World Applied Sciences Journal 32 (8): 1671-1685, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.08.848

Niosomal Drug Delivery Systems: Formulation, Preparation and Applications

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Abstract: Nonionic surfactant vesicles (niosomes) have drawn a lot of attention in the area of modern drug delivery systems due to their salient features such as biodegradability, biocompatibility, chemical stability, low production cost, easy storage and handling and low toxicity. Niosomes are hydrated vesicular systems having a lamellar bilayer structure formed from nonionic surfactants with or without cholesterol and they are capable of encapsulating bioactive compounds with a wide range of solubility. Niosomes have been evaluated as a carrier for delivery of a number of anti-cancer drugs, genes, antigens and other bioactive compounds. Niosomal systems offer advantages over conventional delivery systems via delivering the bioactives in a controlled/sustained manner and delivering the actives to the target site. Niosomes tackle the problem of insolubility, instability, low bioavailability and rapid degradation of drugs. This paper overviews the general advantages of niosomal systems along with their potential applications in pharmaceutical areas. This article also highlights the effect of niosomal formulation on the physicochemical properties of niosomal systems. Various techniques of preparation are detailed and some down-sizing approaches have been identified.

Key words: Niosomes · Nonionic surfactant · Drug delivery system · Targeted delivery · Bioavailability improvement

INTRODUCTION

Niosomes are colloidal particles formed from the self-assembly of non-ionic surfactants in aqueous medium resulting in closed bilayer structures [1-3]. The assembly into closed bilayers is not always spontaneous and requires input of external energy such as heat or shearing forces [4,5]. Niosomes were first reported as a feature of cosmetic industry in the seventies and have been used in cosmetic formulations devised by L'Oreal and since then niosomes were extensively studied as an alternative drug delivery system to liposomes [6]. Niosomes and liposomes are both similar in structure, as well as the manner of entrapping drugs. Niosomes structurally consist of a non-ionic surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle to the aqueous phase, while hydrophobic chains face each other within the bilayer [7]. Like liposomes, niosomes are also capable of entrapping both hydrophilic and hydrophobic drugs. In contrast to liposomes, the bilayer system in niosomes is made up of uncharged single-chain nonionic surface-active agents, while double-chain phospholipids (neutral or charged) seen in the liposomal structures.

Niosomes alleviate the disadvantages associated with liposomes such as chemical instability due to their predisposition to oxidative degradation and variable purity of phospholipids [6, 8]. Niosomes as drug carriers offer remarkable advantages that make them preferable over other conventional and vesicular delivery systems [9, 10]. Biodegradability, biocompatibility, chemical stability, low production cost, easy storage and handling and low toxicity are the main benefits for developing niosomal systems [11-13]. Niosomes can be administrated through various routes such as oral, parenteral, topical, ocular, etc. In recent years niosomal formulations have been extensively used as a carrier to deliver different types of drugs (synthetic and herbal), antigens, hormones and other bioactive compounds [14, 15].

This article presents some advantages of niosomes along with an overview of the preparation techniques and the current applications of niosomes in encapsulation and

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delivery of bioactive compounds. This review also addresses the influence of formulation such as the nature of the surfactant, cholesterol and the encapsulated drug on the characteristics of niosomes.

