Review Article

Niosomes: A Non-Ionic Surfactant Based Vesicles as a Carriers for Drug Delivery

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ABSTRACT

Niosomes are vesicular drug delivery system which is reported in seventies. They are formed by self-assembly of non-ionic surfactants with or without incorporation of cholesterol or other lipid in aqueous media resulting in lamellar structure that encapsulates both lipophilic and hydrophilic drugs. Although structurally similar to liposomes, niosomes offer several advantages over liposomes. They act as drug carriers which can be administered via various routes in controlled manner. This review focuses on all aspects of niosomes including their historical development, structural components, types, method of preparation, loading methods, factors affecting their formation, controlling the size, separation of unentrapped materials, characterization, therapeutic potentials and stability. This review also provides relevant information regarding patents and marketed products. The application of niosomes is widely varied and can be used to treat number of diseases.

Keywords: Niosomes, Non-Ionic Surfactant, Cholesterol, Vesicles, Carriers, Vesicular Drug Delivery System.

INTRODUCTION

Non-ionic surfactant based vesicles are termed as Niosomes, were first reported in the seventies as a feature of the cosmetic industry but have since been studied as drug targeting agents. [1] Niosomes are formed from the self-assembly of non-ionic surfactants in aqueous media resulting in closed bilayer structures (uni-lamellar or multi-lamellar) capable of encapsulating both hydrophilic and lipophilic substances. [2-4] The assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat. [5]

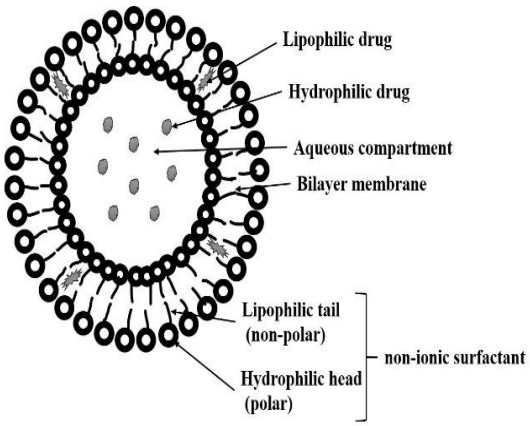


Figure 1: Structure of Niosome

Non-ionic surfactants orient as planar bilayer lattices which forms closed or sealed vesicular shape wherein polar or hydrophilic heads align facing aqueous media while hydrocarbon segments are so aligned that their interaction with aqueous media is minimized (i.e. every bilayer for thermodynamic reasons folds over itself to be a continuous membrane and forms a vesicle so that hydrocarbon-water interface remains no more exposed). [3, 6]

Historical Development of Niosomes

Vesicular drug delivery system are highly ordered assemblies consisting of one or more concentric bilayers formed as a result of self-assembling of amphiphilic building blocks in presence of hydrating media. [7] Advances have since been made in the area of vesicular drug delivery, leading to the development of systems that allow drug targeting in sustained and controlled manner. [8] The biologic origin of phospholipid vesicles or liposomes, prepared with variety of phospholipids were first described by British Haematologist Alec Douglas Bangham. [9] Liposomes exhibit certain disadvantages such as physical and chemical instability, high cost, variable purity of phospholipids and various other factors that militates against their practical adoption as drug carrier system. [2,10-13] Amphiphiles other than natural phospholipids have also been studied and found to form vesicular system similar to liposomes in physical characteristics. [3] Non-ionic surfactant based vesicles termed as Niosomes were first reported in the seventies as a feature of the cosmetic industry but have since been studied as drug targeting agents. [1] Niosomes are now widely studied as an alternative to liposomes. [4]

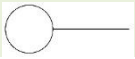
Structural Components of Bilayered Niosomes

Surfactant: [14-16]

Surfactants can be classified to anionic, cationic, amphoteric and non-ionic; according to their hydrophilic functionality polar head group. A non-ionic surfactant has no charge groups in its head. If the head has a negative charge then it is called an anionic surfactant. If the head has a charge positive, it is called a cationic surfactant. If a surfactant contains a head with two

oppositely charged groups, it is termed as a zwitterionic (amphoteric) surfactant. Non-ionic surfactants are preferred due to their superior benefits they impart with respect to stability, compatibility and toxicity compared to their anionic, amphoteric or cationic surfactants. They contain both polar and non-polar segments. The non-ionic surfactant molecules tent to orient themselves in such a way that the hydrophilic ends (head) point outwards, while the hydrophobic ends (tail) face each other to form the bilayer. The head group involves highly solvated hydrophilic functionalities, such as sulfonates, carboxylates, phosphonates and ammonium derivatives. The lipophilic region is chains made up of alkanes, fluorocarbons, aromatic or other non-polar groups.

Table 1: The effect of the surfactant on the properties of niosome dispersion.

Increased Hydrophilicity of Surfactant	Increased Hydrophobicity of Surfactant
	
Low phase transition.	High phase transition
Increased leakage of low molecular weight drugs from the aqueous compartment.	Decreased leakage of low molecular weight drugs from the aqueous compartment.
Decreased stability of the niosomal suspension.	Increased stability of niosome and increased encapsulation.
Improved transdermal delivery of hydrophobic molecules.	Decreased toxicity.

Different types of non-ionic surfactants [17]

1) Alkyl ethers
   (a) Glucoside alkyl ethers: Deacyl glucoside, lauryl glucoside, octly glucoside
   (b) Polyoxyethylene glycol octyl phenol ethers: Triton X-100
   (c) Polyoxyethylene glycol alkylphenol ethers: Nonoxynol-9
   (d) Others ethers: Brij, polyglycerol alkyl ether, glucosyl dialkyl ether, crown ethers, etc
2) Alkyl esters: Spans (sorbitan alkyl esters), polysorbates (polyoxyethylene glycol sorbitan alkyl esters, glyceryl laurate (glycerol alkyl esters)
3) Alkyl amide: Galactoside, glucoside
4) Fatty alcohol: Cetyl alcohol, steryl alcohol, ceto-steryl alcohol, oleyl alcohol
5) Block co-polymers: Poloxamer (block copolymers of polyethylene glycol and polypropylene glycol)

Cholesterol [11,18-21]

Stability of the niosomes can be increased by membrane additives into niosomal formulation along with surfactant and drug. The membrane stability, morphology and permeability of vesicle is affected by membrane additive like cholesterol. Surfactants preferably need additives such as cholesterol to achieve suitable molecular geometry and hydrophobicity for bilayer vesicle formation (i.e. to turn the micellar structure of surfactant aggregates into bilayer arrangements as CHOL is a wedge-shaped molecule). Cholesterol can be incorporated in bilayers but it itself does not form bilayers. Cholesterol being a steroid derivative that fits and adjust itself between the bilayers, possible restricting the freedom of movements of the bilayers thus providing an absolute membrane rigidization and good physical stability by supressing the tendency of the surfactant to form aggregates as vesicle size is increased. It preventing leakage of drug from niosomal vesicle by increasing hydrodynamic diameter along with increase in the viscosity of the formulation, indicating high entrapment efficiency.

