

# Comment on “An Alternative Explanation for Ions Put Forth as Evidence for Abundant Hydroxyl Radicals Formed Due to the Intrinsic Electric Field at the Surface of Water Droplets”

Jinheng Xu, Xiaowei Song, Lecheng Lyu, Xinxing Zhang, and Richard N. Zare\*

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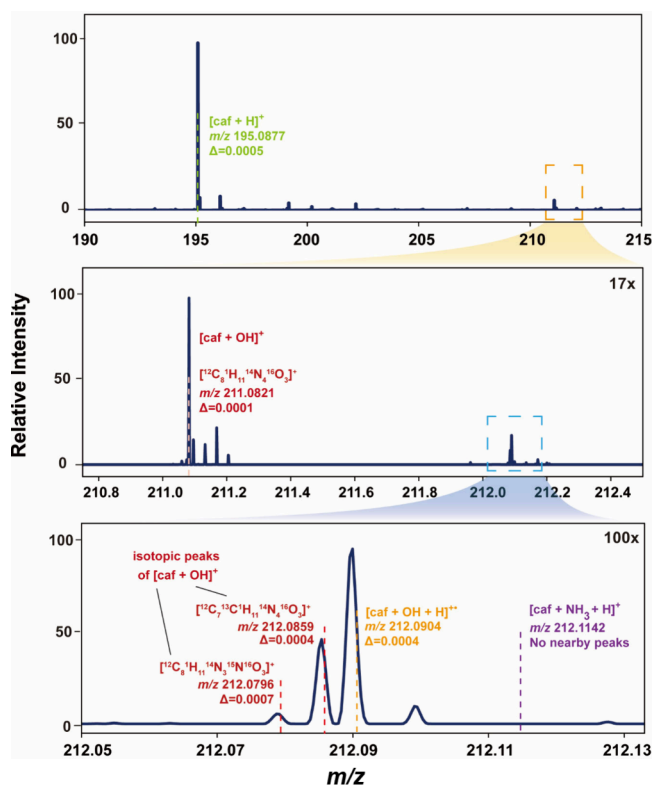
Article Recommendations



Supporting Information

In their article “An Alternative Explanation for Ions Put Forth as Evidence for Abundant Hydroxyl Radicals Formed Due to the Intrinsic Electric Field at the Surface of Water Droplets”, Chen and Williams<sup>1</sup> have suggested that when an aqueous solution of caffeine is sprayed and analyzed by low-resolution mass spectrometry, the observed mass peak at  $m/z = 212$  is not [caffeine + H + OH]<sup>+</sup> as proposed by Xing et al.<sup>2</sup> but rather [caffeine + H + NH<sub>3</sub>]<sup>+</sup>. If correct, this would invalidate many other studies.<sup>3–6</sup> To test this conjecture, we used an Orbitrap high-resolution mass spectrometer (Thermo Scientific, San Jose, CA, USA) to record the mass spectrum of a 10  $\mu$ M caffeine–water solution sprayed at a voltage of 1.0 kV. Parameters of the mass spectrometer are set as follows: capillary temperature, 250 °C; S-lens radio frequency (RF) value, 55%; maximum injection time, 400 ms. Figure 1 shows the resulting mass spectrum obtained by nano-electrospray ionization (nESI), the same method used by Chen and Williams in their study. The nESI sprayer tip was fabricated from a borosilicate glass capillary (Item# B150-86-10, OD: 1.5 mm; ID: 0.86 mm) by placing it into a micropipette puller (P-87, Sutter Instrument Co., Novato, CA, USA; Heat: 730, Pull: NULL, Velocity: 12, Time: 250).