Advantages of Niosomes

Bioavailability Improvement: The term bioavailability refers to the fraction of a dose that is available at the site of action in the body. Niosomes have distinct advantages over conventional formulations since the vesicles can act as drug reservoirs and protects drug from acidic and enzymatic degradation in the gastro-intestinal tract which results in bioavailability enhancement [16]. Further, owing to the ability to cross the anatomical barrier of gastrointestinal tract [16], niosomal systems increase drug uptake as seen in antifungal agent griseofulvin niosomes (poorly water-soluble drug) through oral administration as demonstrated in an *in vivo* study [17]. Another example was an increased uptake of more than 2-fold observed in a niosomal formulation of poorly water-soluble acyclovir compared to the free drug solutions [18]. Similar results were obtained in podophylotoxin, etoposide and methotrexate by transdermal route of their respective niosomes [19-20]. It is thought that vesicles like niosomes enable successful transdermal drug delivery hence improve bioavailability through four different potential mechanisms of action. Vesicles might (a) deliver encapsulated drug molecules into or across the skin; (b) act as penetration enhancers following the penetration of the individual lipid components into the stratum corneum and subsequently the alteration of the intercellular lipid lamellae within the skin layer; (c) serve as a depot for sustained release of dermally active compounds; and (d) serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system [21,22]. Non-ionic surfactants in niosomes can act as penetration enhancers due to their ability to fluidize the stratum corneum lipid bilayers and diffuse through them [23]. Thus permeability and fluidity of biological membrane can be increased in the presence of nonionic surfactants.

Targeted Drug Delivery System: The main roles of a drug delivery system are to: a) concentrate the drug in the site of interest; and b) reduce drug delivery to the remaining tissues [24]. To date, various pharmaceutical carriers have been investigated such as soluble polymers, microcapsules, micro particles, liposomes, micelles and niosomes [25, 26]. Niosome is one of the most promising carriers that can be used in targeted drug delivery systems through different principal schemes of drug targeting such as magnetic targeting (or targeting of a drug immobilized on paramagnetic materials under the action of an external magnetic field), targeting using a specific 'vector' molecules with a high specific affinity toward the area of interest, etc. [12, 25]. The ability of niosomes to deliver drug in a controlled/sustained manner in various applications and therapies has led to bioavailability enhancement and continuous therapeutic effect over a longer period of time [12]. Sathali and Rajalakshmi [27] reported that niosomal formulations of terbinafine hydrochloride showed a gradual increase in antifungal activity due to the controlled release of drug in the zone of inhibition. In another study, sustained and controlled release of timolol maleate in niosomal formulations was reported in ocular administration [28]. The prolonged and controlled drug release prevents the excess drug from pouring into the systemic circulation and therefore, results in better patient compliance and reduced side-effects [28]. Another example is the niosomal gel formulation of tretinoin for treatment of acne vulgaris which showed sustained release pattern of the drug over the treatment span compare to tretinoin solution and tretinoin conventional gel [29]. Niosomes can also be used as an oral controlled release system. For example, anti-tubercular hydrophilic drugs such as isoniazid and pyrazinamide which are orally active showed sustained drug release from the tyloxapol niosome membrane [30]. These results are in accordance with the possibility of depot acting of vesicle to release drug in a controlled manner [15].

Niosome Preparation Techniques: In general, niosomes are prepared upon hydration of a mixture of the surfactant and lipid at an elevated temperature by a hydration medium, followed by optional size reduction process and ultimately obtain a colloidal dispersion [1, 6]. To date, various niosome preparation techniques have been reported and it has been found that niosomal properties can be affected by the preparation techniques. All the common laboratory methods of niosome preparation can be classified into two major strategies. In the first strategy, volatile organic solvent is used to dissolve all the components. Removal of the organic solvent forms a thin film, which is then hydrated by an aqueous medium to obtain niosomes. Thin layer hydration, reverse phase evaporation and ether injection are the most common examples of this strategy. The second strategy involves a direct mixing of lipids and hydration medium at an elevated temperature without using any organic solvents, such as bubbling of nitrogen technique and the proniosomes approach [31].

