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Studying the Permeability of Lipid Membranes

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ABSTRACT

Lipid molecules contribute to about 50% of the cell structure of a cell. Along with membrane proteins, these molecules have a significant role in transporting substances in and out of the cell. Lipid (phospholipid) molecules are arranged in two layers (a bilayer) given their special properties and act as a lipid bilayer protector for the cell. Proteins transport ions and sugars in and out in cases needed, and two lipid layers prevent excessive entry and exit of these substances. However, some of these substances and particles cannot be passed on by proteins, yet the two lipid layers do the specific processes of this.
Firstly, the spherical lipid bilayer membrane was made of phospholipid molecules in the study. These artificial membranes are called a vesicle. Their diameter ranges from a few tens of nanometers to a few tens of micrometers. Holes of several hundred nanometers are used in processes like drug delivery in the body. A few tens of microns model is considered a good one for cell membranes. Vesicles can behave similarly to the lipid portion of a cell membrane. After the production of vesicles, by applying electric potential and creating a pore, they became permeable to ions.
Using an experimental arrangement and taking into account the electrophysiological conditions of ionic current passing through the vesiclehole and the value of its permeability time was measured and by converting this ionic current to electrical potential, the value of membrane potential was measured. It was then seen that the electrical potential of the membrane and the time that the membrane remains permeable increase with the increase in ion concentration. With this measurement, information was obtained about the permeability behavior and the value of membrane changes at various ion concentrations.

Keywords
Lipid bilayer, vesicle, membrane potential, permeability

Introduction

Biological membranes exist in every corner of biology. Every eukaryotic and prokaryotic cell is surrounded by a plasma membrane determining the cell range relative to its surrounding environment. Moreover, intracellular organs (like chloroplasts, mitochondria, or endoplasmic reticulum) are in biological membranes.

Some of the key functions in cells are carried out by biological membranes. They control the entry of nutrients and the excretion of waste products by controlling the structure of cells and cellular components [1]. Understanding the roles of the cell plasma membrane is critical to understanding the cell, its function, and its interaction with the environment, in other words, “life.”

Lipids are the building blocks of cell membranes. Lipids are small amphipathic molecules with a hydrophobic hydrocarbon region and a hydrophilic headgroup. They form spontaneous masses in water, which are mostly bilayer membranes. The lipids of biological organs show the abundance of chemical structures. Although most lipids have two hydrocarbon chains and a hydrophilic headgroup, the composition of the chains and the headgroup can drastically change. Interestingly, the lipid composition varies in various cell types of an organism, or even in various organs of the same cell. Lipid composition responds to changes in thermodynamic variables like temperature, pressure, pH, or solvent concentration [2].

Ten to 20% of lipids exist in biological membranes, but their concentration in mitochondrial membranes can increase by up to 40%. One can see that various membranes have various lipid compositions. The relative abundance of various headgroups in biological membranes can affect their fuzzy behavior and electrical or interaction properties with proteins or drugs as the major binding sites for them. For instance, mitochondria has about 14% of cardiolipin (with two net negative loads), whereas no other organelle has even a small value of this lipid that makes mitochondrial membranes become severely charged. The cholesterol concentration of the myelin sheet of nerve cells is three times that of other membranes [2].

In biological membranes, the chain composition varies for lipids with various headgroups. For instance, it is seen that in this type of cell, phosphatidylcholines (PC) tend to have relatively short chains of 16-18 carbons.

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Sphingomyelin (SM) tends to have long chains with 24 carbons. The chain composition is different in various organs of the same cell [2].

Biological membranes act as a semi-permeable selection wall for the cell by controlling the process of material entry and exit. Moreover, they ease communication with the environment and intercellular signaling (cell-to-cell).

Biological membranes maintain the unbalanced ion distributions along with each living cell, which creates a potential difference between the cell and the environment (inside is more negative) [3].

Nonetheless, cell plasma membranes are not completely insulators as they have channels making them permeable to certain ions like potassium. Thus, a membrane can have a difference in net electrical potential across the membrane, called the membrane-permeable potential. For neurons, the values of a typical membrane resting potential are usually in the range of -70 to -80 mV. A change in membrane permeability like the opening and closing of ion channels ends in an outflow from the resting potential, called depolarization if the electric potential difference increases, and metapolarization if the electric potential difference becomes more negative.

