



Stability aspects of niosome

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Abstract

In the past few decades considerable attention has been focused on the designing of the drug within the vesicular system to overcome the problem associated with old pre-existing drug delivery system and it has also improved the therapeutic efficacy by controlling and sustaining the actions. Niosome vesicular systems are similar to liposomes that can be used as carriers of both hydrophilic and lipophilic drugs. As liposome, niosome is also associated with different stability problems like physical stability, chemical stability and biological stability. But in comparison with liposome niosome shows good stability. Every new delivery system has some advantages as well as disadvantages also. This review focuses on the stability issues of niosome. Vesicular system such as liposomes, niosome, transfersome, and ethosome provide an alternative to improve the drug delivery. Niosome play an important role owing to their nonionic properties, in such drug delivery system.

Keywords: Niosome, Stability aspects, Physical stability, Chemical stability.

INTRODUCTION

Niosomes are multilamellar vesicular structure of nonionic surfactants, like liposomes and are made out of non-ionic surfactant rather than phospholipids which are the parts of liposomes. Thus, niosome or non-ionic surfactant vesicles are presently considered as an alternative device to liposome. Assorted types of surfactants are used to form vesicles, and they have the ability to entrap and hold the hydrophilic and hydrophobic solute particles. Mainly two type of components are used in the formulation of niosome *i.e.*, nonionic surfactant and the additives. The non-ionic surfactant makes the vesicular layer and the improvement in the rigidity of bilayer is due to presence of cholesterol. (1-3). From the premature degradation and inactivation due to unwanted immunological and pharmacological effects this carrier system protects the drug molecules. In recent years, niosomes have been broadly studied for their potential to serve as a transporter for the delivery of drugs, antigens, hormones and other bioactive agents. Besides this, the problem of insolubility, instability and rapid degradation of drugs has been solved by making

niosomes. (4) Niosomes are one of the promising drug carriers that have a bilayer structure and are formed by self-association of nonionic surfactants and cholesterol in an aqueous phase. Niosomes are biodegradable, biocompatible, and non immunogenic. They have long shelf life, exhibit high stability, and allow the delivery of drug at target site in a controlled and/or sustained manner. Different types of nonionic surfactants have been reported to form niosomes and enable the entrapment of a large number of drugs with a wide range of solubility. The composition, size, number of lamellae, and surface charge of niosomes can be varied and optimized to enhance the performance of niosomes for drug delivery. (5) For use in biomedicine these non-ionic surfactants are cheap and safe. (6) Due to their excellent behavior in encapsulating both hydrophilic and hydrophobic agent, non-Ionic surfactant based vesicles, also known as niosomes, have attracted much attention in pharmaceutical fields. In recent years, it has been discovered that these vesicles can improve the bioavailability of drugs, and may function as a new strategy for delivering several typical of therapeutic agents, such as chemical drugs, protein drugs and gene materials with low toxicity and desired targeting efficiency. During the formulation process and storage, compared with liposomes, niosomes are much more stable. By optimizing components or by surface modification the required pharmacokinetic properties can be achieved. This novel delivery system is also easy to prepare and scale up with low production costs. (7)

Classification of stability of niosomes

Physical Stability

The niosomes can change their physical characteristics in several ways.

Due to formation of aggregation and fusion in the formulation particle size can change. During storage phase separation of bilayer components occur. Leakage in the encapsulated substance from niosome occurs.

By the selection of proper charge inducing agents the changes in particle upon storage of niosomes over pharmaceutically relevant time intervals can be minimized. Mostly, negatively charged phospholipids are used to stabilize the niosomes.

When the bilayer composition changes due to chemical degradation reactions or when the bilayer goes through temperature cycles the phase separation may occur. Sometimes, it may occur *in vivo*, when bilayer components are selectively drawn from the bilayer plasma components. If this effect is undesired, the components that form more rigid bilayers are preferred. The permeability of bilayers is highly dependent on the physico-chemical properties of the bilayer, drug and the temperature.

