

Article

Electrochemical Reactions at the Boundary Areas Between Cold Atmospheric Pressure Plasma, Air, and Water

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Abstract: A cold atmospheric-pressure He-plasma jet (CAPPJ) interacts with air and water, producing reactive oxygen and nitrogen species (RONS), including biologically active ions, radicals, and molecules such as NO_x , H_2O_2 , HNO_3 , HNO_2 , and O_3 . These compounds can activate interfacial redox processes in biological tissues. The CAPPJ can oxidize N_2 to HNO_3 and water to H_2O_2 at the interface between plasma and water. It can also induce the oxidation of water-soluble redox compounds in various organisms and in vitro. This includes salicylic acid, hydroquinone, and mixtures of antioxidants such as L (+)-ascorbic acid sodium salt with NADPH. It can react with redox indicators, such as ferroin, in a three-phase system consisting of air, CAPPJ, and water. Without reducing agents in the water, the CAPPJ will oxidize the water and decrease the pH of the solution. When antioxidants such as ascorbate, 1,4-hydroquinone, or NADPH are present in the aqueous phase, the CAPPJ oxidizes these substances first and then oxidizes water to H_2O_2 . The multielectron mechanisms of the redox reactions in the plasma-air/water interfacial area are discussed and analyzed.

Keywords: antioxidants; cold plasma; interfacial catalysis; multielectron reaction; plasma-activated water; reactive oxygen and nitrogen species; surface chemistry



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1. Introduction

Cold atmospheric-pressure plasma (CAPP) is a partially ionized gas that operates at atmospheric pressure. It consists of positively and negatively charged particles, radicals, neutral molecules, and UV-VIS photons. The number of applications of CAPP for the treatment of plants, seeds, fruits, and other materials in agriculture has increased over the past two decades [1–7], as well as its use in medicine [8–13] for the treatment of cancer, dermatology, dentistry, disinfection, and transdermal drug delivery via plasma poration through human skin [2,14]. Additionally, CAPP has been used to improve seed germination by plasma processing of seed shells [1,2].

Poration refers to the formation of pores on the surface of a material or within a structure. This occurs when the magnitude of the electric fields generated by CAPP exceeds the threshold for electroporation. When applied to seeds, CAPP can induce corrugation, hydrophilization, and pore formation, leading to improved germination and water uptake through intracellular penetration of electric fields and reactive oxygen–nitrogen species (RONS) [1,2,10].

RONS can participate in redox and photochemical reactions with biological tissues from various organisms, but these processes are not well understood, as both cold plasma and biological tissues are complex multicomponent systems. Most of RONS generated by the plasma jet are generally unstable and have a short lifespan. The most common and relatively stable products include nitric acid (HNO_3), hydrogen peroxide (H_2O_2), nitrous acid (HNO_2), ozone (O_3), and nitrogen oxides (Figure 1). Additionally, several studies have been conducted on the interaction between cold plasma and individual chemical compounds [15–19]. It will be important to further investigate redox processes between cold

plasma and biologically active molecules such as salicylic acid (a plant hormone), aspirin (a pain reliever), vitamin C, quinones, amino acids, and proteins. Plasma is used in biomedical research directly on multicomponent biological tissues. To clarify the mechanisms, it is necessary to know both the mechanisms of plasma interaction with individual components *in vitro* and the mechanisms of interaction with several components in a mixture. Studies of the mechanisms of plasma-induced processes in biological tissues and surfaces can help to neutralize or prevent side effects in medicine, cosmetology, agriculture, and the food industry.

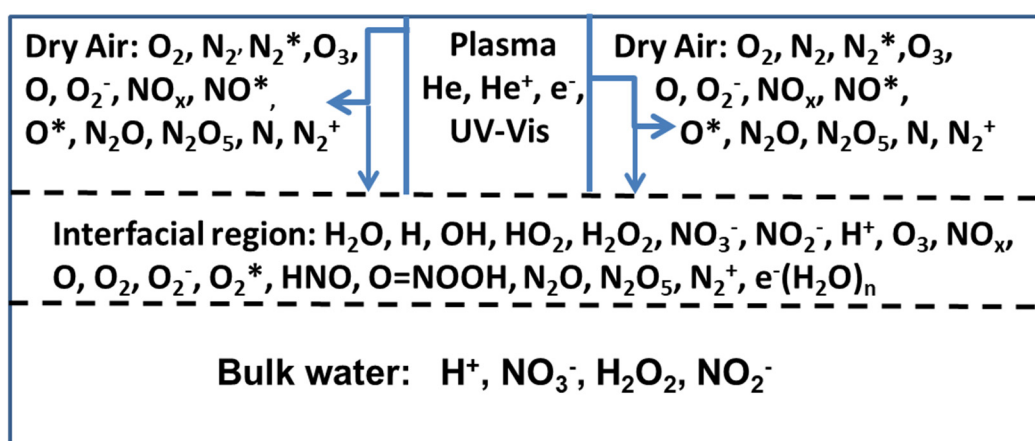


Figure 1. Redox chemicals between cold atmospheric pressure He-plasma jet, air, and water. Asterisks (*) indicate radicals.

