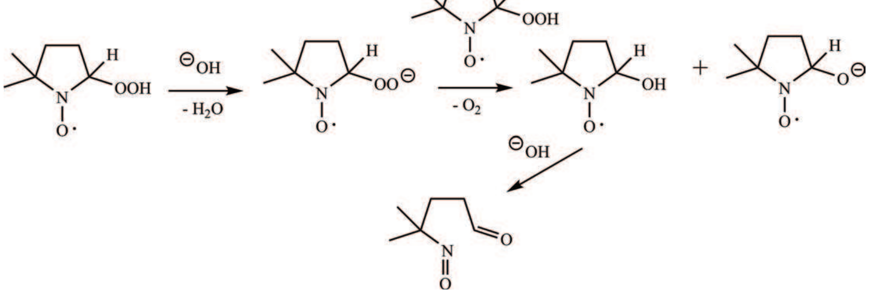
2.2 Limitations of spin trapping

Quantification of the radical adduct (i.e., concentration in the analysed solution) can be achieved by calibrating an EPR spectrometer with solutions of a stable radical compound, e.g., a stable nitroxide (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) or its derivatives [15, 22, 44]. However, obtaining concentration values of radicals (rather than the formed adduct) in liquids is hindered due to various side reactions of the analysed radicals: recombination (e.g., •OH into H_2O_2), reactions with scavengers (solvated electrons, hydrogen atoms, possible physiological media components [22, 37, 42, 48]). Comparison of reaction rate coefficients of these processes needs to be performed. Otherwise, EPR with spin trapping provides only semi-quantitative data: the amounts of a radical adduct formed under different conditions follow the same trend as the initial concentration of the free radical.

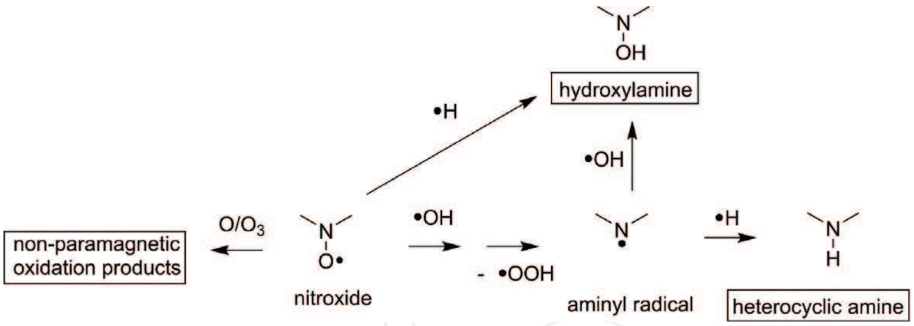
Different spin traps have different affinity towards different radicals expressed by the rate coefficients of the respective reactions [44, 45]. Moreover, the stability of the formed radical adducts varies in orders of magnitude. Under physiological conditions (pH, temperature, etc.), the half-life of the radical adduct with superoxide radical of the DMPO spin trap is 45 s, with DEPMPO, it is 14 min, and with 3,5-dimethyl-5-(iso-propoxycarbonyl)-1-pyrroline *N*-oxide (3,5-DIPPO), it is 55 min [55]. The decay of spin adducts occurs naturally in liquids and depends on many factors: temperature, concentration of the adduct and other components of the solutions, etc. The half-life of the DMPO-OOH radical adduct was shown to be a function of pH by Buettner et al. [56]. The rapid decay of DMPO-OOH proceeds via formation of DMPO-OH, and in the presence of electron acceptors, it can lead to a complete disappearance of the EPR signal due to the loss of the radical moiety (**Scheme 4**). It was also reported that the stability of DMPO-OH was substantially reduced in the presence of nitrogen oxides [57].

Hence, the choice of a spin trap is a very important factor, which may affect both the quantitative and the qualitative results. Selectivity, adduct stability and last but not least commercial availability of a spin trap should be taken into account when preparing for the analysis of radical species in liquids.

The limitations discussed above are known in biological systems, with limited production and diversity of RONS. With CAPs, various atomic and radical reactive species can be simultaneously delivered to the liquid. Our previous work showed that nitroxides can decay via reactions with the same species from which they were formed [58]. Our results demonstrated that the loss of nitroxide moiety in plasma-exposed water occurred via reactions with •OH radicals, H atoms and oxygen species (atomic oxygen and/or ozone) with pseudo-first-order kinetics (**Scheme 5**). Hence, to perform even relative measurements of RONS induced in water by CAPs, a study



Scheme 4.
pH-dependent degradation pathway of the radical adducts of the DMPO spin trap.



Scheme 5.
Degradation of nitroxides (radical adducts) via reactions with CAP-induced species.

of the product (spin adduct, aromatic substitution product, etc.) concentration development over time may be necessary to exclude possibilities of the increasing analyte degradation.

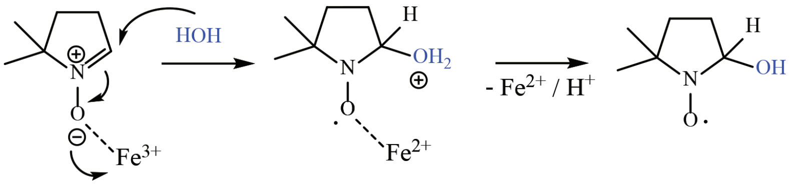
Another factor affecting the analysis is the potential decay of the nitrone spin traps themselves. Upon plasma exposure, it was shown that DEPMPO spin trap was partially degraded, yielding carbon-centred radicals, which were trapped by the remaining DEPMPO [42]. Similarly, PBN can undergo degradation into *tert*-butyl hydronitroxide [59].

A number of 'false-positive' results have been identified for spin trapping. For instance, a nucleophilic addition via the Forrester-Hepburn mechanism, either direct [60] or metal-catalysed [61] (**Scheme 6**), may lead to the formation of nitroxides with the same structure as the radical adducts. It is thus important to perform control experiments with no CAP-induced RONS, to assess the possible interference from such reactions.

2.3 Solvated electrons

Many types of plasma set-ups either have discharges to the surface of the liquid (e.g., floating electrode plasmas with no gas flow), or an electron-rich afterglow (most plasma jets, with the exception of COST jet-type set-ups) [15, 16, 23]. In such cases, not just the RONS, but the electrons too may interact with the CAP-exposed liquid [16, 62]. Solvated electrons contribute to the additional charge in the liquid and induce electrochemical reactions [63], affecting potential substrates.

