Are Hydroxyl Radicals Spontaneously Generated in Unactivated Water Droplets?

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Abstract: Spontaneous ionization/breakup of water at the surface of aqueous droplets has been reported with evidence ranging from formation of hydrogen peroxide and hydroxyl radicals, indicated by ions at *m*/*z* 36 attributed to $OH\bullet\text{-}H_3O^+$ or $(H_2O\text{-}OH_2)^{+\bullet}$ as well as oxidation products of radical scavengers in mass spectra of water droplets formed by pneumatic nebulization. Here, aqueous droplets are formed both by nanoelectrospray, which produces highly charged nanodrops with initial diameters ~100 nm, and a vibrating mesh nebulizer, which produces 2 – 20 μm droplets that are less highly charged. The lifetimes of these droplets range from 10s of μs to 560 ms and the surface-to-volume ratios span ~100-fold range. No ions at *m*/*z* 36 are detected with pure water, nor are significant oxidation products for the two radical scavengers that were previously reported to be formed in high abundance. These and other results indicate that prior conclusions about spontaneous hydroxyl radical formation in unactivated water droplets are not supported by the evidence and that water appears to be stable at droplet surfaces over a wide range of droplet size, charge and lifetime.

Rates of organic reactions can be accelerated by many orders of magnitude in droplets, a phenomenon that has been attributed both to the unusual properties of the surface of droplets[1,2] and to reagent concentration in rapidly evaporating droplets.[3,4] Recent reports claim that water molecules in microdroplets spontaneously produce hydrogen peroxide and hydroxyl radicals, a result that has been attributed to the special properties of the air-water interface at the surface of microdroplets.[5–9] These results have important implications for chemistry that occurs in droplets, such as naturally occurring atmospheric aerosols, emulsions, and droplets produced by nebulization sources, such as humidifiers, and electrospray that is used in thousands of laboratories world-wide. Inhalation of water droplets that spontaneously produce abundant hydroxyl radicals also has important implications in human health.

Production of up to 30 μM hydrogen peroxide was reported in droplets formed by pneumatic nebulization, compared to an undetectable concentration formed in bulk solution, and reactions attributed to the hydrogen peroxide that was produced were observed.[8] Hydrogen peroxide was also reported to be formed by condensing water vapor into droplets, indicating that no catalyst, external electrical field, or precursor chemicals were needed for the spontaneous formation of hydrogen peroxide from water droplets.[10,11] In these studies, the authors proposed that hydrogen peroxide was spontaneously formed by autoionization of water at the droplet surface to produce OH^- that can electron detach to form OH• that reacts to form hydrogen peroxide. They hypothesized that autoionization of water is induced by the high electric field at the surface of water microdrops.[8,10,11] Computational results indicate that the electric field at the surface of water droplets can weaken chemical bonds,[12,13] but even higher electric fields are necessary to dissociate water molecules.[14,15] Computations indicate that strong electric fields can lead to a low probability of electron detachment from OH^- at the droplet surface,[16] indicating that a very low concentration of hydroxyl radical is likely produced from the autoionization equilibrium in water that results in low concentrations of both OH^- and H_3O^+. Colussi proposed that the substantial hydrogen peroxide generation that has been reported is initiated by encounters between oppositely charged droplets leading to electron transfer, with the energy required for spraying water and the limited solvation at the microdroplet surface contributing to hydrogen peroxide production.[17]

Other results indicate that H_2O_2 is not formed at the surface of water droplets.[18–21] Mishra and co-workers showed that condensing water vapor into micron-size droplets in a controlled environment that excluded oxygen resulted in an unmeasurable concentration of H_2O_2 (<0.25 μM), but ~1 μM H_2O_2 was formed when microdroplets were formed by ultrasonic nebulization,[19] a result they attributed to cavitation in solution from the nebulizer.[19,22] No hydrogen peroxide (<50 nM) was detected in microdroplets formed by pneumatic nebulization in an inert environment when dissolved oxygen was removed from water,[21] but hydrogen peroxide was formed when droplets were exposed to gaseous ozone, a result attributed to incorporation of ozone into the droplet through interfacial mass transfer.[20] Zare and co-workers[5] used an ozone scrubber to remove ambient ozone (<3 ppb), and reported hydrogen peroxide formation that was more than an order of magnitude lower than previously reported,[8] but indicated that 0.3 – 1.5 μM hydrogen peroxide was still produced when N_2 was used as a nebulization gas, depending on the flow rate used.

