

UNSTIRRED LAYER EFFECTS ON CALCULATIONS OF THE POTENTIAL DIFFERENCE ACROSS AN ION EXCHANGE MEMBRANE

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ABSTRACT The potential difference between two solutions of the same 1:1 electrolyte bathing an ion exchange membrane has been calculated as the sum of the following components: (a) a Donnan potential at each membrane-solution interface, (b) a diffusion potential within the membrane phase, and (c) a diffusion potential in the unstirred layer on each side of the membrane. For a highly charged ion exchange membrane with at least one surface in contact with a dilute solution, calculated transmembrane potential differences are extremely sensitive to the assumed thickness of the unstirred layers. This sensitivity to unstirred layer thickness is primarily due to changes in the Donnan components of the potential difference.

By this approach, it was possible to fit membrane potential data from Gunn and Curran (1971, *Biophys. J.* 11:559) for a range of bathing solution concentrations from 0.0016 to 4.0 M. If no effort was made to account for the modification of the Donnan potentials by the presence of unstirred layers, the data appeared incompatible with an electrodiffusion equation description.

Suggestions for a more stringent experimental test and a brief discussion of possible implications for electrical measurements on fresh-water giant algal cells are presented.

Gunn and Curran (1971) measured diffusion potentials across an ion exchange membrane, bathed in solutions of sodium chloride, for a large range of concentration differences, and compared their observations with theoretical calculations of the transmembrane potential from two different approaches. An expression derived from irreversible thermodynamics closely approximated the observed potential over the whole concentration range (the maximum deviation was 4.2 mV). A modified Goldman-Hodgkin-Katz equation gave overestimates of the potential differences by amounts that increased to 32.4 mV for the greatest concentration difference. I shall show here that a theoretical approach ascribing the transmembrane potential to Donnan and electrodiffusion components can, however, provide a reasonable fit to the data over the whole concentration range. Although my calculation differs from the Goldman-Hodgkin-Katz approach used by Gunn and Curran in a number of details, which I shall describe later in this paper, by far the most important change lies in my treatment of the effects of unstirred layers near the membrane surface. The importance of unstirred layers in membrane studies is well recognized (see, for example,

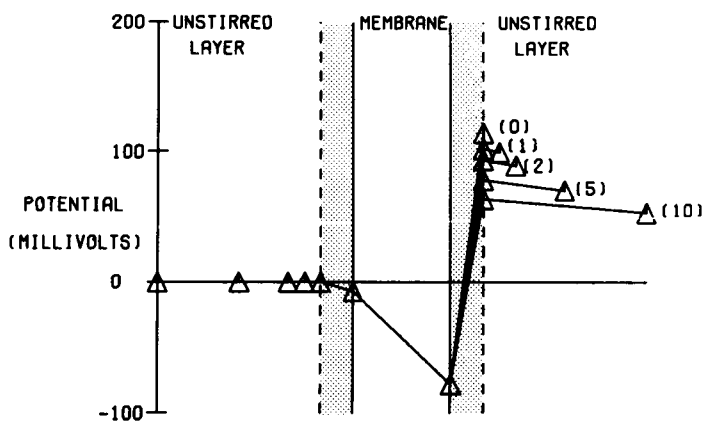


FIGURE 1 Electrical potential profile in the vicinity of a hypothetical ion exchange membrane with 3.0 M of negative fixed charge per liter of membrane water. Internal permeabilities assumed were: cation, $7.619 \times 10^{-6} \text{ cm s}^{-1}$; anion, $0.218 \times 10^{-3} \text{ cm s}^{-1}$. Bathing solutions contained 5.0 M/liter on the left, and 0.0015 M/liter on the right, of a 1:1 univalent electrolyte. The potential profiles illustrate the effect of assuming unstirred layers of 0, 1, 2, 5, and 10 μm thickness on each side of the membrane. The potential profiles superpose from left to right until they enter the Donnan layer on the low concentration (right) side of the membrane. The thicknesses of the membrane and the Donnan layers are not drawn on the same scale as the unstirred layer thickness. The stippled area indicates the Donnan layer.

Barry and Hope, 1969). In this case, however, the distinctive feature is that the influence of the unstirred layers on the Donnan contribution to the transmembrane potential plays a key role.

