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# VESICULAR SYSTEM AS TARGETED DRUG DELIVERY SYSTEM: AN OVERVIEW

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**Abstract:**

There has been interest in the development of a novel drug delivery system. Novel drug delivery system aims to deliver the drug at a rate directed by the need of the body during the period of treatment, and channel the active entity of the site of action. At present, no available drug delivery system behave ideally achieving all the lofty goals, but sincere attempts have been made to achieve them through novel approaches in drug delivery. A number of novel drug delivery system has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved. Consequently a number of vesicular drug delivery system such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting, and the sustained or controlled release of conventional drug medicines. The focus of this review is to bring out the application, advantages, and drawbacks of vesicular system.

**Keywords:** Liposomes, niosomes,transfersomes, pharmacosomes.

## Introduction:

The quest never ends. From the very beginning of the human race; the quest is going on for newer and better alternatives, and in case of drugs it will continue; continue till we find a drug with maximum efficacy and no side

effects. Many drugs, particularly chemotherapeutic agents, have narrow therapeutic window, and their clinical use is limited and compromised by dose limiting toxic effect. Thus, the therapeutic effectiveness of the existing drugs is improved by formulating them in an advantageous way.

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). The NDDS should ideally fulfil two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery [1].

In this article, an attempt has been made to touch upon different aspects related to the vesicular system, including method of preparation, advantages, drawbacks, and applications. Various types of vesicular systems such as liposomes, niosomes, transfersomes and pharmacosomes have been discussed.

**Vesicular System:**

In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering [2],[3],[4]. Vesicles can play a major role in modelling biological membranes, and in the transport and targeting of active agents.

Conventional chemotherapy for the treatment of intracellular infections is not effective, due to limited permeation of drugs into cells. This can be overcome by use of vesicular drug delivery systems. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved [5]. The phagocytic uptake of the systemic delivery of the drug-loaded vesicular delivery system provides an efficient method for delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. Vesicular drug delivery systems delay drug elimination of rapidly

metabolizable drugs, and function as sustained release systems. This system solves the problems of drug insolubility, instability, and rapid degradation. Consequently, a number of vesicular delivery systems such as liposomes, niosomes, pharmacosomes etc, were developed.

**Liposomes**

Liposomes are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecule. There are a number of components present in liposomes, with phospholipid and cholesterol being the main ingredients. The type of phospholipids includes phosphoglycerides and sphingolipids, and together with their hydrolysis products [6].

**Liposomes Preparation Methods**

**A) Multilamellar Liposomes (MLV)**

**i) Lipid Hydration Method**

(a) This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous**.** **[7, 8]**

(b) MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing or sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent **[9,10]**. The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication.

**ii) Solvent Spherule Method**

A method for the preparation of MLVs of homogeneous size distribution was proposed by Kim et al. (1985)[11]. The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

**B) Small Unilamellar Liposomes (SUV)**

**i) Sanitation Method**

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV alongwith SUV. Recently, Oezden and Hasirci (1991) [12] prepared a polymer coated liposomes by this method.

**(ii) French Pressure Cell Method**

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials.[13] The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).

**iii)** A new method for the preparation of SUV was given by Lasic et al. (1987) [14] . They deposited egg phosphatidylcholinc mixed with 1.5 %w/v of cetyl tetramethylammonium bromide (a detergent) in CHCI3/CH3OH on various supports for example silica gel powder, zeolite X, zeolite ZSM5. After the removal of organic phase, the system was resuspended by shaking or stirring in distilled water or 5 mM NaCl. There was some loss of phospholipid (about 10-20%) due to adsorption on the supports. The loss was 70% and

95% in the case of silica gel and zeolite ZSMS respectively. An homogenous population of vesicle with average diameter of 21.5 nm was obtained when zeolite X (particle size of

0.4 mm) was used as a support.

**C) Large Unilamellar Liposomes (LUV)**

They have high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

**i. Solvent Injection Methods**

**a) Ether Infusion Method**

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature [15,16] .

**b) Ethanol Injection Method**

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol [17] .

**ii. Detergent Removal Methods**

The detergents at their critical micelles concentrations have been used to solubilise lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis [18] . The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations which are homogenous in size. The main drawback of the method is the retention of traces of detergent(s) within the liposomes. A commercial device called LIPOPREP which is a version of dialysis system is available for the removal of detergents. Other techniques have been used for the removal of detergents: (a) by using Gel Chromatography involving a column of Sephadex G-25, [19] (b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2 [20]. (c) By binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads [21].

**iii. Reverse Phase Evaporation Method**

First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands [22]. We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented by Handa et al. (1987) [23] and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%). The Reverse Phase Evaporation Method of Szoka and Papahadjopoulos (1978) [22] has also been modified to entrap plasmids without damaging DNA strands.

