



Review Article

Niosome: A Vesicular Weapon for Targeted and Controlled Drug Delivery

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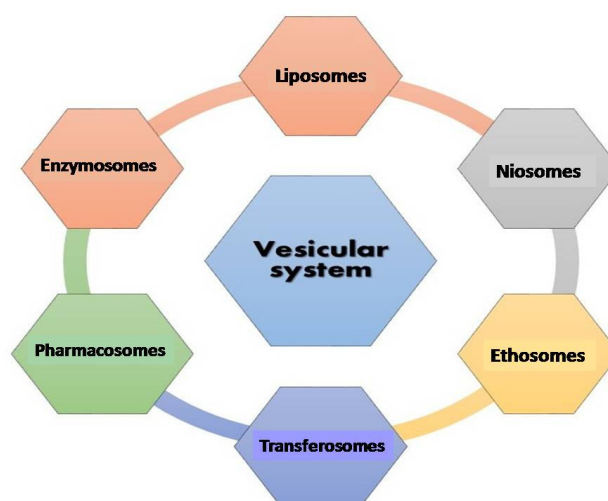
*Keywords:*Niosome,
Nonionic surfactant;
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Stability**ABSTRACT**

An appropriate drug concentration at the site of action for sufficient period of time is desired for cogent pharmacotherapeutics. Vesicular drug delivery system is a mean to achieve this goal with enhanced bioavailability. Niosome is such vesicular mean containing nonionic surfactants and cholesterol. Longer shelf life, stability and ability to deliver drug at target site in a controlled or sustained manner are some of its unique credentials. The present review has been grafted to highlight some of the fascinating features of niosome as drug delivery vehicle. It includes formulation aspect of niosome, its types, preparation methods, stability, toxicity, its characterization and lastly glimpses of its pharmaceutical utilities are being enumerated.

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INTRODUCTION

The formulation scientists tend to find drug delivery systems that are 'patient-friendly' in nature. In the past few decades, formulation of novel drug delivery system (NDDS) has created awe in the pharmaceutical field. The NDDS should ideally fulfill two prerequisites: Firstly, it should deliver the drug at the site of action with appropriate rate for predetermined time period, Secondly; it should be able to deliver maximum amount of drug at the site of action. Targeted drug action can be achieved either through carrier system or chemical derivatization. Vesicular drug delivery systems are one type of carrier system which consist of highly ordered assemblies of one or several concentric lipid bilayers consist of amphiphiles [1]. The vesicular composition affects their physicochemical characteristics such as their size, charge, lamellarity, elasticity and thermodynamic phase. Different vesicular systems are shown in the **Figure 1**. Niosomes were adjudicate its role in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering [2].

**Figure 1:** Vesicular drug delivery systems**Retrospect of Niosome**

Niosomes are a novel carrier system, in which the drug is encapsulated in a vesicles composed primarily of synthetic nonionic surfactants and cholesterol and sometimes charge stabilizers [3]. The formation of such non-ionic surfactant vesicle was first reported in the 70s by researchers in the cosmetic industry. Niosomes are made up of a bilayer of non-ionic surfactant and hence the name niosome. Some Scientific workers [4] described in their prophecy that the nonionic surfactants are preferred as the irritation power of surfactants decreases in the following order: cationic > anionic > ampholytic > non-ionic. In terms of size they are in

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nanometric range. They offer several advantages over liposome despite of structural similarity. Niosomes have great applicability in transdermal drug delivery and in targeted drug delivery. It has been explored for various route of administration including intramuscular [5], intravenous [6], perioral [7] and transdermal [8]. Apart from that, niosomes have been shown to enhance absorption of some drugs across cell membranes [9], to localize in targeted organs [10] and tissues [11] and to elude the reticuloendothelial system [12]. Niosomal formulation can increase the bioavailability [13], stability [14] as well as permeability [15].

Structure of Niosomes

Niosomes are microscopic lamellar structures composed of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol (Cholesterol or 5-cholesten-3 β -ol) which forms upon hydration in aqueous media [16]. It must contains a vesicle forming amphiphile i.e. a non-ionic surfactant, which is usually stabilized by the addition of cholesterol if required and a little albeit of anionic surfactant such as diacetyl phosphate, which also aides in stabilizing the niosome. When a surfactant is immersed in water it will create the micellar structures, but some surfactants can yield bilayer vesicles instead. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them and also the method utilized for size reduction [17]. They are composed of a surfactant bilayer with its hydrophilic ends seems on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer. It is due to this only the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself [18]. The **Figure 2** given below will give a better idea regarding this doctrine.

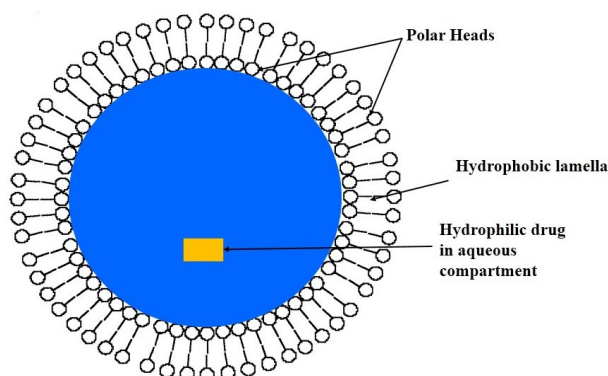


Figure 2: Structure of niosome

Advantages of Niosome [19-22]

- The niosomal dispersion being water based hence offers greater patient compliance over oil/lipid based systems.
- Due to its ability to accommodate all kind of drug moieties, they can be nurtured for a wide variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be tailorable as per the requirement of the delivery.
- The vesicles provides depot action with a controlled release pattern.
- They are osmotically active and stable.
- They increase the stability of the entrapped drug especially protenious agent.
- Handling and storage of surfactants do not require any special conditions
- It can solve the issue regarding oral bioavailability of drugs and also provides controlled release characteristics
- It can enhance the skin penetration of drugs hence fruitful for dermal application
- They can be used for most of the rote of administration with ease
- The nonionic surfactants are biodegradable, biocompatible, no irritant and non-immunogenic
- Reduce the clearance of the drug by its targeting action
- It also protects the drug from biological environment

Why niosome in lieu of liposome?

In contrary to liposomes niosomes does have certain advantages. The susceptibility of phospholipids to oxidative degradation in air is main culprit when liposome is utilized as adjuvant which demands highly purified phospholipids. Apart from that liposomes have to be stored and handled in an inert (e.g. nitrogen) atmosphere [23]. As phospholipids are naturally occurring substances which require a good deal of purification ultimately, increases raw material cost [3]. Alternatively, it can be synthesized de novo though it is even more costly. Due to above mentioned drawbacks, Nonionic surfactants have been investigated to nurture a new vesicular form. The hydrated mixtures of cholesterol and nonionic surfactants such as monoalkyl or dialkyl polyoxyethylene ether yields anew vesicular form "Niosome" [23]. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic, hydrophobic and/or amphiphilic molecules [17]. From a technical point of view, these are more cogent stable drug

carriers with lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids [24]. Another advantage is it is easy to scale up their production without the use of unacceptable solvents.

Types of Niosome

Based on the method of preparation niosome can be classified as shown in **Figure 3**. Some special types of niosomes are mentioned in the literature including discomes, proniosomes, elastic niosomes, surfactant ethosomes, vesicle in water in oil systems and polyhedral niosomes etc., which are in brief discussed here.