Membrane potential has two main functions: Firstly, it enables the cell to act as a battery providing energy for different “molecular devices” embedded in the membrane. Secondly, in the cells like neurons, electrically excitable, they transmit signals between various parts of the cell [4]. Regarding excitable cells (like nerve tissue or nerve cells), the potential difference of the electrons passing through the membrane reaches values even of the order of 100 mV [1,5]. Such potentials are used as an energy source for the cell. In neurons, these potentials are the key to the production and propagation of nerve impulses. A difference of 100 mV electric potential resulted in a large electric field (of the order of 10^7 V / m) at the nanoscale of membrane thickness. It is very unlikely that the lipid substrate remains fully inactive.

While studying electrical phenomena in excitatory cells, the biological membrane is often represented as an electrical circuit. Such a demonstration was first done by Hodgkin and Huxley in the field of neural pulse propagation [6].

Nevertheless, since that time their assumptions on the membrane electrical behavior have turned into the most accepted model. In this model, the production and transfer of action potentials along nerve cell membranes are attributed to ionic currents (mainly sodium and potassium) flowing through the membrane-permeable protein called protein channels. Such protein channels can be opened and closed with complex dependence behavior over time and electrical potential differences, increasing the selective conductivity of various ions. When a channel is open, the ions can penetrate in and out of the membrane given their electrochemical potential gradient. The potential passing through the membrane regulates the opening of the channels, which in turn can change the potential through ionic currents. These currents are measured in Voltage-Clamp electrical voltage tests, where the electric potential difference is kept constant and the currents resulting from a sudden change in electrical potential difference are recorded [7].

In Hodgkin and Huxley's model, two lipid layers have no active role. It is assumed to behave like an insulator given its hydrophobic inside.

Biological membranes are subjected to melting transitions several degrees below physiological temperature [8]. These transitions involve severe structural changes in the membrane (like the changes in area and thickness [9]) able to significantly affect the capacitance. Moreover, the temperature at which membranes melt can be affected by several variables like electrical potential differences. Furthermore, the dependence of the electric potential difference between the capacitance of two lipid layers and biological membranes is a known phenomenon widely examined in the past [10]. After this, the strong electromechanical coupling is seen in experiments with two lipid layers when an external electrical potential difference is applied [11]. As the membrane is subjected to a significant difference in electrical potential under physiological conditions and its proximity to the melting transition at which large fluctuations in mechanical properties occur, this coupling phenomenon is worth studying more.

At first, membrane potentials were recorded using glass electrodes in several preliminary experiments where this was possible, the most know of which was squid giant axon [6]. Far thinner electrodes were required in measuring the membrane potential in other pilot experiments [12]. They produced glass micropipettes with 5 to 10-micrometer tips in diameter that could be used to measure resting membrane potentials in skeletal muscle cells. It was found that

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even pipettes with smaller tips could drastically increase the success and stability of the results [13]. Applications for recording cellular activity have expanded to study synaptic potentials and other changes in membrane potential, whereas using glass micropipettes for pharmaceutical applications is starting too.

Ultimately, although not examined here, it has to be stated that many scholars have combined these methods with other techniques. Examples of such symbioses are combinations with the recording of other physiological parameters like calcium fluorescence or with the recording of single-cell contraction. The evolution of molecular biological techniques has greatly affected the world of electrophysiology scientists. It is possible to explain ion channels or modulatory factors in systems like eggs or cell lines and to understand exactly how they work by stimulating the protein described at the same time on the amino acid.

The incentive for conducting the present study is the lack of understanding of the effects of electrical potential differences on the lipid membrane. Despite its biological significance, the community of biological membrane scientists, focusing most of their efforts on understanding the structure and function of specific membrane proteins have paid little attention to this issue.

Materials and Methods

By applying a voltage to the vesicle membrane and measuring the output current with a microtube having the electrode and converting it to electrical potential by the electrical circuit made, the potential value of the membrane was examined. The layout of the process will be explained below:

Construction of vesicles: The method used to build vesicles was the electrical swelling method. It was carried out by applying an alternating electric field to two glass plates coated with indium tin oxide (ITO). POPC phospholipids were used to construct vesicles.

