ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

NIOSOMES: A PROMISING NANOCARRIER APPROACH FOR DRUG DELIVERY

Aarti Tawani, Gitanjali Chavan, Shraddha Vedpathak, Ritu Chakole, Manoj Charde*

Post Graduate Department of Pharmaceutics, Government College of Pharmacy, Vidyanagar, Karad, Dist. Satara, Maharashtra, India

**Corresponding author: manojudps@rediffmail.com*

ABSTRACT

Niosome is a novel, vesicular drug delivery system meant to deliver drug in a controlled manner to increase its bioavailability, therapeutic efficacy and duration of action thereby minimizing side-effects. Niosomes are structurally similar to liposomes. However, the bilayer of niosomes is made of Non-ionic surfactants rather than phospholipids. Niosomes offer several advantages over conventional drug therapy, but niosomes also have some serious challenges. This paper overviews the types of niosomes, its formulation, effect of individual components on niosomes, various methods of preparation, evaluation and its applications. Finally, patents on niosomal formulations are also discussed.

Keywords: Niosomes, Vesicular system, Nanocarriers, Drug delivery, Non-ionic surfactants.

1. INTRODUCTION

The growth of nanoparticle drug carriers is motivated by the need to diversify methods of administration and distribution and secure delicate pharmaceutical compounds from harsh environments. Traditional, non-invasive drug delivery routes are restricted in their usefulness, such as oral or transdermal. The harsh environments of digestive system denature or degrade many biochemical formulations. Topical (transdermal) formulations are user-friendly, but owing to the low permeability of the dermal membrane, they yield limited dosages and distributed delivery. Research activities have centered in recent decades on the production of new drug delivery carriers that encapsulate and shield pharmaceuticals from the environment and improving treatment results by treating particular types of tissue [1]. Based on the type of molecules that make up their bilayer membranes, vesicle drug carriers are classified into three categories: liposomes, where the shell includes phospholipids, polymersomes, where synthetic block copolymers are formed, and niosomes, formed by non-ionic bilayers [1]. As a novel drug delivery system, niosomes entrap the hydrophilic drug in the central cavity and hydrophobic drugs in the non-polar region found in the bilayer, so both hydrophilic and hydrophobic drugs can be integrated into niosomes. They are amphiphilic in nature, in which the drug is encapsulated in a vesicle formed by non-ionic surfactant and hence the name niosomes. The scale of the niosomes is tiny and microscopic [2]. It is possible to prescribe niosomes via different pathways, such as oral, parenteral, topical. Niosomes are used to carry various types of medicines such as natural, antigens, hormones and other bioactive compounds as a carrier. Niosomes were taken to the spotlight by the cosmetic giant L'Oreal. Niosomes then made their transition to pharmaceutical industry as possible distribution mechanisms to distribute medicines via diverse pathways including oral, buccal, dermal, in the pharmaceutical field [3].

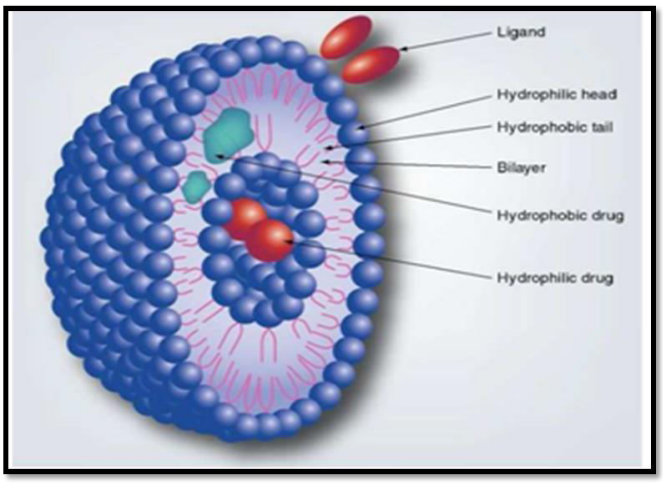


Fig. 1: Structure of Niosome [6]

Due to their ability to form bilayer vesicles such as liposomes, niosomes have created enough interest; however, for different reasons, niosomes are a more desirable alternative than liposomes for drug delivery.

Owing to their predisposition to oxidative degradation and variable purity of phospholipids, chemical instability of liposomes is induced. Chemical stability, biodegradability, biocompatibility, chemical stability, low manufacturing costs, fast storing and handling and low toxicity are the main goals of designing the niosomal system [4, 5].

1.1. Advantages of Niosomes [3, 6]

- Niosomes can serve as a carrier for medicines with a wide range of solubility, as both hydrophilic and hydrophobic components are present.
- Niosomes are bio-degradable, biocompatible, non-immunogenic and nontoxic.
- Niosomes can also be used as a depot formulation.
- Greater patient compliance than oil-based formulations.
- Niosomes not only have good stability, but also have an osmotic property that makes them superior to formulations based on oil and also improves the efficiency of trapping.
- Niosomes are known to be flexible since oral, parenteral and topical pathways can be provided to them.
- Niosomes are suggested to penetrate through the cornea, i.e., it can be used to distribute ocular drugs.
- The stability of the encapsulated drug can be improved by niosomes.
- Niosomes can enhance the drug penetration through the skin.
- The ability of niosomes to overcome BBB and provide the brain with drugs.
- Through surface modification and limiting effects on target cells, they improve the therapeutic performance of the drug, thereby reducing the clearance of the drug.
- Niosomes can enhance the oral bioavailability of drugs.
- The vesicle formulation characteristics, such as scale, lamellarity, surface charge, concentration, and drug sting are controllable.
- No special requirements are needed for the handling, storage and preparation of niosomes.
- Simple methods for the production and large-scale production of niosomes are possible.
- In contrast to liposomes, niosomes are osmotically active, chemical stable and have a long storage time.
- By shielding the medication from the biological environment, they may enhance the therapeutic performance of the drug molecules, resulting in improved accessibility and regulated drug delivery by limiting the drug effects on target cells in targeted carriers and delaying circulation clearance in sustained drug delivery.
- Niosomes can be used for immediate, controlled or sustained release of drug.

1.2. Disadvantages of Niosomes: [7, 8]

- Restricted shelf life due to merging, aggregation, leakage of trapped drugs, and hydrolysis of encapsulated drugs is due to niosomal aqueous suspensions.
- The techniques involved in the niosomal formulation, such as extrusion, sonication, take time and require specialized processing equipment.
- The aqueous suspension of niosomes may have short shelf life due to aggregation, leakage and encapsulated medication hydrolysis.
- Heat sterilization cannot be carried out for niosomal system.
- Multilamellar vesicle preparations are time consuming and require separate instruments.

1.3. Comparison of Liposomes and Niosomes

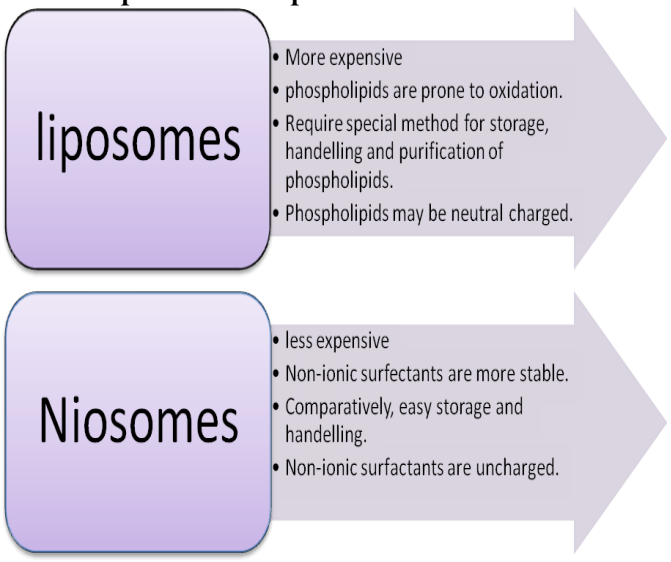


Fig. 2: Difference between liposomes and niosomes [9]

2. TYPES OF NIOSOMES: [3, 7, 9]

- Classification based on number and size

2.1. Multilamellar Vesicles (MLVs)

MLVs are produced individually from certain bilayers adjacent to the aqueous lipid segment. The measurements of these vesicles are calculated to be between 100 and 1000 nm in diameter. Because of easy preparation, multilamellar vesicles are re-excessively stable when maintained for prolonged phases and are commonly used for lipophilic agents.

2.1.1. Large Unilamellar Vesicles (LUVs)

The diameter of these vesicles is approximately 100-250 nm in size. LUV has a high aqueous portion of the lipid segment, so that membrane lipids can absorb bioactive resources.

2.1.2. Small Vesicles Unilamellar (SUVs)

Small unilamellar vesicles have an estimated size of 10-100 nm. Several processes, such as sonication, high-pressure homogenization, and extrusion techniques, consist of small unilamellar vesicles.

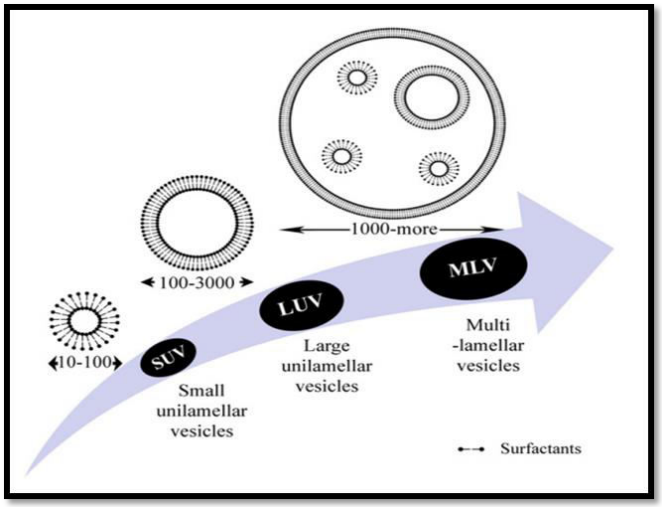


Fig. 3: Structure of SUV, LUV, MUV [10]

2.2. Other types

2.2.1. Bola-surfactant-Containing Niosome

Bola-surfactant compounds need two hydrophilic heads for these kinds of niosomes, which can be connected by one or two long lipophilic spacers. The use of surfactants in niosome-containing bola-surfactants is prepared from omega hexadecyl-bis-(1-aza-18 crown-6) (bola surfactant): span-80/cholesterol 2:3:1 molar percentage.

2.2.2. Proniosomes

Proniosomes are the aggregation of niosomes consisting of carriers and surfactants that are water soluble. The proniosomes are structures of dehydrated niosomes that would have been hydrated for earlier use. Proniosomes can minimize problems with niosomes, such as accumulation, fusion, and leakage of medication after a while.

2.2.3. Aspasome

Cholesterol, ascorbylpalmitate and highly charged lipids such as dihexadecyl phosphate are found in Apsasome (DCP). It is hydrated by water solvent and sonicated to create the final product. The transdermal drug delivery systems can be improved by Aspasome and the conditions created by the use of reactive oxygen species can be minimized.

2.2.4. Discomes

There is low cholesterol concentration in large disk-shaped structures or discomes. Niosomes have been reported to be prepared at 75°C for 1 h from incubation in cholesteryl poly-24-oxyethylene ether (Solulan C24) to obtain spherical niosomes. This contributed to approximately 11-60 μm and multilayered vesicular structures in the construction of large sizes.

