

Electrodifusion in electrolytes and membranes

Author:

Eberl, Maria

Publication Date:

1993

DOI:

<https://doi.org/10.26190/unsworks/4136>

License:

<https://creativecommons.org/licenses/by-nc-nd/3.0/au/>

Link to license to see what you are allowed to do with this resource.

Downloaded from <http://hdl.handle.net/1959.4/54394> in <https://unsworks.unsw.edu.au> on 2024-04-28

ELECTRODIFFUSION IN ELECTROLYTES AND MEMBRANES

BY

MARIA EBERL

A thesis submitted for the degree of
DOCTOR OF PHILOSOPHY
in the Faculty of Science
The University of New South Wales

July, 1993

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

M. Eberl
July, 1993

ABSTRACT

A theoretical treatment of various electrodiffusion regimes for electrolytes and membranes was given. One dimensional analytical solutions to the Nernst-Planck and Poisson equations for the small signal a.c. response of an electrolyte were found for both planar and spherical symmetry and its four terminal electrical impedance was calculated. When the voltage electrodes were situated outside the double layer of the current electrodes, the impedance was an oscillatory function of frequency at low frequencies. The magnitude of the variations in impedance and the frequencies at which oscillations occurred were determined by the diffusion constants of the ion species, the dimensions of the system and the ion concentrations.

Ion concentrations, electric field and ionic currents were also oscillatory functions of position. Calculations suggest the possibility of large errors in ion current measurements through membranes, if these currents are inferred from measurements in the external electrolyte.

Numerical solutions to the Nernst-Planck and Poisson equations were found for a pore of arbitrary shape, but with cylindrical symmetry in an electrolyte. These were used to model the small signal a.c. response of a cylindrical pore and an insulating membrane. It was found that the conductance of the pore was considerably lower than the equilibrium value, while the capacitance was much greater than the geometrical value for both the pore and the insulating membrane, especially at low frequencies. Other non-geometric effects were modelled: i) Including a Born energy term restricted the occupancy of the channel and hence reduced channel conductance. It also increased the effective thickness of the membrane, resulting in a lower channel capacitance. ii) A channel which is impermeable to one ion species significantly reduced the current

of the counter-ion through the channel, resulting in a marked decrease in channel conductance.

In all cases the magnitudes and phases of ion concentrations, electric fields, ion fluxes and impedances were computed at all points in space for frequencies above and below the characteristic frequency of the system.

ACKNOWLEDGEMENTS

I would like to thank Dr. John R. Smith for being my supervisor.

The people who have given me great emotional know who they are.

A very special thanks from the bottom of my heart goes to Associate Professor Joe Wolfe for his very valuable advice and views on the work involved, along with his generous help and support (many thanks for putting up with the "glooms"). He has provided motivation at times when it was difficult for me to proceed. I would like to especially thank Professor Hans G. L. Coster for his interest in my work and his helpful ideas and discussions. His sound "on the spot" advice was much appreciated.

I would also like to thank the fellow Biophysics students and workers who have helped me. They have given me a lot of support and encouragement. Although there have been many, I would like to just mention a few: Tuang, Tohsak, Alfred and Terry. Their friendship was much appreciated. A special thanks to Alfred for his efficient and valuable help during the final stages of my PhD.

I would like to thank Gloria Milmlow for her friendship and understanding and efficient help with administrative matters.

I would like to thank the Costers for their kindness and welcome.

I would like to thank Charles, Kevin Lamberto and Mark for their helpful comments and their proof reading. A special thanks goes to Kevin for his mammoth last minute proof reading effort.

I would like to thank my family for their support, especially during the final stages of my thesis.

TABLE OF CONTENTS

"Admittance to Qualified Personnel Only"

(Anonymous sign affixed to many a locked door and believed to be responsible for the low values of the admittance)

Declaration.....	i
Title Page.....	ii
Abstract.....	iii
Acknowledgements.....	v
Table of Contents.....	vi
 CHAPTER 1 : Introduction.....	 1
1.1 Introduction	
1.2 Membranes	
1.3 Ion Channels	
1.4 Patch Clamping	
1.5 Scope of the Thesis	
 CHAPTER 2 : Literature Review.....	 9
2.1 Introduction	
2.2 a.c. Electrodifussion Theory of Electrolytes	
2.3 Membrane Impedance	
2.4 Double Layer Theory of Electrolyte Solutions	
2.5 Structure and Function of Channel Proteins	
2.6 The Electrical Modelling of Ion Channels	
2.7 Patch Clamping	

CHAPTER 3 : Solutions to the Nernst-Planck Poisson Equations of an Electrolyte for Planar Geometry.....	36
3.1 Introduction	
3.2 Theory	
3.3 Boundary Conditions	
3.4 Four Terminal Impedance	
3.5 Low Frequency Limit	
3.5 High Frequency Limit	
3.7 Binary Electrolyte	
3.8 Results	
3.9 Conclusions	
CHAPTER 4 : Solutions to the Nernst-Planck Poisson Equations of an Electrolyte for Radial Geometry.....	76
4.1 Introduction	
4.2 Theory	
4.3 Boundary Conditions	
4.4 High Frequency Limit	
4.5 Results	
4.6 Conclusions	
CHAPTER 5 : Numerical Solutions to the Nernst-Planck Equations.....	95
5.1 Introduction	
5.2 Methods	
5.3 Results	
5.4 Insulating Membrane	
5.5 A Simple Pore in a Membrane	
5.6 Frequency Spectrum	
5.7 Impedance Calculations	
5.8 Conclusions	

CHAPTER 6 : Decorations to the Model of an Ion Channel..... 134

- 6.1 Introduction
- 6.2 Methods
- 6.3 Results
- 6.4 Equilibrium Distribution for the Born Energy Version
- 6.5 Results for the a.c. Signal Response Including the Effect of the Born Energy
- 6.6 Equilibrium Distributions for a Charged Channel
- 6.7 Small a.c. Signal Calculations for a Charged Channel
- 6.8 Equilibrium Distributions for an Ion Selective Channel
- 6.9 a.c. Calculations for the Ion Selective Channel
- 6.10 Impedance Calculations
- 6.11 Conclusions

CHAPTER 7 : Conclusions and Suggestions for Further Work..... 155

- 7.1 Conclusions
- 7.2 Suggestions for Further Work

Appendices..... 162

References..... 168

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Mental processes (such as my writing and your reading of this thesis) involve complicated electrochemical computers whose active elements are tiny nonlinear conductors in the membranes of nerve cells: Na^+ and K^+ channels. These elements are not nearly as well understood as the transistors which are the active elements of less powerful electronic computers (whose operation was also involved in this thesis, as later chapters will attest).

This work was inspired by biophysics and its application to biological membranes. It began as a theoretical analysis of the questions: What are the electrical properties of ion channels and how do they affect membranes? These questions are of great interest to electrophysiologists with recent contributions from molecular biophysicists. Yet in this area some basic electrical questions remain unanswered because it is often difficult to interpret apparently simple electrophysiological measurements. So the first few chapters of this thesis address more fundamental theoretical questions of how salt solutions behave and how they interact with electrodes.

Although these questions were inspired by biophysics, much or all of the approach used will be of much wider usefulness, and may have applications in electrochemistry and in artificial membrane research. Work in this thesis may prove useful in such diverse areas as the performance evaluation of electrochemical cells and separation

technologies commonly used in food processing.

Put in the most general way, this thesis is a theoretical analysis of the electrical properties of i) electrolyte solutions, ii) electrolyte solutions in contact with electrodes and iii) membranes with holes and electrically interesting structures.

The power of electrical techniques, used *in situ*, suggests that one should use them to obtain structural details. However, until the present time little information is available. Much progress has been made in the development of steady state (d.c. or zero frequency) models of transport mechanisms (see for example Levitt, 1986 and 1991, Jordan, 1986 and Lauser, 1985). Relatively little progress has been made in the analysis of transient or a.c. response, even though such analysis may be able to yield significant information.

In studying the electrical behaviour of biological membranes and conducting channels, electrical measurements such as patch clamping and impedance spectroscopy are obvious tools for obtaining information about the electrical behaviour and electrical structure of these systems. Patch clamping is the study of the electrical properties of a very small region of membrane which may contain one or a few channels. Impedance spectroscopy is the measurement of the frequency dependence of real and imaginary parts of impedance for comparison with calculations based on known or hypothesized structures. Patch clamping has many advantages: it has sufficiently high resolution to allow detection of the tiny current pulses through a single ion channel and it can be used in several different configurations. Recently, patch clamping has revealed some of the detailed behaviour of individual channels. However, it yields relatively few data about any given channel. Impedance spectroscopy on the other hand, produces a whole spectrum for each experimental condition. The disadvantage of using impedance spectroscopy is its lack of resolution in

the plane of the membrane. So far little or no use has been made of a.c. techniques and impedance spectroscopy in patch clamping. However, combining the two techniques has potential, since impedance spectroscopy provides a powerful technique for structural analysis. It should be noted that before applying the a.c. signal, the membrane patch would have to be suitably voltage clamped, so that the ion channel was either permanently closed or, where possible, open for a sufficiently long time. The time has almost arrived for the combination of these techniques since i) the patch clamping technique has become well established and refined, ii) very accurate impedance spectrometers have been developed and iii) almost enough computing power is available for sufficiently detailed modelling of channels.

This thesis is a theoretical analysis of the electrical response of membranes and the surrounding electrolyte solution to small a.c. signals. The a.c. approach has two advantages over the steady state models:

- 1) Exact algebraic solutions to the Nernst-Planck and Poisson equations are possible for the case of one dimensional electrolyte systems.
- 2) Additional information can be obtained from measurements of the impedance spectrum that could provide information about the structure of ion channels.

The remainder of this chapter is used to give a brief description of cell membranes, their electrical properties and patch clamping.

1.2 MEMBRANES

Membranes form a barrier between the interior and the surrounding environment of all living cells. Hence, they allow different environments to exist in the interior and exterior of the cell (e.g. concentration

differences) and control both active and passive transport of ions, nutrients and waste products into and out of the cell. Recently, intensive work on artificial membranes has attempted to reproduce these functions in artificial membranes.

There are many cell types which perform different functions. However, all cell membranes consist of a lipid bilayer, typically 2 to 5 nm thick, into which protein molecules are embedded to various extents, as shown in Figure 1.1. Membranes and their embedded proteins form a dynamic structure, of which the lipid bilayer is a two dimensional fluid. The most common types of lipid are phospholipids (mammalian cells contain four major types), sterols and glycolipids. The basic structure of a lipid has two components. The first is a hydrophilic polar head group and the second contains hydrophobic hydrocarbon chains (usually two per polar head group). In the bilayer the chains meet near the central plane and the head groups are directed towards the surrounding solution. Lipid bilayers can form spontaneously in aqueous media due to their amphipathic nature. They are highly impermeable to ions and charged molecules, due to the low dielectric constant of the central hydrophobic region, i.e. they have low conductances (of the order of mS m^{-2} , depending on electrolyte composition and concentration, Smith *et al.*, 1984) and many different ion species cross the cell membrane through channels formed by protein molecules spanning the bilayer. The passive transport that occurs primarily through channels, greatly increases the conductance of biological membranes to values between 1 and 100 S m^{-2} .

Studies of proteins inserted into artificial bilayers suggest that these protein channels only function fully in the presence of certain phospholipids. However, the functional significance of different lipid types is still largely unknown. The lipid composition of the inner and

FIGURE 1.1

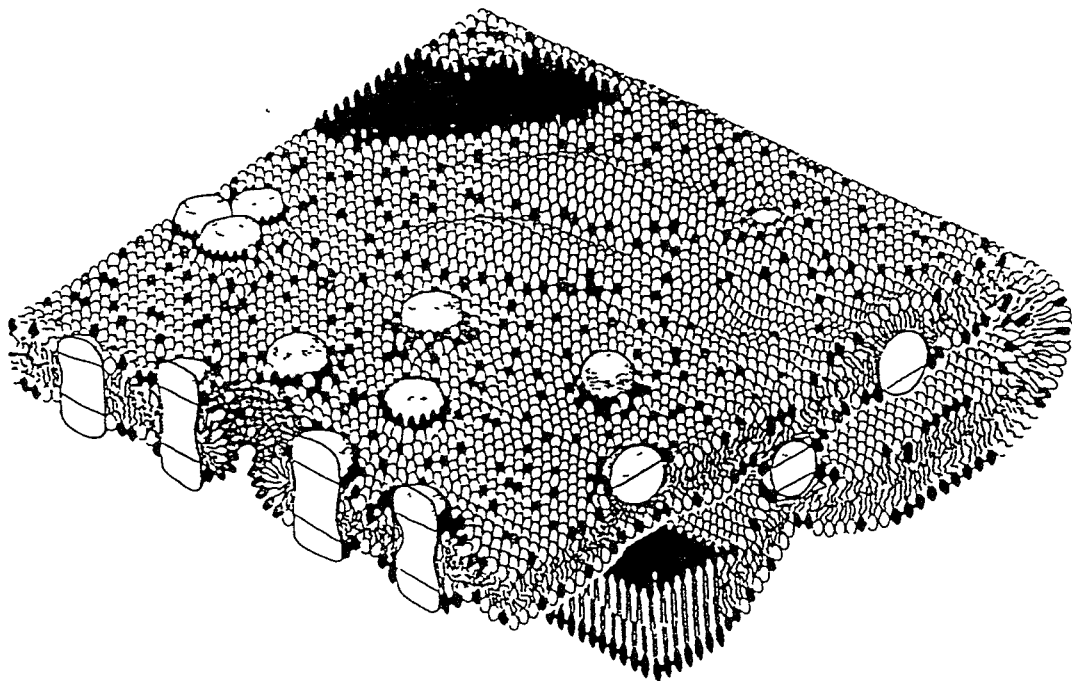


Figure 1.1. A schematic diagram of the fluid mosaic model of a membrane (from Israelachvili, 1978). The large structures represent channel proteins embedded in the lipid bilayer.

outer monolayer of cell membranes differs in some cases, such as the membranes of human red blood cells (see Alberts *et al.*, 1989). This asymmetric structure might assist in maintaining the correct orientations of proteins.

1.3 ION CHANNELS

Channel proteins form aqueous pores through which ions can pass. The transportation through open channels is passive and ions diffuse through the aqueous interior of the protein due to electrochemical potential differences between the cell's interior and exterior. Most channels in animal cells are highly ion selective. A description of some of the better known channel proteins is given in Chapter 2.

Almost all animal cells maintain a potential difference across their cell membranes at equilibrium, with the inside of the cell being usually more negative than the outside. This is called the resting potential. The electric field across membranes is enormous, since membranes are very thin. For example, transmembrane potentials are typically in the range of 10 to 100 mV while membrane thickness is of the order of 5 nm, which results in electric fields of the order of 10^7 V m^{-1} . These fields are perhaps sufficiently strong to play a role in the transition of channels from the non-conducting (closed) to conducting (open) state (Honig *et al.*, 1986).

1.4 PATCH CLAMPING

One of the most successful techniques for studying the electrical properties of ion channels in membranes is patch clamping (see Neher and Sakman, 1992; Neher, 1992; Auerbach and Sachs, 1984 and further

details in Chapter 2). With this technique, single channel currents can be measured with a resolution of fractions of picoamperes.

Ion channels open and close in response to stimuli such as changes in transmembrane potentials and ligand binding. Hence single ion channel currents occur as a series of constant amplitude current steps as shown in Figure 1.2. These are in the range of picoamperes, except for channels with substates which lead to multiple conductance steps. The opening frequency distribution in these cases are probabilistic (Auerbach and Sachs, 1984). The conductance level obtained from the amplitude of the current pulses is a characteristic unique to each type of channel. Conductance levels are typically anywhere from tens to hundreds of pS. They are often used to identify the channel type in individual membrane patches under investigation. These unit conductances for many channel types appear to be species invariant.

Normally patch clamping will only reveal channels with fluctuating currents. This is because of the necessary initial arbitrary zeroing of the current and voltage scales in order to allow detection of the current pulses. These can be very small compared to the d.c. offsets present. In biological membranes, d.c. or steady state currents as well pulsed currents are present. Even though the treatment in Chapter 3 and 4 concentrates on the fluctuating current, the numerical model of Chapter 6 includes the additional possibility of modelling steady currents.

1.5 SCOPE OF THE THESIS

This thesis models passive ion transport through open channels or pores, using a.c. response theory to determine the impedance of the channel. The a.c. signals considered are assumed to be small, and hence do not cause action potentials to occur. In addition, gating mechanisms or

FIGURE 1.2

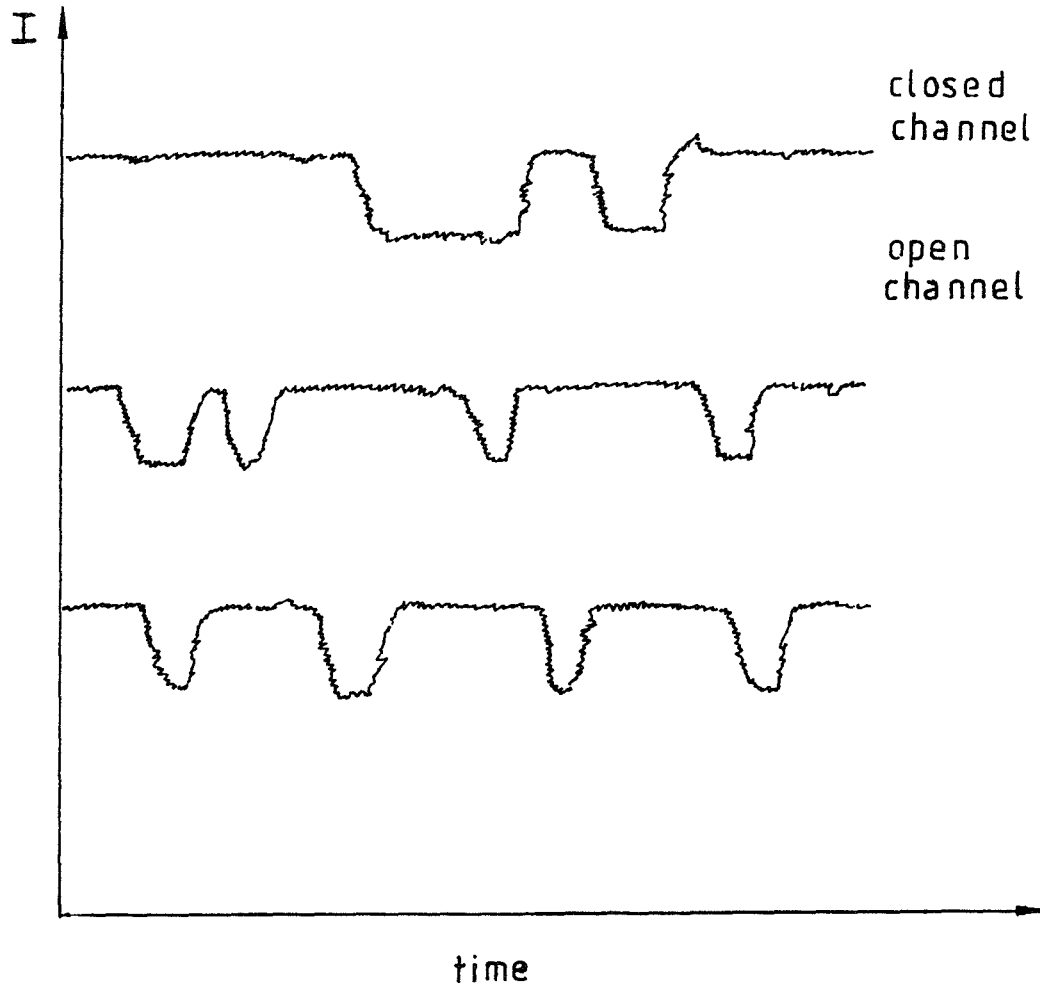


Figure 1.2. A schematic diagram of typical current recordings for single channel patch clamps. Channel opening times are typically of the order of milliseconds and the amplitude of the current pulses is of the order of picoamperes.

carrier facilitated transport are not considered in this work.

Models of ion transportation which use the small signal a.c. response complement information obtained through steady state models, which have been extensively used to calculate channel current voltage characteristics and conductances of channels. Steady state models have already been developed by various authors (e.g. Levitt, 1991, Lauser, 1987 and Jordan, 1986)

To understand how ions pass through aqueous channels, one has to first consider how ions pass through any aqueous medium. This is nontrivial, because the positive and negative ions interact and since, in general, the ion species have different diffusion constants. Their behaviour is particularly complicated when boundaries - electrodes - are imposed. Hence in the first part of this thesis, the electrical properties of electrolyte solutions only are investigated. Chapter 3 gives analytical solutions to the small a.c. signal response of multi-ion (2 or 3) electrolyte solutions in the simplest one dimensional geometry possible: planar geometry.

Ion currents through membranes can be measured directly by positioning a microelectrode on either side of the cell membrane. This is however not possible for some cell types without unacceptable damage to the cell. Hence methods have been developed to determine local ionic currents solely from electrochemical measurements in the external electrolyte. The membrane currents are then assumed to equal these local ion currents, once the necessary geometrical correction have been made. However, in many experiments of this nature, it is quite likely that the ionic currents will vary with distance away from the membrane, especially for time-varying measurements. Hence these techniques may lead to significant errors. This problem is addressed in Chapter 3.

Channels in membranes have cross-sections with dimensions in the

order of nm. Hence they are very small compared to the size of most measuring electrodes, which are usually situated at macroscopic distances away from the membrane. In order to pass through the channel, ions in some cases have to diffuse to the mouth of the channel from a large reservoir of electrolyte solution. The region of electrolyte solution around the channel mouth is therefore often modelled as a hemisphere (e.g. Lauger, 1976 and Levitt, 1987). Hence in Chapter 4, the electrodiffusion equations are solved for spherical geometry. This also allows investigation of geometrical effects, by comparing the solutions in spherical geometry to those for the planar geometry of Chapter 3.

Exact algebraic solutions to the Nernst-Planck and Poisson equations appear to be possible only for one dimensional systems. However, ion channels through membranes are complicated three dimensional structures. Consequently, in Chapters 5 and 6 a numerical approach is developed to solve the electrodiffusion equations. These solutions have the advantage of allowing arbitrary channel geometry and including other important effects such as the surface charge of channels, effects due to the dielectric discontinuity and ion selectivity.

Chapter 7 presents the conclusions and suggestions for further work.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The electrical properties of cell membranes are of fundamental importance in physiology and biology. Since membranes are necessarily surrounded by electrolyte solutions, measurements of membrane properties must include effects of the electrolyte. Furthermore, measurements are made with electrodes which themselves have electrical properties. Hence, the first part of my thesis investigates the electrical properties of electrolyte solutions, in particular, their a.c. impedance. Theories of the a.c. impedance of electrolytes are commonly used in electrochemistry, in order to find equivalent circuits. The reason for introducing equivalent circuits is this: if the equivalent circuits are simple and if they are also good approximations of the system, they enable simple calculations of the system response. It is not always clear, however, that published equivalent circuits satisfy these criteria.

Any charged surface in an electrolyte solution attracts counter-ions and repels co-ions, thus forming a double layer. Some of the theories of the electrical properties of double layers are briefly discussed in this chapter.

Chapters 5 and 6 give a description of a numerical model of the electrical properties of ion channels through membranes based on the finite difference version of the Nernst-Planck electrodiffusion equations. In biological membranes, channels are formed by protein molecules which span the lipid bilayer. The structure of these proteins determines

the characteristics of channels, so a brief review of protein structure and function is included. Much progress has been made in recent years in modelling ion channels. However, most of the conventional theories model steady state systems, whereas the approach presented in this thesis is to investigate the small signal a.c. response of ion channels.

The nature of this study poses problems for the review of relevant literature. A thorough review of electrolyte theory, double layer studies, permeation, membrane pores and channels would produce a volume larger than this thesis in itself. On the other hand, the material which is directly relevant to the thesis - a.c. electrolyte and pore studies, is very scarce indeed. Therefore a brief review of background material will be presented, along with more detail on the relatively few directly related articles.

2.2 A.C. ELECTRODIFFUSION THEORY OF ELECTROLYTES

Electrical measurements of electrolyte solutions almost always involve electrodes, so it is of interest to determine the electrical properties of an electrolyte in contact with an electrode. Although the equilibrium distribution of ions and potential profile of the double layer formed near an electrode have been studied extensively, relatively few analyses of the a.c. response of the double layer appear to be available. A simple system is considered where it is assumed that any molecular effects can be neglected, and that the electrode is blocking or ideally polarizable, i.e. impermeable to ions.

The polarization impedance of a single blocking electrode in an electrolyte solution containing an arbitrary number (N) of ion species was calculated by DeLacey and White (1982) as follows. The system was

described using three equations: Poisson's equation, the continuity equation and a form of the Nernst-Planck equation equivalent to that used in this thesis, which represents the balance of the hydrodynamic drag and the electrical and thermodynamic forces on each ion species.

The effect of applying a small a.c. voltage of frequency $\omega/2\pi$ to the system was investigated. The amplitude was chosen so that all second and higher order terms of the electric field could be neglected. An analytical solution for the potential difference of the double layer of one infinite electrode was obtained for zeta potential (ζ), $\zeta = 0$. This was then used to determine numerically the polarization impedance¹ of the double layer (Z_e) for cases of non-zero zeta potentials². The series resistance (R_s) and capacitance (C_s) of the double layer as a function of frequency were then calculated for different values of ζ and electrolyte concentrations (c)³.

DeLacey and White found that R_s approached a limiting value with increasing zeta potential. This is consistent with the non-linear Gouy-Chapman theory, which predicts that the potential in the double layer at very high ζ varies with ζ only over a very thin region. C_s also increased with increasing ζ , but showed no limiting behaviour. The behaviour of the double layer impedance with increasing concentration was found to be consistent with the concentration dependence of the Debye length⁴ (see

¹Note that the Stern layer, i.e. the layer of adsorbed charge on the electrode surface, was neglected in the polarization impedance calculations.

²The zeta potential is defined as the electrostatic potential at the surface of shear between the solution-associated and the stationary part of the double layer" for electrophoresis measurements (e.g. Cevc, 1990). This is a pragmatic definition inspired by electrophoretic measurement of surface charge.

³For a series combination, R_s and C_s are related to Z_e by: $Z_e = R_s + i/(\omega C_s)$. This choice is arbitrary and DeLacey and White (1982) give the alternate expression for the total impedance in terms of a parallel resistance (R_p) and capacitance (C_p). In this case, $Z_e = (R_p + i\omega C_p R_p^2)/[1 + (\omega C_p R_p)^2]$. The latter definition is used in this thesis.

⁴The Debye length gives an approximate range of the electric field and ion concentration

section 2.4). R_s was approximately proportional to $c^{-1/2}$, while C_s increased as approximately $c^{1/2}$.

The double layer impedances for several electrolytes (KCl, $\text{Ba}(\text{NO}_3)_2$, LaCl_3 and $\text{K}_4\text{Fe}(\text{CN})_6$) were determined for a fixed ionic strength and value of ζ to investigate the effect of ion valency on R_s and C_s . The total number of counter-ions in the double layer increases with increasing valency, as predicted from non-linear Gouy-Chapman theory. Furthermore, the higher valency ions can carry more current for a given applied field. The observed decrease in R_s and increase in C_s with increasing valency was mainly attributed to these two effects.

DeLacey and White (1982) also concluded that the marked sensitivity of the total double layer impedance to valencies could have contributed significantly to the reported irreproducibility of early electrode impedance measurements.

Although their treatment was more general in terms of the number of ion species and range of zeta potentials than the system described in Chapter 3, it differs in other ways: DeLacey and White calculated the a.c. impedance of the double layer of a single infinite electrode in an infinite electrolyte solution. Calculations of Chapter 3 were for the a.c. 4 terminal impedance of a finite electrolyte between two infinite electrodes, i.e. the impedance was calculated between two points at an arbitrary distance away from the current electrodes. There is, however, an overlap for one particular case and a brief comparison will be given in Chapter 3.

The characteristics of electrochemical cells are commonly evaluated using a.c. impedance measurements (see for example Bai and Conway, 1990, Bates and Chu, 1988; Bohnke and Bohnke, 1990; Morita *et al.*, 1992; Muragesamoorthi *et al.*, 1991 and Ratnakumar *et al.*, 1990). However, published theoretical treatments of the a.c. impedance of liquid

gradients in the double layer.

electrolytes used to interpret these measurements differ from the approach used in this thesis. For example, one conventional method is to fit impedance data to well established equivalent circuits, such as the Randles circuit, to extract the time constants for the faradaic and diffusion processes of the system (VanderNoot, 1991). The impedance of simple redox reactions has been calculated for various electrode geometries using simple diffusion equations. The presence of excess support electrolyte is assumed, so that electrical forces may be neglected (Fleischmann *et al.*, 1991).

Macdonald has published many papers on the small a.c. response of both solid and liquid binary electrolytes of arbitrary valencies and mobilities for various boundary conditions and approximations (e.g. Macdonald, 1974 and Macdonald and Franceschetti, 1978). Unlike the approach used in this thesis, Macdonald concentrates on finding equivalent circuits of the different electrolyte systems, in some cases including frequency dependent elements. (e.g. MacDonald, 1987). If the equivalent circuit is sufficiently simple it can provide a useful empirical characterisation of the system's behaviour. In some cases a circuit analog can be intuitively useful. For example the potential difference across an electrode/electrolyte system is, in some cases, equal to $V_{dl} + V_s$, where V_{dl} is the voltage drop across the double layer and V_s is the voltage drop across the bulk solution. Hence the total impedance may be modelled by a double layer impedance in series with an electrolyte impedance. However, equivalent circuits with frequency independent elements may become misleading, especially for mathematically very complicated analysis, since they are at best a representation of the system and not a physical equivalent thereof.

All of Macdonald's papers deal with the total impedance measured between two electrodes (i.e. a two terminal impedance) which is

dominated by the impedance of the double layer near the electrodes. In Chapters 3 and 4, the focus will be on the impedance between two voltage electrodes situated in the electrolyte solution outside the two double layers. This effectively excludes the impedance of the double layers.

An alternative technique to a.c. impedance measurements is to investigate the transient response of an electrolyte solution to small pulses of charge or voltage steps. This has also been calculated using the Nernst-Planck equations (Buck, 1969). However there are technical problems associated with analysing transient response data: fourier transform algorithms use sums of exponentials, hence smaller components often cannot be distinguished from noise and large time constants may be misinterpreted as systematic shifts or errors.

Two terminal impedance measurements essentially measure the double layer plus electrolyte. To investigate the electrolyte solution alone usually requires four terminal measurements using separate electrodes to measure the current and voltage. In order to achieve this, voltage electrodes have to be very small so that they do not perturb ion diffusion. They also have to have a very large impedance, so that they do not change the current and hence do not introduce an additional potential difference. The importance of testing the electronic response of the measuring instrument has been emphasized by VanderNoot *et al.* (1990). All impedance calculations given in this thesis are for four terminal systems. They may be reduced to two terminal results simply by setting the position of the voltage electrodes equal to that of the current electrodes.

2.3 MEMBRANE IMPEDANCE

The small a.c. signal response of lipid bilayers (e.g. Ashcroft *et al.*, 1983 and Laver *et al.*, 1984) and to a lesser extent that of living membranes have been widely studied, using impedance spectroscopy (e.g. Chilcott, 1988 and Coster and Smith, 1977). In principle, these impedance spectra can provide much structural information since they provide many data for any one experimental condition. However, there remains a problem in the interpretation of these results. Most conduction is thought to occur via pores or transient holes in lipid bilayers and through ion channels in living membranes. To date there has been no satisfactory a.c. theory to model the electrical properties of these pores or channels. This thesis attempts to provide such a theory.

2.4 DOUBLE LAYER THEORY OF ELECTROLYTE SOLUTIONS

Electrostatic interactions are of paramount importance for the case of charged surfaces in an electrolyte solution. They are also relevant to electrolyte solutions between electrodes, as well as to membranes and colloids. A combination of Poisson's equation and Boltzmann statistics have commonly been used in equilibrium double layer theories.

At equilibrium the cation (n_+) and anion (n_-) concentrations in an electrolyte solution obey Boltzmann statistics. That is:

$$n_+ = n_0 z_+ \exp(-z_+ q\psi/kT) \quad (2.4.1)$$

$$n_- = n_0 z_- \exp(-z_- q\psi/kT) \quad (2.4.2)$$

where: q is the electronic charge,
 n_0 is the bulk electrolyte concentration
 ψ is the electric potential,
 k is Boltzmann's constant,
 T is the temperature and
 z_+ and z_- are the valencies of cations and anions, respectively.

Poisson's equation relates the space charge (ρ) $\rho = q(z_+n_+ - z_-n_-)$ to the electric potential (ψ) :

$$\nabla^2\psi = -\rho/\epsilon \quad (2.4.3)$$

Combining 2.4.1 to 2.4.3 gives the Poisson-Boltzmann equation:

$$\nabla^2\psi = -q/\epsilon[n_0z_+\exp(-z_+q\psi/kT) - n_0z_-\exp(-z_-q\psi/kT)] \quad (2.4.4)$$

where ϵ is the permittivity of the electrolyte solution.

Debye and Hückel (1923) used the linearised Poisson-Boltzmann equation in spherical geometry to calculate the activity coefficients for non-ideal electrolyte solutions, i.e. the correction to the electrolyte concentration which accounts for the influence of the surrounding counter-ions on the distribution of ions in solution. They treated the ion as a point charge and the charges surrounding it as a uniform ionosphere, when calculating the average potential as a function of radial distance. Since the concentrations and potentials used in this thesis are low, the electrolyte solutions can be approximated by ideal solutions.

The Gouy-Chapman theory of double layers is one of the earliest, simplest and widely used theories and can be found in many reviews and

texts (e.g. Feynmann, 1972; McLaughlin, 1989; Carnie and Torrie, 1984 and Cevc, 1990). In this theory, the potential $\psi(x)$ in an electrolyte solution as a function distance x from an electrode or charged membrane surface can be calculated from the one dimensional Poisson and Boltzmann equation. The surface is assumed to be planar and infinite, which is often applicable because its size is usually much greater than the Debye length.

Solving equation 2.4.4 for $\psi(x)$ using the boundary conditions: $\psi(0) = 0$ and $d\psi/dx = 0$ as x approaches infinity gives:

$$\psi(x) = \frac{2kT}{q} \ln \left(\frac{1 + \alpha \exp(-\kappa x)}{1 - \alpha \exp(-\kappa x)} \right) \quad (2.4.5)$$

where : $\alpha = (eq\psi(0)/2kT - 1)/(eq\psi(0)/2kT + 1)$ and

$\kappa = [2z^2q^2n_o/(\epsilon kT)]^{1/2}$ is the inverse of an effective screening length known as the Debye length.

For cases where $\psi(x)$ is small compared to kT 2.4.5 reduces to :

$$\psi(x) = \frac{\sigma}{\epsilon \kappa} e^{-\kappa x} \quad (2.4.6)$$

In this theory, the effects of discreteness of charge, the finite size of the ions, the inhomogeneities of the charged surface (especially relevant to surfaces such as membranes which have low surface charge density) and hydration are neglected. Furthermore the equivalence of spatial averaging and time averaging is implied.

To allow for adsorption of ions of finite size at the membrane surface, the Gouy-Chapman theory has been extended to include a layer of adsorbed or fixed charge known as the Stern layer. Complications of a

Stern layer and reactions at the electrodes have not been considered in this thesis, since all impedances calculated are four terminal impedances and the potential at the current electrode surfaces is consequently not required. In addition, confining the study to small signals minimises the effects of ion size in activity and dielectric saturation.

A comparison of modern statistical mechanical theories of the double layer by Carnie and Torrie (1984) with computer simulations for a primitive model of electrolyte solution (i.e. ions are hard spheres in a homogeneous dielectric medium) confirmed that despite its simplistic approach, the modified Gouy-Chapman⁵ theory is able to predict properties of double layers as accurately as far more elaborate theories for a significant range of concentrations and surface charge for monovalent ions. Recent experimental tests of the Gouy-Chapman theory, as well as Monte Carlo and molecular dynamics simulations also confirm this (e.g. Honig *et al.*, 1986).

In the limiting case when the surface potential $\psi(0)$ is much greater than kT/q , rearrangement of Gouy-Chapman's expression for $\psi(0)$ shows that the concentration of the counter-ion at the membrane/solution interface is proportional to σ^2 (σ is the surface charge density), and is independent of the counter-ion valency and the concentration of the bulk salt solution. Recently this relation has also been derived from more general considerations, hence providing a possible reason for the success of the Gouy-Chapman theory, despite its crude approximations (McLaughlin, 1989). Cevc (1990) gives criteria for the suitability of simple electrostatic theoretical models and more complicated modern theories for double layers near membranes.

⁵The modified Gouy-Chapman theory is an extension of the simple Gouy-Chapman theory, described in this section. It includes with Poisson's equation the effects of ion-ion and ion-surface interactions via an additional potential term (Cevc, 1990).

2.5 STRUCTURE AND FUNCTION OF CHANNEL PROTEINS

Transmembrane proteins extend across the membrane as single or multiple alpha helices, with hydrogen bonding between overlying peptide bonds. Some of these have covalently bonded fatty acid chains extending into the bilayer. Proteins on the surface are attached either via a fatty acid chain extending into the bilayer or via an oligosaccharide to a minor phospholipid. Other proteins are held by noncovalent interactions to the surface of transmembrane proteins (Eisenberg, 1984). Unlike lipids, proteins are fairly rigid molecules, although parts of them can undergo conformational transitions. These conformational changes include i) the translocation of charged groups perpendicular to the membrane plane, ii) the reorientation of existing dipoles, and iii) the binding of ions, e.g. protons to sites near the channel entrance (e.g. Anderson and Koeppe, 1992).

The proteins involved in transporting substances across membranes can be divided into two groups: channel proteins and carrier proteins.

2.5.1 Channel Proteins

Channel proteins form aqueous pores through which ions can pass. Transport through open channels is passive. Ions diffuse through the aqueous interior of the protein due to electrochemical potential differences between the cell's interior and exterior. Many transmembrane proteins found in animal and higher plant cells are highly selective to certain ion species, due to their structure and the position of fixed charged groups. One commonly accepted reason for this selectivity is that ions temporarily bind very selectively to charged groups of channel

proteins during their passage through the channel (Eisenman and Alvarez, 1991).

Proteins determine most of the specific functions of cells, hence the type and abundance of proteins in cell membranes varies considerably. Examples of some of the commonly studied channel types will now be presented.

2.5.1a) Gated Channels

Gated channels open in response to a membrane perturbation. These perturbations include i) a change in transmembrane potential (voltage gated) ii) the binding of a signalling molecule such as a neurotransmitter (transmitter-gated) or an ion, usually Ca^{2+} (Alberts *et al.*, 1989). Voltage gated channels are thought to have "sensors" or charged structures intrinsic to the channel protein, which move in response to changes in the transmembrane potential (e.g. Honig *et al.*, 1986). This results in conformational changes which open the channel to a specific ion species.

One example, the Acetylcholine receptor (ACH) channel is a well known transmitter-gated channel. It is formed by a glycoprotein, consisting of five subunits, as shown in Figure 2.1. Two of these subunits are identical and each can bind one molecule of ACH. The channel opens as a result of a conformational change in the pentameric complex induced by the binding of ACH. This transition is not stable and the channel closes after approximately one millisecond. The ACH molecule dissociates and is hydrolysed by enzymes. The channel then returns to its original configuration. ACH receptor channels found in membranes of skeletal muscles play a major part in muscle contraction. In this case the ACH molecules needed to activate the channel are released from nerve endings.

Functional acetylcholine channels can be incorporated into artificial

FIGURE 2.1

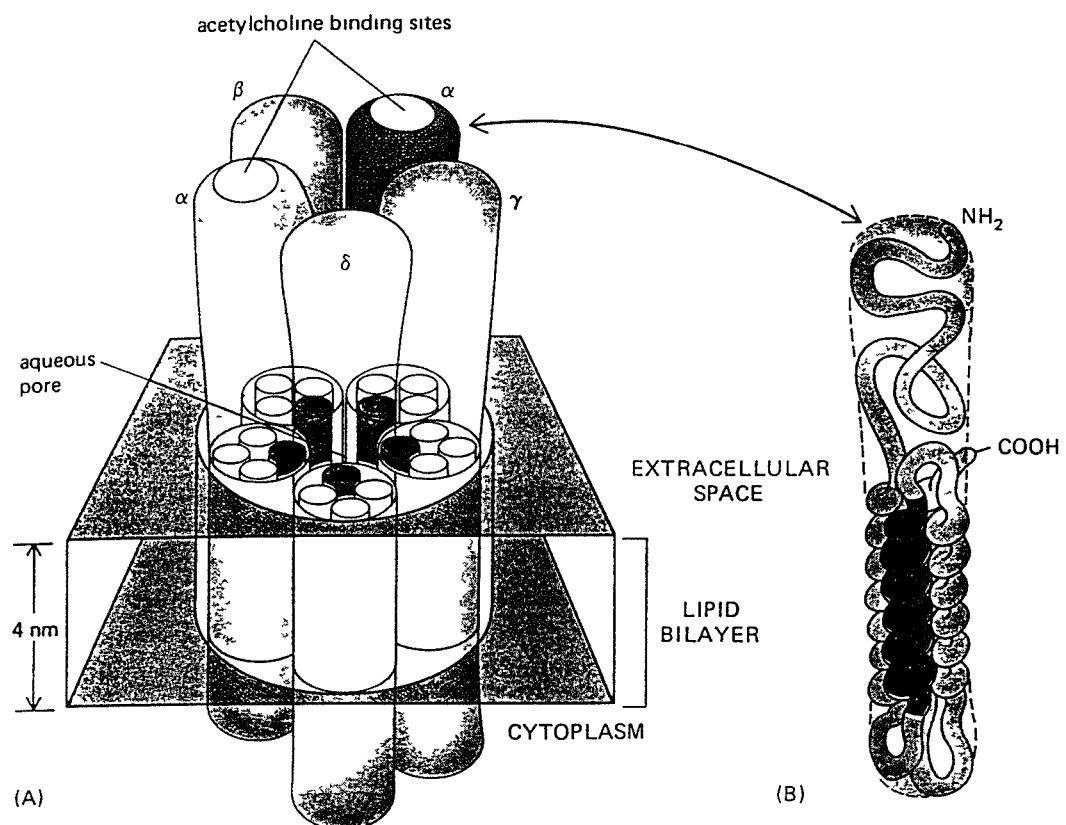


Figure 2.1. An illustration of the proposed structure of the acetylcholine receptor channel (from Alberts et. al., 1989). It consists of five subunits ($\alpha, \alpha, \beta, \gamma, \delta$), two of which (α) can bind an acetylcholine molecule. each subunit contains about 500 amino acid residues, and the channel weighs approximately 300,000 Dalton. The polypeptide chain of each subunit is thought to cross the bilayer as 4 α helices.

lipid bilayers, enabling the measurement of single channel conductances (Montal *et al.*, 1986 and MacNamee *et al.*, 1986). Several models have been proposed to describe the arrangement of ACH channels in bilayers, based on electron microscopy and X-ray diffraction work. The large number of negatively charged amino acid residues at the channel mouth is thought to exclude anions from the channel. Only hydrated cations with radii less than about 0.65 nm can pass through. However, the channel is not selective to cation type, hence the flux of cations depends on their electrochemical gradient.

A second example is the propagation of action potentials along neurons and axons. This process involves a number of different types of channels. In the resting state the external concentration of sodium ions (Na^+) is high, whilst the internal concentration of K^+ is high on the inside. At rest the membrane is more permeable to K^+ than to Na^+ and this results in a transmembrane potential which is negative in the interior of the cell. If the magnitude of the membrane potential difference is reduced below a critical or "threshold" value, a large influx of Na^+ ions occurs via voltage gated channels. The influx continues in a self amplifying manner until the membrane potential is predominantly permeable to Na^+ and approaches the Nernst equilibrium potential⁶ for Na^+ (typically +50 mV). This process is self amplifying because the influx of Na^+ further shifts the transmembrane potential to more positive

⁶Ions flow into and out of cells depending upon the gradient of their electrochemical potential. This is comprised of two major terms: the first is the potential difference (V) between the inside and outside of the cell and the second is the concentration gradient of the ion species across the membrane. When these two terms balance exactly, i.e. the ratio of the internal and external ion concentrations: $C_i/C_o = \exp(-V/kT)$, the electrochemical gradient, and hence the ionic current is zero. The transmembrane potential for this case is known as the Nernst equilibrium potential for that ion species, and can be calculated from the Nernst equation: $V = -kT/q \ln(C_o/C_i)$.

values, causing the opening of even more Na^+ channels.

The permeability of K^+ also increases, but more slowly, resulting in a net outward flow of K^+ . This further reduces the potential, until it reaches a value close to the resting potential. Since the sodium channels have a refractory period of a few milliseconds after the membrane potential returns to its original negative value, the system can return to its original state via the Na^+ - K^+ pump. This change in membrane potential is sufficient to trigger neighbouring membrane patches, enabling signal transmission along the axon. Hodgkin and Huxley (1952) were the first to develop a model for these action potentials.

The resting potential of the cell membrane is maintained as follows. Na^+ - K^+ pumps actively drive K^+ into the cell and Na^+ out. The permanently open potassium (K^+) leak channels, found in the membrane of all animal cells, make the membrane predominantly permeable to potassium ions. Thus the membrane potential approaches the Nernst equilibrium potential for K^+ , known as the resting potential. This potential typically has range of -20 mV to -200 mV, depending on cell type.

Other commonly studied gated channels include the calcium activated potassium channel and voltage dependent anion selective channels (VDAC).

2.5.1b) Antibiotics

Some of the most studied channel proteins, such as gramicidin and alamethicin, are antibiotics. These were among the first proteins whose structure could be determined completely, since they are small and chemically simple compared to other channel forming proteins. They

form cylinders (alpha helixes) whose exterior surfaces are lipophilic, hence they "dissolve" easily in the membranes of microorganism (Gomperts, B.D., 1977 and Wallace, 1990). By dramatically increasing cation permeability, they disturb the concentration gradients of cations between the cell's interior and exterior, leading to cell death. Unlike gramicidin, the conductance of alamethicin is strongly voltage dependent. Gramicidin molecules adopt two distinct families of conformations designated "channel" and "pore" structures, depending on whether they are added to lipid bilayers or organic solutions respectively. The channel consists of a dimer: its two monomers are right handed helixes, linked by hydrogen bonds between the two N-terminal amino acid groups. When the channel closes these bonds break and the monomers are displaced laterally as shown in Figure 2.2. The pore is a double helix. (Note: the "pore" referred to here is not related to pores through lipid bilayers). The gramicidin channel is selective for monovalent cations (alkali metals) and its conductance also depends on the nature of lipid used (e.g. Wallace, 1990).

2.5.1c) Porins

Some bacteria such as *Escherichia coli* have an outer membrane with a high density of different types of channel forming proteins called porins. These channels serve as a "sieve" to molecules in the bacterium's environment. They control the exchange of nutrients and form a barrier to certain antibiotics as well as harmful chemicals, such as detergents and degradative enzymes (Jap and Walian, 1990 and Hancock and Brown, 1992). The exclusion of molecules for the different types of porins is mainly controlled by their channel radii.

FIGURE 2.2

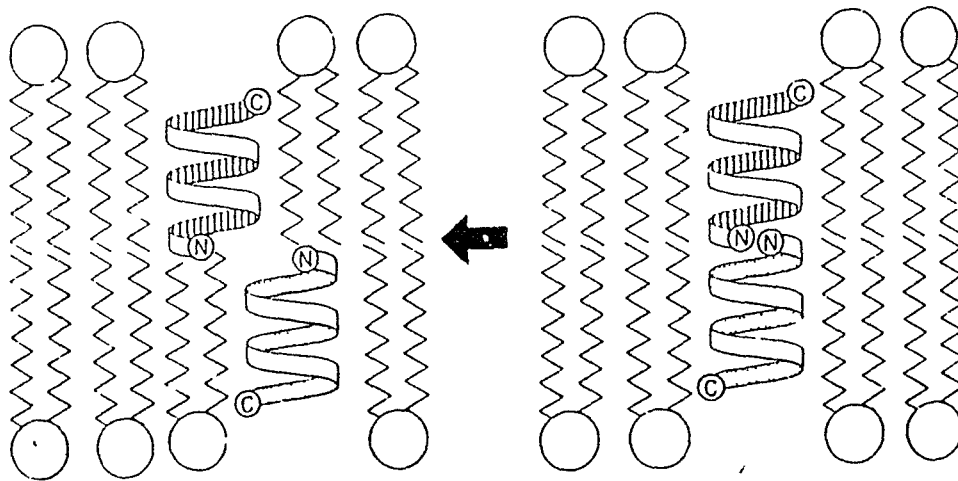


Figure 2.2. A schematic illustration of the proposed opening and closing of the gramicidin channel. When the channel is open, the two monomers are held together by hydrogen bonds. When these bonds break the two subunits are displaced laterally and the channel closes (from Wallace, 1990).

2.5.2 Carriers

Carrier proteins transport molecules across the bilayer by binding the molecule to the protein and translocating it across the membrane by means of conformational changes of the carrier protein. This form of transport can be active, as in pumps, i.e. it requires energy, usually provided by ATP hydrolysis, or it can be passive, i.e. by diffusion down the electrochemical gradient. Carrier facilitated conduction is usually around 100 times slower than that through channels (Alberts *et al.*, 1989). Active transport may also be driven by concentration gradients of other ion species, as for example in kidney epithelial cells (Scott, 1987 and Duchatelle *et al.*, 1992).

For most animal cells the concentration of Na^+ ions varies from being 10 to 20 times higher in the interstitial fluid than in the interior. In contrast, the reverse is true for K^+ ions. Na^+ - K^+ pumps found in these cells maintain this concentration difference. They also play an important role in osmotic regulation and are exploited to facilitate the transport of sugars (e.g. Scott, 1987). Another important pump is the Ca^{2+} -ATPases found in muscle tissues. The Na^+ - H^+ exchange pump is present in all vertebrate cells and plays a major role in regulating the pH of the cell. In addition, bacteriorhodopsin is an example of a light activated proton pump.

2.6 THE ELECTRICAL MODELLING OF ION CHANNELS

When modelling ion channels, the following three factors need to be considered:

- 1) Selection of a suitable model for ion flow through a channel, usually using electrodiffusion equations or reaction kinetics.
- 2) The external electrolyte solution (see sections 2.2 and 2.4)
- 3) Image forces due to the dielectric discontinuity.

The following models describe the steady state conductance of the channel. They are essentially one dimensional, except for simulations. The approach in this thesis differs from these example, since the small signal a.c. response of channels is investigated. Furthermore, the numerical solutions of electrodiffusion through ion channels presented in this thesis are two dimensional.

2.6.1 Steady State Electrodiffusion Theories

The steady state electrodiffusion theories are continuum theories (like the approach used in this thesis), i.e. they do not include any effects of discreteness of charge and they imply the equivalence of space and time averaging. Ion flux is calculated using the Nernst-Planck equation for each ion species, which give the current density as a function of the concentration gradient and the electric field. Solutions to these require a knowledge of the electrostatic potential $\Psi(x)$ and the cross-sectional area available to the ion as a function of position (x). $\Psi(x)$ is usually resolved into two terms : the external applied potential and the electrochemical potential of the channel itself. The latter includes the Born image charging potential (see section 2.6.3), the potential due to the fixed charge

and the surrounding counter-ions, whose potential is calculated from the Debye-Hückel theory, and the potential difference across the mouth of the channel which is assumed to be a hemispherical region of radius equal to the "capture radius" of the ion (Läuger, 1976). The equilibrium ion concentrations are calculated using Boltzmann statistics. The equilibrium solutions for the case of a charged channel have also been used as a starting point to obtain numerical solutions for the nonequilibrium case. These are very difficult to obtain for large values of the applied potential and potential energy wells of the fixed charge because of problems of convergence (Levitt, 1985). From the total potential across the channel and channel mouths and the ion currents, the steady state conductance can be determined.

In the above theory, it is assumed that the dielectric constant of the aqueous channel is equal to that of water and the ratio of the dielectric constant of water to lipid is infinite. The latter approximation implies that the field lines are completely constrained to the channel, and hence the component of the electric field perpendicular to the channel walls can be neglected. This simplifies considerably the calculation of the Born energy potential, which is very difficult to calculate exactly (see section 2.6.3). At the channel mouth the electric field lines are assumed to have spherical symmetry, i.e. the equipotential surfaces are hemispheres (Levitt, 1985). Since the dielectric constant of water is much higher than for the interior of lipid bilayers (of the order of 20-40 times greater), this is a suitable approximation for most aqueous channels, although it may fail in high field regions where dielectric saturation becomes important.

One of the major limitation of the continuum theory is its inability to include strong ion-ion interactions. These effects have been accounted for, to some extent, by the model of single ion channels, i.e. a channel that can be occupied by one ion at most at any one time. This is achieved

by including the probability of channel occupancy in the boundary condition for ion concentration at both ends of the channel. This leads to zero concentration of ions entering the channel, if the channel is already occupied (Levitt, 1986). The theory for single ion channels has been extended to channels occupied by two ions (Levitt, 1987) and multi-ions (Levitt, 1991)

The structural details of both gramicidin and the acetylcholine receptor channel (ACH) are amongst the best known and so electrodiffusion models have been applied to these two channel types in particular. For example, calculations based on the multi-ion channel model for a channel geometry similar to that of the ACH channel are in qualitative and, sometimes quantitative, agreement with measured voltage current characteristics of ACH (Levitt, 1991).

Jakobsson and Chiu (1987) extended Levitt's theory for gramicidin channels to calculate the mean passage time for ions to traverse the channel and the effect of ions that remain in the channel. Calculations based on this theory as well as calculations based on Brownian dynamic simulations gave potential profiles consistent with Na^+ permeation through gramicidin channels.

2.6.2 Reaction Kinetic Schemes

A method of calculating steady state ion flow through channels, is as follows (Läuger, 1987): A potential profile, consisting of a series of maxima and minima, is estimated through the channel. Ion transport is described by "thermally activated jumps" across barriers between local potential minima. Rate constants for these transitions can be calculated from the barrier height and oscillation frequency of an ion in a potential minimum, using classical rate theories (Eyring *et al.*, 1949 and Zwolinski

et al., 1949, as cited by Läuger, 1987). The current can be obtained from these rate constants and the potential difference ($\Delta\psi$) across the channel. At equilibrium, the ion concentrations on both sides are equal and the channel conductance can be calculated from the current for the limit $\Delta\psi \rightarrow 0$.

2.6.2.1 Conductances for two different types of barriers:

a) Time independent barriers:

If a channel has only one ion binding site (i.e. the channel can only be occupied by a single ion at any time), the conductance $\Lambda(c)$ at equilibrium ion concentration (c) can be shown to equal (Läuger, 1987):

$$\Lambda(c) = \Lambda_{\max} \frac{c}{c+K} \quad (2.6.1)$$

where $\Lambda_{\max}$ is the conductance for $c \rightarrow \infty$

$$K = \frac{\mu'}{\rho'} = \frac{\mu''}{\rho''} \quad \text{and}$$

μ' , μ'' are the rate constants for an ion jumping from the binding site to the left and right solution and ρ' , ρ'' are the ratios of rate constants on concentration for an ion entering the binding site from the solution on either side.

When the channel contains more than one binding site, ion transport is complicated by the interaction between ions occupying the channel at the same time. For a two site channel the equilibrium conductance can be written as: (Urban and Hladky, 1979, as cited by Läuger, 1987)

$$\Lambda(c) = \frac{A_1c + A_2c_2 + A_3c_3}{B_0 + B_1c + B_2c_2 + B_3c_3 + B_4c_4} \quad (2.6.2)$$

where the A_i and B_i are combinations of concentration independent rate constants.

b) Channels with conformational changes:

Conformational transitions of the protein may occur. This means that the potential profile of the channel now varies with time. These strongly affect ion fluxes and channel selectivity (Läuger, 1985). If conformational transitions are fast compared to the "dwell time" of the ion, the above relations may still be used, provided the potential profile is replaced by an average profile (the so-called "potential of mean force").

When the frequency of conformational changes is less than or equal to the jumping frequency of the ion, the conductance will explicitly depend on the rate constants of the conformational transitions, as well as those for ion binding. For a channel with two different structural states, the equilibrium conductance is as follows:

$$\Lambda(c) = \frac{z_2 F_2}{RT} \frac{Ac + Bc_2}{C + Dc + Ec_2} \quad (2.6.3)$$

where A , B , C , D and E are again concentration-independent combinations of rate constants, z is the valency, F is Faraday constant, R is the ideal gas constant and T is the absolute temperature.

For this case, coupling between the ion conformational changes and ion passage may occur. Computer simulations of the molecular dynamics of the trajectory of an ion through a protein channel with conformational transitions confirm this (Läuger, 1985).

The total potential profile of the channel arises from various other sources. These include: i) the transmembrane potential, ii) the image potential due to the polarization charge induced by the ion or ions traversing the channel, iii) the applied potential, iv) the charge distribution and conformation changes of the channel protein and v) the effects on ion access to the diffuse electrolyte region near the channel mouth due to long range electrostatic forces (Jordan, 1986).

Ion transport may include various processes such as dehydration of the ion at the channel mouth, translocation through the channel and hydration at the other channel mouth (Eisenman and Dani, 1987). These can be characterised by different rate constants. Expressions for some of these rate constants have been derived using stochastic theories (Cooper *et al.*, 1988). The dimensions of the constriction at each channel mouth (i.e. its effective radius) can be varied independently. Access to the channel mouth is limited by diffusion. The effect of the bilayer is modelled by a surface layer of point dipoles at the membrane/electrolyte solution interface. Jordan (1986) discusses the effect of varying the various geometrical and electrical factors on channel transport.

At low electrolyte concentrations (c), the total channel conductance is limited by diffusion to the channel mouth. Hence the capture radius, i.e. the effective radius of the channel mouth minus the ionic radius (Läuger, 1976), can be estimated from the limit of conductance as c approaches zero.

Reaction kinetic schemes have also been found to be suitable for describing a wide variety of membrane transport systems (e.g. Sanders *et al.*, 1984 and Hansen *et al.*, 1981). The rate equations for multi-occupancy ion channel systems can be conveniently summarized by a state diagram represented by a two dimensional array of all states, connected by all possible transitions. These state diagrams can be transformed into

equivalent circuits, which simplify the calculation of ion fluxes in some cases. (Sandblom and Eisenman, 1982).

2.6.3 Image Forces due to Dielectric Discontinuity

An ion in an infinite dielectric medium with dielectric constant $K = \epsilon/\epsilon_0$ (ϵ is the permittivity of the medium and ϵ_0 is the permittivity of free space) possesses a self energy due to its electric field, which is inversely proportional to the dielectric constant. For the i^{th} ion species this is given by:

$$U_E = \frac{z_i^2 q^2}{8 \pi \epsilon a_i} \quad (2.6.4)$$

where a_i is the radius of the i^{th} ion species. The dielectric constant of lipids is much lower than that of water. Hence the energy required to translocate an ion through a lipid from an aqueous phase is immense, due to the difference in U_E . For typical values of ion radii and dielectric constants of lipids, U_E is much larger than kT , hence ions cannot partition into the lipid and thus ion transport must occur primarily through aqueous channels. For example, $U_E \approx 2.3$ eV for a lipid dielectric constant of 3 and ion radius of .1nm, while $kT \approx .025$ eV. The vicinity of the low dielectric medium to an ion in a channel however alters the self energy of the ion and this affects its transport significantly. It therefore should be included in ion channel models.

The electric field surrounding an ion has the effect of inducing charge at an interface between two media with different dielectric constants. These charges alter the original field of the ion. The true potential can be calculated by summing up the contribution of a set of fictitious image charges, which leads to an infinite series of potential

terms. These charges are situated at positions which are reflections of the original charge through the dielectric interface and would produce the same field as the induced charge. For semi-infinite and finite dielectric media these calculations are in general very complicated. Neumke and Lauser (1969) have calculated the image potential energy of an ion in a thin slab of low dielectric constant (K) surrounded by a semi infinite homogeneous region of high dielectric constant on both sides (applicable to bilayers). Parsegian (1975) has added a cylinder with a different dielectric constant (K_p , $K_p \neq K$) which extends through the slab of low dielectric material as described above. The potential energy due to the image charges as well as the self energy for an ion situated at the centre of the cylinder. This geometry simulates a pore through a bilayer.

2.7 PATCH CLAMPING

One common technique of investigating the behaviour of active channels (e.g. voltage or transmitter gated channels) is the patch clamp technique, which was first developed by E. Neher and B. Sakmann (1976). The success of the patch clamping technique lies in its ability to form a very high resistance seal (of the order of $100\text{ G}\Omega$) between the tip of a very small pipette and an area of the membrane by applying suction. This enables the recording of the current through a single ion channel in biological membranes. Earlier techniques employing microelectrodes failed to achieve this, since the background noise, although small, completely masked the single channel signal. As the resistance of the solution in the pipette is much lower than that of the membrane, the cell can be voltage clamped by applying a voltage to the pipette.

Patch pipettes are mechanically stable, so they can be relatively easily excised from the cell. Another technique, used for whole cell

recordings, is to remove a microscopic patch of membrane inside the pipette without breaking the membrane/pipette seal. The pipette then replaces the conventional intracellular microelectrode. This technique allows detailed electrophysiological study of small mammalian cells. Earlier techniques (e.g. voltage clamping using intracellular microelectrode impalement) were restricted to large cells (e.g. Hodgkin, 1976). Whole cell patch clamping can also be used to modify the composition of the cytoplasm. (e.g. Neher and Sakmann, 1992)

Patch clamping thus allows the conductance of an individual ion channel to be measured as a function of voltage across the membrane and the internal and external ion concentrations, giving information about the electrical behaviour channels. On the other hand, much knowledge of the structure of channel proteins has been gained from progress in deciphering the amino acid sequences of some channel forming proteins.

By combining recent genetic techniques with patch clamping, it has been possible in some cases to make structural changes to critical amino acid sequences. A study of the resultant change in electrical behaviour of such mutagenic channel proteins can provide useful information about the three dimensional structure of these channels (e.g. Anderson and Koeppe, 1992)

Some possible limitations of the patch clamp technique include:

- 1) Errors due to inadequate correction for liquid junction potentials between the pipette and the bathing solution.
- 2) Leakage through the seal. Small cells may have a resistance not significantly less than the seal resistance, hence current leakage through the seal can be considerable and has to be accounted for (especially for the case of whole cell measurements) in order to obtain representative single channel conductances.
- 3) Distortion. Current recordings of intact patches of small cells

may be distorted considerably, due to the change in the cell's potential resulting from the passage of current through the channel. A method of correcting for this effect has been developed by Barry and Lynch (1991).

Patch clamp resolution is ultimately limited by the thermal noise of the patch/seal combination. At high frequencies it also depends on the membrane patch, the seal or the glass. Also, current amplifiers have noise that increases with bandwidth (Auerbach and Sachs, 1984).

In recent years it has been possible to isolate functionally active channel forming proteins from various biological cells and to incorporate them into artificial lipid bilayers so that their function may be analysed in a simple, controlled environment, in order to gain some insight into the possible molecular mechanisms of channel transport. This technique has the advantage of allowing transmembrane potential, electrolyte concentration and composition on both sides to be easily varied over a wide range. Furthermore, the composition of the lipid bilayer can be varied to study the effect of lipid environment on channel function. In addition, the isolated channel proteins can be modified chemically or genetically before being incorporation into bilayers. This allows one to investigate the importance of the various subunits on channel function. Some of the more common channels incorporated into artificial bilayers include: the acetylcholine receptor, Na^+ , K^+ and Ca^{2+} channels, voltage gated anion-selective channels (see for example Montal *et al.*, 1986, Anderson *et al.*, 1986 and Latorre, 1986). Studying single channels in artificial bilayers using the patch clamp technique rather than conventional voltage clamping has the advantage of improving the signal to noise ratio by allowing smaller membrane patches and thus lower membrane capacitances (Auerbach and Sachs, 1984).

As the patch clamp technique is becoming more sensitive and refined, much more detailed channel behaviour can be detected. Hence, more complex kinetic models are required to explain the channel activity. Even though these models fit the experimental results more closely, they may become too complex and involve too many free parameters to aid our understanding of channel function (Auerbach and Sachs, 1984).