**iv. Calcium-Induced Fusion Method-** This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs [24]. The main advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

**v. Microfluidization Method**

Mayhew et al. (1984)[25] suggested a technique of microfluidization/ microemulsification/ homogenization for the large scale manufacture of liposomes. The reduction in the size range can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation. Riaz and Weiner (1995) [26] prepared liposomes consisting of egg yolk, cholesterol and brain phosphatidylserin diasodium salt (57:33:10) by this method. First MLV were prepared by these were passed through a Microluidizer at 40 psi inlet air pressure.

The size range was 150-160 nm after 25 recylces. In the Microluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely definedmicrochannels which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

**vi.** Extrusion under nitrogen through polycarboriatefilters LUV can be prepared by passing MLV under nitrogen through polycarbonate membrane filters. The vesicles produced by this method have narrow size distribution. The extrusion is done under moderate pressures (100-250 psi). A special filter holder is required. Such devices are available commercially under the trade names such as LUVET and EXTRUDER and are equipped with a recirculation mechanism that permits multiple extrusions with little difficulty. Small quantities of liposome preparations (about 10 mL) can be easily prepared by the help of a commercial extruder. The liposomes contained phosphatidylcholine from egg yolk and crude phosphoinositide sodium salt in the ratio of 4:1 and the lipid concentration was 12.5 /mole/ml. MLVs were passed through Extruder Lipex Membrane Inc., Vancouver, Canada) ten times through a stalk of two 100 nm polycarbonate filters employing nitrogen pressures upto 250 psi. Freeze fracture electron microscopy and p31-FT NMR revealed that the liposomes were unilamellar. Photon Correlation Spectroscopy revealed that the size range was 99-135 nm.

**vii.** Lasic et al. (1988) [27] reported a method for the instant formation of a rather homogeneous preparation of LUV by a simple technique. The formation of multilammelar liposomes is prevented by inducing a surface charge (+ ve) on the bilayer while the size of the vesicles is controlled by the topography of the wafer support surface on which phospholipid film was formed. They deposited 0.5-1.0 mg egg yolk lecithin doped with 3 ml of CHCl3/CH3OH on a specially ethched 2 inch silicon wafer. This wafer was put in place of the original bottom of an Erlenmeyer flask, that is bottom of the flask is replaced by wafer. After having dried overnight at 102 tore (about 1 Pa), the film was resuspended by gentle shaking in 1-2 ml water. Liposomes were formed instantly. The contamination of liposomes with large structures such as MLVs, giant vesicles and phospholipid particles was ruled out by video enhanced phase contrast microscopy.

**viii.** A method for the extemporaneous preparation of LUVs has been described by Liautard and Phillippot (1993) [28]. The method was recommended for immediate clinical use of liposomes.

**ix. Freeze-Thaw Method**

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesiclesis due to the fusion of SUV during the processes of freezing and or thawing. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained. [29]

**D. Giant Liposomes**

i) The procedure for the formation of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methylglucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm. [30]

ii) A method for the formation of giant single lamellar liposomes with size in the range of 10 to 20 prn by the removal of sodium trichloroacetate by dialysis. [31]

**E. Multivesicular Liposomes**

i) The water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency (up to 89%). [32]

ii) Cullis et al. (1987) [33] found that when MLV preparations were subjected to five cycles of freeze on liquid nitrogen-thaw and followed by thawing in warm water, the liposomes of high encapsulation efficiency (up to 88%) could be obtained. Freeze fracture electron micrographs revealed vesicles within vesicles.

**F. Assymetric Liposomes**

It has been shown that the phospholipid distribution in natural membranes is asymmetric. For example phosphatidytcholine and sphingomyelin concentrate at the outer half of lipid bilayer whereas phosphatidylethanolamine, phosphatidylinositoland phosphatidylserine are mainly localized in the inner half of bilayer.

Due to this, attempts have been made to prepare LUVs in which phospholipid distribution in both halves of bilayer is different. It appears that as model membranes the asymmetric liposomes are nearer to natural membranes than the conventional unilamellar liposomes. In the latter the phosphofpids distribution is symmetrical in bilayer.

i) Cestaro et al., (1982) described a procedure for the preparation of asymmetric liposomes which contain cerebroside sulfate only at the outer leaflet of phospholipid bilayer. Cerebroside sulfate was adsorbed on to a filter paper (cellulose) support and then the support was incubated with small or large fused unilamellar liposomes. After six hours sulfatide contents reached about 6 mole percentage of the total quantity of phospholipid, corresponding to about 10 mole % of phospholipid present in the outer layer. The sulfatide could not be removed by washing with 1M NaCl or 1M urea. [34]

ii) Pagano et al. (1981) reported the formation of asymmetric phospholipid vesicles which contained fluorescent lipid analogue in either the outer or inner leaflet of the liposome bilayer. The procedure is based on the observation that the lipid analogues undergo rapid exchange (transfer) between the vesicles populations. [35]

iii) Denkins and Schroit (1986) prepared asymmetric liposomes by the enzymatic conversion of the fluorescent lipid-analogue of phosphatidylserine (NBD-PS) in the outer leaflet of LUV to NBD- phosphatidylethanolamine (NBD-PE).

iv) Low and Zilversmit (1980) reported that lipid exchange proteins could be effectively be used so remove phosphatidylinositol at the outer leaflet of unilamellar liposomes. Therefore, it appears that these proteins may be used for the preparation of asymmetric liposomes. [36]

v) Collis et al. (1987) found that in SUV, distribution of lipid was not symmetrical and ratio of lipid in the outer monolayer to lipid in the inner monolayer could be as large as 2:1. Therefore, small unilamellar liposomes can be also be called as asymmetric to some extent.

