

Plasma activated water chemical and antibacterial effects correlate with gaseous and aqueous RONS, plasma source, air and water flow conditions

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Abstract: Control and tunability of the chemical composition and biomedical effects of plasma activated water/media for emerging applications in biomedicine and agriculture is possible by controlling the discharge regime, deposited power, plasma-liquid interface area, and gas flow conditions. We compare gaseous and aqueous RONS of two cold air plasma sources: streamer corona and transient spark, interacting with water in open and closed reactors with water electrospray through these discharges or in water batch treatment.

Keywords: Cold air plasma, plasma activated water, reactive oxygen and nitrogen species

1. Introduction

Non-thermal plasmas generated by electrical discharges in atmospheric pressure air are sources of various *reactive oxygen and nitrogen species (RONS)*. When generated in contact with water, they enable the transfer of RONS formed in the gas-phase plasma into the water or aqueous solutions and so generate the *plasma activated water (PAW)*. PAW is typically a strong antibacterial agent and besides multiple uses in medicine for disinfection it has the potential for food processing or agriculture applications. [1-6]

We prepare PAW by a DC-driven streamer corona (SC) and transient spark (TS) discharges operated in air with/without water electrospray. The production of active species (e.g. O₃, NO, NO₂ and OH) in the gas and consequently the PAW properties can be controlled by the discharge regime and gas-flow and liquid-flow parameters. In low power air corona discharge, O₃ production probably results in enhanced antibacterial effects. In the higher power TS; the dominant gaseous products are NO_x that lead to significant NO₂⁻ and NO₃⁻ in the PAW and practically no O₃. The antibacterial action is then mainly due to the synergy of H₂O₂, NO₂⁻ and acidic milieu (via ONOOH formation) and typically decays in time within several hours post plasma activation, depending on temperature and pH [4,7]. The controlled and selective generation of RONS using air plasmas with water will facilitate targeted applications of cold plasmas and PAW to various fields including disinfection and antimicrobial applications, food processing, agriculture, and even cancer therapies, where the roles of different key reactive species on cancer cell biochemistry is particularly delicate [6,8].

2. Experimental set-up and methods

DC-driven streamer corona and transient spark discharges in positive polarity were generated in point-to-plane configuration in ambient air at atmospheric pressure. Positive streamer corona and transient spark discharge regimes used here has been described in more details in [9-10]. Figure 1 shows a schematic of the set-up used for water electrospray or batch treatment with SC or TS discharge.

We used a high voltage (HV) hollow needle anode opposite to the metallic (stainless steel) grounded mesh or bulk water surface cathode. The inter-electrode spacing between the needle and the mesh (water surface) was kept at 10 mm. A positive high voltage was applied from the power supply *Technix SR20-R-1200* through the ballast resistor *R* (20 MΩ for SC or 10 MΩ for TS). The discharge voltage was measured by the HV probe *Tektronix P6015A* and the discharge current was measured as a voltage drop across 50 or 1.2 Ω resistors for SC and TS, respectively. The electrical parameters were processed and recorded during the experiments by a 200 MHz oscilloscope *Tektronix TDS 2024C*. Typical current and voltage waveforms and other discharge characteristics of SC and TS discharge with water electrospray or water cathode, were documented in detail in our previous publications [3,4,9,10]. Both TS and SC can be operated in the same versatile setup with the same HV power supply, which represents an advantage for practical applications. Both discharges were run either with water electrospray (ES) that enabled the water flow with various flow rates in the range 0.01-1 mL/min by the syringe pump *New Era Pump Systems NE-300* directly through the high-voltage needle electrode into the active discharge region, where it was sprayed to micrometric droplets; or the discharge hit directly the water surface with a submerged grounded cathode in the batch system (BS).

Besides operating in the open ambient air reactor, we operated the same plasma discharges in a closed reactor (50 mL volume) with a defined low air flow rate (0.5 L/min). This air flow rate determines the gas mixing and accumulation of species produced by the plasma.

Gaseous NO and NO₂ concentrations were measured online by electrochemical gas sensors *Membrapor NO2/S-1000* and *NO/SF-1000* (resolution 5 ppm, 0-1000 ppm). Fourier transform infrared (FTIR) absorption spectrophotometer *Shimadzu IRAffinity-1S* was used for the detection of gaseous nitrogen oxides NO, NO₂ and N₂O; nitric and nitrous acids HNO₃, HNO₂, and ozone O₃ inside a 10 cm long gas cell. Ozone concentrations were measured by UV absorption using 253.8 nm mercury lamp and the compact fiber optic spectrometer *OceanOptics SD2000*, employing the Lambert-Beer law

with the absorption cross section $1.14 \times 10^{-21} \text{ m}^2$ [11] in a 12.5 cm gas cell.

