Edge Article

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Recent reports on the formation of hydrogen peroxide (H$_2$O$_2$) in water microdroplets produced via pneumatic spraying or capillary condensation have garnered significant attention. How covalent bonds in water could break under such mild conditions challenges our textbook understanding of physical chemistry and water. While there is no definitive answer, it has been speculated that ultrahigh electric fields at the air–water interface are responsible for this chemical transformation. Here, we report on our comprehensive experimental investigation of H$_2$O$_2$ formation in (i) water microdroplets sprayed over a range of liquid flow-rates, (shearing) air flow rates, and air composition, and (ii) water microdroplets condensed on hydrophobic substrates formed via hot water or humidifier under controlled air composition. Specifically, we assessed the contributions of the evaporative concentration and shock waves in sprays and the effects of trace O$_3$(g) on the H$_2$O$_2$ formation. Glovebox experiments revealed that the H$_2$O$_2$ formation in water microdroplets was most sensitive to the air–borne ozone (O$_3$) concentration. In the absence of O$_3$(g), we could not detect H$_2$O$_2$(aq) in sprays or condensates (detection limit >250 nM). In contrast, microdroplets exposed to atmospherically relevant O$_3$(g) concentration (10–100 ppb) formed 2–30 μM H$_2$O$_2$(aq), increasing with the gas–liquid surface area, mixing, and contact duration. Thus, the water surface area facilitates the O$_3$(g) mass transfer, which is followed by the chemical transformation of O$_3$(aq) into H$_2$O$_2$(aq). These findings should also help us understand the implications of this chemistry in natural and applied contexts.

Introduction

Interfacial mass and energy transfer, chemical transformations/ reactions, and tensions at water interfaces are implicated in numerous natural and industrial processes, such as the atmosphere–hydrosphere exchange,† cloud physics† and chemistry,† thundercloud charging,§ precipitation,§ ion speciation§ and ion-catalyzed proton transfers,§§ aerobic bioreactors,§§ food science,‖ soil physics,‖ underwater insect respiration,‖ and the ubiquitous water evaporation/condensation events. Probing the air–water interface of molecular thickness, however, remains a daunting research area due to the challenges associated with direct experimental interrogation as well as the limitations of the water models.‖ Therefore, investigating the properties of the water surface is a research frontier in chemical science, sometimes invoking vigorous debates.14–26 With this preface, we introduce the latest reports on the chemical transformation of water into hydrogen peroxide (H$_2$O$_2$). Researchers have discovered the spontaneous production of (i) H$_2$O$_2$ (~30 μM) in water microdroplets of diameter ≤20 μm sprayed via pressurized gas27 and (ii) H$_2$O$_2$ (≤115 μM) in condensed water microdroplets of diameter ≤10 μm on common substrates in the relative humidity range 40–70%.28 Crucially, smaller droplets yielded higher H$_2$O$_2$ concentration, pinpointing that the air–water interface is somehow implicated. How could the O–H covalent bonds in water with an approximate strength of ~100 kcal mol$^{-1}$ be broken under normal temperature and pressure (NTP, 293 K and 1 atm; k$_N^0T$ = 0.58 kcal mol$^{-1}$) without the use of a catalyst, significant energy, or co-solvents? There is no explanation available at the moment. Presence of ultrahigh electric fields at the air–water interface has been speculated to be the cause.27,28 These findings challenge our textbook-level understanding of water and perhaps other similar liquids where constituent atoms occupy distant positions on the Pauling’s electronegativity table.29 Given water’s innumerable roles in environmental cycles and phenomena and industrial processes, could these findings herald a reassessment of what...
we know of water; opportunities in green chemistry also deserve a serious consideration.

