

Electronic Supplementary Information

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Electrochemical pH Regulation in Droplet Microfluidics

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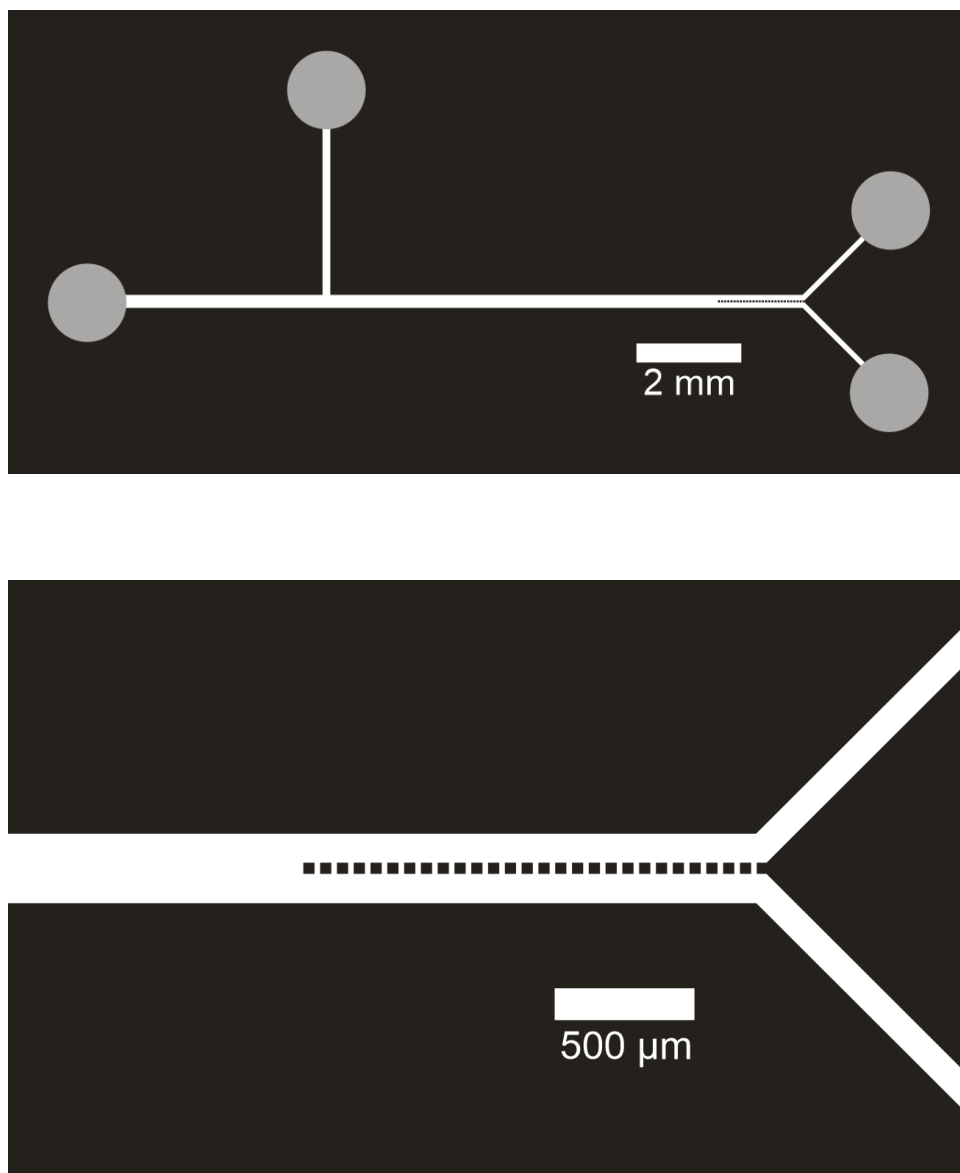


Figure S1. Iteration 2 device geometry. Device inlets and outlets are shown in gray and device channels are shown in white. The lower image is a magnified view of the upper image showing the splitting junction.