Patch clamping has become a powerful tool for investigating the electrical properties of individual ion channels in a variety of environments. However, it provides little information about channel structure and any such information has been mainly obtained by studying the effect of specific changes of critical amino acid sequences on channel function. Techniques using small signal a.c. response have the advantage of providing a whole range of data for any one experimental condition and may therefore prove to be useful in yielding structural information about channel proteins.

CHAPTER 3

SOLUTIONS TO THE NERNST-PLANCK POISSON EQUATIONS OF AN ELECTROLYTE FOR PLANAR GEOMETRY

3.1 INTRODUCTION

Before tackling complicated structures such as ion channels in membranes, one needs to understand how currents flow through electrolyte solutions and the way that they are measured. These questions are of a wider interest than to just bioelectrochemistry. Consider first, however, two situations in which the external electrolyte may influence the measurement of ion transport across cell membranes.

As mentioned, the thicknesses of cell membranes are of the order of nm. However, measuring electrodes employed by physiologists can usually only be positioned to within μm of the cell membrane. It is unavoidable that these measurements will always include some electrolyte solution in series with the membrane. Hence, when studying the electrical properties of membranes and ion channels, the properties of the surrounding medium must also be taken into account. Since these properties are frequency dependent, they can be investigated by measuring the response of the medium to an a.c. signal. By measuring the potential difference between two points in the medium for a given applied current, the impedance can be calculated. This may be useful in characterising the medium. For simple electrolyte systems the impedance

can be calculated analytically. Such systems are discussed in this and the following chapter.

Similar problems occur in electrochemistry generally and in ion exchange and ultrafiltration membranes. Although this thesis is inspired by and takes examples from electrophysiology, it has potentially much broader relevance.

Exact solutions to the Nernst-Planck electrodiffusion equations are possible for one dimensional systems. The simplest such system is that of a fully dissociated binary electrolyte solution. This is rarely of practical importance, except as an approximation. Since water is itself a weak electrolyte (i.e. $\text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^-$), any aqueous salt solution will contain more than two ion species. Hence in this chapter exact one dimensional solutions for the case of three distinct ion species are given. The three ion theory is applicable to strong bases and acids as well as salts, i.e. those for which recombination of the ion species in solution is negligible. Strong bases contain two cation and one anion species (e.g. sodium hydroxide solutions contains Na^+ , H^+ and OH^- ions), while acids contain two anion and one cation species. Because of the difference in the sizes of the ions, the ion species may have very different diffusion constants. The following analysis is for two cation and one anion species, but can be easily modified, by a simple change in notation, for the case of two anions and one cation.

The solutions to the electrodiffusion equations are complicated functions of position and frequency. However they can be simplified for the case of a binary electrolyte (which is a special case of the three ion theory), so the later part of this chapter will present a description of the behaviour of such a system.

One technique of measuring ion fluxes is to insert a microelectrode into the cell and measure the membrane current directly from the current between the internal electrode and a reference electrode in the external medium (e.g. voltage clamp and patch clamp techniques). However this is not always possible without significantly damaging the cell. Consequently, researchers in physiology have developed methods of inferring the properties of cell membranes from measurements at electrodes in the medium surrounding the cell, sometimes far away (of the order of μm) from the cell surface.

One such technique is the vibrating probe, (Jaffe and Nuccitelli, 1974) which measures the a.c. potential difference over the range of alternating displacement of a vibrating metal electrode in the external electrolyte solution. The ion current can then be calculated using the local conductivity of the solution.

Another method employs ion selective microelectrodes to measure the ion activities at different points in the external solution and uses these to estimate the local chemical potential and hence the ion current (Newman *et. al.*, 1987). The local ion currents obtained by these techniques are assumed to equal the current through the membrane, once the necessary geometrical corrections have been made, i.e. in all these techniques it is assumed that the ionic currents are approximately independent of position. This assumption is valid only for one dimensional systems which have reached steady state. However solutions to the Nernst-Planck electrodiffusion equations for the small signal a.c. response of an electrolyte solution presented in this chapter indicate that for some one dimensional cases, the potential difference and ionic currents can vary significantly with position. For 2 and 3 dimensional systems the variations with position may be even more noticeable.

3.2 THEORY

In this section the linearised Nernst-Planck equations and Poisson's equation are used to calculate the ion concentrations, ion current densities and impedance for the following system: a fully dissociated electrolyte solution situated between two infinite plane electrodes. A uniform a.c. current of small amplitude is passed between two current electrodes (Figure 3.1).

The Nernst-Planck equations are obtained from the derivative of the chemical potential in the following manner. The electrochemical potential (μ_i) of an ion of the i th species in a dielectric medium at constant pressure is given by:

$$\tilde{\mu}_i = \mu_i^0 + k T \ln(\gamma_i C_i) + z_i q \Psi \quad (3.2.1)$$

where : z_i is the valency

C_i is the concentration

q is the electron charge

k is Boltzmann's constant

T is the temperature

Ψ is the (electrical) potential

μ_i^0 is the standard chemical potential

γ_i is the activity coefficient

$\gamma_i C_i$ is the chemical activity of the i th ion species

$\gamma_i C_i$ gives the activity - an effective concentration of ions. For all calculations in this thesis, it is assumed the activity coefficient (γ_i) is one, i.e. the electrolyte solution is ideal. This is a valid approximation for

FIGURE 3.1

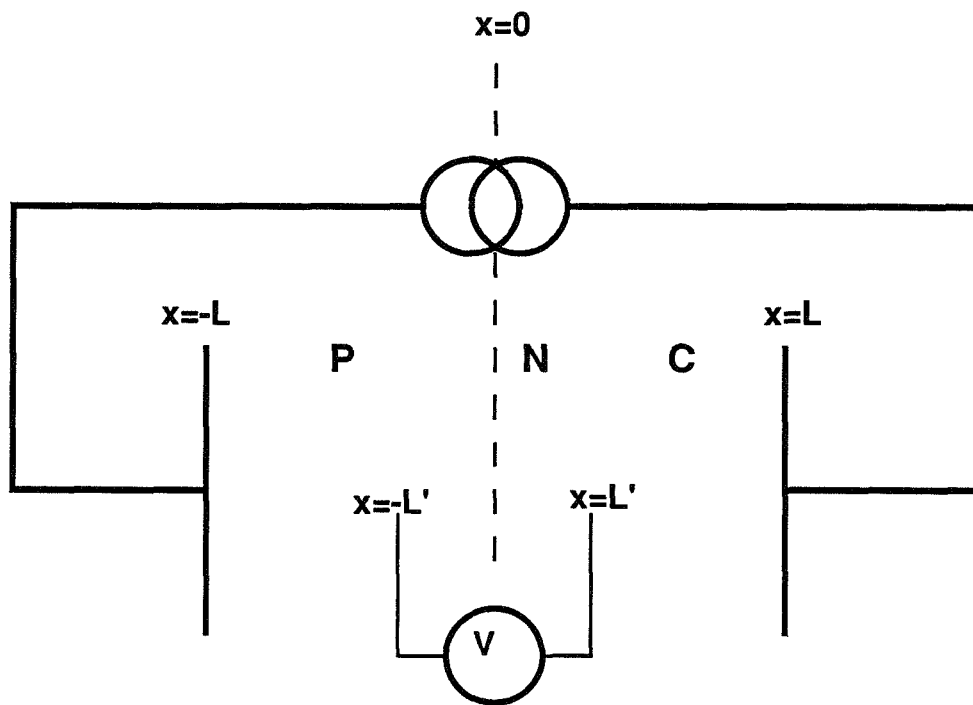


Figure 3.1. The geometry of the system used for chapter 3.

dilute strong electrolytes. The distribution of ions in solution is influenced by the presence of counter ions (see Chapter 2). Debye and Hückel's analysis of this effect gives values for γ_i . The current density ($\mathbf{J}_i$) is related to the negative derivative of its chemical potential (μ_i) as:

$$\begin{aligned}\mathbf{J}_i &= -\frac{q D_i z_i C_i}{k T} \nabla \mu_i \\ &= \frac{q D_i z_i C_i}{k T} (-kT/C_i \nabla C_i - z_i q \nabla \Psi - \nabla \mu_i^0)\end{aligned}\quad (3.2.2)$$

where D_i is the diffusion constant of the i th ion species.

Note that bold characters have been used to indicate vectors.

It is assumed that the electrolyte solution is homogeneous (except for ion concentration variations) and consequently, $\nabla \mu_i^0$ is zero throughout this system. In Chapter 6, variations in other energy terms are considered. For this case the Nernst-Planck equations reduce to :

$$\mathbf{J}_p = -q z_p D_p \nabla P + \frac{q^2 z_p^2 D_p P \mathbf{E}}{k T} \quad (3.2.3)$$

$$\mathbf{J}_n = q z_n D_n \nabla N + \frac{q^2 z_n^2 D_n N \mathbf{E}}{k T} \quad (3.2.4)$$

$$= -q z_c D_c \nabla C + \frac{q^2 z_c^2 D_c C \mathbf{E}}{k T} \quad (3.2.5)$$

where : $\mathbf{J}_p$ is the current density of cations with valency z_p , diffusion constant D_p and concentration P .

$\mathbf{J}_n$ is the current density of anions with valency z_n , diffusion constant D_n and concentration N .

J_c is the current density of the second cation species with valency z_c , diffusion constant D_c and concentration C .

E is the electric field (note bold characters are used to denote vectors).

For a very small a.c. signal of angular frequency ω , the ion concentrations and electric field can be written as (lower case variables denote a.c. variations) :

$$P = P_0 + p e^{j\omega t} \quad (3.2.6)$$

$$N = N_0 + n e^{j\omega t} \quad (3.2.7)$$

$$C = C_0 + c e^{j\omega t} \quad (3.2.8)$$

$$E = E_0 + e e^{j\omega t} \quad (3.2.9)$$

where : $j = \sqrt{-1}$ and P_0 , C_0 and N_0 are the bulk ion concentrations of the first and second cation species and anion species, respectively (i.e. the concentrations for zero a.c. signal). $z_p P_0 + z_c C_0 = z_n N_0$, since the system is electrically neutral.

Substituting equations 3.2.6 to 3.2.9 into equations 3.2.3 to 3.2.5 and retaining only the a.c. terms, expressions for the ionic components of the a.c. current densities now may be expressed as (after dividing by $e^{j\omega t}$):

$$J_p = -q z_p D_p \nabla p + \frac{q^2 z_p^2 D_p}{k T} (e P_0 + p E_0) \quad (3.2.10)$$

$$J_n = q z_n D_n \nabla n + \frac{q^2 z_n^2 D_n}{k T} (e N_0 + n E_0) \quad (3.2.11)$$

$$J_c = -q z_c D_c \nabla c + \frac{q^2 z_c^2 D_c}{k T} (e C_0 + c E_0) \quad (3.2.12)$$

where products of a.c. terms have been discarded, since (for small signals they are negligibly small.

For a one dimensional system with zero d.c. electric field (i.e. $E_0 = 0$) the expressions for the current densities reduce to:

$$J_p = -q z_p D_p \frac{dp}{dx} + \frac{q^2 z_p^2 D_p P_0 e}{k T} \quad (3.2.13)$$

$$J_n = q z_n D_n \frac{dn}{dx} + \frac{q^2 z_n^2 D_n N_0 e}{k T} \quad (3.2.14)$$

$$J_c = -q z_c D_c \frac{dc}{dx} + \frac{q^2 z_c^2 D_c C_0 e}{k T} \quad (3.2.15)$$

Poisson's equation relates e to the a.c. ion concentrations:

$$\frac{de}{dx} = \frac{q}{\epsilon} (z_p p + z_c c - z_n n) \quad (3.2.16)$$

where ϵ denotes the permittivity of the electrolyte solution

The continuity equations give:

$$\frac{dJ_p}{dx} + q z_p \frac{dP}{dt} = 0 \rightarrow q z_p j \omega p + \frac{dJ_p}{dx} = 0 \quad (3.2.17)$$

$$\frac{dJ_n}{dx} - q z_n \frac{dN}{dt} = 0 \rightarrow -q z_n j \omega n + \frac{dJ_n}{dx} = 0 \quad (3.2.18)$$

$$\frac{dJ_c}{dx} + q z_c \frac{dC}{dt} = 0 \rightarrow q z_c j \omega c + \frac{dJ_c}{dx} = 0 \quad (3.2.19)$$

Combining equations (3.2.13) to 3.2.19) produces the following second order differential equations:

$$\frac{d^2p}{dx^2} = \frac{j \omega}{D_p} p + \frac{1}{z_p \lambda_p^2} (z_p p + z_c c - z_n n) \quad (3.2.20)$$

$$\frac{d^2n}{dx^2} = \frac{j \omega}{D_n} n - \frac{1}{z_n \lambda_n^2} (z_p p + z_c c - z_n n) \quad (3.2.21)$$

$$\frac{d^2c}{dx^2} = \frac{j \omega}{D_c} c + \frac{1}{z_c \lambda_c^2} (z_p p + z_c c - z_n n) \quad (3.2.22)$$

where λ_p , λ_n and λ_c are the "effective" Debye lengths associated with the different ion species:

$$\lambda_p = \sqrt{\frac{\epsilon k T}{q^2 z_p^2 P}}$$

$$\lambda_n = \sqrt{\frac{\epsilon k T}{q^2 z_n^2 N}}$$

$$\lambda_c = \sqrt{\frac{\epsilon k T}{q^2 z_c^2 C}}$$

The differential equation 3.2.20 to 3.2.22 can be written as:

$$\frac{d^2p}{dx^2} = A_{11} p + A_{12} n + A_{13} c \quad (3.2.23)$$

$$\frac{d^2n}{dx^2} = A_{21} p + A_{22} n + A_{23} c \quad (3.2.24)$$

$$\frac{d^2c}{dx^2} = A_{31} p + A_{32} n + A_{33} c \quad (3.2.25)$$

where :

$$\begin{aligned}
A_{11} &= \frac{2j}{\xi_p^2} + \frac{1}{\lambda_p^2} & A_{12} &= -\frac{z_n}{z_p \lambda_p^2} & A_{13} &= \frac{z_c}{z_p \lambda_p^2} \\
A_{21} &= -\frac{z_p}{z_n \lambda_n^2} & A_{22} &= \frac{2j}{\xi_n^2} + \frac{1}{\lambda_n^2} & A_{23} &= -\frac{z_c}{z_n \lambda_n^2} \\
A_{31} &= \frac{z_p}{z_c \lambda_c^2} & A_{32} &= -\frac{z_n}{z_c \lambda_c^2} & A_{33} &= \frac{2j}{\xi_c^2} + \frac{1}{\lambda_c^2} \\
\xi_p &= \sqrt{\frac{2D_p}{\omega}} & \xi_n &= \sqrt{\frac{2D_n}{\omega}} & \xi_c &= \sqrt{\frac{2D_c}{\omega}}
\end{aligned}$$

The ξ_i are characteristic rms distances travelled by the ions via diffusion per radian.

The characteristic polynomial for this system is :

$$\mu^3 + a_1 \mu^2 + a_2 \mu + a_3 = 0 \quad (3.2.26)$$

where : $a_1 = -(A_{11} + A_{22} + A_{33})$

$$a_2 = A_{11} A_{22} + A_{11} A_{33} + A_{22} A_{33} - A_{12} A_{21} - A_{13} A_{31} - A_{23} A_{32}$$

$$a_3 = -A_{11} A_{22} A_{33} + A_{11} A_{23} A_{32} - A_{12} A_{23} A_{31} + A_{12} A_{21} A_{33} - A_{13} A_{21} A_{32} + A_{13} A_{22} A_{31}$$

To solve the cubic the following definitions are made:

$$a_4 = \frac{1}{9} (3 a_2 - a_1^2)$$

$$a_5 = \frac{1}{54} (9 a_1 a_2 - 27 a_3 - 2 a_1^3)$$

$$a_6 = \sqrt[3]{a_5 + \sqrt{a_5^2 + a_4^3}}$$

$$a_7 = \sqrt[3]{a_5 - \sqrt{a_5^2 + a_4^3}}$$

The roots of the cubic (i.e. characteristic lengths) are given by:

$$\lambda_1 = \frac{1}{\sqrt{-a_1/3 + a_6 + a_7}} \quad (3.2.27)$$

$$\lambda_2 = \frac{1}{\sqrt{-a_1/3 - (a_6 + a_7)/2 + j\sqrt{3} (a_6 - a_7)/2}} \quad (3.2.28)$$

$$\lambda_3 = \frac{1}{\sqrt{-a_1/3 - (a_6 + a_7)/2 - j\sqrt{3} (a_6 - a_7)/2}} \quad (3.2.29)$$

From the symmetry of the system, $p(x) = -p(-x)$, $n(x) = -n(-x)$ and $c(x) = -c(-x)$, consequently :

$$p(x) = \sum_{i=1}^3 \alpha_i \sinh(x/\lambda_i) \quad (3.2.30)$$

$$n(x) = \sum_{i=1}^3 \alpha_i S_i \sinh(x/\lambda_i) \quad (3.2.31)$$

$$c(x) = \sum_{i=1}^3 \alpha_i T_i \sinh(x/\lambda_i) \quad (3.2.32)$$

The S_i and T_i are calculated by substituting the expressions for $p(x)$, $n(x)$ and $c(x)$ into the differential equations for $\frac{d^2 p}{dx^2}$ and $\frac{d^2 n}{dx^2}$ i.e. :

The α_i are constants to be determined later

$$\frac{1}{\lambda_i^2} = A_{11} + S_i A_{12} + T_i A_{13} \quad (3.2.33)$$

$$\frac{S_i}{\lambda_i^2} = A_{21} + S_i A_{22} + T_i A_{23} \quad (3.2.34)$$

$$S_i = \frac{A_{13} A_{21} + \frac{A_{23}}{\lambda_i^2} - A_{23} A_{11}}{A_{12} A_{23} - A_{22} A_{13} + \frac{A_{13}}{\lambda_i^2}} \quad (3.2.35)$$

$$T_i = \frac{A_{11} A_{22} - A_{12} A_{21} - \frac{A_{11} + A_{22}}{\lambda_i^2} + \frac{1}{\lambda_i^4}}{A_{12} A_{23} - A_{22} A_{13} + \frac{A_{13}}{\lambda_i^2}} \quad (3.2.36)$$

The electric field is obtained from Poisson's equation:

$$e(x) = \int \frac{q}{\epsilon} (z_p p + z_c c - z_n n) dx \quad (3.2.37)$$

$$= \frac{q}{\epsilon} \left[\sum_{i=1}^3 \alpha_i \lambda_i (z_p + z_c T_i - z_n S_i) \cosh(x/\lambda_i) \right] + e_I \quad (3.2.38)$$

where e_I is an integration constant.

The total a.c. current (J) at x is given by:

$$J = J_p(x) + J_n(x) + J_c(x) + J_D(x) \quad (3.2.39)$$

where $J_D(x)$ is the displacement current defined by:

$$J_D(x) = j \omega \epsilon e(x). \quad (3.2.40)$$

J is of course independent of position (from the continuity equations $dJ/dx = 0$ for all x), and equal to the applied current density.

Terms applicable to the case where e is independent of position are now introduced. These have been indicated by subscript K . Equation 3.2.38 shows that this can only occur if $\alpha_1 = \alpha_2 = \alpha_3 = 0$ and the electric field is given by $e_K = e_I$.

The area specific admittance per unit length (y_K) for constant electric field and an applied current density J is given by :

$$y_K = J/e_K = g_K + j\omega\epsilon\epsilon_0 \quad (3.2.41)$$

where g_K is the total conductivity of the electrolyte and is given by:

(using equations 3.2.13 -3.2.15 and 30.32)

$$g_K = g_{pK} + g_{nK} + g_{cK} \quad (3.2.42)$$

$$g_{pK} = q^2 z_p^2 D_p P / kT \quad (3.2.43)$$

$$g_{nK} = q^2 z_n^2 D_n N / kT \quad (3.2.44)$$

$$g_{cK} = q^2 z_n^2 D_c C / kT \quad (3.2.45)$$

Therefore $e_I = e_K = J/y_K$. The ion current densities then reduce to :

$$J_p(x) = q z_p D_p \sum_{i=1}^3 \{ -\alpha_i / \lambda_i + \alpha_i \lambda_i / (z_p \lambda_p^2) (z_p + z_c T_i - z_n S_i) \} \cosh(x/\lambda_i) \\ + \frac{q^2 z_p^2 D_p P_0 J}{k T y_K} \quad (3.2.46)$$

(using equations 3.2.13 -3.2.15, 30.32 and 30.38)

$$J_n(x) = q z_n D_n \sum_{i=1}^3 \{ \alpha_i / \lambda_i + \alpha_i \lambda_i / (z_n \lambda_n^2) (z_p + z_c T_i - z_n S_i) \} \cosh(x / \lambda_i) \\ + \frac{q^2 z_n^2 D_n N_0 J}{k T y_K} \quad (3.2.47)$$

$$J_c(x) = q z_c D_c \sum_{i=1}^3 \{ -\alpha_i / \lambda_i + \alpha_i \lambda_i / (z_c \lambda_c^2) (z_p + z_c T_i - z_n S_i) \} \cosh(x / \lambda_i) \\ + \frac{q^2 z_c^2 D_c C_0 J}{k T y_K} \quad (3.2.48)$$

3.3 BOUNDARY CONDITIONS

Three linearly independent boundary conditions are needed to evaluate the constants α_1 , α_2 and α_3 . The following boundary conditions for perfectly blocking current electrodes, located at $x = \pm L$, have been used. Note that because of the symmetry of the system, the ion concentrations are antisymmetric functions and the electric field and J_p and J_n are symmetric functions, hence boundary conditions at only one of the electrodes are required. These are expressed as:

$$J_p(L) = 0 \quad J_n(L) = 0 \quad J_c(L) = 0 \quad (3.3.1)$$

Rearranging these gives the following:

$$\frac{J_n(L)}{q z_n^2 D_n N_0} - \frac{J_p(L)}{q z_p^2 D_p P_0} = 0 \quad (3.3.2)$$

$$\frac{J_n(L)}{q z_n^2 D_n N_0} - \frac{J_c(L)}{q z_c^2 D_c C_0} = 0 \quad (3.3.3)$$

$$\frac{J_p(L)}{q z_p D_p} = 0 \quad (3.3.4)$$

Equations 3.3.2. to 3.3.4 then reduce to :

$$\frac{1}{z_p P_0} \frac{dp}{dx}|_L + \frac{1}{z_n N_0} \frac{dn}{dx}|_L = 0 \quad (3.3.5)$$

$$\frac{1}{z_p P_0} \frac{dp}{dx}|_L - \frac{1}{z_c C_0} \frac{dc}{dx}|_L = 0 \quad (3.3.6)$$

$$\frac{J_p(L)}{q z_p D_p} - \frac{dp}{dx} + \frac{q z_p P_0 e}{k T} = 0 \quad (3.3.7)$$

Substituting equations 3.2.30 to 3.2.32 and 3.2.38 and 3.2.46, into 3.3.5 to 3.3.7 gives:

$$B \begin{bmatrix} \frac{\alpha_1}{\lambda_1} \cosh\left(\frac{L}{\lambda_1}\right) \\ \frac{\alpha_2}{\lambda_2} \cosh\left(\frac{L}{\lambda_2}\right) \\ \frac{\alpha_3}{\lambda_3} \cosh\left(\frac{L}{\lambda_3}\right) \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ \frac{q z_p P_0 J}{k T y_K} \end{bmatrix} \quad (3.3.8)$$

where the elements of matrix B are given by :

$$B_{11} = \frac{1}{z_p P_0} + \frac{S_1}{z_n N_0} \quad B_{12} = \frac{1}{z_p P_0} + \frac{S_2}{z_n N_0} \quad B_{13} = \frac{1}{z_p P_0} + \frac{S_3}{z_n N_0}$$

$$B_{21} = \frac{T_1}{z_c C_0} + \frac{S_1}{z_n N_0} \quad B_{22} = \frac{T_2}{z_c C_0} + \frac{S_2}{z_n N_0}$$

$$B_{23} = \frac{T_3}{z_c C_0} + \frac{S_3}{z_n N_0}$$

$$B_{31} = 1 - \frac{\lambda_1^2}{z_p \lambda_p^2} (z_p + z_c T_1 - z_n S_1)$$

$$B_{32} = 1 - \frac{\lambda_2^2}{z_p \lambda_p^2} (z_p + z_c T_2 - z_n S_2)$$

$$B_{33} = 1 - \frac{\lambda_3^2}{z_p \lambda_p^2} (z_p + z_c T_3 - z_n S_3)$$

Solving equation 3.3.8 for α_i gives:

$$\alpha_i = \frac{\lambda_i}{\cosh(\frac{L}{\lambda_i})} \frac{q z_p P_0 J F_i}{k T y_K \det} , \quad i = 1 \text{ to } 3 \quad (3.3.9)$$

$$\text{where : } F_1 = B_{12} B_{23} - B_{22} B_{13} \quad (3.3.10)$$

$$F_2 = B_{21} B_{13} - B_{11} B_{23} \quad (3.3.11)$$

$$F_3 = B_{11} B_{22} - B_{21} B_{12} \quad (3.3.12)$$

$$\text{and det is the determinant of matrix B} \quad (3.3.13)$$

3.4 FOUR TERMINAL IMPEDANCE

The potential $\Psi(x)$ is given by:

$$\Psi(x) = -\int e(x) dx \quad (3.4.1)$$

The impedance Z_t times unit area between the voltage electrodes (located at $x = \pm L'$) is given by:

$$Z_t = \frac{\Psi(-L') - \Psi(L')}{J} \quad (3.4.2)$$

$$= Z_K + \sum_{i=1}^3 Z_i \quad (3.4.3)$$

where :

$$Z_K = \frac{2 L'}{y_K} \quad (3.4.4)$$

$$Z_i = 2 (z_p + z_c T_i - z_n S_i) \frac{\lambda_i^3 F_i}{\cosh(\frac{L}{\lambda_i}) z_p \lambda_p^2 y_K \det} \sinh(\frac{L'}{\lambda_i}) \quad (3.4.5)$$

Thus the impedance can be written as the sum of four distinct terms. Z_K is the small signal impedance which would be measured if there were no a.c. concentration variations. For this case e would be uniform (and equal to e_l) and the potential difference between the electrodes would be linear, therefore the impedance would be given by the reciprocal of the geometrical admittance y_K/L . The Z_i 's are impedance terms associated with the three different characteristic lengths λ_i . Z_1 is associated with the space charge produced when cations and anions move in opposite directions due to the presence of an electric field. For blocking electrodes this space charge is highest in the double layer near the electrodes, hence Z_1 dominates the total impedance of this region at low frequencies. Z_2 and Z_3 arise from the charge separation produced when cations and anions diffuse at different rates and they can contribute significantly to the total impedance under appropriate conditions.

The total capacitance (C_t) and conductance per unit area (G_t) can be calculated from the total impedance times unit area (Z_t). C_t and G_t are given by :

$$C_t = \frac{1}{\omega} \text{Im}\left(\frac{1}{Z_t}\right) \quad \text{and} \quad G_t = \text{Re}\left(\frac{1}{Z_t}\right) \quad (3.4.6)$$

where $\text{Im}(1/Z_t)$ and $\text{Re}(1/Z_t)$ denote the imaginary and real parts, respectively, of the admittance per unit area. Note that all calculated impedances and hence conductances and capacitances in this thesis are area specific, except for those for spherical geometry of Chapter 4.

The exact solutions are rather complicated functions of the parameter space and will not be presented in this thesis (to conserve trees). However, some limiting cases of physical importance for which relatively simple explicit expression can be found have been considered.

3.5 LOW FREQUENCY LIMIT

At low frequencies the diffusion lengths in aqueous solutions are much greater than the Debye lengths associated with each ion species, i.e. ξ_p, ξ_n and $\xi_c \gg \lambda_p, \lambda_n$ and λ_c . It can be shown that, to first order in ω , the characteristic lengths λ_i simplify to :

$$\lambda_1 = \left\{ \frac{1}{\lambda^2} + j \omega \left(\frac{\alpha_c}{D_c} + \frac{\alpha_n}{D_n} + \frac{\alpha_p}{D_p} \right) \right\}^{-1/2} \quad (3.5.1)$$

$$\lambda_2 = \left\{ \frac{j \omega}{2} \left(\frac{1}{D^*} - \frac{1}{D} \right) \right\}^{-1/2} \quad (3.5.2)$$

$$\lambda_3 = \left\{ \frac{j \omega}{2} \left(\frac{1}{D^*} + \frac{1}{D} \right) \right\}^{-1/2} \quad (3.5.3)$$

(see eqns 2.2.26 -2.2.29)

where :

$$\alpha_p = \frac{P_0 z_p^2}{P_0 z_p^2 + N_0 z_n^2 + C_0 z_c^2} \quad (3.5.4)$$

$$\alpha_n = \frac{N_0 z_n^2}{P_0 z_p^2 + N_0 z_n^2 + C_0 z_c^2} \quad (3.5.5)$$

$$\alpha_c = \frac{C_0 z_c^2}{P_0 z_p^2 + N_0 z_n^2 + C_0 z_c^2} \quad (3.5.6)$$

$$1/\lambda^2 = \frac{q^2(P_0 z_p^2 + N_0 z_n^2 + C_0 z_c^2)}{\epsilon_0 \epsilon k T} \quad (3.5.7)$$

$$\frac{1}{D^*} = \alpha_c \left(\frac{1}{D_p} + \frac{1}{D_n} \right) + \alpha_n \left(\frac{1}{D_p} + \frac{1}{D_n} \right) + \alpha_p \left(\frac{1}{D_n} + \frac{1}{D_c} \right) \quad (3.5.8)$$

$$\begin{aligned} \frac{1}{D^2} = & (\alpha_c \left(\frac{1}{D_p} - \frac{1}{D_n} \right) + \alpha_n \left(\frac{1}{D_p} - \frac{1}{D_c} \right) + \alpha_p \left(\frac{1}{D_n} - \frac{1}{D_c} \right))^2 - \\ & 4 \alpha_c \alpha_p \left(\frac{1}{D_p} - \frac{1}{D_n} \right) \left(\frac{1}{D_n} - \frac{1}{D_c} \right) \end{aligned} \quad (3.5.9)$$

The zeroth order term of the characteristic length λ_1 is equal to the Debye length (λ) defined above. Z_1 is proportional to $\sinh(L'/\lambda_1)/\cosh(L/\lambda_1)$. Thus Z_1 becomes negligibly small when the current electrode spacing and the separation between current and voltage electrodes are large compared to the Debye length. (For L and $L' \gg \lambda_1$, $\sinh(L'/\lambda_1)/\cosh(L/\lambda_1) \approx e^{-(L-L')/\lambda_1}$ which is very small provided $L - L' \gg \lambda_1$).

Since at low frequencies λ_2 and λ_3 are inversely proportional to $\omega^{1/2}$ (see equations 3.5.2 and 3.5.3), $L' \ll \lambda_2$ and λ_3 for small voltage

electrode spacings. Hence $\sinh(L'/\lambda_i)$ can be approximated by L'/λ_i , $i=2,3$ and equation 3.4.5 reduces to:

$$Z_i \approx \frac{2 L'}{y_K} K_i / \cosh\left(\frac{L}{\lambda_i}\right), \quad i=2,3 \quad (3.5.10)$$

$$\text{where : } K_i = \frac{1}{\lambda_p^2 \det} F_i (z_p + z_c T_i - z_n S_i) \lambda_i^2 \quad (3.5.11)$$

To lowest order in ω , K_i simplifies to :

$$K_i \approx \frac{(D_n - D_c) (D_n - D_p) \alpha_n (D_c \alpha_c + D_n \alpha_n + D_p \alpha_p) D}{D_c D_n D_p^3} K_i^{\text{diff}} \quad (3.5.12)$$

where K_i^{diff} is equal to the low frequency limit of $F_i (z_p + z_c T_i - z_n S_i) \lambda_i^2$, $i = 2,3$ (see equation 3.2.35, 3.2.36, 3.4.5)

The explicit expression for K_i^{diff} , $i=2,3$, is a complicated function of the ion concentrations and the diffusion constants D_p , D_n , D_c , D^* and D and will not be given here. The low frequency limit of K_i^{diff} can, however, be simplified under certain conditions that are described below.

Since λ_i is complex, $\cosh(L/\lambda_i)$ and hence both Z_2 and Z_3 are oscillatory functions of frequency, i.e. both the real and imaginary part of Z_2 and Z_3 will alternate between positive and negative values with increasing frequency. For cases where Z_2 and or Z_3 dominate the total impedance, the behaviour of Z_t may deviate considerably from that expected at or near equilibrium. For example the imaginary part of Z_t will alternately become capacitive and inductive with increasing frequency.

The lowest order terms (in ω) of $1/\lambda_2^2$ and $1/\lambda_3^2$ are imaginary, therefore $1/\lambda_i$ can be written as $(1 + j) k_i$ ($i = 2$ and 3) where :

$$k_2 = \frac{1}{2} \left\{ \omega \left(\frac{1}{D^*} - \frac{1}{D} \right) \right\}^{1/2} \quad \text{and} \quad k_3 = \frac{1}{2} \left\{ \omega \left(\frac{1}{D^*} + \frac{1}{D} \right) \right\}^{1/2} \quad (3.5.13)$$

At low frequencies $L \ll \lambda_i$ ($i = 2$ or 3), $\cosh(L/\lambda_i) \approx \cosh[(1+j)k_i L] \approx 1 + j k_i^2 L^2$.

Let $K_i = K_{rei} + j \omega K_{imi}$ which will then give:

$$K_i / \cosh[(1+j) k_i L] \approx K_{rei} + j \omega (K_{imi} - K_{rei} L^2 k_i^2 / \omega) \quad (3.5.14)$$

Both K_{rei} and K_{imi} are complicated functions of the diffusion constants D_p , D_c and D_n weighted by the relative bulk ion concentrations. The expression for K_{imi} includes second order terms which cannot be simplified. However the ratio $(K_{rei} L^2 k_i^2 / \omega) / K_{imi}$ is proportional to L^2 / λ^2 . λ is an “effective” Debye length typically of the order of nm, thus $(K_{rei} L^2 k_i^2 / \omega) / K_{imi}$ is very large unless L is comparable to λ . Hence the contribution of the K_{imi} term to the total impedance can be neglected for typical values of ion concentrations and macroscopic values of L .

The real part of Z_i (and hence G_i) will be zero for $k_i L = (n - 1/2) \pi$, where $n = 1, 2, 3, \dots$. This occurs for frequencies $f_{ni} = \omega'_{ni} / 2 \pi$ ($i=2, 3$). Using equation 3.5.13 these are given by:

$$\omega'_{n2} = \frac{(n - 1/2)^2 \pi^2}{L^2 \left(\frac{1}{D^*} - \frac{1}{D} \right)} \quad \text{and} \quad \omega'_{n3} = \frac{(n - 1/2)^2 \pi^2}{L^2 \left(\frac{1}{D^*} + \frac{1}{D} \right)} \quad (3.5.15)$$

Since K_{rei} is real, the imaginary part of Z_i (and therefore the corresponding capacitance C_i) is equal to zero for $L k_i = n \pi$, i.e. for $\omega_{ni} = 2\pi f_{ni} = n^2/(n - 1/2)^2 \omega'_{ni}$ ($i=2,3$). (3.5.16)

In order to predict the frequencies for which the real and imaginary parts of the total impedance are zero, the relative magnitudes of Z_2 and Z_3 need to be compared, using the ratio $Z_2/Z_3 = K_2^{diff}/K_3^{diff}$ (see equations 3.5.10 to 3.5.12). K_2^{diff} and K_3^{diff} involve the factor $1/D$ which is equal to the square root of a complicated expression depending on the diffusion constants weighted by the relative bulk ion concentrations (see equation 3.4.9). Therefore useful analytical expressions for Z_2 and Z_3 could only be found for ranges of the diffusion constants where the square root term could be approximated by a simpler expression¹. Three such cases are considered :

Case 1) If D_c is very different from D_p and D_n , the lowest order K_2^{diff} and K_3^{diff} are proportional to $z_c^2 C_0$ and $z_p^2 P_0$ respectively.

If $D_c \ll D_p$ or D_n , then K_2^{diff} and K_3^{diff} simplify to :

$$K_2^{diff} \approx \frac{-D_p^3 \alpha_c (\alpha_n + \alpha_p)}{D_c (D_n - D_p) \alpha_n} \quad (3.5.17)$$

$$K_3^{diff} \approx \frac{-D_p^2 (D_n - D_p) \alpha_p}{D_n (\alpha_n + \alpha_p) (D_n \alpha_n + D_p \alpha_p)} \quad (3.5.18)$$

i.e. Z_2 is much larger than Z_3 , except when C_0 approaches zero.

¹ An exact expressions for the much simpler case of a binary electrolyte has been derived and is presented in section 3.6.

If $D_c \ll D_p$ or D_n , then K_2^{diff} is again inversely proportional to $(D_n - D_p)$, thus for similar D_p and D_n , Z_2 is larger than Z_3 except for low values of C_0 .

Hence for both situations Z_3 dominates the total impedance for low values of C_0 only and Z_2 dominates for all other concentrations.

Case 2) If $D_c \approx D_p$:

$$K_2^{\text{diff}} \approx \frac{\alpha_c \alpha_p (D_c - D_p)^2}{\alpha_n (\alpha_c + \alpha_p)^2 (D_p - D_n)} \quad (3.5.19)$$

$$K_3^{\text{diff}} \approx \frac{D_p^2 (D_p - D_n) (\alpha_c + \alpha_p)^2}{D_n ((\alpha_c + \alpha_p) D_p + \alpha_n D_n)} \quad (3.5.20)$$

It is assumed that the only charges present are the ionic species, hence $z_p P_0 + z_c C_0 = z_n N_0$. Therefore if the two cation species have the same valency z , $\alpha_c + \alpha_p = (z/z_n) \alpha_n$. Hence Z_3 does not vary greatly with C_0 and P_0 , provided the total ion concentration remains the same.

If $D_n \approx D_p$, when the two cation concentrations are approximately equal, Z_2 and Z_3 are of the same order of magnitude. Hence the frequencies for which the real and imaginary parts of the total impedance are zero lie between those predicted for Z_2 and Z_3 alone. For small C_0 and P_0 the total impedance is approximately equal to $Z_3 + Z_K$.

If $D_n \gg D_p$ or D_c , Z_3 will dominate for all concentrations, provided $(D_c - D_p) \ll D_p$.

Case 3) If D_n is much smaller than D_p or D_c :

$$K_2^{\text{diff}} \approx \frac{\alpha_c \alpha_p (D_c - D_p)^2}{D_p \alpha_n (\alpha_c + \alpha_p)^2} \quad (3.5.21)$$

$$K_3^{\text{diff}} \approx \frac{D_p^3 (\alpha_c + \alpha_p)^2}{D_n (D_p (\alpha_c + \alpha_p) + \alpha_n D_n)} \quad (3.5.22)$$

Therefore Z_3 is very large compared to Z_2 (inversely proportional to D_n^2). If the cation valencies are equal, Z_3 again does not vary much with ion concentration, provided the total ion concentration is kept constant. It dominates the total impedance for all concentrations.

At very low frequencies $\cosh(L/\lambda_i)$ is approximately equal to one, hence the real part of Z_i simplifies to $2 L' K_i / y_K \approx 2 L' K_i / g_K$. The total conductance G_t is equal to $\text{Re} \left(1 / \left(Z_K + \sum_{i=1}^3 Z_i \right) \right)$. For the cases where either Z_2 or Z_3 dominates, G_t thus simplifies to :

$$G_t = \frac{g_K}{2 L'} \frac{1}{1 + K_i} \quad (i = \text{either } 2 \text{ or } 3) \quad (3.5.23)$$

The imaginary part of Z_i is proportional to ω and includes second order terms of λ_2 and λ_3 .

3.6 HIGH FREQUENCY LIMIT

At very high frequencies the ions do not have enough time to diffuse far in one cycle, hence the total impedance approaches Z_K . The total capacitance and conductance are therefore approximately equal to C_K and G_K respectively, where $C_K = \epsilon / (2 L')$ and $G_K = g_K / (2 L')$. (3.6.1)

3.7 BINARY ELECTROLYTE

To gain further insight into the physical mechanisms involved in the impedance terms, it is helpful to examine a binary electrolyte. This is because the algebra is considerably simpler than for the three ion case².

3.7.1 General Solution

For the case of a binary electrolyte the characteristic equation reduces to a quadratic, i.e. it involves only two characteristic lengths λ_i .

$$p(x) = \alpha_1 \sinh(x/\lambda_1) + \alpha_2 \sinh(x/\lambda_2) \quad (3.7.1)$$

$$n(x) = \alpha_1 S_1 \sinh(x/\lambda_1) + \alpha_2 S_2 \sinh(x/\lambda_2) \quad (3.7.2)$$

$$\text{where: } \lambda_1 = (\eta_1 + \eta_2)^{-1/2} \quad (3.7.3)$$

$$\lambda_2 = (\eta_1 - \eta_2)^{-1/2}$$

$$\eta_1 = \frac{1}{2\lambda^2} + j\left(\frac{1}{\xi_p^2} + \frac{1}{\xi_n^2}\right) \quad (3.7.4)$$

$$\eta_2 = \sqrt{\frac{1}{4\lambda^4} - \left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2}\right)^2 + \frac{j(z_p - z_n)\left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2}\right)}{(z_p + z_n)\lambda^2}} \quad (3.7.5)$$

² In work, currently in press (Smith and Eberl, 1993) concerned only with a binary electrolyte we use a slightly different notation where S_i are replaced by θ_i and a_i are replaced by $2a_i$.

$$S_i = [z_p + (z_p + z_n) \lambda^2 (\frac{2j}{\xi_p^2} - \frac{1}{\lambda_i^2})]/z_n, \quad i=1 \text{ to } 2 \quad (3.7.6)$$

The Debye length (λ) reduces to: $\lambda = [\epsilon\epsilon_0 kT/q^2(z_p^2 P_0 + z_n^2 N_0)]^{1/2}$, where $z_p P_0 = z_n N_0 = C_0^3$. (3.7.7)

As before the electric field can be obtained by integrating Poisson's equation:

$$\begin{aligned} e(x) &= \int \frac{q}{\epsilon} (z_p p - z_n n) dx \\ &= \frac{q}{\epsilon} \left(\alpha_i \lambda_i (z_p - z_n S_i) \cosh\left(\frac{x}{\lambda_i}\right) + \alpha_i \lambda_i (z_p - z_n S_i) \cosh\left(\frac{x}{\lambda_i}\right) \right) \\ &\quad + J/y_K \end{aligned} \quad (3.7.8)$$

For a binary electrolyte the admittance per unit length y_K is given by:

$$y_K = J/e_K = g_{pK} + g_{nK} + j\omega\epsilon \quad (3.7.9)$$

Substituting equations 3.7.1, 3.7. 2 and 3.7. 8 into 3.2.13 gives, after simplification:

$$J_p(x) = -j \omega q z_p \left(\alpha_1 \lambda_1 \cosh\left(\frac{x}{\lambda_1}\right) + \alpha_2 \lambda_2 \cosh\left(\frac{x}{\lambda_2}\right) \right) + J g_{pK}/y_K \quad (3.7.9b)$$

3 Note that for consistency in notation with chapter 4, I retain C_0 as the symbol for the overall bulk electrolyte concentration. This should not be confused with C_0 used in the theory for the three ion electrolyte of sections 3.2 to 3.4 above, where it denotes the bulk concentration of the third ion species only.

Similarly, combining 3.7.1,2 and 8 and 3.2.14 gives J_n :

$$J_n(x) = -j \omega q z_n \left(H_1 \alpha_1 \lambda_1 \cosh\left(\frac{x}{\lambda_1}\right) + H_2 \alpha_2 \lambda_2 \cosh\left(\frac{x}{\lambda_2}\right) \right) + J_{g_{nK}/y_K} \quad (3.7.10)$$

$$\text{where : } H_i = (D_n/D_p) + j \xi_n^2 (1 + S_i)/(2 \lambda_i^2), i = 1 \text{ to } 2 \quad (3.7.11)$$

The two unknowns α_1 and α_2 can again be determined from the following boundary conditions at the electrodes, situated at $x = \pm L$. However, I now consider the more general boundary conditions than for the three ion case, i.e. the current electrodes can now also be partially blocking as well as completely blocking. The ion current densities are now set equal to an arbitrary fraction of the total applied current density (J) at $x = \pm L$:

$$J_p(L) = t_p J \text{ and } J_n(L) = t_n J \quad (3.7.12)$$

where t_p and t_n are the fractions of J carried by the cations and anions at the current electrodes, respectively.

For this case α_1 and α_2 simplify to:

$$\alpha_1 = \frac{jJ[(t_n - g_{nK}/y_K)/z_n - H_2(t_p - g_{pK}/y_K)/z_p]}{\omega q (H_1 - H_2) \lambda_1 \cosh(L/\lambda_1)} \quad (3.7.13)$$

$$\alpha_2 = \frac{2jJ(t_p - g_{pK}/y_K)/(2\omega q z_p) - \alpha_1 \lambda_1 \cosh(L/\lambda_1)}{\lambda_2 \cosh(L/\lambda_2)} \quad (3.7.14)$$

The a.c. potential ($\Psi(x)$) can be obtained from :

$$\begin{aligned}\Psi(x) &= -\int e(x) dx \\ &= -\frac{q}{\epsilon} \sum_{i=1}^2 [\alpha_i \lambda_i^2 (z_p - z_n S_i) \sinh(x/\lambda_i)] + J/y_K x + \Psi_I \quad (3.7.15)\end{aligned}$$

Again $\Psi_I = 0$, since $\Psi(0)$ is chosen to be zero.

The impedance $Z_T = [\Psi(-L') - \Psi(L')]/J$ measured in between voltage electrodes at $x = \pm L'$ can now be written as the sum of three terms (from equations 3.7.8 and 3.7.9).

$$Z_T = Z_1 + Z_2 + Z_K \quad (3.7.16)$$

As before Z_K is simply the impedance that would be measured in the absence of a.c. space charge.

$$\begin{aligned}Z_K &= \frac{2L'}{y_K} \\ Z_T &= -\frac{2q}{\epsilon} \left(\alpha_1 \lambda_1^2 (z_p - z_n S_1) \sinh\left(\frac{L'}{\lambda_1}\right) + \alpha_2 \lambda_2^2 (z_p - z_n S_2) \sinh\left(\frac{L'}{\lambda_2}\right) \right) \\ &\quad + 2L'/y_K \quad (3.7.17)\end{aligned}$$

Z_1 and Z_2 are the impedances associated with the characteristic lengths λ_1 and λ_2 , respectively. Z_T involves three fundamental characteristic lengths : λ , ξ_p and ξ_n in addition to y_K , the constant field area specific admittance per unit length. Z_1 and Z_2 as well as λ_1 and λ_2 depend on η_2 which includes the square root of a complex function of

frequency. As before, for voltage electrodes situated many Debye lengths away from the current electrodes, the contribution of Z_1 to the total impedance is negligible.

Z_2 is again an oscillatory function of frequency. As before the angular frequencies (ω'_{ni} and ω_{ni}) at which the real and imaginary parts of Z_2 are equal to zero, strongly depend on the differences between D_p and D_n and the current electrode separation L . However these frequencies are now independent of bulk electrolyte concentrations (P_0 and N_0). For this case ω'_{ni} and ω_{ni} reduce to :

$$\omega'_{ni} = 2 (n + 1)^2 \pi^2 (z_p + z_n) D_p D_n / [L^2 (z_p D_p + z_n D_n)] \quad (3.7.20)$$

$$\omega_{ni} = \omega'_{ni} m^2 / (m + 1)^2 \quad (3.7.21)$$

3.7.2 Low Frequency Limit

Measurements of electrical properties of membranes, using a.c. techniques such as the vibrating probe, are usually performed at low frequencies (below 500 Hz). The expressions for arbitrary ω can be simplified considerably by considering the low frequency limit (see Appendix 3.1). This involves neglecting the contribution of the displacement current and assuming that the diffusion lengths are large in comparison with the Debye length λ (given by equation 3.7.7). It is also assumed that measurements are taken outside the electric double layer in the electrolyte at the membrane surface, i.e. $(L-x) \gg \lambda$ where λ is typically of the order of nm. Variables derived for these low frequencies have been indicated by the subscript L.

Equations that describe the low frequency behaviour of $p(x)$, $n(x)$, $e(x)$, $\psi(x)$, $J_p(x)$ and $J_n(x)$ in terms of the constants of the electrolyte (i.e. D_p , D_n , z_p , z_n , P , N and ϵ) and the membrane (t_p and t_n) are now presented. It is convenient to introduce some extra terms: Q_p denotes the difference in cation transference number between the membrane and the bulk electrolyte:

$$Q_p = t_p - z_p D_p / (z_p D_p + z_n D_n) \quad (3.7.22)$$

ξ_s denotes the rms distance through which solute diffuses during a change of one radian in the phase of the applied AC current and is defined by:

$$\xi_s = [2D_s/\omega]^{1/2} = [2(z_p + z_n)D_p D_n / (z_p D_p + z_n D_n)\omega]^{1/2} \quad (3.7.23)$$

where D_s denotes the effective diffusion coefficient for the solute. This is given by:

$$D_s = (z_p + z_n)D_p D_n / (z_p D_p + z_n D_n) \quad (3.7.24)$$

The low frequency variations in cation and anion concentration are respectively given by:

$$p_L(x) = \frac{(j-1)JQ_p \sinh((1+j)x/\xi_s)}{2^{3/2}\omega^{1/2}z_p q D_s^{1/2} \cosh((1+j)L/\xi_s)} \quad (3.7.25)$$

$$n_L(x) = \frac{(j-1)JQ_p \{1 + j\omega\lambda^2(1/D_p - 1/D_n)\} \sinh((1+j)x/\xi_s)}{2^{3/2}\omega^{1/2}z_n q D_s^{1/2} \cosh((1+j)L/\xi_s)} \quad (3.7.26)$$

The concentration changes in $p_L(x)$ and $n_L(x)$ are thus approximately in the ratio z_n/z_p . There is, however, a small difference and this gives rise to an a.c. space or net charge $\rho_L(x)$ given by:

$$\begin{aligned} \rho_L(x) &= z_p p_L(x) - z_n n_L(x) \\ &= \frac{(1+j)JQ_p(D_p - D_n)\omega^{1/2} \lambda^2 \sinh((1+j)x/\xi_s)}{2^{3/2}qD_p D_n D_s^{1/2} \cosh((1+j)L/\xi_s)} \end{aligned} \quad (3.7.27)$$

This space charge produces an additional term in the electric field. The a.c. electric field is then given by:

$$e_L(x) = \frac{J}{g_{KL}} \left(1 + \frac{Q_p(D_n - D_p) \cosh((1+j)x/\xi_s)}{D_s \cosh((1+j)L/\xi_s)} \right) \quad (3.7.28)$$

where g_{KL} denotes the area specific admittance per unit length at low frequencies, assuming a constant electric field (i.e. independent of position) and is given by:

$$g_{KL} = q^2(z_p^2 D_p P + z_n^2 D_n N)/kT \quad (3.7.29)$$

The potential is then given by:

$$\psi_L(x) = - \frac{J}{g_{KL}} \left(x + \frac{(1-j)Q_p(D_n - D_p) \sinh((1+j)x/\xi_s)}{2^{1/2}D_s^{1/2}\omega^{1/2} \cosh((1+j)L/\xi_s)} \right) \quad (3.7.30)$$

where $\psi(0)=0$ at $x=0$.

The a.c. ion currents are:

$$J_{pL}(x) = J \left(\frac{T_p \cosh((1+j)x/\xi_s)}{\cosh((1+j)L/\xi_s)} + \frac{z_p D_p}{z_p D_p + z_n D_n} \left\{ 1 - \frac{\cosh((1+j)x/\xi_s)}{\cosh((1+j)L/\xi_s)} \right\} \right) \quad (3.7.31)$$

$$J_{nL}(x) = J \left(\frac{T_n \cosh((1+j)x/\xi_s)}{\cosh((1+j)L/\xi_s)} + \frac{z_n D_n}{z_p D_p + z_n D_n} \left\{ 1 - \frac{\cosh((1+j)x/\xi_s)}{\cosh((1+j)L/\xi_s)} \right\} \right) \quad (3.7.32)$$

It should be remembered that equations 3.7.25 to 3.7.28 and 3.7.30 to 3.7.32) give the spatial dependence of each parameter. The time dependence can be introduced by multiplying by $\exp(j\omega t)$. Thus for example $p_L(x,t) = p_L(x)\exp(j\omega t)$ etc.

3.8 RESULTS

The results in this chapter are for the behaviour of the electrolyte many Debye lengths away from the electrodes, hence they effectively exclude the double layer impedance of the current electrodes. For the four terminal impedances which exclude these double layers, diffusion effects become important due to the contribution of the impedance terms Z_2 and Z_3 to the total impedance (see section 3.4). Z_2 and Z_3 arise from a component of the space charge which is produced if the cationic and anionic diffusion constants differ. Cations and anions will then tend to move at slightly different rates under the influence of the applied field. They can never move at very different rates, even if they have very different diffusion constants, because the charge separation creates a large electric field which slows down the more mobile and accelerates the less mobile ion. The strong electric field that opposes their independent

motion produces "waves" of solute movement between the current electrode, analogous to standing waves in an air column.

The a.c. impedance of the double layer has been calculated by DeLacey and White (1982) for an arbitrary number of ion species. Although the results presented in this chapter are for impedances which exclude the double layer impedance, the voltage electrode separation (L') can be varied arbitrarily, and hence a comparison was possible for the case of KCl and $\xi = 0$. The impedance calculated for this case approximately agreed with that of Delacey and White's value and showed approximately the same trend with frequency. (It was difficult to determine on the scale of the graphs).

3.8.1 Impedances Ratios for a 3 ion electrolyte

The capacitance and conductance ratios C/C_K and G/G_K were calculated as functions of frequency for various bulk ion concentrations and diffusion constants. C_K and G_K denote the geometrical capacitance and conductance, respectively, i.e. the capacitance and conductance for no a.c. concentration variation ($C_K = \epsilon/L'$ and $G_K = (g_{pK} + g_{nK})/L'$). Because these ratios varied considerably with diffusion constants and ion concentrations, it was difficult to find a suitable set of parameters for representative plots of capacitance and conductance.

Calculations of the impedance require very high numerical precision, since the solution of the three differential equations for the ion concentrations (p , c and n) involves finding complex cubic roots which may include subtracting terms of similar magnitude. Furthermore, finding α_i from the boundary conditions involve the inversion of a matrix whose elements have different orders of magnitude, which can lead to loss

of precision. Hence one needs to use very high precision variables, otherwise the error in the results renders them meaningless. For the results below 36 digits were used per variable (Checks at higher precision showed this to be a suitable precision).

As discussed below, C_l/C_K and G_l/G_K depend strongly on the ratio of the cation to anion diffusion constants (D_p/D_n). To illustrate this effect, plots of C_l/C_K and G_l/G_K for a binary electrolyte for a large range of the ratio D_p/D_n and a wider range of frequencies are presented in Figure 3.2 for the case of a binary electrolyte.

No variations of the ratios C_l/C_K and G_l/G_K with frequency occur above or below the frequency range of the oscillations of C_l/C_K and G_l/G_K : at high frequencies the capacitance and conductance were equal to their geometrical values and at low frequencies they reach a limiting value. Therefore plots demonstrating the effect of the third ion species were limited to this frequency range only.

If the three ion species have different diffusion constants, the capacitance ratio C_l/C_K reaches a value much greater than one at low frequencies. This low frequency limit is a complicated function of diffusion constants and ion concentrations and the dimensions of the system and increases with greater differences in the diffusion constants. C_l/C_K oscillates between negative and positive values at intermediate frequencies, i.e. the total reactance becomes capacitive and inductive alternately. However the magnitude of consecutive oscillations decreases very quickly with frequency. C_l/C_K approaches one at high frequencies.

The conductance ratio G_l/G_K is less than one at very low frequencies. It also oscillates at intermediate frequencies and approaches one at high frequencies. As explained above, the oscillations of the total impedance and hence capacitance and conductance, are due to Z_2 and Z_3 , whose real

FIGURE 3.2

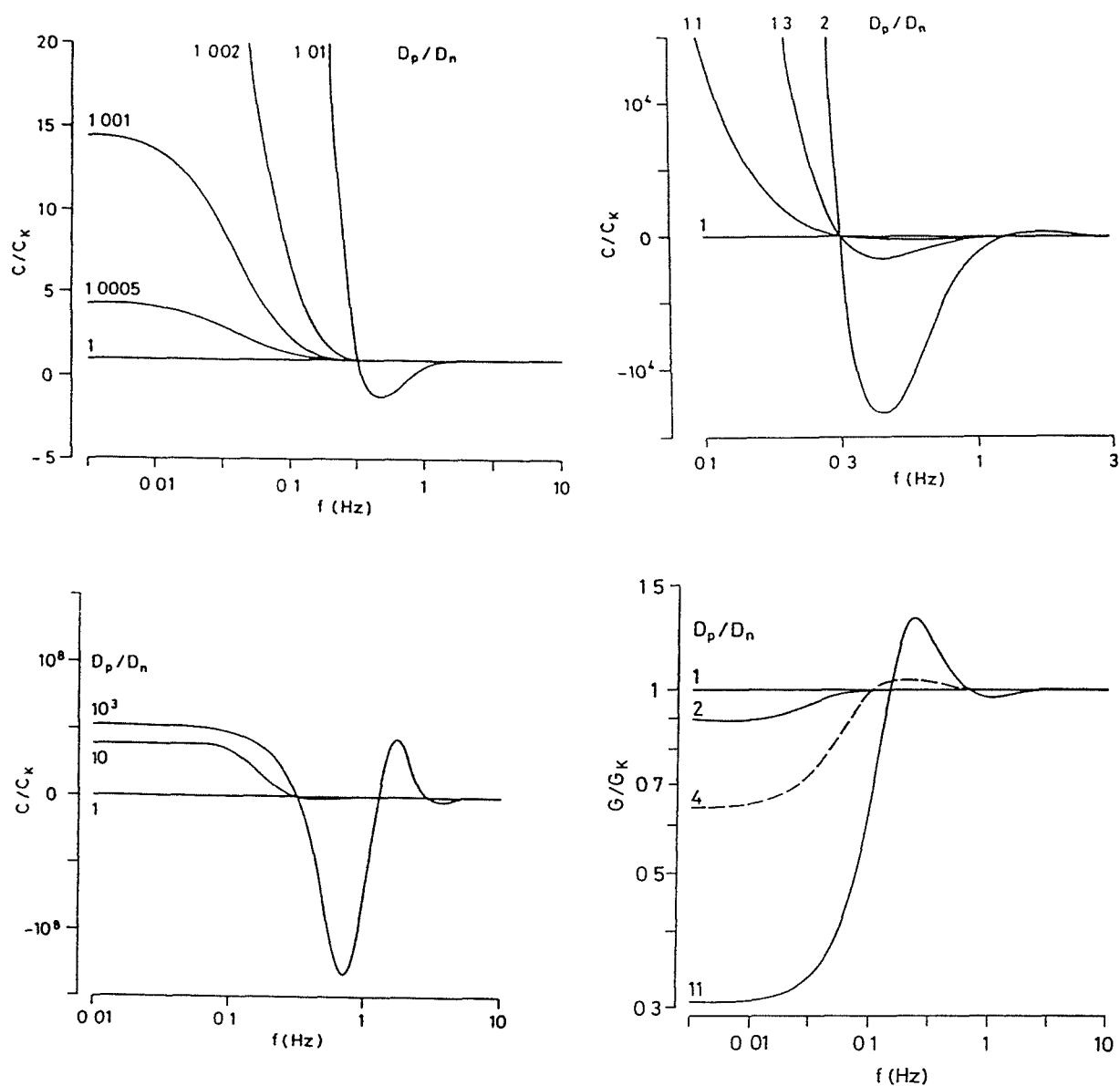


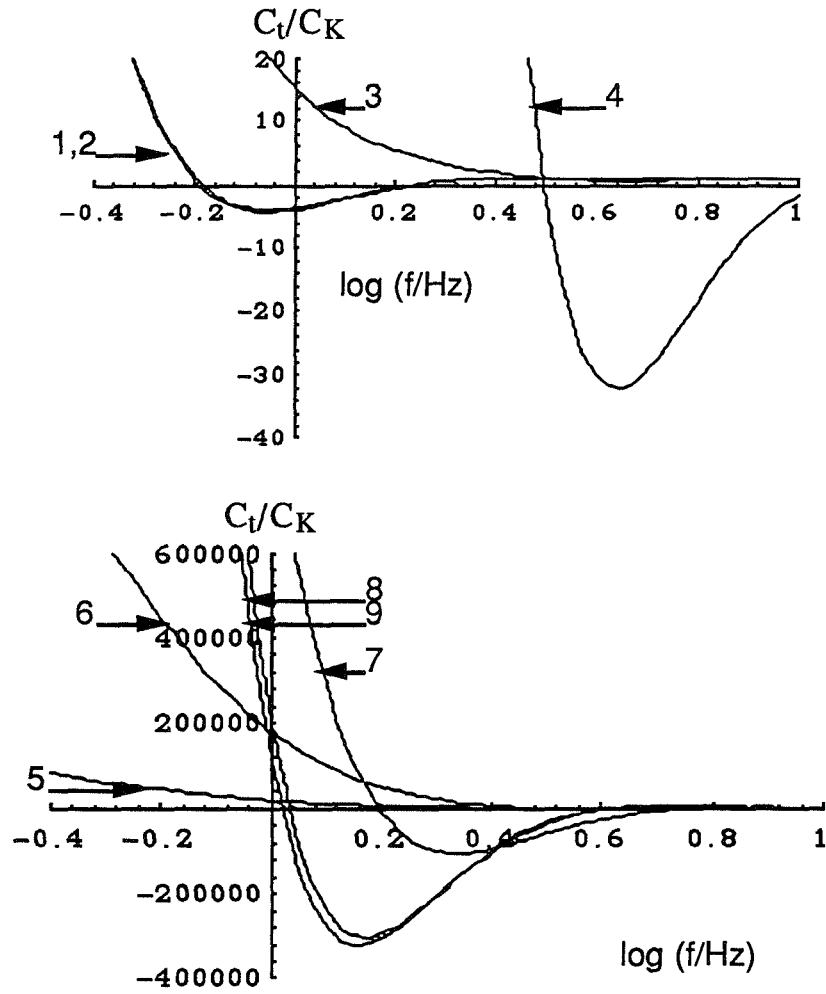
Figure 3.2. The conductance (G/G_K) and capacitance ratio (C/C_K) as a function of frequency for different ratios of the cation to anion diffusion constant (D_p/D_n). For these calculations $L = 100 \mu\text{m}$, $L' = 10 \mu\text{m}$, $C_0 = 1 \text{ mM}$, $\epsilon/\epsilon_0 = 80$ and the average diffusion constant $= 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

and imaginary parts alternately become negative and positive with increasing frequency. Since Z_1 is negligible for the voltage electrode spacings used here (see section 3.4), the real and imaginary parts of the total admittance ($\text{Re}(1/Z_t)$ and $\text{Im}(1/Z_t)$) will equal $\text{Re}(1/Z_K)$ and $\text{Im}(Z_K)$ at frequencies for which $\text{Re}(1/(Z_2 + 1/Z_3))$ and $\text{Im}(1/(Z_2 + 1/Z_3))$, respectively are equal to zero. At these frequencies (denoted by f_{nC} and f_{nG} , respectively) the capacitance and conductance ratios are equal to unity. f_{nC} and f_{nG} vary with diffusion constants and electrode separation, as well as bulk ion concentrations.

While the effect of the impedance terms Z_2 and Z_3 on the capacitance ratios is spectacular, the conductance ratios remain reasonably close to unity, since, although C_t is much greater than C_K , ωC_t is considerably smaller than G_t at low frequencies for the parameters chosen in this chapter (of the order of 1%). However, at very low electrolyte concentrations, the effect on the conductance can become important. The expressions for the ion current densities also include the oscillatory term $\cosh(L/\lambda_i)$. Hence this analysis may help to explain qualitatively some of the peculiarities of ion flux measurements of plant cells reported in the literature, (e. g. Newman *et. al.*, 1987 and Kochian, 1989).

