

Oxidation of *N*-Acetylcysteine (NAC) under Nanosecond-Pulsed Nonthermal Dielectric Barrier Discharge Plasma

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ABSTRACT: NAC solutions in water and phosphate buffer were treated with nonthermal plasma generated by using a nanosecond-pulsed dielectric barrier discharge (DBD) device, and the products generated after plasma treatments were characterized by ¹H nuclear magnetic resonance. Our results demonstrate that NAC was oxidized to acetic acid, 2-amino-3-mercaptopropanoic acid, 2-aminoethanethiol, and 2-aminoethanesulfonic acid. The concentrations of these compounds are time dependent with plasma treatment. Mechanisms of NAC under the DBD plasma are also proposed in this study, and the products and mechanisms differ from those generated by microsecond-pulsed DBD plasma.

KEY WORDS: non-thermal plasma, DBD, acetylcysteine

I. INTRODUCTION

Thermal and nonthermal plasma have been demonstrated to have a wide variety of applications, such as in aerospace,¹ surface treatment,² and microelectronics,³ environmentally and clinically.⁴ Recently, their application in agriculture has attracted a great deal of attention that led to the first international conference in this field in 2016.⁵ During the conference, it was generally recognized that plasma effects on plant growth, root stimulation, and microbial activation, among others, were not always consistent with each other among laboratories. One of the explanations for this was that the chemistries of the plasmas used in different labs also differed. Thus, understanding the chemical species generated from each plasma is required to fully develop the agriculture applications of plasma. It is believed that plasma generated in different environments, such as air, oxygen, and nitrogen, produce different chemical species that result in different subsequent effects on their applications. The chemistries of plasma in the same environment, such as cold plasma in air, are not expected to significantly differ from one another. However, in this report, we demonstrate that similar cold air plasma may also result in significantly different chemistries, suggesting that the chemistries of plasmas may have an important role in their effects on agriculture. This fact may contribute to the inconsistency among labs.

We used dielectric barrier discharge (DBD) plasma in our experiment. DBD is generated when high voltages of sinusoidal waveform or short duration pulses are applied between two electrodes, with at least one electrode being insulated.^{6,7} The plasma can be formed in air or other gases at atmospheric pressure and room temperature. The insulator prevents the build-up of current between the electrodes, creating electrically safe plasma without substantial gas heating. Recently, we reported that *N*-Acetylcysteine (NAC) was converted to cysteic acid in microsecond-pulsed DBD air plasma.⁸ In this work, we applied nanosecond-pulsed DBD air plasma on the NAC solution, and found that the chemical oxidation species from this plasma were totally different from that of the microsecond-pulsed sample.

II. EXPERIMENT

A. Chemicals

NAC was purchased from Alfa Aesar (Tewksbury, MA, USA) and was recrystallized from ethanol-water solution before use. Deuterium oxide (D_2O) was purchased from Sigma-Aldrich (Milwaukee, WI, USA) and phosphate buffered saline (PBS; 10 \times , powder) from Fisher Scientific (Pittsburgh, PA, USA). The PBS buffer solution was prepared immediately before plasma treatment. Dichloromethane (DCM) was purchased from Pharmco-AAPER (Shelbyville, KY, USA). Nitrogen and oxygen gas were obtained from Airgas (Philadelphia, PA, USA).

B. Plasma Setup and Treatment

DBD plasma was produced using the experimental setup shown in our previous publication.⁹ Plasma was generated by applying alternating polarity-pulsed (500 Hz–1.5 kHz) voltage of 20-kV magnitude (peak to peak), 10-ns pulse width, and a rise time of 5 V/ns between the high-voltage electrodes, using a variable-voltage and -frequency power supply (FID Technology, Burbach, Germany). The input energy was 10 mJ/pulse. The parameters of the plasma setup were 11.2 kV ($R = 75 \Omega$) and 690 fHz for all experiments. The NAC D_2O solutions (20 mM) were treated with air plasma for different amounts of time.

C. Sample Preparation and Instrumentation

For 1H nuclear magnetic resonance (NMR) tests, the plasma-treated NAC D_2O solutions were tested directly without dilution. Following plasma treatment, methanol was added to the solutions (1 mM) as an internal standard to quantify the chemical concentrations in the solutions. 1H NMR spectra were recorded on a Varian Gemini 500-MHz spectrometer.