Thin Layer Hydration: In thin layer hydration method, surfactants and lipids are completely dissolved in a volatile organic solvent in a round bottom flask. Then, by using a rotary evaporator, the organic solution of surfactants/lipids is evaporated under reduced pressure to produce a thin lipid film on the wall of the flask. The formed thin layer is hydrated with an aqueous solution at a temperature above the phase transition temperature of the surfactants for a specified time with gentle agitation, leading to the formation of vesicles [9, 32]. Using this method, Guinedi *et al*. [33] prepared multilamellar vesicles encapsulating acetazolamide with an entrapment efficiency of about 32%; while Pardakhty *et al*. [34] prepared a niosomal formulation for encapsulation of insulin. It is noted that addition of the drug to the system depends on the nature of the drug, in which a hydrophilic drug can be added to the aqueous phase while a hydrophobic drug only can be dissolved in organic solvent with other components [10]. In general, lipid layer hydration method is one of the most common methods for multilamellar niosome preparation [17, 35].

Reverse Phase Evaporation: Reverse phase evaporation technique was first described for liposome preparation by Szoka and Papahadjopoulos [36]. This technique involves the formation of water-in-oil emulsions from an organic solution of surfactants/lipids and an aqueous solution containing the drug. The two phases are usually emulsified by mechanical methods or by sonication. The organic solvent evaporated under reduced pressure causes the surfactants coated water droplets to come together to form a gel-like matrix. Further removal of organic solvent causes the gel-like matrix to form a paste of smooth consistency. This paste is an aqueous suspension of large unilamellar vesicles [37]. As an example, spherical large unilamellar vesicles encapsulating salicylic acid were prepared using this technique [38]. Guinedi *et al*. [33] and Aggarwal *et al*. [39] used this technique to prepare niosomal formulation of acetazolamide and both groups also reported the formation of large unilamellar vesicles in spherical shape.

Ether Injection: In ether injection method, surfactant, cholesterol, drug and other components are dissolved in ether and the organic phase was slowly injected into aqueous phase which is kept at a temperature above the boiling point of the organic solvent [40]. The mechanism of large unilamellar vesicles formation is not fully understood. However, presumably it could be ascribed to the slow vaporization of organic solvent due to the temperature difference between the organic and aqueous phases. The vaporization of ether creates a solvent gradient extending across the interfacial lipid/surfactant monolayer at the ether-water interface which subsequently led to the formation of bilayer sheets. The surfactant bilayer sheets then folds onto themselves to form sealed vesicles when they come into contact with water [41]. Lingan *et al*. [42] prepared clobetasol propionate niosomes using Span 60 and cholesterol at a molar ratio of 1:0.5 by ether injection method with an entrapped efficiency of 58%. According to Sankhyan and Pawar [10], controlling the size of vesicles is the advantages of this method while solubility of main component in ether and difficulty of complete removal of ether from final formulation are drawbacks of this method.

Transmembrane pH Gradient Method (Drug Uptake Process): The use of transmembrane pH gradients which is previously used to encapsulate drugs into liposomes is also applicable to niosomal formulation [37]. In this technique, surfactant/lipids are dissolved in organic solvent(s) and evaporated under reduced pressure to form a thin film on the inner wall of a round-bottomed flask. The film is then hydrated with an acidic solution (generally citric acid with pH 4) by vortex mixing to form multilamellar vesicles. Then the resulting vesicles are subjected to freeze thaw cycles and later sonication. An aqueous solution containing the drug was then added to this niosomal suspension [12]. In order to create a pH gradient across the niosomal membrane, the pH of the exterior aqueous compartment must be increased to 7–7.5 using a base, normally disodium hydrogen phosphate solution. A neutral exterior pH results in a mixture of both protonated and un-protonated forms of the drug which are membrane impermeable and membrane permeable, respectively. The un-protonated neutral form of the drug tends to cross the niosome membrane which becomes protonated after entering into the acidic medium and trapped inside the vesicles. This diffuses across the bilayer continues until the interior and exterior concentrations of drug are in equilibrium [43, 44]. Hence, the pH-gradients are responsible for trans-bilayer transport of drug resulting in loading into niosomes [45]. Using this technique, Bhaskaran and Lakshmi [46] developed Span 60 niosomes containing salbutamol sulphate with an entrapment efficiency of 87.5%.

