

4-2022

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Gyanendra Kharel
Old Dominion University

Andrew J. Evans
Old Dominion University

Christopher M. Russo
Old Dominion University

Michael Eason
Old Dominion University

James W. Lee
Old Dominion University

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Kharel, Gyanendra; Evans, Andrew J.; Russo, Christopher M.; Eason, Michael; and Lee, James W., "Investigation of Magnesium Cation-proton Exchange with Transmembrane Electrostatically Localized Protons (TELP) at a Liquid-membrane Interface: Fundamental to Bioenergetics" (2022). *College of Sciences Posters*. 5.
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Investigation of magnesium cation-proton exchange with transmembrane electrostatically localized protons (TELP) at a liquid-membrane interface: Fundamental to bioenergetics

Gyanendra Kharel, Andrew J. Evans, Christopher M. Russo, Michael Eason and James W. Lee
The Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529



Abstract

The Lee transmembrane electrostatic proton localization theory (TELP) is a revolutionary scientific theory that has successfully explained decades long-standing quandary in the field of bioenergetics regarding ATP synthesis in biological systems, specifically alkalophilic bacteria. This study provides experimental support for the TELP theory by further demonstrating evidence of a localized proton layer existing at the liquid-membrane interface in a simulated biological membrane apparatus. Whilst monovalent cations have been studied extensively, divalent cation exchange has not been studied experimentally.

A previous study determined equilibrium constant for Na^+ and K^+ to exchange with localized H^+ layer to be $(5.07 \pm 0.46) \times 10^{-8}$ and $(6.93 \pm 0.91) \times 10^{-8}$ respectively. We discovered that an equilibrium exchange occurs at 0.85 mM Mg^{2+} concentration. The findings here contributed to the successful determination of the equilibrium constant between Mg^{2+} and the localized H^+ layer to be $(1.56 \pm 0.46) \times 10^{-5}$. The equilibrium constant, much smaller than one, thus provides support for Lee's TELP model since so many more Mg^{2+} ions in the bulk liquid phase that are required to even partially delocalize just a single H^+ at the liquid-membrane interface. These results are relevant to further understand how water can act as a proton conductor for proton coupling energy transduction and the implications of different biological organisms' salinity tolerance.

Introduction

Peter Mitchell's work on chemiosmotic theory and its central bioenergetics equation has been incorporated into many textbooks. In one of its forms, this equation is expressed as the proton motive force (pmf) across a biological membrane that drives protons through the ATP synthase:

$$pmf = \Delta\psi - (2.3 RT/F) \Delta pH$$

[1]

where $\Delta\psi$ is the electric potential difference across the membrane, R is the gas constant, T is the absolute temperature, and ΔpH is the pH difference between the two bulk aqueous phases separated by the membrane. However, there are some limitations in Mitchellian pmf equation and thus should be revised. The most well-established observation that disagree with the Mitchellian pmf equation are in alkalophilic bacteria, such as *B. pseudofirmus*. Recently, Lee developed a transmembrane electrostatic proton localization theory that elucidates how free excess protons in an aqueous medium separated by an impermeable membrane alone can be localized spontaneously at the liquid-membrane interface. Furthermore, a newly developed pmf equation by modifying eq. 1 to account for localized protons at a liquid-membrane interface was introduced and shown to result in a large enough pmf for the synthesis of ATP in alkalophilic bacteria.

