INTRODUCTION

The development of different drug delivery systems have conveyed significant innovations in molecular biology and other fields of biological sciences leading to unique prospect for delivering nanoparticles of various dimensions to biological systems. In recent years, substantial considerations have been bred in formulating nano colloidal systems for drug or gene delivery applications.[1] Dendrimers are one such type of nanoparticle, having a high degree of branching, low polydispersity, nanometer size range and controllable architecture.[2] Polyamidoamine (PAMAM) dendrimers, as used in this research study, have demonstrated various biopharmaceutical applications such as antibacterial agents, antiviral drugs, as well as drug or gene delivery vehicle. Potential application of dendrimers as drug delivery vehicle and therapeutic agent require critical understanding of their interaction with the liposome bilayer. However, the molecular and physical complexity of cell membranes have limited the detailed kinetic and mechanistic investigations of dendrimer interactions with living cells.

On the other hand, it should be noted that liposomes have already been applied as cell models for investigating drug transport from unilamellar liposomes when the latter were employed as drug delivery systems.[3] Many reports have covered the interactions between dendrimers and biological membranes. [4] But the main objective of the present work is to study the interaction between dendrimer and liposome and to prepare new drug delivery systems of dendrimer-liposome complex. Although there are different reports on the interaction studies between dendrimers and liposomes,[5, 6, 7] however, limited comprehensive and systematic studies have been carried out previously in order to assess the impact of dendrimer generation, concentration as well as the variation of the liposome type.

1. Amphiphiles:

Amphiphiles are the class of compounds having special structural features as it possess the hydrophilic (water loving) and hydrophobic part (fat loving) shown in Figure 1.[8] The hydrophilic portion generally consists ions (anionic or cationic or uncharged polar groups) whereas lipophilic parts are generally long chain hydrocarbon, such in the form of $CH_3(CH_2)_n$, with $n > 10$.

Carboxylate (COO^-), sulphate (SO_4^-), sulfonate (SO_3^-), phosphate (PO_4^-), etc., are the anionic and amine (NH_4^+) is the cationic group attach to lipophilic hydrocarbon chain. Alcohols are the class of polar uncharged group often attached with long chain hydrocarbon, such as diacyl glycerol. In aqueous medium they spontaneously form self-asembled structure.

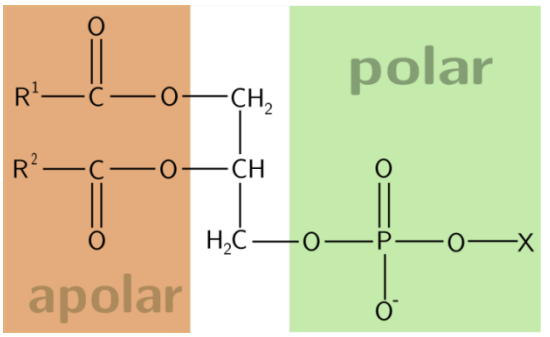


Figure 1: General structure of an amphiphile.

Because of the hydrophilic-lipophilic behavior, it has diverse range of applications: in detergent, paint, pharmaceutical industries to food, etc. The variety of self-assembled structures make the amphiphiles to be useful in large number of areas.[9,10] Throughout the present work, mixed lipid bilayer is the most enlighten/highlighted aggregate structure. Naturally occurring anionic and zwitterionic phospholipids with cholesterol have been used for the preparation of liposome.

1.1. Self-assembly and Aggregate structure: Self-assembly is a spontaneous process, constructs aggregate having various morphology from a variety of chemical building blocks. Being an aggregated form, the process of self-assembly can include verity of complexity. It can be a simple process of dimerization of two simple building blocks or as complicated as a biological membrane. Among various building blocks, amphiphiles draw the attention most over the other as its superiority to form robust assemblies.

Amphiphiles consist with hydrophilic and lipophilic part and thus has dual preference for solvent. Due to their amphiphilicity, the polar head groups interact with water or polar solvent while the lipophilic part tends to stay out of contact with water and thus try to orient in the air water interphase. In water it first dissolves as a monomer, but above certain concentration level, they form micro-structure or assemblies to avoid the unfavorable solvent hating interaction between water and lipophilic parts. This process of self-organization is spontaneous and

accompanied by increased entropy. The increased entropy begins from water-hydrocarbon interaction when amphiphiles are present as monomer. As monomer it forces water molecules in

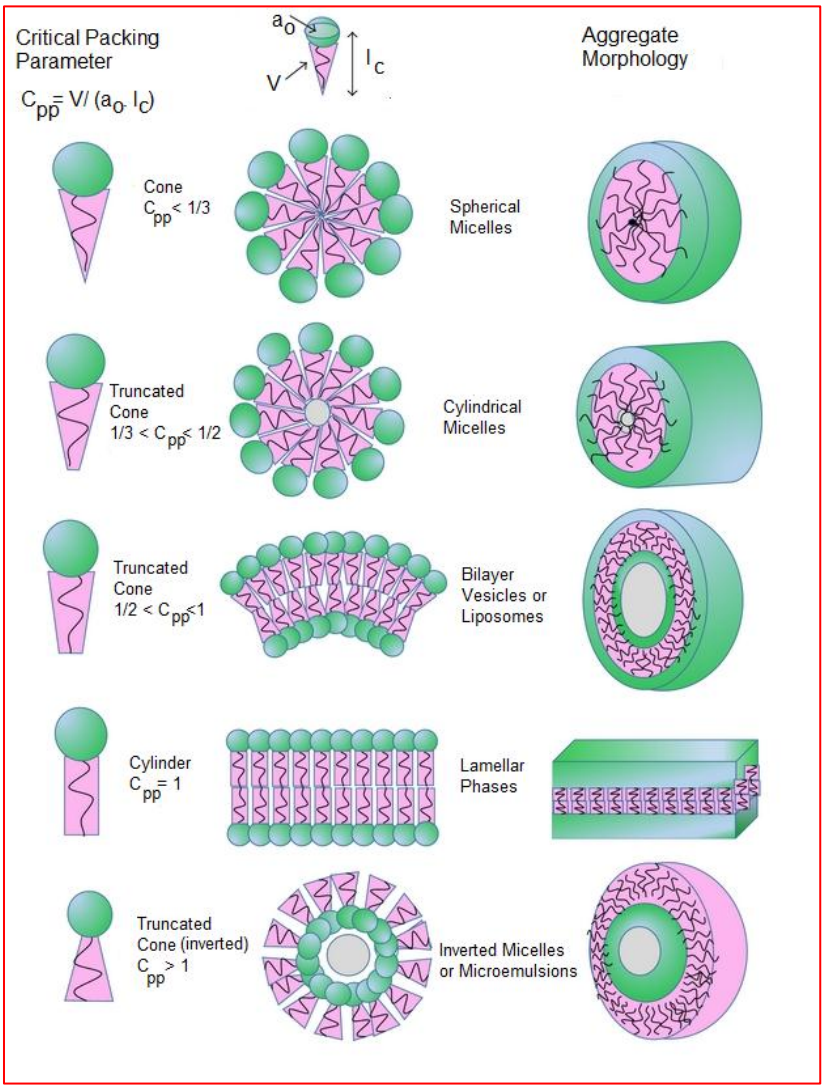


Figure 2: Aggregate structure of lipid assessed from critical packing parameter C_{pp}.

an order state around the lipophilic part. When concentration of amphiphiles is increased two possible scenarios is developed. Either they have to move at air-water interphase or to form aggregate structure. The brakeage of ordered water molecule enhances the process of entropy which leads to an overall gain of free energy that drives the amphiphiles to form aggregate structure by virtue of self-assembly. The aggregation of amphiphiles is generally driven by hydrophobic interaction, hydrogen bond, steric effect and electrostatic interaction

1.2. Critical Packing parameter and aggregates morphology:

The shape and the size, or better to say morphology of the aggregates not only depend on above mentioned forces, but also depend on the structure, molecular geometry and concentration of the amphiphiles, extent of hydration, pH and ionic strength of the of the dispersion medium. Critical packing parameter (C_{pp}) which takes into account parameters such as polar head group cross-sectional area (a_0), hydrocarbon chain length (l_c) and hydrophobic volume (V) is related as: [11,12]

$$C_{pp} = \frac{V}{a_0.l_c} \quad (1)$$

This parameter together holds the information about the geometrical structure of the aggregates. With the increasing C_{pp}, the structure of the aggregated form could be spherical (C_{pp}< 1/3), cylindrical (1/3< C_{pp}< 1/2) and lamellar (C_{pp} = 1). In case of vesicles or liposome, the range of C_{pp} is 1/2 to 1 with a inner cavity encapsulating dispersion medium. A summary of the aggregated structure predicted from critical packing parameter (C_{pp}) is represented in Fig 2. Lipids are the most common amphiphile to formulate the aggregate morphology.

2. Lipid:

The organic compounds, which are insoluble in water consisting of fats and oils, are called lipids. Chemical composition of these lipids includes carbon, hydrogen, and oxygen. Lipids belong to a diverse and large number of non-polar organic compounds having non-polar hydrocarbon chain or acyl chain attached with the polar head group. In aggregation, its head group exposed towards the polar solvent medium, the non-polar acyl chain being shielded inside. They deliver high energy and execute three vital biological functions in the body: to arrange for structure to cell membranes, to accumulation energy, and to role as signaling molecules.[13, 14]

2.1. Biological function of lipids:

Lipids play important roles in the normal function of the body:

1) Lipids works for cell membranes and organelles to serve as structural building materials.

2) Lipids greatly afford energy for the organisms than carbohydrates or proteins.

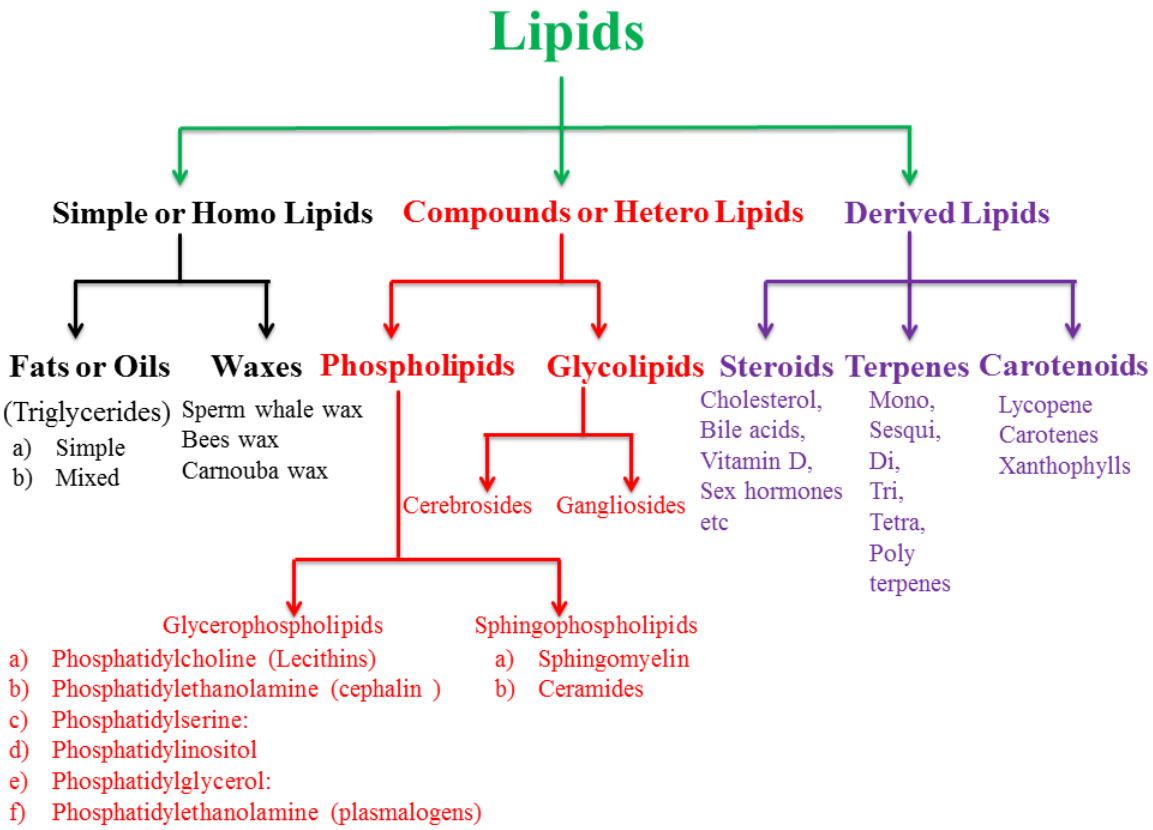
3) To serve as signaling molecules and molecular messengers in the body.

4) Lipids act as biomarkers for certain diseases and play some important role in chronic disease and genetic modification.

2.2. What are the food sources of lipids?

The lipids or fat are the macronutrients important in nutrition. They are mainly produced in dietary food and fisheries, and other foods. The delicious sources of diabetes fat include soybean, nuts and seeds, olive oil, and avocados, which contain essential fatty acids. Contrary to popular belief, lipids are an important part of our diet, and it is necessary to feed a minimum amount. However, many health problems are associated with excessive dietary fat, such as obesity, heart disease, and cancer.[15,16,17]

2.3. General classification and structure of lipids: Lipids are broadly classified into simple, complex and derived which are further subdivided into different groups. The lipid classification is shown in the chart below:[18]



2.3.1. Simple lipids: Simple lipids are esters of fatty acids with different alcohols. Even number of carbon atoms may contain saturation or unsaturation in the fatty acid chain. They are classified as triglycerides (fats and oils) and waxes. The esters of fatty acid with combination with glycerol are called triglycerides (fats and oils). Fat and oil are extensively dispersed among plants and animals. Triglycerides generally known as neutral fat, some of them are insoluble in water and non-polar in nature. The triglycerides of saturated fatty acids, which are present in solid form at room temperature, are called fats. On the other hand, when glycerides are presented in liquid-sized form at room temperature, then it is called oil. They are the unsaturated fatty acids of triglycerides. The degree of unsaturation of fatty acid residue in fat is lower than that of oil. On hydrolysis of oil and fat gives long chain monocarboxylic acid and glycerol.[19,20]

Monoglycerides:

$R_1 = C_nH_{2n}O$

$R_2 = R_3 = H$

Diglycerides:

$R_1 = R_2 = C_nH_{2n}O$

$R_3 = H$

Tryglycerides:

$R_1 = R_2 = R_3 = C_nH_{2n}O$

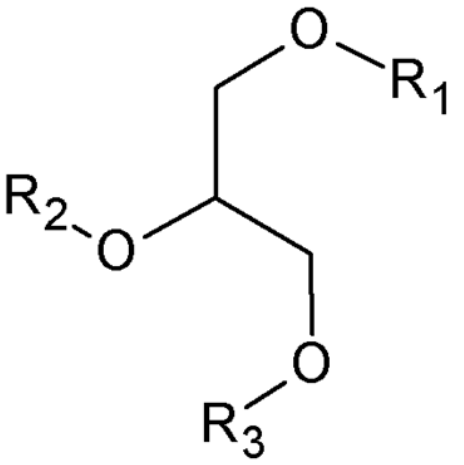


Figure 3: General structure of glycerides.

Waxes are the ester of long chain saturated or unsaturated fatty acid with long chain monohydric alcohol. Waxes are considered as the lipid which on hydrolysis produces long chain fatty acids and long chain alcohols. The fatty acid range between C_{14} and C_{36} and the alcohol rang between C_{16} and C_{36}. Because of the long hydrocarbon chain, it has high molecular mass with relatively high melting point. It is insoluble in water but soluble in organic, non-polar solvents.

Waxes→ hydrolysis→ long chain fatty acids + long chain alcohols

In the case of plankton (aquatic plants) waxes is the chief storage form of metabolic fuel. Biological waxes have different kinds of applications in the pharmaceutical, cosmetics and other industrial level.

2.3.2. Compound or complex or hetero lipids: Complex lipids are commonly found in plants, bacteria and animals. They are the major ingredients of cell membranes but are established also in circulating fluids.[21] On the basis of composition complex lipids are also categorize as: 1) Phospholipids 2) Glycolipids.

2.3.2.1. Phospholipids: The complex lipids comprising phosphoric acid is named as phospholipids. It contains nitrogen base and other elements and they are generally located in the brain and nervous tissue. They also exist in cytoplasmic membrane because of which they are elaborate in cellular activities. [22] They are classified as i) Glycerol phospholipids and ii) Sphingo phospholipids.

2.3.2.1.1. Glycerophospholipids: They are most abundant phospholipids. They comprise 2 fatty acids esterified with first and second hydroxyl group of glycerol. The third hydroxyl group of glycerol makes an ester linkage with phosphoric acid. Glycerophospholipids are more allocated into following groups shown in Figure 4.

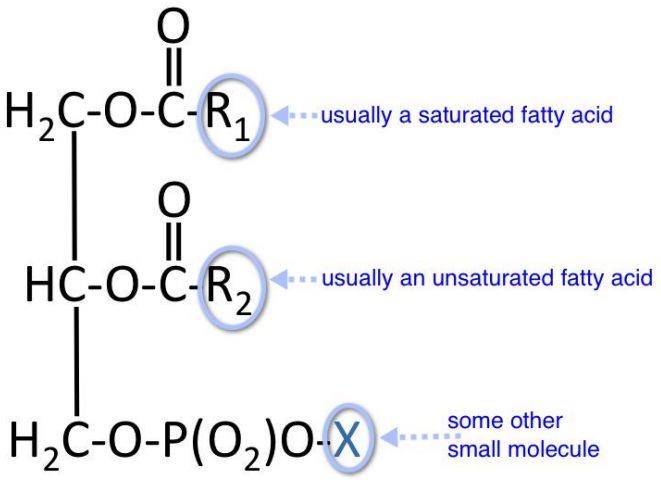


Figure 4: General structure of glycerophospholipid.

Phosphatidylcholine (Lecithins): It is extensively circulated in the body mostly in the liver. [23] In these phospholipids, collin is exist as the base which inhibits the deposition of abnormal fat in the liver. It is also the major elements of acetyl choline.

