Final Report of

Collaborative Research: Atmospheric Pressure Plasma-Biomaterial Surface Interactions - Bridging Understanding of APP Sources to Rational Modification of Biomolecules

(Award# DE SC0012500)

November 2017

Principal and co-principal investigators

University of Maryland, College Park, Profs. G. S. Oehrlein and J. Seog

UC Berkeley, Profs. D. B. Graves and J. A. Reimer

Executive Summary

In this project, we focused on the interactions between low-temperature plasma and aqueous solutions. The primary plasma device used for the experimental work is a positive polarity point-to-plane corona discharge, as reported in the previous document.

We demonstrate that a self-pulsed corona discharge, and specifically the transient spark discharge in air, offers a simple, accessible platform for studying plasma/liquid interactions. Initially, the accumulation of stable plasma-generated species (H_2O_2 and NO_2^-) in the liquid phase proceeds linearly with NEAPP exposure at constant pH in the absence of competing reactions. Under acidic conditions, subsequent reactions in the liquid phase begin to consume significant quantities of NO_2^- and H_2O_2 once these species accumulate to concentrations greater than about 500 M.

We show that the effects of H_2O_2 and NO_2^- on indigo carmine are consistent with the established kinetics of OH• production through the O=NOOH pathway, with 24% of O=NOOH going to OH• generation and the oxidation of indigo carmine. When indigo carmine is added to plasma-activated solutions, the observed decay rate follows that observed when H_2O_2 and NO_2^- are added to solution without plasma exposure. This supports the conclusion that NEAPP treatment does not generate significant concentrations of other, long-lived reactive species in the buffered solutions investigated.

Experiments using indigo carmine as an indicator of oxidative strength during NEAPP exposure demonstrate that the activity of OH• (or other highly oxidizing species) near the plasma-liquid interface dominates the effects of NEAPP treatment. This result is striking given the small area of contact between the discharge and liquid (<1 mm2), and highlights the importance of gas-liquid transport phenomena in these systems. The observed effects could be due to either a) direct reaction with plasma-generated species, most likely OH•, at the interface, b) locally elevated concentrations of H_2O_2, NO_2^-, and/or local reduction in the pH at the surface, leading to acceleration of the O=NOOH pathway in this region; or c) the effect of plasma-generated species such as

UV and/or ions directly on indigo carmine. However, all of our initial results are consistent with OH• being the primary oxidizing agent.

Description of experiments and results

A photograph of the discharge and schematic of the major chemical pathways of interest are shown in Figure 1.