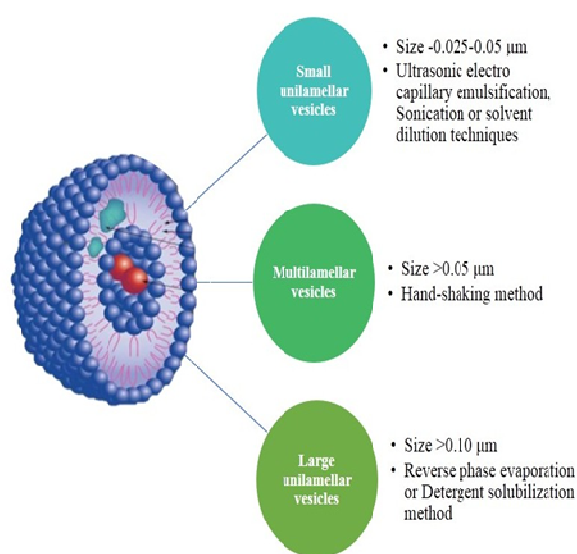


Figure 3: Types of niosome based on method of preparation

Discomes

These are niosomes from hexadecyl diglycerol ether (C16), cholesterol and dicetylphosphate. The solubilization of C16G2 niosomes by Solulan C24 results in the formation of the discome phase. This giant vesicles are of 60 nm in diameter which can encapsulate hydrophilic solutes [25]. Large vesicles that appear ellipsoid in shape and large vesicles that are truly discoid are the two type of discome vesicle. Abdelkader et al., [26] prepared discomes of naltrexone (NTX) for ophthalmic drug delivery which are practically nonirritant. *In vitro* drug release showed that the prepared niosomes significantly controlled rate and extent of naltrexone release. *Ex vivo* transcorneal permeation studies reveals that niosomes were capable of controlling NTX permeation.

Proniosome

Proniosomes are the non-hydrated solid colloidal particles which upon hydration forms niosomes similar to those produced by more cumbersome conventional methods. Physical stability issues associated with niosome such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing are being conquer by proniosomes [27]. Walsh and colleagues [28] developed slurry method to produce proniosomes. Maltodextrin was utilized as a carrier.

Elastic niosome

Nonionic surfactants, ethanol and water forms the building block of this system. They are superior to conventional niosomes because they enhance penetration of a drug through intact skin by passing through pores in the stratum corneum, which are smaller than the vesicles [29]. Van den Bergh et al., [30] developed the first detergent-based elastic nano vesicles called elastic or deformable niosomes consisting of surfactant L-595(sucrose laurate ester) and the micelle forming surfactant PEG-8-L (octa oxyethylene laurate ester).

Aspasomes

It is incarnated from the combination of acorbyl palmitate, cholesterol and highly charged lipid diacetyl phosphate. Aspasomes are first hydrated with water/aqueous solution and then sonicated to obtain the niosomes. Aspasomes finds its main utility in topical drug delivery. Due to its inherent antioxidant property they will decrease the disorder caused by reactive oxygen species [31].

Algosomes

Biologically active 1-O-alkylglycerols (ALKG) forms a new vesicular form called "Algosome" in conjunction with cholesterol which are osmotically sensitive. Its prominent effect on blood brain barrier permeability has markedly improved brain uptake of anticancer agent. Algosomes were formed by film hydration method using ALKG (tetra-, penta-, hexa-, hepta-, octa- or nonadecylglycerols) in combination with cholesterol and dicetyl phosphate (1-O-alkylglycerol : Cholesterol :DCP in 45:45:10 molar ratio). Algosome dispersions on addition of higher concentrations of KI (40-100 mM) brought about increases in vesicle size and at concentrations 60 mM and above showed aggregation [32].

Surfactant ethosomes

These are novel carrier system which improves permeation through skin. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water, which are used mainly for transdermal delivery of drugs [33]. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear it has good permeation through skin than liposomes and conventional niosomes. The synergistic effects of phospholipids and high concentration of ethanol have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers. The size range is in nanometers [34].

Vesicle in W/O system

Niosomes containing span as surfactant have been dispersed in an oil in water emulsion to yield a vesicle in water in oil system v/w/o. The release of 5(6) - carboxy fluorescein from these systems followed the trend v/w/o < water in oil (w/o) emulsions < niosome dispersions. The release was higher in case of span 20 than span 60. Isopropyl myristate was found to be best as oil component [35].

Polyhedral niosome

Low cholesterol regions of the C16G2, cholesterol, Solulan C24 ternary phase diagram is the birth place of polyhedral niosomes. The Polyhedral shape of the vesicle is just because of minimum mobility of hydrocarbon chain in gel phase [36]. These can be used in topical drug delivery as they are extremely viscous (due to the polyhedral niosome shape) and also due to the fact that at 30°C, (skin surface temperature lies between 26 and 30°C) they are non-thermoreponsive and thus are capable of releasing their encapsulated contents once either the ambient temperature increased to 35°C (e.g. in the use of photoprotective agents) or the skin temperature was raised (e.g. in inflammation) [17, 37].

Surface coated niosome

Surface coated niosomes were grafted using α,ω -hexadecyl-bis-(1-aza-18-crown-6) (bola), Span 80 and cholesterol (2:5:2 molar ratio) using hydration followed by sonication. It was first used in the treatment of breast cancer using 5-fluorouracil (5-FU) [38]. The loading capacity was found to be of ~40% with respect to the amount of 5-FU added during the preparation. Similar findings were achieved with PEG coated bola niosomes (bola, Span 80(R), cholesterol, DSPE-

mPEG2000, 2:5:2:0.1 molar ratio respectively). 5-FU-loaded PEG-coated and uncoated bola-niosomes were tested on MCF-7 and T47D cells. Both bola niosome formulations provided an increase in the cytotoxic effect with respect to the free drug. *In vivo* experiments on MCF-7 xenograft tumor SCID mice models showed a more effective antitumoral activity of the PEGylated niosomal 5-FU at a concentration ten times lower (8 mg/kg) than that of the free solution of the drug (80 mg/kg) after a treatment of 30 days.

Niosomes in Carbopol Gel

The first step of this method is to form conventional niosome using hydration method. The niosomes thus obtained were then incorporated in carbopol-934 gel (1% w/w) base containing propylene glycol (10% w/w) and glycerol (30% w/w). It was observed that the mean flux value and diffusion co-efficient were 5 to 7 times lower for niosomal gel as compared to plain drug gels. Moreover, carrageenan induced paw edema inhibition was higher by niosome formulation as compared to plain gel [39].

Formulation Aspects of Niosome

Each and every component of the formulation has its importance as far as stable and effective dosage unit is concern. The cardinal elements of the niosomal preparation include nonionic surfactant, Cholesterol and hydration medium. Now each will be elaborated with its unique credentials.

Nonionic amphiphiles/surfactants

These 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 [40]. It must has a hydrophilic head group and a hydrophobic tail. Nonionic surfactants such as alkyl ethers, alkyl glyceryl ethers, Sorbitan fatty acid esters, Polysorbates, etc., are used in the formulation of various niosomes. Gemini nonionic surfactants are newer class of surfactants used in cosmetics. It has two hydrophobic chains and two hydrophilic head groups linked with spacers. Bola amphiphiles contains bipolar amphiphiles with two polar heads connected by one or two long hydrophobic spacers [17, 18, 24, 36, 40, 41]. The three important features of the amphiphile which needs consideration in the preparation of stable niosome is critical packing parameter, chain length and HLB value are discussed elsewhere in

the article. The nonionic surfactants orient themselves in bilayer lattices where the polar or hydrophobic heads align facing aqueous media while the hydrophobic head align in such a way that the interaction with the aqueous media would be minimized. The hydrophobic moiety may consist of one or two alkyl or perfluoroalkyl groups or in certain cases a single steroidal group. The alkyl group chain length is usually from C₁₂–C₁₈ forms stable vesicle with good entrapment efficiency [17, 35, 36, 42, 43]. Nonionic surfactants with stearyl (C18) chains show higher entrapment efficiency than those with lauryl (C12) chains. Some important classes of amphiphiles are captivated in **Table 1** with cogent examples and applications.

Table 1: Examples of nonionic surfactant classes and their use

Cholesterol content

It mainly influences the extent of aggregation, Permeability of ions, fusion processes, fluidity, elasticity, enzymatic activity, size and shape of the niosome. Hence it is thus usually incorporated in a 1:1 molar ratio in most formulations [35].

