

ION TRANSPORT AND THE VIBRATING PROBE

JACK FERRIER* AND WILLIAM J. LUCAS†

*Medical Research Council Group in Periodontal Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada; and †Department of Botany, University of California, Davis, California 95616

ABSTRACT The theory of ion transport in the vicinity of a vibrating probe is developed. It is shown that the convection loops produced by the probe will not affect the electrical current density, assuming that the action of the probe does not affect the sources of the current in the biological system. However, the convection loops will significantly alter the ion concentration gradients in the unstirred layer near a tissue or cell surface. The concentration gradients within each convection loop will be reduced, while the concentration gradients between the loops and outside of the loops will be increased relative to the gradients existing without the probe. As a consequence, the electrical potential gradients can be changed relative to the potential gradients existing in the absence of the convection caused by the probe. If the mobility of the ion species carrying the electrical current is greater than the average ion mobility in the medium, then a decrease in ion concentration gradient will be accompanied by an increase in electrical potential gradient, while an increase in concentration gradient will be accompanied by a decrease or even a reversal of electrical potential gradient. Thus, the electrical potential gradient measured by the probe will depend on the concentration gradient in the vicinity of the probe, which will depend in turn on the spatial relation of the convection loops to the probe. An example of the effect of the convection loops on ion concentration and electrical potential is obtained from the theory via a numerical computer calculation. Experimental tests of this theory are discussed.

INTRODUCTION

The vibrating probe has been used to measure electrical currents produced by a number of biological systems (e.g., Jaffe and Nuccitelli, 1974, 1977; Lucas and Nuccitelli, 1980; Nuccitelli, 1983). However, the vibrating probe is thought to actually measure the electrical potential gradient. The current density is then calculated by assuming that it is related to the potential gradient by the electrical conductivity of the medium and Ohm's law. That the relationship between current and the electrical potential gradient is not governed by Ohm's law in many systems is well known. For example, in a wide range of solid state systems the existence of charge carrier concentration gradients leads to drastic departures from Ohm's law (e.g., Wannier, 1966; Ferrier, 1973). For ion transport in electrolytic solutions, the conducting system in which the vibrating probe is used, it has also been shown that there can be significant deviations from Ohm's law in the unstirred layer near tissue and cell surfaces (Ferrier and Lucas, 1979; Ferrier, 1980, 1981a, 1981b, 1983; Ferrier et al., 1985).

In this paper, we show that the current density is not affected by the convection loops produced by the vibrating probe. In addition, we develop a relationship between current density, the ion concentration gradients, and the electrical potential gradient. This relationship is not affected by the convection loops that are generated by the vibrating probe. As a consequence, it can be used to show the conditions under which Ohm's law does not hold, and

can also be used to investigate the effect of the convection loops on the electrical potential gradient.

THEORY

The results of Ferrier (1980, 1981b) for extracellular ion transport at steady state, and the investigations in this paper, are based in part on the mathematical property of a vector field that, if the divergence and curl of the vector are zero everywhere inside a closed boundary (i.e., if there are no sources, sinks, or closed loops), then there is a unique solution for the vector inside the boundary that depends only on the normal component of the vector on that boundary (e.g., Jackson, 1962). The flux vector of ion species k can be written as the sum of three terms, involving the concentration gradient, the electrical potential gradient, and the convection velocity

$$J_k = -D_k \text{grad} C_k - (D_k C_k z_k F / RT) \text{grad} V + v C_k, \quad (1)$$

where D_k is the diffusion coefficient for species k , C_k is the concentration, z_k is the valency, V is the electrical potential, v is the convection velocity, and F , R , and T have their usual thermodynamic meanings. At steady state, the divergence of J_k must equal zero (otherwise C_k will change with time). Furthermore, the curl of a gradient is identically zero. Thus, if the curl of v is zero inside the boundary, then the curl of J_k will be zero, and there must be a unique vector field, J_k , which depends only on the normal component of J_k on the boundary. This means that if the boundary is drawn to include the cell membranes, plus an

outer boundary at some distance from the cells, then the J_k for species not transported across the cell membranes will depend only on the normal component of J_k for those species at the outer boundary. If this boundary is sufficiently far from the cells so that ion transport there is not affected by membrane ion transport, then transport of these species anywhere within the boundary will be unaffected by membrane transport at steady state.

