Modelling Water and Solute Transport in *Chara*: A Numerical Study of the Effects of Unstirred Layers on Membrane Parameter Estimation

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Declarations

This thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

Sharon Koh

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Abstract

The cell pressure probe (CPP) is an apparatus used to measure membrane parameters of cells, namely the hydraulic conductivity which indicates the permeability of the membrane to water, the permeability coefficient which indicates the permeability of the membrane to solutes, and the reflection coefficient which indicates the extent to which water and solute transport across the membrane is coupled. This thesis is a numerical exploration of the impact of unstirred layers on the measurement of these parameters. Unstirred layers alter the effective concentration across the membrane, and hence influence the calculation of the membrane parameters which are usually obtained using the concentration value in the external bulk solution and assume a homogeneous internal cell solution.

In CPP experiments, cell pressure dynamics are changed by imposing either: a) a hydrostatic perturbation, where cell sap is injected into or removed from the cell, or b) an osmotic perturbation, where permeable solutes are added to or removed from the external solution. Outputs are pressure-time curves which are termed relaxation curves.

Much of the CPP data has been obtained for *Chara*, a large-celled algae. The model developed here will be applied to two sets of *Chara* data: one previously published, and one unpublished and obtained from collaborators who freely contributed their data to this study. Data from two types of CPP experiments were used to estimate membrane parameters by fitting both the classical and unstirred layer (UL) models. These were: hydrostatic pressure pulse experiments, and osmotic pressure pulse experiments using permeable solutes.

This thesis comprises five chapters. Chapter 1 provides an introduction to the research area, and gives an overview of the cell system, CPP experiments, and membrane transport theory. In Chapter 2 an analysis of predictions and limitations using the classical (i.e. usual) method of parameter estimation is made by applying it to published data. This classical model makes simple assumptions about the system,

allows analytical solutions to the membrane transport equations, and does not include unstirred layers. In Chapter 3, a model based upon the classical model but incorporating unstirred layers, is outlined and its behaviour and predictions examined. In Chapter 4, the unstirred layer model is applied to unpublished CPP data, its predictions compared with those from the classical model, and the overall predictions and behaviour of the unstirred layer model evaluated. Finally, in Chapter 5 an assessment of usual practices and assumptions made in the parameter estimation process using the CPP is carried out, and recommendations for future research are given.

The UL model was found to reproduce the observed CPP data to a high degree of accuracy, and reproduced subtle details in the observed data better than the classical model. Estimated parameters from the two models differed significantly; the relative difference in the parameters with respect to the UL model was up to 50% for osmotic experiments and 5% for hydrostatic experiments. This shows that unstirred layers have a significant impact on estimated parameters, and that the membrane parameters commonly estimated using the classical model may be in error by up to 50%.

Data from three *Chara* cells were fit in Chapter 4. Significant inter-cell variation in estimated parameters was found. Estimated parameters for experiments carried out within the same cell were quite consistent, indicating that the UL model is predicting the membrane parameters well since parameters are expected to characterise a cell and its membrane. The behaviour of the UL model was also consistent with expectations from the Kedem and Katchalsky theory for membrane transport, suggesting that the UL model affects the estimated membrane parameters but not the overall behaviour predicted by the membrane transport equations.

Cell pressure dynamics were found to be very sensitive to the thickness of the unstirred layers in the system, so that estimated membrane parameters are dependent on knowledge of the UL thicknesses. In Chapter 4, the UL model was used to estimate the external UL thickness together with the membrane parameters, while the internal UL thickness was fixed at a value effectively equivalent to assuming the whole cell interior is a UL. The model estimated the external UL thickness to be in the range of 30-50 μm for fits to the unpublished data. Some variation in estimated parameters between types of CPP experiments (e.g. hydrostatic or osmotic experiments; experiments with positive or negative pressure perturbations) were found, but the sample size was not sufficiently large for definite conclusions to be made. The UL model did not predict polarity in the membrane parameters (i.e. differences in parameters between positive and negative pressure perturbations). This suggests that evidence of polarity found in the parameters is likely due to effects of a composite membrane (e.g. presence of a tonoplast) or of dehydration of the membrane, and not due to the presence of ULs.

Data were also available from osmotic experiments where bubbles were used to separate the new and old external solutions during the solution changeover. Fits to experiments where bubbles are present were found to be more straightforward and to give more accurate estimates of membrane parameters, as the time for solution exchange was significantly shortened. Where bubbles were not present, the time for solution exchange could not be as effectively incorporated into the model due to lack of experimental data regarding the duration and shape of the solution changeover.

Results clearly showed that some common assumptions regarding the effects of ULs on CPP experiments are incorrect. External ULs are often assumed to primarily influence only the first few seconds of the relaxation curve, but the UL model shows that internal and external ULs influence the cell dynamics throughout the entire course of a CPP experiment. Furthermore, the extent of the influence on ULs on CPP data can only be quantified numerically. Previous attempts at using solutions to steady-state diffusion equations, or using steady-state equations relating permeability across the membrane to permeability in the ULs to predict the impact of ULs on estimated membrane parameters, are shown to be inaccurate. Published estimates of membrane parameters for *Chara* are deemed to be in error, because even where effects of ULs have been claimed to be taken into account, this has not been done numerically. In addition, it is shown that relaxation curves can be fit using the classical model (which does not incorporate ULs) despite the presence of unstirred layers, because ULs do not change the fundamental shape of the relaxation curves, and therefore the true effects of ULs are hidden.

It is recommended that the classical model no longer be used for parameter estimation, and a more realistic model incorporating ULs be applied. This will lead to a more accurate estimation of membrane parameters. The model developed in this thesis, by taking into account effects of unstirred layers, can help to resolve the extent to which ULs impact on estimated membrane parameters, and also the extent to which ULs influence parameter variation among different types of experiments or experimental conditions. Currently, further experimental data is necessary for a wider application of the UL model and fuller assessment of its predictions. The UL model may also be extended in the future for application to more complicated systems such as root tissues.

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