

Kinetics of peroxyxynitrite formation and its decomposition in air plasma treated liquids

P. Lukes¹, E. Dolezalova¹, M. Clupek¹, H. Jablonowski², Th. von Woedtke² and S. Reuter²

¹ Institute of Plasma Physics AS CR, Prague, Czech Republic

² Leibniz Institute for Plasma Science and Technology (INP Greifswald e.V.), Greifswald, Germany

Abstract: Formation of peroxyxynitrite (ONOOH) in air plasma-treated water and in model mixtures of H₂O₂/HNO₂ was determined using kinetic analysis of the pseudo-second-order reaction between H₂O₂ and NO₂⁻ at various acidic pH values. Decomposition of ONOOH was evaluated by EPR analysis of OH• radical formed by homolysis of ONOOH. Good correlation with OH yields estimated from ONOOH kinetics was determined.

Keywords: plasma-liquid interactions, peroxyxynitrite, hydroxyl radical, EPR

1. Introduction

Interactions between plasma and liquids become of great interest during recent years, and electrical discharge plasmas in liquids and gas-liquid environments is under investigation for a range of applications, especially those focused on biomedical applications of plasma. Various types of discharge plasmas were shown efficient in inactivation of a number of microorganisms (bacteria, spores and viruses) and new successful or potential applications of plasmas in medicine have been demonstrated. However, despite successful results, the detailed mechanisms of plasma-induced microbial and living tissue interactions are still not well understood. Depending on the type of discharge, its energy, and the chemical composition of the surrounding environment various types of physical processes (ultraviolet radiation, overpressure shock waves) and plasma-chemical reactions can be initiated and a number of primary and secondary species can be formed by plasma in the liquid either directly, or transferred from the gas phase discharge plasma being in contact with the liquid. The type and quantity of the reactive species formed by discharge plasma depend on the nature and the composition of the ambient gas and also on the properties of the liquid in the case of contact with plasma.

Among these processes, the oxidative properties of reactive oxygen species (OH radical, atomic oxygen, ozone, hydrogen peroxide) and nitrogen species (nitric oxide, nitrogen dioxide radical) are generally accepted to play central role in the chemical and biological effects of plasma produced in gas-liquid environments - typically discharges produced in humid air touching a wet surface (biofilms, cell tissue, skin) or an aqueous solution. In addition, secondary chemical and biological effects can be induced in the plasma-treated liquid through the post-discharge reactions of chemical species produced by plasma in the liquid either directly, or transferred from the gas phase discharge plasma via gas-liquid interface (e.g., H₂O₂, ozone, nitrite, peroxyxynitrite). Many of these chemical species are not stable in the liquid and

subsequent reactions can take place giving rise to new transient species as OH•, O₂⁻, NO• and NO₂• radicals, which have highly cytotoxic properties and cause prolonged antibacterial activity of plasma-treated solutions even several days after the solution's exposure to the discharge. Therefore, the yield of a total plasma-chemical process is due to synergistic contributions of numerous different elementary reactions taking place simultaneously in a discharge system [1, 2].

In this work we have focused on the chemical analysis of peroxyxynitrite (ONOOH) in water treated by air discharge plasma. Previously, the formation of peroxyxynitrite was proposed by many authors [3-5], however, its experimental evidence was rather limited. Half-life of ONOOH is typically less than 1 s in acidic solutions, which implies its very low steady-state concentrations possibly present in plasma-treated water (PTW) and hence explains the difficulty to detect ONOOH directly in plasma-treated water. Attempts to identify ONOOH in plasma-treated water were made through the analysis of the absorption spectra of PTW [3], using fluorogenic probes [6], and by analysis of the antibacterial activity of model solutions mimicking plasma-treated water [4]. Recently, we have experimentally proved the formation of ONOOH in plasma-treated water and determined quantitatively the formation rate of ONOOH through kinetic analysis of the post-discharge reaction between H₂O₂ and HNO₂ in plasma-treated water at pH 3.3 [7]. In this paper we have expanded our study on peroxyxynitrite formation in plasma-treated water through the reaction of H₂O₂ and HNO₂ into wider range of acidic pH values (2.3 - 4.0) and using more analytical methods. ONOOH formation was evaluated in plasma-treated water and in model water mimicking content of PTW. Formation of OH• radical as a product of ONOOH homolysis was quantitatively determined by electron paramagnetic resonance (EPR) spectroscopy using spin-trap technique [8]. Correlation between the yields of OH• radicals and the rate of ONOOH formation in plasma treated water was performed.