**INDUSTRIAL PRODUCTION OF LIPOSOMES**

The several preparation methods described in the literature, only a few have potential for large scale manufacture of liposomes. The main issues faced to formulator and production supervisor are presence of organic solvent residues,

physical and chemical stability, pyrogen control, sterility, size and size distribution and batch to batch reproducibility.

Liposomes for parenteral use should be sterile and pyrogen free. For animal experiments, adequate sterility can be achieved by the passage of liposomes through up to approximately 400 nm pore size Millipore filters. For human use, precautions for sterility must be taken during the entire preparation process: that is, (1) the raw materials must be sterile and pyrogen free, (2) preparation in sterile system: working areas equipped with laminar flow and (3) use of sterile containers. [37]

Some issues related to phospholipids need attention. The liposomes based on crude egg yolk phospholipids are not very stable. The cost of purified lipids is very high.The liposomes prepared from polymerizable phospholipids are exposed to UV light. The polymerization process takes place in the bilayer(s). Such liposome preparations usually have better storage stability. It should be noted that such materials usually are phospholipid analogues and their metabolic fates have yet to be established.

**i) Detergent Dialysis**

A pilot plant under the trade name of LIPOPREPR II-CIS is available from Diachema, AG, Switzerland. The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml 100 mg/ml then up to many litres of liposomes can be produced. In USA, LIPOPREPR is marketed by Dianorm-Geraete. [38]

**ii) Microlluidization**

A method based on micro0uidization/microemulsiftcation/homogenization was developed for the preparation of liposomes. MICROFLUIDIZERR is available from Micro0udics Corporation, Massachusetts, USA. A plot plant based on this technology can produce about 20 gallon/minute of liposomes in 50-200 nm size range. The encapsulation efficiency up to 75% could be obtained. [26]

**iii)** Aqueous dispersions of liposomes often have tendency to aggregate or fuse and may he susceptible to hydrolysis and or oxidation. Two solutions have been proposed:

**iii a) Proliposomes**

In proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs. [39]

**iii b) Lyaphilization**

Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed that leakage of entrapped materials may take place during the process of freeze-drying and on reconstitution. Recently, it was shown that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original contents. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes.[40] Freeze-driers range in size from small laboratory models to large industrial units are available from Pharmaceutical Equipment Suppliers.

**Advantages and Disadvantages**

Liposomes as a potential delivery system for the oral administration of insulin, have been extensively studied [41],[42]. It was observed by many scientists, that the liposomes had protective effects against proteolytic digestive enzymes like pepsin and pancreatin [43],[44] and they can increase the intestinal uptake of macromolecules and hence are capable of enhancing insulin uptake[45].

Liposomes with a specifically modified design, i.e. long-circulating and especially actively targeting liposomes, stand a better chance in becoming truly tumoritropic carriers of photosensitizers, and can hence be used successfully in photodynamic therapy [46] .

Liposomal drug delivery system is advantageous in the fulfillment of the aspects related to protection of the drug, controlled release of the active moiety along with the targeted delivery, and cellular uptake via endocytosis [47],[48],[49],[50]. Besides the merits, liposomes also pose certain problems associated with degradation by hydrolysis,

oxidation, sedimentation, leaching of drug; and aggregation or fusion during storage. Approaches that can be used to increase liposome stability involve efficient formulation and lyophilization. Formulation involves the selection of the appropriate lipid composition and concentration of the bilayer, in addition to the aqueous phase ingredients, such as buffer, antioxidants, metal, chelators, and cryoprotectants. Charge-inducing lipids, such as phosphatidylglyceride be incorporated into the liposome bilayer to decrease fusion, while cholesterol and sphingomyelin can be incorporated in formulations, in order to decrease the permeability and leakage of encapsulated drugs. Buffers at neutral pH can decrease hydrolysis. Addition of antioxidants such as sodium ascorbate, can decrease the oxidation. Freeze-dried liposome formulations should incorporate a lipoprotectant-like non-reducing disaccharide, such as trehalose, and sucrose. Some problems associated with clinical applications of liposomes, are difficulties experienced in sterilization and large-scale production. Moreover, it is difficult to obtain large quantities of sterile products with defined and reproducible properties, which display adequate chemical and physical stability. The cost and purity of phospholipid is another limiting factor. They are suitable for parenteral administration but oral administration is not possible, because of inability of liposomes to survive to the action of bile salts and phospholipids [51].

**Applications**

Liposomes are spherical, selfclosed vesicles of colloidal dimensions, in which phospholipid bilayer sequesters part of the solvent, in which they freely float, into their interior [52]. In the case of one bilayer encapsulating the aqueous core one speaks either of small or large unilamellar vesicles while in the case of many concentric bilayers one defines large multilamellar vesicles [53].