The detection of aqueous RONS in the PAW is challenging due to the chemical instability of the detected RONS and possible cross-reactivities of the used analytical methods. We tested and adapted colorimetric methods for special PAW conditions, such as colorimetric detection of H_2O_2 by TiOSO_4 reagent, NO_2^- and NO_3^- by Griess reagents, and O_3 by indigo blue dye [12]. Here we focus on the detection of RONS formation induced by air plasma gas-liquid chemistry in PAW, namely to H_2O_2 , NO_2^- and NO_3^- , and dissolved O_3 produced in PAW by the two discharges.

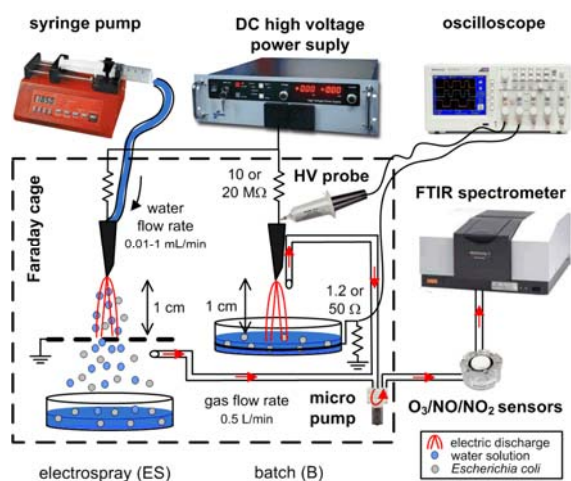


Fig. 1. Schematic diagram of the set-up used for water activation by electrospray or batch treatment through air streamer corona or transient spark discharges and gaseous diagnostics.

Bacterial suspensions of planktonic *Escherichia coli* ATCC25922 with initial concentration $\sim 10^7$ CFU/mL were prepared. We treated bacterial suspension directly by TS/SC with water ES or in BS. The bacterial inactivation was evaluated by classic thermostatic cultivation on Petri dishes with LB agar.

3. Results and discussion

Air discharges

Both discharges used in this study are DC driven, but self-pulsing. Their typical characteristics are:

Streamer corona (SC): the mean power 0.2-0.4 W, the pulse repetition frequency ~ 10 kHz, operated with water electrospray (ES) flow rates 0.01-0.5 mL/min and typical voltage ~ 10 kV and current pulses ~ 20 -30 mA of 100-200 ns duration.

Transient spark (TS): the mean power 1.5-2.3 W, the typical pulse repetition frequency ~ 1 kHz, the typical current pulse amplitude 25 A and duration ~ 25 ns without water. The current pulses were similar for TS operated with water electrospray flow rates 0.5-1 mL/min. In the

batch treatment, the current pulses were of lower amplitude ~ 2.2 A but longer ~ 200 ns due to the additional resistance in the circuit imposed by the liquid.

Gaseous RONS in air

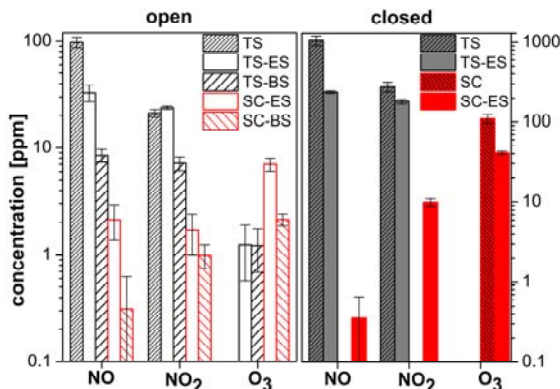


Fig. 2. Gaseous NO, NO₂ and O₃ concentrations generated by TS and SC discharges, without and with water ES and in water BS, in the open and closed (50 mL, 0.5 L/min air flow) reactors. Logarithmic scale. Statistical mean values with standard error of the mean.

