An overview on Niosomes
A novel vesicular approach for Ophthalmic Drug Delivery

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ABSTRACT
Non-ionic surfactant vesicles (Niosomes or NSVS) are widely studied as an alternative to hydrated surfactant monomers. Non-ionic surfactants of wide structural types have been found to be useful alternatives to phospholipids in fabrication of vesicular system. They are the microlamellar structures formed by the mixing of Non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. This review article deals with the detailed use of Niosome as novel drug delivery system for ophthalmic use, since this route is most frequently used for the treatment of eye infection.

Keywords: Niosome, Non-ionic surfactants, Ophthalmic

INTRODUCTION
Non-ionic surfactant vesicles are now widely studied as alternate to liposomes. A wide range of non-ionic surfactant has been found to form vesicles that are capable of entraping hydrophilic and hydrophobic drugs like Acyclovir, Doxorubicin, Methotrexate, Vasopressin, Oestradiol, Sodium stibogluconate etc. Niosomes are unilamellar or multilamellar vesicles formed from synthetic, non-ionic surfactant of alkyl or dialkyl poly glycerol ether class\(^1\), offering an alternative to liposomes as drug carriers. Niosomes have the ability to entrap solutes in a manner analogous to liposomes, They are relatively more stable in vitro and improve the stability of entrapped drug as compared with stability in conventional dosage forms.

In ophthalmic treatment, the site of action may be any ocular tissue, depending on where the disorder is located. Hence the drug should be targeted to many different sites within the eye. Poor bioavailability of drugs from ocular dosage form is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium. Though the topical and localized application are still an acceptable and preferred way to achieve therapeutic level of drugs used to treat ocular disorders but the primitive ophthalmic solution, suspension, and ointment dosage are no longer sufficient to combat various ocular diseases. The use of niosomes in combating the ophthalmic disorders is gaining momentum in the present scenario. This article reveals the importance in using niosomes as a potential ocular drug delivery system and highlights the need for the successful formulation, method of preparation and its characterization etc. to meet the future challenges and thereby rendering the dosage form for ocular therapy more effective.

Advantages of niosomes\(^2\)
1) They can be used for hydrophilic, amphiphilic & lipophilic moieties, Niosomes are widely used for the delivery of drugs like Vincristine, Doxorubicin, Methotrexate, Indomethacin, Diclofenac etc.
2) The various characteristics of vesicle formulation are variable & controllable. Variables like volume of hydrating media used, speed of rotation of the round bottom flask, type of hydrating media, time taken for hydration, amount of cholesterol added etc are the various variables that can effect the type of layer formed and finally the nature of vesicle and it’s entrapment efficiency.
3) They act as depots which release the drug in controlled manner.
4) They are osmotically active and stable.
5) Help to improve Bioavailability.
6) They improve the oral bioavailability of drugs that are poorly absorbed and enhance skin permeation of drugs.
7) They can be made to reach the drug to site of action by oral, parenteral as well as topical route; it has been observed that carrier system can be directed to specific site in the body by the use of antibodies. Immunoglobulins bind frequently to the surface of Niosomic vesicles, thus offering an adequate means of drug targeting.

8) Handling and storage of surfactants require no special conditions.

Why Niosomes are preferred over liposomes?
Niosomes are preferred over liposomes because of several reasons
1. Liposomes are expensive formulations.
2. Ingredients used for liposome preparation like phospholipids are chemically unstable because of their predisposition to oxidative degradation so they require special storage, handling and purity of phospholipids.
4. Niosomes are made up of non-ionic surfactants which are uncharged single chain molecules. Whereas liposomes are made of charged double chain molecules which limits its usage in entrapment of drugs of diverse nature. The surfactants that are used in noisomes formation do not require special storage conditions since they are not subjected to oxidative degradation; moreover they are less expensive & non-toxic.

Structure of Niosome

Constraints to ocular delivery
Topical application of drug to eye is the most popular as well as accepted route of administration for the treatment of various ophthalmic disorders but numbers of limitations are faced in delivering drug to eye for instance:
• Solution drainage
• Lacrimation
• Tear dilution
• Conjunctival absorption

Desirable Properties of Niosomes for ophthalmic use
• Capable of providing targeted drug delivery in the ocular cavity.
• Capable of providing sustained and controlled release of drug to the site of action.
• Capable of overcoming the barriers like solution drainage, lacrimation, tear dilution, conjunctival absorption etc.
• Ease of installation and good patient compliance.
• The degradable products of the vesicles should be non toxic and should not cause irritation in the ocular cavity.
• Formulation should not cause blurring of vision.
• Should be capable of protecting the drug from lysozymes present in the ocular cavity.