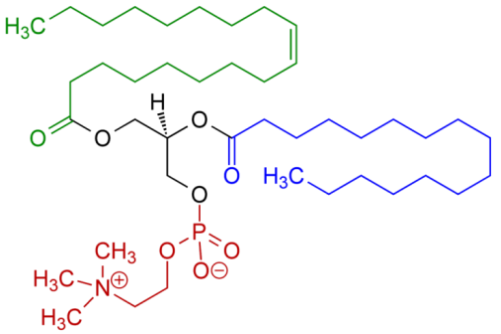


Figure 5: Structure of phosphatidylcholine (SPC).

Phosphatidylethanolamine (cephalin): They are manufactured by the addition of cytidine diphosphate-ethanolamine to diglycerides, liberating cytidine monophosphate. The base ethanolamine is present which composed with lecithin and associated with the brain. It is also exist in RBC.

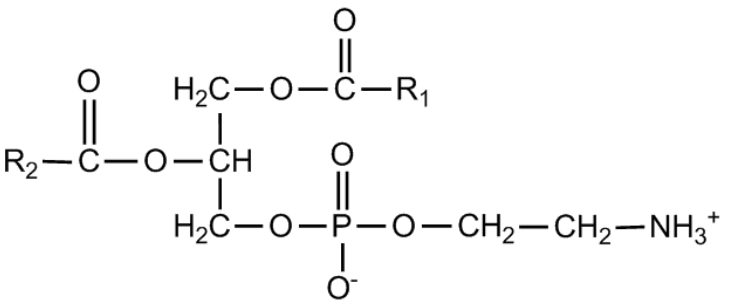


Figure 6. General structure of phosphatidylethanolamine.

Phosphatidylserine: It contains of two fatty acids connected in ester linkage to the first and second carbon of glycerol and on the other hand, serine is connected through a phosphodiester linkage to the third carbon of the glycerol. Phosphatidylserine works for blood coagulation. The base amino acid serine is present. It has similar function with lecithin.

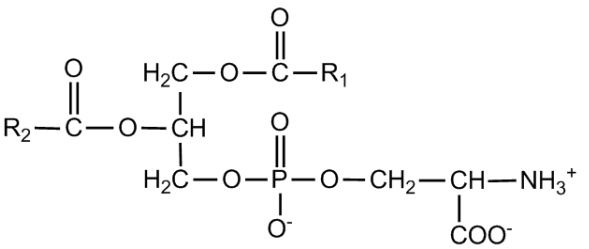


Figure 7: General structure of phosphatidylserine.

Phosphatidylinositol: It contains inositol as the base instead of nitrogen base. It is mainly found in plants and nervous tissue. Some hormones such as oxytocin, vasopressin are modified through phospholipids.

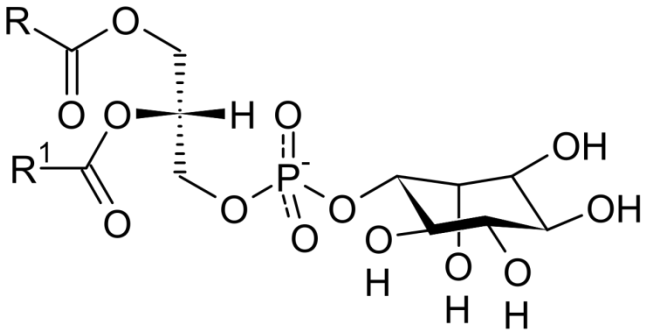


Figure 8: General structure of phosphatidylinositol.

Phosphatidylglycerol: In phosphatidylglycerol, two molecules of phosphatic acids are connected by the accumulation of glycerol to phosphate groups. The common structure of phosphatidylglycerol contains of a L-glycerol 3-phosphate backbone which are ester bonded to either saturated or unsaturated fatty acids on carbons 1 and 2. The head group substituent glycerol is bonded through a phosphomonoester. It is originated in the inner membrane of mitochondria.

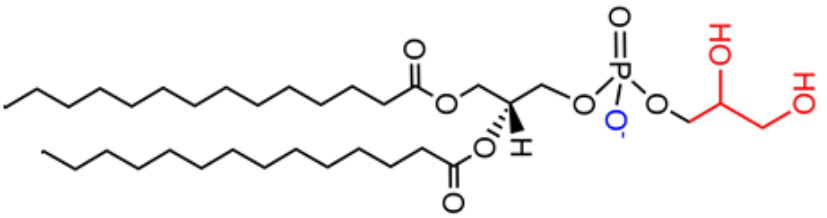


Figure 9: Structure of phosphatidylglycerol.

Phosphatidylethanolamine (plasmalogens): In these phospholipids, fatty acids are linked with carbon and glycerol by an ester linkage. Generally, unsaturated fatty acid is linked at carbon.

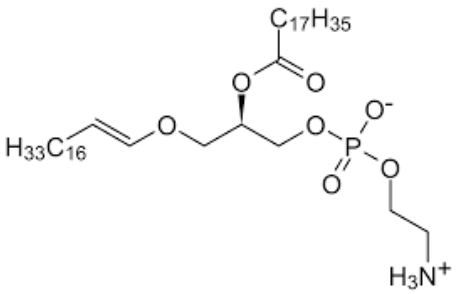


Figure 10: Structure of plasmalogens.

2.3.2.1.2. Sphingophospholipids: They are formed by the combination of long chain fatty acids with long chain amino alcohol sphingosine or one of its derivatives and the polar bond of alcohols. They are originate in the brain or nervous tissue.[24,25] On the basis of composition

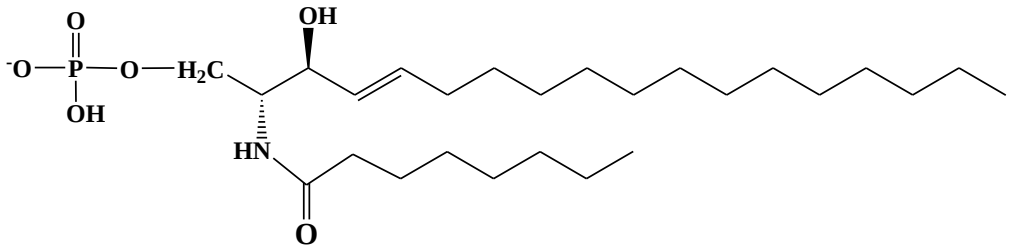


Figure 11: Structure of sphingophospholipid.

Sphingolipids are two kinds, one is sphingomyelin and another is ceramides. Sphingomyelin is the humblest and best abundant lipid which holds phosphocholine or phosphoethanolamine as the polar head region. It is founded in the animal cell as myelin sheath which covers the nerve cell. Ceramides consist of sphingosine molecule and fatty acids. It is equable of sphingosine molecule and fatty acids:

2.3.2.1.3. Functions of phospholipids: Some essential functions of phospholipids are:

1) Phospholipids in the relations with proteins make the structural constituents of a membrane and control membrane permeability.[26]
2) Phospholipids in the mitochondria are accountable for preserving the conformation of electron transport chain mechanisms and thus cellular respiration.[27, 28]
3) Phospholipids contribute in the absorption of fat in the intestine.[29, 30]
4) Phospholipids are important for the production of different proteins *i.e.* lipoproteins and thus take part in the transport of lipids.[31,32]
5) Accumulation of fat in the liver can be stopped by phospholipids; hence they are considered as the lipoprotein factor.[33, 34]
6) Phospholipids take part in the reverse cholesterol transport and thus help in the elimination of cholesterol from the body.[35, 36]
7) Cephalins are the crucial group of phospholipid contribute in the blood clotting.[37,38]
8) Phosphatidylinositol are participated in signal transmission through membrane.[39, 40]
9) Phospholipids performance as an agent for pull down surface tension. [41, 42]

2.3.2.2. Glycolipid: The lipids with combination of carbohydrates attached by a glycosidic bond or covalently bond are called glycolipids. They have no any phosphate group. It comprises C 24 fatty acid molecule like cerebric acid, lignoceric acid. It is generally two types: a) Cerebrosides b) Gangliosides. The simplest form of glycolipids is called Cerebrosides. Glucose and galactose are remains as carbohydrate residue. Generally, 2-20 units carbohydrate chain is present in cerebtosides. On the other hand, the derivative of cerebroside are called gangliosides and they contains one or more molecule of N- acetyl neuraminic (NANA). It is mostly present on grey matter of the brain and neutralized tetanus toxins.[25]

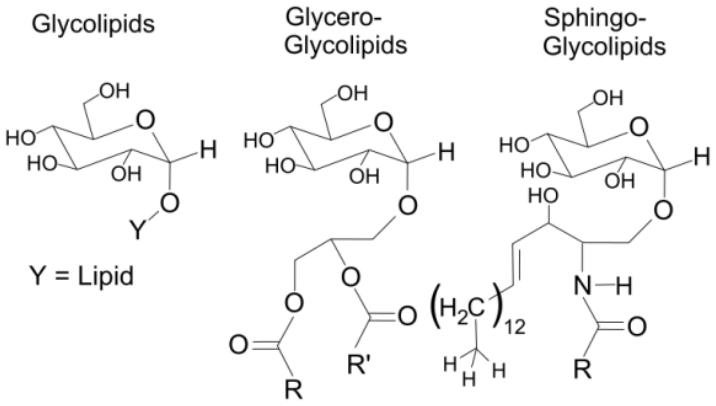


Figure 12: General structure of sphingophospholipid.

2.3.2.2.1. Function of glycolipids:

1) Glycolipids are important components of all membranes in the body.[22]
2) They are situated mainly in the outer part of the plasma membrane where they can interact with the external part of cellular environment.[43]
3) They play an important role for the cellular interaction, growth, and development.
4) They act as an antigen which decide blood group of an individual. [44, 45]
5) They function as cell surface receptors for some viruses, like cholera and diphtheria. [46]
6) It produce the structural constituent of nervous tissues.[47]

2.3.3. Derived Lipids: By the hydrolysis of simple and compound lipids derived lipids are prepared. Fatty acids, alcohols, monoglycerides, diglycerides, steroids, terpenes and carotenoids are the main components of derived lipids. This lipid contains cyclopentane rings which are the complex fat soluble molecule with four fused rings. Steroids are compounds holding a cyclic

steroid nucleus that is Cyclopentanoperhydrophenanthrene. It involves of a phenanthrene nucleus to which a cyclopentane ring is committed. There are a number of steroids in the biological system. These consist of cholesterol. vitamin D, bile acids , sex hormones etc. If the steroids enclose one or more hydroxyl group, it is famous as the sterol (means alcohol).

The best common sterol create in animal tissue is the cholesterol. The cholesterol contains of Cyclopentanoperhydrophenanthrene ring with OH group at carbon number of 3 and the double bond among carbon number 5 and 6. The state at room temperature is solid and insoluble in water but rarely in higher plants. The cell membrane of eukaryotes contains of cholesterol. It is the precursor of a large number of essential steroids comprising bile acids, sex hormones, vitamin D etc. Cholesterol is manufactured by the liver and secreted in the blood.[48, 49] It is likewise circulated in serum and plasma. The normal blood cholesterol level contains 130-250 mg/dl. But cholesterol level is studied by metabolic functions which are prejudiced by nutrition,

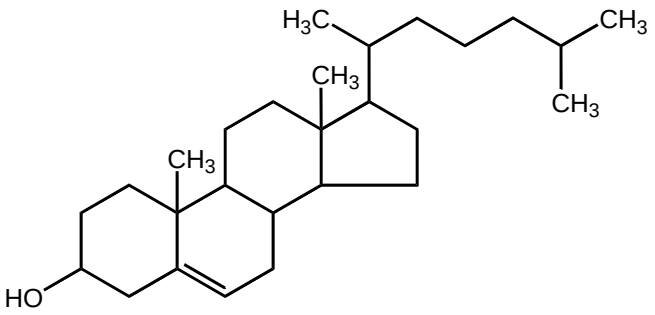


Figure 13: Structure of cholesterol.

heredity, endocrine functions, liver, kidney etc. If the cholesterol level is lower than normal value then it is called hypercholesterolemia.[50] This situation is understood in hyperthyroidism acute infection, anemia, malnutrition etc. If the cholesterol level is higher than the normal value then it is called hypercholesterolemia.[51] This condition is seen in hypothyroidism poison, less controlled diabetes mellitus, chronic hepatitis, nephrotic syndrome, obstructive Jaundice. Thus the purpose of blood cholesterol level is the significant diagnostic parameter from the medicine technology.

2.3.3.1. Function of steroids/ cholesterols:

1) Cholesterol is an important component of cell membrane that modulates the fluidity/rigidity of membrane.[52, 53]

2) Cholesterol acts as an insulating guard for the transmission of electrical impulses in the nerve systems.
3) Steroids/Cholesterol comprise the bile acids, sex hormones and vitamin D.[54]
4) Cholesterol acts as an essential role in fertility for female body. At low concentration of cholesterol in female body, it may lead to sterility. [55]
5) The cardiovascular diseases controlled by cholesterol.[56, 57]
6) Steroids preserves the serum calcium level.[58, 59]

2.3.4. Description of some others phospholipids relevant to the present study: The phospholipids which were used in this research work are given bellow:

2.3.4.1. Zwiterionic phospholipids: Generally phospahtidylcholine (PC) lipids are zwiterionic in nature. PC is derived from natural and synthetic sources. It is also known as lecithin. The two glycerol moieties attached with fatty acyl chain via ester linkage and the remaining one hydroxyl group linked with phosphate ion, which is further, connects with choline group. They are easily available in egg-yolk and soybean but hard to extract form bovine heart and spinal cord. Lecithin is chemically inert and neutral charge. Besides chemical properties, the low cost of lecithin made it as one of the major phospholipid in liposome preparation. Lecithin from vegetable oils contains one or multiple unsaturation in the acyl chain. On the other hand lecithin from animal fat produces fully saturated hydrocarbon chain. Soy phosphatidylcholine (SPC) and dipalmitoylphosphatidylcholine (DPPC) are the two important PC lipids. In case of SPC lipid, one hydrocarbon chain is unsaturated.

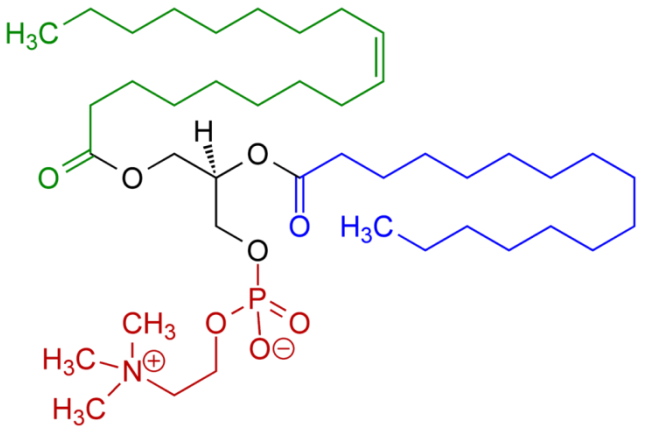
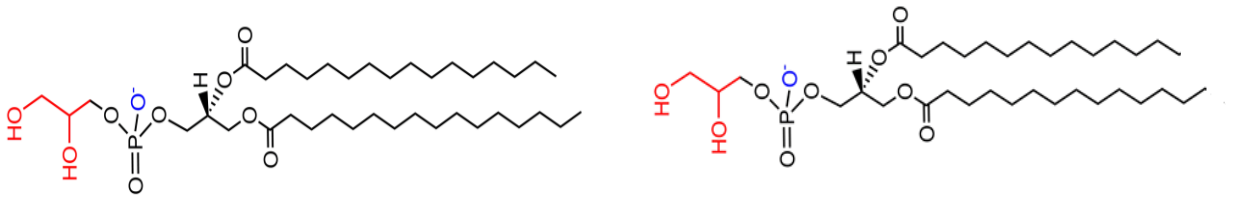


Figure 14: Structure of soy phosphatidylcholine (SPC) lipid.

2.3.4.2. Negatively charged phospholipids: Generally Phosphatidyl glycerol (PG) are anionic in nature. They found in pulmonary surfactant. It is consisting of L-glycerol 3-phosphate backbone. Two fatty acyl chains either saturated or unsaturated are connected with the ester linkage. The head group region constituted with glycerol molecule bonded with phosphomonoester.



DPPG DMPG

Figure 15: Structure of DPPG and DMPG.

Anionic lipids are freely accessible in nature and they are easily manufactured by semi-synthetically form other lipids by means of phospholipase D in the existence of glycerol. These kinds of phospholipids hold uninterrupted negative charge all over the place of body pH range. 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) and 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) are the two important anionic lipids. They are only differing by chain length.

2.3.4.3. Phosphadityl alcohol: They have the same kinds of dipalmitoyl groups, but the phosphate group is directly connected to alkyl group. Phosphadityl alcohols are generally physiologically scare.The small and anionic head group of phosphadityl alcohol has large effect on their membrane properties. They are highly portent promoters on membrane bilayer and their trans bilayer movement is three times higher than that of other naturally occurring phospholipids. 1,2-dipalmitoyl-sn-glycero-3-phospho ethanol (DPPEth) is an example of phosphadityl alcohol.

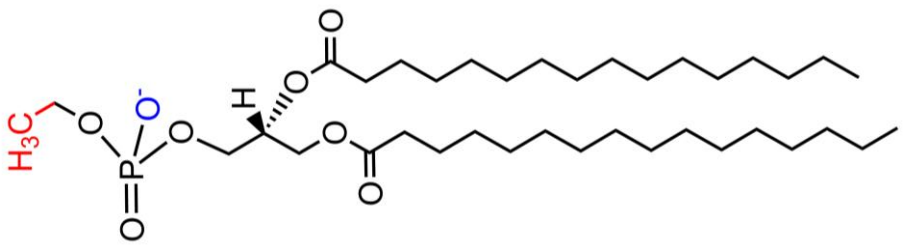
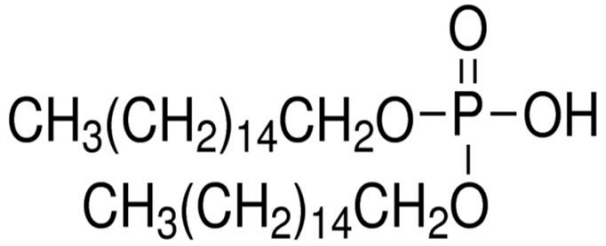
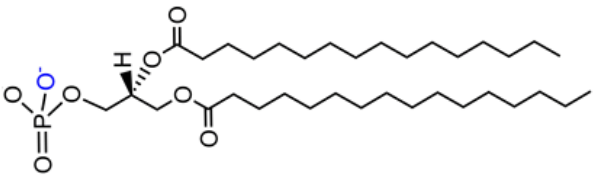


Figure 16: Structure of 1,2-dipalmitoyl-sn-glycero-3-phospho ethanol (DPPEth).