Addition of cholesterol into the niosomal system

- Enables more hydrophobic surfactants to form vesicles.
- Strengthen the non-polar tail of the non-ionic surfactant.
- Increase in the entrapment efficiency.
- Suppresses the tendency of the surfactant to form aggregates.
- Increase the chain order of liquidated bilayer and decrease the chain order of the gel state bilayer.
- Preventing leakage pf drug from niosomal vesicle by increasing hydrodynamic diameter along with increase in the viscosity of the formulation, indicating more membrane rigidity, high entrapment efficiency and good physical stability.
- Provides greater stability to the lipid bilayer by promoting the gel-liquid transition temperature (T_c) of the vesicle [i.e. increase the repulsive stearic or electrostatic forces that lead to the transition from gel state to liquid phase in niosomal system] as the presence of charge tends to increase the interlamellar distance between successive bilayers in multi-lamellar vesicle structure.

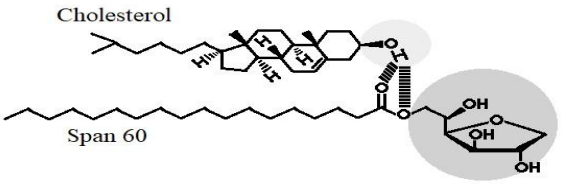


Figure 2: The interaction of cholesterol with Span 60 in the bilayer of niosomes. [14]

Schematic showing the possible interaction between cholesterol and span 60 within the bilayers of the niosome membrane. Being amphiphilic in nature cholesterol aligns itself into the bilayer membrane in such a way that the hydrophilic 3β-hydroxyl (β-OH) group of the cholesterol in the bilayers is able to position itself in the vicinity of the sorbitan monostearate ester group and the hydrophobic steroid ring orients itself parallel to the chains of the non-ionic surfactant. The β-OH group of the cholesterol could from a hydrogen bond with the oxygen at the ester group of the sorbitan monostearate. However, it is also possible to form hydrogen bonds at the other oxygen functionalities of sorbitan monostearate. [20, 22]

Additives: [10, 23-25]

Charge-inducing agent, induces same charge on each vesicle which produces electrostatic repulsion between adjacent vesicles, thus remain discrete and prevent vesicles flocculation, aggregation and fusion. Only 2.5 – 5 mol % concentration of charged molecules is acceptable because high concentration can inhibit the niosome formulation. Dicetyl phosphate (DCP) and Phosphotidic acid being anionic in nature, are negatively charged that induces negative charge. Stearylamine (STR) [also known as octadecylamine] and Stearyl pyridinium chloride being cationic in nature, are positively charged that induces positive charge. Charge inducers are added to stabilize niosomes (stearic stabilization or electrostatic stabilization). An example of stearic stabilization is the inclusion of Solulan C24 (a cholesteryl poly-24-oxyethylene ether) in doxorubicin (DOX) sorbitan monostearate (Span 60) niosome formulations. An example of electrostatic stabilization is the inclusion of Dicetyl phosphate in 5(6)-carboxyfluorescein (CF) loaded Span 60 based niosomes.

Drug [17]

Hydrophilic drug gets entrapped into the aqueous chamber of niosome while lipophilic drug gets entrapped between vesicular bilayer of niosomes. The physicochemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer.

Factors Governing the Self-Assembly of Non-Ionic Surfactants In to Niosomes [16, 20]

Hydrophilic-Lipophilic Balance (HLB) [15, 26]

HLB is a good indicator and a time saving guide for selection of surfactant and the vesicle forming ability of any surfactant. It also plays a key role in controlling drug entrapment and stability of the vesicle it forms.

Impact of HLB value on formulation

- 8.6 HLB: Highest entrapment efficiency of niosome.
- 4 – 8 HLB: Suitable for vesicle formation.
- 14 – 17 HLB: Not suitable for niosome formulation.
- >6 HLB: Need to add cholesterol in order to form a bilayer vesicle.
- Lower HLB value: Addition of cholesterol enhances stability of vesicles.
- Decrease in HLB from 8.6 to 1.7: Decrease entrapment efficiency.

Critical Micelle Concentration (CMC) [27]

It is defined as the concentration of surfactants above which micelles form and all additional surfactants added to the system go to micelles. Upon introduction of surfactants into the system, they will initially partition into the interface, reducing the system free energy by lowering the energy to the interface and removing the hydrophobic parts of the surfactant from contact with water. Subsequently, when the surface coverage by the surfactants increases, the surface free energy (surface tension) decreases and the surfactants start aggregating into micelles, thus again decreasing the system's free energy by decreasing the contact area of hydrophobic parts of the surfactant with water. Upon reaching CMC, any further addition of surfactants will just increase the number of micelles.

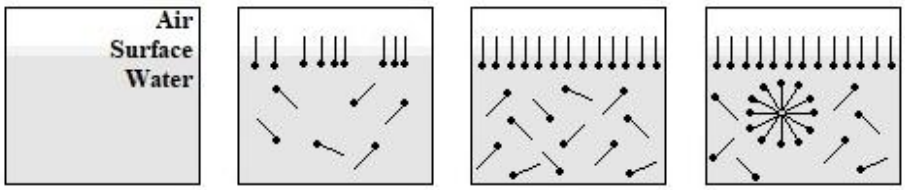


Figure 3: Orientation of surfactant to form micelles at or above CMC

Critical Packing Parameter (CPP) [28]

The geometry of bilayer vesicle to be formed from surfactant is affected by its structure, which is predicted from CPP (i.e. it helps in the prediction of vesicle forming ability of surfactant).