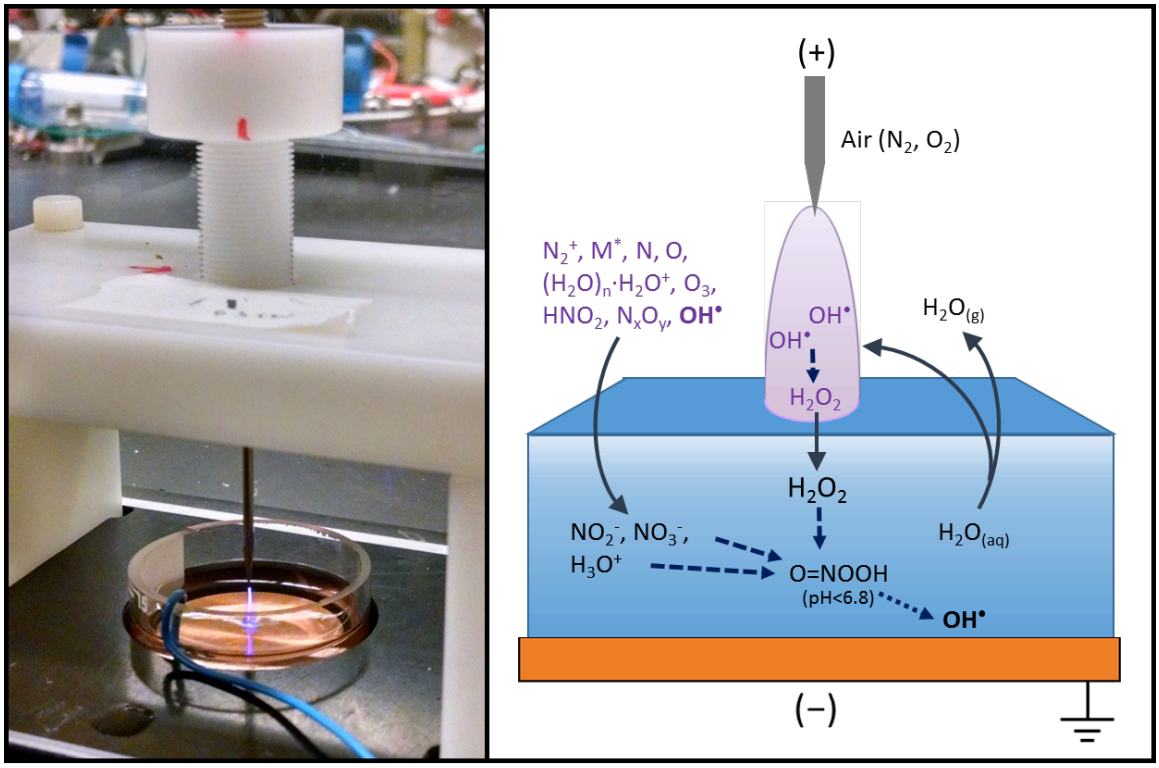

Figure 1. Left: Photograph of corona discharge (transient spark), with gap distance adjustment. Right: Schematic of the flow of major species produced in the discharge (purple) and in solution. Solid lines indicate species transport; dotted lines represent reactions.

Characterization of this discharge was expanded to include the effects of applying a wider range of voltages to the system, spanning the transition between two distinct discharge modes (streamer and transient spark) [2]. In a streamer discharge, the breakdown potential is exceeded but a fully conductive channel is not formed before the end of the pulse. In the transient spark discharge, a conductive plasma region bridges the entire interelectrode gap (a transient "spark" condition). The transition between these two modes is evidenced by a step change in the pulse frequency and the visible appearance of the plasma. The effect of applied voltage on discharge power and H2O2 production were examined over the full range of applied voltages. Discharge power and H2O2 production in the solution are expected to be related to the relative OH• density near the plasma-liquid interface [3]; thus, these parameters are evaluated as a rough indicator of oxidizing strength.

Within the transient spark regime, the power dissipated by the plasma increases with applied voltage, as shown in Figure 2a. The pH of the solution as no apparent effect on the apparent discharge power. However, increasing solution conductivity appears to result in less power dissipated by the plasma. This result is surprising considering that the resistance of the solution is expected to be negligible relative to that of the air.

The concentration of H2O2 generated over a 5-minute interval as a function of discharge power (Figure 2b) suggests that H2O2 production increases linearly with increased power within the transient spark regime. The low power streamer and transition regimes display significantly greater variability. The pH and conductivity of the solution do not appear to affect the generation of H2O2 by discharges of similar power. Despite the more filamentary appearance of the transient spark discharge, it displayed more consistent operating characteristics as measured by power dissipation and H2O2 production.