Hydration medium

It is one of the most critical parameter chosen depending upon the method of preparation and drug solubility. Phosphate buffer at various pH is most commonly used hydration medium for preparation of niosomes. The pH 5.5 phosphate buffer was used in the preparation of ketoconazole niosomes [44] whereas pH 7.4 phosphate buffer was used in the preparation of meloxicam niosomes [42].

Factors to Be Considered For Effective Niosomal Delivery

Nature of drug

It mainly affects the increases vesicular size, probably by interaction of it with surfactant head groups which leads to increase the charge and mutual repulsion of the surfactant bilayers [45]. The hydrophilic lipophilic balance of the drug affects degree of entrapment. The encapsulation of the amphipathic drug doxorubicin has been shown to alter the electrophoretic mobility of hexadecyl diglycerol ether (C16G2) niosomes in a pH dependent manner, indicating that the amphipathic drug is incorporated in the vesicle membrane. Steric stabilizers are added to prevent aggregation [46]. In polyoxyethylene glycol (PEG) coated vesicles, some drug is

entrapped in the long PEG chains, thus reducing the tendency to increase the size [47].

Amount and type of surfactant

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6) as the surface free energy decreases with an increase in hydrophobicity of surfactant [48]. Vesicle aggregation of niosomes may be prevented by the inclusion of compounds that introduce repulsive steric or electrostatic forces. With an optimum level of cholesterol, it seems that niosomes are indeed formed from Polysorbate 20 despite of its higher HLB value [49]. Polyglycerol monoalkyl ethers and polyoxylate analogues are the most widely used single-chain surfactants. However, it must be noted that they possess less encapsulation efficiency in the presence of cholesterol. Etheric surfactants have also been used to form niosomes. The maximum amount of surfactant/lipid used to prepare niosomes is generally 10–30 mmol/L (1–2.5%, w/w) [50]. 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. 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 [17, 51]. The effect of surfactant feature on niosome preparation is summarized in **Figure 5**. The geometry of the vesicles formed during the niosomal preparation also depends upon the critical packing parameter (CPP). According to CPP the geometry of the vesicles can be predicted. CPP can be calculated using following equation [52]:

$$\text{Critical packing parameter (CPP)} = v/l_c \cdot a_0$$

Where;

v = hydrophobic group volume,

l_c = the critical hydrophobic group length,

a_0 = the area of hydrophilic head group

CPP is helpful in predicting the structure of niosome vesicles in following way;

- ✓ Spherical micelles formed if $CPP < \frac{1}{2}$
- ✓ Bilayer micelles is formed if $\frac{1}{2} < CPP < 1$
- ✓ Inverted micelles is formed if $CPP > 1$.

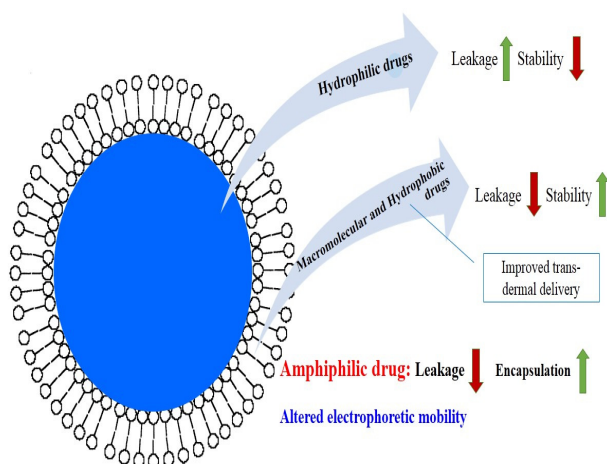


Figure 4: The effect of the nature of the drug on the formation of niosomes

Cholesterol content and charge of the surfactant

Hydrodynamic diameter and entrapment efficiency of niosome gets increased when it contains cholesterol in the formulation [53]. Its interaction with the nonionic surfactants is responsible for such structural attributes [54]. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume. Cholesterol in higher amount brings down the release rate of encapsulated drug with change in fluidity and increased membrane rigidity [18, 53, 55]. The amount of cholesterol to be added in the niosomal preparation depends on the HLB value of the surfactants. As the HLB value increases above 10, it is necessary to increase the minimum amount of cholesterol to be added in order to compensate for the larger head groups [17].

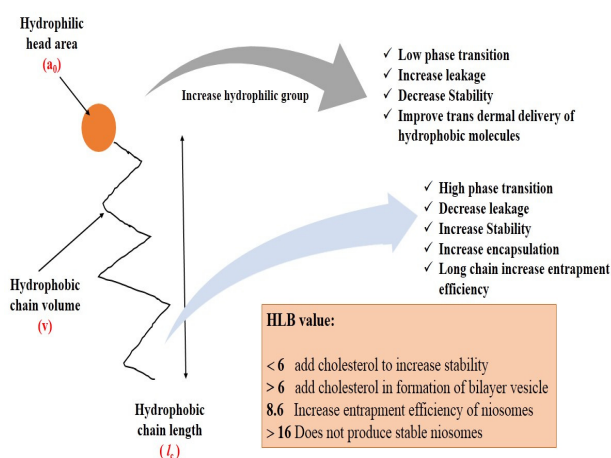


Figure 5: Effect of surfactant Structure on niosome preparation

It interacts with sorbitan ester by virtue of hydrogen bond. It is usually included in a 1:1 molar ratio in most formulations by the inclusion of charge stabilizer molecules (dicetyl phosphate) that stabilize the system against the formation of aggregates by repulsive steric or electrostatic effects the resulted niosomes are less leaky. It leads to the transition from the gel state to liquid phase in niosome systems. Dicetyl phosphate was added to C16G2 niosomes encapsulating hemoglobin in order to achieve similar electrophoretic mobility as that of erythrocytes [56-58].

Methods of preparation

Methods of preparation affect niosome characteristics. It mainly affects size, entrapment efficiency and retention of drug. Hand shaking method forms vesicles with greater diameter (0.35-13nm) compared to the ether injection method (50-1000nm)[59]. Reverse Phase Evaporation (REV) method gives vesicles which has good affinity to targeted site at the same time smaller in size [60]. Microfluidization method gives unilaminar vesicles with greater uniformity[59]. Parthasarathi *et al.*, [61] prepared niosomes by transmembrane pH gradient (inside acidic) drug uptake process with improved entrapment efficiency and better retention of drug.

Resistance to osmotic stress

Hypertonic media cause shrinkage of niosomal vesicle (reduction in diameter) while hypotonic salt solution cause swelling of it with initial slow release, probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress [62].

Temperature of Hydration

The hydration temperature mainly affects the size and shape of the niosome. Polyhydral vesicles of C16G2 : solulan C24 (91 : 9) is formed at 25 °C which was transformed to spherical vesicles at 45 °C and a cluster of smaller spherical niosomes at 49°C [17].

Effect of pH of the hydration medium

The pH of the hydration medium directly affect the entrapment efficiency. High entrapment of flurbiprofen was reported at acidic pH (5.5). The encapsulation efficiency of flurbiprofen increased to about 1.5 times as pH decreased from 8 to 5.5 and decreased significantly at pH > 6.8. The enhancement of encapsulation efficiency of flurbiprofen at lower pH is mainly 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 [63].

Method of Preparation of Niosomes

Most common method of niosomes preparation involves evaporation of organic solution containing surfactant mixture to produce a thin film followed by hydration with the hydration medium. The preparation methods for niosome influence the numbers of bilayers, size, size distribution and entrapment efficiency of the aqueous phase and the membrane permeability of the vesicles. Many methods are available for niosome preparation methods which are as follows:

Ether injection method

The solution of the surfactant is made by dissolving it in diethyl ether or any ether based solvent which is then introduced into warm aqueous media containing the drug maintained at 60°C using appropriate gauze. Vaporization of the ether leads to the formation of single layered vesicles [17]. The particle size of the niosomes formed depend on the conditions used, and can range anywhere between 50-1000µm [50]. Bhaskaran et al., [64] prepared salbutamol niosomes by ether injection with an entrapment efficiency of 67.7%.

