WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 560-586. Review Article ISSN 2277– 7105

PRONIOSOMAL GEL FOR IMPROVED TRANSDERMAL DRUG DELIVERY: AN OVERVIEW

Prashant G. Pandit*, Durgesh R. Parakh, Moreshwar P. Patil

Department of Pharmaceutics, MET'S Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik. 422003. (M. S.) India.

Article Received on
22 May 2015,

Revised on 15 June 2015,
Accepted on 08 July 2015

***Correspondence for Author**
Prashant Gautam Pandit
Department of Pharmaceutics, MET'S Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik, 422003. (M. S.) India.

ABSTRACT

Human skin acts as one of the key sites for non-invasive delivery of therapeutic agents into the body and it has verious numerous advantages over oral drug delivery system.but the molecule or compound with high molecular weight(500 Da) can not cross the skin. For that compound reqires some novel techniques, methods to transfer these compound across the skin easily without any side effects. Vesicular system is one of the systems which have potential to transfer high molecular weight compound (hydrophilic as well as lipophilic) across the skin in control manner. But the major drawback of this system is their stability issue, and hence it can not prepare in large quantity both in laboratory scale and in industrial scale, it required some specific condition when formulation is concerned. Proniosomal is advanced concept over niosome and liposome, which have ability to overcome the problem associated with farmal vesicular drug delivery system. Basically, proniosomal gel is a compact semi-solid liquid crystaline (gel) product of non-ionic surfactants easily formed on dissolving the surfactant in minimal amount of acceptable solvent and the least amount of aqueous phase. This compact liquid crystalline gel can be readily converted into niosomes on hydration. This review provides an important overview of preparation, formulation, evaluation and application of proniosome gel as a drug carrier.

KEYWORDS: Vesicuar drug delivery, niosome, liposome, transdermal drug delivery.

INTRODUCTION

Various novel drug delivery systems now days has been developed to modify drug release and to overcome problem associated with drugs and there formulation. There has been a

tremendous growth in the area of developing various new drug delivery systems. It is capable of providing the drug to particular site of action. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity. Advances have since been made in the area of vesicular drug delivery, leading to the development of systems that allow drug targeting and the sustained or controlled release of conventional medicines. It has also reduced number of toxic, dose related side effects and maintained therapeutic efficacy of drugs for longer time duration by decreasing dosing frequency.Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and liophilic drugs.

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphillic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphillic building blocks. Amphiphilic molecules known as surfactants and they have self assembling properties. They aggregate into variety of shapes like micelles (spherical, cylindrical, rod like) or into a planar lamellar bilayer. By generating new interfaces in solutions, the aggregates show various phases and unique rheological behaviors.[1]

The main aim of vesicular drug delivery system is to control degradation of drug and loss, prevention of harmful side effects and increase the availability of the drug at the disease site. 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. Lipid vesicles are one type of many experimental models of biomembranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective due to limited permeation of drugs into cells. This can overcome by the use of vesicular drug delivery systems.

Advantages of Vesicular drug delivery system[2]

1. Prolong the existence of the drug in systemic circulation and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
2. Improves the bioavailability especially in the case of poorly soluble drugs.
3. Both hydrophilic and lipophilic drugs can be incorporated.

4. Delays elimination of rapidly metabolizable drugs and thus function as sustained release Systems.

Vesicle Drug delivery system

Vesicle drug delivery act as a novel drug delivery system in which the drug is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agent. Vesicles are colloidal particles in which a concentric bilayer made-up of amphiphilic molecules surrounds an aqueous compartment. They are a useful vehicle for drug delivery of both hydrophobic drugs and hydrophilic drugs. Hydrophobic drugs associate with the lipid bilayer and hydrophilic drugs, which are encapsulated in the interior aqueous compartment. Generally, vesicles which are made from natural or synthetic phospholipids are called liposomes whereas those made of nonionic surfactants (e.g. alkyl ethers and alkyl esters) and cholesterol constitutes nonionic surfactant vesicular system called niosomes.[1]

1. Conventional liposomes

Liposomes as vesicular drug delivery system were first introduced in 1965 and have been extensively studied as drug carriers and drug delivery. The basic structure of liposome is shown in Fig. 1. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceutics such as drug targeting, controlled release or increased solubility. Liposomes have been reported to increase drug stability, enhance therapeutic effects, prolong circulation time and promote uptake of the entrapped drugs into target site while drug toxicity is diminished. They may serve as a solubilization matrix as local depot for sustained release or permeation enhancers of dermally active compounds or as a rate- limiting membrane barrier for the modulation of systemic absorption of drugs via the skin.

However, there are significant problems associated with clinical applications of liposomes that include difficulties in sterilization and in large-scale production to obtain a product with adequate physical and chemical stability. Problems with the physical and chemical stabilities of aqueous suspensions of liposomes have been addressed by many researchers who introduced a dry free flowing granular product known as proliposomes in 1986 that could be immediately hydrated before use. It avoided the problems associated with conventional Liposome.[4] Although this is an improvement over conventional liposomes, a vacuum and nitrogen atmosphere is still recommended during preparation and storage to prevent oxidation

of phospholipids. Therefore, the alternative substances are extensively studied to prepare bilayer vesicles instead of phospholipids in order to avoid such problems.[3]