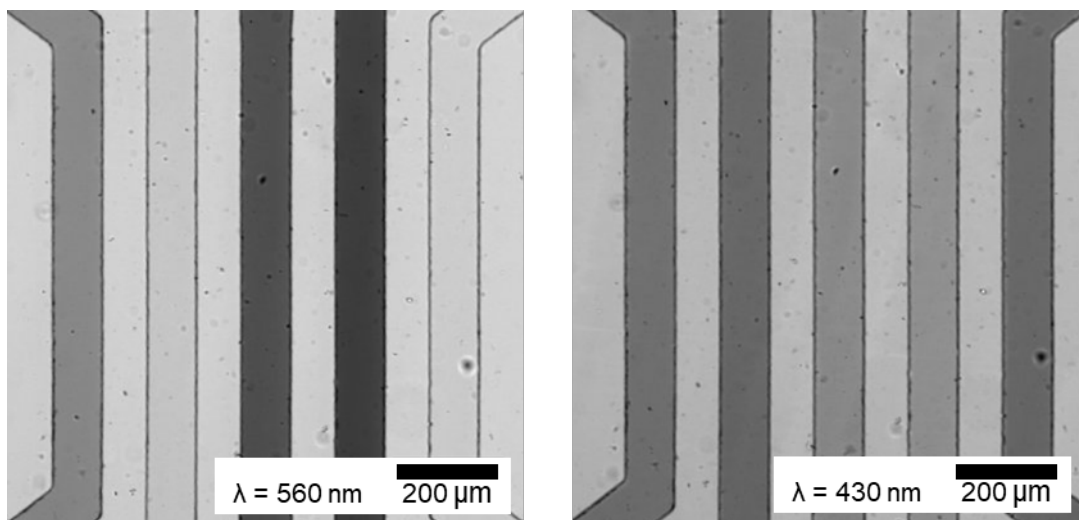


Figure S2. Micrographs of pH calibrant solutions in microfluidic channels (100 μm wide and 40.7 μm high) imaged at different wavelengths. The wavelengths indicated in the legends refer to the pass energy of the optical bandpass filter used to generate monochromatic light for capturing the micrograph. From left to right, calibrant solutions shown have pH values of 7.35, 6.32, 7.93, 8.19, and 5.93. pH calibrant solutions were randomly distributed across four devices with identical channel geometry for measurement of the calibration curve.

