Diffusion Potentials Across an Artificial Membrane

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One of the fundamental concepts taught in introductory cell biology courses is the structure and function of the plasma membrane. However, the relationship between the passive properties of the membrane and membrane potential is commonly misunderstood. In many cases, this misunderstanding has detrimental effects on students' performance in senior courses in cell biology and neurobiology. This exercise attempts to provide students with an understanding of ionic movements and membrane potential using a selectively permeable artificial membrane placed in an Ussing chamber. Application of various concentrations of NaCl solutions to either side of the artificial membrane allows the potential difference across the membrane to be measured with the aid of AgCl-AgCl electrodes and a multimeter. For introductory courses, this experiment clearly demonstrates the movement of ions across a membrane. In more senior courses, the experimental potential difference values can be compared to membrane potential values calculated using the Nernst equation, thereby permitting students to gain an understanding of the effect of ionic concentration gradients on membrane potential. An added advantage of this procedure is that students are introduced to the concept of dilution and use of a micropipettor as they are required to dilute a stock solution to prepare the various concentrations of solutions needed in the experiment. A second advantage of this exercise is that the need for live tissue is eliminated by using an artificial membrane, making this an inexpensive, ethical approach to studying this aspect of biology.

This experiment on diffusion potentials was adapted from Phywe.

Student Outline

Introduction

All cells studied to date bear a potential difference across their membranes. There are a number of common synonyms for this term: "diffusion potential", "membrane potential", and "electrical potential"; they are used more-or-less interchangeably in this lab. Usually the cytoplasm is at a negative potential relative to the outside of the cell, although the magnitude of this potential varies from only a few millivolts (mV) to over 100 mV depending on numerous factors. In the "classical" case (vertebrate nerve and skeletal muscle), the resting membrane potential arises primarily from a K^+ - diffusion potential. The concentration gradient of K^+ across the membrane is such that K^+ tends to diffuse out of the cell without a concomitant inward flow of positive charge. Thus, there develops a slight excess of positive charge outside the cell which, at equilibrium, opposes the further outward diffusion of K^+ and accelerates the inward diffusion of Na^+ and Ca^{2+}. In a living cell, this system is in a dynamic equilibrium due to the sodium/potassium exchange pump that constantly restores the Na^+ and K^+ gradients that maintain the resting potential. These concepts are reviewed in Randall et al. (1997), Chapters 4 and 5.

In this laboratory, you will measure diffusion potentials across an artificial membrane, using an "Ussing chamber" named after the eminent physiologist, Hans Ussing. The measured potentials will be compared to theoretical values calculated from the Nernst Equation.

Resting membrane potentials often arise as "diffusion potentials" of certain ions across the membrane (the classical case is the K^+ - diffusion potential). The size and polarity of the diffusion potential depends on the electrolyte tested, on the **concentration difference across the membrane**, and on the **nature of the membrane's permeability**. If the membrane is permeable to all ions (as is the case with cellophane), the diffusion potential is initially maximum, but progressively declines as the ions diffuse across the membrane and the concentration gradient is reduced. However, if a membrane is cation- or anion-impermeable, an equalization of concentration is inhibited and a permanent potential difference develops.

The diffusion potential (E) can be calculated according to the following formula:

$$E\ (mV) = \frac{u - v}{u + v} \cdot 0.2 \cdot T \cdot \log (a_1 / a_2) \qquad (1)$$

where

- T = absolute temperature (0°K = -273°C)
- u = diffusion rate of the cation (see Table 1)
- v = diffusion rate of the anion (see Table 1)
- a_1 = activity of Chamber 1
- a_2 = activity of Chamber 2

Note: This equation assumes that the cations and anions are monovalent.

The "activity" is equal to the concentration (c) multiplied by the activity coefficient (f), (a = c *x* f; see Table 2). The measurements involve activity and not concentration, since the ions in the more concentrated solution mutually inhibit each other and the solution thus behaves as though there were fewer ions in solution than is actually the case. In very dilute solutions, where the ions no longer hinder each other, the activity coefficient approximates 1.0 (see Table 2).