On the other hand, an ion species that is membrane-transported will have a nonzero normal component of flux at the membrane, and hence the vector field for its flux within the boundary will be clearly dependent on membrane transport. This implies that at steady state an electric current that results from membrane ion transport will be carried only by those ion species that are membrane-transported. Furthermore, it is clear that a species will contribute to carrying the current in the extracellular medium in proportion to its contribution to carrying the current across the membrane. However, these results hold only if the curl of the convection velocity equals zero within the boundary. This requirement can be reasonably approximated for many cases of extracellular ion transport, but not for the case of the vibrating probe, with its attendant convection loops.

The current density can be obtained from Eq. 1 as follows:

$$I = F \sum_i z_i J_i = -F \sum_i z_i D_i \text{grad} C_i - \sigma \text{grad} V, \quad (2)$$

where the summation is over all ion species, and σ ($= \sum_i D_i C_i (z_i F)^2 / RT$) is the electrical conductivity of the medium. The term in v drops out because of the requirement for electroneutrality ($\sum_i z_i C_i = 0$). The electroneutrality condition also implies that the divergence of the current density equals zero, and since the curl of a gradient equals zero we can see from Eq. 2 that the curl of the current density equals zero. Thus, the current density will have a unique solution that depends on the normal component of the current density on the boundary, but that does not depend on the convection velocity, even in the case that the convection velocity has a nonzero curl (forms closed loops).

If all but one of the ion species in the system have about the same diffusion coefficient, say D_o , while the other ion species is designated as species h (because we may specify that it has a higher diffusion coefficient), then we can rewrite Eq. 2

$$I = -(1 - B) F z_h D_h \text{grad} C_h - \sigma \text{grad} V, \quad (3)$$

where $B = D_o/D_h$, and we have used the electroneutrality condition in going from Eq. 2 to Eq. 3.

By combining Eqs. 1 and 3 we can obtain

$$J_h = -D_h [1 - (1 - B)(\sigma_h/\sigma)] \text{grad} C_h + [\sigma_h/(z_h F \sigma)] I + v C_h, \quad (4)$$

where $\sigma_h = D_h C_h (z_h F)^2 / RT$ is the conductivity attributable

to species h . Eq. 4 shows that, if species h carries the current at steady state, if the convection velocity is low, and if C_h is small relative to the total ionic concentration, then the flux of species h will be driven largely by its concentration gradient. Thus, under these conditions, $I = -F z_h D_h \text{grad} C_h$. Putting this relation into Eq. 3 gives $\text{grad} V = -BI/\sigma$. So, we see that in cases where high mobility ions such as protons or hydroxyls carry the current, there can be a significant deviation from Ohm's law, since B will be much less than 1 ($B = 0.17$ for protons, $B = 0.31$ for hydroxyl ions).

Eqs. 3 and 4 can also be used to show that the magnitude of the electrical potential gradient can be greater than would be expected on the basis of Ohm's law. In a situation with a flux of high mobility ions that is balanced by the flux of other ions so that the current density equals zero, and with a low convection velocity, Eq. 4 again shows that the flux of species h would be largely driven by its concentration gradient. Eq. 3 then shows that the electrical potential gradient would be nonzero. This would apply, for example, to a steady state situation where a proton flux through the cell membrane was balanced by an equal but opposite potassium flux. It would also apply to the situation where there had been a steady state current carried by protons that was suddenly switched off.