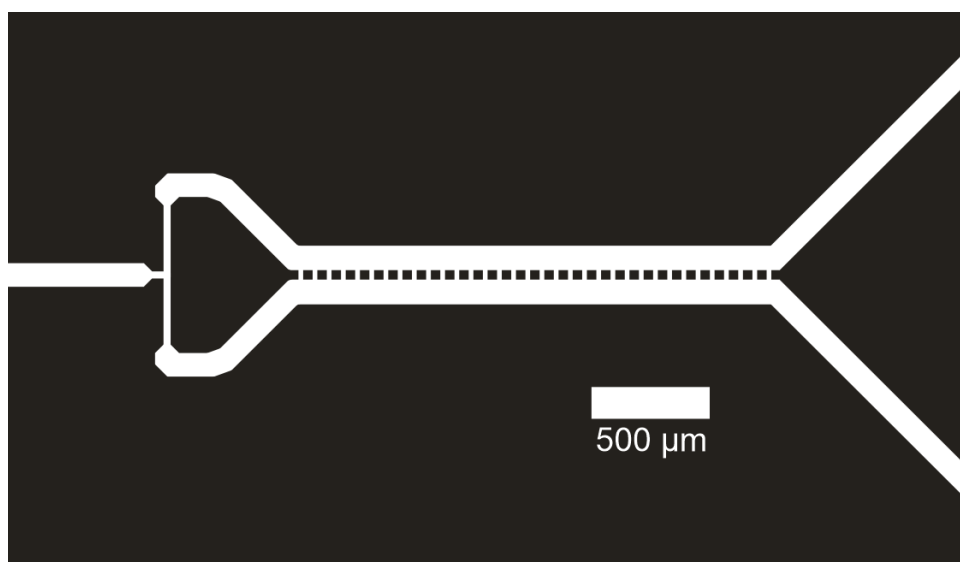
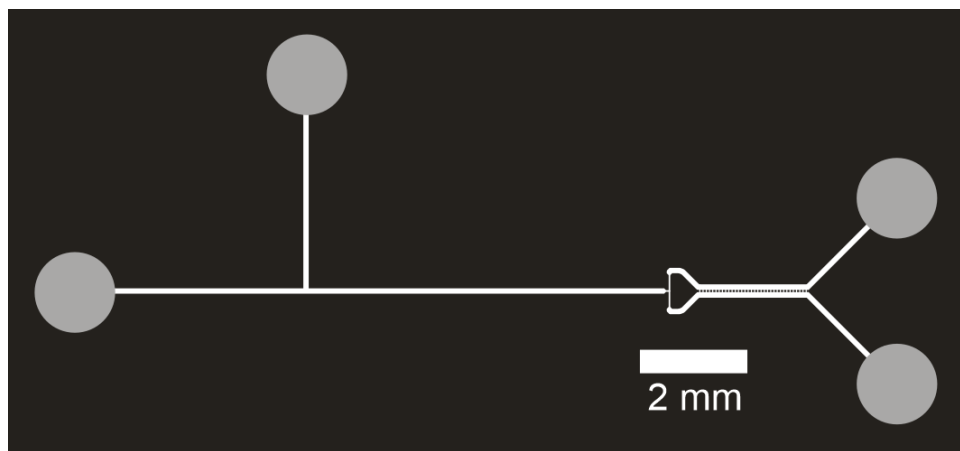


Figure S3. Iteration 1 device geometry. Device inlets and outlets are shown in gray and device channels are shown in white. The lower image is a magnified view of the upper image showing the splitting junction.

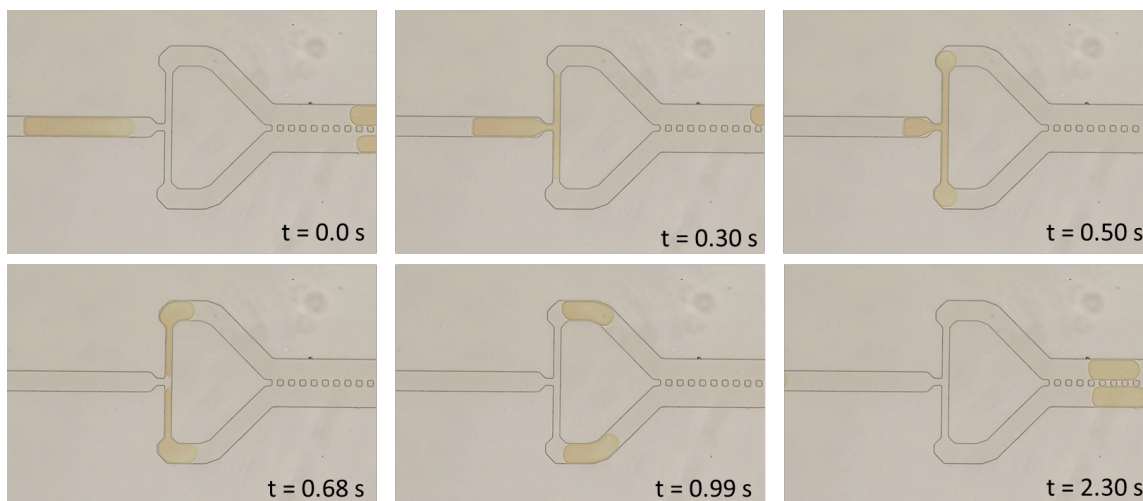
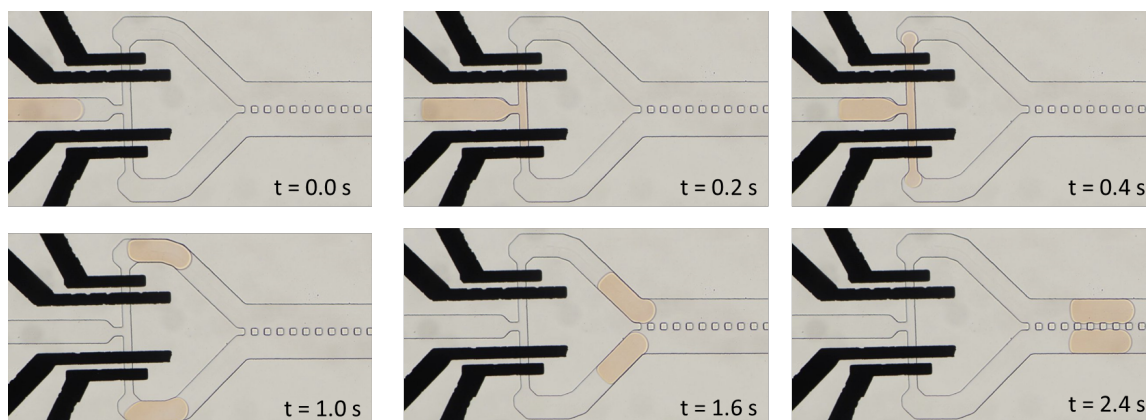