$$\textbf{CPP} = \frac{\upsilon}{lc \; x \; ao}$$

Where,

υ is hydrophobic group volume,

l_c is critical hydrophobic group length,

a_o is area of hydrophobic head group

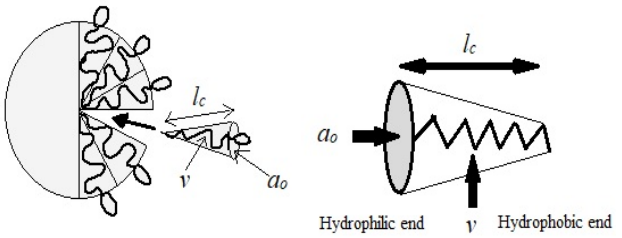
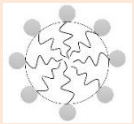
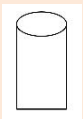
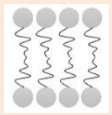
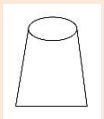
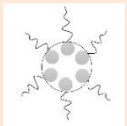


Figure 4: Effect of value of CPP on formulation of vesicles [20]

Table 2: Effect of value of CPP on formulation of vesicles [28]

Critical Packing Parameter	Critical Packing Shape	Structures Formed	Description
< 0.5		 Spherical micelles	Indicating a large contribution from the hydrophilic head group area and is said to give spherical micelles as it favours micellization rather than vesiculization.
0.5 – 1		 Planar bilayers	It indicates that the surfactant is likely to form bilayers micelles.
> 1		 Inverted micelles	Indicating a large contribution from the hydrophobic group volume and should produce inverted micelles, the latter presumably only in an oil phase, or precipitation would occur.

Phase Transition Temperature (T_C) or Gel-Liquid Transition Temperature: [17, 29]

It is defined as the temperature required to induce a change in the lipid physical state from the ordered gel phase (where the hydrocarbon chains are fully extended and closely packed) to the disordered liquid crystalline phase (where the hydrocarbon chains are randomly oriented and fluid). T_C affect the entrapment efficiency, permeability, fusion and aggregation. Span 60, as an example of surfactants with high T_C, exhibits the highest entrapment efficiency.

Salient Features of Niosomes [10, 29-32]

- Targeted, controlled, sustained and site-specific drug delivery can be achieved by administrating through various routes like oral, parenteral, topical and ocular with different dosage forms such as semisolids, powders, suspensions etc.
- They can improve the therapeutic performance of the drug molecules by protecting the drug from biological environment, resulting in better availability and controlled drug delivery by restricting the drug effects to target cells in targeted carriers and delaying clearance from the circulation in sustained drug delivery, thereby improving the bioavailability of drugs.

Advantages of Niosomes [14, 17, 32]

- They are stable as well as they increase the stability of entrapped drug.
- Access to raw materials is convenient, inexpensive an economical.
- Handling and storage of niosomes and its components like surfactants require n special storage condition.
- Their surface formation and modification are very easy.
- They are osmotically active, biodegradable, non-immunogenic and safer.

Disadvantages of Niosomes [17]

- Aqueous suspension of niosomes may exhibit stability problems such as fusion, aggregation and sedimentation of vesicles along with leakage of entrapped drug, thus limiting the shelf life of niosome dispersion.
- Insufficient drug loading.
- Time consuming.

Types of Vesicles [17]

Small Uni-lamellar Vesicle (SUV)

size = 0.025 – 0.050 µm

They are mostly prepared by sonication, french press and extrusion method. They are thermodynamically unstable and are susceptible to aggregation and fusion. Their entrapped volume is small and percentage entrapment is correspondingly low.

Large Uni-lamellar Vesicle (LUV)

size = > 0.100 µm

They are prepared by reverse phase evaporation and ether injection method. Vesicles of this type have a high aqueous lipid compartment ratio, so that larger volumes of bio-active materials can be entrapped with a very economical use of membrane lipids. These provide a number of advantages as compared to multi-lamellar vesicles, including high encapsulation of water-soluble drugs, economy of lipid and reproducible drug release

rates. Because of the larger size of the vesicles attainable a high percentage capture can often be achieved.

Multi Lamellar Vesicle (MLV)

size = > 0.050 µm

They are prepared mostly by thin film hydration method. They show increased trapped volume. It is simple to make and they are mechanically stable upon storage for long periods of time. These vesicles are highly suited as drug carrier for lipophilic compounds.

Method of Preparation of Niosome [14, 16, 17]

Ether injection method (EIM) [33, 34]

- This method was described by Baillie and others in 1985; previously described by Deamer and Bangham in 1976 for the preparation of liposomes.
- Slow injection of niosomal ingredients in diethyl ether through a 14-guage needle at the rate of approximately 0.25 ml/min into a preheated warm aqueous phase (containing drug) maintained at 55 – 65 ^{o}C or at the temperature for vaporizing the organic solvent and under reduced pressure. Vaporization leads to the removal of residual organic solvent (i.e. ether) and results in the formation of ether gradient at aqueous-nonaqueous interface (i.e. water-ether interface) where aqueous phase (i.e. water) is responsible for the formation of the bilayer sheet, which eventually folds on itself to form sealed vesicles. This process forms large uni-lamellar vesicles.
- **Advantages**
  - The captured volume per mole of lipid remains high.
- **Disadvantages**
  - Chances of oxidative degradation provided ether is free from peroxides.
  - Careful control needed for introduction of the lipid solution, regarding a mechanically operated pump.
  - Very slow process.
  - The efficiency of encapsulation is relatively low, although the captured volume per mole of lipid remains high.
  - Small amount of ether may still be present in the vesicles suspension as it is difficult to remove all the organic solvent because complete vaporization of organic solvent is not possible.

Thin Film Hydration Method (THF) / Hand Shaking Method (HSM) [35]

- This method was described by Azmin et al., and Baillie et al., in 1985; previously described by Bangham and others in 1965 for the preparation of liposomes.
- Niosomal ingredients are dissolved in volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed by evaporation under vacuum reduced pressure using rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of the flask which is further hydrated with aqueous phase such a water or PBS at normal temperature, with gentle agitation or shaking that makes the hydrated lipid sheets to swell and detach from the support and self-associate to form vesicles (milky niosomal dispersion). This process forms multi-lamellar vesicles with large diameter.
- **Advantages**
  - Simple and reproducible method.
  - Encapsulation efficiency of lipid soluble drug is very high (up to 100 %).
- **Disadvantages**
  - Encapsulation efficiency of water soluble drug might be low.
  - Produces non-homogeneous vesicles that require further size reduction process.
  - Time consuming.