III. RESULTS AND DISCUSSION

A. Characterization of Compounds in Air-Plasma-Treated NAC Solutions

After NAC solutions were treated with DBD nonthermal plasma in air for varying amounts of time, the compounds (N-acetylcysteine: 2-amino-3-mercaptopropanoic acid; compound 2: 2-aminoethanethiol; compound 3: 2-aminoethanesulfonic acid; compound 4: acetic acid) in the NAC D₂O solutions were analyzed using ¹H NMR spectroscopy. Without further treatment, the solution retained all of the oxidized chemicals after plasma treatment. The NMR spectra provided full information for all of the chemical species in the treated solutions. Figure 1 shows the ¹H NMR spectra of NAC D₂O solutions before and after varying treatment times with nanosecond-pulsed air plasma. In the ¹H NMR spectrum of the untreated NAC (compound 1), the three peaks (a singlet at 2.09 ppm, a doublet doublet at 2.99 and 3.00 ppm, and a triplet [triplet doublet in higher-resolution NMR] at 4.61 and 4.63 ppm) are attributed to the protons of $-\text{COCH}_3$, $-\text{CH}_2\text{SH}$ and $-\text{CH}(\text{NH})-$ of NAC, respectively. ¹H NMR spectra of the plasma-treated solutions in Figure 2 show that the three peaks of NAC disappear after a 3-min air-plasma treatment, suggesting the total decomposition of NAC in the solution.

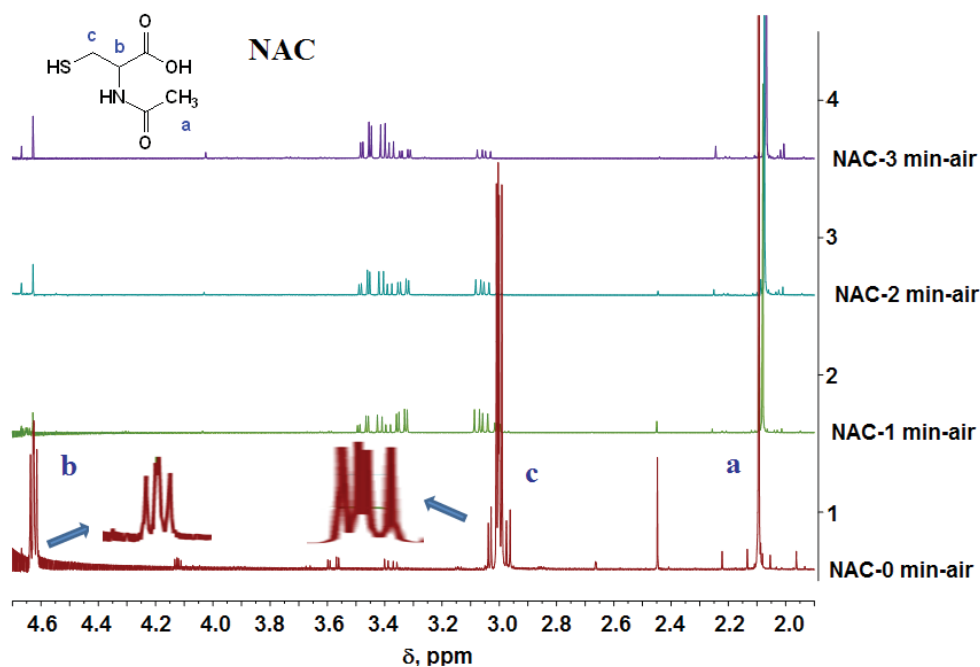


FIG. 1: ¹H NMR spectra of a 1-mL D₂O solution of NAC after treatment with air plasma for 1 min. (N-acetylcysteine: 2-amino-3-mercaptopropanoic acid; compound 2: 2-aminoethanethiol; compound 3: 2-aminoethanesulfonic acid; compound 4: acetic acid)

