

# MICRO- pH CONTROL BY BREAKING WATER AND ITS APPLICATIONS

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## ABSTRACT

A novel strategy is demonstrated for pH regulation in microscale environments by injecting controllable amounts of protons and hydroxide ions via field-enhanced dissociation of water molecules. The dissociation occurs at the ion-depleted junction of reverse-biased bipolar membranes, fabricated by photo-patterning ion-exchange polymers into microfluidic chips. The bipolar membrane generates and separates H<sup>+</sup> and OH<sup>-</sup> ions without gas production or contaminant generation of electron-transfer reactions. Furthermore, it effects pH changes with no need of additional acidic/basic solutions that dilute analyte concentrations. Combining with properly designed microchannel configurations, the approach allows us to sustain robust local on-chip pH and pH gradients to improve several microfluidic operations, such as micro-reactors and molecular separation in microfluidic chips.

**KEYWORDS:** pH Control, Field-Enhanced Water Dissociation, Polyelectrolyte, Membrane

## INTRODUCTION

Control of pH in microscale environments offers many intriguing possibilities for several biological applications such as on-chip microbioreaction and cell or biomolecular separation. Several techniques have been developed for pH actuation in microfluidic devices including carbon dioxide dissolution, regulation of acid and base solutions [1] and electrolysis of water by embedded electrodes. These methods, however, are either difficult to miniaturize or require microvalves to control the flow of different pH solutions.[1, 2] Electrolysis produces hydrogen and oxygen bubbles that impede microfluidic flow and other reaction products that contaminate the sample. To overcome the aforementioned obstacles, we present an alternative microfluidic pH actuator by using field-enhanced water dissociation in microscale bipolar membranes, composed of adjoining cation and anion exchange layers in integrated microfluidic chips. The excess protons and hydroxide ions are generated by directly stripping water molecules apart at the junction of the bipolar membrane, where a high electric field exists due to ion depletion by a reverse voltage-bias.[3] Without any electron-transfer reaction to generate gas, the process produces H<sup>+</sup> and OH<sup>-</sup> ions more efficiently than electrolysis. The resulting H<sup>+</sup> and OH<sup>-</sup> ions can be separated and injected into nanoliter or smaller volumes of fluid in a microfluidic chip. As the generated ions significantly increase the local conductivity of the ion depleted region, the amount of H<sup>+</sup> or OH<sup>-</sup> ions generated can be controlled by monitoring the electrical current, thus allowing precise feedback control of the local pH and pH gradient on the chip. In contrast to the conventional way of changing pH by adding acidic or alkaline solutions, the injection of H<sup>+</sup> or OH<sup>-</sup> ions does not alter the total solution volume in the microfluidic chip and can hence adjust the pH level without diluting the analyte concentration.