In air SC, both O₃ and NO_x are produced [13]. Similar to air surface DBDs, low power discharge leads dominantly to O₃ production, while higher power discharges increase the gas temperature which promotes NO_x production and the thermal depletion of O₃ combined with the chemical decay of O₃ by fast reaction with NO. In addition to O₃ and NO_x gaseous products, water vapors in air significantly influence the plasma induced gas-phase chemistry, especially thanks to highly reactive hydroxyl ($\cdot\text{OH}$) radicals. We detected lower concentrations of the gas phase NO, NO₂ and O₃ in air discharges with water compared to the dry air. When the discharges are operated with water electrospray, there is a strong water evaporation and humidification of the air, which enhanced the $\cdot\text{OH}$ formation. Moreover, the transport of NO, NO₂, O₃, and other species such as HNO₂ into the bulk water, i.e. their solvation driven by the Henry's law equilibria, also decrease NO, NO₂ and O₃ concentrations in the gas phase.

The water electrospray improves the gas-liquid transport of the gaseous NO_x into the liquid resulting in NO_x dissolution in the water. In the batch system, especially with SC discharge, the ionic wind enhanced the RONS transfer into the liquid, despite its significantly lower surface to volume ratio (if compared with ES). NO formation in SC-ES was considerably lower compared to TS-ES due to the much lower delivered power. NO₂ formation in general increased with the discharge power in all systems and SC generated considerably lower NO₂ due to the lower discharge power.

Comparison of the NO, NO₂ and gaseous O₃ concentrations produced by TS and SC, without and with ES and in BS, in open and closed systems together is shown in Fig. 2. Apparently, the closed reactor resulted in considerably higher (~1 order of magnitude) concentrations of all measured species for both TS and SC. The closed reactor with a slow air flow rate enabled accumulation of species, whereas immediate dilution of species with the surrounding ambient air occurs in the open reactor.

It is clear that SC in all systems generated lower NO and NO₂ (due to its lower power) but higher O₃ concentrations than TS. SC corresponds well to the low power ozone mode and TS to the high power NO_x mode of the surface air DBD described by [14]. O₃ was completely absent in the NO_x-dominated TS without water and in TS or TS-ES in the closed reactor.

SC in the closed reactor produced negligible NO concentrations, lower than in the open reactor. At the same time, there was considerably more O₃ produced by SC in the closed reactor. The reaction of NO oxidation by O₃ depleted most of the generated NO that was oxidized to NO₂, while the excess O₃ remained in the gas. Detailed gas-phase air plasma chemistry is described in [9].

Aqueous RONS in PAW

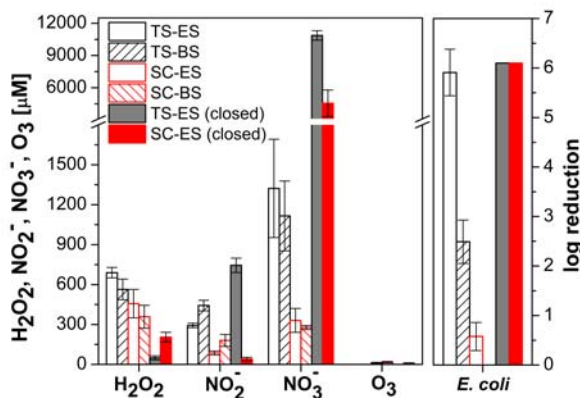


Fig. 3. Concentrations of aqueous H₂O₂, NO₂⁻, NO₃⁻ in PAW and corresponding pH for TS and SC with water ES and in BS, in the open and closed reactor. Statistical mean values with standard error of the mean.

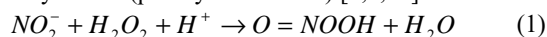
Both air discharges, SC and TS, with water electrospray or in batch treatment in both open and closed reactors were tested and by this way prepared the plasma activated water (PAW). We focused on the detection of long-lived aqueous RONS produced in PAW, namely H₂O₂, NO₂⁻ and NO₃⁻, and dissolved O₃, shown in Fig. 3. Clearly, the aqueous RONS concentrations are related to the plasma formed gaseous RONS.

Aqueous H₂O₂(aq) is produced by extremely fast dissolution of gaseous H₂O₂(g) formed in the gas. The

Henry's law solubility coefficient of H₂O₂ ($k_H \approx 9 \times 10^2$ mol.m⁻³.Pa⁻¹) is about 7 orders of magnitude larger than that of NO or NO₂ or O₃ [46], thus all H₂O₂(g) readily transfers into H₂O₂(aq) through the gas-liquid interface.