Due to their structure, chemical composition and colloidal size, all of which can be well controlled by preparation methods, liposomes exhibit several properties which may be useful in various applications. The most important properties are colloidal size, i.e. rather uniform particle size distributions in the range from 20 nm to 10 nm, and special membrane and surface characteristics. They include bilayer phase behavior, its mechanical properties and permeability, charge density, presence of surface bound or grafted polymers, or attachment of special ligands, respectively. Additionally, due to their amphiphilic character, liposomes are a powerful solubilising system for a

wide range of compounds. In addition to these physico-chemical properties, liposomes exhibit many special biological characteristics, including (specific) interactions with biological membranes and various cells. These properties point to several possible applications with liposomes as the solubilizers for difficult-to-dissolve substances, dispersants, sustained release systems, delivery systems for the encapsulated substances, stabilizers, protective agents, microencapsulation systems and microreactors being the most obvious ones. Liposomes can be made entirely from naturally occurring substances and are therefore nontoxic, biodegradable and non immunogenic. In addition to these applications which had significant impact in several industries, the properties of liposomes offer a very useful model system in many fundamental studies from topology, membrane biophysics, photophysics and photochemistry, colloid interactions, cell function, signal transduction, and many others [54–56].

The industrial applications include liposomes as drug delivery vehicles in medicine, adjuvants in vaccination, signal enhancers/carriers in medical diagnostics and analytical biochemistry, solubilizers for various ingredients as well as support matrix for various ingredients and penetration enhancer in cosmetics.

Applications of liposomes in pharmacology and medicine can be divided into therapeutic and diagnostic applications of liposomes containing drugs or various markers, and their use as a model, tool, or reagent in the basic studies of cell interactions, recognition processes, and of the mode of action of certain substances [54].

Many different liposome formulations of various anticancer agents were shown to be less toxic than the free drug [57]. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and therefore kill predominantly quickly dividing cells.

Since conventional liposomes are digested by phagocytic cells in the body after intravenous administration, they are ideal vehicles for the targetting of drug molecules into these macrophages. The best known examples of this ‘Trojan horse-like’ mechanism are several parasitic diseases which normally reside in the cell of mononuclear phagocytic system. They include leishmaniasis and several fungal infections.

The sustained release system concept can be used in various fermentation processes in which the encapsulated enzymes can greatly shorten fermentation times and improve the quality of the product. This is due to improved spatial and temporal release of the ingredient(s) as well as to their protection in particular phases of the process

against chemical degradation. A classical example is cheesemaking. The first serious attempts to decrease the fermentation time using cell-wall-free bacterial extracts were encouraging enough to stimulate efforts to improve enzyme presentation. After preliminary studies in which liposome systems were optimized the cheese ripening times can be shortened by 30–50% [58-60]. This means a substantial economic profit knowing that ripening times of some cheeses, such as Cheddar, say, are about one year during which they require well controlled conditions. In addition, due to the better dispersal of the enzymes the texture of cheeses was even and bitterness and inconsistent flavour due to the proteolysis of enzymes in the early phase of fermentation was much improved [59, 60].

In ecology, liposomes offer improvements in bioreclamation and various monitoring and analytical-diagnostic applications. For instance, it was shown that in an oil spill, the addition of various bacteria with possible nutrients encapsulated in liposomes improves the degradation rates of carbohydrates, which are otherwise very slow. Due to the surfactant action liposomes also improve the coagulation and sinking of oil spread on the water surface or its cleaning up with floating booms [61]. The Environmental Protection Agency is testing liposomes' ability to deliver nutrients to oil spills to speed up the degradation [62].

Liposomes containing membrane anchored chelators can be used to clean toxic or radioactive metals from solutions. For instance, water contaminated in a nuclear reactor can be purified by addition of such liposomes which could be easily precipitated after binding of the toxic ions.

In addition to the above mentioned liposome applications there are many others which were not mentioned. An interested reader may find more information In ref. [56] and references therein.

In conclusion, it seems that liposomes established themselves as an important model system in several different basic sciences and as a viable alternative in several applications.

**Niosomes [63-77]**

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. Although structurally similar to liposomes, they offer several advantages over them. Niosomes have recently been shown to greatly increase transdermal drug

delivery and also can be used in targeted drug delivery, and thus increased study in these structures can provide new methods for drug delivery.

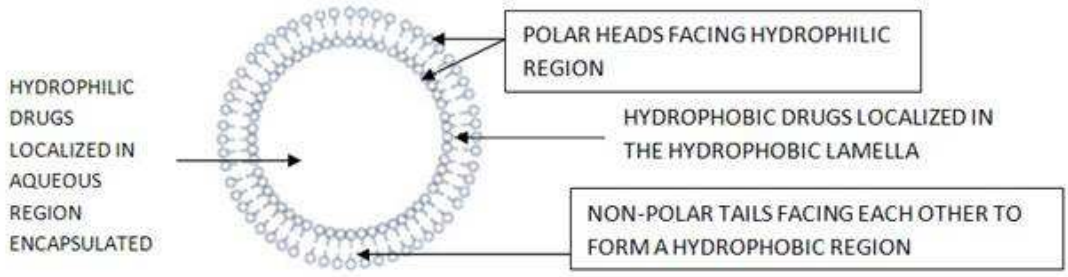
**Structure of Niosomes:**

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media1.