Table 1. Diffusion rates.

Ion	μ (cm/s)	Ion	v (cm/s)
H+	318.0	OH-	174.0
Na^+	43.5	Cl-	65.4
K^+	64.7		

Table 2. Activity coefficient (f).

	0.00l N	0.01 N	0.1 N	1 N
HCl	0.965	0.904	0.796	---
NaCl	0.966	0.906	0.786	0.664
KCl	0.965	0.902	0.771	0.611

Equation (1) given above can also be used to calculate the potential across the **cation-permeable membrane**. However, since the anion diffusion rate (v) is zero, the expression (u - v) / (u + v) is equal to 1.0. The formula thus simplifies to:

$$E\ (mV) = 0.2 \cdot T \cdot \log (a_1 / a_2) \qquad \text{(Nernst Equation)} \quad (2)$$

Note: The cation-permeable membrane differs in two essential respects from the membrane of a non-excited nerve cell: 1) the artificial membrane is equally permeable to K^+ and to Na^+, and 2) the artificial membrane does not possess an (energy-consuming) ion pump.

Materials

Ussing chamber (includes O-ring and butyl rubber; 1 per group)
recording electrodes (AgCl-AgCl; 2 per group)
multimeter (1 per group)
thermometer (1 per group)
100 ml beaker (for storing electrodes; 1 per group)
metal stand with clamp (2 per group)
clamps for latex tubing of Ussing chamber (2 per group)
cation-permeable membrane (1 per group)
air lines for aeration
distilled water
funnel (2 per group)
1000 ml beakers (5 per group)
micropipettors (1 set per group; 0.5-5 ml, 200-1000 µl, 50-200 µl, 5-50 µl)
NaCl (1.0 N; approximately 700 ml per group)
wash bucket for waste water

Procedure for Measuring Potential Differences

Using the 1.0 N solution of NaCl, make each of the following solutions:

- 0.1 N NaCl (200 ml)
- 0.01 N NaCl (200 ml)
- 0.001 N NaCl (400 ml)
- 0.002 N NaCl (200 ml; activity coefficient = 0.94)

Store the two electrodes in a beaker containing 0.1 N NaCl. Since they both sit in the same solution, in principle, there should be no potential difference across these electrodes. In practice, however, one can record a small difference and this is called the **asymmetry potential**. Asymmetry potentials are due to differences between the electrodes themselves, and are not due to differences in potential within the NaCl solution. (Remember, the whole solution in any one vessel is at the same potential. There can be no net difference in charge from one side of the vessel to the other). With the multimeter set at 300 mV, determine the asymmetry potential in your system, and record this value in Table 3. Remember to correct your average diffusion potential values for the asymmetry potential.

After mounting the membrane in the Ussing chamber fill each chamber **simultaneously** with the desired solution. It is best to fill the two chambers **simultaneously** so that there is a minimal hydrostatic pressure difference across the membrane. Fill both chambers to the same level, and be sure to cover the membrane completely. Insert an air line into each chamber and bubble air vigorously through each solution. The purpose of doing this is to ensure that the solutions remain homogeneous, and to minimize "unstirred layers" of solution at each face of the membrane. The membrane itself is inert, and does not require oxygenation.

After allowing about 10-15 seconds for equilibration, read the potential difference on the multimeter. Take another reading 15 and then 30 seconds later. Turn the multimeter off between readings. The average of these 3 readings, corrected for the asymmetry potential, is the

value used for subsequent comparisons to the theoretical potential difference values obtained using the Nernst equation. The three readings should be quite close to each other. If not, there may be some "fault" in your system. (Check for leaks from the Ussing chamber).

