Non-thermal plasma in contact with water: The origin of species

Y. Gorbanev,[a, b] D. O’Connell\*[b] and Dr. V. Chechik\*[a]

[a] Dr. Y. Gorbanev, Dr. Victor Chechik   
Department of Chemistry   
University of York  
Heslington, York, YO10 5DD (UK)  
E-mail: victor.chechik@york.ac.uk

[b] Dr. Y. Gorbanev, Dr. D.O’Connell  
York Plasma Institute, Department of Physics  
University of York  
Heslington, York, YO10 5DQ (UK)

E-mail: deborah.oconnell@york.ac.uk

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**Abstract:** Non-thermal atmospheric pressure plasma has attracted much attention in recent years due to its potential for biomedical applications. Determining the mechanism of the formation of reactive species in the liquid treated with plasma has thus paramount importance for both fundamental and applied research. In this work, the origin of reactive species in the plasma-treated aqueous solutions was investigated using spin trapping, hydrogen and oxygen isotopes and electron paramagnetic resonance (EPR) spectroscopy. The species originating from the molecules of the liquid phase and those introduced with the feed gas were differentiated *via* the use of EPR and 1H NMR analyses of the liquid samples. The effects of water vapour and oxygen admixtures in the feed gas were investigated. All reactive species detected in the liquid sample were shown to be formed largely in the plasma gas phase. Hydrogen peroxide (determined by UV-Vis analysis) was suggested to be formed primarily in the plasma tube, whereas the radical species ·OOH, ·OH and ·H were proposed to originate from the region between the plasma nozzle and the liquid sample.

Introduction

Non-thermal plasmas have attracted increased attention in recent years due to their potential for biomedical applications.[1-4] The interaction of these plasmas with ambient atmosphere results in the formation of a variety of reactive species which exhibit high biological (anti-microbial, anti-cancer, wound healing, etc.) activity.[5-9] A range of spectroscopic techniques have been used to monitor different reactive species in these plasmas, such as IR optical emission spectroscopy for 1O2,[10] diode laser absorption spectroscopy for metastable states of helium,[11] VUV absorption spectroscopy and laser-induced fluorescence for radical and atomic species,[12,13] FT-IR spectroscopy for hydrogen peroxide,[14] mass–spectrometry for ionic species,[15] etc.

Aqueous media is a fundamental part of biological milieu. Two main types of plasma application in research and biomedical trials are pre-treatment of the aqueous media, which is subsequently applied to the tissue or bacteria, and direct exposure of a biological sample to a plasma jet.[16,17] While the first method relies on the formation of relatively long-lived reactive species such as hydrogen peroxide and ozone as well as the secondary radicals generated in the liquid phase,[18] the efficacy of the latter is dependent on the short-lived species such as 1O2 and radicals including hydroxyl radical •OH, superoxide radical O2•-, and atomic radicals directly formed by plasma. Investigating factors that govern the formation of reactive species in plasma-treated liquids is therefore very important for biomedical applications.

Electron paramagnetic resonance spectroscopy (EPR) is the most direct method of radical detection in a liquid. Short-lived radical species are usually detected in liquid solutions using spin traps.[19] Tani *et al.* and Takamatsu *et al.* described the detection of radical species in plasma-treated liquids using various spin traps.[20,21] The concentrations of •OH and •OOH radical adducts of BMPO and DMPO spin traps were measured in liquid samples by Reuter and co-workers.[22] Uchiyama *et al.* performed EPR and flow cytometric studies of free radicals induced in liquid by non-thermal plasma.[23] In many reports, it is proposed that •OH and other radicals are at least partially formed from the dissociation of the liquid phase molecules.[21]

The concentrations of stable molecules in plasma-treated liquids have also been measured. Reuter *et al.* assessed the concentration of H2O2 in the liquid phase as a function of feed gas humidity using microscope analysis of colour test stripes.[14] The authors found a direct correlation between concentration of H2O2 in the liquid media and in the gas phase (the latter was measured by FTIR). Lukes *et al.* determined H2O2 concentration in the liquid phase colourimetrically with titanium sulfate.[24]

Recently, Xu *et al.* measured concentrations of H2O2, O2•-, •OH and •H in argon plasma-treated liquid samples containing cell cultures (colourimetrically and using EPR).[9] The authors proposed *in situ* formation of hydroxyl radical from hydrogen peroxide and superoxide radical anion with iron ions and correlated it with induced cell death. The reports of the identification of reactive species in plasma-treated liquids, however, remain relatively scarce, and our understanding of where the reactive species originate from and how their concentrations depend on the experimental parameters is limited.[25]