Formation of Niosomes from Proniosomes: Niosomes may also be formed from proniosomes which was previously described by Perrett *et al*. [47] in liposome preparation to avoids the use of pharmaceutically unacceptable solvents. In this technique, all the

ingredients of proniosomal formulation including surfactants, drug and alcohol are mixed in a small glass tube. Then the open end of the glass tube is tightly sealed and warmed in water bath (55–60°C) while shaking until complete dissolution of the surfactants. The aqueous phase is then added and warmed on a water bath at a temperature above the mean phase transition temperature until a clear or translucent solution is produced. The mixture is then allowed to cool to room temperature until the dispersion is converted to proniosomal gel [8, 48]. In case of incomplete dissolution of the drug in the prepared formulations, the drug and surfactants are dissolved first in chloroform or ether, followed by evaporation of the solvent [49]. Elhissi *et al*. [50] described the encapsulation of beclometasone dipropionate into niosomes using this method with an entrapment efficiency of 36.4%. According to the work done by Alsarra *et al*. [48], proniosome is a promising carrier for ketorolac; while Ammar *et al*. [49] reported that proniosomes can be used as a carrier for transdermal delivery of tenoxicam.

Bubbling of Nitrogen: This method produces niosomes without using any organic solvents. All the components are dispersed in the appropriate aqueous solutions and then mixed using a homogenizer to obtain a homogeneous dispersion. The dispersion is placed in a round bottom flask with three necks – attached to a water-cooled reflux, thermometer and nitrogen supply – immersed in a water bath. A continuous stream of nitrogen gas bubbles is generated and introduced through the dispersion. In general niosome dispersions formed via this method have a mean particle size between 0.2 and 0.5 μm [51].

Down-Sizing: Niosomes prepared via methods mentioned above are usually in micron size range. Often the hydration step is followed by size reduction since vesicle size has an important bearing on biodistribution and circulation time [1]. As an example sub-200 nm phospholipid vesicles have been shown to avoid splenic but not liver uptake [52]. Size reduction can be achieved by various techniques such as probe sonication, extrusion through filters of defined pore sizes, microfluidization, high-pressure homogenization or combinations of the methods thereof. Ampicillin-loaded vesicles obtained via thin layer hydration technique were probe sonicated which gave the mean vesicle size around 170 nm for different compositions [53]. Nanosized melatonin-encapsulated niosomes composed of Span 60/cholesterol/sodium deoxycholate were prepared by a lipid thin film formation and rehydration method followed by vesicle size reduction using extrusion through a 100 nm polycarbonate membrane. The laser diffraction method showed spherical vesicles with particles sizes of about 84-102 nm [54]. Uchegbu and Vyas [1] studied the effect of microfluidization on the size of niosomes which showed the possibility of downsizing up to sub-50 nm sizes by the use of a microfluidizer. High-pressure homogenization can also be used to reduce the vesicle size. The effect of homogenization of doxorubicin multilamellar niosomes on niosome size and encapsulation efficiency was studied by Uchegbu and Vyas [1]. After homogenization vesicles of below 100 nm in diameter were obtained although drug loading is ultimately sacrificed to achieve this small size. The combination of sonication and filtration (220 nm Millipore filter) resulted in the 200 nm size range of doxorubicin loaded Span 60 niosomes [11].

Effect of Preparation Techniques on the Vesicles Characteristics: It is obvious that the technique used to prepare niosomes influences the niosome characteristics. All the multilamellar niosomes of acetazolamide prepared by thin film hydration method showed higher entrapment efficiency of acetazolamide compared to those of niosomes with identical formulation composition prepared by reverse-phase evaporation method [33]. This was possibly due to the fact that acetazolamide was a hydrophobic drug thus insoluble in water, where the solubility of acetazolamide at pH 7 was approximately 1.01 mg/ml, so multilamellar vesicles were more capable of loading a higher mass of a hydrophobic drug than were the vesicles prepared from reverse-phase evaporation method [33].

