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Review Article

NIOSOMES: AN EXCELLENT TOOL FOR DRUG DELIVERY

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ABSTRACT

Design and development of novel drug delivery system (NDDS) has two prerequisites. First, it should deliver the drug in accordance with a predetermined rate and second it should release therapeutically effective amount of drug at the site of action. Conventional dosage forms are unable to meet these requisites. Niosomes are essentially non-ionic surfactant-based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulting from the organisation of surfactant macromolecules as bilayer. Niosomes are formed on hydration of non-ionic surfactant film which eventually hydrates imbibing or encapsulating the hydrating aqueous solution. The proposed review deals with composition, methods of preparation, and applications of niosomes in the pharmaceutical field. The main aim of development of niosomes is to control the release of drug in a sustained way, modification of distribution profile of drug and for targeting the drug to the specific body site.

INTRODUCTION

Vesicular systems are a novel means of drug delivery that can enhance bioavailability of encapsulated drug and provide therapeutic activity in a controlled manner for a prolonged period of time. Niosomes are nonionic surfactant vesicles in aqueous media resulting in closed bilayer structures that can be used as carriers of amphiphillic and lipophilic drugs [1].Niosomes are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol and the enclosed interior usually contains a buffer solution at appropriate pH[2]. In niosomes, the vesicles forming amphiphile is a non-ionic surfactant stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate[3]. Non-ionic surfactants provide a few advantages over the phospholipids because they are more economical and are chemically more stable as they are not easily hydrolysed or oxidised during storage. The vesicular structure can be modified to provide sustained or controlled drug delivery thus enhancing efficacy of system for prolonged periods [4].

ADVANTAGES

The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems for therapeutic purpose may offer several advantages: -

1. High patient compliance in comparison with oily dosage forms as the vesicle suspension is a water–based vehicle.
2. Accommodate drug molecules with a wide range of solubilities.
3. The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, surface charge and concentration can control the vesicle characteristics.
4. The vesicles may act as a depot, releasing the drug in a controlled manner.
5. They are osmotically active and stable, as well as they increase the stability of entrapped drug.
6. Handling and storage of surfactants requires no special conditions.
7. They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
8. They can be made to reach the site of action by oral, parenteral as well as topical routes.

9. The surfactants are biodegradable, biocompatible and non-immunogenic.

NIOSOME FORMATION

Theoretically, niosomal formation requires the presence of a particular amphiphile and aqueous solvent. The association of amphiphile monomers into vesicles on hydration is the result of a high interfacial tension between water and hydrocarbon portion (or any other hydrophobic group) of the amphiphile, causing them to associate. Simultaneously, the steric hydrophilic and ionic repulsion between the head groups ensure that these groups are in contact with water. These two opposite forces lead to a supramolecular assembly. The essential components in the preparation of niosomes are membrane additives such as cholesterol and non-ionic surfactants.

Cholesterol

Cholesterol is a waxy steroid metabolite found in the cell membranes. Cholesterol is added usually to the non-ionic surfactants to give rigidity and orientational order to the niosomal bilayer[5]. Cholesterol and its derivatives are the most common additives found in niosomal systems. Cholesterol enables the formation of vesicles, reduces aggregation and provides greater stability. Cholesterol is also known to abolish gel to liquid phase transition of niosomal systems resulting in niosomes that are less leaky[6].

Non-ionic surfactants

Non-ionic surfactants act as main component in niosomal formulations. Generally, they possess hydrophilic head group and a hydrophobic tail. The hydrophobic moiety may consist of 1/2/3 alkyl chains or per fluro group or in certain cases a single stearyl group. The alkyl group chain length is from C_{12}- C_{18}. Perfluro alkyl group surfactants have chain length having C_{10}. Crown ether amphiphile bears a steroidal group.

Sorbitan fatty acid esters are most commonly used surfactants found in literature. Most commonly used Spans are Span 20, 40, 60, 65, 80, and 85. All Span types have the same head group and different alkyl chain length. It was reported that increasing alkyl chain length leads to high entrapment efficiency of drug[7].

A number of researchers investigated Tweens as non-ionic surfactants in niosomes formation. Most commonly reported Tweens in niosomal preparations are Tween 20, Tween 40, and Tween 60.Tweens are polysorbates derived from PEGylated sorbitan esterified with fatty acids[8].

Solulan C24 poly-24-oxyethelene cholesteryl ether has been reported to form spherical, tubular or polyhedral niosomal vesicles[9].