The main aim of this work is to study multicomponent interfacial redox reactions of water-soluble biologically active molecules *in vitro* at the fluid interfaces between cold plasma, air, and water.

2. Materials and Methods

2.1. Chemicals and Test Strips

Salicylic acid, β -nicotinamide adenine dinucleotide 2'-phosphate tetrasodium salt hydrate (NADP⁺), β -nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH), hydrogen peroxide, ferriin indicator aqueous solution, silver nitrate, 1,4-hydroquinone, and L (+)-ascorbic acid sodium salt (vitamin C) were purchased from Sigma-Aldrich (Cleveland, OH, USA). HNO₃ was purchased from Thomas Scientific (Swedesboro, NJ, USA). Test strips for H₂O₂ (0–100 ppm), nitrate (0–500 ppm), and nitrite (0–25 ppm) were purchased from Bartovation LLC (New York, NY, USA). These strips were used to determine the content of H₂O₂, NO₂[−] and NO₃[−] in water after treatment with CAPPJ. Ultra-high purity bottled helium was purchased from Sexton Welding Supply (Huntsville, AL, USA).

2.2. Cold Plasma

The CAPPJ source consists of a nested pair of quartz tubes with a central tungsten rod and an outer steel ring (Figure 2). The rod and ring served as the powered and grounded electrodes, respectively. The outer tube, which has a diameter of 4 mm and an outside diameter of 6 mm, is open on both ends. The inner tube, with a 1 mm diameter and a 2 mm outer diameter, has a closed end and is inserted into the outer tube. A tungsten rod, with a diameter of 1 mm, is placed inside the inner tube and rests against the sealed end. The tubes are held in place by a plastic Tee compression fitting (Swagelok, Birmingham, AL, USA, NY-400-3). A grounded ring electrode is placed near the end of the outer tube. Gaseous helium is ionized by the strong electric fields between the electrodes, forming a plasma jet of approximately 4 cm in length, measured from the outlet of the outer tube [2].

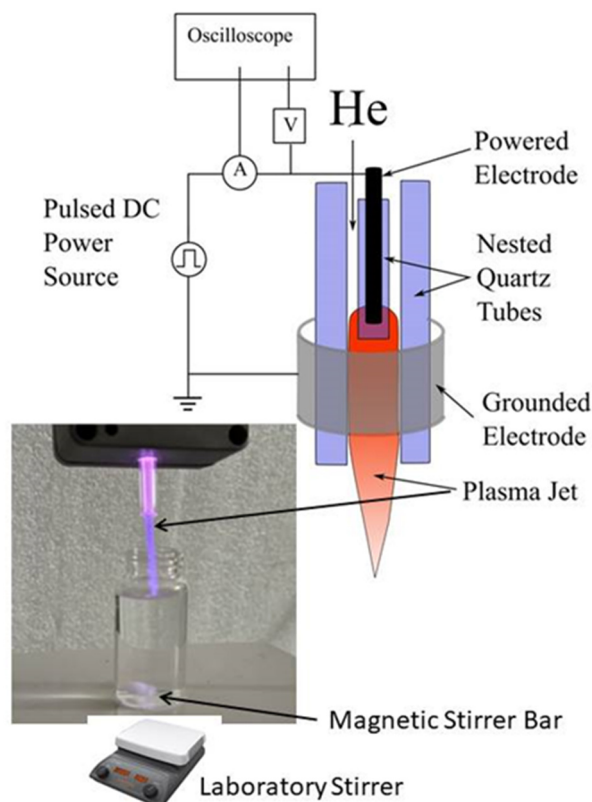


Figure 2. Cold atmospheric pressure He-plasma jet.

The treatment was conducted in close contact between the plasma and the water surface. Each sample of an aqueous solution in a 20 mL clear borosilicate glass vial was thoroughly stirred with a magnetic stirrer during the CAPPJ treatments (Figure 2). The diameter of the water surface was 15 mm.

The plasma was powered with a high-voltage pulsed DC system consisting of a Matsusada AU-10P60 10 kV DC power supply (Matsusada Precision Inc., Shiga, Japan), a IXYS PVX-4110 pulse generator (DEI, Fort Collins, CO, USA), and an SRS DG-645 digital delay generator (Rigol Technologies Inc., Beaverton, OR, USA). The system was operated with an 8 kV pulse amplitude, 6 kHz pulse frequency, 1 μ s pulse width, and approximately 70 ns pulse rise and fall time. The pulse voltage and current were measured from the pulse generator and had a square pulse shape with a slight sloping during the rising edge. The current trace had two distinct current pulses at the rising and falling fronts of the voltage with damping oscillations after the main current peaks due to both displacement and conduction currents.