Micropipette

Other tools are required that can be connected to an electrical circuit in a part of the membrane and by applying various potentials, the degree of permeability of the vesicles can be studied to measure the action potential of biological membranes, so a micropipette with a very small opening was used in the laboratory.

It was constructed such that the openings of glass micropipettes filled with the salt solution were used to contact the surface of the electrode with the outside world, which is microscopically small. The contact area is determined with the diameter of the pipette tip. The pipette is typically made of a glass rod with an outer diameter of 200 to 300 micrometers and an inner diameter of 100 to 200 micrometers. Moreover, it is constructed from 10 to 12 centimeters. Its tip is made by local melting and pulling the rod in a pipette pulling machine. The glass rods used are commercially available in various materials and sizes.

Glass micropipettes or capillaries were pulled with the Narishige Group microtube vertical puller. The device was producing relatively short micropipettes with sharp conical points like the san clock by heating and adjusting the temperature required for thinning by the embedded element.

In the next step, using the microforge device and heating the thinned orifice of the micropipettes in the previous step, one can get the desired diameters. This created micropipettes with openings ranging from 5 to 15 micrometers in diameter. As a rule of thumb, larger openings, with an experimental upper limit of 20 μm, make creating a stable membrane more difficult.

The pipettes were filled with 4 to 5 mm of electrolyte solution, further filling was used to lead to the solution leaking into the pipette holder, producing additional noise.

The electrolyte solution usually has to have a concentration of about a few molar to show good conductivity. The micropipettes produced in the study were generally filled with 3M NaCl electrolyte.

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Microtube holder (micron manipulator)

The main function of the micro-manipulator is to place the tip of the micropipette on the cell membrane in a controlled way. While placing a micropipette on a cell, the manipulation changes rapidly more than several times, from the approximate placement of the pipette on the test (1 to 10 mm) to find the right spot on the cell membrane (1 to 10 μm). Thus, most manipulators have three scaling scales:

- A three-axis mechanical manipulator with a range of 1 to 3 cm to lower the pipette into the solution and approach the cell by about 100 μm, or the range where the pipette and cell can be seen in the same field and the third axis used in the range of 1-10 μm to place the pipette on the cell.

Electrical circuit

To measure the current flowing through the membrane and convert it to voltage, an electrical converter circuit was used. As the current passing through the membrane is in the pico ampere range, an operational amplifier was used in this project to amplify it and convert it to potential in the circuit.

AD549 amplifier (Analog Devices Company) was used in the study in line with the characteristics of low current (in the pico ampere range) with very little noise.

Reverse circuit and converting current to voltage

In the circuit constructed to measure the membrane potential, after applying an electric potential difference reaching the membrane and creating a hole in the membrane, the ionic current enters the electrolyte solution through a silver wire in the microtube, trying to distance the wire and reach the minimum possible distance from the membrane. The positive end of the amplifier by the reference electrode is in the base position of the membrane before the application of the electric potential (reference potential) and the negative one is connected to the surface of the membrane by the silver wire after applying the electric potential and creating a hole. Concentrations and variations are encountered since the resistance of the negative end of the amplifier is very high (the IC introduced in the study is the maximum current passing through the femtoampere range). All current passes through the resistance (Rf). Based on Ohm's law, we have an electric potential at the output, the amplifier shows the change in membrane potential at the output by comparing it with the membrane potential before creating a hole (positive head). This type of circuit is called a reverse circuit.

As the value of this current is of the order of pico amperes, the resistance (Rf) must be of the order of Giga ohms to have a potential value of the order of millivolts in the output.

The input resistance is the total resistance of the microtube and electrolyte solution (niR) of the order of 100 ohms in the project that can be overlooked.

