1. Amphiphile

Amphiphiles have special structural features that simultaneously possess the hydrophilic (water loving) and hydrophobic part (water hating/fat loving). The hydrophilic portion generally consist ions (anionic, cationic, zwitter ionic) or uncharged polar groups whereas lipophilic parts are generally long chain hydrocarbon, such in the form of $CH_3(CH_2)_n$, with n > 8.

Carboxylate (COO^-), sulphates (SO_4^{2-}), sulfonates (SO_3^{2-}), phosphate (PO_4^-), *etc.*, are the anionic and the quaternary ammonium (NH_4^+) ion is the cationic group that attached with the lipophilic hydrocarbon chain. Alcohols are the class of polar uncharged group often attaché with long chain hydrocarbons, such as diacyl glycerol. In aqueous medium they spontaneously form self-assembled structures. Schematic diagram of amphiphile and its self assemble structure in water, commonly known as "micelle" (Figure 1).

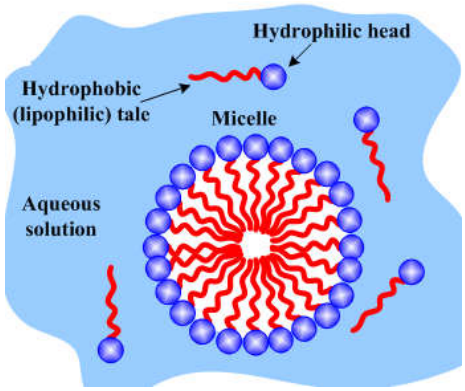


Figure 1. Structure of amphiphile and its self assembled structure (Micelle). Source: www.substech.com.

Because of the hydrophilic-lipophilic charisma, it has diverse range of applications, *viz.,* in detergent[1], paint, pharmaceutics[2-4] food[5,6] to mention a few. The variety of self-assembled structures made amphiphiles to be useful in different areas. Throughout the dissertation work, hybrid lipid bilayer is the most highlighted aggregate structure. Naturally

occurring phospholipids, synthetic amphiphiles and double tail cationic surfactants will extensively be considered in this dissertation in preparing liposome mimetic systems, here in hybrid vesicles.

1.1. Self Assembly and Aggregate Structure

Self assembly of amphiphiles is a spontaneous process, leading to the formation of aggregate having various morphologies from a variety of building blocks. Being the aggregated form, the process of self assembly can include verity of complexities. It can be a simple process of dimeriztion of two amphiphiles or as complicated as biological membranes. Among the various building blocks, amphiphiles draw the attention most over the others as its superiority to form robust assemblies.[7]

Amphiphiles having hydrophilic and lipophilic part renders dual preferences for solvents. Due to amphiphilicity, the polar head group interacts with water (or polar solvent) while the lipophilic part tends to reside out of the contact of water and thus try to orient in the air water interface. In water, it first dissolves as normal solute into the form of a monomer, after a threshold concentration is reached; they form micro-structure or assemblies to avoid the unfavourable solvent hating interaction between water and lipophilic parts. The course of self organization is spontaneous, as the overall entropy increases in the process.[8,9] The increased entropy begins from water-hydrocarbon interaction when amphiphiles are present as monomer. As monomer it forces water molecules in an order state around the lipophilic part. When concentration of amphiphiles is increased two possible scenarios are developed. Either they have to move at air-water interface or to form aggregate structure. The brakeage of ordered water molecules enhance the process of entropy that leads to an overall gain of free energy that drives the amphiphiles to form aggregate structure by virtue of self-assembly.[8,10] The aggregation of amphiphile is generally driven by hydrophobic interaction, hydrogen bond, steric effect and electrostatic interaction.

1.2. Critical Packing Parameter and Aggregate Morphology

Self assembling is a spontaneous process; where amphiphilic building blocks governed by mutual interactions form ordered structure. 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 dispersion medium. Critical packing parameter (C_{pp})[11,12] which takes into account the parameters like polar head group cross-sectional area (a_0), hydrocarbon chain length (l_c) and hydrophobic volume (v) are related as:

$$C_{pp} = \frac{v}{a_0 . l_c} \qquad (1)$$

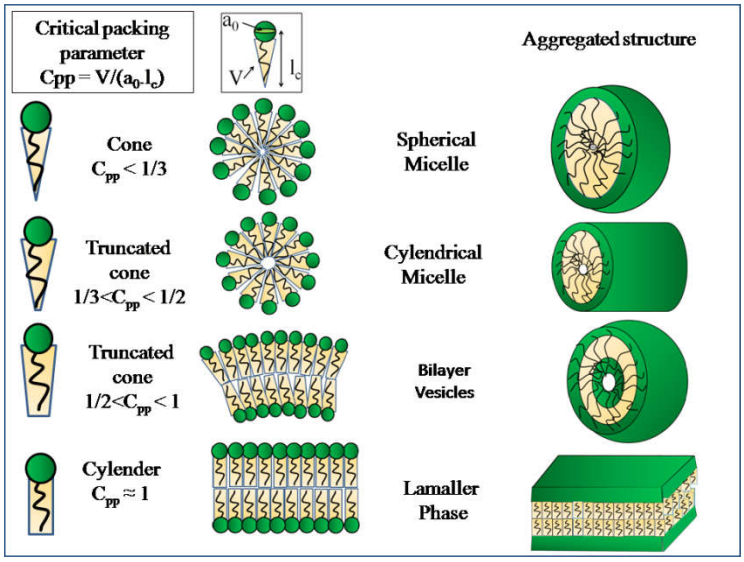


Figure 2: Summary of aggregate structure predicted from critical packing parameter C_{pp}

These parameters together hold the information about the geometrical structure of the aggregates. With 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, the range of C_{pp} is 1/2 to 1 with an inner cavity encapsulating the dispersion medium. A summary of the aggregated structure predicted from critical packing parameter (C_{pp}) is represented in Figure 2.

2. Lipids

Lipid is one of the most significant or important constituents that helps to make the building blocks for all the varieties of animal and plants. Because of its high calorific values, lipids are important dietary components.[13] Lipids are naturally occurring carbon compounds, related to fatty acids and esters of fatty acids. 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 groups. During the process of aggregation, its head group gets exposed towards the polar solvent medium keeping the non polar acyl chain shielded inside. Lipids, along with proteins and nucleic acids, are essential biomolecules for the structure and function of living matter. The common lipids are fats, oils, waxes, steroids, terpins, phospholipids and glycolipids. Phospholipids are the predominant building blocks of biological membranes and herein considered in the dissertation for the preparation of liposome mimetic systems or hybrid vesicles.

2.1. General Classification and Structure of Lipids.

Lipid can be classified into three categories.

2.1.1. Simple lipids (Homolipids)

2.1.2. Compound lipids (Heterolipids)

2.1.3. Derived Lipids (Simple and Compound lipids)

2.1.1. Simple Lipids

Simple lipids are the alcohol esters of fatty acids including neutral fats and waxes. Even number of carbon atoms in the fatty acyl chain may contain saturation or unsaturation. Simple lipids are known as triglycerides or triacyl glycerols. Glyceride bond is formed when the –COOH group of fatty acid and the –OH group of glycerol is attached by the removal of one water molecule to form –C-O-C- bond. Depending upon the extent of esterification, it

can be monoglyceride, diglyceride and triglyceride. Lipids having solid or liquid state at room temperature are known as fat and oil respectively. Schematic structure of simple glyceride is shown in Figure 3.

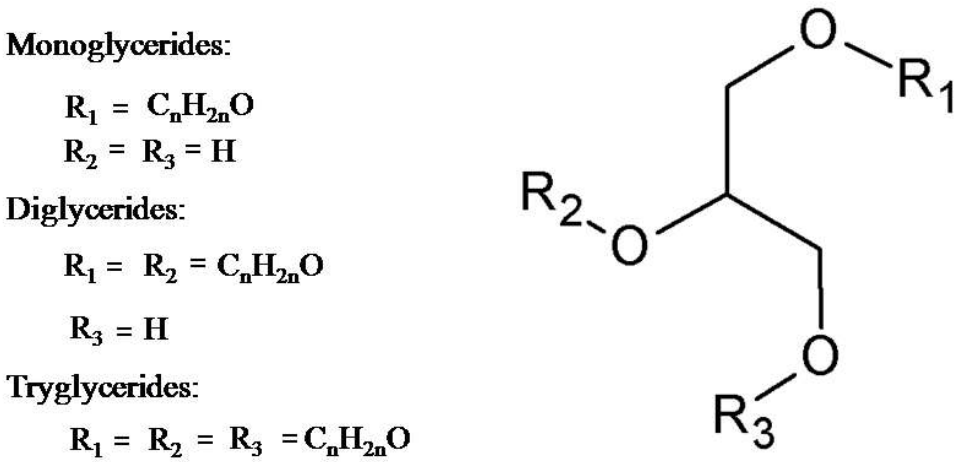


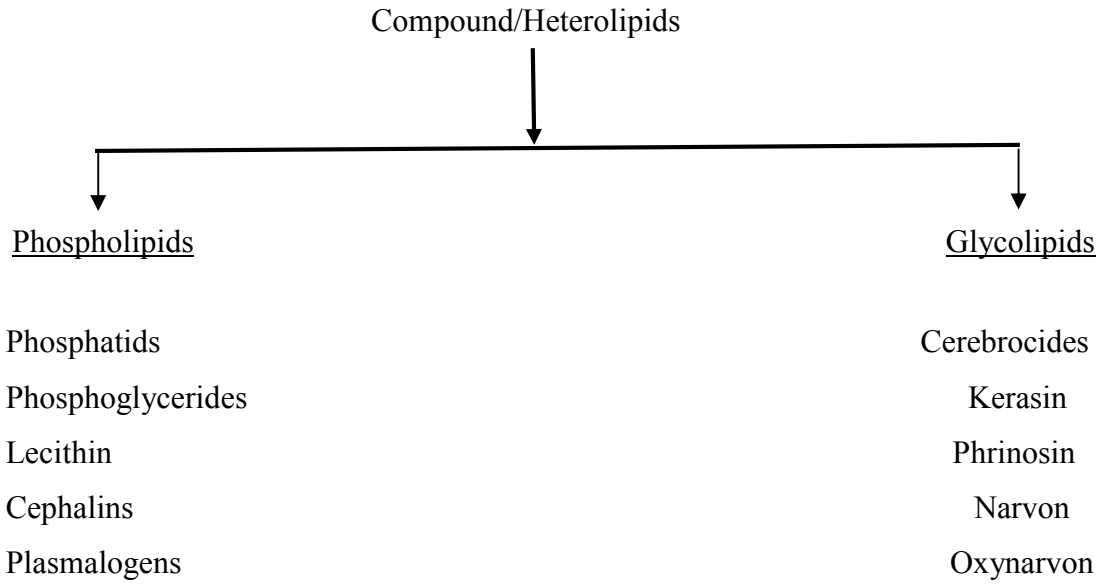
Figure 3. General structure of glycerides.