2.3. Temperature Control

The Etekcity laser grip 800 Digital Laser Temperature Gun (Etekcity, Anaheim, CA, USA) was used to measure the temperature of the plasma jet, water, plants, and air. The temperature of the plasma jet, air, and aqueous solutions or water during plasma treatment was 20 °C.

2.4. Absorption Spectra

Absorption spectra were recorded using 1 cm quartz cuvettes on Genesis 180 spectrophotometers (Thermo Fisher Scientific, Waltham, MA, USA) and the Shimadzu UV-Vis spectrophotometer ISR-260 Plus (Shimadzu, Kyoto, Japan).

2.5. Statistics

All experimental results were reproduced at least 16 times. SigmaPlot 15 software (Systat Software, Inc., San Jose, CA, USA) was used for statistical analysis of the experimental data.

3. Results and Discussion

3.1. Redox Reactions at the Plasma/Air and Plasma/Water Interfacial Regions

When plasma contacts water and air, nitrogen fixation occurs, forming RONS, ions, and molecules in plasma-activated water [17]. The most stable products of these interfacial redox reactions are HNO_3 (Figure 3) and H_2O_2 (Figure 4A) with minor amounts of HNO_2 . The concentration of HNO_3 increased linearly with time of exposure to plasma (Figure 3A,B).

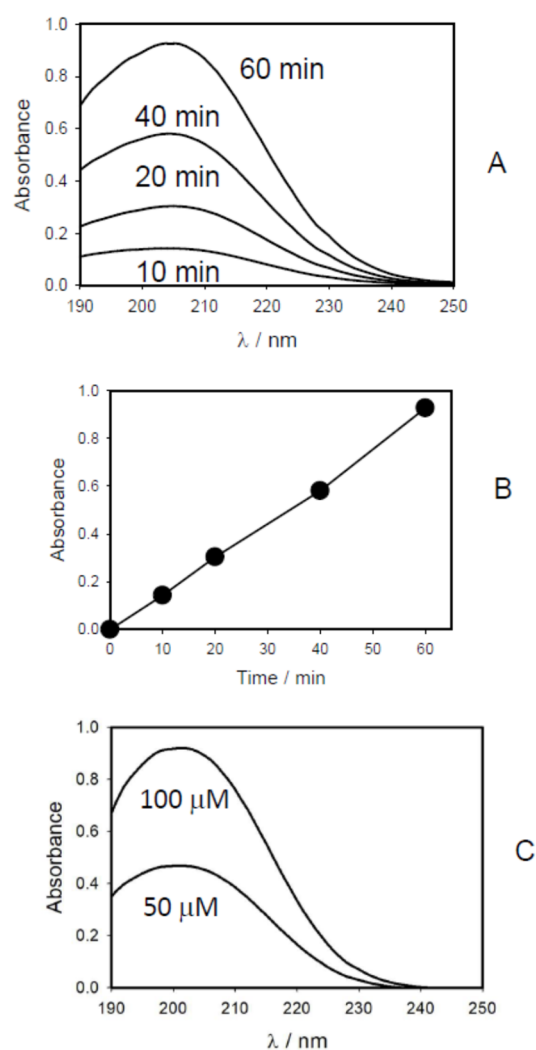


Figure 3. (A): Absorbance of the aqueous phase after treatment with cold He-plasma jet. (B): Time dependence of adsorption of aqueous phase at the wavelength of 204 nm. (C): Absorbance of aqueous solutions of HNO_3 .

Figure 3A shows the absorbance of the aqueous phase after treatment with CAPPJ. Figure 3C shows the absorbance of the aqueous solution of HNO_3 at different concentrations without any treatment with CAPPJ. In our previous paper [15], we used test strips to approximately determine the concentration of NO_3^- (25 ppm) and NO_2^- (1 ppm) water after treatment with CAPPJ. A decrease in the pH of an aqueous solution during plasma treatment occurs at the plasma/water interface. This is indicated by pH indicators such as methyl red, universal indicator solution, and bromocresol violet [15,17]. The pH decreases

from 6 to approximately 4, depending on the volume of the aqueous phase and the surface area of the water [15]. The acidification occurs at the plasma/water interface, and the acid then diffuses into the aqueous phase.

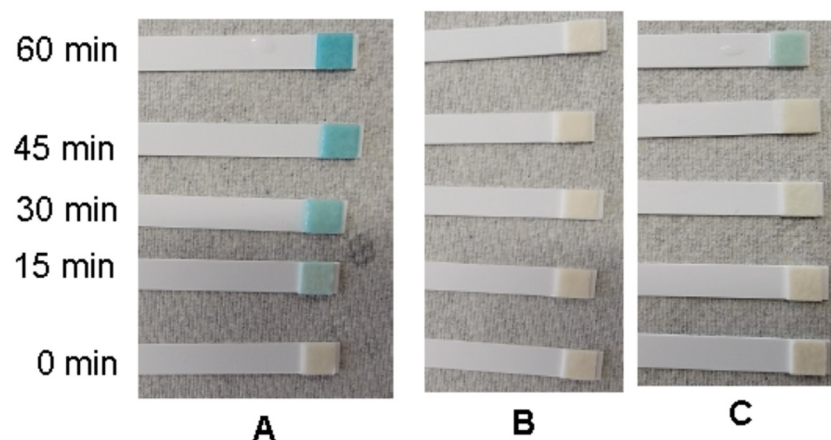
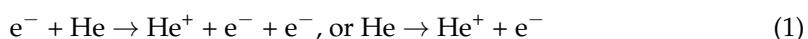