Another class of non-ionic surfactants is polyoxyethylene alkyl ethers (C_nEO_m , Brij ™) which have ability to form bilayer vesicles when mixed with cholesterol. The different grades of Brij used for vesicle formation are Brij 30, 35, 52, 58, 72, 76, 92, and 97. Numerous studies have been reported in literature using these surfactants[10].

New surfactants like bola surfactants have been synthesized with the aim of preparing innovative niosomal systems. Bola forms amphiphiles are composed of two identical AZA- Crown ether units, as polar heads, linked to a long alkyl chain and represent a new class of non-ionic surfactants, which are able to assemble in colloidal structures if associated with cholesterol[11].

Another category of surfactants Wasag®7 (70% stearate sucrose ester, 30% palmitate sucrose ester) and Wasag® 15 (70% stearate sucrose ester, 30% palmitate sucrose ester) have been investigated for entrapment of ovalbumin niosomes. These sucrose ester surfactants are easily biodegradable and posses very low toxicity. Moreover they are able to form gel state bilayer at 37°C[12].

Other additives

Charge inducers are one of the membrane additives which are often included in niosomes because they increase surface charge density and prevent vesicles flocculation, aggregation and fusion. Negatively and positively charged molecules are used for induction of charge in niosomes as reported by number of scientists. Dicetyl phosphate (DCP) and stearyl amine (SA) which induces negative or positive charge is examples of such membrane additives.

METHODS OF PREPARATION

The general method of preparation of niosomes involves evaporation to produce a lipid film followed by hydration with the hydration medium. However, there are variants of this method that are described here in detail.

Lipid film hydration (Hand shaking method)

In this method, surfactants/lipids are casted as layers of film from their organic solution using flask rotary evaporator under reduced pressure (or by hand shaking) and then casted films are dispersed in an aqueous medium. Upon hydration, the lipids swell and peel off from the wall of the round bottom flask at temperature slightly above the phase transition

temperature of surfactants used, for specified period of time (time of hydration) with constant mild shaking. The mechanical energy required for the swelling of the lipids and dispersion of casted lipid film is imparted by manual agitation (hand shaking) or by exposing the film to a stream of water saturated nitrogen for 15 minutes followed by swelling in aqueous medium without shaking (non shaken vesicles). Hand shaking method produces multi lamellar vesicles (MLVs) while vesicles produced by non shaking method are large unilamellar vesicles LUVs[13].

Reverse phase evaporation
The novel key in this method is the removal of solvent from an emulsion by evaporation. The surfactant and cholesterol are dissolved in ether or chloroform or in a mixture of chloroform and ether chloroform with or without drug. The resulting two-phase system is then homogenized using homogenizer. The organic phase is removed under reduced pressure to form niosomes dispersed in aqueous phase. In some cases, resulting suspensions must be further hydrated or homogenized to yield niosomes[14].

The Bubble method It is novel technique for 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 is supplied through the third neck. Researchers dispersed cholesterol and surfactants together in buffer (pH 7.4) at 70°C, the dispersion is mixed for 15 seconds with high shear homogenizer and immediately afterwards “bubbled” at 70°C using nitrogen gas[15].

Microfludisation
This is a recent technique to prepare small multi lamellar vesicles. A microfludizer is used to pump the fluid at a very high pressure (10,000 psi) through a 5 pm screen. Thereafter; it is forced along defined micro channels, which direct two streams of fluid to collide together at right angles, thereby affecting a very efficient transfer of energy. The lipids can be introduced into the fluidizer. The fluid collected can be recycled through the pump until vesicles of spherical dimensions are obtained. This method resulted in niosomes with greater uniformity and small size which shows better reproducibility [16].

Trans membrane pH gradient (inside acidic) Drug Uptake Process (Remote Loading)
In remote loading process surfactants 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 produce niosomes [17].

Multiple membrane extrusion method In membrane extrusion method, the size of niosomes is reduced by passing them through membrane filter. This method can be used for production of multi lamellar vesicles as well as large unilamellar vesicles. It is found as a good method for controlling niosomal size[18].

Ethanol injection method
This method has been reported as one of the alternatives used for the preparation of small unilamellar vesicles (SUVs) without sonication. In this method, an ethanol solution of surfactant is injected rapidly through a fine needle into excess of saline or other aqueous medium. Vaporization of ethanol leads to the formation of vesicles. Enoxacin was entrapped in niosomes using this particular method[19].