Formation of hydrogen peroxide has been used as evidence for hydroxyl radical formation at the surfaces of uncharged microdroplets, which has been reported to occur in the absence of light.[23] Additional recent evidence supporting substantial spontaneous hydroxyl radical formation at the surface of water droplets has been reported by Xing et al., who found that ions at *m*/*z* 36 were generated when water microdroplets were formed by pneumatic nebulization.[7] This ion was attributed to $OH\bullet\text{-}H_3O^+$ or $(H_2O\text{-}OH_2)^{+\bullet}$ formed by the autoionization of water and formation of a hydroxyl radical that recombines with hydronium at the droplet surface.[7,8] Which form of this *m*/*z* 36 ion is lowest in energy depends on the computational method used.[24,25] Additional evidence reported for the spontaneous formation of hydroxyl radicals in water droplets was the formation of oxidation products for the radical scavengers caffeine and melatonin. An ion at *m*/*z* 36 formed by nanoelectrospray of pure water was attributed to a radical cation of a water dimer.[26]

Here, we show that a *m*/*z* 36 ion is not formed in pure water droplets to a measurable extent under a wide range of droplet size, net charge and lifetime, nor are oxidation products of caffeine or melatonin reliably detected. These results indicate that prior evidence used to support the conclusion that substantial hydroxyl radical formation spontaneously occurs in water microdrops is likely due to some additional form of activation. Water appears to be stable at the air-water interface of both microdroplets and nanodroplets to the extent that can be measured.

Nanoelectrospray ionization is a soft ionization method that produces highly charged droplets that have much higher surface-to-volume ratios than micron-size droplets owing to their small size.[4,27,28] Ions corresponding to Na^+, $(H_2O + Na)^+$, and $(2H_2O + Na)^+$ are primarily formed by nanoelectrospray of pure water (Figure 1a; experimental details are provided in SI). K^+ is also formed at <1% relative abundance, but there is no signal at *m*/*z* 36 (Figure 1a, inset). The most abundant ion in the mass spectrum of microdroplets formed by nebulization reported by Xing et al. is at *m*/*z* 36 and sodiated water ions are only ~5% relative abundance.[7] An upper limit to the amount of *m*/*z* 36 produced by nanoelectrospray is 0.01% of that of $(H_2O + Na)^+$ based on the detection limit determined using a method described by Makarov et al.[29] (Table S1). Thus, at least 2000 times less $OH\bullet\text{-}H_3O^+$ (or $(H_2O\text{-}OH_2)^{+\bullet}$) is formed by nanoelectrospray ionization than was reported for microdroplets formed by pneumatic nebulization.[7] The absence of an *m*/*z* 36 ion also indicates that $(H_2O)_2^{+\bullet}$ reported previously is not formed at the surface of unactivated pure water droplets formed by nanoelectrospray. The droplets formed in our nanoelectrospray experiments are highly charged[30] and the initial diameters are ~100 nm. The surface-to-volume ratios of these nanodrops are at least 10x greater than the micron-size droplets formed by pneumatic nebulization. Thus, there is ample surface area for surface reactions to occur.