Jadon *et al*. [17] compared griseofulvin niosomes prepared by ether injection method and thin film method and they found that the former method produced niosomes with a smaller vesicle size but lower entrapment efficiency compared to those prepared by the latter. The higher entrapment efficiency of niosomes prepared by thin film method might be the result of partly uniform vesicle size and due to well-packed bimolecular film formation via this method [17]. Nimesulide niosomes prepared by lipid film hydration method showed higher entrapment efficiency compared to those prepared by ether injection method [72] which is in accordance to Jadon and co-workers' findings [17]. Another work carried out by Rangasamy [55] evaluated the effect of ether injection method and hand shaking method on the vesicle characteristics. The percentage of acyclovir entrapped in the niosomal formulations (at various molar ratio of cholesterol/span 80) prepared by hand shaking was higher than those formulations prepared by ether injection

Table 1: Examples of nonionic surfactant, their properties and applications

Nonionic surfactant main groups	Examples	HLB Values	T_c °C	Application	References
Alkyl ethers and alkyl glyceryl ethers	Polyoxyethelene 4lauryl ether (Brij30)	9.7	>10	Niosomes of tretinion	[59,60]
	Polyoxyethylene stearyl ethers (Brij72)	4.9	44	delivery of insulin	[34]
Sorbitan fatty acid esters	sorbitan monostearate(Span 60)	4.7	56-58	acetazolamide niosomes	[73]
Polyoxyethylene fatty acid esters	polysorbate-80 (Tween 80)	15		Zidovudine niosomes	[70]
Gemini surfactants	Sugar-based nonionic Gemini surfactant			personal care and pharmaceutical formulations and medicinal chemistry	[61]

process. For instance, the entrapment efficiency of 84% was achieved in the niosome formulation prepared by hand shaking method at 1:3 molar ratio of cholesterol: Span 80 while the entrapment efficiency of 69% was achieved in the same formulation that was prepared with ether injection method. It was thought that vortexing during hand shaking process facilitated drug entrapment [55]. The acyclovir niosomes prepared by hand shaking method were larger in size (ranged from 0.5-5ìm, with an average size of 2.7ìm) which could be another reason for increased entrapment of drug [55].

Effect of Formulation on the Properties of Niosomes: Preparation of niosomes requires a deep understanding of the characteristics of the principal ingredients and their effects on the physicochemical properties and stability of the resultant niosomes. Physicochemical properties of niosomes such as particle size, entrapment efficiency and stability are important properties of niosomes that have to be investigated in order to produce high performance niosomes [11]. The influence of some formulation parameters such as the type of surfactants and their properties, the nature of the encapsulated compounds and cholesterol content are reviewed in this article.

Type of Nonionic Surfactant: A variety of nonionic surfactants and their combinations have been reported to have great potential to accommodate many drugs in niosomes [56]. The most common types of niosome-forming nonionic surfactants include alkyl ethers, alkyl esters, alkyl amides, sorbitan fatty acid esters, etc. [1, 10]. All of these surfactants have been reviewed in the literature [6, 40]. These nonionic surfactants are used in various niosome formulations and showed different effects on the properties of niosomes. For instance, polysorbate 80 (Tween 80) as a nonionic surfactant has a specific role in brain targeting which can be due to the interaction between Tween 80 and brain micro-vessel endothelial cells [57]. Some examples of these common surfactants are shown in Table 1. There are some new types of nonionic surfactants such as sucrose esters, bola-form amphiphiles and tyloxapol which have been used to prepare niosomes.

Sucrose esters are nonionic surfactants which structurally consist of sucrose as the hydrophilic head group and fatty acids as lipophilic tail [58]. These natural surfactants have been reported with vesicle formation capabilities and are used to formulate drug delivery systems. For instance, Honeywell-Nguyen and Bouwstra [62] prepared pergolide-loaded vesicles from sucrose laurate and PEG-8-L. Also, rotigotine-loaded vesicles using sucrose ester surfactant was prepared for transdermal delivery [63].