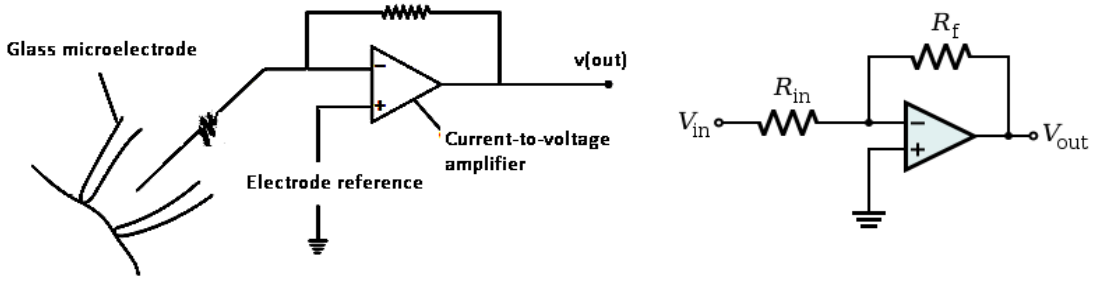

Figure 1. Schematic of an electrical circuit for measuring membrane potential

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Electrode reference

One always needs a reference to measure the potential of various materials compared to each other for specifying the electrical potential. This reference can be the ground potential, as zero. In electrochemistry, this potential is determined by the primary reference electrode that is Standard hydrogen electrode: SHE.

A standard hydrogen half-cell has a platinum blade in an acid solution where the concentration of hydrogen ions is 1 M and hydrogen is blown into it at the pressure of one atmosphere. Conventionally, the potential of this half-cell is considered zero.

Nonetheless, as maintaining the 1 atmospheric pressure of hydrogen and maintaining a constant concentration of 1 M of hydrogen ions in the laboratory is hard and costly, the silver/chloride electrode was designed as an alternative to be used repeatedly in the laboratory.

Silver chloride electrodes have silver wires “chlorinated” forming an AgCl layer on the outside of the wire. Chlorination in solution is an easily reversible equilibrium that is because of the low bonding potential of silver chloride. One of the signs that the silver chloride electrode is worn is an asymmetry between the electrode connection potentials. Moreover, its polarization is shown in the form of slow responses.

Data acquisition and digitization

Tokens must be converted from analog to digital (AD) format to acquire data. The device to perform these tasks is an AD / DA converter or interface. As soon as analog data is digitized and stored, it is no longer vulnerable to degradation. Unlike analog signals, digital data can be interconnected and processed with computer programs. Digitization includes dividing continuous data into discrete numbers by sampling.

Results

An area of the spherical membrane connected to the microbe is de-energized and permeable to ions for a few milliseconds by applying an electric potential. In this short time, the membrane potential increases and normalizes. This mechanism transmits nerve messages well in nerve cells.

In this thesis, an arrangement was designed to measure the increased potential because of the entry and exit of ions in the wells, given in the following results related to this experiment.

In this project, the vesicles produced in the sucrose solution are diluted 4-5 times their volume by sorbitol and loaded into the sample chamber, After being connected to the capillary tube containing Na^{+}ions by a micron vesicle holder, we apply a 75 mV square potential to the membrane at a frequency between 10 and 100 Hz using a potential generator creating a pore in the membrane and the flow of ions in the membrane to the outside and the entry of positive sodium ions into it.

Resting stage:

Before the potential, the resting phase dominates the membrane. At this stage, the membrane is said to be polarized or polar.

Depolarization stage:

By applying a potential difference at this stage, the membrane suddenly becomes permeable to sodium ions and allows many ions to enter the membrane. The natural polarized state has zero potential.

It was seen that the potential increases rapidly in a positive direction. The state is called depolarization. This stage of depolarization differs from zero and +130 mV depending on the value and concentration of ions in the membrane. In

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some small vesicles, the potential shifts only in the zero range and does not reach a measurable positive state (Figure 2).