## THEORY

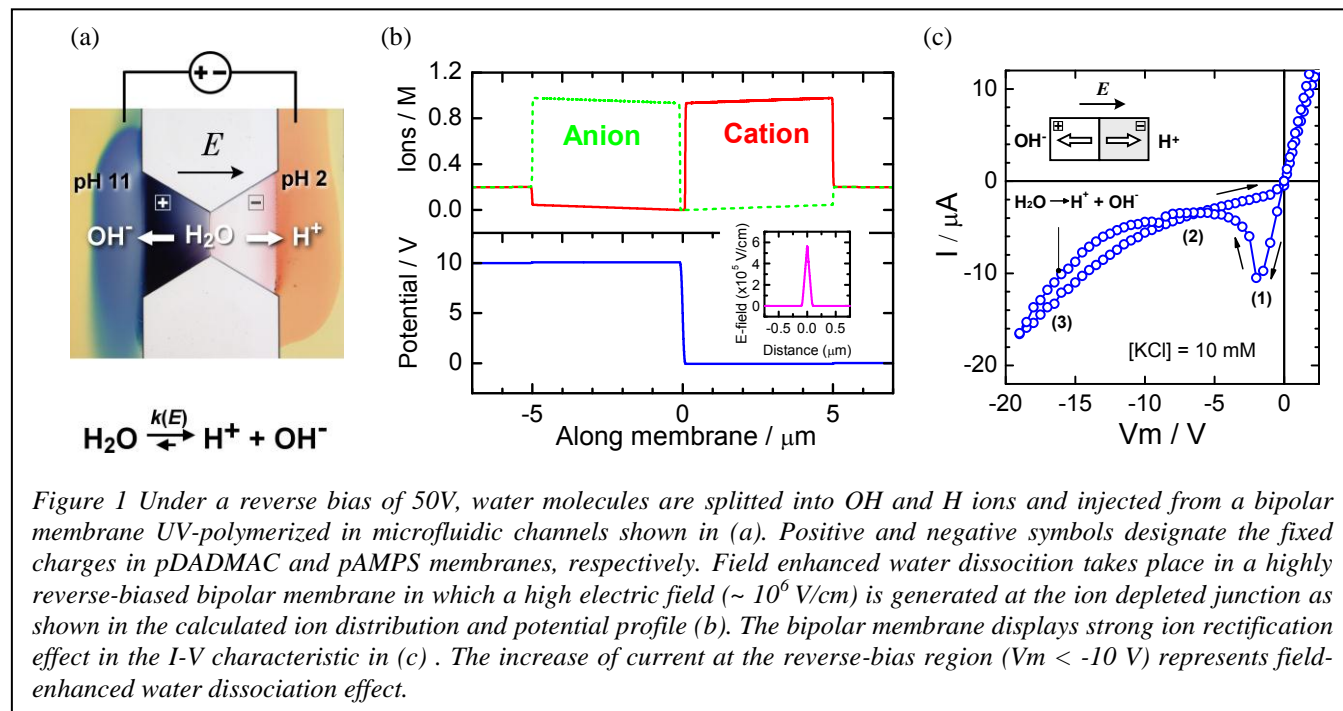
Bipolar membranes consist of an anion-permeable membrane and a cation permeable membrane laminated together. When this composite structure is oriented such that the anion-exchange layer faces the cathode, it is possible to spit water into proton and hydroxyl ions. This results in the production of acidic and basic solutions at the surfaces of the bipolar membranes. Water splitting only commences beyond a threshold reverse bias after the charged mobile species are removed from the junction of the two ion-exchange layer, forming a low ion-conductive depletion region with a thickness of a few Debye lengths. With almost the entire applied voltage falling across this short distance, the bipolar membrane junction possesses a strong electric field of the order of MV/cm. According to Onsager's theory of the second Wien effect,[4] the water dissociation rate is enhanced by a strong electric field while the recombination rate is not. Water molecules dissociate into H<sup>+</sup> or OH<sup>-</sup> ions at the highly reverse-biased bipolar membrane junction only after the salt ions are depleted and hence a high field builds up (see the image of the pH change produced by the water splitting effect and the calculated ion distribution and potential profile in a reverse-biased bipolar membrane in Figure 1(a) and (b)). [5]

## EXPERIMENTAL

To integrate bipolar membranes in a microfluidic device, we started with fabricating microfluidic channels with a depth of 20  $\mu\text{m}$  by wet-etching on a glass substrate and bonding with another piece of glass through sodium silicate solution at 90°C for 3 hours. A layer of negatively charged poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (pAMPS) and a layer of positively charged poly(diallyldimethyl-ammonium chloride) (pDADMAC) were then UV-polymerized in the microchannel by photolithography. Both membranes were synthesized to contain about 2M concentration of fixed charge. With such a high fixed charge density, the membrane is insensitive to the surrounding ionic strength, providing a stable physicochemical property. Figure 1(a) shows a resulting bipolar membrane with a total length of 800  $\mu\text{m}$ , bridging two microfluidic channels.

## RESULT AND DISCUSSION

The bipolar membrane exhibits unique electrical features in reverse bias, as shown in the cyclic I-V curve (Fig. 1(c)) measured in a 10 mM KCl solution with the voltage swept back and forth at a rate of 0.5 V/s. Its I-V characteristic at reverse bias can be divided into three regimes as indicated in the plot: a hysteresis regime (regime 1) at low reverse bias, in which the current amplitude increases linearly and then drops precipitously when the voltage is swept from zero to negative values but decreases monotonically at a lower conductance for a voltage sweep from negative to zero; a low conductance regime (regime 2); and at high voltage, a water splitting regime (regime 3) in which the excess protons and hydroxide ions contribute to the elevated ion current. The hysteresis effect at low reverse bias is most likely due to ion migration transients within the cation and anion exchange membranes. The bipolar membrane preserves the low ionic conductance until the electric field at the depletion region is large enough to generate additional charge carriers by enhancing the dissociation of water molecules into  $H^+$  and  $OH^-$  ions. Greater current amplitude is obtained with the increase of reverse voltage bias, implying more dissociation of water molecules.