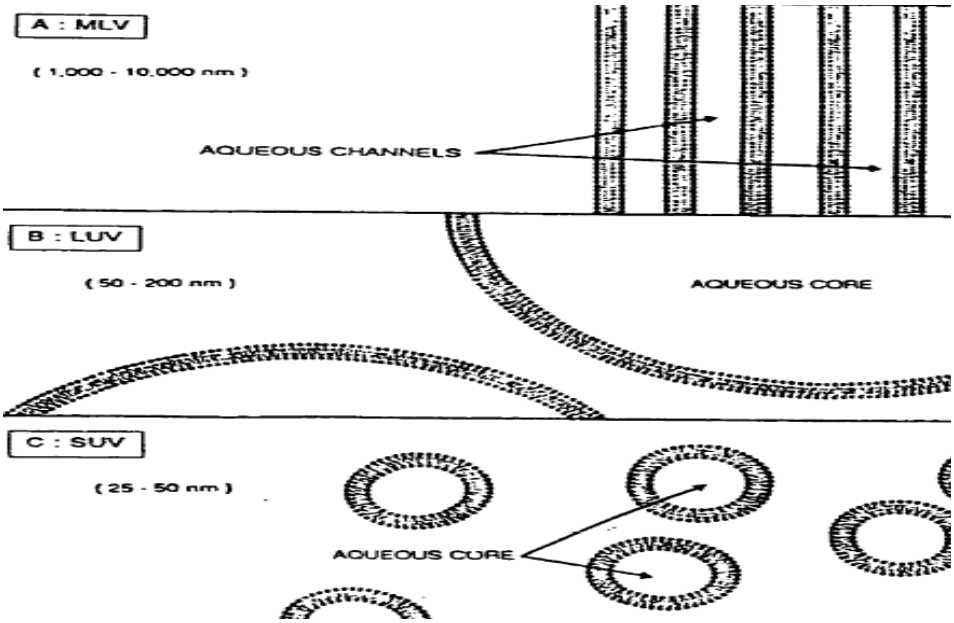

Fig. 1 different type of vesicles MLV (Multilamellar vesicles), LUV (Large unilamellar vesicles), SUV (Small unilamellar vesicles)[5]

Stability of Liposomes

Liposomes face a number of chemical and physical destabilisation processes. So their stability problems are classified in to two types,

1. Physical stability
2. Chemical stability

Physical stability

Physical processes that affect shelf life of liposomes includes loss of liposome associated drug and changes in size, aggregation and fusion.

1. Aggregation is the formation of larger units of liposome material, these units are still composed of individual liposomes.In principle, this process is reversible, e.g., by applying mild shear forces, or by changing the temperature or by binding metal ions that intiaaly induced aggregation.

2. Fusion indicates that new colliodal structures were formed. As fusion is an irreversible process, the original liposomes can never be retrieved. Drug molecules can leak from liposomes. The leakage rate strongly depends on the bilayer composition and the

physiochemical nature of the drug. Bilayers in the gel state or those containig fractions of cholesterol tend to lose assocaited drug slowly; liquid state bilayers are more prone to drug loss and are less stable during storage.

Chemical stability[6]

1. Hydrolyiss of the ester bonds: Phosphatidylcholine possesses for ester bonds. The two acyl ester bonds are most liable to hydrolysis. The glycerophosphate and phosphocholine ester bonds are more stable. The 1-acyl-lysophosphatidylcholine (LPC) and 2- acyl LPC are both formed at comparable rates.

2. Lipid peroxidation of phospholipids: The polyunsaturated acyl chains of phospholipids are sensitive to oxidation via free radical reactions. Cyclic peroxides, hydroperoxides, malonilaldehyde, alkanes are the major degradation products. Low oxygen pressure, absence of heavy metals, addition of anti-oxidants, complexing agents (EDTA, etc), quenchers (beta-carotene) of the photo-oxidatio reactions improve resistance against lipid peroxidation.

Adavantages of Liposomes

1. Liposomes increses efficiency and therapeutic index of drugs
2. It increases stability via encapsulation of drugs
3. Liposomes are non toxic, flexible, biocompatible, completely biodegradable, and non immunogenic for systemic and non systemic administrations
4. Liposomes reduces toxicity of encapsulated agent (amphotericine B, Taxol)
5. It helps reduce the exposure of sensitive tissue to toxic drugs
6. Flexibility to couple with site specific ligands to achieve active targeting

Disadvantages[7]

1. Low solubility
2. Short half life
3. Some times phospholipid undergoes oxidation and hydrolysis reactions
4. Leakage and fusion of encapsulated drug/ molecule
5. Production cost is high
6. Fewer stables

1. Conventional niosomes

Liosome itself acts as promising drug delivery system for various drugs to modify its drug release and overcome problems associated with conventional drug delivery systems. But the major drawback of liposome is its physical and chemical stability and hence Niosome concept came in market.

Basically, Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes. Niosome are vesicular drug delivery system in which drug is incorporated in vesicle. The vesicle is composed of bilayer of non ionic surface active agents and hence the name Niosome it got. Niosome are microscopic in size. They constitute of non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequient hydration in aqueous media. The surfactant molecule tends to orient them self in such a way that hydrophilic ends of non ionic surfactant points outwards, while hydrophobic ends face each other to form bilayer.[4] From a technical point of view, niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids. Another advantage is the simple method for the routine and large scale production of niosomes without the use of unacceptable solvents. One alternative of phospholipids is the hydrated mixture of cholesterol and non-ionic surfactants such as alkyl ethers, alkyl esters or alkyl amides. This type of vesicle formed from the above mixture has been known as niosomes or non-ionic surfactant vesicles. The structure of niosome is shown in Fig. 2. The structure and properties of niosomes are similar to those of liposomes.

The chemical stability as well as the relatively low cost of the materials used to prepare niosomes made the vesicle more attractive than liposomes for industrial productions both in pharmaceutical and cosmetic applications. Furthermore, there are large numbers of non-ionic surfactants available for the design of vesicles on demand. These vesicles have been reported to decrease side effects, give sustain release and enhance penetration of the trapped substances through skin. Several mechanisms have been used to explain the ability of niosomes to modulate drug transfer through skin.

1. Adsorption and fusion of niosomes on the surface of skin leading to high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drug.

2. Reduction of the barrier properties of stratum corneum resulting from the property of vesicles as a penetration enhancer. The encapsulation of drugs in niosomes can decrease drug toxicity, increase drug absorption and retard removal of drug from the circulation due to slow drug release.[3]

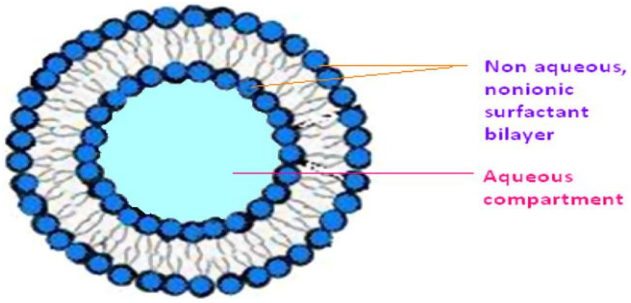

Fig. 2 Structure of niosome

Advantages of Niosomes[8]

1. To improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells
2. To increase the stability of entrapped drug since these are osmotically active and stable.
3. To regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.
4. To improve the oral bioavailability of poorly absorbed drugs and to enhance skin penetration of drugs.
5. To act as a depot, for releasing the drug in a controlled manner.
6. They have better patient compliance and better therapeutic effect than conventional oily formulations.
7. They can be made to reach the site of action by oral, parenteral as well as topical routes.
8. They can show a greater bioavailability than conventional dosage forms.
9. They are biodegradable, biocompatible and non immunogenic to the body.
10. They are more stable than liposomes.
11. No special conditions required for handling and storage of surfactants used in niosomal drug delivery.