2.3.4.4. Others anionic lipids: 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPP) and dihexadecylphosphate (DHP) are the two another negatively charged phospholipids. In case of dihexadecylphosphate (DHP), the phosphate group is directly connected to the long chain hydrocarbon. On the other hand DPP has only phosphatidyl group with two palmitoyl chain length.



DHP



DPP

Figure 17: Structure of DHP and DPP phospholipids.

3. Aggregation behavior of amphiphiles: On the basis of concentration and structural complexity of the lipid (amphiphile) form aggregates like micelles, bilayer or liposomes or other form of lipids.

3.1. Micelle formation: In an aqueous medium, the polar head groups of amphipathic lipids expose the polar towards aqueous environment, while the hydrophobic tail groups decrease their contact with water and have a greater tendency to group organized called as lipid aggregates. Depending on the concentration and structural complexity of the lipid, these lipid aggregates constitute either of micelles, bilayer or liposomes. Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions are shown in Figure 18.[60] Fatty acids form micelles generally contain a single hydrocarbon chain instead of two hydrocarbon tails. This permits them to configure into a spherical shape for lesser steric hindrance within a fatty acid. For instance, salts of fatty acids (such as sodium palmitate, a component of soap), which contain only one chain can easily form micelles.

3.1.1. Biological importance of micelles: Micelles are essential as they transport the weakly soluble monoglycerides and fatty acids to surface of enterocyte or intestinal absorptive cells (are simple columnar epithelial cells found in the small intestine) where fats are absorbed.[61] In the digestive tract, triacylglyceride is hydrolyzed through the lipase enzyme, to produce two free

fatty acids and a monoglyceride. These free fatty acids and monoglycerides along with bile salts and phopholipids create micelles.[62, 63] Also, micelles enclose fat soluble vitamins and cholesterol. Micelles continuously break down and reconstitute, feed a small group of monoglycerides and fatty acids which are present in medium.

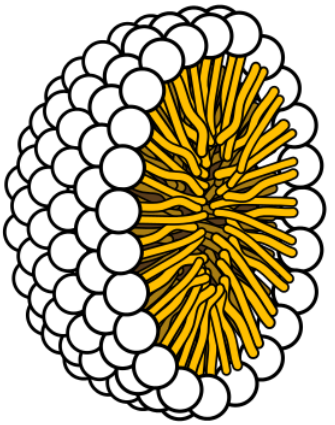


Figure 18. Schematic diagram of micelle.

3.2. Monolayer formation: Due to amphipathic nature, lipid molecules are insoluble in individually polar and apolar media. In these media, they produce monomolecular films termed as monolayers (shown in Figure 19), that decrease the surface tension. The properties of lipid

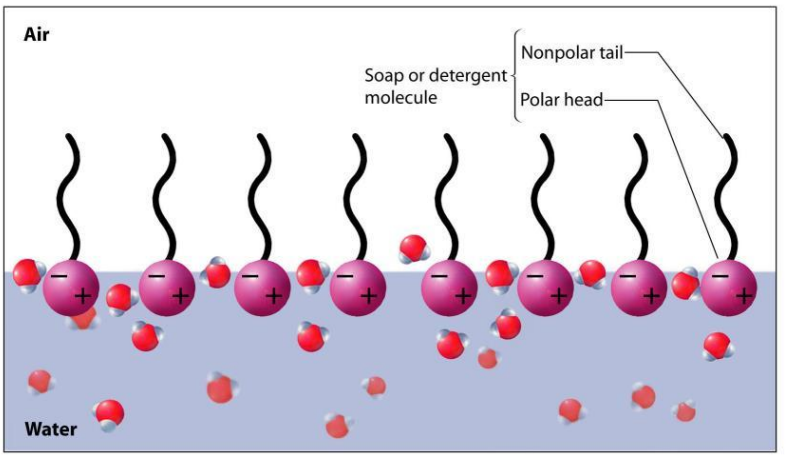


Figure 19: Structure of lipid monolayer at the air-water interface.

monolayers differ with their surface density. For example, when the density is higher, the surface tension becomes lower at the interface. At a definite high surface density further decrease of the surface tension is not likely: the monolayers turn into unbalanced at the interface and collapse.

Besides being of fundamental interest for surface science, lipid monolayer collapse is crucial for sustaining low surface tension at the gas-exchange interface in the lungs for the duration of breathing.[64, 65]

3.3. Bilayer formation: Phospholipids and glycolipids unable to generate micelles as happen in fatty acids. Because phospholipids and glycolipids comprise two hydrocarbon chains which are so large that cannot assemble them into a micelle. So the formation of lipid bilayers from phospholipids is a quick and spontaneous manner in water. A lipid bilayer is also termed as a bimolecular sheet. The hydrophobic tails of each individual plane interact with one another, constituting a hydrophobic core which acts as a permeability barrier. The interaction between hydrophilic head groups with water on both side of the bilayer is predominant. The two opposite sheets are known as leaflets, are shown in Figure 20.

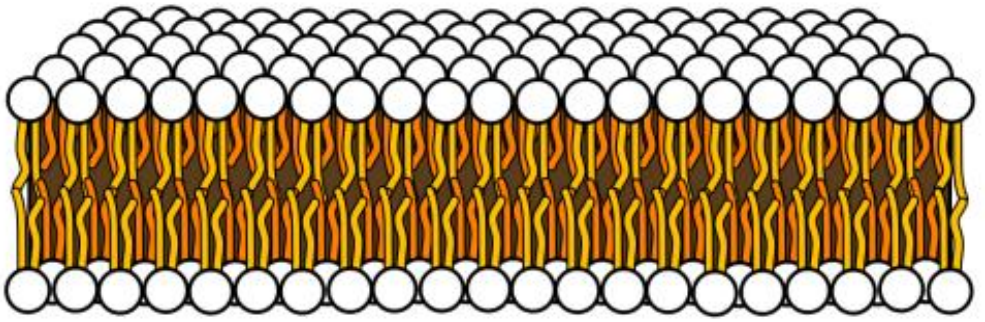


Figure 20: Structure of planar lipid bilayer.

3.3.1. Biological importance of lipid bilayers:

The development of bilayers as a replacement for of micelles by phospholipids has vital biological importance. A micelle is a smaller structure, generally less than 20 nm (200 Å) in diameter. On the contrary, a bilayer sheet can be of larger dimensions, e.g. a millimeter (106 nm or 107 Å). Phospholipids and related compounds are important membrane constituents as they easily form extensive bilayer sheets. Lipid bilayer sheet associated with proteins in biological membranes contains lipid of various structural complexities depending upon the membrane source.[66]

3.3.2. Hydrophobic interactions in lipid bilayer:

The formation of lipid bilayers is an energetically preferred and spontaneous process when the phospholipids are placed in an aqueous medium. This is called as the hydrophobic effect or hydrophobic interactions, shown in Fig 16.[67, 68] Lipid bilayers stabilized through the variety of forces which promote molecular interactions in biological systems which are followed as

1) Molecules of water are released from the hydrocarbon tails of membrane lipids as these tails become sequestered in the nonpolar core of the bilayer.[69]

2) In addition, van der Waals attractive forces among the hydrocarbon tails favor close packing of the tails.[70]

3) There are electrostatic and hydrogen bonding attractions between the polar head groups and water molecules.[71]

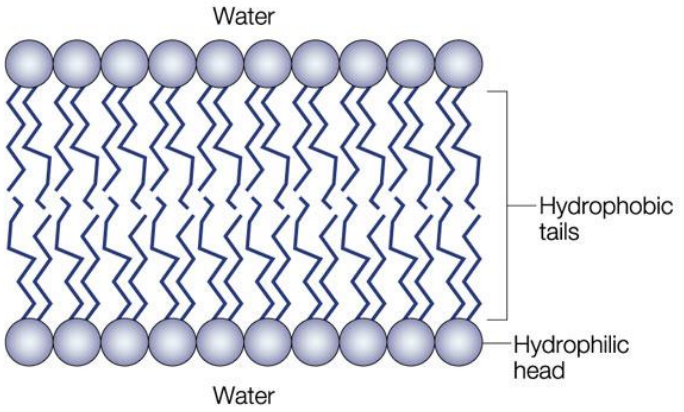


Figure 21: Schematic diagram of hydrophobic interactions in lipid bilayer.

3.3.3. Properties of lipid bilayer:

Because of hydrophobic interactions, lipid bilayer inherits unique properties.

1) Lipid bilayers have an inherent tendency to be extensive.[72]
2) Lipid bilayers tend to close on themselves so that there are no edges with exposed hydrocarbon chains and they constitute compartments.
3) Lipid bilayers are self-sealing since a hole in a bilayer is energetically unfavorable.[73],

3.4. Cubosome formation:

The liquid crystalline form nano-structured cubic states of lipids are called cubosomes. The monooleate, or other amphiphilic large molecules with the similar behaviour were used to form cubosomes. They are formed at definite temperatures where lipid bi-layer form 3-D structure with minimal surface area developing a compacted structure with bicontinuous areas of water and lipid, shown in Figure 22. Cubosomes remain in different phases of cubic structure (like P-surface, G-surface and D-surface for primitive, gyroid and diamond) arrangements correspondingly. This types of structure ultimately use as different drug delivery against verity therapeutic agent or microbial disease. [74]

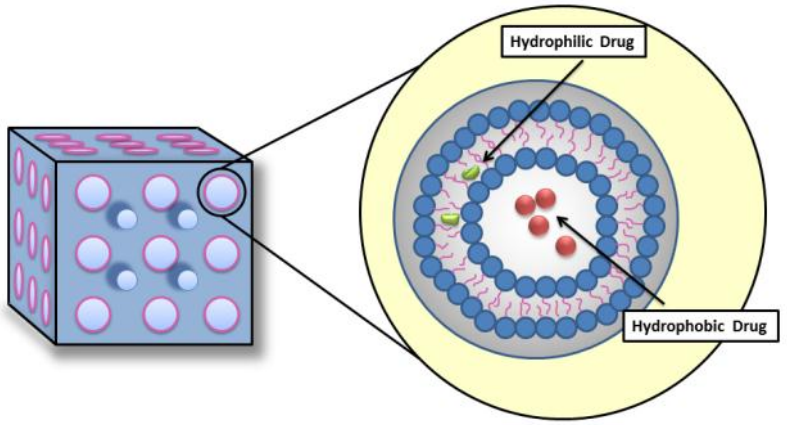


Figure 22: Schematic diagram of cubosome.

4. Further details of liposomes:

A liposome is a spherical vesicle having at least one lipid bilayer. Liposomes are artificially prepared spherical shaped vesicles consisting one or more phospholipids bilayer. Amphiphilic molecules like lipids, cholesterol etc. are the main constituting molecules in the liposome bilayer. The word liposome comes from Greek words: "*lipo*" means fat and "*soma*" means body.[75] Liposomes were first describe in the year of 1961 by British haematologist Alec D. Bangham . Today, liposome become one of the important tools in different scientific discipline, including mathematics, theoretical physics, chemistry, colloid science, biochemistry and in various clinical trial. The representative image of liposome is given below in Figure 23.

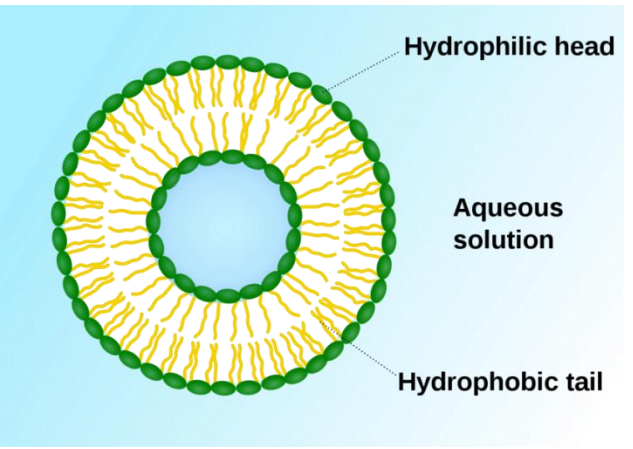


Figure 23: Schematic structure of liposome.

Due to their unique hydrophilic - lipophilic environment, it can host hydrophilic drug into the polar head group region or into the interior whereas lipophilic drug could be trapped inside the hydrocarbon region, *i.e*, acyl chain region.[76, 77] Thus artificially prepared liposome become one of the important agents for improving the delivery of the large number of drugs: enzyme vaccines, genetic material, drugs against microbial agents, anticancer drug, antifungal drugs, peptide hormones etc. [78,79, 80,81]

In many cases with many drugs, the direct oral administration may always not leads to the better result. Most of the drugs are not good enough to tolerate the resistance of enzymes in our mouth and stomach. Bile salt, intestinal flora that produce in our body, alkaline solution, digestive juices and free radicals in human body etc are smart enough to reduce the efficacy of the drug and may promote the side effect. Here liposome made its attention as a protecting vehicle for the drug and transfer it to the desire cell or tissue with controlled release. [82, 83] It also draws attraction because of its unique inert behaviour towards the entrapped drug (hydrophilic and lipophilic), ease of biodegradability, non-toxicity and improved bio-distribution of the drug with fewer side effects.[84, 85] The life time of the drug encapsulated inside of liposome also get increase as it shields the drug from different pH in human body system.

Liposomes act as drug carrier for management of nutrients and pharmaceutical purpose. By disrupting the biological membranes through sonication process liposomes are prepared. [86, 87, 88] The main composition of liposomes are phospholipids, mainly the phosphatidylcholine, besides other lipids, like egg phosphatidyl ethanolamine also used for preparation of liposome as they

easily form lipid bilayer structure. These are some different kinds of liposomes: multilamellar liposomes/vesicles (MLV), small unilamellar liposome/vesicles (SUV), the large unilamellar liposomes/vesicles (LUV), and the cochleate liposomes/vesicles. In case of MLV a number of lipid bilayers present and on the other hand for SUV have one lipid bilayer. A fewer anticipated form of liposomes is multivesicular liposomes in which one vesicle surrounds one or more smaller vesicles. Liposomes and lysosomes are two different things.

4.1. Liposome formulations: Liposomes are made by the hydration of thin lipid films or lipid cakes and stacks of liquid crystalline bilayers turn into fluid and swell. The hydrated lipid sheets separate during agitation and self-close to produce large multilamellar vesicles (LMV) which inhibits the interaction between water and hydrocarbon core of the bilayer at the boundaries. When these elements have made, the size of these particles have reduced by sonic energy (sonication) or another mechanical energy (extrusion).

The main goal in liposome formulation method is to prepare drug loaded liposome bilayer or vesicles with acute accuracy. Narrow size distribution and long term stability of the dispersion are also the primary concern in choosing the method for liposome preparation. Generally all the methods involve the lipid film hydration followed by sizing of the particle and finally removing the non-encapsulated drug. There are two kinds of methods involve in liposome preparation: Passive loading mechanical dispersion method and active loading methods.

In passive loading mechanical dispersion method the drug either in aqueous phase or organic phase is encapsulated by adding it before or during liposome preparation. One would get high drug encapsulation efficiency by using this method. On the other hand in active loading method, a diffusion gradient of the ions or drugs around the inner and outer phase of the aqueous layer is generated. The diffusion of the drugs thus gets loaded into the liposome. The most common used methods for the preparation of the liposome are: thin-film hydration method, injection methods, sonication, membrane extrusion, micro emulsification, reverse phase evaporation method and calcium induced fusion method.

Properties of lipid formulations can vary depending on the composition (cationic, anionic, neutral lipid species), however, the same preparation method can be used for all lipid vesicles

regardless of composition. The different technique for preparation of liposomes are given bellow:

4.1.1. Thin film rehydration: It is one of the most simple and common method for the preparation of liposomes. The desire amounts of lipidic components were taken in round bottom flask chloroform, dichloromethane, ethanol and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v). A thin lipidic layer was formed by the evaporation of organic solvent under the

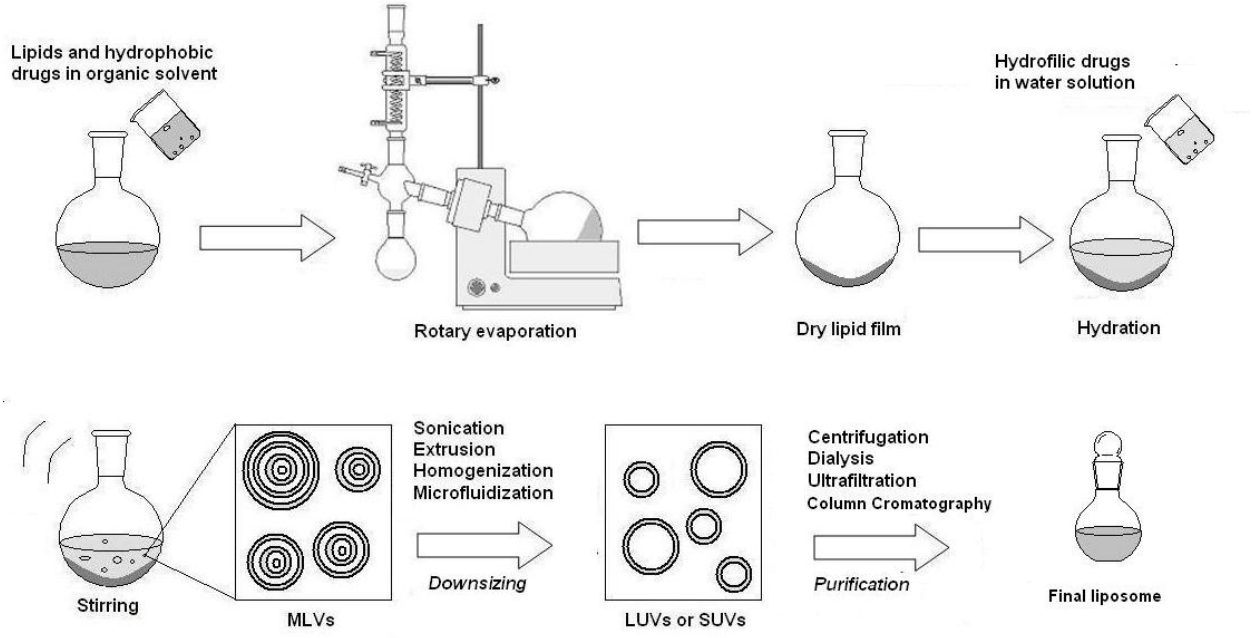


Figure 24: Thin film rehydration method for preparation of liposome.

influence of vacuum. The traces/minute quantity of organic solvent was further removed by the stream of nitrogen at temperature 4 ºC. Distilled water, phosphate buffer solution with varying pH and normal saline buffer solution could be used for the hydration of the thin film. It was hydrated at temperature 60-70 ºC for 1h. Total lipid hydration of the liposomal dispersion was done by allowing the lipid film to hydrate overnight. All kind of lipidic mixture can be used in this method for the preparation of the liposome. One of the important drawbacks of this method is the irregularity of the particle size which leads to the formation of heterogeneous system.