We started exploring this phenomenon soon after the appearance of the first report. A follow-up report revealed that even condensed water microdroplets contained H$_2$O$_2$, in fact, at even higher concentrations $\leq$115 $\mu$M. Let us take a moment to juxtapose these two unrelated experimental methods utilized to study this chemical transformation. Condensation is a gentle process governed by the relative humidity and the substrate’s temperature and chemical make-up. In contrast, pneumatic sprays utilize high shearing gas-flow (e.g., 1–10 L min$^{-1}$) from a microscale capillary/annulus leading to fast gas speeds (e.g., 100–1000 m s$^{-1}$) that cause much turbulence/mixing/forced-convection inside and around the spray plume and can enhance the concentration of H$_2$O$_2$ due to the faster evaporation of the solvent (more details in Results and Discussion). In fact, if the spray-based experiments are conducted inside a controlled environment glovebox, the shearing gas-flow disturbs the atmospheric composition/distribution, especially the relative humidity. Thus, we restricted our preliminary investigation to water condensation, wherein we found that: (i) the condensed microdroplets formed via the vaporization of hot water (50–70 $^\circ$C) did not contain H$_2$O$_2$; (ii) if ultrasonic humidifiers were exploited to form the vapor, $\sim$1 $\mu$M H$_2$O$_2$ was detected. Therefore, we established that the ultrasonic humidifier was a contributor to the H$_2$O$_2$ production and the air–water did not have any detectable effect. The fact that ultrasonic waves can produce H$_2$O$_2$ in water is well-known and has even been exploited in practical applications, including disinfection and water treatment (see ref. 33–35 for further information). Still, however, we remained puzzled – why did researchers in California observe $\sim$115-times and $\sim$30-times higher H$_2$O$_2$ in their condensation and spray experiments, respectively, while we did not find any H$_2$O$_2$ in similar experiments at KAUST? Here, by designing and applying a broad set of experiments, we propose a different explanation for the H$_2$O$_2$ formation in water microdroplets formed via pneumatic spraying or condensation.

**Results**

In this experimental study, we utilized high-purity HPLC-grade water as well as water from the standard MilliQ Advantage 10 set up (Methods). Commercially available hydrogen peroxide (H$_2$O$_2$) 30% solutions (v/v) were diluted using water and utilized for the calibration of the assays and other experiments (Fig. S1†). We utilized the fluorometric Hydrogen Peroxide Assay Kit (HPAK, ab138886, Abcam PLC, Cambridge, UK) that facilitated a detection range for aqueous H$_2$O$_2$ down to $\sim$250 nM, thereby affording a nearly 40-times lower limit of detection than the potassium titanium oxalate (PTO) assay employed in the original reports (Methods). HPAK calibration plots and comparisons with other standard H$_2$O$_2$ quantification methods, including the PTO assay and the hydroxyterephthalic acid (HTA) assay, have already been presented in our recent report.

For spraying water microdroplets, we custom-built pneumatic setups following the details presented in the recent report. Broadly, our spray units comprised of concentric stainless steel tubes (Fig. 1 and S2†), wherein water was pushed...
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Evaporative concentration

As a solution comprised of water and H$_2$O$_2$ evaporates, for instance, inside a vacuum oven or in the form of sprayed microdroplets, the ratio of the formed vapor (H$_2$O(g) : H$_2$O$_2$(g)) is not the same as their bulk concentration (H$_2$O(l) : H$_2$O$_2$(l)). This is so because water evaporates faster due to its lower boiling point (373 K at 1 atm) than that of H$_2$O$_2$ (423 K at 1 atm). Here, we define evaporation ratio as the ratio of the initial volume prior to spraying ($V_0$) to the finally collected volume after spraying ($V_f$). A simple evaporation experiment was performed on bulk aqueous solutions containing 4 and 8 mM H$_2$O$_2$ by placing them inside a vacuum oven set at 20 mbar and 293 K for approximately 2 days. These solutions exhibited evaporation ratios of ~50 and ~17, respectively, such that the H$_2$O$_2$ concentration increased by ~12 and ~7-times, respectively. Next, we quantified evaporation ratios within pneumatic sprays formed via (i) ~0.5 mM H$_2$O$_2$ solutions and (ii) ultrapure water (Fig. 2). The water flow rate was varied in the range of 50–400 µL min$^{-1}$ and the nebulizing gas (N$_2$) flow rate was fixed at 5.3 L min$^{-1}$ (~8 bar in-line pressure). Our experimental set up’s details are presented in Fig. S2, S3 and Table S1† the influence of the in-line gas pressure and the inner/outer capillary dimensions on the gas flow rates is plotted in Fig. S3A and B† and the size distributions of sprayed microdroplets, determined via dynamic light scattering (Methods), as a function of gas flow is shown in Fig. S4B.† Under these experimental conditions, when the liquid flow rate was >70 µL min$^{-1}$, there was hardly any enhancement in the H$_2$O$_2$ concentration. However, as the