Eq. 3 can also be used to investigate the theoretical effect of the vibrating probe on the electrical potential gradient. We will consider the case with an initial steady state with low convection and with current carried by a high mobility ion species. When the vibrating probe is turned on, the resulting convection loops may significantly alter the concentration gradient for species h . Since the vector field for current density will not be affected by the convection loops, Eq. 3 can be used to calculate the change in the electrical potential gradient that results from the alteration of the concentration gradient of the high mobility species. However, to estimate the effect of the convection loops on this concentration gradient, we must resort to a numerical computer calculation.

METHODS

Numerical Calculations

Because of the convection loops produced by the vibrating probe (Jaffe and Nuccitelli, 1974), the vector field for the flux of an ion species will no longer be uniquely determined by the normal component of the flux on the boundary. To calculate the effect of the convection loops on the concentration gradient of species h , Eq. 4 was put into difference equation form and solved on a computer (DEC 10 system; University of Toronto, Toronto, Ontario, Canada). Only the terms involving the concentration gradient and convection velocity were used, since the term with current density will be extremely small for reasonable values of σ_h . We used rectangular coordinates, with spatial elements of $10 \mu\text{m}$ dimension, and time increments of 0.25 ms. The numerical calculation was very close to steady state after 4,000 iterations.

In an attempt to simulate the convection pattern produced by the vibrating probe, the computer calculation was performed with two convection loops, which represent the main features of the pattern observed by Jaffe and Nuccitelli (1974). The magnitude of the convection

velocity and the width of the convection stream were allowed to vary around the loops. The outer boundary of each loop was assumed to be circular. For simplicity, mirror-symmetry of the loops was also assumed. It was found that the numerical results were not sensitive to the velocity used in the central region of each loop. The results reported here are with zero velocity in the central regions. The rectangular components of the convection velocity for each element in the computational grid were calculated according to their position relative to the loops as shown in Fig. 1. The direction of the current and flux of the high mobility ion species is from top to bottom of the figure.

RESULTS

Without the convection loops, the isoconcentration lines shown in Fig. 2 would be equally spaced, straight, parallel, and horizontal. As Fig. 2 shows, these lines are significantly altered by the convection loops. The isoconcentration lines are pushed in the direction of the convection velocity. The concentration gradient is largely eliminated within each loop, but is increased between the two loops, and on the boundaries of the loops.

Using Eq. 3, the resulting electrical potential can be calculated. Fig. 3 shows the resulting equipotential lines for proton transport ($B = 0.17$). Without the convection loops, the equipotential lines would also be equally spaced, straight, parallel, and horizontal. With the convection loops, there is a greatly increased potential gradient within each loop. Near the bottom of the top loop, there is a very low potential valley. There is a very steep upward potential gradient (opposite in direction to the original potential gradient) spanning the interface between the convection loops, with a very high potential peak near the top of the bottom loop.

The concentration and electrical potential profiles along a path through the convection loops, $20 \mu\text{m}$ to the right of the centres of the loops, are shown in Fig. 4. These profiles should be close to those existing in the plane of oscillation of the vibrating probe. The profiles along parallel paths within $20 \mu\text{m}$ are similar to those shown. These results

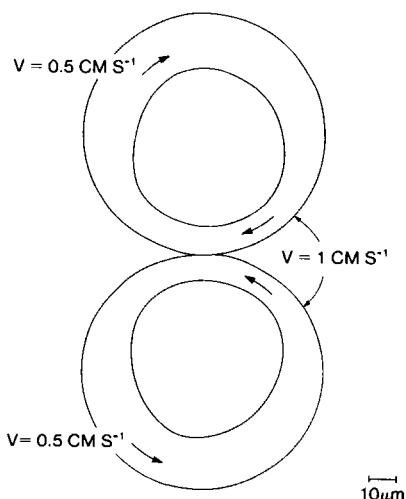


FIGURE 1 The convection loops used in the numerical calculation, showing the velocity and the width of the convection streams.