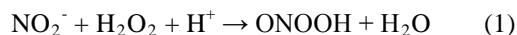
2. Experimental

The gas phase pulsed positive corona discharge in contact with water surface was used as source of air plasma in water. The discharge was generated in the air gap between a planar high voltage electrode made from reticulated vitreous carbon (RVC) and the water surface with an immersed grounded stainless steel plate electrode using the apparatus described in detail in [7]. In this work applied voltage of a positive polarity of 27 kV with a pulse repetition frequency of 50 Hz and the discharge gap height of 12 mm was used. Artificial air prepared from mixture of O₂/N₂ gases flowed continuously through the reactor with a flow rate 2.5 l min⁻¹. Buffered aqueous solutions used in the experiments were prepared from Milli-Q water by addition of NaH₂PO₄ and phosphoric acid in amounts needed for the desired solution pH (2.3, 2.7, 3.0, 3.3, 3.6 and 4.0). The total volume of the aqueous solution treated by the plasma was 900 ml.

The concentration of H₂O₂ was determined colorimetrically using the titanium sulfate method with sodium azide modification to eliminate interferences of nitrites [7]. The concentration of nitrites was measured by ion chromatography using the HPLC system Shimadzu LC-10Avp with UV (210 nm) and suppressed conductivity detection. Analyses were made by means of a 7-μm Allsep A1 anion exchange column (10 cm × 4.6 mm) with 0.85 mM NaHCO₃/0.9 mM Na₂CO₃ as the eluent (flow rate of 1.2 ml min⁻¹). The quantitative detection of OH• radicals in model H₂O₂/HNO₂ mixture was evaluated by electron paramagnetic resonance spectroscopy using an X-band (9.87 GHz) EPR (EMXmicro, Bruker BioSpin GmbH, Germany) with the resonator ER 4119HS and spin trap BMPO (5-tert-Butoxycarbonyl-5-methyl-1-pyrroline-N-oxide) from Dojindo Laboratoire (Kumamoto, Japan). The absolute OH• radical concentrations were determined from the EPR spectra of BMPO/OH• adduct using the alanine spin counting calibration sample for calibration of the EPR spectroscope [8].

3. Results and discussion

The formation rate of peroxyntirite was determined from kinetic analysis of the pseudo-second-order reaction between H₂O₂ and NO₂⁻ under acidic conditions:



This method was described in our previous work [7]. Temporal changes in concentrations of H₂O₂ and NO₂⁻ were monitored in buffered mixtures H₂O₂/HNO₂ of various pH values (2.3, 2.6, 3.0, 3.3, 3.6 and 4.0). Measured concentrations of H₂O₂ and NO₂⁻ were fitted into the second-order rate kinetics as:

$$\ln \frac{[\text{NO}_2^-]_t [\text{H}_2\text{O}_2]_0}{[\text{H}_2\text{O}_2]_t [\text{NO}_2^-]_0} = k_{\text{H}_2\text{O}_2, \text{NO}_2^-} ([\text{NO}_2^-]_0 - [\text{H}_2\text{O}_2]_0) t \quad (2)$$

where $k_{\text{H}_2\text{O}_2, \text{NO}_2^-} = k [\text{H}^+]$, is the pseudo-second-order rate constant for the reaction between H₂O₂ and NO₂⁻, and

subscripts 0 and t denote concentrations of H₂O₂ and NO₂⁻ at times t = 0 and t, respectively.

Fig. 1 summarizes the results obtained at each pH expressed as a plot $\ln([\text{NO}_2^-]_t [\text{H}_2\text{O}_2]_0)/([\text{H}_2\text{O}_2]_t [\text{NO}_2^-]_0)$ versus reaction time using Eq. 2. Consequently, Fig. 2 shows the values of pseudo-second-order rate constant $k_{\text{H}_2\text{O}_2, \text{NO}_2^-}$, which were determined from slopes $k_{\text{H}_2\text{O}_2, \text{NO}_2^-}([\text{NO}_2^-]_0 - [\text{H}_2\text{O}_2]_0)$ for each pH.

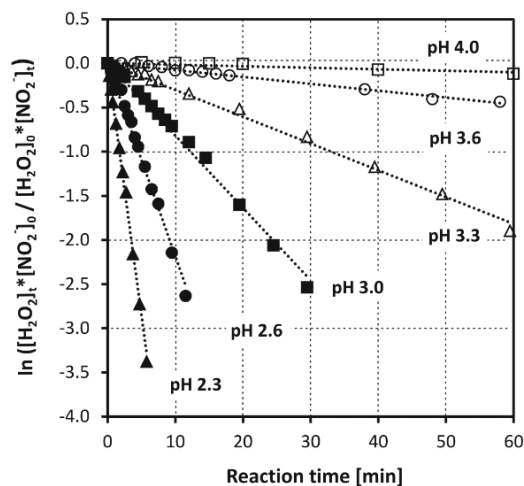


Fig. 1. Fit of H₂O₂/HNO₂ decay into the second order kinetics of peroxyntirite reaction in buffered mixtures of various pH values.