2.2.5. Elastic Niosomes

This type of niosomes can be versatile in the absence of destructive construction, so they have the potential to allow smaller pores from side to side. Such vesicles include surfactants, water, and ethanol that are nonionic. Using this versatile structure, intact skin layers may increase penetration.

2.2.6. Polyhedral Niosomes

The hexadecyldiglycerol ether (C16G2) creates this form of niosome, replacing it with either of the nonionic surfactants and the polyoxyethylene 24 cholesterol ether (C24) without cholesterol. Such vesicles have unconventional structures that can trap particles that are water-soluble.

2.2.7. Vesicles in Water and Oil System (V/W/O)

Vesicles in the water and oil system contain emulsion (v/w/o) niosomes in the water in the oil (as the outer phase). The suspension of niosomes from the blend of sorbitol monostearate, cholesterol, and solulan C24 (poly24-oxyethylene cholesteryl ether) to the oil process at 60°C is the product of this phenomenon. This results in the formation of vesicles using room temperature cooling to form vesicles in oil gel emulsion water (v/w/o) Water (v/w/o gel) This results in the

formation of vesicles in oil (v/w/o) emulsion. This form of niosome has been recruited for the delivery of protein drugs and defense against enzymatic degradation following oral administration and controlled release.

2.2.8. Carbopol Gel Niosomes

Niosomes from the compound, nonionic surfactant, and cholesterol is prepared in this system; then, carbopol-934 gel (percent 1 w/w) is combined with a base consisting of propylene glycol (percent 10 w/w) and glycerol (percent 30 w/w).

3. FORMULATION OF NIOSOMES

Lipids such as cholesterol, nonionic surfactants and charge imparting agents are the essential components of niosomes.

3.1. Cholesterol

Cholesterol is an amphiphilic molecule; it arranges itself such that OH group is towards the aqueous phase and the aliphatic chain is towards the hydrocarbon chain of the surfactant, thereby contributing to the formation of hydrogen bond with non-ionic surfactant [3]. To provide rigidity, cholesterol is added to the non-ionic surfactants. Cholesterol is used to adapt the niosomes to deliver unbending nature and suitable shape. By banning gel to liquid phase transition, cholesterol is also known to prevent leakage. It also improves the entrapment efficacy of Niosomes [9, 11]. The proportion of cholesterol in niosomes affects the structures of niosomes and the efficiency of drug entrapment, time circulation, and payload release. According to previous research, it has been found that the usage and quantity of cholesterol in niosomes must be balanced according to the physical and chemical properties of surfactants and the type of medicine [9].

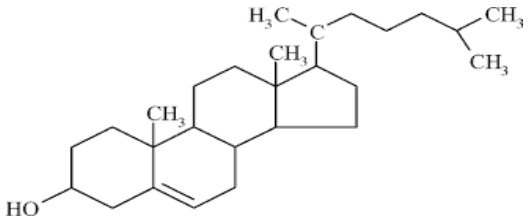


Fig. 4: Structure of cholesterol [8, 12]

The disparity in the rate of release is due to the change in the quantity of cholesterol in various niosomes. The amount of cholesterol needed in a specific formulation depends on the surfactant's HLB value; if the value is greater than 6, it is essential to use cholesterol to shape niosomes.

For Span niosomes, due to their low HLB values, span 60 and 80 can create niosomes requiring little or no addition of cholesterol. With a higher HLB, Tween 60 needs more cholesterol to preserve rigidity of the membrane [12].

3.2. Non-ionic surfactant:

Surfactants are amphiphilic molecules with two very distinct solubility regions, hydrophilic head group (polar) and hydrophobic tail (non-polar). Ether, amide or ester bonds may connect the two portions of such molecules. Surfactants can be categorized as anionic, cationic, amphoteric, and non-ionic according to their hydrophilic head group functionality [10, 13]. In controlled, sustained, targeted and continuous drug delivery, non-ionic surfactants are absolutely one of the smartest polymeric nanocarriers with a large function. A non-ionic surfactant has no charge in its head. Compared to anionic, cationic and amphoteric surfactants, they are therefore more stable, compliant, and less toxic. Such surface-active agents cause cellular surfaces to cause less haemolysis and irritation. These can be used as wetting agents and emulsifiers to increase permeability and to improve solubility [8].

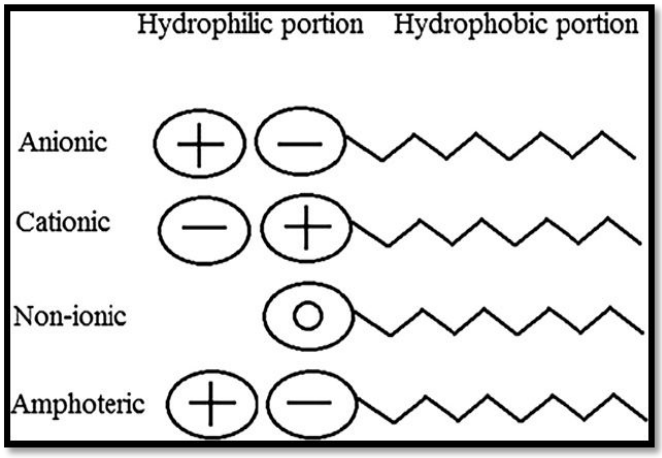


Fig. 5: Types of Surfactants [12]

Non-ionic surfactants inhibit p-glycoprotein, which can improve the absorption and targeting [8] of anticancer drugs such as doxorubicin, daunorubicin, curcumin, and morusin [14-17], steroids such as Hydrocortisone [18], HIV protease inhibitors such as ritonavir [19], and cardiovascular drugs such as digoxin, beta-blockers [20]. The hydrophilic-lipophilic balance (HLB) and critical

packing parameter (CPP) values govern the selection of surfactant. Therefore, as the HLB value increases, the alkyl chain increases, thereby increasing the size of niosomes. (HLB rate 14-17 is also not acceptable for the formulation of niosomes. HLB values 8 shows highest entrapment efficiency [3, 8, 20].

The surfactant's structure played a major role in the stabilization and inhibition of vesicle aggregation of niosomes by repulsion of steric or electrostatic force.

The impact of the structure of the surfactant on the formation of niosomes is explained by the critical packing parameter (CPP) that describes the following equation:

$CPP = V/A_o l_c$

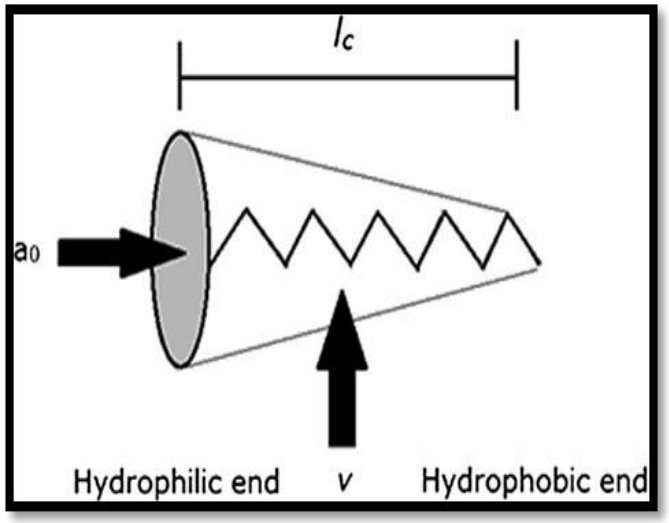
CPP is the critical packing parameter, V is the hydrophobic group volume, l_c is the critical hydrophobic group length, and A_o is the area of the hydrophilic head group. The type of micellar structure was predicted by the critical packing parameter value as assumed:

If CPP <1/2 formation of spherical micelles

If 1/2 < CPP <1 formation of bilayer micelles

If CPP >1 formation of inverted micelles [9, 10, 12]

The surfactant's phase transition temperature (TC) is one of the significant parameters that have a direct effect on the entrapment efficiency. The highest entrapment efficiency is shown by Span-60, as an example of surfactants with high TC [10, 20, 21].



V is the hydrophobic group volume, lc is the hydrophobic group length, and Ao is the area of the hydrophilic head group [12]

Fig. 6: Schematic structure of a single-chain surfactant

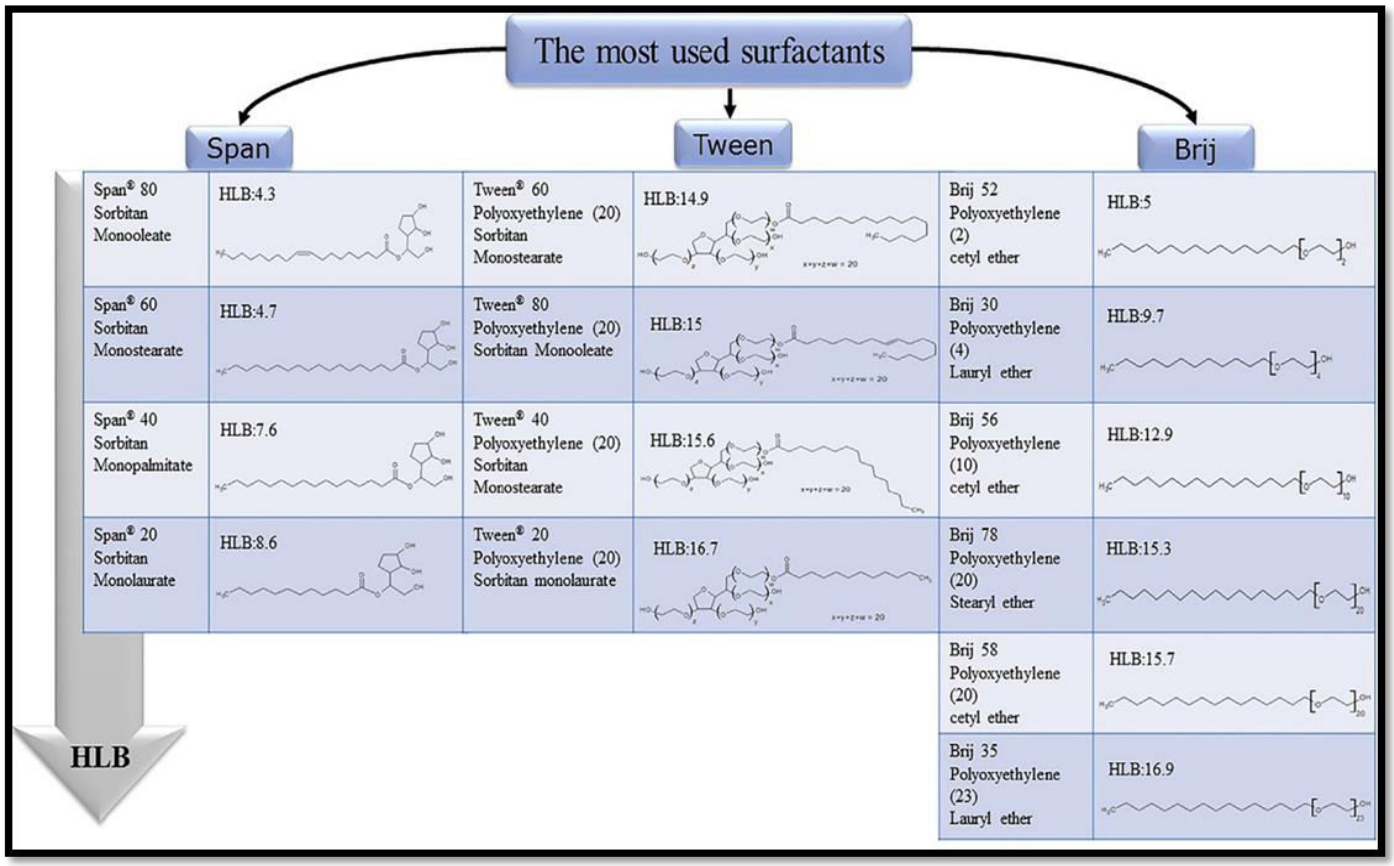


Fig. 7: Commonly used surfactants in niosomes [12]