Sonication [36-38]

- This method was described by Baillie et al., in 1986; previously described by Huang in 1969 for the preparation of liposomes.
- The aqueous phase is added to the mixture of surfactant and cholesterol in scintillation vail and sonicated at 60 ^{o}C for 3 minutes to produce niosomes. This process forms small uni-lamellar vesicles.
- Following types of sonicator can be used for the production of niosomes:

Probe Sonicator

The sonication may be accomplished using probe sonicator when sample size is a small volume.

- Advantages: leads to more rapid size reduction.
- Disadvantages: heat production, metal particle shedding from probe tip and aerosol generation.

Bath Sonicator

For large sample volume, bath sonicator is considered to be suitable.

- Advantages: temperature can be accurately regulated.

Reverse Phase Evaporation Method (REV) [39, 40]

- This method was described by Kiwada and others in 1985, previously described by Szoka and Papahadjopoulosin 1978 for the preparation of liposomes.
- Aqueous phase is added to the volatile organic solvent containing mixture of surfactant and cholesterol dissolved in it. The resulting two phases are emulsified to form W/O emulsion and sonicated at 4 – 5 °C the emulsion is dried down to semisolid clear gel which is further sonicated after the addition of a small amount of PBS. The organic phase is removed at 40 °C under reduced pressure resulting in the conversation of the gel to a homogeneous free flowing suspension. The resulting viscous niosome suspension is diluted with phosphate buffered saline and heated in a water bath at 60 °C for 10 min to yield larger uni-lamellar vesicles.
- **Advantages**
  - Vesicles can entrap larger percentage of the aqueous material.
  - Biologically active macromolecules such as RNA and various enzymes can be encapsulated without loss of activity.
- **Disadvantage**
  - Exposure of the material to be encapsulated to organic solvents and mechanical agitation or sonication which lead to the denaturation of sensitive proteins or breakage of DNA strands.
  - Produce heterogeneous size dispersion of vesicles.

Multiple Membrane Extrusion Method [3,41]

- A mixture of surfactant and cholesterol in chloroform is made into thin film by evaporation. The film is hydrated with aqueous phase and the resultant suspension extruded through the series of sufficient number (up to 8 passages) of the polycarbonate membranes (mean pore size 0.1 µm nucleopore).
- **Advantages**
  - Good method of controlling niosome size.
  - Ease of production, batch-to-batch reproducibility.
  - Freedom from solvent and/or surfactant contamination.
  - Reducing polydispersity.
- **Disadvantages**
  - Clogging of extrusion membrane may occur specially during large scale production.
  - Time consuming.
  - High product loss.

Bubble Method [17, 41]

- Round bottom flask or a glass reactor having three necks positioned into water bath in order to control the temperature. First neck holds the thermometer, nitrogen is supplied through the second neck and water-cooled reflux in the third neck. Mixture of surfactant and cholesterol are dispersed in phosphate buffer saline at 70 °C, the dispersion is homogenized with high shear homogenizer for 15 seconds and immediately bubbled at 70 °C with nitrogen gas. This process forms large uni-lamellar vesicles.
- **Advantages**
  - One step preparation of niosomes without the use of organic solvent.

Micro-fluidization Method [41]

- It is based on submerged jet principle in which two fluidised streams (one containing drug and the other surfactant) are pumped at very high pressure (10,000 psi); hence, move forward through precisely defined micro channel and interact at ultra-high velocities (up to 1700 ft/sec) by colliding together at right angles within the interaction chamber and lead to the formation of small uni-lamellar vesicles.
- **Advantages**
  - Greater uniformity, smaller size and better reproducibility.
  - High rate of production and good aqueous phase encapsulation.
- **Disadvantages**
  - High pressure in the chamber can cause partial degradation of lipids.

Freeze and Thaw method (FAT) [38]

- Niosomal suspensions, prepared by using thin film hydration method, is frozen in liquid nitrogen (-196 °C) for 1 minute and thawed (melted) in a water bath at 60 °C for another 1 minute. It results in the formation of frozen and thawed multi-lamellar vesicles (FAT-MLVs).
- Advantages: Simple and rapid method.
- Disadvantages: Cryoprotectant, divalent metal ions and high ionic strength salt solutions cannot be entrapped efficiently.

Dehydration Rehydration Method (DRM) [42]

- This technique was first described by Kirby and Gregoriadis in 1984.
- The dehydration rehydration vesicles (DRVs), prepared by Thin Film Hydration Method, is frozen in liquid nitrogen then is followed by freeze-drying

overnight. Niosomes powder is hydrated with PBS (phosphate buffered saline) pH 7.4.

Loading Methods (Trapping Techniques) [14, 17, 41]

The encapsulation/entrapment processes of drugs are called drug loading methods (also termed as trapping techniques), which are as follows:

Passive loading or passive trapping techniques (Direct entrapment)

- It is the simplest loading method. Lipophilic drugs are dissolved in organic solvent and hydrophilic drugs are dissolved in aqueous phase and a percent of these dissolved drugs are loaded during the preparation of niosomes. Unloaded drugs can be separated from niosomal suspension by dialysis, centrifugation, column chromatography or gel filtration.

Active loading or active trapping techniques (Remote loading)

- An exciting development concerning loading of lipophilic cationic drugs into niosomes is the active loading or active trapping.
- This method or technique enhances the efficiency drug loading by aid of pH and ion.

Remote loading by using trans membrane pH gradient (in acidic range)

- The mechanism of drug uptake induced by pH gradient is probably similar to pH gradient dependent transmembrane redistribution of other weak bases.
- In this technique, lower pH value inside the niosome is developed which causes pH differential across the niosome barrier. When the pH is higher in the outside of the niosome vesicle, basic drug in unionized state passes the membrane barrier of the niosome. Due to the lower pH inside the niosome, the basic drug becomes ionized and precipitate. Thus, it becomes unable to leave the vesicle, after encapsulation. (i.e. the acidic pH within the niosome interior thus act as an intra-vesicular trap).

Remote loading by suing trans membrane ion gradient

- It is achieved in response to the ion gradient placed across niosomal membranes.
- It allows the drug entrapment after the niosomal carrier has been generated.

Factors Affecting Formation of Niosomes [15-18, 20, 43-45]

Surfactants

- Nature and type of surfactant influences encapsulation efficiency, toxicity, stability and various other properties of niosomes.
- The ether type of surfactant with single chain alkyl as hydrophobic tail is more toxic than corresponding dialkyl ether chain.
- The esters type surfactant is chemically less stable and less toxic than ether type surfactant.
- The chain length of non-ionic surfactants affects entrapment efficiency, such as steryl chain (C18) non-ionic surfactant vesicles show higher entrapment efficiency than lauryl chain (C12) non-ionic surfactant vesicles.
- Diameter of the vesicles is dependent on the length of the alkyl chain of the surfactants. Surfactants with longer alkyl chains generally give larger vesicles. This might be the reason for higher entrapment efficiency of vesicles prepared with stearyl chain surfactants.