Hand shaking method (Thin Film Hydration Technique)

Nonionic surfactant(s) and cholesterol are dissolved in a volatile organic solvent in a round bottom flask. The organic solvent is removed using a rotary evaporator, which leaves a thin film of solid mixture deposited on the walls of the flask which is after drying being hydrated using aqueous phase with or without drug to be entrapped which yield multilamellar niosomes [65]. Sonication, micro fluidization or membrane extrusion techniques were utilized for further size reduction [17]. Laxmi et al., [66] have prepared niosomal gel of methotrexate for the treatment of psoriasis. Methotrexate niosomal gel reduce the in total score from 6.2378 ± 1.4857 to 2.0023 ± 0.1371 in psoriasis at the end of 12 week period.

Sonication

A typical method of production of the vesicles is by sonication of solution. In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes [50]. Sathali et al., [67] developed

multilamellar vesicles containing terbinafine. Small unilamellar niosomes with an entrapment efficiency of about 85% have been produced using sonication.

Micro fluidization

This is most cogent technique to prepare unilamellar vesicles of defined size distribution. It is based on submerged jet principle in which two fluidized streams interact at ultra-high velocities (100 ml/min), in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed [68].

The enzymatic method

Niosomes may also be formed by an enzymatic process from a mixed micellar solution. In this method ester links are cleaved by esterases leading to break down products such as cholesterol and polyoxyethylene, which in combination with dicetylphosphate and other lipids produce multilamellar niosomes. The surfactants used are polyoxyethylene estearyl derivatives and polyoxyethylene cholesteryl sebacetate diacetate [17]. Cholesterol in combination with C₁₆G₂ and dicalcium phosphate yield niosome using esterase as enzyme.

Multiple membrane extrusion method

The first step of this method is using hydration technique to prepare a thin film and then hydrate it with aqueous drug solution. The resultant suspension extruded through polycarbonate membranes, which are placed in series for upto 8 passages which forms niosome of controlled size. It was found that using extrusion method vesicles of mean size diameter 136 nm can be prepared. E.g.: Stilbogluconate Niosome (200nm) [69].

Reverse phase evaporation technique

A mixture of ether and chloroform is utilized to form a solution of nonionic surfactant and Cholesterol in equivalent amount. At the same time drug is solubilized in water with the aid of heat. The resulting two phases are sonicated at 4-5°C until a clear gel is formed which is further sonicated after the addition of phosphate buffered saline (PBS). Temperature is raised up to 40°C with reduced pressure to vanish the organic volatile solvent. This results in a viscous niosome suspension which can be diluted with PBS and heated on a water bath at 60°C for 10

min to yield niosomes [60]. Guinedi et al., [70] also used this method to develop niosomes containing acetazolamide. Gyanendra et al., [71] developed isoniazid niosomes by reversed phase evaporation which has good affinity to targeted site and also capable of maintaining steady drug concentrations for up to 30 h with 61.8 % Cellular uptake (by macrophage).

Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote loading)

A solution of surfactant and cholesterol is being made in volatile organic solvent which is subsequently removed under reduced pressure in round bottom flask so as to get a thin film on the wall. This film is then hydrated using citric acid solution (300mM, pH 4.0) by vortex mixing yields multilamellar vesicles which are treated to three freeze thaw cycles and sonicated for further size reduction. To the above niosomal suspension, aqueous solution containing 10mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 using 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside it is again ionized thus not allowing it to exit the vesicle). The mixture is later heated at 60°C for 10 minutes to give niosomes [42, 72].

The “Bubble” Method

It is a technique allows the preparation of niosomes without the use of organic solvents. The bubbling unit consists of a three neck round bottom flask in a temperature controlled water bath. Water-cooled reflux and thermometer is positioned in the first and second neck, while the third neck is used to supply nitrogen. Cholesterol and surfactant are dispersed together in a phosphate buffer pH 7.4 at 70°C followed by high shear homogenizer for 15 sec and immediately afterwards, it is bubbled with nitrogen gas to yield niosomes [73, 74].

The single pass technique

This is a patented technique. The first step of the process involves formation of solution or suspension of lipid in volatile organic solvent which is then extruded through a porous device and subsequently through a nozzle. High pressure homogenization and high pressure extrusion are utilized to produce niosomes with a narrow size distribution (50–500nm) [75].

The Handjani-Vila method

A homogeneous lamellar phase is produced by mixing a drug and lipid or lipid mixture with an aqueous in the ratio of 1:1. Ultracentrifugation or

mechanical agitation is used to homogenized the above mentioned mixture at controlled temperature [76].

Formation of Proniosomes and Niosomes from Proniosomes

The formation of proniosomes commenced with coating of a water soluble carrier (sorbitol) with the surfactant. The coating is done by preparing a solution of the surfactant and cholesterol in a volatile organic solvent, which is sprayed onto the carrier kept in a rotary evaporator. The resulting coating over carrier is a dry formulation in which a water soluble particle is coated with a thin film of dry surfactant which is nothing but “Proniosome”[28]. Hydration of proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant leads to formation of niosome.

Lipid injection method

The drug was dissolved in water using water bath at the same time mixture of lipids and surfactant is melted which is subsequently injected into above mentioned aqueous phase [77] or the addition of a warmed aqueous phase dissolving the drug to a mixture of melted lipids and hydrophobic drug [78]. This method does not require expensive, hazardous organic phase, which are also difficult to remove from final product.

Size Reduction of Prepared Niosome

Niosomes prepared by most of the above mentioned method are usually in the micron size range [79].

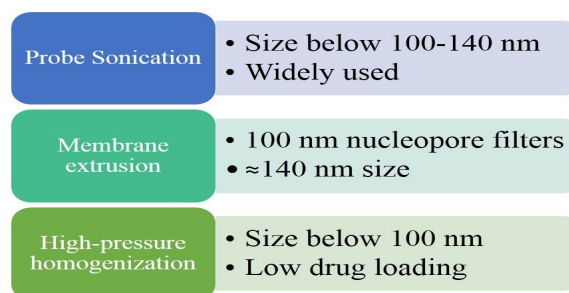


Figure 6: Size reduction techniques for prepared niosomes

Hence a size reduction step must be incorporated so as to make it appropriate for bio distribution. The size of the prepared vesicle is reduced by various methods. (See **Figure 6**)

Table 1: Examples of nonionic surfactant classes and their use

Nonionic surfactant class	Example	Remarks	Application
Alkyl ethers [39]		General: ✓ High stability ✓ Non-irritant, ✓ Compatible with other surfactants ✓ Decrease entrapment efficiency when used with cholesterol	Encapsulate proteins and peptides
	Polyoxyethelene 4 lauryl ether (Brij 30)(40)	✓ Incompatible with iodide, mercury salts, tannins sulphonamides and some drugs ✓ Forms large unilamellar vesicles when combined with 30 mmol/L cholesterol ✓ HLB 9.7	Niosomes of tretinoin
	Polyoxyethylene cetyl ethers (Brij 52, 56 and 58)(41)	✓ Ease of storage ✓ Enhancement of dermal and/or transdermal bioavailability ✓ The HLB value of Brij 58 is 15.7	Brij 58 form inverted vesicles, which are useful for studying ion-pumping activity Minoxidil niosome
Alkyl glyceryl ethers(43)	Polyoxyethylene stearyl ethers (Brij 72 and 76)(42)	✓ High entrapment efficiency ✓ Brij 72 forms multi- lamellar vesicles ✓ HLB value of Brij 72 is 4.7 and for Brij 76 of 12.4	Intracellular chemotherapy
	Surfactant-I (molecular weight (MW 473)) is C16 monoalkyl glycerol ether with average of three glycerol units Surfactant-II (MW 972) is diglycerol ether with average of the seven glycerol units Surfactant III (MW 393) is ester linked surfactant	✓ High stability ✓ Non-irritant, ✓ Compatible with other surfactants ✓ Decrease entrapment efficiency when used with cholesterol	Good cosmetic potential described by L'orealparis Algosome Delivery of insulin and antineoplastic agents
Sorbitan fatty acid esters(44)	Span 20,Span 40,Span 60,Span 85	✓ The molar ratio of cholesterol to Span may affect the entrapment of drugs into niosome ✓ Length of the lipophilic chain increased; Span 20 >Span 40 >Span 60 > Span 85 with increased gel transition	In cosmetics as solubilizer
Polysorbate(45)	Tween 28, 40, 60 and 80are liquids derived from PEG-ylated sorbitan esterified with fatty acids.	✓ Stabilize dispersion ✓ Lower CMC ✓ Harmful to persons with Crohn's disease ✓ Prolong release	Used in injections, vaccines
Alkyl Amides(46)	Fatty acid sulpho alkyl amides	✓ Use of cholesterol to provide rigidity	Transdermal delivery
Acetylenicdiol (Dimeric) type surfactant(47)	Gemini surfactants	✓ Lower CMC value ✓ Nontoxic, more ✓ Non irritating non hemolytic ✓ Excellent wetting	In cosmetics and pharmaceutical formulations
Bola Surfactants(33)	α,ω -hexadecyl-bis-(1-aza-18-crown-6)	✓ Higher solubility ✓ Higher CMC ✓ Lower aggregation number	In cosmetics and pharmaceutical formulations