Rumbach et al. have demonstrated that both electron transfer and neutral reactions occur when CAPs interact with aqueous media. The authors report an optical technique employing a series of individual diode lasers for the spectroscopic detection of solvated electrons [63, 64]. Another (chemical) approach describes the analysis of the pH of the saline solutions in an electrochemical system where a cathode is substituted by a plasma jet. In such system, the pH changes as a result of the chlor-alkali process initiated by plasma electrons [65]. To the best of our knowledge, no other works describe chemical detection of solvated electrons in plasma-liquid systems.



Scheme 6.
Metal-catalysed Forrester-Hepburn mechanism of nucleophilic addition of a water molecule to the DMPO nitrone spin trap, leading to the formation of the nitroxide with the DMPO-OH structure.

Several reviews and reports describe possibilities of chemical detection of solvated electrons [66, 67]. The methods include indirect detection, e.g., EPR analysis of the DMPO-spin trapped benzyl radicals, which were formed upon reaction of benzyl chloride with solvated electrons [68]. These methods could prove very useful in plasma-liquid systems, although their direct applicability (limitations due to selectivity, etc.) needs to be determined.

2.4 Atomic oxygen, singlet oxygen and ozone

Some of the most reactive and biologically relevant species created by CAPs are atomic oxygen O, ozone O_3 and singlet oxygen $^1O_2$ [14, 69]. It has been shown by Benedikt and co-workers that oxygen atoms are delivered to exposed liquid solutions from the gas phase plasma, where they are generated [70, 71], while computational results indicate that these extremely reactive atomic species can also be formed inside the liquid from hydroxyl radicals [18]. Singlet oxygen and ozone are generally considered short-lived (compared to e.g. hydrogen peroxide), although they are more stable than the atomic or radical species.

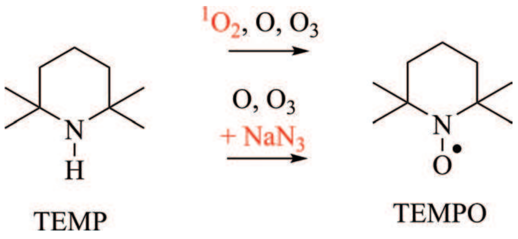
Ozone is often detected colorimetrically using the degradation of coloured dyes, e.g., methylene blue. This method of ozone detection is highly non-selective, since the dyes can be degraded by other CAP-produced RONS, including •OH radicals [32]. Kovačević et al. measured ozone delivery to the plasma-exposed liquid using iodometry, while the solubilised ozone remaining in the liquid after the CAP treatment was detected with decolourisation of indigo trisulphonate [72]. However, recently, Tarabová et al. reported that indigo dyes decay in reactions with other RONS, e.g., secondary •OH radicals produced in liquids after the plasma exposure [73]. The non-selectivity of the iodometric method is due to the other oxidising RONS [72]. Fluorescent probes have also been used to detect ozone in plasma-liquid systems, albeit not without selectivity issues [74].

Benedikt and co-workers detected oxygen atoms in aqueous solutions of phenol, with further MS analysis of the formed hydroxylated products [70, 71]. However, hydroxylation of phenol can also occur in a reaction with hydroxyl radicals [73, 75].

EPR detection of a combination of CAP-induced O, $^1O_2$ and O_3 in aqueous media was performed by Takamatsu et al. The authors used 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide (TPC) as a chemical detector of oxygenated species in liquid [50]. TPC is oxidised to produce a stable nitroxide, which can be detected by EPR. While the oxidation of TPC was non-selective, the addition of sodium azide NaN_3 as a singlet oxygen scavenger allowed to distinguish between the TPC oxidised by $^1O_2$ and by all other species.

We have previously used 2,2,6,6-tetramethylpiperidine (TEMP), another cyclic amine, to detect $O/^1O_2/O_3$ produced by an atmospheric pressure plasma via its oxidation to 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) [22, 44]. Adding NaN_3 let us individually assess the concentrations of $^1O_2$ and O/O_3 induced by CAPs (**Scheme 7**). Our work showed that although TEMPO was not produced in reactions with H_2O_2 or $O_2•^-$, it could be formed by other plasma-induced RONS: ozone and possibly by atomic oxygen [44].

Later, Elg et al. demonstrated that most TEMPO was in fact formed by atomic oxygen by comparing ozone densities in the gas phase with concentrations of the formed TEMPO [76]. However, the main contributor to the production of a stable nitroxide in these reactions would depend on the densities of O and O_3 (and therefore, a specific plasma set-up) in each case. Other limitations of this method are related to the loss reactions of nitroxides [58], as described above (see **Scheme 5**).



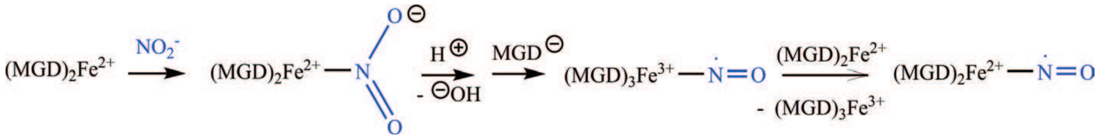
Scheme 7.
Detection of singlet oxygen, atomic oxygen and ozone by oxidation of TEMP. NaN_3 is used as a specific scavenger for $^1O_2$.

2.5 Nitrogen oxides and peroxynitrite

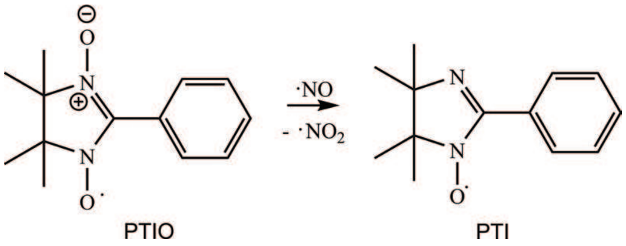
Nitric oxide •NO is considered one of the CAP-induced RONS responsible for bactericidal effects and wound healing. Furthermore, its reactions with and within aqueous media produce a large variety of secondary RONS [77]. Unlike the more persistent products of the •NO transformations such as NO_2^- and NO_3^-, which are detected colorimetrically [20, 48, 72, 73], the radical nitric oxide itself is monitored using spin trapping and EPR. Many EPR methods of detection of nitric oxide are known in biological milieu. Among these are the use of dithiocarbamate metal complexes, oxidation of nitronyl nitroxides, etc. [78].