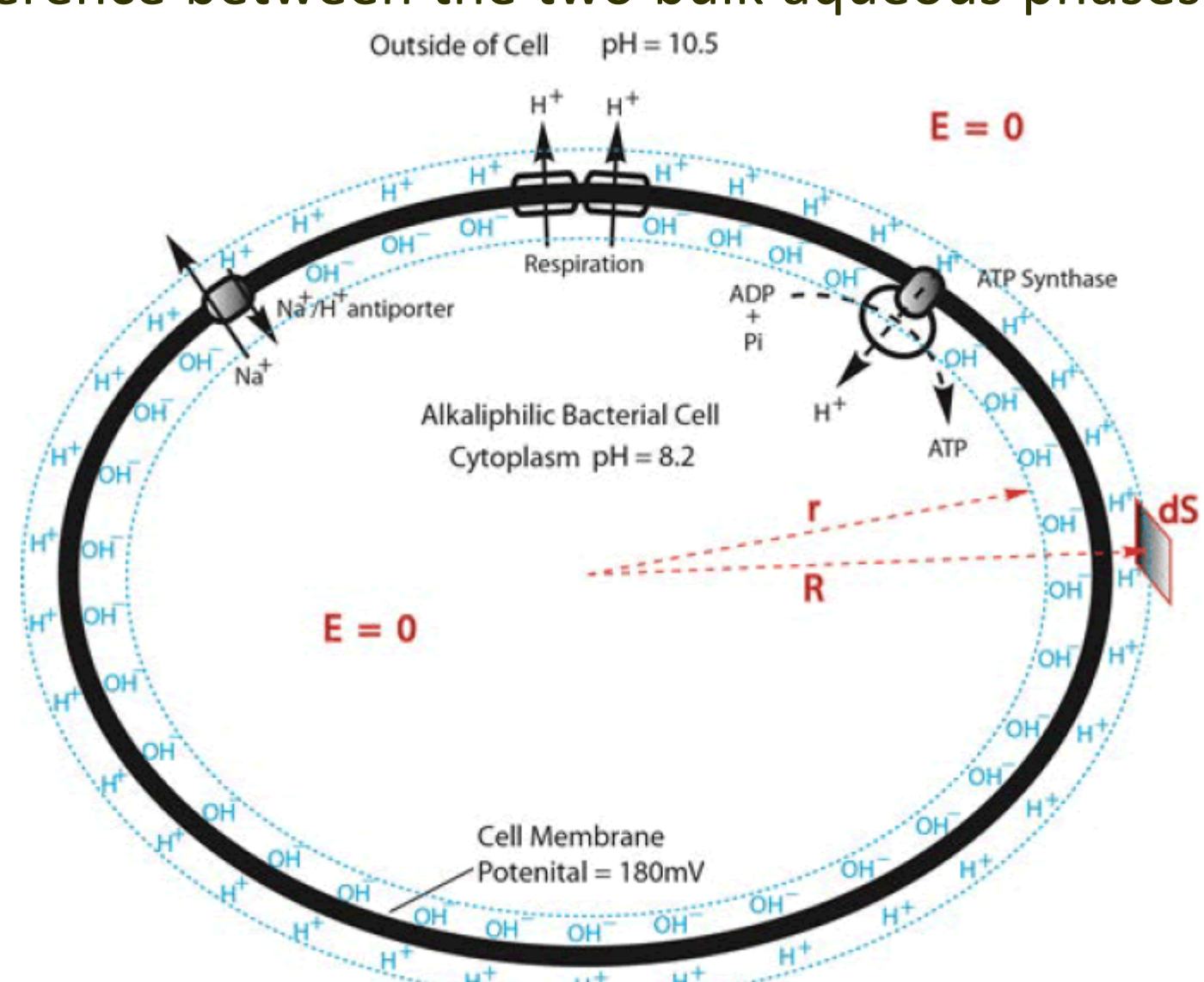


Figure 1. Proton-electrostatic localization model shown as “a proton capacitor-like structure” illustrating how excess H^+ and OH^- are transmembrane electrostatically localized at the water-membrane interfaces along the two sides of the bacterial cell membrane before proton-cation exchange as it would be in a theoretically pure water-membrane-water system.

$$pmf = \Delta\psi + \frac{2.3 RT}{F} \log_{10} \left(\frac{[\text{H}_{pB}^+]}{[\text{H}_{nB}^+]} \right) + \frac{2.3 RT}{F} \log_{10} (1 + [\text{H}_L^+]/[\text{H}_{nB}^+])$$

[2]