To illustrate this point, the results of a model calculation are shown in Fig. 1. The fixed charge on the membrane and the values for ionic permeabilities in the membrane are taken from the range of values experimentally determined by Gunn and Curran. For the five cases shown in the figure, the only condition changing is the thickness of the unstirred layers on either side of the membrane. As unstirred layer thickness changes from 0 to 20 μm , a change of about 60 mV in potential difference across the membrane is predicted, largely due to the change in the Donnan potential at the low concentration side of the membrane. In this example, but not in the following computations on the complete experimental data, constant field was assumed in the membrane to simplify the calculations.

Implicit in the Goldman-Hodgkin-Katz expression used by Gunn and Curran is the assumption that the total transmembrane potential difference is made up of three components: a Donnan potential at each of the membrane-solution interfaces, and a diffusion potential across the membrane phase. In the following calculations I have considered a further component to be added to these three, a diffusion potential across an unstirred layer close to the membrane. This contribution is small, but does amount to a few millivolts for the highest concentration gradients, and so was included. Only the diffusion potential across the unstirred layer on the low concentration side was included in the calculations. The contribution from the unstirred layer on the higher

concentration side is negligible since the concentration ratio across this layer is close to unity.

In calculating the potential generated across the unstirred layer, the Nernst equation for the diffusion potential, based on the electroneutrality assumption, was applied. The use of this equation for diffusion across a "thick" layer may be justified from the arguments of Patlak (1956), MacGillivray (1968), and Agin (1969), and from numerical calculations (French, 1973).

$V_{\text{diffusion}} = V_2 - V_1 = (RT/F)(w - u)/(w + u) \ln (C_2/C_1)$, where w and u are the anion and cation mobilities, respectively, and C_1 and C_2 are the concentrations at each side of the unstirred layer. Barry and Hope (1969) used a similar estimate of the unstirred layer diffusion potential in their discussion of unstirred layer effects.

The intramembrane diffusion potential was evaluated by the numerical solution of the complete Nernst-Planck-Poisson system of equations. No simplifying assumption of constant field or electroneutrality within the membrane was made. Descriptions of the method are given in French's work (1973, 1974). The numerical solutions differed by less than a millivolt from constant field equation predictions with the same permeability values. Thus it was not the application of the constant field assumption that was responsible for the discrepancy between the values observed and calculated by Gunn and Curran.

The Donnan potential between the membrane and solution phases is dependent on the concentration of diffusible ions just inside the membrane and in the aqueous phase immediately outside. If there were a layer of unstirred solution adjacent to the membrane and a steady net flux of salt across the membrane, the concentrations of salt in the aqueous phase at the interface would not be the same as the bulk solution concentrations. On the high concentration side of the membrane the concentrations would be lowered and on the low concentration side concentrations would be raised above the bulk solution values. To a first approximation, for a small uniform steady flux across the membrane and unstirred layers, the changes in concentration in the unstirred layers—lowering on the high concentration side and raising on the low concentration side—should be equal in magnitude. The Donnan potential, π , is determined by the ratio of concentrations of diffusible ions across the interface.

$\pi = (RT/F) \ln (1/r)$, where π is the potential just inside the membrane with respect to that in the solution just outside, and r is the Donnan ratio as calculated by Gunn and Curran.

Thus, the major effect on the transmembrane potential of a given absolute change in unstirred layer concentrations will be because of the modification of the Donnan potential at the low concentration side of the membrane, since there the change in concentration ratio is greater. A similar argument shows that the unstirred layer diffusion potential, also determined by a concentration ratio, is much larger on the low concentration side of the membrane, though still only a minor contribution to the total transmembrane potential difference.

There is empirical as well as logical justification for neglecting the unstirred layer effects at the high concentration side. Gunn and Curran reported that only stirring

TABLE I
INTRAMEMBRANE DIFFUSION COEFFICIENTS USED TO
CALCULATE INTRAMEMBRANE DIFFUSION POTENTIALS The diffusion coefficients were estimated from the intramembrane permeabilities presented by Gunn and Curran (Table III in their paper), assuming a diffusion path length² of 0.056 cm (see text for further details).