Structural components of niosomes

1. Surfactants

Nonionic surfactants are the most common type of surface active agent used in preparing vesicles due to the superior benefits they impart with respect to stability, compatibility and

toxicity compared to their anionic, amphoteric or cationic counterparts. They are generally less toxic, less hemolytic and less irritating to cellular surfaces and tend to maintain near physiological pH in solution. They have many functions including acting as solubilizers, wetting agents, emulsifiers and permeability enhancers. Nonionic surfactants are comprised of polar and non- polar segments and possess high interfacial activity. The forma- tion of bilayer vesicles instead of micelles is dependent on the hydrophilic–lipophilic balance (HLB) of the surfactant, the chemical structure of the components and the critical packing parameter (CPP).

Ether linked surfactants

These are polyoxyethylene alkyl ethers which have hydrophilic and hydrophobic moieties are linked with ether. The general formula of this group is CnEOm, where n can be 12-18 and m can be 3-7. Surfactants with polyhydroxyl head and ethylene oxide units are also reported to be used in niosomes formation. Single alkyl chain surfactant C16 mono alkyl glycerol ether with an average of three glycerol units is one of the examples of this class of surfactants used for the preparation of niosomes.

Ester linked surfactants

These surfactants have ester linkage between hydrophilic and hydrophobic groups and have been studied for its use in the preparation and delivery of sodium stibogluconate to the experimental marine visceral leishmaniasis.

Sorbitan Esters

These are most widely used ester linked surfactants especially in food industry. The commercial sorbitan esters are mixtures of the partial esters of sorbital and its mono and di-anhydrides with oleic acid. These have been used to entrap wide range of drugs viz doxorubicin.

Alkyl Amides

These are alkyl galactosides and glucosides which have incorporated amino acid spacers. The alkyl groups are fully or partially saturated C12 to C22 hydrocarbons and some novel amide compounds have fluorocarbon chains.

Fatty Acids and Amino Acid Compounds: These are amino acids which are made amphiphilic by addition of hydrophobic alkyl side chains and long chain fatty acids which form “Ufasomes” vesicles formed from fatty acid bilayers.

2. Cholesterol

Steroids bring about changes in fluidity and permeability of the bilayer and are thus important components. Cholesterol a waxy steroid metabolite is usually added to the non-ionic surfactants to provide rigidity and orientational order. It does not form the bilayer itself and can be incorporated in large molar ratios. Cholesterol is an amphiphilic molecule; it orients its -OH group towards aqueous phase and aliphatic chain towards surfactant's hydrocarbon chain. Rigidization is provided by alternative positioning of rigid steroidal skeleton with surfactant molecules in the bilayer by restricting the movement of carbons of hydrocarbon. Cholesterol is also known to prevent leakage by abolishing gel to liquid phase transition.

3. Charge Inducers

Charge inducers increase the stability of the vesicles by induction of charge on the surface of the prepared vesicles. It act by preventing the fusion of vesicles due to repulsive forces of the same charge and provide higher values of zeta potential. The commonly used negative charge inducers are dicetyl phosphate, dihexadecyl phosphate and lipoamine acid and positive charge inducers are sterylamine and cetyl pyridinium chloride. The structure of noisome is given in figure 1.[9]

Factors Influencing Niosomes Formation

1. Nature of surfactants: A surfactant used for preparation of niosomes must have a hydrophilic head and hydrophobic tail. The hydrophobic tail may consist of one or two alkyl or perfluoroalkyl groups or in some cases a single steroidal group. The ether type surfactants with single chain alkyl as hydrophobic tail is more toxic than corresponding dialkylether chain .The ester type surfactants are chemically less stable than ether type surfactants and the former is less toxic than the latter due to ester-linked surfactant degraded by esterases to triglycerides and fatty acid in vivo. The surfactants with alkyl chain length from C12-C18 are suitable for preparation of niosomes.

2. Surfactant and lipid amount: The maximum amount of surfactant/lipid used to prepare niosomes is generally 10–30 mmol/L (1–2.5%, w/w). Alterations in the surfactant: water ratio during the hydration step may affect the structure and properties of the niosomes produced. As the surfactant/lipid level increases, the amount of drug to be encapsulated also increases leading to an increase in the viscosity of the system.

3. Effect of cholesterol: Cholesterol influences the physical properties and structure of niosomes possibly due to its interaction with the nonionic surfactants. The interaction is of biological interest since cholesterol is always present in biological membranes where it influences membrane properties such as aggregation, ion permeability, fusion processes, elasticity, enzymatic activity, size and shape. The effect of cholesterol in lipid bilayers is mostly to modulate their cohesion and mechanical strength and their permeability to water. Through the addition of cholesterol, the fluidity of niosomes is changed considerably. Cholesterol imparts rigidity to vesicles, which is very important under severe stress conditions.

4. Nature of encapsulated drug: The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size. Another factor to be considered is whether the drug to be encapsulated is amphiphilic. The best example of such a drug is doxorubicin. When encapsulated in niosomes, aggregation occurred and was overcome by the addition of a steric stabilizer. The increase in encapsulation of a drug that occurs when more is added could be the result of saturation of the medium. This suggests that the solubility of certain poorly soluble drugs can be increased by formulation in niosomes but only up to a certain limit above which drug precipitation will occur. An increase in the encapsulation of flurbiprofen due to saturation of drug in the hydration medium has been reported. However, when niosomes were prepared using higher amounts of minoxidil, optical micro- scopy revealed minoxidil crystals dispersed in between the niosomal particles.

5. Structure of surfactants: The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of surfactants can predicate geometry of vesicle to be formed. Critical packing parameters (CPP) can be defined using following equation,

$$\mathbf{CPP = v / lc \times a0}$$

Where, v = hydrophobic group volume, lc = the critical hydrophobic group length and a0= the area of hydrophilic head group.

From the CPP value, type of miceller structure formed can be ascertained as given below,
If CPP < ½ then formation of spherical micelles, If ½ < CPP < 1 formation of bilayer micelles and If CPP > 1 formation inverted micelles.