Figures 3.3 and 3.4 give examples of the capacitance and conductance ratio respectively as functions of frequency for the case when the diffusion constant of the second cation species (D_c) is much greater than that of the first (D_p). Plots are shown for several different concentrations of salt 1 (P_0). The concentrations of salt 2 (C_0) are chosen so that $z_p P_0 + z_c C_0$ (and therefore the total ion concentration) is kept constant. Simple approximate expressions for the frequencies (f'_{ni} and f_{ni} , $i=2,3$) for which the real and imaginary parts of Z_i , respectively are equal to zero can be found (see theory section above). As explained in the previous section,

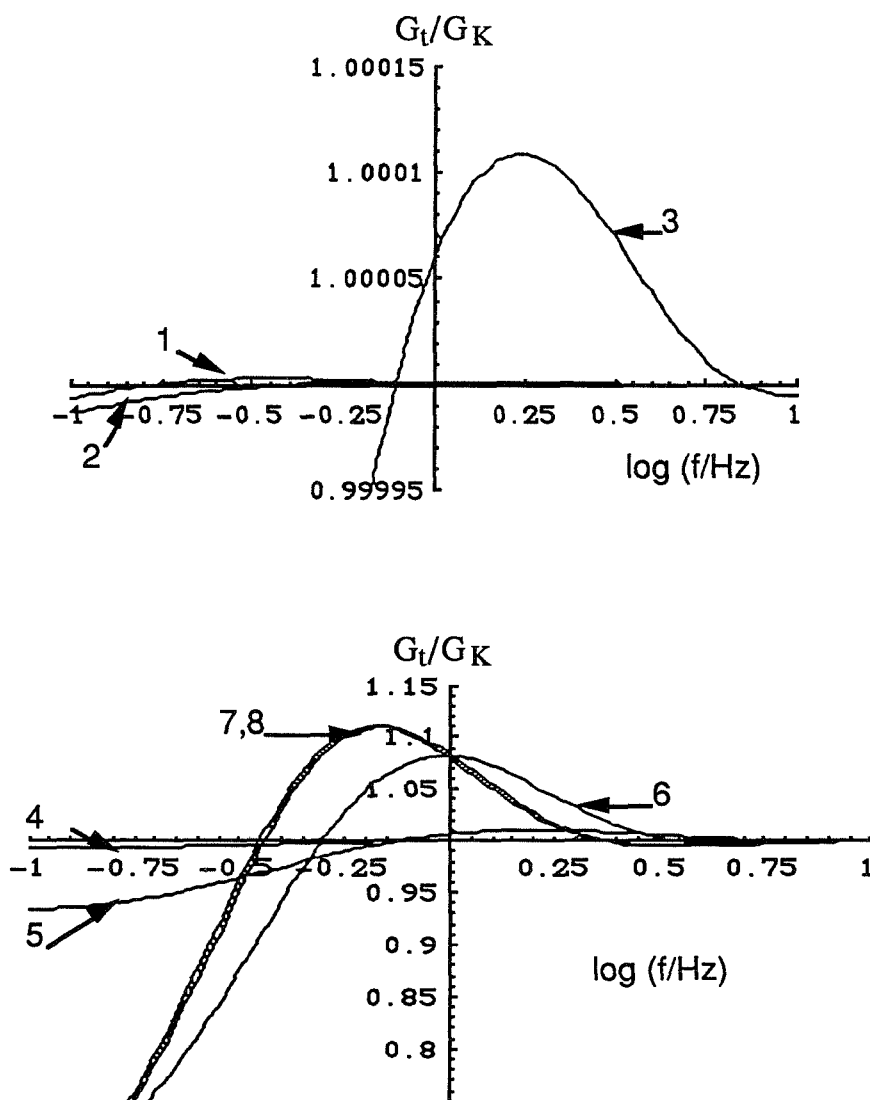
FIGURE 3.3



	1	2	3	4	5	6	7	8	9
P_0 (mM):	2.0	2.0	2.0	2.0	1.99	1.9	1.0	0.1	0.01
C_0 (mM):	10^{-9}	10^{-7}	10^{-5}	10^{-3}	0.01	0.1	1.0	1.9	1.99

Figure 3.3 a and b. The effect on the capacitance ratio of swapping two cation species, whose diffusion constants are very different. C_t/C_K is plotted for different concentrations of the two cation species, P_0 and C_0 , respectively, for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $D_c = 1 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1}$ and $D_n = 1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$. See Table 3.1 for all other parameters. (D_c/D_p used for these calculations is approximately equal to the ratio of the diffusion constant of H_3O^+ to K^+ ions in dilute aqueous solutions). Note a and b use different scales for C_t/C_K for clarity.

FIGURE 3.4



	1	2	3	4	5	6	7	8
P_0 (mM):	2.0	2.0	2.0	1.99	1.9	1.0	0.1	0.01
C_0 (mM):	10^{-7}	10^{-5}	10^{-3}	0.01	0.1	1.0	1.9	1.99

Figure 3.4. The effect on the conductance ratio (G_t/G_K) of swapping two cation species, whose diffusion constants are very different. G_t/G_K is plotted for different concentrations of the two cation species, P_0 and C_0 , respectively, for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $D_c = 1 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1}$ and $D_n = 1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$. See Table 3.1 for all other parameters.

TABLE 3.1

PARAMETER		VALUE
ϵ/ϵ_0	relative dielectric constant of the solution	80
T	temperature	20°C
Z_p	valency of cation 1	1
Z_c	valency of cation 2	1
Z_n	valency of anion	1
L	current electrode separation	10^{-4} m
L'	voltage electrode separation	10^{-6} m

The values of L and L' were chosen as the best realisation of a macroscopic system consist with the very large exponents so produced.

even though the frequencies f_{nC} and f_{nG} (defined above) depend on both Z_2 and Z_3 , usually one of the two terms dominates Z_t , and therefore C_t/C_K and G_t/G_K over large ranges of the bulk ion concentration. Thus approximate values f_{nC} and f_{nG} can be predicted from the expressions for f'_{ni} and f_{ni} , $i=2,3$. ($f_{nC} \approx f_{n2}$ or f_{n3} and $f_{nG} \approx f'_{n2}$ or f'_{n3} , depending on whether Z_2 or Z_3 dominates). For example, if D_c is much larger than D_p , Z_2 dominates the total impedance except for very low concentrations of the second cation (C_0). Hence the frequencies for which the total capacitance equals C_K (f_{nC}) are expected to equal f_{n2} except for very small values of C_0 , (down to $C_0 = 10^{-5}$ mM for the parameters chosen here). For smaller C_0 , f_{nG} should approach f_{n3} . Similarly the calculated frequencies for which the total conductance equals G_K (f_{nG}) are approximately equal to f'_{n3} for very small values of C_0 only and are similar to f'_{n2} for all other concentrations. The results presented in Figures 3.2 and 3.3 are in agreement with this⁴.

For a binary electrolyte with cation diffusion constant D_p , the characteristic frequencies f_{nC} and f_{nG} are similar to f_{n3} and f'_{n3} respectively. Thus this example shows that small concentrations of a second ion species can alter the variation of the total capacitance with frequency considerably, provided that the diffusion constants of the cations differ significantly. The effect on the total conductance is however much smaller. The effect of the third ion may be important in the measurement of the diffusion constant for a given ion.

4 Note: The total conductance G_t is smaller than G_K at f'_{12} (i.e. at the first frequency for which G_2 is zero). Hence f_{1G} is somewhat higher than f'_{12} unless C_0 is small. This difference is due to the imaginary part of Z_2 , which dominates the imaginary part of the total impedance at this frequency, provided C_0 is sufficiently high. ($\text{Im}[\cosh(L/\lambda_2)]$ is nonzero at f'_{12} and K_2 is large unless $C_0 \rightarrow 0$)

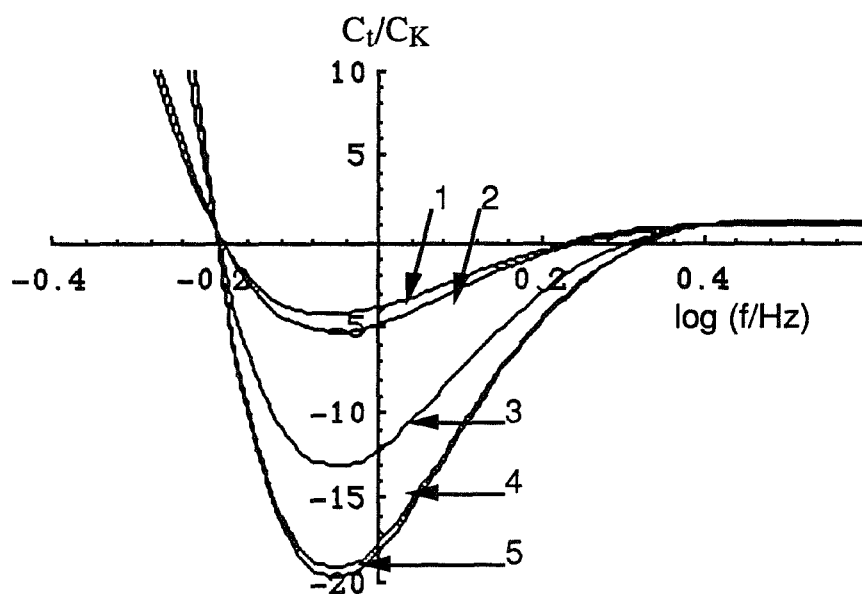
Figures 3.5 and 3.6 show the capacitance and conductance ratios as a function of frequency for similar cation and anion diffusion constants. The magnitudes of the capacitance and conductance oscillations are much smaller than for the previous case. Also f_{nC} and f_{nG} do not vary significantly with ion concentrations. f_{nC} and f_{nG} are approximately equal to f_{n3} and f'_{n3} respectively for low values of C_0 and lie between f_{n3} and f_{n2} , and f'_{n3} and f'_{n2} for most other concentrations.

3.8.2 Spatial Variations at low Frequencies for the Binary Electrolyte

The problem of estimating membrane currents from electrochemical measurements in the external electrolyte is now considered. An instrument that could measure J directly would thus give the correct value at all positions within the electrolyte, since the total a.c. current J in the electrolyte must necessarily be independent of position.

An a.c. technique such as the vibrating current probe, or two closely spaced voltage electrodes, however introduces the possibility of incorrect estimates of time varying J because it essentially measures the local electric field. A knowledge of the local ion concentrations then allows calculation of the local conductivity and thus the current. However the particular relationship used to calculate the conductivity (usually equation 3.7.29 or equivalent) is derived on the basis of an electric field independent of position. Equation 3.7.28 indicates that such a constant field will only occur if $T_p = z_p D_p / (z_p D_p + z_n D_n)$ and/or $D_p = D_n$. The first condition, when the transference number is the same for the membrane and the bulk electrolyte, results in zero a.c. concentration changes throughout the electrolyte, i.e. $p_L(x) = n_L(x) = 0$ for all x . The second

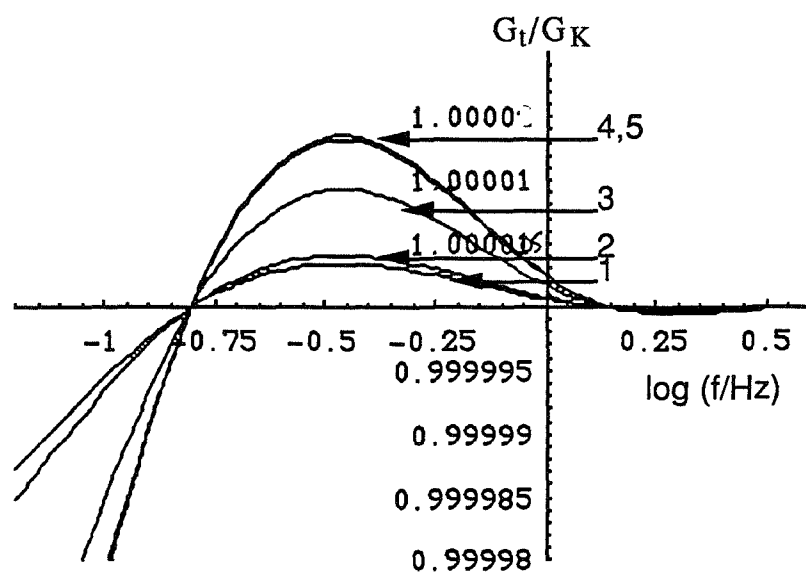
FIGURE 3.5



	1	2	3	4	5
P_0 (mM):	1.999	1.9	1.0	0.1	0.001
C_0 (mM):	0.001	0.1	1.0	1.9	1.999

Figure 3.5. The effect on the capacitance ratio (C_t/C_K) of swapping two cation species, with similar diffusion constants. C_t/C_K is plotted for different concentrations of the two cation species, P_0 and C_0 , respectively, for C_t/C_K for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $D_c = 2.02 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$ and $D_n = 1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$. See Table 3.1 for all other parameters.

FIGURE 3.6



	1	2	3	4	5
P_0 (mM):	1.999	1.9	1.0	0.1	0.001
C_0 (mM):	0.001	0.1	1.0	1.9	1.999

Figure 3.6. The effect on the conductance ratio (G_t/G_K) of swapping two cation species, with similar diffusion constants. G_t/G_K is plotted for different concentrations of the two cation species, P_0 and C_0 , respectively, for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $D_c = 2.02 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$ and $D_n = 1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$. See Table 3.1 for all other parameters.

condition results in $p_L(x)=n_L(x)$ and then the a.c. concentration changes carry no net charge. Both of these conditions are unlikely in experiments and consequently the current calculated from the electric field on the basis of a constant field may be in error depending on t_p , D_p , D_n and the cosh terms (given by the function F below) in equation 3.7.28.

Figure 3.7 gives an example of the behaviour of the oscillatory function F which occurs in both the low frequency limit of the electric field and the ionic current densities.

$$F = \frac{\cosh((1+j)x/\xi_s)}{\cosh((1+j)L/\xi_s)}$$

$$= \frac{\cos(x/\xi_s)\cosh(x/\xi_s) + j\sin(x/\xi_s)\sinh(x/\xi_s)}{\cos(L/\xi_s)\cosh(L/\xi_s) + j\sin(L/\xi_s)\sinh(L/\xi_s)} \quad (3.8.1)$$

This theoretical treatment shows that the inclusion of the Poisson equation has important consequences. It produces an electrical coupling between fluxes because movement of one species will cause concentration changes that produce a local net or space charge that will itself then generate a local electric field that attracts the other species. It is consequently impossible for ionic species to flow independently and after travelling sufficient distance the fraction of the total current carried by each species will eventually be in proportion to their valence, concentration and mobility in the bulk electrolyte. Again because the exponential of a complex variable is a periodic function, $J_{pL}(x)$ and $J_{nL}(x)$ will both be oscillatory functions of ω , D_s and x (see Figure 3.7).

The variation of phase shown in Figure 3.7 indicates that even the apparent direction of a time-varying ionic current can be incorrect. This could provide a possible explanation for the widely varying

FIGURE 3.7

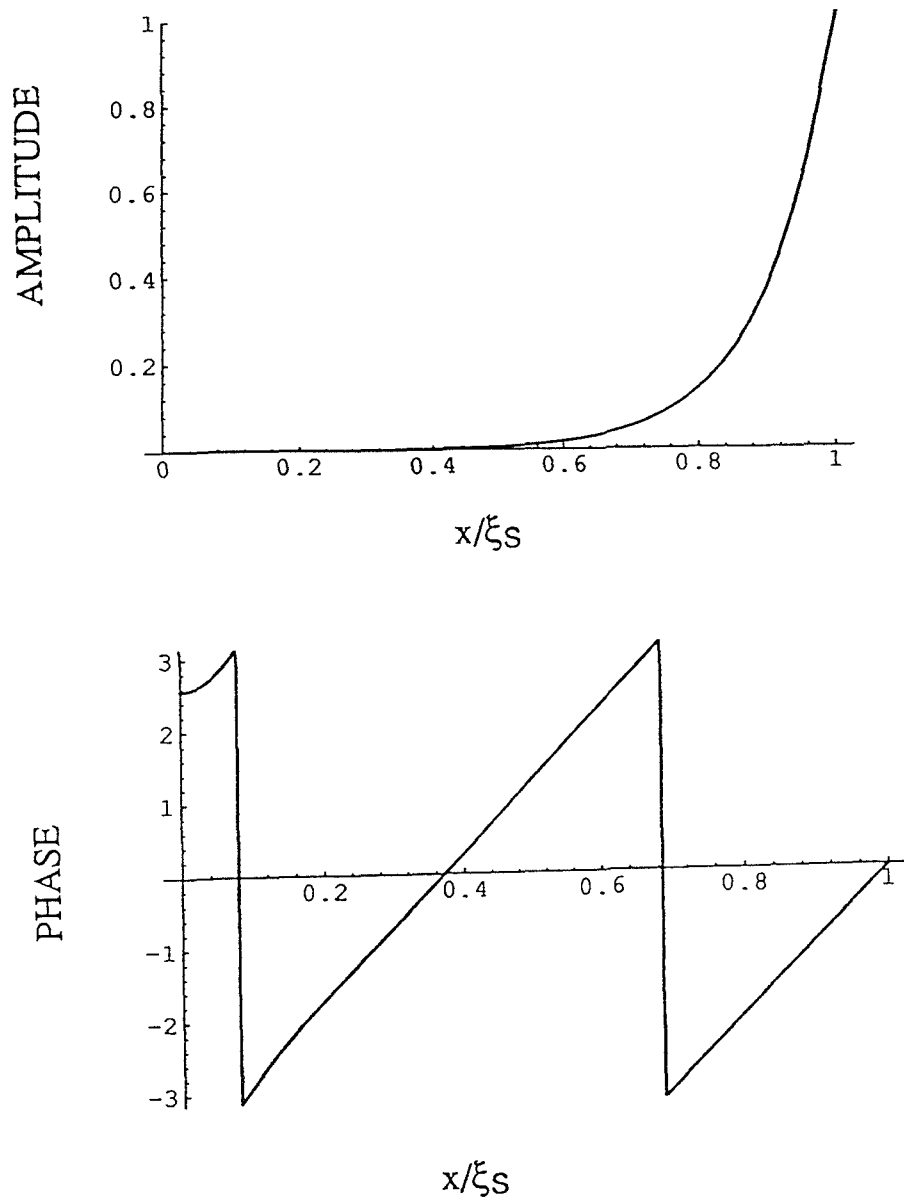


Figure 3.7. The amplitude and phase of the function F as a function of normalized distance x/ξ_S . This term results in the oscillatory variation of the low frequency limit of the electric field and ionic current densities.

stoichiometries sometimes observed between fluxes of different ions and the sometimes perplexing reversals in flux direction seen in measurements on plant cells (Newman *et. al.*, 1987 and Kochian *et. al.*, 1989).

The situation is more complicated if ion fluxes are estimated from the difference in concentration between two positions in the electrolyte as determined by ion-sensitive electrodes. The ion current is usually calculated from the gradient of the ion concentration assuming a linear variation with distance. The oscillatory nature of $p(x)$ means that interpolating between two points could possibly lead to erroneous conclusions, particularly when the distance between measurements becomes comparable to ξ_s . An additional problem might occur if the time taken to move the ion-sensitive-electrode between positions is comparable to the time scale of ion current variations - it is then possible that measurements are taken in different parts of cycle.

It is stressed that this treatment is for a simple, one-dimensional system only. Calculations in Chapter 4 show no qualitative difference from the results for planar geometry, apart from geometrical factors. Results presented should at least be qualitatively applicable to measurements in external electrolyte solutions of biological membranes, whatever the geometry.

3.9 CONCLUSIONS

Exact algebraic solutions to the Nernst-Planck and Poisson equations for planar geometry were presented for an electrolyte containing at most 3 distinct species. Calculations of the impedance of the regions excluding the double layer at the electrodes showed hitherto unexpected behaviour at low frequencies. For this region Z_t was dominated by diffusion effects

(due to the oscillatory nature of the impedance terms, Z_2 and Z_3), which led to large oscillations in the capacitance as a function of frequency. These effects could possibly offer an explanation for the inductances occasionally measured in electrochemical experiments on biological systems. Calculations showed that the presence of even a low concentration of the second cation species could considerably affect the total capacitance, if the diffusion constants of the two cation species were very different.

The large oscillations in the impedance occurred only for four terminal measurements. Even though these oscillations may not have been observed to date, they are not just of academic interest, but could provide the basis for a useful measuring tool, since they allow null measurements. This would be a powerful method of determining diffusion constants for the following two reasons:

- 1) They remove free parameters. For example, fitting measured capacitance values to the zero points of the frequency spectrum, predicted by a theory such as the one described in this chapter, removes the necessity of knowing the electrode area precisely. This area can be difficult or cumbersome to measure in some cases. Furthermore, since the theory predicts more than one zero, the additional zeros could provide an extra check of the goodness of the fit to the data, or enable additional parameters to be determined.
- 2) They are inherently powerful, since measuring systems are usually most sensitive near their zero points.

The varying activity of membrane transport systems produces membrane currents that vary with time. These currents flow through the electrolyte that surrounds the membrane and produce time-dependent

ionic concentrations. This creates several possible sources of error if external electrochemical measurements are used to determine the membrane currents. Two such errors are:

- (i) The relationship between the electric field and total current at any point in the electrolyte depends upon the rate of change of the membrane currents. This raises complications in the interpretation of time-varying measurements with the conventional vibrating probe.
- (ii) The coupling between ionic currents that vary with time means that the current of a species measured in the external electrolyte some distance away from the membrane can be quite different from its value in the membrane.

CHAPTER 4

SOLUTIONS OF THE NERNST-PLANCK POISSON'S EQUATIONS FOR SPHERICAL GEOMETRY

4.1 INTRODUCTION

In this chapter analytical solutions to the Nernst-Planck and Poisson equations for a binary electrolyte with spherical geometry are presented. For this system the two current electrodes are assumed to be a point and a hemispherical shell electrode as shown in Figure 4.1. As it is assumed that ion concentrations and current densities depend only on radial distance, so mathematically this is also a one dimensional system as was the planar geometry of Chapter 3. Membranes are flat on scales of the order of a Debye length and in experimental situations the electrodes are normally situated at macroscopic distances from membranes. Ion channels have radii in the order of a nm and it is therefore reasonable (not to mention analytically convenient) to assume that the electric field lines and ion fluxes will follow approximately radial lines outside the channel.

Spherical geometry has also been used by Bers and Peskoff (1991) to calculate the electrodiffusion of calcium ions in a hemisphere of electrolyte solution centred on the external mouth of a cardiac calcium channel. However, unlike the solutions presented in this thesis, their treatment is for the steady state or d.c. ion concentrations and potentials.

Some steady state theories of the electrical properties of ion channels model the channel mouth by a hemisphere of electrolyte solution (see for example Levitt, 1987; Lauser 1976 and Jordan, 1986). Although this

FIGURE 4.1

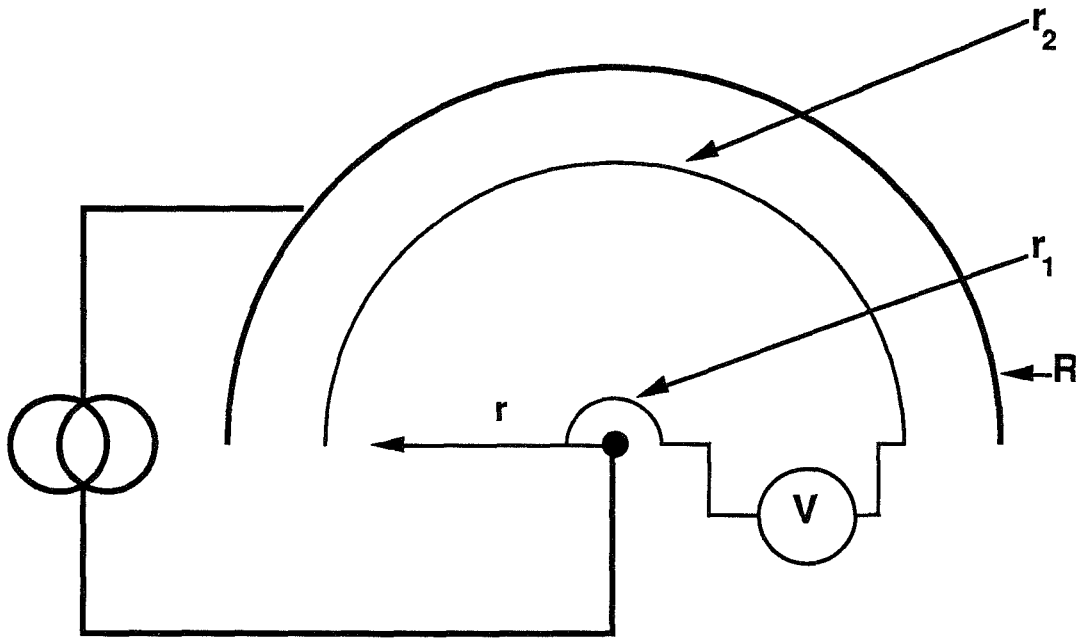


Figure 4.1. The geometry used for the impedance calculations of Chapter 4. The current electrodes are situated at $r=0$ and R , respectively, while the potential and hence impedance is calculated between $r = r_1$ and r_2 .

region has been shown to have significant effects on the total channel conductance in some cases, its influence on a.c. impedance remains undetermined. Solutions for the spherical geometry presented in this chapter may be useful for investigating this effect.

Channel selectivity is thought to be controlled by the narrowest part of the channel, e.g. the acetylcholine receptor channel and sarcoplasmic reticulum K^+ channel both have wide, funnel-shaped outer channel regions which lead to a short narrow section deep inside the pore (Eisenman and Dani, 1987). This may allow the approximation of the pore as a point source or sink used as boundary conditions for this geometry. Note that the infinities in ion concentrations and the potential at the point source do not concern the results calculated for the impedances. As I use four terminal measurements, the potentials at both voltage electrodes are finite and the current has a finite value everywhere over 2π steradians.

Ion flow near biological membranes is, of course, much more complicated than the electrodiffusion model presented here. Current flow near the entrance of the channel is also influenced by effects such as the vicinity of the low dielectric constant of the lipid bilayer (see Chapter 2), the presence any fixed charge on the channel walls and possible steady ion currents. I return to some of these complexities in Chapter 6.

4.2 THEORY

As in Chapter 3, the three dimensional Nernst-Planck equations give the current densities of ions moving under the influence of their concentration gradients and the electric field. For the case where no d.c. electric field is present, the expressions for the a.c. current densities become:

$$\mathbf{J}_p = -q z_p D_p \nabla p + \frac{q^2 z_p^2 D_p P_0 \mathbf{e}}{k T} \quad (4.2.1)$$

$$\mathbf{J}_n = q z_n D_n \nabla n + \frac{q^2 z_n^2 D_n N_0 \mathbf{e}}{k T} \quad (4.2.2)$$

where: $\mathbf{J}_p$ = current density of cations with valency z_p , diffusion constant

D_p and concentration P_0

$\mathbf{J}_n$ = current density of anions with valency z_n , diffusion constant

D_n and concentration N_0

p = a.c. cation concentration

n = a.c. anion concentration

$\mathbf{e}$ = a.c. electric field

q = electron charge

k = Boltzmann's constant and

T = temperature (in Kelvin)

(note characters in bold denote vectors)

Again products of a.c. terms have been discarded, since they are negligibly small, and $z_p P_0 = z_n N_0 = C_0$ = bulk electrolyte concentration.

Poisson's equation relates the electric field to the ion concentrations:

$$\nabla \cdot \mathbf{e} = \frac{q}{\varepsilon} (z_p p - z_n n) \quad (4.2.3)$$

where ε is the permittivity of the medium.

The a.c. continuity equations are given by :

$$q z_p \frac{\partial p}{\partial t} = q z_p j \omega p + \nabla \cdot \mathbf{J}_p = 0 \quad (4.2.4)$$

$$-q z_p \frac{\partial p}{\partial t} = -q z_n j \omega n + \nabla \cdot \mathbf{J}_n = 0 \quad (4.2.5)$$

Combining equations 4.2.1 to 4.2.5 gives :

$$\nabla^2 p - \left(\frac{1}{\lambda_p^2} + \frac{2j}{\xi_p^2} \right) p + \frac{z_n}{z_p \lambda_p^2} n = 0 \quad (4.2.6)$$

$$\nabla^2 n + \frac{z_p}{z_n \lambda_n^2} p - \left(\frac{1}{\lambda_n^2} + \frac{2j}{\xi_n^2} \right) n = 0 \quad (4.2.7)$$

$$\text{where } \lambda_p^2 = \frac{\varepsilon k T}{z_p^2 q^2 P_0}, \quad \lambda_n^2 = \frac{\varepsilon k T}{z_n^2 q^2 N_0}, \quad (4.2.8)$$

$$\text{and } \xi_p = \sqrt{\frac{2D_p}{\omega}} \quad \text{and} \quad \xi_n = \sqrt{\frac{2D_n}{\omega}} \quad (4.2.9)$$

As for Chapter 3, λ_p and λ_n are the "effective" Debye lengths associated with the cations and anions, while ξ_p and ξ_n are the characteristic rms distances travelled via diffusion in the characteristic time $1/\omega$ by the cations and anions, respectively, via diffusion per radian.

The equations for p and n are now solved assuming spherical symmetry, i.e. the equations are now one dimensional.

For spherical symmetry, ∇^2 reduces to $\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 \frac{\partial}{\partial r})$ and equations 4.2.6 and 4.2.7 then have solutions of the form :

$$p(r) = \frac{1}{r} (a_1 e^{m_1 r} + a_2 e^{-m_1 r} + b_1 e^{m_2 r} + b_2 e^{-m_2 r}) \quad (4.2.10)$$

$$n(r) = \frac{1}{r} (a_1 S_1 e^{m_1 r} + a_2 S_1 e^{-m_1 r} + b_1 S_2 e^{m_2 r} + b_2 S_2 e^{-m_2 r}) \quad (4.2.11)$$

$$\text{where: } m_1 = (\eta_1 + \eta_2)^{1/2} \quad (4.2.12)$$

$$m_2 = (\eta_1 - \eta_2)^{1/2} \quad (4.2.13)$$

$$\eta_1 = \frac{1}{2\lambda^2} + j\left(\frac{1}{\xi_p^2} + \frac{1}{\xi_n^2}\right) \quad (4.2.14)$$

$$\eta_2 = \sqrt{\frac{1}{4\lambda^4} - \left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2}\right)^2 + \frac{j(z_p - z_n)\left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2}\right)}{(z_p + z_n)\lambda^2}} \quad (4.2.15)$$

Note: m_i is used here instead of $1/\lambda_i$, $i=1,2$ of Chapter 3.

S_1 and S_2 can be found by substituting the expressions for $p(r)$ and $n(r)$ back in to the differential equations. They simplify to:

$$S_i = [z_p + (z_p + z_n) \lambda^2 \left(\frac{2j}{\xi_p^2} - m_i^2\right)] / z_n \quad i=1,2 \quad (4.2.16)$$

$$\text{where } \lambda^2 = \frac{\epsilon k T}{q^2 (Z_p + z_n) C_0} \quad (4.2.17)$$

The values for the complex coefficients a_i and b_i ($i=1,2$) can be obtained from the boundary conditions at each of the two electrodes.

The electric field can be obtained from Poisson's equation (4.2.3) which for a small a.c signal is :

$$\frac{1}{r^2} \frac{\partial}{\partial r} r^2 e_r = \frac{q}{\epsilon} (z_p p - z_n n) \quad (4.2.18)$$

The electric field can be obtained by integrating equation 4.2.18: :

$$e(r) = \frac{q}{\epsilon r^2} \int r^2 (z_p p - z_n n) dr \quad (4.2.19)$$

substituting equations 4.2.10 and 4.2.11 into equation 4.2.19 gives :

$$\begin{aligned} e(r) = \frac{e_I}{r^2} + \frac{q}{\epsilon r^2} \Big[& (z_p - z_n S_2) \left(\frac{m_2 r - 1}{m_2^2} b_1 e^{m_2 r} - \frac{m_2 r + 1}{m_2^2} b_2 e^{-m_2 r} \right) \\ & + (z_p - z_n S_1) \left(\frac{m_1 r - 1}{m_1^2} a_1 e^{m_1 r} - \frac{m_1 r + 1}{m_1^2} a_2 e^{-m_1 r} \right) \Big] \end{aligned} \quad (4.2.20)$$

where e_I is an integration constant.

Substituting equations 4.2.10 and 4.2.11 into equations 4.2.1 and 4.2.2 gives:

$$\begin{aligned}
J_p(r) = & \frac{q D_p z_p}{r^2} \left[a_1 e^{m_1 r} (1 - m_1 r) + a_2 e^{-m_1 r} (1 + m_1 r) \right. \\
& + b_1 e^{m_2 r} (1 - m_2 r) + b_2 e^{-m_2 r} (1 + m_2 r) \\
& + \frac{1}{(z_p + z_n) \lambda^2} \left\{ (z_p - z_n S_2) \left(\frac{m_2 r - 1}{m_2^2} b_1 e^{m_2 r} - \frac{m_2 r + 1}{m_2^2} b_2 e^{-m_2 r} \right) \right. \\
& + (z_p - z_n S_1) \left(\frac{m_1 r - 1}{m_1^2} a_1 e^{m_1 r} - \frac{m_1 r + 1}{m_1^2} a_2 e^{-m_1 r} \right) \left. \right\} \left. \right] \\
& + \frac{q^2 z_p D_p C_0 e_I}{k T r^2} \tag{4.2.21}
\end{aligned}$$

$$\begin{aligned}
J_n(r) = & \frac{q D_n z_n}{r^2} \left[-S_1 a_1 e^{m_1 r} (1 - m_1 r) - S_1 a_2 e^{-m_1 r} (1 + m_1 r) \right. \\
& - S_2 b_1 e^{m_2 r} (1 - m_2 r) - S_2 b_2 e^{-m_2 r} (1 + m_2 r) \\
& + \frac{1}{(z_p + z_n) \lambda^2} \left\{ (z_p - z_n S_2) \left(\frac{m_2 r - 1}{m_2^2} b_1 e^{m_2 r} - \frac{m_2 r + 1}{m_2^2} b_2 e^{-m_2 r} \right) \right. \\
& + (z_p - z_n S_1) \left(\frac{m_1 r - 1}{m_1^2} a_1 e^{m_1 r} - \frac{m_1 r + 1}{m_1^2} a_2 e^{-m_1 r} \right) \left. \right\} \left. \right] \\
& + \frac{q^2 z_n D_n C_0 e_I}{k T r^2} \tag{4.2.22}
\end{aligned}$$

The total current density is equal to:

$$J_t = J_p + J_n + j \omega \varepsilon e \tag{4.2.23}$$

$\nabla \cdot J_t = 0$, hence the total current $I_t = 4 \pi r^2 J_t$ is independent of r .

Using this, e_I can be found :

$$e_I = \frac{I_t}{2\pi Y_k} \quad (4.2.24)$$

$$\text{where } Y_k = \frac{q^2 C_0}{kT} (z_p D_p + z_n D_n) + j \omega \varepsilon \quad (4.2.25)$$

The electric potential Ψ is given by :

$$\nabla \Psi = -e \quad (4.2.26)$$

For spherical symmetry equation 4.2.26 becomes:

$$\Psi(r) = - \int e(r) \cdot dr \quad (4.2.27)$$

$$\begin{aligned} &= \frac{-q}{\varepsilon r} \left[\frac{z_p - z_n S_2}{m_2^2} (b_1 e^{m_2 r} + b_2 e^{-m_2 r}) + \right. \\ &\quad \left. \frac{z_p - z_n S_1}{m_1^2} (a_1 e^{m_1 r} + a_2 e^{-m_1 r}) \right] + \frac{I_t}{2\pi Y_k r} + \Psi_I \end{aligned} \quad (4.2.28)$$

where Ψ_I is a constant of integration.

The impedance between $r = r_1$ and r_2 (r_1 and $r_2 > 0$) is given by :

$$Z = \frac{\Psi(r_1) - \Psi(r_2)}{I_t} \quad (4.2.29)$$

As for section 3.7, Z can be written as:

$$Z = Z_I + Z_2 + Z_K \quad (4.2.30)$$

$$\text{where : } Z_i = \frac{q}{\varepsilon I_t r} \frac{z_p - z_n S_i}{m_i^2} (b_1 \exp[m_i r_2] + b_2 \exp[-m_i r_2])$$

$$-b_1 \exp[m_i r_1] - b_2 \exp[-m_i r_1]) , i=1,2 \text{ and} \quad (4.2.31)$$

$$Z_K = \frac{1}{2\pi Y_k} \left(\frac{1}{r_1} - \frac{1}{r_2} \right) \quad (4.2.32)$$

The conductance G and capacitance C can be calculated from the real and imaginary part of $1/Z$, respectively:

$$G = \text{Re}\left(\frac{1}{Z}\right) \text{ and } C = \text{Im}\left(\frac{1}{Z}\right)/\omega \quad (4.2.33)$$

Note that Z , G and C are not area specific, unlike those of the preceding chapter, since the area is a function of r for this geometry. Italics are used to distinguish this case.

4.3 BOUNDARY CONDITIONS

The current electrodes are situated at $r = 0$ and $r = R$, and are assumed to be partially blocking (see Figure 4.1). As this system is no longer antisymmetric as was the case in Chapter 3, four coefficients (a_1 , a_2 , b_1 and b_2) rather than only two for planar geometry have to be determined. So boundary conditions at both electrodes must be used:

$$I_p(o) = I_p(R) = t_p I_t \quad (4.3.1)$$

$$I_n(o) = I_n(R) = t_n I_t \quad (4.3.2)$$

where I_p and I_n denote the total cation and anion currents, respectively and t_p and t_n are fractions of the total current I_t carried by the cations and anions at the current electrodes.

The total current is equal to $(2 \pi r^2)$ times the current densities for a hemisphere. Note that the r^2 term in $r^2 J_p(o)$ and $r^2 J_n(o)$ cancels, since both current densities are proportional to $1/r^2$. Therefore:

$$2 \pi r^2 J_p(o) = t_p I_t \quad \text{and} \quad 2 \pi R^2 J_p(R) = t_p I_t \quad (4.3.3)$$

$$2 \pi r^2 J_n(o) = t_n I_t \quad \text{and} \quad 2 \pi R^2 J_n(R) = t_n I_t \quad (4.3.4)$$

Substituting equations 4.2.21 and 4.2.22 into 4.3.3 and 4.3.4 and solving for the complex coefficients a_i and b_i ($i=1,2$) gives:

$$a_1 = -\frac{r_a}{S_1} \frac{-1 + e^{m_1 R} - R m_1}{1 - e^{2m_1 R} + R m_1 + R e^{2m_1 R} m_1} \quad (4.3.5)$$

$$b_1 = \frac{r_b}{S_2} \frac{-1 + e^{m_2 R} - R m_2}{1 - e^{2m_2 R} + R m_2 + R e^{2m_2 R} m_2} \quad (4.3.6)$$

$$a_2 = \frac{r_a}{S_1} - a_1 \quad \text{and} \quad b_2 = -\frac{r_b}{S_2} - b_1 \quad (4.3.7)$$

$$\text{where : } r_a = \frac{k_{1p} k_n - k_{1n} k_p}{k_{1p} k_{2n} - k_{1n} k_{2p}} \quad (4.3.8)$$

$$r_b = \frac{k_{2p} k_n - k_{2n} k_p}{k_{1p} k_{2n} - k_{1n} k_{2p}} \quad (4.3.9)$$

$$k_p = \frac{z_p q C_0 I_t}{k T 2 \pi Y_k} - \frac{t_p I_t}{2 \pi q D_p} \quad (4.3.10)$$

$$k_n = \frac{z_n q C_0 I_t}{k T 2 \pi Y_k} - \frac{t_n I_t}{2 \pi q D_n} \quad (4.3.11)$$

$$k_{1p} = z_p b - \frac{z_p b + z_n}{z_p \lambda_p^2 m_1^2} \quad \text{and}$$

$$k_{2p} = z_p a - \frac{z_p a + z_n}{z_p \lambda_p^2 m_2^2} \quad (4.3.12)$$

$$k_{1n} = z_n - \frac{z_p b + z_n}{z_n \lambda_n^2 m_1^2} \quad \text{and}$$

$$k_{2n} = z_n - \frac{z_p a + z_n}{z_n \lambda_n^2 m_2^2} \quad (4.3.13)$$

$$a = \frac{\lambda_p^2}{2} \left[\frac{1}{\lambda_p^2} - \frac{1}{\lambda_n^2} + 2j \left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2} \right) + \eta_2 \right] \quad (4.3.14)$$

$$b = \frac{\lambda_p^2}{2} \left[\frac{1}{\lambda_p^2} - \frac{1}{\lambda_n^2} + 2j \left(\frac{1}{\xi_p^2} - \frac{1}{\xi_n^2} \right) - \eta_2 \right] \quad (4.3.15)$$

k_p , k_n , k_{ip} and k_{in} are introduced for mathematical convenience only.

k_{ip} and k_{in} simplify to :

$$k_{1p} = \frac{j \omega z_p b}{m_1^2 D_p} \quad \text{and} \quad k_{2p} = \frac{j \omega z_p a}{m_2^2 D_p} \quad (4.3.16)$$

$$k_{1n} = z_p \left(1 - b + \frac{j \omega b}{m_1^2 D_p} \right) \quad \text{and} \quad k_{2n} = z_n \left(1 - a + \frac{j \omega a}{m_2^2 D_p} \right) \quad (4.3.17)$$

As for the binary electrolyte of Chapter 3, the total impedance comprises of three terms: the impedance terms Z_1 and Z_2 which are associated with the characteristic lengths λ_1 ($=1/m_1$) and λ_2 ($=1/m_2$) and the impedance at equilibrium Z_K , i.e. the impedance for no a.c. signal (see equation 4.2.28). λ_1 and λ_2 are the same as for the two ion case of Chapter 3. However, because of the asymmetry of this geometry, the solutions include four coefficients, a_1 , a_2 , b_1 and b_2 rather than the two of

Chapter 3 and the exponential terms $e^{\pm m_i R}$ cannot be combined into a single cosh term, unlike those in the previous chapter.

4.4 HIGH FREQUENCY LIMIT

At high frequencies the ions do not have sufficient time to travel far per cycle, resulting in negligible space charge in the bulk solution and the impedance approaches the equilibrium value, i.e. the value for zero a.c. concentration changes.

For spherical symmetry, the high frequency limit of the capacitance C_K and conductance G_K can be shown to equal:

$$C_K = \frac{2 \pi \epsilon r_1 r_2}{r_2 - r_1} \quad (4.4.1)$$

$$G_K = \frac{2 \pi r_1 r_2 q^2 C_0 (z_p D_p + z_n D_n)}{k T (r_2 - r_1)} \quad (4.4.2)$$

Note that C_K and G_K are not area specific and that in the limit of r_2 approaching infinity, both C_K and G_K are independent of r_2 and proportional to r_1 , as is the case for an ohmic conductor.

4.4.1 Electrical Characteristics of "a Pore in a Membrane" at High Frequencies.

For the case of a pore in a membrane, the impedance of the pore (Z_p) as well as that of the surrounding lipid bilayer (Z_m) has to be considered, in addition to that of the surrounding electrolyte medium (Z_s) as shown in Figure 4.2. At high frequencies, Z_p , Z_m and Z_s can be

FIGURE 4.2

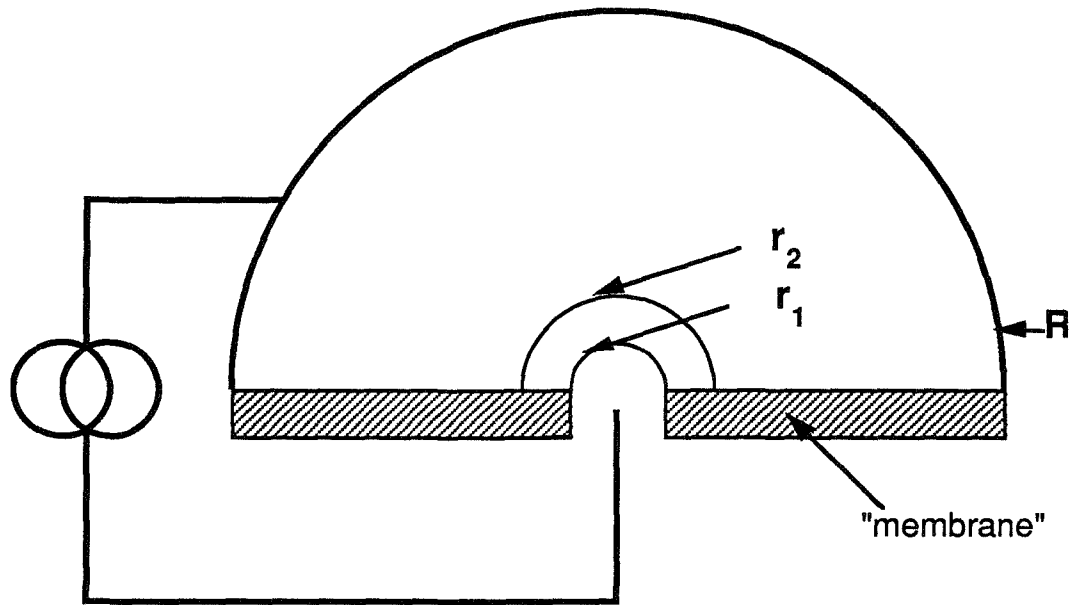


Figure 4.2. The configuration used for the high frequency calculations.

approximated by their geometrical values, since there is negligible space charge in the electrolyte solution. The presence of the bilayer leads to distortions of the electric field near the pore due to the small but finite displacement or polarization current through it, especially at high frequencies. However, even though the impedances of the different regions cannot strictly speaking be added as separate elements, it is still useful to determine their magnitudes at high frequencies to obtain an estimate of the relative importance of the electrolyte at the channel mouth to the total impedance of a pore at frequencies where the conductances of the various components are given by simple expressions. The high frequency limit of the total conductance of a pore and the capacitance of a membrane are now compared to C_K and G_K for a spherical shell of electrolyte solution of inner radius, r_1 equal to the radius of the pore (see equations 4.4.1 and 4.4.2). For these calculations, the pore is approximated by a cylinder of electrolyte solution of concentration C_0 , length d and radius equal to r_1 . Hence the high frequency limit of its conductance is given by:

$$G_p = \frac{q^2 C_0 (z_p D_p + z_n D_n) \pi r_1^2}{k T d} \quad (4.4.3)$$

The ratio of the high frequency limit of the "pore" to the hemispherical shell of electrolyte at the channel mouth (G_p/G_K) is obtained from equations 4.4.2 and 4.4.3:

$$G_p/G_K = \frac{r_1(r_2 - r_1)}{2 d r_2} \quad (4.4.4)$$

Usually $r_2 \gg r_1$, hence the conductance ratio G_p/G_K can be approximated by:

$$G_p/G_K = \frac{r_1}{2d} \quad (4.4.5)$$

Hence, for typical values of pore radii and pore length, G_p is comparable in magnitude to G_K . Table 4.1 gives some typical values of the high frequency limit of the conductance and capacitance of the "pore" and the hemispherical shell of electrolyte as well as typical measured values of the capacitance and conductance of a bilayer. The bilayer capacitance C_b dominates the total capacitance for typical membrane areas.

Because of the awkwardness of the assumptions made in the spherical symmetrical model, the solutions presented in this chapter can only resemble electrical properties of a pore. However, exact algebraic solutions to the electrodiffusion equations appear only to be possible for one dimensional systems. Hence numerical solutions of the Nernst-Planck equations for a channel had to be resorted to. These are discussed in Chapters 5 and 6.

4.5 RESULTS

4.5.1 Comparison of Spherical and Planar Geometries

Again the results are shown as the ratios G_t/G_K and C_t/C_K , where C_t and G_t are the total capacitance and conductance, respectively and C_K and G_K denote the geometrical capacitance and conductance when ionic concentration changes do not occur. C_K and G_K are equal to the high frequency limits of the capacitance and conductance given by equations 4.4.1 and 4.4.2, respectively. At low frequencies Z_I makes a negligible

TABLE 4.1

The high frequency limit of the conductance (G) and capacitance (C) for a cylinder of electrolyte solution of radius r_1 and a hemispherical shell of electrolyte of inner radius r_1 and outer radius r_2 . These values are for a univalent binary electrolyte with diffusion constants D_p and D_n and permittivity ϵ :

$$D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$$

$$D_n = 1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$$

$$\epsilon = 80 \epsilon_0$$

$$r_1 = 1 \text{ nm}$$

$$r_2 \geq 1 \text{ } \mu\text{m}$$

4.1a) The electrolyte concentration: $C_0 = 1 \text{ m M}$:

	$G \text{ (S)}$	$C \text{ (F)}$
Hemispherical shell of electrolyte:	$9.55 \cdot 10^{-11}$	$4.45 \cdot 10^{-18}$
Cylinder of electrolyte of length 5 nm:	$9.55 \cdot 10^{-12}$	$4.45 \cdot 10^{-19}$

4.1b) The electrolyte concentration: $C_0 = 100 \text{ mM}$:

	$G \text{ (S)}$	$C \text{ (F)}$
Hemispherical shell of electrolyte:	$9.55 \cdot 10^{-9}$	$4.45 \cdot 10^{-18}$
Cylinder of electrolyte of length 5 nm:	$9.55 \cdot 10^{-10}$	$4.45 \cdot 10^{-19}$
Measured bilayer values for a circular area of radius = 0.1 mm: (from Smith <i>et. al.</i> , 1984)	$2.1 \cdot 10^{-10}$	$3.1 \cdot 10^{-10}$

contribution to the total impedance (Z_t), calculated many Debye lengths away from the current electrodes, since $\lambda_1 (=1/m_1)$ is equal to the Debye length to first order. The contribution of Z_2 thus dominates Z_t and the variation of G_t/G_K and C_t/C_K with frequency is expected to be similar to that for the binary electrolyte for planar geometry. The differences due to geometrical factors are demonstrated in Figures 4.4. to 4.7 and a comparison to the results of Chapter 3 is given in Appendix 4.1. Even though the calculated capacitances and conductances for spherical geometry are necessarily extensive rather than area-specific, this comparison is still valid since in each case the ratio of total capacitance and conductance to their geometrical values (i.e. to their high frequency limits) for both geometries are calculated.

4.5.2 Results for Small Values of the "Pore" Radius r_1

At low frequencies the contribution of the impedance term associated with the double layer (Z_I) to the total impedance (Z_t) is now considerable. The imaginary part of Z_I , $\text{Im}(Z_I)$, now dominates $\text{Im}(Z_t)$. For the case of perfectly blocking electrodes (i.e. $t_p = t_n = 0$) at low frequencies the total conductance ratio G_t/G_K approaches zero as expected. C_t/C_K again reaches a plateau at low frequencies. This limiting value is, however, much less for impedance calculations far away from both current electrodes (within one order of magnitude of unity), since the large capacitances of the previous section are attributable to diffusion effects (see Figures 4.4 to 4.7). Neither C_t/C_K nor G_t/G_K vary significantly with bulk electrolyte concentration (C_0) nor with r_2 for values of $r_2 \gg r_1$.

For the case of partially blocking electrodes, (i.e. t_p and/or $t_n \neq 0$), diffusion effects again became important. The low frequency limit of the

FIGURE 4.3

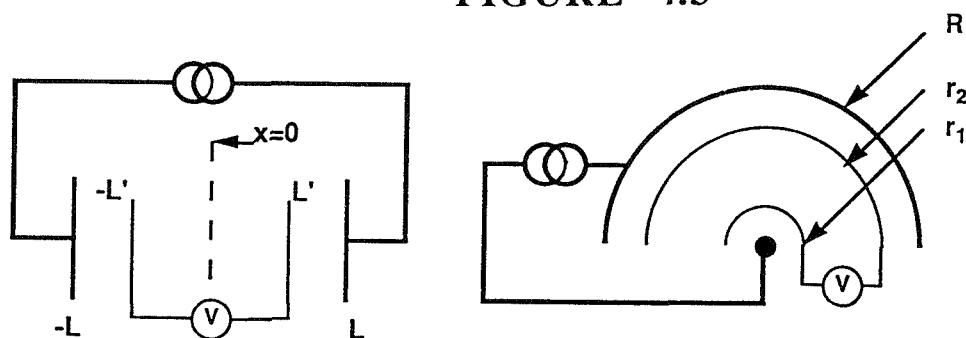


Figure 4.3. The configurations used for the calculations for planar and spherical geometries.

FIGURE 4.4

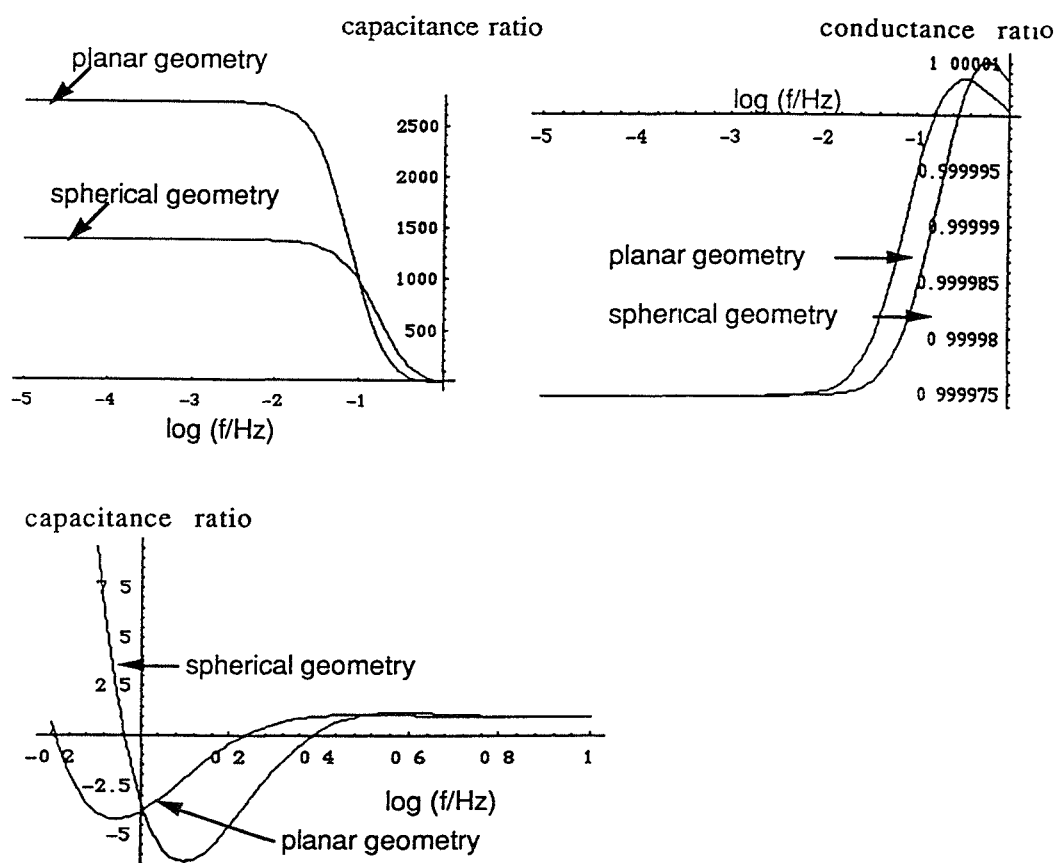


Figure 4.4. The capacitance and conductance ratios for planar and spherical geometries, for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $R = 200 \text{ } \mu\text{m}$, $r_1 = 99 \text{ } \mu\text{m}$, $r_2 = 101 \text{ } \mu\text{m}$, $L = 100 \text{ } \mu\text{m}$ and $L' = 1 \text{ } \mu\text{m}$. The variations in the conductance ratios are too small to be usefully displayed graphically for intermediate frequencies (see Table 4.2 for all other parameters, see Figure 4.3 for the geometry used).

FIGURE 4.5

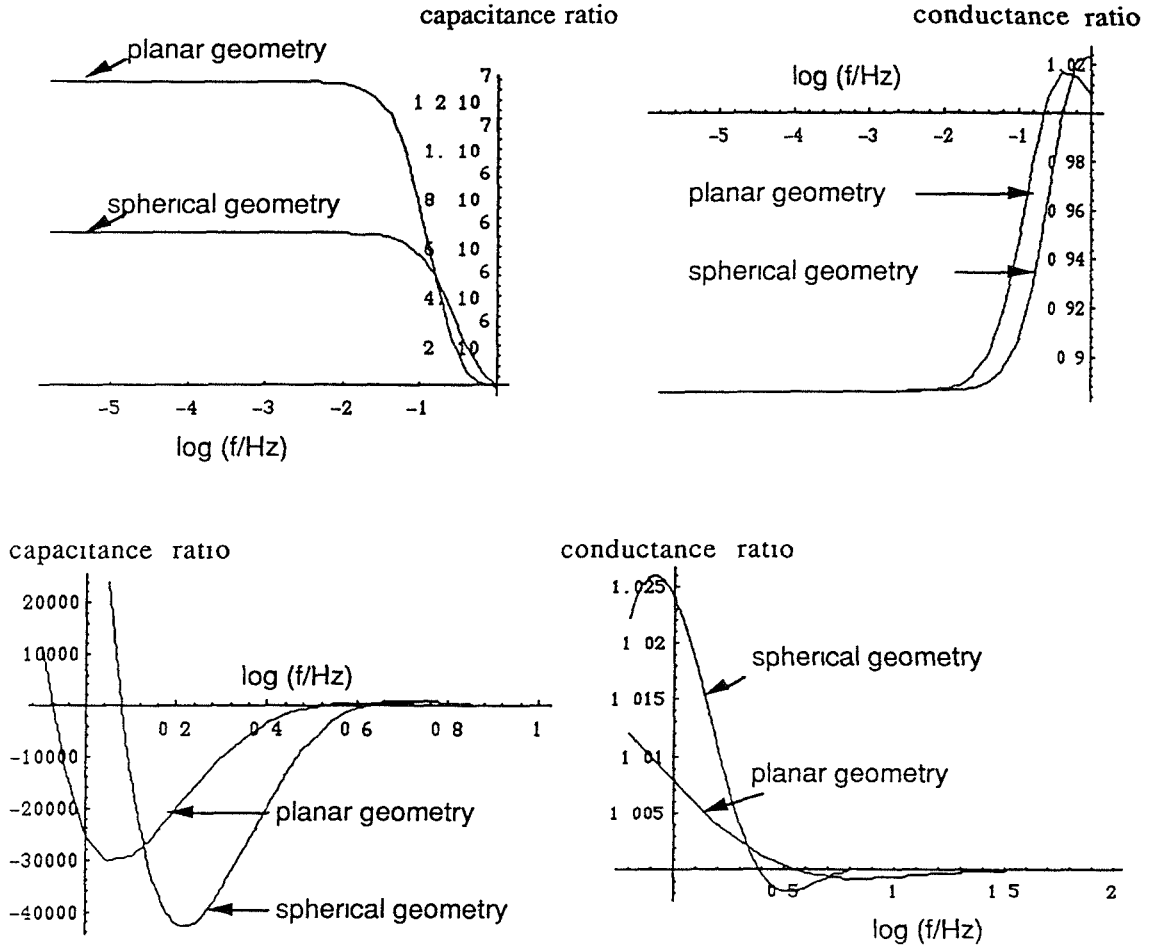


Figure 4.5. A comparison of the capacitance and conductance ratios for planar and spherical geometries, for $D_p = 4 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $R = 200 \text{ } \mu\text{m}$, $r_1 = 99 \text{ } \mu\text{m}$, $r_2 = 101 \text{ } \mu\text{m}$, $L = 100 \text{ } \mu\text{m}$ and $L' = 1 \text{ } \mu\text{m}$ (see Table 4.2 for all other parameters, see Figure 4.3 for the geometry used).

FIGURE 4.6

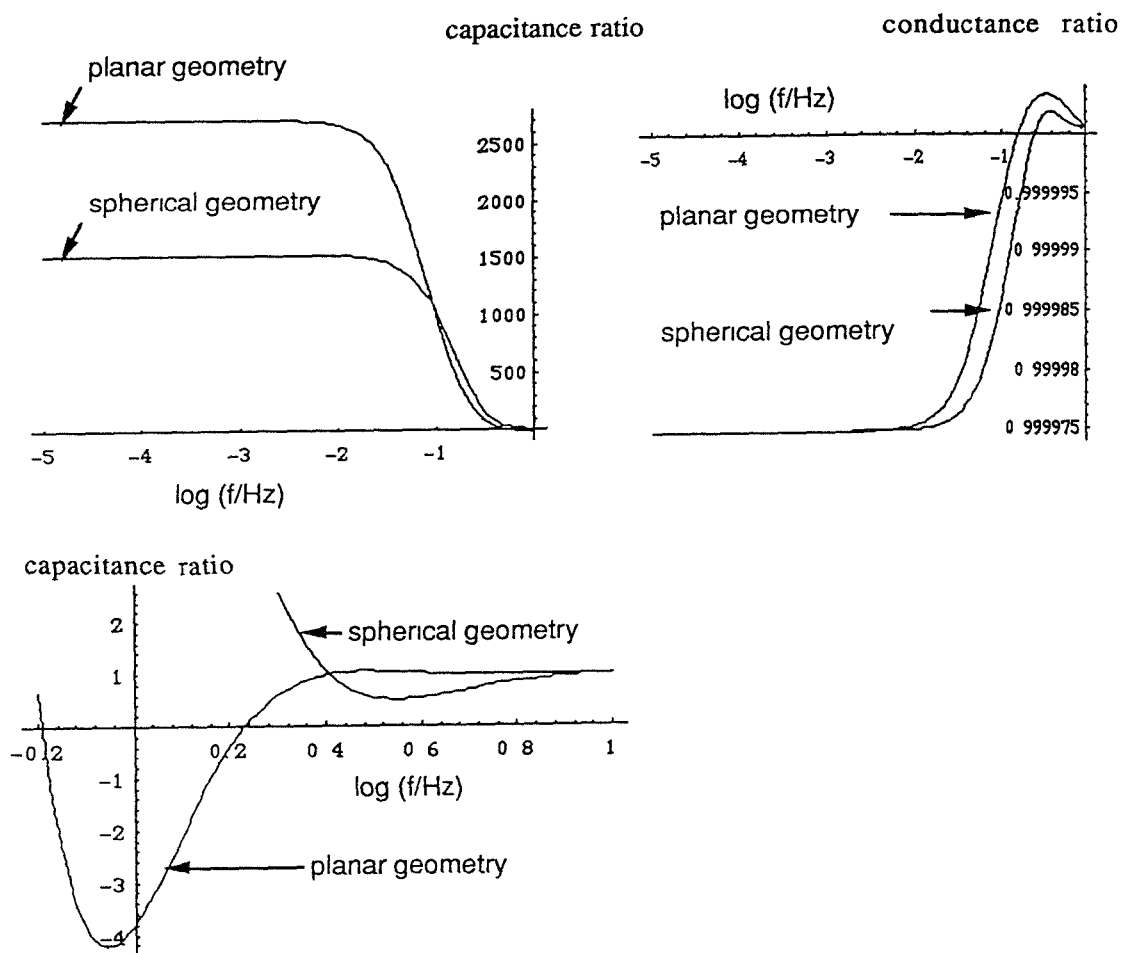


Figure 4.6. The capacitance and conductance ratios for planar and spherical geometries, for $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $R = 200 \text{ } \mu\text{m}$, $r_1 = 149 \text{ } \mu\text{m}$, $r_2 = 151 \text{ } \mu\text{m}$, $L = 100 \text{ } \mu\text{m}$ and $L' = 1 \text{ } \mu\text{m}$. The variations in the conductance ratios are very small - too small to be usefully displayed graphically for intermediate frequencies (see Table 4.2 for all other parameters, see Figure 4.3 for the geometry used).