Turn off the multimeter, rinse the electrodes in distilled water, and return them to the vessel of 0.1 N NaCl. Empty the chambers via the draining ports underneath (latex tubing held closed with metal clamps). Replace clamps on the latex tubing and then **add a SMALL volume (10 - 20 ml) of the solution to which the chambers will subsequently be exposed**. Swirl the Ussing chamber gently, making sure to completely rinse the insides of the chambers and the membrane with the solution. Drain the “rinse solution” from the chambers. Fill the chambers with the next pair of solutions to be tested, and record the potential difference as before. There are 8 pairs of solutions to be tested (see Table 3).

Treatment of Results

For your report, compare your measured values of E with those calculated from the Nernst equation. Comment on any significant differences. Plot your measured potential differences as a function of concentration gradient. On the same graph, include the curve of the calculated Nernst potential differences as a function of concentration gradient.

Does the sign of the potential difference ("+" or "-") change between tests 1-4 and 5-8? If so, give an explanation for why you think this happens.

Is the potential difference influenced by the absolute concentration of salt or only by the concentration gradient (i.e., compare tests 1 with 8, 2 with 7, and 3 with 6)?

Table 3. Solutions used in tests 1 through 8.

Test Number	1	2	3	4	5	6	7	8
Asymmetry potential _____ mV								
	Concentration of NaCl (Normality)							
Chamber 1	0.001	0.001	0.001	0.002	1.0	1.0	1.0	1.0
Chamber 2	0.001	0.01	0.1	1.0	0.002	0.01	0.1	1.0
Concentration difference	1 X	10 X	100 X	500 X	500 X	100 X	10 X	1 X
Diffusion potential reading a:								
b:								
c:								
Corrected average reading *								
Calculated diffusion potential **								

* Average reading minus asymmetry potential.
** Calculated from the Nernst equation.

Notes for the Instructor

1. When mounting the artificial membrane in the Ussing chamber, tighten opposite wing nuts in sequential order (similar to tightening the lug nuts on a tire). It is important that the Ussing chamber is mounted tightly as spaces between the 2 chambers of the apparatus may cause air to bubble around the membrane, thus reducing the surface area available for diffusion, resulting in incorrect values.
2. The volume of each chamber, as per the specifications provided, is approximately 70 ml. This volume will completely cover the membrane.
3. The butyl rubber (1/16" thick) is placed between the two sides of the Ussing chamber, followed by the cation permeable membrane and O-ring. The butyl rubber can be purchased at any hardware store. The piece of rubber should be cut with 4 corner holes (for the Redi-rods) and a center hole, identical to the holes cut in the square acrylic B in Figure 2.
4. It is important that the tests be performed in sequential order from test 1 to test 8.
5. The highest concentration difference these membranes can handle is 500 X. A difference higher than that results in unstable potential difference recordings.
6. Theoretical diffusion potential values calculated from the Nernst equation (at 24°C) are as follows:

Test 1	0 mV
Test 2	-57.57 mV
Test 3	-113.48 mV
Test 4	-151.35 mV
Test 5	151.35 mV
Test 6	110.78 mV
Test 7	55.05 mV
Test 8	0 mV

7. Membranes are purchased from:

The Electrosynthesis Company Incorporated.
72 Ward Road
Lancaster, NY
14086-9779 USA
Tel: (716) 684-0513
Fax: (716) 684-0511

The membrane is the Nafion 450. The cost is $175 US per square foot. This amount results in 36 square membranes (2" X 2" or 55 mm^2). The membranes are completely reusable. Before use, the membranes should be soaked in distilled water for 30 minutes. After use, soak the membranes in 6 N nitric acid for 2 hours, rinse with double distilled water, let dry, and store until required.

Figures 1 and 2 provide details of the construction and assembly of the Ussing chamber.