In plasma-liquid systems, •NO has been detected intracellularly using fluorescent probes [51, 79] and directly in media by EPR with iron complexes of *N*-methyl-D-glucamine dithiocarbamate (MGD) [50]. In the latter reaction, chelated Fe^{2+} ions form paramagnetic complexes with •NO. However, it was shown by Tsuchiya et al. [80] that this reaction is not selective: $(MGD)_2Fe^{2+}$ complex reacts with the nitrite anion NO_2^- with the oxidation of Fe^{2+} to Fe^{3+}, eventually leading to the formation of the $(MGD)_2Fe^{2+}$ adduct with •NO (**Scheme 8**). Another limitation is the possible oxidation of the iron ion to Fe^{3+} (which forms a non-paramagnetic complex with •NO) by other plasma RONS, and thus the necessity to use large amounts of a reducing agent [58].

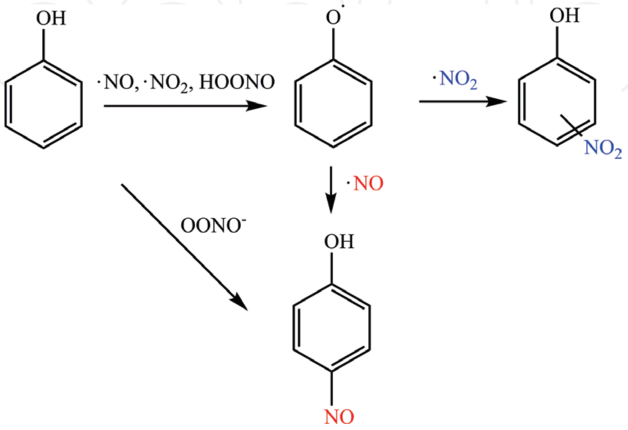
Another method to detect nitric oxide in CAP-liquid systems is the transformation of nitronyl nitroxides such as 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) or its derivatives [48, 51, 58]. PTIO, a stable nitroxide radical, reacts with nitric oxide to form 2-phenyl-4,4,5,5-tetramethylimidazoline 1-oxyl (PTI), an imino nitroxide radical (**Scheme 9**). EPR analysis with deconvolution of the spectra via radical signal simulations allows differentiating between the two radicals [48, 78]. We previously showed that the limitations of the method are related to the nitroxide decay pathways (see above). The other issue is the reverse transformation (oxidation) of PTI to PTIO in oxygen-containing plasmas, even in the absence of nitrogen [58]. This makes detection of nitric oxide in plasma-liquid systems an extremely difficult task, when both the absence of the detectable •NO (PTIO) and its presence (a 'false positive' with dithiocarbamates) can be due to the limitations of each method.



Scheme 8.
Reaction pathway of the $(MGD)_2Fe^{2+}$ complex interaction with the NO_2^- anion to yield a paramagnetic adduct $(MGD)_2Fe^{2+}$-NO.



Scheme 9.
Reaction of nitronyl nitroxide PTIO with nitric oxide, yielding imino nitroxide PTI.



Scheme 10.
Reactions of nitration and nitrosation of phenol by nitric oxide, nitrogen dioxide and peroxynitrite.

Lukes et al. used nitrosation and nitration reactions of phenol as a technique to detect post-plasma discharge formation of nitrogen oxides (•NO and •NO_2) in aqueous media [75] (**Scheme 10**). It can be used to detect these two radicals directly during plasma exposure, or the secondary radicals formed during the decay of peroxynitrite (see below). As far as we know, no other methods of •NO_2 detection have been reported specifically in plasma-treated solution, although other ways of detecting nitrogen dioxide in aqueous media are available in literature: e.g., spin trapping with nitrones [81] or nitroalkanes [82].

The peroxynitrite anion $ONOO^-$ is another reactive species induced by CAPs in water-based media [14, 72, 83]. Lukes et al. were the first to evaluate its formation and stability in aqueous solutions exposed to plasma. Peroxynitrite decayed rapidly in acidic conditions via peroxynitrous acid decomposition into •OH and •NO_2, which were detected via phenol derivatisation [75]. The instability of peroxynitrite in neutral pH was later demonstrated by Weltmann and co-workers [84, 85]. Here, peroxynitrite was detected by exposing solutions of L-tyrosine to CAP and further MS analysis of the formed 3-nitrotyrosine. The authors have also assessed the interferences from the •NO radicals by introducing an •NO donor [85]. When L-tyrosine was added to plasma-treated solutions after the exposure, no nitration product was detected, suggesting the short-lived nature of peroxynitrite under the applied conditions. Girard et al. developed a method of direct spectrophotometrical detection of peroxynitrite based on its absorption properties in the UV region [86]. Here, peroxynitrite was only detected in highly basic solutions, confirming its short life at neutral pH. Xu et al. used 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine (a hydroxylamine) to detect both superoxide radical anion and peroxynitrite anion via EPR analysis of the formed 4-oxo-2,2,6,6-tetramethylpiperidine 1-oxyl [87] (distinguishing between the two is not possible with this method [46, 87]).

2.6 Hypochlorite formed in physiological media

Both the physiological media used in research in vitro (PBS, DMEM, etc.) and the blood and tissues in vivo have high concentration of chloride salts. Chloride anions can act as scavengers of the CAPs-generated RONS in media, e.g., the •OH radicals [28, 88]. It has been suggested by different research groups that chlorinated species such as hypochlorite ClO^- (short-lived under physiological conditions [84]) can be formed in solutions upon interaction with the plasma RONS [79, 85, 89]. Wende et al. have assessed the effects of the potential ClO^- presence in situ on the overall biomedical efficacy of CAPs [85]. Recently, Piskarev et al. showed that the ClO^- anion is formed in plasma-treated saline solutions via reactions of the Cl^- anion with superoxide or hydroxyl radicals from plasma [90].

The detection of hypochlorite in solutions was performed by direct UV detection [90] or by exposure of a solution of L-tyrosine to CAPs [84, 85]. The latter method is based on the MS detection of the chlorinated product of L-tyrosine. The UV detection requires large concentrations of hypochlorite, while the MS method is highly sensitive with a low detection limit. However, Bekeshus et al. detected no hypochlorite formation with their plasma set-up [84]. The formation of hypochlorite (formed from the initial CAP-generated RONS in liquid media), therefore, depends on the particular plasma set-up and application conditions.