Vesicle aggregation of niosomes may be prevented by the inclusion of compounds that introduce repulsive steric or electrostatic forces. An example of steric stabilization is the inclusion of Solulan C24 (a cholesteryl poly-24-oxyethylene ether) in doxorubicin sorbitan monostearate (Span 60) nio- some formulations. Examples of electrostatic stabilization are the inclusion of dicetyl phosphate in 5(6)-carboxyfluorescein loaded Span 60 based niosomes and the inclusion of stearyl amine in rifampicin loaded niosomes.

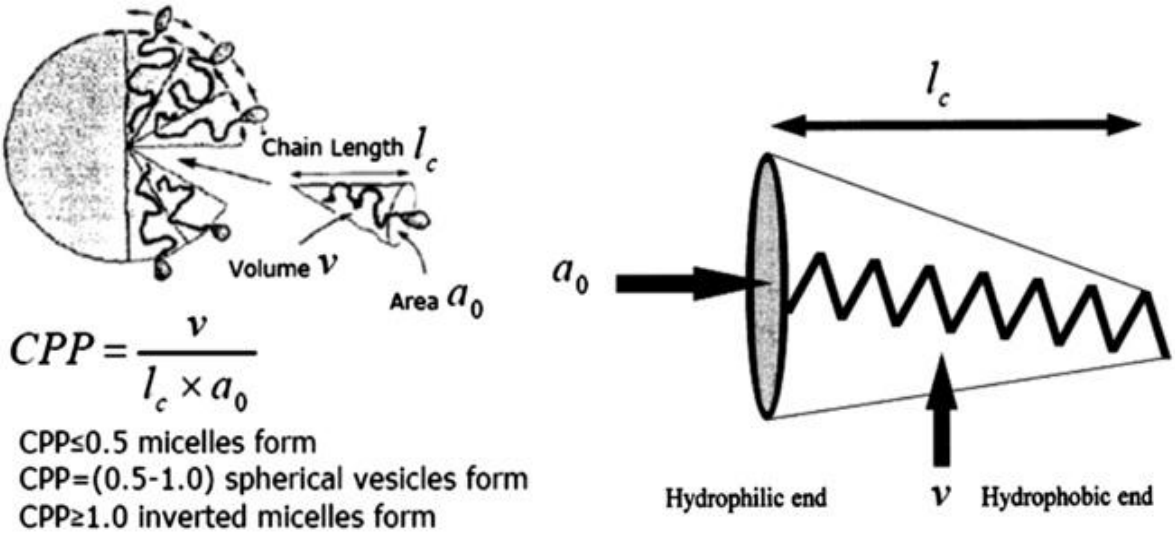

Fig 3: Relationship between CPP and structure of surfactant. CPP(Critical packing parameter), V(Volume), l_c(Chain length), a_0(area of hydrophilic head group).

6. Temperature of hydration: Hydration temperature influences the shape and size of the noisome. For ideal condition it should be above the gel to liquid phase transition temperature of system. Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformation.

Temperature of the hydration medium plays a major role in the formation of vesicles and affects their shape and size. The temperature should always be above the gel to liquid phase transition temperature of the system. Temperature affects the assembly of surfactants into vesicles and also induces changes in vesicle shape. For example, polyhedral vesicles formed by C16:solulan C24 (91:9) at 25Ċ transformed into spherical vesicles upon heating to 48Ċ but, on cooling from 55Ċ, formed a cluster of smaller spherical niosomes at 49Ċ and changed to a polyhedral structure at 35Ċ. In contrast, vesicles formed by C16: cholesterol: solulan C24 (49:49:2) showed no shape transformations on heating or cooling. Volume of the hydration medium and duration of hydration of the lipid film also affect vesicle structure and yield.

7. Effect of pH of the hydration medium

Entrapment efficiency of niosomes is greatly affected by the pH of the hydration medium. High entrapment of flurbiprofen was reported at acidic pH with a maximum encapsulation efficiency of 94.6% at pH 5.5. The fraction of flurbiprofen encapsulated increased to about 1.5 times as pH decreased from 8 to 5.5 and decreased significantly at pH4. The lowest entrapment of flurbiprofen occurred at pH 7.4 and 8 with no significant difference between them. The increase in encapsulation efficiency of flurbiprofen at lower pH is pre- sumably due to its ionizable carboxylic acid group. At lower pH, the proportion of unionized flurbiprofen increases and partitions more readily into the lipid bilayer than the ionized species. At lower pH, niosome formulations should be examined by optical microscopy for the presence of drug precipitates both before and after centrifugation and washing. This will help to determine the concentration of drug in the hydration medium giving optimum encapsulation in niosomes.

Membrane composition: The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives. In case of polyhedral niosomes formed from C16G2, the shape of these polyhedral niosome remains unaffected by adding low amount of solulan C24 (cholesteryl poly-24-oxyethylene ether), which prevents aggregation due to development of steric hindrance.[13]

3. Proniosome

Niosomes are osmotically active and are stable chemically because chemical stability related with phosphotidylcholine is completely replaced by non ionic surfactants for vesicle formation in noisome. Although niosomes as drug carriers have shown advantages such as being cheap and chemically but they are associated with some problem as written:

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of dispersion.

So, to increase shelf life and stability of niosomes Proniosomes are developed. Proniosomes are dry formulations of surfactant coated carriers which can be rehydrated by brief agitation

in hot water. The resulting niosomes are very similar to conventional niosomes and more uniform in size. Proniosomes are dry solid in form which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing so the proniosome can be provided in capsule form.

Advantages of proniosomes

1. Proniosomes have potential for the entrapping wide range of active compounds.
2. Convenient for transportation, sterilization, distribution, storage and dosing.
3. Problem of degradation by hydrolysis or oxidation which is usually seen in liposomes is avoided.
4. Requires no special conditions for storage and handling.
5. Sedimentation, aggregation or fusion is not seen.
6. Uses acceptable solvents in the preparation.

Type of Proniosome

According to their physical behaviour proniosomes are classified in to two categories as follows,

1. Dry granular proniosome
2. Liquid crystalline proniosome

1. Dry granular proniosome

According to type of carrier and method of preparation, proniosomes are further classified in to two types,

a. Sorbitol based proniosomes
b. Maltodextrin based proniosomes

Sorbitol based proniosome is dry formulation that involves sorbitol as carrier which is further coated with non ionic surfactant and is used as noisome within minute by addition of hot water followed by agitation. These are normally made by spraying surfactants mixture prepared with organic solvent on to the sorbitol powder and then evaporating the solvent. Finaly we will get dry free flowing granular powder.