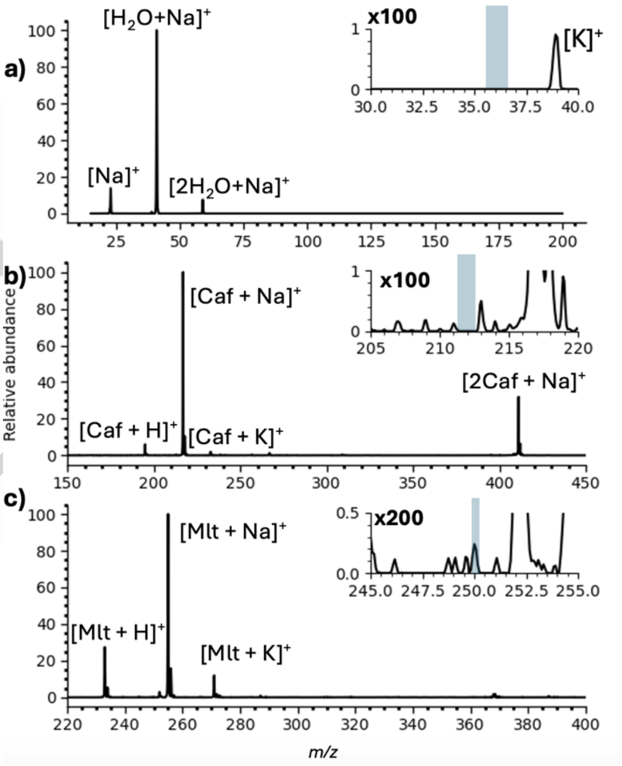

Figure 1. Electrospray ionization mass spectra of a) pure water b) 100 μM aqueous caffeine (Caf), and c) 100 μM aqueous melatonin (Mlt) obtained using borosilicate emitters with inner tip diameters of 1.7 μm. Insets show expansions around a) *m*/*z* 36 previously attributed to spontaneous formation of $OH\bullet\text{-}H_3O^+$ or $(H_2O\text{-}OH_2)^{+\bullet}$ in pure water microdrops, b) around *m*/*z* 212 (oxidized protonated caffeine), and c) around *m*/*z* 250 (oxidized protonated melatonin). The *y*-axis expansions for the insets are indicated in the figure. These data were acquired in the ion trap of an Orbitrap mass spectrometer with an inlet capillary temperature of 50 °C in order to closely replicate prior experiments where these spontaneous reaction products were reported.[7]

Caffeine readily reacts with hydroxyl radicals[31] and this reaction was used by Xing et al. to confirm OH• formation at the droplet surface.[7] A nanoelectrospray spectrum acquired from an aqueous 100 μM caffeine solution is shown in Figure 1b. The most abundant ions are protonated caffeine, sodiated caffeine, and sodiated caffeine dimer. Xing et al. reported that the abundance of protonated oxidized form of caffeine was ~45% that of protonated caffeine. There is no detectable signal corresponding to oxidized protonated caffeine at *m/z* 212 leading to an upper limit to the oxidation of caffeine of less than 0.03%, a value that is ~1400 times less than reported by Xing et al.[7] Ion signal at *m/z* 234 (10.4% abundance of *m/z* 233; expansion in Figure S1) is not an oxidation product of sodiated melatonin but corresponds to the A+1 isotope peak of potassium adducted melatonin (theoretical value 10.3%).

Melatonin was also used previously as a radical scavenger to show the capture of hydroxyl radicals spontaneously produced in microdroplets.[7] A nanoelectrospray spectrum of a 100 µM aqueous melatonin solution is shown in Figure 1c. Sodiated (*m/z* 255), protonated (*m/z* 233), and potassium-adducted (*m/z* 271) melatonin are primarily produced. There is signal at *m/z* 250 that could be oxidized melatonin with an abundance ~0.8% of protonated melatonin. The ion at *m/z* 272 (14.4% abundance of *m/z* 271) is not an oxidation product of sodiated melatonin but corresponds to the A+1 isotope peak of potassium adducted melatonin (theoretical value 14.2%). Xing et al. reported protonated melatonin is ~30% of oxidized melatonin and the sodiated oxidized melatonin is ~10% of the sodiated melatonin.[7] Thus, at least 400 times less oxidized product are formed in nanodroplets.