Fig. 2  Evaporative concentration in pneumatic sprays formed with ultrapure water (blue and pink) and 0.5 mM H$_2$O$_2$ solutions (green and orange) using a fixed N$_2$ gas flow rate 5.3 L min$^{-1}$. (A) Sharp rise in the H$_2$O$_2$ concentration of (spiked) 0.5 mM solution when the liquid flow rate was <70 µL min$^{-1}$; minimal H$_2$O$_2$ production in sprays formed with ultrapure water. (B) Relationships between the final H$_2$O$_2$ concentration and the evaporation ratios. Note: the error bars correspond to the standard deviations in the spectrophotometer readings. All slopes in (B) were statistically greater than zero ($p < 0.05$; ANOVA test).
liquid flow rate was reduced below 70 µL min⁻¹, the H₂O₂ concentration began to rise exponentially. Remarkably, within the range of 400–50 µL min⁻¹, the H₂O₂ concentration in the ~0.5 µM standard solutions increased by 9.1 × (0.7–6.2 µM) and 13.6 × (0.4–5.2 µM), respectively, and the evaporation ratios were 14.2 and 21.3, respectively. Similarly, the H₂O₂ concentrations in sprays formed with ultrapure water also increased when the liquid flow rate was within the range 400–50 µL min⁻¹; the enhancement factors were 6.6 × 1 and 1.1 × − both <0.34 µM − while the evaporation ratios were 14.2 and 8.6, respectively. ANOVA tests confirmed that even the slopes of the ultrapure water samples (#1 and #2) were statistically greater than zero (p < 0.05). The average time for sample collection (~1 mL for analysis) at 50 µL min⁻¹ flow rate was >5 h per datum point due to evaporation rates being almost as high as liquid injection rates; so, we did not pursue investigation of evaporation at lower liquid flow rates. Taken together, these experiments established that while evaporative concentration could enhance the H₂O₂ concentration in sprayed water microdroplets slightly, this effect is nearly a factor 100× too short to account for the ppm-level (1 ppm = 29.4 µM) H₂O₂ concentrations noted recently.²⁷

**Mechanical vibrations and shock waves**

We combined experiment and theory to probe the effects of mechanical vibrations on the formation of shock waves during spraying. High-speed videos of pneumatically driven sprays were recorded using a Kirana-05M camera at 5 million frames per second (fps). Its 200 ns time-resolution enabled us to observe microdroplets trajectories before, during, and after their interaction with the shearing gas (Fig. 3A and ESI Movie S1†); their size, number, and velocity were also monitored (Methods). We found that as the slow-moving water droplets got hit by the fast-moving gas, they accelerated and frequently broke into smaller ones (Fig. 3A). We deduced that the speed of the N₂ gas to be ~800 m s⁻¹.

Before computational fluid dynamic (CFD) simulations, a theoretical calculation was conducted based on the Rankine–Hugoniot conditions, because from a CFD simulation point of view H₂O₂ could only be generated by high-temperature reactions (Section S1†). Shock waves could generate high temperatures and pressures. When the high-speed gas hits the water droplet, almost all of the momentum energy is converted to heat. If we assume the heat capacity does not change, then, the temperature rise of the static gas can be estimated as, $\frac{u^2}{2c_p}$, where $u \approx 800$ m s⁻¹ and $c_p = 4.2$ J g⁻¹ K⁻¹. This yields $a \approx 301$ K rise in temperature at the droplet front.

Next, we probed the gas–water interactions via three-dimensional CFD simulations using the converge code, wherein the turbulence is simulated by the renormalization group k-ε model.⁴⁶ The Eulerian void of fluid (VOF) method was adopted to capture the in- and near-nozzle spray details (ESI Section S1, Fig. S5 and S6†). Simulations revealed the formation...
of bowl-shaped shock waves at the impact point of the droplets that led to an increase in the local temperature (~327 °C) and pressure (~7 bar) (Fig. 3B and C). However, these conditions are too mild to produce a chemical transformation; a relatively high concentration of H$_2$O$_2$ was generated only with a temperature over 1000 K and a residence time over 10 μs (Fig. S7†) that are unachievable in our experiments. Thus, this hypothesis cannot explain the formation of H$_2$O$_2$ in sprayed water microdroplets.

**Effects of atmospheric ozone on the H$_2$O$_2$ formation in water**

Next, we exploited a glovebox to investigate the effects of ozone (O$_3$) gas concentration in the air on the H$_2$O$_2$ formation in water microdroplets (Fig. 4). The O$_3$ concentration in the air was varied in the range 2–4900 ppb and measured using an ozonometer with a 2 ppb detection limit and a range of 2–5000 ppb (Methods). Subsequently, the H$_2$O$_2$ concentration in the water microdroplets was quantified using the HPAK.