- **Waxes**

Waxes are the ester of long chain saturated or unsaturated fatty acid with long chain monohydric alcohol. The fatty acids range between C_{14} and C_{36} and the alcohols range between C_{16} and C_{36}. Because of the long hydrocarbon chains, it has high molecular mass with relatively high melting point (above 40 ^{o}C). They are mainly lipophilic in nature. Few examples are: Bee's wax secreted by bees is an ester of palmitic acid and aliphatic alcohol ($C_{30}H_{61}OH$), Lanoline wool (or) fat: Palmitic acid (or) Stearic acid (or) Oleic acid ester of cholesterol, obtained from wool.

2.1.2. Compound Lipids

Compound lipids are the fatty acid esters of glycerol in combinations with the additional groups such as phosphoric acid, nitrogen containing bases and other substituent. It can be categorized into two classes.



2.1.2.1. Phospholipids

Phospholipids are a class of lipids that are the major components of all cell membranes. It usually has two fatty acid chains attached to a glycerol backbone. The third carbon atom in the glycerol moiety is attached with the phosphate ion via C-O-P bond. The number of carbon atoms in the acyl chain is even and can be varied. Different phospholipids have different modified functional groups attached with the phosphate ion which eventually controls the functions of lipid. Few examples are: a nitrogen containing compound (choline), an amino acids contain phosphate (serine) an ethanol amine group linked with phosphate ion (ethanolamine) *etc*. The general structures of phospholipids with different head groups are given Figure 4.

Lecithins and cephalins are the common example of phospholipids found in the nerve cell[14] and animal liver. Egg yolks, yeast, soybeans, *etc.,* are also rich in phospholipids. Soylecithin (SLC) was one of the major components in the dissertation because of its natural abundance in the cell membrane. The structure of soylecithin is shown in Figure 5.

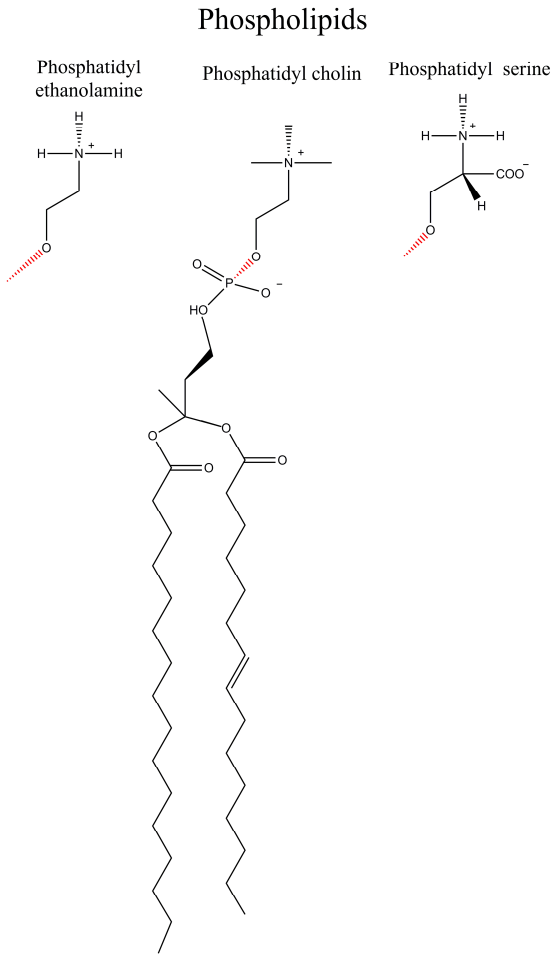


Figure 4. Molecular structure of Phospholipid with different head groups.

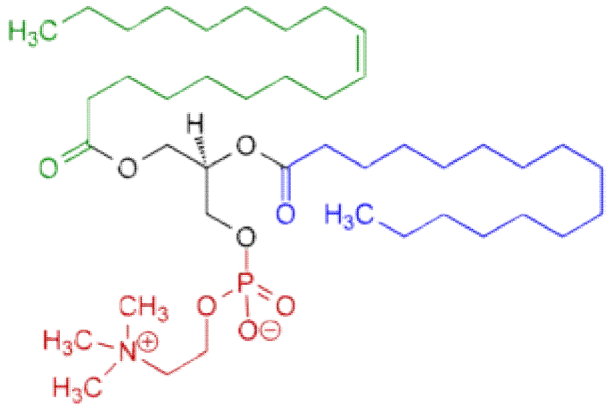


Figure 5. Structure of Soylecithin.

A general description about few phospholipids having different head groups are discussed next.

- **Phospahtidylcholine (PC)**

PC is derived from natural and synthetic sources. It is also known as lecithin. The two glycerol moiety attached with fatty acyl chain via ester linkage and the remaining one hydroxyl group linked with phosphate ion, which is further, connect with choline group. Common source of PC includes egg-yolk and soybean; although bovine heart and spinal cord are rich in PC, however its extraction is not so convenient. Lecithin is chemically inert and zwitter ionic. Besides the chemical properties, the low cost of lecithin has made it as one of the major phospholipid in liposome formulation. Lecithin from vegetable oils contains one or multiple unsaturation in its fatty acyl chain. On the other hand lecithin from animal fat produces fully saturated hydrocarbon chain.

- **Phosphatidylglycerol (PG)**

PG or glycerophospholipid are generally found in pulmonary surfactant, in bacteria and others[15]. It consist an L-glycerol 3-phosphate backbone. Two fatty acyl chains, either saturated or unsaturated, are connected through ester linkages. The head group region constituted with glycerol molecule bonded with phosphomonoester (Figure 6).

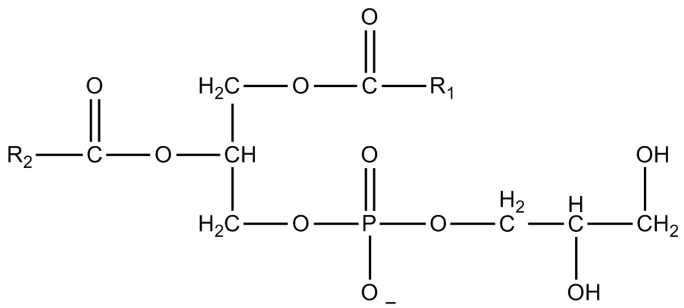


Figure 6. Structure of phosphatidylglycerol.

It is readily available in natural sources and can readily be prepared semi-synthetically form other lipids by means of phospholipase D in presence of glycerol. This type of phospholipids carries a net negative charge in the biological pH range.[16]

- **Phosphatidylethanolamine (PE)**

PE is also found in almost all the living cells and amounts to ~ 25% of phospholipids. In human body, they are found particularly on nerve tissue and spinal cord.[17] It has similar structure to the PC except three hydrogen atoms are directly attached with nitrogen of the ammonium group. These hydrogen atoms allow interaction with other molecules around the membrane via hydrogen bonding. At low or neutral pH, the ammonium group becomes protonated, leading to a resultant zwitter ionic lipid.

- **Phosphatidylserine (PS)**

Ps is another important component of mammalian cell membrane. Hydrocarbon chains are similarly attached to first two carbon atoms of glycerol via ester linkages. The phosphate group linked with the remaining hydroxyl group is also attached to serine moiety. The head group carries net negative charge due to the presence of the negative charge on phosphate. PS collected from plants and animals are different in terms of their fatty acyl chain length.

2.1.2.2. Glycolipids

Glycolipids are the glycoconjugates of lipids and generally found on the extracellular eukaryotic cell membrane. It functions to maintain the stability of membrane and to facilitate cell-cell interaction. (Source: https://www.nature.com/subject/glycolipids). Here glycolipids tail connected with one or more hydrophilic sugar head group through glycosidic bond. It acts as a rcognizing site for specific chemicals and also maintain the stability of the membrane by attaching with tissue. The general structure of glycolipids is shown in Figure 7.