Nitrites NO₂⁻ and nitrates NO₃⁻ are generated in the PAW from the dissolved gaseous NO and NO₂ [10,24,62]. The solubility coefficients of NO or NO₂ are much smaller than that of H₂O₂ [15], thus NO(g) and NO₂(g) would not readily transfer into water to form NO₂⁻(aq) and NO₃⁻(aq). Enhancement of NO_x dissolution by increasing the surface area of the plasma-liquid interface by spraying water into fine droplets in the ES system is helpful. Since protons H⁺ are released in the PAW by the above reactions, acidic pH~3.3 is typical for PAW prepared by air plasmas.

The reaction between H₂O₂ and NO₂⁻ occurs under acidic PAW conditions and leads to the formation of peroxynitrites (peroxynitrous acid) [4,7,12]:



ONOOH then decomposes at acidic pH to •OH and •NO₂ radicals [7,12]:

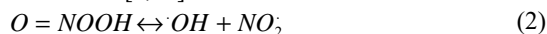


Fig. 3 shows the measured concentrations of H₂O₂, NO₂⁻, NO₃⁻ in PAW and corresponding antibacterial effect for TS and SC with water ES or in BS in the open and closed reactor. The low power SC generates less H₂O₂ and much less NO₂⁻ + NO₃⁻. The higher power TS generates both H₂O₂ and NO₂⁻ + NO₃⁻, with the ratio of H₂O₂/NO₂⁻ approximately 2 in the open system. On the other hand, after TS water activation in the closed system, much less H₂O₂ and much more NO₂⁻ and particularly high NO₃⁻ were detected, which resulted in very acidic pH (2.4). In such case, the antibacterial effects might have been enhanced.

NO₂⁻ was higher in TS than SC, and mostly increased with energy density per water volume. Closed TS resulted in very high NO₂⁻ and NO₃⁻. SC in the closed reactor also generated high NO₃⁻ (yet lower than TS-ES closed) at pH 3.1, while very low NO₂⁻. This might be possibly due to the NO₂⁻ depletion with ozone.

H₂O₂ is dominantly formed from •OH radicals in the gas and solvates extremely. We should note that in the ES, there is a good gas-liquid mixing that might enhance the aqueous peroxynitrite chemistry (Eq. 1 and 2), which would then faster deplete the produced H₂O₂(aq). The measured H₂O₂(aq) concentrations after treatment may be then lower with respect to the H₂O₂(aq) really produced during the plasma-water interaction. Higher NO₃⁻ were reached in TS than SC, which can be certainly related with considerably higher gaseous NO and NO₂ production in TS (Fig. 2).

O₃(aq) concentrations in PAW increased as a function of energy density in the open reactor with SC-ES. Dissolved ozone concentrations O₃(aq) in PAW were detected in SC. There was almost negligible gaseous O₃ detected in TS, and consequently undetectable O₃ dissolved in PAW either.

4. Summary

The potential use of plasma activated water and liquids is constantly growing in various biomedical and agriculture applications. The PAW chemical properties and effects strongly depend on the plasma sources, discharge regimes and their interaction with water. We compared two non-thermal atmospheric plasma sources operating in air and interacting with water: a lower power streamer corona (SC, 0.4 W) and a higher power transient spark (TS, 2 W). We analyzed gaseous and aqueous RONS in the air and the PAW. Water was activated in the electrospray system (ES) with fine aerosol droplets sprayed through the plasma zone or in water batch system (BS) where ionic wind plays a role. We also compared the open air and closed small volume reactor at different air flow rates to understand the effects of reactor volume and air flow rate on the gaseous and aqueous chemistry.

Both SC and TS represent inexpensive and easy to operate nonequilibrium air plasma sources that can be run in the same versatile setup. Switching between them can be easily managed by changing the resistor in the circuit. Each of these discharge regimes generates air plasma of different properties that results in different gaseous products: dominated by O₃ in lower power SC and NO_x in higher power TS. The gaseous products, their production and solvation rates determine the aqueous RONS in the activated water, which then control the antibacterial effects of such PAW. Gas flow conditions in the reactor, as well as the type of interaction with water (electrospray vs. batch) strongly influence gaseous and aqueous RONS production. The water electrospray through the discharges is more efficient to transfer the plasma gaseous species into the PAW. Other ways of water interaction with air plasmas can lead to different transfer mechanisms of some species (especially those with low solubility), which may influence the aqueous chemistry and antibacterial effects and are being subjected to our future studies.

5. Acknowledgement

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6. References

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