Below, we discuss the various scenarios in this chronology: (i) condensing the vapor formed using heated water under ~85% RH (Methods), (ii) condensing water from a commercial ultrasonic humidifier with 15 W output power and RH varying in the range 70–80% (Methods), and (iii) pneumatic sprays formed with a water flow rate of 1 mL min$^{-1}$ and shearing gas (N$_2$) flow rate of 2.3 L min$^{-1}$. Single crystal SiO$_2$/Si wafers silanized with perfluorodecyltrichlorosilane (FDTS) using a molecular vapor deposition technique$^{49}$ were used as substrates. For the condensation experiments, the substrates were cooled down to 3–4 °C. Next, it is crucial to note that the O$_3$(g) concentration in our laboratory during the course of this study was <2 ppb. Thus, the glovebox filled with ambient air served as the control case. In this scenario, the water collected from the pneumatic sprays or the condensates formed via the vapor of hot water (50–70 °C) did not contain H$_2$O$_2$ (Fig. 2 – blue and pink data points and ref. 30. Curiously, when the water vapor was derived from a 15 W commercial ultrasonic humidifier, ~1 μM H$_2$O$_2$ was detected in the condensate. We have explained its origins in a recent report$^{30}$ as well as in the introduction above.

O$_3$(g) was produced using an ozone generator and diluted with air (or N$_2$ gas) prior to its entry in the glovebox equipped with an ozonometer (Methods). In the condensation experiments, as the O$_3$(g) and the RH reached the desired levels, the O$_3$(g) supply was terminated (ESI Section S2†). We monitored the time-dependent loss of O$_3$(g) concentration as we waited for 40 minutes to collect adequate condensate volume to be able to perform the HPAK analysis. Fig. 5 (green and red datapoints) presents the final H$_2$O$_2$ concentration in the condensates (from the humidifier and hot water, 40 °C) against the initial O$_3$(g) concentration. Note that these data should not be mistaken to represent thermodynamic equilibrium, which is not achieved in our system. Specifically, a number of factors influence O$_3$(g) depletion, e.g., reactions with and/or adsorption onto the glovebox surface and materials/instruments inside it, reactions with condensed water on the substrates as well as the cold surfaces underneath them, and the leakage from the glovebox. To appreciate this further, we point out that when a metallic heating plate was used to produce water vapor inside the glovebox for condensation experiments, O$_3$(g) depleted rapidly (Fig. S8B†). In comparison, when an ultrasonic humidifier with a plastic surface was utilized, the decay was gradual. This also is the reason for the lower H$_2$O$_2$(aq) concentration in the condensates of heated water in comparison with that of the humidifier (Fig. 5).

To distinguish the O$_3$ consumption by the water from the parasitic losses in the glovebox, we compared the time-dependent depletion of O$_3$(g) with and without water. In this...
experiment, 120 mL of water was placed inside a shallow container presenting 390 cm² of the air–water surface area. The O₃(g) depletion was faster in the presence of water (Fig. S9†). These experiments and the controls establish unambiguously that (i) water contributes to the O₃(g) depletion and (ii) the higher the initial O₃(g) concentration, the higher is the formation of H₂O₂ in the condensates (Fig. 5 – green and red datapoints). We explain in the Discussion section what happens to the depleted O₃(g) inside water.

Next, we discuss our spray experiments under controlled O₃(g) environment (Fig. 4 (left) and S2†). To offset the dilution of the gas-phase O₃(g) due to the shearing gas flow from the sprays (N₂, 2.3 L min⁻¹ at NTP), we manually controlled the flow of O₃(g) and air via separate lines (ESI Section S2†). Thus, we managed to achieve relatively stable O₃(g) concentration (Fig. S8C†) and RH in the range of 30–60%. At the above-listed water and shearing gas flow rates, we were able to collect adequate sample volumes for the HPAK analysis within 5 min. Fig. 5 (blue datapoints) presents the H₂O₂ concentration in the water collected from the sprays; note-in these experiments, the O₃(g) does not deplete with time (Fig. S8C†) unlike the case for the condensation experiments (Fig. S8A and B†). To probe the effects of the sprayed microdroplet size distribution on the formation of H₂O₂, we varied the shearing gas flow rate keeping the water flow rate fixed under 100 ± 20 ppb O₃(g) concentration. The mean droplet diameter decreased non-linearly within

![Fig. 6](image_url) Influence of nitrogen shearing gas flow rates on the formation of H₂O₂ in water microdroplets produced by pneumatic sprays under ozone atmosphere (100 ± 20 ppb). The liquid flow rate was fixed at 1 mL min⁻¹.