Table 2: Marketed Niosomal Cosmetic products

Brand Name	Company Name	Application
Beyond Paradise	Estee Lauder Companies	Beyond Paradise After Shave Lotion 100ml/3.3oz
Prototype#37-C Lancome	L-oreal Paris	Anti Aging Cream
White Shoulders	White Shoulders	White Shoulders Eau De Cologne Spray 130ml/4.5oz
Orlane - Lip color and Lipsticks	Orlane	Lip gloss
Suractif	Lancaster	Suractif Non Stop Lifting Advanced Night Cream 50ml/1.7oz
Jean Paul Gaultier	Jean Paul Gaultier	Le Classique Eau De Toilette Spray 100ml/3.3oz
Love in Paris	Nina Ricchi	Deodorant Spray 100ml/3.3oz
Realities	Liz Claiborne	Realities Shower Gel 200ml/6.7oz
Blanc Parfait	Givenchy	Blanc Parfait W4-L Universal Brightening Spots Corrector SPF 45 1.6ml/0.05oz
Foundation and Complaxions	Lancome	Flash Retouche Brush On Concealer
Britny Spears - curious	Britny Spears	Curious Coffret: Edp Spray 100ml+ Dual-ended Parfum& Pink Lipgloss+ Body Souffle 100ml 3pcs
Elene – Eye care	Elene	Day & Night Eye Programme 15mlx 2
Guinot – night care	Guinot	Deep Action Whitening Serum 30ml/1.07oz
Gatineau – Moderactive - Cleanser	Gatineau	Moderactive Almond Make-Up Remover 250ml/8.3oz
Shieseido – Bioperformance – Night care	Shieseido	Bio-Performance Intensive Clarifying Essence 40ml/1.3oz
Hugo Boss – Boss Soul	Hugo Boss	Boss Soul After Shave 90ml/3oz
Givenchy - Amarige	Givenchy	Amarige Eau De Toilette Spray 100ml/3.3oz
LorizAzzaro - Chrome	LorizAzzaro	Chrome Eau De Toilette Spray 200ml/6.8oz
Guinot – Cleanser	Guinot	Gentle Face Exfoliating Cream 50ml/1.7oz

Separation of untrapped drug

The hydration of formulation component does not remove the entire amount of drug, regardless of the drug loading optimization steps taken. The removal of untrapped drug from the niosomes can be accomplished by various techniques, which include;

Exhaustive dialysis

Dialysis tubing against phosphate buffer or normal saline or glucose solution was used in the dialysis of niosomal dispersion^[80]. It is suitable for large vesicles >10 nm and for highly viscous system. One of biggest disadvantage of this method is, extremely slow (up to a day) nature of method. Large volumes of dialysate required which dilutes the niosomal dispersion^[50].

Gel Filtration

Sephadex-G-50 column was utilized for gel filtration of niosomal dispersion followed by elution with phosphate buffered saline or normal saline^[48]. The process become slow (1–2h) when using Sepahrose 2B:4B for macromolecule separation is used. One of the biggest disadvantage of this method is gels are expensive if not reused and also dilutes the niosome dispersion. It is not suitable for highly viscous

formulations and for formulations with a large particle size (>10–20 nm).^[48]

Centrifugation

After centrifugation of niosomal suspension the supernatant is separated, which is washed and then resuspended to obtain a niosomal suspension free from untrapped drug^[27]. The process is quick (30 min), but fails to sediment the sub-micron niosomes. Ultracentrifugation Sediments all size particles, but expensive instrumentation and long centrifugation times (within 2 h) which may lead to aggregation needs attention^[65].

Stability of Niosomes

The main problems associated with storage of vesicles are aggregation, fusion and leakage of drug. So as to ascertain the stability of niosomes, the optimized batch was stored in airtight sealed vials at different temperatures. The samples were characterized for color change, surface characteristics and tested for the percentage drug retained after being hydrated to form niosomes and analyzed by suitable analytical methods (UV spectroscopy, HPLC methods etc)^[81]. Apart from that niosomal stability was ascertained for its osmotic activity^[58] as well as to light.

Stability in phosphate Buffer

The stability of alkyl glycoside vesicles (niosomes) was compared with phosphatidylcholine based (liposomes) vesicles in buffer. It was observed that the phosphatidylcholine based vesicles (liposomes) disintegrated in vitro after 22 weeks; while niosomes prepared using alkylglycosides remained at least for 25 weeks.

Stability in hypertonic media

Addition of a hypertonic media to a suspension of niosomes brings It was interpreted that the osmotic gradient induces the reduction in vesicle diameter by effectively pumping out the vesicular contents. Thus, apparent resistance to osmotic shrinkage presumably is related to higher permeability efflux of solute(s).

Stability in hypotonic media

In hypotonic media, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

Photo degradation Study

To promote photo degradation exposure to UV irradiation and fluorescent light has been utilized. For the former, drug is analyzed after the drug solution and vesicle preparation are maintained at room temperature and exposed to UV radiation for 1h at 25°C. Such studies have been reported for niosomes loaded with tretinoin, a metabolite of vitamin A [82]. For the latter, the samples are exposed to artificial light at room temperature for a specific period and the drug concentration was determined[83]. It may be possible to stabilise niosomes by a variety of methods such as the addition of polymerised surfactants to the formulation [58], the use of membrane spanning lipids and the interfacial polymerisation of surfactant monomers in situ [84]. The entrapment of hydrophobic drugs [17] or macromolecular prodrugs [65] also increases the stability of these dispersions.

Toxicity of Niosomes

Despite of immense work done in the area of niosome the toxicity studies must as far as regulatory perspective is in its infancy. Proliferation of keratinocytes was observed in one of the topical niosome formulations[85]. Safety profile of the ester type surfactants was albeit higher due to enzymatic degradation of ester bounds than ether type surfactants[86]. In

general, the physical form of niosomes did not influence their toxicity. Though nasal applications of these formulations caused toxicity in the case of liquid crystal type niosomal formulation. Niosomal delivery decreases toxicity E.g. niosomes containing vincristine^[61]. It decreased the neurological toxicity, diarrhoea and alopecia following the intravenous administration of vincristine and increased vincristine anti-tumor activity in S-180 sarcoma and Erlich ascites mouse models.