Structurally, niosomes are similar to liposomes, in that they are also made up of a bilayer. However, the bilayer in the case of niosomes is made up of non-ionic surface active agents rather than phospholipids as seen in the case of liposomes. Most surface active agents when immersed in water yield micellar structures, however some surfactants can yield bilayer vesicles which are niosomes.

Niosomes may be unilamellar or multilamellar depending on the method used to prepare them.

The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself. The figure below will give a better idea of what a niosome looks like and where the drug is located within the vesicals.




A typical niosome vesicle would consist of a vesicle forming ampiphile i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of anionic surfactant such as diacetyl phosphate, which also helps in stabilizing the vesicle

**Method of Preparation of Niosomes**

Niosomes can be prepared by a number of methods which are as follows:

Ether injection method: In this method, a solution of the surfactant is made by dissolving it in diethyl ether. This solution is then introduced using an injection (14 gauge needle) into warm water or aqueous media containing the drug maintained at 60°C. Vaporization of the ether leads to the formation of single layered vesicles. The particle size of the niosomes formed depend on the conditions used, and can range anywhere between 50-1000µm.

**Hand shaking method (Thin Film Hydration Technique):** In this method a mixture of the vesicle forming agents such as the surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether or chloroform in a round bottom flask. The organic solvent is removed at room temperature using a rotary evaporator, which leaves a thin film of solid mixture deposited on the walls of the flask. This dried surfactant film can then be rehydrated with the aqueous phase, with gentle agitation to yield multilamellar niosomes. The multilamellar vesicles thus formed can further be processed to yield unilamellar niosomes or smaller niosomes using sonication, microfluidization or membrane extrusion techniques.

**Reverse phase evaporation technique:** This method involves the creation of a solution of cholesterol and surfactant (1:1 ratio) in a mixture of ether and chloroform. An aqueous phase containing the drug to be loaded is added to this, and the resulting two phases are sonicated at 4-5°C. A clear gel is formed which is further sonicated after the addition of phosphate buffered saline (PBS). After this the temperature is raised to 40°C and pressure is reduced to remove the organic phase. This results in a viscous niosome suspension which can be diluted with PBS and heated on a water bath at 60°C for 10 mins to yield niosomes.

**Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote loading):** In this method, a solution of surfactant and cholesterol is made in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask, similar to the hand shaking method. This film is then hydrated using citric acid solution (300mM, pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To the niosomal suspension, aqueous solution containing 10mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 using 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside

it is again ionized thus not allowing it to exit the vesicle). The mixture is later heated at 60°C for 10 minutes to give niosomes.

**The "Bubble" Method:** It is a technique which has only recently been developed and which allows the preparation of niosomes without the use of organic solvents. The bubbling unit consists of a round bottom flask with three necks, and this is positioned in a water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck, while the third neck is used to supply nitrogen. Cholesterol and surfactant are dispersed together in a buffer (pH 7.4) at 70°C. This dispersion is mixed for a period of 15 seconds with high shear homogenizer and immediately afterwards, it is bubbled at 70°C using the nitrogen gas to yield niosomes.

**Formation of Proniosomes and Niosomes from Proniosomes:** To create proniosomes, a water soluble carrier such as sorbitol is first coated with the surfactant. The coating is done by preparing a solution of the surfactant with cholesterol in a volatile organic solvent, which is sprayed onto the powder of sorbitol kept in a rotary evaporator. The evaporation of the organic solvent yields a thin coat on the sorbitol particles. The resulting coating is a dry formulation in which a water soluble particle is coated with a thin film of dry surfactant. This preparation is termed Proniosome.

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

**Advantages of Niosomes**

- Use of niosomes in cosmetics was first done by L'Oreal as they offered the following advantages:
- The vesicle suspension being water based offers greater patient compliance over oil based systems
- Since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used for a variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be varied depending on the requirement.
- The vesicles can act as a depot to release the drug slowly and offer a controlled release.

**Other advantages of niosomes are**:

- They are osmotically active and stable.
- They increase the stability of the entrapped drug.
- Handling and storage of surfactants do not require any special conditions.
- They can increase the oral bioavailability of drugs.
- They can enhance the skin penetration of drugs..
- They can be used for oral, parenteral as well topical use
- The surfactants are biodegradable, biocompatible, and non-immunogenic.
- Improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
- The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.

**Comparison of Niosome v/s Liposome**

Niosomes are different from liposomes in that they offer certain advantages over liposomes. Liposomes face problems such as –they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes do not have any of these problems. Also since niosomes are made of uncharged single-chain surfactant molecules as compared to the liposomes which are made from neutral or charged double chained phospholipids, the structure of niosomes is different from that of liposomes.