The range 105–14 μm as the shearing gas flow was varied from 1–5 L min⁻¹ for 100 μL min⁻¹ liquid flowrate (Fig. S4A and B†). However, the H₂O₂ concentration increased linearly with nitrogen flowrate (Fig. 6). This indicates that, despite the higher surface, the main reason for the increase in H₂O₂ concentration with nitrogen flowrate has to do with enhanced mixing between the low-ozone spray region and with the surrounding ozone-containing atmosphere in the glovebox. Some other factors that influence the droplet-O₃(g) interactions and the H₂O₂(aq) formation include, the life-span of the sprayed water droplets, that in pneumatic sprays of pure water at 1 mL min⁻¹, the H2O2 concentration in water microdroplets produced by pneumatic sprays under ozone atmosphere (100 ± 20 ppb). The liquid flow rate was fixed at 1 mL min⁻¹.

**Discussion**

Here, we draw together our results to pinpoint the key factors and mechanisms responsible for the H₂O₂ formation in water microdroplets. First of all, the fact that we do not see the H₂O₂ formation in water microdroplets, sprayed or condensed, in the absence of O₃ proves that this chemical transformation is not a property of the air–water surface (Fig. 2 – blue and pink data points and ref. 30). While the evaporative concentration of trace quantities of H₂O₂ already present in water is possible, in principle, in sprays, neither is it adequate to explain the ppm-level H₂O₂ concentration in the previous report nor is it relevant to the condensation experiments. Similarly, shock waves and temperature rise during pneumatic spraying under our experimental conditions simply cannot contribute to ppm-level H₂O₂; also, they are irrelevant to condensation-based reports. These experiments, however, facilitate valuable insights into

![Fig. 5](image_url) H₂O₂ concentration in water microdroplets formed inside the glovebox via condensation from two humidity sources, (i) ultrasonic humidifier (18–20 °C; RH 70–80%) and (ii) water heated beaker (40 °C; RH ~ 85%), and via pneumatic sprays of pure water at 1 mL min⁻¹ with 2.3 L min⁻¹ N₂ flow rate (18–20 °C; RH 30–60%). As the O₃(g) concentration in the glovebox atmosphere increased, the H₂O₂ concentration in the microdroplets also increased. The condensation experiments were performed by setting the initial concentration in the gas phase and then stopping all in and out flows, this led to the gradual depletion of ozone from the glovebox, while for the spray was performed by controlling the ozone concentration with manual adjustments in the dilution of ozone by nitrogen gas and clean air (Fig S8†). Horizontal error bars in the sprays represent the range of ozone concentration fluctuation during the time of sample collection.
relevant physical and chemical processes that could, under extreme conditions, contribute to the H$_2$O$_2$ concentration/formation in water. For instance, it is worth exploring how those effects could influence gas-phase reactions between O$_3$(g) and H$_2$O(g) followed by the repartitioning of H$_2$O$_2$(g) in the condensed phase.

Next, we discuss the scope and significance of our investigation of the H$_2$O$_2$ formation in condensed and sprayed microdroplets under controlled O$_3$(g) environment. These experiments unambiguously pinpoint the H$_2$O$_2$ formation in water is extremely sensitive to the ambient O$_3$ concentration (Fig. 5 and S8†); if the O$_3$(g) is present, then the enhancement in the air–water surface area combined with higher mixing with the surrounding ozone containing atmosphere leads to higher H$_2$O$_2$ formation (Fig. 6 and S4†). Based on this insight, below, we present an alternative explanation for the H$_2$O$_2$ formation phenomenon reported by researchers based in California, i.e., the original reports.27,28 The Environmental Protection Agency (EPA)49 live database reports that the average O$_3$ concentrations in Santa Clara County in California during the years 2018–2020 were ~26 ppb with a maximum daily average in the range 48–64 ppb; the highest O$_3$(g) concentrations on some days could even exceed 80 ppb. We submit that this high regional O$_3$(g) is the primary reason behind the H$_2$O$_2$ formation in those reports. Next, we provide some qualitative insights into this chemical transformation.