Cholesterol

- Addition of cholesterol increases vesicle size, entrapment efficiency and rigidity of bilayers thereby preventing leakage of drug leading to increase in the stability.

Charge Inducers

- Charge inducing agent induces charge on the vesicle that tends to increase the interlamellar distance between successive bilayers in multi-lamellar vesicle structure and leads to greater overall entrapped volume along with stability.

Drug

Table 3: Effect of nature of drug on properties of niosomal vesicles.

Nature of the drug	Leakage from the vesicle	Stability	Other properties
Hydrophobic	Decreased	Increased	Improved transdermal delivery
Hydrophilic	Increased	Decreased	-
Amphiphilic	Decreased	-	Increased encapsulation
Macromolecule	Decreased	Increased	-

Surfactant/Lipid ratio

- Increasing the surfactant/lipid level increases the total amount of drug encapsulated. However, if the level of surfactant/lipid is too high, then it results in highly viscous systems.

Surfactant/Water ratio

- Altering the surfactant/water ratio during the hydration step may affect the system's microstructure and hence the system's properties.

Method of Preparation

- Method of preparation of niosomes affects its size, entrapment efficiency and stability.
- Sonication and micro-fluidization method yield SUVs.
- Ether injection and reverse phase evaporation method yield LUVs with high entrapped volume and high encapsulation efficiency without any loss of solute.
- Thin film hydration method yields larger diameter MLVs while freeze and thaw method yield FAT-MLVs (frozen and thawed multilamellar vesicles).

Temperature of hydration

- Hydration temperature affects the assembly of surfactants into vesicles along with vesicular shape and size. For ideal condition the hydration temperatures used to make niosomes should usually be above the gel to liquid phase transition temperature.

Resistance to Osmotic Stress

- Niosomes prepared from pure surfactant are osmotically more sensitive in contrast to vesicles containing cholesterol.
- In hypertonic salt solution, diameter of vesicular structure was found to be decreased.
- In hypotonic salt solution, there is slight swelling of vesicles resulting in slow release of drug followed by faster release, which may be due to mechanical loosening of vesicular structure under osmotic stress.

Methods for Controlling Niosome Size [46-49]

Niosomes prepared as described above are usually in the micron size range although some of the methods produce niosomes in the sub-micron size range. The vesicle size can have dramatic effects on the in-vivo behaviour of niosomes. Therefore, their size will have to be controlled within reasonable and verifiable limits. Often a size reduction step must be incorporated into the niosome reduction procedure. Various possibly approaches have been explored for achieving defined particle size distributions of niosomes. They are as follows:

Fractionation

Two methods have become popular for fractionating defined sized viz, centrifugation; and size extrusion chromatography. Both can be used to obtain the product with the desired particle size but are limited in terms of the volumes that can be easily handled.

Centrifugation

Niosomes sediment in a centrifugal field at a rate that is related to their size and density. Large niosomes composed of neutral lipids such as phosphatidyl choline can easily be pelleted at fairly low gravitational forces in conventional centrifuge. Under proper conditions the smaller niosomes will remains in the supernatant.

Size extrusion chromatography

Column chromatography has been used for many years as an analytical method to measure the particle size of niosomes. Preparative scale chromatography has also been applied to isolate niosomes of fairly homogenous sizes. This method is particularly useful for separating small uni-lamellar vesicles from larger structure. Typically, a column of sepharose 48 is used. As the column is washed with buffer, larger niosomes elute in the void volume. Larger pore size chromatographic media have been used in a similar fashion to fractionate populations of larger particles. In general, such chromatographic separations are quite limited in terms of volume and through put must be carried out in batches, resulting in significant dilution of the product and require strict controls to ensure that no microbial contamination of gel beds occur.

Homogenization

When fairly small particles are desirable homogenization has proven to be a useful approach. The average particle size of niosome dispersions can be reduced by passage under high pressure through a homogenizer. High-pressure homogenization also yields vesicles of below 100 nm in diameter although drug loading is ultimately sacrificed to achieve this small size. Conventional homogenizers and high sheer mixers have also been reported to be useful for down-sizing niosomes.

Extrusion

Capillary Pore Membrane Extrusion

A technique that has gained widespread acceptance for the production of niosomes of defined size and narrow size distribution. It is based on the extrusion of fairly large niosomes through polycarbonate membrane under pressure. Polycarbonate membranes have uniform straight through capillary pores of defined size and normally do not bind niosomes. This simple technique can reduce multi-lamellar vesicles and reverse phase evaporation vesicles to a much more homogenous suspension of vesicles exhibiting a mean particle size that approaches the diameter of the pores through which they are extruded.

Ceramic Extrusion

If the size of the niosome is greater than the membrane pore diameter, the pores of the membrane tend to clog. The clogged membranes cannot easily be cleared because the filter housing configuration does not allow back-flushing. One approach that overcomes the above-

mentioned limitations makes use of a ceramic membrane. The suspension may be alternately passed through the membrane in an outside to inside direction to maintain the membrane in an unclogged condition. The average size of niosomes may further be reduced by passage through similar types of ceramic filters that have been rated at smaller inner surface pore diameters.

Nucleopore filters Extrusion

Extrusion through 100 nm Nucleopore filters which yields sodium stibogluconate C16G3 niosomes in the 140 nm size range.

Separation of Unentrapped Materials

The hydration of surfactant/lipid mixtures rarely leads to the entire drug being encapsulated, regardless of the drug loading optimization steps taken. It is thus essential to separate the drug, which has remained unentrapped after preparation of niosomes. The removal of unentrapped material from the vesicles can be accomplished by various techniques, which include:

Dialysis [50, 51]

- Dialysis separates the free drug from dispersion. The niosomal dispersion is exhaustively dialyzed by placing it in previously washed and soaked (in PBS or distilled water) dialysis membrane or cellophane bag/tubing (donor compartment) and it is suspended or immersed in a beaker (receptor compartment) containing dialysis medium like phosphate buffered saline or distilled water or normal saline or glucose solution which is constantly stirred on a magnetic stirrer at suitable temperature until entire free drug is separated from system.
- **Merits**
  - Easy to perform and inexpensive.
  - Also suitable for highly viscous systems.
- **Demerits**
  - Extremely slow (5 – 24 hours).
  - Large volumes of dialysate may be required.
  - Chances of dilution of the niosomal dispersion.
- Reverse Dialysis It is similar but opposite to dialysis. Dialysis medium is filled in small bags or tubing made from dialysis membrane or cellophane membrane and are suspended in the beaker containing niosomal dispersion, which is stirred continuously on magnetic stirrer at suitable temperature for particular until entire free drug is been separated from system.
- **Merits**
  - Easy to perform and inexpensive.
- **Demerits**
  - Extremely slow and tedious.
  - Chances of dilution of the niosomal dispersion.