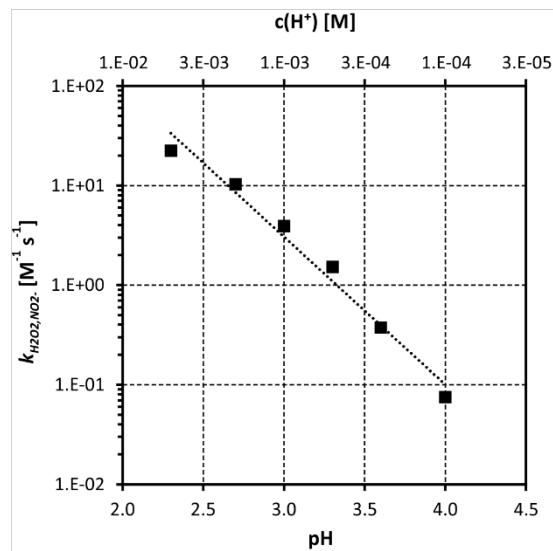


Fig. 2. Effect of pH on the second order rate of the reaction between H₂O₂ and NO₂⁻.

Figs. 1 and 2 show strong effect of acidity on the rate of the reaction between H₂O₂ and NO₂⁻, i.e., formation of ONOOH. The rate of ONOOH formation linearly increased with [H⁺] and was more than two orders of magnitude higher by lowering the pH of the solution from 4.0 to 2.3. Moreover, the linear relationship $k_{\text{H}_2\text{O}_2, \text{NO}_2^-} = f(\text{pH})$ proves the validity of the

pseudo-second-order kinetics of ONOOH formation by the reaction (1) across a wide range of acidic pH values.

Peroxynitrite, however, is not stable under acidic conditions and two processes occur simultaneously: the formation of ONOOH (in our case by the reaction between H_2O_2 and NO_2^- (Eq. 1)) and ONOOH decomposition. The formation of ONOOH (Eq. 1) has been shown to follow the pseudo-second order kinetics, the decomposition of ONOOH is more complex [9]. Nevertheless, using the values of $k_{\text{H}_2\text{O}_2, \text{NO}_2^-}$ the temporal evolution of peroxynitrite formation can be estimated. Fig. 3 shows the ONOOH rates approximated from decay kinetics of H_2O_2 and NO_2^- in buffered mixtures of pH 2.3-3.3. The formation rates of ONOOH decrease with longer reaction times as the concentrations of H_2O_2 and NO_2^- available in the liquid for ONOOH formation are decreasing. Fig. 3 also shows that ONOOH can exist significantly longer time in less acidic liquids since the rate of ONOOH formation is lower at higher pH.

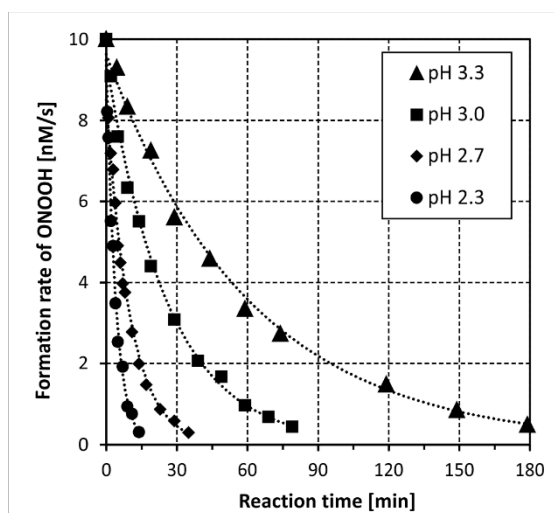
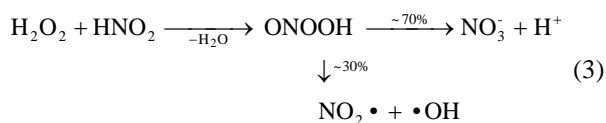


Fig. 3. Temporal evolution of ONOOH rates approximated from decay kinetics of H_2O_2 and NO_2^- in buffered mixtures of pH 2.3-3.3.

Concerning the ONOOH decomposition the half-life of ONOOH is typically less than 1 s. The mechanism of the decomposition of ONOOH has been studied in detail elsewhere [9]. Approximately 30% of ONOOH leads into the formation of free $\text{OH}\cdot$ and $\text{NO}_2\cdot$ radicals while the remainder collapses to nitric acid immediately followed by dissociation into NO_3^- . This process is schematically shown in Eq. 3.