Several computational approaches have been used to model the reactive species in plasmas. For example, a global model for the discharges in helium with the admixtures of H2O was described by Bruggeman and co-workers.[26] Murakami *et al.* developed models of chemical kinetics[27] and the afterglow (effluent) chemistry for helium plasma with oxygen admixtures and water vapour impurity.[28] Kushner and co-workers presented a model in which the plasma effluent was in contact with liquid water.[29] The authors described the formation and distribution of reactive species in a plasma effluent in contact with a liquid sample. More recently, Lindsay *et al.* predicted the distribution of reactive species in the liquid treated by plasma using a neutral mass transport model for convective gaseous plasma/liquid water systems.[30] In general, plasmas in contact with liquids are extremely complex systems, making modelling very challenging. Kinetic models sometimes include hundreds of rate coefficients obtained from literature. The accuracy of the modelling thus relies on the accuracy of the original data. At the plasma-liquid interface, other factors such as diffusion coefficients, sample evaporation, convection must be considered. Therefore, further experimental work is needed to improve our understanding of plasma-liquid systems and benchmark models and simulations.

In this work, an experimental study of the origin of the reactive species induced in a liquid sample by non-thermal plasma treatment is presented. The effect of H2O and O2 admixtures in plasma feed gas on the generation of •OH, O2•-, O3 and H2O2 in plasma-treated liquid samples was investigated. These data, and the use of isotopically-labelled water (H2O/H217O, H2O/D2O) allowed us to distinguish between the species generated in the liquid phase, those that diffused into the liquid from the plasma gas phase, and those formed either in the plasma core or close to the gas-liquid interface.

Results and Discussion

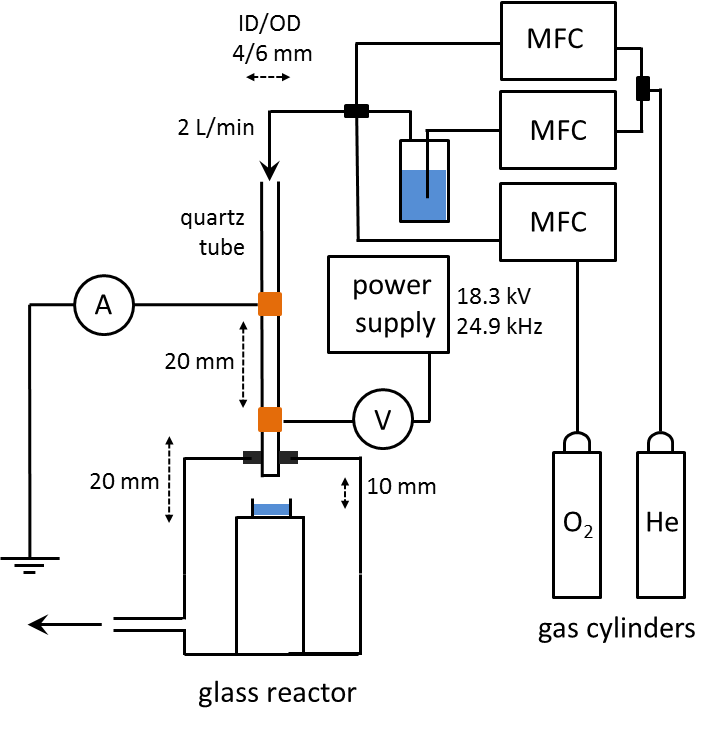
**Experimental setup**

In biomedical applications of cold plasma, tissue or bacteria are exposed to plasma in ambient atmosphere. The interaction between plasma and air leads to the formation of species which introduce additional reaction pathways and post-discharge reactions.[6,24,31,32] In order to elucidate the origin of reactive species in solution, a simplified system is required to exclude the uncontrollable local concentrations of oxygen, nitrogen, water vapour and other components of ambient atmosphere.

In the presented investigation, an in-house designed reactor was used in which the plasma jet was in direct contact with the liquid (aqueous) sample (Figure 1). The atmosphere inside the reactor was composed exclusively of the plasma feed gas and additional vapour originating from the evaporation of the liquid sample. Sample evaporation was observed even at 100% humidity of the feed gas, thus indicating the increased temperature of the feed gas compared to room temperature (20 oC). Indeed, the temperature measured in the liquid sample immediately after plasma exposure was 24 oC and 26.8 oC for the dry and water-saturated helium, respectively.

The plasma used was a kHz frequency parallel field jet. The plasma was ignited in a quartz tube with two copper electrodes positioned around the tube and operated with a sinusoidal voltage of *ca.* 18 kV. Helium was used as a carrier gas with molecular admixtures. Detailed experimental description is found in Supporting Information (SI).