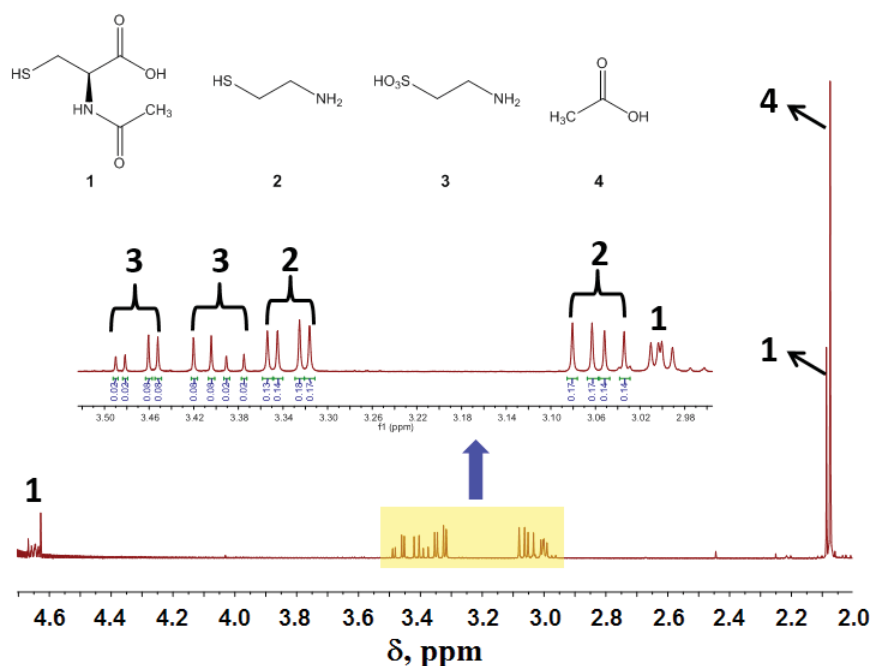


FIG. 2: ^1H NMR spectra of the untreated and air-plasma-treated NAC D_2O solutions. The initial concentration of NAC is 20 mM. (N-acetylcysteine: 2-amino-3-mercaptopropanoic acid; compound 2: 2-aminoethanethiol; compound 3: 2-aminoethanesulfonic acid; compound 4: acetic acid)

New peaks appeared in the ^1H NMR spectra of the air-plasma-treated NAC solutions. Figure 2 shows the enlarged ^1H NMR spectra of the NAC solution after a 1-min treatment with air plasma. A singlet peak at 2.08 ppm is assigned to acetic acid (compound 4). A doublet doublet at 3.03 and 3.06 ppm and a doublet doublet at 3.32 and 3.34 ppm belong to 2-aminoethanethiol (compound 2). Another two peaks, a doublet doublet at 3.38 and 3.40 ppm and a doublet doublet at 3.45 and 3.48 ppm, are from 2-aminoethanesulfonic acid (compound 3). The assignments of these peaks are based on the reported chemical shifts, splitting patterns, and coupling constants of chemicals (compounds 2–4).^{10,11} ^1H NMR spectra show that compounds 2–4 were produced in the solution after treated with air plasma for 1, 2, and 3 min.

B. Kinetics Study of NAC Decomposition in Air Plasma

The decomposition kinetics of NAC solutions was obtained from eight equal aliquots of NAC in D_2O (20 mM) that were air-plasma treated for different amounts of time (0, 10, 20, 60, 80, 100, 120, and 180 s). After treatment, each solution was immediately analyzed using ^1H NMR to measure the concentrations of compounds 1–4. Figure 3 shows that the relationship between the concentration of NAC and air-plasma treatment time can be well fitted to first-order degradation kinetics $\ln(c_t/c_0) = -kt$, where c_t

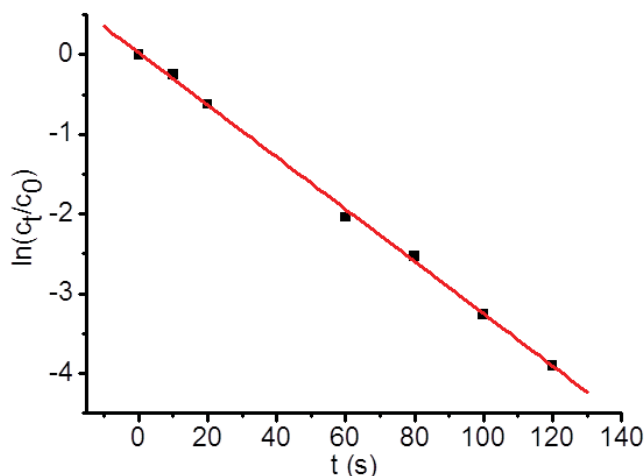


FIG. 3: First-order degradation kinetics curves of NAC under air-plasma treatment