New classes of surfactants have been synthesized for development of innovative niosomal systems, for example, the bola-form amphiphiles. Bola-form amphiphiles consist of two similar azacrown ether units (polar heads) linked to a long alkyl chain and represent a new group of nonionic surfactants, which are able to form vesicles if prepared by thin film layer hydration method in the presence of cholesterol [64]. Bola surfactant niosomes encapsulating 5-fluorouracil formulated for skin cancer treatment [65].

Tyloxapol is a liquid polymer of 4-(1,1,3,3-tetramethylbutyl)phenol with ethylene oxide and formaldehyde. It is a nonionic biological surfactant of the alkyl aryl polyether alcohol type with a HLB value of 12.5. It is mostly used in marketed ophthalmic products and as a mucolytic agent for treating pulmonary diseases. Tyloxapol molecules assemble in aqueous solution to form vesicles [30, 66].

Phase Transition Temperature and Alkyl Chain Length of Surfactant: Vesicle formation along with vesicle structure is highly affected by the phase transition temperature of the surfactant [67]. The inherent phase transition behavior of surfactants plays important role in the properties of the dispersion particularly the membrane permeability, bilayer rigidity, stability, entrapment efficiency, etc. [1]. It is conceivable that niosome-forming surfactants with the higher phase transition temperature yield vesicles with less fluidity. Phase transition temperature and the length of alkyl chain of nonionic surfactants are interchangeably affecting the niosomal formulation since both are related to each other [67].

In general, increasing the number of carbons in the alkyl chain length leads to an increase in the gel to liquid phase transition temperature; and the higher the gel to liquid phase transition temperature the less leaky niosomes can be produced [48]. In other words, the bilayer fluidity (permeability) of long chain length surfactants is less than that of surfactants with shorter chain length. This is due to interactions between adjacent molecules and consequently decreasing permeability [68]. Thus Span 20 (C12) is liquid at room temperature; Span 40 (C16) has a gel transition temperature of 46–47°C and Span 60 (C18) has a gel transition temperature of 56–58°C [69]. Span 60 (C18) and Span 40 (C16) produce less leaky niosomes because these Span surfactants have a higher phase transition temperature compare to Span 20 [69]. Regarding the effect of chain length on the release rate, zidovudine niosomes prepared with Tweens of various carbon chain lengths are a good example in which the drug release rate from the niosomes followed the trend of Tween 80 (C9=9)>Tween 20 (C12)>Tween 40 (C16)>Tween 60 (C18) and it is in agreement with the concept that longer the chain length, the slower the release rate [70].

Encapsulation efficiency is another dispersion property of nonionic vesicles which is strongly affected by the intrinsic phase transition temperature of surfactant as well as the saturated alkyl chain length. A higher gel to liquid phase transition temperature (less fluid the bilayer) results in a higher encapsulation efficiency which possibly explains the higher entrapment efficiency of the Span 60 formulation of colchicine and nimesulide compared to formulations prepared from other type of Span series [71,72]. Furthermore, the entrapment efficiency of flurbiprofen (a poorly soluble drug) followed the trend of Span 60 (C18)>Span 40 (C16)>Span 20 (C12)>Span 80 (C18) and it is in accordance with the fact that the Span species having the higher phase transition temperature provide the higher entrapment of drug and vice versa (Mokhtar *et al*., 2008). For Span surfactants with the same hydrophilic head group, increasing the alkyl chain length resulting in a higher phase transition temperature, thus higher entrapment efficiency [69].