4.1.2. Ether injection: Lipids are dissolved in ether or ether/methanol combination solution. Then it is gradually mixed into an aqueous solution of encapsulated resources at 55-65 ºC with decrease pressure. Under the vacuum, the evaporation or elimination of the organic layer leads to

the liposome preparation. Reports suggest the drawbacks for this method are the exposure of the encapsulated material to the organic solvent and high temperature. Irregular particle size distribution also leads to the heteroginaty.[90]

4.1.3. Ethanol Injection Method: A lipid solution of ethanol is quickly injected to a large amount of buffer or distilled water. Instantly the liposomes are made in the mixture. The drawbacks of this method are the heterogeneity in liposome size. Liposomes are very dilute and the exclusion of the ethanol is very tough as it produce azeotrope with water. Formation of the azeotrope restricted this kind of liposomes to apply in biological system. [91, 92, 93]

4.1.4. Ultrasonic homogenization: This method includes the transformation of size of the liposomes. MLV prepared in thin film rehydration technique, is sonicated by bath or probe sonicator to prepare homogeneous dispersion of small unilamellar vesicles. The difficulties associated with this method are: low encapsulation of the drug due to lesser volume of each particle, the overheating could possibly degrade the liposomal dispersion. The tip of the probe sonicator tends to release Ti particle that may contaminate the dispersion. [88, 90]

4.1.5. Reverse phase evaporation: This method involves the formation of water-in-oil emulsion by means of brief sonication of a two phase system. The two phase system containing a lipidic solution of organic solvent (diethyl ether, isopropyl ether or mixture of isopropyl ether-cholroform) and aqueous phase. Phosphate buffer solution or citric-Na_2HPO_4 buffer is added to the aqueous phase to improve the efficiency of the liposomal dispersion. The final liposomal gel was formed by the evaporation of the organic solvent by rotary evaporator under constant vacuum. The residual solvent was removed by continued rotary evaporation under reduced pressure. The principle advantage of this method is high encapsulation efficiency. However a disadvantage is also there. Possible reaming of the solvent in the formulation may restrict its application. [87, 94, 95]

Due to the curve like structure, it enclosed some part of the solvent into their interior. The size of the vesicles starts from 20 nm up to several micrometers depending upon the pattern of aggregations between the amphiphiles. However liposome's properties could be changed by altering or tuning the charge of the head group, acyl chainlength, addition of other amhiphiles etc. The properties of liposome could be altered by altering the type of PL, head group charge, size, hydrocarbon chain saturation-unsaturation. Liposome of various types has been formulated

so far.[96, 97] Apart from the precursor, different ways of liposome preparation also leads to create different type of liposomes.

4.2. Classification of liposome: Liposome can be classified into two categories: on the basis of composition and hydrodynamic size.

4.2.1. On the basis of composition: Liposomes are the aggregative product of natural and/or synthetic lipids along with 30% cholesterol. Other hydrophilic polymer conjugate lipids could also play the role in formation of liposomes. Thus liposome with different surrogates generate liposomes with different properties. The major focus on liposomes relate to its capability as a drug delivery agent. Being a drug delivery agent its efficacy towards the target cells and effect on normal cell, tissues is primary concern. Depending upon the terms of composition and mechanism of drug delivery, it could be classified into five different types. (i) Conventional liposome (ii) pH-sensitive liposome (iii) Cationic liposomes (iv) Immunoliposomes and (v) Long-cerculating liposomes (LCL)

4.2.2. On the basis of hydrodynamic size: The size of the liposome can range from 50 to 1000 nm. The size of the liposome is vital parameter to determine its circulation life time. Bilayer controls the incorporation of the drug; which is a direct size dependent phenomenon.[98] Depending on the size, liposome is divided into three categories: (i) small unilamellar vesicles (SUV) (ii) large unilamellar vesicles (LUV) and (iii) multilamellar vesicles (MLV). For SUV, the size is less than 50 nm, whereas for LUV it's higher than 50 nm. MUV consisted of more than one bilayers, size ranged from 500 to 1000 nm.

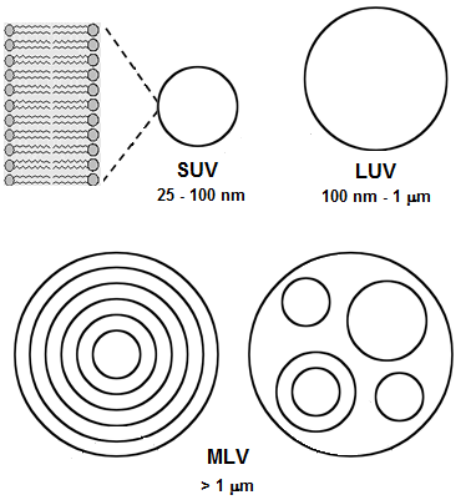


Figure 25. Representative images of different liposomes.

4.3. Advantages and disadvantages of liposome:

Advantages	Disadvantages
Liposomes increase the stability and therapeutic index of drug (actinomycin-D)	Solubility of liposomes is very low
Liposomes enhance the stability via encapsulation	Liposomes show short half-life
Liposomes are non-toxic and flexible in nature. They are biocompatible, biodegradable, and non-immunogenic for widespread.	Sometimes liposome containing phospholipid go through oxidation and hydrolysis-like reaction
When drugs/molecules/toxic agents (amphotericin B, Taxol) are encapsulated by liposomes, it reduces the toxicity.	Leakage and fusion of encapsulated drug/molecules
Liposomes support to reduce the exposure of sensitive tissues to toxic drugs.	Liposome manufacture cost is high
Liposomes show site avoidance effect.	Fewer stables
Elasticity to couple with site-specific ligands to attain active targeting	

4.4. Applications of liposome:

Liposomes are used as pharmacology and medicine technology which can be distributed into therapeutic and diagnostic applications of liposomes comprising drugs carrier or usage as a model or any reagent in the undeveloped studies of cell membrane. Unluckily various drugs have a very constricted therapeutic window, significance that the therapeutic application is not considerably lower than the toxic one. In a number of cases the toxicity can be decreased or the efficacy improved by the use of a suitable drug carrier which modifies the temporal and spatial scattering of the drug, i.e. its pharmacokinetics and biodistribution. Various applications of liposomes are specified bellow.

1. Liposomes are used in chelation therapy for treatment of heavy metal poisoning.
2. Liposomes performance as protein carriers in immunology.
3. They used as sustained or controlled delivery.
4. Liposomes used as diagnostic imaging of tumours.
5. They show intracellular drug delivery properties.
6. They used as site-avoidance delivery.
7. Enzyme replacement
8. Liposomes are used to study the membrane property.
9. They are used as oral drug delivery systems.
10. Liposomes are used as gene therapeutic agent.
11. Liposomes are used to prepare various cosmetics in our recent days.

4.5. Loading of Drugs by Liposomes: There are two types of drug loading capacity were seen for liposome.

4.5.1. Encapsulation of Hydrophilic Drugs: Encapsulation of hydrophilic drugs effects in hydration of lipids hydrophilic drugs mixture. By this method, drugs can move in the liposome core and other materials persist in exterior part of the liposome. Stayed materials will eliminate drug entrapment in liposome. In order to clean these two parts (drugs and remained outside materials), gel filtration column chromatography and dialysis are used. In addition, dehydration and rehydration method may be applied for high encapsulation of the DNA and proteins.

4.5.2. Encapsulation of Hydrophobic Drugs: The phospholipid bilayer of liposomes is a region of hydrophobic drug encapsulation. By entrapment of this type of drugs (such as curcumin), movement of drug will be reduced towards the exterior aqueous and interior parts of liposomes. These drugs are encapsulated through solubilizing of drug in the organic solvent and phospholipids. Region of drug entrapement in liposome is the hydrophobic part of liposome.

After that, it is promising to use laser light for activation of drug due to the treatment of wet macular degeneration.

4.6. Advantage of liposome drug delivery systems:

1) Liposomes are suitable for delivery of hydrophobic, hydrophilic and amphipatic drugs and agents.
2) They are chemically and physically well characterized entities
3) Liposomes are bio compatible.
4) Liposomes are used as carrier for suitable for controlled release drug delivery.
5) They are suitable to give localized action in particular tissues.
6) Liposomes are suitable to administer via various routes.
7) They increase the efficiency and therapeutic index.
8) Liposomes improve the pharmacokinetics properties.
9) They can be made into verity of drug.
10) They show lowest antigenicity properties.

4.7. Disadvantages of liposome drug delivery systems:

1) Their rapid clearance from circulation due to uptake
2) By the reticuloendothlial system (RES), primarily in the order
3) Leakage of encaptulation drug delivery during storage
4) Batch to batch variation
5) Once administered, cannot removed
6) Difficult in large scale manufacture and sterilization
7) Physical and chemical stability

8) Very production cost

9) Possibility of dumping due to faulty administration.

5. **Dendrimer:**

Dendrimers are synthetically prepared hyper branched macromolecules which contain high number of active termini that describe their properties and functions.[8-9] The term dendrimers arises from Greek words "dendron" meaning "tree" and "meros" meaning "branches". Because of perfect branching; dendrimers have the maximum number of terminal functionalities of any polymeric material at a given molecular weight and are perfectly mono dispersed (shown in Figure 26). Dendrimers compared to the corresponding linear polymers possess architectural advantages in terms of drug delivery: (i) reproducible pharmacokinetic behavior (due to mono disperse nature);[10] ii) globular shape provides superior biological and rheological properties;[11-12] and iii) controlled multivalency can attach several molecules (drugs, imaging agents, cell-penetrating peptides, targeting groups, and solubilizing moieties etc.

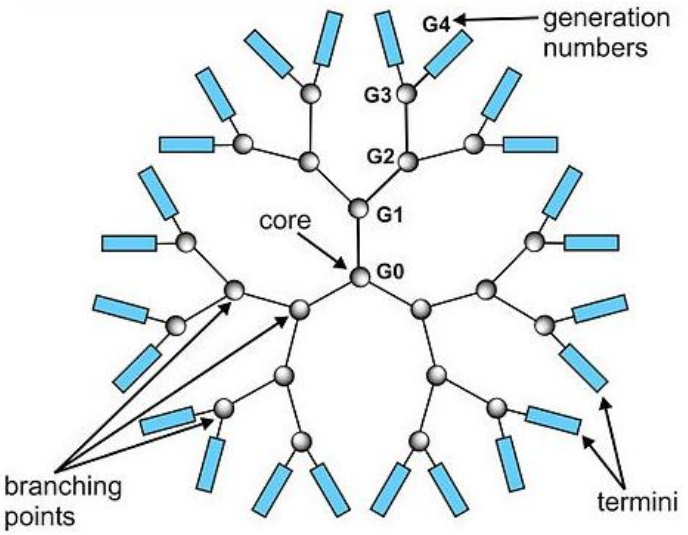


Figure 26. Schematic diagram of dendrimer.

Dendrimers are polymeric, hyper branched three dimensional tree-like architecture widely used in the field of nanotechnology.[99] These synthesised macromolecules can be fabricated by controlling its molecular weight, degree of branching, globular shape and surface functionalities and the size measured as reported ranging from 2.5 nm to 10 nm.[99-100] It exhibits homogeneous

symmetrical monodisperse structure with a typical core inside, involving an inner shell and outer shell structure. Its solubility in the solvent is highly dependent on the nature of the surface groups. Hydrophilic surface groups of dendrimers are soluble in polar solvent like water whereas hydrophobic surface group are soluble in non-polar solvent. Interaction can be proceeds either with the groups inside called "endorecepters" or the groups on the periphery well-known as "exorecepters" liable on the type of host-guest. Dendrimer structure can be categorized into three parts. (i) a multifunctional core moiety connecting the dendrons, (ii) radially linked repeating layer of branches connected with the core and (iii) a terminal surface groups as represented in the figure. Dendrimers have grown a wide-ranging of applications in supra molecular chemistry, mostly in host-guest reactions and self-assembly methods. Dendrimers are described by special features that make them auspicious aspirants for a lot of applications.[101] The role of dendritic molecules for anticancer therapies and diagnostic imaging is outstanding. The benefits of these well-defined materials make them the latest class of macromolecular nanoscale delivery procedures. Dendritic macromolecules have a tendency to linearly rise in diameter and espouse a more globular shape with enhancing dendrimer generation. Hence, dendrimers act as model delivery vehicle for clear study of the properties of dendrimer size, charge, and composition on biologically related properties.[102], [103]

5.1. Structure and chemistry of dendrimer: The structure of dendrimer molecules generates with a central atom or group of atoms termed as the core. From this central groups or atoms, the subdivisions of other atoms entitled 'dendrons' generates via various chemical reactions. There continues to be a argument about the proper structure of dendrimers, Especially whether they are fully extended with maximum density at the surface or whether the end-groups fold back into a densely packed interior different chemical events taking place at the dendrimer surface. [101] The unique building block structure of dendrimers gives a special prospects for host-guest type chemistry and is mainly well prepared to comprise in multivalent interactions. At the same time, one of the first recommended applications of dendrimers was as carrier like compounds, wherein small substances are bound inside the core side of the dendrimer

5.3. Physical properties of different generation of dendrimer.

Generation	Molecular weight	Diameter	Surface group
0	517	15	4
1	1,430	22	8
2	3,256	29	16
3	6,909	36	32
4	14,215	45	64
5	28,826	54	128
6	58,048	67	256

5.4. Synthesis of dendrimer: Dendrimers are not only the material from molecular chemistry but also polymer chemistry. As they prepared by the feature of their step-by-step well-ordered synthesis thus they tell to the molecular chemistry substance. On the other hand as dendrimers have the repetitive structure made from monomers so they are class of polymeric substances. They are in three types macromolecular architectural classes like linear, cross-linked, and branched. Dendrimer have definite size and molecular weight. Dendrimers are usually synthesized using two different methods like divergent and convergent. In the altered methods, dendrimer produces outward from a multifunctional core molecule. After reaction between core molecules and monomer molecule which is enclosed by one reactive and two dormant groups, form the first-generation dendrimer. After that again the new molecule is activated for reactions with more monomers. Dendrimer can be producing by changing functionality in each of this portion to categorize properties such as solubility, thermal stability, and addition of compounds for meticulous application. Synthetic procedure can also correctly manage the size and number of branches on the dendrimer. There are two different methods of dendrimer synthesis, divergent synthesis and convergent synthesis. [101]

5.4.1. Divergent Method: The dendrimer is manufacturing to from a multifunctional voids, which prepared by the subsequent of reactions, commonly a Michael addition type of reaction.