Table 3: Characterization of Niosome

Assay		Methodology
Physical Characterization/Stability		
1	Vesicle size, surface morphology and size distribution	Transmission electron microscopy (TEM), freeze fracture electron microscopy, Dynamic light scattering, TEM, zeta sizer, Laser light scattering, gel permeation, gel exclusion, Cryo electron microscopy, Confocal Microscopy
2	Osmotic pressure	Osmometer
3	Phase behavior	Differential scanning calorimetry
4	Lamellarity	Small angle X-ray scattering, ³¹ P-NMR
5	Surface charge	Free-flow electrophoresis
6	Entrapment efficiency	Exhaustive dialysis, gel filtration and centrifugation.
7	Relevant body fluid induced leakage	Protamine precipitation and GEC
8	Dilution dependent drug release	Retention loss on dilution
9	<i>In vitro</i> release	Hu's method, Dialysis through a semipermeable membrane and its measurement using suitable analytical method
Chemical Characterization/Stability		
1	pH	pH meter
2	Cholesterol Concentration	Cholesterol oxidase assay
3	Cholesterol Auto oxidation	HPLC, TLC
4	Electric surface potential and surface pH	Zeta potential measurements and pH sensitive probes
Biological Characterization/Stability		
1	Sterility	Aerobic and Anaerobic culture
2	Pyrogenicity	SAM or LAL test
3	Animal toxicity	Monitor survival, histology and pathology

Characterization of Niosomes

Once the niosomes are prepared with appropriate method the next step come is the characterization of prepared vesicles for its physical, chemical and biological stability. The characterization elements of niosome are depicted in **Table 2**.

Applications of Niosomes

This drug delivery has been explored for administration including intramuscular, intravenous, peroral, and transdermal. One of the cardinal impact in pharmafield by niosome is targeted drug delivery. The following are some glimpses regarding research on niosome since last two decades.

Niosomes as a novel vaccine delivery system

Development of new safe and effective vaccines is a major thrust area for researchers all around the world. The oral vaccination has been successfully done using niosome as carrier [7, 87, 88]. Various lyophilized ovalbumin niosome preparations consisting of sucrose esters, cholesterol and dicetyl phosphate were incarnated. Only encapsulation of ovalbumin into Wasag®7 (70% stearate sucrose ester, 30% palmitate sucrose ester (40% mono-, 60% di:tri-ester)) has provide a significant increase in antibody titres [89]. Vyas et al., [14] developed mannosylated tetanus toxoid (TT) containing niosomes by the reverse-phase evaporation method as oral vaccine delivery carrier and adjuvant for the induction of humoral, cellular, and mucosal immunity. Mannosylation by modified polysaccharide o-palmitoyl mannan (OPM) make it inert to bile salts and gut enzymes. It also enhance their affinity toward the antigen presenting cells of Peyer's patches which signifies its potential for vaccine delivery as well as an adjuvant [14]. SPANosomes [90], composed of cationic lipid and Span 80 were synthesized and evaluated as small interfering RNA (siRNA) vectors by Zhou and co-workers. Ferro et al., have studied oral vaccination of tetanus toxoid encapsulated in niosome with bile salt for systemic as well as mucosal immunity [91]. Perri and co-workers have done subcutaneous DNA vaccination using niosome [92]. Various studies are available for topical immunization [93, 94]. Hassan et al., [95] reported better immunogenicity with herpes simplex virus 1 antigen encapsulated in l-mono palmitoyl glycerol (MP)/CHOL/DCP niosomes in mice. Yoshioka et al. [18] formulated Span/CHOL/DCP niosomes containing tetanus toxoid (TT) emulsified in an

external oil phase to form a vesicle-in-water-in-oil (v/w/o) formulation. Initial studies of the system *in vivo* using cottonseed oil as the external oil phase, showed enhanced immunological activity over the free antigen or vesicles. Ferro et al., [96] used a gonadotrophin releasing hormone (GnRH) analogue, GnRH-glycs, linked to different carrier molecule and encapsulated in NSV formulations to immune-neutralisation of GnRH in male Sprague-Dawley rats. The results were encouraging to use NSVs as a nontoxic immune adjuvant. Then, a modified GnRH peptide (CHWSYGLRPG-NH₂) was conjugated to TT and was formulated with different adjuvants such as C18EO2/CHOL/DCP niosomes [97]. The best castration effect, depicted in production of IgG2b antibody, was not as well by nano-niosomes as compared to sustained release poly(lactide-co-glycolide)/triacetin (PLGA) formulation. Chattaraj et al., [7] entrapped haemagglutinin antigens from three different influenza A strains in Span 40 or 60 niosomes for nasal mucosal delivery. Niosomes containing Span 60, Tween 61, cholesterol, and dicetyl phosphate were conjugated with a purified monoclonal antibody to CD44 (IM7) through a cyanuric chloride (CC) linkage on the polyoxyethylene group of the Tween 61 molecule. The immuno-niosomes were incubated with synovial lining cells expressing CD44. Attachment of niosomes was evident and showed selectivity and specificity compared to controls when such florescent tagged vesicles were detected using UV absorbance [98].

Niosomes for non-invasive genetic immunization and in gene delivery

Gene delivery as well as genetic immunization shows dignified potential for the treatment of many different diseases even though hindered by many technical as well as ethical challenges. However, few candidate DNA vaccines have progressed past preclinical development and none have been approved for human use [99]. DNA encoding Hepatits B Surface antigen was moulded in the form of niosomes as DNA vaccine carriers for effective topical immunization by reverse phase evaporation method [41]. Brewer et al., [100] have checked the adjuvant activity of niosome on Balb/C humoral response. They deduced that niosomes were potentially better stimulators of the Th1 lymphocyte subset than was Freund's complete adjuvant and by inference, potent stimulators of cellular immunity. Huang et al., [101] used cationic niosomes of sorbitan monoesters for delivery of

antisense oligonucleotides (OND) in a COS-7 cell line among which Span 40 and 60 vesicles had more significant effect. Niosomes composed of non-ionic surfactants (i.e., Tween and Span) and cholesterol was mixed with novel synthesized spermine-based cationic lipids to prepare cationic niosomes that could act as gene carriers^[10]. Huang *et al.* hypothesized using PEGylated cationic niosomes. They used DSPE-mPEG 2000 for PEGylation of cationic niosomes and the resultant OND-vesicle complexes showed a neutral zeta potential with particle size about 300 nm. These complexes had less serum-protein binding affinity and particle aggregation in serum. On the other hand, the PEGylated niosomes showed a higher efficiency of OND cellular uptake in serum when compared with cationic niosomes with protection from serum nuclease^[102]. Basiri *et al.*,^[103] have prepared and characterized a negatively-charged niosomes as gene-delivery vectors in presence of Ca^{+2} . Vyas *et al.*^[104] formulated Span 85/CHOL niosomes encapsulating DNA encoding HBsAg and applied them topically in Balb/c mice. Elevation of serum anti-HBsAg titer and cytokines level (IL-2 and IFN- γ) indicated the efficacy of used topical vesicular vaccine delivery. A series of positively charged micron-sized niosomal formulations containing sorbitan esters, CHOL and CTAB for the entrapment of autoclaved *Leishmania major* (ALM)^[105]. In spite of large diameter of prepared vesicles, the results obtained showed that the niosomes containing ALM have a moderate effect in the prevention of cutaneous leishmaniasis in BALB/c mice. Herring sperm DNA has successfully being encapsulated using niosome which is superior than liposome.^[106]