Concentration of bathing solution (high concentration side)*	Diffusion coefficients	
	$10^7 \times D_{Na}$	$10^5 \times D_{Cl}$
<i>M/liter</i>	<i>cm² s⁻¹</i>	
0.014	3.36	2.12
0.04	3.36	1.60
0.01	3.39	1.15
0.5	3.40	0.53
1.0	3.7	0.31
4.0	5.7	0.105

*Lower concentration 0.0016 in all cases.

rate changes on the low concentration side of the membrane had observable effects on the potential readings.

One assumption implicit in the calculations that I present is that, for a given pair of solutions, the diffusion coefficients of the ions are constant across the membrane (see Table I for values used). This assumption cannot be justified in a strict sense, as experimentally measured permeabilities show a marked concentration dependence. However, as the chosen permeabilities strongly affect only the intramembrane diffusion potential, and as this is less than 10% of the total transmembrane potential even for the largest concentration differences, the error involved in considering the intramembrane diffusion coefficients to be constant at suitably chosen values is unlikely to be serious. The measured permeabilities were plotted against the intramembrane concentrations, and for each calculation the permeabilities at the arithmetic mean of the boundary concentrations within the membrane were used. Gunn and Curran also assumed that one could define fixed ionic permeabilities for each pair of external solutions, the assumption being implicit in their Goldman-Hodgkin-Katz expression for total transmembrane potential difference.

My complete calculation procedure was as follows:

(a) Assuming the bulk solution concentrations held right up to the membrane solution interface, the transmembrane potential was calculated as a sum of two Donnan potentials and a diffusion potential within the membrane. The program used to perform the calculations evaluated the steady state ion fluxes as part of the solution.

(b) From the fluxes, the concentration difference, ΔC , across an unstirred layer of arbitrary thickness was estimated. $\Delta C = (Jd/D_{NaCl})$, where J is the flux of salt, d is the thickness of the layer, and D_{NaCl} , the diffusion coefficient, was taken to be $1.55 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. A Nernst diffusion potential for the unstirred layer and the corrected concentrations in the unstirred layers at the interfaces were then estimated.

(c) The numerical routine was then reapplied with the new concentrations to obtain an improved estimate of the Donnan and intramembrane diffusion components of the potential.

In principle, one could obtain different estimates of the fluxes at this step, but in all examples, the calculations from steps (a) and (c) agreed to at least four significant figures and the procedure was stopped. Further iterations could be made if needed, until the flux estimates ceased changing.

In Fig. 2 A I have plotted my calculation of the total potential difference against concentration¹ for several different unstirred layer thicknesses. I present these results together with the Goldman-Hodgkin-Katz calculations of Gunn and Curran, and their published experimental data. Fig. 2 B graphically shows the contributions of each component of the transmembrane potential difference for the case most closely approximating the data. The difference between the Gunn and Curran calculation and my own for a zero thickness unstirred layer is attributable largely to the different method in which the experimentally measured membrane permeabilities were used in the calculations, as explained earlier. This cannot explain the discrepancy between the data and the calculated values from the Goldman-Hodgkin-Katz approach.

Most striking was the predicted effect of varying the unstirred layer thickness. By assuming an unstirred layer of 5 μm on the low concentration side, potential predictions that were within less than 1% of the tabulated observations for the highest concentrations were obtained. The figure for the unstirred layer is considerably smaller than the estimate of 51 μm on each side of the membrane given by Gunn and Curran in their paper, but within the range of values cited by Bircumshaw and Riddiford (1952) and Tetenbaum and Gregor (1954). However, the 5- μm value should not be expected to correspond to the steady-state thickness of the unstirred layer, since the potential values, although steady for several seconds after first observation, were the earliest measurable values. For the highest concentration differences, the potentials drifted down and apparently reached steady-state values after periods of 1 min or so, though little time dependence was seen for the lower concentration differences. Gunn and Curran deduced that the transient was caused by film control due to unstirred layers adjacent to the membrane surface, but in their treatment, assumption of unstirred layer thicknesses from 10 to 58 μm did not significantly change the prediction from their Goldman-Hodgkin-Katz expression.