Where $[\text{H}_{pB}^+]$ is the bulk liquid phase proton concentration at the p -side of the membrane, $[\text{H}_{nB}^+]$ is the bulk liquid phase proton concentration at the n -side of the membrane, and $[\text{H}_L^+]$ is the effective concentration of electrostatically localized protons at the liquid membrane interface along the membrane surface at the p -side. The first two terms of eq. 2 represent the Mitchellian bulk phase-to-bulk phase proton electrochemical potential gradient that Lee now calls the “classic” pmf, whereas the last term is the “local” pmf that is contributed by transmembrane electrostatically localized protons. In actual biological systems, nonproton cations such as Na^+ , K^+ and Mg^{2+} in the aqueous media may exchange with the localized protons at the liquid-membrane interface and therefore reduce their concentration. It is therefore vital to determine equilibrium constant K_p for the cations to exchange with the localized protons.

Results

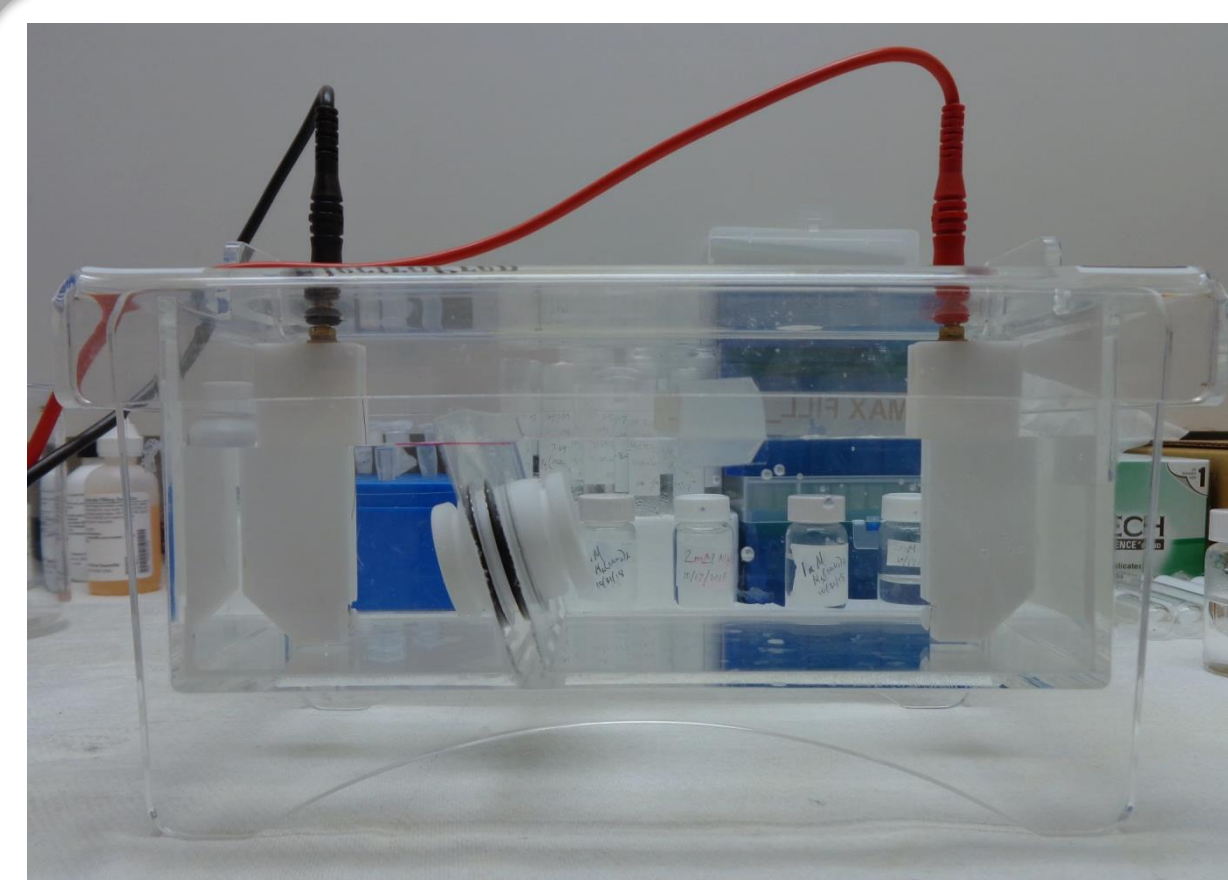


Figure 1. Experimental Setup.

