

MEMBRANE POTENTIAL MEASUREMENT AT NERNST EQUILIBRIUM

The aim of the practical:

The aim of the practical is to present the electrodiffusion and Nernst equilibrium in the system consisting of ion solutions and ion-selective membrane. Students perform the measurements of the voltage that exist on cation-selective membrane at equilibrium.

The experimental set-up:

Voltmeter, two chambers separated by cation-selective membrane, two electrodes (Ag/AgCl), NaCl solutions: 0.01 mol/dm³ and 0.001 mol/dm³.

General description

When two salt solutions of different concentrations are separated by a membrane permeable to only one type of ions (ion-selective), the diffusion flux of these ions will flow through the membrane (from the chamber of higher ion concentration to the chamber of lower ion concentration). This ion flux will generate a voltage across the membrane – membrane voltage. The electric field which is the consequence of membrane voltage will generate the ion flux in the direction opposite to the diffusion flux. Membrane voltage will increase (due to the diffusion) until the electric flux will balance the diffusion. At this certain membrane voltage (Nernst potential) these two fluxes will balance and effective (diffusion and electric) ion flux through the membrane will be zero. The equilibrium that is reached in this situation is called a Nernst equilibrium. Using the formulas for the individual fluxes (diffusion and electric) the following equation can be obtained for the Nernst potential:

$$\Delta V = \frac{RT}{zF} \ln \frac{c_1}{c_2} \quad [1]$$

where c_1, c_2 – equilibrium sodium ions concentrations in chambers #1 and #2, respectively, R – gas constant 8.31 J/mol·K, T – absolute temperature, z – ion valence, F – Faraday constant (96 500 C). By putting into the eqn. [1] $z = 1$, $T = 293$ K (20 °C), and changing natural logarithms to decimal the following formula is obtained:

$$\Delta V = 58 \log \frac{c_1}{c_2} \quad [2]$$

In the measurements we assume that due to the relatively small area of membrane, small amounts of ions passing the membrane ensure equilibrium of the system. Therefore the initial ion concentrations (as applied to the chambers) are considered as equilibrium concentrations. In spite of small area of the membrane, however, it takes a few minutes until the system reaches the equilibrium state.

Course of the measurements

1. Using the graduated cylinder measure off 85 ml of 0,001 molar NaCl solution and fill with this solution the right chamber (P). Next, fill the left chamber (L) with 85 ml of 0,01 molar NaCl solution.
2. Place electrodes in the holder of the stand.
3. Rinse the electrodes with distilled water and dry them with absorbing paper.
4. By lowering the position of electrode holder immerse the electrodes in the chambers. Avoid the situation in which the electrodes touch the bottom of the chambers.
5. Check if the voltmeter is switched off. Connect the electrode wires with the voltmeter. Connect left electrode to the plug COM, and right electrode to the plug V/Ω.

6. Set the measuring range of the voltmeter to 200 mV DCV.
7. Switch on the voltmeter and read the voltage (mV).
8. Observe the voltage and wait till the constant value is reached (i.e. the reading will be stable for at least 30 seconds). Put this value of voltage into the table.
9. Switch off the voltmeter, rise the electrode holder, rinse electrodes with distilled water and dry with absorbing paper.
10. Using the automatic pipete (F100) add to the right chamber (P) 0,1 ml of 1 molar NaCl solution and stir the solution in the chamber. Do not change the concentration of left chamber solution (L). Measure the voltage again as described above.
11. Add 0,1 ml of 1 molar NaCl solution to the right chamber 4 more times. Measure the voltage after each addition.
12. Now, using the automatic pipete (F200) add to the right chamber (P) 0,2 ml of 1 molar NaCl solution and stir the solution in the chamber. Measure the voltage.
13. Add 0,2 ml of 1 molar NaCl solution to the right chamber 3 more times. Measure the voltage after each addition.
14. After completing the measurements switch off the voltmeter, disconnect the electrodes and put them into tubes containing KCl.
15. Remove the solutions from chambers and rinse the chambers several times with distilled water. After last rinsing leave the distilled water in the chambers.
16. The subsequent concentrations of salt in the right chamber can be calculated using the following formula:

$$c_2 = \frac{c_0 V_0 + c_d V_d}{V_0 + V_d} \quad [3]$$

where c_0 – initial salt concentration in right chamber, c_d – concentration of the added solution, V_0 – initial volume of the solution in the right chamber, V_d – sum of the volumes of already added solutions (total volume of added solution).

17. Using the plotting paper plot the dependence of voltage on the logarithm of concentration ratio:
 $\Delta V = f(\log c_1/c_2)$

Required theoretical knowledge:

1. Passive membrane transport – electrodiffusion.
2. Nernst equilibrium.
3. Nernst equilibrium potential.
4. Membrane voltages in living cells.
5. Goldman equation.

Wroclaw Medical University Department of Biophysics and Neuroscience	Practical No 13 Membrane potential measurement at Nernst equilibrium
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Grade:	Tutorial signature

1. Put the measured voltage values into table:

No.	Concentration c_2 [mol/dm ³]	Concentration ratio c_1/c_2	Log (c_1/c_2)	Measured voltage ΔV [mV]
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

2. Using the plotting paper plot the dependence $\Delta V = f(\log c_1/c_2)$.