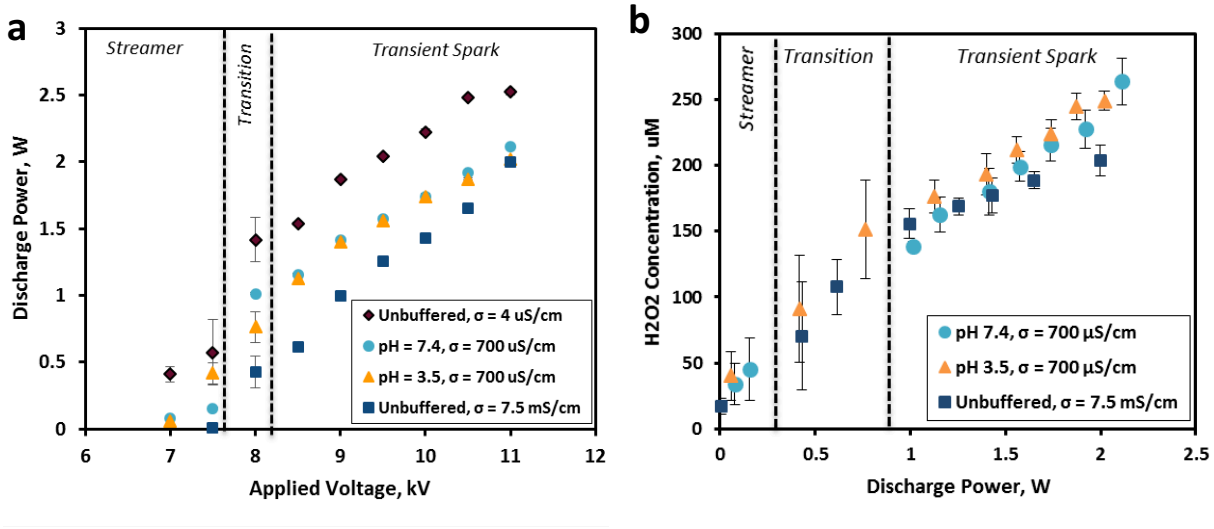

Figure 2. Effects of applied voltage on power dissipated by the plasma and related H2O2 generation.

Previous experiments in our group looked only at the production of reactive oxygen and nitrogen species (RONS) in ultrapure water. Under these conditions, the pH, solution conductivity and RONS concentrations all vary significantly with time. The evolution of pH and relatively stable RONS (H2O2, NO2-, NO3-) in ultrapure water with time shows that the concentrations of H2O2 and NO2- initially increase, then level off or decline as the solution acidifies and consumption reactions become significant (Figure 3). The major reactions through which these species are consumed are described in reference [4].

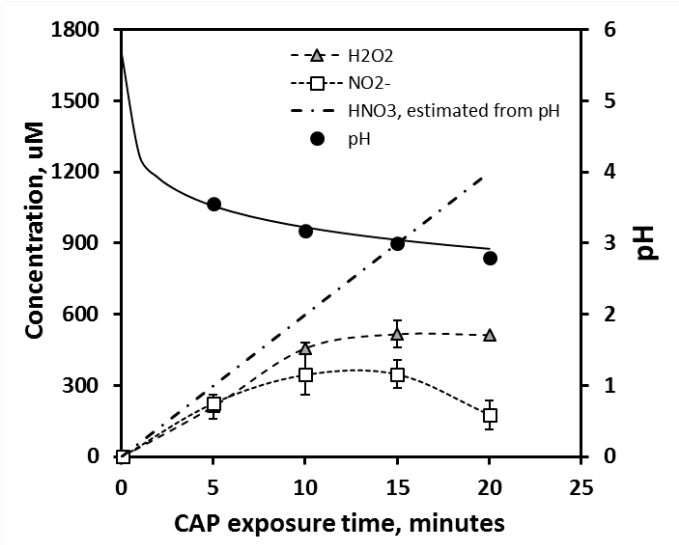

Figure 3. Evolution of H2O2, NO2-, and NO3- in ultrapure water with time. As the solution becomes acidified, the rates of consumption of NO2- and H2O2 by acid-catalyzed reactions increase.

The change in pH during CAP treatment was eliminated by buffering the target solution to a pH of 3.5 or 7.4. The evolution of H2O2 and NO2- during plasma treatment in buffered and unbuffered solutions is shown in Figure 4. Note that at pH 7.4, the concentrations of both species continue to increase linearly out to 20 minutes. The rate of plasma-induced species generation in the absence of consumption reactions was estimated from this data and used in subsequent models of this system.