It is well-known that O$_3$(g) is minimally soluble in water (Henry’s law constant, $H_{O_3}^{CP} = 10^{-3}$ M atm$^{-1}$ at NTP50) and it would take a much longer time for bulk water to equilibrate with the ambient atmosphere than the duration of our experiments (5–40 min). Thus, water microdroplets in our experiments will have always have an aqueous ozone concentration that is lower than the equilibrium concentration specified by the Henry’s law, i.e., $C_0 = P_0 \times H_{O_3}^{CP} \approx 43 \times 10^{-9} \times 10^{-2} = 0.4$ nM. Furthermore, sprays had lower H$_2$O$_2$(aq) concentrations than the condensates because (i) the sample collection for the sprays (~5 min) was faster than that for the condensates (~40 min); and (ii) the fast flow of the shearing gas (e.g., N$_2$ gas at 2.3 L min$^{-1}$) created a N$_2$-rich region around the spray that reduced O$_3$(g) uptake in the microdroplets. Under these circumstances, how could H$_2$O$_2$(aq) concentrations rise to 1–10 μM in sprayed microdroplets, i.e., a factor $>2500$–25 000× higher than that highest possible O$_3$(aq) concentration (0.4 nM)? Here, the microscale of droplets comes into play: in comparison to bulk water, microdroplets have a dramatically larger surface-to-volume ratio in comparison to the bulk. For instance, if a spherical water droplet of volume 1 mL and diameter $D = 1.24$ cm is sprayed into microdroplets of diameter $d = 10$ μm, the surface area increases by a factor of $D/d = 1240$. This enhancement in the air–water surface area facilitates higher mass transfer of O$_3$(g) into the water within their limited life-span. Next, O$_3$(aq) undergoes chemical transformations in water leading to a variety reactive species such as OH, HO$_2$, HO$_2^-$, O$^-$, O$_2^-$, OH$_2$, and O$_3^-$ and a stable product, $i.e.$, H$_2$O$_2$.44,59,61 The decomposition of ozone in water is complex, but the following representative reactions provide a qualitative description:

$$O_3(g) \rightarrow O_3(aq) \quad (1)$$

$$O_3(aq) + OH^-(aq) \rightarrow HO_2^-(aq) + O_2(aq) \quad (2)$$

$$HO_2^-(aq) + H_2O^+(aq) \rightarrow H_2O_2(aq) + H_2O(aq) \quad (3)$$

Since H$_2$O$_2$(aq) is miscible in water ($H_{H_2O_2}^{CP} \sim 10^3$ M atm$^{-1}$ at NTP), it accumulates in the bulk, i.e., interfacial mass transfer of O$_3$(g) into microdroplets continues to produce H$_2$O$_2$(aq). Next, we note the limitations of this description: kinetics of the reactions (eqn (1)–(3)) may not be viable to quantitatively explain the observed H$_2$O$_2$(aq) concentrations within 5–40 min; thus, other reaction pathways of O$_3$ might be implicated.44,50–52 Additionally, discrepancies between the H$_2$O$_2$(aq) concentrations in sprays reported here and those in the original reports27,28 are attributed to the differences in experimental spray setups’ dimensions. Minute differences can lead to significantly different gas flow rates (Fig. S3†) and aerodynamic conditions that influence the mass transfer of ozone to the liquid and the formation of H$_2$O$_2$ (Fig. 6, S3 and S4†). Lastly, in the presence of organics in water, such as in the natural and industrial contexts, products of ozone autodissociation participate in numerous reactions and get consumed;51,54 solid surfaces such as silica and perfluorocarbons can also influence the rates of these reactions;44,50 we also note that ozone generators could produce NO$_x$ species that could interfere with reactions.55 An in-depth analysis of these factors as well as the rates of reactions and mechanisms, the mass transfer considerations, and the non-equilibrium effects listed in the Results section is warranted. A microdroplet-level analytical assay with an HPAK-like detection limit, if developed, could help immensely. We close the discussion by noting that this O$_3$–H$_2$O$_2$ coupling inside water has led to misinterpretations in the past also44,53,56 and strategies for curtailing these effects have been devised.27 Furthermore, for a reader interested in simulations of the mass transfer of O$_3$(g) and H$_2$O$_2$(g) at the water surface and subsequent chemistries, we refer to this excellent report.27

