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A Comprehensive Understanding of Model Lipid Membranes: Concepts to Applications

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ABSTRACT. The cell membrane, also known as the biological membrane, surrounds every living cell. The main components of cell membranes are lipids and therefore called as lipid membranes. These membranes are mainly made up of a two-dimensional lipid bilayer along with integral and peripheral proteins. The complex nature of lipid membranes makes it difficult to study and hence artificial lipid membranes are prepared which mimic the original lipid membranes. These artificial lipid membranes are prepared from phospholipid vesicles (liposomes). The liposomes are formed when self-forming phospholipid bilayer comes in contact with water. Liposomes can be unilamellar or multilamellar vesicles which comprises of phospholipids that can be produced naturally or synthetically. The phospholipids are non-toxic, biodegradable and are readily produced on a large scale. These liposomes are mostly used in the drug delivery systems. This paper offers comprehensive literature with insights on developing basic understanding of lipid membranes from its structure, organization, and phase behavior to its potential use in biomedical applications. The progress in the field of artificial membrane models considering methods of preparation of liposomes for mimicking lipid membranes, interactions between the lipid membranes, and characterizing techniques such as UV-visible, FTIR, Calorimetry and X-ray diffraction are explained in a concise manner.

Key words: Liposomes, Phospholipids, Bilayers, Fluidity, Lipid membrane

INTRODUCTION

Cell membrane is mainly formed of lipids and proteins.[1] The relative proportions of proteins and lipids in it vary with the type of membrane, and for well characterized functional membranes, their ratio by weight (proteins to lipids) ranges from about 1.5 to 4.[3] The lipids in the cell membrane are arranged themselves as bilayer called lipid membrane, *Fig*. 1. The biological lipid membrane is essential for maintaining cell physiology and architecture. It is flexible, dynamic, fluid like, self-healing A, B, C, and D, and selectively permeable to polar solutes.[1] Lipid membranes are essential for physiological activities like cell protection and cell-to-cell communication.[4]

Morphology of lipid membrane is due to their fluidity i.e., ability to move laterally along membranes. With changes in temperature, lipid membrane undergoes thermally excited bending undulations or shape fluctuations. Whether lipids present in the lipid bilayer exist in a fluid, liquid-crystalline phase or an ordered gel phase, depend upon the type of lipids involved and the temperature. The temperature at which a lipid changes its phase from the gel to liquid-crystalline is known as melting temperature. Above this temperature, the hydrocarbon chains are tilted in a nearly all-trans conformation and disordered, and they acquire a gauche conformation which weakens the van der Waals chain interactions and it also affects the polar interaction and hydration of the phospholipid head groups.[5]

Classification of Membrane Lipids

Cell membranes mainly consist of phospholipids (in abundance, ~50%), sterol (cholesterol, ~5%) and glycolipids (~2%) as represented in *Fig.* 2.[6]

Phospholipids

Phospholipids contain phosphoglycerides (PG) or glycerophospholipids which are most abundantly found phospholipids in cell membranes. Glycerophospholipids are made up of a phosphate-containing head group and saturated or unsaturated hydrocarbon chains that are linked to a glycerol through ester bonds.[5]

Phospholipids are classified depending on the head group present; if head group is choline, then lipid is phosphatidyl choline as shown in *Fig.* 3(a), if head group is ethanolamine, then lipids is phosphatidyl ethanolamine as in *Fig*. 3(b), if head group is serine group, then lipids is phosphatidyl serine as shown in *Fig*. 3(c). Phosphatidylcholine (PC) phospholipids are mostly present abundantly in cell membranes.

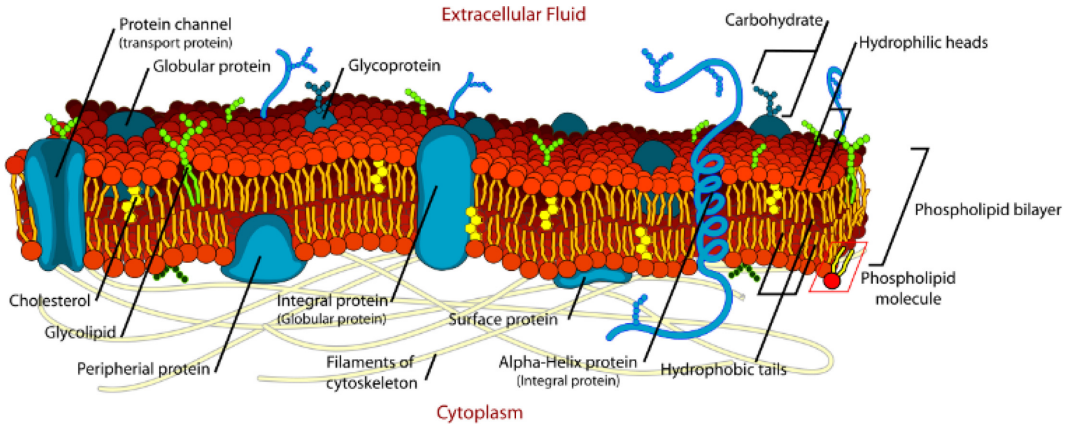


***Figure* 1.** Diagrammatic representation of cell membrane.[2] Reproduced from ref. 2., copyright@Université Paris-Saclay, France.