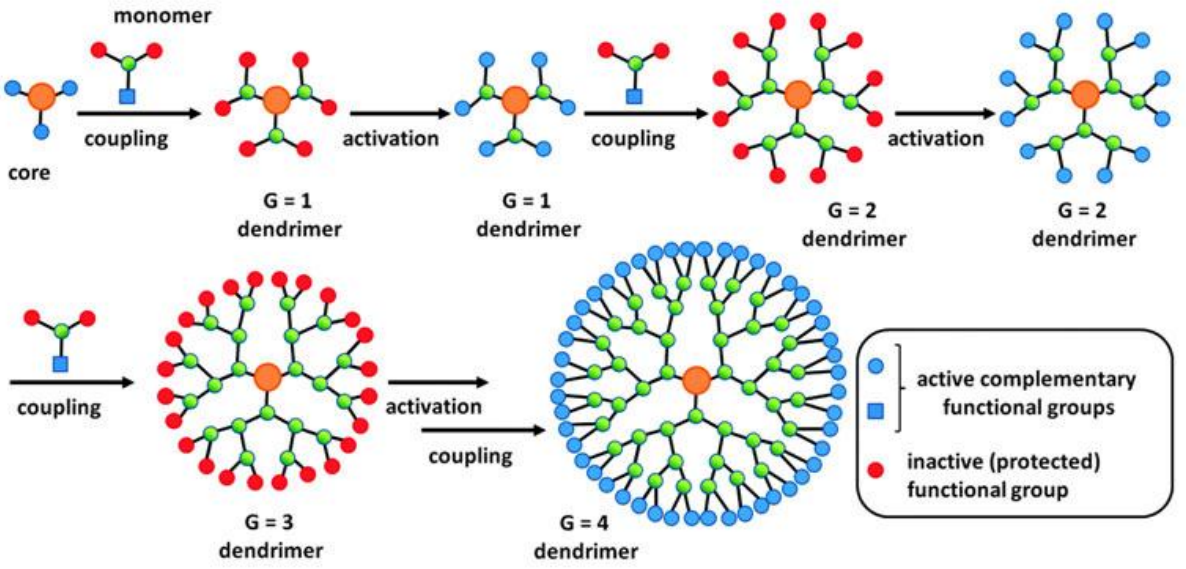


Figure 27. Divergent growth method for dendrimer synthesis. ***J. Chem.*, 2014, 38, 2168-2203**

For a single step of the reaction must be strong-minded to full completion of the reaction to inhibit mistakes in the dendrimer, that can grounds trailing generations (few branches are smaller than the others). These impurities can pileup the functionality and structure of symmetry of the dendrimer, but are terrifically hard to cleanse out because the virtual size variation between pure and impure dendrimers are very small. The main disadvantage of this method is that the incomplete development and the side reactions lead to defect of dendrimers. To decrease these side reactions and imperfections needs to recommend to usage a higher amount of reagents. The divergent growth reaction of dendrimer is shown bellow. [104]

5.4.2. Convergent Method: Dendrimers are constructed from starting point of small molecules which finished up at the surface of the sphere. The reactions precede deepest building internal and are suddenly attached to a core side. This types of method creates it very easier to remove the impurities, so that the final dendrimers are the type of mono-disperse. Therefore, the dendrimers completed by this way are not so high as those made by other method (divergent methods). Because by this method it is crowding due to stearic property of the core is restrictive 10. The convergent growth reaction are shown show in figure 28. Main problem of convergent method is that we cannot manufacture a large molecule for drug loading due to crowding and stearic effect. [105]

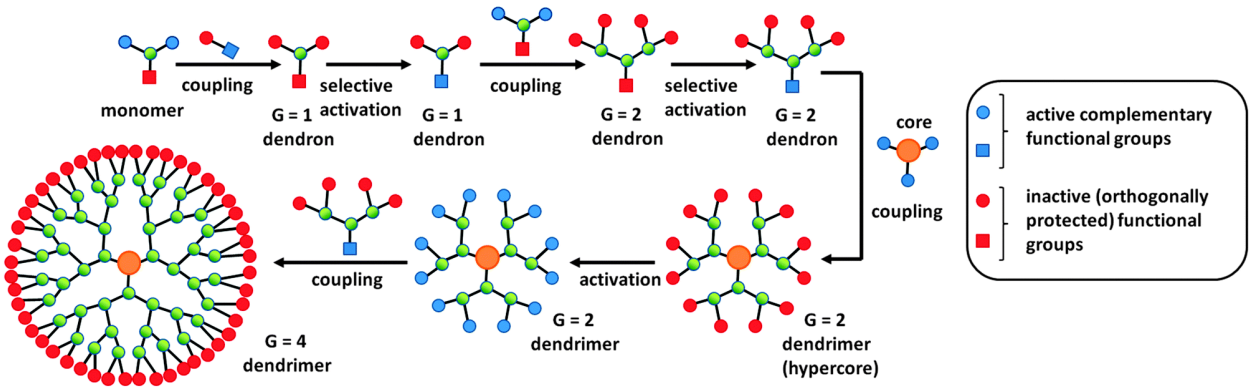


Figure 28: Convergent growth method for dendrimer synthesis. *New J. Chem.*, 2014, 38, 2168-2203

5.5. Classification of dendrimer:

Classifications of dendrimer sort out through the basis of dendrimer generation. Generation depend on the number of repeated branching cycles that are accomplished throughout its synthesis. For example when a dendrimer is prepared by convergent synthesis by performing the branching reactions onto the core molecule three times, then it is called 3rd generation of dendrimer. For each consecutive generation affects in a dendrimer approximately twice the molecular weight of the former generation. Higher generation of dendrimers having higher number of end group can later be used to modify the dendrimer for a particular application.

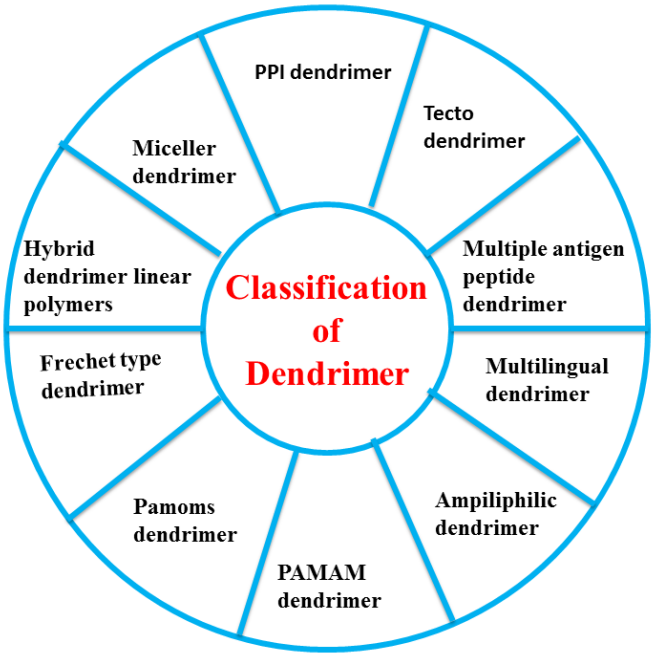


Figure 29: Classification of dendrimer.

5.6. PAMAM dendrimer: Poly (amidoamine), or PAMAM dendrimers are the fourth class of synthetic polymers, characterized as highly branched, globular, monodispersed (single molecular weight distribution) and reactive molecule which is prepared of repetitively branched subunits of amide and amine functionality. Since the first successful synthesis of poly(amidoamine) (PAMAM) dendrimer in the mid-1980s by Tomalia's group, dendrimers have received much attention, in particular, the biomedical applications of dendrimers in various medical and pharmaceutical field including drug delivery, gene transfection, nanoscale drug and diagnostic tools have regarded to be highly promising and have drawn eminent interest and studies in these few areas. PAMAM dendrimers, sometimes referred to by the trade name Starburst and represent the most well-characterized dendrimer. Similar to other dendrimers, they have a sphere-like structure having the internal molecular construction of tree-like branching, with each outward 'layer'. This branched architecture differentiates PAMAMs and other dendrimers from other normal polymers, as it permits for low polydispersity and a high level of structural control for the duration of synthesis, and gives increase to a huge number of surface

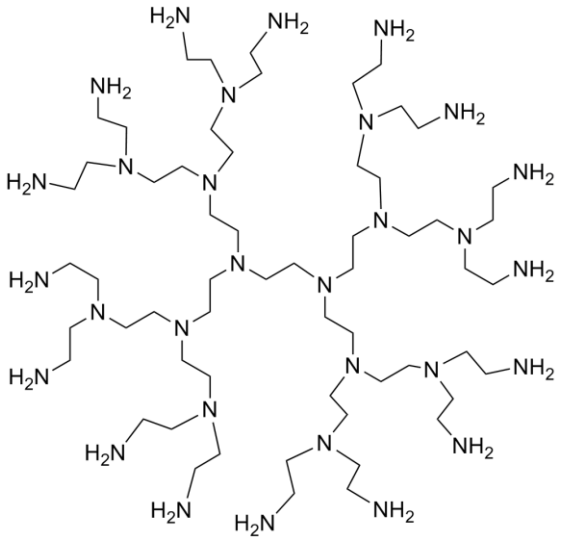


Figure 30: Molecular structure of PAMAM dendrimer.

sites comparative to the total molecular volume. Furthermore, PAMAM dendrimers display higher biocompatibility than other dendrimers, possibly due to the organization of surface amines and interior amide bonds; these bonding themes are greatly important for biological chemistry as PAMAM dendrimers shows similar properties with globular proteins. Comparatively ease/little cost of production of PAMAM dendrimers (mainly comparative to

similarly-sized biological molecules like proteins and antibodies), with their biocompatibility, functional property, structural control and, have ready PAMAMs viable candidates for use in drug improvement, biochemistry, and nanotechnology. [106]

5.7. Application of dendrimer: Now a day's dendrimers have numerous medicinal and practical applications.

5.7.1. Dendrimers in biomedical field: Dendrimers have improvement in different biomedical uses. They are equivalent to protein, enzymes, and viruses, and are simply functionalized. Dendrimers can either be close to the outside edge or can be encapsulated in their internal cavities. Present drug uses a variety of this substance as potential blood substitutes, e.g., polyamidoamine dendrimers.[106]

5.7.2. Anticancer drugs: Possibly the best auspicious potential of dendrimers is in their probability to execute controlled and definite drug delivery, which concerns the area of nanomedicine. One of the best important problems that are established toward recent medicine is to recover pharmacokinetic assets of drugs for cancer. Drugs are conjugated with these polymers which are described by half-life, higher stability, water solubility, reduced immunogenicity, and antigenicity. Distinctive pathophysiological behaviors of tumors like extensive angiogenesis causing in hypervascularization, the enlarged penetrability of tumor vasculature, and restricted lymphatic drainage allow impassive targeting, and as a consequence, selective addition of macromolecules in tumor tissue.[107], [108],

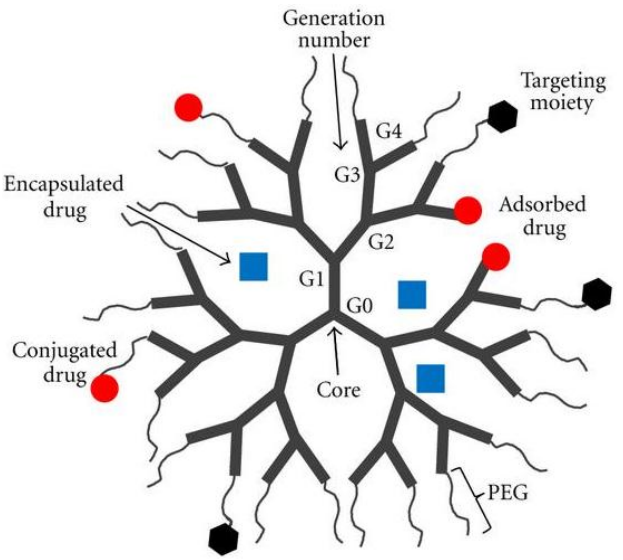


Figure 31. Schematic diagram of dendrimer as drug delivery.

5.7.3. Dendrimers in drug delivery: In 1982, Maciejewski planned, fat first, the use of these highper branched polymeric molecules as molecular containers. Host-guest properties of dendritic polymers are currently under scientific investigation and have gained crucial position in the field of supramolecular chemistry. Host-guest chemistry is based on the reaction of binding of a substrate molecule (guest) to a receptor molecule (host) shown in Figure 31. Transdermal drug delivery Clinical have used the NSAIDs, which is limited due to antagonistic reactions such as GI side special effects and renal side special effects when specified orally. Transdermal drug delivery incapacitates these corrupt effects and also conserves therapeutic blood level for a long time. Transdermal delivery undergoes lowly rates of transcutaneous delivery because of barrier function for the skin. Dendrimers have originate variety of applications in transdermal drug delivery schemes. Commonly, in bioactive drugs which contains hydrophobic parts in their structure and low soluble in water, dendrimers are a good carrier in the field of different delivery system. [109], [108], [108]

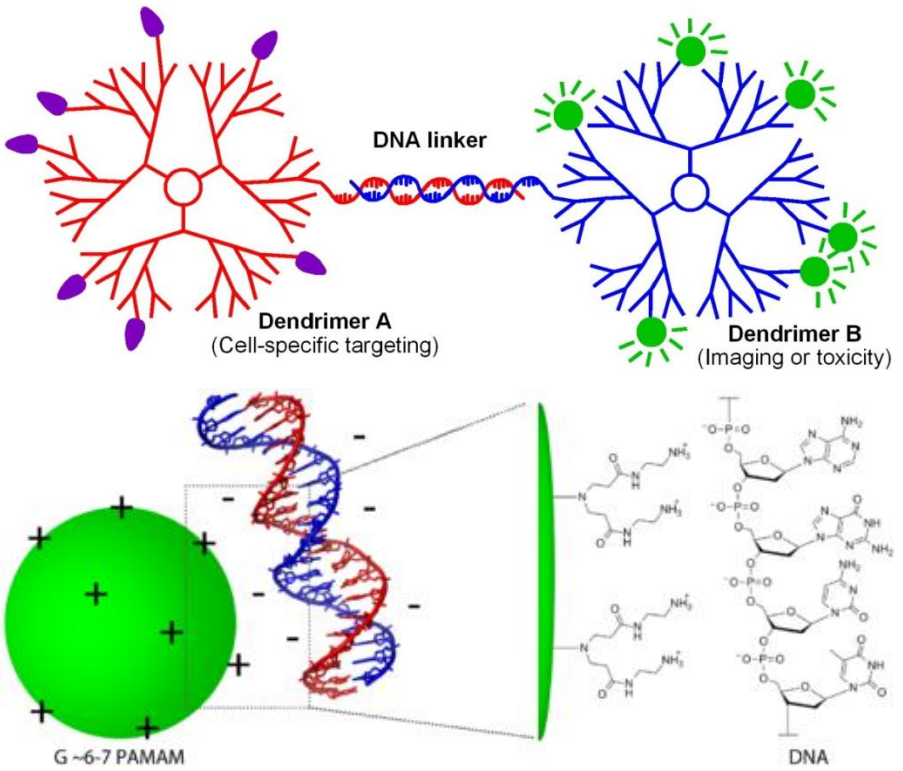


Figure 32. Schematic diagram of dendrime as gene delivery.

5.7.4. Gene delivery: The primary potential that the mixture of thoughtful the molecular paths of infection and the whole human genome arrangement would produce nontoxic and more effective medicines and change the way we pleasure patients has not been satisfied to date. Conversely, there is tiny hesitation that genetic therapies will create a important support to our therapeutic armamentarium when some of the key contests, such as definite and well-organized delivery, have been resolved. The capability to deliver parts of DNA to the requisite parts of a cell comprises many contests. Present investigation is being completed to discovery ways to custom dendrimers to transportation genes into cells without harmful or disengaging the DNA. To conserve the activity of DNA during dehydration, the dendrimer/ DNA complexes were captured in a water soluble polymer and then dropped on or sandwiched in well-designed polymer films with a fast degradation speed to facilitate gene transfection. PAMAM dendrimer/DNA complexes were used to capture functional biodegradable macromolecule polymer films for substrate-mediated gene delivery. Investigation has shown that the fast-degrading functional polymer has abundant potential for restricted transfection. [110], [111], [112]

5.7.5. Dendrimers as magnetic resonance imaging contrast agents:

Dendrimer-based metal chelates act as magnetic resonance imaging contrast agents. Dendrimers are extremely appropriate and used as image contrast media because of their properties. [113]

5.7.6. Dendritic sensors:

In a single molecule, dendrimers have large numbers of functional groups on their surface side. This makes them outstanding for applications where covalent linking or close proximity of a large number of classes is significant. Balzani and coworkers inspected the fluorescence of a 4^{th} generation of poly (propylene amine) dendrimer containing 32 dansyl units at the boundary. As this dendrimer contains 30 number of aliphatic amine units in the structure, it is appropriate for metal ions which are able to coordinate. It has been seen that when a Co2+ ion is amalgamated into the dendrimer, the heavy fluorescence for dansyl units is quenched. Little concentrations of Co2+ ions ($4.6 \times 10{-}7$ M) can notice by a dendrimer concentration of $4.6 \times 10{-}6$ M. The many fluorescent groups on the surface help to intensify the sensitivity of the dendrimer as a sensor. [114, 115]

5.7.7. Dendrimers used for enhancing solubility:

PAMAM dendrimers are probable to have potential uses in increasing solubility for drug delivery schemes. Dendrimers have hydrophilic surfaces, which are accountable for its unimolecular micelle like properties. Dendrimer-based transporters offer the chance to increase the oral bioavailability of difficult drugs. Thus, dendrimer nano transporters suggest the potential to increase the bioavailability of drugs that are slightly soluble and/or substrates for efflux transporters.[116], [117], [118]

5.7.8. Photodynamic therapy:

Photodynamic therapy (PDT) trusts on the initiation of a photosensitizing mediator with visible or near-infrared (NIR) light. By excitation, a extremely energetic state is made which, by the reaction with oxygen, gives a extremely sensitive singlet oxygen accomplished of making necrosis and apoptosis in tumor cells. Delivery of PDT agents has been examined within the last few years in order to progress upon tumor choosiness, holding, and pharmacokinetics. [119], [120],

6. Other drug delivery systems:

Colloidal drug carrier systems such as micelle, vesicles, liquid-crystalline system as well as nanoparticle dispersion etc with diameter 10 to 400 nm show promising agent as drug carrier. With the aid of advance technologies, researchers are developing drugs with high and promising activity. Most of the drug mainly consists with DNA and/ or protein and are characterized on the basis of its bioactivity; liberate their bioactive material right time with right concentration and limited toxicity. When developing the formulation of the drug carrier, the aim is to get an optimized system that has well encapsulation efficacies, long shelf life and low toxicity with controlled release of the drug. So carrier such as colloidal medium, nanoparticle gets special attraction due to the biocompatibility, biodegradability and biomimiking phenomenon. General description about different drug carrier system is discussed below.

6.1. Microemulsions and nanoemulsions: Microemulsions is composed of water, oil, surfactant and co-surfactant[121] are transparent and optically isotropic single –phase liquid solution. The system is bicontinuous with low viscosity and is thermodynamically stable. The solubilisation of both hydrophilic and lipophilic drug in microemulsions could be achieved. The

limited use of microemulsions for dermal and oral application is a significant drawback. Nanoemulsions come into the picture in 1950's to the need of parenteral nutrition. It has been introduced into the market as various pharmaceutical products, such as: disoprivan, Etomidat lipuro, diazepam lipure, steasolidand lipotalon. Nanoemulsions are the two component heterogeneous system where one liquid is dispersed as droplets in the other one. As a drug delivery agent, it reduces the local and systematic side effect. However it has some limitation when lipophilic drug is concerned as the presence of oil solubilizes the drug.

