NIOSOMES: FORMULATION AND EVALUATION

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ABSTRACT
Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. This review article focuses on the preparation methods, characterizations, factors affecting release kinetic, advantages, and applications of niosomes.

KEY WORDS: Niosomes, Proniosomes, Non-ionic surfactant, Cholesterol.

INTRODUCTION
Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. The method of preparation of niosome is based on liposome technology. The basic process of preparation is the same i.e. hydration by aqueous phase of the lipid phase which may be either a pure surfactant or a mixture of surfactant with cholesterol. After preparing niosomal dispersion, unentrapped drug is separated by dialysis centrifugation or gel filtration. A method of in-vitro release rate study includes the use of dialysis tubing. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants. They are very similar to the liposomes. Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. Niosomes have shown promise in the release studies and serve as a better option for drug delivery system. This class of vesicles was introduced by Handjani – Vila et al. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs.(Thersa 1998) One of the reasons for preparing niosomes is the assumed higher chemical stability of the surfactants than that of phospholipids, which are used in the preparation of liposomes. Due to the presence of ester bond, phospholipids are easily hydrolysed.(Breimer 1985) Unreliable reproducibility arising from the use of lecithins in liposomes leads to additional problems and has led scientist to search for vesicles prepared from other material, such as nonionic surfactants. Niosomes are promising vehicle for drug delivery and being non-ionic; it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent
hydration in aqueous media. (Malhotra) In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as Span – 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate. The first report of non-ionic surfactant vesicles came from the cosmetic applications devised by L’Oreal (Buckton et al., 1995). The concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Niosomes represent a promising drug delivery module. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure (Don et al., 1997). Niosomes are thought to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc. Various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parenteral etc (Handjani et al., 1979).

ADVANTAGES OF NIOSOMES

The niosomal drug delivery is a potential drug delivery method for controlled and targeted drug delivery, the major advantages of these vesicular drug carriers are;

- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external nonaqueous phase.
- The vesicle suspension is water–based vehicle. This offers high patient compliance in comparison with oily dosage forms.
- They are osmotically active and stable, as well as they increase the stability of entrapped drug.
- Handling and storage of surfactants requires no special conditions.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.
- Niosomes possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

APPLICATION OF NIOSOMES

Niosomes as a novel drug delivery system: A number of workers have reported the preparation, characterization and use of niosomes as drug carriers. The major research in this field has made niosomes a very unique, yet simple, technique for the delivery of many drugs;

- Niosomes containing anti-cancer drugs, like, methotrexate and doxorubicin increases drug delivery to the tumor and tumoricidal activity of the drug.
- Carter et al. reported that multiple dosing with sodium stibogluconate loaded niosomes was found to be effective against parasites in the liver, spleen and bone marrow as compared to simple solution of sodium stibogluconate (Carter et al., 1989).
- Azmin et al. reported the preparation and oral as well as intravenous administration of Methotrexate loaded Niosomes in mice. They observed significant prolongation of plasma levels and high uptake of Methotrexate in liver from niosomes as compared to free drug solution (Azmin et al., 1985).
- Chandraprakash et al. reported the formation and pharmacokinetic evaluation of Methotrexate niosomes in tumor bearing mice (Chandraprakash et al., 1992).
- Cable et al. modified the surface of niosomes by incorporating polyethylene alkyl ether in the bilayered structure. They compared the release pattern and plasma level of Doxorubicin in niosomes and Doxorubicin mixed with empty Niosomes and observed a sustained and higher plasma level of doxorubicin from Niosomes in mice (Cable et al., 1989).
- D’ Souza et al. studied absorption of Ciprofloxacin and Norfloxacin when administered as niosome encapsulated inclusion complexes (D’Souza et al., 1997).
- Namdeo et al. reported the formulation and evaluation of Indomethacin loaded niosomes and showed that therapeutic effectiveness increased and simultaneously toxic side effect reduced as compared with free Indomethacin in pawoedema bearing rats (Namdeo et al., 1999).
• Parthasarathi et al. prepared niosomes of vincristine sulfate which had lesser toxicity and improved anticancer activity. Jagtap and Inamdar prepared niosomes of Pentoxifylline and studied the in-vivo bronchodilatory activity in guinea pigs. The entrapment efficiency was found to be 9.26 ± 1.93% giving a sustained release of drug over a period of 24 hrs (Parthasarathy et al., 1994).

• Raja Naresh et al. reported the anti-inflammatory activity of niosome encapsulated Diclofenac sodium in arthritic rats. It was found that the niosomal formulation prepared by employing a 1:1 combination of Tween 85 elicited a better consistent anti-inflammatory activity for more that 72 hrs after administration of single dose (Raja et al., 1994).

• Niosomes can be used for targeting of drug in the treatment of diseases in which the infecting organism resides in the organ of reticulo-endothelial system. Leishmaniasis is such a disease in which parasite invades cells of liver and spleen. The commonly prescribed drugs are antimonials, which are related to arsenic, and at high concentration they damage the heart, liver and kidney.