In category first, the permeability reduces in the presence of cholesterol in the bilayer of niosome. The permeability is low with or without cholesterol for gel state bilayers. From *in vivo* performance it is clear that if it allows “gel state” bilayers to be used, the shelf life of the niosomes in aqueous media with the proper pH might easily meet industrial demands. In the second category, the drug tends to be difficult to keep entrapped over periods of months as long as outside sink conditions exist. In the third category, strongly lipophilic drugs have high affinity for the bilayers and therefore these drugs stay there over a long period of time, independently of the state of the bilayer.

Niosomes stored in freeze dried form: The proper in vivo performance of niosomes with long term stability can be achieved by the niosomes that are stored in freeze dried form. A cryoprotectant needs to be added to maintain the particle size distribution after freeze drying-rehydration cycle. Different types of cryoprotectants and their possible mechanisms of action have been discussed for niosome stabilization. Usually, sugars are used as cryoprotectant, although other types of excipient also have been found to exert cryoprotective effects.

- 1) During the freeze drying process the formation of amorphous glass structures may avoid mechanical damage inflicted by ice crystals. It is suggested to store these cakes below the glass transition temperature.
- 2) When the bilayer stabilizing water is removed by sublimation, the sugars may interact with the polar head groups of the phospholipids and stabilize the membranes. (8)

Chemical stability

The chemical stability of niosome depends on the lipid components and the bilayer components of niosomes. Usually, the two degradation processes which occur with phospholipids are hydrolysis and peroxidation.

The hydrolysis reactions are influenced by pH as well as other experimental conditions like temperature, ionic strength, buffer species, and ultra sonication. Many investigators choose the formation of lysophosphatidyl choline as a standard measure for the chemical stability to phospholipids. As, in lipid bilayer the presence of lysophosphatidyl choline greatly enhances the permeability of niosomes; the proper sourcing of the phospholipid is the most important method for minimizing this problem.

Lipid peroxidation: Unsaturated acyl chains as a part of their molecular structures is present in most of the phospholipid niosomal dispersions. These chains are vulnerable to oxidative degradation (lipid peroxidation). During preparation, storage or actual use the peroxidation can occur. The formation of cyclic peroxides and hydro peroxides are produced by Peroxidation of phospholipids. Peroxidation of the phospholipids may be minimized by a number of ways such as:

- Minimum use of unsaturated phospholipids.
- Nitrogen or argon can be used to minimize exposure to oxygen.
- Use of light resistant container
- Removal of heavy metals (EDTA)
- Antioxidants such as tocopherol or BHT are used

It was reported that niosomes of different lipid composition could be steam sterilized without substantial hydrolytic or oxidative degradation. (8)

Stability in biological fluids

The inability of niosomes to retain entrapped substances when incubated in blood or plasma has been known for a decade. The instability of niosomes in plasma appears to be the result of transfer of bilayer lipids to albumin and high density lipoproteins. With the membrane of red blood corpuscle both lecithin and cholesterol also exchanges. To high density lipoprotein attack at their gel to liquid crystalline phase transition temperature niosomes are most susceptible. On the size and type of niosomes the susceptibility of niosomal phospholipids to lipoprotein and phospholipase attack is strongly dependent. Usually, small lamellar vesicles are least stable where as multilamellar vesicles are most stable. The bilayer membrane structure is also destabilized by the bile salts, thereby, leading to release of the entrapped material. (8)