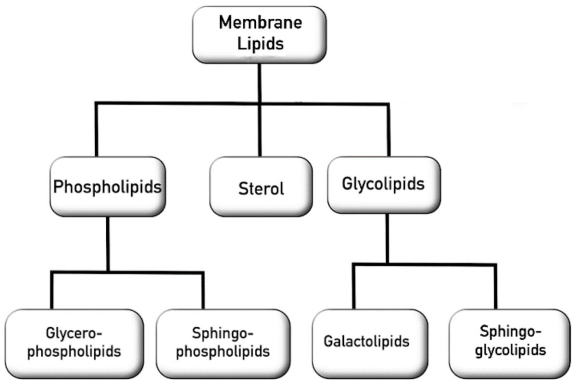


***Figure* 2.** Classification of membrane lipids.

Sphingolipids

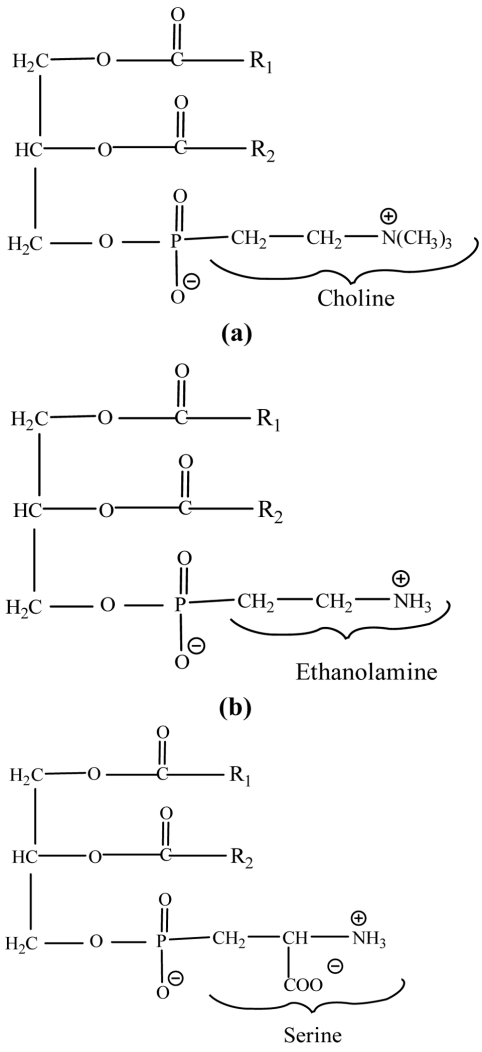
Sphingolipids are another kind of natural lipids found abundantly in mammalian cell membranes. Sphingolipids are comprised of sphingosine back bone with an 18-carbon amino-alcohol. e.g. Sphingomylein (SM). Ceramides are a kind of sphingolipid which include sphingosine and a single fatty acid chain as shown in shown in *Fig*. 4.[7]

Glycolipids

Cerebrosides and gangliosides are examples of glycosphingolipids, a type of lipid in the membrane in which the lipid chains are connected to sphingosine rather than glycerol as in phospholipids. Gangliosides consist of one or more than one units of negatively charged sialic acid, whereas cerebrosides are neutral glycosphingolipids.[1]

Phase Behavior of Lipids Membranes

Lipid bilayers can produce a number of polymorphic phases depending on pH, molecular structure, ionic strength, water content, and temperature. The hydrophobic acyl chains of lipid bilayers are conformationally disordered in fluid



where R_1 & R_2 are acyl carbon chains.