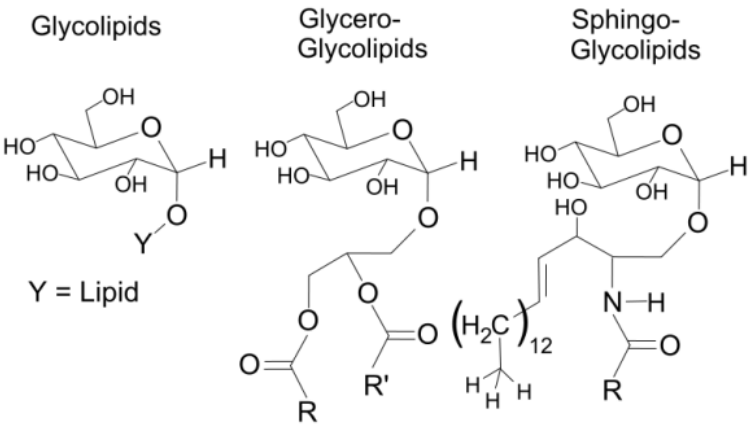


Figure 7. General structure of glycoplipids.

2.1.3. Derived Lipids

These are the class of lipids derived from simple or compound lipids via hydrolysis. Examples include cholesterol, coprostanol, cholestanol, ergosterol, different types of terpenes, lycopene, carotenese, xanthophylls, *etc*., Steroids, terpenes and carotenoieds are the most common class of derived lipids.

- **Steroids**

Steroids are the class of organic compound having four rings arranged in a specific orientation. Nucleus of the steroids has 17 carbon atoms bonded to form four fused rings; three six member cyclohexane rings (A, B and C) and one cyclopentane ring (D) as shown in Figure 8.

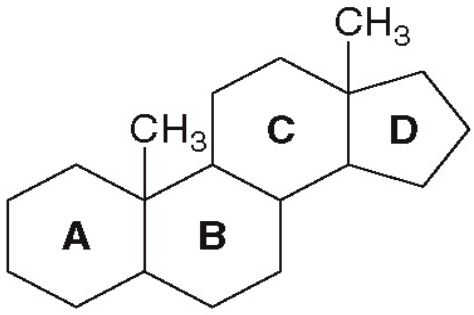


Figure 8. Steroid ring system.

Steroids do not contain any fatty acid and are hydrolyzed on heating.

Sterols are the derivatives of steroid with a hydroxyl group attached in position 3. It is also known as solid alcohol.

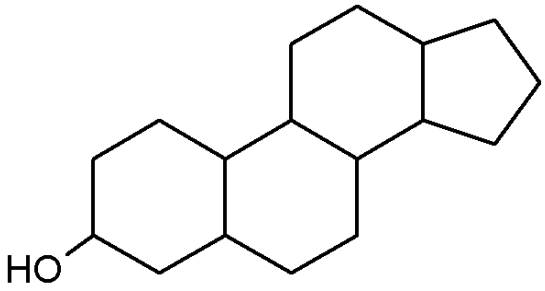


Figure 9. Core structure of sterol.

They occur naturally in the cell membrane of all type animals, plant fungi. One of the most important and familiar type of animal sterol is cholesterol which is vital to animal cell membrane as it regulates the membrane rigidity and fluidity.[18,19] It also acts as a precursor to fat-soluble vitamin and steroid hormones. Throughout this research work cholesterol have been used in preparing the liposomes. Cholesterol along with ergosterol, sex harmons, bile acids are all sterol derivatives. The structure of cholesterol is shown in Figure 10.

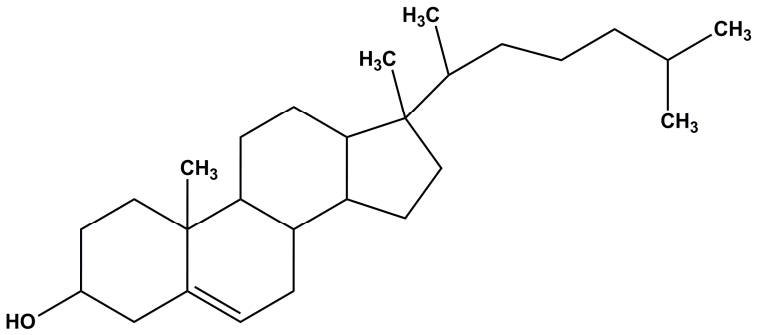


Figure 10. Structure of cholesterol.

Animal cell membrane is composed of cholesterol with a maximum of 30 mol%. It controls the membrane fluidity at all physiological temperature. Cholesterol is essential in our body as it produce hormones, vitamin D and other substances that help us to digest food. It is usually produces in our body, only a limited amount of body cholesterol comes from the diet. Unsaturated fatty acids from vegetable oil help to reduce cholesterol synthesis in our body system. On the other hand, saturated fatty acids from animal fat enhance the amount of

cholesterol in the blood as well as triglycerides. Brain, nervous tissues, solid alcohol from bile, adrenal glands, *etc*., are the sources of cholesterol.

2.2. Biological Role of Lipids

The biological functions of lipids are the following:

- Phospholipids act as the structural material to form cell wall.
- Fat is the important food storage for animals and plants.
- It acts as an important energy source through food supply.
- Transport of fat within the body is directly not possible; lipids act as detergent to emulsify the fat and thereby transport to the different parts of the body.
- Simple lipids protect intracellular organism as a heat insulators. It is also essential for fat soluble vitamin like A, D, E and K.

3. Synthetic Amphiphiles

Naturally occurring phospholipid based liposomes are common in clinical[20,21] and cosmetic formulations.[22] However liposomes formulation draw considerable issues as per as stability is concerned. Areal oxidation, bacterial attack and limited room temperature stability are some of the major concern.[23,24] Ion pair amphiphile (IPA), structurally resemble with double tail phospholipids are considered to be novel substitute that can be prepared in laboratory by stoichiometric mixing of aqueous solution of cationic and anionic surfactants.[25,26] Reports suggest that in aqueous medium few of these lipids like IPA molecules form vesicle structure, better known catanionic vesicles or "catanosome".[28] Ionic surfactants are easily available and commercially cheap relative to the phospholipids. Thus IPA prepared in combination of various type of cationic and anionic surfactants draw much attention of the researchers. However for long term storage, catanionic vesicles from IPA

easily fuse together and cannot maintain their uniformity in size. Investigation pointed out two aspects, inter-vesicles and intra-vesicle interaction that assist the process of fusion.[29] In order to improve the physical stability of catanionic vesicles, IPAs were designed by changing the hydrocarbon chain length and/or by altering the polar head group of the constituent ionic surfactant as is shown in Figure 11.

(HTMA-DS) is one of the widely investigated IPA prepared by mixing stoichiometric amount of hexadecyltrimethylammonium bromide (HTMAB) and sodium dodecylsulphate (SDS) in water. Although it possesses poor vesicular stability, however introduction of specific molecule such as phospholipid, cholesterol or double tail ionic surfactants can impact additional stability. IPA designed with different alkyl chain length or polar head groups were widely investigated in the form of ^{1}H-NMR, X-ray diffraction and FTIR.[26] Due to its similarities to the phospholipid, HTMA-DS has been frequently used to prepare hybrid vesicles throughout the dissertation.

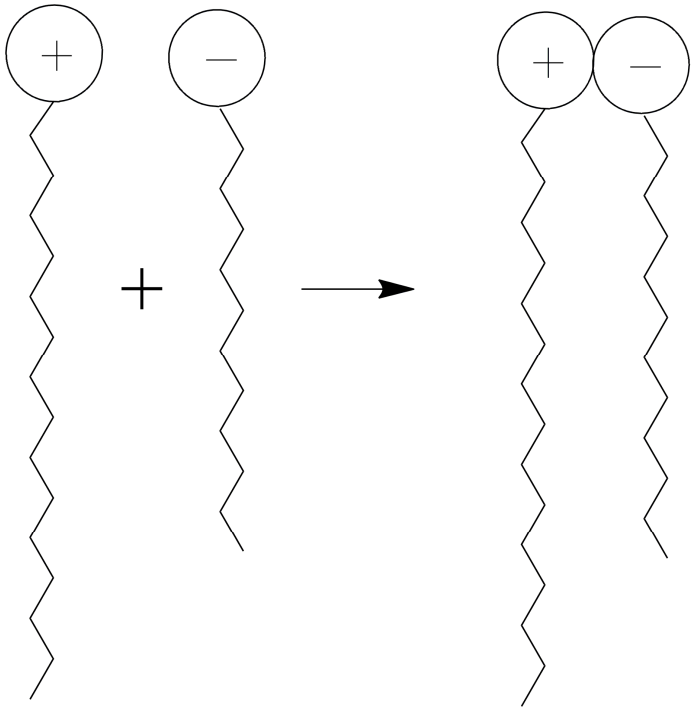


Figure 11. Schematic diagram of IPA.

4. Liposomes

Liposomes are the spherical shaped vesicles consisting one or more phospholipid bilayer. Amphiphilic molecules like lipids, cholesterol, *etc.,* are the main constituents. The word liposome comes from Greek words: “lipo” means fat and “soma” means body. Liposomes were first described in the year of 1961 by British haematologist Alec D. Bangham.[30-32] Today, liposomes become one of the important tools in different scientific discipline, including mathematics, theoretical physics, chemistry, colloid science, biochemistry and in various clinical trials.[33,34] Schematic diagram of liposome is shown in Figure 12.

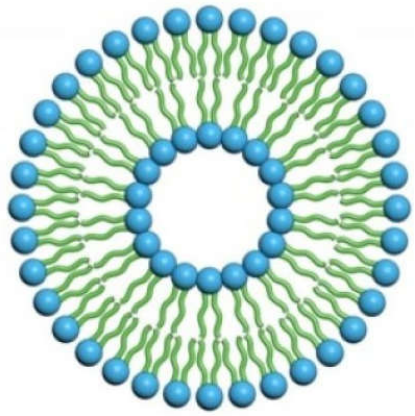


Figure 12. Structure of a liposome.