Figure 4. Detection with test strips H_2O_2 production during the treatment of water (A), 0.25 mM sodium ascorbate (B), and 50 μM 1,4-hydroquinone aqueous solution (C), with cold atmospheric pressure He-plasma jet. These strips were used for the detection of H_2O_2 in water after the treatment with cold atmospheric pressure He-plasma jet. The plasma treatment time of the aqueous solution in experiments (A–C) is indicated for each detection strip case (A–C). The test strips acquire the specific color only in the presence of specific substrates, such as H_2O_2 .

Figure 4 demonstrates that the presence of reducing compounds in the aqueous phase, such as ascorbate (Figure 4B) or hydroquinone (Figure 4C), temporarily inhibits the formation of hydrogen peroxide caused by the oxidation of water using a cold plasma. Ascorbate and hydroquinone act as powerful antioxidants due to their ability to reduce oxidized forms of the compounds that can be formed during plasma treatment.

Cold atmospheric pressure He-plasma jet ionizes He-gas and produces plasma:



Plasma interacts with dry air:

Nitrogen oxidation at the cold plasma/air interfacial region:



Oxygen reduction at the cold plasma/air interfacial region:



Nitrogen oxides formation at the cold plasma/air interfacial region:



Ozone formation from oxygen:

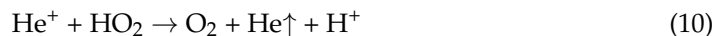
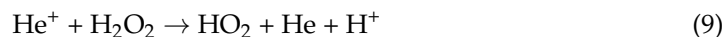
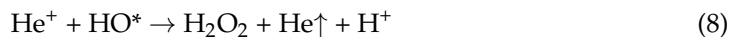
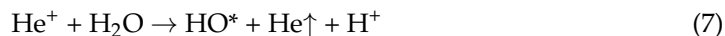


Ozone can oxidize nitric oxides NO_x :



The equations for redox reactions (1–4, 6–31) and Figure 5 are consistent with the thermodynamic data on redox potentials found in reference books. Figure 5 shows thermodynamics of possible pathways of water oxidation and/or water reduction induced

by CAPPJ. The source of protons according to the thermodynamic diagram is H₂O only (Figure 5). Possible reactions of water oxidation and acidification at the plasma/water interface are as follows:



or synchronous 2-electron reaction of hydrogen peroxide formation:

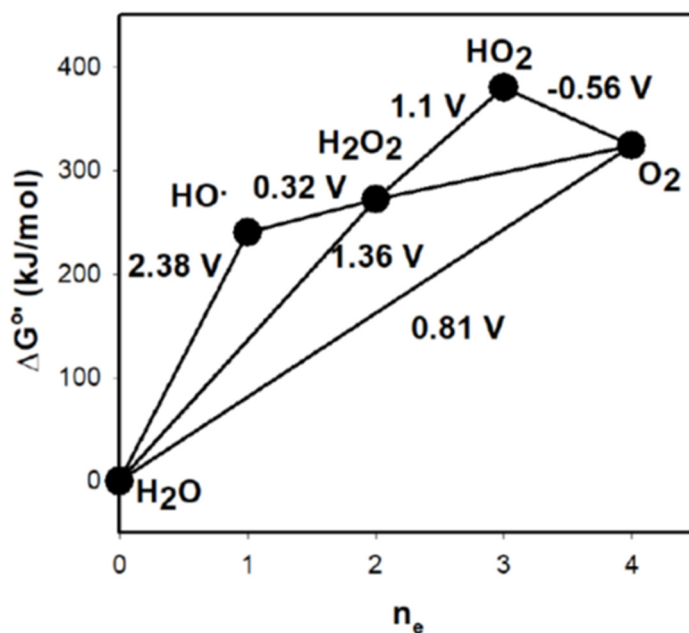
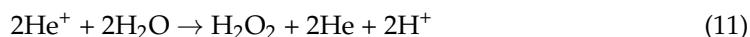
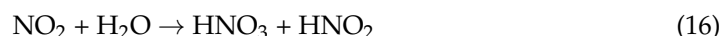
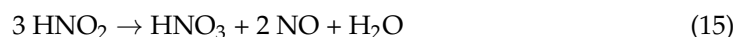
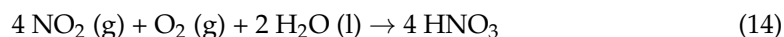
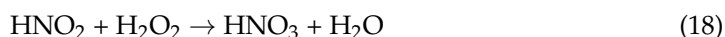


Figure 5. Gibbs energy and redox potentials scheme for the water oxidation at pH 7 are shown. The number of involved electrons is n_e . Redox potentials were taken from the Handbooks of Chemistry and Physics. The Gibbs energy was calculated as $\Delta G = -n_eFE$, where n_e is the number of electrons, F is the Faraday constant, and E is the redox potential at pH = 7.