Figure S4. A series of micrographs showing a droplet traveling through the splitting junction of an Iteration 1 device. The aqueous phase inlet flow rate was $20 \text{ nL}\cdot\text{min}^{-1}$ and the oil phase inlet flow rate was $120 \text{ nL}\cdot\text{min}^{-1}$. Channel height was $18.2 \text{ }\mu\text{m}$.

a.



b.

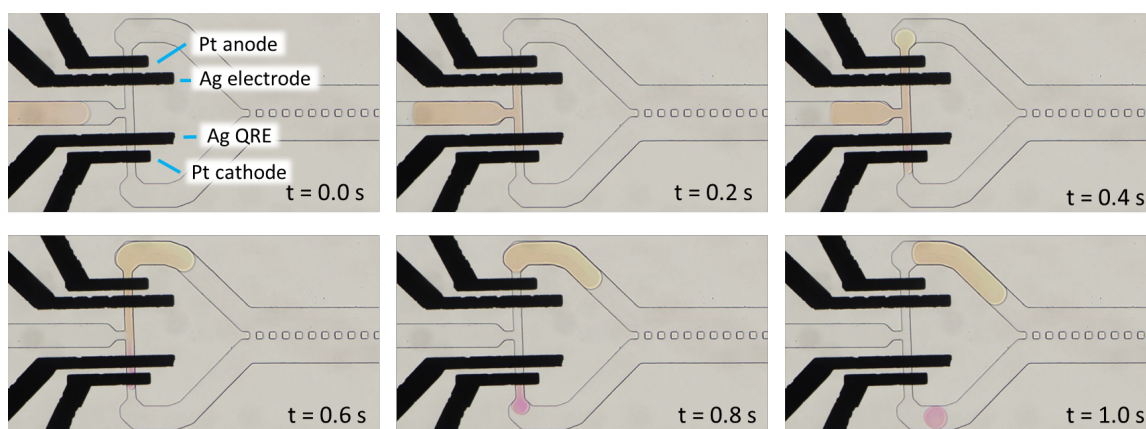


Figure S5. (a) A series of micrographs showing a droplet traveling through the splitting junction of an Iteration 1 device. No voltage was applied to the electrodes. (b) A series of micrographs showing a droplet traveling through the splitting junction of an Iteration 1 device. In this case, the cathode was held at -1.4 V vs. Ag quasi-reference electrode (Ag QRE). The width of the Pt working and counter microelectrodes and the Ag QRE (positions indicated in the figure) was $50 \mu\text{m}$. The upper Ag electrode was not connected to the potentiostat in this