	pH before	pH after
Cathode chamber	6.02	6.08
Anode chamber	6.02	5.89
Center chamber	6.02	8.03

	Conductance before	Conductance after
Cathode chamber	1.113	1.370
Anode chamber	1.113	1.054

Aluminum	Initial mass (mg)	Final mass (mg)	Mass change (mg)
P'	27.77	27.85	+0.08
P	27.73	27.68	-0.05

Table 1. pH and conductance of the liquids and mass of Al films for 0.0 mM $\text{Mg}(\text{HCO}_3)_2$

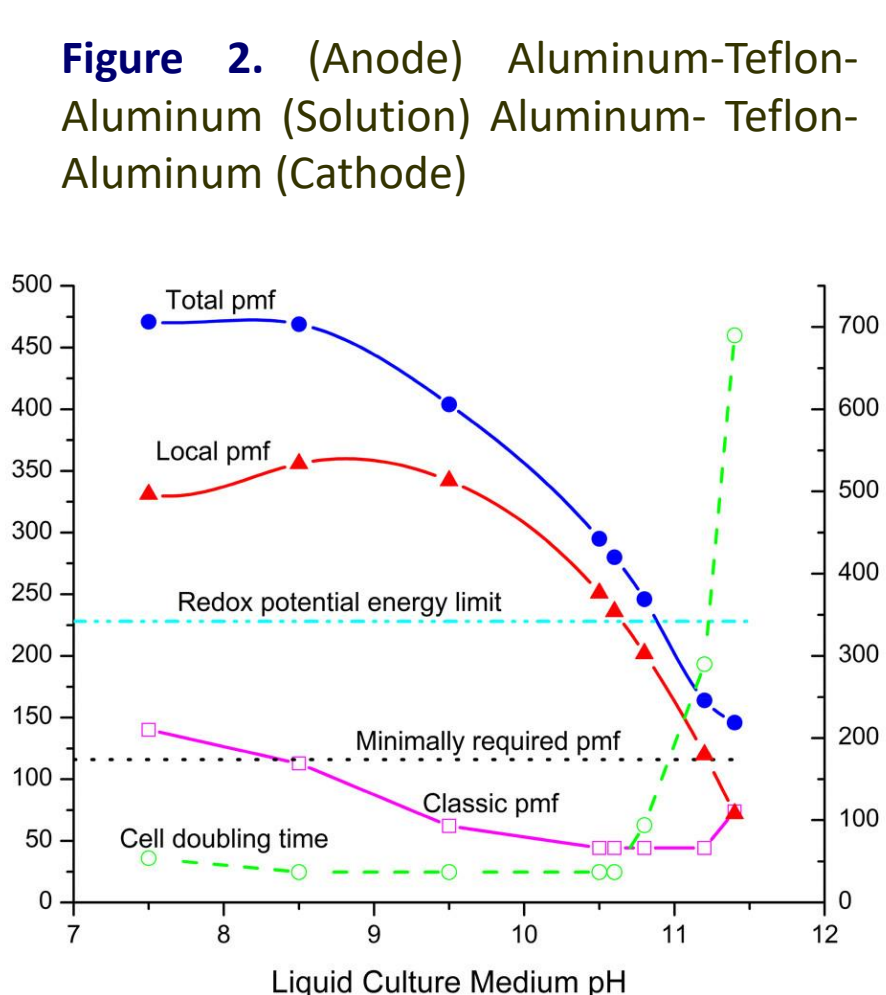


Figure 3. Effect of surface localized protons on PMF

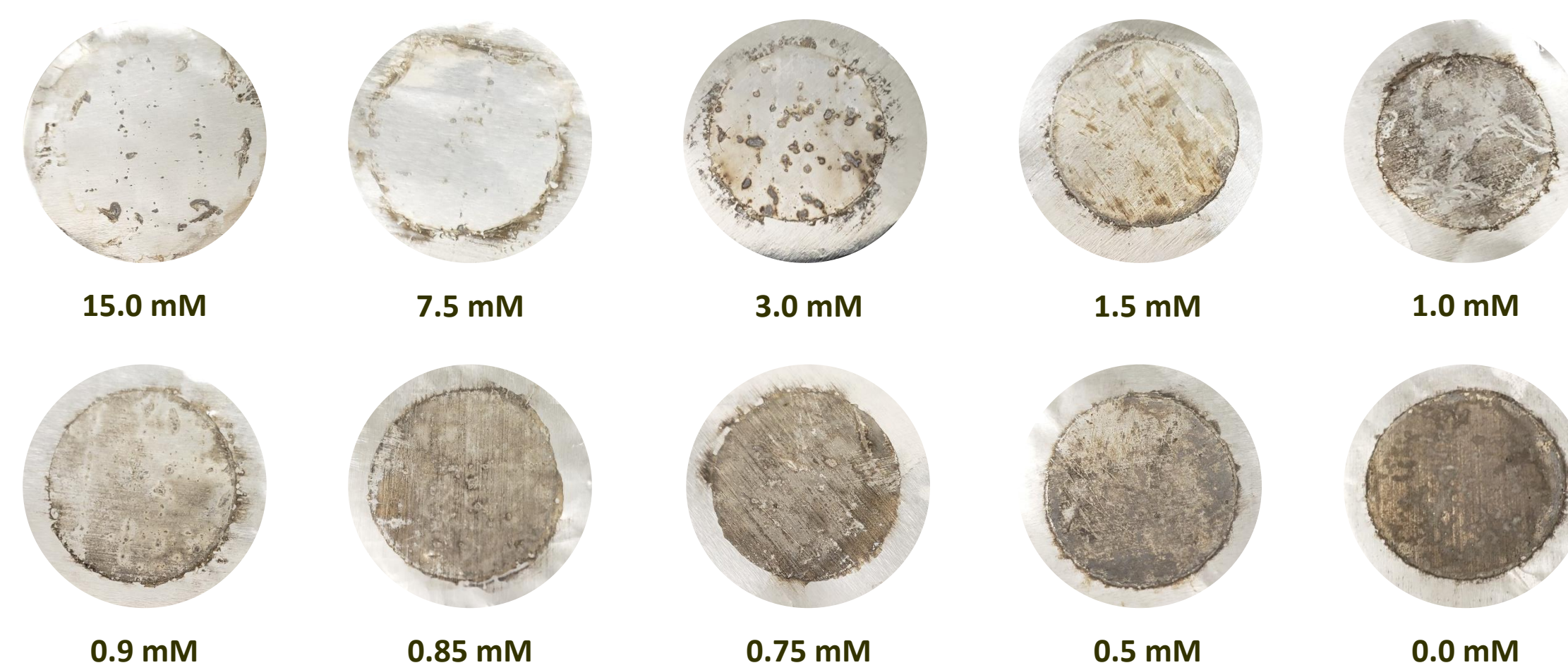


Figure 4. P' membranes compiled.

