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Review Article

NON-IONIC PROVESICULAR DRUG CARRIER: AN OVERVIEW

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ABSTRACT

Non ionic surfactant vesicles are ideal means of drug delivery that can enhance bioavailability of encapsulated drug by various mechanisms and provide therapeutic activity for prolonged period of time. However they are suffered with aggregation, fusion, leaking, sedimentation of vesicles, difficulty in sterilization, so to overcome these problems a newer approach was employed known as pro vesicular carriers here in this review we elaborate one of the pro vesicular carrier, widely known as proniosome which is semisolid liquid crystal (gel) products of non ionic surfactants converted into niosomes (non ionic surfactant vesicular system) upon hydration. This review covers all aspects of proniosomes including mechanism, formulation variables and their affects, methods of preparation, parameters for characterizations and reported formulations with emphasize on transdermal route of delivery.

Keywords: Proniosomes, niosomes, liquid crystals.

INTRODUCTION

Vesicular carriers are colloidal particles in which a concentric bilayer made up of amphiphilic molecules surrounds an aqueous compartment. These amphiphilic molecules viz phospholipid, surfactant (Non ionic, ionic in combination) are either present separately or in combination along with cholesterol as fluidity buffer. Vesicular carriers shows a very promising role in permeability improvement[1] and solubility enhancement[2] and therefore they can also improve the bioavailability of drug by enhancing stability, absorption, targeting to site of action which is actually the resultant of improved solubility, stability and permeability. The stability of peptide drugs have been reported viz. insulin loaded niosomes[3] showed enhancement in their absorption as they become more resistant to proteolytic enzymes and gastric pH due protective sheath of non ionic surfactants. Vesicular carrier viz. liposome, niosomes provides a alternative path for drug delivery by enhancing permeability and increasing occlusion time on skin. Some drugs Hydralazine[4], methotrexate[5], gallic acid[6] levonorgestrol[7], estradiol[1], flurbiprofen[8], tenoxicam[9], captopril[10], ketorolac[11], carvediol[12], minoxidil[13], ellagic acid[14] have been evaluated for transdermal application in niosomal or proniosomal carriers. Niosomes has been also tested as ophthalmic administration of acetazolamide[15]. Vesicular carriers are further exploited in vaccination as adjuvant for enhancing the presentation of immunogens[16]. These carriers, not only provides alternative routes they also provide a sustained action due to prolonged release. All mentioned studies showed that vesicular carriers are very promising and effective for drug delivery. However, on other hand these carriers also suffer from some shortcomings viz. liposomes have poor shelf life, less purity of ingredients, high cost, poor yield, restricted storage condition, difficulty in sterilization; niosomes also show aggregation, fusion, leaking, sedimentation of vesicles, difficulty in sterilization. So a new approach of provesicular carriers has been introduced. Proniosomes are semisolid liquid crystal (gel) products of non ionic surfactants[7], prepared by techniques such as coacervation phase separation, slurry method and spray drying, upon subsequent hydration by means of incorporation in hydrophilic gel or by absorbing moisture from site of administration turns to niosome. Proniosomes are more stable and convenient than niosomes and also provide following advantages viz. ease in transportation, distribution, storage, dosing, sterilization. The release profile of both proniosomes and niosomes indicate that proniosomes derived niosomes are at least effective as conventional niosomes[17]. Some researchers have also showed some results for particular drug where proniosome show better permeation than niosomes. This may be justified on the basis of less relative concentration of non ionic surfactants[1].

Mechanisms[18, 19]

following are the possible mechanisms of penetration and release proposed by various authors-

The vesicles in contact with stratum corneum aggregates, fuse and adhere to cell surface. It is believed that this interaction leads to a high thermodynamic activity gradient of the drug at the vesicle stratum corneum interface, which may be the driving force for penetration of lipophilic drug across the stratum corneum.

-The stratum corneum-vesicles interaction involves the changes in ultra structures of the intercellular lipid regions of the stratum corneum and its deeper layers at maximum depth of about 10 µm as revealed by freeze fractured microscopy and single angle X-Ray scattering (SAXS).