Reactive species in the plasma-treated liquids could form not only through reactions with the gas phase constituents of the plasma, but also through photolysis by plasma-emitted UV and VUV photons.[33] In order to test whether UV/VUV irradiation in our setup affects the formation of reactive species in the liquid sample, control experiments were carried out in which the sample was covered with a MgF2 window (Crystran Ltd., > 40%



**Figure 1.** Setup used in the plasma exposure experiments. Feed gas flow was controlled by the mass flow controllers (MFCs). In all experiments the distance between the nozzle and the sample was 10 mm unless stated otherwise.

transmittance at 121 nm) and then exposed to plasma. In these experiments, the surface of the sample was in direct contact with the window to prevent UV quenching in the gas between the window and the sample (helium operated plasma is transparent to the UV). The obtained results (see SI, Figure S4) revealed that neither radicals nor hydrogen peroxide were formed in the liquid due to the photolysis reactions.

For reactive species formed in the plasma gas phase (*e.g.*, in the plasma core), the efficiency of plasma treatment critically depends on the diffusion of these species into the liquid. In order to assess the diffusion properties of our setup, we investigated the delivery of molecules from the gas phase into the liquid sample using isotopically labelled water. In these experiments, the delivery of H2O vapour to a liquid sample of D2O (and *vice versa*) was studied using 1H NMR with sodium tosylate as an internal standard. The use of isotopes made it possible to distinguish the water molecules originally present in the sample (D2O) from those delivered by the plasma (H2O). The results are presented in Table 1.

The amount of H2O introduced into the sample during the setup of the experiment was subtracted from the recorded data. This amount was measured experimentally when a 100 L sample of D2O was placed in a well on top of the glass stand inside the reactor pre-flushed with helium. The sample was kept for 80 s without the plasma or gas flow exposure, after which it was analysed by 1H NMR.

With the dry He feed gas, a small amount of H2O was delivered to the liquid sample (Table 1, entries 1, 4). A clear increase in the amount of H2O in D2O was observed at prolonged plasma exposure time (see SI, Figure S5). This is likely due to the physisorbed water from the gas tubing[34] or H2 and H2O impurities present in the feed gas.

The introduction of water vapour into the feed gas clearly increased the amount of H2O delivered to the liquid D2O sample. This increase was observed under both plasma off (Table 1, entries 1-3) and plasma on conditions (entries 4-6). The system with the plasma ignited exhibited an enhanced delivery of water vapour to the liquid sample. This can be ascribed to several factors, including temperature increase and turbulent flow of the plasma jet.[35,36]

In order to confirm the validity of these results, a control experiment was carried out with a H2O liquid sample exposed to a plasma operated with D2O-saturated feed gas. The amount of D2O delivered to the liquid H2O sample was very similar to the amount of H2O delivered into the liquid D2O sample (Table 1, entries 6 and 9).

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| **Table 1.** Table Caption. | | |
| Entry | Relative humidity (H2O) of the feed gas [%] | H2O delivered to the liquid sample[a][mol%] |
| ***Plasma OFF*** | | |
| 1 | 0[b] | <0.05 |
| 2 | 10 | 0.7 |
| 3 | 100 | 5.3 |
| ***Plasma ON*** | | |
| 4 | 0[b] | 0.1 |
| 5 | 10 | 1.7 |
| 6 | 100 | 13.5 |
| 7[c] | 10 | 1.8 |
| 8[c] | 100 | 13.1 |
| 9[d] | 100[d] | 13.8[e] |
| [a] The data are corrected for the initial concentration of H2O in the D2O sample and the amount of H2O introduced by the handling of the sample (*ca.* 0.2 mol% together). [b] Feed gas contained trace amounts of water vapour. [c] At 4 mm distance from the nozzle to the sample. [d] Using H2O liquid sample and D2O vapour in the feed gas. [e] D2O delivered to the liquid sample of H2O. | | |
|  | | |

We were interested to see whether the delivery of molecules from the gas into the liquid is influenced by the distance between the nozzle and the sample. This was tested by comparing the delivery of H2O into the sample located at two different distances under the nozzle: 10 and 4 mm. The distance of 4 mm was chosen as the minimal possible distance between the nozzle and the sample which did not result in significant liquid surface disturbances by the feed gas flow. Virtually identical amounts of H2O were measured in the D2O sample at the two distances (Table 1, entries 7, 8 and 5, 6).

These results clearly demonstrate that both H2O and D2O are delivered into the liquid sample with equal efficiency at both 10 and 4 mm distances from the jet nozzle to the liquid sample, thus allowing direct use of these conditions in the investigation of the origin of species induced in the liquid sample by plasma.



**Figure 2.** H2O2 concentration in the liquid sample as a function of feed gas humidity: He (●), He with 0.5% O2 (○) at 10 mm and He at 4 mm () distance from the nozzle to the sample. In this figure and further the lines are added to guide the eye.

**Reactive species in the liquid sample**

**Hydrogen peroxide**. H2O2 is considered to be a key component responsible for the wound healing, anti-microbial and anti-cancer properties of cold plasma.[8,37] A multitude of possible reactions can lead to the formation of H2O2[7,26-28] from water vapour and oxygen; the most straightforward pathway is shown in Equations 1-3. The effects of humidity and oxygen admixture in the feed gas on the H2O2 content in the plasma-treated liquid were investigated.

