6th international conference on plasma medicine

Bratislava, Slovakia
September 4–9, 2016

icpm6

BOOK OF ABSTRACTS

Edited by Karol HENSEL, Barbora TARABOVÁ, Katarína KUČEROVÁ,
Zuzana KOVAĽOVÁ, Mário JANDA, and Zdenko MACHALA

Title: 6th International Conference on Plasma Medicine (ICPM-6)
Subtitle: Book of Abstracts
Editors: K. Hensel, B. Tarabová, K. Kučerová, Z. Kovaľová, M. Janda, and Z. Machala
Cover design: L. Jeuffrault
Publisher: KEC FMFI UK, Bratislava
Printing: Neumahr s.r.o., Bratislava, 2016
ISBN 978-80-8147-066-0

Title: 6th International Conference on Plasma Medicine (ICPM-6)
Subtitle: Book of Abstracts
Editors: K. Hensel, B. Tarabová, K. Kučerová, Z. Kovaľová, M. Janda, and Z. Machala
Cover design: L. Jeuffrault
Publisher: KEC FMFI UK, Bratislava
Printing: Neumahr s.r.o., Bratislava, 2016
ISBN 978-80-8147-066-0

Challenges of RONS detection in air plasma activated solutions by colorimetric and fluorescent based assays

B. Tarabová[1], P. Lukeš[2], M. U. Hammer[3], H. Jablonowski[3], T. von Woedtke[4], S. Reuter[3], Z. Machala[1]

[1]Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská dolina, 842 48 Bratislava, Slovakia

[2]Institute of Plasma Physics of the CAS, v.v.i., Za Slovankou 3, 182 00 Prague, Czech Republic

[3]Centre for Innovation Competence "*plasmatis*", at INP Greifswald e.V., Felix-Hausdorff.-Str. 2, 174 89 Greifswald, Germany

[4]INP Greifswald e.V., Felix-Hausdorff.-Str. 2, 17489 Greifswald, Germany

e-mail: tarabova@fmph.uniba.sk

Air plasmas activated water (PAW) and liquids posses bactericidal or therapeutic properties given by the formation of reactive oxygen and nitrogen species (RONS) [1-2]. RONS such as hydrogen peroxide H_2O_2, nitrites/nitrates NO_2^-/NO_3^-, peroxynitrites/peroxynitrous acid $ONOO^-$/ONOOH, superoxide $O_2^{\cdot-}$, ozone O_3 or hydroxyl radical $OH^{\cdot}$ are formed in plasma activated solutions through the gas-liquid interface. The detection of RONS in the plasma activated solutions is challenging either due to the chemical instability of detected RONS or the presence of highly reactive species, which may cause interferences or cross-reactivities of the used methods. Therefore it is crucial to test and adapt the methods for special conditions in plasma activated solutions.

Solutions (water, phosphate buffer and phosphate buffered saline, or saline) with different buffering capacity, pH or salt content were electrosprayed directly through the air transient spark (TS) discharge. The accuracy of the colorimetric Griess assay for NO_2^- detection was confirmed by comparison measurement with the ion chromatography. Due to the possible interference of Griess assay with H_2O_2, we attempted to increase the detection limit by using enzyme catalase as a H_2O_2 scavenger. We showed that H_2O_2 has no influence on the Griess assay. Dissolved ozone in PAW was detected by standardized colorimetric Indigo blue assay, which unfortunately resulted in false positive response. Results were confirmed by the experiment with the synthetic PAW (prepared without plasma) and by the detection of no muconic acid in PAW as a specific product of phenol oxidation with ozone. A hypothesis that $OH^{\cdot}$ as a product of peroxynitrite decay in acidic PAW is responsible for the false positive results was confirmed by using RONS scavengers. The presence of superoxide $O_2^{\cdot-}$ was detected indirectly as the increase of H_2O_2 concentration. By using the superoxide dismutase enzyme, $O_2^{\cdot-}$ was dismutated into the H_2O_2 and O_2. The fluorescent probe 2,7-dischlorodihydrofluorescein diacetate (H_2DCFDA) was tested for qualitative detection of peroxynitrites in combination with several RONS scavengers. We demonstrated that H_2DCFDA assay is a promising method for peroxynitrite detection in plasma activated solutions under considering some limitations, especially the composition of the used solutions and potential ClO^- presence. The peroxynitrite detection by H_2DCFDA was compared with the kinetic study of post-discharge processes based on the detection of NO_2^-/NO_3^- and H_2O_2.

This work was supported by Slovak Research and Development Agency APVV-0134-12, Comenius University Grant UK/477/2016, COST Action TD1208 and the Ministry of Education and Sports of the Czech Republic (project LD 14080).

References

[1] Z. Machala, B. Tarabová, K. Hensel, et al., *Plasma Process. Polym.*, **10**, 649-659 (2013).

[2] H. Tanaka, M. Mizuno, K. Ishikawa, et al., *Clinical Plasma Medicine*, **3**, 72-76 (2015).