Spans (sorbitan fatty acid esters) and tweens (polyoxyethylene fatty acid esters) are the most commonly used non-ionic surfactants in niosomal formulations. Alkyl ether type surfactants such as brij surfactants are also used. Another type of non-ionic surfactants are sucrose esters (SEs); they have a sugar substituent, sucrose, as the polar head, and fatty acids as non-polar groups. Since sucrose comprises eight hydroxyl groups, it is possible to manufacture compounds ranging from sucrose monoesters to octaesters. These esters contain distinct fatty acids in various ratios (stearic, palmitic, myristic and lauric acid). The HLB value and the melting point of these materials are determined by the fatty acid type and the degree of esterification. They are used as emulsifiers, solubilizing agents, lubricants, penetration enhancers and pore forming agents in many areas of pharmaceutical and cosmetic technology. SEs has drawn a global attention. Because of their high safety and excellent properties; they are approved as food additives by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), Japan, the USA and Europe. Sucrose stearate and sucrose palmitate are approved by the Food and Drug Administration as inactive ingredients and listed in the Inactive Ingredients Database for oral dosage forms and sucrose distearate and sucrose polyesters are listed for administration by topical route [22]. Most applications of SEs are found in transdermal drug delivery although they are also being analyzed for oral antigen and sucrose stearate administration. Pulmonary delivery of cromolyn sodium has been studied for baseline proniosomes [23]. The newer generations of surfactants that have been synthesised to generate niosomes with optimal properties are bola and Gemini surfactants [12, 24].

Table 1: **Non-ionic surfactants used for formation of niosomes [8, 12, 25]**

Non-ionic surfactant	HLB Value	Physicochemical properties	Preferable use	Drawback
Sorbitan fatty acid Esters	4-8	Less leaky vesicles with high entrapment efficiency. Non-toxic, non-irritating.	In cosmetics as solubilizer of essential oils in water-based products.	The molar ratio of cholesterol to Span may affect the entrapment of drugs into niosomes.
Polyoxyethylene fatty acid esters	9-15	derived from fatty acid esterified ethoxylated sorbitans. Non-toxic, non-irritating.	Usually, Tween 20, 40, 60 and 80 are used.	-
Alkyl ethers and alkyl glyceryl ethers		High stability	To encapsulate proteins and peptides	Decrease entrapment efficiency when used with cholesterol
Poly oxy ethylene 4 lauryl ether (Brij 30)	9.7	TC below 10°C	Forms large unilamellar vesicles	Causes oxidation with some medications leading to discoloration.
Poly oxy ethylene acetyl ethers (Brij 58)	15.7	Ability to form inverted vesicles due to its large head group.	Useful for studying ion-pumping activity (Hþ-ATPase and Ca2þ-ATPase) at the plasma membrane.	-
Poly oxy ethylene fatty acid esters (Brij 72)	4.7	Ability to forms multi-lamellar vesicles with high encapsulation efficiency	For vesicle formation having high encapsulation efficiency	-
Sucrose esters	1-16	low toxicity and less hemolytic, biocompatible	Mostly for transdermal drug delivery	-
Gemini surfactants	6-9	Lower CMC value, non toxic, more stable, non irritating non hemolytic	Personal care and pharmaceutical formulations	-

Bola Surfactants	9.7	Higher solubility, higher CMC, lower aggregation number	Personal care and pharmaceutical formulations	-
Tyloxapol	12.9	composed of ethylene oxide and formal-dehyde and forms niosomes in water,	ophthalmic preparations and as a mucolytic agent for pulmonary diseases	-
Polysorbate	14.9-16.7	Stabilizer, lower CMC, good water solubility	Used in injections, vaccines	Harmful to persons with Crohn's disease

3.3. Charge imparting agents:

Charge imparting agents may be positive or negative charge inducers. By producing charges on the surface of the prepared vesicles, the stability of the vesicles is enhanced. It works by preventing vesicle aggregation and coalescence due to repulsive forces of the same charge and by providing higher zeta potential values [24]. Stearylamine and cetylpyridinium chloride are the most widely used positive charge inducers, and dicetyl phosphate, dihexadecyl phosphate, phosphatidic acid and lipoamine acid are negative charge inducers [26].Molar concentration of charged molecules 2.5-5 percent is appropriate as high concentration will prevent the development of niosomes [27, 28].

4. FACTORS AFFECTING PHYSICOCHEMICAL PROPERTIES OF NIOSOMES:

4.1. Type of surfactant

Etherlinked surfactants are more chemically stable but toxic than esterlinked surfactants because ester bonds are broken to triglyceride and fatty acids by esterases [3,8,29].

It is the surfactant's HLB, gel transition temperature and CPP that affects the type of vesicle that will be formed i n a process [8].

Table 2: Effect of surfactant HLB on Niosome [21, 25, 30-32]

HLB VALUE	IMPACT ON NIOSOME FORMULATION
14-16	Does not produce niosomes
8.6	Increase in entrapment efficiency
1.7-8.6	Decrease in entrapment efficiency
>6	Cholesterol addition for formation of bilayer vesicle
Lower value	Cholesterol addition to increase stability

As HLB of surfactant increases such as Span 85 (HLB 1.8) to Span 20 (HLB 8.6), the mean size of niosomes increases proportionally, since surface free energy reduces due to increase in surfactant hydrophobicity [3,33]. Entrapment efficiency of niosomes is also affected by Phase transition temperature (TC) of surfactant, for example Span 60 has higher TC thus it provides better entrapment efficiency [3,33]. Alkyl chain length surfactants from C12-C18 are sufficient for the preparation of niosomes. Surfactants like C16EO5 (poly-oxyethylenecetyl ether) or C18EO5 (polyoxy-ethylenesteryl ether) are used to make polyhedral vesicles. Span surfactants with HLB between 4 to 8 can form Niosomes [3, 33].

4.2. Nature of encapsulated drug

The drug entrapment is influenced by drug properties such as molecular weight, structural characteristics, hydrophilic or lipophilic character and balance between the two [34]. The size of the vesicle can increase due to the interaction of drugs and surfactants [20, 21, 35, 36].

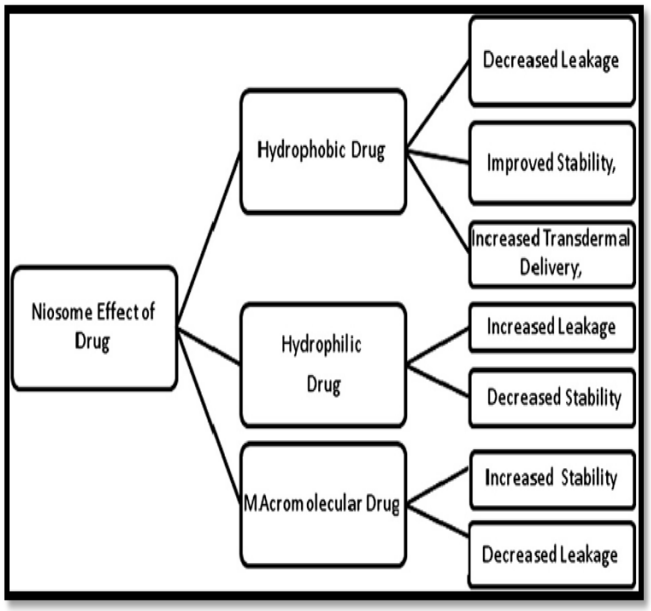


Fig. 8: Effect of drug on Niosomes [25]

Previously, it was stated in some of the researches that a hydrophilic drug's maximum trapping efficiency in niosomes could be 10-20 percent [37-39] But Manosroi et al. mentioned that up to 45 percent entrapment can be achieved due to the interaction between negatively charged niosome and positively charged hydrophilic drug gallidermin in anionic niosomes [40].

Marinecci prepared hydrophobic glucocorticoid niosomes for pulmonary delivery. High loading drug concentration reduces the entrapment efficiency owing to the interference due to high concentration of drug with vesicle formation [41]. Whereas lipophilic drug entrapment relies on the thermodynamic properties of the supramolecular aggregates, hydrophobic drug trapping in the bilayer occurs until the bilayer is saturated.

4.3. Temperature of hydration:

The shape and size of the Niosome are determined by hydration temperature. Niosomal system temperature shift influences the arrangement of surfactants into vesicles by which the alteration of vesicle shape may happen. The hydration temperature for niosome formation should preferably be above the system's gel to liquid phase transition temperature [8, 29, 42, 43].

4.4. Resistance to osmotic stress

The addition of a hypertonic salt solution to the niosomal suspension results in a decrease in diameter. There is initial slow release with mild vesicle swelling in hypotonic salt solution, possibly due to obstruction of eluting fluid from the vesicles, followed by faster release, which may be due to mechanical slackening of the vesicle framework under osmotic stress [3, 8, 44, 45, 46].

4.5. Surfactant / lipid volume:

The surfactant/lipid level that is necessary for niosomal formulation is usually maintained between 10-30 mM (1-2.5 percent w/w). Variation of the surfactant, water ratio during the hydration process affects the niosomal dispersion. The total amount of encapsulated drug also rises by raising the surfactant/lipid amount [8, 20, 25, 26, 29].

4.6. Cholesterol content

Due to interaction of cholesterol with nonionic surfactants, cholesterol may affect the physical properties and structure of niosomes. Cholesterol is present in biological membranes; here it influences membrane properties such as aggregation, ion permeability, elasticity, enzymatic activity, size and shape of niosome.

In lipid bilayers, the function of cholesterol is mainly to modify their cohesion and mechanical strength and their water permeability. The fluidity of niosomes is considerably changed by the addition of cholesterol. Cholesterol imparts vesicle rigidity, which is very significant under conditions of extreme stress. Cholesterol interacts with span 60 by hydrogen bonding within the niosome bilayer. The amount of cholesterol to be added depends on the surfactants' HLB value. In order to make up for larger head groups, as the HLB value increases above 10, it is important to raise the minimum amount of cholesterol to be added [25,47].

The addition of cholesterol to niosomal formulation enhances the entrapment of drug. It also increases the vesicles' hydrodynamic diameter. The chain order of liquid state bilayers is also increased by cholesterol and the chain order of gel state bilayers is decreased. By increasing the concentration of cholesterol, gel-state bilayers can be converted into a liquid state [29]. However, increasing cholesterol above a certain level decreases entrapment of drug which might be due to decrease in volume diameter ($CPP<0.05$) [25]. Bovine serum albumin niosomes were prepared using varying levels of cholesterol (0%, 20%, 40%,60%, 80%) by Moghassemi et al. He observed that adding 60% cholesterol gives best entrapment efficiency. Above this concentration, entrapment efficiency decreases [48].