The concentration of H2O2 in the liquid sample was measured by UV-Vis spectroscopy using a solution of potassium oxotitanate dihydrate in H2O/H2SO4.[38] The evaporation of water from the liquid sample was included in the calculation of the final H2O2 concentration. As the H2O2 vapour pressure was at least 10 times lower than that of H2O under all experimental conditions,[39,40] evaporation of H2O2 was disregarded. The results are presented in Figure 2.

The results in Figure 2 show that in the absence of H2O vapour in the feed gas, only a minor amount of H2O2 is detected in the liquid sample. The concentration of H2O2, however, increases dramatically with increased feed gas humidity. This observation suggests that H2O2 is not formed in the liquid by dissociation of H2O (Equations 1, 2; this would not show such a strong dependence on the feed gas humidity), but instead is formed in the gas phase and then diffused into the liquid sample.



The plot of H2O2 concentration (Figure 2) flattens out at high humidity. This is attributed to a reduced electron density in the plasma with increased molecular content.41 A similar effect was observed for introducing oxygen admixture (0.5%) to the feed gas (Figure 2), however in this case the amount of H2O2 in the liquid decreased at elevated humidity (> 70%) of the feed gas. Here, besides a drop in electron density of plasma, the amount of H2O2 could also be reduced due to side reactions involving some of the species which form H2O2 (*e.g.* Equation 4) or secondary reactions, *e.g.* peroxone process.42 Varying O2 admixture at low humidity, however, did not result in significant changes of H2O2 concentration in the liquid sample (Figure S6).

In order to test the origin of the minor amount of H2O2 observed with dry feed gas, the distance between the nozzle and the sample was decreased to 4 mm. This short distance significantly reduces the interaction of the plasma jet with the wet ambient gas inside the reactor (the gas inside the reactor contains evaporated water). The diffusion of the species from the ambient gas into the plasma effluent was demonstrated by performing an experiment in which NO radical was detected with the (MGD)2Fe2+ spin trap in a plasma with air as ambient gas and He as feed gas. The amount of trapped NO reduced drastically at 4 mm compared to 10 mm distance from the nozzle to the sample (data not shown).

The experimental data revealed that the amount of H2O2 formed with dry helium at 4 mm distance was somewhat lower than at 10 mm, thus supporting the hypothesis that this minor amount was formed from the ambient humidity in the reactor (see expanded region in Figure 2). This amount increased with the addition of oxygen, possibly due to reactions of atomic oxygen (*e.g.* Equation 3).

The amount of H2O2 detected with wet feed gas was almost the same for the 10 and 4 mm distances between the nozzle and the sample (Figure 2) and hence was not affected by the interaction of plasma jet with the wet gas in the reactor. As the delivery of the species from the gas phase into the liquid sample was nearly equal at these distances (Table 1) and strongly dependent on the feed gas humidity, we propose that H2O2 induced in the liquid was largely formed from the species generated inside the plasma tube (but not in the region below the nozzle of the plasma tube as observed for the •OH and •H radicals, *vide infra*) and subsequently delivered to the liquid sample.

**•OH** **radical**.Biologicaleffects of cold plasma treatment are often attributed to the formation of hydroxyl radicals,[9,43] an important precursor of hydrogen peroxide (Equation 2). Hydroxyl radical is a very short-lived species and in most cases cannot be detected in liquids directly.[44] In this work, spin trapping in conjunction with EPR spectroscopy was used for the detection of such reactive radicals.

The concentration of •OH radical in liquid samples was assessed by exposing aqueous solutions of DMPO spin trap to plasma. The subsequent EPR analysis of the solutions revealed the trapping of both •OH and •H radicals (Figure 3). Here, the DMPO-OH radical adduct in the liquid sample was observed in concentrations up to *ca.* 23.5 M, whereas the concentration of DMPO-H in most cases did not exceed 2.3 M (SI, Figure S7).







**Figure 3.** Typical experimental and simulated EPR spectra of DMPO radical adducts formed in plasma-exposed aqueous solutions of DMPO in H2O (a) and in H217O (b). DMPO-H: aN = 16.4 G, aH = 22.6 G (×2); DMPO-OH: aN = 15.0 G, aH = 14.7 G; DMPO-17OH: aN = 14.9 G, aH = 14.8 G, a17O  = 4.7 G.