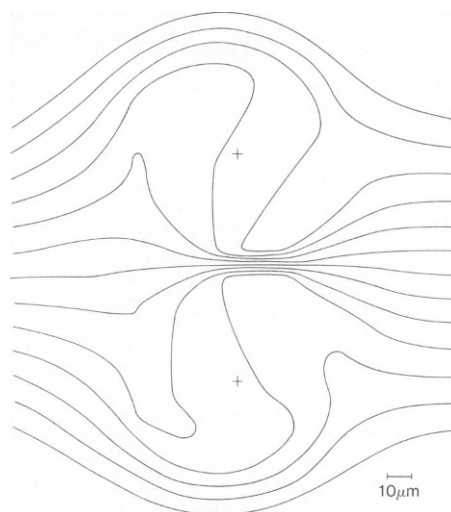


FIGURE 2 Isoconcentration lines in the presence of the convection loops. These lines are $1 \times 10^{-6} \text{ mol litre}^{-1}$ apart, for a current density of $10 \mu\text{A cm}^{-2}$, a flux for the high mobility ion species in the absence of convection of $100 \text{ pmol cm}^{-2} \text{ s}^{-1}$, and $D_h = 1.0 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$. However, the concentration gradient, current density, and ion flux can all be scaled by the same factor for a given D_h . For example, the lines would be $1 \times 10^{-7} \text{ mol litre}^{-1}$ apart for a current density of $1 \mu\text{A cm}^{-2}$ and a flux of $10 \text{ pmol cm}^{-2} \text{ s}^{-1}$. For C_h much less than the total ion concentration, the concentration gradient scales inversely with D_h for a given current density and ion flux, and the concentration gradient does not depend on C_h . The centers of the convection loops are indicated by crosses.

imply that, if the vibrating probe actually oscillates between the two convection loops, the measured electrical potential gradient would be smaller than, or even of opposite sign to, the potential gradient without convection. In this case, the current density would be significantly underestimated, or even taken as being of opposite direction to the actual current, if Ohm's law were used to calculate the current density. If the probe oscillates within

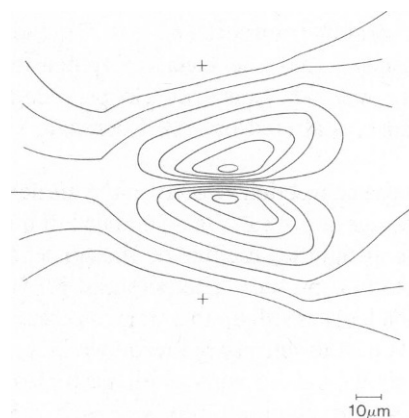


FIGURE 3 Equipotential lines in the presence of the convection loops. The lines are $20 \mu\text{V}$ apart, for a current density of $10 \mu\text{A cm}^{-2}$, an electrical conductivity of $3 \times 10^{-4} \text{ S cm}^{-1}$, and a B value of 0.17. The electrical potential gradient will scale with the same factor as the concentration gradient and the current density, for a given B value. For example, the lines would be $2 \mu\text{V}$ apart for a current density of $1 \mu\text{A cm}^{-2}$. The centers of the convection loops are indicated by crosses.