3. Conclusion and perspectives

A plethora of methods are available for the detection of reactive oxygen and nitrogen species induced in liquids by plasma. Chemical 'detector' systems based on colorimetry, fluorescence, (LC-)MS analysis of the products and EPR spin trapping are some of the techniques used in the detection of atomic, radical, molecular and ionic short-lived RONS. Each method has its potential and limitations; the latter associated with the decay of the products, low selectivity and other factors.

Despite several limitations, spin trapping coupled with EPR analysis as an analytical method of radical detection in CAPs systems has a very high value as the most direct method of radical detection in liquids. Aside from the liquid media itself, it has a potential to be used in plasma-gel systems, which mimic interaction of CAPs with tissue [91], if gels are formed from nitrone molecules [92]. The availability of EPR equipment is not necessarily crucial to perform spin trapping of RONS. An interesting alternative to EPR analysis of radical adducts was demonstrated by Guo et al. and Tuccio et al., who used liquid chromatography and mass spectrometry systems to analyse the adducts of oxygen-centred radicals of the DMPO and DEPMPO spin traps [93, 94].

The emerging role of the chlorinated species in CAP-treated media is gaining attention. ClO^- anion, a biomedically relevant species, has been monitored using UV absorption spectroscopy and modification of tyrosine. However, other detection methods may need to be used, especially in cases when hypochlorite is not detected. For example, colorimetric analysis on the oxidation of 3,3′,5,5′-tetramethylbenzidine is a simple technique [95], albeit with yet unknown limitations due to the presence of other oxidising RONS. Moreover, the highly oxidative nature of the hypochlorite anion in plasma-treated physiological solutions is often emphasised, but the possibility of formation of other anions such as ClO_2^-, ClO_3^- and ClO_4^- has not been addressed. Since these species are cytotoxic, monitoring them in physiological media exposed to plasma can provide valuable information on the biomedical effects of CAPs. Possible analyses can include ion chromatography [96, 97].

Finally, some other short-lived yet highly reactive species are overlooked in the current research. Among these is the carbonate radical anion CO_3•.: a very potent oxidising agent causing DNA damage [98], which can be formed in a reaction of peroxynitrite with ambient CO_2 [99, 100]. The determination of this and other reactive species will aid in completing the picture of the plasma-produced 'cocktail' of reactive species. It can facilitate both the understanding of the existing CAP devices and their effects, and the development of new plasma systems with dedicated RONS concentrations for specific applications.

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Conflict of interest

The authors have no conflict of interest to declare.

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References

[1] Adamovich I, Baalrud SD, Bogaerts A, Bruggeman PJ, Cappelli M, Colombo V, Czarnetzki U, Ebert U, Eden JG, Favia P, Graves DB, Hamaguchi S, Hieftje G, Hori M, Kaganovich ID, Kortshagen U, Kushner MJ, Mason NJ, Mazouffre S, Mededovic Thagard S, Metelmann HR, Mizuno A, Moreau E, Murphy AB, Niemira BA, Oehrlein GS, Petrovic ZL, Pitchford LC, Pu YK, Rauf S, Sakai O, Samukawa S, Starikovskaia S, Tennyson J, Terashima K, Turner MM, Van De Sanden MCM, Vardelle A. The 2017 plasma roadmap: Low temperature plasma science and technology. Journal of Physics D: Applied Physics. 2017;**50**:323001. DOI: 10.1088/1361-6463/aa76f5

[2] Bogaerts A, Neyts EC. Plasma technology: An emerging Technology for Energy Storage. ACS Energy Letters. 2018;**3**:1013-1027. DOI: 10.1021/acsenergylett.8b00184

[3] Kakiuchi H. Atmospheric-pressure low-temperature plasma processes for thin film deposition. Journal of Vacuum Science & Technology, A: Vacuum, Surfaces, and Films. 2014;**32**:030801. DOI: 10.1116/1.4828369

[4] Vanraes P, Nikiforov AY, Leys C. Electrical discharge in water treatment technology for micropollutant decomposition. In: Mieno T, editor. Plasma Science and Technology. London: IntechOpen; 2016. pp. 429-478. DOI: 10.5772/61830

[5] Vanraes P, Wardenier N, Surmont P, Lynen F, Nikiforov A, Van Hulle SWH, Leys C, Bogaerts A. Removal of alachlor, diuron and isoproturon in water in a falling film dielectric barrier discharge (DBD) reactor combined with adsorption on activated carbon textile: Reaction mechanisms and oxidation by-products. Journal of Hazardous Materials. 2018;**354**:180-190. DOI: 10.1016/j.jhazmat.2018.05.007

[6] West A, van der Schans M, Xu C, Cooke M, Wagenaars E. Plasma Sources Science and Technology. 2016;**25**:02LT01. DOI: 10.1088/0963-0252/25/2/02LT01

[7] Rezaei F, Gorbanev Y, Chys M, Nikiforov A, Van Hulle SWH, Cos P, Bogaerts A, De Geyter N. Investigation of plasma-induced chemistry in organic solutions for enhanced electrospun PLA nanofibers. Plasma Processes and Polymers. 2018;**15**:e1700226. DOI: 10.1002/ppap.201700226

[8] Weltmann K-D, von Woedtke T. Plasma medicine—Current state of research and medical application. Plasma Physics and Controlled Fusion. 2017;**59**:014031. DOI: 10.1088/0741-3335/59/1/014031

[9] Bazaka K, Jacob MV, Chrzanowski W, Ostrikov K. Anti-bacterial surfaces: Natural agents, mechanisms of action, and plasma surface modification. RSC Advances. 2015;**5**:48739-48759. DOI: 10.1039/C4RA17244B

[10] Shintani H, Sakudo A, editors. Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives. Vol. 156. Poole: Caister Academic Press; 2016. DOI: 10.21775/9781910190258

[11] Zhang Z, Xu Z, Cheng C, Wei J, Lan Y, Ni G, Sun Q, Qian S, Zhang H, Xia W, Shen J, Meng Y, Chu PK. Bactericidal effects of plasma induced reactive species in dielectric barrier gas–liquid discharge. Plasma Chemistry and Plasma Processing. 2017;**37**:415-431. DOI: 10.1007/s11090-017-9784-z

[12] Aboubakr HA, Gangal U, Youssef MM, Goyal SM, Bruggeman PJ. Inactivation of virus in solution by cold atmospheric pressure plasma: Identification of chemical inactivation pathways. Journal of Physics D:

Applied Physics. 2016;**49**:204001. DOI: 10.1088/0022-3727/49/20/204001

[13] Yan D, Sherman JH, Keidar M. Cold atmospheric plasma, a novel promising anti-cancer treatment modality. Oncotarget. 2017;**8**:15977-15995. DOI: 10.18632/oncotarget.13304

[14] Lu X, Naidis GV, Laroussi M, Reuter S, Graves DB, Ostrikov K. Reactive species in non-equilibrium atmospheric-pressure plasmas: Generation, transport, and biological effects. Physics Reports. 2016;**630**:1-84. DOI: 10.1016/j.physrep.2016.03.003

[15] Gorbanev Y, Verlackt CCW, Tinck S, Tuenter E, Foubert K, Cos P, Bogaerts A. Combining experimental and modelling approaches to study the sources of reactive species induced in water by the COST RF plasma jet. Physical Chemistry Chemical Physics. 2018;**2018**(20):2797-2808. DOI: 10.1039/C7CP07616A

[16] Bruggeman PJ, Kushner MJ, Locke BR, Gardeniers JGE, Graham WG, Graves DB, Hofman-Caris RCHM, Maric D, Reid JP, Ceriani E, Fernandez Rivas D, Foster JE, Garrick SC, Gorbanev Y, Hamaguchi S, Iza F, Jablonowski H, Klimova E, Kolb J, Krcma F, Lukes P, Machala Z, Marinov I, Mariotti D, Mededovic Thagard S, Minakata D, Neyts E, Pawlat J, Petrovic ZL, Pflieger R, Reuter S, Schram DC, Schröter S, Shiraiwa M, Tarabova B, Tsai PA, Verlet JRR, von Woedtke T, Wilson KR, Yasui K, Zvereva G. Plasma-liquid interactions: A review and roadmap. Plasma Sources Science and Technology. 2016. DOI: 25:053002. DOI: 10.1088/0963-0252/25/5/053002

[17] Alves LL, Bogaerts A, Guerra V, Turner MM. Foundations of modelling of nonequilibrium low-temperature plasmas. Plasma Sources Science and Technology. 2018;**27**:023002. DOI: 10.1088/1361-6595/aaa86d

[18] Verlackt CCW, Neyts EC, Bogaerts A. Atomic scale behavior of oxygen-based radicals in water. Journal of Physics D: Applied Physics. 2017;**50**:11LT01. DOI: 10.1088/1361-6463/aa5c60

[19] Verlackt CCW, Van Boxem W, Bogaerts A. Transport and accumulation of plasma generated species in aqueous solution. Physical Chemistry Chemical Physics. 2018;**20**:6845-6859. DOI: 10.1039/c7cp07593f

[20] Van Boxem W, Van der Paal J, Gorbanev Y, Vanuytsel S, Smits E, Dewilde S, Bogaerts A. Anti-cancer capacity of plasma-treated PBS: Effect of chemical composition on cancer cell cytotoxicity. Scientific Reports. 2017;**7**:16478. DOI: 10.1038/s41598-017-16758-8

[21] Yan D, Nourmohammadi N, Bian K, Murad F, Sherman JH, Keidar M. Stabilizing the cold plasma-stimulated medium by regulating medium's composition. Scientific Reports. 2016;**6**:26016. DOI: 10.1038/srep26016

[22] Privat-Maldonado A, Gorbanev Y, O'Connell D, Vann R, Chechik V, van der Woude MW. Non-target biomolecules Alter macromolecular changes induced by bactericidal low-temperature plasma. IEEE Transactions on Radiation and Plasma Medical Sciences. 2018;**2**:121-128. DOI: 10.1109/TRPMS.2017.2761405

[23] Lin A, Truong B, Patel S, Kaushik N, Choi EH, Fridman G, Fridman A, Miller V. Nanosecond-pulsed DBD plasma-generated reactive oxygen species trigger immunogenic cell death in A549 lung carcinoma cells through intracellular oxidative stress. International Journal of Molecular Sciences. 2017;**18**:966. DOI: 10.3390/ijms18050966

[24] Norberg SA, Tian W, Johnsen E, Kushner MJ. Atmospheric pressure

plasma jets interacting with liquid covered tissue: Touching and not-touching the liquid. Journal of Physics D: Applied Physics. 2014;**47**:475203. DOI: 10.1088/0022-3727/47/47/475203

[25] Laroussi M. Plasma medicine: A brief introduction. Plasma. 2018;**1**:5. DOI: 10.3390/plasma1010005

[26] Sasaki S, Kanzaki M, Kaneko T. Calcium influx through TRP channels induced by short-lived reactive species in plasma-irradiated solution. Scientific Reports. 2016;**6**:25728. DOI: 10.1038/srep25728

[27] Gorbanev Y, Leifert D, Studer A, O'Connell D, Chechik V. Initiating radical reactions with non-thermal plasmas. Chemical Communications. 2017;**53**:3685-3688. DOI: 10.1039/C7CC01157A

[28] Xu D, Liu D, Wang B, Chen C, Chen Z, Li D, Yang Y, Chen H, Kong MG. In situ OH generation from O_2^- and H_2O_2 plays a critical role in plasma-induced cell death. PLoS One. 2015;**10**:e0128205. DOI: 10.1371/journal.pone.0128205

[29] Takemura Y, Yamaguchi N, Hara T. Decomposition of methylene blue by using an atmospheric plasma jet with Ar, N_2, O_2, or air. Japanese Journal of Applied Physics. 2013;**52**:056102. DOI: 10.7567/JJAP.52.056102

[30] Attri P, Yusupov M, Park LLP, Koduru JR, Shiratani M, Choi EH, Bogaerts A. Mechanism and comparison of needle-type non-thermal direct and indirect atmospheric pressure plasma jets on the degradation of dyes. Scientific Reports. 2016;**6**:34419. DOI: 10.1038/srep34419

[31] Garcia MC, Mora M, Esquivel D, Foster JE, Rodero A, Jimenez-Sanchidrian C, Romero-Salguero FJ. Microwave atmospheric pressure plasma jets for wastewater treatment: Degradation of methylene blue as a model dye. Chemosphere. 2017;**180**:239-246. DOI: 10.1016/j.chemosphere.2017.03.126

[32] Zhao YY, Wang T, MacGregor SJ, Wilson MP, Given MJ, Timoshkin IV. Investigation of plasma-induced methylene blue degradation using dielectric barrier discharge. In: Proceedings of the 20th International Conference on Gas Discharges and their Applications (GD2014). 6-11 July 2014; Orleans, France. pp. 566-569