***Figure* 3.** Types of phospholipids a) Phosphatidyl choline b) Phosphatidyl ethanolamine c) Phosphatidyl serine.

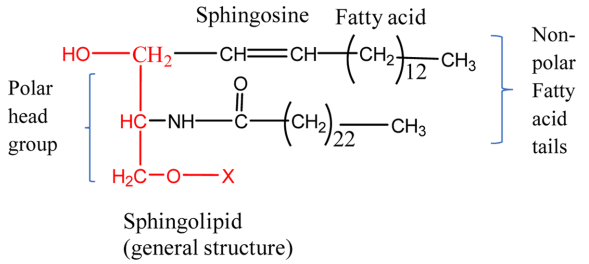


***Figure* 4.** Structure of Sphingolipids.

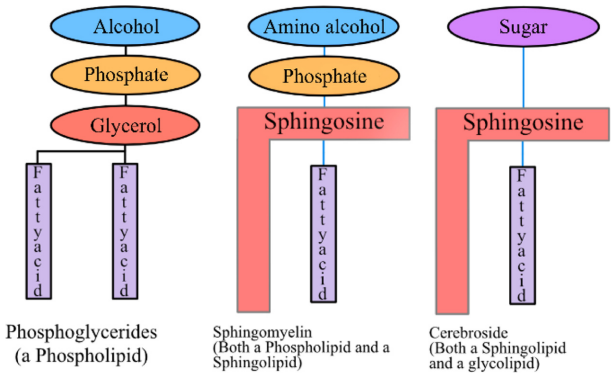


***Figure* 5.** Diagrammatic representation of phospholipid, sphingolipid and cerebroside.

phase, but they are more stretched and well organized in the gel phase. The lipid state at very low temperatures is called as the 'gel' phase because the hydrocarbon chains are mainly arranged in an all-trans arrangement. The 'fluid' state is a high-temperature condition with unordered chains. The words 'fluid' and 'gel' phase are a little ambiguous because they do not describe the type of order that is changing. The solid-liquid transition is the change in lateral order (from crystalline to random) in physics. Internal degrees of freedom exist in lipids, which can be switched from an all-trans chain conformation (ordered) to a random chain arrangement (disordered) through trans gauche isomerization. As a result, the phases in the lipid-cholesterol model membranes can be classified as gel phase L_{β}' or solid-ordered (all-trans chains arranged on a crystalline lattice), liquid-ordered L_0 (all-trans chains arranged in an ordered or glass-like lateral arrangement), or liquid-disordered L_{α} (all-trans chains arranged in an unordered or glass-like lateral arrangement) (random chains with random lateral arrangement) as shown in *Fig.* 6. The first phase is similar to the 'gel' phase, whereas the second phase is similar to the 'fluid' phase.[8] Lipids when present in aqueous environment will form multilamellar vesicles (vesicle is a closed, spherical lipid bilayer that creates a cavity inside that can hold aqueous solution), by changing temperature the lipid membranes change from liquid ordered to liquid disordered phase.

Melting point of unsaturated lipids containing double bonds is significantly lesser than saturated lipids. So, melting point decreases when unsaturation increases and melting point of trans is more than cis. The general melting point trend is, Saturated fatty acid > trans unsaturated > cis unsaturated fatty acid. Rigidity of the membrane increases, when saturation of acyl chains increases as shown below. Saturated fatty acid < trans unsaturated < cis unsaturated fatty acid.

Charitat *et al*. reported in his study that by introducing saturated fatty acid, the fluidity of the membrane can be decreased or rigidity of the membranes can be increased e.g., by adding dipalmitoylphosphatidylcholine (DPPC) or mixed lipids rather than pure lipid such as mixing cholesterol with DPPC.[9] On the other hand, fluidity can be increased through using unsaturated lipids, for example, 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or mixing of saturated and unsaturated lipids. Phosphatidylcholine lipids possess different hydrophobic fatty acids chain length, with the increase in fatty acid chain length of the lipid, the melting point of the phase transition of the lipid membrane increases owing to increase in hydrophobic interactions among fatty acid acyl chains and the melting point of the phase transition of the membrane decreases with decrease in chain length. A phospholipid bilayer containing polyunsaturated fatty acids (PUFAs) is especially vulnerable to oxidative stress, resulting in changes in membrane characteristics. The cell membranes act as a barrier and controls various functions of the cell such as transport of chemicals into and

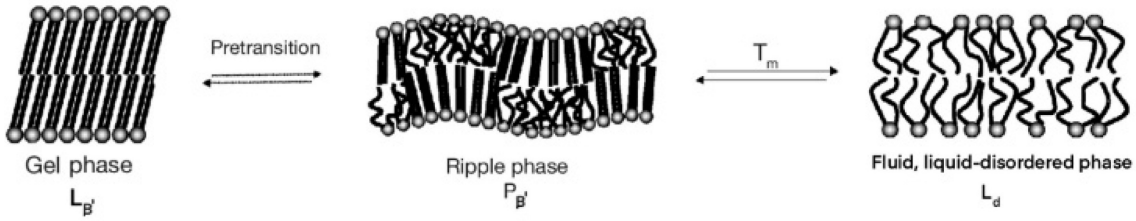


***Figure* 6.** Phase transitions in lipids.

out of the cell, structural support, protection, cell to cell communication, and cell signalling. When the integrity, elasticity and fluidity of a lipid membrane decreases, it loses its ability to operate as a barrier.[10]

Cholesterol and Its Effect on Lipid Membranes

Cholesterol (~5% of cell membrane lipid) has four rings that are fused together with polar hydrophilic OH group and non-polar hydrophobic hydrocarbon chains as shown in *Fig*. 7.