experiment. The droplets contained 2.00 mM phenol red, 1.00 mM KCl, and 100 mM K_2SO_4 . The aqueous phase inlet flow rate was 20 $nL \cdot min^{-1}$ and the oil phase inlet flow rate was 120 $nL \cdot min^{-1}$. The channel height was 18.2 μm .

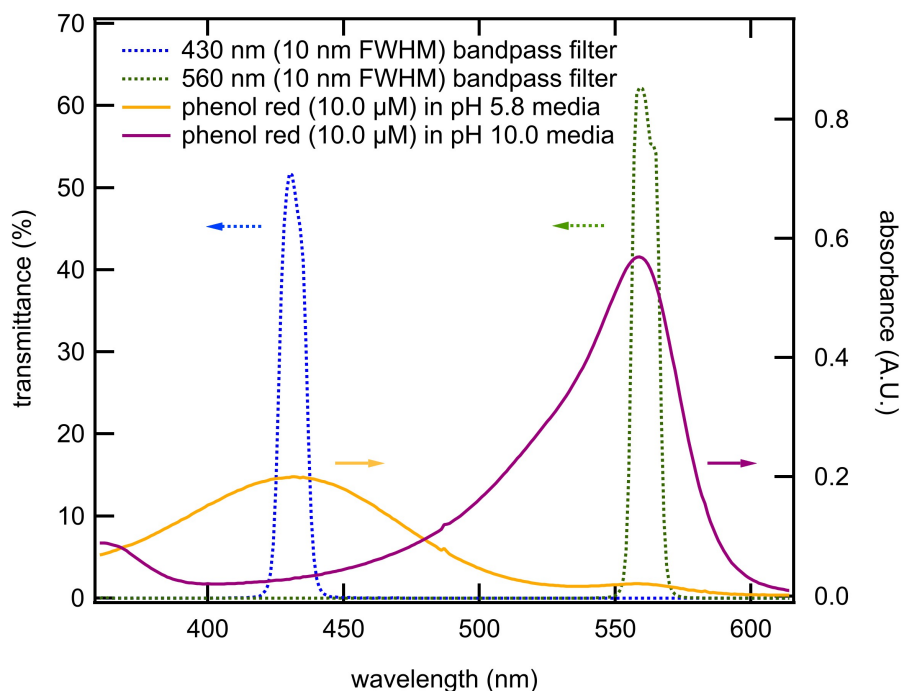


Figure S6. UV-vis absorption spectra of 10.0 μM phenol red in acidic (pH 5.8) and basic (pH 10.0) media (pathlength 1.00 cm) and UV-vis transmission spectra of the 430 nm (10 nm FWHM) and 560 nm (10 nm FWHM) bandpass filters used for the microscopy experiments. UV-vis absorption and transmission spectra were obtained using a UV-vis spectrometer (product G1103A, Hewlett-Packard, Palo Alto, CA).

Discussion of calculation of measured pH in droplets. The theoretical basis for microdroplet pH measurement by ratiometric colorimetry is as follows. Beer's law states that absorption is proportional to concentration. Moreover, according to the Henderson-Hasselbalch equation (eq S1) the logarithm of the concentration ratio of a base to the corresponding conjugate acid is directly proportional to solution pH.

$$\text{pH} = \text{pK}_a + \log_{10} \left(\frac{[A^-]}{[HA]} \right) \quad (\text{S1})$$