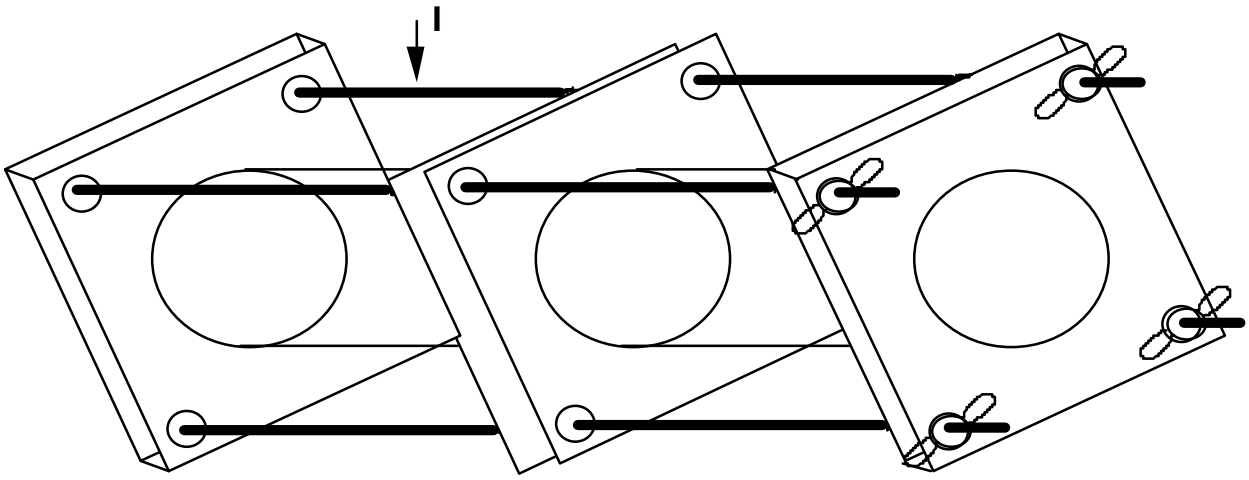

FIGURE 1

The apparatus consists of 2 identical chambers. Each chamber is made up of two ends, A and B in Figure 2 and a center tube, C in Figure 2.

A: 1/2" X 3 3/8" X 3 3/8" square acrylic. Drill 4 - 1/4" holes on a 1 1/2" radius and in line with the corners (H, Figure 2).

B: 3/8" X 3 3/8" X 3 3/8" square acrylic with a 1 1/8" hole drilled in the center. Drill 4 holes in the same location as in "**A**".

C: 2" diameter - 1/4" wall X 1 3/4" long cast acrylic tubing. 1/2" hole drilled at the mid-point on the tubing (D, Figure 2). Two 1/4" holes drilled 1/2" center to center on opposite ends of "**D**" along the axis of tubing (E, Figure 2). Drill and tap a hole opposite "**D**" for 1/8" NPT (F, Figure 2).

Note: Do not use extruded acrylic tubing; wall variance occurs and it easily cracks.

D: Hole for filling the chamber and for electrodes.

E: Holes for aeration tubes.

F: 3/16" tubing to 1/8" NPT brass male connector. Threads seal with Teflon tape.

G: Latex tubing (4" to 7") connected to brass male hose connectors for draining the solutions from each chamber.

H: 4 - 1/4" holes for 1/4" NC brass Redi-Rod.

I: 4 - 6" long 1/4" brass Redi-Rod.

Assembly:

Permanently attach Redi-Rod with hex nuts to "**A**" of one chamber (Figure 1). Slide second chamber over Redi-Rod with cation permeable membrane between "**B**" of each chamber and hold the membrane in place with 4 wing nuts.

