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Specificity of detection methods of ROS/RNS in plasma activated water by air transient spark discharge

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Abstract

Plasmas generated in air and in contact with liquids generate a number of primary reactive species in the gas phase, which induce formation of secondary reactive species in the liquid phase through the gas-liquid interface. Reactive oxygen and nitrogen species such as hydrogen peroxide H_2O_2, hydroxyl radical ·OH, nitrites NO_2^-, nitrates NO_3^-, hypochlorite OCl^- and peroxynitrites $ONOO^-$ induce chemical changes in water solutions and leads to various biocidal effects on microorganisms or therapeutic effects for biomedical applications. It was shown that the acidic environment with plasma agents (especially hydrogen peroxide, nitrites, nitrates) lead to the strong bacterial inactivation [1]. The detection of reactive species in the plasma activated water (PAW) is not simple due to the presence of highly reactive species and possible cross-reactivities. Therefore is crucial to either develop new methods of their detection or adapt the methods known from analytical chemistry and biology for special conditions in PAW.

In this work we focused on the formation of reactive oxygen and nitrogen species (ROS/RNS) induced by plasma gas-liquid chemistry in treated aqueous solutions. Hydrogen peroxide, nitrites, nitrates and dissolved ozone were detected in solutions treated directly by DC-driven positive transient spark discharge generated in ambient air. Aqueous solutions with different initial pH and electrolytic conductivities were electro-sprayed through the active zone of discharge:

- **water** = NaH_2PO_4 solution (pH 5-5.5 and σ = 0.6 mS/cm) with no buffering capacity
- **PB** = Na_2HPO4/KH_2PO_4 buffer (pH 6.9 and σ = 0.56 mS/cm)

First, we focused on the nitrite detection in the PAW. Due to the possible interaction of NO_2^- and H_2O_2 in acidic condition of the PAW (peroxynitrite chemistry [1,2]), we tested the accuracy of the Griess assay. The standardized colorimetric method based on the reaction of nitrites with Griess reagents was compared with the high precision ion chromatography (IC). In addition, to avoid possible interference of hydrogen peroxide on Griess assay, we attempted to increase the detection limit of Griess assay by using the enzyme catalase as a H_2O_2 scavenger. In PAW H_2O_2 progressively reacts with NO_2^- by forming peroxynitrites and so the concentration of NO_2^- decreases in time (very fast in acidic pH). The comparison of the nitrites concentration measured by Griess assay (we tested two assays: Griess 1 prepared according [3] and Griess 2 (Nitrate/Nitrite Colorimetric Kit, Cayman Chemical)), and by ion chromatography showed no significant difference in the nitrite concentration. By addition of the enzyme catalase we showed that hydrogen peroxide does not have any influence on the Griess reagent colorimetric method for nitrites detection. The Griess assay is precise enough and it is reliable and suitable for nitrite detection in PAW.

The presence of dissolved ozone in PAW was primarily detected by standardized colorimetric method based on the bleaching process of the indigo dye in the presence of ozone [4]. Indigo was strongly decolorized without any difference not only in plasma treated solutions, but even in the simulated plasma treated water solution with similar chemical composition and pH as our PAW (1 mM H_2O_2 + 1 mM $NaNO_2$ at pH 3.3) where no ozone could have been present. Furthermore, phenol was used as a chemical probe to characterize the specific products of its degradation by the reactive species during plasma treatment [2]. Contrary to seemingly detected ozone by indigo method, the results from the phenol degradation product analyses showed no muconic acid as a specific product with ozone. This result supports the fact that the indigo method is not selective to ozone in the PAW and it seems that the hydroxyl radical as a product of peroxynitrite decay at acidic pH is responsible for the false positive reaction of the indigo method.

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