Due to its unique hydrophilic - lipophilic environment, liposome can host both the hydrophilic drug into the polar head group region or into the interior whereas lipophilic drug could be trapped inside the hydrocarbon region.[35,36] Thus artificially prepared vesicles become one of the important agents to improve the delivery of the large number of molecules; like enzyme vaccines,[37,38] genetic material,[34] drugs against microbial agents, anti cancer drug,[39,40] antifungal drugs,[34] peptide hormones, *etc*.[41]

In many cases with many drugs, the direct oral administration may always not lead to the better result. Most of the drugs are not good enough to tolerate the resistance of enzymes that it encounters during its journey through the digestive tracts. Bile salt, intestinal flora produced in human body, alkaline solution, digestive juices and free radicals in human body, *etc.*,[34] can substantially reduce the efficacy of a drug and may promote the side effect. In such cases liposome can make its mark as a protecting vehicle in transferring the drug to its desire target with controlled release. Its unique inert behaviour towards the entrapped drug (hydrophilic and lipophilic) along with ease of biodegradability and non-toxicity results in the improved bio-distribution of the drug with fewer side effects. Life time of the entrapped drug is also increased as the liposome shields the drug from different physiological environment.

4.1. Preparation of Liposome

Aforementioned lipids can be considered as the major components of cell mimetic system like liposome. The main goal in liposome formulation would be to prepare drug loaded liposome or hybrid vesicles with superiority. Narrow size distribution and long term stability of the dispersion are 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 that eventually follows the removal of the non-encapsulated entities. Two major methods are involved 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 can expect high drug encapsulation efficiency by such liposomes. 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. Drugs, through the process of diffusion thus get loaded into

the liposome. Some of the commonly used methods in preparing the liposome are: thin-film hydration method, injection methods, sonication, membrane extrusion, micro emulsification, reverse phase evaporation method and calcium induced fusion method.[23,34,42]

4.1.1. Thin Film Rehydration Method

It is one of the most commonly used methods in preparing liposomes. Requisite quantities of lipidis are taken in a round bottom flask; dissolved in solvent like chloroform, dichloromethane[43], ethanol[44] and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v).[45,46] A thin lipidic layer is generated by evaporating the solvent under vacuum. Traces/minute quantity of organic solvent was further removed by the stream of nitrogen at 4 ºC[47]. Distilled water, phosphate buffer solution with varying pH and normal saline buffer solution is subsequently added for the hydration of the thin film. It is then hydrated at 60-70 ºC for 1h (usually above the chain melting temperature). Total lipid hydration of the liposomal dispersion was done by allowing the lipid film to hydrate overnight.

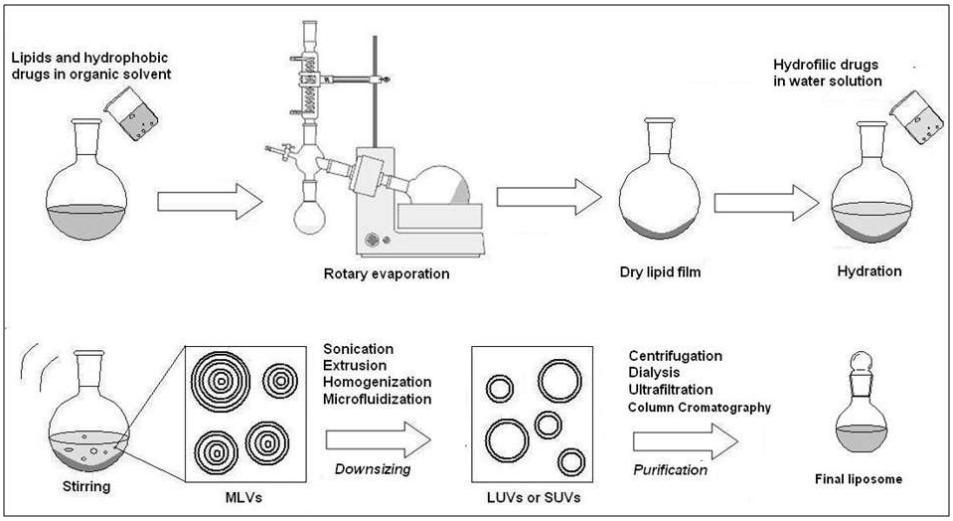


Figure 13. Schematic diagram of thin film rehydration technique.

All kinds of lipid mixture can be used in this method for the preparation of 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. Injection Method

- **Ether Injection Method**

Solution of lipids dissolved in ether or ether/methanol mixture is slowly mixed into an aqueous solution of encapsulated materials at 55-65ºC with reduced pressure. At higher temperature (above the boiling point of the solvent) evaporation or removal of the organic layer leads to the formation of liposome. Major drawbacks of this method include the exposure of the encapsulated material to the organic solvent and high temperature. Irregular particle size distribution also leads to the heterogeneity.[16,48]

- **Ethanol Injection Method**

A lipid solution of ethanol is rapidly injected to a huge amount of buffer or distilled water. Immediately, the liposomes are formed in the mixture. Major disadvantages of this method are the heterogeneity in liposome size. Liposomes are very dilute and the removal of ethanol is very difficult as it forms azeotrope mixture with water. Formation of the azeotrope restricts this kind of liposomes to apply in biological system.[49,50]

4.1.3. Sonication Method

This method includes the size transformation of liposomes. Multi lamellar vesicles (MLV), prepared by in thin film rehydration technique, is sonicated by bath or probe sonicator to prepare homogeneous dispersion of small unilamellar vesicles. Major difficulties

associated with this method include low drug encapsulation due to smaller size, overheating that can degrade the components.[51]

4.1.4. Reverse Phase Evaporation Method

This method involves the formation of water-in-oil emulsion by means of brief sonication of a two phase system. The two phase system comprises the lipids dissolved in an organic solvent (diethyl ether, isopropyl ether or mixture of isopropyl ether-chloroform) and aqueous phase. Phosphate buffer solution or citric-Na_2HPO_4 buffer is sometimes 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 under vacuum. The residual solvent is then removed by continued rotary evaporation under reduced pressure. The principle advantage of this method is high encapsulation efficiency. However a disadvantage includes the possible existence of the reminiscent solvent in the formulation restricts its application.[52,53]

Size of the vesicles ranges from 20 nm to several micrometers depending upon the pattern of aggregations. However liposome's properties could be changed by altering or tuning the charge of the head group, acyl chainlength, addition of other amphiphiles, *etc*. The properties of liposome could be altered by altering the type of phospholipid charge on the polar head group, size, hydrocarbon chain saturation-unsaturation, *etc*. Liposome of various types can thus be formulated. Apart from the precursor, few different ways of liposome preparation also leads to create different type of liposome.

4.2. Classification of Liposome

4.2.1. On the basis of composition

Liposomes are the aggregates product of natural and/or synthetic lipids along with cholesterol. The major focus on liposome relates 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 are the primary concerns. Depending upon the composition and mechanism of drug delivery, it could be classified into five different types:

(1) Conventional liposome (ii) pH-sensitive liposome (iii) Cationic liposomes

(iv) Immunoliposomes and (v) Long-circulating liposomes (LCL).

4.2.2. On the Basis of Hydrodynamic Size

The size of the liposome can range from 50 to 1000 nm. Size of the liposome is a vital parameter to determine its circulation life time. Bilayer controls the incorporation of the drug; a direct size dependent phenomenon.[16] 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. MLV consists of more than one bilayer, size range from few hundred nanometre to microns.

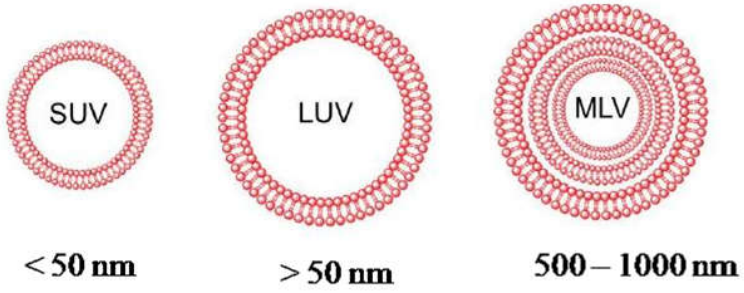


Figure 14. Representative images of SUV, LUV and MLV.

4.3. Lipid Bilayer and Lamellar Phases

Throughout the dissertation, lipid bilayer is the most frequently used system. Bilayer aggregate is formed when lipids are dispersed in water. According to the geometrical packing concept, cylindrical structure of some lipids prefer to self-aggregate into bilayer in water.[54] Higher lipid concentration usually forms two dimensional bilayer with lamellar phase. The

lamellar phase of phospholipid bilayer may exist in different states, depending on bilayer component and the environmental temperature.[55,56] For example, liposome with unsaturated phospholipids like soylecithin (SLC) produces more permeable and less stable bilayer due to its fluid (or liquid crytaline) nature. Whereas the bilayers of saturated lipid like DPPC are less permeable and more stable due to rigidity.[57,58] Temperature certainly does control the physical state of the lamellar phase. At low temperature, it exists in the gel phase (L_{β}')[59,60] where the hydrocarbon chains are closely attached with each other that forms more or less like a frozen state. When the temperature is increased, thermal energy after a threshold limit, becomes sufficient enough to transform the rigid bilayer moieties into a fluid like state. At higher temperature the randomness of the hydrocarbon chain get increased and thus form more permeable liquid-crystaline or fluid phase (L_{α}).[61,62]

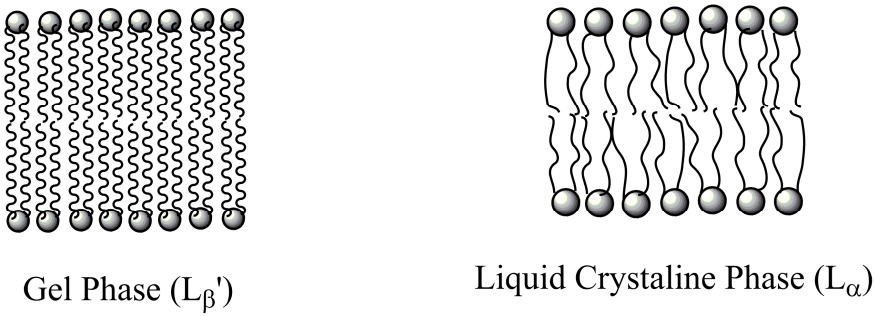


Figure 15. Gel and liquid crystalline phase of bilayer aggregate.