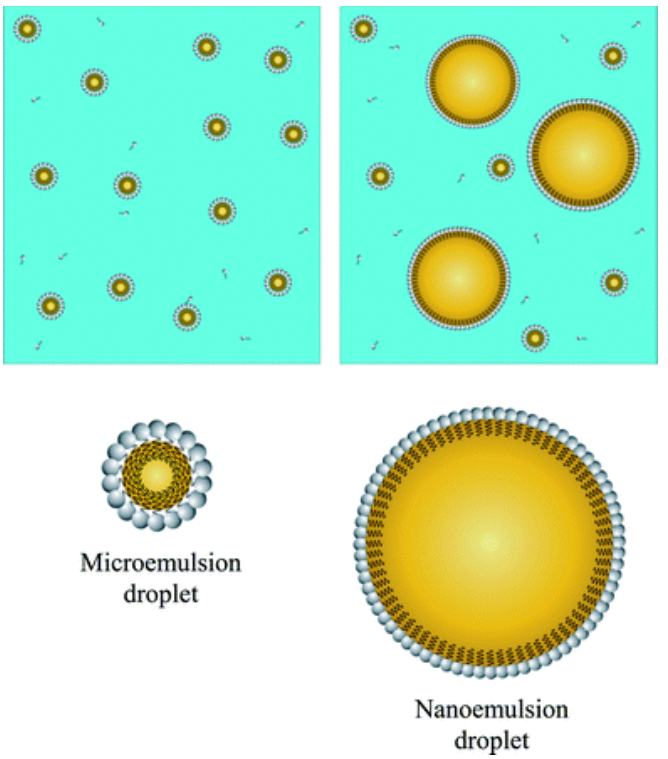


Figure 33. Scheme representative of microemulsion and nanoemulsion.

6.2. Hydrogels. Hydrogels are hydrophilic three dimensional polymeric networks composed of homopolymers and co-polymers and can absorb large quantity of biological fluid or water. [122] Due to the chemical or physical crosslinks between the polymers, they are insoluble in water. Hydrogels are on the forefront of control drug delivery as it shows thermodynamic stability in water and can modulates the drug release with respect to temperature, ionic strength, pH, electric field etc. This versatility of hydrogel makes it as an superior agent for the specific drug delivery.

6.3. Nanoparticles. It includes nanocapsuls and nanospheres having size 10 – 200 nm and is crystalline or amorphous. They have the capabilities to encapsulate or absorb the drug and act as a protective barrier. In the recent years it draws researcher's attention because of the controlled release of the drug and targeting drug delivery. It has the ability to deliver genes, proteins and peptide through the peroral route. [123-124]

6.4. Solid lipid nanoparticles. Solid lipid nanoparticles (SLN) are composed with solid lipid core matrix that can solubilise lipophilic molecules. It is spherical shape and has the diameter 10 – 1000 nm. Lipids such as triglycerides, diglycerides, monoglycerides, faty acids, wasex, and steroids are used to prepare SLN. The lipid core is stabilized by adding surfactants based on the charge and molecular weight. SLN is used in variousapplications such as parental, pulmonary, ocular deliver of drugs. [125]

7. Importance of monolayer study:

The mutual miscibility and stability of the binary monolayers of zwitterionic phospholipids with negatively charged phospholipids at the air-buffer interface and the interaction of positively charged dendrimer with the stable monolayers have been studied in detail using surface pressure area isotherms. The surface pressure-area isotherms indicated that the binary monolayers of zwitterionic lipid and negatively charged lipid at the air-water interface were miscible and more stable than the monolayers of the two individual components. Based on the regular solution theory, the miscibility and stability of the two components in the monolayer were analyzed in terms of compression modulus and excess Gibbs free energy. Our main object is to recognize which binary composition is considered to be liposome formulation. Also the kinetic behavior of liposome disintegration to monolayer is carried out by monolayer study.

8. Dendrimer-liposome interaction:

Since the first successful synthesis of poly (amidoamine) (PAMAM) dendrimer in the mid-1980s by Tomalia's group, dendrimers have received much attention, in particular, the biomedical applications of dendrimers in various medical and pharmaceutical field including drug delivery, gene transfection, nanoscale drug and diagnostic tools have regarded to be highly promising and have drawn eminent interest and studies in these few areas . A few numbers of these dendrimer molecules are already now available on the market (e.g. Polyfect® and SuperFect® as in vitro

transfection agent) or have proceeded to clinical trial as dendrimer-based pharmaceutical (e.g. Viva Gel TM as microbicide against HIV or other sex transmitted diseases) , and the numerous successful studies of dendrimers in various other applications including Boron neutron capture therapy for cancer treatment have allowed dendrimer to replace other traditional polymers as the revolutionary agent in the biomedical field. They include development as: inherently active drugs (e.g., as antiviral and antimicrobial agents and modulators of angiogenesis); drug-carriers for targeting and controlled release (particularly as anticancer agents); non-viral vectors to promote oligonucleotide and gene delivery. Dendrimers are also being studied as medical imaging agents, as components of tissue engineering scaffolds and as adjuvants for vaccine delivery.

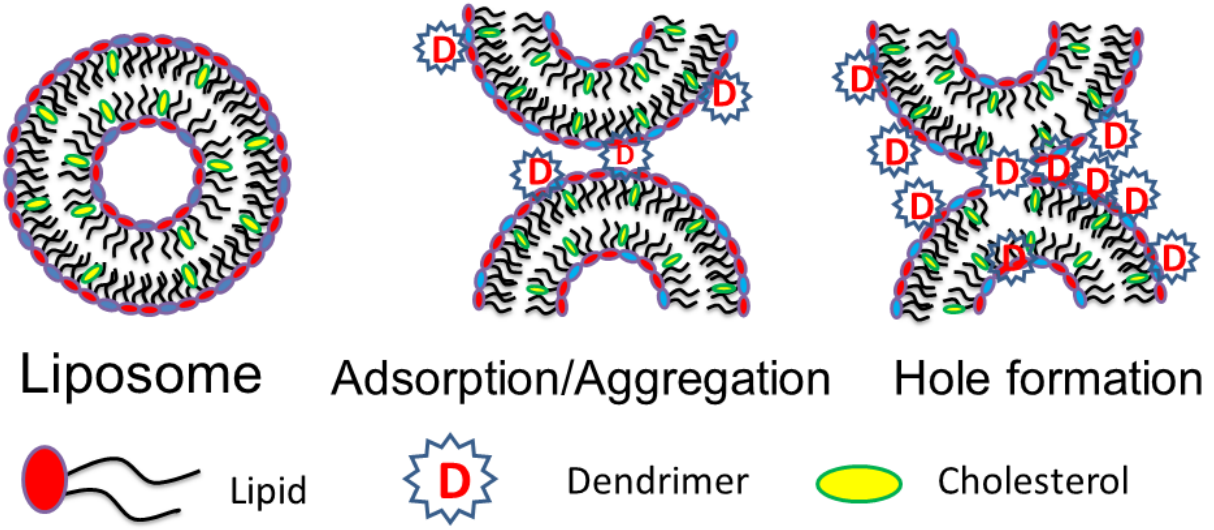


Figure 34. Schematic diagram for the formation of dendrimer-liposome aggregates

The field of biomedical dendrimers is yet not completely studies, but the eruption of attention in dendrimers and dendronised polymers as fundamentally active therapeutic mediators, as vectors for engaged delivery of drugs, peptides and oligonucleotides, and as permeability enhancers capable to stimulate oral and transdermal drug delivery creates it timely to analysis current understanding concerning the toxicology of these dendrimer chemistries (at present under improvement for biomedical applications). Apart from their antiviral properties, dendrimers have also been demonstrated to have antibacterial properties. In general, the mechanism of action of antibacterial dendrimer is based on the electrostatic interaction between the ammonium groups

on the dendrimers with the anionic charged membrane of bacteria, which in turn caused their membrane to lose integrity and thus lysis.

The physical properties of liposomes are highly dependent on the type of phospholipids constituted (i.e. negative charged DPPG or neutral charged DPPC phospholipids). intermolecular interactions as well as the conditions of the environment which surround it. Nevertheless, due to their easy generability and readily modifiable nature in terms of its lipid contents and structure (*i.e.*, unilamellar and multilamellar), liposomes have received particular attention as the simplified version of cell membrane and have been commonly employed in the investigation of drug-lipid bilayer interaction. In this proposed program, liposomes will be employed in order to study the interaction between dendrimer and lipid bilayers.

Adsorption of dendrimers on liposomes and the formation of dendrimer-liposome aggregates are the most common aspect in the interaction between dendrimers and liposomes. It is believed that the dendrimers could act as glue for the vesicles. Most of the recent studies have focused on the interactions of positively charged dendrimers with cell membranes, since greater interaction potency is expected between these dendrimers with the negatively charged cell membrane compared to other neutral or negatively charged dendrimers. However, it has also been shown that besides the surface charge of the dendrimer molecules, there are many other physicochemical properties of dendrimers which influence the interaction between dendrimers and lipid bilayers. These properties of dendrimers include the dendrimer type, generation, surface charge and also solution conditions (e.g. pH and salt) as well as the composition of membrane lipids.

Studies on the change of the zeta-potential of liposomes are assumed to provide better understanding of the nature of dendrimer-liposome interaction. It is known that the zeta-potential of liposomes would change as a result of the adsorption of charged dendrimers, while remain constant if dendrimers are internalized from the interaction with the liposomes.

The current barrier to the application of dendrimers as various therapeutic agents as mentioned above, however, is the lack of established knowledge on biocompatibility and toxicity of dendrimers in biological systems. The kinetics and mechanisms of dendrimer-lipid bilayer interaction have received considerable attention as cells contain many layers of membranes

which dendrimers need to recognize, interact and internalized before it could reach its intracellular targets. As discussed, the knowledge on this area has been expanding over the past few years, but there are still many questions left to be answered before dendrimers could be safely employed as a novel therapeutic agent.

As part of the effort to provide a better understanding of the mechanism of dendrimer interactions with lipid bilayers, this research is aimed to investigate the mechanism involved in the interaction between different generation and types of PAMAM dendrimers and liposomes (i.e. electrostatic and hydrophobic interaction), and also to provide an understanding on how the various physicochemical properties of dendrimers and liposomes could affect the dendrimer-liposome interaction. Specifically, in this research, different types of phospholipids including 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), dihexadecyl phosphate (DHP), 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPP), 1,2-dipalmitoyl-sn-glycero-3-phospho ethanol (DPPEth), L-α-phosphatidylcholine (soy Lecithin) and 1,2-Dipalmitoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DPPG) and cholesterol (CHOL) will be employed to synthesize a range of liposomes of varying chemical composition, structural and physical properties. The information obtained from their interaction, including adsorption isotherm, packing bilayer and palisade layer, changes in surface charge as a result of adsorption process and aggregate formation (changes in particle size) are believed to be useful as they would provide a basis for future studies which investigate the interaction between dendrimers and cell membranes, which structurally and chemically more complex than simple lipid bilayer (*i.e.* liposomes).

Also, as dendrimers with different physical and chemical properties would be employed as therapeutic agent in future according to the need of the specific therapeutic purpose, the studies are also assumed to investigate the influence of dendrimer types on its interaction with liposomes. Three different types of PAMAM dendrimers that contain a similar surface group (amine), but different generations will be employed. Besides some polymer anchored dendrimers and dendrimers with different end functional groups will also be explored. The influences of the dendrimer type and generation on the dendrimer-liposome interaction will be studied in terms of variation in the adsorption isotherm, changes in zeta potential as well as particle size. The information obtained would be useful as they would provide an idea in future for the rational

design of dendrimers which are biocompatible and are optimized for therapeutic application. In another attempt, efforts will be put to study the kinetic and mechanistic aspects of dendrimer-liposome interaction. To meet up the final goal of the proposed work, dendrimers of different kinds and generations will be employed to perform cell (bacterial/ cell lines) growth studies. This will eventually be helpful in obtaining a proper denderimer based formulation for therapeutical uses.

9. Characterization of monolayer and bilayer:

Preparation of samples with high purity is the most important criteria. Once the sample was prepared and purified one could measure the particle size, surface charge (or zeta potential) and polydispersity of the medium by using DLS studies. Monolayer studies another important one to get knowledge about the mutual misicibility among the bilayer components. Electron microscopy (TEM and FF-TEM) and AFM studies also helpful determining the bilayer thickness. The effect of temperature on vesicles bilayer could be scrutinized. Apart from this inclusion of drug in its subsequent effect on the bilayer could also be viewed on such studies. Vesicles hosted drug also be scrutinized by measuring their entrapment efficiency, release kinetics and cytotoxicity effect. In case of vesicles/dendrimers interaction, apart from the above mentioned studies, one could perform vesicles disintegration kinetics and Isothermal carometric (ITC) measurement.

9.1. Dynamic light scattering (DLS) technique:

Liposomes are known as colloidal particles render surface charge and have hydrodynamic size in nano dimension. Being in dispersion state, it involves in Brownian motions. DLS technique is useful in determining the hydrodynamic size, zeta potential as well as polydispersity of the medium. It is worthy to mention that the hydrodynamic diameter is the hypothetical diameter of the hydrated particles that diffuse through the medium. In practice the colloidal particles are non-spherical, solvated and tumbling. Hence based on diffusion properties of particles, DLS calculates the apparent dynamic hydrated size of the particles; known as hydrodynamic size. Figure 18 represent particle size measurement curve.

A He- Ne leaser light with an emission wavelength 628 nm at an angle 90º collide with particles involved in the process of diffusions. It measures the translational diffusion coefficient (D) by collecting the light scattered by the diffused particles. Based on Stokes-Einstein's equation.

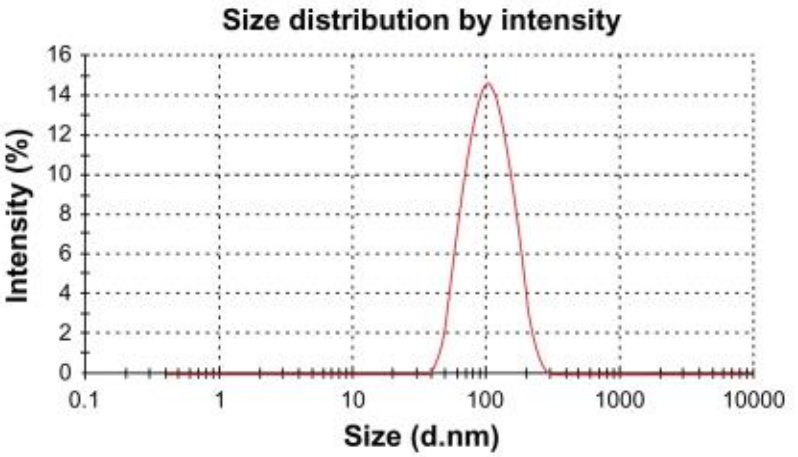


Figure 35. Hydrodyanmic size distribution as function of intensities.

$$d_h = \frac{kT}{3\pi\eta D} \quad (1)$$

where, k, T and η indicate the Boltzmann constant, temperature and viscosity of water respectively.

Hydrodynamic diameter (d_h), polydispersity index (PDI) and zeta potential (Z.P.) measured by Dynamic Light Scattering (DLS) technique. DLS measures the autocorrelation function $g^1(\tau)$ that is correlated with the size distribution of the particles by the given equation:

$$g^1(\tau) = \int_0^\infty m_\Gamma^2 P(q,\Gamma) G(\Gamma) e^{-\Gamma\tau} d\Gamma \quad (2)$$

where, $G(\Gamma)$ is the normalized number distribution function for the decay constant Γ, m_Γ is the particle mass, $P(q,\Gamma)$ is the particle scattering factor, the decay constant $\Gamma = q^2 D_T$, the scattering vector $q = \left(4\pi n/\lambda\right)\sin(\theta/2)$, θ is the angle between the incident and scattered beam, n is the refractive index of the medium and D_T is the translational diffusional coefficient. D_T is related to the hydrodynamic radius (R_h) of the particles by the given equation[126]:

$$D_T = \frac{k_B T}{6\pi\eta R_h} \quad (3)$$

where, k_B is the Boltzmann constant, T is the temperature of the dispersion medium and η is the viscosity of the medium. The zeta potential was measured using folded capillary cells. The PDI and zeta potential measurements were carried out at the temperature of 25 °C; however the size

of the liposome systems was measured in the temperature range between 25 to 50 °C. DLS studies were undertaken for three different samples of liposome formulations. The DLS results of each sample were the average of four consecutive measurements.

ZP is one of the crucial parameter that maintains the stability of the vesicles. It prevents particle collision wing to the repulsion and hence it could be understood, higher the ZP, higher is the stability of the particles. Substitution of other substances in vesicle bilayer may often change the value of ZP. Thus measurement of ZP could be useful in determining the role of other components in the bilayer. Like any charge particles, vesicles can also involve in of electrophoresis under the influence of an applied electric field. The speed of the particles depends on the size, ZP, viscosity and dielectric of the dispersion medium. Figure 18 describes a representative image of ZP of an vesicle medium.

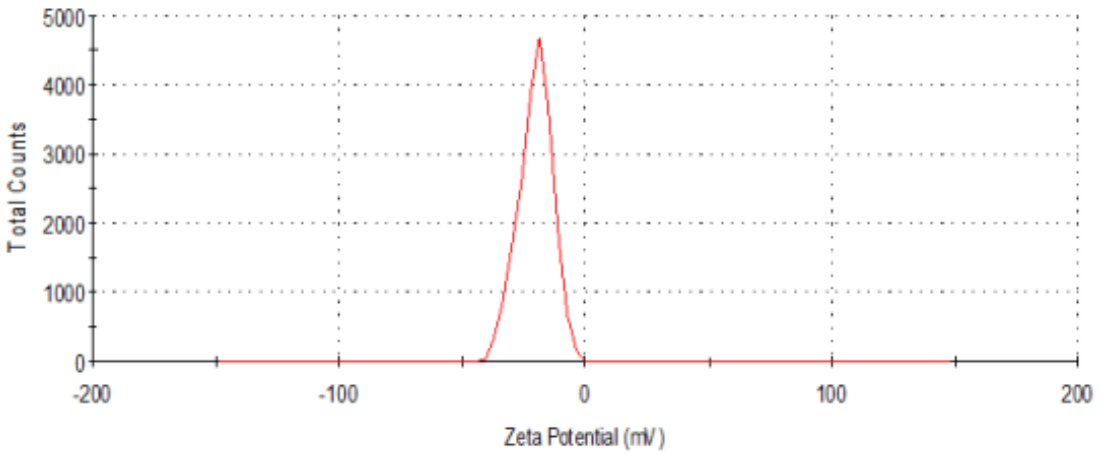


Figure 36. Representative image of ZP of a vesicular dispersion.