At high temperature, hydrophobic part of cholesterol present in membrane interacts with phospholipid hydrophobic tails making the membrane more rigid and if temperature is low then cholesterol's hydrophobic part interacts with phospholipid hydrophobic tails and make the membrane more fluid as depicted in *Fig*. 8.[3] "The umbrella model assumes that the hydrophilic portion of cholesterol is minimal and that lipids must contribute to the screening of cholesterol molecules for hydrophobic interactions with water. Only if the phospholipid molecules straighten to make room for cholesterol they can form this umbrella".[11] The phospholipid head groups serve as "umbrellas" for the cholesterol molecules beneath them because they share the restricted area beneath the phospholipid head groups, acyl chains and cholesterol become more closely packed as cholesterol concentration rises. The solubility limit is reached when the head groups can no longer cover more cholesterol molecules and cholesterol precipitates to form a distinct cholesterol monohydrate crystalline phase.

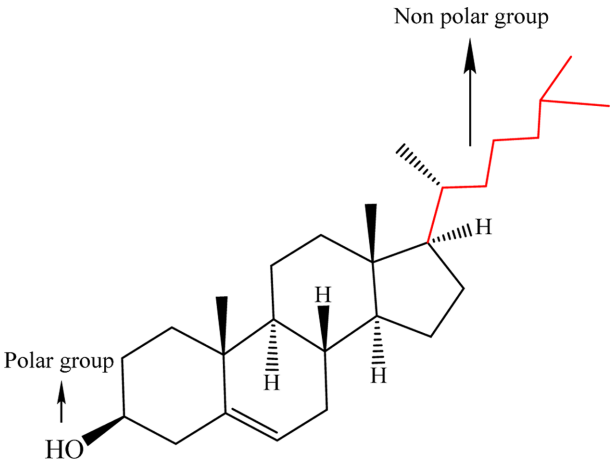


***Figure* 7.** Structure of Cholesterol.

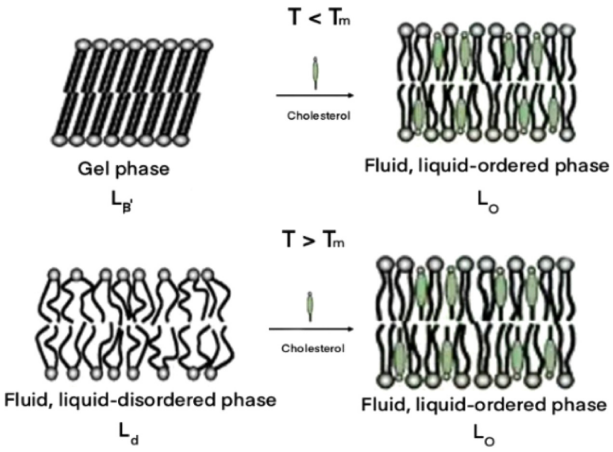


***Figure* 8.** Effect of cholesterol on lipid membranes (T is temperature, T_m is melting temperature of lipid or main phase transition temperature of lipid).

The following is the order in which glycerophospholipids interacts with cholesterol, phosphatidylcholine > phosphatidylserine > phosphatidylethanolamine. When compared to lipids having one or more unsaturated chains, cholesterol prefers to interact with those that have entirely saturated aliphatic chains. In both monolayer and bilayers, multiple unsaturation of the fatty acyl chain reduces the interaction with cholesterol and a lot of evidence shows that cholesterol interacts more favorably with Sphingomyelin (SM) than with other phospholipids like PC. In binary solutions with other phospholipids, cholesterol preferentially eradicates the phase transition of SM, showing a preferred interaction with the SM molecules. SM/cholesterol bilayers have a lower water permeability than PC/cholesterol membranes.[12] Cholesterol prefers ordered acyl lipid chains, but it is more soluble in the fluid phase. This problem has become more evident as studied by various authors and has been resolved by creating an intermediate phase, a phase that is ordered from the perspective of the lipid chains conformational structure but disordered from the perspective of the molecule's lateral orientations.[13] Cholesterol's impacts on membrane characteristics, particularly permeability, have been studied extensively. Cholesterol-containing lipid membranes are thicker and more rigid, and they also have a greater chain order and a smaller membrane area. The membranes permeability to smaller solutes is consequently decreased. As cholesterol concentration increases, a membrane's permeability to solutes typically decreases monotonically.[14] By creating a more bulklike environment for interfacial water molecules, increasing local water density, shortening the interfacial layer, flattening the orientational distribution, and increasing both the number of hydrogen bonds and the complexity of the hydrogen bond networks, cholesterol minimizes the disruptive effect of the membrane on water-water hydrogen bonding.[15]

Interactions Between Lipid Membranes

Van der Waals attraction, short-range repulsion that decays exponentially and long-range entropic repulsion caused by thermal undulations of lipid bilayers are the intermembrane interactions involved in lipid bilayer stacking.[16] Hydrophobic and hydrophilic interactions are two types of non-covalent interactions, hydrophobic interactions are between non-polar groups and water e.g. immiscibility of hydrocarbons and water. Hydrophilic interactions are between ionic or polar group and aqueous environment are at the exterior phase of bilayer. Hydrogen bonding and electrostatic interactions are other non-covalent interactions which also contribute to determine macromolecule structure. Phospholipids bilayer structure exemplify the combined effects of both these effects. Non-polar fatty acid chains of phospholipids are away from contact of water increasing hydrophobic interactions; dipole-dipole interactions stabilize bilayer structure of zwitterionic phospholipids such as phosphatidylcholine.[3]