As Figure 1 clearly illustrates, the mass peak ( $m/z$  212.0900) agrees within 2 ppm with that of [caffeine + H + OH]<sup>+</sup> ( $m/z$  212.0904) but not that of [caffeine + H + NH<sub>3</sub>]<sup>+</sup> ( $m/z$  212.1142), which lies 110 ppm away. The detected signal intensity of [caffeine + H + OH]<sup>+</sup> might be significantly lower than its real quantity owing to measurement loss, as reported previously,<sup>5</sup> because a portion of the fragile radical-adduct ions may decompose or fail to survive the transit through the mass spectrometer before reaching the detector, leading to an underestimation of their true abundance. Nonetheless, this peak is still readily detected under our operating conditions. We also find several nearby mass peaks which are identified as isotopic isomers of [caffeine + OH]<sup>+</sup>, in particular, [<sup>12</sup>C<sub>8</sub><sup>1</sup>H<sub>11</sub><sup>14</sup>N<sub>4</sub><sup>16</sup>O<sub>3</sub>]<sup>+</sup>,  $m/z$  211.0821; [<sup>12</sup>C<sub>8</sub><sup>1</sup>H<sub>11</sub><sup>14</sup>N<sub>3</sub><sup>15</sup>N<sup>16</sup>O<sub>3</sub>]<sup>+</sup>,  $m/z$  212.0796; and [<sup>12</sup>C<sub>7</sub><sup>13</sup>C<sup>1</sup>H<sub>11</sub><sup>14</sup>N<sub>4</sub><sup>16</sup>O<sub>3</sub>]<sup>+</sup>,  $m/z$  212.0859. One plausible route for the formation of [caffeine + OH]<sup>+</sup> is the capture of OH<sup>•</sup> by [caffeine]<sup>+</sup> ( $m/z$  194.0798), as evidenced by the peak at  $m/z$  194.0796, which is consistent with the known tendency of nitrogen-containing aromatics to stabilize radical cations. Another possibility is fragmentation of [caffeine + H + OH]<sup>+</sup>. We examined the baseline level of



**Figure 1.** Mass spectrum of 10  $\mu$ M caffeine–water solution (caffeine is abbreviated as ‘caf’ in labels). The top panel shows the full spectrum; and the middle and bottom panels are enlargements of parts of the mass spectrum by factors of 17 and 100, respectively.

ammonia in our laboratory air using a dehumidifier to collect condensed water vapor for 3 h. The ammonia concentration was checked by a commercial colorimetric test kit (API Ammonia Test Kit), showing a maximum presence of 0–0.25

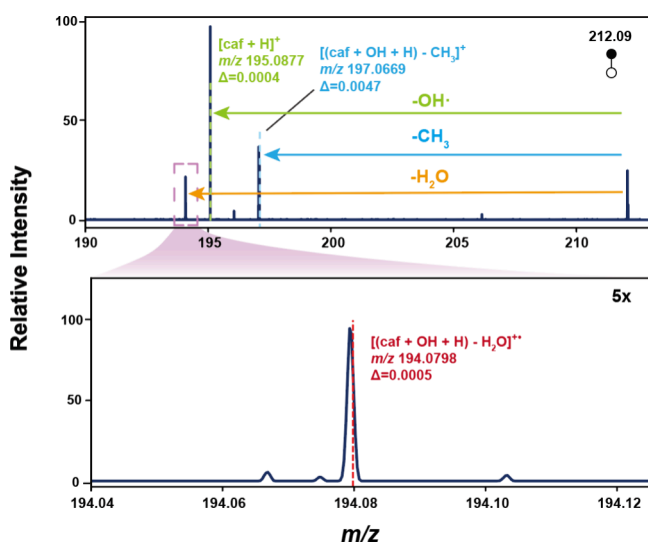
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ppm ammonia in the indoor environment (Figure S1). We also mounted a 1000 ppm  $\text{NH}_4\text{OH}$  solution beside the nESI tip. We still did not detect the  $[\text{caffeine} + \text{H} + \text{NH}_3]^+$  ion, meaning that the lower detection limit for the ammonia should be higher than 1000 ppm. Only when we mounted a 5%  $\text{NH}_4\text{OH}$ -soaked Kimwipe paper at 1 cm away from the nESI tip, a peak corresponding to  $[\text{caffeine} + \text{H} + \text{NH}_3]^+$  ( $m/z$  212.1142) indeed appeared, confirming that the high-resolution mass spectrometer is fully capable of detecting such adducts when ammonia is present at sufficiently high concentrations. As a result, we do not intend to dispute the possible formation of  $[\text{caffeine} + \text{H} + \text{NH}_3]^+$  ions under high ammonia contamination; rather, our results suggest that the dominant species at  $m/z$  212 when spraying aqueous caffeine solution into air is more consistent with  $[\text{caffeine} + \text{H} + \text{OH}]^{+\bullet}$  than  $[\text{caffeine} + \text{H} + \text{NH}_3]^+$  with normal atmospheric ammonia concentrations. We were unable to find the ammoniated species using our laboratory air. Notably, the presence of  $\text{NH}_4\text{OH}$  solution largely suppressed the signal intensity of  $[\text{caffeine} + \text{H} + \text{OH}]^{+\bullet}$  and  $[\text{caffeine} + \text{OH}]^{+\bullet}$  (see Figures S2 and S3)