9.2. Turbidity measurements: Interaction of dendrimers with different liposomes was studied by measuring the % transmittance (%T) at 420 nm. It was studied by measuring the absorbance by U.V. spectrophotometer with varying concentration of ligand at a particular wavelength. At this wavelength, the turbidity of a solution is assumed to be proportional to (100-%T). [127]

9.3. TEM and FF-TEM technique: DLS study delivers the information about apparent dynamic size of the particles but the morphology of liposome can not be seen by DLS technique.

However, the details size and morphology were studied by TEM and FF-TEM technique. [128] Morphology of the sample was studied by the transmission electron microscopy by taken a drop of dilute dispersion solution was placed on Formver™ carbon-coated 200 mesh copper grid and dried under air. It was then analyzed to obtain TEM images using TEM instrument (like Hitachi H-600 transmission electron microscope (Japan) operating at 80 kV). In case of FF-TEM studies, a drop of the sample was placed onto the sample holders and frozen in liquid propane. FR-7000A (Hitachi High Technologies Ltd., Japan) was used at -150 °C for the freeze fracturing. Samples were then replicated by evaporation using platinum carbon. The replica was placed on 300- mesh copper grid, dried, and examined in a transmission electron microscope (H-7650, Hitachi High Technologies Ltd., Japan) with an accelerating voltage of 120 kV.

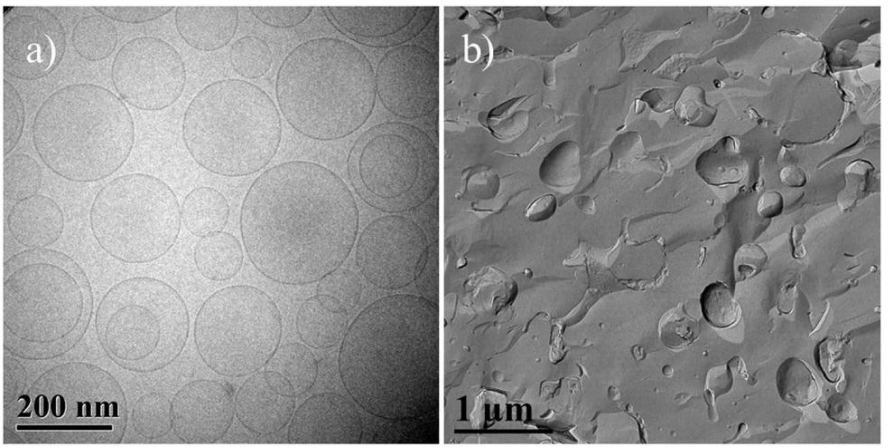


Figure 37. TEM and FF-TEM images of liposomes.

9.4. Atomic Force Microscopy (AFM) technique: A supported bilayer could be generated by way of vesicle fusion on mica/glass/gold substrate. Vesicles have the tendency to get fused over glassy substrates. Thus if a vesicular solution is placed over glass or freshly cleaved mica, it will fuse to form bilayers. These solid supported bylayers could also be considered as model membranes. Feature of the bilayers, generated onto freshly cleaved mica, will be visualized by way of atomic force microscopic (AFM) measurements. Bilyaer disruption can then be studied by way of addition of dendrimers. Bilayer images are grabbed at different resolutions. Then dendrimer solutions of desired concentration is used to rinse the bilayer. After certain time, it is again scanned to visualize the effect of dendrimers. [129, 130, 131]

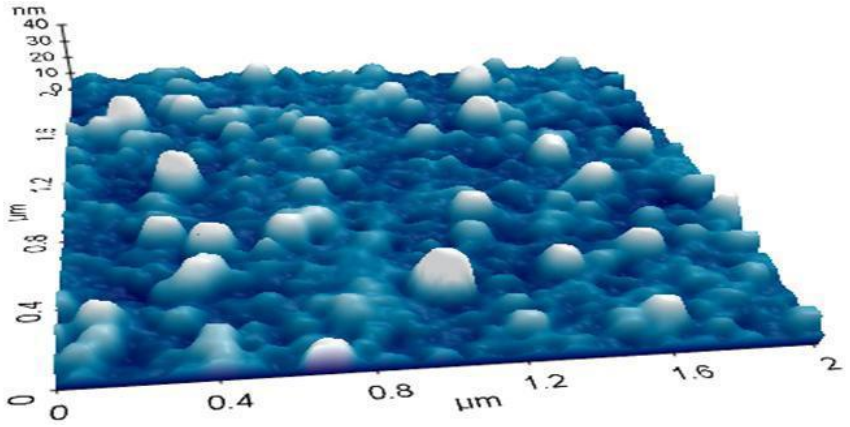


Figure 38. AFM image of liposome.

9.5. Fluorescence Spectroscopy: It is known that the dendrimers have the capability to rupture cell membranes. Therefore, if a dendrimer is added to a liposomal solution, where some fluorescent dye (like curcumin, 7-hydroxy coumarine, *etc.*) is entrapped, the dye will start leaking out of the liposome. Such leakage of fluorescent dyes will be monitored through fluorescence spectroscopic measurements. Extent of leakage would then be correlated with the membrane rupture. Steady state fluorescence spectra, as well as fluorescence anisotropy and fluorescence life time studies were carried out by a bench-top spectrofluorimeter (Quantummaster-40, Photon Technology International Inc., NJ, USA). Curcumin, used as the probe, was excited at 415 nm. Fluorescence anisotropy value was determined using equation mentioned below:

$$r = \frac{I_{VV} - G.I_{VH}}{I_{VV} + 2G.I_{VH}} \tag{4}$$

where, I_{VV} corresponds to the intensity obtained when the excitation and the emission polarizers are oriented vertically. I_{VH} is the intensity obtained for vertical excitation polarizer and horizontal emission polarizer. G is the grating correction factor which is the ratio of sensitivities of the instrument for vertically and horizontally polarized light. Thus:

$$G = \frac{I_{HV}}{I_{HH}} \tag{5}$$

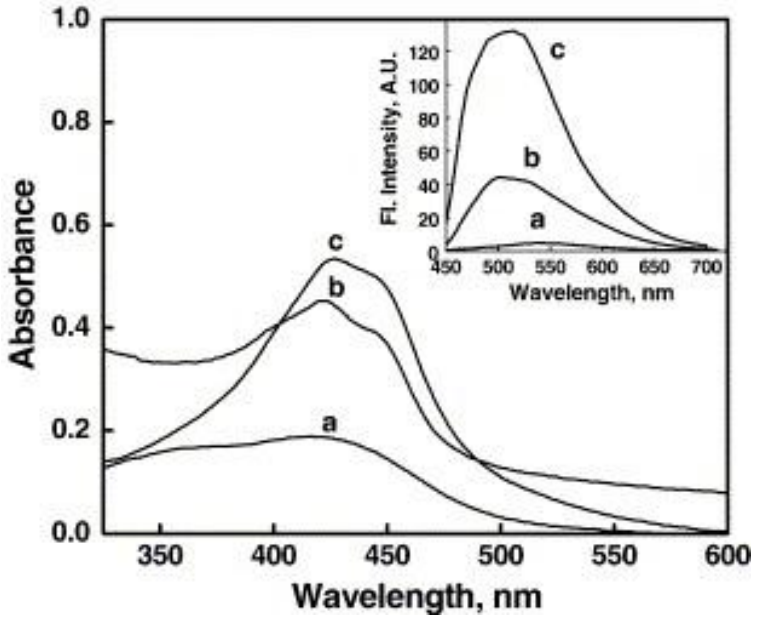


Figure 39. Absorbance and fluorescence spectra of curcumin.
https://doi.org/10.1016/j.bbagen.2006.06.012

Fluorescence lifetime measurements for fluorescent dye were recorded with the same fluorimeter where a nano LED (Photon Technology International Inc.) of different wavelength was used to excite the dye. Fluorescence decays were obtained by Strobe technique. [132]

9.6. Differential Scanning Calorimetry (DSC). DSC is useful techniques that can detect the hydrocarbon chain melting process also known as phase transition. The temperature at which chain melts, known as chain melting temperature (T_m). By measuring T_m one could also get other thermodynamic parameters like enthalpy change, change in heat capacity, crystalinity of hydrocarbon chain etc. In this experiment, the vesicle bilayer were heating or cooling with desire temperature range. In chain melting process, the orientation of hydrocarbon chains get tilted leading the creation of endothermic event as reflected in the Figure 2. Hence bilayer strength and other thermodyanmical entities are heavily depend on bilayer composition and the process of chain melting.

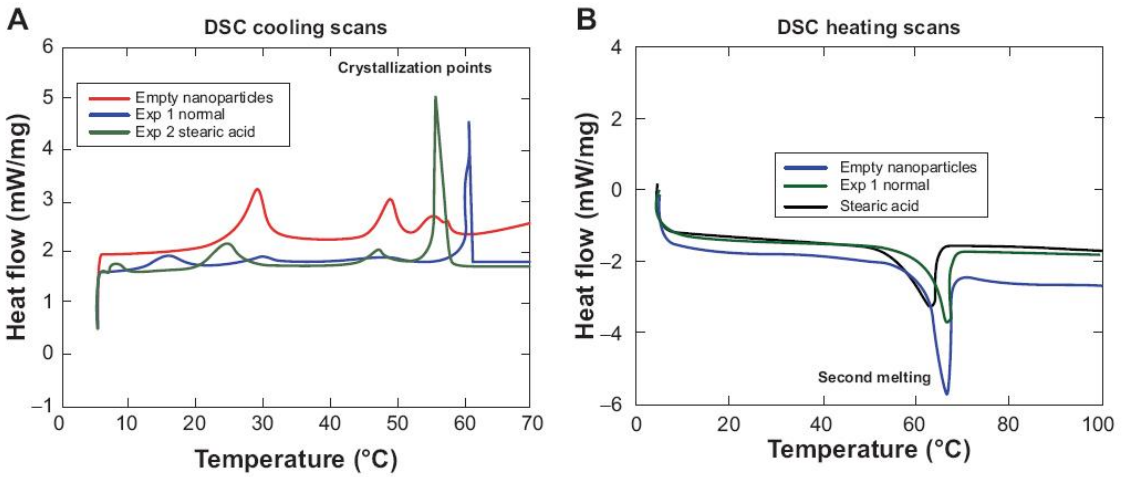


Figure 2 (**A**) DSC cooling scans; (**B**) DSC heating scans.
Notes: Empty nanoparticles refers to solid lipid nanoparticles that do not contain drug; Exp 1 normal refers to primaquine-loaded solid lipid; Exp 2 refers to stearic acid alone, which was used as the matrix in forming the solid lipid nanoparticles.
Abbreviations: DSC, differential scanning calorimetry; Exp 1, experiment number 1; Exp 2, experiment number 2.

Figure 40. DSC thermogram of SLN. https://doi.org/10.2147/IJN.S62630

9.7. Isothermal Titration Calorimetry (ITC). ITC measurements were performed in a VP-ITC titration calorimeter (MicroCal, Northampton, MA). Before loading, the solutions were thoroughly degassed. The preformed samples were kept in a sample cell, and a syringe of aliquot volume sample was filled by the ligand in the same buffer. The sequential titrations were performed to ensure full occupancy of the binding sites by loading and titrating with the same ligand without removing the samples from the cell until the titration signal was essentially constant. The respective heat of dilution was subtracted from the corresponding binding experiments prior to curve fitting. Typically three replicate titration experiments were performed.

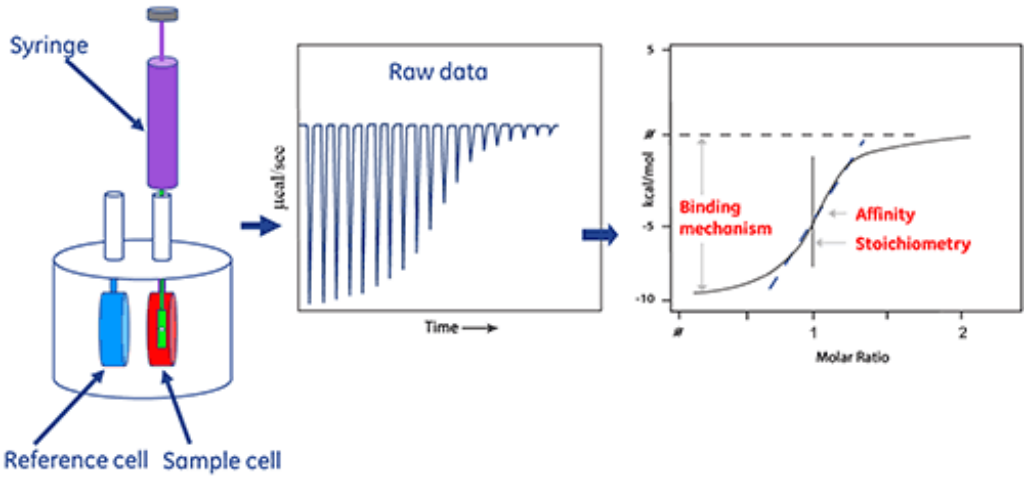


Figure 41. Schematic diagram of ITC technique.

9.8. Entrapment efficiency (EE) and release kinetics: It is known that liposome can accommodate both hydrophilic and lipophilic drug. Hence Drug loading capacity of vesicles need to be determined. Once drug loaded liposome dispersion was prepared, it filled into the cellulose dialysis bag (MWCO 12 KDa) and dialyzed in PBS or water with constant stirring (50 rpm) under sink conditions for about 20 min. The un-entrapped drug was removed from the formulations and the absorption spectra of collected samples were recorded through UV-VIS spectrophometer. The EE was calculated by considering the following equation: [133, 134] where,

$$\text{E.E. (\%)} = \frac{T-C}{T} \times 100 \qquad (6)$$

T is the total amount of drug presents both in the sediment and supernatant, C is the amount of drug identified only in the supernatant. T and C values were determined colorimetrically.

9.9. Drug release Kinetics. Liposomes are marked to be specific when they liberate the drug in target cell with sustained release. Hence drug incorporated vesicles often undergo such kinetics measurement. Dialysis method is generally used where the drug loaded samples were placed in dialysis bag and immersed in the same medium or solvent that was used in liposome preparation. [135, 136, 137] UV-VIS spectra of the sample were recorded with time which reveals the release pattern of the drug. Figure 40 put on a view of such release kinetics of drug loaded liposomes.

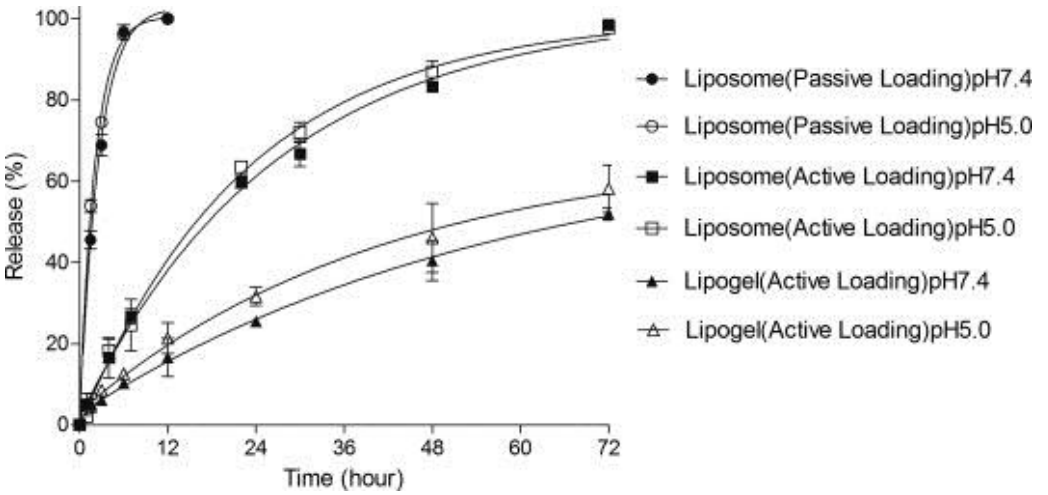


Figure 42: Representative image of % of drug release with time form liposome bilayer.

9.10. Antibacterial activity: Antibacterial activity of drug loaded systems was assessed by the conventional cup-plate method. Nutrient broth and agar were used as the culture growing and solidifying agent respectively. In the incubated plates, bacterial suspension (0.1 mL of 5×10^5 CFU mL^{-1}) was homogeneously spread over the agar surface and then grooves of equal diameter (0.8 cm) were made. The plates were incubated at 37°C for 24 h.

9.11. Studies on the behavior of monomolecular films: Surface pressure (π) – area isotherm (A): Bilayer could be viewed as superimposition of two monolayer's. Hence through investigation of monolayer would shed light on hydrocarbon chin miscibility, bilayer compressibility, free energy of chain mixing which helps to understand the role of hydrocarbon wall in bilayer.[138] Excess area of mixed monomolecular film where more than one amphiphiles are present are often guide to understand the associative or repulsive type interaction between the hydrocarbon chains of the amphiphiles. Π-A Isotherm is measured in a A Langmuir–Blodgett trough (LB trough that is used to compress monolayer of amphiphiles molecules on the surface of a given subphase (usually water) and measures surface phenomena (viz. surface pressure, surface potential etc.) due to this compression. Water must be purified to remove impurities. Impurities as small as 1ppm can radically change the behaviour of a monolayer. Representative image of Π-A isotherm is given in Figure 42.

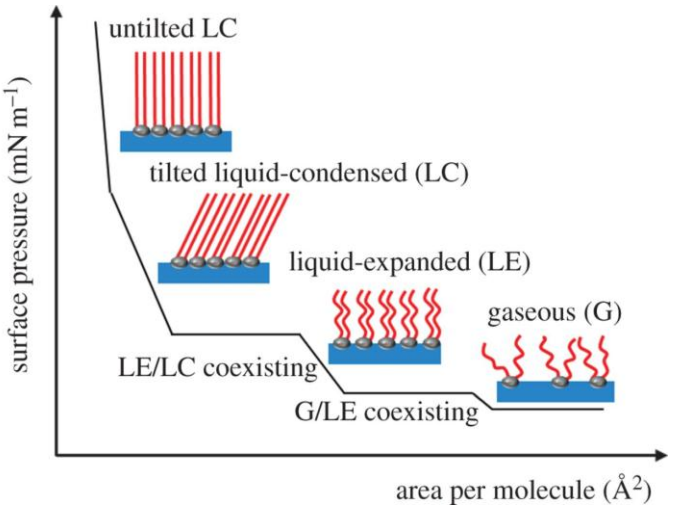


Figure 43: Representative surface pressure – area isotherm of lipid.