Asymmetry In Lipid Membranes

Lipid membrane is asymmetric in nature and lateral composition of phospholipids along the membrane is non-uniform as two leaflet of the lipid membrane don't have similar phospholipid composition.[4,17] By using any routinely employed preparation techniques, lipid asymmetry i.e. lipid composition difference between inner and outer leaflets are not controlled. Sphingolipids and phosphatidylcholine (PC) are abundant in the plasma membrane outer leaflet (exofacial monolayer) of mammalian cells, whereas phosphatidylethanolamine (PE) and phosphatidylserine (PS) are abundant in the inside leaflet (cytofacial monolayer). Asymmetry can be produced by standard vesicle preparation processes (e.g., sonication) in some circumstances, but it can be difficult to regulate. Asymmetry of modest amounts of anionic lipids has been obtained using pH gradients. The capacity to interchange lipids in one bilayer leaflet can be used to make asymmetric vesicles with regulated asymmetry. A phospholipid exchange protein was utilized in one investigation to successfully transfer marked phosphatidylcholines to the outer leaflet of model membranes. A monolayer-by-monolayer building approach for creating asymmetric vesicles has also been described.[18] Only the outer leaflet of cellular membranes is replicated by lipid monolayers. Monolayers also have a lower thermodynamic stability than lipid bilayers.

The lipid composition of the model lipid membrane must be different depending on which portion of the membrane is being imitated. In reality, negatively charged phospholipids can be used to mimic the inner leaflet of the cell membrane, while positively charged phospholipids can be used to simulate the outer leaflet. The most acceptable lipids are PC, SM, and glycosphingolipids.[19] Lipid peroxidation lead to changes in membrane structure that compromise their integrity, as seen by changes in critical membrane properties as surface roughness, elasticity, fluidity and thickness. These qualities are all necessary for biological membranes to function effectively.[10] "The purpose of Exchange (Asymmetric) Vesicle Preparation is to create asymmetric vesicles with SM in the outer leaflet and glycerophospholipids with at least one unsaturated acyl chain in the inner leaflet".[18] Water permeability was found to be strongly correlated with membrane area, when phosphatidylglycerol (PG) lipid membranes with saturated and unsaturated lipid tails were analyzed. Some researchers revealed that membrane thickness and permeability may be interrelated. It has been discovered that longer chain membranes have greater, wider energy barriers, resulting in decreased permeability. The thermal stability of the outer leaflet SM was comparable to that of pure SM vesicles and much higher than that of symmetric vesicles with the same overall lipid composition.[18]

Organization of Lipid Membranes

Various models such as lipid monolayers, micelles, and liposomes can be used to investigate a variety of biophysical properties using different methodologies.[19] Depending on the nature of lipids and particular conditions, amphipathic lipids, when mixed with water form 3 types of lipid aggregates: Micelles, bilayers and liposomes as shown in *Fig*. 9.[1] Lipid monolayers over air-water interfaces are effective models for examining the interactions of solutes, drugs, enzymes, and water-soluble and amphipathic peptides with the lipid component of cell membranes in a well-defined environment.[13]

Micelles, as shown in *Fig.* 9(a) are the most basic 3D model for studying drug and membrane interactions. Other exclusive characteristics of micelles make them appealing tools, such as the ability to alter the shape and size of the micelles by selecting the lipid content and aqueous medium. Additionally, different surface charges can be prepared to study the electrostatic forces involved in drug-membrane interactions depending on the kind of amphiphilic molecules used in the micelle's composition.

These molecules spontaneously organize in aqueous solution, similar to bilayers and micelles, with their hydrophilic parts in contact with the water and their hydrophobic parts inside, the forces that hold these structures together

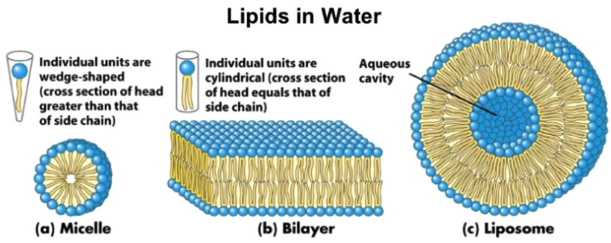


***Figure* 9.** Organization of Lipids in water[1] (Reproduced from ref. 1, copyright@W H Freeman & Co, New York, NY).

include weak van der Waals, hydrophobic, hydrogen-bonding, and electrostatic interactions. The monomolecular film's in-plane and lateral structure, which can be If surrounding media is changed, such as ionic strength, pH, and temperature, can impact the interactions between the molecular in these structures, causing them to change size and shape.[5] The morphology of the structures being generated is controlled by the effective shapes of the molecules.