METHOD OF PREPARATION OF NIOSOMES
Niosomes can be prepared by a number of methods which are as follows:

Ether injection method: In this method, a solution of the surfactant is made by dissolving it in diethyl ether. This solution is then introduced using an injection (14 gauge needle) into warm water or aqueous media containing the drug maintained at 60°C. Vaporization of the ether leads to the formation of single layered vesicles. The particle size of the niosomes formed depend on the conditions used, and can range anywhere between 50-1000µm.
Hand shaking method (Thin Film Hydration Technique) \cite{6}. In this method a mixture of the vesicle forming agents such as the surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether or chloroform in a round bottom flask. The organic solvent is removed at room temperature using a rotary evaporator, which leaves a thin film of solid mixture deposited on the walls of the flask. This dried surfactant film can then be rehydrated with the aqueous phase, with gentle agitation to yield multilamellar niosomes. The multilamellar vesicles thus formed can further be processed to yield unilamellar niosomes or smaller niosomes using sonication, microfluidization or membrane extrusion techniques.

Reverse phase evaporation technique \cite{7}: This method involves the creation of a solution of cholesterol and surfactant (1:1 ratio) in a mixture of ether and chloroform. An aqueous phase containing the drug to be loaded is added to this, and the resulting two phases are sonicated at 4-5°C. A clear gel is formed which is further sonicated after the addition of phosphate buffered saline (PBS). After this the temperature is raised to 40°C and pressure is reduced to remove the organic phase. This results in a viscous niosome suspension which can be diluted with PBS and heated on a water bath at 60°C for 10 mins to yield niosomes.

Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote loading) \cite{8}. In this method, a solution of surfactant and cholesterol is made in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask, similar to the hand shaking method. This film is then hydrated using citric acid solution (300mM, pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To the niosomal suspension, aqueous solution containing 10mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 using 1M disodium phosphate (this causes the drug that is outside the vesicle to become non-ionic and can thus cross the niosomal membrane, and once again inside the vesicle it is ionized thus not allowing it to exit the vesicle). The mixture is later heated at 60°C for 10 minutes to give niosomes.

The “Bubble” Method \cite{9}: It is a technique which has been recently developed and allows the preparation of niosomes without the use of organic solvents. The bubbling unit consists of a round bottom flask with three necks, and this is positioned in a water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck, while the third neck is used to supply nitrogen. Cholesterol and surfactant are dispersed together in a buffer (pH 7.4) at 70°C. This dispersion is mixed for a period of 15 seconds with high shear homogenizer and immediately afterwards, it is bubbled at 70°C using the nitrogen gas to yield niosomes.

Formation of Proniosomes and Niosomes from Proniosomes \cite{10}: To create proniosomes, a water soluble carrier such as sorbitol is first coated with the surfactant. The coating is done by preparing a solution of the surfactant with cholesterol in a volatile organic solvent, which is sprayed onto the powder of sorbitol kept in a rotary evaporator. The evaporation of the organic solvent yields a thin coat on the sorbitol particles. The resulting coating is a dry formulation in which a water soluble particle is coated with a thin film of dry surfactant. This preparation is termed as Proniosome. The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

**TYPES OF NIOSOMES**

- Multilamellar vesicles (MLV, size >0.05 μm)
- Large unilamellar vesicles (LUV, size >0.10 μm)
- Small unilamellar vesicles (SUV, size 0.025-0.05 μm)

**CHARACTERIZATION OF NIOSOMES**

**Entrapment efficiency** \cite{10,11,12}

Entrapment efficiency largely depends on the preparation method. Non-ionic surfactant vesicles prepared by ether injection method demonstrate higher entrapment efficiency as compared to those prepared by hand shaking method. The Analysis of entrapment efficiency can be done by dialysis or ultracentrifugation methods. The niosome entrapped drug could be separated from the free drug by dialysis method. Fill the prepared noisome into the dialysis bags and dialyze the free drug for 24
hrs into 100 ml of phosphate buffer saline, pH 7.4. The absorbance (A) of the dialysate can be measured against phosphate buffer saline using UV spectrophotometer and the absorbance (A) of the corresponding blank niosome would be measured under the same condition. The concentration of free drug could be obtained from absorbance difference (ΔA = A - A) based on standard curve. The entrapment efficiency of the drug is defined as the ratio of the mass of niosome associated drug to the total mass of drug.

In the ultracentrifugation method, the prepared niosomal suspension will be subjected for centrifugation at high rpm for 30 mins to 60 mins. Analyze the clear supernatant liquid by using spectrophotometer and calculate the amount of un-entrapped drug. Amount of entrapped drug can be obtained by subtracting amount of un-entrapped drug from the total drug incorporated.

Percent entrapped = \frac{\text{[Entrapped drug (mg) / Total drug added (mg)]}}{100}

Size, Shape and Morphological characterization

Vesicular structure of surfactant based vesicles can be visualized and established using freeze fracture electron microscopy while photon correlation spectroscopy could be successfully used to determine mean diameter of the vesicles. Electron microscopy can be used for morphological studies of vesicles while master sizer based on laser beam is generally used to determine size distribution, mean surface diameter and mass distribution of niosomes.

Drug release studies \[^{[12]}\]

The release of drug from niosomes is determined using the membrane diffusion technique. Suspend an accurately measured amount of drug niosomal formulation in 1ml phosphate buffer saline and transferred to a glass tube covered at its lower end by a soaked cellulose membrane. Suspend the glass tube in the dissolution flask of a dissolution apparatus containing 75 ml phosphate buffer saline and rotate it at 50 rpm. Keep the temperature at 37º C. Draw the aliquots of the dialysate at predetermined time and replenish immediately with the same volume of fresh simulated fluid. Analyze the withdrawn samples using spectrophotometer.