However Niosomes are similar to liposomes in functionality. Niosomes also increase the bioavailability of the drug and reduce the clearance like liposomes. Niosomes can also be used for targeted drug delivery, similar to liposomes. As with liposomes, the properties of the niosomes depend both- on the composition of the bilayer, and the method of production used.

**Applications of Niosomes**

The application of niosomal technology is widely varied and can be used to treat a number of diseases. The following are a few uses of niosomes which are either proven or under research

**Drug targeting;**

One of the most useful aspects of niosomes is their ability to target drugs. Niosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelial system (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of drugs can also be used for treating parasitic infections of the liver.

Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulins bind readily to the lipid surface of the niosome) to target them to specific organs. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells.

**Anti-neoplastic Treatment:**

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism, prolong circulation and half life of the drug, thus decreasing the side effects of the drugs. Niosomal entrapment of Doxorubicin and Methotrexate (in two separate studies) showed beneficial effects over the unentrapped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination.

**Leishmaniasis:**

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

**Delivery of Peptide Drugs:**

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an invitro study conducted by Yoshida et al, oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

**Use in Studying Immune Response:**

Due to their immunological selectivity, low toxicity and greater stability; niosomes are being used to study the nature of the immune response provoked by antigens.

**Niosomes as Carriers for Haemoglobin:**

Niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anemic patients.

**Transdermal Drug Delivery Systems Utilizing Niosomes**:

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics; in fact, it was one of the first uses of the niosomes. Topical use of niosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug.

Recently, transdermal vaccines utilizing niosomal technology is also being researched. A study conducted by P. N. Gupta et al has shown that niosomes (along with liposomes and transfersomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in niosomes allows only a weak immune response, and thus more research needs to be done in this field.

**Other Applications**:

Niosomes can also be utilized for sustained drug release and localized drug action to greatly increase the safety and efficacy of many drugs. Toxic drugs which need higher doses can possibly be delivered safely using niosomal encapsulation.

**Transferosomes:**

liposomes and niosomes are the vesicular carrier systems which have received a lot of attention over the last decade as a means of transdermal drug delivery, in most cases transdermal drug penetration has not been achieved[9]. To overcome these problems a new type of carrier system called "transferosomes" was introduced for the effective transdermal delivery of number of low and high molecular weight drugs.It consist of both hydrophilic and hydrophobic properties,high deformablity gives better penetration of intact vescicles. A transferosomes, i n functional terms, may be described as lipid droplets of such deformability that permits its easy penetration through the pores much smaller than the droplets size. They protect the encapsulated drug from metabolic degradation. In thermodynamics terms this typically corresponds to an aggregate in the quasi-metastable state, which facilitates the formation of highly curved bilayers. From the composition point of view, a transferosomes is a self adaptable and optimized mixed lipid aggregate. They act as depot,releasing their content slowly and gradually [78].

Transferosomes have been developed in order to take advantage of phospholipids vescicles as transdermal drug carrier. These self optimized aggregates,with ultraflexible membrane, are able to deliver the drug reproducibily either into or through the skin, depending on the choice of administration or application, with high efficiency. These vescicular transferosomes are several orders of magnitude more elastic than the standard liposomes and thus well suited for the skin penetration. Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. There is provision for this, because of the high vescicle deformability, which permits the entry due to mechanical stress of surrounding, in a self adapting manner. Flexibility of transferosomes membrane is achieved by mixing suitable surface active agents in the proper ratios. The resulting flexibility of transferosome membrane minimize the risk of complete vescicle rupture in the skin and allow transferosomes to follow the natural water gradient across the epidermis ,when applied under non occlusive condition. Transferosomes can penetrate the intact stratum corneum spontaneously either through intracellular route or transcellular route [79].

The high and self-optimizing deformability of typical composite transferosomes membrane, which are adaptable to ambient stress allow the ultra deformable transferosomes to change its membrane composition locally and reversibily, when it is pressed against and attracted into narrow pore.

Some of the limitations which have been encountered is that transferosomes are chemically unstable because of their predisposition to oxidative degradation as well as Purity of natural phospholipid is another criteria militating against adoption of transferosomes as drug delivery vehicles.

**Materials and Methods**: Materials which are widely used in the formulation of transferosomes are various phospholipids, surfactants, alcohol, dye, buffering agent etc. [79] different additives used in the formulation of transferosomes are summarized in table no. 1.

First of all phospholipids and surfactants are dissolved in organic solvent. Any lipophilic drug could also be encorporated in these organic solvent. Then prepare thin film using rotary evaporator then keep under vacuum for 12 hrs, after that hydrate it with buffer (pH 6.5) at 60 rpm, any hydrophilic drug can be encorporated in these buffer. Then sonicate for 30 min using probe sonicator at 380 W, and then homogenize it using polycarbonate membranes (extrusion 10 times through a sandwich of 200 and 100 nm). Then finally we got the transferosomes.