Micelles would be stabilized by molecules with a conical shape, whereas lamellar bilayers would be formed by molecules with a cylindrical shape.[13] The disadvantages of micelles are; Because of the background noise from dispersed light, micelles are not optimal for various spectroscopic experimental procedures. Furthermore, small molecule partitioning into micelles is frequently ill-defined. Other challenge of adopting micelles as membrane biomimetic models is that, unlike biological membranes, micelles also include monolayers rather than bilayers, as in biological membranes.[19] Simple models of lipid membranes are lipid monolayers dispersed on oil–water or air–water interfaces. Such models have the advantage of being simple to study and control in a Langmuir trough, where thermodynamic connections between surface area and surface tension can be quantified. Several spectroscopy techniques can easily reveal the in-plane and lateral structure of the monomolecular film, which can be made up of lipids, proteins, and lipid-protein recombinants.

Liposomes (Vesicles) and Its Types

A liposome is a closed, spherical lipid bilayer that creates a cavity inside that can hold aqueous solution as shown in *Fig*. 9(c).[20] Phospholipids have a property of self-assembly; so, they assemble into spherical vesicles (liposomes) with bilayer membranes.[21] Liposomes can be categorized based on their size and number of bilayers. Unilamellar vesicles may be small (20-40 nm), medium (40-80 nm), large (100-1000 nm) and Giant Unilamellar Vesicles (GUV) (>1000 nm).[7] GUV's have diameter 1–10 mm (*Fig*. 10),

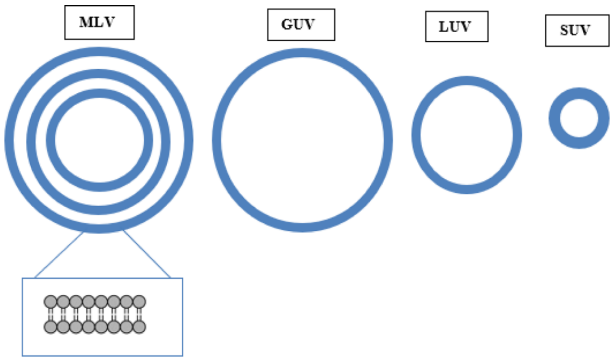


***Figure* 10.** Types of Liposomes (Vesicles) - Multi Lamellar Vesicles (MLV), Giant Unilamellar Vesicles (GUV), Large Unilamellar Vesicles (LUV), and Small Unilamellar Vesicles (SUV).

Giant vesicle formed by electro-formation having 30-100 mm; where multilamellar vesicles have numerous layers as shown in *Fig*. 10.[4]

Multilamellar vesicles in aqueous medium are the most extensively studied and reported systems for studying membrane physical interactions.[22] Giant lamellar vesicles may be free standing, interact directly with solid substrate or tethered (thiolipids, hydrogels, silanelipids, gold or even DNA oligomers) to the substrate.[3] Liposomes, despite being the most prominent lipid membrane model, have several shortcomings that are shared by all of the biophysical models. The complexity of biological membranes is significantly more enhanced in vivo and many models are unable to replicate it completely. Although the membrane bilayer's simplicity is crucial for studying particular interactions at the molecular level, it also makes it challenging to understand membrane activities precisely. These models do not accurately imitate many aspects of biological membrane functioning because they lack normal cell membrane characteristics such as cytoskeletal elements, receptors, membrane proteins, and others.[19]

The majority of the liposomes employed in drug-membrane interaction investigations contain only three or four distinct phospholipid molecules. Despite the fact that cholesterol and SM are important components of cell membranes, they are rarely included in models. Furthermore, despite the fact that negatively charged phosphotidyl serine phospholipids are a substantial component of cell membranes, negatively charged models were only used in a tiny percentage of the studies. Lopes et al employed cardiolipin, a significant component of the inner mitochondrial membrane, to simulate those.[23] It is exceedingly difficult to fully replicate the lipid asymmetry between the two leaflets that characterizes biological membranes. Since lipo-

somes have a three-dimensional shape that resembles both the outer and inner leaflets of cell membranes, this is a popular model.[19] One or more phospholipid bilayers are assembled in a concentrically organized ring around an aqueous compartment to form the vesicles known as liposomes (*Fig*. 10). A number of lipid ingredients that closely resemble the composition of bodily membranes can be used to create liposomes.[19] Azithromycin is antibiotic drug that interacts with phospholipids but not with sphingomyelin and cholesterol. But according to Peetla *et al*.[24] not all drugs can interact with lipid membrane, some will increase the lipid membrane fluctuations, leading to disruption of giant unilamellar vesicle.