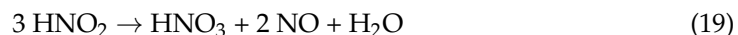
Thermodynamically possible reactions of nitric acid formation at the interfacial area between plasma, air, and water are as follows:



The lifetime of the majority of RONS in the aqueous solution is very short. The most stable product is the nitric acid with a small amount of HNO₂ and H₂O₂, which can interact during the redox reaction between them:



In water, nitrous acid also can be decomposed into nitric oxide and nitric acid:



Salicylic acid is a plant hormone that plays a role in regulating many physiological processes. It is involved in seed germination, flowering, and the synthesis of other hormones such as gibberellins. It also helps plants respond to pathogen infections and environmental stressors, such as drought and heat. This hormone was found in the bark of white willow trees, and it has antiseptic properties that make it widely used in medical and food preservation applications. Topical applications of salicylic acid are commonly used due to its keratolytic (skin-softening), bacteriostatic (bacteria-stopping), fungicidal (fungus-killing), and photoprotective (sunscreen) properties. Salicylic acid, which is used in the pharmaceutical industry for the synthesis of aspirin (acetylsalicylic acid $\text{C}_9\text{H}_8\text{O}_4$), exists in plants in the form of esters. Under stress, it interplays with reactive oxygen species (ROS) as a signaling molecule, inducing genetically controlled defense-related mechanisms and expression of genes that provide defense against stress.

The main industrial method for its synthesis is phenol carboxylation. Collado et al. [20] proposed a mechanism for the noncatalytic oxidation of salicylic acid through decarboxylation to phenol. Cold atmospheric He-plasma jet oxidizes salicylic acid very slowly (Figure 6).

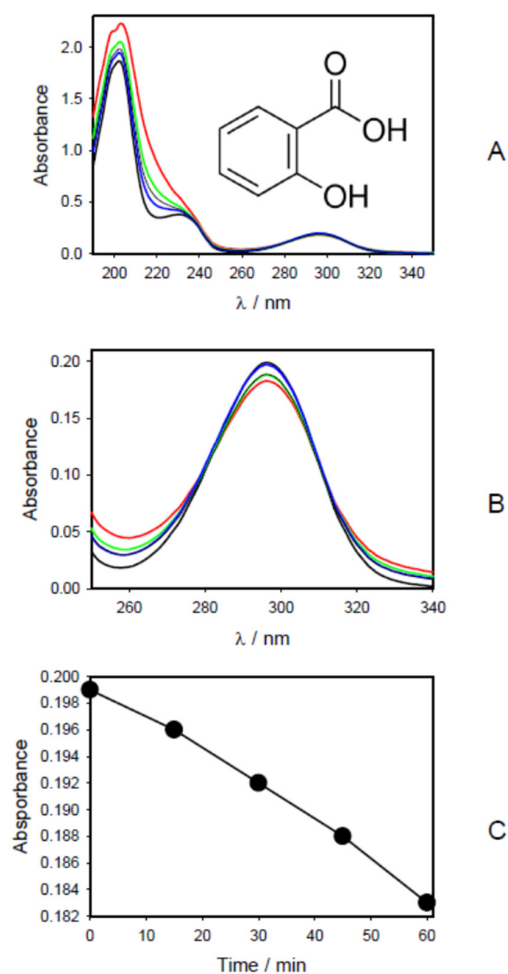


Figure 6. Absorption spectra of an aqueous solution containing 50 μM of salicylic acid after treatment with CAPPJ. Lines: black 0 min, blue 15 min, grey 30 min, green 45 min, red 60 min. Figure (B) shows an enlarged portion of the upper graph (A). The dependence of the absorption at 296 nm on the treatment time of the aqueous solution with CAPPJ is shown in Figure (C).

Ferriin/ferritin is known as a redox indicator system. Figure 7 shows the absorption spectra of aqueous solutions of ferriin before and after treatment with CAPPJ. There is an isosbestic point of 388 nm (Figure 7). CAPPJ oxidizes ferriin slowly:

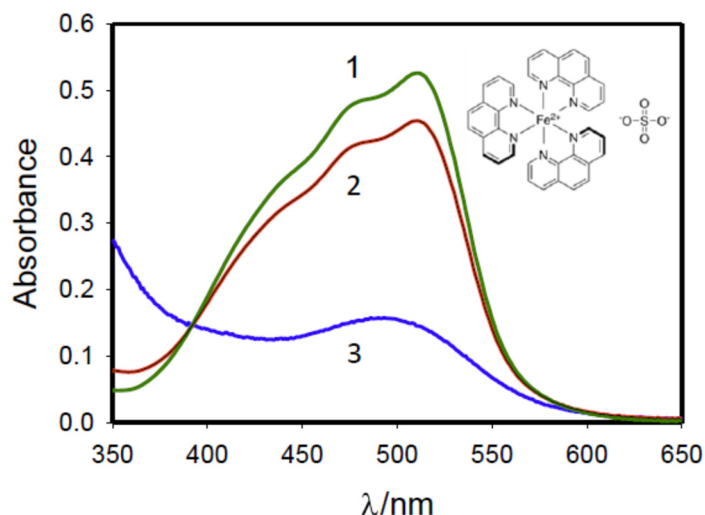
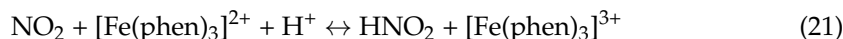
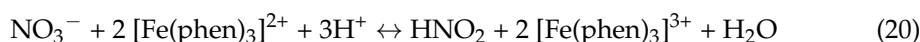
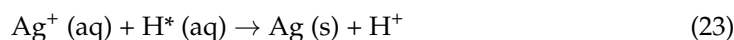
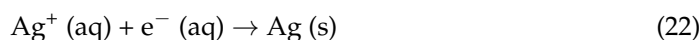


Figure 7. Absorption spectra of an aqueous solution containing ferriin at a concentration of 50 μM ferriin before (1) and after (2,3) treatment with cold atmospheric pressure He-plasma jet. Time of treatment: 1—0 min, 2—1 h, 3—5 h.

3.2. Traps for Plasma Electrons

It is interesting to investigate the presence of hydrated electrons in the surface layer of water, as plasma contains free electrons. AgNO_3 and NADP^+ were chosen as electron traps. Nakamura et al. [21] produced silver nanoparticles by highly intense laser irradiation of aqueous solutions of silver nitrate. The authors [21] proposed a mechanism for the reduction of Ag^+ by hydrated electrons and/or hydrogen radicals formed during the laser irradiation of an aqueous solution.

The reaction for the formation of a metallic mirror is well known in analytical chemistry, where Ag^+ can be reduced to metallic silver by exposure to weak reducing agents:



Ag^+ can be also oxidized:



The redox potentials of He^+ and some components of RONS are sufficient to oxidize silver into oxides Ag_2O , AgO , and Ag_2O_3 (Figure 8B).

Free electrons in cold plasma (Equation (1)) can reduce oxygen (Equation (3)), but the reduction of Ag^+ was not detected (Figure 8A). Figure 8A shows that there is no influence of Ag^+ on HNO_3 production (maximum at 204 nm) and there is also no Ag^+ reduction by plasma. The acidification of the AgNO_3 aqueous solution during plasma treatment is similar to the decrease in pH during treatment without AgNO_3 [17].