In gel phase all the hydrocarbon chain is in trans form whereas in liquid crystalline phase, the lipid chain is in gauche conformation.[63-65] Being in gauche conformer, the randomness of the bilayer hydrocarbon region increased which eventually holds the fate of the bilayer. Transformation of the bilayer from gel to liquid crystalline phase is known as phase transition and very much substantial to understand the thermodynamics associated with it.[66-68] The temperature at which the physical state of the bilayer transforms from L_{β}' to L_{α} is known as the phase transition temperature (T_m). Transformation or the existence of liquid crystalline

phase involves the process of interdigitation of the lipid acyl chain. Alteration in hydrocarbon chain and head group greatly affects the interdigitation and the physical property of the bilayer. Herein the bilayers that have extensively been studied are the mixture of SLC, IPA and surfactant in combination with cholesterol.

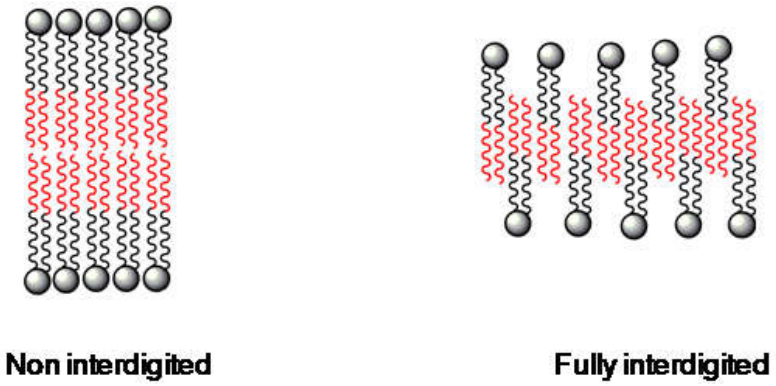


Figure 16. Schematic representation of non-interdigited and fully interdigited lamellar phase.

Interdigitation could occur in multiple ways. Structure and symmetry of the hydrocarbon chain deeply holds the nature and extent of interdigitation. Lipid often conflict its characteristics and ability to form interdigited system and thus so far no general rule has developed to fully understand the process of interdigitation.

4.4. Liposome in Drug Delivery

A drug delivery system (DDS) is capable to conduct therapeutic substances or drugs to the target tissue and thus can improve its life time and efficacy by controlling the release of the drug.[2,23, 33-35,37,45,69-71] With the help of advance technologies, several new drug-therapies have been invented, but efficacy and steady concentration of the drug in *in vivo* condition is still a challenging issue. On the other hand, some drugs have an optimum concentration range in which it shows maximum efficacy, below or above this concentration it may produce toxic effect or may not show any therapeutic activity. Liposome formulations have widely been used as an effective drug delivery system.

Table 1. Commercially available liposome based drug formulation.

Product Name	**Drug**	**Drug form/Storage time**	**Approved indication**
Ambisome	Amphotericin B	Liposome powder/36 months	Sever fungal infections
Doxil	Doxorubicin	PEGylated liposome suspension/20 months	Kaposil's sarcoma, ovarian/breast cancer
DepoDur	Morphine sulphate	Liposome suspension/24 months	Pain management
Myoset	Doxorubicin	Liposome powder/18 months	Metastatic breast cancer
Visudyne	Vertiporfin	Liposome powder/48 months	Age related molecular degradation

4.4.1. Drug Delivery Routes

Several drug delivery routes have been adopted to introduce the drug in human body. Choices of the routes of drug administration depend on the type of disease, infected zone of the body. A classification of systematic drug delivery by anatomical route is summarized as:

- **Gastrointestinal System:**
  - Oral
  - Rectal
- **Parenteral:**
  - Subcutaneous injection
  - Intramuscular injection
  - Intravenous injection
  - Intra-arterial injection
- **Transmucosal:** Buccal and through mucosa lining the rest of gastrointestinal track
- **Transnasal**
- **Pulmonary: drug delivery by inhalation**
- **Transdermal drug delivery**
- **Intra-osseous infusion**

4.4.1.1. Oral Delivery

Oral route of drug delivery has been the most acceptable and widely used process for drug administration. It is more convenient because of the easy route of administration and is also widely accepted by the patients.[72-74] However, there are some drugs that suffer major problems during oral administration, like

(i) Presence of digestive enzyme may damage the activity of the drug.

(ii) The high acid content and presence of digestive enzyme in the digestive tract can degrade some drug before they reach to the target cell or tissue.

(iii) Many drugs having structure like macromolecules or polar compound are incapable to effective traverse across the cell of the epithelial membrane in the small intestine to reach the blood stream.

(iv) Many drugs are insoluble in low pH and there by non effective towards digestive track as they are not absorbed by bloodstream.

4.4.1.2. Parenteral Delivery

This involves the administration of drugs to the body by other routs than gastrointestinal tract.[34,69,75-78] The drug is injected to the body through intramuscular, intravenous, subcutaneous and intra-arterial routes. Some advantages and disadvantages of parenteral administration are presented in Table 2.

4.4.1.3. Transdermal Delivery

The drug is administrated into the body surface such as skin or mucous for therapeutic use.[79] This route of delivery is alternative to oral, intravascular, subcutaneous and transmucosal route. It is significantly associated with local effect rather than systematic

effect.[79] The mode of drug delivery is advantageous the drug can directly affect the active site avoiding gastrointestinal and liver metabolism.

Table 2. Advantages and Disadvantages of Parental and Nasal Drug Delivery.

Routes	Advantage	Disadvantage
. Parenteral Delivery	Gastrointestinal track is avoided	Involvement of pain complaint by the patient
	Rapid onset action	For the subcutaneous process the size of the molecule does matter. Bigger molecule has slower penetration rate than smaller one.
	Almost complete bioavailability	Diffusion of the drug into the body fluid may get hamper by the viscosity.
	Proficient route for comatose patient who are unable to ingest drug by oral administration	To avoid the accumulation of the drug the sites of injection have to be changed.
Nasal Delivery	Compared to epidermis or gastrointestinal mucosa, nasal mucosa shows higher permeability.	Dose is limited as the available area for the absorption of the drug is small
	Highly vascularised sub epithelial tissue	Time available for absorption is limited
	. Rate of absorption high, usually within half an hour	Diffusion of the drug into the body fluid may get hamper by the viscosity.
	Gastric stasis and vomiting can be avoided.	During common cold or a nose with surplus watery rhinorrhea, the nasal routes get blocked.
	Most feasible roué for the delivery of peptides	Degradation of the drug due to enzymatic effect

4.4.1.4. Nasal Delivery

For systematic and topical effect, drug can be administrated nasally for several years. For nasal delivery the drug is adsorbed via the aqueous channel of the membrane. As long as the drug is in solution phase and small molecular size, it will be absorbed rapidly via aqueous path of the membrane.[80,81] With increasing molecular size of the drug, the absorption through nasal cavity decreases. The rate and extent of absorption of the drug through nasal cavity depends on several factors. Some advantages and disadvantages of parenteral administration are presented in Table 2.

5. Different Drug Delivery System

Colloidal drug delivery systems like micelle, vesicle, liquid-crystalline system and nanoparticle with diameter 10 to 400 nm are considered to be promising agents as drug carrier.[82-86] With the aid of advance technologies, researchers have been developing drugs with high and promising activity. Most of the drug, DNA and/ or protein and are characterized on the basis of its bioactivity; liberation of their bioactive material at right time with right concentration and limited toxicity.[87-89] While developing a drug carrier, the aim is to get an optimized system with better encapsulation efficiency, long shelf-life and low toxicity with controlled release of the drug. Carriers like colloids get special attraction due to the biocompatibility, biodegradability and biomimiking phenomena. General description about different drug carrier system is discussed in the following section.

5.1. Microemulsions and Nanoemulsions

Microemulsions is composed of water, oil, surfactant and co-surfactant are transparent and optically isotropic single –phase liquid solution.[90-92] The system is bi-continuous 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.

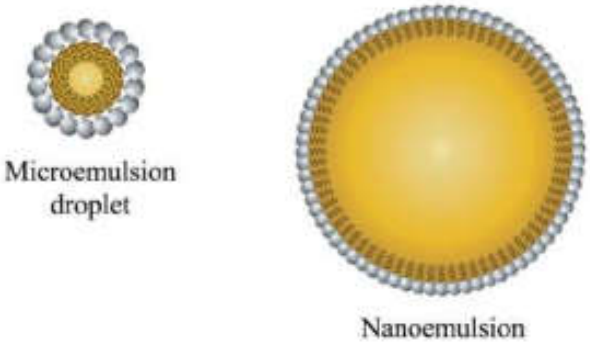


Figure17. Schematic representation of Micro and Nano emulsion. Source: Nano emulsions versus micro emulsions: Terminology, differences, and similarities, Soft Matter, 2012.