**Conclusion**

In this contribution, we investigated the factors and mechanisms responsible for the formation of H$_2$O$_2$ in water microdroplets produced via spraying or condensation. We found that in the absence of ozone gas, H$_2$O$_2$ does not form regardless of condensation/spraying or the droplet size or the substrate. This unambiguously establishes that the air–water interface does not spontaneously produce H$_2$O$_2$. The H$_2$O$_2$ formation in microdroplets only happens in the presence of O$_3$(g); as the O$_3$(g) concentration increases in the air, the H$_2$O$_2$ concentration also increases in the microdroplets – condensed or sprayed. The higher the gas–liquid surface area, mixing, and contact time, the higher is the O$_3$(g) uptake in the water; this O$_3$(aq) then undergoes chemical transformations yielding H$_2$O$_2$(aq). Through the Environmental Protection Agency (EPA) database,49 we found that the average O$_3$ concentration in California during the years 2018–2020 was ~26 ppb, with a maximum daily average in the 48–64 ppb range. These facts and our findings...
therefore present an alternative explanation for the spontaneous formation of H$_2$O$_2$ in water microdroplets (reported from California). The primary role of the air–water interface is to facilitate the mass transfer of ozone into the water. Of course, reactions of O$_3$ with the water surface could be implicated, and they should be probed via surface-specific platforms in conjunction with theory and computation. To sum up, our findings disprove the latest claims for the water surface’s ability to spontaneously produce H$_2$O$_2$.$^{27,28}$ The speculation for the presence of the mysteriously high electric fields at the air–water interface, responsible for transforming H$_2$O into H$_2$O$_2$, thus also appears untenable. H$_2$O$_2$ formed in water microdroplets can also help explain the origins of the recently reported bactericidal properties of pneumatically sprayed water and help assess their practical and environmental relevance.$^{58-60}$

Materials and methods

Chemicals

Standard hydrogen peroxide (H$_2$O$_2$) 30% and high-performance liquid chromatography (HPLC LC-MS) grade water were purchased from VWR Chemicals (Catalogues #270733 and #23622.298). Deionized water produced from a MilliQ Advantage 10 set-up was also used in this study.

Quantification of H$_2$O$_2$ in water

Hydrogen peroxide assay kit (HPAK) assay. H$_2$O$_2$ concentration present in both condensed and sprayed water microdroplets was quantified by the Hydrogen Peroxide Assay Kit (Fluorometric-Near Infrared, Catalogue #ab138886). It can detect H$_2$O$_2$ by the fluorescence produced when in contact with the AbiIR peroxidase indicator, and its maximum excitation and emission wavelengths are 647 nm and 674 nm, respectively. This method also contains a horseradish peroxidase enzyme that catalyzes the reaction and increases the fluorescence signal, having a linear range of detection from 250 nM to 10 μM. Samples containing higher concentrations than the detectable range were diluted with deionized water. The analyses were conducted in a 96-well black/clear bottom microtiter-plate, adding 50 μL of each sample within 50 μL of HPAK, using a SpectraMax M3 microplate reader (Molecular Devices LLC) and the software SoftMax Pro 7 for fluorescence reading. The H$_2$O$_2$ concentration was calculated using the calibration curve obtained on the same day.

Peroxide test strips for semi-quantitative analysis. We used peroxide test strips (Baker Test Strips, VWR International) with a detection limit of 1 ppm (29.4 μM) for a qualitative estimation of H$_2$O$_2$ in aqueous samples. They work through a colorimetric reagent which gives blue color when exposed to H$_2$O$_2$(aq) and the color deepens with the H$_2$O$_2$ concentration.

Water microdroplets generation via sprays

We built a spray to generate water microdroplets by injecting water through an inner tube of 100 μm of diameter using a syringe pump (PHD Ultra, Harvard Apparatus), and nitrogen coaxial sheath gas through a 430 μm diameter tube, both tubes made of stainless steel. For the concentration by evaporation experiments, HPLC grade water was used, and a glass reactor (a tube of three glass pieces connected with only a small opening for gas release) was connected to the spray to reduce the evaporation while collecting all the sprayed droplets. The water flow rate used varied from 50 to 400 μL min$^{-1}$, while the nitrogen gas flow rate was set at 5.3 L min$^{-1}$. For the experiments inside the glovebox, deionized water was injected at a flow rate of 1 mL min$^{-1}$ and the nitrogen gas flow rate varied from 1.1 to 5.9 L min$^{-1}$, with most of the experiments set at 2.3 L min$^{-1}$.

Sprayed water microdroplets diameter acquisition

The distribution of microdroplets-size was measured with a Spraytec system (Malvern Instruments). The interaction between the laser beam with the spray produces a diffraction pattern, from which the derived parameter Sauter mean diameter (SMD) was calculated. Our spray was positioned at ~2 cm from the laser.

Substrates for condensation

Silicon wafers (p-doped, <100> orientation, 4" diameter, thickness of 500 μm and a 2 μm-thick oxide layer) were purchased from Silicon Valley Microelectronics (Catalogue #SV010).