FIGURE 4.7

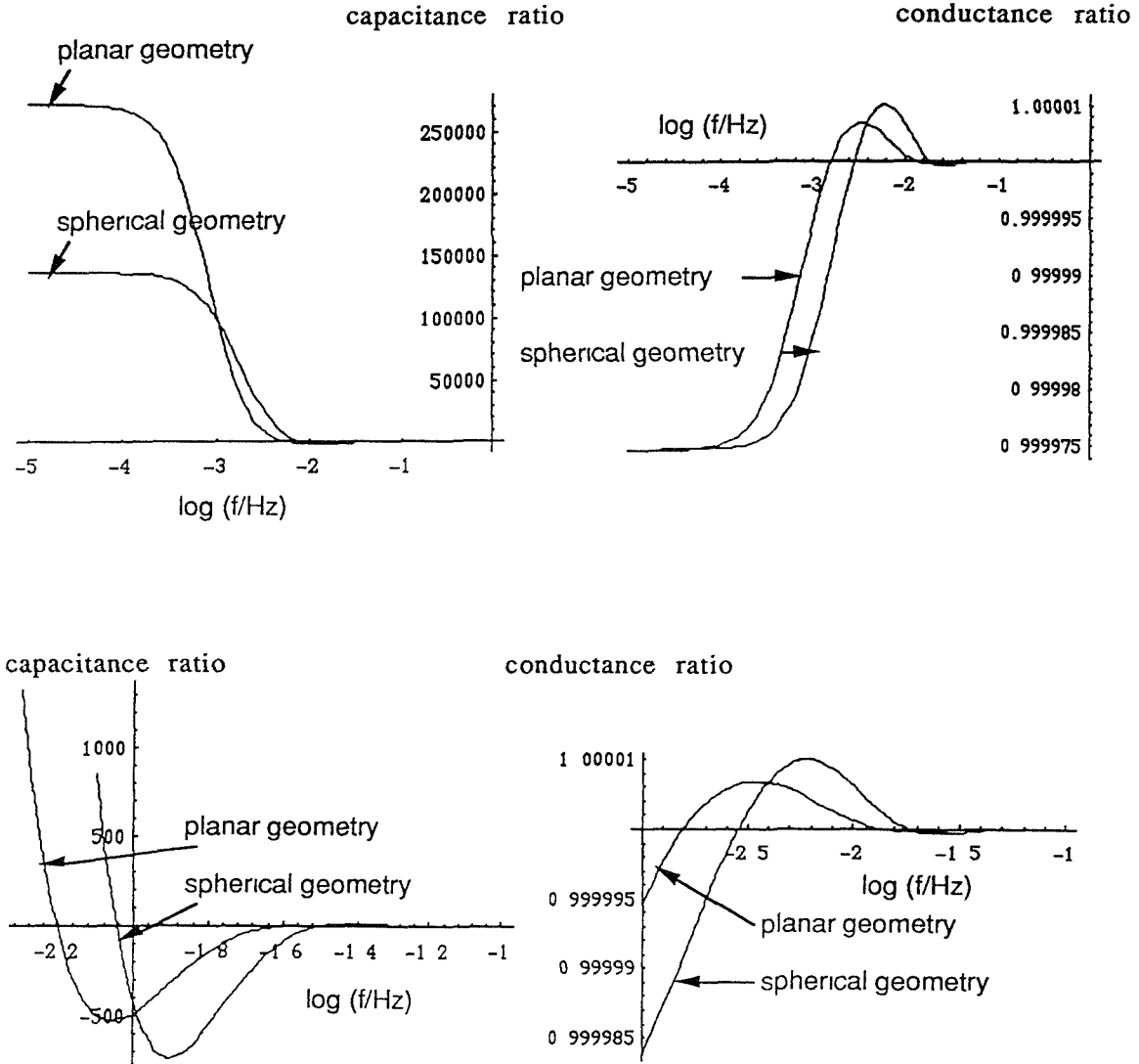


Figure 4.7. The capacitance and conductance ratios for planar and spherical geometries, for $D_p = 2 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$, $R = 2 \text{ mm}$, $r_1 = 999 \text{ }\mu\text{m}$, $r_2 = 1001 \text{ }\mu\text{m}$, $L = 1 \text{ mm}$ and $L' = 1 \text{ }\mu\text{m}$ (see Table 4.2 for all other parameters, see Figure 4.3 for the geometry used).

TABLE 4.2

PARAMETER		VALUE
T	temperature	293 K
D_n	anion diffusion constant	$1.98 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$
ϵ/ϵ_0	dielectric constant of the solution and channel	80
z_n	anion valency	1
z_p	cation valency	1
C_0	bulk electrolyte concentration	2 mM

The current electrodes are assumed to be perfectly blocking for the comparison between spherical and planar geometry.

capacitance ratio C_l/C_K increases with r_2 and is a strong function of t_p and t_n . If the fraction of current carried by the more mobile ion species at the electrodes is greater than that for the slower one, the impedance can become inductive, i.e. the low frequency limit of the C_l/C_K is negative, while for the case when the current electrodes are completely blocking to the more mobile ion only, C_l/C_K is positive (see Figure 4.8 for an example). This is a diffusion effect as discussed in the Chapter 3. However, this case differs from the results presented in Chapter 3, which concentrated on the variation of the impedance resonances as a function of frequency for perfectly blocking electrodes.

This can be explained in two ways. First, recall the analogy with the standing waves in a pipe: blocking electrodes are like closed ends - displacement nodes. Non blocking electrodes are more like anti-nodes. The consequent shifts in the resonance positions when changing from blocking to non blocking electrodes can give rise to inductive behaviour in four terminal measurements. A more physical explanation is as follows: The faster ion drags the slower ion species through the electrolyte against the field. If the cations are more mobile and the current electrodes are completely non blocking to cations, then anions initially accumulate at the electrodes, and start to diffuse away from the current electrode. Since they are negatively charged, the direction of the current remains (briefly) unchanged and the impedance becomes inductive.

As for calculations of Chapter 3, although the diffusion constants and t_p and t_n have a considerable effect on the capacitance, the variation in the conductance ratio is much smaller, since $\text{Re}(Z_l)$ is dominated by $\text{Re}(Z_K)$, except at low bulk electrolyte concentrations. The low frequency conductance is greater than G_K (within one order of magnitude).

FIGURE 4.8

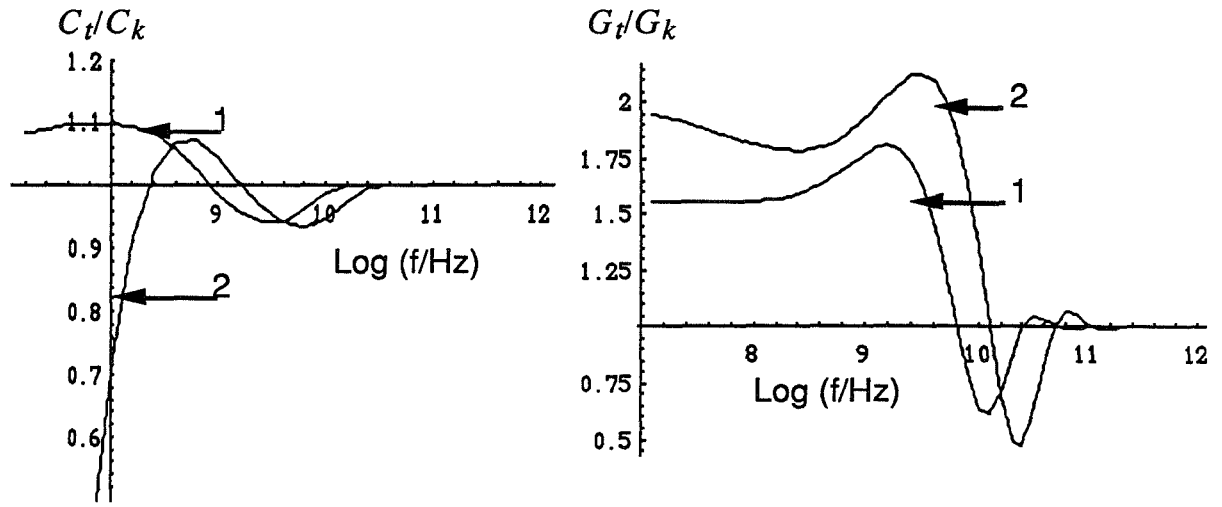


Figure 4.8. The capacitance (C_t/C_k) and conductance (G_t/G_k) ratios of a univalent binary electrolyte of concentration $C_0 = 100$ mM for different cation diffusion constants :

- 1 : $D_p = 2 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$
- 2 : $D_p = 4 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

$R = 200 \text{ } \mu\text{m}$, $r_1 = 1 \text{ nm}$, $r_2 = 1 \text{ } \mu\text{m}$
 $t_p = 1$ and $t_n = 0$.

The low frequency limits of the capacitance and conductance ratios for these parameters are:

C_t/C_k	G_t/G_k
1 : -5.38	1.56
2 : -654.1	2.07

The capacitance and conductance approach their geometrical values at frequencies above the characteristic frequency¹ of the electrolyte solution. G_l/G_K and oscillates about unity at high frequencies below the characteristic frequency. So too does C_l/C_K , although the effect is less spectacular. For partially blocking electrodes the conductance ratio may become negative at these high frequencies, due to resonance effects of solute movement between the current electrodes. The negative conductances occur at very low bulk electrolyte concentrations for which the contribution of Z_K to the total impedance is significantly reduced. This is not however, a perptuum mobile. Recall that this is a 4 terminal impedance, so negative conductance merely means that the potential difference between the voltage electrodes is out of phase with the current. To return to the analogy of the resonant pipe: depending on the choice of placement of two pressure sensors in the pipe, the phase of the pressure difference could be in or out of phase with particle velocity at the reed. Unlike at low frequencies, the two ion species can now essentially move independently (both the real and imaginary part of λ_1 and λ_2 are of the order of the diffusion length of the cations and anions respectively at high frequencies). This effect, as well as the large variations in the total capacitance, may play a role in channel gating which has not been considered in steady state models.

Note that for t_p and/or $t_n = 1$, the outer current electrode (at R) is completely non blocking to either or both ion species. This, regrettably, is

¹The characteristic frequency (f_c) is defined as frequency for which the geometrical conductance (G_K) equals the geometrical capacitance (C_K):

$$f_c = \omega_c/2\pi = G_K/C_K, \text{ where } G_K \text{ and } C_K \text{ are given by equations 4.4.1 and 4.4.2.}$$

only a poor approximation of the current electrodes commonly used for impedance measurements.

For small r_1 and for non blocking electrodes, the total impedance is dominated by a small region surrounding the hemisphere of radius r_1 and the strong radial dependence means that variations in the space charge, and hence in the field near the origin, dominate the behaviour. Consequently the values for t_p and t_n at the outer current electrode have little effect on the impedance. These and other important pore effects may be masked by the double layer impedance of the electrode at R in measurements with only two electrodes.

4.5.3 The Effect of Access Impedance on Membrane Channels

At very low concentrations of the permeant ion, channel conductance is ultimately limited by diffusion to the channel mouth, and the resistance of this region can contribute significantly to the total resistance (Levitt, 1987). This access resistance is usually approximated by the convergence resistance to a hemisphere of electrolyte of radius r_1 equal to the radius of the pore* (Hille, 1968, 1970, as cited by Hall, 1975). The conductance is strongly dependent on r_1 . A similar result has been reported by Levitt (1987 and 1991). The treatment given in this chapter allows the a.c. access resistance to be calculated. The high frequency limit of the conductance of a hemispherical shell of electrolyte, G_K , given by equation 4.4.2, is equivalent to $1/R_h$ for $r_1 \ll r_2$. The pulsed nature of current flow through channels means the a.c. impedance could be more relevant than the d.c. value. In addition to R_g , the resistance of the hemisphere of electrolyte solution at the channel mouth (R_h) also has to be taken into account. The d.c. value of R_h has been found to be up to 60 % of R_g (Hille, 1968, 1970, as cited by Hall, 1975).

* Strictly the convergence radius is equal to pore radius minus ion radius.

4.6 CONCLUSIONS

i) Solutions to the Nernst-Planck and Poisson's equations in spherical geometry include no new impedance terms and they differ from the planar solutions of the previous chapter by the expected geometrical factors (i.e. the radial dependence of the ion concentrations, electric field and current densities).

ii) The calculated values of the impedance differ quantitatively between planar and spherical geometries, thus calculations performed in the simpler planar geometry (e.g. Chapter 3) should at least be qualitatively applicable to other membrane electrolyte geometries in between the two extreme geometries considered in Chapters 3 and 4.

iii) The total conductance of a channel necessarily includes a region of electrolyte solution surrounding the channel mouth. This region extends over a small distance only and the contribution of the electrolyte solution outside this region is small. The contribution to the total channel conductance of this region can become important, especially for channels with a high intrinsic channel conductance and at very low electrolyte concentration, since ion transfer becomes diffusion limited in these cases (Levitt, 1987). The a.c. impedance of this electrolyte has been shown to be a complicated function of frequency.

The exact solutions presented in this chapter cannot deal with other important effects such as fixed charges, which are thought to play a fundamental role in the operation of channels. Accordingly in Chapters 5 and 6 a new approach is used to overcome the restrictions of Chapters 3 and 4. Of necessity, this approach must be numerical, since Nernst-Planck Poisson's equations cannot be solved exactly for these cases.

CHAPTER 5

NUMERICAL SOLUTIONS TO THE NERNST-PLANCK EQUATIONS

5.1 INTRODUCTION

In this chapter a method for solving the Nernst-Planck equations numerically is described, and the advantages and disadvantages of analytical and numerical solutions are discussed.

First numerical solutions for an electrolyte in simple geometry are found. This allows qualitative comparisons with the analytical solutions as such comparisons are not possible for interesting geometries. The simpler geometries provide "controls" for comparison when the order of complexity is increased. In this chapter, the models considered are the case of a disc of insulating membrane and a simple transmembrane pore. In chapters 5 and 6 the term "pore" is used to denote an aqueous hole through an insulating membrane, while "channel" is used for holes with more elaborate structure or features. This choice is arbitrary.

For systems where both analytical and numerical solutions are possible, the analytical calculations are preferable to numerical ones, unless they prove to be so complex that their implementation becomes difficult. Analytical solutions have the following advantages:

- 1) They introduce no errors by differentiation,
- 2) In some cases with simple geometry, i.e. systems with a high degree of spatial and temporal symmetry they are often faster (always assuming they are possible),
- 3) They give arbitrary time and spatial resolution.

However when substituting values for the various parameters to obtain numerical results, care has to be taken to allow for enough precision, otherwise numerical answers for some values of the parameters may become meaningless. For example, the analytical solutions described in Chapter 3 involve matrix inversions, which can lead to loss of precision due to rounding errors associated with each type of computer.

Analytical solutions for simple electrolyte systems in only one dimension are complicated, as was shown in Chapters 3 and 4. It is extremely difficult to find analytical solutions to the three dimensional Nernst-Planck equations.

Consequently, many three dimensional systems can only be solved numerically. These calculations have the advantage of yielding solutions for the arbitrary geometries and boundary conditions that need to be considered for modelling the small signal response of an ion channel in a membrane surrounded by an electrolyte solution.

Numerical solutions have finite temporal and spatial resolution, as well as limited parameter space for stability. The new values of the variables for each iteration depend on the accuracy of the values of the previous iteration. In this model the ion concentrations for each iteration are calculated from the ionic current of the previous iteration from the continuity equations: the rate of change of ion concentrations in each finite volume element, or voxel is equal to the total ionic current entering or leaving it. This can be written as:

$$\Delta q_p = \Delta t \Delta I_p \quad (5.1.1)$$

where: Δq_p is the change in total charge carried by the ion species in each voxel,

Δt is the time step between consecutive iterations and

ΔI_p is the total ion current entering or leaving the voxel, i.e. each unit element of volume.

For stability, $\Delta t \Delta I_p$ has to be very small compared to the total charge in each voxel, otherwise the concentrations are forced to vary too quickly with time and the calculations diverge ($\Delta q_p / \Delta I_p < 10^{-10} \text{s}$, for the parameters chosen for this thesis). Since the thickness of membranes and the diameter of ion channels are typically of the order of nm, the voxel size also should be smaller than these dimensions. Hence the total charge carried by each ion species in each voxel is small. This limits the time interval between consecutive iterations to very small values and since Δt is inversely proportional to frequency, the criterion for stability limits the lowest frequency feasible for finite calculation times. The total number of voxels is limited by memory size and iteration time which is proportional to the square of the number of voxels for the method used.

The computational power available means that the numerical calculations described here are a feasibility study that gives an indication of the precision and iteration step size that are required. Strong assumptions and crude approximations are used, so the purpose of these calculations illustrate qualitatively and semi-quantitatively the behaviour of the system. Even though these calculations take a long time on the Macintosh IIfx used throughout this thesis, in future larger and faster machines could be employed to allow greater resolution calculations in shorter time periods. Due to these limitations a quantitative comparison with experimental results would be premature and will not be given in this thesis. Not only the computational methods are immature, the detailed

structure of channels is still poorly known. In the future, the method described here could be used to calculate the electrical properties of ion channels as their structures are determined. Nevertheless, the current study is expected to show the qualitative behaviour and give order of magnitude calculations of the important effects in real membrane channels.

5.2 METHODS

An ion channel in a uniform dielectric slab, immersed in a binary fully dissociated electrolyte solution with the same dielectric constant is modelled in the following manner. The response of this system to a small applied a.c. signal is considered. The boundary conditions chosen are as follows. A uniform surface charge density $\sigma_s(t)$, which varies sinusoidally with time, is assumed to be situated at opposite ends of the chamber. The region between the boundaries is divided into finite elements or voxels. Constrictions or ion channels are introduced by blocking ion current flow completely (or partially) into the appropriate group of voxels, i.e. setting the ion current densities equal to zero (or some small fraction) at the channel boundaries. Note that since the electric field is continuous through the blocked region, displacement current (i.e. the current due to time varying polarization charges at the interface) is still allowed in this region.

In practice electrodes are conductors, hence equipotential lines would be a more realistic boundary condition, rather than assuming a uniform charge density at the boundaries. However there are problems with this.

One could obtain the potential directly from Poisson's equation. This however, leads to second order differential equations or involves a double

integration, which makes convergence much more difficult. In two dimensions, the equations involve x and y (or r and z components) of the electric field. These are difficult to adjust independently in order to keep the total potential between the two boundaries constant. Changes in the electric field lead to changes in currents and ion concentration. During further iterations all variables eventually reach a steady a.c. value (i.e. the transients have decayed away). Having to converge on a constant potential difference between the two boundaries would add an order of magnitude of difficulty to the problem. Hence one needs to be content with using uniform surface charge density as a boundary condition. Fortunately for the interesting geometries, the equipotential lines are nearly parallel near the electrodes, so the difference is small - smaller indeed than that introduced by the quantization in finite elements.

Two obvious methods are possible for calculating the electric field: i) calculate the potential V in each voxel and obtain the electric field (e) from the gradient of V or ii) calculate e directly from the charge distribution and obtain the potential V by integration.

Method ii) has been chosen for the system described here to determine the electric field at each voxel surface. V can then be obtained by numerical integration of e . There are obvious reasons for preferring integration to differentiation in numerical calculations.

V is assumed to vary linearly with position throughout each voxel. This may not be a valid approximation, especially in the double layer near the charged boundaries, where V and e vary rapidly with distance (y or z) and where available memory has constrained me to use sizes which are not much smaller than the Debye length.

Both method i) and ii) introduce quantization errors - the error of setting the cord equal to the curve and of taking average values. In method 2, they produce a "noise" current which is not necessarily zero at

"equilibrium". The size of this error depends on the size of the applied charge (a.c. case) as well as on the nonlinearity of V , as discussed later.

It is assumed that a sinusoidally varying charge density $\sigma_s(t)$ of small amplitude σ_0 and angular frequency ω is applied, i.e.

$$\sigma_s(t) = \pm \sigma_0 \sin(\omega t) \quad (5.2.1)$$

This charge density produces a current density $J_t(t)$ given by:

$$J_t(t) = J_{t0} \cos(\omega t) = \omega \sigma_0 \cos(\omega t) \quad (5.2.2)$$

The continuity equations give the variation in time of the ion concentrations :

$$\nabla \cdot \mathbf{J}_p = -q z_p \frac{\partial p}{\partial t} \quad \text{and} \quad \nabla \cdot \mathbf{J}_n = q z_n \frac{\partial n}{\partial t} \quad (5.2.3)$$

The Nernst-Planck equations are used to calculate the cation and anion current densities from the ion concentrations and the electric field :

$$\mathbf{J}_p = -q z_p D_p \nabla p + q^2 z_p^2 D_p P_0 \mathbf{e}/kT \quad (5.2.4)$$

$$\mathbf{J}_n = -q z_n D_n \nabla n + q^2 z_n^2 D_n N_0 \mathbf{e}/kT \quad (5.2.5)$$

where: p is the a.c. cation concentration

n is the a.c. anion concentration

D_p is the cation diffusion constant

D_n is the anion diffusion constant

z_p is the cation valency

z_n is the anion valency

P_0 is the cation concentration for zero current

N_0 is the anion concentration for zero current

q is the electron charge.

Each a.c. cycle is divided into n_T time iterations, hence the time interval between iterations (Δt) equals $2\pi / (\omega n_T)$. Initial values of the ion concentrations and a.c. ion current densities are chosen. The new a.c. ion concentrations can then be calculated from the finite difference version of the continuity equations. (see 5.2.1-5.2.3)

The whole procedure is iterated until the solution converges (i.e. the concentrations, electric field and ion currents vary sinusoidally with time with constant amplitude and phase). The amplitude and phase of all a.c. variables can be determined by fitting a sine wave to their variation with time (the sine wave fitter was developed by T. Chilcott and J.R. Smith).

This method is applied to the following two systems. A simple case is first considered where rectangular geometry is assumed, i.e the channels are either rectangular holes or constrictions of infinite width in the z (third) dimension. This provides a geometry where the numerical solutions can be compared to the analytical ones. The second system uses cylindrical coordinates, since pores have an approximately circular cross-sectional area.

In experiments the electrodes are typically spaced at distances of μm or more, thus a large number of voxels are required to cover these distances, provided the voxels are equally spaced between the two boundaries. However the important variations in ion concentrations, electric field and ion currents presumably occur close to the ion channel and near the system boundaries. Reducing the total number of voxels between the two boundaries, will not introduce a significant error, provided that the voxel size is kept small enough to show these variations

(of the order of nm or less). All calculations described later in this chapter are for relatively few voxels in both the x and y, or r and z directions.

The electric field is due to the vector sum of the space charge in every voxel, hence the array size for these calculations is proportional to the square of the total number of voxels, again making calculations for many voxels lengthy.

Analytical one dimensional solutions for an electrolyte solution between two infinite electrodes as discussed in Chapter 3, can be compared to the corresponding numerical calculations for both rectangular and cylindrical geometry for the case where the region between the charged boundaries contains only electrolyte solution. However there are important differences between the one dimensional system and the numerical calculations. These include:

- 1) The dielectric/electrolyte system for the numerical solutions is now of finite width and therefore the electric field and ion currents are not everywhere parallel to the central axis
- 2) Both the applied charge on the boundaries and the a.c space charge of the electrolyte solution are assumed to be discrete.

Due to these differences, the expected agreement between the analytical solutions and the numerical calculations discussed later in this chapter is only approximate.

Both systems (rectangular and cylindrical) assume that the dielectric constant of the membrane is equal to that of the liquid electrolyte. However the dielectric constant of lipid bilayers and cell membranes is typically of the order 2 to 5, while that of water is approximately 80. This dielectric continuity alters the self energy of the ions in its vicinity and induces a polarizations charge at the interface, which give rise to

"image forces". For simplicity any effects due the dielectric discontinuity in these initial calculations are ignored. The channels are also assumed to be uncharged. Surface charges can significantly alter the current flow of the two ion species (i.e. increase one and reduce the other). Both the presence of fixed charge and the effect of image forces will be dealt with in Chapter 6. Note that the assumption of uniform dielectric constant has also been used in other methods of calculating the electrical properties of electrolytes (e.g. Lamperski, 1991 has made this assumption in his Monte Carlo simulation of the properties of solvent molecules adjacent to charged interfaces).

5.2.1 Rectangular Geometry

The dielectric/electrolyte system is assumed to be two dimensional, i.e. it extends infinitely in the z direction. Two infinite strips of width L_{tx} , carrying a uniform surface charge per unit length $\Lambda_s(t)$ are situated at $y = 0$ and L_{ty} . The region between these boundaries is divided into n_x by n_y voxels of dimension h_x and h_y in the x and y direction respectively, as shown in Figure 5.1. The concentrations are calculated in the centre of each voxel, whereas the x and y components (e_x and e_y) of the electric field (e) and cation and anion current densities (J_{px} , J_{py} , J_{nx} and J_{ny}) are calculated at the voxel boundaries.

The a.c. ion concentrations at time $t+\Delta t$ in voxel ij are given by :

$$p_{ij}(t+\Delta t) = -\frac{\Delta t}{q z_p} [(J_{py} i(j+1)(t) - J_{py} ij(t))/h_y + (J_{px}(i+1)j(t) - J_{px} ij(t))/h_x] + p_{ij}(t) \quad (5.2.6)$$

FIGURE 5.1

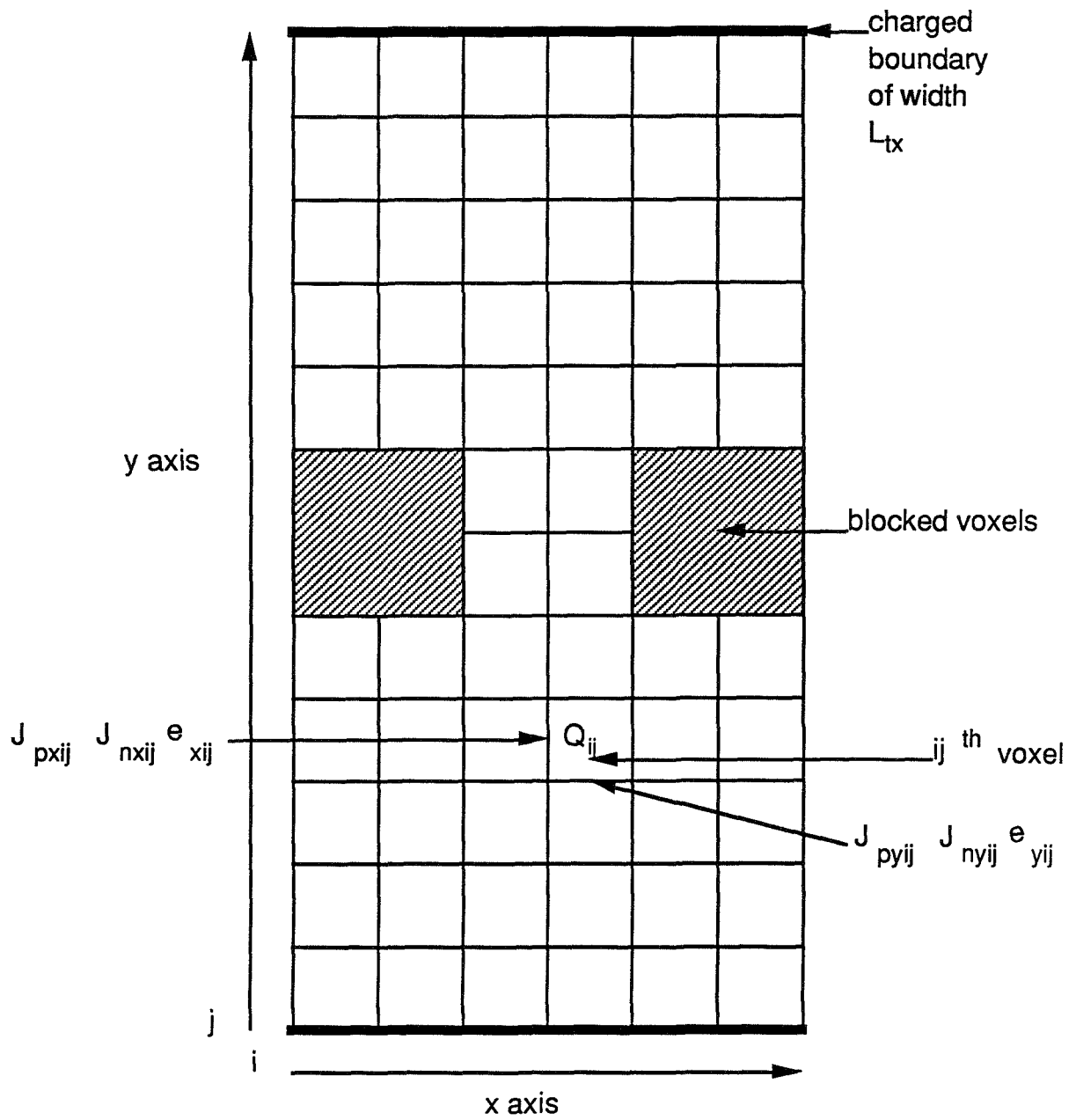


Figure 5.1. The geometry used for the numerical calculations using rectangular coordinates.

$$n_{ij}(t+\Delta t) = -\frac{\Delta t}{q z_n} [(J_{ny} i(j+1)(t) - J_{ny} ij(t))/h_y + (J_{nx}(i+1)j(t) - J_{nx} ij(t))/h_x] + n_{ij}(t) \quad (5.2.7)$$

When calculating the electric field at the voxel boundaries the space charge in the electrolyte and the surface charge at the boundaries are treated as lines of charge located at the centre of each voxel. Hence the x and y components of the electric field at point P due to the ij^{th} voxel are given by (see Figure 5.2) :

$$e_x = \frac{Q_{ij} x}{2 \pi \epsilon r^2} \quad \text{and} \quad e_y = \frac{Q_{ij} y}{2 \pi \epsilon r^2} \quad (5.2.8)$$

where: ϵ is the permittivity of the electrolyte and

Q_{ij} is the charge in the ij^{th} voxel and is given by:

$$Q_{ij} = (z_p p - z_n n) h_x h_y. \quad (5.2.9)$$

Q_i due to the surface charge $\Lambda_s(t)$ at the boundaries at time t is given by:

$$Q_i = \pm \Lambda_s(t) h_x, \quad \text{for } i=1 \text{ to } n_x \quad (5.2.10)$$

FIGURE 5.2

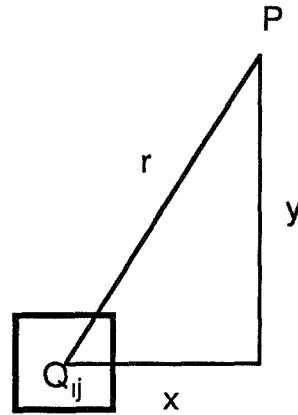


Figure 5.2. The geometry used for the electric field calculation at point P.

The total electric field at each voxel boundary can then be obtained by summing over all voxels, including the surface charge on both boundaries. These new values are then substituted into the following equations to obtain the new values of cation and anion current densities.

$$J_{pyi(j+1)} = qz_p D_p \left\{ \frac{p_{ij} - p_{i(j+1)}}{h_y} + \frac{qz_p}{2kT} e_{yi(j+1)} (p_{ij} + p_{i(j+1)} + 2P_0) \right\} \quad (5.2.11)$$

$$J_{nyi(j+1)} = qz_n D_n \left\{ \frac{n_{i(j+1)} - n_{ij}}{h_y} + \frac{qz_n}{2kT} e_{yi(j+1)} (n_{ij} + n_{i(j+1)} + 2N_0) \right\} \quad (5.2.12)$$

$$J_{px(i+1)j} = qz_p D_p \left\{ \frac{p_{ij} - p_{(i+1)j}}{h_x} + \frac{qz_p}{2kT} e_{x(i+1)j} (p_{ij} + p_{(i+1)j} + 2P_0) \right\} \quad (5.2.13)$$

$$J_{nx(i+1)j} = qz_n D_n \left\{ \frac{n_{(i+1)j} - n_{ij}}{h_x} + \frac{qz_n}{2kT} e_{x(i+1)j} (n_{ij} + n_{(i+1)j} + 2N_0) \right\} \quad (5.2.14)$$

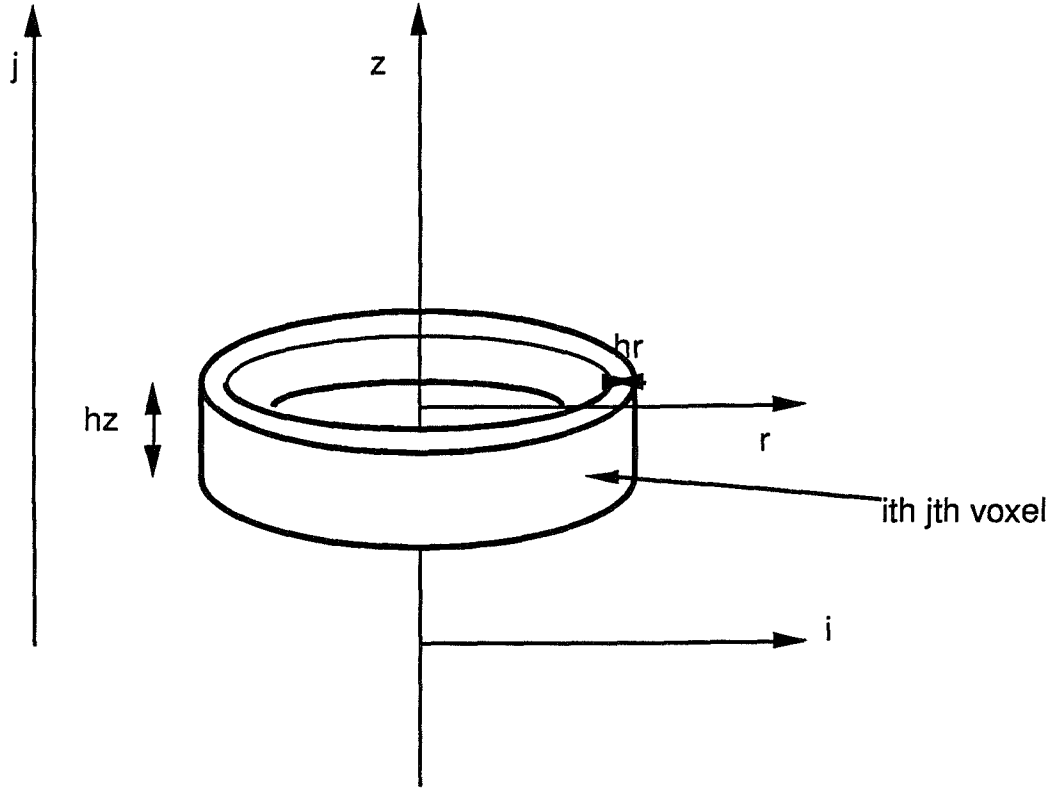
Note : The y component of the electric field need not be calculated at the y boundaries, since the ion current is set equal to zero at the boundaries.

5.2.2 Cylindrical Geometry

Channels through biological membranes resemble cylindrical holes more closely than wide rectangular slabs. So the finite difference Nernst-Planck equations are now solved using cylindrical geometry.

The voxels are now concentric annular prisms of thickness h_r in the radial direction and h_z in the z direction. Two circular charged boundaries are situated at $z = 0$ and L_t . There are n_r voxels in the radial direction, and n_z sets of such voxels along the z axis (see Figure 5.3).

FIGURE 5.3

Figure 5.3. An illustration of the ij^{th} voxel.

As before, the r and z components (e_r and e_z) of the electric field (e) and cation and anion current densities (J_{pr} , J_{pz} , J_{nr} and J_{nz}) are defined at the centre of the four surfaces of each voxel. However the a.c. ion concentrations are calculated at an area weighted centre.

The total a.c. cation and anion current entering or leaving each voxel give the change with time of the a.c. cation and anion concentrations and hence the new concentrations at time $t+\Delta t$ are given by:

$$p_{ij}(t+\Delta t) = -\frac{\Delta t}{qz_p} [\{J_{pz} i(j+1)(t) - J_{pz} ij(t)\}/h_z +$$

$$\frac{2}{r_{i+1}^2 - r_i^2} \{r_{i+1} J_{pr(i+1)j}(t) - r_i J_{pr ij}(t)\} + p_{ij}(t) \quad (5.2.15)$$

$$n_{ij}(t+\Delta t) = \frac{\Delta t}{qz_n} [\{J_{nz i(j+1)}(t) - J_{nz ij}(t)\}/h_z + \frac{2}{r_{i+1}^2 - r_i^2} \{r_{i+1} J_{nr(i+1)j}(t) - r_i J_{nr ij}(t)\}] + n_{ij}(t) \quad (5.2.16)$$

The electric field at each voxel surface due to the ions in the electrolyte and the surface charge on the boundaries is determined in the following way.

The radial and axial (z) components of the electric field (e_r and e_z respectively) due to a ring of charge with linear charge density Λ at a point A can be shown to equal:

$$e_r = \frac{\Lambda}{4\sqrt{2} \pi \epsilon_0 \epsilon \sqrt{R} \sqrt{r}} \int_0^\pi \frac{d\theta}{(\alpha - \cos\theta)^{3/2}} - \frac{\Lambda \sqrt{R}}{4\sqrt{2} \pi \epsilon_0 \epsilon r^{3/2}} \int_0^\pi \frac{d\theta \cos\theta}{(\alpha - \cos\theta)^{3/2}} \quad (5.2.17)$$

$$e_z = \frac{\Lambda z}{4\sqrt{2} \pi \epsilon_0 \epsilon R^{1/2} r^{3/2}} \int_0^\pi \frac{d\theta}{(\alpha - \cos\theta)^{3/2}} \quad (5.2.18)$$

$$\text{where } \alpha = \frac{R^2 + r^2 + z^2}{2 R r} \quad (5.2.19)$$

$\Lambda = \frac{Q_{ij}}{2 \pi R_i}$ where Q_{ij} is the total charge in the ij^{th} voxel and R_i is the radius where this charge is assumed to be located.

This is shown diagrammatically in Figure 5.4.

FIGURE 5.4

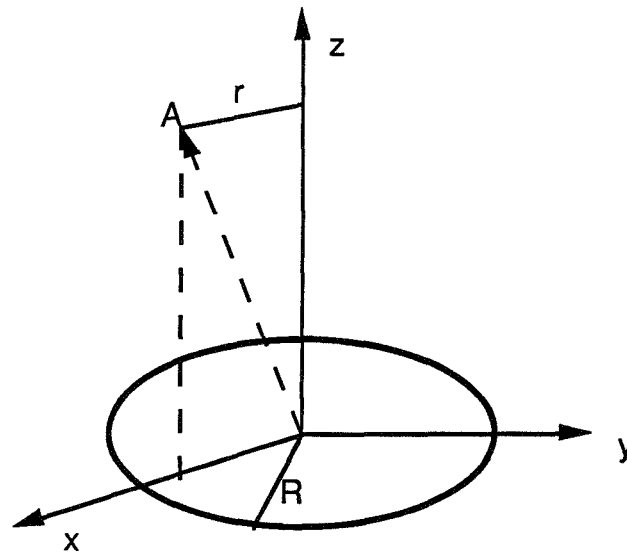


Figure 5.4. The geometry used for calculating the electric field due to each voxel.

Since the cross-sectional area of a cylindrical shell increases with r , R_i is taken to be the radius at half the cross-sectional area:

$$R_i = \frac{1}{\sqrt{2}} \sqrt{r_i^2 + r_{i+1}^2} \quad (5.2.20)$$

This choice of R_i becomes important for non-negligibly small radial voxel thicknesses. Q_{ij} due to the space charge in the electrolyte solution is given by :

$$Q_{ij} = q (z_p p - z_n n) (r_{i+1}^2 - r_i^2) \pi h_z \quad (5.2.21)$$

where r_i and r_{i+1} are the inner and outer voxel radii respectively.

Q_i due to the surface charge $\sigma_s(t)$ at the boundaries at time t is given by:

$$Q_i = \sigma_s(t) \pi (r_{i+1}^2 - r_i^2) \quad (5.2.22)$$

The integrals with respect to θ of equations 5.2.17 and 5.2.18 are calculated numerically for appropriate values of n_r , n_z and ratio h_z/h_r .

The new a.c. ion current densities can then be calculated from these values for the a.c. ion concentrations and electric field :

$$J_{pzi(j+1)} = qz_p D_p \left\{ \frac{p_{ij} - p_{i(j+1)}}{h_z} + \frac{qz_p}{2kT} e_{zi(j+1)} (p_{ij} + p_{i(j+1)} + 2P_0) \right\} \quad (5.2.23)$$

$$J_{nzi(j+1)} = qz_n D_n \left\{ \frac{n_{ij} - n_{i(j+1)}}{h_z} + \frac{qz_n}{2kT} e_{zi(j+1)} (n_{ij} + n_{i(j+1)} + 2P_0) \right\} \quad (5.2.24)$$

$$J_{pr(i+1)j} = qz_p D_p \left\{ \frac{p_{ij} - p_{(i+1)j}}{h_r} + \frac{qz_p}{2kT} e_{r(i+1)j} (p_{ij} + p_{(i+1)j} + 2N_0) \right\} \quad (5.2.25)$$

$$J_{nr(i+1)j} = qz_n D_n \left\{ \frac{n(i+1)j - n_{ij}}{h_r} + \frac{qz_n}{2kT} e_{r(i+1)j} (n_{ij} + n(i+1)j + 2N_0) \right\} \quad (5.2.26)$$

(J_{pr} and $J_{nr} = 0$ along the central axis).

5.2.3 Summary of the Iteration Procedure

An iterative procedure has been used to solve the Nernst-Planck equations numerically. The main steps are outlined below:

- 1) The ion concentrations are set equal to the equilibrium, i.e. bulk electrolyte, concentrations. The cation and anion current densities through the unblocked region are set equal to half of the applied current densities.
- 2) The new ion concentrations are calculated from the divergence of the ion current densities.
- 3) The electric field at each voxel boundary is calculated from the sum of the electric field due to the space charge in each voxel plus the contribution from the charged boundaries.
- 4) These values of cation and anion concentrations and electric field are then used to determine new values of the cation and anion current densities at the voxel boundaries from the Nernst-Planck equations.
- 5) The applied charge density ($\sigma_s(t)$) at the boundaries is set equal to the value for the next time step, i.e. $\sigma_s(t+\Delta t) = \sigma_0 \cos(t+\Delta t)$, where Δt is the time increment.
- 6) Steps 2 to 5 are then repeated until all transients have become negligibly small for one half cycle.

5.3 RESULTS

In this section the results of the numerical calculations for different geometries in order of increasing complexity will be presented. The effects of quantization as well as limitations on voxel size and total voxel number are also discussed. For the simpler configurations, the results are compared qualitatively to the analytical solutions.

All the cases described are cylinders of electrolyte with various materials and internal geometries between two small circular boundaries (e.g. Figure 5.14), except for section 5.3.5, where a comparison is made with analytical solutions. The electric field is imposed by a sinusoidally varying uniform surface charge density on both plates. Both these and the cylindrical boundary are perfectly blocking i.e. at these points the ionic current is set equal to zero. Even though using equipotentials as boundary conditions might be more appealing due to the problems discussed earlier, other methods are preferred.

Simpler configurations are considered first to serve as "controls" before the level of complexity is increased. These may show the peculiarities of each new feature such as completely blocking the ion flow in all or the outer voxels in the central plane, simulating a membrane or a simple pore through a membrane (see Figures 5.11 and 5.14).

Note that when varying the frequency (f), the amplitude of the total applied current density (J_{t0}) is kept constant rather than that of the applied charge density (σ_0) on the plates. They are related by $\sigma_0 = J_{t0}/(2\pi f)$. σ_0 for the high frequency (f_h) calculations is lower than that for low frequency (f_l) calculations by a factor of f_l/f_h and hence the amplitude of the ion concentrations and the electric field at high frequencies can be expected to also decrease by approximately the same factor.

In each case reported the logistics of obtaining a solution are described. All calculations were carried out on a Macintosh IIfx, with a clockspeed of 40 MHz and memory size of 8 Mbytes. This meant that calculations required, approximately 5 minutes for every 100 iterations. For the high frequency calculations 1000 iterations per cycle were been used (this gives time steps between iteration = 10^{-11} s for a frequency of 100 MHz). All transients decay to negligible levels after a few cycles. For all floating point variables and calculations 20 digits precision was used. Several tests were been performed at higher precision to check for round off errors. In "real life", time steps are of the order of 10^{-12} s ($\approx$ typical collision time of water molecules) and "voxel size" of the order of 10^{-11} m ($\approx$ distance between water molecules). As explained earlier, convergence depends on the time step between iterations. The iteration step size (Δt) has been varied for the different frequencies to achieve convergence. Δt is upper bound, since the calculations diverge if Δt is too large, and the values of Δt chosen for the calculations in this thesis are not greatly less than maximum. The low frequency calculations required more iterations per cycle for convergence due to the much lower frequency and hence 24000 iterations per cycle were used in that situation.

5.3.1 A Nonconducting Solvent

The medium between the boundaries contains only non-conducting solvent. For this configuration, calculations of the electric field due to the applied charge at the boundaries only (i.e. in the absence of space charge) are investigated. Although this is not a very realistic model, since even water molecules form hydrogen bonds and partially dissociate, it still serves as a starting point for this analysis, from which further levels of

complexity will be introduced. Complications such as water being a nonlinear dielectric at the high fields obtained in the double layer have been neglected.

In this case, the solvent is by hypothesis an insulator, so there is no rearrangement of charge and only one iteration is required for the electric field calculations. The calculation only takes seconds and is essentially a d.c. calculation. However it can be easily extended to a.c. - the electric field and potential are in phase with the applied signal and all ion currents are equal to zero.

Figure 5.5a presents the results. The z component of the a.c. electric field (e_z) decreases rapidly with distance (z) away from the charged boundaries. Close to these e_z is considerably lower in the outer voxels ($i=4$) than in the centre because of the edge effects as a result of the finite diameter of the charged boundaries. The radial component (e_r) in the region of counter charge increases with radial distance and is comparable in magnitude to e_z near the edge as expected for finite systems (Figure 5.5.b).

Since the diameter of the cylinder is less than its length, the electric field lines near the edge are not parallel. However near the middle, the field is approximately uniform, and thus the system provides a reasonable “control” for the later configurations in which a membrane or pore is located at the centre.

The electric field can be integrated numerically to obtain the potential difference (V) (V is set equal to zero at the centre of the system). The effect of the finite size can be seen in the equipotential lines shown in Figure 5.5c. One or two equipotential lines intersect the circular boundaries, therefore the electrodes are only approximately equipotentials (they are in fact surfaces carrying uniform charge density $\sigma_s(t)$ as mentioned before, see equation 5.2.2). Again the approximately

FIGURE 5.5

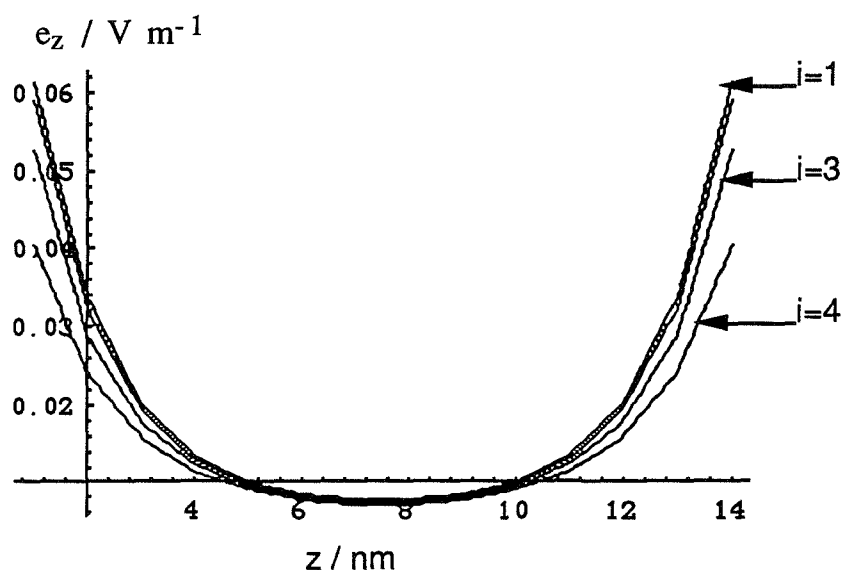


Figure 5.5a. The z component of the electric field (e_z) as a function of z for the different voxel radii (r_i , $i=1,4$) for the non-conducting solvent.

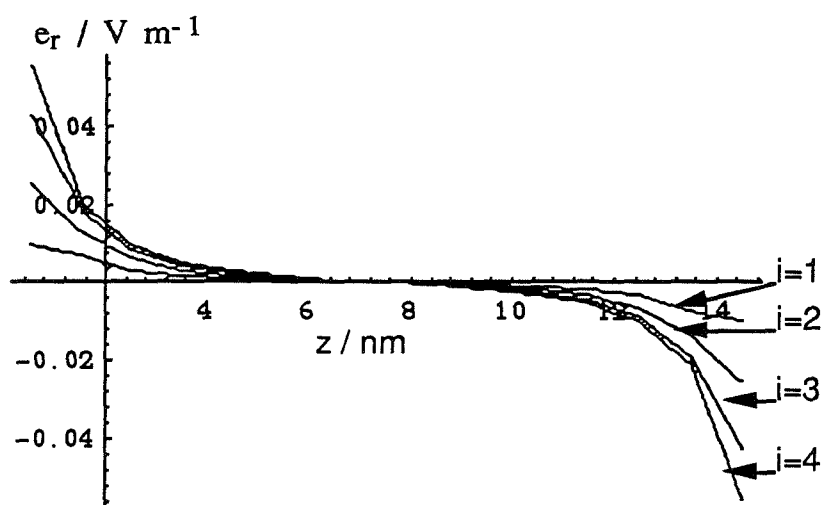


Figure 5.5b. The radial component of the electric field (e_r) as a function of z for the different voxel radii (r_i , $i=1,4$) for the non-conducting solvent.

FIGURE 5.5 continued

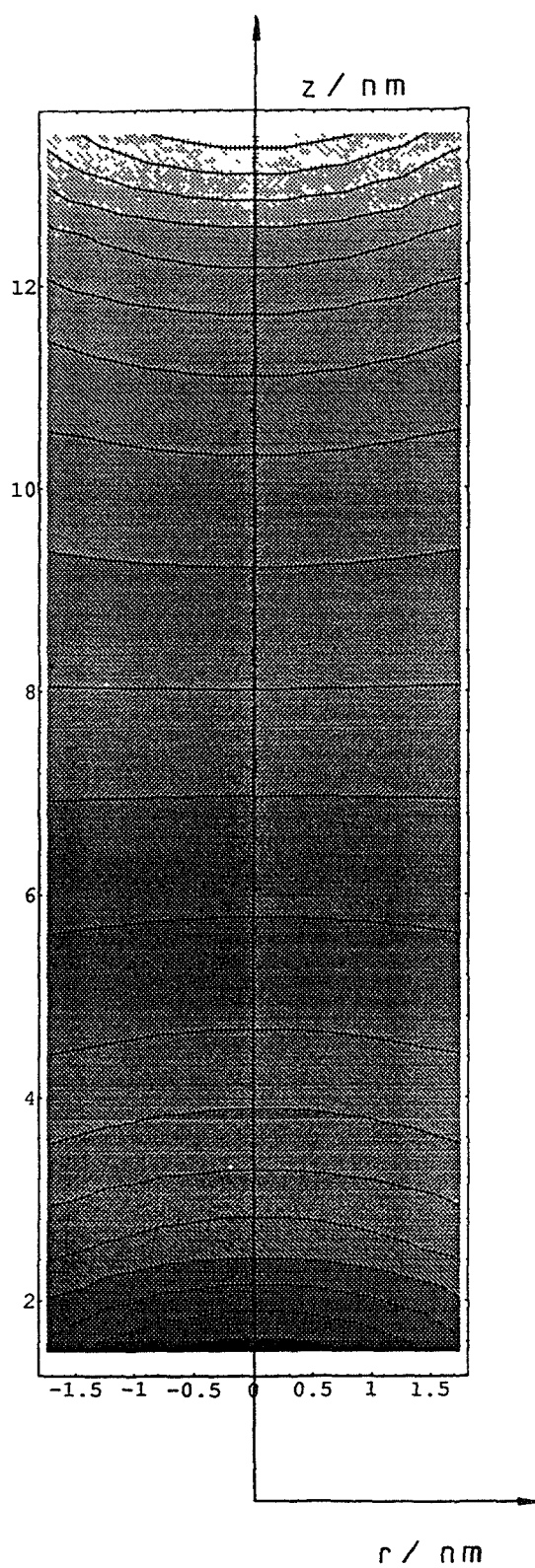


Figure 5.5c. A plot of the equipotential lines for the non-conducting solvent.

parallel equipotential lines near the centre show that the field is approximately uniform in this region.

5.3.2 The Effect of Finite Size and Discreteness of Charge

The geometry of section 5.3.1 is used to obtain a quantitative (order of magnitude) estimate of the effect of finite size as well as discreteness of charge on the electric field. Figure 5.6a shows two circular plates of diameter L_r carrying equally spaced rings of charge. The cylinder of dielectric in between is assumed to contain no net charge and the circular boundaries are separated by a distance L_z . The electric field due to these rings of charge is calculated along the central axis (i.e. z axis) of the system and is compared to that for two infinite plates of the same separation L_z and carrying the same charge per area. For values of L_r and L_z equal to those of section 5.3.1 above, the electric field along the central axis is considerably smaller than that for infinite plates. To investigate the effect of size, the diameter (L_r) of the charged plates is increased by adding extra rings of charge at the same separation (h_r) as those used for the calculations in section 5.3.1, while keeping the total charge constant. This has a marked effect on the electric field (Figure 5.6b) for small ratios of L_r/L_z . Similarly the effect of the discreteness of charge can be estimated by dividing the circular plates into a larger number of more closely spaced rings of charge (for a fixed value of L_r and the same total charge). It was found that this produces considerably less error in the electric field than the effect of finite size described above for the dimensions used here (except at the region close to the rings of charge).

FIGURE 5.6

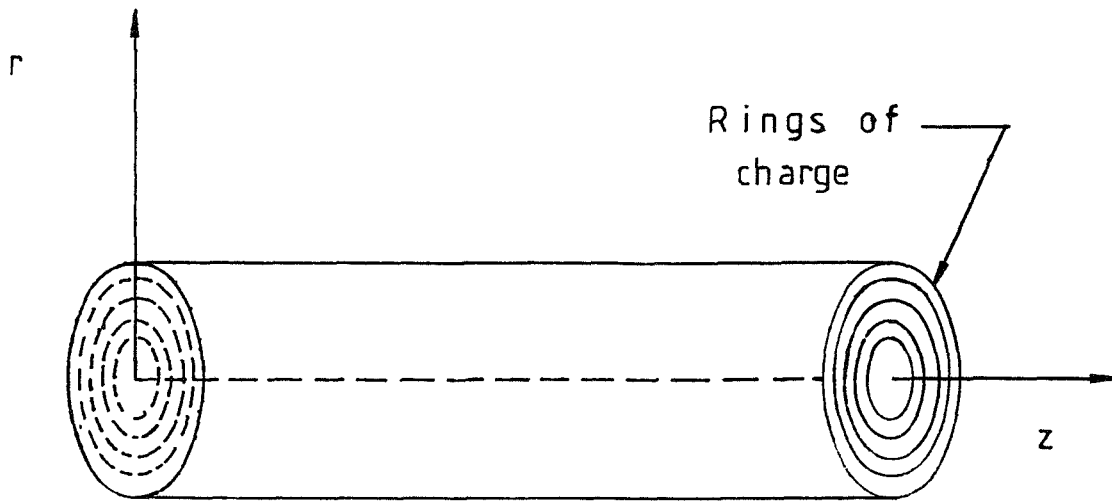


Figure 5.6a. The geometry used for the estimates of the effect of size on the electric field.

electric field
ratio

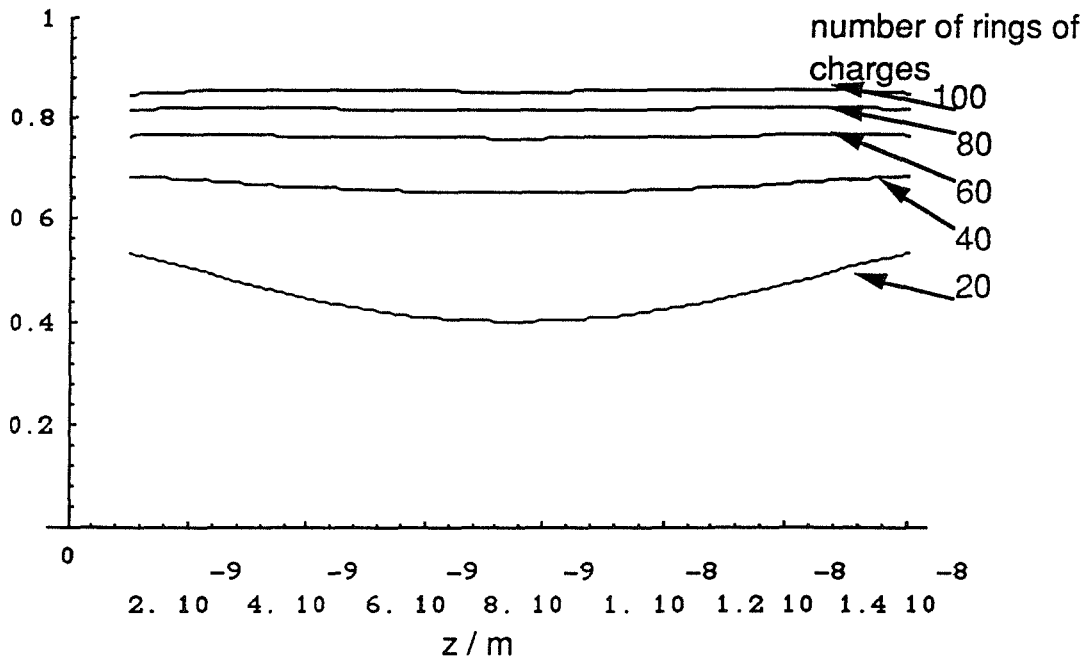


Figure 5.6b. The ratio of the electric field due to discrete rings of charge to that for a uniform charge density on infinite plates along the z axis for a constant total charge and a separation (L_z) between the plates of 15 nm. The separation between each ring of charge (h_r) is kept constant at 1/2 nm. Both L_z and h_r have the same value as used for the calculations for cylindrical geometry in this chapter.

5.3.3 A Cylinder of Electrolyte

All calculations of the small a.c. signal response presented in the remainder of this chapter were performed at two frequencies: one above and one below a characteristic frequency f_c . I define f_c as the frequency at which $G_K = 2\pi f_c C_K$:

$$f_c = G_K / (2\pi C_K) \quad (5.3.1)$$

where G_K is the equilibrium conductance of a region of bulk electrolyte of thickness L_G . G_K can be derived from Boltzmann's transport theory and is given by:

$$G_K = q^2 (z_p D_p + z_n D_n) C_0 / (k T L_G) \quad (5.3.2)$$

C_K the capacitance of an ideally polarised dielectric slab of thickness L_C and permittivity ϵ).

$$C_K = \epsilon / L_C \quad (5.3.3)$$

The choice of L_C and L_G depends on the characteristics and/or geometry of the system studied.

The effect of adding a fully dissociated binary electrolyte between the two charged circular plates of section 5.3.1 is now considered. Given enough time the ions will redistribute in response to the a.c. signal applied at the boundaries. In the region near the applied charge counter ions accumulate forming a double layer. The range of variation of charge and electric field in the double layer is of the order of a Debye length λ_D given by :

$$\lambda_D = [\epsilon kT/q^2 C_0(z_p + z_n)]^{1/2} \quad (5.3.4)$$

where ϵ denotes the permittivity of the electrolyte solution and C_0 is the bulk electrolyte concentration (see Table 5.1a for all other variables).

Hence when calculating the the characteristic frequency, denoted by f_{dl} for this system, L_G and L_C were set equal to λ_D .

Unless stated otherwise, the plots presented show the magnitude of the amplitude of each variable, which by definition is always positive. For antisymmetric functions such as the a.c. ion concentration and potential, which change sign at the origin, the symmetry of the system is conveyed by the phase.

The spurious points in the phase plots for values of z for which the amplitude is very small are artefacts, since it is difficult to fit a phase to variables with close to zero amplitude. This occurs near the centre for the case of antisymmetric functions.

5.3.3 a) High frequency calculations for a Cylinder of Electrolyte Solution

The frequency (f) chosen for these calculations higher than f_{dl} (see Table 5.1a). As the frequency is very high, the a.c. charge carried by the counter-ions is small compared to the applied charge. Hence there is very little screening of the applied charge and geometrical effects dominate: the a.c. cation concentration (p) as well as e_z in the double layer are considerably lower in the outer voxels ($i=4$ and 3) than near the central axis (see Figures 5.7a and 5.7b). The peculiarities of the variation of amplitude and phase of p are possibly due to spatial resolution problems.

TABLE 5.1a

PARAMETER		VALUE
T	temperature	293 K
D _n	anion diffusion constant	$2 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$
D _p	cation diffusion constant	$1.98 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$
ϵ/ϵ_0	dielectric constant of the solution	80
z _n	anion valency	1
z _p	cation valency	1
J ₀	magnitude of the total current density at the boundaries	1 A m^{-2}
C ₀	bulk ion concentration	100 M m^{-3}
n _T	points per cycle	1000
f	frequency - section 5.3.3: section 5.4, 5.5 and chapter 6:	1 GHz 100 MHz
n _r	total number of voxels in the r direction	4
n _z	total number of voxels in the z direction	15
h _r	voxel size in r direction (i.e. width of ring)	0.5 nm
h _z	voxel size in the z direction	1 nm

FIGURE 5.7

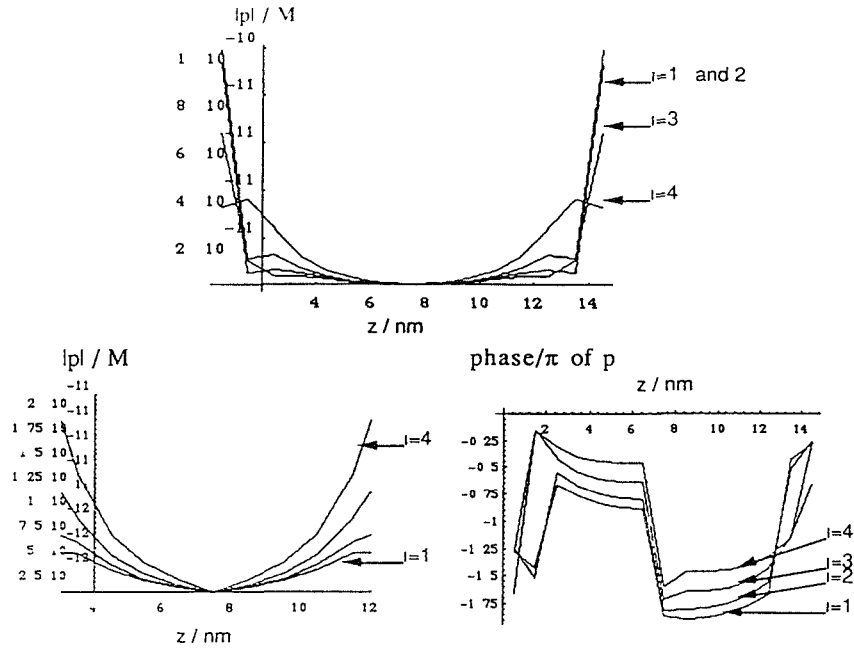


Figure 5.7a The amplitude ($|p|$) and phase/ π (with respect to the applied charge) of the a.c. component of the cation concentration (p) as a function of z for the different voxel radii r_i , $i=1$ to 4, for a cylinder of electrolyte for a frequency above f_{dl} . (The top diagram includes all voxels, while the two lower ones shows only the voxels near the central plane. (see Table 5.1a for all other parameters).

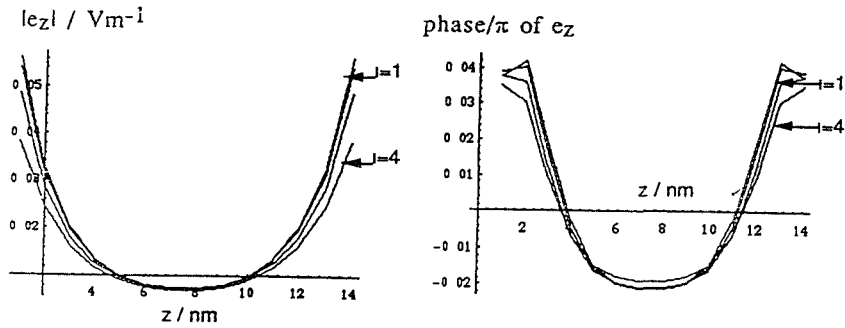


Figure 5.7b. The amplitude ($|e_z|$) and phase/ π of the z component of the a.c. electric field e_z for the different voxel radii r_i , $i=1$ to 4, for a cylinder of electrolyte for a frequency above f_{dl} .