[33] Hayyan M, Hashim MA, AlNashef IM. Superoxide ion: Generation and chemical implications. Chemical Reviews. 2016;**116**:3029-3085. DOI: 10.1021/acs.chemrev.5b00407

[34] Choudhury FA. Fluorophore-based sensor for oxygen radicals in processing plasmas. Journal of Vacuum Science & Technology, A: Vacuum, Surfaces, and Films. 2015;**33**:061305. DOI: 10.1116/1.4930315

[35] Sahni M, Locke BR. Quantification of hydroxyl radicals produced in aqueous phase pulsed electrical discharge reactors. Industrial and Engineering Chemistry Research. 2006;**45**:5819-5825. DOI: 10.1021/ie0601504

[36] Kanazawa S, Kawano H, Watanabe S, Furuki T, Akamine S, Ichiki R, Ohkubo T, Kocik M, Mizeraczyk J. Observation of OH radicals produced by pulsed discharges on the surface of a liquid. Plasma Sources Science and Technology. 2011;**20**:034010. DOI: 10.1088/0963-0252/20/3/034010

[37] Attri P, Kim YH, Park DH, Park JH, Hong YJ, Uhm HS, Kim K-N, Fridman A, Choi EH. Generation mechanism of hydroxyl radical species and its lifetime prediction during the plasma-initiated ultraviolet (UV) photolysis. Scientific Reports. 2015;**5**:9332. DOI: 10.1038/srep09332

[38] Thiruvenkatachari R, Kwon TO, Jun JC, Balaji S, Matheswaran M, Moon IS. Application of several advanced oxidation processes for the destruction of terephthalic acid (TPA). Journal of Hazardous Materials. 2007;**142**:308-314. DOI: 10.1016/j.jhazmat.2006.08.023

[39] Zhang X, Zhou R, Bazaka K, Liu Y, Zhou R, Chen G, Chen Z, Liu Q, Yang S, Ostrikov K. Quantification of plasma produced OH radical density for water sterilization. Plasma Processes and Polymers. 2018;**15**:e1700241. DOI: 10.1002/ppap.201700241

[40] Ma Y, Gong X, He B, Li X, Cao D, Li J, Xiong Q, Chen Q, Chen BH, Liu QH. On the quantification of the dissolved hydroxyl radicals in the plasma-liquid system using the molecular probe method. Journal of Physics D: Applied Physics. 2018;**51**:155205. DOI: 10.1088/1361-6463/aab379

[41] Shirai N, Matsuda Y, Sasaki K. Visualization of short-lived reactive species in liquid in contact with atmospheric-pressure plasma by chemiluminescence of luminol. Applied Physics Express. 2018;**11**:026201. DOI: 10.7567/apex.11.026201

[42] Bekeschus S, Schmidt A, Niessner F, Gerling T, Weltmann K-D, Wende K. Basic research in plasma medicine - a throughput approach from liquids to cells. Journal of Visualized Experiments. 2017;**129**:e56331. DOI: 10.3791/56331

[43] Kohno M. Applications of electron spin resonance spectrometry for reactive oxygen species and reactive nitrogen species research. Journal of Clinical Biochemistry and Nutrition. 2010;**47**:1-11. DOI: 10.3164/jcbn.10-13R

[44] Gorbanev Y, O'Connell D, Chechik V. Non-thermal plasma in contact with water: The origin of species. Chemistry - A European Journal. 2016;**22**:3496-3505. DOI: 10.1002/chem.201503771

[45] Chechik V, Carter E, Murphy D. Electron Paramagnetic Resonance. Oxford, UK. Oxford University Press; 2016. p. 128 ISBN: 9780198727606

[46] Torfs E, Vajs J, Bidart de Macedo M, Cools F, Vanhoutte B, Gorbanev Y, Bogaerts A, Verschaeve L, Caljon G, Maes L, Delputte P, Cos P, Košmrlj J, Cappoen D. Synthesis and in vitro investigation of halogenated 1,3-bis (4-nitrophenyl)triazenide salts as antitubercular compounds. Chemical Biology and Drug Design. 2018;**91**: 631-640. DOI: 10.1111/cbdd.13087

[47] Pan J, Sun P, Tian Y, Zhou H, Wu H, Bai N, Liu F, Zhu W, Zhang J, Becker KH, Fang J. A novel method of tooth whitening using cold plasma microjet driven by direct current in atmospheric-pressure air. IEEE Transactions on Plasma Science. 2010;**38**:3143-3151. DOI: 10.1109/TPS.2010.2066291

[48] Chauvin J, Judée F, Yousfi M, Vicendo P, Merbahi N. Analysis of reactive oxygen and nitrogen species generated in three liquid media by low temperature helium plasma jet. Scientific Reports. 2017;**7**:4562. DOI: 10.1038/s41598-017-04650-4

[49] Tani A, Ono Y, Fukui S, Ikawa S, Kitano K. Free radicals induced in aqueous solution by non-contact atmospheric-pressure cold plasma. Applied Physics Letters. 2012;**100**:254103-254105. DOI: 10.1063/1.4729889

[50] Takamatsu T, Uehara K, Sasaki Y, Miyahara H, Matsumura Y, Iwasawa A, Ito N, Azuma T, Kohno M, Okino A. Investigation of reactive species using various gas plasmas. RSC Advances. 2014;**4**:39901-39905. DOI: 10.1039/C4RA05936K

[51] Uchiyama H, Zhao Q-L, Ali Hassan M, Andocs G, Nojima N, Takeda K, Ishikawa K, Hori M, Kondo T. EPR-spin trapping and flow Cytometric

studies of free radicals generated using cold atmospheric argon plasma and X-ray irradiation in aqueous solutions and intracellular milieu. PLoS One. 2015;**10**:e0136956. DOI: 10.1371/journal.pone.0136956

[52] Tresp H, Hammer MU, Winter J, Wetlmann K-D, Reuter S. Quantitative detection of plasma-generated radicals in liquids by electron paramagnetic resonance spectroscopy. Journal of Physics D: Applied Physics. 2013;**46**:435401-435408. DOI: 10.1088/0022-3727/46/43/435401

[53] Burgett RA, Bao X, Villamena FA. Superoxide radical anion adduct of 5,5-Dimethyl-1-pyrroline N-oxide (DMPO). 3. Effect of mildly acidic pH on the thermodynamics and kinetics of adduct formation. The Journal of Physical Chemistry A. 2008;**112**:2447-2455. DOI: 10.1021/jp7107158