Nano emulsions come into the picture in 1950's to the need of parenteral nutrition.[93] It has been introduced into the market as various pharmaceutical products, such as: disoprivan, Etomidat lipuro, diazepam lipure, steasolid and lipotalon.[93,94] Nano emulsions 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 solubilises the drug.

5.2. Nanoparticle

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 oral route.[95]

5.3. 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 various applications such as parental, pulmonary, ocular deliver of drugs.[96-98]

5.4. Liposome

The details of the liposome and its superiority as a drug delivery agent have already been discussed. Apart from the aforementioned DDS, a new class of hyper branch, polymeric molecule known as dendrimer have been using for some while. In the dissertation, dendrimer is one of the important aspects to study the interaction with liposome. The detail of dendrimer is described below.

6. Dendrimer

Dendrimers are three dimensional, hyperbranched tree-like polymeric architectures whose size and shape can be precisely controlled.[99] Dendrimers are fabricated from monomers by step growth polymerisation process. The term dendrimer arises from Greek words “dendron” meaning “tree” and “meros” meaning “branches”.[100] Dendrimer can be fabricated by controlling its molecular weight, degree of branching, and surface functionalities whose measured size reported as ranging from 2.5 nm to 10 nm.[101] Representative molecular structure of dendrimer is shown in Figure 18. Size of dendrimers can be regulated by controlling the polymeric branch which is extended outward like a tree producing spherical shape nanometre size particle leaving cavities inside.[33] Thus

encapsulation of drug inside a dendrimer cavities and transport to the target cell can be achieved.

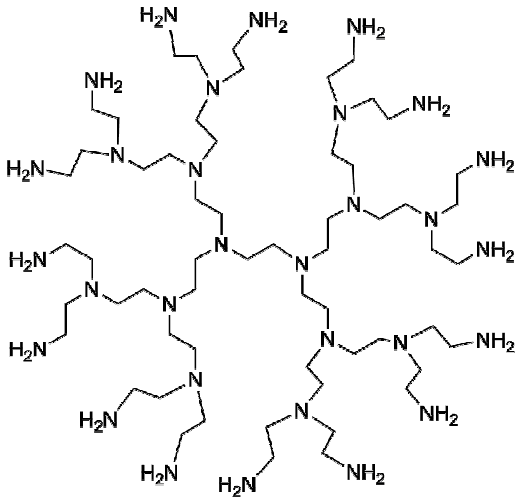


Figure 18. Schematic structure of dendrimer.

Besides the internal cavities, surface end group of dendrimer can also actively conjugate with other molecules. Molecule having amide containing cascade of polymers with amine surface group is known as PAMAM dendrimer works effectively as antibacterial agent[102], antifungal, drug[103] and gene delivery.[104]

6.1. General Properties of Dendrimer

As discussed earlier, dendrimers are polymeric, hyperbranched three dimensional tree-like architectures widely used in the field of nano science. The properties of dendrimers are mainly controlled by the surface groups; however internal core can also play a decisive role.[105-107] It is also possible to make dendrimer water soluble unlike some other polymers by introducing polar groups. It also possesses other controllable properties like toxicity, crystallinity and chirality.[106,108] It exhibits homogeneous symmetrical monodisperse structure with a typical core inside, involving an inner shell and outer shell structure. Dendrimers with hydrophilic surface groups are soluble in polar solvent like water whereas surface group with hydrophobic moieties are soluble in non-polar solvent.[109] Interaction with other entities can

takes place either with the groups inside called “endorecepters” or the groups on the periphery known as “exorecepters” depending on the type of host-guest.[110] 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) terminal surface groups.

6.2. Classification of Dendrimer

The layer of the branches represents dendrimer generation. Higher the number of the branches, higher is the generation of the dendrimer. Synthesis of each new generation increases the molecular weight exponentially and doubles the number of the terminal groups.

Table 3. Generation based physicochemical properties of dendrimers.

Generation	Molecular weight	Measured 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

The generation of the dendrimers are represented as G1, G2, G3 etc indicating generation 1, 2 and 3 respectively. Generation of the dendrimer governs the morphology as lower generation (G0 to G4) dendrimer exhibited planner or elliptical shape whereas higher generation shows spherical conformation.[111] PAMAM is an example of dendrimers having polyamide branch into the core with different amine terminal group and can be replaced with alcohols,

carboxylate ions *etc*. Classification of dendrimer based on generation and their subsequent properties are summarized in Table 3.

6.3. General Methods of Dendrimer Synthesis

The first report on the synthesis of dendrimer was given by Vogtle and co-workers. Relying on the same protocol, amide containing cascade of polymers also known as PAMAM dendrimer was synthesised by Tomalia's group.[112] It can be categorized it three parts; a core, an inner shell and outer moieties. Different functionalities like solubility and thermal stability can be induced during dendrimer synthesis. Commonly use two methods for dendrimer synthesis are given below.

6.3.1. Divergent Method

This method was first reported by Tomalia group[112]. The method involves growth of dendrimer from the core that builds out in step wise fashion towards the periphery through polymerization process. Repetition of each steps generate a new branch which define the number of generation of dendrimer. As the growth of dendrimer originates form the core, alteration of surface group can easily be achieved. It is one of the popular methods for the synthesis of dendrimers as it leads to the formation of highly symmetrical molecules.[113]

6.3.2. Convergent Method

This process follows the opposite route of the divergent method, where the growth of dendrimer starts at the terminal functional groups and proceeds into the core. This process was pioneered by Hawker and Frechet in 1994.[114] The dedritic segment is prepared by coupling the monomers by "one to one" fashion. Different segment can be coupled to the core in this method and hence widely used in formulation of asymmetric dendrimers.

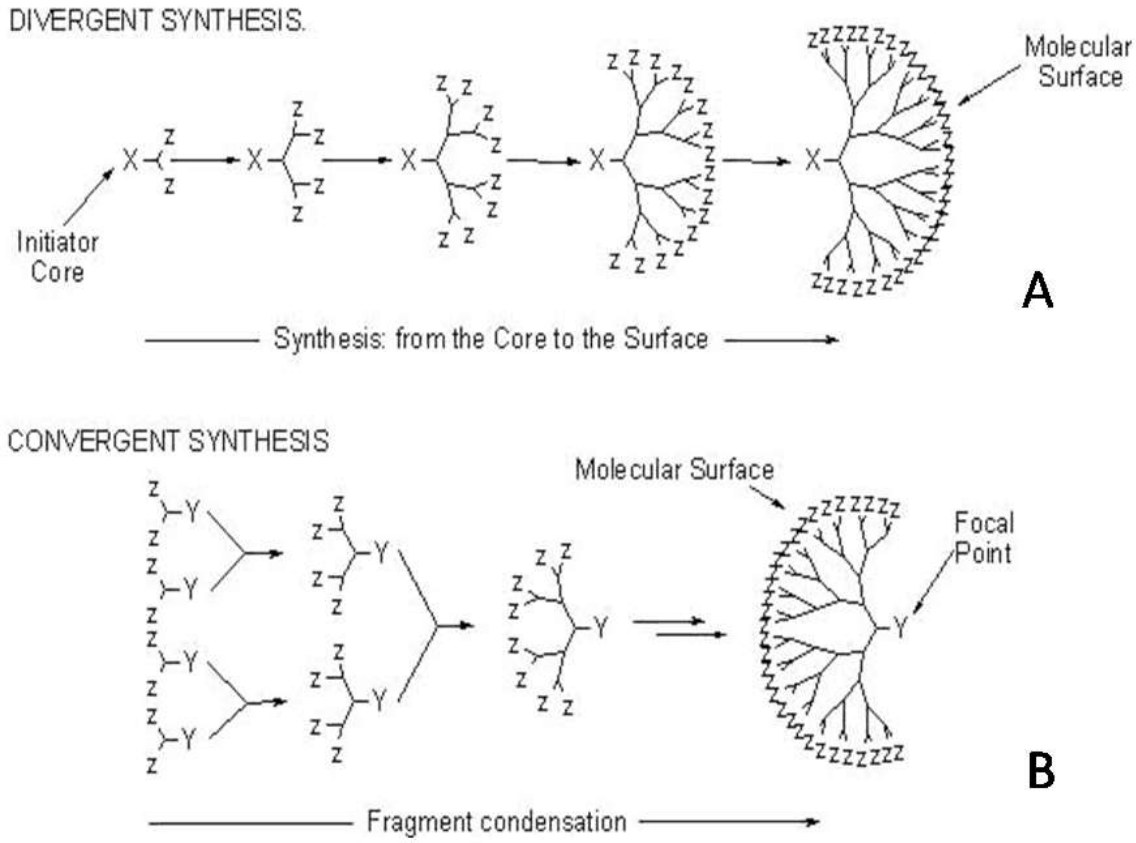


Figure 19. Synthetic routes of dendrimer. (A) Divergent synthesis and (B) Convergent synthesis. Source: http://www4.utsouthwestern.edu/jdebralab/dendrimer.html