Preparation of Lipid Membranes from Multilamellar, Unilamellar and Giant Unilamellar Vesicles

Lipid membranes are prepared from multilamellar vesicles by dissolving various kinds of lipids (*Table* 1) in water; then mutual interactions between these molecules form bunches, stacks and myelin sheaths.[17] To prepare multilamellar vesicles (MLV), firstly the lipids are dissolved in organic solvent, then the solvent is dried by evaporation under nitrogen stream, then lipid suspensions are prepared in respective buffer solutions, after five-six freeze–thaw and vortex cycles, the homogeneous lipid dispersions produces multilamellar vesicles in aqueous medium. The unilamellar vesicles (ULV) are formed from multilamellar vesicles that are prepared by above method. The unilamellar vesicles are formed by passing a solution of multilamellar vesicles about 15-20 times through polycarbonate filters of desired size through extrusion process.[22] If the MLVs are passed through a polycarbonate filter of pore size of <0.04 μm then small unilamellar vesicles (SUV) are formed, on the other hand if the pore size of the polycarbonate filter is >0.1 μm and <1 μm then large unilamellar vesicles (LUV) are formed.

GUV are formed using the electroformation approach.

***Table* 1.** List of commonly used Lipids used for liposome preparation having net charge 0 at pH 7.4

Name of lipid	Fatty acid	Transition temperature (℃)
DLPC	12:0	-1
DMPC	14:0	23
DPPC	16:0	41
DSPC	18:0	55
DOPC	18:1	-20
DMPE	14:0	50
DPPE	16:0	63
DOPE	18:1	-16

Firstly, lipid stock solution of appropriate concentration is prepared and Pt wire is dipped in it. After that the nitrogen gas is passed through it followed by lyophilization to remove the organic solvent completely. The Pt wire is subsequently placed in water that has been heated to a temperature greater than the lipid's melting point. In recent years, microfuidic technology has been used to create well-defined cell-sized lipid vesicles. To generate gigantic lipid vesicles that are monodispersive, unilamellar, and have a highly concentrated encapsulation with an asymmetric lipid distribution like natural bio membranes, Microfuidic technologies for droplet generation with a T-junction device or fow-focusing device are used.[5]

Preparation of Solid Supported Lipid Membranes by Spreading Organic Solution and Spin Coating Method

The Solid supported lipid membranes for X-ray reflectivity measurements can be prepared on a silicon wafer having dimensions of about 15 × 15 mm^2. Before preparing lipid membranes, the silicon substrates are cleaned with piranha solution [mixture of 30% H_2O_2 in H_2SO_4 (30:70 v/v)] by heating at 90 ℃ followed by rinsing with ultrapure water and then drying it with a nitrogen flow to evaporate the water. The cleaned substrates thus obtained possess good hydrophilicity to mount lipid bilayers on to it.

To prepare multilamellar bilayers, the spreading by organic solution method is used. In this method, around 200 μL of the desired lipid dissolved in a suitable solvent is pipetted out on to a silicon substrate for uniform distribution. After that excess of solvent is kept near the substrate in a closed chamber for 12 hours. To remove all the traces of solvent, the substrate is dried under vacuum for 24 hours. By this method, a stack of 100 or more multilamellar bilayers are produced.[5]

To prepare a smaller number of bilayers spin coating method is used. In this method, around 200 μL of lipid solution is spread on to a substrate then allowing the substrate to rotate at 3000 rpm on a spin coater. This leads to purple-colored aligned bilayers on the substrate during rotation. To remove all traces of solvent, the substrates are dried under vacuum for 24 hours. By this method lipid bilayers from 3 to 30 can be prepared on solid support by varying the frequency of rotation during deposition.[5]

Characterisation Techniques

Characterization of multilamellar vesicles bulk was mostly done using various techniques such as calorimetry, X-ray diffraction (XRD) and spectroscopic methods (*Table* 2).[5]

Synchrotron XRD is frequently used to determine lipo-

***Table* 2.** Main in vitro lipid membrane models, respective biophysical techniques applied and evaluated parameters

Membrane Model	Biophysical Techniques	Studied parameters
Liposomes	Column chromatography	Extent of drug trapped[31]
	Nuclear magnetic resonance	Drug location, phase transition, and lipids mobility
	Small angle & wide-angle X-ray scattering	Thickness of the bilayer, membrane order, structure, and fluidity, molecular interactions[6]
	Isothermal titration calorimetry	Thermodynamic Properties of drug membrane binding
	Fluorescence anisotropy	Membrane order & fluidity, & lipids mobility
	Fluorescence quenching	Partition coefficient, drug membrane binding, drug location, and membrane integrity
	Fluorescence methods (titration, fluorescence resonance energy transfer)	Drug location & drug membrane binding Partition coefficient & drug location
	Electrophoretic light scattering	Membrane fluidity, order & fluidity, & lipids mobility
	Electron paramagnetic resonance spectroscopy	Phase transition temperature, lipids cooperativity, & membrane integrity
	Dynamic light scattering	Phase transition temperature, & phase transition enthalpies
	Differential scanning calorimetry	Partition coefficient & drug membrane binding
	Derivative spectrophotometry	

some structure and mainly used to measure the thickness of lipid membrane by knowing the difference between reflected and incident light. Small-angle X-ray scattering (SAXS) is used to determine thickness of the bilayer, membrane order, structure, fluidity and molecular interactions.[25] From XRD we can know about number and thickness of bilayers. If bilayers are less, peaks are broad and if bilayers are more, peaks are very sharp known as Bragg's peaks.