Ether injection method
This method was reported in 1976 by Deamer and Bangham. It is similar to ethanol injection method; however it differs with ethanol injection method in many ways. It involves injection of immiscible organic solution containing surfactant or surfactant-cholesterol or surfactant cholesterol-diacetyl phosphate or surfactant cholesterol- drug solution mixture very slowly into an aqueous phase through a narrow needle at vaporization temperature of organic solvent. Vaporization of ether leads to the formation of single layered vesicles (SLVs). It has a little risk of causing oxidative degradation provided ether is free from peroxides. The disadvantage of this method is that a small amount of ether is often present in the vesicles suspension and is difficult to remove very often[20].

Formation of niosomes from proniosomes
Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol

with non-ionic surfactants. 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 as “proniosomes”. The niosomes are recognized by the addition of aqueous phase at (T > Tm) and brief agitation simultaneously.
T=Temperature.
Tm = mean phase transition temperature [21].

Sonication Method

The size range of niosomes has a major effect on their *in-vitro in-vivo* fate. Hence, size reduction of niosomes is essential after hydration stage. Niosomes prepared by reverse phase evaporation and hand-shaking method are usually in micron size range (1.15 and 2.75mm). By using sonication technique, size of niosomes formed by hand shaking can be reduced to 100-140 nm. In this method, average size of vesicles can be reduced by providing high energy by sonication. This is done by exposure of multi lamellar vesicles to ultrasonic irradiation. Sonication method is most widely used for producing small vesicles. There are two types of sonication based on the use of either probe or bath ultrasonic disintegrators. The probe is employed for dispersions, which require high energy in small volume while bath is more suitable for large volume[22].

APPROACHES OF NIOSOMES

The administration of niosomes by various routes has been reported and it is clear that the route is important in designing a vesicular formulation.

Oral Route Delivery

Niosomes can be proposed as a potential oral delivery system for the effective delivery of drugs. The delivery of biopharmaceuticals to the systemic circulation through oral administration is hindered by numerous barriers, including pH gradients, proteolytic enzymes and low epithelial permeability. The oral delivery of recombinant human insulin using niosomal formulations was demonstrated by a study involving polyoxyethylene alkyl ethers based niosomes. Entrapment of insulin in bilayer structure of niosomes protected it against proteolytic activity of α-chymotrypsin, trypsin and pepsin in vitro. Significantly higher protection activity was seen in Brij 92/cholesterol (7:3 molar ratios) in which only 26.3±3.98% of entrapped insulin was released during 24 h in simulated intestinal fluid (SIF) [10]. Encapsulation of Ganciclovir in lipophilic vesicular structure may be expected to enhance the oral absorption and prolong the existence of the drug in the systemic circulation. Niosomes were prepared from Span40, Span60, and Cholesterol using reverse evaporation method. The in vitro release study signifies sustained release profile of niosomal dispersions. Release profile of prepared formulations has shown that more than 85.2±0.015% drug was released in 24 h with zero-order release kinetics. The results obtained also revealed that the types of surfactant and Cholesterol content ratio altered the entrapment efficiency, size, and drug release rate from niosomes[23]. Niosomal formulation could be promising delivery system for gliclazide with improved bioavailability and prolonged drug release profile. Gliclazide-loaded niosomes were formulated and evaluated for their *in-vitro* as well as *in-vivo* characteristics in an attempt to improve the oral bioavailability of the drug. The *in-vitro* release studies of drug from niosomes exhibited a prolonged drug release as observed over a period of 24 hrs[24].

Transdermal Delivery

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. Niosomes of terbinafine hydrochloride was formulated by thin film hydration method using different ratios of non-ionic surfactants (Tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. Niosomal preparations were tested for *in-vitro* antifungal activity using the strain *Aspergillus niger* and compared with pure drug solution (as standard). All the niosomal formulations showed gradual increase in zone of inhibition due to the controlled release of medicament. The studies revealed that gel containing total niosomes possess maximum zone of inhibition values (12mm) initially followed by sustained release (12mm-16mm) compared to gel containing drug entrapped niosomes, gel containing pure drug and marketed preparation[25]. Proniosomal gels of flurbiprofen were developed using different spans with and without cholesterol. Niosomes were formed by hydrating proniosomes. Results indicated that the entrapment efficiency followed the trend Sp 60 (C_{18})>Sp 40 (C_{16})>Sp 20 (C_{12})>Sp 80 (C_{18}). Cholesterol increased or decreased the entrapment efficiency depending on either the type of the surfactant used or its concentration within the formulae. Increasing total lipid or drug concentration also increased the entrapment efficiency of flurbiprofen into niosomes[26]. Benzoyl peroxide was entrapped