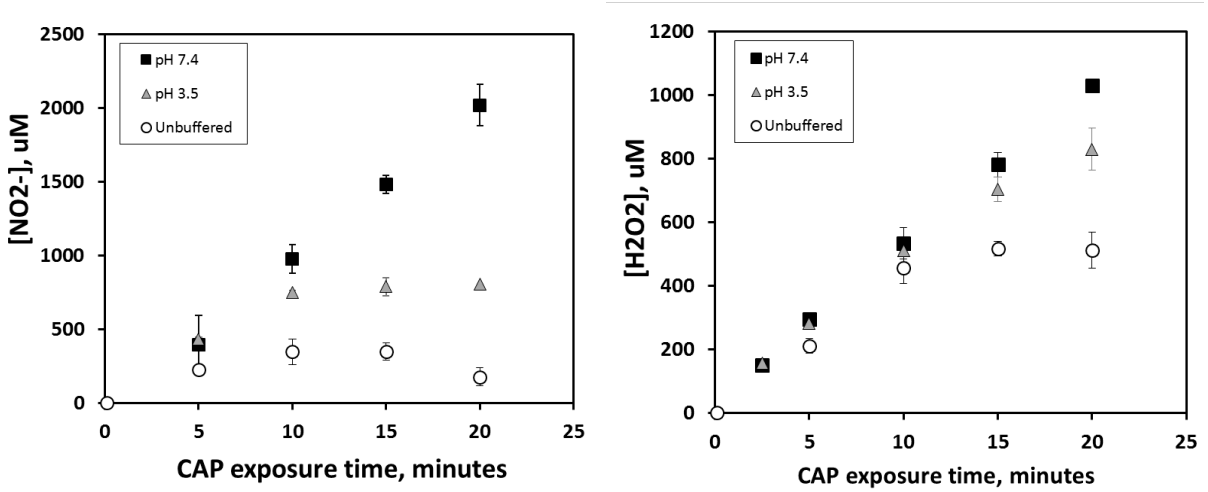

Figure 4. Evolution of nitrite (left) and H2O2 (right) in buffered and unbuffered solutions as a function of CAP treatment duration.

There is currently much debate about the mechanisms through which treatment with plasma-activated water (PAW) causes biological effects [5]–[7]. In such treatments, a solution (often cell culture media) is treated with CAP and then applied to the target. We compared the ability of PAW (initially at pH 3.5 or 7.4) to oxidize a model organic dye (indigo carmine), relative to solutions H2O2, NO2-, and NO3- that had not been treated with plasma ("artificial PAW"). We determined that the oxidation of indigo carmine by both PAW and artificial PAW are due to the production of OH• through the O=NOOH pathway by comparing our data with a model based on the published kinetics (Figure 5). These experiments confirmed the absence of other reactive species (specifically O3) lingering in solution following CAP treatment.

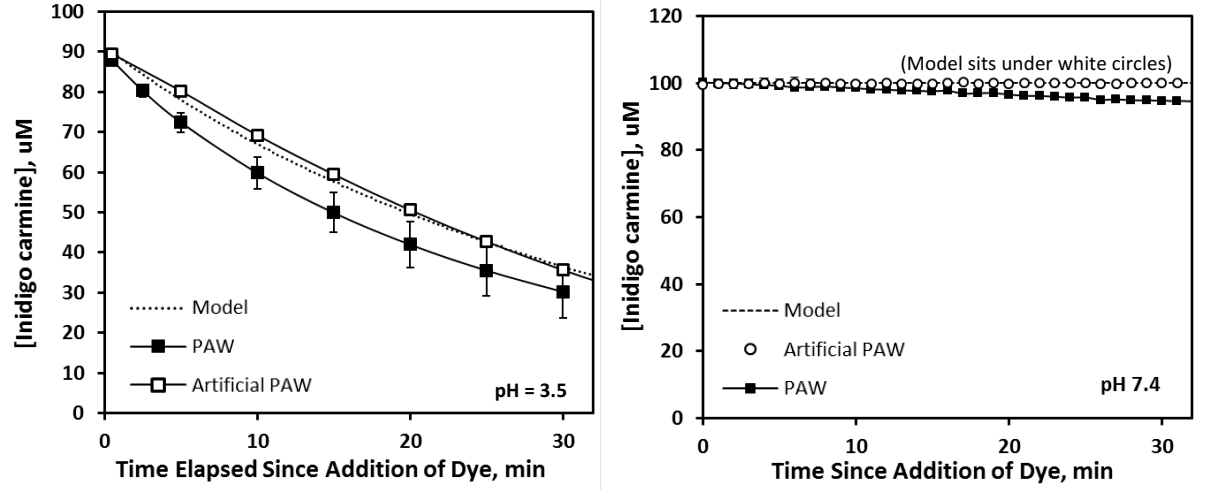

Figure 5. Oxidation of indigo carmine, a model organic compound and pollutant from the textile industry, due to PAW and solutions containing similar concentrations of H2O2, NO2-, and HNO3-. Solutions were buffered to a pH of 3.5 (left) or 7.4 (right).