- The bilayers of vesicles also function as rate limiting membrane barrier for drug release.

FORMULATION ASPECTS

Non ionic surfactants are better candidates for vesicular formation than other surface active agents viz cationic, anionic, as they are less toxic, less haemolytic and less irritating to cellular surface and tend to maintain near physiological pH in solution[21]. They also inhibit p-glycoprotein, this leads to better targeting of specific tissues and the expulsion of drug from tissues is also inhibited. This property was studied for anticancer drug doxyrubicin[22]. Non-ionic surfactants might increase the membrane fluidity of the intercellular regions of the stratum corneum (e.g., Brij_) and may extract lipid components and additionally, though of minor importance, they might interfere with keratin filaments and create a disorder within the corneocytes[2].

Surfactants

Surfactants consists of two segments ionic and non ionic and so possess good interfacial activity at oil water interface. The formation of bilayer vesicles instead of micelles is dependent on the HLB of the surfactant, the chemical structure of the components and the critical packaging parameter.

CPP[23] can be calculated by following formula

CPP= V/L*A

Where:

V= Volume of hydrophobic group, L=Length of hydrophobic group, A= area of hydrophilic group

At

CPP≤0.5 micelles formation, CPP=(0.5-1.0) spherical vesicles formation, CPP≥1.0 inverted micelles form

Polyoxyethylene Alkyl Ether

The polyoxyethylene alkyl ethers are a series of polyoxyethylene glycol ethers of n-alcohols (lauryl, oleyl, myristyl, cetyl, and stearyl

alcohol).Polyoxyl ethers having high HLB value such as Brij 78 (HLB=15.3) do not form vesicles as it is dispersible in water although Polyoxyl 2 stearyl ether also known Brij 72 (HLB=4.7,T_c=53^{0}c) produced proniosomal gel of carvediol[12]. Polyoxyethylene alkyl ethers are stable at acidic as well as basic pH. They also exhibit incompatibility with some chemical as iodides, mercury salts, phenolic substances, salicylates, sulfonamides, and tannins and benzocaine, tretinoin and oxidizable drugs[24].

Sorbitan Esters (Sorbitan Fatty Acid Esters)

Sorbitan monoesters are a series of mixtures of partial esters of sorbitol and its mono- and dianhydrides with fatty acids. Sorbitan monolaurate (span 20), sorbitan monopalmitate(span 40), Sorbitan monostearate (span 60), Sorbitan monooleate (span 80) have HLB values 8.6, 6.7, 4.7 and 4.3 respectively. They show higher entrapment as their chain length increases but sorbitan mono laurate is the exception because it has double bond in its carbon chain so this double bond may produce a tilt in carbon chain that's why bilayer packing is not compact and it forms leaky vesicles so the final entrapment order may be as follows span80<span20<span<40<span60 at moderate conc. of cholesterol[8].

Polyoxyethylene Sorbitan Fatty Acid Esters

Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 mole of ethylene oxide for each mole of sorbitol and its anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound. Tween is one of the most common name of polyoxyethylene sorbitan fatty acid esters. Tweens are reported to produce vesicles in presence of high concentration of cholesterol so that the drug entrapment of lipophilic drug eg. Estrdiol. It may be possible that cholesterol competes with the drug in bilayer[1], on the other hand tween with different type of lecithin showing very good entrapment for tenoxicam in comparison to spans[9]. Tweens formed vesicles are more leaky due to hydrophilic nature of tween so sometimes gives a higher flux value but this trend is not follows in case of proniosomes[1].

Lecithin

The USPNF 23 describes lecithin as a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates as separated from a crude vegetable oil source. The composition and so the physical properties depends upon the source from where it is obtained. Egg lecithin, for example, contains 69% phosphatidylcholine and 24% phosphatidylethanolamine, while soybean lecithin contains 21% phosphatidylcholine, 22% phosphatidylethanolamine, and 19% phosphatidylinositol, along with other components[25]. Various types of Lecithin with unsaturation have more permeability enhancing properties which may be due to tilt in side chain that's why soya lecithin shows more permeability then egg lecithin.

