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0521301513 - The Biophysical Basis of Excitability
Hugo Gil Ferreira and Michael W. Marshall
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Introduction and overview

This book is not a textbook of biophysics, cell biology or the electrophysiology of excitable cells, as there are already a number of excellent books available which deal with these subjects. The book instead is an attempt to describe the origins and derivations of the principles upon which these other books are based.

To understand and apply the principles of excitability requires a knowledge of subjects as diverse as physiology, physics, mathematics, statistics, signal and system analysis. It is a difficult task to obtain this knowledge because the jargon in other fields is often obscure, mathematical proofs are frequently abstruse and generally many original manuscripts have to be consulted. We can both testify to the frustrations that accompany such efforts and this has therefore been written in an attempt to enable the reader to acquire more easily this knowledge. Half of the book is appendices which deal with many of the key concepts from a fairly basic level.

We have assumed that the reader has only a modest mathematical background (about G.C.E. 'O' level) and most formulae are derived from first principles. For people with mathematical ability this approach may be somewhat tedious but we make no apologies for this. We consider it necessary that most of the steps in the derivation of an important equation are left in. Far too often have we struggled to follow mathematical proofs that are presented by an author in two lines which in reality take pages to derive.

The title of the book should not be taken too literally. The principles of excitability apply to many other biological fields. For example, Chapters 2–6, which deal with the movements of ions in solution and across barriers, could be useful to those who study ion transport in non-excitable cells. Chapters 7–9 give a good basis for research workers interested in a more

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analytical study of excitability. Chapters 7–9 should also provide a good basis for those wishing to study more advanced electrophysiological texts (such as Jack, Nobel and Tsien's *Electric Current Flow in Excitable Cells*).

The book is intended to be as self-contained as possible and it is for this reason that there are extensive appendices. In the appendices we have attempted to lay out most of the fundamental concepts in a simple way and by separating them from the remainder of the text to avoid interrupting the flow of the main text with derivations.

The appendices can be broadly categorized into three groups. These are mathematics, signal analysis (harmonic analysis) and physical chemistry. The mathematical appendices cover calculus (differential and integral), number theory, probability, solutions of differential equations and some special mathematical relationships such as Stirling's formula and Wallis's product. In the appendices on signal analysis the aim is to introduce the harmonic analysis of stochastic signals. Physical chemistry covers some key concepts such as free energy and Debye layers.

At all times we have tried to develop the theory presented in the appendices in a semi-intuitive way, and this is often at the expense of mathematical rigour. The reader approaching the book for the first time should realize that the appendices tend to build on one another so that there is a gradual increase in both mathematical and conceptual difficulty This occurs throughout the book. Conceptually the content of Chapters 2–6, which deals with the principles of excitability, is easier to understand than the content of Chapters 7–10 which deals with the applications. This is not surprising, since Chapters 7–10 integrate and extend the ideas and theory developed in the previous chapters.

There are a large number of diagrams and figures and, rather than borrowing from original experimental records, journals or books, we have presented almost exclusively our own computed curves.

When we think of the long-term usefulness of this book we are aware that not all the basic principles presented here will still obtain in some years' time. We believe that concepts developed in the earlier chapters (like chemical potential, free energy, the Nernst–Planck equation and Fick's Laws) will remain of value for some time. Chapters 6–9 deal with more recent mid-twentieth-century advances that have survived more or less intact to this day. Chapter 10 deals with some of the most recent additions to the field of excitability and these have yet to withstand the test of time. This chapter was included as it is about an exciting, rapidly expanding subject and also because we think some of the concepts it develops are rather difficult. We have tried to present our analysis as clearly as possible, but some of the ideas are not easy to comprehend and we can only hope we have succeeded.

Dimensional arguments are often used in the book, not only to show that the derivations are correct, but also to derive some equations. It is our experience that dimensional analysis can be an excellent way of checking equations that set out to describe the physical world.

It may be helpful to provide a more detailed overview of the book. Chapter 2 sets out to show how to solve the problem of establishing electrical continuity between measuring equipment and biological compartments, which are aqueous solutions. Such continuity is achieved by means of metal electrodes. The chapter then develops such concepts as electrochemical potentials, standard electrode potentials and molar conductances of solutions.