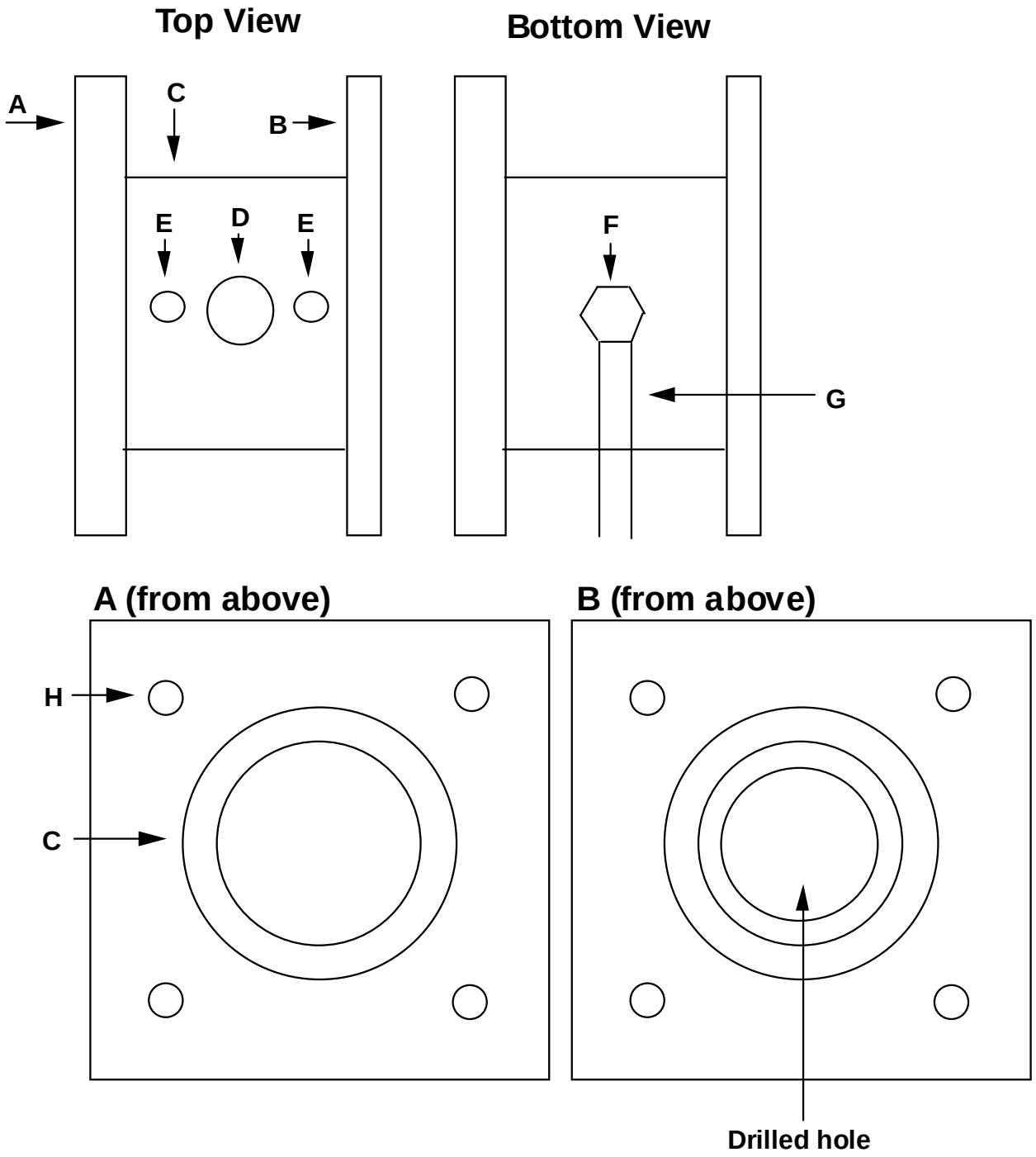

FIGURE 2

References

Phywe A. G. *Laboratory Experiments in Physiology.* D-3400 Göttingen, P.O. Box 3044. West Germany.

Randall, D., W. Burggren, and K. French. 1997. *Eckert Animal Physiology: Mechanisms and Adaptations.* 4th Edition. W.H. Freeman. New York.