The intensity (I) of light traveling through a droplet can be measured from a CCD image. At a later timepoint when the droplet has moved away from the measured region, the blank intensity (I_0) can be obtained from a CCD image of the fluorocarbon oil phase in the same region of the channel. The measurements of I and I_0 correspond to absorption as shown in eq S2.

$$A = \log_{10} \frac{I_0}{I} \quad (\text{S2})$$

By imaging microdroplets through optical bandpass filters with pass energies corresponding to the absorption maxima of the protonated (430 nm) and deprotonated (560 nm) forms of phenol red, measurements of I and I_0 can be obtained. Combining eqs S1 and S2 into eqn S3 reveals the relationship between solution pH and the measured values I and I_0 at wavelengths corresponding to the deprotonated and protonated forms of phenol red.

$$\text{pH} \propto \log_{10} \left(\frac{\log_{10} \frac{I_0}{I_{(560 \text{ nm})}}}{\log_{10} \frac{I_0}{I_{(430 \text{ nm})}}} \right) \quad (\text{S3})$$

In our experiments, near-monochromatic light was obtained by using a white LED and optical bandpass filters with pass energies of 560 nm (10 nm FWHM) and 430 nm (10 nm FWHM). Transmission spectra of the optical bandpass filters are shown in Figure S6.

The values of I and I_0 of the calibrant solutions, like those shown in Figure S2 and Figure 1b of the main text, were measured as follows. The intensity of light, I , passing through the calibrant solutions contained within microfluidic channels was measured from a CCD image. Specifically, the average pixel intensity value of pixels in a 60 μm by 900 μm box drawn in the middle of each microfluidic channel was measured. The blank intensity I_0 was measured from light passing through the PDMS monolith directly adjacent to the microfluidic channel. Specifically, the average pixel intensity value within a 60 μm by 900 μm box drawn 50 μm to the left of the leftmost microfluidic channel was measured, and the average pixel intensity value within a 60 μm by 900 μm box drawn 50 μm to the right of the rightmost microfluidic channel was measured, and these intensity values were averaged to obtain I_0 .

To measure I of droplets, the intensity of light I passing through the middle of a droplet was determined from a CCD image. Specifically, the average pixel intensity value within a 40 μm by

300 μm box, drawn in the middle of each microdroplet, was measured. Droplets in rectangle-shaped microfluidic channels having horizontal dimensions greater than the vertical dimension will exhibit rounded edges and flat, parallel top and bottom surfaces.¹ Thus, selecting only the middle of a droplet in the analysis of a CCD image (avoiding the corners of the droplet which are curved) ensures measurement of light which has traveled through the droplet with an optical path equal to the channel height.

The blank intensity I_0 was measured as the average pixel intensity within a box with the same position and dimensions as the box used for determining I , placed on a CCD image obtained at a timepoint directly after the droplet moved away from the location of the box. The measurement of I_0 represents the light intensity that passed through the channel when the non-absorbing fluorocarbon oil phase was present.

Discussion of calculation of charge injected per unit volume in droplets. The calculation of charge per unit volume injected into droplets was made as follows. All current is assumed to drive water electrolysis (eqs 1 and 2 of the main text). Charge delivered to the separate branches of a splitting droplet during residence of the droplet in the splitting junction is determined by the applied current, the cross-sectional area of the branch

of the splitting droplet, and the flow rate of the leading edge of the splitting droplet as it travels over and past the anode or cathode microelectrode. The cross-sectional area calculation assumed the shape of the cross section of the branch of the splitting droplet traveling over the microelectrode is a rectangle with rounded corners which occupies a rectangular channel with dimensions of $40.5 \mu\text{m} \times 105 \mu\text{m}$.¹ The dimensional analysis is shown in eq S4.

$$\frac{\text{current} \left(\frac{\text{nC}}{\text{s}} \right)}{\text{cross-sectional area} (\mu\text{m}^2) \times \text{flow rate} \left(\frac{\mu\text{m}}{\text{s}} \right) \times 10^{-6} \left(\frac{\text{nL}}{\mu\text{m}^3} \right)} = \text{charge per volume} \left(\frac{\text{nC}}{\text{nL}} \right) \quad (\text{S4})$$