Differential scanning calorimetry (DSC) is a thermodynamic method used to study the thermotropic phase behaviors of liposomes that measures heat changes that occur during increase or decrease in temperature.[26] DSC is used to study a wide range of thermal transition in biological systems, to determine melting temperature as well as thermodynamic parameter associated with these changes. In advanced DSC equipment, the sample volume required is approx. 0.5 mL & sample concentration required is very low i.e. 0.2-2 mg/mL & temperature range is 5-120 ℃. Protein interactions with liposomes have been studied using DSC.[27] High scanning DSC will help in detecting phase separation in the gel state of binary lipid mixtures.[28]

Pressure perturbation calorimetry (PPC) has been extensively used since early 2000s to study the biological materials. PPC is easy to use technique that works when pressure cap is connected to a sample cell through nitrogen cylinder and by applying low pressure (3-5 atm) to sample and reference solutions in calorimetry cells simultaneously, the change in energy to regulate the temperature is measured. PPC is used to determine thermal expansion coefficient, volume transitions of lipid transitions and molecular compressibility.[29,30]

Fourier transform-infrared (FT-IR) spectroscopy used to get information about sub-molecular structure of liposomes.[26]

To analyze bio membranes, complimentary approaches such as scanning tunnelling microscopy (STM), atomic force microscopy (AFM), and high-resolution transmission electron microscopy (TEM) are also used. In virtually all circumstances, complementary probes are very effective for investigating surfaces. While FT-IR and DSC investigations can be performed at a wide variety of temperatures, procedures that determine the drug's partition and position within the membrane, as well as membrane integrity, should be performed at 37 ℃ to better replicate physiological settings. The fluidity of the membrane, for example, is modified by temperature; hence the experimental temperature may have an impact on drug partition.

RESULTS AND DISCUSSION

Artificial lipid membranes can be created, stabilized and functionalized in a variety of ways and widely used in the development of sensors, drug discovery, drug testing, research probes, and molecular tools to better understand the mechanisms underlying the mechanics of biological membranes.[4]

Model lipid membranes can interact with many biomolecules like proteins, drugs, ion channels, enzymes, and DNA.[24,13] Vesicles with an asymmetric shape could be utilized to encapsulate drugs. The development of vesicles with an outer leaflet lipid composition compatible with the surrounding biological milieu and an inner leaflet lipid composition suited with the encapsulated medicine would be made possible by the availability of asymmetric vesicles,

especially big vesicles.[18] Giant vesicles are used as carrier for encapsulating proteins, drugs to carry out specific cell processes, as biochemical reactors for macromolecule syntheses.[21]

LUV liposomes are the best choice for drug membrane interaction.[19] Lipid biomimetic models are helpful in vitro tools for examining membrane properties and medication interactions. These models can mimic the lipid content of cell membranes in both healthy and diseased conditions, which enables them to predict drug transport, distribution, accumulation, efficacy, and toxicity in vivo because they can imitate the lipid composition of cell membranes under both healthy and pathological situations.[32,19,11] Liposomes are an effective way to deliver small gold nanoparticles intracellularly. The cellular absorption of tiny gold nanoparticles will increase when liposomes are used as a carrier.[33] A complex of liposomes and gold nanoparticles has practical uses as nanomedicines with both therapeutic and diagnostic potential.[34]

Liposomes being biodegradable, non-toxic and non-immunogenic has more advantages over other delivery systems.[25] Liposomes are thus extremely adaptable biomimetic models that may be employed in a wide range of investigations. Liposomes can also be used to research membrane processes like fusion, membrane trafficking, cell adhesion, molecular recognition, and pore creation. One of the most advanced drug delivery methods now in use is with liposomes. As liposomes are believed to be the greatest carriers for the introduction of a wide range of agents, including anticancer drugs, antibiotics, anti-inflammatory, genes, and antifungal agents, liposome research has gained popularity in the pharmaceutical, biological, and medical industries.[35]

CONCLUSION

Phospholipids are amphipathic in nature as they possess hydrophilic (water-loving) polar phosphate ester head and two hydrophobic (water-hating) non-polar acyl tails so when they come in contact with water, they will form bilayer or micelles or vesicles (liposomes). A variety of methods are used to prepare model lipid membranes that are freely suspended in aqueous medium. By increasing cholesterol concentration, rigidity of lipid membrane increases. Lipid bilayers can act as interface for biochemical reactions. Liposomes can encapsulate biomolecules such as drugs, proteins and gold nanoparticles. Using model membranes, researchers discovered that the lipids surrounding receptor molecules have a significant impact on biomolecular interactions, increased chain length lipids, for example, reduce binding ability and may influence binding moieties exposure.

Declaration of Competing Interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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