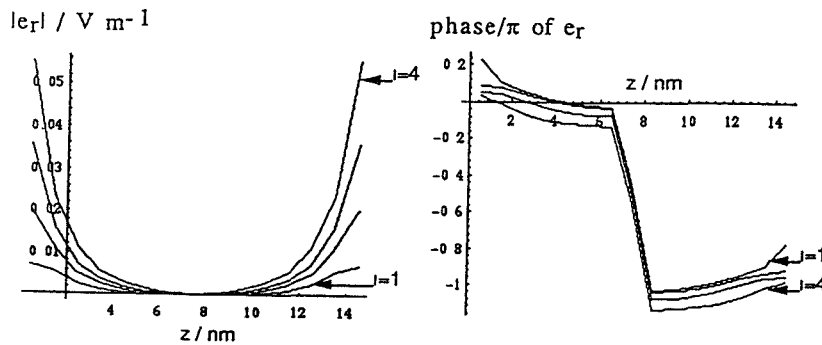


Figure 5.7c. The amplitude and phase/ π of the radial a.c. electric field (e_r) for the different voxel radii r_i , $i=1$ to 4, for a cylinder of electrolyte for a frequency above f_{dl} .

The voxel size is of the order of λ_D and there is very little counter charge.

As before, e_r in the region of counter charge increases with radial distance (Figure 5.7c). Both e_z and e_r are approximately in phase with the applied charge (e_z is symmetric while e_r is antisymmetric). The electric field as well as the equipotential lines (Figure 5.7d) are similar to those for the case of the nonconducting solvent of section 5.3.1, because of limited screening of the applied charge.

The z component of the a.c. cation current density (J_{pz}) is highest in the double layer and its phase leads the applied charge (note the phase of J_{pz} is zero at the boundary, only because J_{pz} is set equal to zero there. J_{pz} should approach $\pi/2$ near the boundaries) This current charges the double layer (Figure 5.7e). The radial a.c. cation current density (J_{pr} , Figure 5.7f) in this region is lower than J_{pz} and increases with r . J_{pz} is fairly uniform along the central axis and approximately in phase with the applied charge.

Only plots of the concentrations (p) of the cations have been included, since this model is charge symmetric. Hence the a.c. anion concentrations (n) are equal in magnitude but have opposite phase to those of the cations. Similarly, plots for anion current densities have been omitted, since the anion and cation currents densities are approximately equal. The next chapter includes a description of asymmetric systems and both p and n and current densities for both ion species will be shown. Plots for anions where n is equal to $-p$ are not presented.

FIGURE 5.7 continued

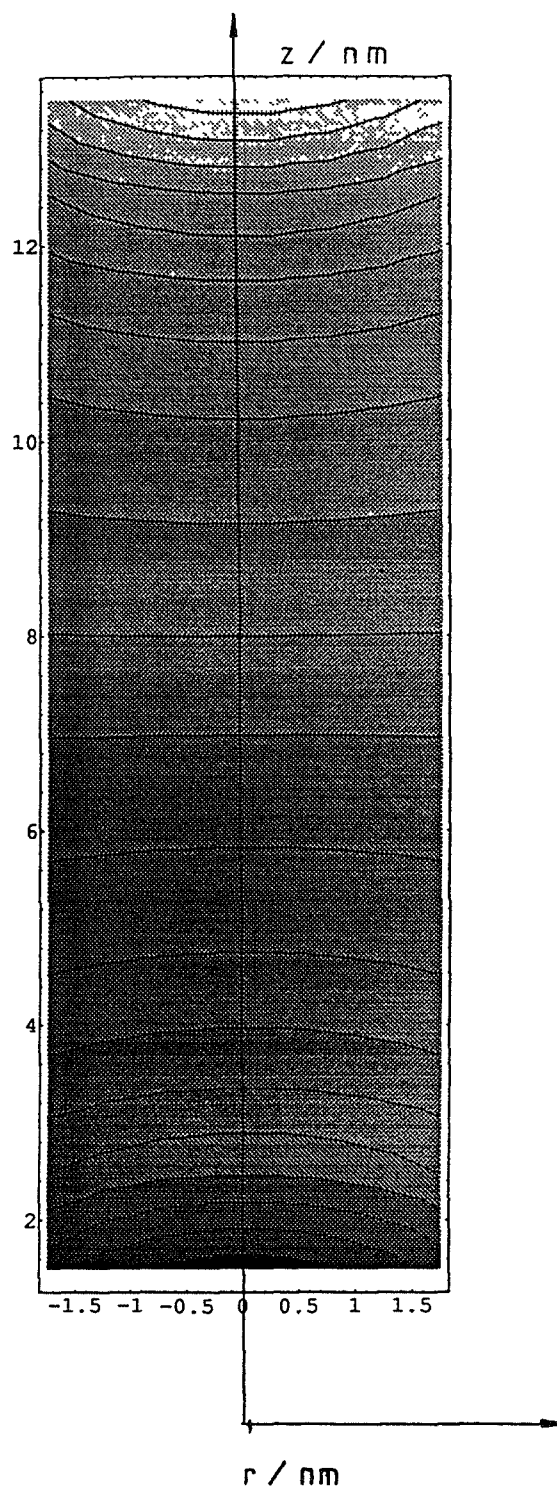


Figure 5.7d. A plot of the equipotential lines for a cylinder of electrolyte at a frequency above f_{d1} .

FIGURE 5.7 continued

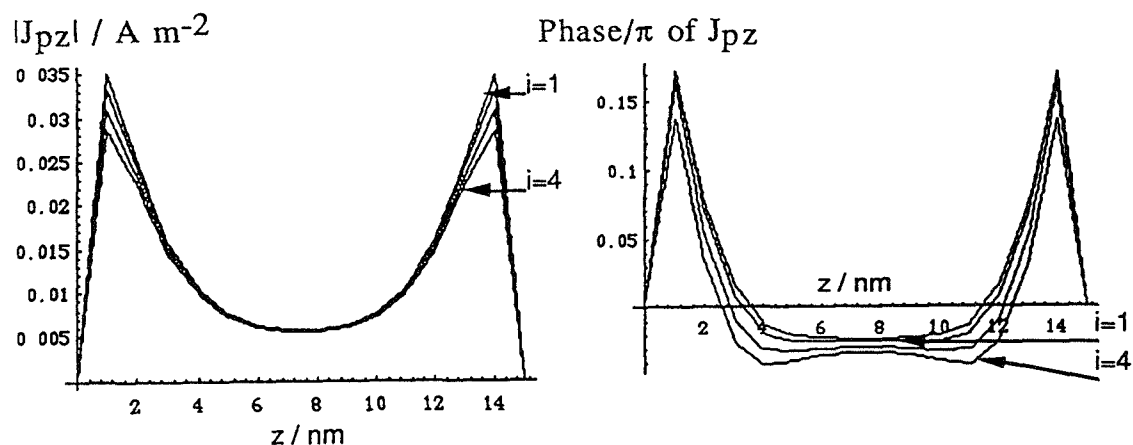


Figure 5.7e. The amplitude and phase/ π of the z component of the a.c. cation current densities (J_{pz}) as a function of z for the different voxel radii r_i , $i=1$ to 4, for a cylinder of electrolyte for a frequency above f_{dl} .

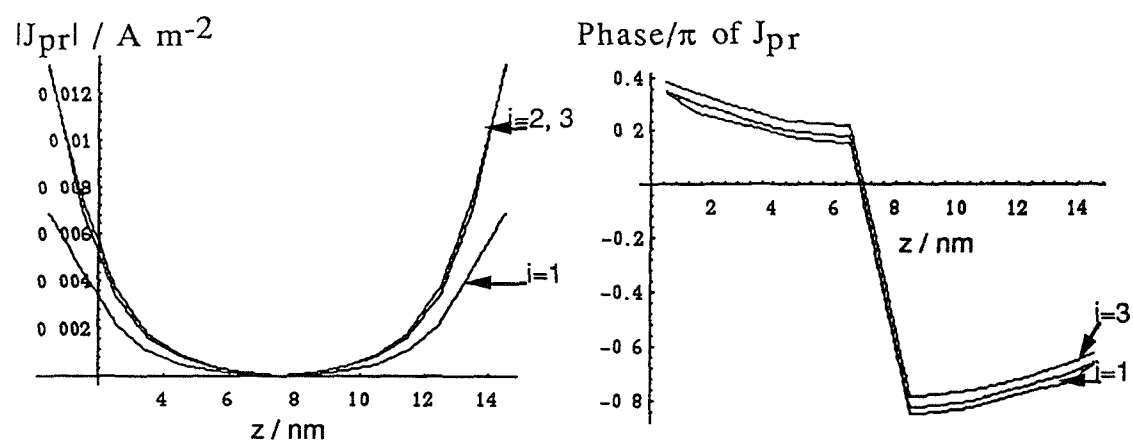


Figure 5.7f. The amplitude and phase/ π of the radial component of the a.c. cation current density (J_{pr}) as a function of z for the different voxel radii r_i , $i=1$ to 4, for a cylinder of electrolyte for a frequency above f_{dl} .

5.3.3 b) The frequency of the a.c. applied charge at the boundaries is much lower than f_{dl} .

To show the effect of frequency and to contrast with the previous results a frequency much lower than f_{dl} is now used. At this frequency, the results are approaching those for d.c., since there is now sufficient time during each cycle for counter ions to accumulate near the charged boundaries to form a double layer.

The frequency used for these calculations is 1/1000 that for the high frequency calculations of the previous section. When changing frequency, the current density is kept constant rather than the applied charge density (σ_0). Thus σ_0 is now 1000 times greater (see equation 5.2.2). The ratio of the amplitude of the a.c. cation concentration ($|p|$) to σ_0 in the double layer is higher than for the high frequency calculations of section 5.3.3a. The total space charge in this region is approximately half of the applied surface charge on the plate. In an infinite system the total space charge is equal to the total charge on the plates, however in such a system all field lines are confined to the region between the plates. In this model the field lines leave the system in both the r and z direction, resulting in reduced space charge. p also decreases less rapidly with radial distance, than for the calculations at high frequencies (5.3.3a) and is in phase with the applied charge but is antisymmetric (Figure 5.8a, see Table 5.1b).

Figure 5.8d shows the equipotential lines for low frequency calculations for a cylinder of electrolyte. The appearance is very different to the equipotential lines due to the surface charge alone (i.e. in the absence of space charge as shown in Figure 5.5b. The differences arise mainly from edge effects in such a small system.

The electric field is strongest near the charged boundaries as also occurred for the cylinder of non-conducting solvent (section 5.3.1).

TABLE 5.1b

PARAMETER		VALUE
T	temperature	293 K
D_n	anion diffusion constant	$2 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$
D_p	cation diffusion constant	$1.98 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$
ϵ/ϵ_0	dielectric constant of the solution	80
z_n	anion valency	1
z_p	cation valency	1
J_{i0}	magnitude of the total current density at the boundaries	1 A m^{-2}
C_0	bulk ion concentration	100 M m^{-3}
n_T	points per cycle	24000
f	frequency	1 MHz
n_r	total number of voxels in the r direction	4
n_z	total number of voxels in the z direction	15
h_r	voxel size in r direction (i.e. width of ring)	0.5 nm
h_z	voxel size in the z direction	1 nm

FIGURE 5.8

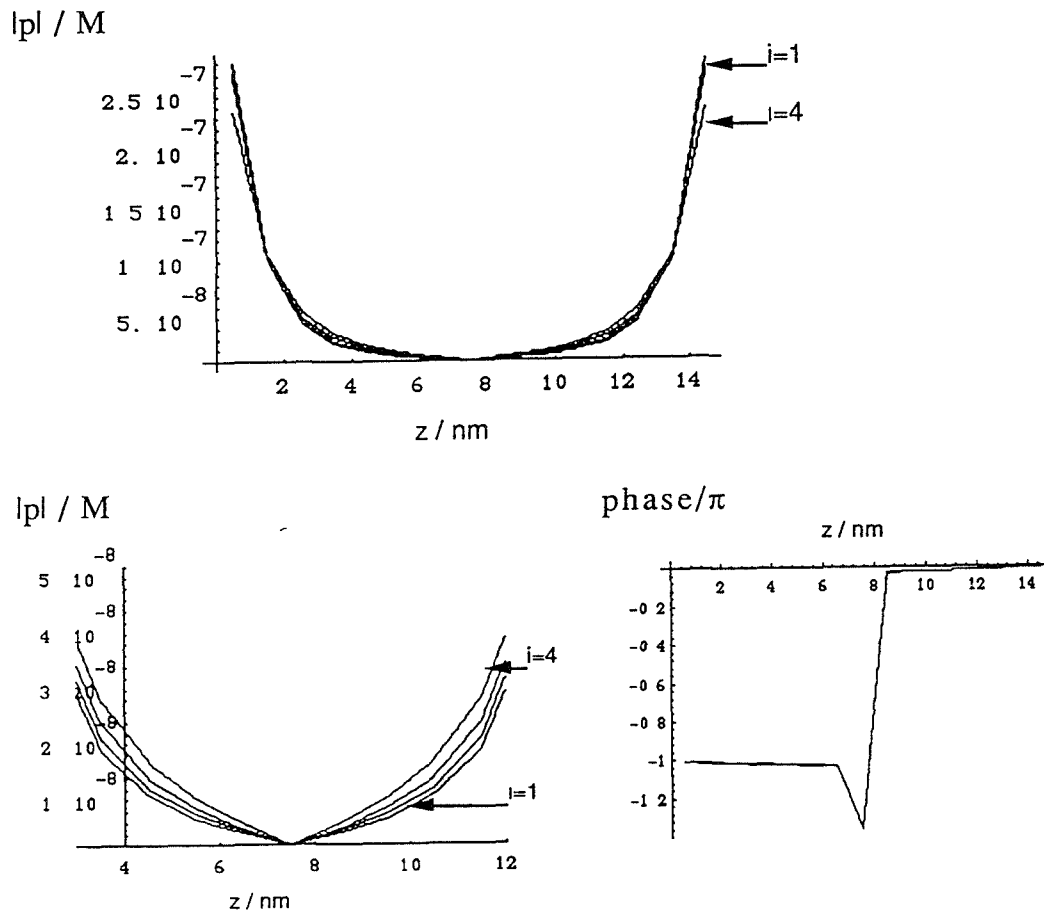


Figure 5.8a. The amplitude and phase/ π of the cation concentration (p) for the different voxel radii (r_i , $i=1,4$) for a cylinder of electrolyte at low frequencies.

FIGURE 5.8 continued

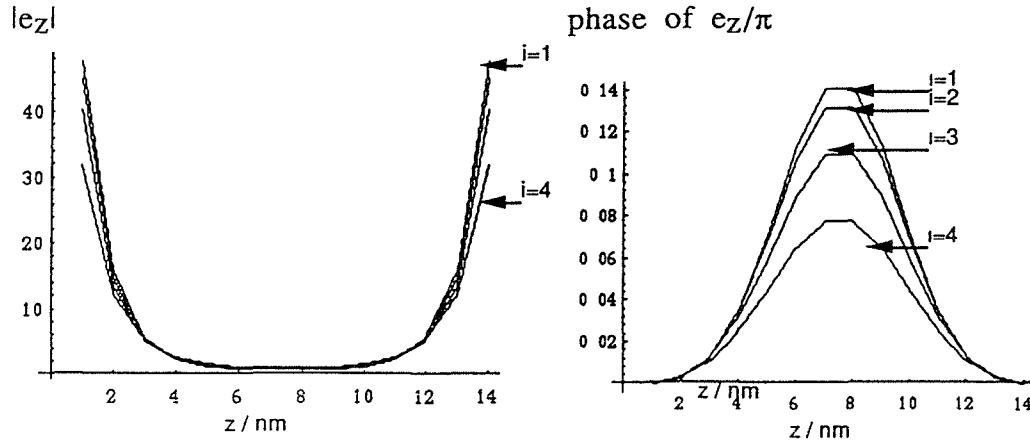


Figure 5.8b. The amplitude and phase/ π of the z component of the electric field (e_z) for voxel radii r_i , $i=1,4$ for a cylinder of electrolyte at low frequencies.

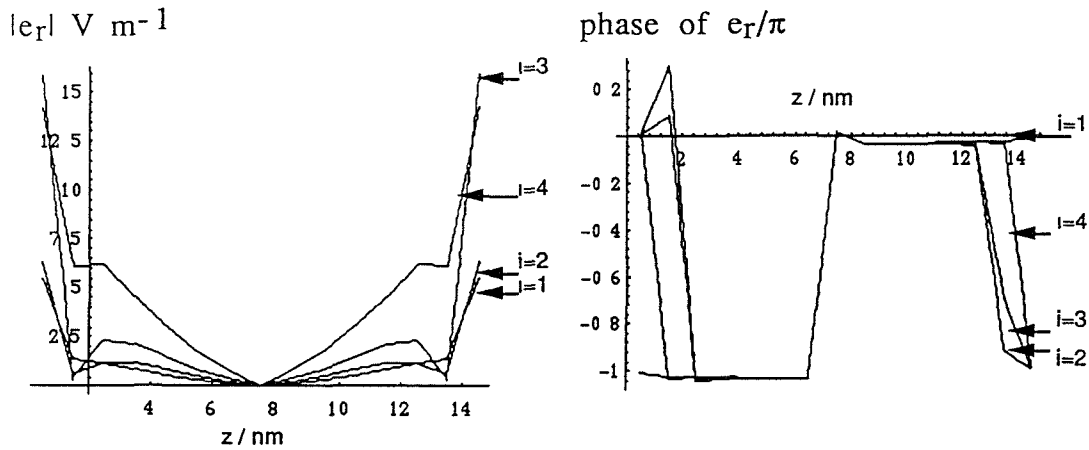


Figure 5.8c. The amplitude and phase/ π of the radial electric field (e_r) for voxel radii r_i , $i=1,4$ for a cylinder of electrolyte at low frequencies.

FIGURE 5.8 continued

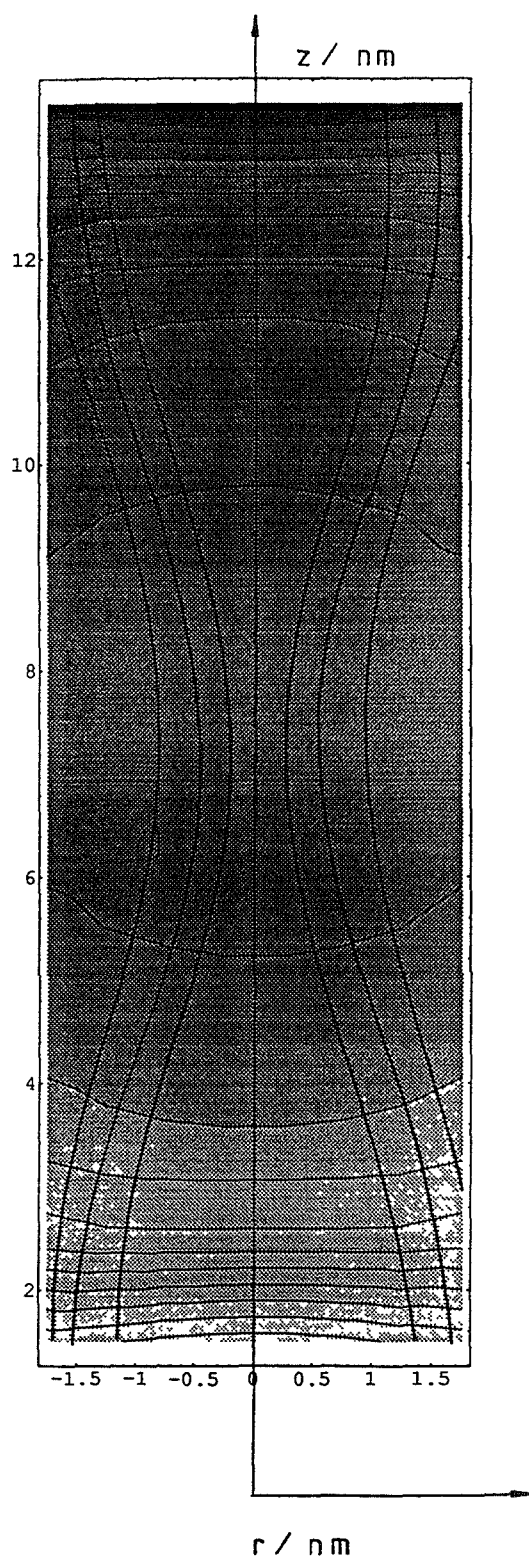


Figure 5.8d. A plot of the equipotential lines for a cylinder of electrolyte solution at low frequencies. A sketch of the electric field lines is included.

Away from the charged plates the curvature of the electric field lines changes sign. (Figures 5.8b and 5.8c) This change in curvature is due to the space charge, which accumulates at the cylindrical edges rather than in the centre as a consequence of the electric field lines leaving the cylinder, while the boundary conditions constrain the charge to remain inside the cylinder. (Figure 5.8d includes an approximate sketch of the electric field lines). The phase of e_z now reaches a maximum near the centre.

5.3.4 Quantization Errors

All numerical calculations have finite resolution. In this section the effect of voxel size for the parameters used in this thesis is investigated.

One consequence of having a finite voxel size is that Gauss's law is only approximately satisfied by the numerical solutions. The electric field is calculated at the centre of each voxel surface and is assumed to be constant over the whole surface. This oversimplification can introduce an error. An order of magnitude estimate of this can be obtained by comparing E_{centre} to $\int E \cdot dA$, where E is the electric field due to a point charge in the voxels along the z axis and A is the surface area of the voxel (see Appendix 5.1).

However this difference does not affect the internal consistency of the calculations, which use Coulomb's law instead of Gauss's law. The electric field (e) and the current density (J_p and J_n) are calculated at the same point. e is used only to calculate J_p and J_n for each iteration. The ion concentration in each voxel for the next iteration is then calculated from the net current entering or leaving each voxel.

As discussed in the introduction to this Chapter, the use of the electric field rather than the potential (V) means that the chemical

potential may not be constant in regions where e and therefore V are varying rapidly and non-linearly in space. Thus a quantization noise, consisting of stable circulating currents are possible in the numerical solutions to the electrodiffusion equations. These currents decrease rapidly with distance away from the charged boundaries. Plots of the current densities at low frequencies will not be presented because of this quantization "noise".

5.3.5 Comparison of the One Dimensional Analytical Theory with the Numerical Calculations

The electrodiffusion equations can be solved analytically for the case of a binary electrolyte between two infinite electrodes (see Chapter 3). These will now be compared to numerical results for an electrolyte between two charged boundaries for two different geometries described in the methods section of this chapter:

Configuration 1) Infinite lines of charge in the z direction.

Configuration 2) Annular prisms of charge.

The analytical case is one dimensional, while the two numerical configurations are two and three dimensional respectively. This difference is important. Since the width of the charged boundaries is smaller than their separation, they do not retain the parallel field of large, closely-spaced plates. Indeed, they resemble a dipole as much as they do two infinite plates. The comparison will show the effect of this geometry, as well as the smaller effect of discrete versus continuous charge.

For all of the following numerical calculations the total width and separation of the charged boundaries is kept constant. All numerical plots in this section show the instantaneous a.c. component of each variable. The cation concentration (p) and the electric field (e) are plotted at the

time of maximum applied charge, while the cation current density (J_p) is shown at the time of minimum applied signal. For the frequency (f) chosen here p and e are approximately in phase with the applied charge and the phase of J_p is approximately $\pi/2$ ahead of the applied charge, hence they are similar to the magnitude of the a.c. amplitude of these variables.

Configuration 1) This is a slab of electrolyte solution (infinite in the z direction) between two boundaries of width L_{tx} (parallel to the x axis) and separation L_{ty} , carrying equally spaced infinite lines of charge. The amplitude of this charge varies sinusoidally with time. The region is divided into rectangular voxels, which are also infinite in the z direction.

Figure 5.9a shows the cation concentration (p) as a function of y for configuration 1 as well as the results of the infinite slab (analytical solution). p for configuration 1 is somewhat lower than that for the one dimensional analytical calculations, especially near the charged boundaries mainly due to the effects of finite size.

The a.c. electric field (e_y) in the y direction is lower for configuration 1 than that of the infinite slab calculated analytically (see Figure 5.9b). This difference can be mainly attributed to geometrical factors (i.e the small ratio of L_{tx}/L_{ty}) rather than the discreteness of charge assumed for the numerical calculations, as discussed earlier. The lower electric field results in a smaller space charge for configuration 1. Ion concentrations and electric field vary most rapidly near the charged boundaries, hence the limited resolution of the numerical calculations (the voxel size is of the order of one Debye length) also affects the magnitude of these quantities.

Figure 5.9c shows the a.c. component of cation current density as a function of y . The cation current density is lower than the analytical value as explained below.

FIGURE 5.9

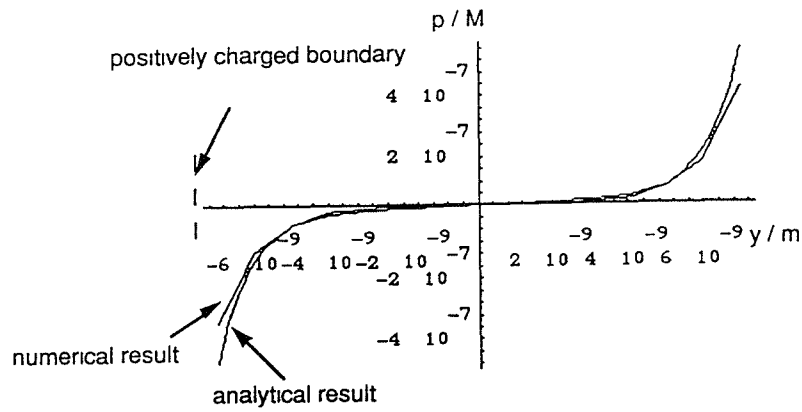


Figure 5.9a. A comparison of the a.c. cation concentrations (p) for rectangular geometry (configuration 1) with the analytical solution of chapter 3.

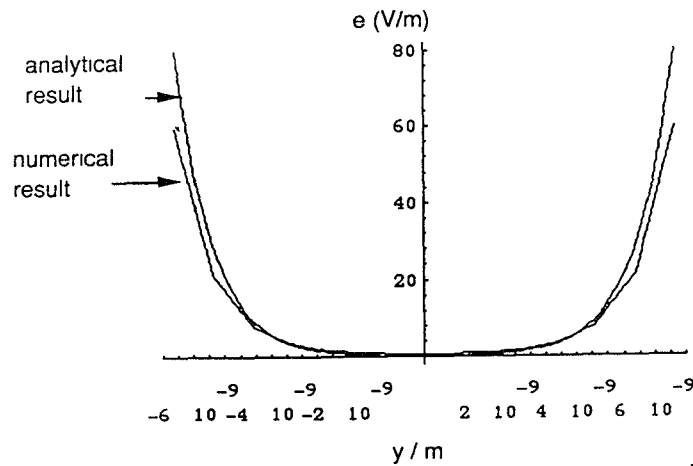


Figure 5.9b. A comparison of the electric field (e) along the y axis for rectangular geometry (case 1) with the analytical solution of chapter 3.

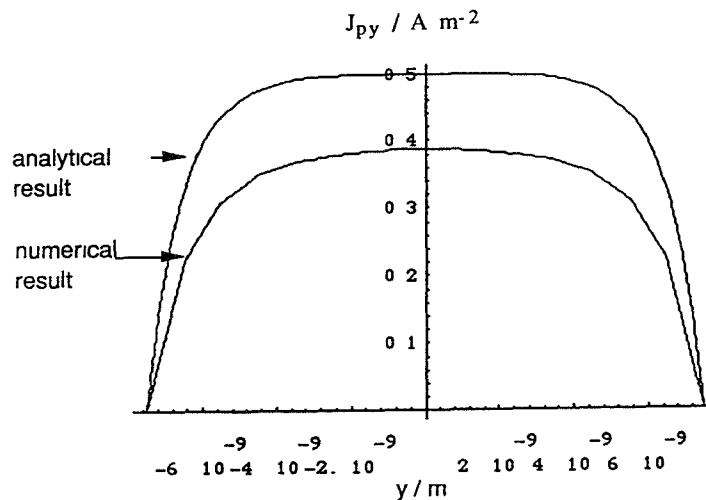


Figure 5.9c. A comparison of the cation current density (J_{py}) along the y axis for rectangular geometry (case 1) with the analytical solution of chapter 3.

Configuration 2 : A cylinder of electrolyte (the voxels are now annular prisms as shown in Figure 5.3). Figure 5.10a shows the cation concentration p along the central axis. The difference between the numerical results and those of the infinite slab is greater than for configuration 1, since this system is finite in all three dimension, while configuration 1 (rectangular geometry) is finite in only two dimensions. As before, the electric field is lower than that of the infinite slab due to differences in geometry. (Figure 5.10b and 5.10c).

For an infinite slab, the electric field half way between the two charged plates is equal to the ratio J_{t0}/Y_K , where J_{t0} is the applied current density and Y_K is the admittance per unit length, as given by equation 3.2.41. This ratio is very small, compared to that near the electrodes at low frequencies, so the current is almost purely ionic. In both configurations 1 and 2 the numerical calculations give a higher electric field (e_z) in the centre, due to limited resolution and finite size, hence a smaller fraction of the total current is ionic. Thus the cation current is lower than that of the analytical solutions.

The effect of blocking some of the voxels to ion flow is considered in the following two geometries.

5.4 INSULATING MEMBRANE

A disc of insulating material in the centre of the system is now considered (Figure 5.11). This disc is initially assumed to have the same dielectric constant as the electrolyte (see section 5.2). So the system resembles an impermeable membrane, except that biological membranes have a dielectric constant much lower than water. This has two effects: i) it makes the membrane effectively "thicker" since it changes the electric field lines and ii) it ignores effects due to the Born energy, as explained

FIGURE 5.10

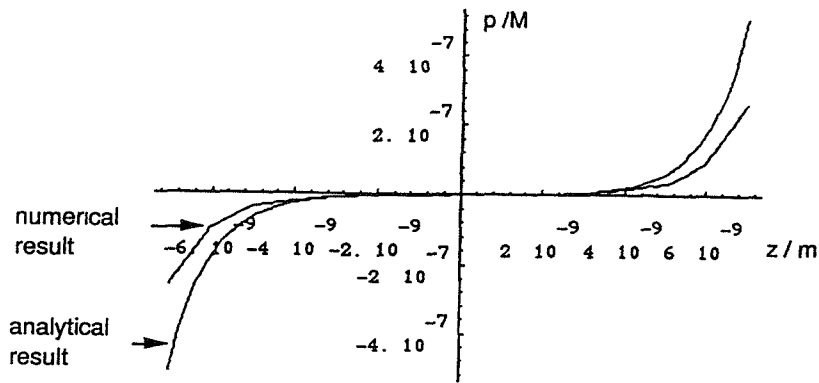


Figure 5.10a. A comparison of the cation concentration p for cylindrical geometry (configuration2) and the analytical solution of chapter 3 (see Table 5.1b for a list of the parameters chosen for this comparison).

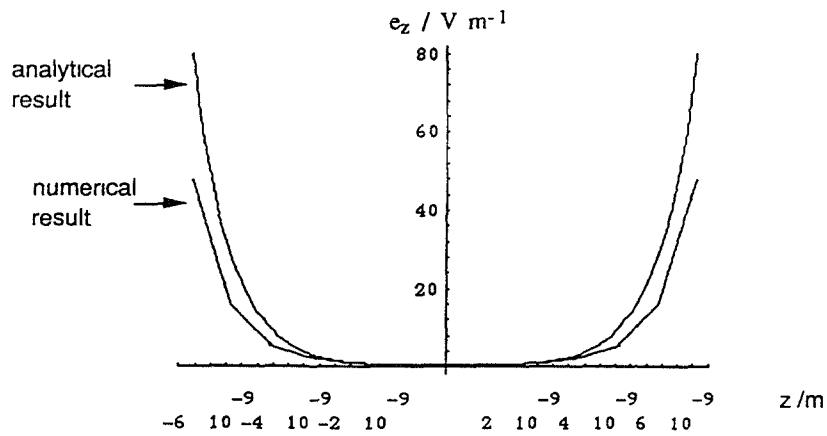


Figure 5.10b. A comparison of the z component of the electric field e_z for cylindrical geometry (configuration2) with the analytical solution of chapter 3 (see Table 5.1b for a list of the parameters chosen for this comparison).

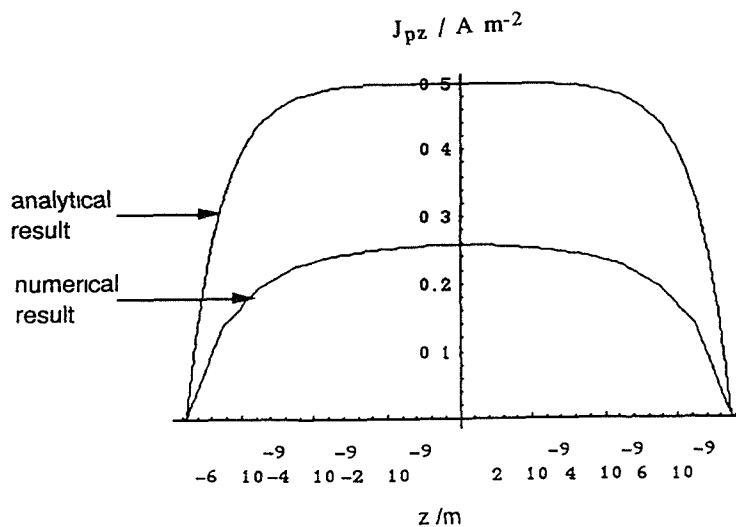


Figure 5.10c. A comparison of the cation current density (J_{pz}) for cylindrical geometry (configuration2) with the analytical results of chapter 3 (see Table 5.1b for a list of the parameters chosen for this comparison).

Figure 5.11

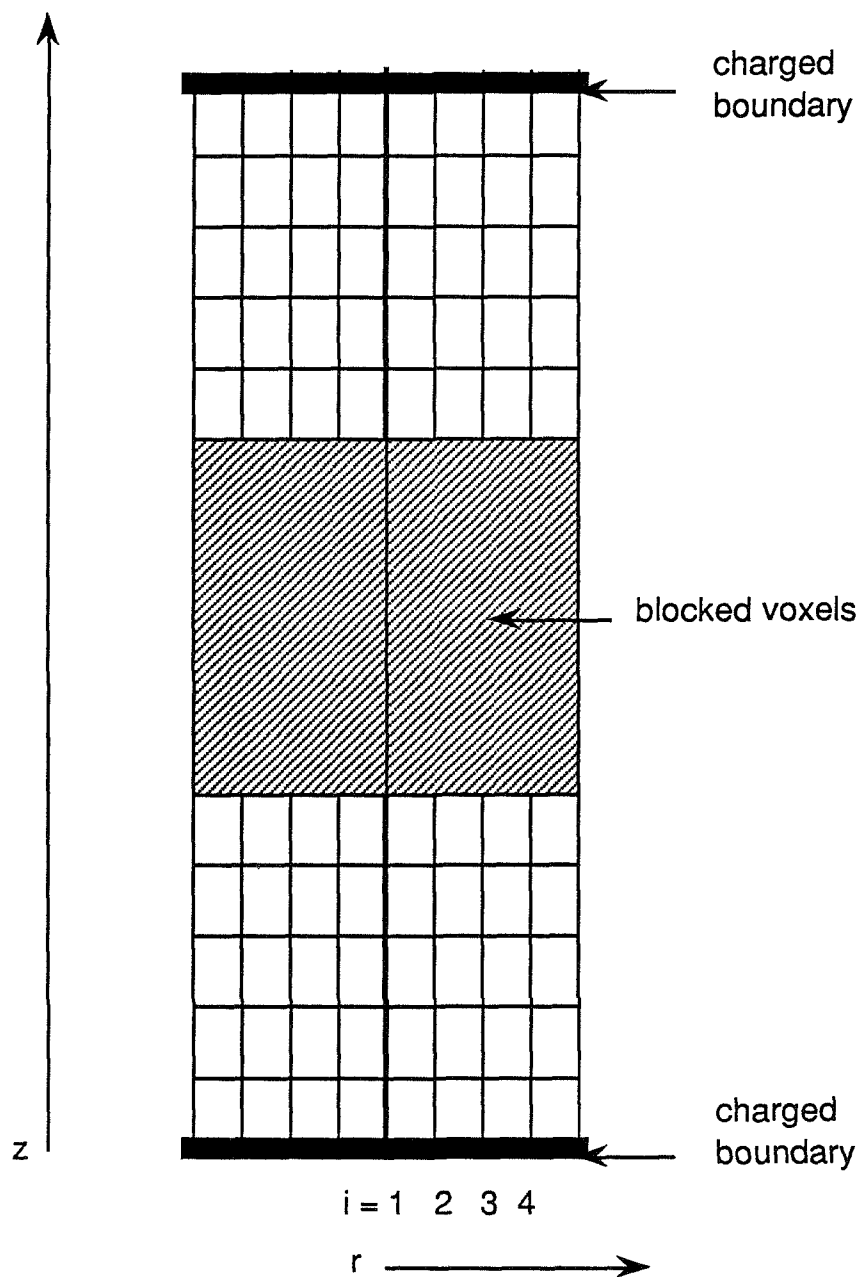


Figure 5.11. The configuration used for the calculations for the insulating disc.

in section 2.6.3. The presence of a region with a lower dielectric constant increases the self energy of ions in its proximity and the ions "induce" charge at the dielectric discontinuity. Restriction (ii) is removed in Chapter 6.

The characteristic frequency of the insulating membrane is the same as for the cylinder of electrolyte solution of section 5.3. However, in the next section the effect of adding a pore through the insulating membrane will be discussed and this pore has a characteristic frequency (f_c), which is lower than f_{dl} . In order to allow comparison between these two configurations f_c is used for calculations above the characteristic frequency, rather than f_{dl} .

5.4.1 Low Frequency Calculations

The following results are for a frequency below f_c , hence these are "near equilibrium" calculations. Therefore plots of ion current densities will not be shown, since the ionic current densities are small and comparable to those due to quantization artefacts as discussed earlier in this chapter.

Figure 5.12a shows that space charge accumulates near the membrane/electrolyte interface. This charge on the membrane is of opposite sign, but smaller in magnitude, to that of the nearest charged boundary. The charge build up at the membrane/electrolyte interface gives rise to an increase in the electric field in the voxels near the two membrane/electrolyte interfaces (Figure 5.12b and 5.12c). The a.c. concentrations do not vary much in the r direction, i.e. parallel to the interface. Any differences can mainly be attributed to the finite size of the system. The effect of the finite size of the system can be more clearly seen in Figure 5.12d, which shows the equipotential lines.

FIGURE 5.12

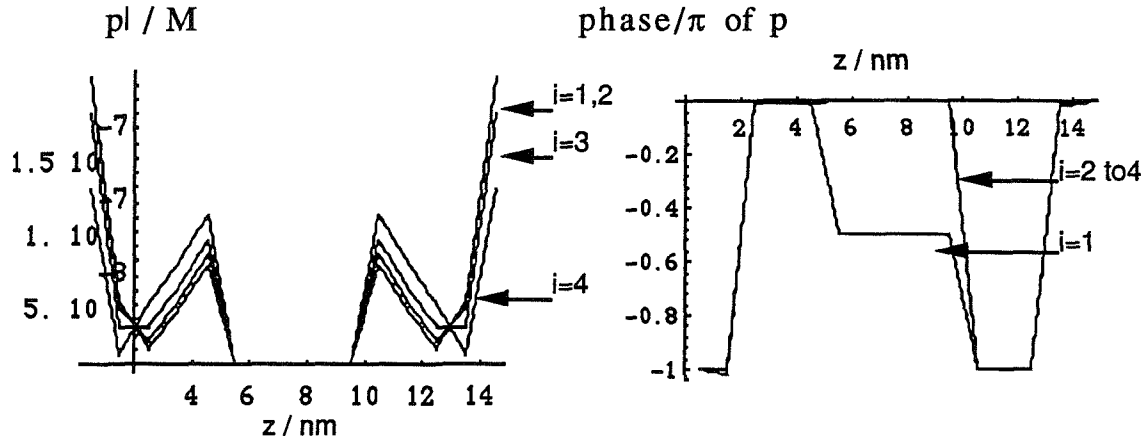


Figure 5.12a. The amplitude and phase/ π of the cation concentration (p) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at low frequencies.

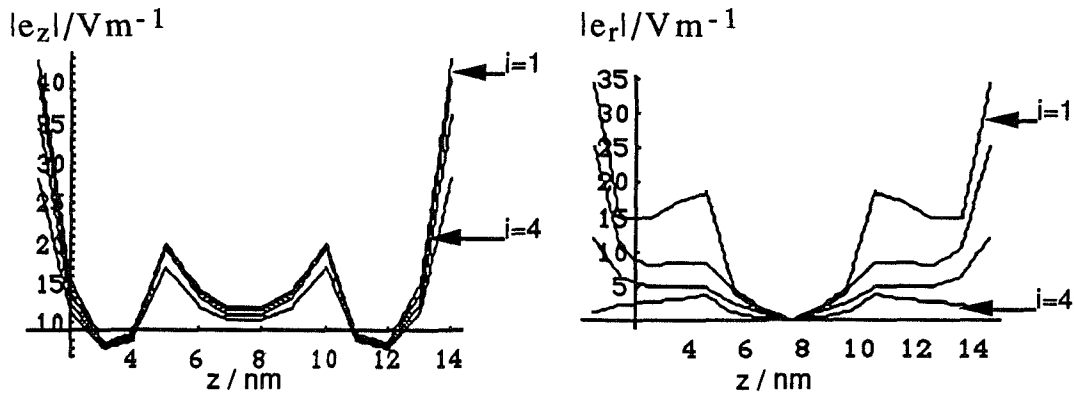


Figure 5.12b and c. The amplitude of the axial (b) and radial (c) component of the electric field ($|e_z|$ and $|e_r|$) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at low frequencies. The phase of $e_z = 0$, and that of $e_r = 0$ and π .

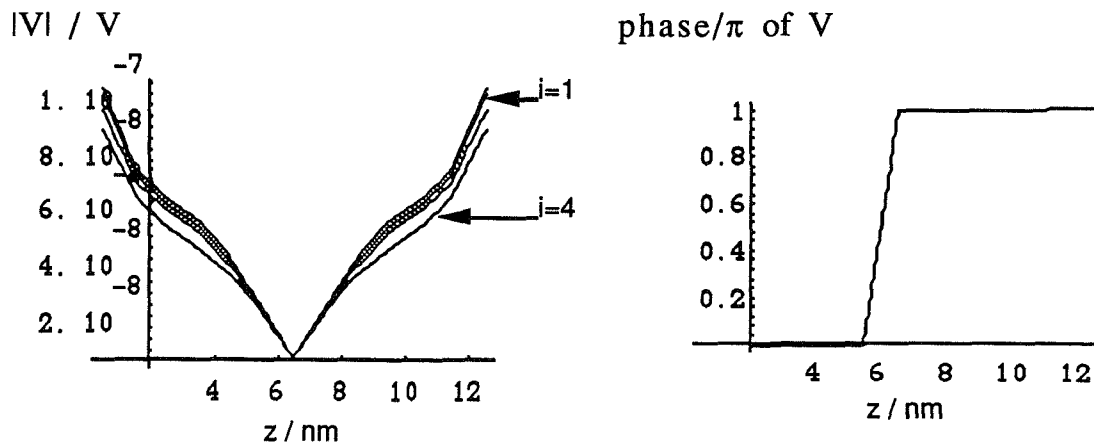


Figure 5.12e. The amplitude and phase of the potential difference (V) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at low frequencies.

FIGURE 5.12 continued

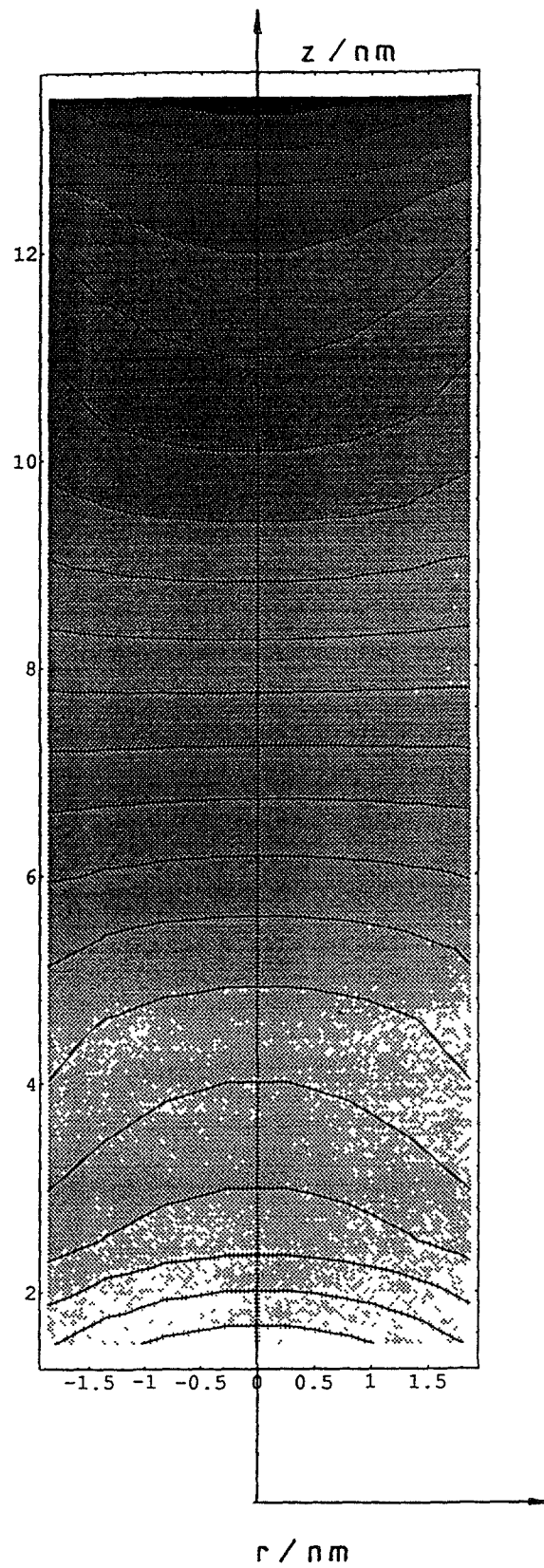


Figure 5.12d. A plot of the equipotential lines for an insulating membrane at low frequencies.

For the parameters chosen, the potential difference near the membrane/electrolyte interface is comparable in magnitude to that of the double layer at the charged boundaries. (Figure 5.12e).

5.4.2 Calculations at a frequency greater than f_c

The variation of the a.c. cation concentration (p) with z at high frequencies appears similar to that at low frequencies (Figure 5.13a). (Note that the applied charge density (σ_0) at this frequency is equal to 1/100 that at the lower frequency). The amplitude of p/σ_0 is now slightly smaller when compared to that for the low frequency calculations. As the frequency is higher, there is less time per cycle for ions to accumulate near the insulating membrane. This leads to a reduction in e_z/σ_0 (and therefore V/σ_0) in the double layer near the electrolyte/membrane interface. The phase of e_z in the region half way between the charged boundary and the membrane now leads the applied charge by a considerably larger angle (Figure 5.13b and c, Figure 5.13d shows the plots of the potential difference).

The z component of the cation current density (J_{pz}) is approximately constant in the region between the charge boundaries and the membrane. This current charges both double layers, hence its phase leads the applied charge approximately $\pi/2$. The amplitude of J_{pz} near the charged boundaries does not vary significantly in the radial direction. The phase is set equal to zero at the charged boundaries as well as the surface of all voxels of the membrane, since the ionic current densities are set equal to zero there (Figure 5.13e).

The radial a.c. current density (J_{pr}) is now much lower than for the low frequency calculations everywhere. (Figure 5.13f). J_{pr} is $\approx 1/2 \pi$ out of phase with the applied charge half way between the charged

FIGURE 5.13

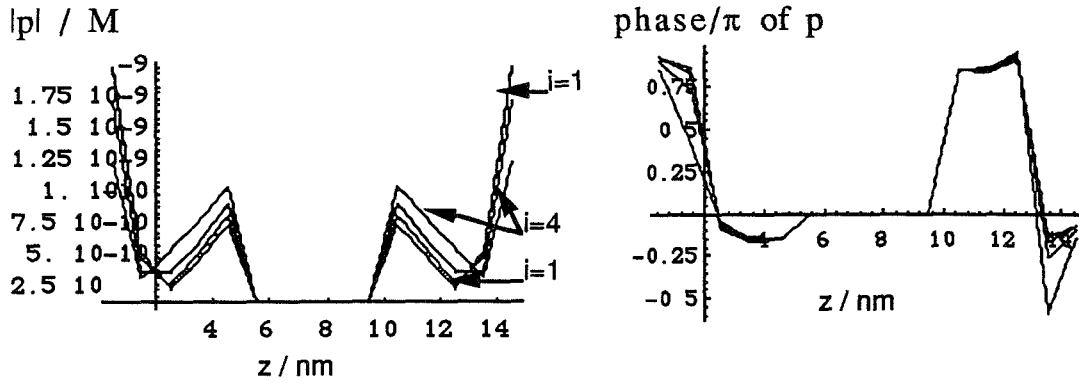


Figure 5.13a. The amplitude and phase/ π of the a.c. cation concentration for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies.

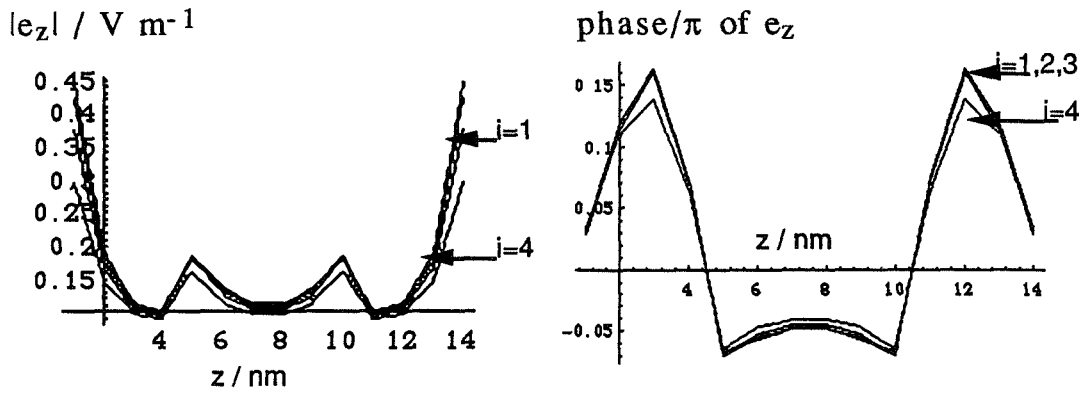


Figure 5.13b. The amplitude and phase/ π of the axial components of the a.c. electric field (e_z) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies.

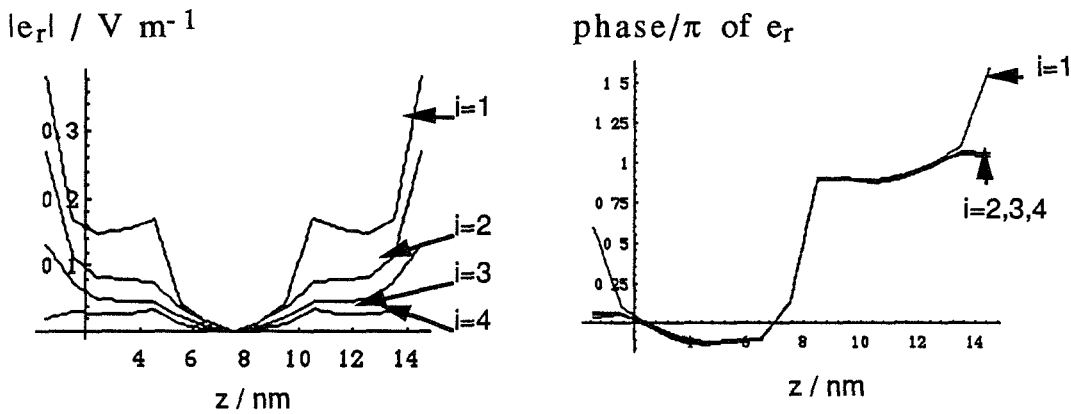


Figure 5.13c. The amplitude and phase/ π of the radial components of the a.c. electric field (e_z) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies.

FIGURE 5.13 continued

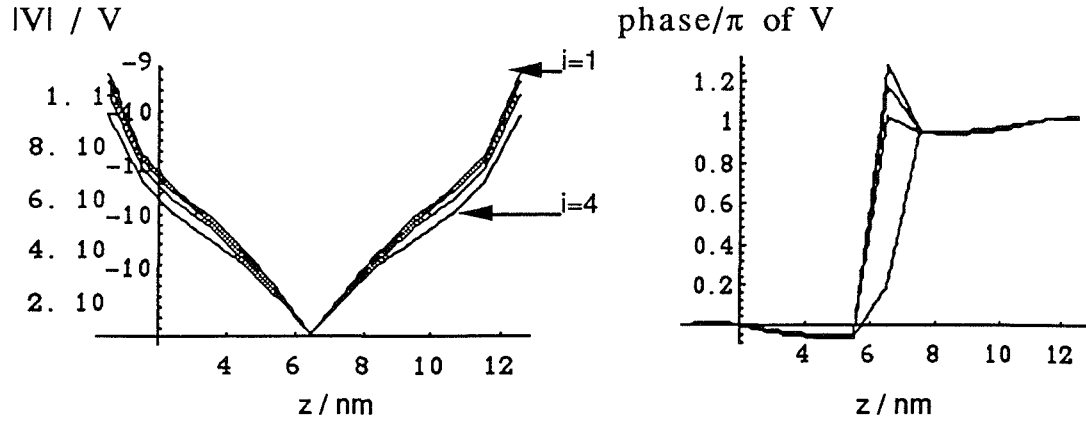


Figure 5.13d. The amplitude and phase/ π of potential difference (V) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies.

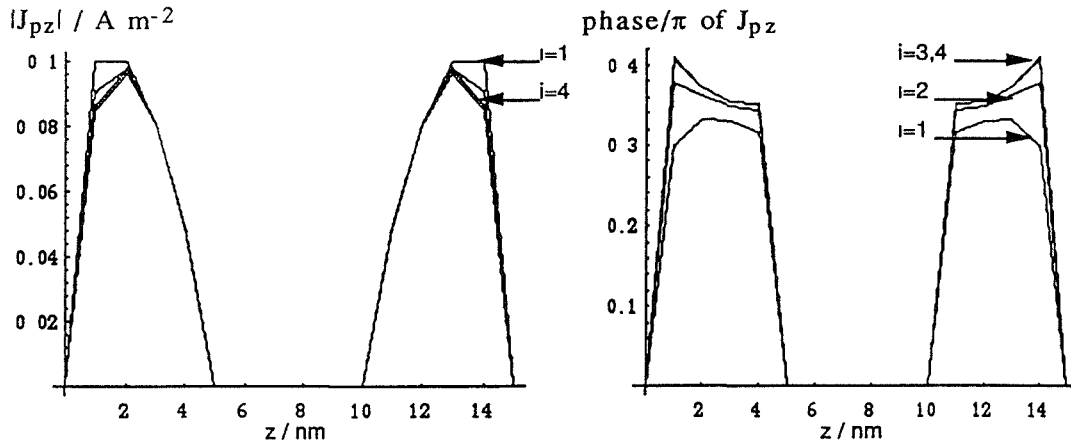


Figure 5.13e. The amplitude and phase/ π of the axial component of the cation current density (J_{pz}) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies

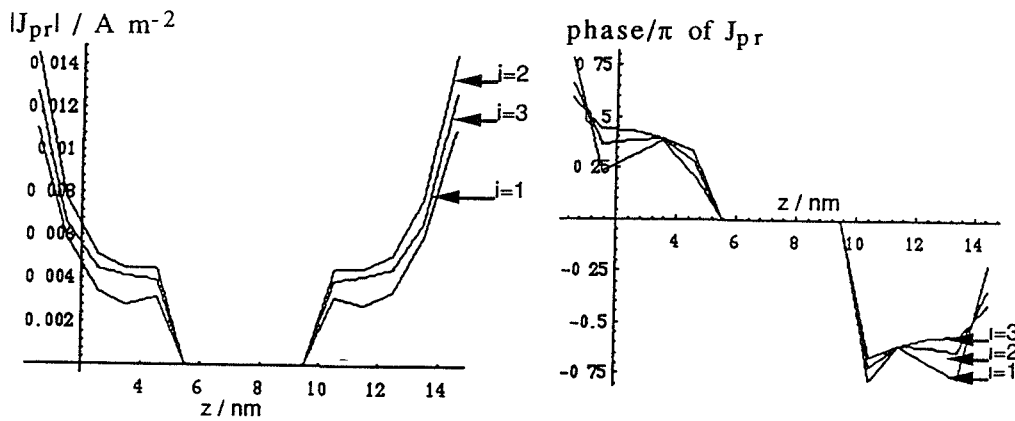


Figure 5.13f. The amplitude and phase/ π of the radial component of the cation current density (J_{pr}) for the different voxel radii (r_i , $i=1,4$) for the insulating membrane at high frequencies.

boundaries. It leads the applied charge in the region between the positively charged boundary and the insulating membrane and lags in the region between the insulating membrane and the negatively charged boundary.

5.5 A SIMPLE PORE IN A MEMBRANE

The effect of putting a pore through the insulating membrane is now investigated. The calculations presented for this and the following chapter are for a pore radius approximately equal to one Debye length, and the configuration for these calculations is depicted in Figure 5.14. Note that the dielectric constant of the membrane is still the same as that of the electrolyte in this chapter. The geometrical conductance (G_K) for the pore is lower than G_K of the double layer of the electrolyte cylinder of section 5.3.3 above, hence its characteristic frequency f_c is lower than f_{dl} . (G_K for the pore is given by 5.3.2, for a value of L_G = length of the pore)

This system is quantitatively different from most biological membranes, whose surface area is very large compared to that of the pore. In such membranes, a large membrane capacitance can be considered to be approximately in parallel with the conductance of a single pore. This calculation is qualitatively similar, but because of the small capacitance of the pore the characteristic frequency of this "membrane" is relatively high. Also, experimental electrodes are situated at macroscopic distances away from the membrane rather than nm away. Therefore the conductance of the electrolyte is considerably lower than for this case, resulting in a characteristic frequency much less than f_c (see equation 5.3.2). The logistical difficulties in these calculations prohibit either large membrane areas or large electrode distances, so the

FIGURE 5.14

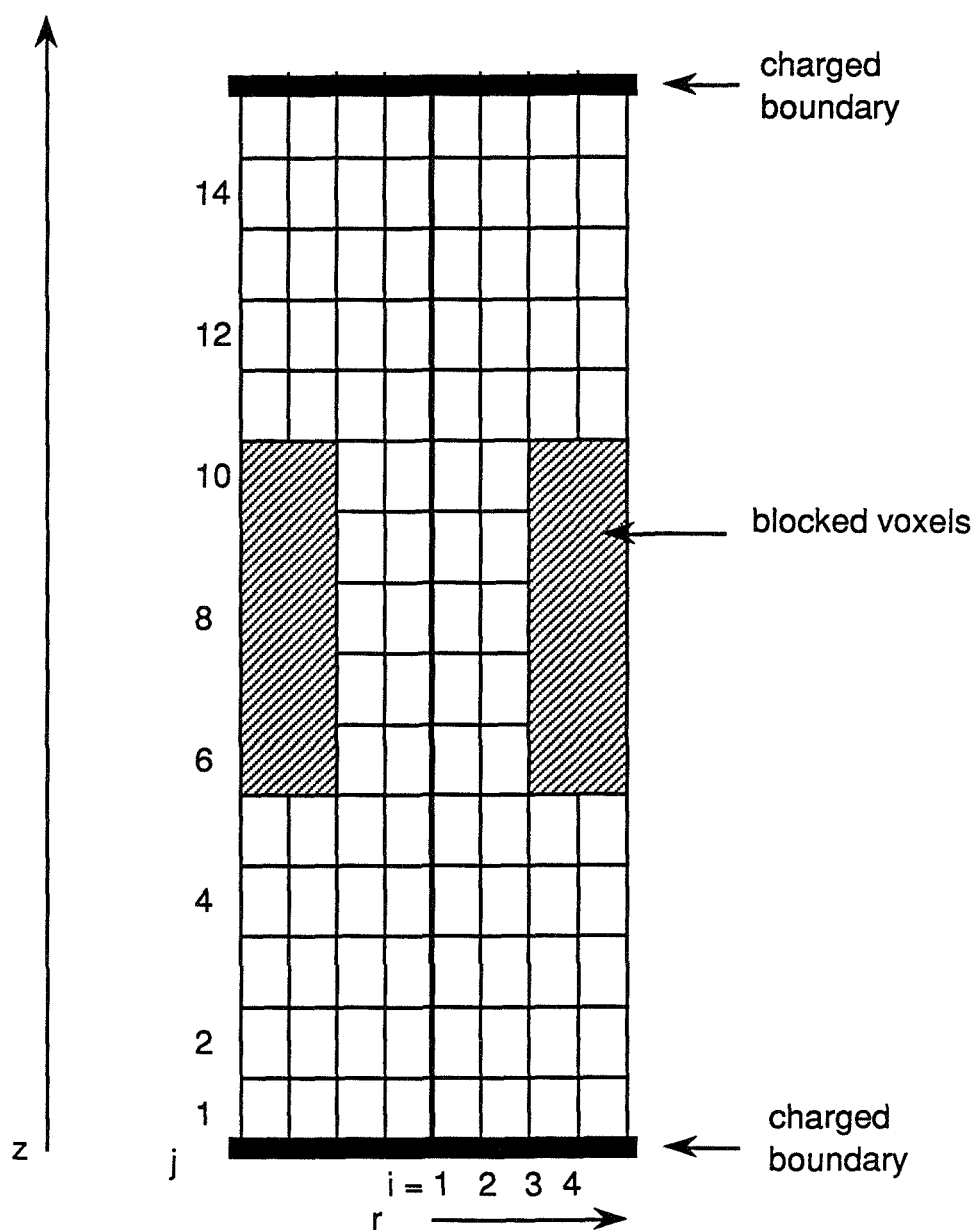


Figure 5.14 The configuration used for the calculations for the simple pore.

characteristic frequency in these calculations will seem very high to experimentalists. On the positive side, however, the fact that pores are far apart, means that it is unlikely that they interact with each other, and hence the characteristics of only a single pore needs to be studied.

5.5.1 Low Frequency Calculations

The following results are for a frequency lower than f_c . The cation concentration (p) in the voxels surrounding the insulating membrane increases in comparison to that for a cylinder of electrolyte solution in section 5.3.3b (Figure 5.15a). However this space charge is very small compared to that near the charged boundaries (the latter is approximately the same as before). It is also very different compared to the insulating membrane of the previous section, i.e. it is smaller in amplitude and p near the insulating region does not change sign. The increase in space charge results in an increase in the electric field (e) in the central region compared to that of the electrolyte cylinder of section 5.3.3b (Figure 5.15b and c). This leads to an increase in the potential difference (V) in the central region, which is however much less than the potential difference across the insulating membrane. The phase of V with respect to the applied charge approaches $\pi/4$ near the centre and decreases in the r direction (Figure 5.15e). This variation would be large if the membrane were large.

Plots of the current densities have again been omitted for this frequency, because artefacts due to quantization noise.