In maltodextrin based proniosome maltodextrin acts as carrier. Generaly it is prepared by fast slurry method. Time required to produced proniosome by slurry method is independent of ratio of surfactant solution to carry out.Since maltodextrin morphology is preserved, hollow,

blown maltodextrin particles can be used for signifigant gain in surface area. The higher surface area results in thinner surfactant coating, which makes the rehydration process efficient.

2. Liquid crystalline proniosome

When surfactant molecules are kept in contact with water, There are three possibilities or way through which lipophilic chains of surfactant can be transformed into disorder liquid state called lyotropic liquid crystalline state(neat phase). These three ways are increasing temperature at kraft point (Tc), addition of solvent which dissolve lipids, and use of both temperature and solvent. Neat phase or lamellar phase contains bilayers arranged in sheet over one another within intervening aqueous layer. For ternary lecithin, non ionic surfactant as monoglyseride and alcohol system, lamellar liquid crystals are formed at kraft temperature in presence of alcohol. The lamellar crystalline phase can be converted into dispersion on noisome by adding high amount of water.[10]

Mechanism of Drug Transport through skin

There is a direct contact of proniosomes formulation with skin after applies so it is better to discuss the potential interactions between skin and vesicle formed in proniosome/ noisome formulation. It is still not clear which factors influence the vesicle skin interactions. But it is clear that Proniosomes should be hydrated to form niosomal vesicles before the drug is release and permeates across skin.

Two types of vesicle skin interactions observed during in vitro studies using human skin.

1. Adhesion, Fusion, and Aggregation: When vesicles came in contact with stratum corneum, they aggregate, fuse and adhere to the surface of cell. It is believed that this type of interaction leads to high thermodynamic activity gradient of the drug at the interface of vesicle and stratum corneum which is driving force for penetration of lipophillic drug across the stratum coerneum.

2. Cellular modification/Changes: This type of interaction involves the ultrastructural changes in the intercellular lipid regions of the skin and its deeper layer at maximum depth of about 10 nm as revealed by Freeze Fracture Electron Microscopy and Small Angle X-Xay Scattering. The other factor which could also explain the ability of vesicles to modulate drug transfer across the skin is

a. Nature of drug

b. The lipid bilayer of niosomes acts as a rate limiting membrane barrier for drugs.

c. Dehydration of vesicles

d. The vesicles act as penetration enhancer to reduce the barrier properties of the skin.

e. Skin and composition of vesicles.

f. Biophysical factor[11]

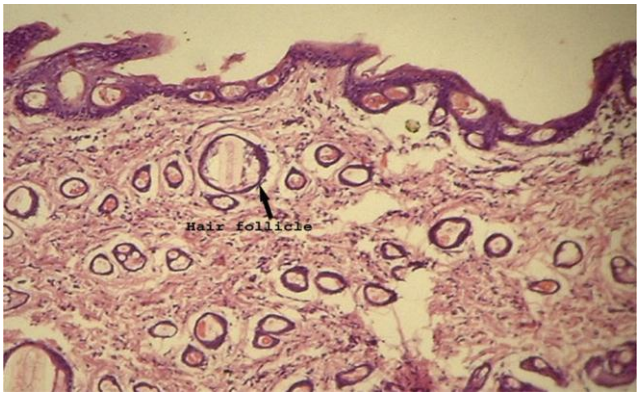

Fig. 4 Photomicrograph of control skin section (not treated with inhancer) showing normal skin structure. Epidermal and dermal layers are well defined.

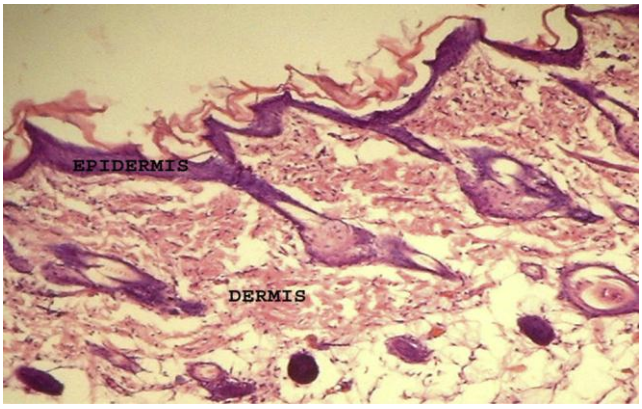

Fig. 5 Photomicrograph of test skin section(enhancer treated) showing generally normal skin structure except for absent keratin layer.[12]

Preparation of proniosome

There are basically 3 methods for preparation of proniosomes which are

1. Slurry Method
2. Coacervation Phase Separation Method
3. Slow Spray coating method.

1. Slurry Method: Proniosomes can be prepared from a stock solution of surfactants and cholesterol in a suitable solvent. The required volume of surfactant and cholesterol stock solution per gram of the carrier and drug should be dissolved in the solvent in a round bottom flask containing the carrier (maltodextrin or lecithin). Additional chloroform can be added to

form the slurry in case of lower surfactant loading. The flask has to be attached to a rotary flash evaporator to evaporate solvent at 50- 60 rpm at a temperature of 45±20 C and a reduced pressure of 600mm of Hg until the mass in the flask had become a dry, free flowing product. Finally, the formulation should be stored in a tightly closed container under refrigeration in light.

2. Coacervation Phase Separation Method: This is widely used method for preparation of proniosomal gel. Proniosomal gel is basically mixtures of many phases of liquid crystals like Lamellae, cubical or hexagonal which upon hydration forms unilameller to multilameller and spherical structures. Precisely weighed amount of drug, surfactant, lecithin, cholesterol take place and suitable alcohol is taken in clean, dry wide mouth glass vial and to it, 0.5 ml alcohol is added (minimum amount of alcohol is added so that micelle formation does not take place). All the ingredients are mixed well with the help of glass rod and covered with a lid to prevent loss of solvent. Further it is warmed on a water bath at 60-70°C for 5 min until all the surfactant dissolved completely. Then aqueous phase (glycerol, isotonic phosphate buffer or distilled water) is added in small amount so as to ensure only the gel formation and not the dispersion. Again it is heated further for 2 min to give clear dispersion which on cooling to room temperature gives formation of proniosomal gel. In this formulation, water addition leads to swelling of bilayer due to interaction of water and polar groups of surfactant.