4.7. pH of the hydration medium

Another factor that can affect drug's entrapment efficiency is the pH of the hydration medium. Flurbiprofen, for instance, at acidic pH, exhibits greater entrapment of drug (maximum 94.6 percent at pH 5.5) [8, 49].

4.8. Hydration time

Methylene blue niosomes were prepared using thin film hydration and later probe sonicated to achieve uniform sized vesicles by Yeo et al. The impact of hydration time and hydration volume on entrapment efficiency and vesicular size was studied. Short hydration time resulted in larger vesicles with less entrapment of drug. 60 minutes was found to be an ideal hydration time and 5 ml Volume of hydration for complete hydration of span 60 and vesicular formation according to them. Longer hydration time produces vesicles in small sizes [8, 50].

4.9. Method of preparation

The niosome preparation method can affect its size and efficiency of entrapment [43]. Abdelkader et al. [51] formulated naltrexone niosomes by thin-film hydration technique, freeze and thaw, dehydration-rehydration and reverse-phase evaporation methods. He stated in the results that the entrapment efficiency significantly depends on the method of preparation.

Abdelkader et al. [52] developed prednisolone ethoniosomes (ethanol-based niosomes) using thin film hydration and ethanol injection method. Small sized niosomes can be made by ethanol injection, reverse-phase evaporation, microfluidization method while niosomes made by thin film hydration, and transmembrane pH method shows better entrapment efficiency.

5. METHOD OF PREPARATION OF NIOSOMES:

5.1. Ether injection method

Slow injection of surfactant; cholesterol (150micro. mol.) is inserted into 20 ml ether via a 14-gauze needle (25 ml/min.) in a previously heated 4 ml aqueous phase maintained at 60°C. The ether solution was evaporated using a rotary evaporator, creating single layered vesicles after evaporation of the organic solvent [3, 9, 29, 25].

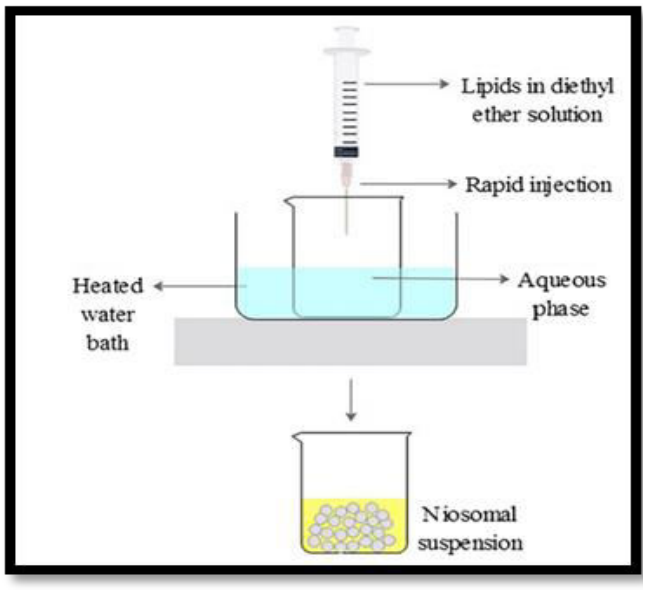


Fig. 9: Niosome preparation by ether injection method [12].

SUVs and LUVs formed by the solvent injection technique have high entrapped aqueous volume. The diameter of the final vesicle varies from 50 to 1000nm, depending on the conditions. EIM has been used for preparation of niosomes containing diclofenac sodium (DCS) [53], fluconazole [54], rifampicin [55], etc.

5.2. Hand shaking method/ Rotary evaporation method/ Thin film hydration method

Surfactant; cholesterol (150micro.mol.) was dissolved in organic solvent in an RBF. At room temperature, the organic solvent is evaporated under vacuum by using rotary evaporator to form a thin, dry film. The surfactant swells after hydration and is peeled into a film from the support. Swollen amphiphiles fold to form vesicles eventually. Only 5-10% liquid volume can be entrapped in such vesicles [3, 56].

HSM has been used for preparation of niosomes that entrap diclofenac sodium (DCS) [53] Zidovudine [57], benzyl penicillin [58], paclitaxel [59], gallidermin [60], prednisolone [61], tenofovir disoproxil fumarate [62], green tea extract [63], and lornoxicam [64] are prepared by film hydration technique [8,10].

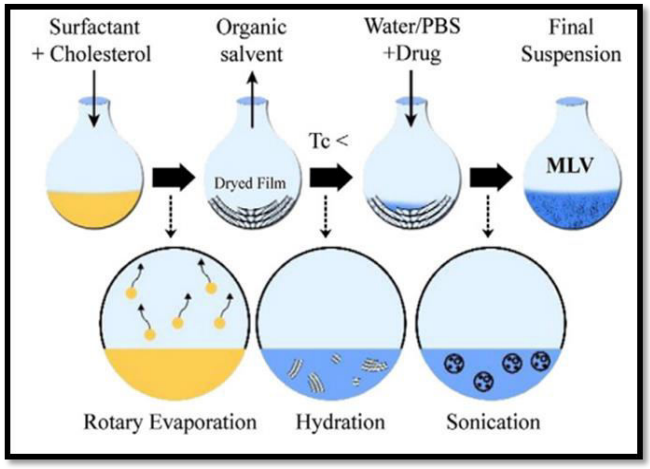


Fig. 10: Niosome preparation by thin-film hydration method [10].

5.3. Sonication

Baillie et al prepared niosomes using the sonication process in 1986. Surfactant; cholesterol (150micro. mol.) mixture was added in the vial in a 2ml aqueous phase in this system. For 3 minutes at 60°C, the dispersion is subjected to probe sonication. This technique involved the development of MLVs exposed to ultrasonic vibration. Probe and bath are the two types of sonicators. If the sample volume is small, probe sonicator is used and the bath sonicator is used when the sample volume is large [3, 9, 56]. Sonication method

has been used for preparation diallyl disulfide (DADS) loaded Niosomes [65], rifampicin and ceftriaxone dual drug loaded niosomes [66], cefdinir niosomes [67].

5.4. Multiple membrane Extrusion

In this process, C16G2, a chemically specified non-ionic surfactant by extrusion, was used to prepare niosomes by extrusion through a polycarbonate membrane. These experiments not only display the impact of the amount of extrusions on the size of the vesicles, but also the effect of the size on the drug's encapsulation [3, 56].

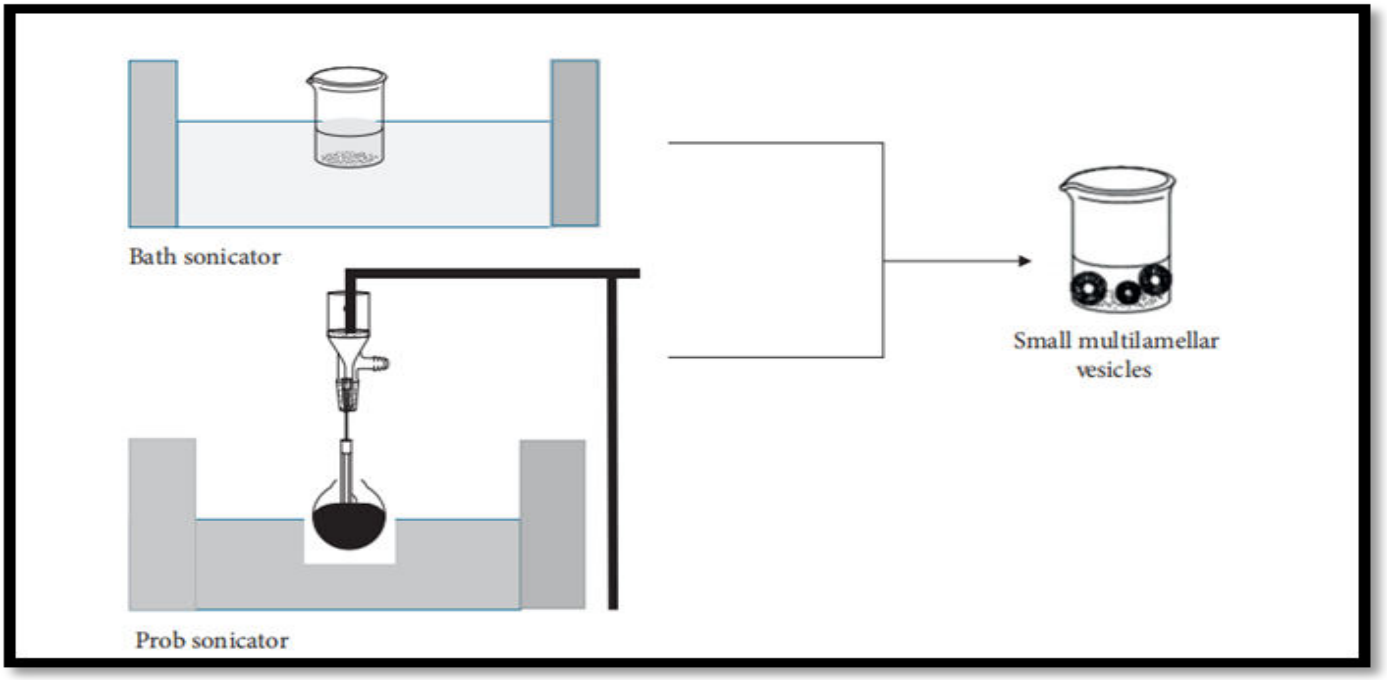


Fig. 11: Preparation of niosomes by sonication method [9]

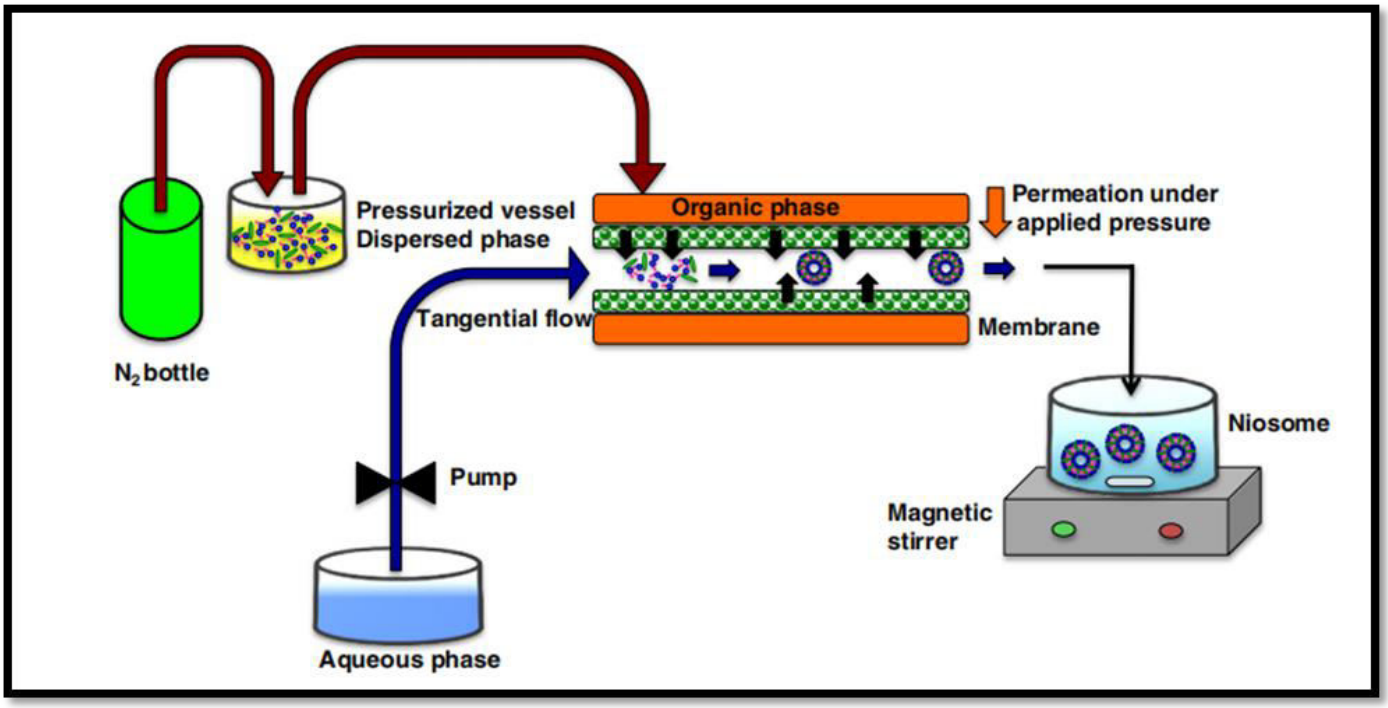


Fig. 12: Preparation of niosomes by membrane extrusion method [24]