6.4. Application of Dendrimers

Dendrimers are the promising macromolecules effectively used in drug delivery or gene delivery. Compared to other polymeric drug delivery architectures, dendrimers offer number of advantages like nanometer size morphology that allows them to pass through vascular endothelial tissue.[110] PAMAM dendrimers efficient in the field of transdermal drug delivery as reported by Cheng *et al*., where PAMAM-NSAIDs complex shows improved permeation of the drug through the skin.[115] Chauhan *et al*. has studied PAMAM-indomethcin complex for transdermal drug delivery.[116] D'Emanuele and his research group had made an

investigation on the monolayer of human colon adenocarcinoma cell line and the impact of dendrimer generation and concentration on it.[117] Bai *et al*. has shown that positively charged dendrimers are suitable agent for enoxaparin pulmonary delivery.[118]

Apart from these, dendrimers are also the point of interest for the targeted drug delivery. Thomas *et al*. has reported the use of dendrimers in the field of targeted delivery as they are able to couple with multiple components such as targeting tissue, drug and cancer imaging agent.[119] DNA assembled dendrimer conjugates have also been developed for targeting drug delivery.[120] Not only DNA, dendrimers are also employed as a gene transfection device as extensively reviewed by Broeren *et al.*[121] Dendrimer-based MRI contrast agents were used first time in *in vivo* diagnostic imaging applications.[122] G5 dendrimers with 64 Gd(III) ions gives lowest concentration detection limit made G5 as an promising dedritic MRI contrast agent.[123]

7. Characterisations of Liposome (or vesicle) and Their Aggregates with Dendrimer

Once a liposome is formulated and purified one could measure its size, surface charge (or zeta potential) and polydispersity by using dynamic light scattering (DLS) studies. Surface pressure (π)-area (a) isotherm measurement assists to understand the mutual miscibility among the components of mixed monolayer at air-water interface. Electron microscopy, small angle neutron scattering (SANS), small angle x-ray scattering (SAXS) and atomic force microscopy (AFM) studies are also helpful in determining the morphology and bilayer thickness. Bilayer phase transition and subsequent thermodynamics could be assessed by differential scanning calorimetry (DSC). Vesicles hosted drug molecules can also be scrutinized by measuring their entrapment efficiency, release kinetics and cytotoxicity. In case of vesicle/dendrimer interaction, apart from the above mentioned studies, one could

perform vesicles disintegration kinetics and Isothermal calorimetry (ITC) measurement to shed light on such interaction.

7.1. Dynamic Light Scattering

Vesicles 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 20 represents particle size measurement curve.

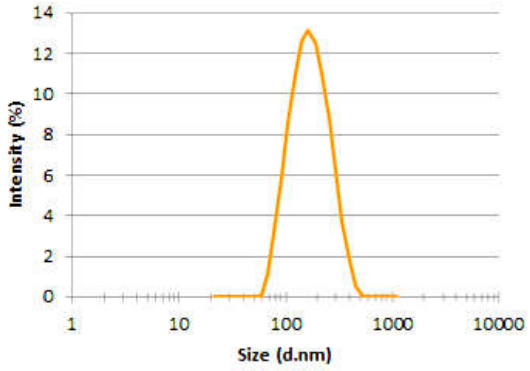


Figure 20. Hydrodynamic size distribution as function of intensities. Source: https://inanobotdresden.github.io/results.html.

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.

$$d_h = \frac{kT}{3\pi\eta D} \quad (2)$$

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

Z. P. is one of the crucial parameter that maintains the stability of the vesicle. It prevents particle collision wing to the repulsion and hence it could be understood, higher the Z. P., higher is the stability of the particles. Substitution of other substances in vesicle bilayer may often change the value of Z. P. Thus measurement of Z. P. 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, Z. P., viscosity and dielectric of the dispersion medium.

7.2. Surface Pressure (π) – Area Isotherm (a) Measurement

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.[124] Excess area of mixed monomolecular film where more than one amphiphiles are present are often lead to understand the associative or repulsive interaction between the hydrocarbon chain 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 21.

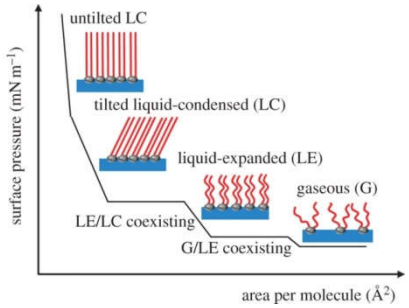


Figure 21. Representative surface pressure – area isotherm of lipids. {Schöne, 2017 #340}Source: Anne-Christin Schöne, Toralf Roch, Burkhard Schulz, Andreas Lendlein. J. Royal Soc. Interface **2017**, 14, 83-110.

After careful addition of lipidic materials to the subphase 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 characterisation of bilayer would not be successful unless one performed monolayer study.

7.3. Morphology Measurement

Although DLS provides the information regarding apparent hydrodynamic size of the particle, however fully not adequate to tell the story of size and shape in detail. In the filled of nanoparticles, they are often scrutinized by using microscopic technique. Transmission Electron microscopy (TEM), Scanning Electron microscopy (SEM) and Atomic Force Microscopy (AFM) are useful in determining vesicles morphology, size, topology as well as

bilayer thickness and phase behaviour of the hydrocarbon wall.[125,126] Representative TEM, SEM and AFM images of vesicles are given in Figure 22.

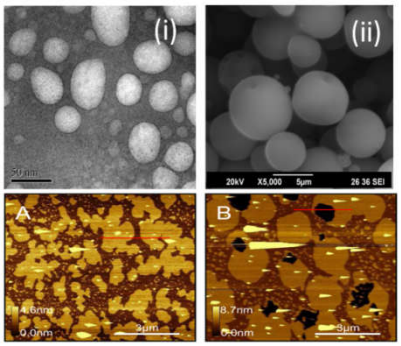


Figure 22. (i) TEM and (ii) SEM image of vesicles. Lower panel: AFM micrograph and height analysis of solid supported bilayer. Source: AFM: S. J. Attwood, Y. Choi and Z. Leonenko, *Int. J. Mol. Sci.*, **2013**, 3514-3539

Thus the micrographs of TEM and SEM provide exact morphology of the vesicles and its type of distribution. Side by side AFM also shed light on bilayer aggregation and its thickness as measured from height profile diagram. Not only microscopic analysis, small angle neutron scattering (SANS)[127-129] and small angle x-ray scattering (SAXS)[130-132] studies also help full in deterring the state of the bilayer thickness.

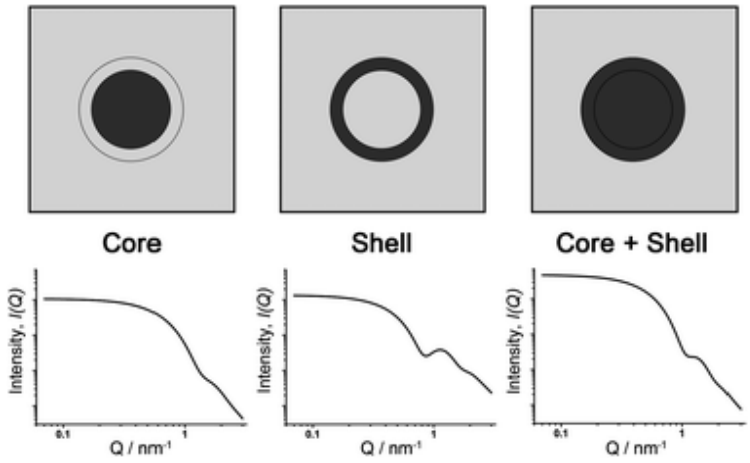


Figure 23. SANS plot of different kind of lipidic aggregates. Source: Martin J. Hollamb, *Phys. Chem. Chem. Phys.*, **2013,** 10517- 11144.

In SANS, neutron particles are made to collide with lipid bilayer or shell. In Figure 23, the intensity of the scattered neutron was plotted against scattering. The pattern of plot tells the architect of the lipidic assembly. Similarly in SAXS, electrons are being used instead of neutron that can scattered by the electron cloud around the bilayer, and hence present the bilayer morphology.

7.4. Differential Scanning Calorimetry

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 24. Hence bilayer strength and other thermodynamical entities are heavily depending on bilayer composition and the process of chain melting.

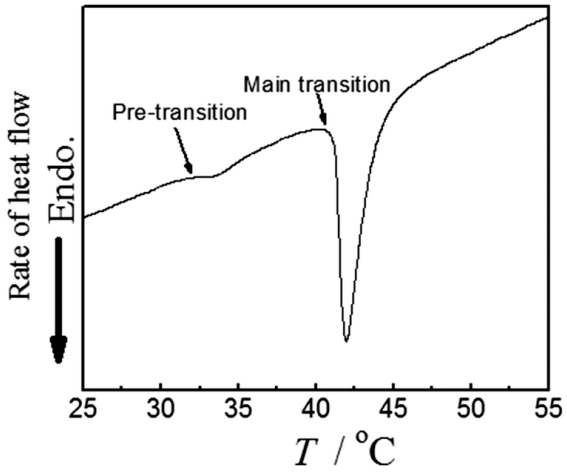


Figure 24. DSC thermogram of SLC vesicle. Scan rate: 2 ºC/min. Source: S. K. Kundu, S. Choe, K. Sasaki, R. Kita, N. Shinyashiki and S. Yagihara, *Phys. Chem. Chem. Phys.***2015**, *17*, 18449-18455.