Di Cetyl Phosphate(DCP)/ stearyl amine(SA)(charged lipids/ stabilizer):

DCP and SA induce charge in vesicles leading to less aggregation of vesicles and increases stability of dispersion, but due to presence of charge, the entrapment of charged drugs in that environment may be decreased due to possible electrostatic repulsion between same charges as reported by[26]. Use of these charge inducing agent in bilayers induce the hydrophilicity so enhanced water uptake takes place[27]. Moreover charge species in bilayer induces repulsion among adjacent layers to produce large size vesicles in case of niosomes.

Solutol HS 15

Solutol HS 15 is polyglycol mono- and di-esters of 12-hydroxystearic acid with about 30% polyethylene glycol. In niosomes it provide stability by hydrophilic ethylene oxide chain and act as a steric stabilizers. Studies reveal that addition of steric stabilizer in niosomes can only produce a stable dispersion[28].

Effect of hydration medium

Hydration medium is selected on the basis of solubility of drug commonly phosphate buffer of different pH is being used for this purpose. Here we would like to report some results which are reported in case of tenoxicam[20], with phosphate buffer formulation with span60 and cholesterol showed highest entrapment rather than other formulation with lecithin. In case of proniosomes prepared with distilled water lecithin free formulation using tween 60 showed highest entrapment. And 0.1% glycerol formulation with tween 60 and soya bean lecithin possessed best entrapment[9]. However considering other results reported earlier one thing can be concluded that the entrapment and release is affected by combination of various factors, more study is needed to establish a clear cut theory.

Effect of alcohol

Vesicles with ethanol are of highest size due to slowest phase separation because of its greater solubility in water. Isoprpopanol results in vesciles of smallest size which may be due to branched chain present in it. The effect of different alcohol on drug permeation profile was obtained in order: Isopropanol>butanol>propanol>ethanol, thus permeation increases from C_2 to C_4 (ethanol to butanol). But levonorgestrel showed maximum permeation with isopropanol may be due to branched chain it works as a cosurfactant and so loosen the bilayer packing to enhance overall permeation[7].

Carrier material

Many water soluble materials have been exploited for preparing proniosomes preparation by slurry technique in which dry proniosomes formulation is produced which on hydration yields niosomes thus eliminating the physical and chemical stability problem viz. aggregation, precipitation, hydrolysis. Sorbitol (describes maltodextrin as a nonsweet, nutritive saccharide mixture of polymers that consist of D-glucose units, with a dextrose equivalent (DE) less than 20) and maltodextrin (describes maltodextrin as a nonsweet, nutritive saccharide mixture of polymers that consist of D-glucose units, with a dextrose equivalent (DE) less than 20) are the two most widely used carriers but former one have some problems with regard of entrapment of drug and carrier concentration[17]. On the other hand use of maltodextrin provides a wide range of flexibility in the amount of surfactants and other components, therefore also useful in scale-up production[29].

Cholesterol

Cholesterol is integral part of biological membrane where it influences several membrane properties such as aggregation, ion permeability, fusion process size and shape[30]. Cholesterol acts as fluidity buffer in bilayer membrane providing stability and rigidness to vesicle membrane. It also causes a change in transition temperature producing change in stability and other characteristic change in vesicles such as release and entrapment[31]. At higher concentration cholesterol causes decreases in entrapment, may be it competes with drug in bilayers. Effect of cholesterol concentration was reported by several workers one of them is mentioned here, entrapment into span 20 is not significantly altered by cholesterol. while in case of span 80 significant increase in entrapment is found. This can be explained on the basis of lowest phase transition temperature due to unsaturation present in oleate side chain. Cholesterol turns the bilayer membrane more ordered and abolish the gel to liquid phase transition above the phase transition temperature in niosome system, hence it prevents the leakage[2]. However, low entrapment in case of span 20 might be justified on the basis of small side chain. However, a significant increase in entrapment efficiency of flurbiprofen was obtained when 10% of cholesterol was incorporated into niosomes prepared from Sp 40 and Sp 60 Further increase leads to decrease in entrapment efficiency[8] this may be due to the following factors i) with increase in cholesterol content, the hydrophobicity and stability increases[32] ii) at higher ratio cholesterol may compete for packing space with drug in bilayer. Beyond a certain concentration cholesterol may disrupt the regular linear structure of vesicular membranes[33]. Apart from