3. Spraying Method: A 100 ml round bottom flask containing desired amount of carrier can be attached to the rotary flash evaporator. A mixture of surfactants and cholesterol should be prepared and introduced into round bottom flash on rotary evaporator by sequential spraying of aliquots onto carrier's surface. The evaporator has to be evacuated and rotating flask can be rotated in water bath under vacuum at 65-70 oC for 15 – 20 min. This process has to be repeated until all of the surfactant solution had been applied. The evaporation should be continued until the powder becomes completely dry.[13]

Formulation aspects of proniosome[21,22]

Proniosomal gel is comprised of ingredients like membrane stabilizer (lecithin, cholesterol), non-ionic surfactants (sorbitans and polysorbitans), alcohol and aqueous phase.

1. Surfactants[23,24]

Hydrophillc Lipophillic Balance (HLB) is the basis for the selection of surfactant. The HLB value indicates that the surfactant will form vesicle or not. It is reported thatthe HLB value

between 4 and 8 are good candidates for vesicle formation. Hydrophillc surfactants, due to their high aqueous solubility on hydration, cannot attain a concentrated system in order to allow free hydrated units to exist aggregates and coalesced to form a lamellar structure. High HLB value reduces the surface free energy and allows vesicle formation of large size. Span 40 and span 60 have high HLB value, which results in reduced surface free energy, hence large size vesicles are formed, which gives a larger area exposed to skin and dissolution medium. HLB value and Phase Transition Temperature affects the encapsulation efficiency of surfactant. All spans have high Phase Transition Temperature hence good encapsulation efficiency, less leakage of drug. Encapsulation efficiency of tween is low as compared to spans. List of surfactants is given in Table 1.

Table 1: Surfactant and their properties. Tc- Phase transition Temperature; HLB- Hydrophilic Lipophilic Balance[14]

Surfactant	Synonyms	Properties
Sorbitan monolourate	Span 20	Tc: 16Ċ, HLB value:8.6
Sorbitan monopalmitate	Span 40	Tc:42Ċ, HLB value:6.7
Sorbitan monostearate	Span 60	Tc:53Ċ, HLB value:4.7
Sorbitan monooleate	Span 80	Tc:12Ċ, HLB value:4.3
Polyoxyethylene(20)sorbitan Monolourate	Tween 20	HLB value: 16.7
Polyoxyethylene(20)sorbitan Monopalmitate	Tween 40	HLB value: 15.6
Polyoxyethylene(20)sorbitan Monostearate	Tween 60	HLB value: 14.9
Polyoxyethylene(20)sorbitan Monooleate	Tween 80	HLB value: 15

Chemical structure of surfactants influences drug entrapment efficiency. Increasing the alkyl chain length is leading to higher entrapment efficiency. It had also been reported that spans having highest phase transition temperature provides highest entrapment for the drug and vice- versa. Drug can be entrapped into proniosomes composed of tweens; however the encapsulation efficiency was relatively low as compared to those composed of spans. Most of Surfactants used to make nonionic surfactant vesicles have a low aqueous solubility. However, freely soluble nonionic surfactants such as tween can form the micelles on hydration in presence of cholesterol.

2. Lecithin [25,26]

Lecithin as a complex mixture of acetone insoluble phosphatides that consists chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates as separated from a crude vegetable oil source.

The composition of lecithin (and hence also its physical properties) varies enormously depending upon the source of the lecithin and the degree of purification. Egg lecithin, for example, contains 69% phosphatidylcholine and 24% phosphatidylethanolamine, while soybean lecithin contains 21% phosphatidylcholine, 22% phosphatidylethanolamine, and 19% phosphatidylinositol, along with other components.

In proniosomal gel, lecithin plays important role like Lecithin acts as a penetration enhancer Increases entrapment efficiency due to high phase transition temperature Prevents leakage of the drug from vesicle Reduces vesicle size due to increase in the hydrophobicity (vesicle composed of soya lecithin is of larger size than that composed of egg lecithin) Egg lecithin contains saturated fatty acid while soya lecithin contains unsaturated fatty acids, oleic acid and linoleic acid, hence soya lecithin is having good penetrability over egg lecithin.

3. Cholesterol[29,30]

Cholesterol is an important component of proniosomal vesicle. As it influences stability and permeability of vesicle. It was found that entrapment efficiency increase with increase in cholesterol content up to a certain limit; at higher concentration it has lowring effect on entrapment efficiency. This is because the cholesterol molecule acts as vesicular cement which accommodates itself in the molecular cavities formed when surfactant monomers are assembled into bilayers to form niosomal membranes, these results in increased rigidity and decreased permeability as compared to cholesterol free niosomal membrane. On further increase in cholesterol concentration, it competes with drug for accommodation between bilayers and disrupts the regular structure of vesicular membrane.

4. Solvent [27,28]

Alcohol used has great influence on vesicle size and permeability of the drug. Vesicles formed from different alcohols have different size and they follow the order
Ethanol > Propanol > Butanol > Isopropanol.

Ethanol gives the highest size due to high aqueous solubility and lowest size with usual is due to branched chain present in it. Selection of solvent also affects the rate of spontaneity of formation of niosomes. Formulation in which isopropanol and butanol is used; niosomes are formed more spontaneously because of faster phase separation of isopropyl alcohol and butanol due to their low aqueous solubility.

5. Aqueous phase[36]

0.1% Glycerol, phosphate buffer pH 7.4 or distilled water is used as an aqueous medium for preparation of proniosomal gel. Selection and pH of aqueous system affects the entrapment efficiency and particle size of proniosomes.

6. MISCELLANEOUS[34]

a. Dicetyl Phosphate (DCP)[33]**:** It is a charged molecules used to impart negative charge to the niosomal vesicles. Formulations containing DCP shows slightly greater amount of drug than those containing surfactant and cholesterol only. It is reported that drug release was maximum for the proniosomes containing DCP due to the charge present in the DCP containing bilayers, which is responsible for an increase in the curvature and decrease vesicle size. DCP decreases the entrapment efficiency of drug into vesicles.

b. Stearylamine (SA)[32,31]**:** This is a charged lipid used to impart a positive charge to the vesicle. SA decreases the entrapment efficiency.

c. Solutan: Solutan C24 a poly-24 oxyethylene cholesteryl ether, is added to formulations to give homogeneous nature and devoid of aggregates.