5.5. Reverse phase evaporation

A definite cholesterol and surfactant ratio is dissolved in organic solvents such as ether or chloroform and evaporated to form a thin dried film under reduced pressure. By vortex mixing, the resulting film is hydrated with 300 mM of citric acid (pH 4.0).

Upon addition of phosphate buffer solution, the formed gel is further sonicated and the organic phase is eliminated at 40°C, which forms high viscosity niosomes, which are diluted with phosphate buffer [9, 25, 29, 68]. The reverse phase method has been reported to encapsulate large hydrophilic macromolecules with relatively higher EE than other methods [12, 69].

REV method has been used for the preparation of niosomes [8, 10] such as diclofenac sodium(DCS) [53], anti-HBsAg [70], naltrexone (NTX) [71], ellagic acid (EA) [72], acetazolamide (ACZ)[73], isoniazid [74] and bovine serum albumin [75] etc.

5.6. Bubble method

It is the one-step preparation of liposomes and niosomes without using organic solvents. To regulate the temperature, it consists of a round-bottomed flask with three necks, positioned in the water bath. The water-cooled reflux and thermometer are placed via the first and second neck while third neck is for nitrogen supply. Cholesterol and surfactant are added to buffer of pH 7.4 maintained at 70°C. A continuous stream of bubbles of nitrogen gas is introduced through the dispersion and niosomes are produced. By this method, large unilamellar vesicles are formed [3, 9, 10, 12, 29, 43].

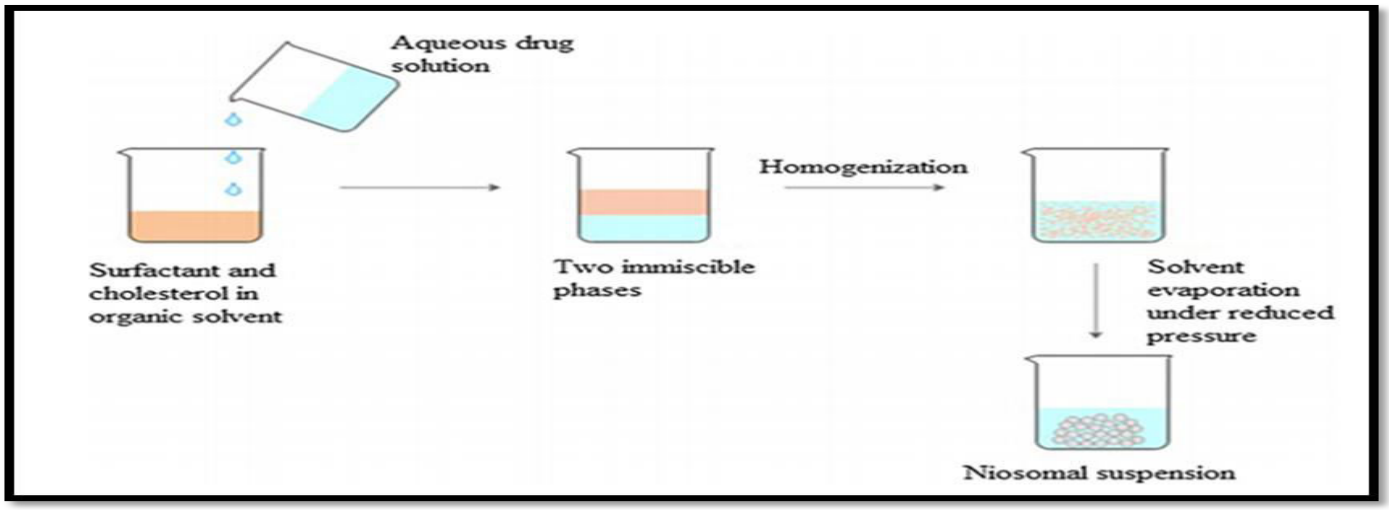


Fig. 13: Preparation of niosomes by reverse phase evaporation method [12]

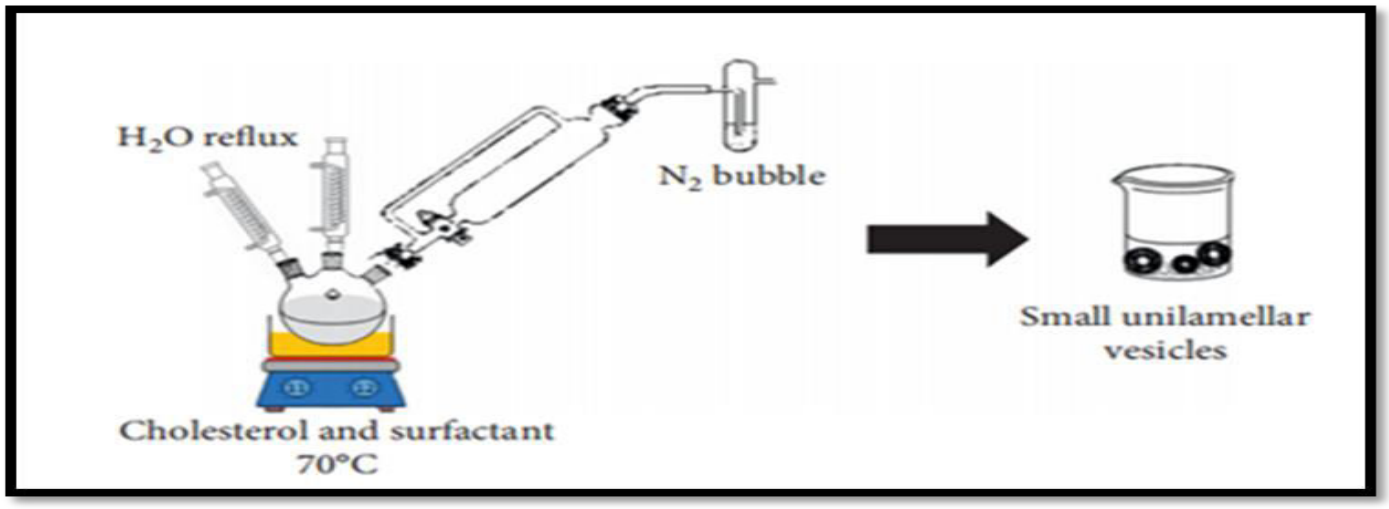


Fig. 14: Niosome formation by bubbling of nitrogen method [9]

5.7. Microfluidization method

The method of microfluidization is based on the concept of submerged jets. This process consists of an interaction chamber filled with ice, two fluidized streams (one containing surfactant and the other drug) interact at ultra-high velocities. To remove heat generated during this process, the fluid collected is passed through a cooling loop. Now, before vesicles of spherical dimensions are collected, fluid is recycled via the pump [7, 8, 12, 76]. This approach resulted in more uniformity, greater reproducibility and smaller size of unilamellar niosomes [77].

5.8. Transmembrane pH gradient method

A definite cholesterol and surfactant ratio is dissolved in organic solvents such as ether or chloroform and evaporated to form a thin dried film under reduced pressure. The resulting film is hydrated by vortex-mixing with 300 mM citric acid (pH 4.0). The vesicles undergo freezing and thawing for several times and are sonicated to get niosomes. Above niosomal suspension is transferred to an aqueous solution of the drug and is vortexed. With 1 M disodium phosphate, the niosomal suspension pH is modified to 7.0-7.2, heated to 60°C for 10 minutes to form the desired multilamellar vesicles [8, 9, 12, 20, 25, 29, 44]. Bhaskaran and Lakshmi [63] reported that this process can generate niosomes with entrapment efficiency up to 87.5% [9].

In a mixture of both unprotonated and protonated forms of the compounds, which are membrane permeable and membrane impermeable, a neutral exterior pH concludes. The drug's unprotonated neutral form continues to move through the niosome bi-layer, which is protonated and stuck within the vesicles after entering the acidic medium. This diffuses across the membrane and persists until the drug's internal and external concentrations are in equilibrium [7, 63, 78].

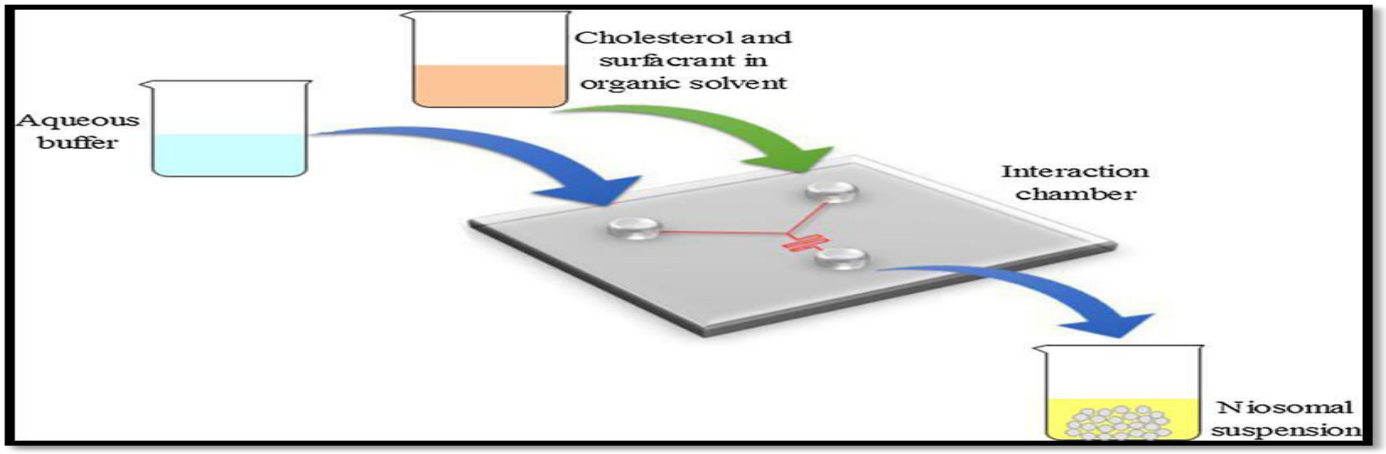


Fig. 15: Preparation of niosomes by the microfludization method [12]