• The study of antimony distribution in mice, performed by Hunter et al. showed high liver level after intravenous administration of the carriers forms of the drug (Hunter et al., 1988).

• Baillie et al. reported increased sodium stibogluconate efficacy of niosomal formulation and that the effect of two doses given on successive days was additive (Baillie et al., 1986).

• Yoshida et al. investigated oral delivery of desglycinamide, 8-arginine vasopressin entrapped in niosomes in an in-vitro intestinal loop model and reported that stability of peptide increased significantly (Yoshiaka et al., 1992).

• Niosomes can be used as a carrier for hemoglobin. Niosomal suspension shows a visible spectrum superimposable onto that of free hemoglobin. Vesicles are permeable to oxygen and hemoglobin dissociation curve can be modified similarly to non-encapsulated hemoglobin (Moser et al., 1990; Jayaraman et al., 1996).

• Slow penetration of drug through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes. Jayraman et al. has studied the topical delivery of erythromycin from various formulations including niosomes or hairless mouse. From the studies, and confocal microscopy, it was seen that non-ionic vesicles could be formulated to target pilosebaceous glands (Hunter et al., 1988).

COMPOSITION OF NIOSOMES
The two major components used for the preparation of niosomes are, Cholesterol Nonionic surfactants. Cholesterol is used to provide rigidity and proper shape, conformation to the niosomes. The role surfactants play a major role in the formation of niosomes. The following non-ionic surfactants are generally used for the preparation of niosomes the spans(span 60,40,20,85,80), twnens(tween 20,40,60,80) and bij (bij 30,35,52,58,72,76). The non ionic surfactants possess a hydrophillic head and a hydrophobic tail (Parthasarathy et al., 1994, Raja et al., 1994).

Surfactants Used In Formation of Niosomes
Niosomes are non-ionic surfactant unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants. The surfactants that are reported to form niosomes are as follows:

1. Ether linked surfactant
These are surfactants in which the hydrophilic hydrophobic moieties are ether linked, polyoxyethylene alkyl ethers with the general formula (CnEOm), where n; i.e. number of carbon atoms varies between 12 and 18 and m; i.e. number of oxyethylene unit varies between 3 and 7 (Rogerson et al., 1988).

2. Di-alkyl chain surfactant
Surfactant was used as a principal component of niosomal preparation of stibogluconate and its potential in delivering sodium stibogluconate in experimental marine visceral leishmaniasis has been explored.

\[
\text{C}_{16}\text{H}_{31}\text{CH-O[-CH}_{2}\text{-CH-O]_3-H} \\
\text{CH}_2\text{CH}_2\text{OH} \\
\text{C}_{12}\text{H}_{25}\text{-O (mol. Wt. 972)}
\]

3. Ester linked
These are the surfactants in which hydrophilic and hydrophobic moieties are ester linked. Ester linked surfactant,

\[
\text{C}_{15}\text{H}_{31}\text{CO[O-CH}_{2}\text{-CH-CH}_2\text{]_2-OH} \\
\text{OH (mol. Wt. 393)}
\]

This surfactant was also studied for its use in the preparation of stibogluconate bearing niosomes and in delivery of sodium stibogluconate to the experimental marine visceral leishmaniasis following administration of niosomal system. (Rogerson et al., 1988) The commercial sorbitan esters are H-C-OH mixtures of the partial esters of sorbital.
4. Sorbitan Esters:
CH₂ where, R is H or an alkyl chain.
| H-C-OH  |
| RCOO- C-H  |
| H-C-OH  |
| H-C-OOC-R  |
| CH₂OOC-R  |

The formula of a representative component is shown above. Sorbitan esters based niosomes bearing methotrexate were prepared and evaluated for pharmacokinetics of the entrapped methotrexate in tumor bearing mice.

5. Polysorbates
The typical structural formula of polysorbates is

\[
\text{CH}_2 \quad \text{H-C-O(CH}_2\text{-CH}_2\text{-O)} \quad \text{H}
\]
| (OCH}_2\text{-CH}_2\text{-O-C-H) | 
| H-C-O(CH}_2\text{-CH}_2\text{-O)y H} |
| CH₂-O(CH₂-CH₂-O)z OCR |

When \( n = x + y + z + 2 \) and \( R \) is an alkyl chain this series of surfactants has been used to study the pharmacokinetics of niosomal entrapped methotrexate.

FORMULATION OF NIOSOMES
The preparation methods should be chosen according to the use of the niosomes, since the preparation methods influence the number of bilayers, size, size distribution, and entrapment efficiency of the aqueous phase and the membrane permeability of the vesicles.

A. Ether injection method
This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14-gauge needle into an aqueous solution of material. Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions used, the diameter of the vesicle range from 50 to 1000 nm (Mayer \textit{et al.}, 1985).

B. Hand shaking method (Thin film hydration technique)
The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes (Baillie \textit{et al.}, 1986).

C. Sonication
A typical method of production of the vesicles is by sonication of solution as described by Cable. 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 (Baillie \textit{et al.}, 1986).