is the concentration of NAC at time t , and c_0 is the initial concentration of NAC before exposure to plasma. This suggests that the decomposition of NAC in air plasma follows the first-order kinetic law; that is, the rate of decomposition is dependent on NAC concentration: $\text{rate}_{(\text{NAC})} = k[\text{NAC}]$. The slope of the plot indicates that the rate constant k of NAC decomposition under air-plasma treatment is $3.28 \times 10^{-2} \text{ s}^{-1}$. The decomposition of a 20-mM NAC solution in air plasma is thus elucidated with the equation $\ln(c_t/c_0) = -3.28 \times 10^{-2}t$ or $\text{rate}_{(\text{NAC})} = 3.28 \times 10^{-2}[\text{NAC}]$.

The changes in concentration of intermediates and products 2–4 depend on air-plasma treatment time, shown in Fig. 4. The concentrations of 2-aminoethanesulfonic acid and acetic acid increased from 0 to 3 min air-plasma treatment time in the solution; the concentration of 2-aminoethanethiol increased from 0 to 1 min and then subsequently decreased with longer plasma treatments. These results suggest that the intermediate 2-aminoethanethiol is further converted to other compounds, mostly 2-aminoethanesulfonic acid in 3 min (discussed in the mechanisms study section below). It is possible that 2-aminoethanesulfonic acid and acetic acid will become further decomposed; however, we did not observe their decomposition within the 3 min air-plasma treatment time.

C. Decomposition Mechanisms of NAC under Air-Plasma Treatment

In addition to ultraviolet photons, plasma also contains a many active species including electrons, ions, electronically excited atoms and molecules, and reactive oxygen species (ROS) and nitrogen species (RNS) such as ozone (O_3), $\cdot\text{NO}$, $\cdot\text{OH}$, and singlet oxygen ($^1\text{O}_2$).¹² When a solution is exposed to air plasma, observed in the solution are hydrogen peroxide (H_2O_2), ozone (O_3), the charged species, and radicals such as hydroperoxyl radicals ($\text{HO}_2\cdot$), peroxyxynitrite (ONOO^-), superoxide ($\text{O}_2^{\cdot-}$), nitric oxide ($\cdot\text{NO}$), and hydroxyl radicals ($\cdot\text{OH}$). Therefore, when dissolved in D_2O and exposed

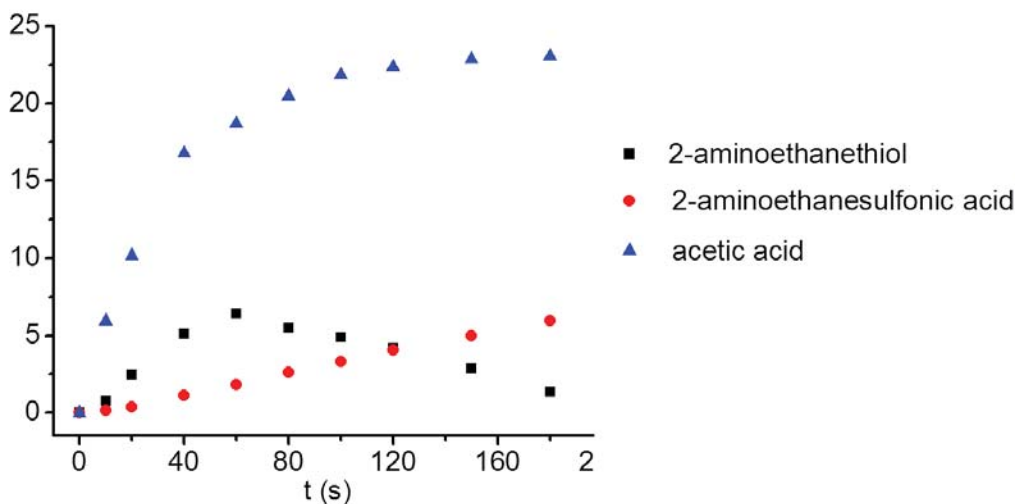


FIG. 4: Concentration changes of compounds 2–4 with air-plasma treatment time

to air plasma, the NAC molecule will hydrolyze by the break of the amide bond and then decompose by reacting with these highly oxidative species. Possible reactions of NAC with some of these species have been studied and the reaction mechanisms are proposed (Fig. 5). First, the NAC molecule hydrolyzes into acetic acid (compound 4) and 2-amino-3-mercaptopropanoic acid (compound 1). With the elimination of a CO_2