[54] Gorbanev Y, Soriano R, O'Connell D, Chechik V. An atmospheric pressure plasma setup to investigate the reactive species formation. Journal of Visualized Experiments. 2016;**117**:e54765. DOI: 10.3791/54765

[55] Stolze K, Rohr-Udilova N, Rosenau T, Stadtmüller R, Nohl H. Very stable superoxide radical adducts of 5-ethoxycarbonyl- 3,5-dimethyl-pyrroline N-oxide (3,5-EDPO) and its derivatives. Biochemical Pharmacology. 2005;**69**:1351-1361. DOI: 10.1016/j.bcp.2005.01.019

[56] Buettner GR, Oberley LW. Considerations in the spin trapping of superoxide and hydroxyl radical in aqueous systems using 5,5-dimethyl-1-pyrroline-l-oxide. Biochemical and Biophysical Research Communications. 1978;**83**:69-74. DOI: 10.1016/0006-291X(78)90398-4

[57] Reszka KJ, McCormick ML, Buettner GR, Hart CM, Britigan BE. Nitric oxide decreases the stability of DMPO spin adducts. Nitric Oxide. 2006;**15**:133-141. DOI: 10.1016/j.niox.2006.03.004

[58] Gorbanev Y, Stehling N, O'Connell D, Chechik V. Reactions of nitroxide radicals in aqueous solutions exposed to non-thermal plasma: Limitations of spin trapping of the plasma induced species. Plasma Sources Science and Technology. 2016;**25**:055017. DOI: 10.1088/0963-0252/25/5/055017

[59] Moreno SNJ, Stolze J, Janzen EG, Mason RP. Oxidation of cyanide to the cyanyl radical by peroxidase/H_2O_2 systems as determined by spin trapping. Archives of Biochemistry and Biophysics. 1988;**265**:267-271. DOI: 10.1016/0003-9861(88)90127-0

[60] Forrester AR, Hepburn SP. Spin traps. A cautionary note. Journal of the Chemical Society C: Organic. 1971;**0**:701-703. DOI: 10.1039/J39710000701

[61] Rhodes CJ, editor. Toxicology of the Human Environment: The Critical Role of Free Radicals. Vol. 512. Boca Raton, Florida: CRC Press; 2000. ISBN: 9780748409167

[62] Richmonds C, Witzke M, Bartling B, Lee SW, Wainright J, Liu C-C, Sankaran RM. Electron-transfer reactions at the plasma-liquid interface. Journal of the American Chemical Society. 2011;**133**:17582-17585. DOI: 10.1021/ja207547b

[63] Rumbach P, Bartels DM, Sankaran RM, Go DB. The effect of air on solvated electron chemistry at a plasma/liquid interface. Journal of Physics D: Applied Physics. 2015;**48**:424001. DOI: 10.1088/0022-3727/48/42/424001

[64] Rumbach P, Witzke M, Sankaran RM, Go DB. Decoupling interfacial reactions between plasmas and liquids: Charge transfer vs plasma neutral reactions. Journal of the American

Chemical Society. 2013;**135**:16264-16267. DOI: /10.1021/ja407149y

[65] Rumbach P, Bartels DM, Sankaran RM, Go DB. The solvation of electrons by an atmospheric-pressure plasma. Nature Communications. 2015;**6**:7248. DOI: 10.1038/ncomms8248

[66] Murai H. Spin-chemical approach to photochemistry: Reaction control by spin quantum operation. Journal of Photochemistry and Photobiology, C: Photochemistry Reviews. 2003;**3**:183-201. DOI: 10.1016/S1389-5567(02)00038-2

[67] Fessenden RW. ESR of reacting radicals. Applied Magnetic Resonance. 2014;**45**:483-503. DOI: 10.1007/s00723-014-0535-5

[68] Šima J, Brezova V. Mechanism of photoinduced processes in solutions of iodo iron (III) complexes containing Schiff base ligands. Monatshefte für Chemie/Chemical Monthly. 2001;**132**:1493-1500. DOI: 10.1007/s007060170005

[69] Bekeschus S, Wende K, Hefny MM, Rödder K, Jablonowski H, Schmidt A, von Woedtke T, Weltmann K-D, Benedikt J. Oxygen atoms are critical in rendering THP-1 leukaemia cells susceptible to cold physical plasma-induced apoptosis. Scientific Reports. 2017;**7**:2791. DOI: 10.1038/s41598-017-03131-y

[70] Hefny MM, Pattyn C, Lukes P, Benedikt J. Atmospheric plasma generates oxygen atoms as oxidizing species in aqueous solutions. Journal of Physics D: Applied Physics. 2016;**49**:404002. DOI: 10.1088/0022-3727/49/40/404002

[71] Benedikt J, Hefny MM, Shaw A, Buckley BR, Iza F, Schakermann S, Bandow JE. The fate of plasma-generated oxygen atoms in aqueous solutions: Non-equilibrium atmospheric pressure plasmas as an efficient source of atomic O(aq). Physical Chemistry Chemical Physics. 2018;**20**:12037-12042. DOI: 10.1039/c8cp00197a

[72] Kovačević VV, Dojčinović BP, Jović M, Roglić GM, Obradović BM, Kuraica MM. Measurement of reactive species generated by dielectric barrier discharge in direct contact with water in different atmospheres. Journal of Physics D: Applied Physics

[73] Tarabová B, Lukeš P, Janda M, Hensel K, Šikurová L, Machala Z. Specificity of detection methods of nitrites and ozone in aqueous solutions activated by air plasma. Plasma Processes and Polymers. DOI: 10.1002/ppap.201800030

[74] Castelló Beltrán C. Fluorescent probes for selective detection of ozone in plasma applications [thesis]. Loughborough: Loughborough University; 2015

[75] Lukes P, Dolezalova E, Sisrova I, Clupek M. Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: Evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H_2O_2 and HNO_2. Plasma Sources Science and Technology. 2014;**23**:015019. DOI: 10.1088/0963-0252/23/1/015019

[76] Elg DT, Yang I-W, Graves DB. Production of TEMPO by O atoms in atmospheric pressure non-thermal plasma–liquid interactions. Journal of Physics D: Applied Physics. 2107;**50**:475201. DOI: 10.1088/1361-6463/aa8f8c