The concentration profile of DMPO-OH adduct in plasma-treated water is shown in Figure 4. Although these concentrations do not represent the exact amount of •OH radical generated by the plasma due to side reactions and limited selectivity of DMPO as a spin trap for •OH, changes in relative concentrations of the DMPO-OH adduct match those of the •OH radical. The concentration of DMPO-OH adduct in the liquid sample was significantly affected by the feed gas humidity. In particular, the adduct concentration detected at 4 mm distance between the sample and the nozzle was much lower for dry helium than for wet helium. This observation strongly suggests that the trapped •OH radical was not formed in the liquid, as in the latter case its concentration in the liquid would have been highest with dry feed gas. The decrease in DMPO-OH concentration observed with increased humidity, and an even more rapid decay for oxygen-containing feed gas (Figure 4; see also Figure S8) can be attributed to a reduced electron density of the plasma with increasing molecular content.



**Figure 4.** DMPO-OH adduct concentration in the plasma-treated liquid samples as a function of the feed gas humidity: He (●), He with 0.5% O2 (○) at 10 mm and He at 4 mm () distance from the nozzle to the sample.

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| **Table 2.** Concentration of DMPO-16OH and DMPO-17OH radical adducts in a liquid H217O sample after 60 s of plasma exposure. | | | | |
| Entry | Relative humidity (H216O) of the feed gas [%] | Distance[a][mm] | Adduct concentration [M] | |
|  |  |  | •17OH | •16OH |
| 1 | 0[b] | 10 | 5.6 | 4.8 |
| 2 | 10 | 10 | 8.3 | 18.3 |
| 3 | 10 | 4 | 2.9 | 9.1 |
| [a] Distance from the nozzle to the sample. [b] Feed gas contained trace amounts of water vapour. | | | | |
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The very significant difference between the DMPO-OH adduct concentrations for samples treated at 4 and 10 mm distances (Figure 4) suggests that the concentration of •OH in the liquid sample is strongly affected by the interaction of the plasma jet with the wet gas inside the reactor. This is in contrast with the trends observed for H2O2. We conclude therefore that while H2O2 is brought into the liquid from the plasma core in the quartz tube, the •OH radical in the liquid phase originates from the plasma jet, *i.e.* the region below the nozzle of the quartz tube.

In order to further confirm that the hydroxyl radicals are formed in the gas phase and not in the liquid, isotopically labelled water (H217O) was employed. This made it possible to differentiate between the •OH radicals created in the liquid phase and those formed in the gas phase and subsequently delivered into the liquid sample. Samples of DMPO solution in H217O were exposed to He plasma, dry and with 10% humidity (H216O) (Table 2). DMPO-16OH and DMPO-17OH adducts have distinct EPR signals (Figure 3), and the concentration of each species can hence be independently determined.

With the dry feed gas, the only major source of •OH radicals is the sample (either liquid or evaporated sample), but with the wet feed gas, the •OH radicals could come from either the sample or the feed gas. Introduction of H2O vapour to the feed gas makes it possible to study the origin of the•OH radicals and the effect of mixing the feed gas with the wet gas inside the reactor. For example, let us consider an experiment with DMPO solution in liquid H217O and H216O vapour in the feed gas. Here, •16OH radicals (if any) could only originate from the gas phase, and •17OH radicals could be formed in either liquid phase or the atmosphere in the reactor which contains evaporated H217O.

An additional factor, however, must be taken into account. The composition of liquid phase changes during plasma treatment, due to sample evaporation and condensation of water vapour from the feed gas. The composition of the liquid sample (H216O to H217O ratio) was determined using the hydrolysis of cinnamoyl chloride by water. The reaction product, cinnamic acid, was analysed by high-resolution mass spectrometry to obtain the ratio of cinnamic acid molecules with 16O and 17O isotopes, *i.e.* those hydrolysed by H216O and H217O, respectively. The H216O content in the plasma-treated liquid H217O sample was in all cases low (see SI, Table S4).

With dry He feed gas, the relative amount of DMPO-16OH radical adduct was quite significant, *ca.* 45% (Table 2, entry 1). This DMPO-16OH adduct here probably originated from H216O impurity in the He feed gas and the H216O content of the liquid sample (*ca.* 18%, see SI, Table S4). This result alone strongly suggests that the •OH radical is not formed in the liquid phase (in this case over 80% of the DMPO-17OH adduct would have been formed). When a small amount of water vapour was introduced in the feed gas, the relative amount ofDMPO-16OH radical adduct increased to 70% (Table 2, entry 2), while only a minor increase of H216O content was observed in the liquid phase (Table S4). This, again, is consistent with •OH radical formation in the gas phase. At 4 mm distance (which results in reduced interaction of plasma jet with the wet gas inside the reactor, *vide supra*), the concentration of both DMPO-OH adducts decreased, with the DMPO-17OH adduct most affected (entry 3), in a good agreement with the proposed formation of the •OH radical in the gas phase, in the region below the nozzle of the plasma tube.