Chen and Williams claimed that a neutral loss of  $\text{H}_2\text{O}$  ( $m/z$  194) was not observed in their experiment. However, we successfully obtained a mass peak ( $m/z$  194.0793) in the  $\text{MS}^2$  spectrum at  $m/z$  212.09, as shown in Figure 2, within 3 ppm



**Figure 2.**  $\text{MS}^2$  spectrum of  $m/z$  212.09 (caffeine is abbreviated as ‘caf’ in labels). The mass peak at  $m/z$  196 is the isotopic isomer of  $[\text{caf} + \text{H}]^+$ . The top panel is the full mass spectrum; the bottom panel is an enlargement of a part of the mass spectrum by a factor 5.

$[(\text{caffeine} + \text{OH} + \text{H}) - \text{H}_2\text{O}]^{+\bullet}$  ( $m/z$  194.0798). This  $\text{MS}^2$  spectrum was taken with an isolation width of 0.2  $m/z$  and collision-induced dissociation with a normalized collision energy of 30.00. The prominent fragment corresponds to  $[\text{caf} + \text{H}]^+$ , which is the result of losing  $\text{OH}^\bullet$ . Thus, we conclude that when an aqueous caffeine solution is sprayed, caffeine does capture the  $\text{OH}^\bullet$  radical. Recently, an independent report<sup>7</sup> successfully characterized the water dimer radical cations ( $m/z$  36.0206,  $(\text{H}_2\text{O})_2^{+\bullet}$ ) by multiple ionization techniques and high-resolution mass spectrometry, which supports our conclusion. These results highlight that the observed  $[\text{M} + 18]^+$  species arise from interfacial redox processes rather than solely from ammonium adduct

formation, providing new insights into the fundamental redox chemistry within water microdroplets. This is also consistent with previous reports.<sup>8,9</sup>

## ■ ASSOCIATED CONTENT

### Data Availability Statement

Original data can be found at <https://github.com/Jinheng-Xu/Caffeine>.

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.5c04123>.

Supporting Figures S1–S3 (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

Richard N. Zare – Department of Chemistry, Stanford University, Stanford, California 94305, United States; [orcid.org/0000-0001-5266-4253](https://orcid.org/0000-0001-5266-4253); Email: [rnz@stanford.edu](mailto:rnz@stanford.edu)

### Authors

Jinheng Xu – Department of Chemistry, Stanford University, Stanford, California 94305, United States; [orcid.org/0009-0007-1187-5389](https://orcid.org/0009-0007-1187-5389)

Xiaowei Song – Department of Chemistry, Stanford University, Stanford, California 94305, United States; [orcid.org/0000-0003-3611-2816](https://orcid.org/0000-0003-3611-2816)

Lecheng Lyu – Department of Chemistry, Stanford University, Stanford, California 94305, United States; [orcid.org/0009-0009-0599-700X](https://orcid.org/0009-0009-0599-700X)

Xinxing Zhang – College of Chemistry, State Key Laboratory of Advanced Chemical Power Sources, Tianjin Key Laboratory of Biosensing and Molecular Recognition, Frontiers Science Centre for New Organic Matter, Nankai University, Tianjin 300071, China; [orcid.org/0000-0001-5884-2727](https://orcid.org/0000-0001-5884-2727)

Complete contact information is available at:

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### Notes

The authors declare no competing financial interest.

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