NON-IONIC SURFACTANT BASED VESICLE 'NIOSOME' AS A POTENTIAL OCULAR DRUG DELIVERY SYSTEM

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ABSTRACT
The common principle for the success of pharmacotherapy is that the suitable drug should be present at the site of action in an effective concentration for a desired period of time. 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 form 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.

Keywords: Ocular drug delivery system, Niosomes, Niosomes preparation, Non-ionic surfactant vesicle.
INTRODUCTION

Ocular diseases were widely noticed since the inception of human race and animals. Reference to diseases of the eye in dogs and cattle has been found in 4000-year-old papyri of Ancient Egypt. Between 450 and 510 AD\(^1\), eight chapters devoted to the eye diseases of the horse and related therapies were translated from Greek by Publius Vegetius Renatus in Artis Veterinariae sive Mulomedicinae. Most veterinary topical ophthalmic drugs and delivery systems (solutions, suspensions and ointments) are derived from human ocular formulations, since in animals the main diseases are similar (e.g. inflammation, keratoconjunctivitis sicca, glaucoma) and exhibit similar pathologies to those of the human eye. In human, various disease states such as keratitis, glaucoma, iritis and cataract affect the anterior segment of the eye. Similarly, the posterior segment of the eye is affected by disease states such as diabetic retinopathy, viral and bacterial infections, malignancies, proliferative vitreal disorders and macular degeneration.

The tendency today is to find ocular delivery systems that are ‘patient-friendly’. Most ocular diseases are treated with a topical application of drug solutions administered as eye-drops. The relative percentages are 62.4% for solution, 8.7% suspensions and 17.4% ointments\(^3\). These conventional dosage forms account for nearly 90% of the currently accessible marketed formulations.

One of the major problems encountered with the topical delivery of ophthalmic drugs is the rapid and extensive precorneal loss caused by the high tear fluid turnover\(^5\) as well as the relatively large volume of the administered eye drop (~50 µl versus 7 µl of corneal tear film), lead to a high rate of lacrimal drainage. Due to the resulting elimination rate, the precorneal half life of drugs applied by these pharmaceutical formulations is considered to be between about 1-3 min. As a consequence, only the very small amount of about 1-3% of the dosage actually penetrates through the cornea and is able to reach intraocular tissues\(^6, 7, 8\).

Anatomical and physiological parameters in the human eyes\(^9\) were summarized in Table No.1. The poor productive absorption, on the other hand, results in a high amount of drug that is drained into the nose or into the gut. Especially the nose but also the gut is very efficient absorption organs of the body. This in turn leads to an extensive systemic absorption and may result in unwanted side effects and toxicity of the drug.

Lacrimation and blinking are actually efficient protective mechanisms which keep the eye free from foreign substances, but they prevent efficient ocular therapy. As a consequence, the
ocular residence time of conventional eye drops is limited to a few minutes and the ocular absorption of a topically applied drug is reduced to approximately 1-10%. Furthermore, drug uptake occurs as a massive pulse entry followed by a rapid decline. The drug is mainly absorbed systemically via conjunctiva and nasal mucosa, which may result in some undesirable side effects. Although these problems have been recognized for a long time, surprisingly little effort has been made by drug companies to improve the situation, and only very few alternative ocular drug delivery systems are on the market. To overcome these problems, various ophthalmic vehicles such as ointments, suspensions, micro- and nanocarrier systems, Inserts, and liposomes have been investigated.

**Vesicular System**

In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering. Vesicles can play a major role in modeling biological membranes, and in the transport and targeting of active agents. Vesicular systems not only help in providing prolonged and controlled action at the corneal surface but also help in providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. The rate of drug penetration depends not only on the physicochemical properties of the drug itself, such as its solubility, and particle size, in case of suspensions but also on those of its vehicle. In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can cross cell membrane. Vesicles, therefore, can be viewed as drug carriers and as such they change the rate and extent of absorption as well as the disposition of the drug. Vesicular drug delivery systems used in ophthalmics broadly include liposomes and Niosomes.

Drug delivery systems using colloidal particulate carrier such as liposomes have distinct advantages over conventional dosage forms. This carrier can act as drug reservoir, and modification of its composition or surface can adjust the drug release rate and/or the affinity for the target site. Liposomes can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains. The first study to utilize liposomes for ophthalmic therapy was reported by Smolin et al. They compared the
therapeutic efficacy of idoxuridine in solution and liposomal form in the treatment of acute and chronic herpetic keratitis in the rabbit eye.