We next investigated the extent to which the oxidation of indigo carmine during CAP treatment can be attributed to OH• produced in the bulk solution (through the O=NOOH pathway) relative to other sources of oxidation. The O=NOOH pathway is not active at pH 7.4 (as confirmed in Figure 5). However, significant oxidation of indigo carmine is observed in solutions buffered to 7.4 that are treated with CAP (Figure 6). This suggests that other sources of oxidizing species play a dominant role. We suspect, although cannot prove, that OH• produced by the discharge at the plasma-liquid interface is responsible. Our these data are consistent with a near-surface concentration of OH• on the order of 0.1-1 μM, assuming the reaction of OH• and indigo carmine proceeds at the rates published in the literature. This simple estimate assumes that there is no significant depletion of indigo carmine in this region.

Based on the data to this point, a model of the chemistry occurring in the liquid phase during CAP treatment was developed. This model includes both surface and bulk solution production of OH•. Under acidic conditions (pH=3.5), the rate of indigo carmine exceeds the model predicted rate, suggesting that unexplored surface effects and the change in pH play an important role in these systems. A publication on this body of work, along with additional analyses of these observations, is in preparation.

We are currently developing the capabilities to identify the radical species produced at the plasma-liquid interface through electron paramagnetic resonance (EPR) and the use of radical-selective spin traps (OH• vs O2•-, NO•, or CO3•). These experiments should provide more conclusive evidence of the species responsible for the oxidation of indigo carmine at the plasma-liquid interface.