7.5. FTIR Spectroscopy

IR spectroscopy is one of the important techniques towards chemist. Bilayer constituting lipidic molecules contains numbers of functional groups that are responsive to IR field. Group Such as –CH, -CH_2, -PO^{4-}, CO *etc*., are important that eventually control the fate of the bilayer.[133] Each of this functional groups have their own stretching frequency and slight deflection of it would tell us the situation of the concerning region of the lipids that eventually make impact on bilayer. Figure 25 describe a FTIR spectrum of DMPC liposome on the values of stretching frequencies of the functional groups. $-CH_2$ stretching frequency of the hydrocarbon chain is one of the primary concerned as it depicts the trans/gauche isomerisation of the acyl chain. The stretching frequency of $-CH_2$ at 2853 and 2924 cm^{-1} represent the antisymmetric and symmetric stretching frequency respectively.

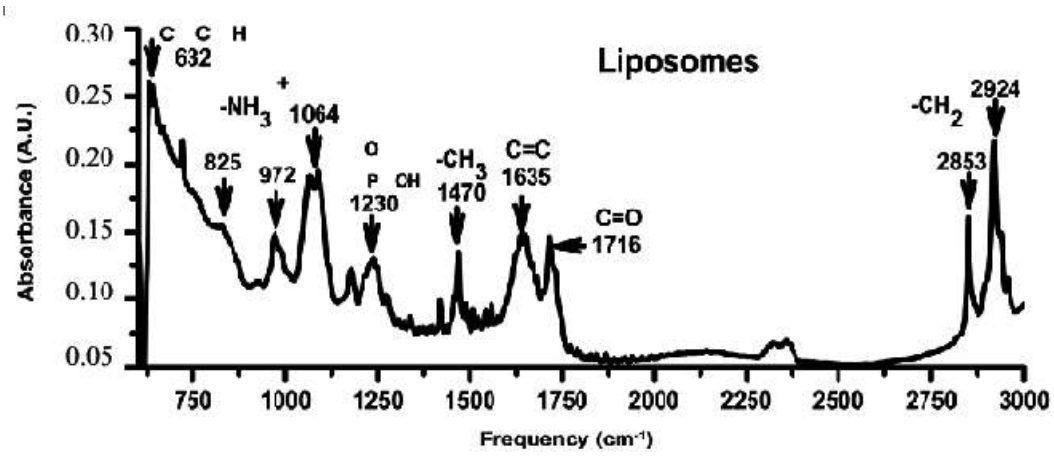


Figure 25. FTIR spectra of DMPC liposome. Source: F. R. M. Julieta, S. Macarena, I. Daniela, P. M. Jimena, A. S. D. Valle, C. N. Silvial, *Open Journal of Medicinal chemistry*, **2013**, *3*, 31-39.

Upshift of the frequencies indicates more disorderness into to the bilayer hydrocarbon zone. Head group polarity could also be checked from such studies. Phosphate stretching frequency appears at 1230 cm^{-1} and may alter depending on the surrounding medium and bilayer components. Increase in the value of stretching frequency indicates less hydration in aqueous medium and vice-versa.[133]

7.6. Fluorescence Study

Fluorescence study can be used to get idea about bilayer packing. Exogenously added compound may often changes the bilayer packing, caused either fluidity or rigidify of the membrane. Hence judicially chosen fluorescence probe like DPH or 7-hydroxycumarine (7-HC) can assert the rigidity of the non-polar chain as well as palisade layer of the membrane respectively. Steady state fluorescence spectra of 7-HC loaded vesicles would tell the polarity of the head region. Also anisotropy measurement of liposome loaded with either DPH or 7-HC would reflect the micro viscosity of the hydrocarbon and polar head region respectively. Anisotropy value of the embedded probe inside the bilayer was determined by using following equation.

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (3)$$

where, I_{VV} and I_{VH} were the fluorescence intensities, the subscripts indicate the position of the excitation and emission polarizer. $G = \frac{I_{HV}}{I_{HH}}$ was the grating correction factors.

Apart from the anisotropy, one could measure membrane polarity through steady state fluorescence spectra by incorporating fluorescence probe into the palisade layer.

7.7. Drug Entrapment Efficiency (E. E.).

It is known that vesicle can accommodate both hydrophilic and lipophilic drug. Hence Drug loading capacity of vesicles need to be determined. Once drug loaded vesicle 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.[134] 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:

$$\text{E.E. (\%)} = \frac{T-C}{T} \times 100 \quad (4)$$

where, 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.

7.8. Drug Release Kinetics

Vesicles 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 vesicle preparation. UV-VIS spectra of the sample were recorded with time which reveals the release pattern of the drug. Figure 26 put on a view of such release kinetics of drug loaded vesicles.

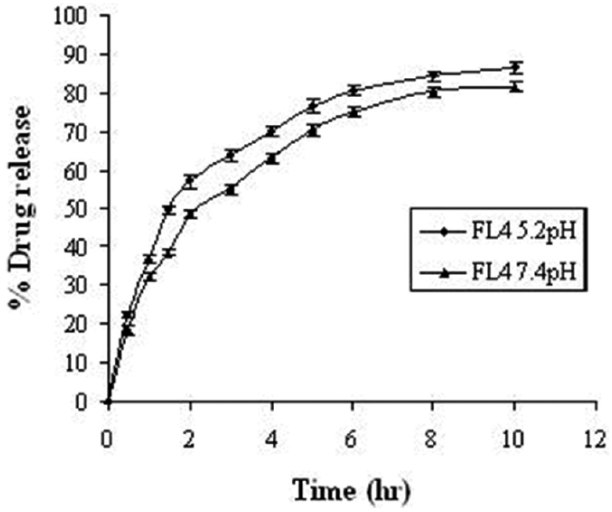


Figure 26. Representative image of % of drug release with time form vesicles bilayer. Source: K. Sasaki, R. Kita, N. Shinyashiki and S. Yagihara, *Phys. Chem. Chem. Phys.* **2012**, 13, 1442-1449

From Figure 26, one could easily get the idea of maximum drug release and the times to achieve sustain drug release.

Although several investigations have been made on liposome and dendrimers, however the exact mechanism of their interaction fully not understood. With a motive to get knowledge about physicochemical properties of vesicles and dendrimers and in particularly their aggregates, investigations have been carried out and the dissertation work has divided into three parts.

Vesicles were prepared at different combination of soylecithin (SLC) and IPA with 30 mol% cholesterol. Impact of IPA on SLC was studied by Langmuir-Blodget and spontaneity of the hydrocarbon chain mixing was found to be dependent on the amount of IPA. Hydrodynamic size (d_h), zeta potential (Z. P.) and polydispersity index (PDI) which describes the dispersion behaviour vesicles were measured through dynamic light scattering (DLS) technique. Vesicles Morphological properties also successfully recognized by electron microscopic (normal TEM as well as FF-TEM) studies. Thermotropic behaviours of the bilayer were scrutinized by differential scanning calorimetry (DSC). Structural changes of bilayer, caused by IPA, were further scrutinized by using fluorescence spectroscopy using 1, 6-diphenyl-1, 3, 5-hexatriene (DPH) and 7-hydroxycoumarin (7HC) as the fluorescent probes to get knowledge about the micro viscosity of the bilayer wall. Entrapment efficiency (E.E.) of the vesicles using cationic dye methylene blue (MB) was also evaluated. Such systems are expected to have superior properties as potent vectors for drug delivery.

As biological cell membranes are negatively charged, non-toxic, biodegradable vesicles could be served as an excellent drug delivery agent. Cationic vesicles were prepared using bi-tail cationic surfactants with varying hydrocarbon chain length (bis-C_{12} to C_{18}) in combination with soy lecithin (SLC) and ion pair amphiphile (IPA). Bi-tail cationic surfactants were chosen to progressively substitute with previously established three sets of SLC/IPA combinations. Interaction between hybrid membrane and piroxicame (Px), a Non Steroidal anti inflammatory Drug were analyzed in the form of monolayer, bilayer and

solid supported bilayer. Finally optimised Px encapsulated formulations were analysed for biological activity. Mutual miscibility among the components was studied by way of the surface pressure – area measurements. Physicochemical characterizations of the different hybrid vesicles with and without Px were assessed by combined dynamic light scattering, zeta potential, electron microscopy, atomic force microscopy, differential scanning calorimetry, FTIR, UV-VIS absorption and emission spectroscopic studies. Entrapment efficiency and the release kinetics of Px from the vesicles were analyzed by conventional dialysis bag approach. Finally the toxicity and biocompatibility of the drug loaded formulations were assessed. And could shed further light in the development of drug delivery systems in the treatment of brain – tumors targeted drug delivery.

The last chapter gives us an idea of physic-chemistry between the interaction of cationic vesicles and PAMAM succinamic acid, 1, 4-diaminobutane core dendrimer generation 5 (G5-SA) which is negatively charged. Previously prepared cationic vesicle comprised of SLC, IPA and DHDAB in three different combinations was taken to investigate the impact of dendrimer. Increasing hydro dynamic size and reduced Z.P. measurement suggests the formation of vesicle/dendrimer aggregates. The formation of aggregates was further confirmed by turbidity measurement. Morphological state of the vesicles with and without dendrimer was analysed via TEM studies. Vesicles disintegration kinetics measurement also has been done to understand the pattern of interaction using varying concentration of dendrimer. A surface pressure – time isotherm developed due to the vesicle disintegration upon the inclusion of dendrimer. The rate kinetics of such disintegration process was found to be depending on the dendrimer concentration. The effect of dendrimer on solid supported cationic bilayer was further scrutinized via AFM studies that help to understand stoichiometry depended aggregate formation. Finally DSC studies was performed which specifically enlighten the features of bilayer in presence of dendrimer. Overall

interaction studies put IPA on the map as it tries to restore the bilayer morphology by providing hydrophobic interaction.

7. Reference.

References are given in BIBLIOGRAPHY under Introduction (pp.142-150).