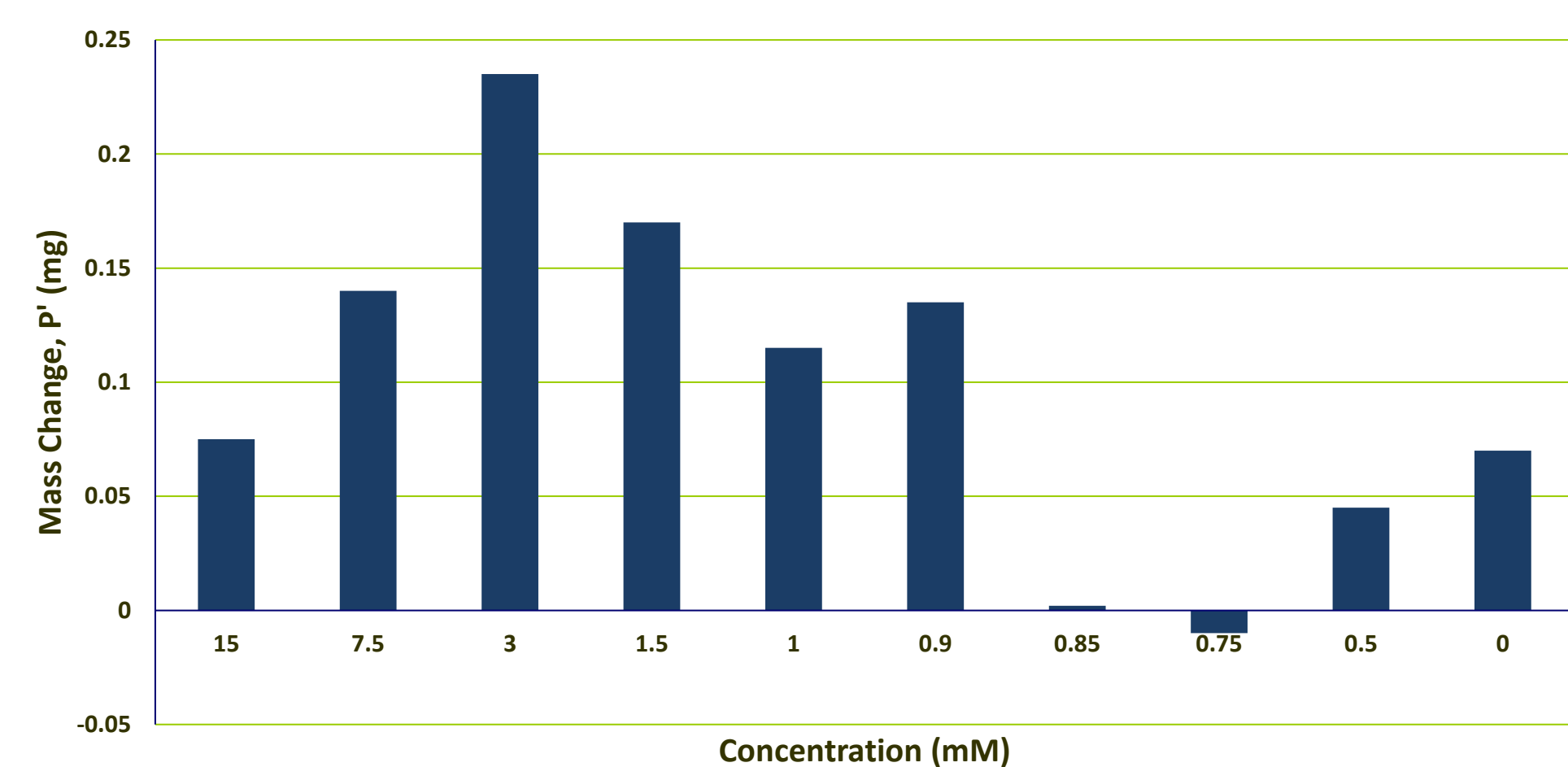


Figure 5. Mass change (P') vs concentration.