D. Micro fluidization
Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, 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 (Mayer \textit{et al.}, 1985).

E. Multiple membrane extrusion method
Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes, solution and the resultant suspension extruded through which are placed in series for up to 8 passages. It is a good method for controlling niosome size (Mayer \textit{et al.}, 1985).

F. Reverse Phase Evaporation Technique (REV)
Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes (Raja Naresh \textit{et al.}, 1994). Raja Naresh \textit{et al.} have reported the preparation of Diclofenac Sodium niosomes using Tween 85 by this method.
G. Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading)

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes (Chauhan et al., 1989)

H. The “Bubble” Method

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards “bubbled” at 70°C using nitrogen gas (Blazek et al., 2001).

I. Formation of niosomes from proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed “Proniosomes”. The niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation (Blazek et al., 2001).

T=Temperature.
Tm = mean phase transition temperature.

Blazek-Walsh A.I. et al have reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.

Separation of Unentrapped Drug

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: - (Mayer et al., 1985; Blazek et al., 2001).
1. Dialysis
2. Gel Filtration
3. Centrifugation

EVALUATION OF NIOSOMES (Chauhan et al., 1999)

a) Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analysing the resultant solution by appropriate assay method for the drug. Where, Entrapment efficiency (EF) = (Amount entrapped total amount) x 100

b) Vesicle diameter

Niosomes, similar to liposomes, assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy and freeze fracture electron microscopy. Freeze thawing(keeping vesicles suspension at −20°C for 24 hrs and then heating to ambient temperature) of niosomes increases the vesicle diameter, which might be attributed to fusion of vesicles during the cycle (Mayer et al., 1985).

c) In-vitro release

A method of in-vitro release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer is analyzed for the drug content by an appropriate assay method (Parthasarthy et al., 1994).

Methods for the evaluation of niosomes:

FACTORS AFFECTING NIOSOMES FORMULATION

a) Drug

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. In polyoxyethylene glycol (PEG) coated vesicles, some drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size. The hydrophilic lipophilic balance of the drug affects degree of entrapment (Raja et al., 1994).

b) 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)
because the surface free energy decreases with an increase in hydrophobicity of surfactant (Raja et al., 1994; Khandare et al., 1994).
The bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such as cholesterol. In the gel state, alkyl chains are present in a well-ordered structure, and in the liquid state, the structure of the bilayers is more disordered. The surfactants and lipids are characterized by the gel-liquid phase transition temperature (TC). Phase transition temperature (TC) of surfactant also effects entrapment efficiency i.e. Span 60 having higher TC, provides better entrapment (Khandare et al., 1994).

c) Cholesterol content and charge
Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. In general, 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. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase (Hunter et al., 1988).
An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

d) Resistance to osmotic stress
Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, 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 (Malhotra et al., 1994).

e) Membrane Composition
The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives. In case of polyhedral niosomes formed from C16G2, the shape of these polyhedral niosome remains unaffected by adding low amount of solulan C24 (cholesteryl poly-24- oxyethylene ether), which prevents aggregation due to development of steric hindrance. In contrast spherical Niosomes are formed by C16G2: cholesterol:solulan (49:49:2). The mean size of niosomes is influenced by membrane composition such as Polyhedral niosomes formed by C16G2: solulan C24 in ratio (91:9) having bigger size (8.0 ± 0.03mm) than spherical/tubular niosomes formed by C16G2: cholesterol:solulan C24 in ratio (49:49:2) (6.6±0.2mm). Addition of cholesterol molecule to niosomal system provides rigidity to the membrane and reduces the leakage of drug from noisome (Chauhan et al., 1989).

Table no.1 methods for evaluation of niosomes

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Method</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>SEM, TEM, freeze fracture technique</td>
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<tr>
<td>Size distribution, polydispersity index</td>
<td>Dynamic light scattering particle size analyzer</td>
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<tr>
<td>Viscosity</td>
<td>Ostwald viscometer</td>
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<tr>
<td>Membrane thickness</td>
<td>X-ray scattering analysis</td>
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<tr>
<td>Thermal analysis</td>
<td>DSC</td>
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<tr>
<td>Turbidity</td>
<td>UV-Visible diode array spectrophotometer</td>
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<tr>
<td>Entrapment efficacy</td>
<td>Centrifugation, dialysis, gel chromatography</td>
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<tr>
<td>In-vitro release study</td>
<td>Dialysis membrane</td>
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<tr>
<td>Permeation study</td>
<td>Franz diffusion cell</td>
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CONCLUSION AND FUTURE SCOPE
Drug incorporation in the niosomes to target the niosomes to the specific site is a promising drug delivery model. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure. Niosomes are considered to be better candidates for drug delivery as compared to liposomes due to various factors like cost, stability etc. Niosomes are promising vehicles at least for lipophilic drugs. These advantages over the liposomes make it a better targeting agent. Ophthalmic, topical, parental and various other routes are used for targeting the drug to the site of action for better efficacy.
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