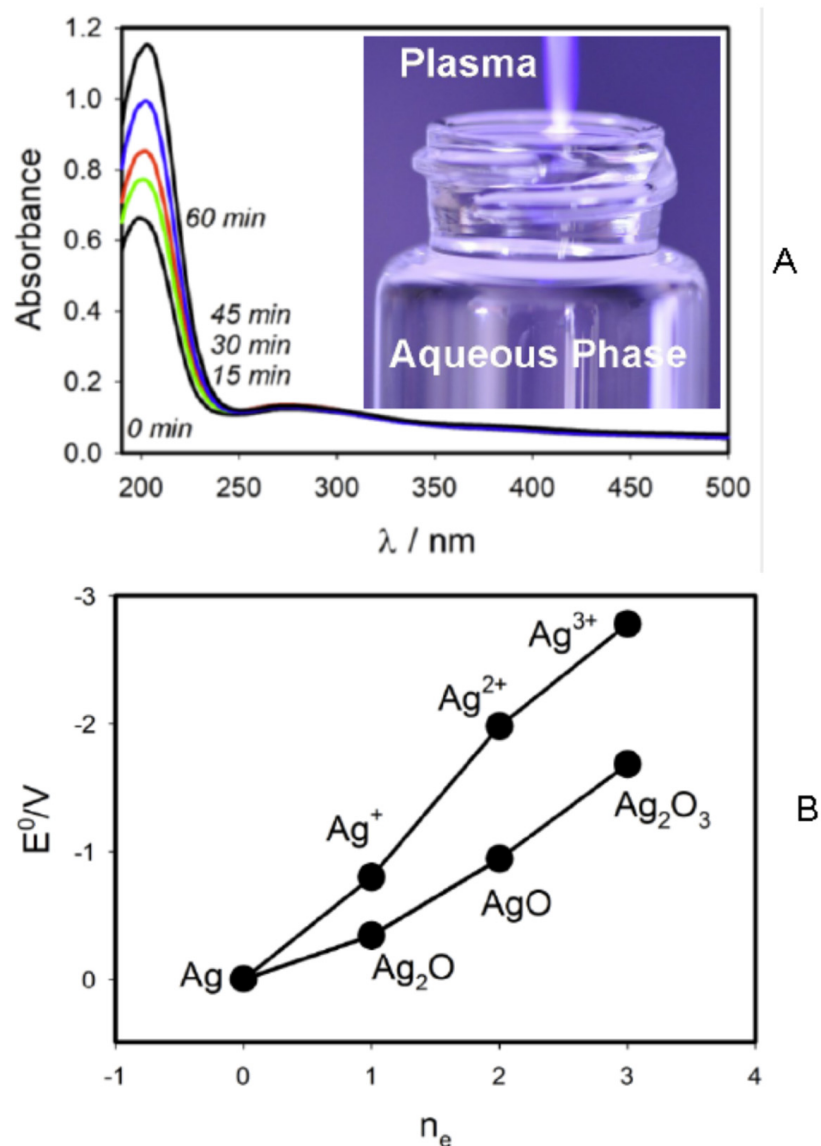


Figure 8. (A): Absorption spectra of aqueous solution containing 50 μM AgNO₃ before and after treatment with cold atmospheric pressure He-plasma jet. (B): Standard redox potentials scheme for silver redox reactions at pH 7; n_e is the number of electrons.

The redox pair NADP⁺/NADPH plays a role in various reactions in photosynthetic organisms. β-nicotinamide adenine dinucleotide 2'-phosphate tetrasodium salt hydrate (NADP⁺) is the terminal electron acceptor in the photosynthetic electron transport chain and is converted to β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) under light conditions. NADP⁺ can also be considered as a trap for electrons. However, absorption spectra (Figure 8) do not show the reduction of NADP⁺ to NADPH according to the following equation:



CAPPJ is unable to reduce NADP⁺ and Ag⁺ despite the presence of free electrons in cold plasma (Figures 8A and 9).

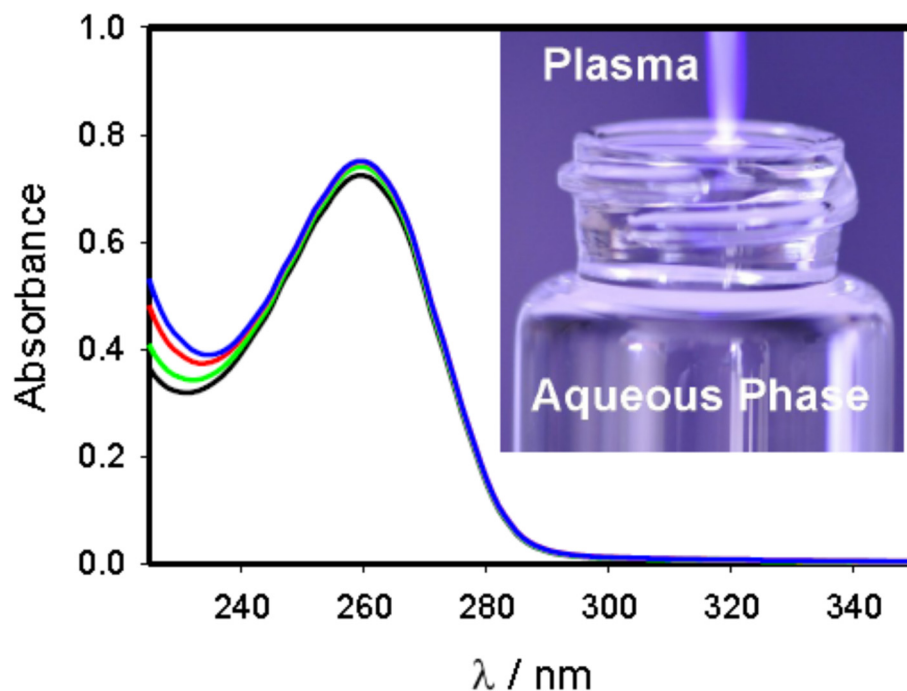
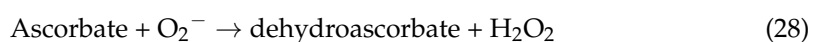


Figure 9. Absorption spectra of 50 μM NADP^+ aqueous solution before (black) and after treatment with CAPPJ for 15 min (green), 30 min (red), and 45 min (blue).

3.3. Treatment of Antioxidants Mixture with Cold Plasma

In nature, there are always several systems to balance oxidative impacts. Moreover, in a multi-component system, various reaction partners are present and will interact with plasma-generated RONS [15–17,22–29]. It is well documented that vitamin C, NADH/NAD^+ , $\text{NADPH}/\text{NADP}^+$, as well as O_2 , and other redox components, are required to balance oxidative stress and protect cellular structures. It is important to study the reactions between individual biologically active molecules and cold plasma to understand the mechanisms of plasma-induced redox processes in biological tissue and evaluate possible side effects. Perhaps the treatment of mixtures of different components with cold plasma could result in different kinetics of oxidation depending on the redox potentials of components. Cold plasma is used directly in biomedical research on multicomponent biological tissues. To clarify the mechanisms, it is necessary to know both the mechanisms of interaction between plasma and individual components *in vitro*, and the mechanisms for several components in combination. Plasma oxidation of ascorbic acid was detected in previous studies, but its degradation may be much less in mixtures with other antioxidant components in biological systems [3,15].