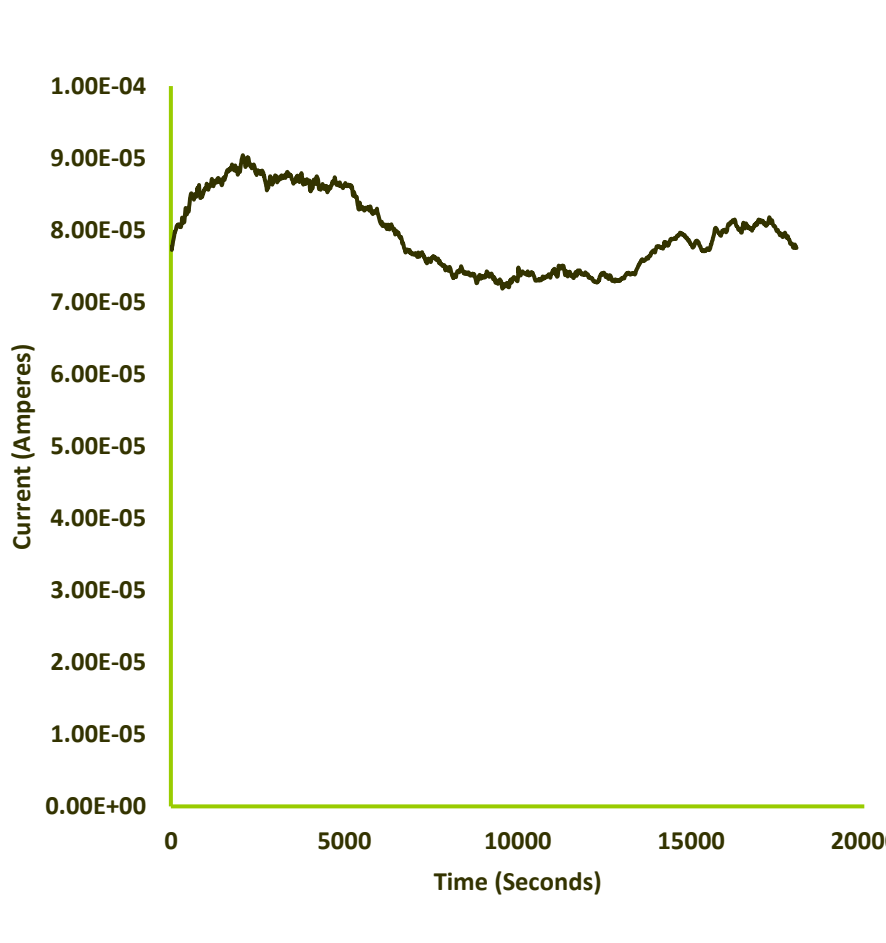


Figure 6. Typical Current vs Time graph.

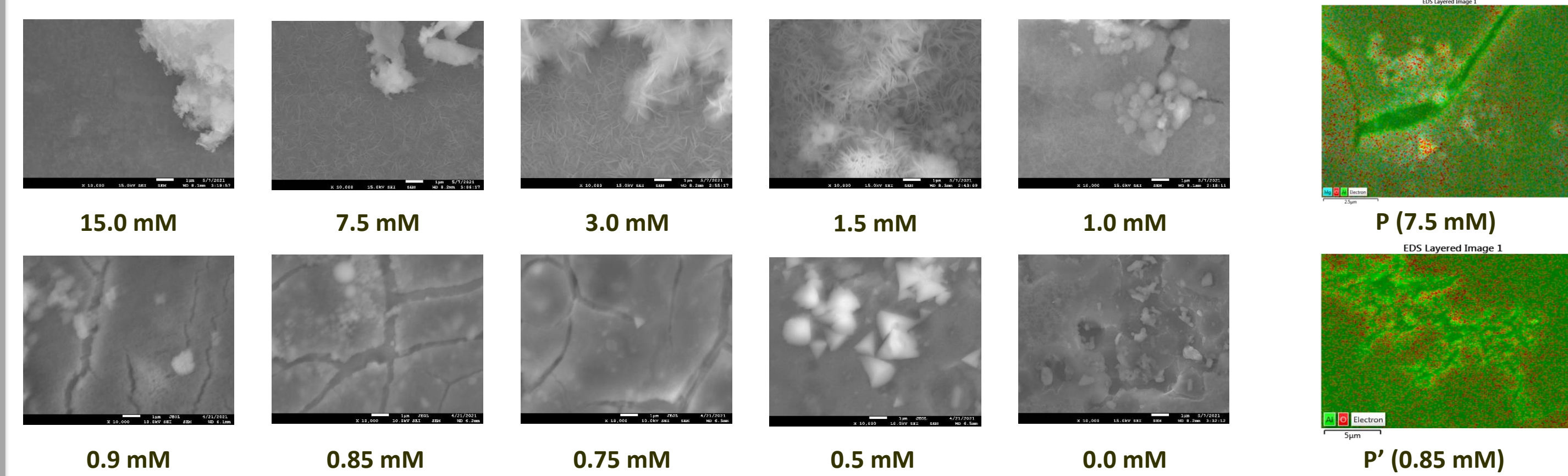


Figure 7. SEM images of P' aluminum ($\times 10,000$).