Two significant assumptions are inherent in this calculation. First, we assume that the microelectrodes are active during the entire droplet splitting event. The distance from the leading edge of the splitting junction to the leading edge of the cathode is $120 \mu\text{m}$, and the distance from the leading edge of the splitting junction to the leading edge of the anode is $20 \mu\text{m}$. As this is the case, in reality, the microelectrodes are disconnected and therefore not producing water electrolysis products during a portion of the time the descendant droplets are in contact with the microelectrodes. Second, an assumption is made that the leading and trailing ends of the droplet are flat. In reality, the droplets exhibit rounded end caps.

Discussion of calculation of predicted pH as a function of charge injected per unit volume in a droplet containing phenol red. The calculation of predicted pH as a function of charge injected per unit volume in droplets containing phenol red is as follows. The indicator dye phenol red was assumed to be the primary contributor to pH buffering in the system. Electrochemically generated H^+ and OH^- were assumed to react completely with deprotonated (PR^-) and protonated (PRH) phenol red species, respectively. The concentrations of PR^- and PRH initially present in the droplet prior to pH change were calculated from the formal concentration of phenol red (1.96 mM) and the initially measured droplet pH using eq S1.

The charge-per-volume delivered to droplets was assumed to contribute only to the generation of H^+ or OH^- in the droplet (eqs 1 and 2 of the main text). As shown by eq 1 of the main text, the ratio of H^+ generated to e^- delivered is -1:1, and thus the amount of H^+ generated per nL ($[H^+]_{\text{generated}}$) was calculated by dividing the absolute value of charge-per-volume by the Faraday constant. Similarly, as shown by eq 2 of the main text, the ratio of OH^- generated per nL ($[OH^-]_{\text{generated}}$) to e^- delivered is 1:1, and thus the amount of OH^- generated per nL was calculated by dividing charge-per-volume by the Faraday constant. In the case of H^+ generation, the final concentrations of PR^- and PRH were calculated according to eqs S5 and S6.

$$[PR^-]_{\text{final}} = [PR^-]_{\text{initial}} - [H^+]_{\text{generated}} \quad (\text{S5})$$

$$[PRH]_{\text{final}} = [PRH]_{\text{initial}} + [H^+]_{\text{generated}} \quad (\text{S6})$$

In the case of OH^- generation, the final concentrations of PR^- and PRH were calculated according to eqs S7 and S8.

$$[PR^-]_{\text{final}} = [PR^-]_{\text{initial}} + [\text{OH}^-]_{\text{generated}} \quad (\text{S7})$$

$$[PRH]_{\text{final}} = [PRH]_{\text{initial}} - [\text{OH}^-]_{\text{generated}} \quad (\text{S8})$$

The pH in both cases was then calculated from eq S1 based on the final concentrations of PR^- and PRH .

Discussion of calculation of predicted pH as a function of charge delivered per unit volume in a droplet containing no buffering species. The calculation of predicted pH as a function of charge injected per unit volume in droplets containing no buffering species is as follows. In this calculation, the only contributor to pH is assumed to be electrochemically generated H^+ or OH^- . As shown by eq 1 of the main text, the ratio of H^+ generated to e^- delivered is -1:1, and thus the amount of H^+ generated per nL was calculated by dividing the absolute value of charge-per-volume by the Faraday constant. Similarly, as shown by eq 2 of the main text, the ratio of OH^- generated to e^- delivered is 1:1, and thus the amount of OH^- generated per nL was calculated by dividing the charge-per-volume by the Faraday constant.

Reference

- 1 M. Musterd, V. van Steijn, C. R. Kleijn and M. T. Kreutzer,
RSC Adv., 2015, **5**, 16042–16049.