**Table No-1: Method of preparation of transferosomes:**

| CLASS | EXAMPLE | USES |
|---|---|---|
| Phospholipids | Soya phosphatidyl choline,egg phosphatidyl choline,dipalmitoyl phosphatidyl choline | Vesicles forming component |
| Surfactant | Sod.cholate,Sod.deoxycholate,Tween-80,Span-80 | For providing flexibility |
| Alcohol | Ethanol,methanol | As a solvent |
| Buffering agent | Saline phosphate buffer (pH 6.4) | As a hydrating medium |

**Charecterization Of Transferosomes [79-81]**

**Entrapment efficiency:** Entrapment efficiency was determined by first separation of unentraped drug by the use of mini-column centrifugation method (Fry et al, 1978; New, 1990). After centrifugation, the vesicle was disrupted

using 0.1%Triton X-100 or 50% n-propanol and then followed by suitable analytical technique to determine the entrapped drugs.

**Vesicle diameter**

Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filterd saline and then size measurement done by using photon correlation spectroscopy or dynamic light scaterring measurements

**Confocal scanning laser microscopy (CSLM) study**

In this technique lipophilic flouresence markers are in corporated into the transferosomes and the light emited by these markers are used for the investigation of mechanism of penetration of transferosomes across the skin, for determining histological organization of the skin and for comparision and differentiation of the mechanism of penetration of transferosomes with liposomes, niosomes and micelles.

**Degree of deformability or permeability measurement**

The deformability study is done against the pure water as standard. Transfrosomes preparation is passed through a large number of pores of known size through a sandwhich of different micropores filters with pore diameter between 50 nm and 400 nm, depending on the starting transferosomes suspension. Particle size and size distribution are noted after each pass by dynamic light scattering (DLS) measurements

**In vitro drug release**

The information from in-vitro studies are used to optimize the formulation before more expensive in vivo studies is performed. For determining in vitro drug release, beaker method is used in which transferosomes suspension is incubated at 320°C using cellophane membrane and the samples are taken at different times and then detected by various analytical techniques (U.V., HPLC,HPTLC) and the free drug is separated by minicolumn centrifugation (Fry et al,1978), then the amount of drug release is calculated.

**Vesicle shape and type**

Transferopsomes vesicles can be visualized by TEM, with an accelerating voltage of 100 kv. Transferosomes vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope.

**Number of vesicle per cubic mm**

This is an important parameter for optimizing the composition and other process variables. Transferosome formulations (without sonication) can be diluted five times with 0.9% of sodium chloride solution and studied with optical microscopy by using haemocytometer.

**Penetration ability**

Penetration ability of transferosomes can be evaluated using fluorescence microscopy

**Turbidity measurement**

Turbidity of drug in aqueous solution can be measured using nephelometer

**Surface charge and charge density**

Surface charge and charge density of transferosomes can be determined using zetasizer

**Application of Transferosomes:**

Transferosomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract. These are the reasons why these peptides and proteins still have to be introduced into the body through injections. Various approaches have been developed to improve these situations. The bioavaibility obtained from transferosomes is some what similar to that resulting from subcutaneous injection of the same protein suspension. The transferosomal preparations of this protein also induced strong immune response after the repeted epicutaneous appilication, for example the adjuvant immunogenic bovine serum albumin in transferosomes, after several dermal challenges is as active immunologically as is the corresponding injected proteo-transferosomes preparations.

Delivery of insulin by transferosomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient.

Encapsulation of insulin into transferosomes (transfersulin) overcomes these entire problems. After transfersulin application on the intact skin, the first sign of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition.

Transferosomes have also been used as a carrier for interferons, for example leukocytic derived interferone-α (INF-α) is a naturally occurring protein having antiviral, antiproliferive and some immunomodulatory effects. Transferosomes as drug delivery systems have the potential for providing controlled release of the administred drug and increasing the stability of labile drugs. Hafer et al studied the formulation of interleukin-2 and interferone-α containing transferosmes for potential transdermal application .they reported delivery of IL-2 and INF- α trapped by transferosomes in sufficient concentration for immunotherapy **[82].**

Another most important application of transferosomes is transdermal immunization using trnsferosomes loded with soluble protein like integral membrane protein, human serum albumin, gap junction protein. These approach offers at least two advantages, first they are applicable without injection and second, they give rise to rather high titer and possibly, to relatively high IgA levels.

Transferosomes have also used for the delivery of corticosteroids. Transferosomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose [83]. Transferosomes beased cortiosteroids are biologically active at dose several times lower than the currently usd formulation for the treatment of skin diseases **[83]**.

Application of anesthetics in the suspension of highly deformable vesicles, transferosomes, induces a topical anesthesia, under appropriate conditions, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transferosomal anesthetics last longer. Transferosmes has also been used for the topical analgesics, anaesthetics agents, NSAIDS and anti-cancer agents.