Functionalization of SiO$_2$/Si wafers

To make our SiO$_2$/Si wafers hydrophobic, we functionalized them by silanization with perfluoroctyltrichlorosilane (FDTS). Firstly, we removed any organic contaminants and hydroxylated the surface by treating it with oxygen plasma for 2 min. Then by using a molecular vapor deposition process (Applied Microsystems MVD100E), we grafted our silicon wafers with FDTS by applying one of our previous methodologies reported.$^{61}$

Ozone generation and experiments inside the glovebox

A portable isolated glovebox (Cleatech, Catalogue #2200-2-B) was used as a controlled-environment chamber for ozone concentration. An ozone generator (Mainstayae; O$_3$ production rate: 24 g h$^{-1}$) was placed outside the glovebox inside a bucket with a hole for plastic tubing with an air flow. To control the ozone concentration inside the glove box, this mixture of air and ozone was further mixed with a different channel containing more air and nitrogen, all of them controlled by valves to change the gas flows. A portable ozone meter (GoolRC) with detection range of 2–5000 ppb and detection limit at 2 ppb was positioned inside the glovebox for real time monitoring; its proper functioning required RH <85%. When the ozone concentration was reached, varying from 10 to 4900 ppb, the silicon wafers used as substrates for water microdroplets formation were placed onto an ice–water bag with uniformly distributed temperature at ~3 °C, which quickly achieved thermal equilibrium, and left for 40 min of condensation time. The temperature on the substrates was checked via a non-contact digital infrared thermometer (Laser-grip 774). In the case of the sprays, the nitrogen gas was already flowing considering its great effect on the ozone concentration inside the glove box, and as soon as the ozone concentration was
stabilized, the water injection was started for collection of the microdroplets into a glass funnel inside a beaker during 3–13 min. The relative humidity inside the chamber was kept in the range 70–80% using the ultrasonic humidifier, at ~85% by heating water and 30–70% when using the spray (a bigger range as the air and nitrogen gas flows were affecting it more). The ambient air temperature was in the 18–20 °C range. To collect the samples, we poured the droplets from the silicon wafers into a clean glassware, and all the samples were transferred into a 15 mL centrifuge tube (VWR International).

Water vapor generation via ultrasonic humidifier
An ultrasonic humidifier (Proton PHC 9UH) with 15 W of power was used in this study. This equipment produces mist from water by ultrasonic waves generated from the piezoelectric disk located on its bottom. Deionized water was used and the ultrasonic humidifier was positioned far away from the silicon wafers (~40 cm apart) to avoid direct mist deposition.

Water vapor generation via a heating plate
Deionized water was heated at 40 °C using a heating plate (IKA RCT, Catalogue #3810000). The plate was positioned ~30 cm apart from the substrates, and the temperature was controlled by the coupled temperature sensor (PT 1000.60), which was in contact with the water.

High speed imaging
The rapid interaction between nitrogen gas and water requires observation with an ultra-high-speed video camera (Kirana-05M, Specialized Imaging, Tring UK) acquiring 180 images at capture speeds of up to 5 × 10^6 fps, with a full resolution of 924 (W) × 768 (H) px irrespective of the frame rate used and magnification with Leica long-distance microscope at magnification up to 29.4. The short- and long-term dynamics of this interaction can be captured using several different frame rates. The framing is synchronized with red diode-lasers with adjustable pulse duration of 50–170 ns per frame to minimize motion smearing. The smallest detectable diameter of the droplets is around 4 μm.

Computational methods
Three-dimensional (3D) CFD simulations were performed using the converge code to simulate the sprays. The turbulence was simulated by the renormalization group k-ε model. The Eulerian void of fluid (VOF) method was adopted to capture the in- and near-nozzle spray details. In this method, the gas and liquid fuel are considered as a single compressible fluid mixture, and the void fraction (α_v) is used to represent the volume fraction of liquid. Details of the related models are available in the ref. 62.

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Data availability
All the data is presented with the article and the ESL†

Author contributions
HM conceived the research plan and oversaw its execution with assistance from collaborators HI and ST. AGJ, NHM, and JP designed and built experimental rigs for sprays; NHM collected experimental data from sprays and humidifiers inside the glovebox; PZ contributed to analytical measurements. AGJ, NHM, and HM analyzed the data. XL and HI performed computational simulation of the spray experiments; ZY and ST conducted ultra-fast imaging of sprays. HM and AGJ wrote the manuscript with assistance from co-authors.

Conflicts of interest
There are no conflicts to declare.

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References


49 USA Environmental Protection Agency (EPA), *Daily ozone concentrations in California*, 2021.