No reliable evidence for spontaneous oxidation of the radical scavengers caffeine and melatonin or formation of *m/z* 36 in highly charged pure aqueous nanodrops was observed. The lifetime of these nanodrops is in the low 10's of µs range[4,32,33] making them much shorter lived than the larger microdroplets (~7 µm)[7] used in the earlier work. A lifetime for these larger droplets was not reported. To evaluate whether the small droplet size, short lifetime, and high charging of nanodrops adversely affects formation of hydroxyl ions at the air-water interface, a mesh screen nebulizer was used to generate a distribution of droplets that range from 2 to 20 µm in diameter. The nebulizer was placed perpendicular to the mass spectrometer inlet at a distance of ~10 cm (Figure S2). Video tracking of the droplets indicate that it takes approximately 160 ms for these large micron size droplets to reach the mass spectrometer inlet establishing a minimum lifetime of greater than 160 ms. No external voltage was used, but mechanical breakup of water is well known to produce charged droplets of both polarities.[34] Mass spectra of pure aqueous micron size droplets produced by the mesh nebulizer are shown in Figure 2a. Abundant ions at *m/z* 46, 60, 70, and 74 appear to be contaminants originating from the plastic housing of the water reservoir of the nebulizer. Na^+ and $(H_2O + Na)^+$ are produced, but there is no ion signal at *m/z* 36. An aqueous solution of 100 µM caffeine was nebulized, and the resulting mass spectrum is shown in Figure 2b. Protonated and sodiated caffeine are formed, as is sodiated caffeine dimer. There is signal at *m/z* 212 near the detection limit that is ~0.04% that of protonated caffeine, but this value is more than 1100 times lower than that reported by Xing et al.[7] Results for 100 µM aqueous melatonin are shown in Figure 2c. Both protonated and sodiated melatonin are formed, but protonated oxidized melatonin (*m/z* 250) was not detected. The upper limit of oxidized melatonin formed is <0.3%, more than 1000x less than reported by Xing et al.[7] The mesh screen nebulizer was positioned an additional 10 cm from the inlet (20 cm total distance) increasing the droplet lifetime to ~560 ms. No oxidation product of melatonin was observed (Figure S3), indicating that droplet lifetime does not appear to be a factor in our inability to observe these reactions.

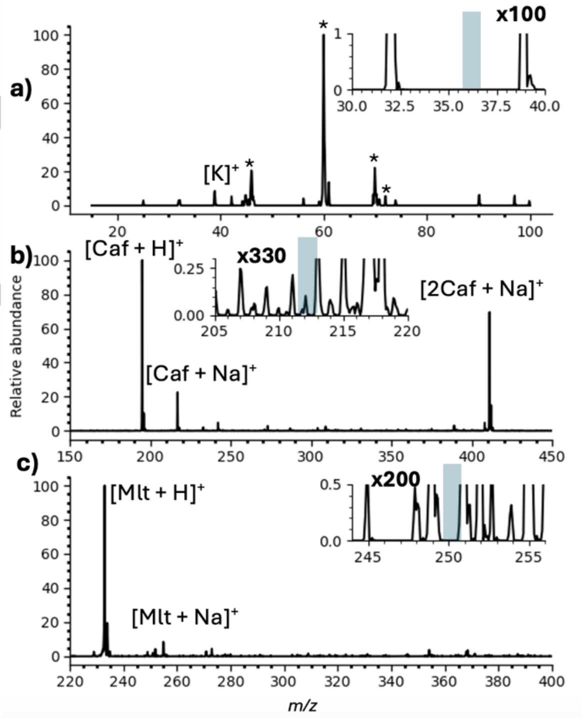

Figure 2. Mass spectra obtained using a mesh screen nebulizer to produce micron size droplets from a) pure water, b) 100 µM aqueous caffeine, and c) 100 µM aqueous melatonin. No other ionization source was used. The nebulizer was placed 10 cm orthogonal to the mass spectrometer inlet resulting in droplet lifetimes of ~180 ms. Insets show the region where ions at a) *m/z* 36 corresponding to $OH\bullet\text{-}H_3O^+$ or $(H_2O\text{-}OH_2)^{\bullet+}$, b) *m/z* 212, corresponding to oxidized protonated caffeine, and c) *m/z* 250 corresponding to oxidized protonated melatonin. The *y*-axis expansions for the insets are indicated in the figure. Starred peaks are contaminants likely originating from the plastic housing of the nebulizer. These data were acquired in the ion trap of an Orbitrap mass spectrometer with an inlet capillary temperature of 50 °C in order to closely replicate prior experiments where these spontaneous reaction products were reported.[7]