*Figure 1 Under a reverse bias of 50V, water molecules are splitted into OH and H ions and injected from a bipolar membrane UV-polymerized in microfluidic channels shown in (a). Positive and negative symbols designate the fixed charges in pDADMAC and pAMPS membranes, respectively. Field enhanced water dissociation takes place in a highly reverse-biased bipolar membrane in which a high electric field ( $\sim 10^6$  V/cm) is generated at the ion depleted junction as shown in the calculated ion distribution and potential profile (b). The bipolar membrane displays strong ion rectification effect in the I-V characteristic in (c). The increase of current at the reverse-bias region ( $V_m < -10$  V) represents field-enhanced water dissociation effect.*

Generation and separation of  $H^+/OH^-$  ions by bipolar membranes and their distinct electrical signatures suggest a new microfluidic pH regulation strategy. The pH regulation device consists of two bipolar membranes sandwiching the upstream microchannel and a microfluidic mixer in the downstream as shown in the microscopic image in Fig. 2(a). Functioning as a  $H^+$  ion pump and a  $OH^-$  ion pump, the two sets of bipolar membrane were arranged in such a way that  $H^+$  or  $OH^-$  ions can be injected independently from one of the two bipolar membranes to the fluid delivered from the left side. Each amount of ions pumped into the microchannel is tunable by controlling the voltage biases,  $V_H$  and  $V_{OH}$ . The corrugated pattern fabricated by wet etching was designed to create chaotic mixing of solutions in microchannels. With different combination of  $V_H$  and  $V_{OH}$ , the device produces various ratios of  $H^+$  and  $OH^-$  ions in the upstream and mixes them up well through the mixer to achieve divergent pH conditions uniformly distributed in the downstream. Under a flow rate of 2  $\mu l/min$ , five particular pH conditions, approximately pH 3, 5, 7, 9 and 11, were successfully demonstrated in Fig. 2(b) by applying corresponding  $V_H$  and  $V_{OH}$  listed in the figure to electrically adjusting the  $H^+ / OH^-$  ion currents. The variation of fluid pH level can be observed by the color of pH indicator as  $H^+$  or  $OH^-$  ions are added and mixed in the microchannel.

Aside from constant pH control, the development of stable pH gradients under flow conditions in microfluidic devices can be important for several applications such as isoelectric focusing for molecular separation. We demonstrated in Fig. 3 a robust variable-range, linear pH gradient across a 500  $\mu m$  wide microchannel. With simultaneous injection of both  $H^+$  and  $OH^-$  ions ( $V_H = 32V$ ,  $V_{OH} = 35V$ ), two extreme pH conditions separated by an abrupt boundary were form in the upstream microchannel (Fig. 3a). When the liquid passed through a 50  $\mu m$  wide, necked microchannel, its pH profile redistributed to form a linear gradient due to dispersion. The narrowed microchannel was designed to reduce the lateral diffusion length. Depending on the

flow rate and the length of the narrowed channel, the lateral diffusion time can be adjusted to create pH gradient over different ranges. Fig. 3b and 3c show two pH profiles produced after passing through a 0.8 mm and 2 mm long narrowed channels, respectively, under the same flow rate of 2  $\mu\text{l}/\text{min}$ . With a shorter diffusion time in the 0.8 mm long channel, the acidic and alkaline solutions were less mixed, yielding a wide-range pH gradient covering approximately from pH 2 to 11. The 2 mm long narrow channel provided longer diffusion time and hence created smoother pH gradient (about pH 5- 9). The pH values across the microchannel are summarized in Fig 3c.

## CONCLUSION

Electrokinetic control of fluid pH and generation of stable pH gradients were successfully demonstrated by using field-enhanced water dissociation in microfluidics. Split directly from water molecules at a reverse-biased bipolar membrane junction,  $\text{H}^+$  and  $\text{OH}^-$  ions are produced without suffering from gas and contaminant generation during water electrolysis. By controlling two independent  $\text{H}^+$  and  $\text{OH}^-$  ions injection rates into a single stream, various pH conditions can be created without the addition of acidic or basic solutions, which requires sophisticated flow control and eventually tends to dilute the sample solution. Offering several advantages over conventional methods, the novel strategy of pH regulation outlined in this work should enable better control of chemical reactions in microreactors and the robust pH gradient generation should improve the performance of molecular separation in microfluidic isoelectric focusing devices.

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