After careful addition of lipidic materials to the sub-phase the trough was closed by a glass box to avoid the entrance of dust. 30 min after addition the compression starts with a speed 5 mm per

sec. Due to the compression, surface pressure increases as the lipidic molecules come closer. As reflected from the figure, the isotherm consist of four differ parts and each describe the state of the monolayer. In gaseous state, the molecules are apart from each other whereas in liquid expanded and in liquid condense phase, they orient themselves in a same fashion. Finally in the solid state, most of the molecules are very close to each other that beyond this no further addition of molecule would not be possible and will break the pattern. Thus characterization of bilayer would not be successful unless one performed monolayer study. [139, 140]

The physical properties of liposomes are highly dependent on the type of phospholipids constituted, intermolecular interactions as well as the conditions of the environment which surround it. Nevertheless, due to their easy generability and readily modifiable nature in terms of its lipid contents and structure (i.e. unilamellar and multilamellar), liposomes have received particular attention as the simplified version of cell membrane and have been commonly employed in the investigation of drug-lipid bilayer interaction. In this proposed program, liposomes will be employed in order to study the interaction between dendrimer and lipid bilayers.

Adsorption of dendrimers on liposomes and the formation of dendrimer-liposome aggregates are the most common aspect in the interaction between dendrimers and liposomes. It is believed that the dendrimers could act as glue for the vesicles. Most of the recent studies have focused on the interactions of positively charged dendrimers with cell membranes, since greater interaction potency is expected between these dendrimers with the negatively charged cell membrane compared to other neutral or negatively charged dendrimers. However, it has also been shown that besides the surface charge of the dendrimer molecules, there are many other physicochemical properties of dendrimers which influence the interaction between dendrimers and lipid bilayers. These properties of dendrimers include the dendrimer type, generation, surface charge and also solution conditions as well as the composition of membrane lipids.

Studies on the change of the zeta-potential of liposomes are assumed to provide better understanding of the nature of dendrimer-liposome interaction. It is known that the zeta-potential of liposomes would change as a result of the adsorption of charged dendrimers, while remain constant if dendrimers are internalized from the interaction with the liposomes.

The current barrier to the application of dendrimers as various therapeutic agents as mentioned above, however, is the lack of established knowledge on biocompatibility and toxicity of dendrimers in biological systems. The kinetics and mechanisms of dendrimer-lipid bilayer interaction have received considerable attention as cells contain many layers of membranes which dendrimers need to recognize, interact and internalized before it could reach its intracellular targets. As discussed, the knowledge on this area has been expanding over the past

few years, but there are still many questions left to be answered before dendrimers could be safely employed as a novel therapeutic agent.

As part of the effort to provide a better understanding of the mechanism of dendrimer interactions with lipid bilayers, this research is aimed to investigate the mechanism involved in the interaction between different generation and types of PAMAM dendrimers and liposomes (i.e. electrostatic and hydrophobic interaction), and also to provide an understanding on how the various physicochemical properties of dendrimers and liposomes could affect the dendrimer-liposome interaction. Specifically, in this research, different types of phospholipids including 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), soyphosphatidylcholine (SPC), 1,2-Dipalmitoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DPPG), dihexadecyl phosphate (DHP), 1, 2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1, 2-dipalmitoyl-*sn*-glycero-3-phosphate (DPP) and 1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanol (DPPEth) and cholesterol (CHOL) will be employed to synthesize a range of liposomes of varying chemical composition, structural and physical properties. The information obtained from their interaction, including adsorption isotherm, packing density and affinity, changes in surface charge as a result of adsorption process and aggregate formation (changes in particle size) are believed to be useful as they would provide a basis for future studies which investigate the interaction between dendrimers and cell membranes, which structurally and chemically more complex than simple lipid bilayer (i.e. liposomes).

Also, as dendrimers with different physical and chemical properties would be employed as therapeutic agent in future according to the need of the specific therapeutic purpose, the studies are also assumed to investigate the influence of dendrimer types on its interaction with liposomes. Different types of PAMAM dendrimers that contain a similar surface group (amine), but different generations will be employed. The influences of the dendrimer type and generation on the dendrimer-liposome interaction will be studied in terms of variation in the adsorption isotherm, changes in zeta potential as well as particle size and other physiochemical parameters. The information obtained would be useful as they would provide an idea in future for the rational design of dendrimers which are biocompatible and are optimized for therapeutic application. In another attempt, efforts will be put to study the kinetic and mechanistic aspects of dendrimer-liposome interaction. To meet up the final goal of the proposed work, dendrimers of different kinds and generations will be employed to perform cell (bacterial/ cell lines) growth studies. This

will eventually be helpful in obtaining a proper denderimer based formulation for therapeutical uses.

In 1st chapter we have studied the different physicochemical properties of liposomes with respect to lipid composition, pH, time and temperature. The liposome were prepared by conventional thin film hydration technique by using soy phosphatidylcholine (SPC), dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG) and a 7:3 (M/M) mixture of DPPC+DPPG along with 30 mole% cholesterol in each combination. Hydrodynamic diameter (d_h), polydispersity index (PDI) and zeta potential (Z.P.) of liposome were determined with respect to time, temperature and pH by DLS. Except SPC, hydrodynamic diameter of other liposomes did not vary significantly up to 100 days indicating its substantial stability. The size of liposomes depended on lipid composition, pH and temperature. On the other hand the zeta potential was found to be independent of the pH of the medium, although it varied with liposome type. Lower polydispersity index (PDI) values (0.15 to 0.4) of all the liposomes indicate that liposomes are monodisperse nature in the entire pH range. Liposomes were thermally reversible in the temperature range 25 to 50°C. All the liposomes exhibited size constriction at ~40°C, both during the heating and cooling cycles, which correspond to the chain melting temperature of the phospholipid bilayer. But PDI values did not change appreciably with temperature. Spherical morphology was observed from electron microscopy for all the liposomes. The size of the liposomes, as obtained by the TEM measurements was found to be comparable with the DLS data. Bilayer thickness of the liposomes was found to be ~5.6 nm, comparable with the literature values. Hydration on the surface of liposome could also be noticed. DSC studies on the liposomes were performed with an aim to understand the bilayer chain melting process. The presence of higher amount of cholesterol (30 mole%) resulted in the broadening of DSC peaks. The main phase transition temperature of the liposomes was in the range of 38 to 50°C for the different studied pH. Influence of pH on the transition temperature (also known as the temperature of the maximum heat flow, T_m) was more significant among the negatively charged liposomes. The phase transition temperature increased with decreasing pH. To understand the physicochemistry of membrane property, curcumin were used as probe. Incorporation of curcumin into liposomes caused fluorescence enhancement with significant blue shift in λ_{em} values indicating its transfer to the less polar region of liposome. Curcumin, predominantly in its neutral form at lower pH, binds strongly to liposomes through hydrophobic

interactions; thus the fluorescence intensity of curcumin is enhanced in liposomes at lower pH. However, at higher pH, curcumin decomposes from its conjugated diene; leading to its poor adherence to the liposomes. The membrane viscosity of liposomes was studied by anisotropy measurement. Anisotropy value followed the order: SPC > DPPC+DPPG > DPPG, irrespective of the pH of the medium. The movement of curcumin in SPC liposomes is hindered to a greater extent compared to other systems which explains the highest anisotropy value of curcumin for SPC liposome. Lowest 'r' value for DPPG could be due to the similarity in the charge of the probe and the lipid. Anisotropy value for all the systems (except DPPC) increased with pH. In conclusion, membrane micro viscosity unveiled highest value for SPC, whereby the rigidity of membranes increased with increasing pH. Entrapment efficiency of liposomes with reference to curcumin followed the order: DPPC > DPPC+DPPG > DPPG > SPC. *In vitro* release kinetics of curcumin were studied to compare the four different liposomal systems and also to define the nature of the release processes. Curcumin release from the liposomes at pH 7.4 followed the order: SPC> DPPG> DPPC+DPPG> DPPC. Sustained release of curcumin was noticed for all the liposomes. The antibacterial efficacy of the liposomes, loaded with and without curcumin, was assessed on gram negative (*Klebsiella pneumoniae* and *Pseudomonas putida*) and gram positive bacteria (*Bacillus amyloliquefaciens* and *Bacillus subtilis*). No antibacterial activity was recorded with the liposomes in the absence of curcumin. Curcumin loaded liposomes exhibited substantial antibacterial activity on the gram positive bacteria *Bacillus amyloliquefaciens*. The comprehensive studies could provide a deeper understanding of the effect of formulation variables on the physicochemical stability of the liposomes.

In second chapter the interaction of different generation poly(amidoamine) (PAMAM) dendrimers and with the combinations of liposomes were studied. Second, fourth and sixth (2G, 4G, and 6G) generation PAMAM dendrimers were used, which are cationic under normal conditions. Liposomes comprised of soy lecithin + cholesterol (SLC+CHOL) (negative surface charge), DPPC+CHOL (positive surface charge), DPPG+CHOL (negative) and a biologically simulated mixture of DPPC + DPPG (7:3) + CHOL (negative) were used as model bilayers. Silica was used as a negatively charged hard sphere model to make a comparative study. The interaction between dendrimer-liposome systems can be assessed by turbidity measurements. There is a threshold concentration after which the absorbance increases, reaches a maximum and decreases again. Similar observation was found in case of size analysis by DLS measurements.

Initial size increment and attainment of maxima is probably due to aggregation/association of liposomes, assisted by dendrimers. Dendrimers being oppositely charged, compared to the surface charge of liposome, get easily bound/attached to the liposome surface. The extent of interaction between dendrimer and liposome depends on dendrimer generation and follow the order 6G>4G>2G. Interaction between negatively charged surface (liposome bilayer) and the positively charged dendrimer were further explored by zeta potential measurements. It has been found that a negative value of zeta potential gets decreased to the positive side upon addition of dendrimers. At a certain concentration zeta potential of the mixture attains zero value, which suggests the charge neutralization. Further increase in zeta potential suggests that it is possible for the non-charged liposomal particles to further interact and aggregate with the PAMAM. After a certain concentration limit, the zeta potential attains a platue, indicating the saturation of liposomes. Interaction after the charge neutralization is probably driven through hydrogen bond and/or hydrophobic interaction. Presence of secondary and tertiary amino groups might induce such interaction. Also bilayer disruption, induced by dendrimers, can also be visualized by AFM studies. Bilayer structures of vesicles, fused on freshly cleaved mica surface, were supported by height analysis. When dendrimer added, get adsorbed onto the bilayer, preferably near the bilayer edges, while at higher dendrimer concentration, the bilayer structure gets completely disrupted. At higher concentration of dendrimers, possibly dendrimers forms some aggregates with the liposomes. Further details about dendrimer-liposome interaction were studied in 3rd chapter.

In 3rd chapter, interaction of liposomes carrying net negative charges with cationic polyamidoamine dendrimers (PAMAM) of different generations were investigated by combined size, zeta potential, turbidity, electron microscopy, atomic force microscopy, fluorescence spectroscopy and calorimetric studies. Firstly, the stability of the liposomes comprising 1, 2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) + dihexadecyl phosphate, DPPC+1, 2-dimyristoyl-*sn*-glycero-3-phosphoglycerol, DPPC+1, 2-dipalmitoyl-*sn*-glycero-3-phosphate and DPPC+1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanol were checked through their size and zeta potential with the variation of time. Hydrodynamic diameter (d_h), polydispersity index (PDI) and zeta potential (Z. P.) of four different liposomes (DHP+DPPC), (DMPG+DPPC), (DPP+DPPC) and (DPPEth+DPPC) were measured at pH 7.4 as a function of time by dynamic light scattering. The size remained almost constant up to 60 days for all the systems, indicating their substantial

stability. The lower PDI values designate that all the liposomes are fairly monodispersed. Size of the liposome depends on the lateral packing of lipid molecules within the membrane bilayer and is mainly determined by the van der Waals interactions between the hydrocarbon chains. The PDI values follow the sequence DPPEth+DPPC> DMPG+DPPC> DPP+DPPC> DHP+DPPC. The zeta potential for different liposome follows the sequence DHP+DPPC > DPPEth+DPPC > DPP+DPPC > DMPG+DPPC. The interaction efficiency between dendrimers and liposomes can be assessed by the turbidity measurements. Turbidity values of the dendrimer-liposome complexes pass through maxima likewise the size after a threshold dendrimer concentration. Initial size or turbidity increments and the attainment of maxima are due to the formation of liposome aggregates assisted by dendrimers. The dendrimers being oppositely charged, get adsorbed on to the liposome surface. Decrease in size and turbidity upon further addition of dendrimers are due to the formation of water soluble dendrimer-liposome aggregates. The ability in imparting turbidity or size enhancement depends on the dendrimer generation. PAMAM dendrimers follow the order 5G>4G>3G while considering the size and turbidity variation. The interactions between a negatively charged surface and the positively charged dendrimers were further explored by zeta potential measurements. Similar effects were studied in 2nd chapter. Spherical morphology with smooth surfaces were observed for all the liposomes; the sizes were comparable to the DLS data. To double check the morphological informing FF-TEM studies were performed. It was observed that with increasing concentration of PAMAM, liposomes surfaces lost their homogeneity with the ultimate formation of aggregated heterogeneous entities. These results further support the proposition of the formation of dendrimer-liposome complexes are discussed in the 2nd chapter. State of polarity of the liposome surface and the packing of bilayer are the two important parameters while considering the dendrimer-liposome interaction processes. Solvatochromic dye 7-hydroxycoumarine (7-HC) was used as the molecular probe to understand the state of polarity as well as the rigidity/fluidity of the palisade layer of the liposome and the effect of dendrimers. Additionally, the hydrophobic probe 6-diphenyl-1,3,5-hexatriene (DPH) were used to understand about the bilayer packing of dendrimer-liposome complexes. Fluorescence anisotropy value of 7-HC loaded liposomes was lower than DPH loaded liposomes in absence of dendrimer. These fluorescent probe monitors interactions between the external and internal regions of the membrane with dendrimers. Increase in the fluorescence anisotropy was due to the adsorption of dendrimer liposome surface; further

addition of dendrimer leads to mild decrease in the fluorescence anisotropy probably due to the formation of holes in the bilayer, reflecting the fact that some dendrimers probably move through the liposome bilayer. Time-resolved fluorescence decay studies of 7-HC were carried out to further understand the emission decay parameters. Initially, with the progressive addition of dendrimer, fluorescence life time increased, and then attained constancy. Rotational diffusion of 7-HC decreased with increasing dendrimer concentration due to the formation of stable dendrimer-liposome complex that lead to overall increase in the viscosity of the medium as also revealed from the anisotropy studies. DSC studies were carried out in order to understand the thermal behavior of dendrimer-liposome interaction process. With increasing dendrimer concentration, the pre-transition temperature of lipids declined and the main transition peak became shorter and wider the high concentrations of dendrimers in DPPEth+DPPC lipid bilayer, that led to the abolishion of the transition peak indicating the perturbation of lipid bilayer. A decrease in the pre-transition peak of liposomes suggests that dendrimers can interact with this liposome surface, whereas alteration of the main transition peak suggests that dendrimers can lead to the generation of holes in the bilayer. With increasing dendrimer concentration, the membrane fluidity increases and the endothermic heat change decreases that lead to the lowering of the enthalpy changes of the chain melting processes. Binding constant is another important parameter to quantify the extent of the interaction processes between the dendrimer and liposome. The binding tendency of dendrimer on liposomes follows the order DMPG+DPPC> DPP+DPPC~ DPPEth+DPPC > DHP+DPPC which were found from ITC and colorimetric technique. With increasing dendrimer generation the binding constant values decreases. Due to presence of larger number of polymeric amino groups, higher generation of dendrimers interact effortlessly than that of lower generation of dendrimer. The understanding of the mechanism of dendrimer-liposome interactions is important as the cytotoxicity issue is vital for acceptance and development of dendrimer-liposome aggregates as pharmaceutical agents. The cytotoxicity results obtained from MTT assay clearly demonstrate that the all the liposome formulations and dendrimer-liposome aggregates were completely non-toxic towards normal human blood lymphocyte as well as in human RBCs suggesting the potential of such aggregates as drug delivery systems against microbial diseases.

In the last chapter, it contains two parts. One is the mutual miscibility and stability of the mixed monolayers of zwitterionic phospholipid, dipalmitoylphosphatidylcholine (DPPC) with

negatively charged phospholipids (dihexadecyl phosphate (DHP), 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPP) and 1,2-dipalmitoyl-sn-glycero-3-phospho ethanol (DPPEth) and another part is the interaction between the positively charged dendrimer with the monolayers which has been studied in detail using surface pressure-area isotherms. Thermodynamic analysis indicates miscibility of the binary mixtures when spread at the air/buffer interface with synergistic interaction between the components. The surface pressure-area isotherms of the binary monolayers of DPPC and negatively charged lipids at the air-water interface showed maximum deviation for DPPC : anionic lipid at 7:3 M/M ratio mixed monolayer was more stable than the monolayers individual components. Based on the regular solution theory, the miscibility and stability of the two components in the monolayer were analyzed in terms of compression modulus (C_s^{-1}) and excess Gibbs free energy ($\triangle G^0$) and these physiochemical parameters dependent on phospholipids composition. Stable liposomes were formulated by the binary mixture in 7:3 molar ratio of DPPC with negatively charged phospholipids. Subsequently adsorbed monolayers were generated through vesicles disruption technique. Effect of polyamidoamine (cationic) dendrimers on the adsorption kinetics at the vesicles was followed. Bylayer disintegration and subsequent interfacial adsorption of lipids were followed up through the surface pressure. Time analysis bilayer disintegration kinetics was governed by the lipid head groups, chain length as well as the dendrimer generation and concentration.