In prior work, droplets formed by pneumatic nebulization were assumed to be uncharged,[7] yet a mass spectrometer was used to detect ions originating from these droplets with no other ionization source described. Sonic spray, a pneumatic nebulization source without an external voltage supply has been previously shown to produce gaseous ions, consistent with charged droplets being formed.[35,36] To obtain some information about the charge density on the large microdroplets formed by the mesh screen nebulizer at late stages of the droplet lifetime where gaseous ions are produced, a mass spectrum of a nebulized solution of 10 μM ubiquitin was obtained. Charge states between 4+ and 9+ are produced (Figure S4), a range that is similar to that formed by nanoelectrospray ionization from aqueous solution. Thus, the low charge density on the initially formed droplets increases as solvent evaporation from the charged droplets occurs, resulting in nanoscale droplets that are charged similarly to those formed by electrospray.

Despite the high surface-to-volume ratios for both the nanodrops and microdroplets, no evidence for spontaneous hydroxyl radical formation from pure water that was reported earlier was observed. The *m/z* 36 ion was reported to have limited stability.[7] We observe abundant (H_2O + Na)$^+$, a weakly bound complex, under these conditions, and the major signal from collision induced dissociation of the oxidized form of caffeine, both protonated and sodiated, were the molecular ions[7] indicating their high stabilities.

To investigate whether hydrogen peroxide or hydroxyl radicals lead to abundant ions at *m/z* 36, a nanoelectrospray spectrum of a 10 μM aqueous H_2O_2 solution was obtained. A low abundance *m/z* 36 ion that is three orders of magnitude lower in abundance than what was reported by Xing et al.[7] without added H_2O_2 was observed (Figure S5). Hydroxyl radicals can be produced by the Fenton reaction. A low abundance *m/z* 36 ion is formed from a solution containing both 10 μM of H_2O_2 and 10 μM $FeCl_2$, but this ion is also observed from an aqueous solution containing just 10 μM $FeCl_2$ along with more abundant (FeOH + H_2O)+ (Figure S5). Reduction of Fe^{2+} at late stages of gaseous ion formation to form a metal hydroxide and protonated water is commonly observed in electrospray ionization of multiply charged metal ions,[37] consistent with protonated water dimer as the major ion formed when $FeCl_2$ is present. These experiments were repeated using the vibrating mesh nebulizer. No signal at *m/z* 36 was observed with 10 μM of H_2O_2, but as was the case with nanoelectrospray ionization, addition of 10 μM $FeCl_2$ both with and without 10 μM H_2O_2 (Figure S5) resulted a low abundance of ions at *m/z* 36.

The abundance of *m/z* 36 ions in solutions containing H_2O_2 with and without $FeCl_2$ enabled more accurate mass measurements of this ion using a Waters QTOF Premier mass spectrometer. The mass of this ion formed by nanoelectrospray from solutions containing H_2O_2 with and without $FeCl_2$ (with 1 μM NaCl for internal calibration) is 36.0437 Da and 36.0458 Da, respectively (Figure S6 and Table S2). The measured mass indicates that the *m/z* 36 ion is (NH_3 + H_2O + H)$^+$ (exact mass: 36.0444 Da; $\Delta m \leq 0.0014$ Da) and not OH•-H_3O^+ (exact mass: 36.0206 Da; $\Delta m \geq 0.0231$ Da). The mass resolution of ~2000 is sufficient to distinguish the two different elemental compositions (~1500 resolution required; Figure S6a). A low abundance ion at nominal *m/z* 39 that was not used in the calibration has a measured mass of 38.9645 Da and 38.9635 Da in these two solutions, indicating that this ion is likely K^+ (exact mass: 38.9632 Da; $\Delta m \leq 0.0013$ Da), a ubiquitous ion often found in trace quantities in borosilicate capillaries. To provide additional support for the *m/z* 36 ion being (NH_3 + H_2O + H)$^+$, a nanoelectrospray mass spectrum of aqueous 1 mM ammonium acetate with 1 μM NaCl was obtained and resulted in a predominant ion at *m/z* 36.0452 Da (Figure S6). The mass of this ion is within 0.0015 Da of the measured mass of the *m/z* 36 ions formed from solutions containing H_2O_2 with and without $FeCl_2$. Ammonia may originate from exhaled breath, or it could be in the form of a residual salt often used in mass spectrometry analysis. Interactions of charged droplets with surfaces[3,38,39] of the mass spectrometer interface could lead to incorporation and ionization of ammonium salts leading to formation of (NH_3 + H_2O + H)$^+$. Ammonia is also more easily ionized (lower ionization energy) and is more readily protonated (higher gas-phase basicity) than water, all of which leads us to conclude that it is the origin of the low abundance signal at *m/z* 36.