Centrifugation [21, 52, 53]

- Separation of unentrapped drug performed by cooling centrifugation with rotation speed below 7000 x g for 30 min time duration (depending on MW of component) at a temperature of 4 ^{o}C It results in the formation of two sections, niosomal pellets and supernatant liquid containing free drug. The supernatant liquid is collected separately. The separated vesicles or the niosomal pellets are washed twice with distilled water or PBS to remove any drug and the washings are mixed with supernatant liquid. The vesicles were resuspended in small amount of PBS to obtain a niosomal suspension free from unentrapped drug.
- **Merits**
  - Quick (approx. 30 min)
  - Inexpensive instrumentation.
- **Demerits**
  - Fails to sediment the sub-micron niosomes.
  - May lead to the destruction of fragile systems.
  - Often weight density differences between niosomes and the external phase are smaller than in case of liposomes, which makes separation by centrifugation very difficult. A possibility is to add protamine to the vesicle suspension in order to facilitate separation during centrifugation.
- Newer advancement in above mentioned technique is ultra-centrifugation, in which the separation of unentrapped drug performed by ultra-centrifugation with rotation speed below 150000 x g for 1 – 1.5 hour time duration (depending on MW of component).
- **Merits**
  - Sediments all size populations.
- **Demerits**
  - Expensive instrumentation.
  - Long centrifugation times (1 – 1.5 hours)
  - May lead to the destruction of fragile systems and formation of aggregates.

Column chromatography or Gel filtration [3]

- The unentrapped drug is removed by passing the niosomal dispersion through a SephadexG-50 column which is pre-saturated with constructive surface and lipids (i.e. pre-treatment of the column with empty or blank niosomes is required). It is also pre-equilibrated with hydrating buffer which is used for the elution. The column allows frequently separation of unentrapped drug from vesicular suspension.

Vesicles elutes out first as a slightly dense, while opalescent suspension; followed by free drug as it is preferentially retained for some time by SephadexG-50 column.

- **Merits**
  - Quick (4 – 5 min with Sephadex G-50).
- **Demerits**
  - Slow (1 – 2 hours when using Sepharose 2B/4B for macro-molecule separation).
  - Pre-treatment of the column with empty niosomes is required.
  - Gels are expensive if not reused.
  - Dilutes the niosome dispersion.
  - Not suitable for highly viscous formulations with a large particle size (>10 – 20 µm).

Characterization of Niosomes [16, 17, 20]

Visual appearance

Niosomal dispersion is placed in transparent containers for checking turbidity, flocculation and sedimentation.

Bilayer formation

- X-cross formation under Light Polarization Microscopy.

Membrane rigidity

- Mobility of Fluorescence Probe as a function of temperature.

Size, shape, morphology, lamellarity and size distribution [3]

- Optical Microscopy/Light Microscopy:
  - Compound Microscope: size (>1 µm).
  - Fluorescence Microscope: lamellarity.
- Electron Microscopy (EM): morphological studies of vesicle.
  - Scanning Electron Microscope (SEM): size, shape and surface characteristics.
  - Transmission Electron Microscope (TEM): structure, size shape, number of bilayers and surface morphology.
  - Negative Staining Transmission Electron Microscopy: shape and lamellarity.
  - Freeze Fracture Replication Electron Microscope or Freese Fracture Transmission Electron Microscopy (FF-TEM): visualization of vesicular structure, size, shape, lamellarity, number of bilayers, surface morphology and phase behaviour. [4]
  - Cryogenic Transmission Electron Microscopy: arrangement of atoms.
- Laser Light Scattering/Photon Correlation Spectroscopy/Dynamic Light Scattering (Master Sizer): size distribution, mean diameter and poly-dispersity index (PDI).
- Small Angle X-ray Scattering (SAXA): size, lamellarity.
- Atomic Force Microscopy (AFM)/Scanning Force Microscopy (SFM) [type of Scanning Probe Microscopy (SPM)]: shape and surface characteristics.
- Phosphorous-31 Nuclear Magnetic Resonance (^{31}P-NMR): size, shape and lamellarity.

Surface charge

- Free Flow Electrophoresis.
- Zeta Potential Measurement (Zeta Sizer)

Entrapment efficiency

After preparing niosomal dispersion, unentrapped material from niosomes is separated by suitable techniques described previously and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100, thereby release of entrapped drug. The sample is filtered and the filtrate is analysed by appropriate spectroscopic method. The following formula is used to calculate the entrapment efficiency:

$$\text{Entrapment efficiency (EE \%)} = \frac{\text{Amount of entrapped drug (mg)}}{\text{Total amount of added drug (mg)}} \times 100$$

In-vitro drug release [54]

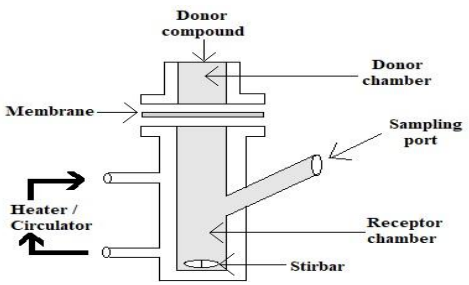


Figure 5: Franz Diffusion Cell

In-vitro drug release study is carried out by using a set of Franz diffusion cells. The receptor chamber is filled with receptor medium (distilled water, buffer, alcohol or suitable solvent). The receptor medium is stirred continuously and its temperature is kept at 37 °C ± 1 °C by circulating water through a jacket surrounding the cell body throughout the experiment. A cellophane membrane or dialysis membrane which is already soaked in receptor medium is clamped between two chambers. Suitable amount of formulation is placed in the donor cell. Subsequently, samples are collected at

predetermined time interval from the receptor cells. The same volume of fresh medium is added after each collection to keep the volume constant. The withdrawn samples are diluted if required and subjected to spectrophotometric analysis using fresh receptor medium as blank. Concentration of drug released at particular time interval [percentage cumulative drug release (% CDR)] is calculated by using an equation generated from standard calibration curve by taking into account of the dilution that occurred throughout the experiment.

