Lab exercise 6 - Osmotic and electric properties cell membrane

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1 Introduction

In this laboratory exercises the electrical and osmotic properties of cell membrane have been studied. This was done by two different experimental setups. In this text the questions from the lab-text will be answered.

2 Tasks

2.1 Explain

2.1.1 a) What is permeability

Permeability is a property that explains the ability of transportation a particle across a substance, like a membrane

2.1.2 b) What is a capacitor

A capacitor is in electromagnetic theory described as a component that may store electrical energy in an electric field. One of the most common examples are two metal plates separated with some distance. In a circuit, a capacitor can be charged and discharged, this gives the circuit different properties. One of these properties is a frequency filter

2.1.3 c) What is electrical impedance

Electrical impedance is the resistance alternating currents experiences. Mathematically impedance is the combination of reactace from capacitors and inductors and resistance from resistors. The reactance gives an imaginary contribution and the resistance gives a real contribution

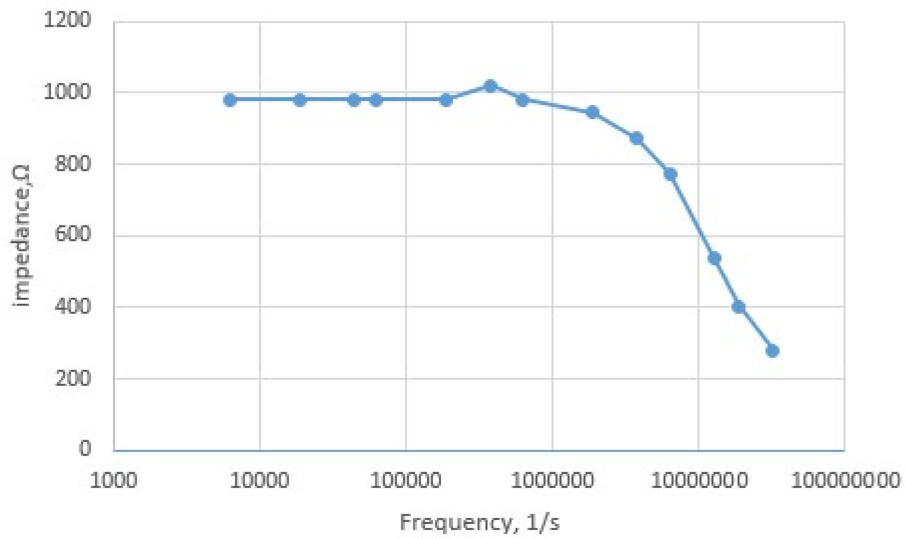


Figure 1: This figure presents the measured potential across the cells for different frequencies.

2.2 Why are you centrifuging the centrifuge tube with the blood several times?

The result of a centrifugation is a supernatant and and some denser pellet in the bottom. By resuspending several times, the dense substance will be "cleaner" and denser and a greater fraction of the pellet will be red blood cells.

2.3 Plot on a semi-log scale the amplitude $Z = R|\frac{V_{cell}}{V_{in}}|$ as a function of ω for the packed red blood cells.

The plot of the amplitude as a function of angular frequency can be seen in figure 1

2.4 Show the calculations estimating the membrane thickness. Comment on your results by comparing it to literature values.

The goal of this calculation is to calculate the membrane thickness, d.

$$d = \frac{\epsilon_m \epsilon_0 a}{c_m} \tag{1}$$

ϵ_m is the relative permittivity of the membrane, while ϵ_0 is the permittivity in vacuum. Both these values are given. c_m is the capacitance of the membrane.

This capacitance can be expressed as

$$c_m = \frac{2NC}{n}. \tag{2}$$

C is the total capacitance of the system. N is the number of cell layers, and N is the number of cells per layer. N can be expressed as

$$N = \frac{L}{l}. \tag{3}$$

L is the distance between the two metal plates and l is the length of the sides in the cube-estimated blood cell.

n can be written as

$$n = \frac{A}{l^2} \tag{4}$$

A represents the area of the metal plates. The red blood cells are approximated to cubes with sides of 4.4μm, the area of the metal plates are $3.0cm^2$, while the distance between the plates is 3mm. By inserting these values into the equations 3 and 4, we get the values $n = 1.5 \cdot 10^7$ and $N = 681, 8$

To find the value of C, it is necessary to determine the limits of the voltage across the cells as w goes to zero and infinity.

By the assumptions in the lab-text it is shown that the impedance when the frequency goes to zero is approximated to be R_1, while in the case of frequencies going to infinity will give $Z \approx \frac{R_1 R_2}{R_2 + R_1}$. When R_2 is calculated, C can be calculated by

$$C = \frac{1}{R_2 \omega_2}. \tag{5}$$

ω_2 corresponds to the point where the change of impedance reduces significantly. By evaluating the data R_1 is approximated to 980Ω. By reading the data it is not possible to observe any specific value the potential converges to. On the other hand it is possible to observe that the data is flattening. Because of this, the last value measured will be used for further calculations.

$280 = \frac{980\mathrm{R}_2}{980 + \mathrm{R}_2} \longrightarrow \mathrm{R}_2 = 392$

By inserting this value into equation 5, the equation returns $C = 7.96 \cdot 10^{-11} F$. This value is then inserted in to equation 2. The values calculated for n and N are substituted into the expression. This gives that $c_m = 7.23 \cdot 10^-15$F. By substituting this result in equation 1, we get that the thickness of the membrane is $d = 71$nm. In this calculation we have assumed that $a = l^2$. The reason for this is that only the sides fronting the metal plates are approximated to be capacitors.

This value is about one order of magnitude larger than literature values(1). The reason we have overestimated our value is might because the blood did not cover the metal plates good enough, or the pellet was not dense enough. It is also possible with some errors in the data and the measurements. We did not get any clear convergence as vi had expected this gave an inaccurate calculation.