It is noted that phase transition depends on the degree of unsaturation in the carbon chain which in turns alters the chain fluidity, membrane permeability and drug release rate [68]. Unsaturation in the chain lowers the phase transition temperatures, thus increases chain fluidity and permeability [70]. Span 60 (C18) and Span 40 (C16) have the same head groups while Span 80 has an unsaturated alkyl chain which is responsible for faster drug release rate and more leaky membrane compare to Span 60 in encapsulation of zidovudine and 5(6)-carboxyfluorescein [69,70]. An unsaturated double bond makes the chains bend resulting in disruption of the regular periodic structure and hence lowers the transition temperature and drug entrapment efficiency [71].

HLB Value: Hydrophilic lipophilic balance (HLB) value of nonionic surfactants is the proportion between the weight percentages of hydrophilic and lipophilic groups first defined by Griffin in 1949 and 1954 [74,75]. The HLB value of nonionic surfactants can be a representative of the vesicle forming ability. Surfactants with a HLB value between 14 and 17 (e.g. Tweens 20, 60 and 80) are not able to form niosomes without lipid additives [76]. However, surfactants of HLB values between 4 and 8 (e.g. Span surfactants) were reported to be compatible with vesicle formation [1]. Surfactants with HLB values higher than 6 show difficulties to form bilayer vesicles and need to add cholesterol in order to form bilayer vesicles [6]. The minimum amount of cholesterol necessary to form vesicles increases for surfactants with higher HLB values in order to compensate the larger hydrophilic head group which increases the lipophilic behavior of the lipid bilayer [40]. It has been found that the addition of cholesterol to lower HLB value surfactants enhances the stability of vesicles [40].

Encapsulated Drug: Generally, drugs can be classified into three major categories in terms of their water solubility: highly hydrophilic drugs, drugs with some lipophilicity, strongly lipophilic drugs [77]. The nature of the incorporated drug influences the properties of niosomes in various aspects including stability, leakage, vesicle formation and encapsulation efficiency [7]. Niosomes encapsulating hydrophobic drugs and macromolecules showed higher stability than those encapsulating low molecular weight and hydrophilic drugs [6].

Encapsulation efficiency can be altered by varying the drug concentration irrespective to the nature of the drug. For example, the entrapped amount of flurbiprofen (a poorly soluble drug) increased by increasing its added amount during the formulation [73]. Similar observations have been reported in the niosomal encapsulation of colchicine (soluble drug), silymarin and minoxidil (poorly soluble drugs) which are in consistent with the fact that increasing the amount of added drug leads to the saturation of the hydration medium with the drug, that forces the drug to be encapsulated into the vesicles [71, 78-79].

Cholesterol Content: Cholesterol is commonly used as an additive in niosomal systems. The basic idea behind the use of cholesterol is to influence the stability and permeability of the membrane, which is mainly due to the interaction between surfactant and cholesterol [80]. Stable vesicles with reduced permeability of water into the vesicle core can be achieved by cholesterol inclusion into the niosomal formulation [80]. Cholesterol as an amphiphilic molecule interacts with surfactants through hydrogen bonding between its â-OH group and surfactant's hydrocarbon chain which increase the mechanical stiffness of the membranes and membrane cohesion [81]. Condensing effect of cholesterol by accommodation in the molecular cavities formed by surfactant monomers assembled into vesicles has been demonstrated by surface pressure measurements on monolayers of surfactant and cholesterol mixtures [82]. This space-filling function of cholesterol restricts the movement of carbons of hydrocarbon which results in a decrease in permeability of cholesterol-containing membranes compared to cholesterol-free membranes [83]. It has been reported that the value of surface elasticity (which is a measure of membrane strength) increases by addition of cholesterol which makes the membrane more rigid and reached at a maximum value of around 47.5 mol% cholesterol. However, further cholesterol addition can cause the formation of cholesterol clusters thus disturbing the uniformity, strength and permeability of bilayers [81]. In general, it has been reported that 1:1 molar ratio of surfactants and cholesterol is an optimal ratio for the preparation of physically stable niosomal formulations [71, 84-85].