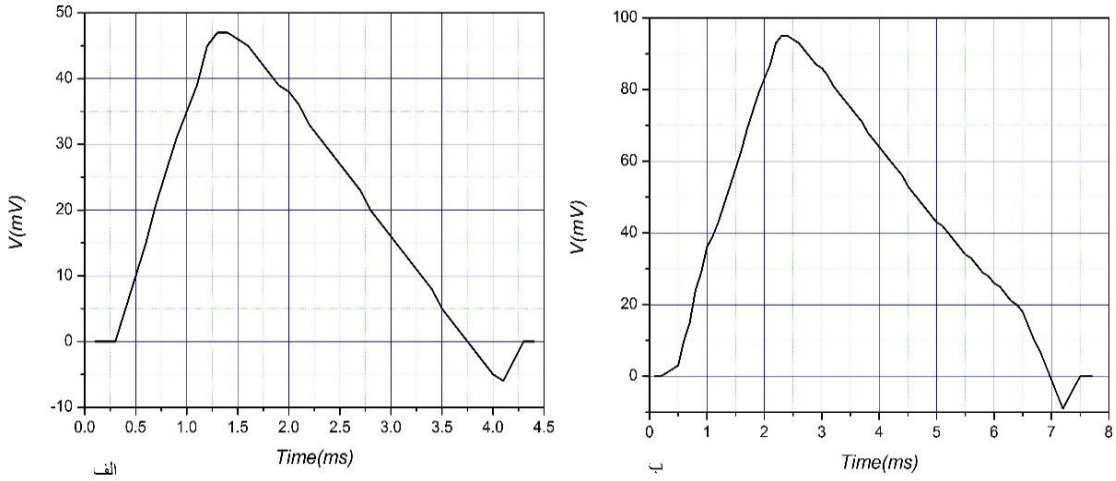

Figure 2. Diagram of membrane potential with 75 mV voltage application, frequency 100 Hz, a: internal concentration of sucrose membrane 100 mM and b: internal concentration of membrane: 200 mM

Starting incremental potential:

As long as the lipid membrane is intact, no potential increase happens in it. Any incident leading to the membrane potential sliding from zero to positive values will cause the membrane attached to the microtube opening to open. Thus, the other stages of incremental potential occur.

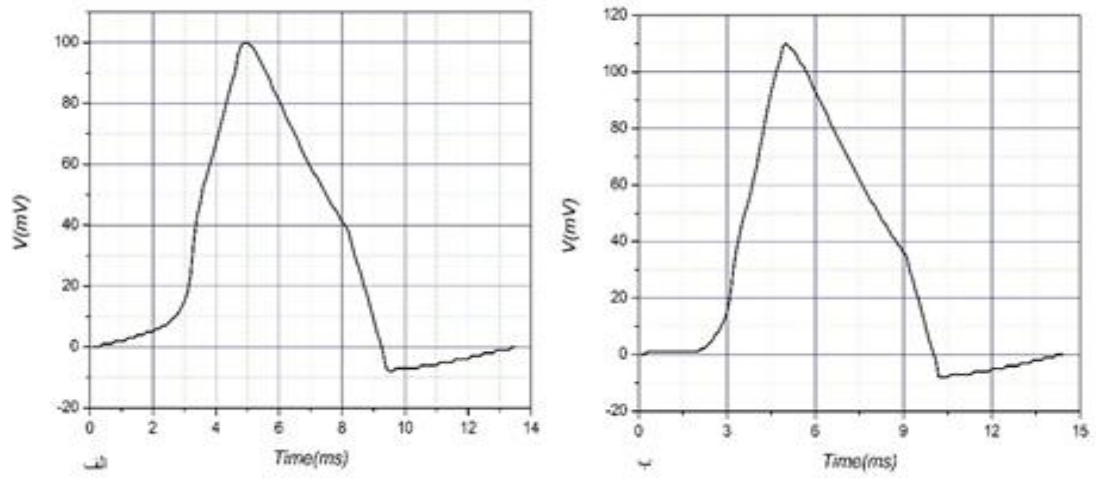

Figure 3. Diagram of membrane potential with 75 mV voltage application, frequency 100 Hz, a: Internal concentration of membrane (sucrose 250 mM) b: Internal concentration of membrane (sucrose 300 mM)

As is seen, the electrical potential increases with an increase in the internal concentration of the membrane. This concentration comparison gives a good scale for the value of ionic current passing through the membrane and thus increasing the membrane potential. In transmitting cellular and neural messages, the value of this current differs depending on the manner and functions of the membrane in exchanging information and transmitting cellular messages.

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Incremental potential excitation threshold:

No incremental potential is created as long as the initial potential of the membrane is not large enough to excite the membrane components. This usually needs a sudden increase of 30 to 100 mV in the membrane potential. In this study, with various measurements, the optimal potential for pore formation in the membrane was 75 mV. The potentials below this value do not affect the membrane well, and higher potentials cause the membrane to disintegrate and deform.