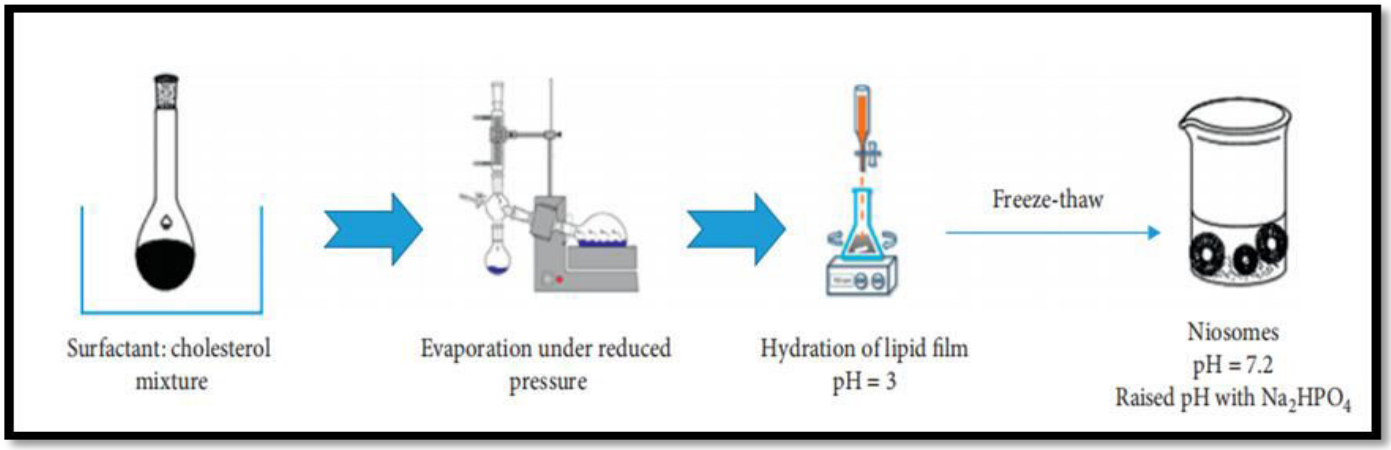


Fig. 16: Preparation of niosomes by transmembrane pH gradient method [9]

5.9. Supercritical CO_2 fluid method

Supercritical carbon dioxide (scCO2) is a one-step method and does not use organic solvent. $ScCO_2$ has strong solvating properties (Tc = 31.1°C, Pc = 73.8 bar), it is cheap and does not adversely affect the environment [7, 79]. Niosomes prepared by this method have the size in the range of 100-440 nm [8].

5.10. Emulsion method

Separately, an aqueous solution of the drug is prepared, and this solution is combined with surfactant and cholesterol solution dissolved in an organic solvent to form oil in water emulsion. Niosomes are dispersed in the aqueous phase after the organic solvent is evaporated [12, 29].

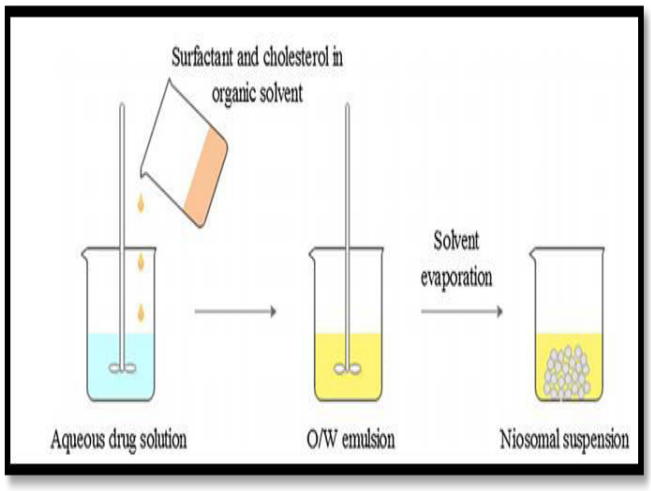


Fig. 17: Preparation of niosomes by emulsion method [12]

5.11. Lipid injection method

Organic solvents are not used in this process. To make a niosomal suspension, surfactant and cholesterol are melted and then injected into a highly agitated heated aqueous phase containing dissolved drug molecules [12, 29].

5.12. Co-acervation phase separation

In a wide mouth glass tube, a mixture of surfactant, cholesterol, medication, and phosphatidylcholine is dissolved in absolute ethanol. This tube's open end is sealed with a lid and warmed in a water bath at 70°C for 5 minutes. After that, an aqueous phase is incorporated and warmed over a water bath until a clear solution is obtained. Cool the mixture to room temperature until proniosomal gel is obtained [12, 80].

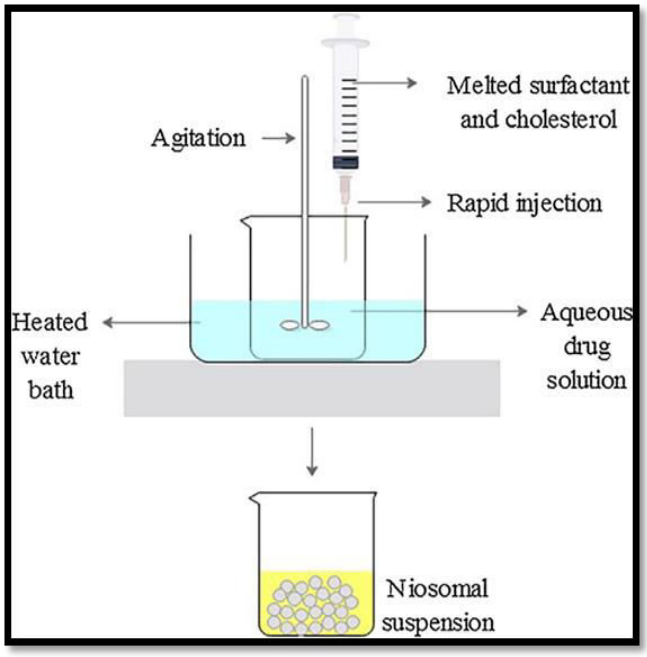


Fig. 18: Preparation of niosomes by lipid injection method [12]

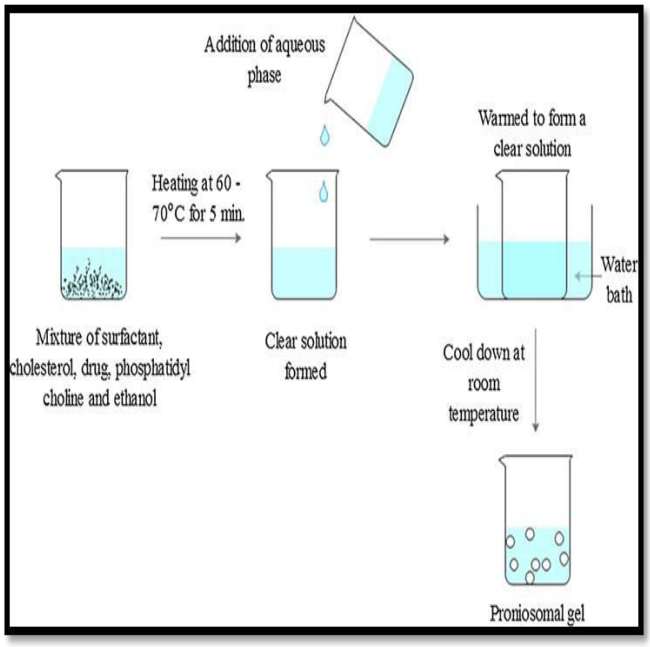


Fig. 19: Preparation of niosomal gel by co-acervation phase separation method [12]

6. NIOSOME PURIFICATION

To avoid the burst release of niosomes when applied in *in-vitro* and *in-vivo* studies, the free drug must be removed, which can be accomplished by different methods, such as dialysis, gel filtration and centrifugation [3, 12]

6.1. Dialysis

In this technique, the aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer or normal saline or glucose solution [3, 9, 29].

6.2. Gel filtration

Purification of niosomes from the unencapsulated drug can be carried out by gel filtration chromatography on Sephadex G75, G50 or G25, which allows efficient separation of free drug molecules [8, 9, 12, 29].

6.3. Centrifugation

According to recent studies, it is the most favoured method for the purification of niosomes. In particular, this method was used to separate the unentrapped genetic material from niosomes by gradient density centrifugation. By using this method 92-100% recovery of niosomes can be achieved without dilution [8, 24]. The niosomal suspension was centrifuged, and the above phase was discarded. The pellet was resuspended to give a niosomal suspension free from unentrapped medication [9].

7. EVALUATION OF NIOSOMES [6, 8, 29]

7.1. Size, morphology and size distribution of Niosomes

Different methods can be used to determine the size of niosomes and their morphology, such as light microscopy, coulter counter, photon correlation spectroscopy, electron microscope examination, SEM (scanning electron microscope), TEM (transmission electron microscope), freeze fracture replicator, light scattering, zeta sizer and metasizer. The double-sided tape that is affixed to aluminium stubs is sprinkled on niosomes. Using a gaseous secondary electron detector, the morphological characteristics of samples are studied. Particle size determined by the transmission electron microscope is smaller than the dynamic light scattering (DLS) process because of the various measuring criteria used by the two.

7.2. Measurement of vesicle size

Vesicles dispersions are diluted about 100 times in the same medium used for their preparation. Vesicle size can be measured by using a particle size analyzer. The apparatus consists of a 632.8 nm He-Ne laser beam centered using a Fourier lens [R-5] to a point at the middle of the multielement detector and a small volume sample keeping cell with a minimum power of 5 mW. Until deciding the vesicle scale, the sample is stirred using a stirrer.

7.3. Entrapment efficiency

The efficiency of niosomal dispersion can be accomplished by extracting the untrapped compound by exhaustive dialysis, filtration, gel chromatography or centrifugation techniques, and the drug stayed stuck in niosomes by using 50 percent n-propanol or 0.1 percent Triton X-100 to assess total vesicle destruction and analyse the resulting solution by suitable assay process. The percentage of entrapment efficiency can be determined using the following equation.

% Entrapment Efficiency = (Quantity of drug-loaded in the Niosome/Total quantity of drug in the suspension) x 100

7.4. Zeta potential analysis

Colloidal properties of the prepared formulations have been accessed by using zeta potential analysis. Using the zeta analyzer based on electrophoretic light scattering and laser Doppler velocimetry mechanism, suitably diluted niosomes extracted from proniosome dispersion are calculated. Charge on vesicles and their mean zeta potential values of normal measurement deviation are derived from the measurement directly. To assess the zeta potential, various instruments are used such as, the Zeta potential analyzer, mastersizer, microelectrophoresis, pH-sensitive fluorophores, high-performance capillary electrophoresis etc.

7.5. Bilayer formation and number of lamellae

The formation of bilayers of niosomes is characterized by X-cross formation under the light polarization microscopy. Different methods, such as AFM, NMR, small-angle X-ray spectroscopy and electron microscopy are preferred for estimation of the number of lamellae.