5. CHARACTERIZATION

a. Morphology

Vesicle structure and shape can be characterized by various types of microscopy such as optical, freeze fracture elec- tron, surface electron, scanning electron, negative staining transmission electron, cryo-electron, fluorescence. The interfacial surface tension of a vesicular system determines the structure of the supramolecular elements of multilamellar vesicles.

b. Size and vesicle charge

Size and charge of vesicles have a significant effect on their stability and drug encapsulation. Size and charge can be assessed using a multifunctional zeta potential analyzer where size of vesicles is the result of repulsion forces between the bilayers and the entrapped drug.

c. Entrapment efficiency[15,39]

This is determined by measuring the difference between the unentrapped and total amounts of drug. Unentrapped drug is determined by various techniques such as exhaustive dialy- sis, gel filtration6, and centrifugation. The drug remained entrapped in noisomes is determined by

complete vesicle disruption using 50% n propanol or 0.1% Triton X-100 and analysing resultant solution by appropriate assay method for the drug.

Percent Entrapment = (Total drug - diffusion drug)/Total drug× 100

c. In vitro release

In vitro drug release can be done by (chen DB et al)

1. Dialysis tubing
2. Reverse dialysis
3. Franz diffusion cell

Dialysis Tubing [39,40]: Muller et al 2002 studied in vitro drug release could be achieved by using dialysis tubing. The proniosomes is placed in dialysis tubing which can be hermitically sealed. The dialysis sac is then dialysed against a suitable dissolution medium at room temperature; the sample are withdrawn from the medium at suitable intervals, centrifuged and analysed for drug content using suitable method.

Reverse dialysis [37,38]: In this technique number of small dialysis as containing 1 ml of dissolution medium are placed in proniosomes. The proniosomes are then displaced in to the dissolution medium. The direct dilution of the proniosomes is possible with this method; however the rapid release can not be quantified using this method.

Franz diffusion cell [40,41]: The invitro diffusion studies can be performed by using Franz diffusion cell. Proniosome is placed in the donar chamber of Franz diffusion cell fitted with a cellophane membrane. The proniosome is then dialysed against a suitable dissolution medium at room temperature; the sample are withdrawn from the medium at suitable intervals, and analysed for drug content using suitable method.

d. Stability

The main problems associated with storage of vesicles are aggregation, fusion and leakage of drug. At the end of each month, only stable formulations were selected to continue for another month. It was found that there was no significant change in the mean size of vesicles after 90 days when compared with those of freshly prepared sucrose stearate niosomes. However, the entrapment efficiency was affected (10%) following storage. The stability of niosomes is also assessed under conditions, which promote photodegradation such as exposure to UV irradiation and fluorescent light. For the former, drug is analyzed after the

drug solution and vesicle preparation are maintained at room temperature and exposed to UV radiation for 1 h at 25Ċ. Such studies have been reported for niosomes loaded with tretinoin, a metabolite of vitamin A. For the latter, the samples are exposed to artificial light at room temperature for a specific period and the drug concentration determined.

f. Osmotic shock

The change in vesicle size can be determined by osmotic studies. Niosomal formulation is incubated with hypotonic, isotonic, hypertonic solution for 3 hr. Then the changes in the size of vesicles in the formulations are viewed under optical microscopy.

Applications of Niosomes

The application of niosomal technology is widely used in treatment of number of diseases. The following are the 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 immunoglobulin 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.

Uses in Studying Immune Response

(Brewer and Alexander in 1992) studied niosomes are used 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 Haemoglobin46

(Moser P. and Marchand Arvier M. in 1989) reported that 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 anaemic 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

a) Sustained Release

Azmin *et al* suggested the role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation.

b) Localized Drug Action

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. The evolution of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and anti-leishmanial therapy. (Sudhamani, T. et al, 2010).[15]

CONCLUSION

Niosome is an advanced vesicular drug delivery system which overcome all the drawback associated with liposome and proliposome.Proniosomes are the modified niosomes which overcome the major problem of vesicular drug delivery system i.e. Stability problem. Availability of various non ionic surfactant make possible to formulate niosome of various sizes, having different entrapment efficiency, and different control release patternTheir vesicular membrane is mainly composed of nonionic surfactants and cholesterol and the enclosed interior usually contains a buffer solution at appropriate pH. Niosomes may be prepared by various methods, which affect their formations along with the properties of the drug, cholesterol content and amount, structrue and type of surfactant. As a drug delivery device, niosomes are osmotically active and stable. They also improve the stability of the entrapped drug during delivery. They do not require special conditions for handling, protection, storage or industrial manufacturing. In addition, they can be prepared with different structural characteristics (composition, fluidity and size), and can be designed for particular routes of administration. Overall, niosomes are a very effective tool for drug delivery and targeting of numerous therapeutically active moieties. They have the potential to provide

better treatment than conventional drug delivery systems. For site specific, cancer treatment and peptide delivery system it have wide approach to deliver the drug.

ACKNOWLEDGEMENT

I am great thankful to MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik.

REFERENCES

1. Kumar GP, Rao, PR, Ultra deformable niosomes for improved transdermal drug delivery: The future scenario. Asian Journal of Pharmaceutical Sciences, 2012; 7(2): 96–109.
2. Argan N, Harikumar SL, Topical Liposomal Gel: A Novel Drug Delivery System. International Journal of Research in Pharmacy and Chemistry, 2012; 2(2): 383–391.
3. Kumar GP, Rajeshwarrao P, Nonionic surfactant vesicular systems for effective drug delivery: an overview. Acta Pharmaceutica Sinica B, 2011; 1(4): 208–219.
4. Abhinav K, Pal K, Jaiswal A, et al, Review on niosomes as novel drug delivery system. International Research Journal of Pharmacy, 2011; 2(May): 61–65.
5. Patel MC, Patel HK, Suthar RM, et al, Liposomes : As A Topical Drug Delivery System. International Journal of Pharmaceutical and Chemical Sciences, 2012; 1(1): 1–10.
6. Kulkarni PR, Yadav JD, Vaidya K, et al, Liposomes : a Novel Drug Delivery System. International Journal of Current Pharmaceutical Research, 2011; 3(2): 10–18.
7. Akbarzadeh A, Rogaie RS, Soodabeh D, et al, Liposome: classification, preparation, and applications. Nanoscale Research Letters, 2013; 8(1): 102.
8. Mansoori M, Agrawal S, Jawade S, et al, A review on liposome. Internathinal Journal of Advanced Research in Pharmaccutical and Bio Sciences, 2012; 1(4): 453–464.
9. Gangwar M, Singh R, Goel RK, et al, Recent advances in various emerging vescicular systems: An overview. Asian Pacific Journal of Tropical Biomedicine, 2012; 2.
10. Walve JR, Rane BR, Gujrathi NA, et al, Transfersomes : a Surrogated Carrier for Transdermal Drug Delivery System. International Journal of Applied Biology and Pharmaceutical Technology, 2011; (1): 204–213.
11. Saroj S, Baby DA, Sabitha M, et al, Current trends in lipid based delivery systems and its applications in drug delivery. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5: 4–9.