By increasing the square voltage, if it does not disrupt the overall structure of the membrane and allows the membrane potential to be measured by creating a hole in the microtube opening, it is seen that it does not affect the membrane potential and just acts as a stimulus to permeate the membrane. Comparing the diagrams in Figures 3 and 4 shows that by increasing the electrical potential from an optimal 75 mV to 90 mV, the diaphragm diagram of the membrane potential is similar and within the maximum range of 110 mV.

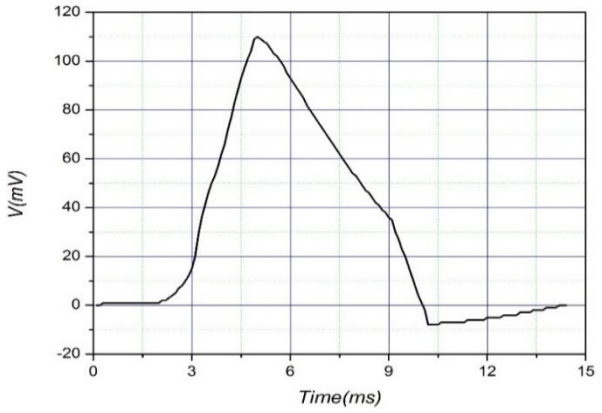

Figure 4. Diagram of membrane potential with the application of 90 mV voltage, frequency 100 Hz, the internal concentration of membrane (sucrose 300 mM)

Given the results, as Figure 5 shows, the electrical potential of the membrane increases almost linearly and the length of time that the membrane remains permeable with an increase in the ionic concentration by applying a constant electric potential. At higher concentrations, ions are higher than that of lower concentrations of ions increasing almost linearly.

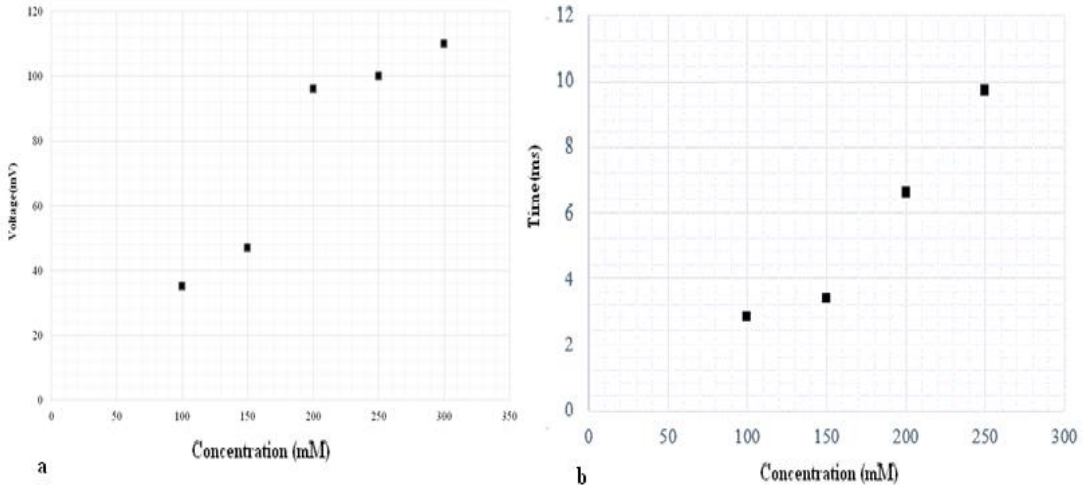

Figure 5: a) Electrical potential diagram of membranes at various concentrations of sucrose b) Diagram of membrane permeability time at different concentrations of sucrose

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Conclusion and Discussion

Biological membranes have always been of great significance as a key structure for maintaining and organizing cell life. These structures have turned into a very interesting topic for most scholars to study in recent years. As stated in the first chapter, the membrane structure can be examined as a very attractive system model. Besides all the activities and biological characteristics, these structures have dynamic electrical and electrochemical properties in an interesting way given their chemical structure and their subunits.

Two various approaches exist on working with model membranes called stabilized lipid bilayers and free lipid bilayers. As its name shows, two stabilized lipid layers use a solid substrate like mica, silicon, glass, under the lipid layer. This gives us the advantage of a flat bi-layer, facilitating fluorescence microscopy. Furthermore, the solid bed allows us to use Scanning Probe Microscopy (SPM). Nonetheless, this has its downsides in that it is primarily important to consider the forces between the bed and the membrane. Those forces can be minimized by using a suitable substrate and aqueous buffer that can protect the membrane against the substrate [14].