7.6. Membrane rigidity and homogeneity

Membrane rigidity affects bio-degradation and bio-distribution of niosomes. The determination of niosomal suspension rigidity is carried out as a function of temperature by using fluorescence probe. For assessing membrane homogeneity, P-NMR, Differential Scanning Calorimetry (DSC), Fourier Transform-infra red spectroscopy (FTIR) and Fluorescence Resonance Energy Transfer (FRET) are used.

7.7. *In-vitro* drug release

In vitro drug release can be done by Dialysis tubing, Reverse dialysis and Franz diffusion cell method.

7.7.1. Dialysis tubing

The niosomes are inserted in prewashed dialysis tubing in this process, which can be hermetically sealed. The dialysis sac is then dialyzed against an appropriate dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, centrifuged and tested for drug content using suitable procedure (U.V. spectroscopy, HPLC etc). It is important to maintain the sink condition.

7.7.2. Reverse dialysis

A number of tiny dialysis containing 1 ml of dissolution medium are inserted in proniosomes in this process. Then the proniosomes are displaced into the medium of dissolution. With this approach, immediate dilution of the proniosomes is feasible; however, it is not possible to measure rapid release using this process.

7.7.3. Franz diffusion cell

Using the Franz diffusion cell, *in vitro* diffusion experiments may be carried out. Proniosomes are mounted in a Franz diffusion cell equipped with a cellophane membrane in the donor chamber. The proniosomes are then dialyzed at room temperature against an acceptable dissolution medium; the samples are collected at reasonable intervals from the medium and tested for drug content using the appropriate procedure (U.V spectroscopy, HPLC, etc.).

7.8. Tissue distribution/*in-vivo* study

In-vivo tests for niosomes rely on the delivery route, drug dosage, drug impact and duration of presence in tissues such as liver, lung, spleen and bone marrow. Using animal models, tissue dissemination of a drug can be studied. Animals are slaughtered and separate tissues such as liver, kidney, breast, lungs, spleen should be separated, washed with buffer, homogenized and centrifuged to research the delivery pattern. For the drug material, the supernatant is analyzed.

7.9. Stability studies

In storage, because of aggregation and fusion, the drug can leak from the niosomes. Various humidity and light (UV) conditions are also exposed to niosomes. Parameters such as size, shape and entrapment performance are routinely tested during stability studies.

8. APPLICATION OF NIOSOMES IN DRUG DELIVERY [10]

Niosomes were first used in the cosmetics industry, and then pharmaceutical companies became involved. They have a great deal of potential for clinical uses, and lately they've been the focus of research studies. Several medications such as Doxorubicin, insulin, monoxide, ovalbumin, oligonucleotide, EGFP, hemagglutinin, DNA vaccine, -interferon, bovine essential pancreatic inhibitor, etc. can be encapsulated into niosomes [81]. Niosomes can be used for antioxidant, anticancer, anti-inflammatory, anti-asthma, antimicrobial, anti-amyloid, anti-Alzheimer, anti-bacterial, and several other applications. Ocular [12, 24, 51], intravenous [82-84], intramuscular [85], oral [53, 83, 86], subcutaneous [87], pulmonary [9, 24, 41, 88], intraperitoneal [90], transdermal [40, 72, 91-95], etc. routes have been used to deliver niosomes.

9. PATENTS ON NIOSOMES

Niosomal formulations are now commercially available, and Patents on niosomal formulations are also on the rise. Table 4 shows a list of some of the patents issued in the last decade [28].

Table 3: Drug delivery by niosomes

Application	Surfactant	Method	Drug	Reference
Pulmonary delivery	Tween 60	Thin layer hydration	Ciprofloxacin	9,24
	Span 60	Thin layer hydration	Clarithromycin	
	Span 60	Sonication	Rifampicin	
Ocular delivery	Span 60	Solvent injection	Gatifloxacin	12,24
	Span 60	Reverse phase evaporation	Naltrexone	
	Poloxomer 188, lecithin	Coacervation phase separation	Tacrolimus	
	Tween 60		Gentamicin	
Protein delivery	Brij 92	Thin layer hydration	Insulin	9
	Span 60	Thin layer hydration	Insulin	
	Span 40	Thin layer hydration	N-acetyl glucosamine	
	Span 60	Thin layer hydration	Bovine serum albumin	

Cancer chemotherapy	Span 60	Thin layer hydration	Cisplatin	9,24
	Span 60	Thin layer hydration	5-Flurouracil	
	Span 80	Sonication	Curcumin	
	Bola surf.	Thin layer hydration	5-Flurouracil	
	Span 60	Thin layer hydration	5-Flurouracil	
Carrier for Hb	Span 60	Thin layer hydration	Hemoglobin	9
Treatment of HIV	Span 60	Thin layer hydration	Lamivudine	9
	Span 60	Ether injection	Stavudine	
	Span 60	Thin layer hydration	Stavudine	
	Span 80	Ether injection	Zidovudine	
Vaccine and antigen delivery	Span 60	Thin layer hydration	Teatanus toxoid	9
	Span 20	Thin layer hydration	Newcastle vaccine	
	Span 60	Thin layer hydration	Ovalbumin	
	Span 60/span85	Reverse phase evaporation	Bovine serum albumin	
	Tween 20		Influenza vaccine	24
Dermal and Transdermal delivery	Span 60	Thin layer hydration	Acetazolamide	12
	Span 20,40,60,80	Coacervation phase seperation	Tramadol	
	Span 60	Thin layer hydration	Roxithromycin	
Bioactive delivery	Span 60	Thin layer hydration	Rutin	12, 24
	Span80, tween 80	Thin layer hydration	Ginkgo Biloba extract	
	Span 40	-	Gymnema extract	
Gene delivery	Tween 80	Reverse phase evaporation	pCMSEGFP	10

Table 4: Description of patents on niosomes

Sr. No.	Patent Number	Title	Reference
1	RU2582290C2 RU2582290C2	Dental gel having niosomes for treatment of inflammatory and dystrophic periodontal diseases.	96
2	US20160184228A1	Unilamellar niosomes having known pharmacological compounds solvated therein and a method for the preparation	97
3	RU2539397C2	Method for making transdermal patch containing peg-12 dimethiconeniosomes.	98
4	RU2600164C2	Doxorubicin and organosilicon nanoparticles-niosomes-based pharmaceutical gel for skin cancer treating.	99
5	FR3032115B1	Composition comprising an association of niosomes and c glycoside derivative, *crocus sativus* extract and/or *crocus sativus* flower extract, for regulating skin pigmentation	100
6	RU2541156C1	Transdermal anthelmintic agent of silicone niosomes with Albendazole.	101
7	RU2583135C1	Method of producing niosomal form of Ofloxacin.	102
8	RU2627449C2	Pharmaceutical niosomal gel based on n-hydroxy-2-(2-(naphtalene-2-yl)-1h-indole-3-yl)-2-phenylacetamide with anti tumour activity to glioblastoma.	103
9	US9522114B1	Enhanced targeted drug delivery system via chitosan hydrogel and chlorotoxn.	104
10	US9572795B2	Drug delivery system and method of treatment of vascular diseases using photodynamic therapy.	105

10. CONCLUSION

The field of vesicular drug delivery system is still in its infancy and increasing gradually during the past few decades. It is expected that this trend will continue to increase further. Niosome is a promising vesicular delivery system compared to liposomes as it is convenient, prolonged, targeted and effective drug delivery system with the ability of loading both hydrophilic and lipophilic drugs. They are a very useful tool for targeted drug delivery and diagnostic approaches. It has expanded to various areas like vaccine delivery system, tumor targeting agents, ophthalmic, and transdermal delivery systems. Despite many promising proof of concept studies there is still a long road ahead for niosomes to become a clinical reality. The potential of niosome can be enhanced by using novel preparations, loading and modification methods. Thus, these areas need further exploration and research so as to bring out commercially available niosomal preparations.

11. ACKNOWLEDGEMENT

The authors of this review article are thankful to the Principal, Government College of Pharmacy, Karad for allowing us to present this paper. We are also thankful to the AICTE for providing research fellowship to complete our research work and convert our research work into the publications.

12. REFERENCES:

1. Sanklecha VM, Pande VV, Pawar SS, Pagar OB, Jadhav AC. *Austin Pharmacol Pharm,* 2018; **3(2)**:1-7.
2. Shakya V, Bansal BK. *International Journal of Research and Development in Pharmacy & Life Sciences,* 2014; 3**(4)**:1036-1041.
3. Hunter CA, Dolan TF, Coombs GH, Baillie AJ. *J Pharm Pharmacol.,* 1988; **40**:161–165.
4. Katare R, Gupta PN, Mahor S, Rawat A, Khatri K, Katare Y, Panda AK, Vyas SP. *Journal of Drug Delivery Science and Technology,* 2006; **16(3)**:167-172.
5. Shahiwala A, Misra A. *Journal of pharmacy and pharmaceutical sciences*, 2002; **5(3):**220-225.
6. Sharma D, Ali AAE, Aate JR. *Pharmatutor*, 2018; **6(3):**58.
7. Mahale NB, Thakkar PD, Mali RG, Walunj DR, Chaudhari SR. *Adv Colloid Interface Sci.*, 2012; **183-184**:46-54.
8. Brewer JM, Alexander J. *Immunology,* 1992; **75**:570-575.
9. Gharbavi M, Amani J, Kheiri-Manjili H, Danafar H, Sharafi A. Niosome: A Promising Nanocarrier for Natural Drug Delivery through Blood-Brain Barrier. Vol. 2018, Advances in Pharmacological Sciences. 2018.
10. Moghassemi S, Hadjizadeh A. *J Control Release,* 2014; **185(1):**22-36.
11. Sankhyan A, Pawar P. *Journal of Applied Pharmaceutical Science*, 2012; **2(6):**20-32.
12. Tampa N, Falahat R, Wiranowska M, Toomey R. US9522114B1
13. Steed JW, Turner DR, Wallace K. *John Wiley & Sons*; 2007.
14. Sharma V, Anandhakumar S, Sasidharan M. *Materials Science and Engineering*: C. 2015; **56**:393-400.
15. Shtil AA, Grinchuk TM, Tee LI, Mechetner EB, Ignatova TN. *International Journal of Oncology,* 2000; **17(2):**387-479.
16. Agarwal S, Mohamed MS, Raveendran S, Rochani AK, Maekawa T, Kumar DS. *RSC advances,* 2018; **8(57):**32621-32636.
17. Naderinezhad S, Amoabediny G, Haghiralsadat F. *RSC advances*. 2017; **7(48):**30008-30019.
18. Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, et al. *Journal of Biological Chemistry*. 1992; **267(34)**:24248-24252.
19. Perloff MD, Von Moltke LL, Marchand JE, Greenblatt DJ. *Journal of Pharmaceutical Sciences,* 2001 Nov 1; **90(11):**1829-1837.
20. Kumar GP, Rajeshwarrao P. *Acta Pharmaceutica Sinica B,* 2011; **1(4):**208-219.
21. Biswal S, Murthy PN, Sahu J, Sahoo P, Amir F. *International journal of pharmaceutical sciences and nanotechnology,* 2008; **1(1):**1-8.
22. Valdés K, Morilla MJ, Romero E, Chávez J. *Colloids and Surfaces B: Biointerfaces.* 2014; **117:1**-6.
23. Abd-Elbary A, El-Laithy HM, Tadros MI. *International journal of pharmaceutics*, 2008; **357(1-2)**:189-198.
24. Marianecci C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhaique F, et al. *Advances in Colloid and Interface Science,* 2014; 205:187-206.
25. Moser P, Arvier MM, Labrude P, Vignerson C. *Pharm ActaHelv.,* 1990; **65**:82–92.
26. Uchegbu IF, Vyas SP. *International Journal of Pharmaceutics,* 1998; **172(1-2):**33-70.
27. Hu C, Rhodes DG. *International journal of pharmaceutics,* 1999; **185(1):**23-35.
28. Junyaprasert VB, Teeranachaideekul V, Supaperm T. *AapsPharmscitech*. 2008 Sep; **9(3):**851-859.
29. Sudheer P, Kaushik K. *J Pharm Res.*, 2015; **14(1):**20.