Factors affecting Vesicle size, entrapment efficiency & release characteristics

a) Drug

Both types of drugs i.e. hydrophilic and hydrophobic can be encapsulated in the niosomes. Molecular weight of drug affects entrapment efficiency, higher the molecular weight, lower the entrapment efficiency. The rigidity and charge of the niosome is influenced by the physical and chemical properties of encapsulated drug. The vesicle size of niosome is increased by entrapment of drug, most likely by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thus increasing vesicle size.(9-10) In the vesicles the drug is entrapped vigorously(actively) or inertly(passively). In passive trapping, drug and lipids are codispersed with part of drug being entrapped, according to hydrophobicity and electrostatic charge. In the internal aqueous phase hydrophilic drug will be entrapped and in the lipid region hydrophobic drug will be entrapped By ion gradients placed across the niosomal membranes, active trapping can be achieved (11-13) The hydrophilic lipophilic balance of the drug affects degree of entrapment. (14). hydrophobic drugs are entrapped in the hydrophobic region of the bilayer while hydrophilic drugs are encapsulated in the corresponding core. (13) Another factor to be considered is whether the drug to be encapsulated is amphiphilic. The best example of such a drug is doxorubicin. When encapsulated in niosomes, aggregation occurred and was overcome by the addition of a steric stabilizer. The increase in encapsulation of a drug occurs when more is added, could be the result of saturation of the medium. The solubility of certain poorly soluble drugs can be increased by formulation in niosomes but only up to a certain limit above which drug precipitation will occur. (15)

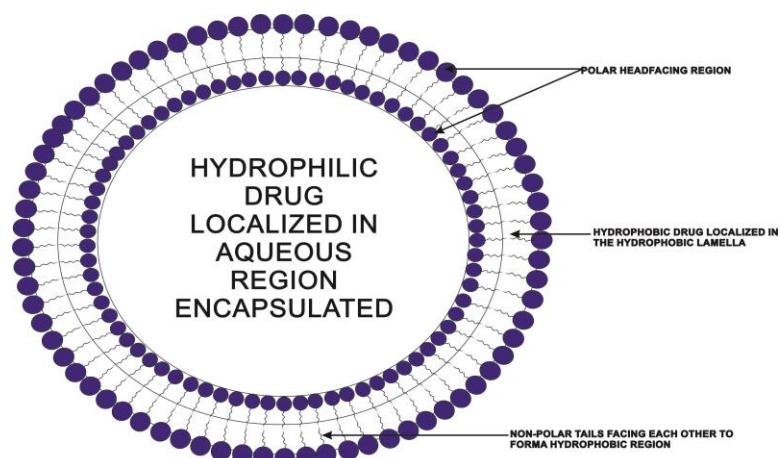


Fig.1. Niosome structure

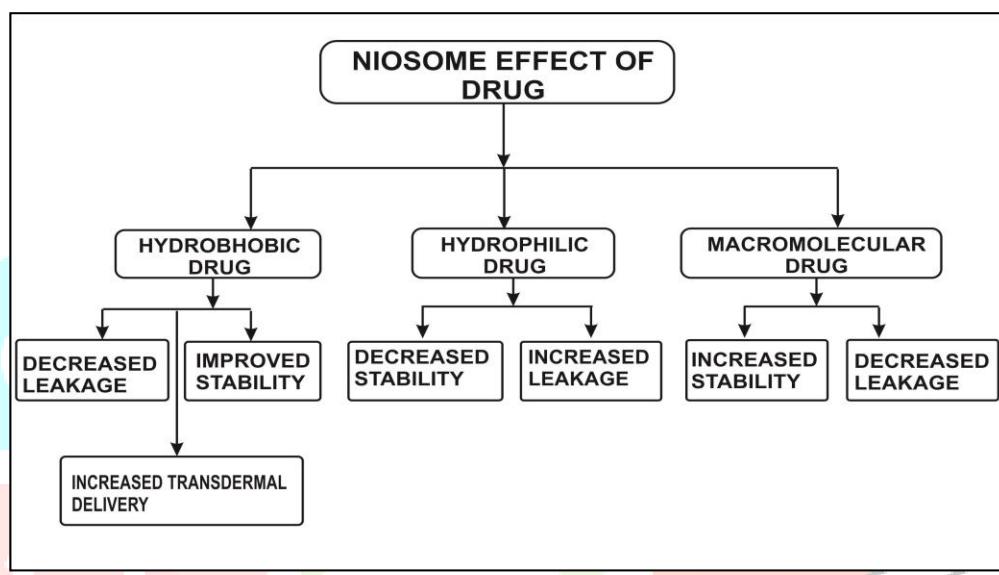


Fig. 2. The effect of nature of encapsulated drug on the properties of the niosome dispersion.