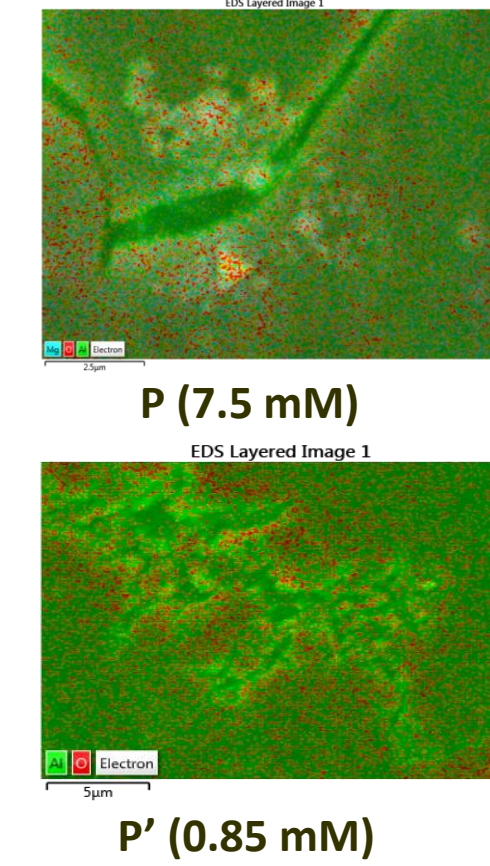


Figure 8. EDS element mapping.

Materials and Methods

Two ElectroPrep electrolysis systems purchased from Harvard Apparatus Inc. were used in this experimental study with one of them as a control. Each system comprised a cathode chamber, a small Teflon center chamber and an anode chamber as illustrated in Figure 1 & 2. The small Teflon center chamber was inserted to the middle O-ring fitting channel of the inter-chamber wall that separates the cathode and anode water chambers. To test the effect of Mg^{2+} salt concentration on localized excess protons, 1.5 ml of pure water or salt solution was placed inside a 1500 μl Teflon center chamber as shown in Figure 1 & 2. The Teflon center chamber was sealed at each of its two ends by Al-Tf-Al membrane assembly that is formed by sandwiching an impermeable 75- μm thick Teflon membrane with two pieces of 25- μm thick proton-sensing aluminum (Al) films. The two compartments were then filled with ultrapure water to create a “cathode water-membrane (Al-Tf-Al)-water-membrane (Al-Tf-Al) water anode” system. After the apparatus was set up, an electrolysis voltage of 200 V was applied to the system for 5 hours using a digital source-meter system. pH and conductance of all the liquids and the mass of aluminum films were measured before and after the experiment.

Conclusions

- Both the proton-sensing film detection and bulk liquid pH measurement demonstrated that protons can be localized at a liquid-membrane interface through electrostatic induction at the P' site in a “cathode water-membrane (Al-Tf-Al)-water-membrane (Al-Tf-Al) water anode” system
- The extent of corrosion for P aluminum is almost same in all Mg^{2+} concentrations whereas decreases with increase in concentration for P' aluminum
- The midpoint with 50-50% cation-proton exchange was achieved when the Mg^{2+} salt concentration was 0.85 mM which was concluded visually, with mass change of the films and SEM imaging along with the SEM-EDS analysis data.
- The equilibrium constant for Mg^{2+} to exchange with the transmembrane electrostatically localized protons was determined to be $(1.53 \pm 0.38) \times 10^{-5}$ at 0.85 mM

Future Work

- To study the effect of various voltage and membrane thickness during the experiment
- To conduct similar sets of experiments with various Ca^{2+} salt concentrations

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Acknowledgements

- I would like to thank my advisor Dr. James W. Lee and all the present and past Lee lab members.
- I would like to thank Dr. Yuan Zhang for her collaboration in conducting the SEM and EDA experiments.
- I would like to thank the ODU's Department of Chemistry and Biochemistry for providing us with its vast supply of valuable instruments.