into niosomes by thin film hydration technique, and various process parameters were optimized by partial factorial design. The optimized niosomal formulation was incorporated into HPMC K15 gel and extensively characterized for percentage drug entrapment (PDE) and in vitro release performance. The present study demonstrated prolongation of drug release, increased drug retention into skin, and improved permeation across the skin after encapsulation of benzoyl peroxide into niosomal topical gel[27]. Entrapment of drug Erythromycin into niosomes showed prolongation of drug release, enhanced drug retention into skin and improved permeation across the skin after encapsulation[28].

Leishmaniasis

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. Liver and serum concentrations of antimony in the mouse have been determined after administration of sodium stibogluconate in the free, liposomal and niosomal form. High liver and low serum values were attained by the use of both vesicular formulations. Niosomal sodium stibogluconate was shown to be more active than free drug against experimental murine visceral leishmaniasis, an effect apparently dependent on maintaining high drug levels in the infected reticuloendothelial system[29].

Neoplasia

Numerous attempts have been made to enhance the selectivity of antinioplastic agents by linking them to a cancer moiety. Novel vesicular systems, niosomes containing anticancer drugs if suitably designed will be expected to accumulate within tumors. Niosomal formulations of paclitaxel (PCT), an antineoplastic agent, were preared using different surfactants by film hydration method. PCT was successfully entrapped in all of the formulations. The slow release observed from these formulations might be beneficial for reducing the toxic side effects of PCT. The efficiency of niosomes to protect PCT against gastrointestinal enzymes (trypsin, chymotrypsin, and pepsin) was also evaluated for PCT oral delivery. Among all formulations, gastrointestinal stability of PCT was well preserved with Span 40 niosomes[30]. Niosomes of cisplatin were prepared using Span 60 and cholesterol and investigated for antimetastatic activity in experimental metastatic model of B16F10 melanoma. Treatment with niosomal cisplatin (1 mg/kg) and combination of the same with theophylline (15 mg/kg) showed significant reduction in the number of lung nodules as compared to untreated control as well as free cisplatin (1 mg/kg). The treatment with activated macrophages (activated by using Muramyl dipeptide) significantly reduced the secondary growth of tumor in lung. Niosomal cisplatin showed a significant protection against weight loss and bone marrow toxicity as compared to free cisplatin[31].

Delivery of peptide drugs

The ability of non-ionic surfactant vesicles to enhance antibody production against bovine serum albumin (BSA) was compared with Freund's complete adjuvant (FCA), in the BALB/c mouse. The adjuvant activity of niosomes was wholly dependent on the BSA entrapped within vesicles although mixing free BSA with vesicles was not effective. Analysis of anti BSA IgG subclasses induced by niosomes and FCA showed that niosomes were generally better stimulators of IgG2a than was FCA, but poorer stimulators of IgG1[32]. In another study, the structure behaviors of hemoglobin (Hb) and the interactions of the niosome with Hb are studied in PEG 6000/Tween 80/Span 80/H_2O niosomal system. The obtained results showed that Hb can be adsorbed and outspread on the surface of the niosome membrane. The behaviors of Hb are partially stabilized and protected in Hb/niosome system. This study can be helpful to understand deeply the structure and functions of biological membrane in life system, and the relation of protein properties with vesicles[33]. PEGylated niosomes showed a promising drug delivery systems for improved oligonucleotides potency. Polyethylene glycol (PEG) modified cationic niosomes were used to improve the stability and cellular delivery of oligonucleotides (OND). PEG-modification significantly decreased the binding of serum protein and prevented particle aggregation in serum. The loaded nuclear acid drug exhibited increased resistance to serum nuclease. Compared with cationic niosomes, the PEGylated niosomes showed a higher efficiency of OND cellular uptake in serum[34].

Hormone delivery

The *in-vitro* permeation of estradiol from vesicular formulations through human stratum corneum was studied. The vesicles were composed of non-ionic *n*-alkyl polyoxyethylene ether surfactants (C_nEO_m). Two mechanisms are proposed to play an important role in vesicle–skin interactions, *i.e*., the penetration enhancing effect of surfactant molecules and the effect of the vesicular structures caused by their adsorption at the stratum corneum–suspension interface[35].