**Niosomes In Lieu Of Liposomes – Reasons**

One of the most significant problems associated with the use of liposomes as adjuvant is the susceptibility of phospholipids to oxidative degradation in air. This requires that purified phospholipids and liposomes have to be stored and handled in an inert (e.g. nitrogen) atmosphere. Phospholipid raw materials are naturally occurring substances and as such require extensive purification thus making them costly. Alternatively, phospholipids can be synthesised de novo, however this approach tends to be even more costly than using naturally occurring lipids. Because of liposomes above mentioned drawbacks, alternative nonionic surfactants have been investigated. This involves formation of liposome-like vesicles from the hydrated mixtures of cholesterol and nonionic surfactant such as monoalkyl or dialkyl polyoxyethylene ether. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes. From a technical point of view, niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids. Another advantage is the simple method for the routine and large-scale production of niosomes without the use of unacceptable solvents. Cholesterol, 5-cholesten-3β-ol is used in combination with nonionic surfactant for the formation of niosomes.

**Points To Meet Successful Niosomes Formulation**

The fundamental requirements for the success of successful niosomes used in ocular delivery may be summarized as follows.

Physician acceptance, User acceptance, Ease of handling and application, Patient comfort, Lack of expulsion during application, Lack of toxicity, Noninterference with vision and oxygen permeability, Reproducibility of release kinetics, Applicability to a variety of drugs, Sterility, Stability, Ease of manufacture, Reasonable price and Duration of action.

**Prerequisites of Controlled Ocular Delivery Systems**

1. To overcome the side effects of pulsed dosing produced by conventional therapy.
2. To provide sustained and controlled release.
3. To increase the ocular bioavailability of drug by increasing corneal contact time. These can be achieved by effective coating or adherence to corneal surface, so that the released drug effectively reaches the anterior chamber.

4. To provide targeting within the ocular cavity so that prevents the loss to other ocular diseases.

5. To circumvent the protective barriers like drainage, lacrimation and diversion of exogenous chemicals into the systemic circulation by the conjunctiva.

6. To provide comfort and compliance to the patient and yet improve the therapeutic performance of the drug over conventional systems.

7. To provide the better housing of the delivery system in the eye so as the loss to other tissues besides cornea is prevented.

**Advantages Of Niosomes In Ocular Drug Delivery**

1. **Niosomes** are quite stable structures, even in the emulsified form. They require no special conditions such as low temperature or inert atmosphere for protection or storage.

2. They are chemically stable as compare to liposomes.

3. They can entrap both hydrophilic and hydrophobic drugs.

4. Nontoxic, biodegradable, biocompatible and non-immunogenic.

5. Flexible in their structural characterization (composition, fluidity and size).

6. They can improve the performance of drug molecules via delayed clearance from circulation, better bioavailability and controlled drug delivery at the desired site.

7. A number of non-ionic surfactants have been used to prepare vesicles viz. polyglycerol alkyl ether, glucosyl dialkyl ethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether, Brij, and a series of spans and tweens.

8. Relatively low cost of materials makes it suitable for industrial manufacture.

9. Handling and storage of surfactants require no special conditions.

10. No tissue irritation and damage as caused by penetration enhancers.

11. They prevent the metabolism of drugs from the enzymes present at tear / corneal epithelium interface.

12. Provide a prolonged and sustained release of drug.

**Retrospect Of Niosome**

**Niosomes**: were first reported in the seventies as a feature of the cosmetic industry by Vanlerberghe et al, Handjani-vila et al., ; Van Abbe explained that the non – ionic
Surfactants are preferred because the irritation power of surfactants decreases in the following order: cationic > anionic > ampholytic > non-ionic. Green and Downs, Keller et al. Burstein, Kaur and Smitha reported that an increased ocular bioavailability of water soluble, entrapped in niosomes, may be due to the fact that surfactants also act as penetration enhancers as they can remove the mucus layer and break functional complexes. Handjani-Vila et al. reported that vesicular systems were formed when a mixture of cholesterol and single alkyl chain, non-ionic surfactants was hydrated. The resultant vesicles, termed as niosomes can entrap solute. Okhata et al. suggested vesicle formation by some members of dialkylpoly oxyethylene ether non-ionic surfactant series. Singh and Mezei stated that niosomes are a suitable delivery system for both hydrophilic and lipophilic drugs. Baillie et al. reported that niosomes are osmotically active and relatively stable. Lasic stated that the assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same. The non-ionic surfactant vesicles have been reported successfully by Saettone et al. as ocular vehicle for cyclopentolate. Carafa et al. stated that niosomes are the non-ionic surfactant vesicles and like liposomes are bilayered structures, which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane, made up of lipids. Vyas et al. prepared both niosomes and disomes of water-soluble drug timolol maleate and found that disomes entrapped comparatively a higher amount of drug (25% as compared to 14% in case of niosomes). Moreover, an increase in ocular bioavailability was found to be approximately 3.07-fold compared to 2.48-fold in case of niosomes with respect to timolol maleate solution. Carafe et al. reported that niosomes are biodegradable, biocompatible, and non-immunogenic. Indu P. Kaur et al. gave an impression on vesicular system in ocular drug delivery. Deepika Aggarwal et al. studied the improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. Deepika Aggarwal et al. studied the ocular absorption of acetazolamide by microdialysis sampling of aqueous humor. Ghada abdelbary and Nashwa el-gendy investigated the feasibility of using non-ionic surfactant vesicles as carriers for the ophthalmic controlled delivery of a water soluble local antibiotic, Gentamicin sulphate.
**Method Of Preparation Of Niosomes:** To date, different methods have been reported on preparation of niosomes by Azmin et a., Baillie et al., Chopineau et al., Handjani- Vila et al., Kiwada et al., Niemec et al., Talsmae et al., Wallach and Philippot, Yoshioka et al. Small unilamellar vesicles (SUV, size 0.025-0.05 μm) are commonly produced by sonication, and French Press procedures. Ultrasonic electrocapillary emulsification or solvent dilution techniques can be used to prepare SUVs.

