Presence and Dynamics of Exclusion Zone Projections

Noa Gang and Gerald H. Pollack
Department of Bioengineering, University of Washington

Introduction

Exclusion Zones (EZ) develop at an interface and are defined by characteristically unique properties, such as solute exclusion, increased viscosity and charge separation. Growths of finger-like projections of EZ water have been noted, but their characteristics and dynamics remain largely unknown. Salt has been reported to diminish EZ formation, however, a paradoxical effect is observed at lower concentrations. As the majority of biological water is interfacial, alterations in EZ size, shape or dynamics accompanying solute concentration change may imply a role in extracellular environments. Nafion, a highly hydrophilic cation exchange membrane, provides clear images of EZ projection formation when paired with a pH indicator dye (figure 1). In this manner EZ projections were investigated in the presence of various NaCl salt concentrations.

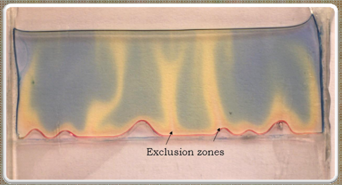

Figure 1. EZ projections seen with pH dye

Methods

Glass experimental chambers (7.5 cm x 3.0 cm x 5.0 cm) were custom made with a quick drying epoxy and fitted with a Nafion membrane floor (figure 2). NaCl salt solutions of 0 mM, 1 mM, 5 mM and 10 mM were mixed in NANOpure Diamond deionized water and diluted Universal pH Indicator dye. Aliquots of the resulting solution were added to chambers layered with vegetable oil to discourage evaporation. Both oil sealed and unsealed measurements were made of 0 mM NaCl (deionized water). Diluted India ink was also employed to image solutions. Images of the chambers were taken every 2.5 minutes over three hours while positioned over a mm x mm grid, which was referenced during quantification. Height, length, number of projections and basal movement were measured.

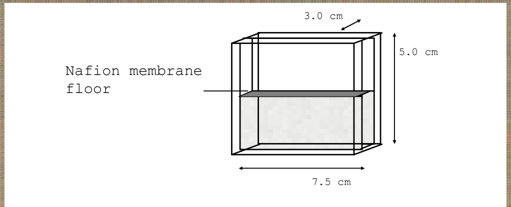

Figure 2. Glass experimental chamber with Nafion membrane floor

Results

Most rapid EZ projection formation occurred in air-exposed pure water. Typically, a comparably uniform EZ front was observed in all other conditions, with distinctive projections distinguishable between the first and second hours of measurement (figure 3).

Results Cont.

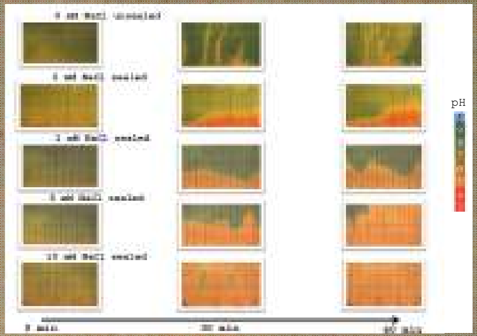

Figure 3. EZ development as visualized by pH indicator dye

Correspondingly, images formed with India ink also showed discrepancies between the EZ formation in pure, air-exposed water and salt solution. Less localized movement was seen in salt solution.

Although EZ formation was found in all conditions, dimensions (height and width) were varied (figure 4).

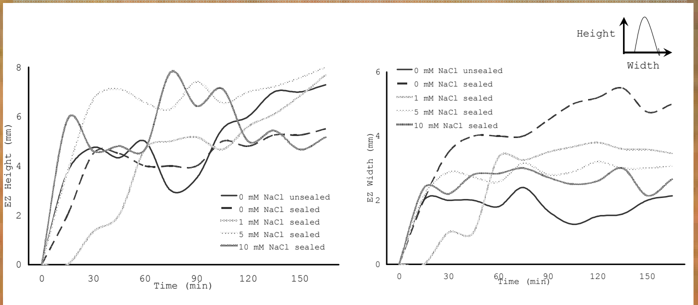

Figure 4. EZ physical characteristics over time

Results Cont.

EZ projections were found earliest in air-exposed pure water and in 5 mM NaCl salt solution, however, during the third hour of measurement a one projection minimum was seen across conditions (figure 5).

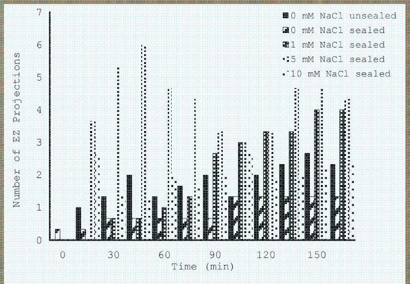

Figure 5. Number of EZ projections over time

Projection speeds were measured during the third hour. Fastest speeds were found in air-exposed pure water and 5 mM NaCl salt solution (figure 6).

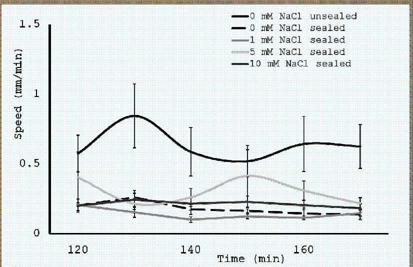

Figure 6. EZ speeds over time

Discussion

Although the salt solutions were not concentrated enough to inhibit EZ formation, the number of projections and movement speed observed in 10 mM NaCl salt solution were comparable to pure water solutions. The increased number of EZ projections, and enhanced movement found in 1 mM and 5 mM NaCl salt solutions is indicative of a window effect. All conditions showed dynamic tendencies, which suggests the presence of a non-evaporative convective force. This was found to be enhanced in the 5.0 mM NaCl salt solutions. The innately dynamic nature of EZ projections provokes the question: what drives the EZ projection movement?