b) **Amount and type of surfactant**

Nonionic Surfactant:-

Nonionic surfactants are the basic components of niosomes which upon hydration form lamellar microscopic and nanoscopic vesicles. Nonionic surfactants are preferred because of their properties and abilities to form stable formulations. The properties like compatible and non toxic, ability to maintain pH up to physiological pH along with their functions such as solubilizer, wetting agents, and permeability enhancers makes them to use in the formulation of niosome. Nonionic surfactants such as alkyl ethers and alkyl glyceryl ethers, poly oxy ethylene 4 lauryl ether (Brij 30), poly oxy ethylene acetyl ethers (Brij 58), Sorbitan fatty acid esters, etc., are used in the formulation of various niosomes. The values of hydrophilic-lipophilic balance (HLB) and critical packing parameter (CPP) plays an essential role in the selection of surfactant molecules for niosome preparation. (5)

Hydrophilic-Lipophilic Balance (HLB):-

HLB is a dimensionless parameter, which is the indication of the solubility of the surfactant molecule. The balance between the hydrophilic portions to the lipophilic portion of the nonionic surfactant is described by the HLB value. The range of HLB value is from 0 to 20 for nonionic surfactants. The lower HLB value refers to more lipophilic surfactant and the higher HLB to more hydrophilic surfactant. Surfactants having HLB value between 4 and 8 can be used for vesicle preparation. Due to high aqueous solubility the surfactants which are hydrophilic in nature having HLB value ranging from 14 to 17 are not suitable to form a bilayer membrane. However niosomes are indeed formed from polysorbate 80 (HLB value = 15) and Tween 20 (HLB value = 16.7) with the addition of an optimum level of cholesterol. In the presence of equimolar cholesterol concentration Tween 20 forms stable niosome. The interaction occurs between the hydrophobic part of the amphiphile next to head group and the 3-OH group of cholesterol at an equimolar ratio and this interaction could explain the effect of cholesterol on the formation and hydration behavior of Tween 20 niosomal membranes. The HLB value of surfactant also affects the drug entrapment efficiency of the niosomes. (5)

Critical Packing Parameter (CPP):-

During the preparation of niosome, the geometry of the vesicle depends upon the critical packing parameter. The shape of nanostructures formed by self-assembly of amphiphilic molecules can be predicted on the basis of the CPP of a surfactant. Critical packing parameter depends on the symmetry of the surfactant and can be defined using following equation:

$$CPP = \frac{V}{l_c \times a_0}$$

Where

V is hydrophobic group volume,

l_c is the critical hydrophobic group length, and

a_0 is the area of hydrophilic head group

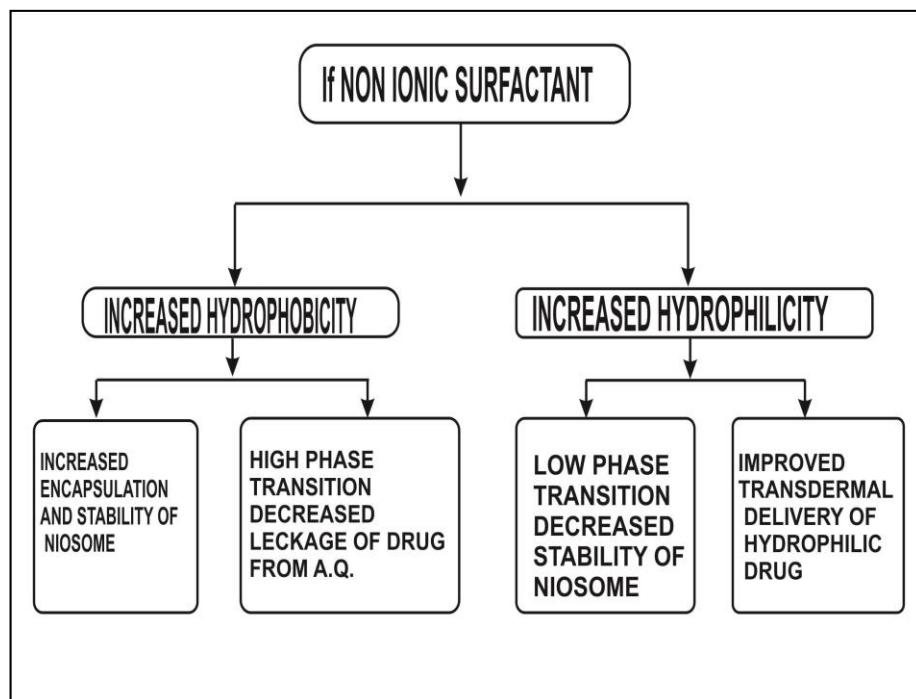
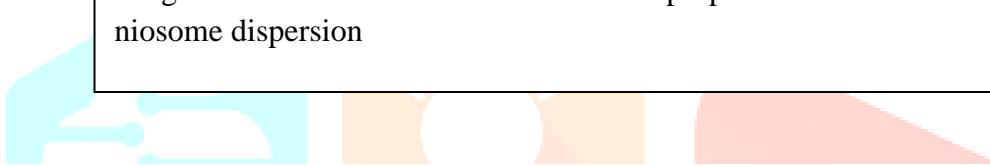