30. Yoshioka T, Stermberg B, Florence AT. *Int J Pharm*, 1994; **105:**1-6.
31. Lawrence MJ, Chauhan S, Lawrence SM, Barlow DJ. *STP Pharm Sci,* 1996; **1**:49-60.
32. Shahiwala A, Misra A. *J Pharm Sci,* 2002; **5**:220-225
33. Keshav J. *International Journal of Pharmaceutical, Chemical &Biological Sciences,* 2015; 5**(4)**.
34. Fathi M, Mozafari MR, Mohebbi M. *Trends in food science &technology*, 2012; **23(1):**13-27.
35. Hao Y, Zhao F, Li N, Yang Y. *International journal of pharmaceutics*, 2002; **244(1-2):**73-80.
36. Stafford S, Ballie AJ. *J. Pharm. Pharmacol.*, 1988; **40:** 26.
37. Hauser H, Gains N, Müller M. *Biochemistry*. 1983; 22**(20)**:4775-4781.
38. Peltonen L, Koistinen P, Karjalainen M, Häkkinen A, Hirvonen J. *Aaps Pharmscitech. 2002 Feb*; **3(4):**52-58.
39. Ferreira LS, Ramaldes GA, Nunan EA, Ferreira LA. *Drug Development and Industrial Pharmacy*. 2004; **30(3):**289-296.
40. Manosroi A, Khanrin P, Lohcharoenkal W, Werner RG, Götz F, Manosroi W, et al. *Int J Pharm.,* 2010; **392(1–2):**304–310.
41. Marianecci C, Paolino D, Celia C, Fresta M, Carafa M, Alhaique F. *Journal of Controlled Release.* 2010; 147**(1)**:127-135.
42. Uchegbu IF, Vyas SP. *International journal of pharmaceutics*, 1998; **172(1-2):**33-70.
43. Rajera R, Nagpal K, Singh SK, Mishra DN. *Biological and Pharmaceutical Bulletin,* 2011 Jul 1; **34(7):**945-53.
44. Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, Kuotsu K. *Journal of advanced pharmaceutical technology &research,* 2010; **(4):**374.
45. Sunilkumar M. *International Research Journal of Pharmaceutical and Applied Science,* 2015; **5:1**-7.
46. Tarekegn A, Joseph NM, Palani S, Zacharia A, Ayenew Z. *Int J Phar Sc Res.*, 2010; **(9):**1-8.
47. Liu T, Guo R, HUa W, Qui J. *Colloid Surf A PhysicochemEng Aspects,* 2007; **293**:
48. Moghassemi S, Hadjizadeh A, Omidfar K. *AapsPharmscitech*, 2017; **18(1):**27-33.
49. M. Mokhtar OA, Sammourb MA, Hammad NA. *Int. J. Pharm.,* 2008; **361**:104-111.
50. Yeo LK, Chaw CS, Elkordy AA. *Pharmaceuticals,* 2019; **12(2):**46.
51. Bhardwaj P, Tripathi P, Gupta R, Pandey S. *Journal of Drug Delivery Science and Technology,* 2020; **56:**101581.
52. Gaafar PM, Abdallah OY, Farid RM, Abdelkader H. *Journal of liposome research,* 2014; 24**(3)**:204-215.
53. Almira I, Blazek-welsh IA, Rhodes GD. *AAPS PharmSciTech.,* 2001; **3:**1–8
54. Sharma SK, Chauhan M, Anilkumar N. *Asian journal of pharmaceutical research and health care*, 2009; **1(2):**142-156.
55. Jain CP, Vyas SP. *Journal of microencapsulation*, 1995; 12**(4):**401-407.
56. Vyas S, Khar R. *CBS publication*, 2007; **1**:249-279.
57. Ruckmani K, Sankar V. *Aaps Pharmscitech*, 2010; **11(3):**1119-1127.
58. Onochie IT, Nwakile CD, Umeyor CE, Uronnachi EM, Osonwa UE, Attama AA, Esimone CO. *Journal of Applied Pharmaceutical Science*, 2013; **3(12):**66.
59. Master AM, Rodriguez ME, Kenney ME, Oleinick NL, Gupta AS. *Journal of Pharmaceutical Sciences,* 2010; 99**(5):**2386-2398.
60. Balasubramaniam A, Kumar VA, Pillai KS. *Drug DevInd Pharm.,* 2002; **28**:1181-1193.
61. Gaafar PM, Abdallah OY, Farid RM, Abdelkader H. *Journal of liposome research*, 2014; 24**(3):**204-215.
62. Kamboj S, Saini V, Bala S. *The Scientific World Journal*, 2014; 2014.
63. Isnan AP, Jufri M. *International Journal of applied Pharmaceutics*, 2017; 38-43.
64. Onochie IT, Nwakile CD, Umeyor CE, Uronnachi EM, Osonwa UE, Attama AA, et al. *Journal of Applied Pharmaceutical Science*, 2013; **3(12):**66.
65. Alam M, Zubair S, Farazuddin M, Malik A, Mohammadm O. *Nanomedicine 9,* 2013; 247-256
66. Khan DH, Bashir S, Khan MI, Figueiredo P, Santos HA, Peltonen L. *Journal of Drug Delivery Science and Technology,* 2020; **58:**101763.
67. Bansal S, Aggarwal G, Chandel P, Harikumar SL. *Journal of pharmacy & bioallied sciences*, 2013; **5(4):**318.
68. Bhaskaran S, Lakshmi PK. *Acta Pharmaceutica Sciencia*, 2009; **51(1)**.
69. Jain S, Singh P, Mishra V, Vyas SP. *Immunology letters*, 2005; 101**(1):**41-9.
70. Vyas SP, Singh RP, Jain S, Mishra V, Mahor S, Singh P, et al. *International Journal of Pharmaceutics,* 2005; 296**(1-2):**80-86.
71. Abdelkader H, Ismail S, Hussein A, Wu Z, Al-Kassas R, Alany RG. *International Journal of Pharmaceutics,* 2012; **432(1-2):**1-10.
72. Zidan AS, Hosny KM, Ahmed OA, Fahmy UA. *Drug Deliv.*, 2014; **11:**1-14.
73. Guinedi AS, Mortada ND, Mansour S, Hathout RM. *International Journal of Pharmaceutics*, 2005; **306(1-2):**71-82.
74. Singh G, Dwivedi H, Saraf SK, Saraf SA. *Tropical Journal of Pharmaceutical Research,* 2011; **10(2)**.
75. Karki R, Mamatha GC, Subramanya G, Udupa N. *Rasayan J Chem.,* 2008; **1**:224-227.

76. Sorgi FL, Huang L. *International Journal of Pharmaceutics*, 1996; **144(2)**:131-139.
77. Khandare JN, Madhavi G, Tamhankar BM. *Eastern Pharmacist*, 1994; **37:**61.
78. Pawar SD, Pawar RG, Kodag PP, Waghmare AS. *International journal of biology, pharmacy and allied sciences,* 2012; **3(11):**406-416.
79. Manosroi A, Chutoprapat R, Abe M, Manosroi J. *International Journal of Pharmaceutics,* 2008; 352**(1-2):**248-255.
80. Zeng W, Li Q, Wan T, Liu C, Pan W, Wu Z, Zhang G, Pan J, Qin M, Lin Y, Wu C. *Colloids and Surfaces B: Biointerfaces,* 2016; **141:**28-35.
81. Shilpa S, Srinivasan BP, Chauhan M. *International Journal of Drug Delivery,* 2011; **1**:3(1).
82. Waddad AY, Abbad S, Yu F, Munyendo WL, Wang J, Lv H, Zhou J. *International Journal of Pharmaceutics,* 2013; 456**(2)**:446-458.
83. Leroux JC, Cozens R, Roesel JL, Galli B, Kubel F, Doelker E, et al. *Journal of Pharmaceutical Sciences,* 1995; 84**(12):**1387-1391.
84. Hong M, Zhu S, Jiang Y, Tang G, Pei Y. *Journal of Controlled Release,* 2009; 133**(2):**96-102.
85. Azmin MN, Florence AT, Handjani-Vila RM, Stuart JF, Vanlerberghe G, Whittaker JS. *J Pharm Pharmacol.,* 1985; **37**:237-242.
86. Gurrapu A, Jukanti R, Bobbala SR, Kanuganti S, Jeevana JB. *Advanced Powder Technology,* 2012; 23**(5):**583-590.
87. Rajeswari TS. *Journal of Applied Pharmaceutical Science*, 2011; **1(08)**:12-20.
88. Arunothayanun P, Bernard MS, Craig DQ, Uchegbu IF, Florence AT. *Int J Pharm.,* 2000; **201**:7–14.
89. Walker W, Brewer JM, Alexander J. *European journal of Immunology*, 1996; 26**(7):**1664-1667.
90. Ammar HO, Ghorab M, El-Nahhas SA, Higazy IM. *International Journal of Pharmaceutics,* 2011; 405**(1-2):**142-152.
91. El-Laithy HM, Shoukry OL. *European Journal of Pharmaceutics and Biopharmaceutics,* 2011; **77**:43-55.
92. Balakrishnan P, Shanmugam S, Lee WS, Lee WM, Kim JO, Oh DH, et al. *International Journal of Pharmaceutics*, 2009; 377**(1-2):**1-8.
93. Shahiwala A, Misra A. *J Pharm Pharm Sci*., 2002; 5**(3):**220-225.
94. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. *International Journal of Pharmaceutics*, 2001 Mar 14; **215(1-2):**91-99.
95. Rogerson A, Cummings J, Willmott N, Florence AT. *J Pharm Pharmacol.,* 1988; **40:**337-342.
96. Morrison E, inventor. *United States patent application* US **14/985,570**.2016.
97. Bhardwaj P, Tripathi P, Gupta R, Pandey S. *Journal of Drug Delivery Science and Technology*, 2020; **56:**101581.
98. Pawar SD, Pawar RG, Kodag PP, Waghmare AS. *International Journal of Biology, Pharmacy and Allied Sciences,* 2012; **3(11):**406-416.
99. Guerin S, Aygatcano C. FR3032115B1
100. Aleksandrovich B, Dalkhatovich B, Nasyrovich A, Zakirovich V. RU2541156C1.
101. Anatolevich K, Nikolaevich K, Evgenevna M, Vladimirovich P, Veniaminovna L. RU2583135C1.
102. Aleksandrovich B, Viktorovich A, Aleksandrovich A, Nikolaevich M, Nikolaevich S. RU2627449C2.
103. Alcantar A, Falahat T, Wiranowska M, Toomey R. US9522114B1.
104. Davalian D, Trollsas M, Stankus J, Khong Y, Wan J. US9572795B2.