We have previously found [15,17], using indicators, that redox reactions between plasma, air, and aqueous solutions occur on the aqueous surface, but not in the bulk liquid phase. The presence of RONS requires water, oxygen, nitrogen, and plasma. Plasma oxidation of ascorbic acid has also been observed *in vivo* [3,18], although the degradation may even be less when combined with other antioxidants in biological systems. It may degrade even less when mixed with other antioxidant molecules in biological systems:



and reverse reduction reaction in the presence of antioxidants:



Semidehydroascorbate radical can be reduced to ascorbate by NADPH:

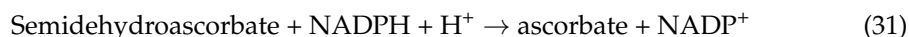


Figure 10 shows that in a mixture of NADPH and sodium ascorbate, NADPH is oxidized first, preventing the oxidation of ascorbate. Ascorbate in an aqueous solution protects water from oxidation (Figure 4B).

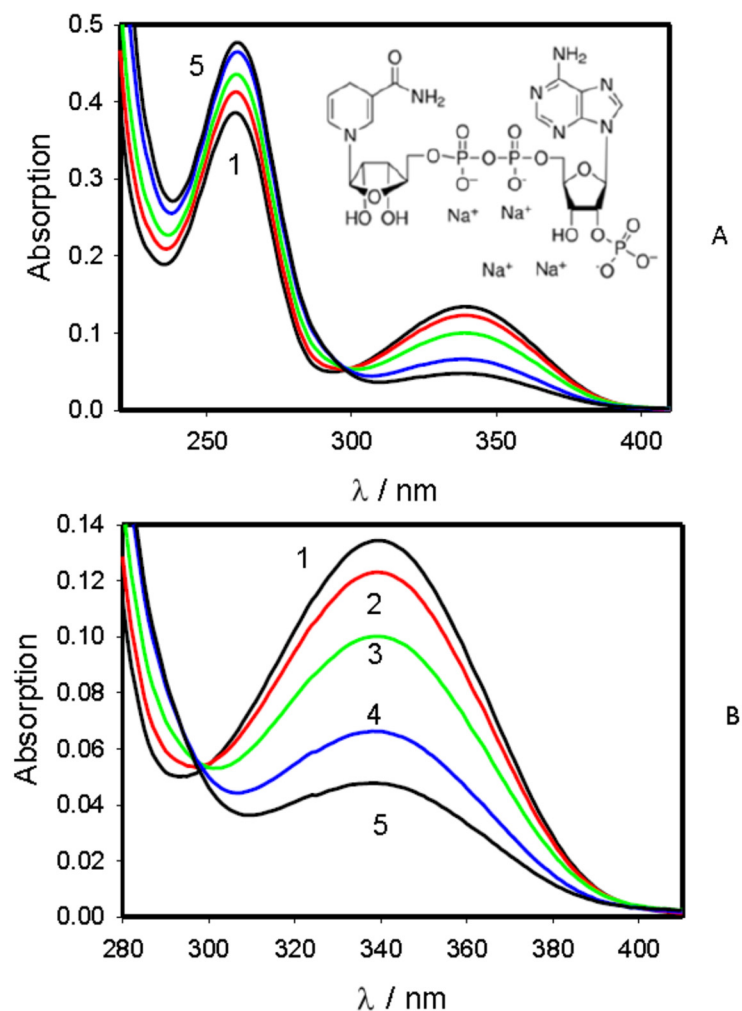


Figure 10. (A) Absorption spectra of 25 μM NADPH and 0.125 mM sodium ascorbate aqueous solution before (1) and after treatment with cold atmospheric pressure He-plasma jet for 15 min (2), 30 min (3), 45 min (4), and 60 min (5). (B) A magnified part of the upper graph (A).

4. Conclusions

We found earlier [17], using indicators, that reactions between plasma, air, and aqueous solutions take place at the aqueous interface, but not in the bulk of liquid phase. The presence of RONS, water, oxygen, nitrogen and plasma are mandatory. Despite the presence of free electrons and potential electron donors, such as O_2^- , H_2O_2 , HO^* , and HO_2 produced at the plasma/water interfaces, the CAPPJ does not reduce certain electron traps. When a mixture of antioxidants, such as ascorbate, 1,4-hydroquinone, or NADPH, is present in the aqueous phase, CAPPJ first oxidizes these substances with more negative redox potentials and then oxidizes the water to form H_2O_2 .

This field of research is topical, and the results of this study are of interdisciplinary interest.

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Conflicts of Interest: The authors declare no conflicts of interest.

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