**Multilamellar vesicles** (MLV, size >0.05 μm) exhibit increased-trapped volume and equilibrium solute distribution, and require hand-shaking method. They show variations in lipid compositions.

**Large unilamellar vesicles** (LUV, size >0.10 μm), the injections of lipids solubilised in an organic solvent into an aqueous buffer, can result in spontaneous formation of LUV. But the better method of preparation of LUV is Reverse phase evaporation, or by Detergent solubilisation method.

However, the more commonly used laboratory methods of niosome preparation and drug loading identified in the literature are listed below.

1. The ether injection method is essentially based on slow injection of surfactant : cholesterol solution in ether through a 14 gauge needle at the rate of approximately 0.25 ml/min into a preheated aqueous phase maintained at 60°C. The mechanism whereby relatively larger unilamellar vesicles are formed however is not understood, presumably it could be ascribed to the slow vapourization of solvent resulting into a ether gradient extending across the interfacial lipid / surfactant monolayer at ether-water interface. The latter subsequently may result into the formation of a bilayer sheet, which eventually folds on itself to form sealed vesicles.

2. Surfactant and cholesterol mixture is dissolved in diethylether in a round bottomed flask. The ether is evaporated under vacuum at room temperature in a rotary evaporator. Upon hydration the surfactant swells and is peeled off the support in to a film, like lipids in lipid based film. Swollen amphiphiles eventually fold to form vesicles.

3. The formation of oil in water (o / w) emulsion from an organic solution of surfactants / lipids and an aqueous solution of the drug. The organic solvent is then evaporated to leave
niosomes dispersed in the aqueous phase. In some cases, a gel results which must be further hydrated to yield niosomes. (reverse phase evaporation).

4. The injection of melted lipids / surfactants into a highly agitated heated aqueous phase in which presumably the drug is dissolved or the addition of a warmed aqueous phase dissolving the drug to a mixture of melted lipids and hydrophobic drug. To this method, do not require the use of organic solvents, which are expensive, difficult to remove in their entirety and hazardous.

5. The addition of the warmed aqueous phase to a mixture of the solid lipids / surfactants. This also does not require the use of organic solvents.

6. Niosomes may also be formed from a mixed micellar solution by the use of enzymes. A mixed micellar solution of C16 G2, DCP, polyoxyethylene cholesteryl sebacate diester (PCSD) converts to a niosome dispersion when incubated with esterases. PCSD is cleaved by the esterases to yield polyoxyethylene, sebacic acid and cholesterol. Cholesterol in combination with C16 G2 and DCP then yields C16 G2 niosomes.

7. The homogenization of a surfactant / lipid mixture followed by the bubbling of nitrogen gas through this mixture. Apparently the homogenization step may be omitted from the procedure without affecting particle size, although a longer bubbling time was required.

Characterization Of Niosomes Involved In Ocular Drug Delivery

Entrapment efficiency

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 60mins. Analyse 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 = \[ \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \] X 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**

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**

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 - 8ºC 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**

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**

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 microciscosity 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**

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 25º 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**

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 haematoxylin and eosin. Corneal histological examinations can be completed after photographing the stained sections using optical microscopy.

**Intraocular pressure**

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, ΔIOP = IOPdosed eye – IOPcontrol eye.

**Aqueous humor analysis**

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 -20ºC until analysis carried out. Measure the levels of drug in the aqueous humor samples by using HPLC with UV detector.

**Future Prospects**

Currently the available conventional dosage forms for ophthalmic use induce the absorption of drugs only to the extent of 1-3% through the cornea. 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. The non ionic surfactant vesicles fulfill all the requirements for the controlled ocular delivery system and in addition, it has the advantage of drug to be administered in the form of a drop, which increases patient compliance. There is very good scope for the non-ionic surfactant vesicles in ocular drug delivery system. However, indepth knowledge about the physicochemical characteristics of the drug molecule and expected interaction and implications of entrapping the same into a vesicular system is important.

REFERENCES