¹Gunn and Curran referred to identical numerical values as concentration (Table IV) and activity (Fig. 2). For the calculation of the small diffusion components of the potential, the error introduced by this procedure is not significant. Some effect on the Donnan components would be expected, though for the intermediate concentrations, effects at the two membrane-solution interfaces would be oppositely directed and tend to cancel. As a worst case, one can consider the highest concentration difference. The major change in activity coefficient would be expected at the low concentration interface where the concentrations change from 0.0016 M to about 3 M. From Robinson and Stokes, 1959, Fig. 8.12, one sees that the activity coefficient for sodium chloride changes peculiarly little with changes in concentration. Assuming a possible change in Donnan ratio by a factor of 0.7, the upper limit to the error introduced would be about 9 mV—still insufficient to account for the deviation between the Goldman-Hodgkin-Katz calculations and the data. Activity coefficients were assumed invariant in the calculations presented here, owing to their small likely influence, and to the difficulty of defining them in both membrane and solution phases.

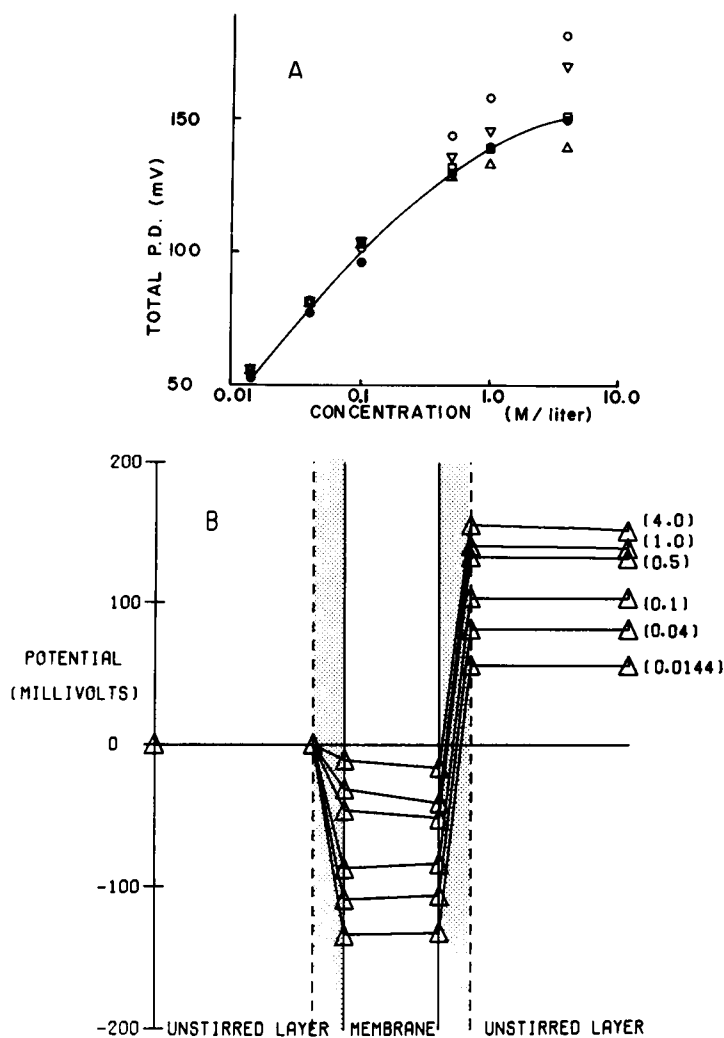


FIGURE 2 (A) Plot of measured transmembrane potential difference (P.D.) from Gunn and Curran, 1971 (●), the Goldman-Hodgkin-Katz equation predictions of Gunn and Curran (○), and the calculated values from the present study for unstirred layer thicknesses of 0 (▽), 5 (◻), and 10 (△) μm on each side of the membrane. The higher concentration is shown on the abscissa; the lower concentration was fixed at 0.0016 M/liter. The smooth curve was drawn by eye through the experimental data points. (B) Graphical depiction of the components of the total transmembrane potential from the present calculations for the case of a 5-μm unstirred layer. Curves are identified by a number in parentheses, indicating the concentration of the more concentrated bathing solution in moles per liter. The right hand solution was 0.0016 M/liter in all cases. Thicknesses of the Donnan layer and the membrane are not represented on the same scale as the unstirred layers. The stippled area indicates the Donnan layer.