FIGURE 5.15

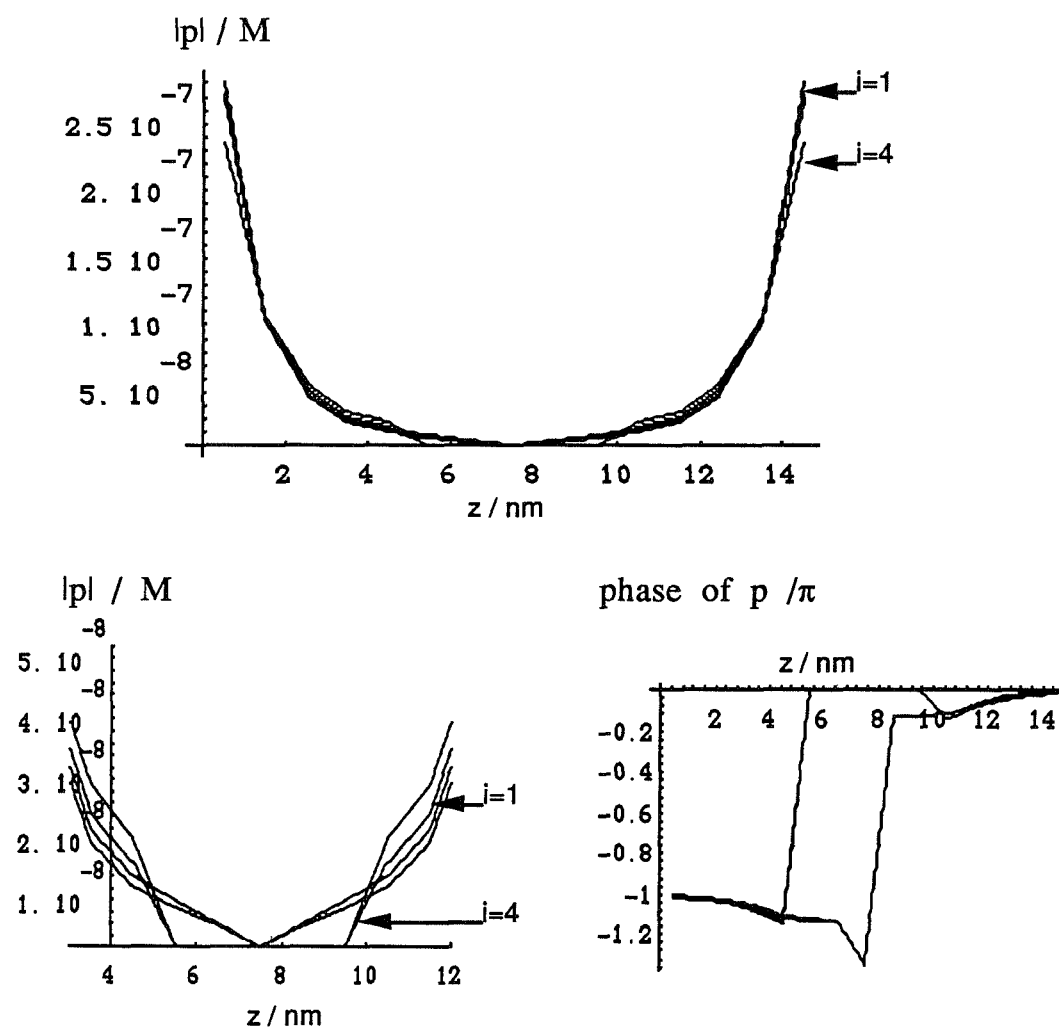


Figure 5.15a. The amplitude ($|p|$) and phase/ π of the a.c. cation concentration (p) for the different voxel radii r_i , $i=1$ to 4 , for a simple pore at low frequencies.

FIGURE 5.15 continued

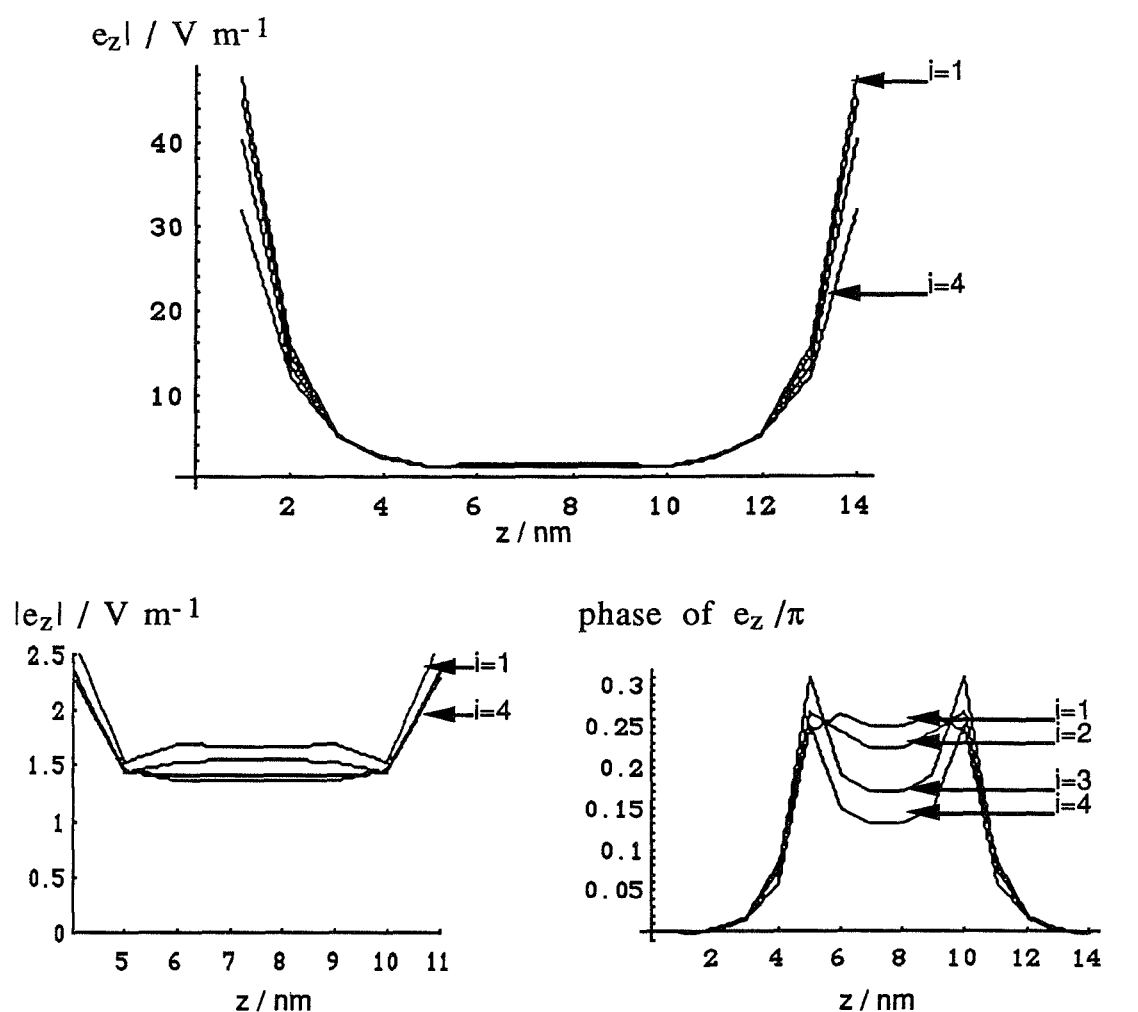


Figure 5.15b. The amplitude and phase/ π of the z component of the a.c. electric field (e_z) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at low frequencies.

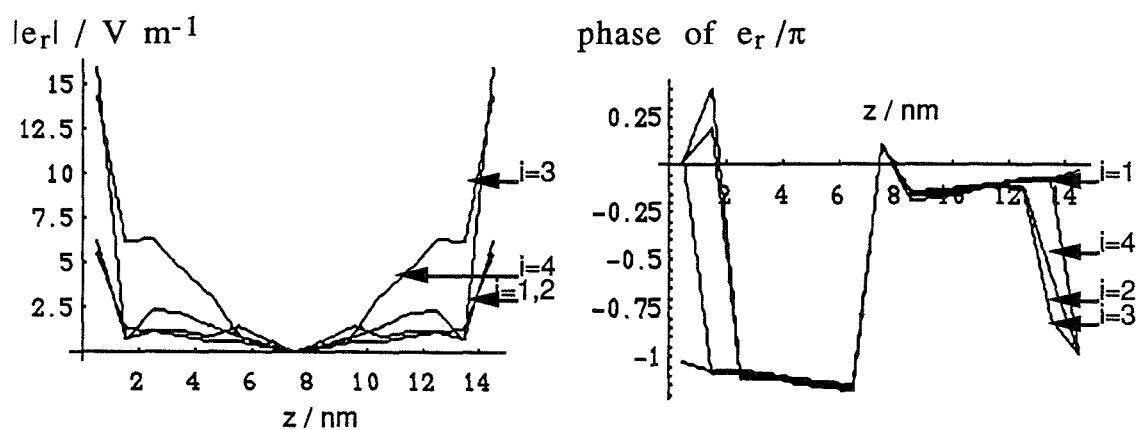


Figure 5.15c. The amplitude and phase/ π of the radial a.c. electric field (e_r) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at low frequencies.

FIGURE 5.15 continued

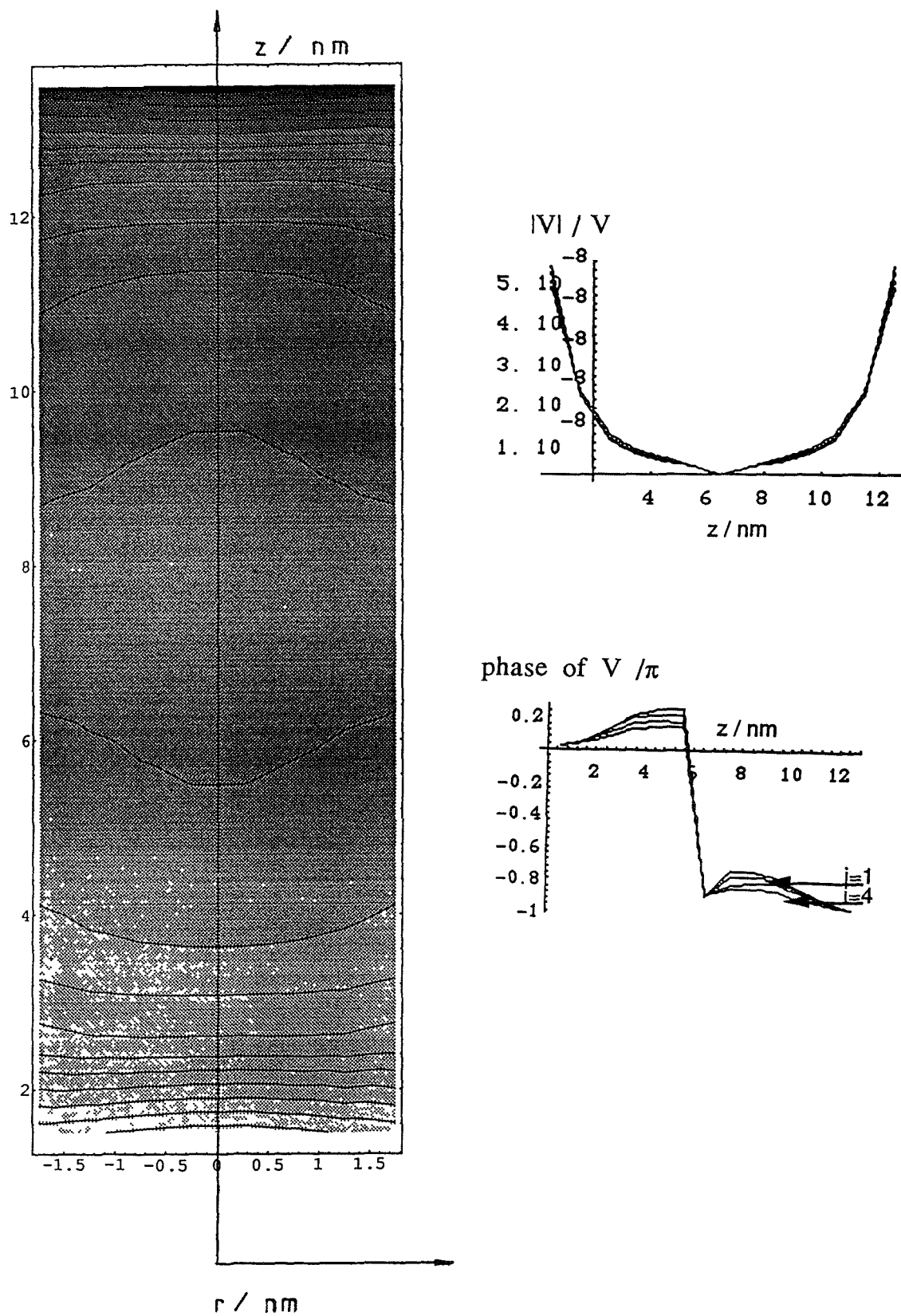


Figure 5.15 d and e. The low frequency potential calculations for the simple pore: d) shows the equipotential lines and e) gives the amplitude and phase/ π of the potential (V).

5.5.2 Calculations at a Frequency above f_c .

When the frequency is high, there is little time for charge transfer, hence ions accumulate near the membrane/electrolyte interface forming a double layer similar to that for the insulating membrane without a pore (i.e. the space charge in this region is smaller in magnitude but of opposite sign than that of the nearest charged boundary). Ions also accumulate at the pore entrance (Figure 5.16a). This charge leads to an increase in the electric field (and therefore potential (V)) in the region near the pore entrance and near the electrolyte/membrane interface (Figures 5.16b to d). As for the insulating membrane, p/σ_0 in the double layer near the charged boundaries is lower than that for the low frequency case. σ_0 is again a factor 1/100 lower than σ_0 for the low frequency calculations.

As for the low frequency calculations, the cation current density (J_{pz}) is highest inside the pore but decreases rapidly with distance in the bulk solution before it increases again near the charged boundaries. This increase near the charged boundaries is due to the current that charges the double layer (Figure 5.16e). J_{pz} now has a considerably lower magnitude in the central region than that for the low frequency case. The phase of J_{pz} leads the applied charge in the double layer near the charged boundaries (by up to $.4 \pi$ for these calculations). J_{pz} charges the double layer, hence phase difference between J_{pz} and the applied charge approaches $\pi/2$ near the boundaries. This phase difference decreases with distance away from the boundaries. In the central region the phase of J_{pz} slightly lags the applied charge.

As ions cannot pass through the insulating membrane, the radial component of the cation current density (J_{pr}) is highest near the electrolyte/membrane interface, as well as near the channel entrance

FIGURE 5.16

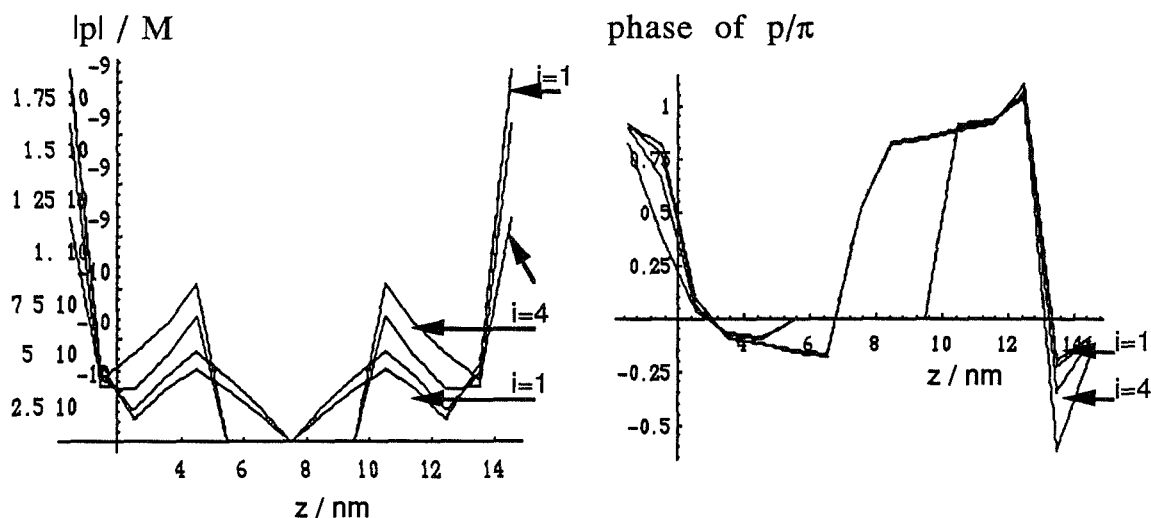


Figure 5.16a. The amplitude and phase/ π of the a.c. cation concentration (p) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

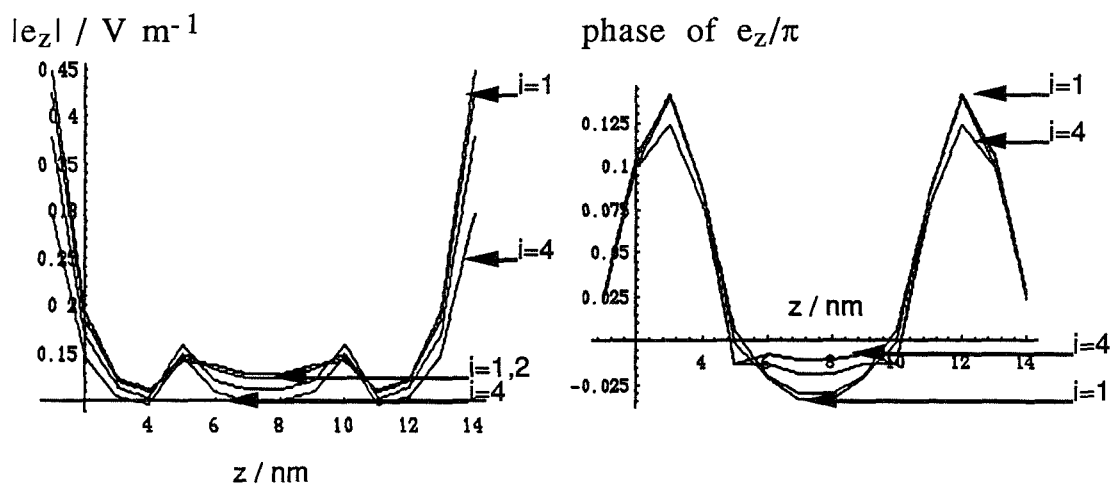


Figure 5.16b. The amplitude and phase/ π of the z component of the a.c. electric field (e_z) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

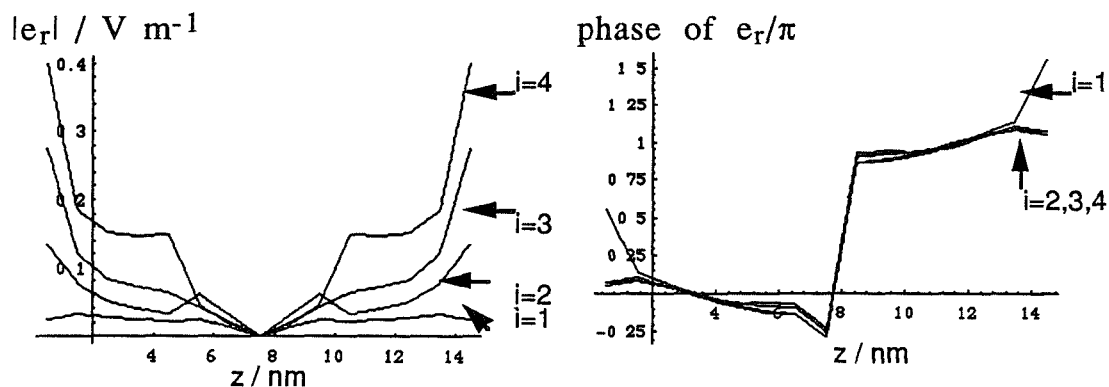


Figure 5.16c. The amplitude and phase/ π of the radial component of the a.c. electric field (e_r) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

FIGURE 5.16 continued

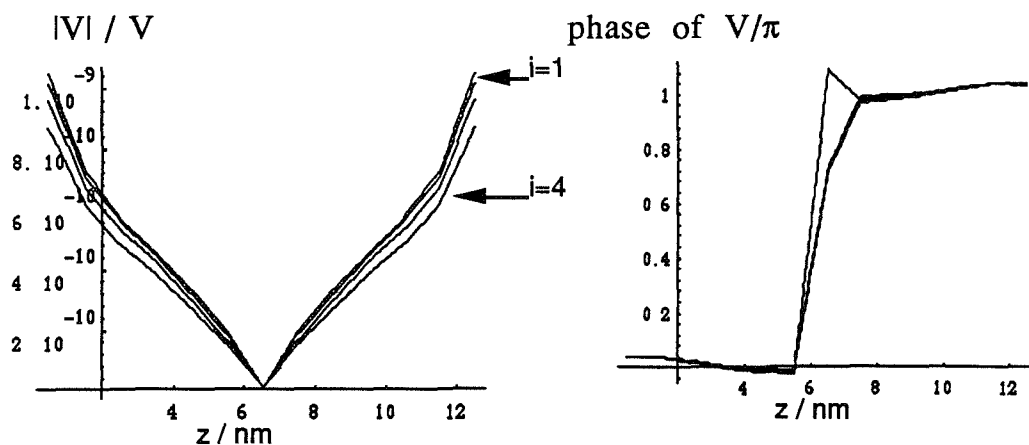


Figure 5.16d. The amplitude and phase/ π of the potential difference (V) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

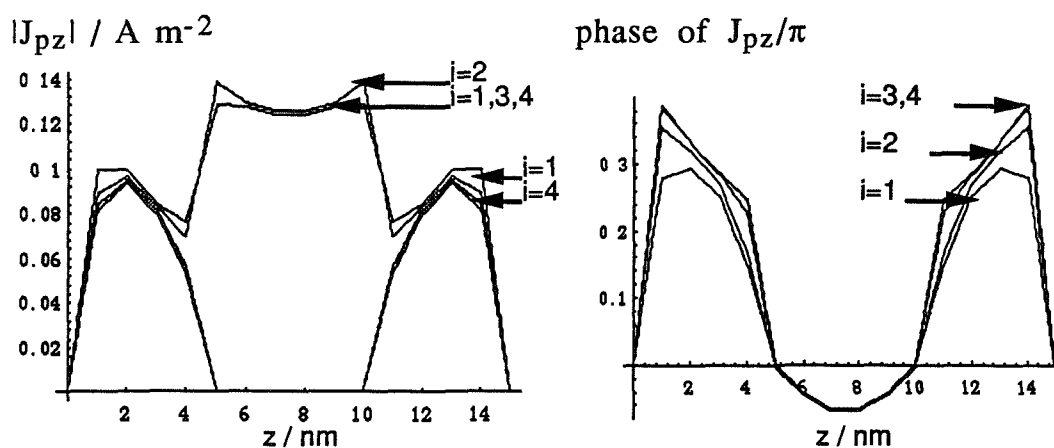


Figure 5.16e. The amplitude and phase/ π of the axial component of the a.c. cation current density (J_{pz}) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

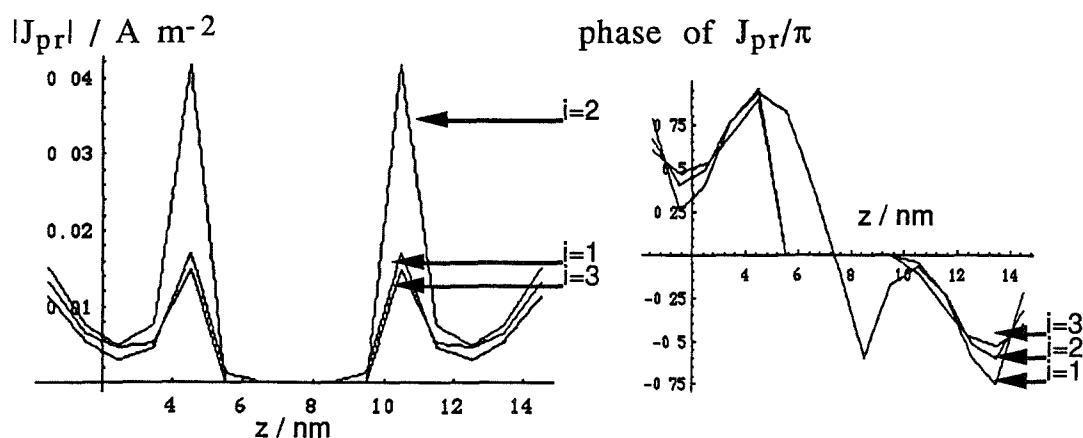


Figure 5.16f. The amplitude and phase/ π of the radial component of the a.c. cation current density (J_{pr}) for the different voxel radii r_i , $i=1$ to 4, for a simple pore at a frequency above f_c .

(Figure 5.16f). The largest increase occurs near the edge of the channel ($i=2$). J_{pr} is in phase with the applied charge in the central region, but changes sign (J_{pr} is antisymmetric). The increase in J_{pr} in the double layer near the charged boundaries can be mainly attributed to the finite size of the system.

Some of the peculiarities of the phase plots (e.g. "spikes") for the high frequency calculations for both the insulating layer and the pore may be due to limited resolution (see section 5.3.3).

5.6 FREQUENCY SPECTRUM

Because calculations at low frequency take such a long time, the effect of frequency was investigated using a very low resolution system with the same overall size and geometry as the system described above. The model uses 2 voxels in the r direction and 6 in the z direction. The reduction in total voxel number led to an almost 50% decrease in a.c. space charge near the charged boundaries as well as the total potential difference between them compared to those above - the voxel size in the z direction is approximately 2.5 Debye lengths.

The same numerical calculations as above were carried out for four different frequencies f : 1 kHz, 10 kHz, 100 KHz and 1 MHz. All are lower than the characteristic frequencies of both the pore and the insulating layer. The magnitude of the total potential difference $|V|$ decreased with increasing frequency, while its phase with respect to the applied charge increased. $|V| \propto 1/f$ to a very good approximation - it is linear up to 100 kHz and deviates from linearity by less than 1% at 1 MHz.

5.7 IMPEDANCE CALCULATIONS

The area specific impedance (Z) and hence the conductance (G) and capacitance (C) for the different geometries can be determined from the amplitude ($|V|$) and phase (ϕ) of the total potential difference :

$$Z = |V| e^{j\phi} / J_{t0} \quad (5.7.1)$$

where J_{t0} is the total applied current density.

Note: ϕ is calculated with respect to J_{t0} rather than the applied charge, unlike the previous phase plots. The conductance (G) and capacitance (C) can then be calculated from the real and imaginary parts of $1/Z$.

$$G = \text{Re}(1/Z) \quad (5.7.2)$$

$$C = \text{Im}(1/(Z 2\pi f)). \quad (5.7.3)$$

For a cylinder of electrolyte at a high frequency (section 5.3.3a), the conductance (G) and capacitance (C) approach their geometrical values (G_K and C_K , respectively) given by equations 5.3.2 and 5.3.3. (G is somewhat higher and C is lower). Any differences can be mainly attributed to geometrical factors (see earlier sections of this chapter). In addition the calculated total potential difference does not include the set of voxels closest to the charged boundaries, since for this model the electric field at the charged boundaries is not defined. This leads to a lower total impedance, especially for low resolution calculations. G , for the cylinder of electrolyte solution is much lower than G_K at low frequencies, while C approaches C_K .

The insulating membrane in the central region completely blocks both cation and anion flow through that region, therefore the conductance approaches zero at low frequencies, and is considerably reduced when compared to that of the cylinder of electrolyte of the same thickness at high frequencies. This non zero conductance is due to the finite charging current in the region next to the electrolyte/membrane interface. Blocking voxels to ion flow (simple pore) reduces the high frequency value of the calculated conductance of the central region compared to that for a cylinder electrolyte of the same thickness, especially at low frequencies, as expected (Figure 5.17).

The capacitance ratio C/C_K for the insulating membrane is higher than for a region of bulk electrolyte of the same thickness, due to the accumulation of charge in the double layers, especially at low frequencies. This increase is much greater for the case of a simple pore, since the potential difference across the pore is considerably lower than for the insulating membrane. Since there is little charge transfer at high frequencies, C/C_K for the insulating layer and the simple pore are approximately the same (Figure 5.17b). The capacitance of a small channel in a membrane is dominated by that of the surrounding insulating layer.

5.8 CONCLUSIONS

Numerical solutions to the Nernst-Planck equations were presented for different geometries at both high and low frequencies. Numerical solutions have the advantage of allowing arbitrary channel configuration. However, spatial and temporal resolution are limited. Both the total number and size of the voxels are limited by feasible computation times on the computer available for these calculations. The resulting effects of

FIGURE 5.17

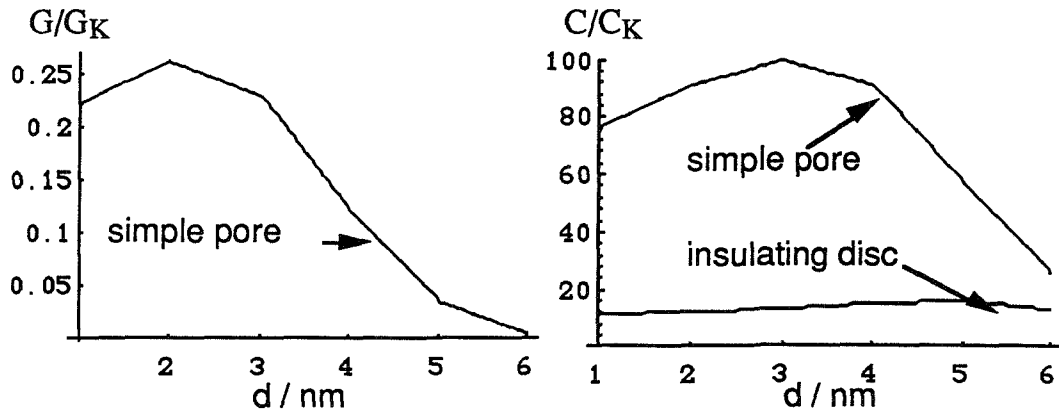


Figure 5.17a. The conductance (G/G_K) and capacitance ratios (C/C_K) as a function distance (d) from the plane of symmetry for the low frequency calculations of chapter 5. G_K and C_K are the geometrical conductance and capacitance defined by equations 5.3.2 and 5.3.3 for a thickness $L = 2d$.

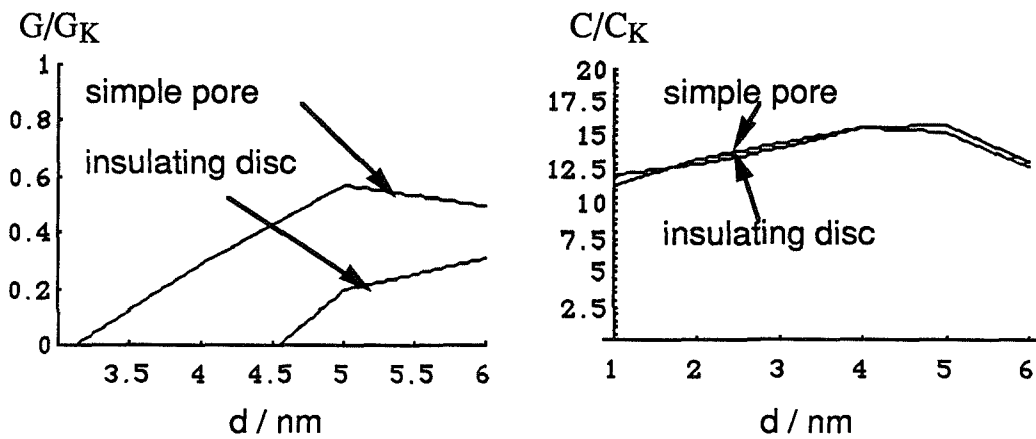


Figure 5.17b. The conductance (G/G_K) and capacitance ratios (C/C_K) as a function distance (d) from the plane of symmetry for the high frequency calculations of chapter 5. G_K and C_K are the geometrical conductance and capacitance defined by equations 5.3.2 and 5.3.3 for a thickness $L = 2d$.

finite size and quantization errors are discussed. For the model chosen for this thesis, effects of geometry have been found to be more important than those due to the finite voxel in the in the boundary.

Calculations for the simpler geometries (i.e. for the case of an infinite slab and a cylinder of electrolyte) show approximate agreement with the analytical solutions of Chapter 3 and the differences are mainly attributable to effects of the finite size of the systems used for the numerical calculations.

Cylindrical geometry has been used except for part of the comparison with analytical solutions. Calculations presented in this chapter assume that the dielectric constant is constant everywhere, to avoid complications due to image charges. Effects due to the difference in dielectric constant are included in Chapter 6.

Results were presented for the following configurations:

- i) A cylinder of electrolyte: At high frequencies the ions cannot move far during each cycle, resulting in only a slight screening of the applied charge. At low frequencies there is sufficient time for ions to accumulate near the charged boundaries to form a double layer.
- ii) An insulating membrane: At low frequencies ions accumulate near the electrolyte/membrane interface forming a double layer with space charge of opposite sign to that of the nearest charged boundary. This leads to an increase in the electric field, and hence potential difference in this region.

The results for the high frequency calculations are similar, except that the space charge in the double layers is reduced. Because the membrane completely blocks ion flow through it, the conductance of the central region is zero. The accumulation of

charge leads to large capacitances of the central region, especially at low frequencies.

iii) A simple pore through a membrane: At low frequencies ions can move sufficiently far per cycle to pass through the pore, leading to only a slight space charge near the membrane/electrolyte interface, hence the electric field is also lower in the central region compared to that of the insulating membrane.

The membrane blocks a significant region to ion flow, leading to a reduction in the conductance of the central region compared to the geometrical value, especially at low frequencies. As for the case of a membrane, the capacitance of the central region is considerably greater than the geometrical value at both high and low frequencies. At low frequencies the capacitance of the pore is considerably higher than that of the insulating membrane due to the lower electric field in the central region.

CHAPTER 6

DECORATIONS TO THE MODEL OF AN ION CHANNEL

6.1 INTRODUCTION

The ion channels through biological membranes that are formed by protein molecules are much more complex than the simple model of the pore described in Chapter 5. In this chapter an extension of the model to mimic more closely biological channels is presented. The embellishments to the basic model include the influence of Born energies, fixed charge and ion selectivity as a result of differences of diffusion constants of the different ion species inside the channel and the possibility of phenomenological flux coupling.

6.1.1 Born Energy

In the calculations of Chapter 5 it was assumed that all unblocked voxels were equally accessible to ions. This would not be the case in a pore in a biological membrane, due to the effect of image forces. It is energetically unfavourable for ions to enter a region of low permittivity (ϵ), because of the consequent increase in their self energy, i.e. the energy stored in the electric field surrounding them which is inversely proportional to ϵ (see Chapter 2). In the previous chapter the assumption of a uniform ϵ throughout the system was made, so that Born energy effects, i.e. the effects of image charges could be excluded. However the dielectric constants of water and the hydrocarbon interior of lipid

membranes differ by a factor of 30 or more. This effect will be included by adding an energy term due to the difference in Born energy in the voxels at the boundary of dielectric discontinuity.

6.1.2 Fixed Charges

The ion distribution in channels is also affected by charges fixed in the channel itself. Researchers in molecular biology (e.g. Fox and Richards, 1982) have now determined the amino acid sequence and structure of some proteins that form ion channels in membranes. Some of the amino acids forming these proteins carry a net charge at physiological pH. Therefore they attract ions of opposite charge (counter-ions) and repel ions of the same sign (co-ions). For example, one of the most widely investigated channel forming proteins is gramicidin A (Wallace, 1990). When incorporated into lipid bilayers, it can significantly increase the flux of cations through the membrane. Negatively charged carbonyl groups are situated at the most constricted part of a gramicidin A channel. It is believed that the interaction between ions and these groups aids the passage of cations through the channel.

The model of ion channels described in the previous chapter assumes that the surface of the cylindrical hole through the dielectric slab is uncharged. The model is now modified to allow for fixed surface charges.

6.1.3 Selectivity

For the calculations of Chapter 5, it has been assumed that the diffusion constant of each ion species is the same everywhere inside and outside the channel. However, some ion channels may hinder or

completely block the passage of specific ions through them, presumably via local geometry or fixed charges. To study this, the diffusion constant of each ion species inside the channel is allowed to differ from that of the external solution.

Selectivity could also arise from the geometry of the fixed charge sites and the different hydration energies (Eisenman and Horn, 1983).

Another effect which could be allowed for in the model is phenomenological coupling between the two ion species, i.e. the flux of one ion species affects the flux of another species.

6.2 METHODS

6.2.1 The Effect of the Difference in Dielectric Constant.

The effect of the Born energy between the lipid bilayer and the electrolyte solution is accounted for in the following way.

The ion current density J_i of the i th ion species can be determined from the negative derivative of the total chemical potential (see Chapter 3):

$$J_i = \frac{-q_i D_i z_i C_i \nabla \mu_i}{kT} \quad (6.2.1)$$

where : z_i is the valency of the i th ion species

D_i is its diffusion constant

C_i is its concentration

The chemical potential ($\tilde{\mu}_i$) of an ion species in a dielectric medium is given by:

$$\tilde{\mu}_i = \mu_i^0 + kT \ln a_i + PV + \text{other energy terms}, \quad (6.2.2)$$

where : μ_i^0 is the standard chemical potential, a_i is the activity of the i^{th} ion species, P is the pressure and V the volume.

The Born energy can be added as an explicit term, or included in μ_i^0 - the effect is the same, only the accounting is different. The difference in chemical potential ($\Delta\mu_i^0$) then appears in the calculations because J_i is proportional to $\nabla \mu_i$ (see equation 6.2.1).

The Born energy comprises of two terms:

The first term is the "self energy" (U_E) of an ion in a dielectric medium due to the electric field of the ion. For the i^{th} ion species in an infinite dielectric medium of permittivity ϵ U_E is equal to (see Chapter 2):

$$U_E = \frac{z_i^2 q^2}{8 \pi \epsilon a_i} \quad (6.2.4)$$

where a_i is the radius of the the i^{th} ion species.

The difference in Born energy between two semi-infinite media with permittivities ϵ and ϵ_p is:

$$\Delta U_E = \frac{z_i^2 q^2 (1/\epsilon_p - 1/\epsilon)}{8 \pi a_i} \quad (6.2.3)$$

The second term arises from dielectric discontinuities. For a dielectric slab of finite width, the energy due to the polarization charges at the boundary surfaces of the slab (U_P) has to be added to the Born energy. One method of estimating this extra term is to sum the contribution of the image charges, i.e. charges located at positions which

are reflections of the position of the actual ion through the boundary surfaces. This infinite set of charges allows calculation of the force on the test charge. These calculations are, however, very complicated (Neumke and Lauser, 1969).

Parsegian (1975) has calculated this term numerically for the following case (these results are given relative to the energy of an ion in an infinite pore): A slab of width L , (infinite in the other two dimensions) and permittivity ϵ_m is surrounded by a medium with much higher permittivity ϵ . The slab contains a cylindrical hole of radius b ($b \ll L$) and permittivity ϵ_p . An ion is situated at the centre of this cylinder.

A value for the Born energy was chosen of the same order of magnitude as the ones calculated by Parsegian. (The geometry here differs from that of Parsegian's calculations, which apply to ions at the centre of the channel, so the exact values were not used.

The difference between the Born energy ($U_B = U_E + U_P$) of an ion inside the channel and that of an ion in the electrolyte solution gives an estimate of $\Delta\mu_i^0$ ($\Delta\mu_i^0 = U_B/q$) which can then be included in the ion current density equations for the voxels shown in Figure 6.1.

The proteins forming the channel effectively lower the dielectric constant inside the channel by varying amounts. The effect of this could be included by adding different Born energy terms to the current density in all channel voxels. On the other hand, the surface groups of lipids also have a dielectric constant, which is greater than the one in their interior. In the absence of detailed information about structure, and in the interest of simplicity, this has not been pursued.

6.2.2 Charged Channels

According to Coulomb's law, any net fixed charge on the channel surface will attract ions of the opposite sign and repel ions of the same sign, therefore altering the ion distributions and current densities in the region surrounding the charge. Unlike all previous cases this system is not charge symmetric.

Charges are introduced by smearing one (or $1/n$) ion charges over a ring in one or more of the voxels comprising the channel surface. The radius of the ring for this "surface" charge is the same as that used for the electric field calculations due to the space charge (see section 5.2.2), i.e. it includes a cross-sectional area weighting factor. The electric field due to these charges at each voxel surface is added to that due to the a.c. space charge and surface charge at the circular boundaries.

The polar groups of some channel forming proteins are believed to form electric dipoles. Even though the computer program used for these calculations allows for an arbitrary number of surface charges, the results presented in this chapter are for a single charge only.

Even though the structure of some protein forming channels have been studied extensively, their configuration in the lipid bilayer may differ from that of their crystallized form. Furthermore, the detailed structure of only a few relatively simple channel proteins is known to date. Because of the assumptions used in the model and the rather crude voxel size, the calculations presented in this thesis only give qualitative results. It will, however, be relatively simple to extend these calculations when better data and faster machines are available.

6.2.3 Asymmetric Ion Distributions

Even though ions can pass through channels or pores easily in comparison to the lipid bilayer, the presence of these constrictions may still affect the diffusion rate of the ions passing through them. The extent of this effect can differ for the two ion species. For example, the ion radius relative to the pore radius, the degree of hydration of the ion and the presence of charged groups on the channel surface could all contribute to the rate of diffusion of a particular ion species throughout the channel.

This effect has been introduced by assuming that the diffusion constant of one (or both) of the ion species is now decreased by an arbitrary fraction for the voxels inside the channel and near the channel surface.

6.2.4 Coupling between the two Ion Species

Experiments on some types of channels indicate that the flux of one ion species through the channel influences the flux of the other species.

One method of accounting for this dependence would be to add a fraction of the anion current density at time t to the new cation current density (i.e. at time $t+\Delta t$) and subtract it from the new anion current density (or vice versa).

This has not been pursued in this thesis because it is, in a sense, contrary to the philosophy of this work. Flux coupling constants are empirical constants which throw no light on the physical process. Once the physical reason for a particular coupling process is understood, it can be incorporated explicitly in computations such as these. One such mechanism, for instance, could be the reduction of the electrical potential by the associated transport of ions of opposite charge.

6.3 RESULTS

The first three modifications to the model discussed are added to the simple pore as previously described in Chapter 5.

Using the method described in section 5.2 all variables are iterated until they cease to vary with time, for the case of zero applied a.c. signal. These equilibrium ion distributions and electric fields (for the cases where it is non zero) are used as initial values for the a.c. calculations.

As before, the a.c. calculations are performed at two different frequencies: the first frequency (f_l) is very much lower than the characteristic frequency (f_c) and the second (f_h) above f_c . For the former, the same frequency and number of points per cycle as for the low frequency calculations of Chapter 5 are used. The calculations take approximately the same time as those in Chapter 5.

Each of the modifications to the simple model affects the characteristic frequency of the system. These new characteristic frequencies are still of the same order of magnitude as f_c of Chapter 5. To allow comparison with the results of Chapter 5, the same values of the frequency were used for the calculations. As in the previous chapter the applied current density is kept constant rather than the applied charge (Q_0), when changing frequency. Therefore, for all high frequency calculations of this chapter the applied charge is smaller than for the calculations below f_c by a factor f_h/f_l . The total size of the system, pore geometry, applied charge and bulk electrolyte concentration etc. are identical to the simple pore described in the previous chapter (see Table 5.1a and 5.1b of Chapter 5).

6.4 EQUILIBRIUM DISTRIBUTION FOR THE BORN ENERGY VERSION

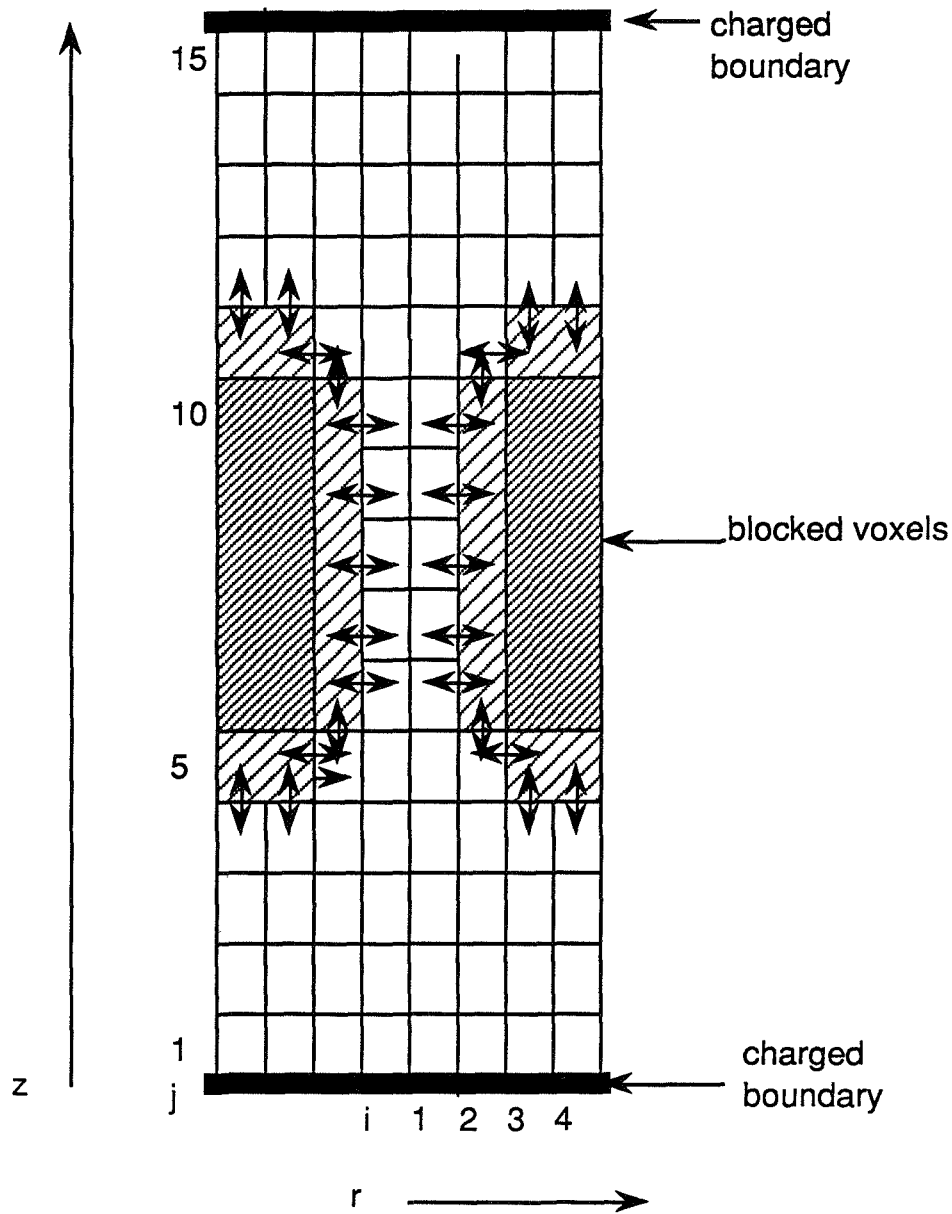
Ions have lower energy in a medium of high dielectric constant, hence their concentration is reduced in and near regions of low dielectric constants, such as bilayers. As described in section 6.2.1, the effect of the difference in dielectric constant between the membrane and the electrolyte solution is modelled by adding an extra term to the chemical potential proportional to the Born energy (U_B) (see Figure 6.1).

This of course reduces the equilibrium ion concentrations in the voxels surrounding the dielectric by $e^{-U_B/kT}$, as given by Boltzmann statistics. The calculations in this section are for a Born energy of 1 kT for both ion species.

Initially the ion concentrations are set equal to the bulk electrolyte concentration in all unblocked voxels. The iterations for zero applied signal produce the expected equilibrium concentrations (Figure 6.2). At equilibrium the ion current density term due to the concentration gradient and the Born energy gradient term for the voxels surrounding the dielectric are equal in magnitude but opposite in sign. The calculated values of both ion current densities and electric field are negligibly small at equilibrium.

Note that plots of current densities for calculations at a frequency below f_c have again been omitted for the three complications considered, because of uncertainties due to quantization noise, as discussed in section 5.3.4.

FIGURE 6.1



The lightly shaded regions indicate voxels where the Born energy term is considered.



Born energy term affects current densities in the z direction



Born energy term affects current densities in the r direction

Figure 6.1. The configuration used for the calculations which include Born energy terms.

FIGURE 6.2

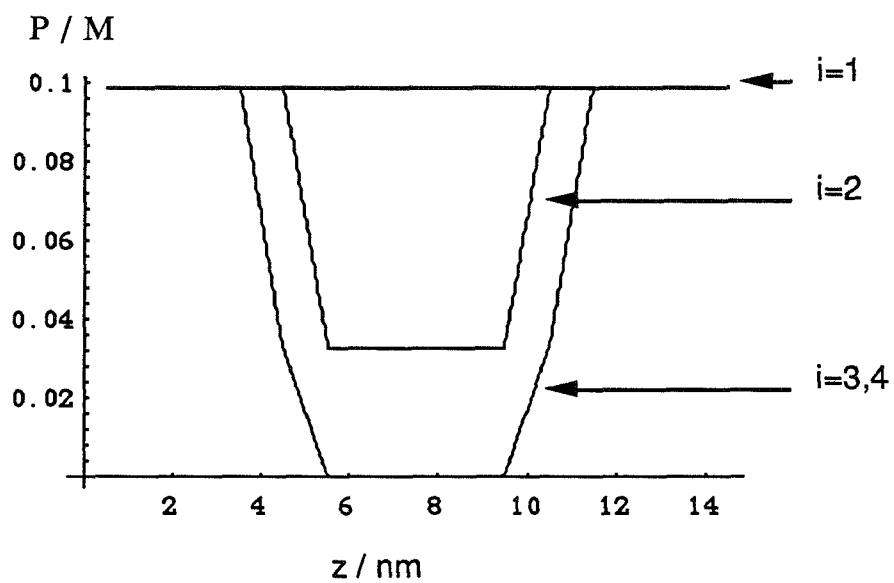


Figure 6.2. The equilibrium cation concentration (P) for a Born energy (U_B) of 1 kT.

6.5 RESULTS FOR THE A.C. SIGNAL RESPONSE INCLUDING THE EFFECT OF THE BORN ENERGY

The effect of applying a small a.c. signal (of magnitude equal to that used in the previous chapter) to the two circular boundaries is now investigated.

6.5.1 Low Frequency Calculations

The amplitude of the a.c. cation concentration (fig. 6.3a) is considerably lower than those for the simple pore in the voxels for which the "Born energy" term (i.e. the image forces) affects the ion currents, since the image forces increase the ion flux out of the region surrounding the dielectric. It is somewhat higher in the channel voxels closest to the z axis as well as near the channel entrance (approximately 10% for the parameters chosen). Near the charged boundary it is approximately the same as for the simple pore of Chapter 5.

Compared to the simple pore, the phase of the a.c. cation concentration lags the applied charge by a larger amount near the centre. This is due to the lower concentrations: the fluxes are smaller, which means that the time taken to shift a given amount of charge is greater. The phase does not vary with radial distance.

Adding a Born energy term effectively increases the resistance of the pore. Thus the axial component of the a.c. electric field (e_z) (Figure 6.3b) in the channel and the potential difference (V) across it increase (Figure 6.3d). e_z for the simple pore is approximately constant inside the pore and then increases with distance away from the channel entrance. For the case discussed here, the constant region includes the voxels at the channel entrance. The radial component of the electric field (e_r) is similar to that

Figure 1 consists of two plots. The top plot shows the amplitude of the pressure field p/M versus z/nm for $l=1$ and $l=4$. The y-axis is labeled 'amplitude of p/M ' and has a scale from 1 to 10 on a logarithmic scale. The x-axis is labeled ' z/nm ' and ranges from 0 to 14. The plot shows two curves, one for $l=1$ and one for $l=4$, both showing a minimum at $z=8$ nm. The bottom plot shows the phase of the pressure field p/π versus z/nm for $l=1, 2, 3, 4$. The y-axis is labeled ' p/π ' and ranges from -1 to 1. The x-axis is labeled ' z/nm ' and ranges from 4 to 12. The plot shows four curves, one for each l , all showing a minimum at $z=8$ nm. A region between $z=6$ and $z=8$ nm is labeled 'blocked voxels'.

Figure 1 consists of two plots. The left plot shows the normalized electric field $|e_z| / \text{Vm}^{-1}$ versus the normalized distance z / nm . The y-axis ranges from 0 to 40, and the x-axis ranges from 0 to 14. Two curves are shown: one for $l=1$ (solid line) and one for $l=4$ (dashed line). Both curves show a minimum value of approximately 5 at $z \approx 4$ nm and a maximum value of approximately 45 at $z \approx 14$ nm. The right plot shows the phase of the electric field phase/π of e_z versus z / nm . The y-axis ranges from 0 to 0.35, and the x-axis ranges from 0 to 14. Two curves are shown: one for $l=1$ (solid line) and one for $l=4$ (dashed line). Both curves show a minimum value of approximately 0.17 at $z \approx 4$ nm and a maximum value of approximately 0.34 at $z \approx 14$ nm.

Figure 6.3b. The amplitude and phase/ π of the z component of the electric field (e_z) for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at low frequencies.

FIGURE 6.3 continued

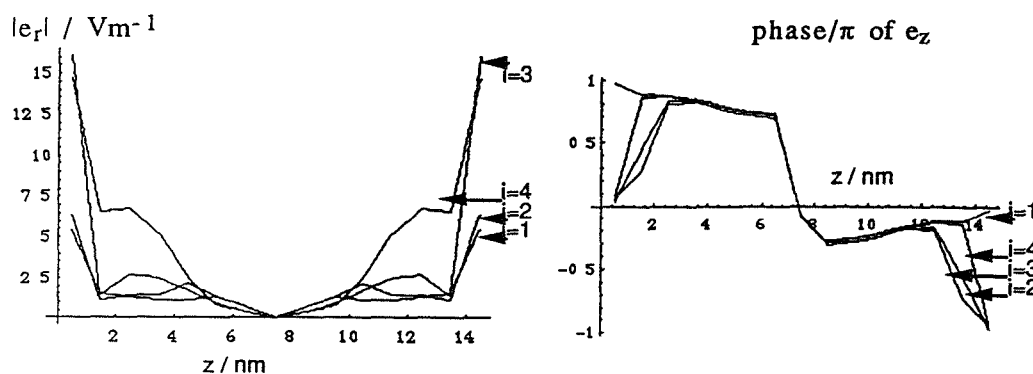


Figure 6.3c. The amplitude and phase/ π of the radial component of the electric field (e_r) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at low frequencies.

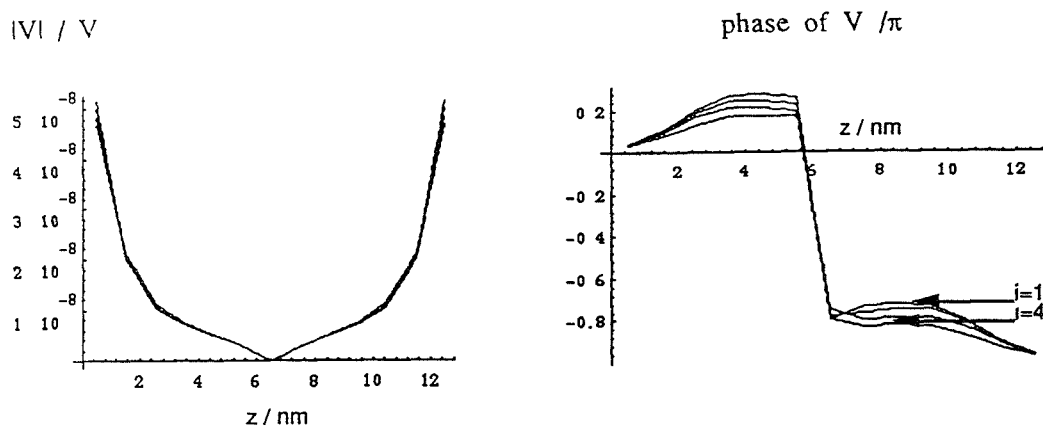


Figure 6.3d. The amplitude and phase/ π of the potential difference (V) for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at low frequencies.

for the simple pore, except close to the central axis, where it is somewhat lower (Figure 6.3c). Near the charged boundaries electric field is similar to those obtained for the simple pore. The phase of e_z and V leads the applied charge by a larger angle in the central region.

This system is charge symmetric, and so only cation concentrations and have been shown.

6.5.2 Calculations for a Frequency above f_c .

As for the case of the simple pore of the previous chapter, ions accumulate near the electrolyte/membrane interface, since charge transfer is much reduced at high frequencies. This space charge is of lower magnitude and opposite sign compared to that of the double layer near the charged boundaries. Adding the Born energy term results in a reduction of the cation concentration (p) in the voxels next to the channel wall. This leads to an increase in p near the channel entrance as well as in the channel voxels closest to the central axis. In the double layer near the charged boundaries p is similar to that for the simple pore (Figure 6.4a).

The reduction in p near the channel wall leads to a slight decrease in the z component of electric field (e_z) in this region, while the accumulation of charge at the channel entrance results in a higher e_z there. The difference is small, since the reduction of p in the channel is partially compensated by an increase near the channel entrance. The phase of e_z is similar to the case of a simple pore (Figure 6.4b). Near the charged boundaries, the radial component of the electric field e_r is similar to the case of the simple pore. In the channel and near the channel entrance, it is lower in the voxels closest to the central axis and higher near the channel wall. The phase of e_r is approximately the same as for the case of the simple pore (Figure 6.4c). The potential difference is also

FIGURE 6.4

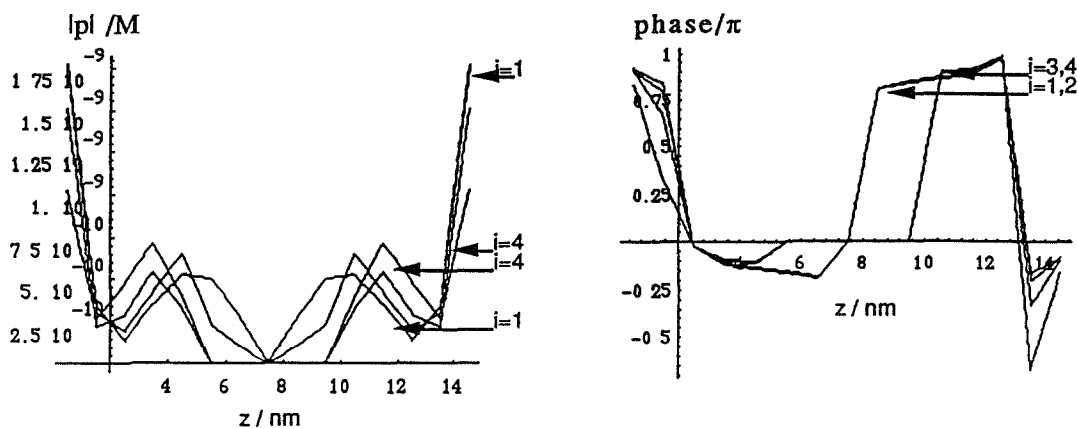


Figure 6.4a. The amplitude and phase/ π of the cation concentrations (p) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

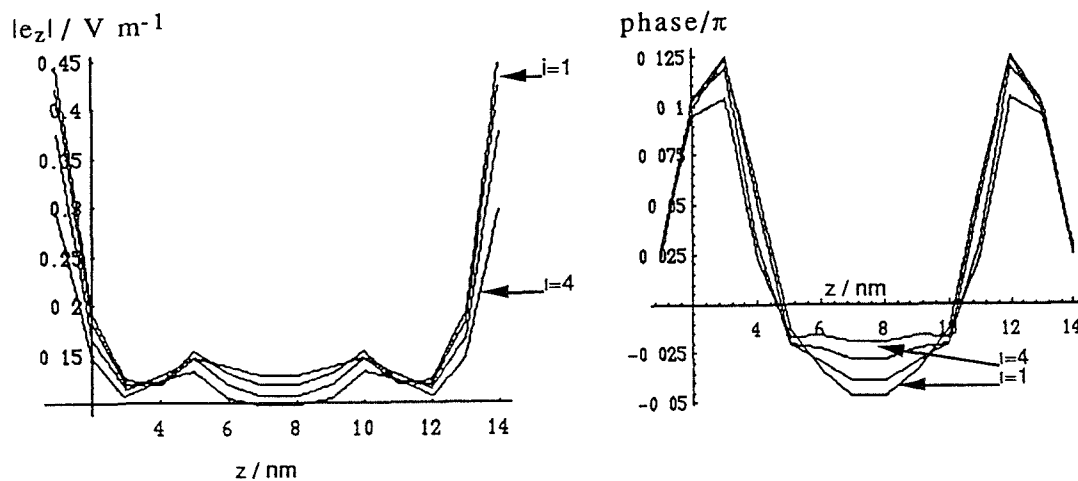


Figure 6.4b. The amplitude and phase/ π of the cation concentrations (p) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

FIGURE 6.4 continued

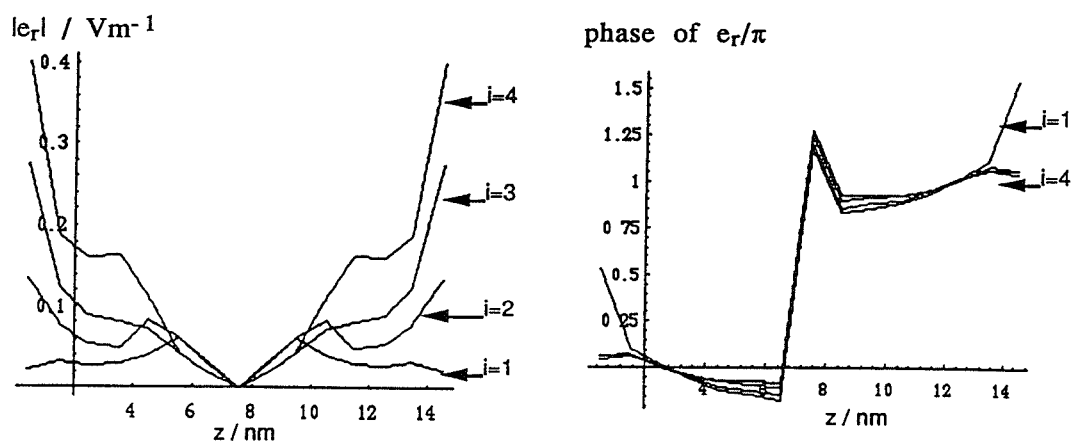


Figure 6.4c. The amplitude and phase/ π of the radial component of the electric field (e_r) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

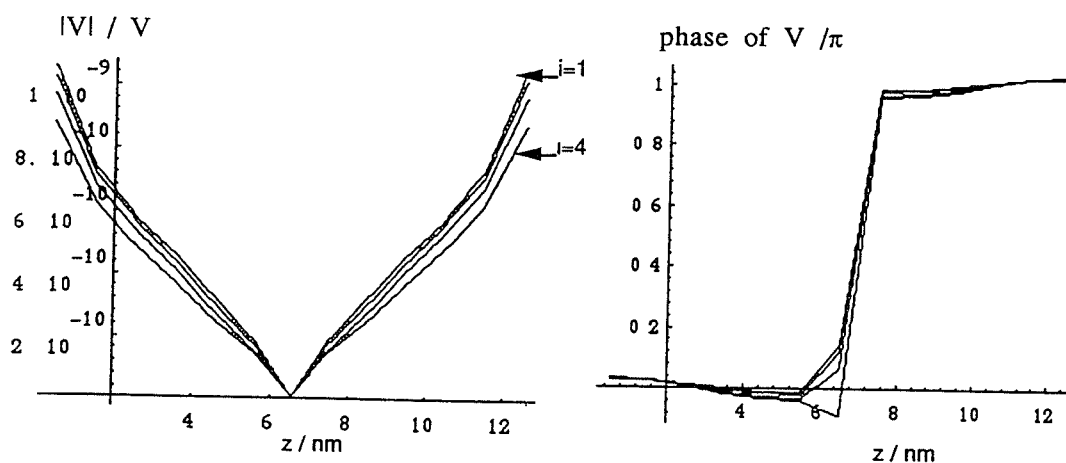


Figure 6.4d. The amplitude and phase/ π of the potential difference (V) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

similar to that of the basic model (Figure 6.4d).

The resistance of the channel increases in the voxels where the Born energy term is included, which leads to a significant reduction in the z component of the current density (J_{pz}) in the voxels next to the channel wall, and a slight increase near the central axis. Adding the Born energy term lowers the cation concentration (p) and therefore also the gradient of p , since p is zero in the centre. J_{pz} involves two terms; one proportional to the concentration gradient and one proportional to the product of p and the e_z . The former dominates, hence J_{pz} is lower in the voxels closest to the channel wall. J_{pz} near the charged boundaries is similar to that for the simple pore (Figure 6.4e). The variation of p with r inside the channel results in a non zero radial component of the cation current density (J_{pr}) near both ends of the channel. Including the effect of the Born energy for the voxels next to the electrolyte/membrane interface reduces J_{pr} near that region compared to J_{pr} of the simple pore (Figure 6.4f). The phase of both J_{pz} and J_{pr} is approximately the same as that for the simple pore everywhere.