Niosomes for Topical Delivery

Drug targeting to the different skin layers and appendages is a key goal of research made in dermatology^[107]. Niosome as drug carrier is fascinating choice to achieve localized drug action due to its smaller size and low penetrability through epithelium and connective tissue which keeps the drug localized at the site of administration^[108]. Sub-micron size niosomes are mostly suited for transdermal and parenteral applications. Niosomes have been valuable in terms of improving bioavailability and skin penetration rather than conventional mean for minoxidil. Higher entrapment efficiency (80% up to 3 months) was obtained when Span 60 and cholesterol were utilized in 1:1 molar ratio with 25mg drug^[15]. Dithranol is the magic bullet in the topical treatment of psoriasis though it is

inconvenient and troublesome, as it has irritating, burning, staining and necrotizing effect on the normal as well as the diseased skin. The entrapment efficiency of dithranol niosomes was optimized by altering the proportion of span 60 and cholesterol. The mean niosome size was $5 \pm 1.5 \mu\text{m}$ ^[5]. Ning and co-workers have carried out the study on insulin to prepare niosome. Two kinds of entrapped insulin vesicles with Span 40 and Span 60 were prepared by either lipid phase evaporation or sonication methods with particle sizes of 242.5 nm and 259.7 nm, respectively. They concluded that vaginally administered nano-niosomes might be a good carrier for protein drugs such as insulin^[109]. Vaginal administration of clotrimazole niosome prepared by lipid hydration method provide sustained release for local vaginal therapy^[110]. The *in vitro* evaluation has been carried out using rabbit vaginal mucosa with vertical Franz diffusion cells which shows increased the clotrimazole total penetration through the vaginal mucosa by 1.6 fold within a day. Rofecoxib containing niosomal gel provided sustained therapeutic action^[111]. In a study, niosomal benzoyl peroxide incorporated into HPMC gel designed and optimized by partial factorial design. Ex vivo release study on human cadaver skin showed increase in drug skin retention, extended drug release and improved permeation of drug across the skin which in turn will reduce the toxicity of drug and enhance the therapeutic efficacy^[112]. Permeation of lidocaine hydrochloride-loaded vesicles through mouse abdominal skin presented a higher flux and a shorter lag time^[9]. A modified proniosomal formulations of hydroxyzine hydrochloride were appropriate for topical drug delivery system for the treatment of localized urticarial as compared to conventional delivery provide CNS side effects^[113]. Niosomes of aceclofenac, a potent analgesic, anti-pyretic and anti-inflammatory agent^[114]. Gugulipid incorporated proniosomal gel showed good antiinflammatory activity but not as good as commercial product diclofenac (Voveran®Emulgel). The authors state that proniosome formulation improve antiinflammatory activity of gugulipids comparable to topical NSAIDs^[8]. In addition to nanovesicle encapsulation, some other methods were developed for enhancing transdermal transport of large molecules such as insulin. A combination technique of charged nano-liposome encapsulation of insulin and iontophoresis through rat skins with microneedle-induced microchannels were resulted in 713.3 times higher transport of the

protein than that of its passive diffusion [115]. Aceclofenac niosomal gel was prepared for topical with improved penetration and therapeutic efficacy.[116] Enhancing the anti-inflammatory activity with prolonged release has been achieved with niosomal gel of nimesulide as compared to plain gel.[117] Ketoprofen was encapsulated in niosomes of span 60 for topical application with sustained release.[118] Niosome of flurbiprofen and Meloxicam has successfully prepared and evaluated.[119, 120] Antiplatelet effect of indomethacin have been improved using niosome which reached in the interior of the platelets and acting directly on the cyclooxygenase enzyme to prevent thromboxane formation.[121] Lidocaine hydrochloride entraped niosomes were prepared for local action.[122] Niosomes of baclofen a centrally acting muscle relaxant have been prepared to improve skin penetration with improved stability, entrapment efficiency and bioavailability.[123] Topical enoxacin was successfully nurtured in the form of niosomal gel with studies carried out on physicochemical property, stability and *in vitro* percutaneous absorption. Gallidermin containing niosomes were prepared in order to increase its stability and efficiency for pharmaceutical and cosmeceutical uses.[124]

Niosome for ophthalmic drug delivery

To minimize the problems associated with conventional eye drops, different ocular drug delivery devices have been investigated such as niosomes for brimonidine tartarate delivery in glaucoma management [12]. Niosomes contain nonionic surfactants which are non-antigenic and nontoxic to the eye were prepared containing gentamycin sulfate as drug [125]. Vyas et al., [126] demonstrated 2.48 times increase in the ocular bioavailability of timolol maleate in the form of niosome as compared to timolol maleate solution. The Carbopol or Chitosan coated niosome of timolol maleate has achieved the desired effect in just half concentration up to 8 h [127]. Niosome has been proved as cogent ocular vehicle for cyclopentolate [128]. The *in vivo* study showed that the niosomes were independent of their pH, significantly improved the ocular bioavailability of cyclopentolate, with respect to reference buffer solution.

Niosome in the delivery of Peptide Drugs

Enzymatic degradation is main culprit when one considered oral peptide drug delivery. Engulfment in niosomes to successfully protect it after oral administration is being investigated. In an *in vitro* study conducted by Yoshida et al, [55]

for the oral delivery of a vasopressin derivative using niosome demonstrated fair bit of peptide stability. Niosomes made up of Brij92 and cholesterol (7:3 ratio) with entrapped insulin showed high degree of *in vitro* stability against proteolytic activity of α -chymotrypsin, trypsin and pepsin [22]. Niosomes composed of various sorbitan monoesters as surfactant were grafted using the film hydration method without sonication for insulin delivery. The quantitative insulin released from Span 40 and 60 was lower than Span 20 and 80 vesicles while one containing Span 60 showed the highest protection of insulin against proteolytic enzymes in simulated intestinal fluid and good stability in the presence of sodium desoxycholate at storage temperature[129]. Cationic niosomes grafted with PEG were used to improve the stability and cellular delivery of oligonucleotides[130]. The n-terminal tat-GFP fusion protein loaded in elastic niosomes have enhanced the cellular uptake and chemical stability of the peptide[131]. Hence ought to be an useful tool for efficient delivery of many therapeutic proteins. Bacitracin (BCT), insulin and bovine serum albumin (BSA) are some of the charge peptide molecules. With modified niosomal composition with respect to charge they can be efficiently given[132]. Sustained release luteinizing hormone releasing hormone polyhedral niosomes were formulated from Hexadecyl diglycerol ether, cholesterol, and poly-24-oxyethylene cholesteryl ether (Solulan C24) in the ratio 91:0:9.[133]

Niosome for nasal and pulmonary route

Drug delivery appears to be quite critical when it comes to nasal and pulmonary route. As a matter of fact, the large surface area of the alveolar region attracts formulation scientist to nurture vesicular delivery for it[134]. Proniosomes of the anti-asthma steroid beclomethasone dipropionate were developed to generate niosomes that were suitable for aerosolization by either air-jet or vibrating-mesh nebulization methods[135]. Priperm *et al.*, [136] prepared melatonin encapsulated niosomes composed of Span 60/CHOL/sodium deoxycholate. Intranasally administered nanovesicles could distribute melatonin to the liver, hypothalamus and testis of male rats. Ciprofloxacin and norfloxacin niosomes were prepared for sustain intranasal delivery with improved stability. Although the systemic availability of these niosome-encapsulated antibacterial compounds was not increased after nasal administration, intestinal absorption was significantly higher in

comparison with that of plain inclusion complexes^[137]. Controlled release proniosome derived niosomes, using sucrose stearates as non-ionic biocompatible surfactants for the nebulisable delivery of cromolyn sodium have been prepared and evaluated ^[73]. Anti-tuberculosis drugs were incorporated in niosomes for pulmonary delivery. High encapsulation efficiency was obtained and should an advantage to solve the problem of multi-drug resistance in case of tuberculosis ^[138]. Ammonium glycyrrhizinate was formulated as bola-niosome which showed improvement of the in vivo anti-inflammatory activity. ^[139] Triton X 100 niosomes containing anti-tubercular drugs (Rifampicin, Isoniazid and pyrazinamide) were studied. Rifampicin and isoniazid had release by fickian diffusion and pyrazinamide had non-Fickian release mechanism.^[140]

Niosome in drug Targeting

The concept of drug targeting is grafted for concentrating the drug in the tissues with minimal dose. This localized drug action doesn't affect surrounding tissues ^[21]. With the advancement of biotechnology and genetic engineering an emphasis has been made to effectively deliver these biologicals at targeted site ^[73].

To reticulo-endothelial system (RES)

The cells of RES preferentially take up the vesicles. The uptake of niosomes by the cells is also by circulating serum factors known as opsonins, which mark them for clearance. Such localized drug accumulation has, however, been exploited in treatment of animal tumors known to metastasize to the liver and spleen and in parasitic infestation of liver ^[141]. Niosomal haemoglobin circulate within the blood which is permeable to oxygen and act as effective carrier for anemic patients ^[56, 57].