Stability: [17,55]

The stability study of formulation is performed as per ICH guidelines. Freshly prepared formulation is divided into groups and kept at different storage conditions as per ICH guidelines. Sample is withdrawn periodically and tested for various evaluation parameters mentioned above. Stable formulation must retain the evaluation parameters at different storage conditions over a period of time.

Storage conditions recommended for stability Study as per International Council for Harmonisation (ICH) guidelines.

- Long term stability study: 25°C ± 2°C / 60% RH ± 5% RH or 30°C ± 2°C / 65% RH ± 5% RH
- Intermediate stability study: 30°C ± 2°C / 65% RH ± 5% RH
- Accelerated stability study: 40°C ± 2°C / 75% RH ± 5% RH

Stability of Niosomes [17,56,57]

Niosomes sized between 1 and 10 µm have been found to be more stable than those in the sub-micron range. Thermodynamically, smaller niosomes have higher surface free energy and tend to aggregate more than larger ones in order to lower the excess free energy.

The following are the four different forces that contribute towards the ability of niosomal vesicles:

1. Van der Waals forces among surfactant molecules.
2. Repulsive forces emerging from the electrostatic interactions among charged groups of surfactant molecules.
3. Entropic repulsive forces of the head groups of surfactants.
4. Short acting repulsive forces.

The stability of niosomes is affected by various factors which are classified into the following three types:

Physical Stability

Niosomes can change their physical characteristics in several ways.

- Particle size can change because of aggregate formation and fusion upon storage, minimised by selection of proper charge inducing agents.
- Phase separation of bilayer components, upon storage can occur, as the bilayer composition changes because of chemical degradation reactions or when the bilayer goes through temperature cycles. Proper selection of bilayer components can avoid these problems.
- Leakage of encapsulated material from niosomes, prevented by freeze drying along with the addition of cryoprotectant like sugar which interact with head group and stabilize the membrane when the bilayer stabilizing water is removed by sublimation.
- Proniosomes are another option for freeze drying, where the niosomes are formed by hydrating the lipids at the time of administration. The dry lipids which are coated as a film on the glass vessel wall in the form of a freeze-dried cake are hydrated by shaking with an aqueous medium just before administration.

Chemical Stability

The stability of niosomes depend on the chemical stability of the lipid components. Commonly, hydrolysis and peroxidation are the two degradation process which occur with lipids.

- Lipid hydrolysis: pH alterations, other experimental conditions like temperature, ionic strength, buffer species, ultra-sonication results in hydrolysis reactions.
- Lipid peroxidation: can occur during preparation, storage or actual use. Unsaturated chains are vulnerable to oxidative degradation (lipid peroxidation) leading to the formation of cyclic peroxides and hydroperoxides.
- Peroxidation of the phospholipids may be minimized by a number of ways: minimum use of unsaturated lipids, use of nitrogen or argon minimize exposure to oxygen, use of light resistant container, removal of heavy metals (EDTA), use of anti-oxidants such as α-tocopherol or BHT.

Stability in Biological Fluids

The instability of niosomes in plasma appears to be the result of the transfer of bilayer lipids to albumin and high-density lipoproteins. Both lecithin and cholesterol exchanges with the membrane of red blood corpuscle. Niosomes are most susceptible to high density lipoprotein attack at their gel to liquid crystalline phase transition temperature. The susceptibility of niosomal phospholipids to lipoprotein and phospholipase attack is strongly dependent on niosome size and type. Generally multi-lamellar vesicles are most stable and small lamellar vesicles are least stable. The bile salts will also destabilize the bilayer membrane structure, hereby leading to the release of the entrapped material.

The Mode of Action of Niosomal Vesicles

Vesicle-skin interactions can occur either at the skin surface or in the deeper layers of the stratum corneum. Several mechanisms can be used to explain the ability of niosomes in drug delivery across the skin:

- Niosomes diffuse from the stratum corneum layer of skin as a whole.
- New smaller vesicles are formed in skin (re-formation of niosome vesicles). The water content of skin is crucial issue for interpreting and establishing this mechanism. Smaller diameter of lipid lamellar spaces of the stratum corneum than niosome vesicles makes this mechanism more meaningful. [58]
- Niosomes interact with stratum corneum with aggregation, fusion and adhesion to the cell surface which causes a high thermodynamic activity gradient of the drug at the vesicle-stratum corneum surface, which is the driving force for the penetration of lipophilic drugs across the stratum corneum. [59]
- Scanning electron microscopy conformed the fusion of niosome vesicles of estradiol on the surface of skin. [60]
- Niosomes may modify stratum corneum structure which makes the intercellular lipid barrier of stratum corneum looser and more permeable. [61]
- Non-ionic surfactant itself, the composing ingredient of niosome, acts as a permeation enhancer and might partly contribute to the improving of drug permeation from niosomes by enhancing the distribution of the drugs and decreasing surface tension, improving and wetting of skin. [62]

***In-Vivo* Behaviour of Niosomes [10]**

In-vivo niosomes have been found equiactive to liposomes in improving the therapeutic performance of drug and their distribution in body follows the pattern of their colloidal drug delivery systems. Although tissues of extravasation like liver, lung, spleen and bone marrow are responsible for disposition of a major part of niosome disposal from blood. [63] It appears that, like liposomes, niosomes are also taken up intact by liver, and break down substantially to release the free drug which eventually re-enters the circulation and maintains the plasma drug level. [51] Niosomes were found to be stable in plasma. However, non-ionic surfactants in higher concentration delipidize the low-density lipoproteins. [64] Niosomes bearing haemoglobin were found to be physically stable with plasma protein component; albumin and transferrin were identified and determined to absorb on vesicles without destabilizing them. Erythrocytes donate cholesterol to niosomes, particularly to cholesterol-free and cholesterol-poor niosomes, maintaining their integrity in body as well as keeping them less vulnerable to destabilization. [65,66]

Types of Niosomes

Various types of niosomes include:

Proniosomes [17,20]

- Proniosomes are dry formulations of surfactant-coated carrier (sorbitol), which are rehydrated by brief stirring in hot water prior use, to yield aqueous niosome dispersion.