6.6 EQUILIBRIUM DISTRIBUTIONS FOR A CHARGED CHANNEL

The effect of adding a positive ring of charge near one of the channel entrances is now investigated (see Figure 6.5). Initially the ion concentrations are set equal to the bulk electrolyte solution in all unblocked voxels. The equilibrium ion distribution and electric field are obtained for the system as before. However for this case an increase in the bulk anion concentration is included to ensure electroneutrality in the whole system (i.e. the total charge of the anions add up $-1q$) before a current flows through the system.

FIGURE 6.4 continued

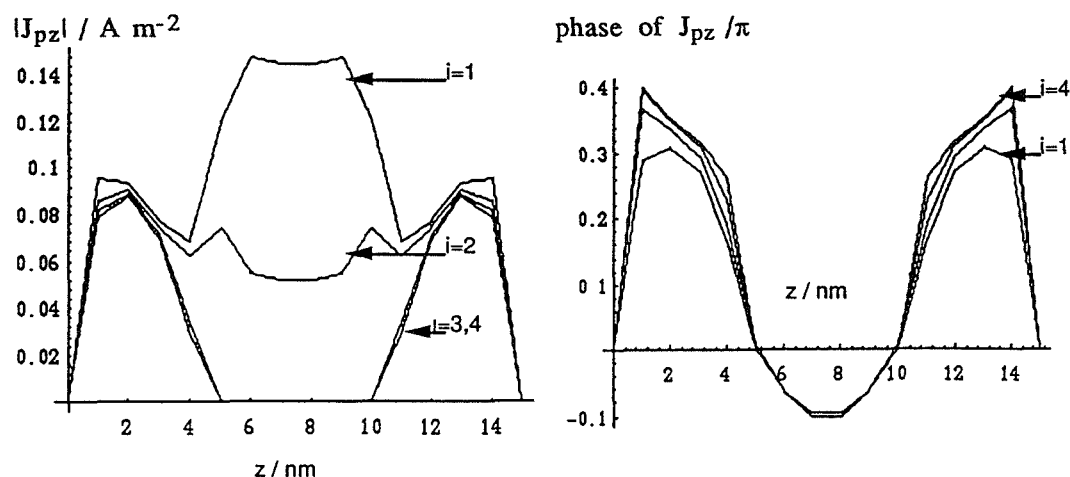


Figure 6.4e. The amplitude and phase/ π of the z component of the cation current density (J_{pz}) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

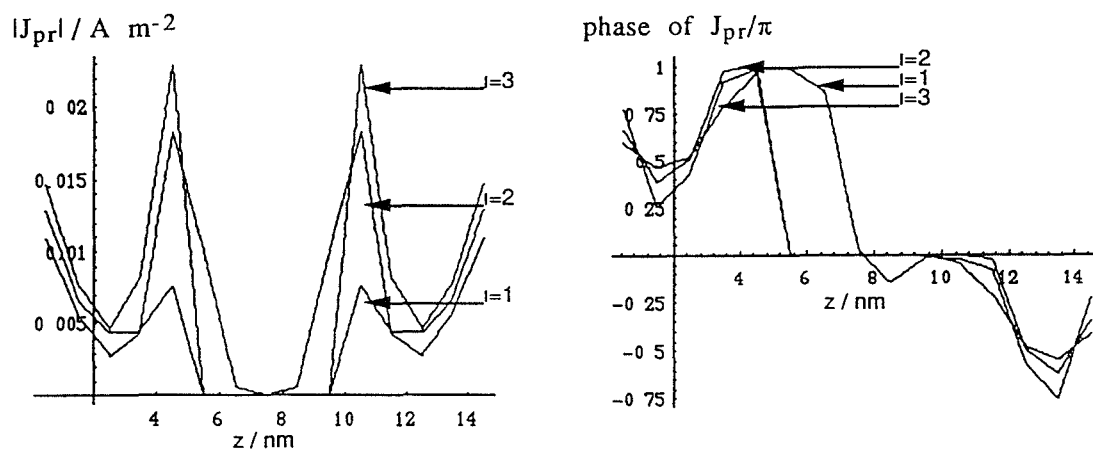


Figure 6.4f. The amplitude and phase/ π of the radial component of the cation current density (J_{pr}) as a function of z for voxel radii r_i $i = 1$ to 4, for a Born energy term of 1 kT at high frequencies.

FIGURE 6.5

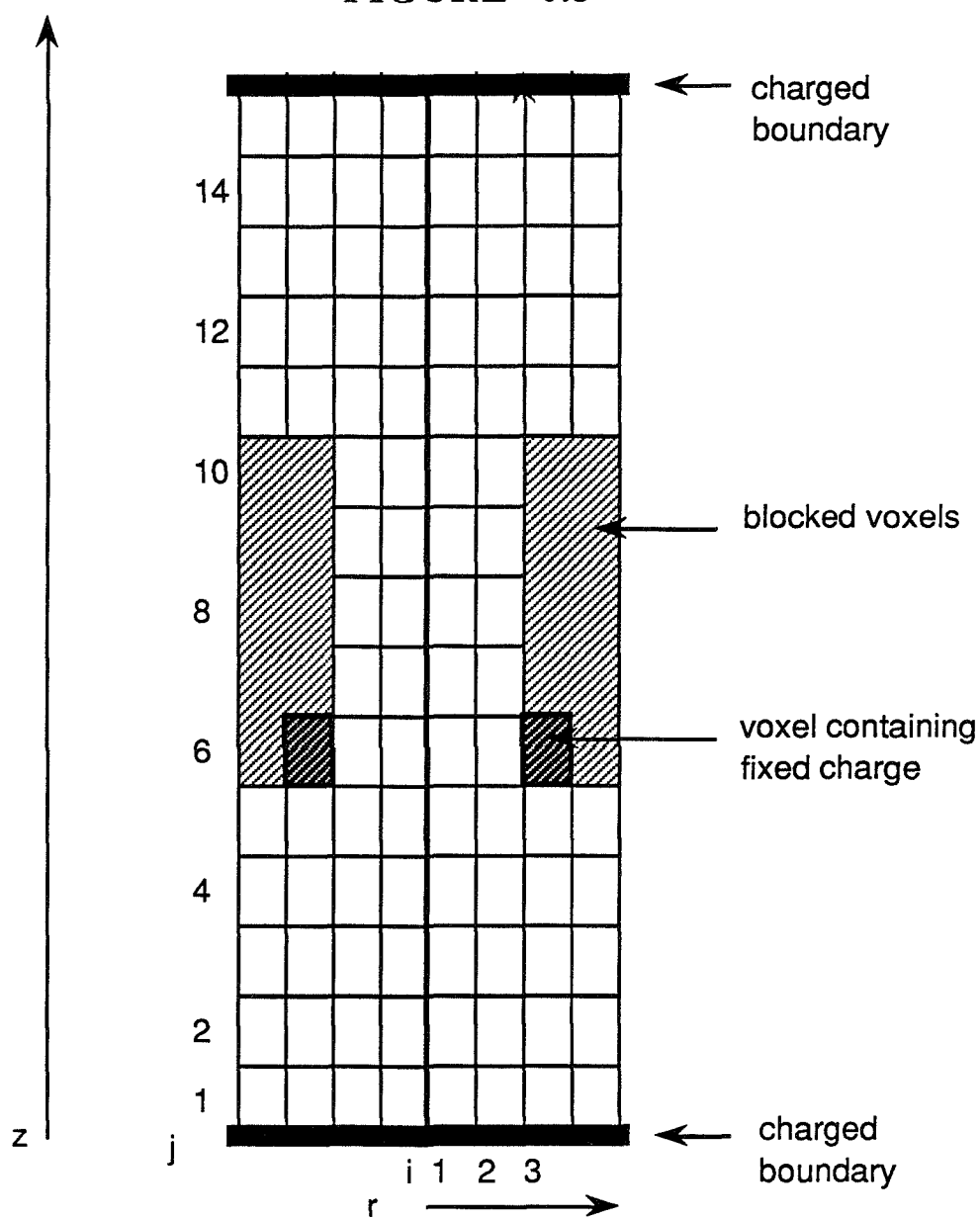


Figure 6.5. The configuration used for calculations for a channel with surface charge. The voxel at the channel mouth contains a ring of fixed charge of magnitude equal to $+1 q$. The initial (bulk) anion concentration is increased to give a net charge of $-1 q$ i.e. to ensure electroneutrality.

The concentration of anions increases near the fixed (positive) charge, while that of the cations decreases, hence partially screening the fixed charge (Figures 6.6a and 6.6b). The separation of charge results in high electric fields in the voxels surrounding the fixed charge, which oppose further movement of charge (Figures 6.6c and 6.6d).

At equilibrium, one would expect the current due to the concentration gradient and that due to the electric field to be equal in magnitude but opposite in sign. However the calculations presented include a small quiescent (i.e. not transient) circulating current densities near the voxel containing the fixed charge. These are possible numerical solutions because of quantisation errors as explained in section 5.3.4.

The percentage difference between the current density term due to the concentration gradient and that due to the electric field at equilibrium gives an estimate of the "error" in the current densities. The maximum difference is approximately 30% for the radial current density and 10% for the axial component for the parameters chosen here.

6.7 SMALL A.C. SIGNAL CALCULATIONS FOR A CHARGED CHANNEL

The effect of adding a small a.c. signal to the charged channel of section 6.6 is discussed. The calculations are again performed at two different frequencies, one above and one below f_c .

6.7.1 Results for a Frequency below f_c .

The amplitude of the a.c. component of the cation concentration, p , in the central region is lower than for the simple pore with the greatest reduction in the voxels near the fixed charge. In the double layer p is

FIGURE 6.6

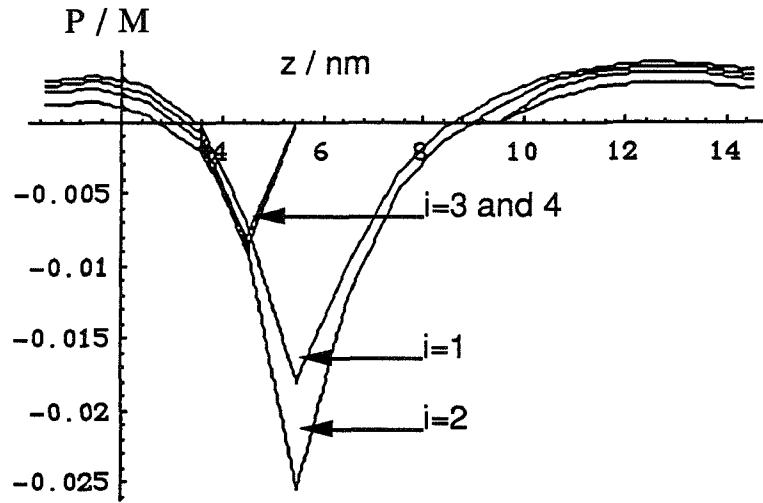


Figure 6.6a. The equilibrium net cation concentration, P for the charged channel (i.e. total cation concentration - bulk electrolyte concentration for zero applied a.c. signal) for the different voxel radii r_i , $i=1$ to 4.

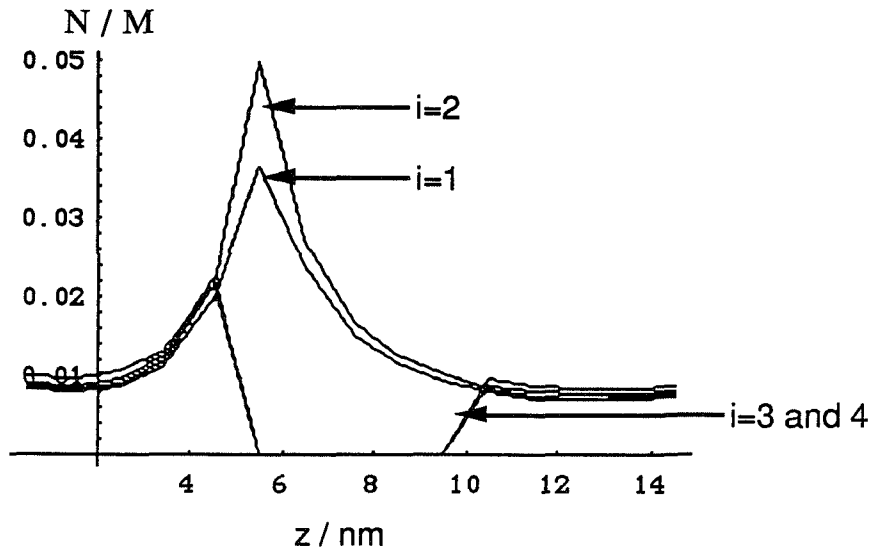


Figure 6.6b The equilibrium net anion concentration N for the charged channel (i.e. total anion concentration - bulk electrolyte concentration for zero applied a.c. signal). for the different voxel radii r_i , $i=1$ to 4.

FIGURE 6.6 continued

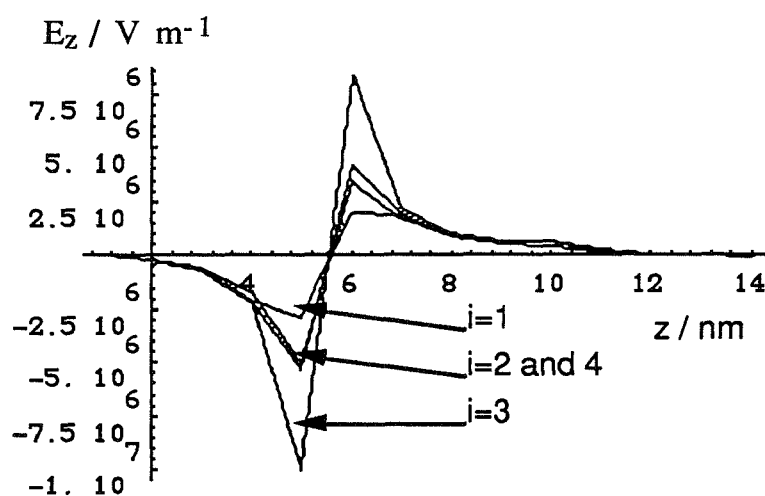


Figure 6.6c. The equilibrium z component of the electric field (E_z) of the charged channel for the different voxel radii r_i , $i=1$ to 4.

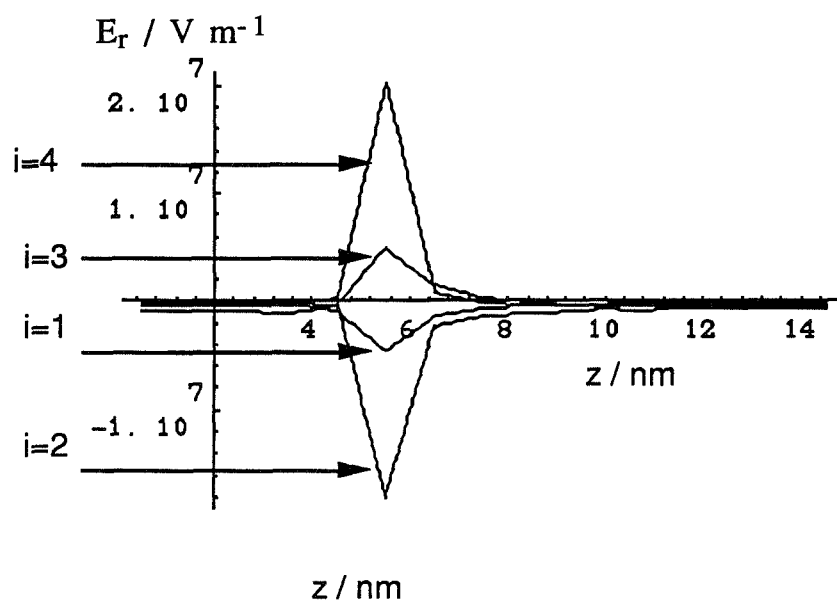


Figure 6.6d. The equilibrium r component of the electric field (E_r) of the charged channel for the different voxel radii r_i , $i=1$ to 4.

approximately the same as before (Figure 6.7a).

The amplitude of the a.c. component anion concentration (n) in the voxels surrounding the fixed charge increases compared to that for a simple pore of Chapter 5. $|n|$ for the charged channel is also higher in the channel voxels closest to the central axis as shown in Figure 6.7b. The phase of both p and n lags the applied boundary charge by a slightly larger angle.

The decrease in p and corresponding increase in n around the fixed charge gives a small a.c. screening. This leads to a slight reduction of the amplitude of e_z and e_r compared to the pore in the central region and hence an increase in the effective conductance of the charged channel compared to the pore. These effects, however, are of size smaller than the quantisation error, so caution should be used in their interpretation. The phase of e_z and e_r are approximately the same as for the simple pore (Figures 6.7c and 6.7d, Figure 6.7e shows the corresponding potential difference plots).

6.7.2 Results for a Frequency above f_c .

As for the simple pore of Chapter 5, the lack of charge transfer through the channel results in a relative increase of the a.c. ion concentrations (p and n) in the region near the electrolyte/membrane interface and the channel mouth, compared to that of the double layers near the charged boundaries, forming a double layer of opposite phase to that of the nearest charged boundary. As for the previous section the fixed charge attract counter-ions and repels ions of the same sign, resulting in an asymmetric charge distribution near the charge and inside the channel (Figures 6.8a and 6.8b).

The electric field and the potential difference is approximately the

FIGURE 6.7

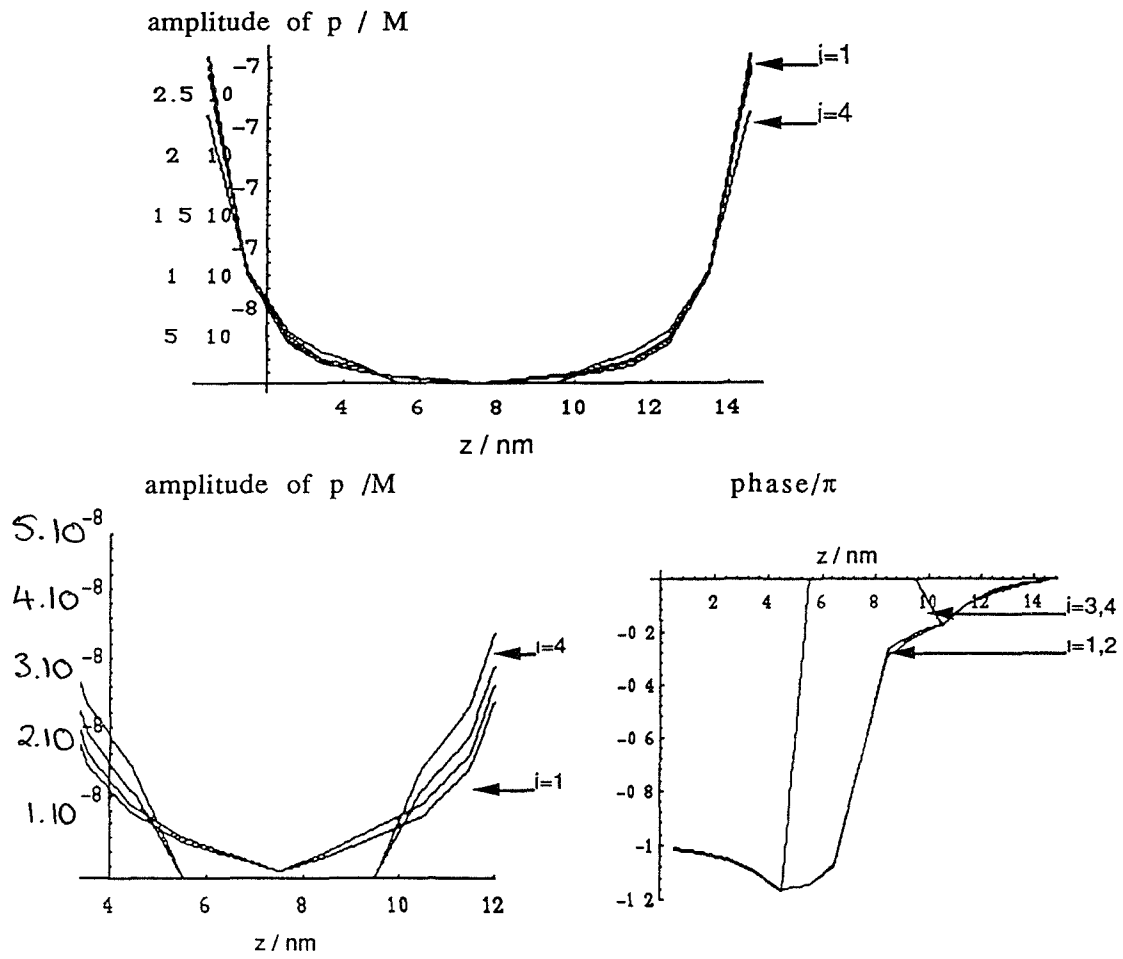


Figure 6.7a. The amplitude and phase/ π of the cation concentrations (p) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency below f_c .

FIGURE 6.7 continued

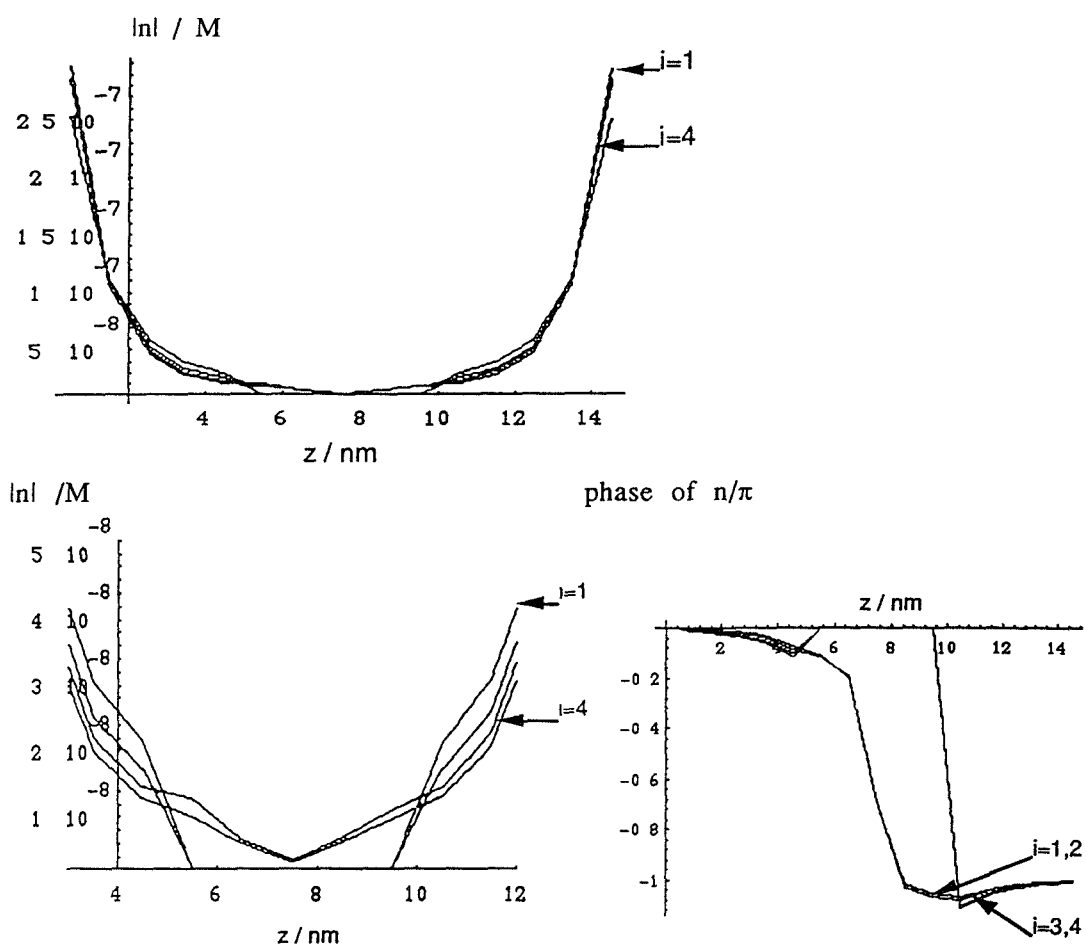


Figure 6.7b. The amplitude and phase/ π of the anion concentrations (n) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency below f_c .

FIGURE 6.7 continued

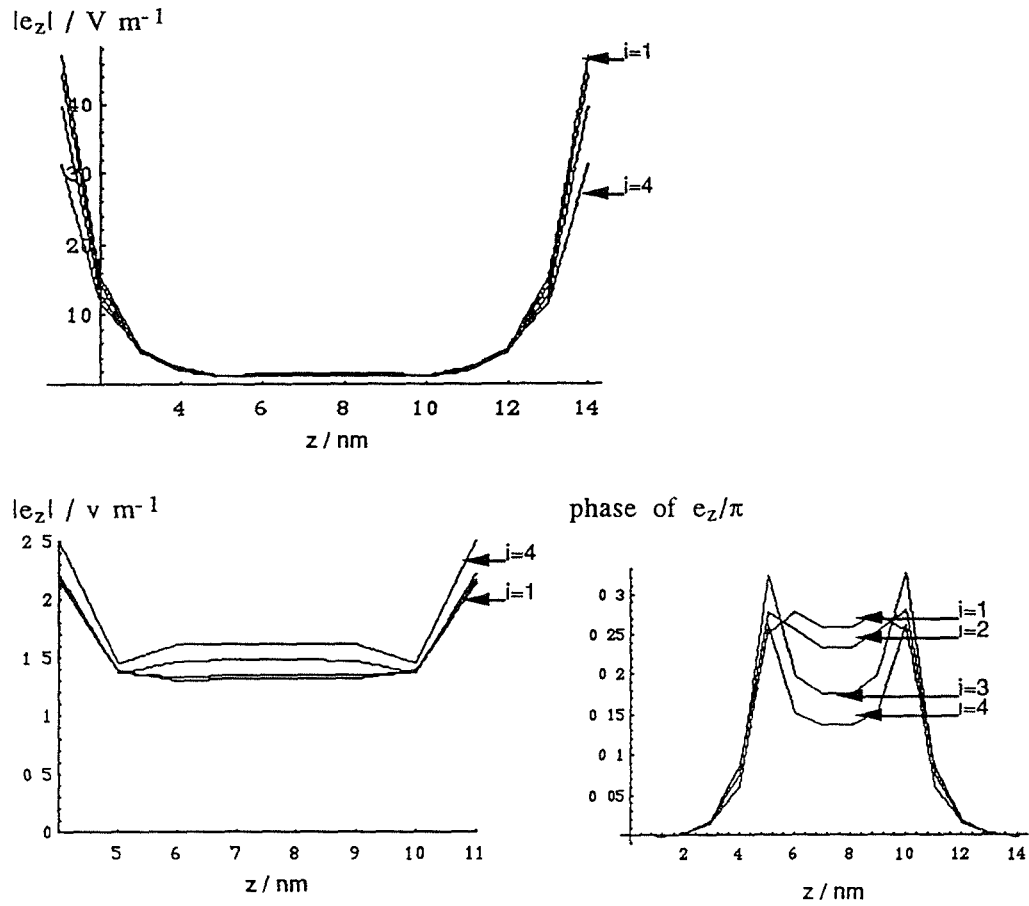


Figure 6.7c. The amplitude and phase/ π of the axial component of the electric field (e_z) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency below f_c .

FIGURE 6.7 continued

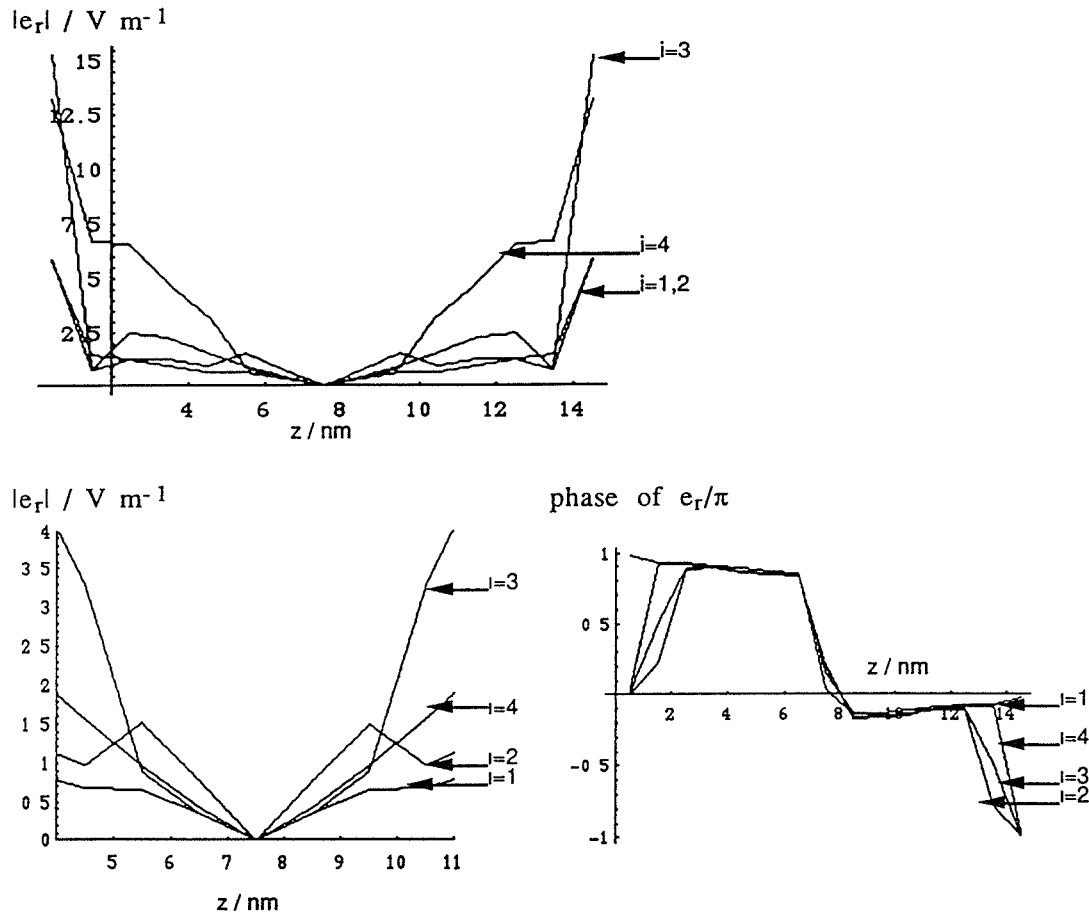


Figure 6.7d. The amplitude and phase/ π of the radial component of the electric field (e_r) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency below f_c .

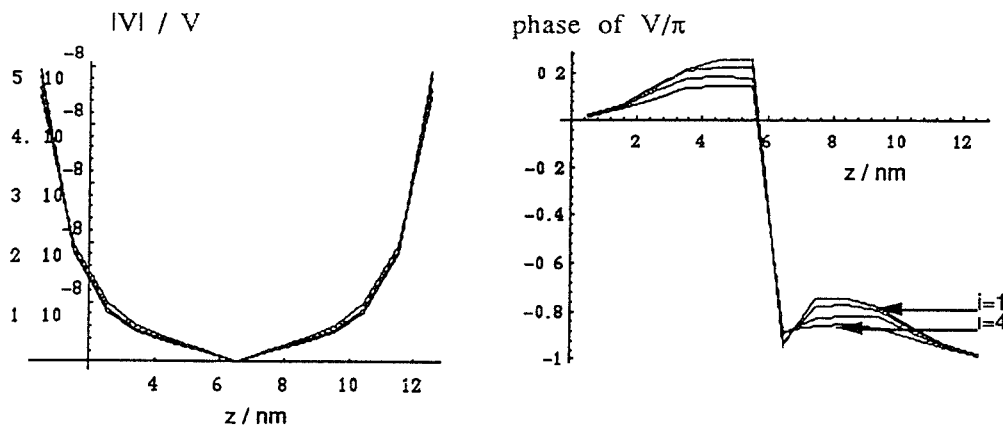


Figure 6.7e. The amplitude and phase/ π of the potential (V) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency below f_c .

FIGURE 6.8

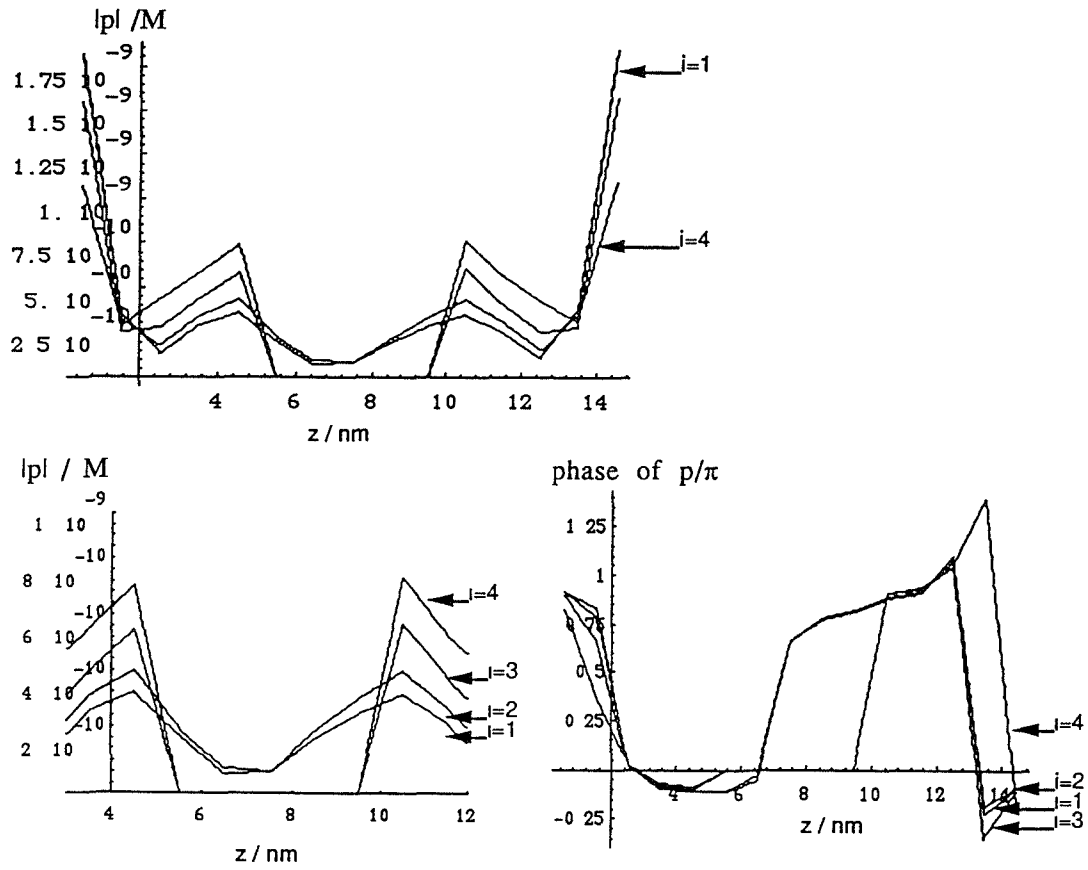


Figure 6.8a. The amplitude and phase/ π of the cation concentrations (p) as a function of z for voxel radii r_i $i = 1$ to 4 , for the charged channel at a frequency above f_c .

FIGURE 6.8 continued

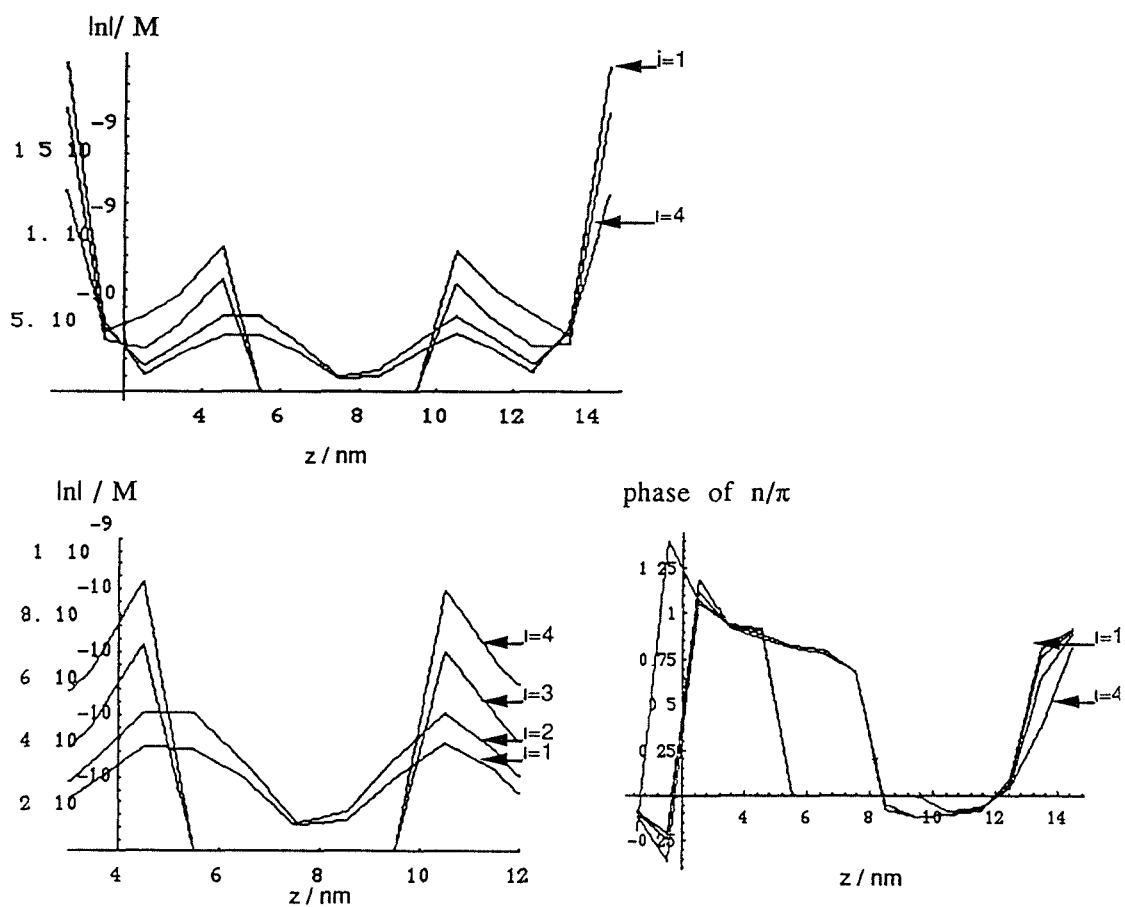


Figure 6.8b. The amplitude and phase/ π of the anion concentrations (n) as a function of z for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

same as the simple pore, due to the screening of the charge (Figure 6.8c to 6.8e).

The fixed charge results in an asymmetric current distribution in the surrounding region. Both the axial and radial components of the cation current density (J_{pz} and J_{pr}) are reduced near the fixed charge (Figure 6.8f and 6.8h), while the anion current density (J_{nz} and J_{nr}) increases in this region, compared to those for the simple pore (Figures 6.8g and 6.8i). These currents maintain the asymmetric charge distribution.

6.8 EQUILIBRIUM DISTRIBUTION FOR THE ION SELECTIVE CHANNEL

As explained earlier in this chapter (section 6.1.3) the diffusion constants for both ion species inside the channel may not be equal to those in the bulk solution. The case where the anion diffusion constant in the channel is set equal to zero is now considered, while the cation diffusion constant remains the same (analogous to a cation selective channel).

Initially the concentration of both ion species is set equal to zero inside the channel and equal to bulk concentration external to the channel. The equilibrium distributions are obtained from iterations for zero applied signal as before.

Cations diffuse into the channel initially because of the concentration gradient. No anions can enter the channel, therefore they accumulate at its entrance. The charge separation results in an electric field which opposes further cation diffusion into the channel. At equilibrium, the current due to the concentration gradient and that due to the electric field should be equal in magnitude and opposite in sign. However, as for the charged channel, the numerical solutions give a residual non-zero ion current density at equilibrium, due to quantisation errors. Figures 6.9a and 6.9b

FIGURE 6.8 continued

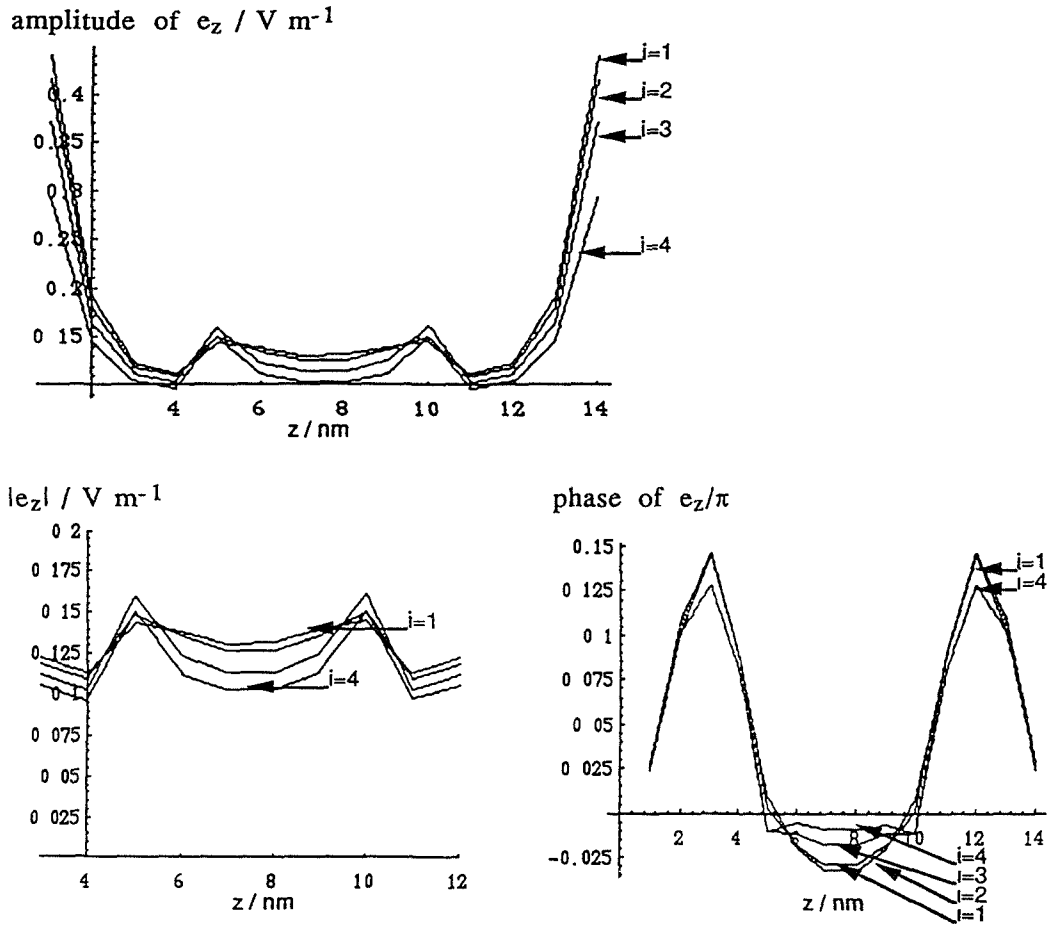


Figure 6.8c. The amplitude and phase/ π of the axial component of the electric field (e_z) for voxel radii r_i $i = 1$ to 4 , for the charged channel at a frequency above f_c .

FIGURE 6.8 continued

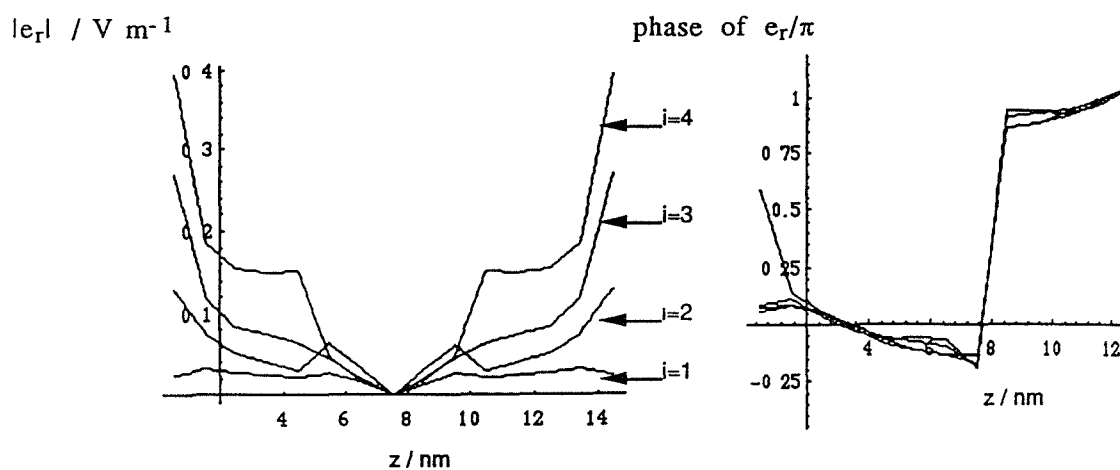


Figure 6.8d. The amplitude and phase/ π of the radial component of the electric field (e_r) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

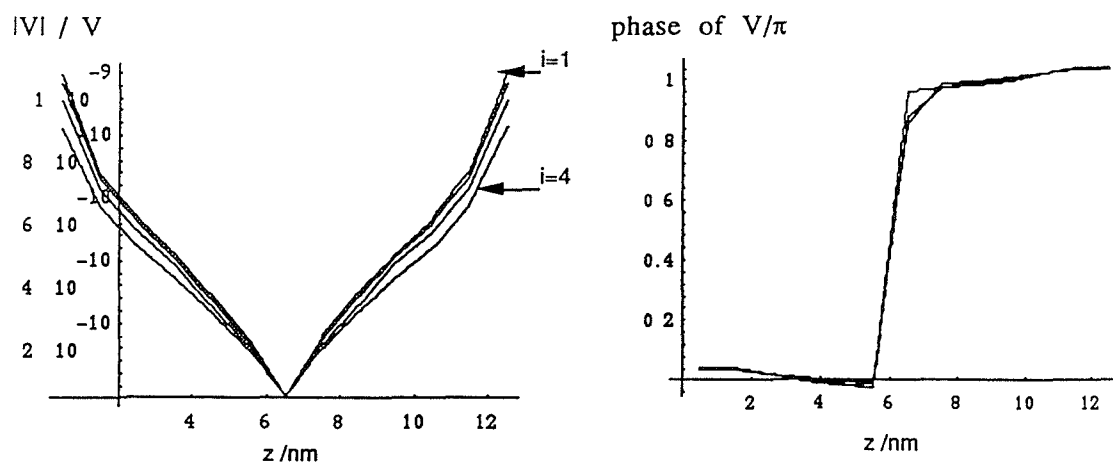


Figure 6.8e. The amplitude and phase/ π of the potential (V) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

FIGURE 6.8 continued

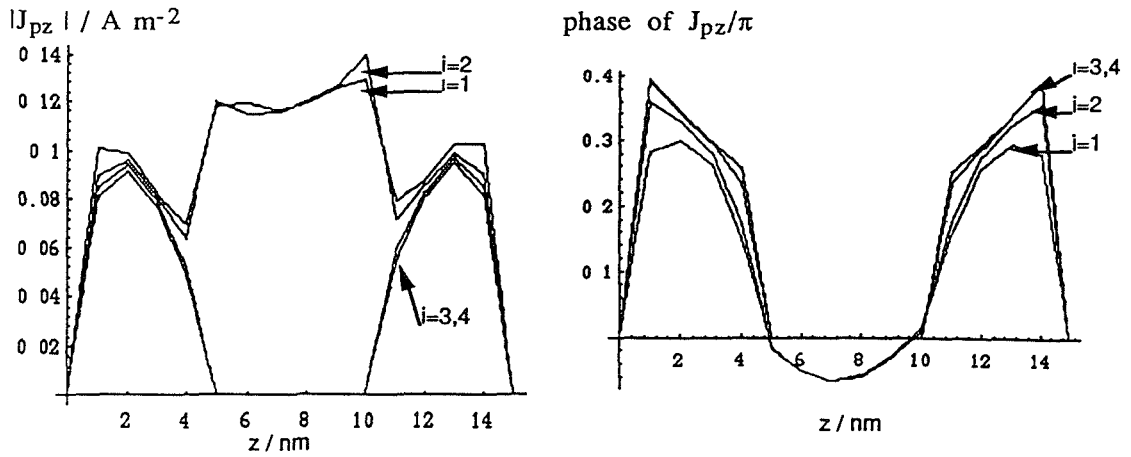


Figure 6.8f. The amplitude and phase/ π of the axial component of the cation current density (J_{pz}) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

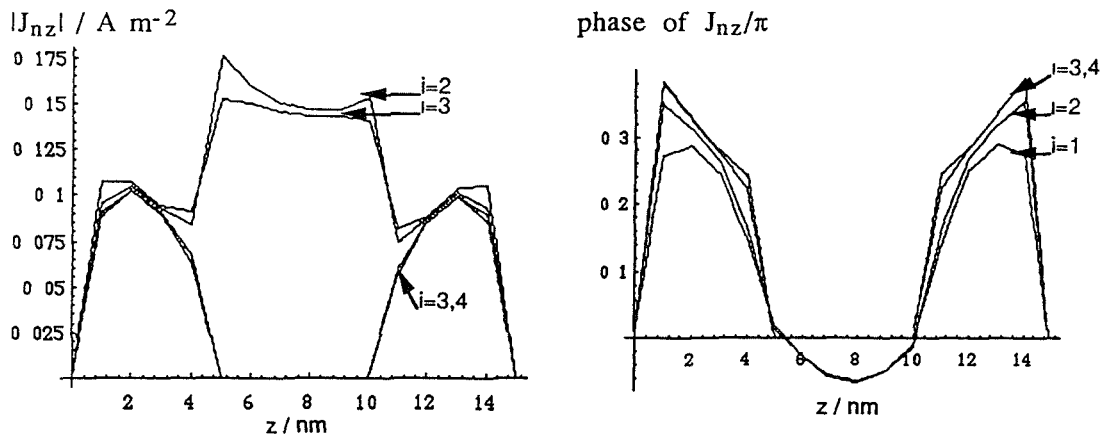


Figure 6.8g. The amplitude and phase/ π of the axial component of the anion current density (J_{nz}) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

FIGURE 6.8 continued

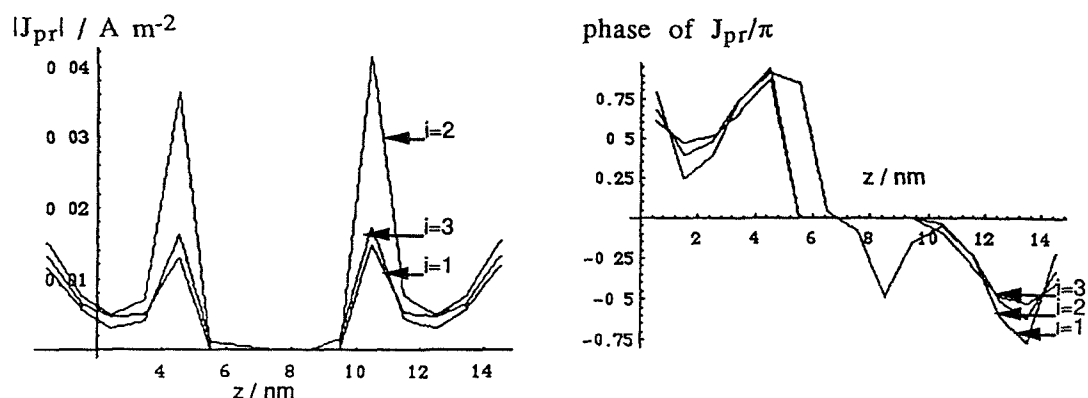


Figure 6.8h. The amplitude and phase/ π of the radial component of the cation current density (J_{pr}) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

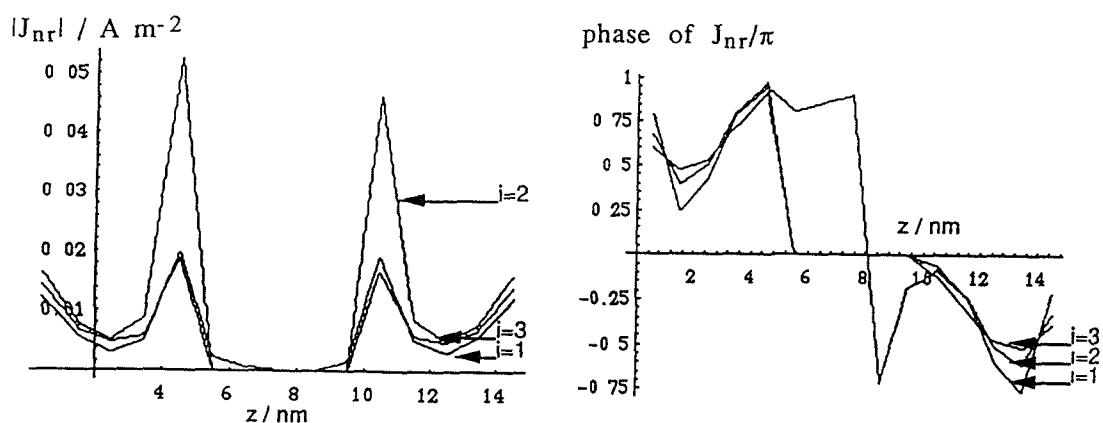


Figure 6.8i. The amplitude and phase/ π of the radial component of the anion current density (J_{nr}) for voxel radii r_i $i = 1$ to 4, for the charged channel at a frequency above f_c .

show the cation (P) and anion (N) concentrations at equilibrium. Note that capital letters denote equilibrium values, while lower case letter are used for the a.c. values. P is lowest inside the channel and approaches the bulk electrolyte concentration (C_0) near the circular boundaries. N is greater than C_0 at the channel entrance and next to the insulator/solution interface. There is very little variation of ion concentration in the radial direction, since all voxels of the insulator and pore are blocked to ion flow. Inside the channel P is slightly higher next to the channel wall than near the central axis.

The space charge is positive inside the channel and slightly negative outside (it is symmetric). This results in an electric field in and near the channel. The axial component of the electric field (e_z) is antisymmetric (Figure 6.9c), while the radial component (e_r) is symmetric (Figure 6.9d).

6.9 A.C. CALCULATIONS FOR THE ION SELECTIVE CHANNEL

Results of the small signal a.c. response of the ion selective channel at a frequency above and below f_c are presented below.

6.9.1 Low Frequency Calculations

The following calculations are for a frequency much lower than f_c . As before, the response of this system to a sinusoidally varying charge applied at both circular boundaries is determined.

The a.c. anion concentration (n) is similar to that for the insulating membrane (except there is variation of the amplitude with radial distance). As the anions cannot pass through the channel or the insulator they accumulate, as for the case of $f > f_c$, near interface between the

FIGURE 6.9

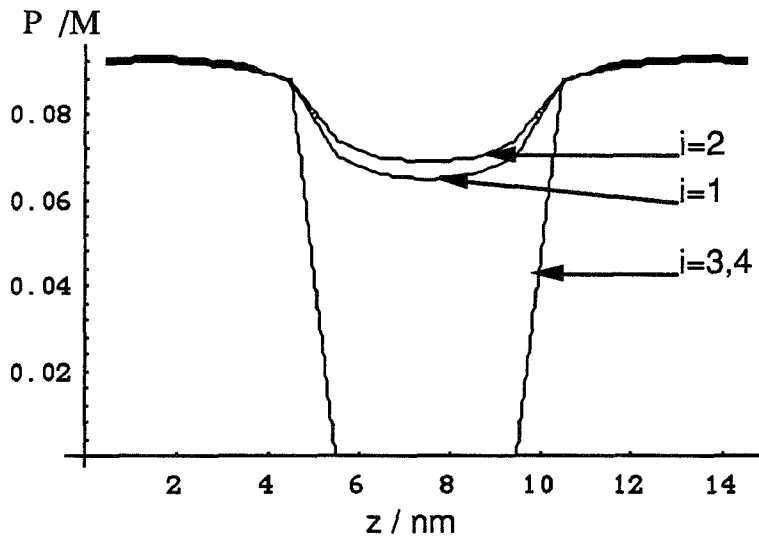


Figure 6.9a. The equilibrium cation concentration (P) of the ion selective channel for voxel radii r_i $i=1$ to 4.

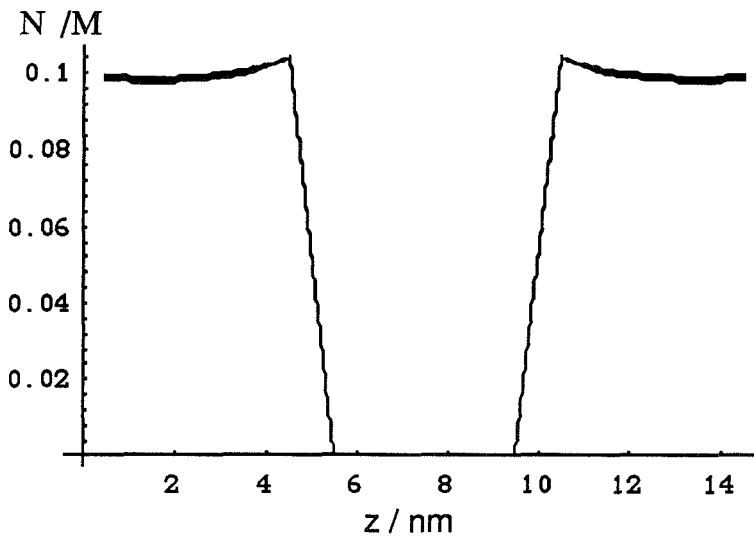


Figure 6.9b. The equilibrium anion concentration (N) of the ion selective channel for voxel radii r_i , $i=1$ to 4.

FIGURE 6.9 continued

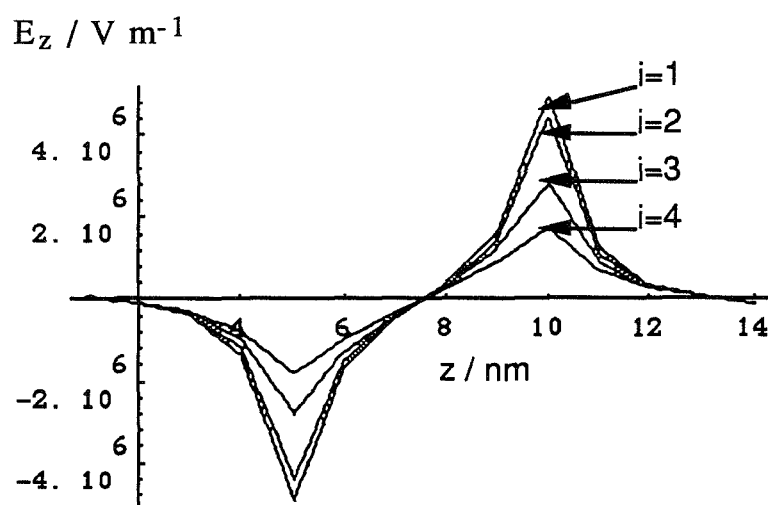


Figure 6.9c. The z component of the electric field at equilibrium (E_z) of the ion selective channel for voxel radii r_i , $i=1$ to 4.

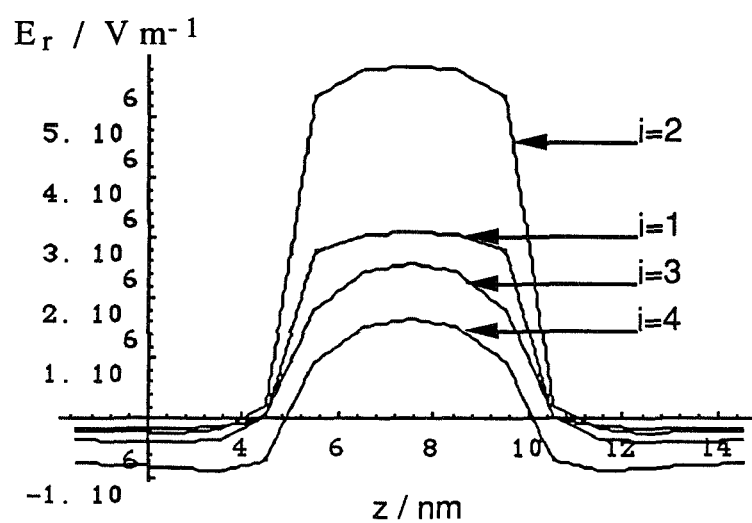


Figure 6.9d. The radial component of the electric field at equilibrium of the ion selective channel for voxel radii r_i , $i=1$ to 4.

electrolyte solution and the membrane containing the channel. Consequently a second double layer forms there of opposite sign and lower magnitude to that near the charged boundaries (Figure 6.10b).

The a.c. cation concentration (p) is higher in comparison to the simple pore, since the anions are excluded from the pore. (Note that initially before the a.c. signal is applied, both the total cation and anion charge in the system is lower than in the case of the simple pore). The greatest increase occurs near the channel entrance next to the insulator/electrolyte interface where n is also high (p and n are in phase in this region). In the radial direction, p does not vary significantly. The phase of p is approximately the same as for the simple pore (Figure 10a).

The accumulation of anions near the channel mouth and electrolyte/membrane interface leads to an increase in e_z in that region. The plots of e_z look similar to those for the insulating membrane, however the amplitude in the central region is much lower than for the membrane. The values of the electric field, and hence the potential, are closer in magnitude to those for the simple pore (Figures 6.10c to 6.10e), since, unlike for the case of the insulating membrane, significant transfer of cations through the channel still occurs at this frequency. The phase of e_z is similar to that for the simple pore, but leads the applied charge by a considerably lower angle, especially in the near channel entrance and membrane/electrolyte interface.

6.9.2 Calculations at a Frequency above f_c .

The cation concentration (p) resembles that for the high frequency calculations of the simple pore of the previous chapter (i.e. charge build up at electrolyte/membrane interface of opposite sign to that of the double

FIGURE 6.10

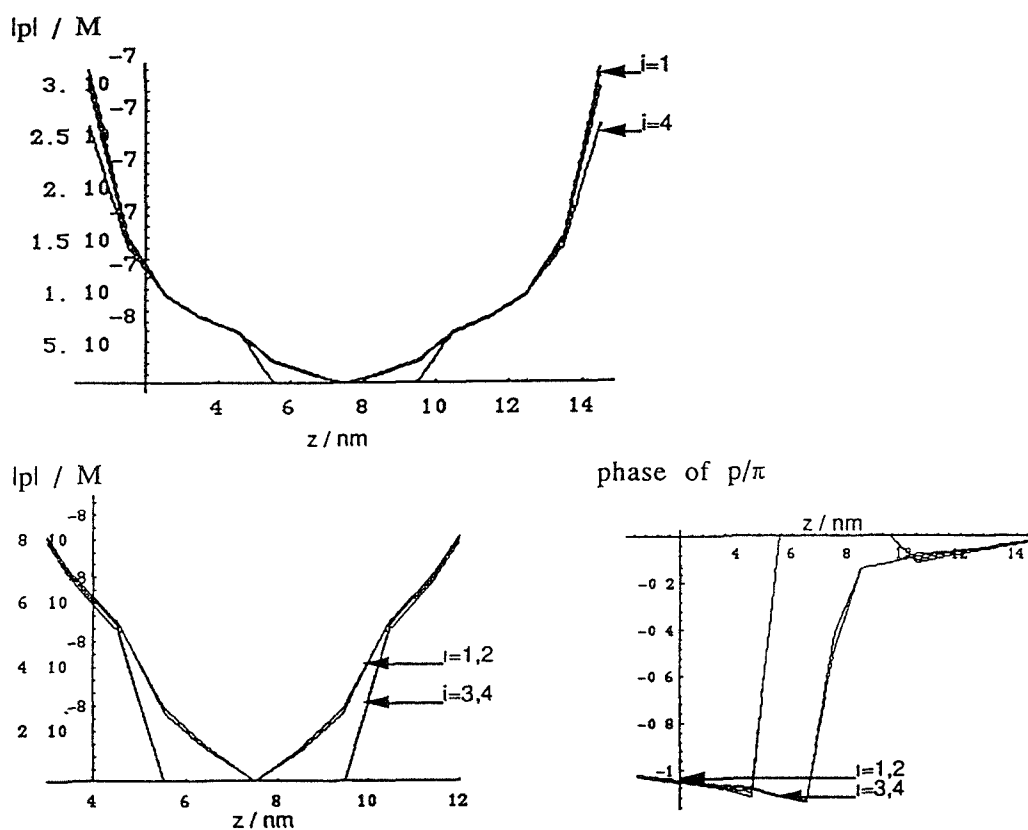


Figure 6.10a. The amplitude and phase/ π of the cation concentrations (p) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency below f_c .