Fig. 3. The effect of the surfactant on the properties of the niosome dispersion



c) Cholesterol content

The hydrodynamic diameter and entrapment efficiency of niosome is increased by inclusion of cholesterol. Generally, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. Cholesterol forms hydrogen bonds with hydrophilic head of a surfactant in the bilayer structure of niosomes. (5).The gel state is transformed to a liquid ordered phase at a high cholesterol concentration. An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and reduction in EF and therefore an increase of the rigidity of the bilayers obtained.(9). Drug entrapment efficiency plays an important role in niosomal formulations and it can be altered by varying the content of cholesterol. (5)

d) Charge inducers

By the addition of charged groups to the bilayer of vesicles charged molecules increase the stability of the vesicles. They increase surface charge density and thus prevent vesicles aggregation. For niosome preparation dicetyl phosphate and phosphatidic acid are most used negatively charged molecules and similarly, stearylamine is well-known positively charged molecules used in niosomal preparations. Usually, the charged molecule is added in niosomal formulation in an amount of 2.5–5mol%. However due to increase in the amount of charged molecules can inhibit niosome formation. (7)Stearyl amine and dicetyl phosphate are positive and negative charge inducers respectively, and are frequently incorporated in the bilayer membranes. These charge inducers have been mainly utilized to improve the physical stability of the vesicular dispersions against aggregation to prolong the half life of circulating surfactant

vesicles in plasma and to prolong the ocular residence time of topically instilled ophthalmic surfactant vesicles. For example, incorporation of positively charged lipids such as stearyl amine is necessary for successful delivery of negatively charged polynucleotides and efficient transfection into the cells. In the prepared niosomes for ocular delivery compared with the neutral niosomal formulations, incorporation of dicetyl phosphate in niosomes significantly improved gentamycin sulfate entrapment efficiency. In prolonging gentamycin release rate and better permeation through excised bovine corneas negatively charged niosomes have been also found most effective. (16)

e) Methods of preparation

Different sized niosome vesicles are yield by different method of preparation such as thin film hydration method, ether injection method and sonication. The vesicle with greater diameter is formed by thin film hydration as compared to the ether injection method (50-1000nm). Sonication of multilamellar vesicles prepared by above methods, either with probe sonicator or bath type sonicator forms unilamellar vesicles with considerably reduced diameter. Increase in sonication time results in hydration help to reduce the size of vesicles prepared by hand shaking method. Reverse Phase Evaporation (REV) method produced small sized niosomes. Niosomes with greater uniformity and small size vesicles are formed by microfluidization method. Niosomes obtained by transmembrane pH gradient (inside acidic) drug uptake process showed greater entrapment efficiency and better retention of drug.(17).