To Brain and Tumor

An alternative to developing new drugs is to focus on delivering drugs of potential therapeutic value to the target site. The brain is one of the organs where the targeting of drugs is difficult. Transport of drugs from circulating blood into the central nervous system (CNS) is restricted by the blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier, which are formed by tight junctions connecting the cerebral endothelial and epithelial cells of the choroid plexus, respectively. Several strategies have been developed to circumvent the BBB, of which

chemical delivery Systems and novel delivery systems are the most important ^[142]. Dufes et al., ^[143] niosomes bearing glucose or transferrin ligands for drug targeting. Glucose bearing vesicles bind Con-A to their surface. Chitosan based vesicles are taken up by A431 cells and transferrin enhances this uptake. The proportion of FITC-dextran positive A431 cells was 42% (FITC-dextran solution), 74% (plain vesicles) and 90% (transferrin vesicles). Doxorubicin brain targeted niosomal formulation functionalized with the glucose-derivative N-palmitoylglucosamine (NPG) was developed by Bragagni and colleagues with doxorubicin brain concentration of $2.9 \pm 0.4 \mu\text{g/g}$ in an hour ^[141]. Doxorubicin shows a dose dependant irreversible cardio toxic effect used in cancer treatment. Niosomal delivery of this drug to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma ^[144]. Intravenous administration of methotrexate entrapped in niosomes to S-180 tumor bearing mice resulted in total regression of tumor and also higher plasma level and slower elimination ^[145]. Dimethylsuberimidate containing doxorubicin niosome with transferrin covalently bound to the surface showed higher uptake and cytotoxicity as compared to glucose targeted niosomes of Npalmitoyl glucosamine also the effect of cholesterol was studied which resulted in more sustained release and reduced tumor growth in presence of cholesterol.^[146] A glucose bearing niosome was prepared as a brain targeted delivery to vasoactive intestinal peptide in mice ^[147]. It provide a new horizon for targeted delivery to brain. Cisplatin-loaded niosomes (CP-NMs) were prepared under optimized conditions with Span 40 and cholesterol as the excipients, and then lyophilized and characterized. Their anticancer efficacy was tested in rabbits bearing VX2 sarcoma. The obtained spherical-looking vesicles showed a diameter of $7.73 \pm 1.49 \mu\text{m}$ with a zeta-potential of 0 mV. The entrapment efficiency was $76.93 \pm 2.67\%$, and drug loading $2.96 \pm 0.10\%$. In vitro release tests gave a $t_{50\%}$ of 8.36 h. The rabbits locally injected with the CP-NMs gave significantly superior results of inhibition of tumour growth, much lower mortality and improved results of body weight change and inhibition of tumour metastasis to inguinal lymph nodes and liver compared to those treated in the same way with the drug solution ^[148]. Niosomal plumbagin was less toxic than free drug with improved anti-tumor activity evaluated in solid tumour (sarcoma-180) and Ehrlich ascites model ^[149]. Encapsulation of

Vincristine Sulfate has been done in niosomes improved Anticancer Activity with Reduced Toxicity in Mice [150]. Cytarabine hydrochloride niosome showed physical stability up to 4 weeks. The drug entrapment efficiency was about 80% with Tween 80, Span 60 and Tween 20 which was 67.5% for span 60 [151]. A study carried out by Raja and co-workers, provided quantitative bleomycin delivery to the tumor using niosome which is possible after macrophage activation [152]. Paclitaxel niosomes with negative charge showed sustained release with reduced side effects after oral administration. [153] 5-fluorouracil (5-FU) used for the treatment of actinic keratosis and non-melanoma skin cancer. Its biggest drawback, poor percutaneous permeation has been improved by encapsulating it in niosome. [154] Reduced photo degradation of adriamycin has been achieved through niosome with delayed growth of tumor volume in human lung tumor cells, monolayered and spheroid cultures and in xenografted nude mice. [155] Combination of the stealth action and active targeting function of polyethylene glycol cyanoacrylate-co-hexadecyl cyanoacrylate (PEG-PHDCa) and transferrin (Tf) containing niosome of hydroxycamptothecin was used to promote drug delivery to solid tumor. [73]

To other organs

Antibodies can be used as pathfinder for a specific site when conjugated with niosome [156]. These seem to bind on niosomal surface, thus offering a convenient means for targeting of drug carrier [157]. Many cells have the intrinsic ability to recognize and bind particular carbohydrate determinants (paratops) and this can be exploited to direct carriers system to particular cells. Leishmaniasis is a parasitic disease of the liver and spleen. Antimonials are the most common treatment choice, which in higher concentrations can cause cardiac, liver and kidney damage. The study of niosomal antimonial distribution in mice, performed by Hunter et al showed high liver level after intravenous administration of the carriers forms of the drug [158]. Amarogentin, a secoiridoid glycoside, [159] harmine, [160] Paromomycin, [161] Bacopasaponin C, [162] quercetin [163] and 14-deoxy-11-oxo-andrographolide [164] have been evaluated for its antileishmanial property using niosomes. Fe-deferrioxamine trioxethylene cholesterol niosomes for i.v. use were prepared which demonstrate greater distribution in liver and spleen. Niosomal doxorubicin reduces its cardiac toxicity upon i.v. administration [165]. Niosome has improved oral bioavailability of

gliclazide with sustained action over a period of 24 hours for better therapeutic efficacy. The high values of zeta potential indicate stabilization of niosomes by electrostatic repulsive forces [166]. The anti-fertility effect of cantchroman was enhanced by incorporation into niosomes with 83.3% protection against pregnancy shown by *in vivo* studies [167].

Niosome for Diagnosis

Not only in therapeutics but a good amount of success has been cherished in diagnostic field since last decade. Antimetastatic activity in experimental metastatic model of B16F10 melanoma was checked using cisplatin niosome with Theophylline and with Activated Macrophages [6]. Erdogan et al., [168] prepared positive charged, ¹³¹I labeled iopromide niosomes (gel or liquid crystal) so as to enhance contrast during CT in rats. Kidneys is the major site for its accumulation with activity up to a day. In another study, Korkmaz et al., [169] used ^{99m}Tc labeled DTPA containing niosomes for spleen and liver targeting with improved stability to light, temperature and oxidation. C₁₆G₃ niosomes containing cholesterol and stearylamine encapsulating the radio-opaque agent iopromide were found to concentrate in the kidneys on intravenous administration. Iobitridol niosomes were formulated as diagnostic agent with improved stability used for X-ray imaging. [170]

Niosome for Cosmetics

Lancome and L'Oreal (Paris) have come out with many anti-ageing cosmetic products [171]. The oil spreads uniformly over the surface of the skin; vesicles penetrate the stratum corneum in fractionated form while the water of continuous phase evaporates which gives a special sensation to touch, freshness, even essence, hydration and a feeling of protection because of the oily film. If the envelope is made of sphingolipids, vesicles are named sphingosomes [172]. Ellagic acid is a BCS class IV solute which is a potent antioxidant. Ellagic acid niosomes with added solubilizers enhance its permeation through the skin [173]. Androgenic alopecia has been treated with finasteride niosomes for successful delivery to the pilosebaceous unit [174]. Elastic niosome of gallic acid showed greater promise for topical anti-ageing application [175]. N-acetyl glucosamine (NAG) niosomes have been prepared in the treatment of hyperpigmentation disorders due to its inhibitory effect on tyrosinase enzymes in melanocytes and good skin penetration ability [176].

CONCLUSION

Since a last decade the vesicular delivery has just enhanced the other drug delivery vehicles. A huge amount of researches have been done in this field. All the formulation aspects of niosomal preparations are given in chronological manner with copula elements and some acid facts with respect to drug delivery. Well it is worth to say that it is still greatly in its infancy yet it is showing great promise in the fields of cancer and infectious disease treatments. The system is well established for cosmetic products which are also described in this manuscript. It certainly has provide potential avenues for future research in drug delivery technology.

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