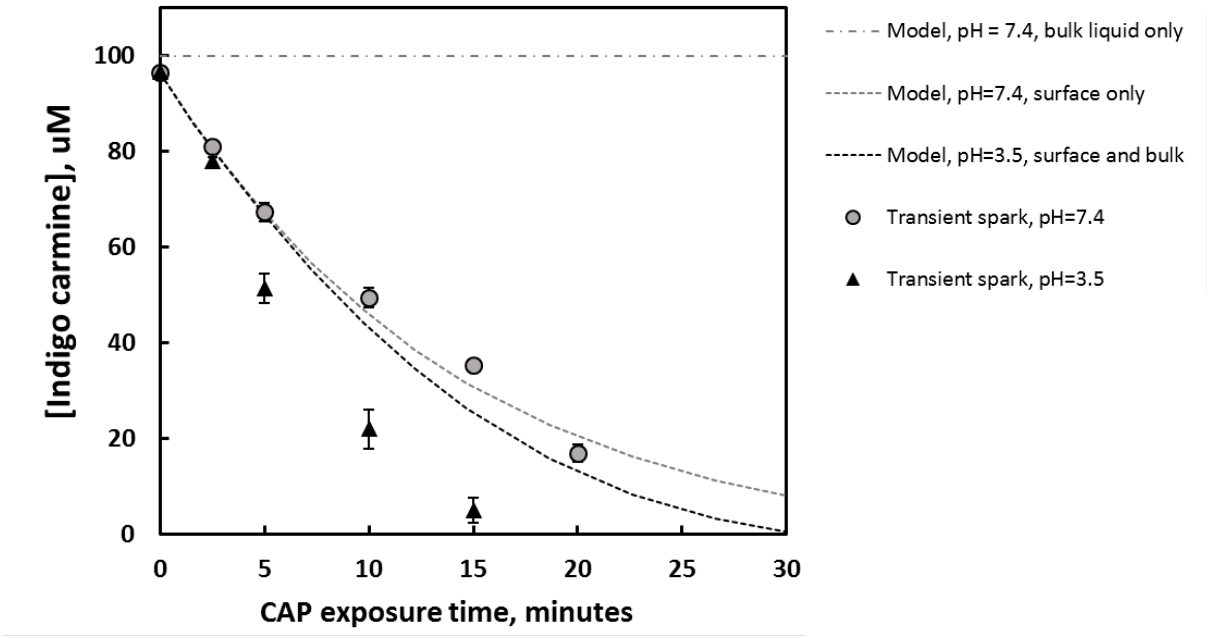

Figure 6. Oxidation of indigo carmine dye during CAP exposure under acidic (pH 3.5) and neutral (pH = 7.4) conditions, experimental vs model data.

The degradation products of CAP-treated indigo carmine were analyzed with mass spectrometry and 1H NMR to ascertain whether multiple oxidation reactions were occurring per

molecule. The analytical results from both techniques were consistent with isatin sulfonic acid (ISA) as the dominant degradation product. However, 1H NMR analyses indicated an ISA content of CAP-treated solutions that was only ~60% of the expected value, given the observed drop in indigo carmine concentration (Figure 7). This suggests that the rate of degradation of ISA to the next intermediate is limiting, and that further oxidation completely to CO2 occurs rapidly.