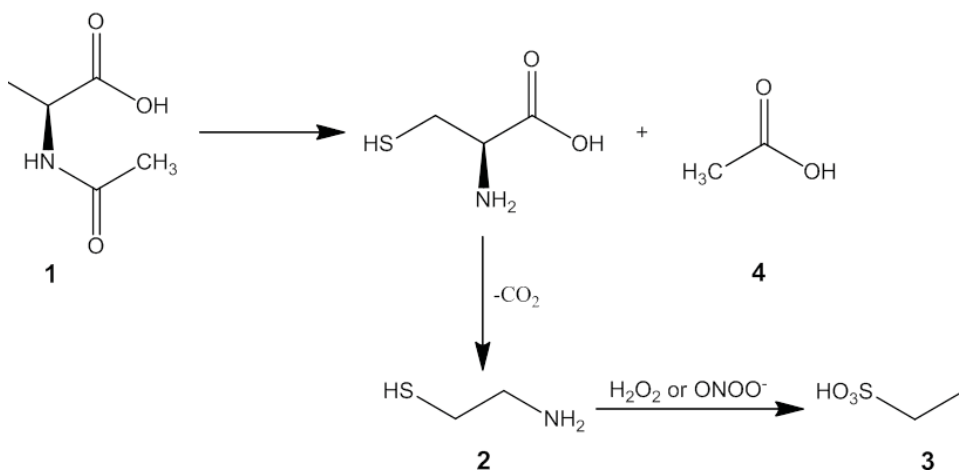


FIG. 5: Proposed mechanisms of NAC hydrolyzation to form acetic acid and 2-aminoethanethiol and the oxidation of 2-aminoethanethiol by air plasma to form 2-aminoethanesulfonic acid. (Compound 1: 2-amino-3-mercaptopropanoic acid; compound 2: 2-aminoethanethiol; compound 3: 2-aminoethanesulfonic acid; compound 4: acetic acid)

molecule, the latter is converted to 2-aminoethanethiol (compound 2), which is further oxidized by the oxidants, for example, H_2O_2 or ONOO^- , to form 2-aminoethanesulfonic acid (compound 3).¹³

D. Decomposition of NAC in PBS D_2O under Air-Plasma Treatment

The ^1H NMR spectra of the products formed in the PBS D_2O solution of NAC after air-plasma treatment are the same as those of the products formed in the D_2O solution of NAC without PBS buffer. Moreover, the decomposition rates of NAC in these two solutions under air-plasma treatment are comparable: $3.28 \times 10^{-2} \text{ s}^{-1}$ in D_2O and $4.08 \times 10^{-2} \text{ s}^{-1}$ in PBS D_2O . This suggests that the decomposition mechanisms of NAC under air-plasma treatment were not affected by the buffer or the pH of the solutions.

IV. CONCLUSIONS

Our work provides insight on the products formed from nanosecond-pulsed nonthermal plasma treatment of NAC, including acetic acid, 2-amino-3-mercaptopropanoic acid, 2-aminoethanethiol, and 2-aminoethanesulfonic acid. The mechanisms of NAC under the DBD plasma were proposed.

The products differ from cysteic acid produced from microsecond-pulsed DBD plasma. The difference indicates that the oxidative species, or the composition of oxidative species in the microsecond- and nanosecond-pulsed air plasma, are different. Although the exact ROS or RNS species that contribute to the reaction in each mechanism are still under investigation, the work demonstrates that the study of chemicals in each plasma system may be critical to explain the results from each plasma system. This study aids in understanding the mechanisms and overall effects of the plasma acting on biological species and in expanding the applications of nonthermal plasma in agriculture.

ACKNOWLEDGMENT

We thank the Keck Foundation for their support of the project.

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