The treatment described here predicts a sensitive dependence of the steady-state transmembrane potential on the thickness of the unstirred layer due to its influence on concentrations immediately adjacent to the membrane. ("Immediately adjacent" in the context means distances on the order of the thickness of the ionic atmosphere of Debye-Hückel theory—in the neighborhood of 10^{-7} – 10^{-6} cm, much less than the unstirred layer thickness of 10^{-4} – 10^{-3} cm). Fitting potential data taken before the steady state was reached would be expected to give an erroneously low value for the unstirred layer thickness. Incomplete buildup of concentration in the unstirred layer on the side of the low concentration bathing solution could result in a larger Donnan component and larger total potential difference than for the steady state.

Unfortunately, a complete set of the long-time steady potentials is not available, but an indication of the trend is given by the time-course of the potential change for the highest concentration difference (Gunn and Curran, Fig. 1). It was in this case that the transient was most dramatic (Curran, personal communication).² The potential appears to be approaching a steady-state value of approximately 120 mV. Under the model that I have assumed, this would require an unstirred layer thickness of about 20 μ m.

As already mentioned, the potential transient was attributed by Gunn and Curran to film control in the unstirred layer. Although the accumulation layer does seem to be a prime factor in determining the magnitude of the membrane potential difference, the half-time for diffusion equilibration in a 20- μ m layer would be on the order of 0.25 s. For this reason it seems more likely that the rate-limiting process in the approach to the steady state must be diffusion of material across the membrane. In principle, this should enable one to estimate the approximate values for the intramembrane diffusion coefficients. In practise, the following uncertainties mean that such an estimate would be highly unreliable: (a) partial equilibration of the unstirred layers would occur during the rinsing procedure used by Gunn and Curran for the crucial high concentration experiments; (b) the diffusion path length in the membrane is not precisely known; and (c) the cross-sectional area of the diffusion path in the membrane is not precisely known, though, given that the membrane water content was only 35–40%, it must have been significantly less than the exposed membrane area.

Ideally, one would like to test the approach I have described against a full set of steady-state data. A much more critical examination of the calculations would be possible if the following quantities were determined on the same membrane: (a) fixed charge concentration, (b) internal potential as a function of the bathing solution concentration (see Nagai and Kishimoto, 1964), and (c) net flux and transmembrane voltage, preferably measured simultaneously. There is no a priori reason to suppose that the Donnan potential expression, derived for equilibrium conditions, should accurately describe the interface potentials when there is a net flux across the interface.

²I am grateful to the late Dr. Curran for providing a thickness measurement (0.056 cm) of the sheet from which the experimental membrane was cut, and for his willing discussion of the experiments in a telephone conversation.

While it may not be possible to test this assumption directly, it seems that sufficient experimental information could be brought to bear to make a more rigorous test than is possible at present.

Nonetheless, the analysis I have presented makes the interesting point that the unstirred layer may have a significant effect on measurements of transmembrane potential difference even though its resistance to ion flow is substantially less than that of the membrane itself. When this is taken into account, the combined ionic diffusion-Donnan equilibrium approach does indeed seem to provide a good description of the transmembrane voltage. The effects of such an unstirred layer would be highly significant when, as in this case, one of the solutions bathing a highly charged membrane contains a very low concentration of ions.

Similar effects could significantly influence electrical measurements made on fresh-water organisms under physiological conditions. The cell wall of fresh-water giant algal cells is a functional ion exchanger. In *Chara*, Dainty and Hope (1959) estimated the fixed charge concentration to be about 0.6 eq/liter of membrane water. Penetration of the cell wall by microelectrodes has revealed, in *Nitella*, an electrical potential inside the wall matrix of about -100 mV with respect to an external solution containing 10^{-4} M KCl (Nagai and Kishimoto, 1964). The potential difference decreased in magnitude by about 25 mV/10-fold increase in the bath [KCl].

Hope and Walker (1975, Chap. 7) point out that, if the cell wall were closely apposed to the plasmalemma, changes in the Donnan potential of the cell wall would be reflected in the cytoplasmic potential measured with respect to the external solution. Such a close apposition of cell wall and plasmalemma is consistent with the data of Nagai and Kishimoto mentioned above. Since the cell wall, as an ion exchanger, does not distinguish measurably between Na and K, one can eliminate changes in the Donnan potential by using bathing solutions in which the sum of [Na] and [K] is constant (Spanswick et al., 1967; Hope and Walker, 1961). However, if the total external [Na] + [K] were changed, the functional dependence of the cytoplasmic potential on the external concentrations would be in part determined by the actual thickness of the unstirred layer at the cell wall-external solution interface.

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