12. Vashisth I, Abdul A, Agrawal S, et al, Investigating the potential of essential oils as penetration enhancer for transdermal losartan delivery: Effectiveness and mechanism of action. Asian Journal of Pharmaceutical Sciences, 2014; 9(5): 260–267.
13. Patel NN, Gupta A, Roopchandani K, et al, Proniosomes for improved transdermal drug delivery – a review. The Pharma, A Journal of Pharmacy Research.
14. Rawat AS, Kumar MS, Khurana B, et al, Proniosome Gel : A Novel Topical Delivery System. International journal of Rescent Advances in Pharmaceitical Research, 2011; (July): 1–10.
15. Sudhamani T, Priyadarisini N, Radhakrishnan M, et al, Proniosomes: Promising Drug Carriers. International Journal of Pharmatech Research, 2010; 2(2): 1446–1454.
16. Akhtar N, Vesicular ocular drug delivery system: preclinical. International Journal of Biopharmaceutics, 2013; 4(1): 38–48.
17. Mehta AK, Dubal AP, Mane PD, et al, Recent trends in niosomes as nanocarriers.Unique Journal of Pharmaceuitical and Biological Scinces, 2013; 01(02): 12–17.
18. Alsarra I, Bosela AA, Ahmed SM, et al, Proniosomes as a drug carrier for transdermal delivery of ketorolac. European Journal of Pharmaceutics and Biopharmaceutics, 2005; 59(3): 485–490.
19. Anosroi A, Wongratul P, Manosroi J, et al, Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. Colloids and Surfaces B: Biointerfaces, 2003; 30(1-2): 129–138.
20. Daniela Stan, Tataringa G, Gafitanu C, et al, Preparation and characterization of niosomes containing metronidazole. Farmacia, 2013; 61(6): 1178–1185.
21. El Maghraby GM, Ahmed A, Osman M, et al, Penetration enhancers in proniosomes as a new strategy for enhanced transdermal drug delivery. Saudi Pharmaceutical Journal, 2014; 61(6): 1178.
22. Gandhi A, Sen SO, Paul A, et al, Structural components of niosomes. Asian Journal of Pharmacy and Life Science, 2012; 2(2): 339–353.
23. Maya W, Ashar S, Proniosomal drug delivery systems : An overview. International Journal of Pharmaceutical and Chemical Sciences, 2012; 1(3): 1044–1056.
24. Lohumi A, Rawat S, Sarkar S, et al, A Novel Drug Delivery System: Niosomes Review. Journal of Drug Delivery and Therapeutics, 2012; 2(5): 129 –135.
25. Marianecci C, Marzio LD, Rinaldi F, et al, Niosomes from 80s to present: The state of the art. Advances in Colloid and Interface Science, 2014; 205: 187–206.

26. Nazia K, Alam MI, Sachan AK, et al, Recent trends in drug delivery by niosomes: A review. Asian Journal of Pharmaceutical Research and development, 2013; 1(June): 115–122.
27. Ning M, Guo Y, Pan H, et al, Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. Drug development and industrial pharmacy, 2005; 31(4-5): 375–383.
28. Madhav NVS, Saini A, Niosomes : a Novel Drug Delivery System. International Journal of Research in Pharmacy and Chemistry, 2011; 1(3): 498–511.
29. Patil HN, Hardikar SR, Bhosale AV, Formulation development & evaluation of proniosomal gel of carvedilol. International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(1): 191–197.
30. Pravina G, Naik N, Chouksey S, Niosome: a Promising Pharmaceutical Drug Delivery. International Journal of Pharmaceutics and Drug Analysis, 2014; 425–431.
31. Ramesh YV, Jawahar N, Jakki SL, Proniosomes : A Novel Nano Vesicular Transdermal Drug Delivery. Journal of Pharmaceutical science and Research, 5(8): 153–158.
32. Sankhyan A, Pawar P, Recent trends in niosome as vesicular drug delivery system. Journal of Applied Pharmaceutical Science, 2012; 2(6): 20–32.
33. Singla S, Harikumar SL, Aggarwal G, Proniosomes for effective topical delivery of clotrimazole: Development, characterization and performance evaluation. Asian Journal of Pharmaceutical Sciences, 2012; 7(4): 257–262.
34. Shamsheer AS, Sabareesh M, Patan RK, et al, Formulation and evaluation of Lisinopril dihydrate transdermal proniosomal gels. Journal of Applied Pharmaceutical Science, 2011; 1(8): 181–185.
35. Shashi K, Satinder K, Bharat P, A Complete Review on: Liposomes. International Research Journal of Pharmacy, 2012; 3(7): 10–16.
36. Singh S, Trivedi S, Jain S, Design and Development of Proniosome Based Transdermal Delivery of Ondansetron. International Journal of Pharmaceutical and Biological Research, 2012; 3(5): 191–201.
37. Thakur R, Anwer MK, Shams MS, et al, Proniosomal transdermal therapeutic system of losartan potassium: development and pharmacokinetic evaluation. Journal of drug targeting, 2009; 17(6): 442–449.
38. Thomas L, Viswanad V, Formulation and optimization of clotrimazole-loaded proniosomal gel using 3 by 2 factorial design. Scientia Pharmaceutica, 2012; 80(3): 731–748

39. Yadav K, Yadav D, Saroha K, et al, Proniosomal Gel : A provesicular approach for transdermal drug. Der Pharma Chemica, 2010; 2(4): 189–198.
40. Yadav U, Prajapati SK, Singh R, et al, Development, Optimization & Characterization of topical Nanoemulsion Gel of an Antifungal Agent for Effective Treatment of Onychomycosis. Asian Journal of Medical and Pharmaceutical Sciences, 2013; 1(1): 12–21.
41. Chandel A, Saroha K, Nanda S, Preparation and Evaluation of Proniosomal Gel of Neem Seed Oil. International journal of Pharmaceutical sciences and Nanotechnology, 2012; 5(3): 1780–1784.