We conclude that the ion signals reported by Xing et al. that were used as evidence for spontaneous formation of OH•-H_3O^+ or (H_2O-OH_2)$^{+\bullet}$ and hydroxyl radicals do not originate from unactivated pure water droplets and that these species must have been formed by an unrelated mechanism. Mechanical breakup of water, whether pneumatically or ultrasonically, leads to charged droplets of both polarities,[34] and the charge density at the surface of the droplets must increase as water evaporates to form smaller droplets. Despite the presence of net H_3O^+ at the droplet surface, no ions at *m/z* 36 nor oxidation products of the previously used radical scavengers were reliably detected from pure water. These results indicate that any hydroxyl radical that is formed must be several orders of magnitude lower in concentration than what has been previously reported. Thus, water appears to be stable at the surface of droplets over a wide range of droplet size, net charge and lifetimes.

While we do not know what caused the formation of abundant ions at m/z 36 or oxidation products of caffeine and melatonin reported by Xing et al., we speculate that this may be due to some form of energy transfer from the nebulization source to the water droplets or water vapor, consistent with an increase in the m/z 36 ion with increasing nebulizing gas pressure that was reported. Activation could be from electrical discharge, field ionization or cavitation that may occur in pneumatic nebulization sources. Production of charged droplets of opposite polarity may lead to an electrical discharge[40] or field ionization that can produce water radical cations in vapor. An electric discharge generated by a needle in the presence of water vapor resulted in ionized water dimer at m/z 36 as the most abundant ion in the mass spectrum.[41] Cavitation at the end of a silica capillary in sonic spray ionization can occur by formation of a vortex from the sheath gas flow.[34] An exact mass of this ion has not been previously reported, leading to the possibility that the m/z 36 ion measured in earlier experiments was misidentified. Recently, ions at m/z 28 and 32 formed by pneumatic nebulization of pure water were reported, and these ions were attributed to ionized N_2 and O_2 gas, respectively.[42, 43] The ionization energies of these gases are 15.58 eV and 12.07 eV, respectively.[44] No ions at these m/z values are observed with nanoelectrospray ionization of pure water in air without an electrical discharge (Figure 1a) or from water with 10 µM $FeCl_2$ and 10 µM H_2O_2 (Figure S6a). This indicates that significant electronic excitation, either from electrical discharge or field ionization as a result of the close proximity of oppositely charged microdroplets, can occur when water droplets are formed in pneumatic nebulization sources in which m/z 36 ions and oxidation products have been reported previously. Formation of ionized gases that have high ionization energies in these nebulizers may also explain some of the unusual reactivity that has been previously attributed to the high electric field at the surface of microdroplets. Based on our results, water appears to be stable at the air-water interface, indicating that some additional form of activation is necessary to produce the reactions that have been previously reported as evidence for substantial spontaneous formation of hydroxyl radicals at the surface of water microdroplets.

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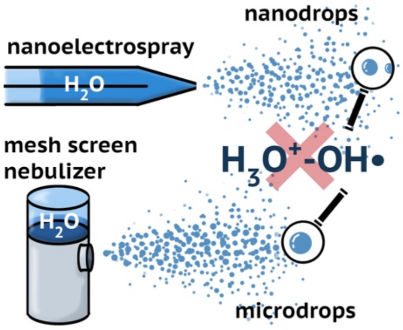

Spontaneous reactions of water in either highly charged nanodrops formed by electrospray ionization, or less highly charged microdroplets formed by a vibrating mesh screen nebulizer were investigated. No evidence for ionized water dimer, nor oxidation products of the radical scavengers caffeine or melatonin was observed over a wide range of droplet size, net charge, and lifetime, indicating that water is stable at the surface of unactivated water droplets.