•**H radical**.Similarly to the investigations of H2O vapour delivery (Table 1) and isotopically labelled •OH radicals (Table 2), the use of hydrogen isotopes made it possible to differentiate between the •H radicals created in the liquid phase and those which were delivered into the liquid sample from the gas phase.

The PBN spin trap in either D2O or H2O was treated with plasma using a feed gas with admixtures of either H2O or D2O. Different spin traps have different affinity towards a certain group of radicals; treatment of PBN solutions with an oxygen-free plasma led to predominant formation of PBN-H (or D) adducts in contrast to DMPO results where under most conditions DMPO-OH adduct was formed predominantly. The EPR spectrum of a typical PBN-H and PBN-D radical adduct mixture is shown in Figure 5.



**Figure 5.** Typical experimental and simulated EPR spectra of PBN radical adducts formed in a plasma-treated H2O or D2O solutions. PBN-H: aN = 16.6 G, aH = 10.8 G (×2); PBN-D: aN = 16.7 G, aH = 10.8 G, aD = 1.6 G.

Here, similarly to H217O experiments, the change in the liquid sample composition due to sample evaporation and feed gas condensation must be considered. This was accounted for by using the data from Table 1. Additionally, the rates of cleavage of the O-H and O-D bonds in H2O and D2O are different due to the primary kinetic isotope effect (KIE). The KIE would thus lead to potentially different concentrations of •H and •D radicals in otherwise identical conditions, and hence must be taken into account. The KIE in our system was estimated by the following method. PBN spin trap dissolved in the liquid samples containing different ratios of H2O/D2O was treated with plasma using a feed gas which was fully saturated with the vapour of the same composition. The apparent KIE had a value of 3.0 as calculated from the ratio of the PBN-H and PBN-D adducts (see Table S5 and related discussion in SI). This value is in a typical range of the KIE for H/D systems. Our method of the KIE estimation is valid regardless of whether the •H/•D radicals are formed in the liquid sample or the feed gas.

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| **Table 3.** Concentrations of PBN-H and PBN-D radical adducts after plasma exposure with H2O and D2O in the feed gas and the liquid sample. | | | | |
| Entry | Plasma exposure conditions | | Adduct concentration [M] | |
|  | Distance[a] [mm] | Relative humidity [%] | •H | •D |
| **D2O liquid sample / H2O vapour in the feed gas** | | | | |
| 1 | 10 | - | 1.9 | 9.3 |
| 2 | 10 | 8.1 | 2.3 |
| 3 | 50 | 3.6 | 0.3 |
| 4 | 100 | 3.2 | 0.2 |
|  |  |  |  |  |
| 5 | 4 | - | 4.3 | 6.6 |
| 6 | 10 | 7.4 | 1.2 |
| 7 | 50 | 4.1 | 0.2 |
| 8 | 100 | 3.1 | 0.2 |
| **H2O liquid sample / D2O vapour in the feed gas** | | | | |
| 9 | 10 | - | 12.9 | 0 |
| 10 | 10 | 9.2 | 6.2 |
| 11 | 50 | 3.9 | 7.5 |
| 12 | 100 | 1.3 | 6.2 |
|  |  |  |  |  |
| 13 | 4 | - | 13.6 | 0 |
| 14 | 10 | 4.3 | 8.5 |
| 15 | 50 | 2.5 | 16.3 |
| 16 | 100 | 1.1 | 6 |
| [a] Distance from the nozzle to the sample. [b] Additional PBN adducts such as *e.g.* PBN-OH were also detected (data not shown). | | | | |
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The results of experiments with different isotopes in the gas and liquid phases show that a minor amount of PBN-H radical adduct is formed in the absence of H2O in both the feed gas and liquid (Table 3, entries 1 and 5). The amount of PBN-H adduct was higher at 4 mm distance than at 10 mm distance. Similar results were obtained using DMPO spin trap (Figure S7). Therefore, we conclude that this •H adduct originates from the impurities in the feed gas (*e.g.* H2). The total radical concentration decreased with increased feed gas humidity (Table 3; see also SI, Table S6). This can be explained by a decreased electron density under these conditions, similar to the results for the •OH radical.

The data in Table 2 clearly show that the trapped H radical does not originate from the liquid phase. The clearest evidence for this comes from entry 12, which describes plasma treatment of H2O using D2O-saturated feed gas. At the end of the plasma exposure, the liquid phase is still dominated by H2O (the concentration of D2O is below 15%). At the same time, little •H is trapped; the trapped radical is over 80% •D. If one takes into account the KIE and the fact that some trapped •H comes from impurities in the feed gas, it becomes clear that only a very small amount (if any) of trapped •H/•D radical originates in the liquid phase. Additional calculations to support this are found in Table S7 and related discussion in SI.