Chapter 3 presents the two Laws of Fick along with the general flux equation and the concept of a driving force. The Nernst–Planck equation is derived from first principles, and (based upon the treatment used by Finchelstein & Mauro) the equation is integrated in such a way as to allow the representation of an electrodiffusional process by electrical equivalent circuits. This approach, using electrical equivalent circuits to model biophysical processes, is a powerful technique often used by electrophysiologists and we use it extensively in the later part of the book.

Chapter 4 deals with a special case of the integration of the Nernst–Planck equation. This is when the electric field is constant throughout the membrane and this constant-field assumption leads to the derivation of the Goldman–Hodgkin–Katz equation. This equation is then analysed in some detail. Membrane permeability and membrane conductance are also presented along with the idea of rectification in membranes. In Appendix 27 (which can be thought of as an extension of this chapter) liquid junction potentials are discussed.

Chapter 5 provides an introduction to the lipid component of biological membranes and to specialized transport systems that are important for setting up membrane potentials.

Our development of electrical equivalent circuits for membranes is presented in Chapter 6 where membrane capacitance is also discussed. A more detailed discussion of membrane capacitance can be found in Appendix 24.

Chapter 7 is entirely devoted to the development of the Hodgkin–Huxley model of excitable membranes. This chapter draws extensively on material given in the previous chapters and shows how action potentials can be modelled using digital computers.

Chapter 8 is an extension of Chapter 7 and deals with the so-called ‘cable properties’ of cells. The propagated action potential is introduced at this point.

Chapter 9 describes in a semi-quantitative way the events that take place at synapses. In this chapter the probabilistic behaviour of synaptic transmission is examined and used as a starting point for an analysis of membrane noise.

Chapter 10 is a more formal treatment of membrane noise and should be read in connection with the appendices on signal analysis.

In conclusion, the aim of the book is not to set out to derive any new fundamental physical laws or propose any new physiological models. Rather, our intention is to present existing knowledge in a way that clearly explains the basic principles underlying excitability in biological tissues.

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Ions in solution

Introduction

A living organism consists of a very large number of interdependent fluid compartments. These compartments are bounded by lipid barriers (membranes) and they contain mainly water and exist in an environment that is mostly water. Communication and exchanges between these compartments are carried out by molecules or ions that are able to move through the water and the lipid phases. In order to study these compartmental exchanges it is thus necessary to examine the way in which molecules and ions move through homogeneous liquid phases. We shall consider homogeneous liquid phases because the membrane lipids are in a liquid state and can be treated in the same way as water – that is, as a homogeneous liquid phase (see Chapter 5). Let us first start by an examination of the way in which ions are formed and then go on to analyse the way in which they move through aqueous solutions under the influence of an electric field.

Formation of ions

Ions are formed when neutral molecules are dissociated or when salts are dissolved. To dissolve a salt, or dissociate a molecule, requires a medium with a high dielectric constant (see Appendix 24 where dielectric constants are discussed) which is then able to weaken ionic bonds. An example of an ionic bond is the bond that holds Na^+ and Cl ions together in a NaCl crystal. Methane, for example (CH_4), is held together by covalent bonds. Ionic and covalent bonds are extreme cases; most chemical bonds are a mixture of the two types. These ionic bonds arise from the coulombic attraction that exists between charged particles and, as shown in Appendix 23, these forces depend on the dielectric coefficient of the medium. The larger the dielectric coefficient of the medium the smaller the attraction.

Water, which is a good solvent for electrolytes, has a large dielectric coefficient (around 80).