Discomes [67]

- Large discoid or disc shaped structures were observed during niosomes to mixed micelles transitions under the light microscope existing under certain conditions of the phase diagram of non-ionic surfactant vesicles prepared from a hexadecyl diglycerol ether, cholesterol and dicetyl phosphate (DCP) (69:29:2) by mechanical disruption and sonication followed by incubation with various proportions of Solulan C24 at 74 ^{o}C they have found to entrapped water soluble solute effective.

Deformable niosomes [68]

- Elastic niosomes are prepared of non-ionic surfactants, ethanol and water. They show superior to conventional niosomes due to their capability to increase penetration efficiency of a compound through intact skin by passing through pores in the stratum corneum, which are smaller than the vesicles.

Bola-surfactant containing niosomes [69]

- Niosomes made of alpha, omega-hexadecyl-bis-(1-aza-18-crown-6) (Bola-surfactant)-Span 80-cholesterol (2:3:1 molar ratio) are named as Bola-Surfactant containing niosomes.

Polyhedral niosomes [70]

- Polyhedral niosomes formed by mixtures of hexadecyl diglycerol ether ($C_{16}G_2$): cholesterol: polyoxyethylene 24 cholesteryl ether (Solulan C24) were previously shown to form spherical, tubular, polyhedral and disc-like vesicles, depending on the molar ratio.

Aspasomes

- Combination of acorbyl palmitate, cholesterol and highly charged lipid diacetyl phosphate leads to the formation of vesicles called aspasomes.

Non-ionic Surfactant Vesicle-in-Water-in-Oil (V/W/O) System [71]

- They are modified versions of vesicular system. V/W/O system are those in which the aqueous suspensions of vesicles are dispersed in a continuous oil phase (i.e. external phase is non-aqueous). The system is an emulsion prepared by dispersion of niosomes in water, followed by re-emulsification of

an oil using a surfactant mixture of low HLB to achieve a stable W/O emulsion.

Therapeutic Potential of Niosomes

Niosomes in oncology [72]

Niosomes based doxorubicin formulation were administered as a bolus via tail caudal vein of mice and plasma levels were found to be higher for entire period of study; confirming sustained release characteristics along with a higher tumour drug concentration which reflected an improved anti-tumour activity, increasing the life span of tumour bearing mice.

Niosomes for the treatment of Leishmaniasis [63]

In-vivo studies on niosomes bearing stibogluconate an anti-leishmaniasis agent were carried out on infected mice and proven the ability of vesicular system in effective and radical eradication of leishmaniasis.

Niosomes as immunological adjuvant

Niosomal-BSA (bovine serum albumin) enhance antibody production comparable to those produced by FCA (Freund's complete adjuvant) by subcutaneous or intraperitoneal route of inoculation in the BALB/C mouse.

Niosomes as carrier for Haemoglobin [17]

Haemoglobin niosomes were studied for functional and physical properties along with compatibility and interaction with blood; and found to be at acceptable levels and limits.

Niosomes for delivery of peptide drugs [73]

The potential of niosomes carrying DGAVP (9-desglycinamide-8-arginine vasopressin) was determined by in-vitro studies. The stability and absorption of DGAVP was found to be increased after incorporation into the niosomes.

Niosomes for diagnostic imaging: [17, 74]

DTPA carrying niosomes were studied for the in-vitro release, radio labelling, in-vivo distribution and to perform scintigraphy imaging studies. It was found that niosomes can act as a carrier for radio pharmaceuticals and site-specific vehicle for spleen and liver imaging.

Niosomes as carrier in dermal delivery

Niosomes act as a carrier for delivery of topical and transdermal products.

- Local anaesthesia: Lidocaine hydrochloride entrapped in niosomes showed better penetration of drug. [75]
- Psoriasis: niosomal Methotrexate gel showed 3 times increase in activity. [76]
- Hyperpigmentation: reduction in appearance of facial hyperpigmentation by topical niosomal preparation containing N-acetyl glucosamine (NAG). [77]
- Arthritis: niosomal Celecoxib (NSAID) gel shoed 6.5 times higher drug deposition in deep skin layer and good permeation through skin. [78]
- Acne: niosomal Benzyl peroxide (Macrolide antibiotic) incorporated into HPMC gel showed good drug skin retention, extended release and reduced toxicity of the drug with improve drug permeation. [79]

Patents of Niosomes [19]

- Handjani et al., Cosmetic and pharmaceutical compositions containing niosomes and water-soluble polyamide, and a process for preparing these compositions: Patient number: US 4,830,857; Date of patient: May 16, 1989.
- Alcanter et al., Niosome hydrogel drug delivery. Pub. No.: US 2010/0068264 Al; Pub. Date: Mar. 18, 2010.
- Smith et al., Adjuant and vaccine compositions. Pub. No.: US 2010/0226932 Al; Pub. Date: Sep. 9, 2010.
- Carafa et al., Niosomes, freeze-dried powder thereof and their use in treatment; Pub. No.: US 2012/0288540 Al; Pub. Date: Nov. 15, 2012.

Marketed Products of Niosomes [19]

1. Lancôme Paris Niosome+ clear whitening foundation cream by Lancôme (L'Oréal).
2. Lumigan® (ophthalmic solution) by Allergen containing Bimatoprost for open – angle glaucoma.
3. Truspot® (ophthalmic solution) by Merck containing Dorzolamide hydrochloride for open – angle glaucoma.
4. Estee Lauder Beyond Paradise after shave lotion by Estee Lauder.
5. Nio-active potent anti-oxidant and skin moisturizer by Naturals Life Technologies.

CONCLUSION

In recent era Niosomes was concluded to be a promising vesicular drug delivery system. It can be effectively used to improve solubility, permeability and bioavailability. The approach of niosomal drug delivery possesses many superior advantages over conventional as well as vesicular drug delivery system. The limitations of liposomes like oxidation, instability, lack of purity etc can be minimised by Niosomes. It is obvious that niosomes appears to be a well preferred candidate over liposomes. The selection of surfactant (nature, type and quantity) and method of preparation is the key parameter during formulation of niosomes, as it plays an important role in formation of vesicles along with their stability. Niosomes offers a convenient, controlled, targeted and effective delivery of drug with the ability of loading both hydrophilic and lipophilic drug. The potential of niosomes can be enhanced by various modifications. Niosomes serve as a better aid in therapeutic applications, as they have proven their application in delivery of anti-infective

agents, anti-inflammatory, anti-cancer agents and vaccine adjuvants.

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