Entries 1, 5, 9, 13 in Table 2 also show that most of the trapped •H/•D did not originate in the quartz tube. These experiments were carried out with dry He gas. A significant amount of trapped ·D (entries 1, 5) suggests that it originates from the evaporated liquid, *e.g.* in the plasma jet mixed with the atmosphere in the reactor (which contains evaporated D2O), just like the •OH radical. This conclusion is further supported by the high concentrations of the •H adduct (entries 9, 13) observed with dry He feed gas and H2O liquid sample.

Comparison of entries 1 and 5 (Table 3) leads to an unexpected conclusion. In this experiment, a D2O sample is treated with H2O-saturated plasma, and hence •D radical must originate from the evaporated sample. Surprisingly, however, only a moderate reduction in the trapped •D is observed at 4 mm distance between the nozzle and the sample, as compared to the 10 mm distance. This is very different from the •OH radical (which also originates from the plasma effluent, and has a greatly reduced concentration at 4 mm, see Figure 4). We infer that the •H radical trapping is less affected by the distance between the nozzle and the sample, possibly because it is formed closer to the plasma-liquid interface.

**Superoxide O2**•**-**. Another important radical formed in oxygen containing plasmas is the superoxide radical anion O2•-.[20,45] It can be formed from the reaction of molecular oxygen with electrons or deprotonation of hydroperoxyl radical •OOH which can be produced *e.g.* in a reaction of ozone and hydroxyl radical (Equation 4).[7,26] Other pathways leading to the formation of superoxide radical include secondary post-exposure reactions (*e.g.* peroxone process[42]).

For the detection of the O2•- radical, DEPMPO spin trap was employed which forms a much more stable radical adduct DEPMPO-OOH compared to DMPO-OOH. The EPR analysis of plasma-treated DEPMPO (Figure 6) showed spin adducts with •H, •OH, and O2•- (calculated as a sum of two conformers) radicals in most experiments. Additionally, C-centred radicalswere observed(the exact structure of the adduct was not determined; its simulated hyperfine values matched literature values for various C-centred radical adducts of DEPMPO[46]). The carbon-centred adduct is likely a degradation product of DEPMPO: it was not observed in the presence of molecular

oxygen in the feed gas (Figure S11), consistently with the high reactivity of carbon-centred radicals with oxygen.[47] In any case, the amount of this adduct was substantially lower than for the other observed species, such as hydroxyl and superoxide radical adducts (see SI, Figure S9-S11).

The results of the spin trapping experiments with DEPMPO at 10 and 4 mm distances from the nozzle to the sample are presented in Figure 7. The amount of both DEPMPO-OH and DEPMPO-OOH radical adducts decreased when the experiments were performed at the 4 mm distance, thereby suggesting that O2•- radical was largely formed in the gas phase inside the reactor, similarly to •OH (as was also demonstrated for the DMPO spin trap, *vide supra*).



**Figure 6.** Typical experimental and simulated EPR spectra of DEPMPO radical adducts formed in a plasma-treated aqueous solutions. DEPMPO-OOH (conformer 1): aN = 14.0 G, aH = 13.1 G, aP = 47.3 G; DEPMPO-OOH (conformer 2): aN = 12.0 G, aH = 9.7 G, aP = 48.7G; DEPMPO-OH: aN = 14.0 G, aH = 13.0 G, aP = 47.2 G; DEPMPO-H: aN = 15.3 G, aH = 20.7 G (×2), aP = 50.5 G; DEPMPO adduct of C-radical: aN = 14.9 G, aH = 19.3 G, aP = 50.7 G.

It is worth noting that the relative amounts of both •OH and O2•**-** adducts depend on the feed gas humidity. For instance, DEPMPO-OH is the dominant adduct with dry feed gas, whereas DEPMPO-OOH dominates at high feed gas humidity. This suggests that not just the amount, but the selectivity of radical generation can be controlled *via* different plasma conditions, such as plasma feed gas humidity and oxygen content (Figure S12). Such ability to tune the nature of reactive species in the liquid sample presents great potential for possible cold plasma applications.[48]

**O3/1O2/O**. Ozone and 1O2 are readily available in oxygen-rich plasma systems.[34,49,50] These species may lead to the formation of other reactive species, *e.g.* •OH and O2•- radicals *via* post-exposure reactions in the liquid sample. Thus, we have performed an assessment of the concentrations of ozone, atomic oxygen and 1O2 in the liquid sample.



**Figure 7.** DEPMPO-OOH ( and ) and DEPMPO-OH (● and ) adduct concentration in the liquid at the 10 mm and at the 4 mm distance from the nozzle to the sample, as a function of feed gas humidity.