3 Task 2- Observation of osmosis with the light microscope

3.1 Definitions

We are to give definitions of the following terms:

3.1.1 Osmosis

Osmosis is the spontaneous movement of solvent molecules through a semi-permeable membrane and to a region with higher solute concentration. A semi-permeable membrane is a membrane that allows some types of particles to enter it, while other types are not permitted to do so. These semi-permeable can be used to separate two solutions. The difference in particle concentration will give a pressure difference that is commonly referred to as osmotic pressure. Osmotic pressure leads to solvent molecules like water to go from one side to an another. The solute molecules migration will continue until an equilibrium is reached.

3.1.2 Hypotonic solution

A hypotonic solution is a solution that has lower osmotic pressure (or has less solutes) than another solution to which it is compared. An another important term is the osmolarity of a solution. This term gives a measure of the molar concentration of particles in the solution that cannot pass the membrane. If the osmolarity of non-permeable molecules in the solution is lower, the solutions are hypotonic (as already partly explained above)

3.1.3 Hypertonic solution

As in the previous assignment, a hypertonic solution is the opposite of a hypotonic solution. Meaning that it has a higher osmotic pressure that the other solution which it is compared with.

3.1.4 Isotonic solution

Generally in biology, it can be said that an isotonic solution is two solutions that have similar solute concentration. In our regard (meaning permeable membrane) we have that if the solution has the same osmolarity of molecules as those that are not able to pass the membrane, then it is called isotonic.

3.2 What to expect when you mix blood to a hypotonic, isotonic and hypertonic solution

First we take a look at placing red blood cells in a hypertonic solution. If you do that, the water molecules will diffuse from inside the cell and through the cell membrane and will continue to do so until the osmolarity is the same on both sides of the membrane. This leads to the volume of the cell shrinking, while the area of the cell membrane will remain the same.

For the hypotonic solution the reaction will be the opposite. Here the water molecules will diffuse into the cell until you get equilibrium. The volume of the cell will increase and its shape will become sphere-like.

By inserting red blood cells into a isotonic solution we do not expect any big changes. We would here refer to the figure below that is taken from the labexercise compendium.

hypertonic and isotonic.png

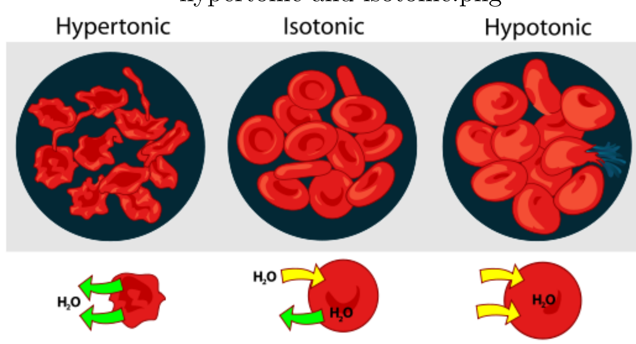


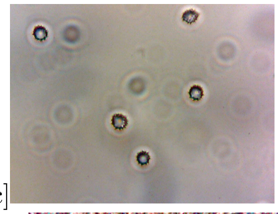
Figure 2: Shows how the reaction will go and the size of the cells

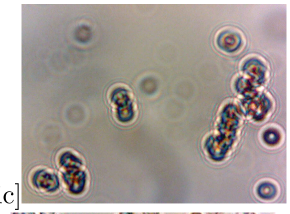
3.3 Observations

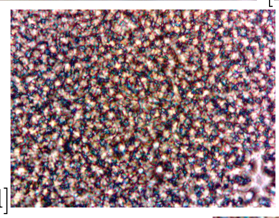
3.3.1 Images of our observations from the lab

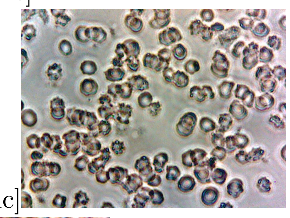
We took images of five samples in the lab exercise. They will all follow below:

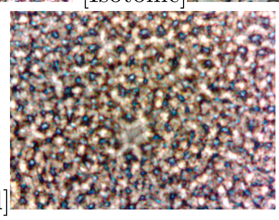
Figure 3: Shows the red blood cells with hypertonic, hypotonic and isotonic solutions

[Hypertonic] 

[Hypotonic] 

[Fresh blood] 

[Isotonic] 

[Hospitalblood] 

3.3.2 Comparison of the geometry of the red blood cells

If we compare the bloodcells and their different classifications in figure 2 and figure 3, we can see that they are roughly the same. This of course indicates that our observations matches our expectations. We can see that the hypertonic ones have sharp edges around them, while the blood cells in the hypotonic solution are more round, sphere-like.

3.3.3 Differences between hospital-blood and fresh blood

We can see that sample 4 contain the fresh blood. They have better colouring and compared to sample 5 look healthier. The older blood cells (the ones that have been storaged in a hospital) have cell membranes that have lost flexibility, something that impedes movement, as we can clearly see from the images that indicates stiffening in sample 5.

3.4 Information about the permeability of Na, Cl and Sucrose

When red blood cells are taken out of their natural environment and placed in a isotonic NaCl solution the osmotic equilbrium continues. This gives no net osmosis. If we put red blood cells in either hypotonic or hypertonic, we see cell-size tendencies (with either swelling or shrinking) as explained above. And we also see that Na+, Cl- and sucrose are impermeable through the membrane. While all inorganic ions are incapable of penetrating biological membranes, they do however contribute to the osmotic pressure of the fluids.

References

[1] https://en.wikipedia.org/wiki/Lipid_bilayer