Ions can also be formed at an interphase between a metal and a salt solution. If, for example, we immerse a solid plate of metal zinc ($Zn_{(s)}$) in a copper sulphate solution a spontaneous reaction occurs where zinc ions are passed into the solution and copper ions ($Cu^{2+}_{(aq)}$) are deposited as solid metal copper ($Cu_{(s)}$) in place of the zinc. The reaction can be described by the chemical equation:

$$Zn_{(s)} + Cu^{2+}_{(aq)} \rightarrow Zn^{2+}_{(aq)} + Cu_{(s)} \tag{2.1}$$

which can be split into two partial reactions

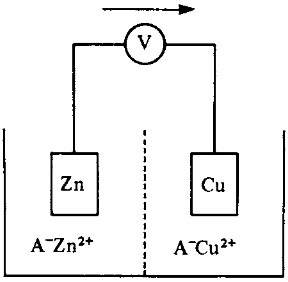
$$Zn_{(s)} \rightarrow Zn^{2+}_{(aq)} + 2e^- \quad \text{(oxidation)} \tag{2.2}$$

$$Cu^{2+}{}_{(aq)} + 2e^- \rightarrow Cu_{(s)} \quad \text{(reduction)} \tag{2.3}$$

Electron affinity

Chemical equation (2.1) tells us that the affinity of zinc for electrons (e^-) is less than that of copper. We can set up an experiment in which we are able to compare quantitatively the attraction of any two metals for electrons. An example is shown in Figure 2.1. Here the zinc ($Zn_{(s)}$) and the copper ($Cu_{(s)}$) plates are placed in their respective equimolar salt solutions (Figure 2.1). The zinc and the copper plates (called electrodes) and their salt solutions have to be separated by a porous barrier. If it were not for this barrier the zinc electrode would give its electrons directly to the copper ions in the solution and there would not be a net flow of electrons through the wire that connects the two electrodes, and we would be unable to measure a voltage between the two cells. It is necessary for the barrier to be porous so that there is a flow of ions, and thus of current, through the solution. We should emphasize here that in solution current is carried by mobile ions; this is because, unlike metals, solutions do not contain free electrons. At

Fig.2.1



(Arrow indicates electron flow)

both metal–solution interphases the following reaction takes place

$$M_{(s)} = M^{2+}_{(aq)} + 2e^{-} \tag{2.4}$$

When an atom of metal (M) loses two electrons and goes into solution it goes from energy state $M_{(s)}$ into energy state $M^{2+}_{(aq)}$, this is because the energy (W_1) required to remove a metal atom from the metal is different from the energy (W_2) required to remove its ion (M^{2+}) from the solution.

Equilibrium at the electrode/solution interphase

When equilibrium is reached we should be able to relate the number of particles in energy state $M_{(s)}$ (atoms of the metal) to the number of particles in energy state $M^{2+}_{(aq)}$ (ions in solution). This is done by considering the 'concentration' of metal atoms ($[M_{(s)}]$) in the metal. In a pure metal the atoms are bound to each other by sharing electrons. These electrons are responsible for the electrical conductivity of metals and can be thought of as a sea (gas) of electrons that link together the metal atoms. We can think of $[M_{(s)}]$ as the number of atoms per unit volume of metal. However, this definition is not used and later on in this chapter we will describe the convention that is now widely accepted. Through the Boltzmann's factor (see Appendix 21) we can relate the concentration of atoms in state $[M_{(s)}]$ to the concentration of ions $[M^{2+}_{(aq)}]$ by the equation

$$[M^{2+}_{(aq)}]/[M_{(s)}] = \exp(-(W_2 - W_1)/RT) \tag{2.5}$$

where W_1 and W_2 are the potential energies of a metal atom and the metal ion respectively (see Appendix 33, where the relationship between potential energy and work is discussed).

By taking logarithms we obtain

$$(W_1 - W_2)/RT = \ln([M^{2+}_{(aq)}]/[M_{(s)}]) \tag{2.6}$$

or

$$(W_1 - W_2)/RT = \ln([M^{2+}_{(aq)}]) - \ln([M_{(s)}]) \tag{2.7}$$

As we are taking logarithms the concentration terms in equation (2.7) have to be defined as dimensionless quantities. This is because if $c = b^x$ from the definition of a logarithm $x = \log_b(c)$. Since b is dimensionless (because it is a base) and an exponent is always dimensionless, we can think of c as a dimensionless quantity, that is, the number of concentration units. This number can be obtained by dividing the concentration $[M_{(s)}]$ (or $[M^{2+}_{(aq)}]$) by the corresponding unit concentrations. Rearranging and collecting terms for the metal and the aqueous phases, equation (2.6) becomes

$$\underbrace{W_1 + RT\ln([M_{(s)}])}_{\text{metal}} = \underbrace{W_2 + RT\ln([M^{2+}_{(aq)}])}_{\text{aqueous}} \tag{2.8}$$

Equation (2.8) was derived from the Boltzmann factor, which requires that the system be in equilibrium. An electrode/solution system is in equilibrium when no current passes through the electrode. This is when the rate at which the metal goes into the solution as metal ions is equal to the rate of deposition of metal at the electrode.