Although there are more possible routes of peroxynitrite decomposition, we have previously demonstrated using phenol as chemical probe [7] that above mechanisms

(Eq. 3) fits very well with the kinetics of ONOOH decomposition in the plasma-treated water and it gives about 10%-15% of $\text{OH}\cdot$ radical yield. Fig. 4 shows an example of temporal evolution of formation rate of $\text{OH}\cdot$ radical estimated from kinetics of ONOOH in water treated by air plasma. The PTW buffered at pH 3.3 contained initially 200 μM H_2O_2 and 90 μM NO_2^- .

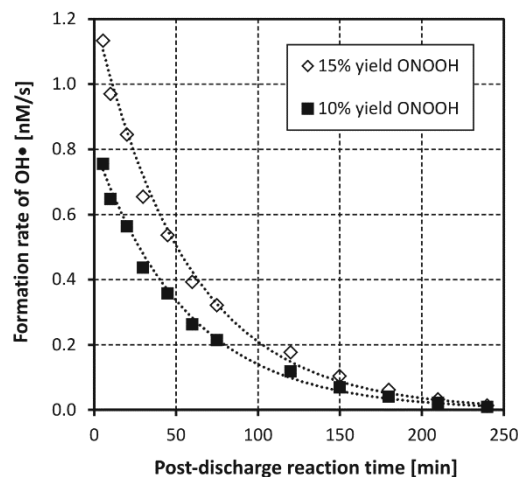


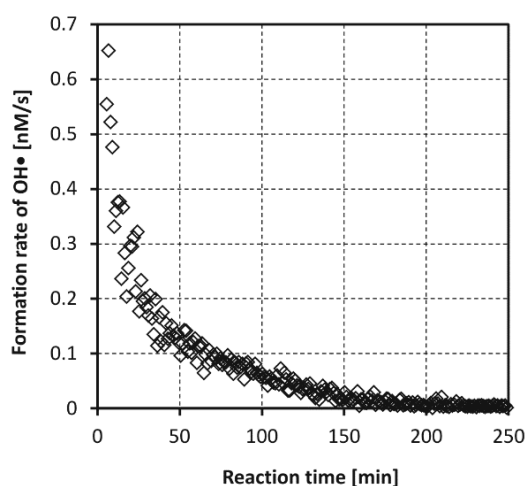
Fig. 4. Approximation of formation rate of $\text{OH}\cdot$ radicals from ONOOH (10%-15% yield to $\text{OH}\cdot$). Source of ONOOH: PTW (pH 3.3).

Formation of $\text{OH}\cdot$ radicals from ONOOH was further quantitatively determined using EPR spectroscopy [8]. Buffered mixture of $\text{H}_2\text{O}_2/\text{HNO}_2$ (pH 3.3) mimicking content of plasma-treated water as in Fig. 4 was prepared for EPR analysis. The spin-trap BMPO was dissolved directly in the investigated solution and EPR signal of BMPO/ $\text{OH}\cdot$ adduct was monitored.

Fig. 5 shows the temporal evolution of the effective formation rates of $\text{OH}\cdot$ radicals in model $\text{H}_2\text{O}_2/\text{HNO}_2$ mixture calculated from BMPO/ $\text{OH}\cdot$ signal overlapped with the slow decay of the spintrap adduct, which is neglected for the present study. Obtained data were in good agreement with the rates of $\text{OH}\cdot$ radicals, which were estimated from kinetic analysis of ONOOH formation through the second-order reaction between H_2O_2 and nitrite ions in PTW (Fig. 4). These results indicate that such a combined chemical/physical analytical approach can be successfully used for the study of transient chemical species produced by plasmas in the gas/liquid environment.

4. Conclusions

Formation of peroxynitrite in air plasma-treated water and in model mixtures of $\text{H}_2\text{O}_2/\text{HNO}_2$ was determined using kinetic analysis of the pseudo-second-order reaction between H_2O_2 and NO_2^- . The validity of this analytic method was proved for a wide range of acidic pH values. The rate of ONOOH formation linearly increased with $[\text{H}^+]$ and was more than two orders higher by lowering the



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Fig. 5. Evaluation of ONOOH decomposition through EPR analysis of OH• radical formation in H₂O₂/HNO₂ mixture (pH 3.3).

pH of the solution from 4.0 to 2.3. Decomposition of ONOOH was evaluated by EPR analysis of OH• radical produced in model H₂O₂/HNO₂ mixture by homolysis of ONOOH. Good correlation with OH yields, which were approximated from ONOOH rates through decay kinetics of H₂O₂ and NO₂⁻, was determined.

5. Acknowledgements

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6. References

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