proniosomes some findings regarding niosomes were also discussed as ultimate carrier is niosomes, cholesterol and span 60 (surfactant) in molar ratio 1:1 would be optimal to get highest entrapment[13, 26]. Chaw CS (2012) with the help of DSC analysis reported that cholesterol with span 60 in niosomes shows a higher transition temperature, endothermic peak was reported at 60^0 c in place of 53^0 c so it partially abolished the gel to liquid phase transition at relatively low temperature thus promoting the formation of a less ordered liquid-crystalline state as vesicles. So these vesicles contain feature of sustained drug delivery.

Phase transition temperature

Here it is the temperature at which ordered gel state turns to less ordered liquid phase. Formulation having higher phase transition temperature showed higher entrapment may be due to formation of less leaky vesicles[28]. We mentioned this property due to its importance in our topic, it provides a good idea regarding entrapment and release.

METHODS OF PRODUCTION

Slurry Method

A stock solution of cholesterol, di cetyl phosphate and suitable surfactant, in optimized ratio is agitated in a rotor evaporator at 60 rpm. Vacuum is applied till the material dries. Product is stored in a sealed bottle at 4^o c. Different load may be prepared by varying stock solution and carrier material ratio. Chengjiy Hu et al. prepared proniosomes of ibuprofen by this method and conclude that proniosomes derived niosomes are as effective as conventional niosomes by comparing release data.

Spray Drying Method

In this method carrier material is taken in a flask attached to rotator evaporator. A mixture of surfactant, cholesterol and other stabilizer is prepared in optimized ratio. This solution is added to sorbitol powder placed in round bottom flask connected with rotator evaporator by sequential spraying of aliquots in a controlled manner to prevent over drying of bed. Then evaporator is evacuated and rotating flask, lowered in into water bath maintained at 65-70^{o}c and rotated in water bath for 15-20 min. till whole volume of surfactant is applied. Evaporation is continued till the powder gets dried completely. This proniosomes formulation is stored in sealed container at 4^o c.

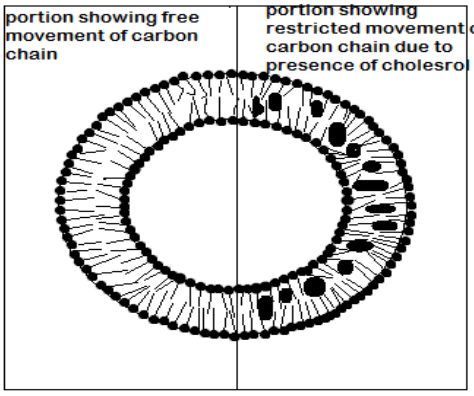
Coacervation Phase Separation

In this method optimized quantity of drug, lecithin, surfactant, cholesterol is dissolved in absolute alcohol keeping mouth of the container covered. warmed in a water bath at 65^o(+-3) c for 5 min. Optimized quantity of buffer pH 7.4 is added and still warmed on water bath for about 2 min. till the solution appeares clear. Then mixture is allowed to cool down at room temperature and proniosomal gel is obtained by change in temperature[4]. Ammar et al. prepared tenoxicam proniosome by employing this method as tenoxicam is a non steroidal anti-inflammatory drug used in rheumatic diseases while show good efficacy in disease management but suffers from marked gastrointestinal side effects on prolonged use so proniosomes were developed using various non ionic surfactants with other aids best formulation in terms of release and entrapment was lecithin free having Tween 20 and cholastrol in 9:1 ratio. Optimized formulation showed significant improvement in anti inflammatory and analgesic properties then oral marketed formulations.