**But like liposomes, transfersomes have certain limitations-**

1. Transfersomes are chemically unstable because of their predisposition to oxidative degradation,

2. Lack of purity of the natural phospholipids comes in the way of adoption of transfersomes as drug delivery vehicles and

3. Transfersomes formulations are expensive to prepare.

**Pharamcosomes [84-97]**

The limitations of transfersomes can be overcome by the "pharmacosome" approach. The prodrug conjoins hydrophilic and lipophilic properties, and therefore acquires amphiphilic characters, and similar to other vesicle forming components, was found to reduce interfacial tension, and at higher concentrations exhibits mesomorphic behavior. These are defined as colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of drug-lipid complex [84]. Many constraints of various classical vesicular drug delivery systems, such as problems of drug incorporation, leakage from the carrier, or insufficient shelf life, can be avoided by the pharmacosome approach. The idea for the development of the vesicular pharmacosome, is based on surface and bulk interactions of lipids with drug. Any drug possessing an active hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid, with or without spacer chain. Synthesis of such a compound may be guided in such a way that strongly result in an amphiphilic compound, which will facilitate membrane, tissue, or cell wall transfer, in the organism. The salient features of pharmacosomes are -

• Entrapment efficiency is not only high but predetermined, because drug itself in conjugation with lipids forms vesicles.

• Unlike liposomes, there is no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.

• Since the drug is covalently linked, loss due to leakage of drug, does not take place. However, loss may occur by hydrolysis.

• No problem of drug incorporation

• Encaptured volume and drug-bilayer interactions do not influence entrapment efficiency, in case of pharmacosome. These factors on the other hand have great influence on entrapment efficiency in case of liposomes

• The lipid composition in liposomes decides its membrane fluidity, which in turn influences the rate of drug release, and physical stability of the system. However, in pharmacosomes, membrane fluidity depends upon the phase transition temperature of the drug lipid complex, but it does not affect release rate since the drug is covalently bound.

• The drug is released from pharmacosome by hydrolysis (including enzymatic).

• Phospholipid transfer/exchange is reduced, and solubilization by HDL is low.

• The physicochemical stability of the pharmacosome depends upon the physicochemical properties of the drug-lipid complex.

• Due to their amphiphilic behavior, such systems allow, after medication, a multiple transfer through the lipophilic membrane system or tissue, through cellular walls piggyback endocytosis and exocytosis.

• Following absorption, their degradation velocity into active drug molecule depends to a great extent on the size and functional groups of drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized in vivo pharmacokinetics.

• They can be given orally, topically, extra-or intravascularly.

Mantelli et al., [98] compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and observed similar effect on lowering of surface tension. Above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behaviour, and assembles in supramolecular structures. The prepared prodrugs are generally characterized for their structural conformation (by IR, NMR spectrophotometry, thin layer chromatography (TLC), melting point determination), partition coefficient [99], and surface tension [100], and prodrug hydrolysis. Hand-shaking method and ether injection method have been utilized for preparing vesicles. In hand-shaking method, the dried film of the drug-lipid complex (with or without egg lecithin) deposited in a round bottom flask upon hydration with aqueous medium, readily gives a vesicular suspension. In ether injection method, organic solution of the drug-lipid complex was injected slowly into the hot aqueous medium, wherein the vesicles are readily formed. Like other vesicular systems, pharmacosomes are characterized for different attributes such as size and size distribution, nuclear magnetic resonance (NMR)

spectroscopy, entrapment efficiency, in vitro release rate, stability studies, etc. The approach has successfully improved the therapeutic performance of various drugs i.e. pindolol maleate, bupranolol hydrochloride, taxol, acyclovir, etc [101],[102]

Pharmacosomes bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicles. The system yet requires greater efforts towards investigating the non-bilayer phases, and exploring the mechanism of action. Furthermore, the effect of covalent linkages and addition of spacer group on rate of in vivo hydrolysis and subsequent pharmacokinetics is to be exhaustively studied, in order to exploit more advantages of this system. Like other vesicular drug delivery systems, pharmacosomes, on storage, undergo fusion and aggregation, as well chemical hydrolysis.

**Method of Preparation:**

In general two methods have been employed to prepare pharmacosomes. They are:

1. Hand-shaking method.
2. Ether-injection method.

In the hand-shaking method, the dried film of the drug– lipid complex (with or without egg lecithin) is deposited in a round-bottom flask and upon hydration with aqueous medium, readily gives a vesicular suspension. In the ether-injection method, an organic solution of the drug– lipid complex is injected slowly into the hot aqueous medium, wherein the vesicles are readily formed. At low concentration the amphiphiles exists in the monomer state. Further increase in monomers may lead to variety of structures i.e., micelles of spherical or rod like or disc shaped type or cubic or hexagonal shape. Mantelli et al., compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and found similar effect on lowering of surface tension. Above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behavior, and assembles in supramolecular structures [103],[104].

**Conclusion**

Vesicular systems have been realized as extremely useful carrier system in various scientific domains. Over the year, vesicular systems have been investigated as a major drug delivery system, due to their flexibility to be tailored

for varied desirable purpose. In spite of certain drawbacks, the vesicular delivery system still plays an important role in the selective targeting, and the controlled delivery of various drugs. Researchers all over the world continue to put in their effort in improving the vesicular system by making them steady in nature, in their leaching of content, oxidation, and their uptake by mechanisms. Current research trends are generally based on using different approaches (like prgylation, biotinylation etc.) for cellular targeting.

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