Physical stability study \[^{[13]}\]

Physical stability study is required to investigate the leaching of drug from niosomes during storage. Seal the prepared niosomes in 20 ml glass vials and store at a temperature of 2 – 8 ºC for a period spread over 60 – 90 days. Withdraw samples from each batch at definite time intervals and determine the residual amount of the drug in the vesicles after separation from un-entrapped drug by ultracentrifugation or dialysis method.

Zeta potential analysis \[^{[14]}\]

The presence of surface charge in vesicular dispersions is critical. Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and there by reducing the electrostatic repulsion. Vesicle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the zeta potential which can be calculated using the Henry equation.

\[ \zeta = \frac{\mu_E 4\pi \eta}{\Sigma} \]

Where, \( \zeta \) = zeta potential, \( \mu_E \) = electrophoretic mobility, \( \eta \) = viscosity of the medium, \( \Sigma \) = dielectric constant.

Microviscosity of bilayer membrane \[^{[15]}\]

The microviscosity of niosomal membrane can be determined by fluorescence polarization (P) and can be calculated according to the following equation.

\[ P = \frac{(I_p - G I_v)}{(I_p + G I_v)} \]

Where, \( I_p \) and \( I_v \) are the fluorescence intensity of the emitted light polarized parallel and vertical to the exciting light, respectively and \( G \) is the grating correction factor. The fluorescence intensities \( I_p \) and \( I_v \) are measured at various temperatures with spectrofluorophotometer. The microviscosity of vesicular membrane could be measured by DPH (1, 6 diphenyl-1,3,5-hexatriene) (fluorescent probe) method. DPH normally exists in hydrophobic region in the bilayer membrane. According to this technique, the mobility of DPH could be monitored as a function of temperatures. Fluorescence polarization correlates to microviscosity near the probe. High fluorescence polarization means high microviscosity of the membrane. Increase of cholesterol contents result in an increase of microviscosity of the membrane indication more rigidity of the membrane. However, membrane formed with stearyl chain surfactants will be more rigid even without cholesterol. The bilayer
membrane with very low microviscosity could not stably carry water soluble substances in the vesicles.

Rheological properties \[16\]
The viscosity of ophthalmic products is most important parameter because it is generally agreed that an increase in vehicle viscosity increases the residence time in the eye, although there are conflicting reports in the literature to support the optimal viscosity for ocular bioavailability products formulated with a high viscosity are not well tolerated in the eye, causing lacrimation and blinking until the original viscosity of the tear is regained. Drug diffusion out of the formulation into the eye may also be inhibited due to high product viscosity. Finally, administration of high viscosity liquid products tends to be more difficult.
The rheological properties of niosomes can be studied using Ostwald-U-tube at 25° C. Dilute the samples with water to the required concentrations and leave it to equilibrate for 1 hr. Relative viscosity can be calculated by comparing efflux time with that of water.

Ocular irritancy of niosomes \[17\]
The potential ocular irritancy and/or damaging effects of the formulations under test could be evaluated by observing them for any redness, inflammation, or increased tear production. The healthy rabbits weighing 2.5-3 kg should be selected. Introduce the test and control samples into the left and right eyes respectively, once a day for a period of 40 days. Separate the eyes, fix them and cut vertically, dehydrate, clear, impregnate in soft and hard paraffin, section at 8µm thickness with the microtone and stain with haemotoxylin and eosin. Corneal histological examinations can be completed after photographing the stained sections using optical microscopy.

Intraocular pressure \[4, 17, 18\]
Adult male normotensive rabbits weighing 1.5 – 2 kg can be used for the study. Measure the IOP using a tonometer after instilling a drop of a local anaesthetic in both the eyes immediately prior to the instillation of the drug. Change in IOP (ΔIOP) for each eye is expressed as follows,

\[
\Delta \text{IOP} = \text{IOP}_{\text{dosed eye}} - \text{IOP}_{\text{control eye}}.
\]

Aqueous humor analysis \[19, 20\]
The albino rabbits weighing 2.5 kg can be used for the study. Keep the rabbits under anesthesia throughout the experiment by intramuscularly injecting 50/50 mixture of ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (10 mg/kg). To reduce the discomfort further, anaesthetize the eyes using one to two drops of oxybuprocaine. Insert the 25 G needle across the cornea, just above the corneoscleral limbus, so that it traverses through the center of the anterior chamber to the other end of the cornea. Collect the samples and can be stored at - 20°C until analysis carried out. Measure the levels of drug in the aqueous humor samples by using HPLC with UV detector.

CONCLUSION
Niosomes alone or in combination with mucoadhesive polymers can be used as an efficient vesicular system that can deliver drug to eye, most of the formulations present in market at present are not capable of overcoming the problems of tear dilution, lacrimation, solution drainage where as Niosomes serve The main aim of ophthalmic preparation is to give the maximum drug absorption through prolongation of the drug residence time in the cornea and conjunctival sac as well as to slow drug release from the delivery system and minimize precorneal drug loss.

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