Using Giant Unilamellar Vesicles (GUVs) presents a different system model for biological membranes. There is no ideal flat area for the microscope and the ability to use SPM in this system model. They are still very popular as a system model for, say, fluorescence microscopes, despite the lack of a flat surface [15]. The system model has problems with its spherical shape and structure but can be controlled. Using a spherical sample enables studying the curvature of the membrane.

The significant benefit of working with model membranes is the ability to control the contents and thus the membrane complexity. Model membrane applications range from a simple single-component system of normal glycerophospholipids to complex cell extracts with proteins. This diversity enables designing the model membrane for a specific or case study. While designing a system model, it is essential to consider the limitations, and most lipid model membranes are isolated systems allowed to equilibrate during the experiment compared to cell membranes. In the cell membrane, there is a constant flux of lipids and proteins and it is a dynamic system in every way. Does this mean that a statically isolated model membrane cannot display anything on a cellular plasma membrane? No, this does not mean that the results obtained can be transferred directly to the cell membrane. Overall, while working with a system model, any result has to be interpreted based on that model, and then a comparison must be made between the results from the model system and the actual system.

The membrane of each cell surrounds the organs but is impermeable. Cells require sources of sugar, amino acids, oxygen, and so on to survive. Moreover, they must get rid of waste. Lipid membranes are very thin. Nonetheless, lipid membranes are usually considered as complete insulators in the biological literature. This is especially true for neural conduction theories where ion conduction is attributed exclusively to protein ion channels. On the other hand, it is clear that in thermodynamics, there is always a limited probability of solute passing through the membrane. Ionic channel-like events can be found even in the complete absence of protein.

The integral proteins passing through the membrane are composed of some subunits and believed that they control the flux of ions across the membrane and can have multiple states (open/closed). These states can be opened and closed with voltage, ligands, light, and so on, which means that changes in these parameters control the protein state. Voltage-sensitive sodium and potassium channels are examples of very important components in the Hodgkin and Huxley model for describing nerve actions [6].

Most membrane transport proteins open and close with sensitivity to external parameters. Ligand-sensitive channels act like a lock for which a specific ligand acts as a key. If the membrane potential exceeds a specific threshold, voltage-sensitive ion channels open/close because of the movement of the Gating Unit in the ion channel. Some MTPs are temperature-sensitive and act by changing to a high entropy composition with increasing temperature, thereby altering the structure of the protein to the open state [16].

The melting temperature of negatively charged lipid membranes increases when the charge is neutralized with protons, proteins, or dual-capacity ions. Indeed, even the melting points in ion polar membranes increase greatly. Hence, it is not unexpected that the presence of calcium affects the occurrence of spontaneous currents within lipid membranes.

The protons concentration (pH) is another variable in a thermodynamic system. Similar to calcium concentrations, proton concentrations affect the melting points of lipid membranes by binding to lipid phosphate groups. A great part of the ion penetration into biological membranes is ascribed to proteins called ion channels. Currents within such molecules have been identified by Nahr & Sakman (1976) in recording data of connected membrane range experiments.

The delivery of such proteins seems to be highly relying on the lipid medium. It was found that the persistence of opening depends on the lipid mixture. At the fuzzy boundary of the POPE-POPC phase diagram (at 80% POPE), they approach a maximum where the additional heat capacity is expected to be maximal as well. The effect of lipids on these potassium channels has been examined by Thornheim et al. (1999) and Schmidt et al. (2006). Clear dependencies of channel activity on lipid composition have been revealed and suggested that lipids regulate channel opening and closing. Schmidt et al. (2006) indicated that voltage-dependent potassium channels (KvAP) show conductivity in negatively charged lipid membranes yet not in positively charged membranes. Hence, the performance of such proteins usually is not independent of the lipid substrate of the environment and its associated thermodynamics.

Pursuing this line of study, other studies can be done as well:
1) Studying the effect of some proteins increasing membrane permeability like some bacterial proteins
2) Studying the mechanism and activity of membrane channels
3) Studying drug delivery through membrane channels
4) The mechanism of neuronal synapses.

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