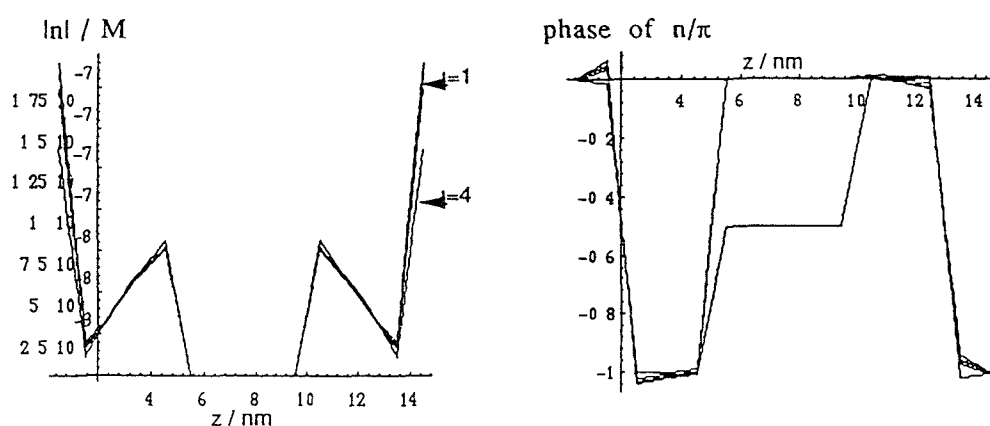


Figure 6.10b. The amplitude and phase/ π of the anion concentrations (n) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency below f_c .

FIGURE 6.10 continued

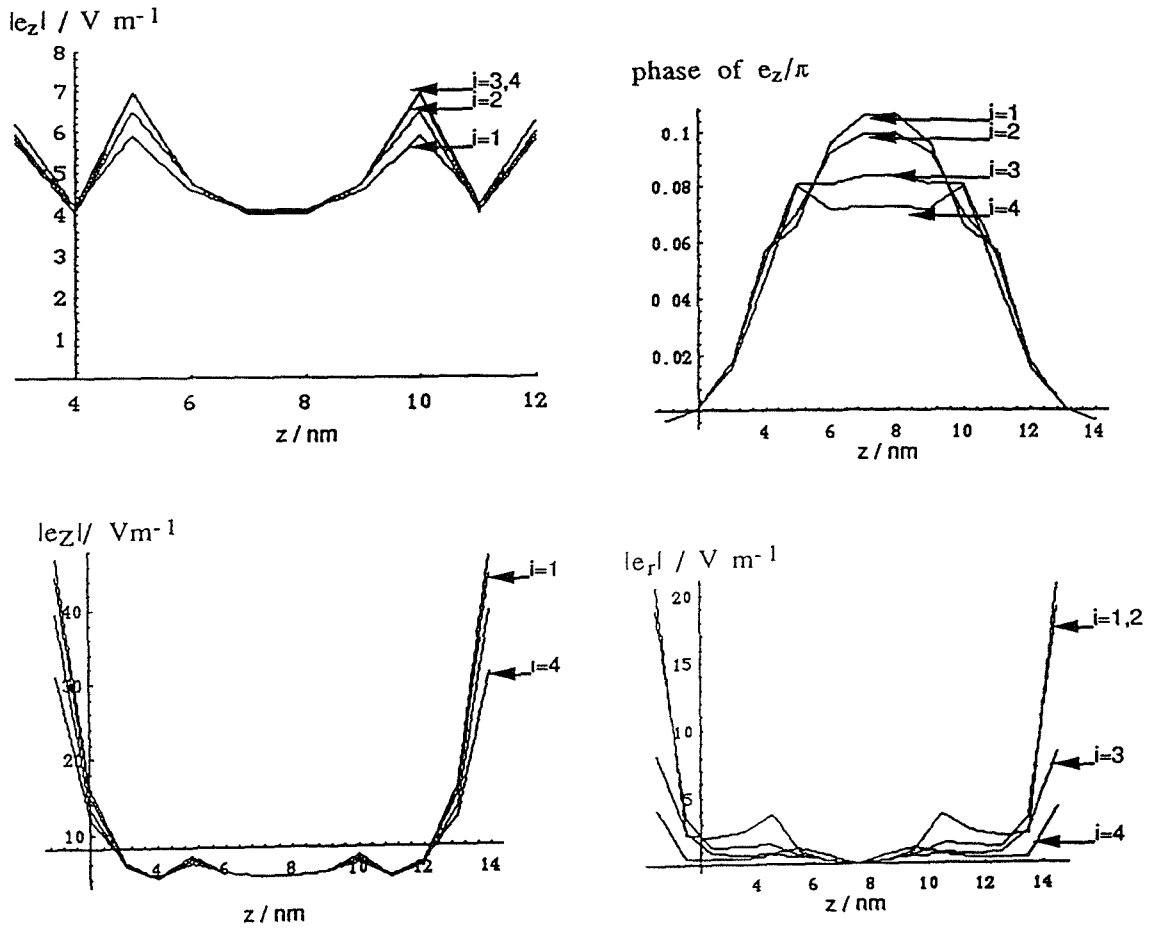


Figure 6.10c and d. The amplitude and phase/ π of axial component of the electric field (e_z) and the amplitude of the radial component of the electric field (e_r) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency below f_c . The phase of e_r has been omitted due to resolution artefacts.

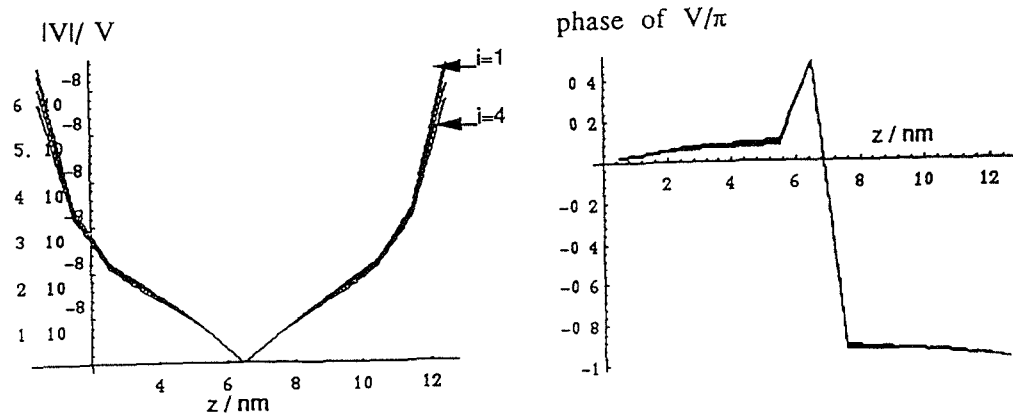


Figure 6.10e. The amplitude and phase/ π of the potential difference (V) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency below f_c .

layer of the nearest charged boundary). However, unlike for the low frequency calculations, it is somewhat lower than for the simple pore in the central region, where n is higher but approximately π radians out of phase with p (Figure 6.11a). As for the low frequency calculation of the previous section, the anion concentration (n) resembles that of the insulating membrane of Chapter 5, except for a slight increase near the channel mouth (Figure 6.11b).

Again e_z , e_r and therefore V have values in between those for the simple pore and an insulating layer (see Figures 6.11c to 6.11d). At this frequency, double layers form near the channel entrance and electrolyte/insulator interface for the case of the simple pore and ion selective pore, as well as the membrane, hence e_z for all three cases is comparable in magnitude.

The anions which accumulate in the central region attract cations, resulting in a small reduction in the cation current density through the pore, as well as J_{pr} in the central region (see Figures 6.11f and 6.11h). J_{nz} remains approximately the same as in the case of the insulating membrane (Figure 6.11g). The phase of both J_{pz} and J_{pr} is similar to that of the simple pore, while that of J_{nz} and J_{nr} is similar to that for the insulating membrane (Figure 6.11h and 6.11i).

6.10 IMPEDANCE CALCULATIONS

As for the previous chapter, the conductance (G) and capacitance (C) can be calculated from the amplitude and phase of the potential difference as a function of distance. Note that the phase used for these calculations lags the phase shown in Figures 6.3, 6.4, 6.7, 6.8, 6.10 and 6.11 by approximately $\pi/2$, since the impedance is calculated with respect to the applied current and not charge. In order to determine the effect of the

FIGURE 6.11

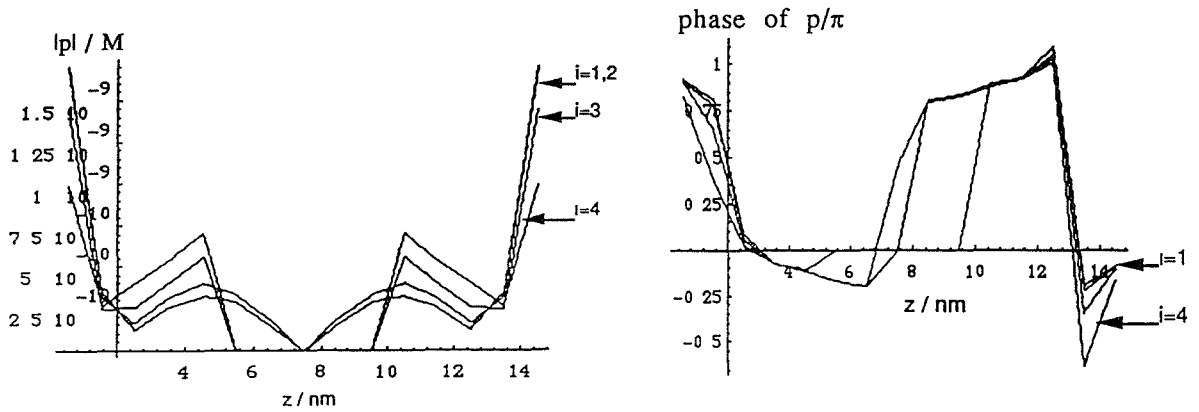


Figure 6.11a. The amplitude and phase/ π of the cation concentrations (p) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

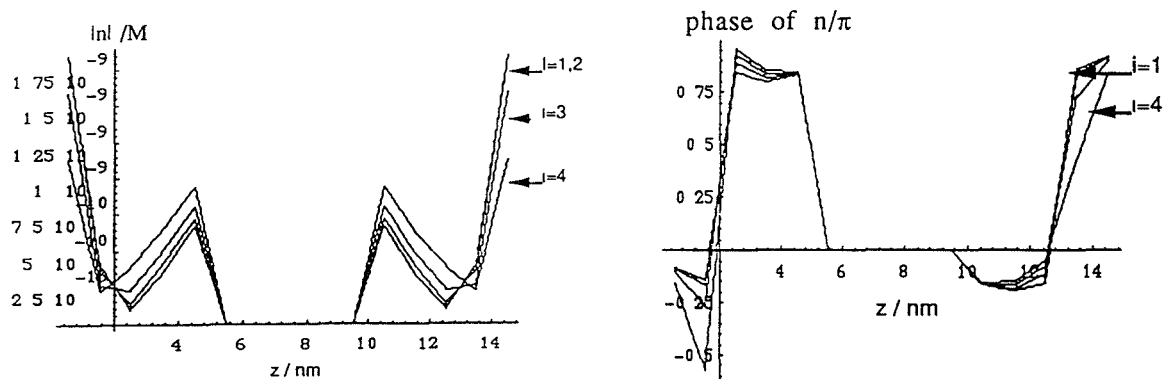


Figure 6.11b. The amplitude and phase/ π of the anion concentrations (n) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

FIGURE 6.11 continued

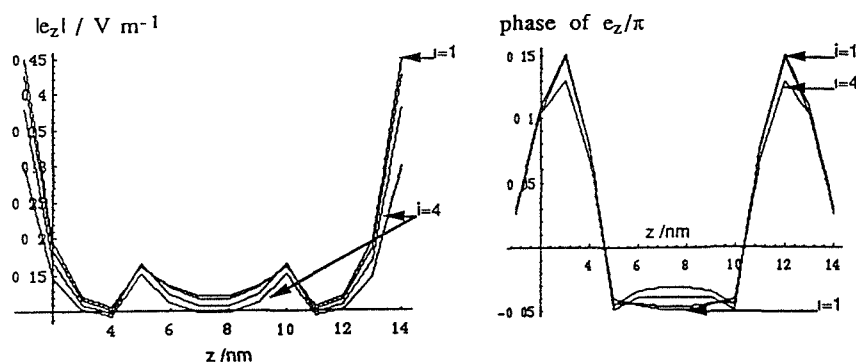


Figure 6.11c. The amplitude and phase/ π of axial component of the electric field (e_z) for voxel radii $r_{i1} = 1$ to 4, for the ion selective channel at a frequency above f_c .

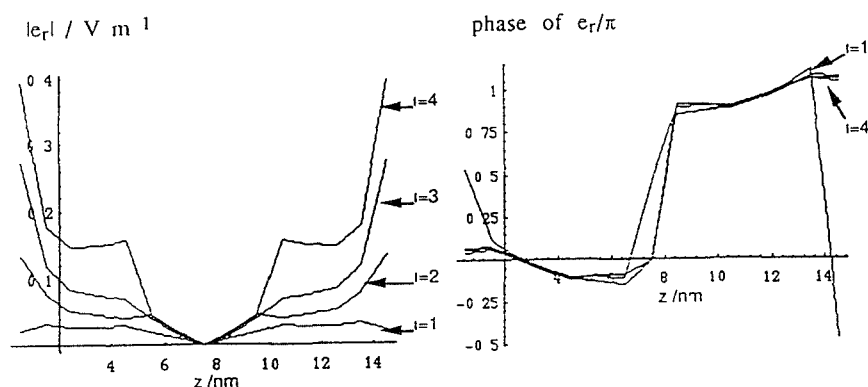


Figure 6.11d. The amplitude and phase/ π of radial component of the electric field (e_r) for voxel radii $r_{i1} = 1$ to 4, for the ion selective channel at a frequency above f_c .

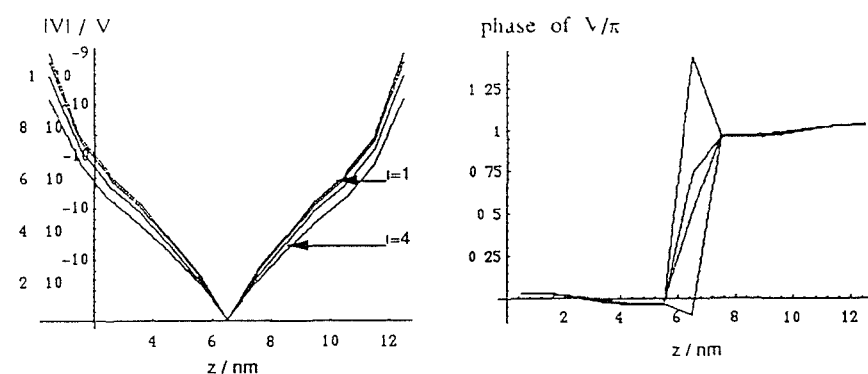


Figure 6.11e. The amplitude and phase/ π of the potential difference (V) for voxel radii $r_{i1} = 1$ to 4, for the ion selective channel at a frequency above f_c .

FIGURE 6.11 continued

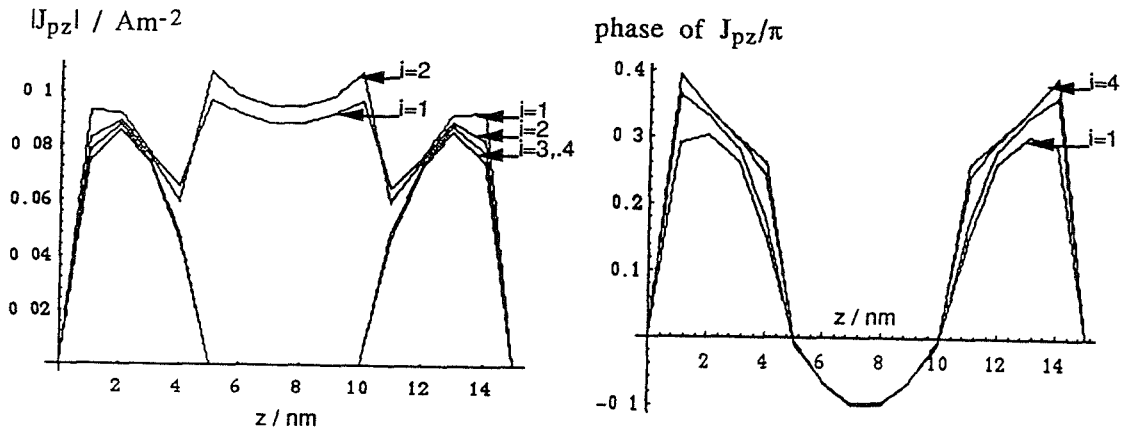


Figure 6.11f. The amplitude and phase/ π of axial component of the cation current density (J_{pz}) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

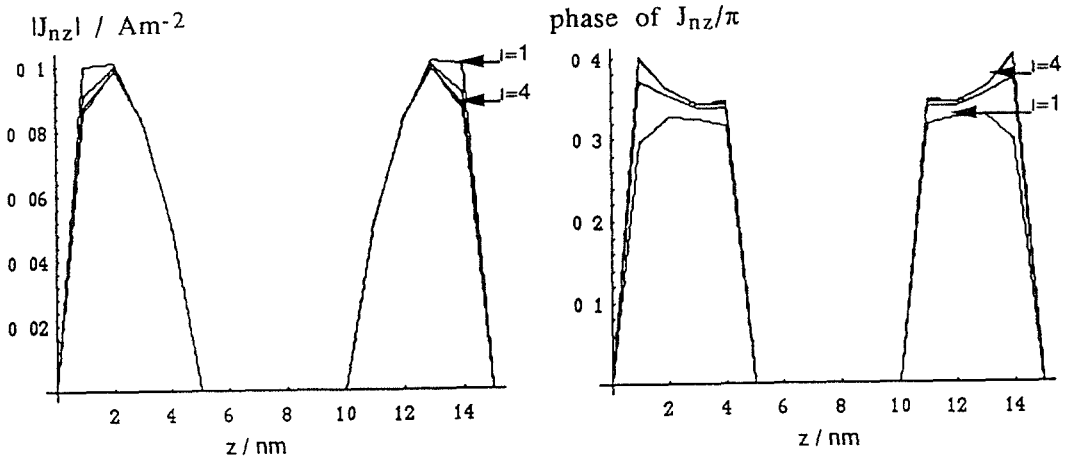


Figure 6.11g. The amplitude and phase/ π of axial component of the anion current density (J_{nr}) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

FIGURE 6.11 continued

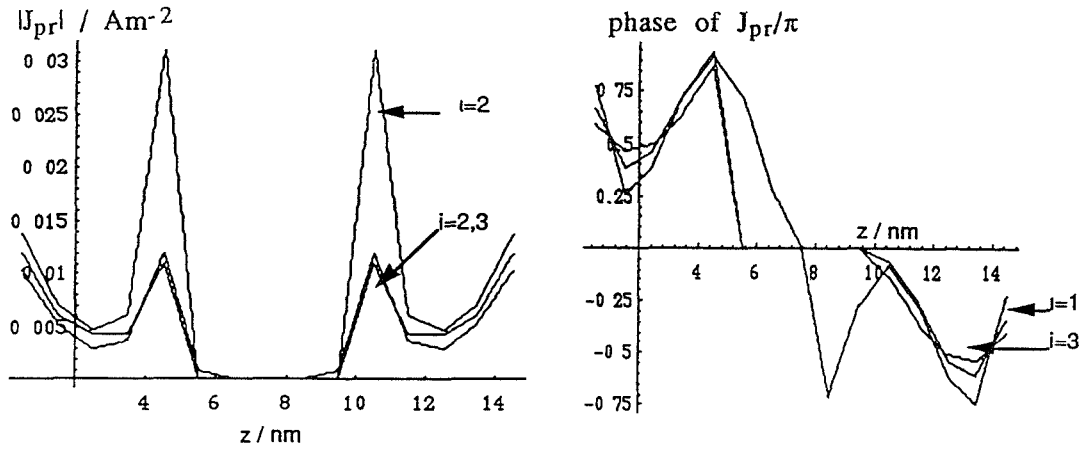


Figure 6.11h. The amplitude and phase/ π of radial component of the cation current density (J_{pr}) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

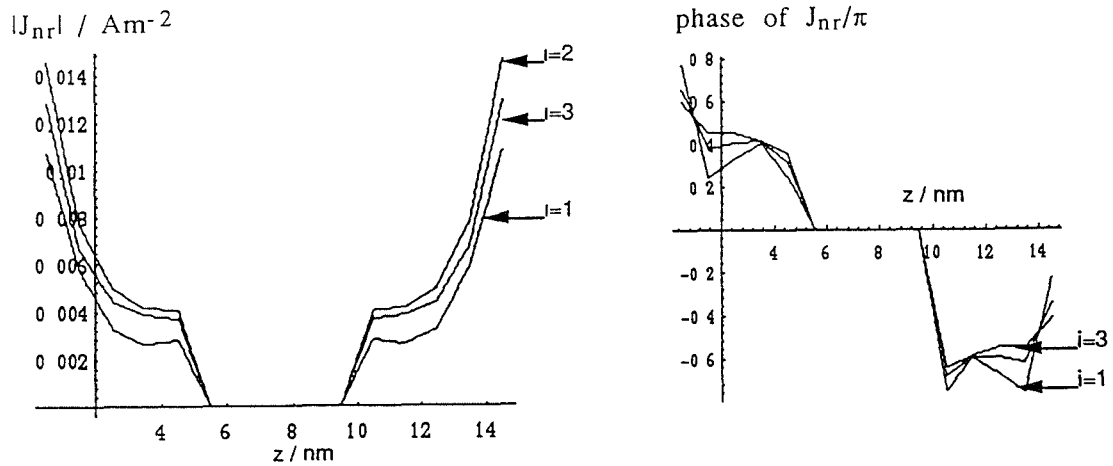


Figure 6.11i. The amplitude and phase/ π of radial component of the anion current density (J_{nr}) for voxel radii r_i $i = 1$ to 4, for the ion selective channel at a frequency above f_c .

various complications, the conductance (G/G_K) and capacitance (C/C_K) ratios are again compared to the corresponding ratios for the simple pore of Chapter 5. G_K and C_K are the geometrical conductance and capacitance, respectively, for a homogeneous dielectric of the same thickness and are given by equations 5.3.3 and 5.3.4.

The Born energy term drives ions out of the voxels surrounding the electrolyte/insulator interface, thus considerably reducing the conductance of the channel at both high and low frequencies (Figure 6.12).

The screening of the fixed charge on the channel surface leads to a very slight reduction in the electric field. Hence the conductance of the channel increases slightly at low frequencies compared to the conductance of the simple pore. At high frequencies, the channel conductance decreases slightly due to the smaller number of charge carriers in the channel.

The example of an ion selective channel used here is one which is completely impermeable to anions. As a result, anions accumulate at the channel mouth. This attracts cations to this region and tends to reduce cation current flow through the channel. This leads to a significant decrease in the channel conductance at both high and low frequencies.

As in the previous chapter, the capacitance at low frequencies is much higher than the equilibrium value for all three types of ion channels. Adding the Born energy term effectively decreases the occupancy of the channel and thus leads to an increase in the potential difference across the channel. This also increases the effective thickness of the membrane and hence the capacitance decreases.

At low frequencies the capacitance of the charged channel increases slightly compared to that of the simple pore, due to the slight decrease in the electric field. This effect may however be small enough to lie within the range of quantisation errors.

FIGURE 6.12

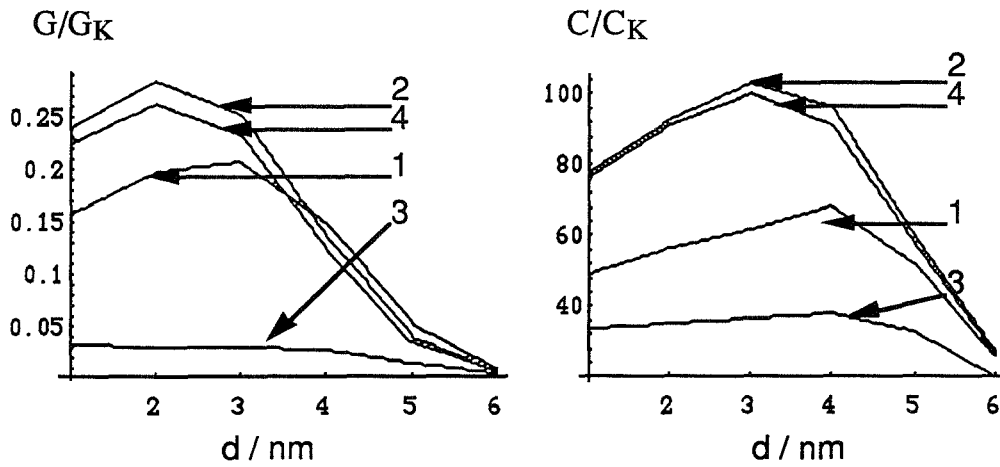


Figure 6.12a. The conductance (G/G_K) and capacitance ratios (C/C_K) as a function distance (d) from the plane of symmetry for the low frequency calculations of chapter 6 for the following cases:

- 1 : Channel including the effect of Born energy - section 6.5.1
- 2 : Charged channel - section 6.7.1
- 3 : Ion selective channel - section 6.9.1
- 4 : Simple pore of chapter 5 - section 5.5.1

G_K and C_K are the geometrical conductance and capacitance defined by equations 5.3.3 and 5.3.4.

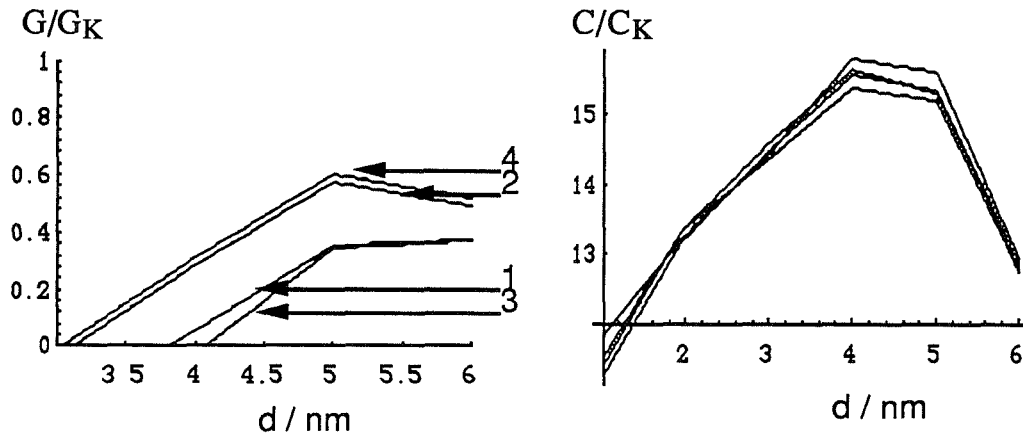


Figure 6.12b. The conductance (G/G_K) and capacitance ratios (C/C_K) as a function distance (d) from the plane of symmetry for the high frequency calculations of chapter 6 for the following cases:

- 1 : Channel including the effect of Born energy - section 6.5.2
- 2 : Charged channel - section 6.7.2
- 3 : Ion selective channel - section 6.9.2
- 4 : Simple pore of chapter 5 - section 5.5.2

G_K and C_K are the geometrical conductance and capacitance defined by equations 5.3.3 and 5.3.4.

The accumulation of charge in the central region leads to a marked increase in the electric field in the central region. Consequently the capacitance of the ion selective channel decreases significantly compared to that of simple pore. At high frequencies, the capacitance is approximately the same as for the simple pore for all three cases.

6.11 CONCLUSIONS

Since ion transport through channels of cell membranes depends on effects other than geometry, extensions of the simple model of Chapter 5, which include some of these effects, have been investigated. The results presented in this chapter serve primarily to demonstrate the order of magnitude of the effects and the feasibility of the model. By increasing the resolution and using a more detailed channel geometry, one could easily use this model to perform much more computationally intensive calculations for specific channel types. Calculations for this model show that these modifications significantly affect the impedance of the channel as described below:

- * Effect of the Born energy: Ions tend to be driven out of voxels surrounding the insulating membrane, as a result of the Born energy term. This effectively restricts the occupancy of the channel and hence significantly reduces the channel conductance. The accumulation of charge near the channel mouth results in an increase in the electric field and potential difference across the channel and a consequent reduction of the capacitance of the channel at low frequencies.

- * Effect of surface charges: The fixed charge attracts counter-ions and repels co-ions. The resulting decrease in cation concentration and corresponding increase in anion concentration leads to a very slight decrease in the electric field in the central region, thus effectively

increasing the conductance of the channel by a very small amount.

* Effect of ion selectivity: A channel impermeable to anions is used as an example in this thesis. Anions accumulate at the channel mouth. This attracts cations and reduces the cation flow, resulting in a significant decrease in the channel conductance, especially at low frequencies.

CHAPTER 7

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

7.1 CONCLUSIONS

Much can be learnt about the mechanisms of ion transport from studies of the electrical properties of channels through membrane. This thesis is a theoretical analysis of the a.c. electrodiffusion for such systems. Electrical measurements of ion channels in membranes necessarily include part of the surrounding medium, which is usually an electrolyte solution. Therefore in Chapter 3 exact algebraic solutions to the Nernst-Planck and Poisson equations for the small signal response of an electrolyte solution were found. The calculations presented in Chapter 3 showed that diffusion effects become important for values of impedances (Z) between voltage electrodes situated many Debye lengths away from the current electrodes. If the ion species have different diffusion constants, the impedance is an oscillatory function of frequencies at low frequencies. The frequencies and magnitude of these oscillations depend on the diffusion constants of the ion species, the spacing of the current electrodes and the ion concentration. The oscillations of the capacitance can be very great for large differences in the diffusion constants of the different ion species. However, since these oscillations occur at low frequencies, the ratio $\text{Im}(Z)/\text{Re}(Z)$ is not very large and typically varies between -0.4 and 0.1 for the parameters chosen for this thesis.

The capacitance alternated between positive and negative values with

increasing frequency. Hence this theory can possibly be applied to the method of null measurements, which is a very powerful measuring technique for the following two reasons: i) it can remove free parameters and ii) it is inherently powerful, since many measuring instruments are most sensitive near zero.

Ionic currents, electric field and ion concentrations are also oscillatory functions of position. Calculations of their spatial variation suggests the possibility of errors in measurements for some small signal a.c. techniques commonly used in physiology, which infer ion fluxes through membranes solely from measurements in the external electrolyte solution relatively far away (μms) from the membrane.

In experimental situations, ion fluxes are approximately radial. Hence in Chapter 4, the Nernst-Planck and Poisson equations were solved for spherical geometry: the current electrodes were assumed to be a very small source (the channel) and a hemispherical shell electrode. The comparison of the four terminal impedances for spherical symmetry with those for the planar geometry of Chapter 3 showed no qualitative differences and the quantitative differences were approximately those expected from geometrical factors. It is possible that calculations performed in the simpler planar geometry (e.g. Chapter 3) can be expected at least to be qualitatively applicable to other membrane electrolyte geometries in between the two extreme geometries considered in Chapters 3 and 4.

The total conductance of a channel necessarily includes a small region of electrolyte solution surrounding the channel mouth, which has often been modelled by a hemisphere (e.g. Levitt, 1985). The contribution to the total channel conduction can be considerable, especially for channels with a high "intrinsic" channel conductance. At very low electrolyte concentrations, the total conductance ultimately

becomes limited by diffusion of ions to the channel mouth (Läuger, 1976 and Levitt, 1987). The spherical geometry employed in this chapter enabled the calculation of this "access impedance".

In Chapters 5 and 6 numerical methods were used to solve geometries which did not yield analytical solutions. Although numerical solutions allow arbitrary channel geometry, they have limited spatial and temporal resolution. Ion channel dimensions are of the order of nm and hence voxel sizes need to be smaller than these. The time steps required for convergence also need to be very small. This made the calculations computationally intensive. Hence, limitations of the available equipment meant that only low resolution calculations could be performed in reasonable computation times.

The results presented in Chapters 5 and 6 show that even with modest computational power, electrical properties of interesting systems could be analysed for useful geometries. No attempt was made to fine-tune the model to allow a comparison with experimental data of which there are as yet virtually none.

The calculations in this thesis required 20 digits of precision. The calculation time per a.c. cycle for one voxel required for convergence (t_c) depends on the frequency (f), the total number voxels (n_v) and the clock speed of the computer. It can be represented by: $t_c = K/(f n_v^2)$. For the calculations in this thesis K is approximately equal to 2×10^7 for a clockspeed of 40 MHz. It was found that for these calculations the effects of the finite size of the system were more important than those of quantisation errors arising from the finite voxel size.

Even though the numerical results presented in this thesis have very limited resolution, they could still provide estimates of the magnitude of some of the important effects on ion transport through channels.

For the simpler geometries, the results were compared with the

analytical solutions of Chapter 3. This comparison showed approximate agreement between the analytical and numerical results and the differences were mainly attributable to the effects of the finite size of the slab and cylinder of electrolyte used for the numerical calculations.

For an insulating membrane, ions accumulated near the membrane/electrolyte interface forming a space charge of sign opposite to that of the nearest charged boundary. The conductance was essentially zero, while the capacitance was much larger than the geometrical value, especially at low frequencies.

For a simple pore at low frequencies, ions can diffuse sufficiently far for significant charge transfer through the channel to occur. Hence a considerably smaller amount of charge accumulated at the electrolyte/membrane interface, resulting in a lower electric field than for the insulating membrane and thus a larger capacitance than for the membrane with no pore. At high frequencies there is little transfer of charge through the pore, hence ions accumulated near the membrane/electrolyte interface and the entrance of the pore. At these frequencies the capacitance of the insulating membrane and the simple pore were approximately the same.

Channels in biological membranes are complicated and ion transport through them depends on effects other than geometry, which have not been considered in the model of the simple pore presented in Chapter 5. Hence in Chapter 6, the simple model was modified to include some of the more important effects. These were:

- 1) Born energy
- 2) Fixed charges on the surface of the channel wall.
- 3) Ion selectivity due to differences in the diffusion constants of the different ion species inside the channel.

These complications had significant effects, especially at low frequencies. Adding the Born energy restricted channel occupancy and hence it reduced the conductance of the channel. The capacitance of the channel also decreased, since the Born energy increased the effective "thickness" of the membrane, by reducing ion concentration in the voxels surrounding the membrane/electrolyte interface.

The fixed charge attracts counter-ions and repels co-ions. This screening of the fixed charge resulted in a slight decrease in the electric field in the central region, and hence an effective increase in channel conductance. The slight decrease in potential difference across the channel led to a slight increase of the capacitance.

The example of an ion selective channel presented in this thesis is a channel impermeable to one ion species only. The consequent accumulation of the impermeable ion species led to a decrease in the ion current for counter-ions. This resulted in a considerable reduction of the conductance of the channel. The increase in electric field across the channel resulted in a reduction in the capacitance of the channel. For the example presented, the value of the capacitance was approximately twice that for the insulating membrane.

At very high frequencies, the capacitance was approximately the same for all three cases.

7.2 SUGGESTIONS FOR FURTHER WORK

Other exact algebraic solutions of the Nernst-Planck and Poisson equations are possible for the following electrodiffusion regimes:

- 1) An electrolyte solution containing four distinct charge species or three charge species and a neutral species formed by recombination of the ions.

2) Reaction kinetic schemes (Walkerden, 1987).

Reaction kinetic schemes have been used extensively in steady state models of ion channels in which the electrical properties of the channel are modelled by a series of rate constants (e.g., Lauser, 1987 and Sanders *et al.*, 1984)

In Chapter 6, the effects of various important factors such as fixed charge and Born energy have been added separately. Although this gives order of magnitude estimates and qualitative understanding, it would be of interest to study the interaction of two or more of these effects. For example, fixed charges can compensate for the effect of Born energies only for the case of the counter-ion. For the case of the co-ion, it presumably would make the situation more severe. This would be especially of interest in particular cases when molecular information will be good enough to allow more detailed modelling.

The model could be extended further to allow for other effects such as coupling currents from the different ion species. A steady d.c. current could also be included explicitly.

Even though Chapters 5 and 6 only give low resolution representative results, much more computationally intensive calculations for specific channels could be performed on more powerful computers, especially as more detailed channel protein structures become available. Currently the expense of array processors is not justified for such problems, given the lack of knowledge of the exact geometries.

Some channel proteins, e.g. the acetylcholine receptor channel, have a narrow charged constriction which is believed to play a major role in the transport mechanism of the channel. This can be simulated by varying the number of blocked voxels in the radial direction as a function of distance through the channel, since the model presented in this thesis

allows an arbitrary number of blocked voxels in both directions.

Voxel size in the z direction for the model presented in Chapter 5 and 6 was kept constant. Since most of the variations of concentration, electric field and ionic current occurs near the charged boundaries and in the region containing the channel and membrane, it may prove useful to include voxel sizes which vary with z and/or with r . This would be particularly useful when modelling large systems, at regions far away from the channel.

This method could also be applied to one dimensional nonlinear systems for which exact algebraic solutions are not possible. An example of this would be a more detailed model of the double layer near an infinite electrode or charged surface which includes fixed charges in the Stern layer, non-zero zeta potentials, recombination of ion species and a dielectric constant that varies with distance.

As a final comment I cannot resist advising, however, that the implementation of such calculations is rather more difficult than their description. To anyone undertaking such future work, I would be willing to provide computer codes and any advice required. Further I would like to wish him/her good luck and as much sanity as is consistent with work of this nature.

APPENDIX 3.1

The slow response time of miniature ion-sensitive electrodes and typical vibration frequencies of probes means that measured changes of ionic activities and electric fields will only occur at relatively low frequencies (typically < 1 Hz). Variables derived at this low frequency limit will be indicated by the subscript L. At these low frequencies the contribution of displacement current will be negligible. The characteristic lengths simplify to:

$$\lambda_{1L} = \lambda \quad (A3.1.1)$$

$$\lambda_{2L} = (1-j) \xi_s/2 \quad (A3.1.2)$$

where ξ_s denotes the rms distance through which the solute diffuses during a phase change of one radian in the applied current. Therefore:

$$\xi_s = [2D_s/\omega]^{1/2} = [2(z_p+z_n)D_pD_n/(z_pD_p+z_nD_n)\omega]^{1/2} \quad (A3.1.3)$$

where D_s denotes the effective diffusion coefficient for the solute which is given by:

$$D_s = (z_p+z_n)D_pD_n/(z_pD_p+z_nD_n) \quad (A3.1.4)$$

Thus at very low frequencies the magnitudes of the two characteristic lengths are simply the Debye length λ and the diffusion length for the solute ξ_s . λ is independent of frequency, whereas ξ_s varies as $\omega^{-1/2}$.

Although λ_{1L} is real, λ_{2L} has real and imaginary components of equal magnitude.

$$S_{1L} = -1 + 2j\lambda^2(\xi_p^{-2} - \xi_n^{-2}) \quad (A3.1.5)$$

$$S_{2L} = (z_p/z_n) [1 + 2j\lambda^2(\xi_p^{-2} - \xi_n^{-2})] \quad (A3.1.6)$$

$$H_{1L} = 1 \quad (A3.1.7)$$

$$H_{2L} = -(z_p/z_n) \{1 - j\omega\lambda^2(D_p - D_n)/D_s D_p\} \quad (A3.1.8)$$

In the low frequency limit, $H_{1L} = 1$ and $H_{2L} = -z_p/z_n$. Thus the ionic currents carried by cations and anions associated with the terms with subscript 1 will be in the ratio z_p/z_n because they move in opposite directions under the influence of the electric field. However the ionic currents carried by cations and anions via terms with subscript 2 will be in the ratio -1. This is because they move in the same direction, effectively as movement of solute. After substituting equations A3.1.1 to A3.1.8, equations 3.5.13 and 3.5.14 become:

$$\alpha_{1L} = JQ_p(1 - D_n/D_p)\lambda/2D_s q(z_p + z_n)\cosh(L/\lambda) \quad (A3.1.9)$$

$$\alpha_{2L} = (j-1)JQ_p/2^{3/2}\omega^{1/2}qz_pD_s^{1/2}\cosh((1+j)L/\xi_s) \quad (A3.1.10)$$

The ionic currents are given by:

$$J_{pL}(x) = J\{z_p D_p/(z_p D_p + z_n D_n) + Q_p \cosh((1+j)x/\xi_s)/\cosh((1+j)L/\xi_s)\} \quad (A3.1.11)$$

$$J_{nL}(x) = J\{z_n D_n / (z_p D_p + z_n D_n) - Q_p \cosh((1+j)x/\xi_s) / \cosh((1+j)L/\xi_s)\}$$

(A3.1.12)

APPENDIX 4.1 COMPARISON OF THE IMPEDANCE OF A BINARY ELECTROLYTE FOR PLANAR AND SPHERICAL GEOMETRY.

The coefficients b_1 and b_2 involve the exponential terms $e^{\pm R/l_2}$ ($l_2=1/m_2$). Since l_2 is complex (see equation 4.2.15 to 4.2.17) these exponential terms, and hence Z_2 , are again oscillatory. Since the contribution of Z_1 is negligibly small for the values of r_1 and r_2 chosen for these calculations, the real and imaginary parts of Z_t will equal Z_K at frequencies for which the real and imaginary parts of Z_2 are equal to zero. At frequencies (f_{nG}) at which the real part of Z_2 is zero, G_t is approximately equal to the geometrical value G_K . Also at frequencies (f_{nC}) at which the imaginary part of Z_2 is zero, C_t approaches C_K . Since this system is not antisymmetric and Z_t is a rather complicated function of $e^{\pm R/\lambda_2}$, it proved impossible to find a simple expression for f_{nG} and f_{nC} as well as Z_t , unlike for the two ion case of chapter 3.

Qualitatively the variation of C_t/C_K and G_t/G_K with frequency is similar to those for two ions in a planar geometry, i.e. C_t/C_K approaches a very large frequency independent value at low frequencies and oscillates at intermediate values. G_t is less than G_K at low frequencies and oscillates about unity at intermediate frequencies. Both C_t and G_t approach their geometrical values at high frequencies. As before the magnitude of the oscillations of C_t/C_K is much larger than that of G_t/G_K . However the values of the frequencies f_{nG} and f_{nC} differs significantly from those for planar geometry of the previous chapter (see Figures 4.4 to 4.7).

As has been found for planar geometries C_t/C_K and G_t/G_K are functions of the difference between the cation (D_p) and anion (D_n) diffusion constants as well as current electrode separation R . The effects of R and the difference between diffusion constants on the capacitance and

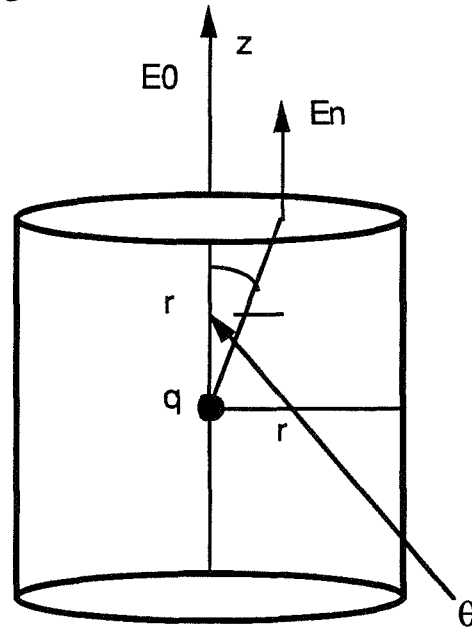
conductance ratios are much more spectacular in the case of C_t/C_K than G_t/G_K , since the real part of Z_t is dominated by Z_K , except for large ratios of D_p/D_n . As the difference between D_p and D_n becomes larger, it considerably increases the low frequency limit of C_t as well as the magnitude of the capacitance oscillations at intermediate frequencies. This increase in the difference between D_p and D_n also lowers the low frequency limit of the conductance (Figures 4.4 and 4.5). As for the two ion case of the previous chapter, f_{nC} and f_{nG} do not vary with bulk electrolyte concentration.

Increasing R shifts f_{nG} and f_{nC} to lower frequencies, as well as significantly increasing the value of C_t/C_K . For planar geometry both f_{nG} and f_{nC} are inversely proportional to the square of the current electrode separation. The fractional increase in the low frequency limit of C_t/C_K for spherical symmetry is approximately the same as that for the binary electrolyte for planar geometry (Figures 4.4 and 4.6).

The frequencies f_{nC} and f_{nG} as well as Z_t now also depend on the positions of the voltage electrodes r_1 and r_2 . Increasing r_1 and r_2 , while keeping $r_2 - r_1$ constant results in an increase in the low frequency limit of C_t . f_{nC} shifts to higher frequencies and the magnitude of the oscillations of C_t/C_K decreases. The low frequency G_t limit remains approximately unchanged (Figures 4.4 and 4.7).

APPENDIX 5.1

Consider a voxel along the central axis as shown below.



Assume that the total charge in the voxel is confined to a point at the centre of the voxel.

$$E_n = E_0 \cos^2\theta$$

$$\begin{aligned} \int_0^{\pi/4} E_n \cdot dA &= \int_0^{\pi/4} 2\pi r \sin\theta / \cos\theta E_0 \cos^2\theta r / \cos\theta d\theta \\ &= 2\pi r^2 E_0 [-\cos\theta]_0^{\pi/4} \\ &= 2\pi r^2 E_0 (1 - 1/\sqrt{2}) \end{aligned}$$

Hence the integral of the electric field is ≈ 0.6 of that assuming uniform electric field on the voxel boundary.

REFERENCES

- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D., (1989). *Molecular Biology of the Cell*. Garland Publishing, Inc. New York & London
- Anderson, O.S., and R.E. Koeppe, (1992). Molecular determinants of channel function. *Physiol. Rev.*, 72(Supp1) 89-158
- Arwin, H., I. Lundstroem A. Palmqvist, (1982). Electrode adsorption method for determination of enzymatic activity. *Med. Biol. Eng. Comput.*, 20, 362-374 - from Kell, 1987*?
- Archer and Cafiso, D. S., (1991). Voltage-dependent Conductance for Alamethicin in Phospholipid Vesicles. A test for the Mechanism of Gating. *Biophys. J.*, 60, 380-388
- Ashcroft, R.G., H.G.L. Coster and J.R. Smith, (1983). The Effects of Cholesterol Inclusion on the Molecular Organisation of Bimolecular Lipid Membranes. *Biochim. Biophys. Acta*, 730, 231-238
- Auerbach, A. and Sachs, F., (1984). Patch Clamp Studies of single ionic channels., *Ann. Rev. Biophys. Bioeng.*, 13, 269-302
- Bai, L. and Conway, B. E., (1990). Complex behavior of Al dissolution in non-aqueous medium as revealed by impedance spectroscopy. *J. of the Electrochem. Soc.* 137, 3737-3747
- Barry, P. and Lynch, J.W., (1991). Liquid Junction Potentials and Small Cell Effects in Patch-Clamp Analysis. *J. Membrane Biol.*, 121, 101-117
- Bates, J.B. and Chu, Y.T., (1988). Surface topography and electrical response of metal-electrolyte interfaces. *Solid State Ionics, Diffusions and Reactions*, 28-30, 1388-1395

- Bers, D.M. and A. Peskoff, (1991). Diffusion around a cardiac calcium channel and the role of surface bound calcium. *Biophys. J.*, 59, 703-721
- Bezanilla, F., Perozo, E., Papzian, D. M. and Stefani, E., (1991). Molecular basis of Gating Charge Immobilization in Shaker Potassium Channels. *Science*. 254,679-683
- Bohnke, C. and Bohnke, O., (1990). Impedance Analysis of Amorphous WO₃ Thin Films in Hydrated LiClO₄-propylene carbonate electrolytes. *Solid State Ionics, Diffusion & Reactions*. 39,195-204
- Buck, R. P. (1969). Diffuse Layer Charge Relaxation at the Ideally Polarized Electrode. *J. Electroanal. Chem.*, 23,219-240
- Cannell, M. B. and Nichols, C. G., (1991). Effects of Pipette Geometry on the Time Course of Solution Change in Patch Experiments. *Biophys. J.*, 60,1156-1163
- Carnie, S.L. and G.M. Torrie,(1984). The Statistical Mechanics of the Electrical Double Layer. *Adv. Chem. Phys.*, 56,141-253
- Cevc, G., Membrane Electrostatics, (1990). *Biochim. et Biophys. Acta*, 1031-3,311-382
- Chilcott, T.C., (1988). Admittance Tomography of Cells of *Chara Corallina*: a Study of the Spatial Structures Associated with Photosynthesis. PhD. thesis, U.N.S.W.
- Cooper, N. E., Gates, P.Y. and Eisenberg, R. S., (1988). Surmounting Barriers in Ionic Channels, *Quarterly Rev. of Biophys.*, 3, 331-364
- Coronado, R., (1986), Recent Advances in Planar Phospholipid Bilayer Techniques for Monitoring Ion Channels, *Ann. Rev. Biophys. Biophys. Chem.*,15, 259-277

- Coster, H.G.L., and J.R. Smith, (1977). Low-frequency Impedance of *Chara corallina*: Simultaneous Measurements of the Separate Plasmalemma and Tonoplast Capacitance and Conductance. *Aust. J. Plant Physiol.*, 4, 667-674.
- Debye, P. and E. Hückel, (1923). Zur Theorie der Electrolyte. *Phys. Z.*, 24, 185-206
- DeLacey, E. H. B. and White, L. R., (1982). The Polarization Impedance of an Ideally Polarizable Plane Electrode, *J. Chem. Soc., Faraday Trans.2*, 78, 457-479
- Duchacelle, P., Ohara, A., Ling, B.N. Kenedy, A.E., Kokko, K.E. (1992). Regulation of renal epithelial sodium channels. *Mol. cell. Biochem.*, 114(1-2), 27-34
- Eisenberg D., (1984). Three-dimensional structure of membrane and surface proteins. *Ann. Rev. Biochem.*, 53, 595-623
- Eisenman, G., and Alvarez, O., (1991). Structure and function of channels and channelogs as studied by computational chemistry. *J. Membr. Biol.*, 119, 109-132
- Eisenman, G., and J.A. Dani, (1987). An Introduction to Molecular Architecture and Permeability of Ion Channels. *Ann. Rev. Biophys. Biophys. Chem.*, 16, 205-226
- Eisenman, G., and R. Horn, (1983). Ionic Selectivity Revisited: The Role of Kinetic and Equilibrium Processes in Ion Permeation Through Channels. *J. Membrane Biol.*, 76, 197-225
- Feynman, R.P., Leighton, R.B. and Sands, M., (1964). The Feynman Lectures on Physics, Vol. 2. Reading, Mass: Addison-Wesley, 7-8 to 7-10
- Fleischmann, M., Pons, S. and Daschbach, J., (1991). The ac Impedance of Spherical, Cylindrical, Disk, and Ring Microelectrodes. *J. Electroanal. Chem.*, 317, 1-26

- Fox, R.O. and Richards, F.M., (1982). A voltage-gated ion channel model inferred from the crystal structure of alamethicin at 1.5 Å resolution. *Nature*, 300, 325-330
- Gates, P., Cooper, K., Rae, J. and Eisenberg, R., (1989). Predictions of diffusion models for one-ion membrane channels. *Progress in Biophys. & Molecular Biology*, 53, 153-196
- Gomperts, B.D., (1977). *The Plasma Membrane: Models for Structure and Function*. 109-219 New York: Academic Press
- Hall, J.E., (1975). Letter to the editor. Access Resistance of a small Circular Pore. *J. Gen. Physiol.*, 66, 531-532
- Hancock, R.E.W. and M.H. Brown, (1992). Bacterial Porins. *Today's Life Science*, Nov., 24-32
- Hansen, U.P., D. Gradman, D. Sanders and C.L., Slayman, (1981). Interpretation of current-voltage relationships for "active" ion transport systems: I Steady-state reaction kinetic analysis of class-I mechanisms. *J. Membrane Biol.*, 63, 165-190.
- Hartmann, H.A., Kirsch, G.E., Drewe, J.A., Taglialatela, M., Joho, R.H. and Brown, A.M. (1991). Exchange of conduction pathways between two related K⁺ channels. *Science*, 25, 942-944
- Hedrich, R. and A. Jeromin, (1992). A new scheme of symbiosis: ligand- and voltage-gated anion channels in plants and animals. *Philos. Trans. R. Soc. Lond. Biol.*, 338(1283), 31-38
- Hodgkin, A.L., (1976). Review Lecture. Chance and design in electrophysiology: an informal account of certain experiments on nerve carried out between 1934 and 1952. *J. Physiol (Lond.)*, 263,
- Hodgkin, A. L. and A.F. Huxley, (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117, 500-544
- Honig, B. H., Hubbell, W. L. and Flewelling, R. F., (1986). Electrostatic

- Interactions in Membranes and Proteins, *Ann. Rev. Biophys. Biophys. Chem.* 15, 163-193
- Horber, J. K. H., Schuler, F. M., Witzemann, V., Schröter, K. H., Müller, H. and Ruppersberg, J. P., (1991). Imaging of Cell Membrane Proteins with a scanning Tunneling Microscope. *J. of Vacuum Science and Technology B*, 9, 1214-1218
- Irvine, J. T. S., Sinclair, D. C. and West, A. R., (1990). Electroceramics: Characterization by Impedance Spectroscopy. *Advanced Materials*, 2, 132-138
- Israelachvili, J.N., (1978). The packing of lipids and proteins in membranes. In: *Light Transducing Membranes: Structure Function and Evolution*. 91-107, D.W. Deamer, Academic Press, New York
- Jakobsson, E. and Chiu, See-Wing, (1987). Stochastic Theory of Ion Movement in Channels with Single-Ion Occupancy. *Biophys J.*, 52, 33-45
- Jaffe, L.F., and R. Nuccitelli, (1974). An ultrasensitive vibrating probe for measuring steady extracellular currents. *J. Cell Biol.*, 63, 614-628.
- Jap, B. K. and Walian, P. J., (1990). Biophysics of the structure and function of porins, *Quarterly Reviews of Biophysics*, 23-24, 367-403
- Jauch, P., Petersen, O.H. and Lauger, P., (1986). Electrogenic properties of the sodium-alanine cotransporter in pancreatic acinar cells: 1. Tight seal whole cell recordings. *J. Membr. Biol.*, 94, 99-115
- Jordan, P.C., (1986). Ion Channel Electrostatics and the Shapes of Channel Proteins. In: *Ion channel reconstitution*. 37-55 C. Miller, Plenum Press, New York and London
- Jorge, G., Durand, R., Faure, R. and Yavari, R., (1988). On the penetration and solution of hydrogen in amorphous Pd₈₀Ge₂₀ and Pd₈₀Si₂₀ before and after crystallization. *J. of the Less- Common Metals*, 145, 383-393

- Kochian, L.V., J.E. Schaff and W.J. Lucas., (1989). High-affinity K⁺ uptake in maize roots: a lack coupling with H⁺efflux. *Plant Physiol.*, 91, 1202-1211.
- Koksbang, R., Olsen, I. I., Tonder, P. E., Knudsen, N. and Fauteux, D., (1991). Polymer electrolyte lithium batteries rechargeability and positive electrode degradation: an ac impedance study. *J. of Applied Electrochem.*, 21,301-307
- Lamperski, S., (1991). A Monte Carlo study of intermolecular electrostatic interactions at electrode/electrolyte interface. *J. Electroanal. Chem.*, 318,39-52
- Läuger, P., (1976). Diffusion-limited ion flow through pores. *Biochim. Biophys. Acta*, 455, 493-509
- Läuger, P., (1985). Mechanisms of Biological Ion Transport-Carriers, Channels, and Pumps in Artificial Lipid Membranes. *Angew. Chem. Int. Ed. Eng.*, 24, 905-923
- Läuger, P., (1987). Dynamics of Ion Transport Systems in Membranes. *Physiol. Rev.*, 67, 1296-1331
- Latorre, R., (1986). The Large Calcium-Activate Potassium Channel, In: *Ion channel reconstitution*. 431-467, Miller, C.
- Laver, D.R., J.R. Smith and H.G.L. Coster, (1984). The Thickness of the Hydrophobic and Polar Regions of Glycerol Monooleate Bilayers Determined from the Frequency-Dependence of Bilayer Capacitance. *Biochim. Biophys. Acta*, 772, 1-9
- Levitt, D. G., (1978). Electrostatic Calculations for an Ion Channel. I Energy and Potential Profiles and Interactions between Ions. *Biophys. J.*, 22, 209-219
- Levitt, D. G., (1985). Strong Electrolyte Continuum Theory Solution for Equilibrium Profiles, Diffusion Limitation, and Conductance in Charged Ion Channels. *Biophys. J.*, 48, 19-31

- Levitt, D. G., (1986). Interpretation of Biological Ion Channel Flux Data- Reaction Rate versus Continuum Theory. *Ann. Rev. Biophys. Chem.*, 15, 29-57
- Levitt, D. G., (1987). Exact Continuum Solution for a Channel that can be occupied by Two Ions. *Biophys. J.*, 52, 455-466
- Levitt, D. G., (1991). General Continuum Theory for Multi-ion Channel. II. Application to Acetylcholine Channel. *Biophys. J.*, 59, 278-288
- Lim, C., Pyun, S.-Il. and Ju, J.-B., (1991). Impedance Analysis of Hydrogen Adsorption on Palladium in 0.1M NaOH Solution. *J. Alloys and Compounds*. 176, 97-103
- McLaughlin, S., (1989) The electrostatic properties of membranes. *Ann. Rev. Biophys. Biophys. Chem.*, 18, 113-136
- McNamee, M., O.T Jones, and T.M. Fong, (1986). Function of Acetylcholine Receptors in Reconstituted Liposomes. In: *Ion channel reconstitution*. 231-273, Miller, C.
- Meves, H. and K. Nagy, (1989). Multiple conductance states of the sodium channel and of other channels. *Biochim. et Biophys. Acta*, 988, 99-105
- Montal, M. R. Anholt and P. L. Abarca, (1986). The reconstituted acetylcholine receptor, In: *Ion channel reconstitution*. 157-204, Miller, C.
- Morita, M., Aoki, S. and Matsuda, Y., (1992). ac impedance behaviour of lithium electrode in organic electrolyte solutions containing additives. *Electrochimica Acta*, 37, 119-123
- Muragesamoorthi, K. A., Scinivasan, S. and Appleby A. J., (1991). ac Impedance Studies on Nickel Oxide Electrodes to Determine Self-discharge Characteristics of Ni-H₂ Batteries. *J. Appl. Electrochem.*, 21, 95-98

- MacDonald J. R., (1974). Binary Electrolyte Small-Signal Frequency Response. *J. Electroanal. Chem.*, 53, 1-55
- Macdonald J. R., (1987). Impedance spectroscopy and its use in analyzing the steady-state ac response of solid and liquid electrolytes. *J. Electroanal. Chem.*, 223, 25-50
- Macdonald J. R., D. R. Franceschetti, (1978). Theory of small-signal ac response of solids and liquids with recombining mobile charge. *J. Chem. Phys.*, 68, 1614-1637
- McNamee, M., O.T Jones, and T.M. Fong, (1986). Function of Acetylcholine Receptors in Reconstituted Liposomes. In: *Ion channel reconstitution*. 231-273, Miller, C.
- Montal, M. R. Anholt and P. L. Abarca, (1986). The reconstituted acetylcholine receptor, In: *Ion channel reconstitution*. 157-204, Miller, C.
- Muragesamoorthi, K. A., Scinivasan, S. and Appleby A. J., (1991). ac Impedance Studies on Nickel Oxide Electrodes to Determine Self-discharge Characteristics of Ni-H₂ Batteries. *J. Appl. Electrochem.*, 21, 95-98
- Neher, E., (1992). Ion channels for communication between and within cells. *Biosci. Rep.*, 12(1), 1-14
- Neher, E. and B. Sakmann, (1976). *Nature*, 260 799-802
- Neher, E. and B. Sakmann, (1992). The patch clamp technique. *Scientific American*, 266, 28-35
- Neumke, B., and P. Luger, (1969). Nonlinear electrical Effects in Lipid Bilayer Membranes. II. Integration of the Generalized Nernst-Planck Equations. *Biophys. J.*, 9, 1160-1170

- Newman, I.A., L.V. Kochian, M.A. Grusak, and W.J. Lucas, (1987).
Fluxes of H^+ and K^+ in corn roots: characterization and
stoichiometry using ion-selective microelectrodes. *Plant Physiol.*, 84,
1177-1184
- Parsegian, V.A., (1975). Ion-membrane interactions as structural forces.
Ann. NY Acad. Sci., 264, 161-174
- Petersen, O.H. and Finlay, I., (1987). Electrophysiology of the pancreas.
Physiol. Rev., 67, 1054-1116
- Polymeropoulos, E.E. and Brickmann, J., (1985). Molecular Dynamics of
Ion Transport through Transmembrane Model Channels. *Ann. Rev.*
Biophys. Biophys. Chem., 14,315-330
- Ratnakumar, B. V., Di Stefano and Halpert, G., (1990). Electrochemistry
of metal chloride cathodes in sodium batteries. *J. of the*
Electrochem. Soc., 137,2991-2997
- Reeves, R., The Double Layer in the Absence of Specific Adsorption,
(1980). In: *Comprehensive Treatise of Electrochemistry*. Vol.1, 83-
133, Bockris, J. O'M., Conway, B. E. and Yeager, E., Plenum Press,
New York
- Robertson, N.L. and Michaels, J.N., (1991). Double layer capacitance of
porous platinum electrodes in zirconia lectrochemical cells. *J. of the*
Electrochem. Soc., 138,1494-1499
- Robertson, R.N., (1983). *The Lively Membranes*. Cambridge University
Press
- Sakmann, B., and G. Boheim, (1979). Alamethicin-induced single channel
conductance fluctuations in biological membranes. *Nature*, 282, 336-
339
- Sandblom, A.R. and G. Eisenman, (1982). Linear Network
Representation of Multistate Models of Transport. *Biophys. J.*, 38,
93-104

- Sanders, D., U.P. Hansen, D. Gradman and C.L. Slayman, (1984).
Generalized Kinetic Analysis of Ion-Driven Cotransport Systems: A
Unified Interpretation of Selective Ionic Effects on Michaelis
Parameters. *J. Membrane Biol.*, 77,123-152.
- Scott, D.M., (1987). Sodium cotransport systems : cellular, molecular and
regulatory aspects. *Bioessays*, 7, 71-78
- See-Wing Chiu and Jakobsson, E., (1989). Stochastic theory of singly
occupied ion channels. II. Effects of access resistance and potential
gradients extending into the bath. *Biophys. J.*, 55,147-157
- Smith, J.R., H.G.L. Coster and D. R. Laver, (1984). The dependence of
the conductance of phosphatidylcholine bilayers upon the
concentration and composition of the external electrolyte. *Biochim.
Biophys. Acta*, 812, 181-192
- Smith, J.R. and N.A.Walker, (1985). Effects of pH and light on the
membrane conductance measured in the acid and basic zones of
Chara. *J. Membr. Biol.*, 83, 193-205
- VanderNoot, T.J., (1991). Limitations in the analysis of ac impedance
data with poorly separated faradaic and diffusional processes.
300,199-210
- VanderNoot, T.J., Schiffrin, D. J. and Whiteside, R. S., (1990). Design
and Evaluation of a Four-Electrode Potentiostat/Voltage Clamp
Suitable for ac Impedance Measurements at the Interface of
Immiscible Electrolytes. *J. Electroanal. Chem.*, 278,137-150
- Walkerden, F., (1987). Electrical Behaviour of Reaction-Kinetic schemes.
Honours thesis, U.N.S.W.
- Wallace, B. A., (1990). Gramicidin Channels and Pores. *Ann. Rev.
Biophys. Biophys. Chem.*, 19,127-157
- Yellen, G., Jurman, M.E., Abramson, T. and MacKinnon, R., (1991).
Mutations affecting internal TEA blockade identify the probable

pore-forming region of a K⁺ channel. Science 251,939-942