The chemical potential and the electrochemical potential

Since we are dealing with collected terms and an equilibrium situation, we should be able to write an expression based upon equation (2.8) for two-state equilibrium situations in general. From equation (2.8) the expression which applies to a single compartment can be written as follows

$$\mu = W + RT\ln(c) \tag{2.9}$$

Since the meaning of the concentration term c is different for different phases (that is, a gas, liquid or solid) we must adopt some convention for the expression of c. Since it is a rule that any two different conventions can be related by multiplying one of the conventions by a constant (a),

$$c' = ac''$$

where c' is one convention and c'' is a different convention. For example, c' may be expressed in grams per litre and c'' in moles per litre. If this were the case a will be the molecular weight of the solute.

Equation (2.9) can be written more generally as

$$\begin{aligned} \mu &= W + RT\ln(ac) \\ &= W + RT\ln(a) + RT\ln(c) \end{aligned} \tag{2.10}$$

If we define

$$\mu^0 = RT\ln(a) \tag{2.11}$$

then

$$\mu = \mu^0 + RT\ln(c) + W \tag{2.12}$$

In expression (2.12), μ^0 is a constant which takes into account the definition of c; that is, if we define c differently equation (2.12) will still be valid but with a different μ^0. The ease with which a metal goes into solution as an ion depends upon its concentration in the solution and the electrical potential that exists between the solution and the metal. This means that in expression (2.12) W represents the work that has to be done against the electrical potential. To express this work term (W) in terms of an electrical potential V we write

$$W = ZFV \tag{2.13}$$

This relationship comes from the definition of the electrical potential. The electrical potential (in volts) at a point in an electrical field, is the amount of work (i.e. number of joules) required to bring 1 coulomb of charge from outside the electrical field (i.e. zero potential) to that point. Since W is the

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work in joule/mole, F is the charge in faraday (in coulomb per gr-eq) and Z is the valence (gr-eq · mol^{-1}).

Equation (2.13) is dimensionally correct because

$$\text{joule/mol} = (\text{gr-eq/mol}) \cdot (\text{coul./gr-eq}) \cdot (\text{joule/coul.})$$

From this definition an electrical potential difference can be defined. Thus one volt electrical potential difference between two points in an electrical field exists when a joule of work is required to transfer one coulomb of charge between those two points within the electrical field. Substituting (2.13) in (2.12)

$$\mu = \mu^0 + RT\ln(c) + ZFV \qquad (2.14)$$

At unit concentration ($c = 1$) and $V = 0$, then

$$\mu = \mu^0 \qquad (2.15)$$

μ is called the electrochemical potential and μ^0 is the standard electrochemical potential. The standard electrochemical potential μ^0 of an ion is its electrochemical potential when the electrical potential is zero and its concentration is 1. We can now substitute equation (2.14) into equation (2.8) and obtain

$$\underbrace{\mu^0_{(1)} + RT\ln([\mathrm{M}_{(s)}]) + ZFV_{(1)}}_{\mu_{\mathrm{M}(1)}} \underset{=}{=} \underbrace{\mu^0_{(2)} + RT\ln([\mathrm{M}^{2+}_{(aq)}]) + ZFV_{(2)}}_{\mu_{\mathrm{M}(2)}} \qquad (2.16a)$$

where the left side refers to the electrode and the right-hand side to the solution. Equation (2.16a) states that at equilibrium the electrochemical potential of a substance in phase 1, $\mu_{\mathrm{M}(1)}$, is equal to the electrochemical potential of the same substance in phase 2, $\mu_{\mathrm{M}(2)}$. Thus, at equilibrium,

$$\mu_{\mathrm{M}(1)} = \mu_{\mathrm{M}(2)} \qquad (2.16b)$$

The adopted convention is that the concentration of a pure metal in its natural state is 1, which means that the term

$$RT\ln([\mathrm{M}_{(s)}])$$

in equation (2.16a) becomes zero and the equation can now be written as

$$\mu^0_{(1)} + ZFV_{(1)} = \mu^0_{(2)} + RT\ln([\mathrm{M}^{2+}_{(aq)}]) + ZFV_{(2)} \qquad (2.17)$$