f) Resistance to osmotic stress

The reduction in diameter is due to addition of a hypertonic salt solution to a suspension of niosomes. In hypotonic salt solution, initially there is 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. (18). When niosomal suspension is kept in contact with hypertonic salt solution, diameter of niosomal vesicles was found to be decreased. There is slow release with slight swelling of vesicles, which is due to inhibition eluting fluids from vesicles, followed by faster release, which may be due to decrease in mechanical strength under osmotic stress. Volume of hydration medium and time of hydration of niosomes are also critical factors which affects the niosomal assembly along with the above mentioned factors. Formation of fragile niosomes or creation of drug leakage problems may be due to improper selection of these factors. (1)

g) Temperature of hydration

Temperature of the hydration medium plays a major role in the formation of vesicles and affects their shape and size.(16). For ideal condition the temperature should always be above the gel to liquid phase transition temperature (T_m) of the system. Change in (T_m) affects the assembly of surfactants into vesicles and also induces changes in vesicle shape. (19)

Comparison of different types of surfactants and cholesterols based on entrapment efficiency and particle size

- Many researchers reported that the surfactants which have low HLB value, higher lipophilicity, and higher phase transition temperature and longer alkyl chain length shows higher entrapment. Upon above properties niosomes prepared with span 40 and span 60 showed higher entrapment efficiency.(20)
- Cholesterol and span 60 used in formulation which interacts with lipid bilayers, showed a direct effect on vesicle size. Researchers observed an increase in average size of the vesicles with increased concentration of span 60 and cholesterol. However further increase in the span 60 concentration leads to decrease in size of vesicles due to the formation of a micellar structure instead of the vesicles, which are relatively smaller in size. Increasing the phospholipid content also contributed an increase in the hydrophobicity. (21)
- According to many researchers, vesicle size was found to be in decreasing order as span 60>span 40>span 80.HLB value of span 60 and span 40 is 4.7 and 6.7 respectively. More HLB value means more hydrophobic character, therefore, due to more hydrophobicity, span 40 resulted in smaller vesicular size than span 60.Increasing in cholesterol content resulted in increased vesicle size. Span 60 and span 40 showed maximum entrapment efficiency.(22)
- Niosomes were prepared using 1:1 molar ratio of various surfactants and cholesterol. There was increase in entrapment efficiency with various surfactants in the order of tween 20<span 80<tween 80<span 20<span 60. Particle size was reduced in the order of tween 80<span 80<tween 20<span 20<span 60.Span 60 had the least particle size with the highest entrapment efficiency. (23)
- On higher cholesterol level a reduction in entrapment efficiency resulted probably due to the competition between cholesterol and drug for packing space within the bilayer.(24)
- Effect of cholesterol on entrapment varied according to the nonionic surfactant used. As the HLB value of the surfactant increases above 10, minimum amount of cholesterol necessary to form vesicles increases. In many studies it was found that Brij 76 has the highest HLB value of 12.6. Thus increased cholesterol content might have increased the lipophilic behavior of Brij 76 niosomes. Hence higher drug entrapment of Brij 76 niosome was observed in the presence of higher content of cholesterol. However Brij 52 with HLB value 5.3 did not show a significant increase in the entrapment efficiency with respect to higher cholesterol content. Span 60 showed the maximum entrapment efficiency at 1 cholesterol molar ratio.(25)

Table 1:- Comparison between different types of surfactant

Surfactant	Entrapment efficiency	Particle size
Span 20	86.33%	329.12 nm
Span 40	77.81%	346.23 nm
Span 60	89.80%	942.73 nm
Span 80	85.06%	756.93 nm
Tween 20	82.05%	928.78 nm
Tween 80	87.00%	931.63 nm
Brij 52	74.83%	933.21 nm
Brij 76	76.06%	934.19 nm

Conclusion:-

Niosomes are novel nano drug carriers to design effective drug delivery systems. They offer a immense opportunity for loading hydrophilic, lipophilic drugs, or both drugs together. The incorporation of the drug into niosome provides better stability. Niosomes are thoughts to be better candidate drug delivery due to various factors like cost, stability etc. different types of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parenteral, etc. Niosomes represent a promising drug delivery module and much research has to be inspired in this to extract out all the potential in this novel drug delivery system.s

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