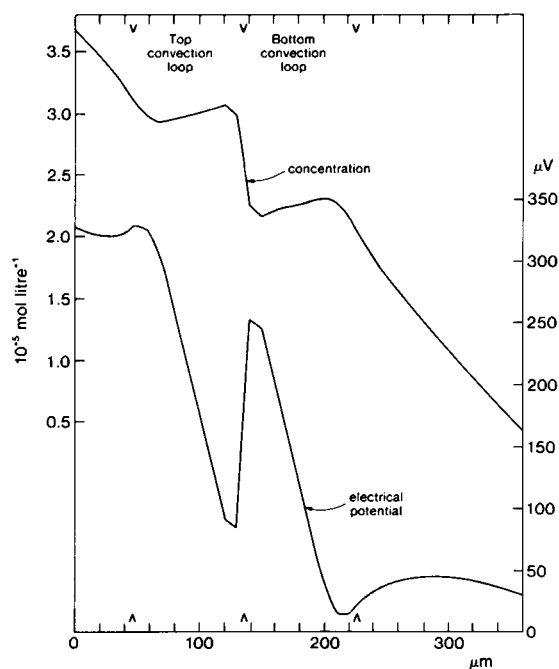


FIGURE 4 Concentration and electrical potential profiles along a path through the convection loops, 20 μm to the right of the centers of the loops, for a current density of $10 \mu\text{A cm}^{-2}$, and $B = 0.17$. The gradients of concentration and electrical potential will scale with the same factor as the current density.

one convection loop, as appears to be the case from the observations of Jaffe and Nuccitelli (1974), then the measured electrical potential gradient would be greater than the potential gradient without convection. The electrical potential gradient within each loop is almost 9 times greater than the gradient without convection. In this case, the current density calculated from Ohm's law would be greater than the actual current density. For hydroxyl transport ($B = 0.31$), although the electrical potential gradient outside of the convection loops is almost twice as great as for proton transport ($B = 0.17$), the computer calculation shows that the electrical potential gradient within and between the convection loops, along the paths discussed above, is altered by $<10\%$ compared to that for $B = 0.17$.

Reducing the convection velocity in the numerical calculation by a factor of 5 results in a smaller but qualitatively similar and still significant effect on the concentration and electrical potential profiles. The potential gradient within the convection loops is still up to 3 times as great as without convection. With the velocity reduced by a factor of 50, the calculation shows only a very small perturbation of the concentration and potential profiles, with the potential gradient changing by no more than 20% compared to the gradient without convection.

DISCUSSION

The vibrating probe has been used to evaluate the apparent current density produced by a large number of different

kinds of cells and tissues (Nuccitelli, 1983). In a situation where the main ion species carrying the current at steady state has a mobility that is not much different from the average in the electrolytic medium in which the measurement is carried out, the theory presented in this paper shows that the current density calculated from the measured electrical potential gradient and the conductivity of the medium using Ohm's law should be numerically correct. In this situation, B in Eq. 3 would equal 1, and Ohm's law would be numerically correct with or without the convection loops. However, Eq. 3 also shows that Ohm's law is not correct if B is not close to 1. This would be the case if a high mobility ion species is involved in carrying the current at steady state. The results of the numerical computer calculations based on Eqs. 3 and 4, indicate that the convection loops produced by the vibrating probe will have a profound effect on the ion concentration gradients, and hence on the electrical potential gradients, in the case that B is not close to 1. The strength of this effect will depend on the convection velocity. If the vibrating probe measures the electrical potential gradient within one of the convection loops, then the measured potential gradient will be increased relative to the gradient without convection. If the measured potential gradient is increased by a factor greater than the reciprocal of B , then the current density calculated using Ohm's law will be greater than the actual current density. Thus, the current density obtained with the vibrating probe depends in a complex manner on the convection velocity and on the identity of the ion species carrying the current. This latter aspect depends on the ion transport processes occurring in the cell membrane. A precise determination of the relationship between the electrical potential gradient measured by the vibrating probe and the current density will require more experimental work on the pattern and velocity of the convection loops produced by the probe, as well as studies on the nature of the underlying ion transport processes occurring in particular biological systems.