[77] Suschek CV, Opländer C. The application of cold atmospheric plasma in medicine: The potential role of nitric oxide in plasma-induced effects. Clinical Plasma Medicine. 2016;**4**:1-8. DOI: 10.1016/j.cpme.2016.05.001

[78] Hogg NN. Detection of nitric oxide by electron paramagnetic resonance spectroscopy. Free Radical Biology & Medicine. 2010;**49**:122-129. DOI: 10.1016/j.freeradbiomed.2010.03.009

[79] Li Y, Kang MH, Uhm HS, Lee GJ, Choi EH, Han I. Effects of atmospheric-pressure non-thermal bio-compatible plasma and plasma activated nitric oxide water on cervical cancer cells. Scientific Reports. 2017;**7**:45781. DOI: 10.1038/srep45781

[80] Tsuchiya K, Yoshizumi M, Houchi H, Mason RP. Nitric oxide-forming reaction between the iron-*N*-methyl-D-glucamine dithiocarbamate complex and nitrite. The Journal of Biological Chemistry. 2000;**275**:1551-1556. DOI: 10.1074/jbc.275.3.1551

[81] Nash KM, Rockenbauer A, Villamena FA. Reactive nitrogen species reactivities with nitrones: Theoretical and experimental studies. Chemical Research in Toxicology. 2012;**25**:1581-1597. DOI: 10.1021/tx200526y

[82] Reszka KJ, Bilski P, Chignell CF. Spin trapping of nitric oxide by aci anions of nitroalkanes. Nitric Oxide. 2004;**10**:53-59. DOI: 10.1016/j.niox.2004.03.001

[83] Girard P-M, Arbabian A, Fleury M, Bauville G, Puech V, Dutreix M, Santos Sousa J. Synergistic effect of H_2O_2 and NO_2 in cell death induced by cold atmospheric he plasma. Scientific Reports. 2016;**6**:29098. DOI: 10.1038/srep29098

[84] Bekeschus S, Kolata J, Winterbourn C, Kramer A, Turner R, Weltmann K-D, Bröker B, Masur K. Hydrogen peroxide: A central player in physical plasma-induced oxidative stress in human blood cells. Free Radical Research. 2014;**48**:542-549. DOI: 10.3109/10715762.2014.892937

[85] Wende K, Williams P, Dalluge J, Gaens WV, Aboubakr H, Bischof J, von Woedtke T, Goyal SM, Weltmann K-D, Bogaerts A, Masur K, Bruggeman PJ. Identification of the biologically active liquid chemistry induced by a nonthermal atmospheric pressure plasma jet. Biointerphases. 2015;**10**:029518. DOI: 10.1116/1.4919710

[86] Girard F, Badets V, Blanc S, Gazeli K, Marlin L, Authier L, Svarnas P, Sojic N, Clément F, Arbault S. Formation of reactive nitrogen species including peroxynitrite in physiological buffer exposed to cold atmospheric plasma. RSC Advances. 2016;**6**:78457-78467. DOI: 10.1039/C6RA12791F

[87] Xu H, Chen C, Liu D, Xu D, Liu Z, Wang X, Kong MG. Contrasting characteristics of aqueous reactive species induced by cross-field and linear-field plasma jets. Journal of Physics D: Applied Physics. 2017;**50**:245201. DOI: 10.1088/1361-6463/aa7118

[88] Liao CH, Kang SF, Wu FA. Hydroxyl radical scavenging role of chloride and bicarbonate ions in the H_2O_2/UV process. Chemosphere. 2001;**44**:1193-1200. DOI: 10.1016/S0045-6535(00)00278-2

[89] Hänsch MAC, Mann M, Weltmann K-D, von Woedtke T. Analysis of antibacterial efficacy of plasma-treated sodium chloride solutions. Journal of Physics D: Applied Physics. 2015;**48**:454001. DOI: 10.1088/0022-3727/48/45/45400

[90] Piskarev IM. Effects of cold plasma and UV-C radiation on isotonic solution. High Energy Chemistry. 2017;**51**:297-301. DOI: 10.1134/S0018143917040129

[91] Szili EJ, Bradley JW, Short RD. A 'tissue model' to study the plasma delivery of reactive oxygen species. Journal of Physics D: Applied

Physics. 2014;**47**:152002. DOI: 10.1088/0022-3727/47/15/152002

[92] Richards JE, Philp D. A reactive nitrone-based organogel that self-assembles from its constituents in chloroform. Chemical Communications. 2016;**52**:4995-4998. DOI: 10.1039/c6cc01259k

[93] Guo Q, Qian SY, Mason RP. Separation and identification of DMPO adducts of oxygen-centered radicals formed from organic hydroperoxides by HPLC-ESR, ESI-MS and MS/MS. Journal of the American Society for Mass Spectrometry. 2003;**14**:862-871. DOI: 10.1016/S1044-0305(03)00336-2

[94] Tuccio B, Lauricella R, Charles L. Characterisation of free radical spin adducts of the cyclic β-phosphorylated nitrone DEPMPO using tandem mass spectrometry. International Journal of Mass Spectrometry. 2006;(1):47-53. DOI: 10.1016/j.ijms.2006.02.009

[95] Guo Y, Ma Q, Cao F, Zhao Q, Ji X. Colorimetric detection of hypochlorite in tap water based on the oxidation of 3,3′,5,5′-tetramethyl benzidine. Analytical Methods. 2015;**7**:4055-4058. DOI: 10.1039/C5AY00735F

[96] Stahl R. Ion chromatographic determination of chloride, chlorate, and perchlorate in sulfuric acid solutions. Chromatographia. 1993;**37**:300-302. DOI: 10.1007/BF02278638

[97] Seiler MA, Neist U, Deister UK, Schmitz F. Trace perchlorate determination by ion chromatography. The Column. 2016;**12**:21-25

[98] Shafirovich V, Dourandin A, Huang W, Geacintov NE. The carbonate radical is a site-selective oxidizing agent of guanine in double-stranded oligonucleotides. The Journal of Biological Chemistry. 2001;**276**:24621-24626. DOI: 10.1074/jbc.M101131200

[99] Bonini MG, Radi R, Ferrer-Sueta G, Ferreira AMDC, Augusto O. Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide. The Journal of Biological Chemistry. 1999;**274**:10802-10806. DOI: 10.1074/jbc.274.16.10802

[100] Medinas DB, Cerchiaro G, Trindade DF, Augusto O. The carbonate radical and related oxidants derived from bicarbonate buffer. IUBMB Life. 2007;**59**:255-262. DOI: 10.1080/15216540701230511