Cosmetic delivery

The first report of non-ionic surfactant vesicles came from the cosmetic applications devised by L'Oreal. Niosomes were developed and patented by L'Oréal in the 1970s and 80s. The first product 'Niosome' was introduced in 1987 by Lancôme. The advantages of using niosomes in cosmetic and skin care applications include their ability to increase the stability of entrapped drugs, improved bioavailability of poorly absorbed ingredients and enhanced skin penetration[36].

Diagnostic markers

Encapsulation of radio opaque agent, iopromide into niosomes using cholesterol and stearylamine which were found to concentrate in the kidneys on intravenous administrationwas studied. The positive charge on the niosomal surface was found to be responsible for kidney targeting. $C_{16}G_3$ niosomes resulted in highest kidney iopromide concentration owing to less fluid bilayer than the $C_{16}C_{12}G_7$. Although, the niosomal formulation enhanced the opacity of this contrast agent, but clinically relevant enhancement of opacity was not achieved in this study due to poor encapsulation efficiency of the agent[37].

A diagnostic agent, iobitridol used for X-ray imaging was encapsulated in niosomes by film-hydration method. The niosomal vesicles were prepared using appropriate mixtures of D-alpha tocopheryl polyethylene glycol 1000 succinate, polyoxyethylene glycol 4000 stearate, sorbitan monostearate, cholesterol and dicetylphosphate and further characterized for the physico-chemical and morphological properties[38].

Vaccine delivery

An interesting group of vaccine carrier systems are formulations based on non-ionic surfactant vesicles (niosomes) which themselves are only weekly immunogenic. Niosomes are gaining wide attention as peroral vaccine delivery system and for topical immunization. Non-ionic surfactant vesicles of influenza antigen for nasal mucosal delivery were studied. The study described the encapsulation of viral influenza vaccine antigen in non-ionic surfactant vesicles using dehydration-rehydration technique. Influence of the varying proportion of surfactant, cholesterol, and dicetyl phosphate on the morphology, particle size, entrapment efficiency, and *in-vitro* antigen release from niosomes was investigated. The stability of the antigen was studied using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and immunoblotting. Researchers also studied the effect of cholesterol concentration and the method of lyophilization on antigen loading and *in-vitro* release of antigen from surfactant vesicles[39]. Niosomes for topical DNA delivery of Hepatitis B surface antigen (HBsAg) were prepared by reverse phase evaporation method using Span 85 and cholesterol. The immune stimulating activity was investigated and it was observed that topical niosomes elicited a comparable serum antibody titer and endogenous cytokines levels as compared to intramuscular recombinant HBsAg and topical liposomes[40].

Advances in niosomal delivery

Glucose-targeted niosomes to deliver vasoactive intestinal peptide (VIP) to the brain was investigated. VIP/^{125}I-VIP-loaded glucose-bearing niosomes were intravenously injected to mice. Brain uptake was determined by measuring the radioactivity of ^{125}I-labeled VIP using γ-counting, after intravenous administration of VIP in solution or encapsulated in glucose-bearing niosomes or in control niosomes. Brain distribution of intact VIP after injection of glucose-bearing niosomes, indicated that radioactivity was preferentially located in the posterior and the anterior parts of the brain, whereas it was homogeneously distributed in the whole brain after the administration of control vesicles[41]. Polyoxyethylene monostearyl ether niosomes using super critical carbon dioxide were prepared and characterized. The niosomes were formed in super critical carbon dioxide fluids without any organic solvent like ethanol that is essential for their preparation by conventional methods. Higher entrapment efficiency was achieved near phase transition temperature. When CO_2 was introduced into the cell kept at a constant volume, the entrapment efficiency of niosomes showed a maximum value on the $C_{18}EO_6$ solubility boundary line in CO_2, while it increased with increase in preparation pressure at varying cell

volume and a constant CO_2 amount as $C_{18}EO_6$ completely dissolved in supercritical CO_2[42].

CONCLUSION

The concept of incorporating the drug into liposomes or 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 presents 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. The usefulness of niosomes in the delivery of proteins and biologicals can be unsubstantiated with a wide scope in encapsulating toxic drugs such as anti-cancer drugs and anti-viral drugs. Niosomes are thoughts to be better candidates drug delivery due to various factors like cost, stability etc. Various types of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parentral, etc.

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