CHARACTERIZATION

Surface Morphology

Vesicle structure and shape can be characterized by various types of microscopy such as optical, freeze fracture electron72, surface electron, scanning electron, negative staining transmission electron, cryo-electron, fluorescence and confo-cal. The interfacial surface tension of a vesicular system determines the structure of the supramolecular elements of multi lamellar vesicles.



Comparitive art of niosomes showing restriction of free movement of carbon chain by presence of chlesterol

Figure: 1

Size and vesicle charge

Size and charge of vesicles have considerable effect on stability and drug encapsulation. Size and charge can be assessed using a multifunctional zeta potential analyzer where size of vesicles is the result of repulsion forces between the bilayers and the entrapped drug. Particle size analysis can be done by Malvern Mastersizer[35], Optical microscopy[10], Laser diffraction particle size analyzer[17, 36], Coulter submicron size analyzer[1].

Aerodynamic Behavior

Aerodynamic behavior is important for study with regard of nasal route. Particles behavior in air stream also state about nebulisation efficiency of the particles. Twin-Stage Impinger may be used to carry out such studies[35].

Entrapment efficiency

This is determined by measuring the difference between the unentrapped and total amounts of drug. Unentrapped drug is determined by various techniques such as exhaustive dialysis, gelfiltration and centrifugation. Total amount of drug can be determined by rupturing a specific amount of a preparation and analyzing it, with a suitable analytical method. Percent entrapment can then be calculated by the usual formula.

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped} \times 100}{\text{Total Amount of drug}}$$

Spontaneity

Spontaneity is defined as rate of hydration that is number of proniosomes converted into niosomes after hydration of proniosomes for 15 minutes. Minute quantity of proniosomes placed at the bottom of a small stoppered glass tube, is spread uniformly. One mL saline (0.154 M Nacl) is added carefully along the walls of test tube and kept aside without agitation. After 15 minutes a drop of above solution is placed on neubaur's chamber. The numbers of niosomes formed are counted with the help of compound microscope[7].

In vitro release

In-vitro release studies are carried out by dialysis through a semi permeable membrane. A niosome preparation is incorporated in an open end dialysis membrane and placed in a receptor compartment containing buffer. Samples are periodically collected and analyzed using a suitable analytical method.

Skin irritancy test

Irritancy test is important to be carried out as safety issues are primarily concerned with developed formulations in transdermal drug delivery. The dose is applied on the skin and the development

of erythema is monitored daily for 6 days. Extents of development of erythema may be indicated on the basis of the following[37].

0: Noerythema development; 2: barely visible few blood vessels and 3:light erythema development;

4: main blood vessels visible and 5: slight erythema development; 6: main blood vessels more obvious and slight erythema development.

Irritation potential was calculated using the following equation:

Resultant index = A · B/number of observation days,

where A and B represent erythema value and corresponding day, respectively.

CONCLUSION

Proniosomal formulation which is converted into niosomes found novel and efficient approach of drug delivery. Proniosomes were also found free from aggregation, fusion, leaking and sedimentation of vesicles with good stability profile. These are actually non aqueous form which is converted into niosomes upon hydration though some formulation factors behave differently for niosomes and proniosomes such as effect of alcohol. Proniosomes can be prepared easily by various techniques they show promising role in enhancing stability of formulation, ease in handling, enhancing bioavailability, controlled and prolonged drug delivery by various routes of drug delivery. But there is lack of uniform pattern with different drugs may be due to drug related factors such as hydrogen bonding, physiochemical properties of drug molecules so there is more research needed to explore more facts. New emerging trend with this is vaccine and antigen delivery as niosomes may work as a adjuncts and enhance over all presentation of antigen to antigen recognizing cell. As niosomes first exploited as cosmetic delivery system they still have potential in that field too need to be explored.

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