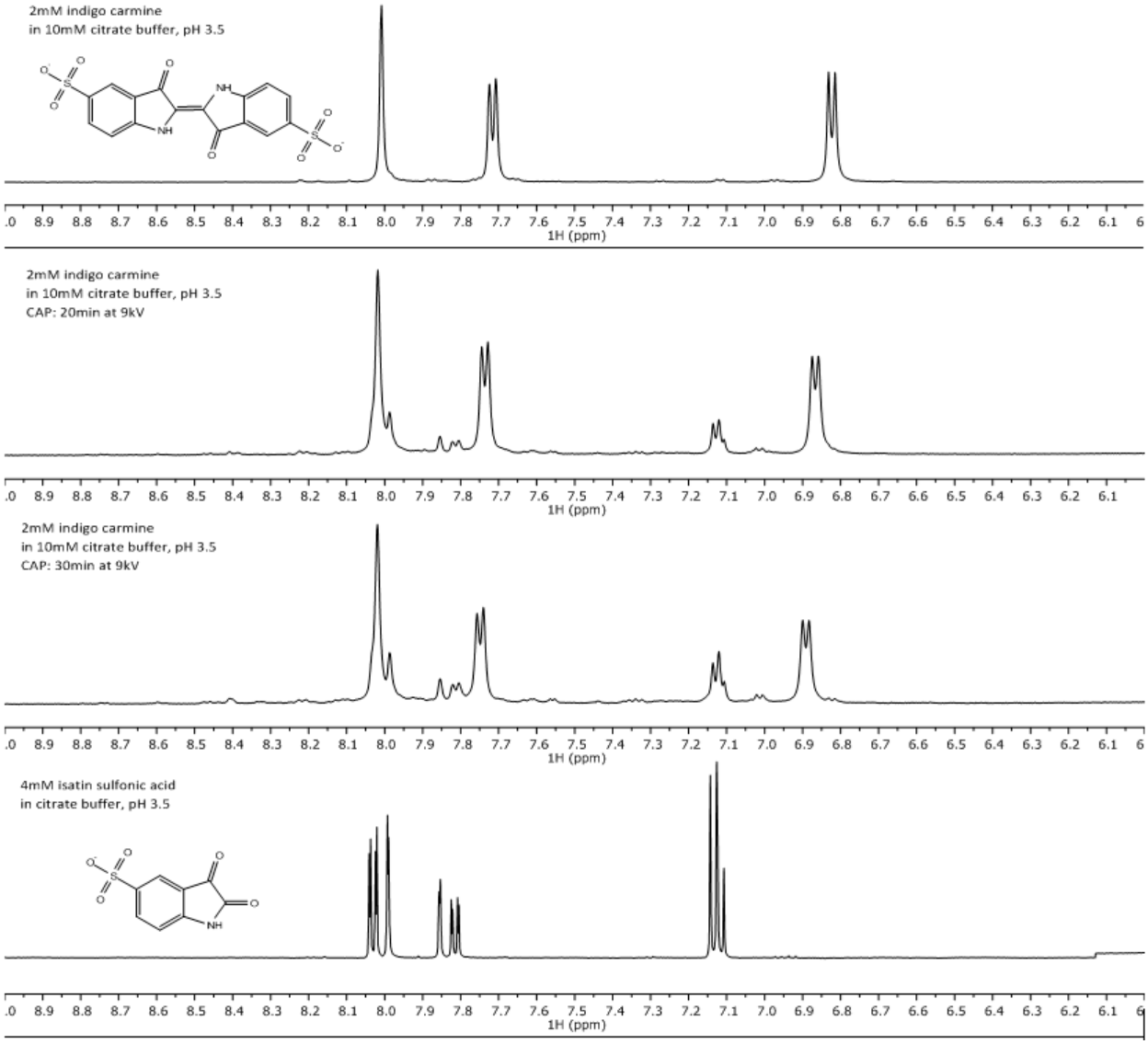

Figure 7. Transient spark (9kV) CAP-treatment of indigo carmine solutions in 10mM citrate buffer, pH 3.5. The concentration of indigo carmine decreased from 18.5mM to 15.5mM (after 20min CAP) or 1.05mM (after 30min CAP). The corresponding concentrations of ISA are 0.6 and 1.0 mM, compared to expected concentrations of 1 and 1.6 mM ISA.

Similar analyses will be used to investigate the effects of low-temperature plasma on simple biologically relevant small molecules. This work is expected to be completed over the coming months, in addition to the previously mentioned work on the identification of radicals (through EPR) and the studies of negative polarity corona discharges. Control experiments for the EPR studies are currently underway and indicate that sufficient sensitivity is available to detect OH• at plasma-generated concentrations. Future work will apply the techniques developed to date to investigate the interaction

of negative polarity streamer and glow discharges with aqueous solutions. In this configuration, electrons and negative ions strike the liquid surface rather than positive ions; this type of interaction is expected to produce solvated electrons that are known to be important in many physical processes.

References:

[1] Z. Machala, L. Chládeková, and M. Pelach, "Plasma agents in bio-decontamination by dc discharges in atmospheric air," J. Phys. D. Appl. Phys., vol. 43, no. 22, p. 222001, 2010.

[2] Z. Machala, I. Jedlovský, and V. Martĭsovitš, "DC discharges in atmospheric air and their transitions," IEEE Trans. Plasma Sci., vol. 36, no. 4, pp. 918–919, 2008.

[3] R. J. Wandell and B. R. Locke, "Hydrogen peroxide generation in low power pulsed water spray plasma reactors," Ind. Eng. Chem. Res., vol. 53, pp. 609–618, 2014.

[4] P. Lukes, E. Dolezalova, I. Sisrova, and M. Clupek, "Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H 2 O 2 and HNO 2," Plasma Sources Sci. Technol., vol. 23, no. 1, p. 015019, Feb. 2014.

[5] H. Tanaka, M. Mizuno, K. Ishikawa, K. Nakamura, H. Kajiyama, H. Kano, F. Kikkawa, and M. Hori, "Plasma-Activated Medium Selectively Kills Glioblastoma Brain Tumor Cells by Down-Regulating a Survival Signaling Molecule, AKT Kinase," Plasma Med., vol. 1, no. 3–4, pp. 265–277, 2011.

[6] S. Kalghatgi, C. M. Kelly, E. Cerchar, B. Torabi, O. Alekseev, A. Fridman, G. Friedman, and J. Azizkhan-Clifford, "Effects of non-thermal plasma on mammalian cells.," PLoS One, vol. 6, no. 1, p. e16270, Jan. 2011.

[7] M. Naïtali, J.-M. Herry, E. Hnatiuc, G. Kamgang, and J.-L. Brisset, "Kinetics and Bacterial Inactivation Induced by Peroxynitrite in Electric Discharges in Air," Plasma Chem. Plasma Process., vol. 32, no. 4, pp. 675–692, May 2012.