A number of experimental tests of the theory presented here are possible. In principle, the ion concentration gradients, the electrical potential gradient, and the current density can all be measured in ways that do not involve the vibrating probe. Thus, Eqs. 3 and 4 could be tested experimentally. Some preliminary experiments along these lines have been done using the giant algal cells of *Chara corallina* (Ferrier and Lucas, 1979). In these experiments, the extracellular pH gradient was measured using a small pH electrode, and the extracellular electrical potential gradient was measured using a microelectrode. These measurements showed that, near the surface of the cell, the hydroxyl chemical potential gradient between the "alkaline" and "acid" regions was ~ 35 times greater than the electrical potential gradient. This means that the flux of hydroxyl ions was carried almost entirely by diffusion. Current carried by diffusion of hydroxyl ions along the cell surface in the alkaline regions can be calculated to be

about twice that driven by the electrical potential gradient. These results agree quite well with the theory underlying the present work (Ferrier, 1980, 1981a, 1981b), and with the equations developed here.

Another kind of experimental test would involve altering the convection loops during a series of measurements with the vibrating probe. One way this can be done is by changing the viscosity of the medium, thereby changing the convection velocity. An experiment of this kind has been carried out by Lucas and Nuccitelli (1980) during the course of vibrating probe measurements on *Chara corallina*. They increased the viscosity of the medium by a factor of 1,500, which should decrease the velocity in the convection loops by a factor of ~ 500 (Jaffe and Nuccitelli, 1974). This had the effect of reducing the measured electrical potential gradient near the alkaline regions by a factor of ~ 4.5 . As indicated above, for hydroxyl transport the numerical calculation gives an electrical potential gradient within the convection loop that is ~ 4.5 times greater than the potential gradient without convection. This experimental result is, thus, in accord with the theory presented here. The measured electrical potential gradient near the acid regions was reduced by a factor of ~ 2 , indicating that the current there may be carried partly by HCO_3^- , for which B is close to 1, and partly by protons.

This work was supported by the Medical Research Council of Canada (Jack Ferrier) and the National Science Foundation (PCM 81-17721, William J. Lucas).

Received for publication 26 June 1985 and in final form 28 October 1985.

REFERENCES

- Ferrier, J. M. 1973. Junction theories of photoelectric effects at dye-semiconductor interfaces. Ph.D. thesis. Ohio State University, Columbus, OH. 88 pp.
- Ferrier, J. M. 1980. Extracellular ion transport. *J. Theor. Biol.* 85:739–743.
- Ferrier, J. M. 1981a. Time-dependent extracellular ion transport. *J. Theor. Biol.* 92:363–368.
- Ferrier, J. M. 1981b. On a boundary condition of extracellular ion transport. *J. Theor. Biol.* 93:495–496.
- Ferrier, J. 1983. A mechanism for the regulation of ligament width based on the resonance frequency of ion concentration waves. *J. Theor. Biol.* 102:477–486.
- Ferrier, J. M., and W. J. Lucas. 1979. Plasmalemma transport of OH^- in *Chara corallina*. II. Further analysis of the diffusion system associated with OH^- efflux. *J. Exp. Bot.* 30:705–718.
- Ferrier, J. M., J. Dainty, and S. M. Ross. 1985. Theory of negative capacitance in membrane impedance measurements. *J. Membr. Biol.* 85:245–249.
- Jackson, W. D. 1962. Classical Electrodynamics. John Wiley & Sons, Inc., New York. 12–17.
- Jaffe, L. F., and R. Nuccitelli. 1974. An ultrasensitive vibrating probe for measuring steady extracellular currents. *J. Cell Biol.* 63:614–628.
- Jaffe, L. F., and R. Nuccitelli. 1977. Electrical controls of development. *Annu. Rev. Biophys. Bioeng.* 6:445–476.
- Lucas, W. J., and R. Nuccitelli. 1980. HCO_3^- and OH^- transport across the plasmalemma of *Chara*. Spatial resolution obtained using extracellular vibrating probe. *Planta (Berl.)* 150:120–131.
- Nuccitelli, R. 1983. Transcellular ion currents: signals and effectors of cell polarity. *Mod. Cell Biol.* 2:451–481.
- Wannier, G. H. 1966. Statistical Physics. John Wiley & Sons, Inc., New York. 532 pp.