Electrode potentials

The electrical potential difference ($V_2 - V_1$) between the two phases can be expressed as

$$V_{(2)} - V_{(1)} = (\mu^0_{(1)} - \mu^0_{(2)})/(ZF) - (RT/ZF)\ln([\mathrm{M}^{2+}_{(aq)}]) \qquad (2.18)$$

If we define

$$E = V_{(2)} - V_{(1)}$$

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and

$$E^0 = (\mu^0_{(1)} - \mu^0_{(2)})/(ZF)$$

where E^0 is the electrical potential difference between the solution and the electrode when the ion concentration ($[\mathrm{M}^{2+}_{(aq)}]$) is one unit, then

$$V_{(2)} - V_{(1)} = E = E^\circ - (RT/ZF)\ln([\mathrm{M}^{2+}_{(aq)}]) \qquad (2.19)$$

We can write an equation like (2.19) for each of two electrodes (Cu and Zn). If we substitute 2 for Z we obtain

$$E_{\mathrm{Zn}} = (V_{(2)} - V_{(1)})_{\mathrm{Zn}} = E^0_{\mathrm{Zn}} - (RT/2F)\ln([\mathrm{Zn}^{2+}_{(aq)}]) \qquad (2.20)$$

$$E_{\mathrm{Cu}} = (V_{(2)} - V_{(1)})_{\mathrm{Cu}} = E^0_{\mathrm{Cu}} - (RT/2F)\ln([\mathrm{Cu}^{2+}_{(aq)}]) \qquad (2.21)$$

Junction potentials

In practice if we want to measure the potential difference between the zinc and the copper electrodes dipped in their respective salt solutions, an electrical continuity between the two solutions has to be established but at the same time mixing of the solutions must be prevented. Experimentally this is achieved by separating the solutions by a porous barrier. However, since the ionic concentrations of the two solutions will be, in general, different, ions will migrate across the porous barrier at different speeds until an electrical potential (diffusion potential) E_{j} reaches a value which prevents a further net charge movement across the barrier. In order to compute the potential difference that exists, for example, between the zinc electrode and the copper electrode equation (2.21) is subtracted from equation (2.20) and a term corresponding to E_{j} is included. So

$$E_{\mathrm{Zn}} - E_{\mathrm{Cu}} = (E^0_{\mathrm{Zn}} - E^0_{\mathrm{Cu}}) - (RT/2F)\ln([\mathrm{Zn}^{2+}_{(aq)}]/[\mathrm{Cu}^{2+}_{(aq)}]) + E_{\mathrm{j}} \qquad (2.22)$$

Equation (2.22) tells us that the electrical potential difference between the two electrodes is the sum of three terms. These are the difference between the two standard potentials ($E^0_{\mathrm{Cu}} - E^0_{\mathrm{Zn}}$), the electrical potential difference across the porous barrier (E_{j}) and a term which depends on the concentrations of $[\mathrm{Zn}^{2+}_{(aq)}]$ and $[\mathrm{Cu}^{2+}_{(aq)}]$.

We can *measure* the potential difference between the electrodes ($E_{\mathrm{Zn}} - E_{\mathrm{Cu}}$) with a voltmeter. If the compartments are big enough the concentrations of the ions (Zn^{2+} and Cu^{2+}) will be fixed and so be known. But it is not possible to *compute* the absolute values of $Z_{\mathrm{Zn}} - E_{\mathrm{Cu}}$ since we are unable to measure or compute independently the diffusion potential E_{j}. This is because in order to measure the electrical potential across the porous barrier we require another electrode to be inserted into the solution. But this electrode would have its own unknown E^0. E_{j} is known as the junction potential. E_{j} is in this case the electrical potential difference across the porous barrier. Since the barrier allows the flow of ions across it, in time both solutions will mix completely and there will be no concentration