Although several methods for measuring the concentration of ozone and 1O2 in liquids are available in the literature, many are not selective in the case of plasma-treated liquids. In a recent report, Kohno and co-workersdemonstrated determination of the oxidising species in solutions by oxidation of 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide (TPC) to form a stable radical which was analysed by EPR. The authors were able to estimate the concentration of singlet delta oxygen 1O2 by employing sodium azide which acts as a selective scavenger for 1O2.[21,51]

In the present study, 60 mM aqueous solutions of 2,2,6,6-tetramethylpiperidine (TEMP)[52] with and without the addition of NaN3 were exposed to plasma and the concentration of oxidising species was estimated from the EPR signal intensity of TEMPO. Control experiments with H2O2 and superoxide (added as KO2), separately and combined, showed that these compounds do not produce TEMPO at up to 60 mM concentrations, and hence do not contribute to the observed signal. Ozone, on the other hand, did produce TEMPO. The reactivity of atomic oxygen with TEMP is unknown but it is reasonable to assume that it contributes to the formation of TEMPO.

The data obtained in the preliminary experiments revealed that the concentration of trapped O3/1O2/O decreased dramatically in all cases when water vapour was introduced into the feed gas. For instance, TEMP solution treated by plasma containing 0.5% of oxygen in helium and humidity above 20% yielded TEMPO at a concentration below 15 M, whereas in the case of dry feed gas it increased to *ca.* 70 M (SI, Figure S13). This is in agreement with results reported by Reuter and co-workers, where the amount of ozone in the gas phase decreased substantially when water vapour was introduced into the feed gas even at low levels.[34] This is most likely due to the decay of formed ozone (water is an extremely effective quencher of ozone, producing *e.g.* hydroxyl radical and molecular oxygen).

Only negligible amounts of TEMPO were formed in the absence of added molecular oxygen (Figure S13). The amount of TEMPO increased somewhat when humidity was introduced into the dry feed gas. This suggests that small amount of O3/1O2/O can be formed from water molecules. The concentration of TEMPO, however, increased dramatically with increased oxygen admixture in the feed gas (Figure 8). This unambiguously demonstrates that the bulk of O3/1O2/O originates from the added molecular oxygen in the feed gas.

At 4 mm distance from the nozzle to the sample, the concentration of TEMPO increased approximately twice for all oxygen concentrations in the feed gas. The decreased concentration of O3/1O2/O at the longer distance (10 mm) can be tentatively attributed to their reactions with •OH and other species present in the plasma jet mixed with the evaporated liquid sample.

Addition of 1O2 scavenger NaN3 did not significantly affect the concentration of TEMPO (Figure 8). Similar results were obtained when the feed gas saturation with water vapour was 20% (SI, Figure S14). This indicates that the contribution of 1O2 to the oxidation of TEMPO is negligible in our investigation, and the data in Figure 8 are largely attributed to O3/O in the liquid sample.

Conclusions

Treatment of aqueous samples with non-thermal atmospheric pressure plasma jets results in the generation of a number of reactive species. This work aimed at understanding where these compounds originate from, and whether experimental parameters (such as the feed gas composition and the distance between the nozzle and the sample) have the potential to tune their concentrations. A combination of spin trapping/EPR spectroscopy and conventional analytical methods made it possible to assess relative concentrations of H2O2, •OH, O2•-, •H, 1O2, O3/O in solutions treated with a parallel field kHz driven atmospheric pressure plasma jet. The ambient atmosphere was controlled using an in-house built reactor.

For the first time, the possibility to experimentally distinguish between the reactive species generated from the liquid sample and the feed gas was demonstrated. It was performed using (a) specific labelling of one phase with the hydrogen or oxygen isotopes (*i.e.*, deuterium oxide or H217O), and (b) variation of the distance between the plasma jet nozzle and the sample (the interaction of the plasma with the evaporated liquid is significantly reduced at short distances between the nozzle and the sample). This method allows to perform such analysis with various plasma jets operated under different conditions.

The results showed that different reactive species detected in the plasma-treated liquid sample originate in different regions of the plasma interaction setup. H2O2 delivered to the sample is almost exclusively created from the species in the plasma tube. On the other hand, •H, •OH and superoxide radicals originate from the plasma effluent, *i.e.* the volume between the plasma nozzle and the sample where some interaction of the plasma jet with the evaporated sample takes place. Different radicals, however, showed different trends, with •H radicals observed even at the short distances between the nozzle and the sample; it was hypothesised that it originates from the volume close to the plasma-liquid interface.

The data obtained in this study make it possible to rationally design certain plasma treatment conditions. For instance, we found that ozone/atomic oxygen/singlet oxygen is only delivered to the liquid sample if O2 is present in the feed gas (*e.g.*, only negligible amount of these species can be formed from the water molecules). In another example, variation of the feed gas composition significantly changed the relative amounts of •OH and O2•- radicals trapped in the liquid phase.

Experimental Section

Full experimental description and methodological details are found in Supporting Information.

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**Keywords:** reactive oxygen species • cold plasma • isotopically labelled water • EPR • radicals

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