Cholesterol also plays a key role in controlling the vesicle formation by modifying the total HLB value and packing parameter of the system [73]. Addition of cholesterol completes the lipophilic moiety of nonionic surfactants with high HLB values to form vesicles [86]. HLB value of surfactants determines the minimum amount of cholesterol needed to form vesicle. As the HLB value of the niosomal systems increases, the minimum amount of cholesterol required to regulate the larger hydrophilic head groups also increases [31]. For instance, a nonionic surfactant belongs to Pluronic surfactant category (L64) can form niosomes only by addition of cholesterol [64]. For insulin encapsulation into niosome, Brij 35 (HLB 16.9) and Brij 58 (HLB 15.7) were not able to form vesicles without the presence of cholesterol due to the dominance of large hydrophilic head groups over the low volume of the lipophilic hydrocarbon chain. The application of cholesterol as a bilayer inducing agent is to regulate the hydrophilicity of the surfactants (with high HLB values) through changing the micellar structure of aggregates to bilayer morphology [34].

In the case of hydrophilic compounds, increasing the cholesterol concentration within the formulae results in an increase in the drug entrapment efficiency since it provides more compact packing which reduces the permeability of the drug the compound from the core of the vesicle [80]. Yoshioka *et al*. [69] reported that increasing cholesterol content in the niosomal formulation of 5(6)-carboxyfluorescein prepared by hand-shaking method led to an increase in the percentage of entrapped drug. However, the influence of the cholesterol addition into the niosomal formulation on the entrapment efficiency of the lipophilic compounds is more complex and two conflicting factors controlling the entrapment efficiency. In one hand, increasing cholesterol results in increasing hydrophobicity [87] and stability [88] of the bilayer but decreasing membrane permeability [89] which ultimately leads to improvement in encapsulating of lipophilic drug into bilayers as vesicles assembled. In the other hand, cholesterol added may compete with the lipophilic drug for packing space between the bilayer thus causes reduction in percentage of entrapped drug [82]. This theory explains why in niosomal formulations of minoxidil (poorly water-soluble drug) a higher entrapment efficiency was achieved at 1:1 cholesterol/surfactant molar ratio compare to the higher cholesterol molar ratio of 1.5 or niosomal formulations without any added cholesterol [79]. Other studies reported similar scenarios for ibuprofen [82] and flurbiprofen [73] which both are poorly water soluble drugs.

Applications of Niosomes: Although niosomes have been applied in various fields such as medicine, diagnostics and cosmetics, it seems that drug delivery application is the best well-studied area. Niosomes can be used in a wide range of pharmaceutical applications due to their inherent advantages. Table 2 summarizes some therapeutic compounds with their biological activities and their niosomal formulation applications. Some of the applications of niosomes are detailed below.

Immuno-Niosomes: Niosomes can be conjugated to antibodies on their surface to form immune-niosomes. Conjugation of the monoclonal IgG antibodies to the vesicle surfaces was carried out through incorporation of a cyanuric chloride derivatized Tween 61 in the niosome formulation formed using thin film hydration techniques followed by sonication [97]. The presence of cyanuric

Table 2: Application of Niosome as a Drug Carrier

Drugs	Applications of Niosomal Formulations	Biological activity	Method of Preparation	Route of Administration	Reference
Indomethacin	Enhanced inhibition of platelet aggregation	Antiplatelet activity	Lipid hydration method	*In-vitro*	[90]
Hyaluronic acid	Improve Endocytosis	Tumor therapy	Emulsion-evaporation method	*In vitro/ in vivo*	[98]
Hemoglobin	Stabilizing and protection of structure behaviors of Hb		Sonication	In -vitro	[91]
-Colchicine -5-fluorouracil	Prolonged release profile	-treat rheumatic complaints -treatment of cancer	Evaporation-sonication method	*In vitro*	[71]
Silymarin	Increase drug bioavailability	Treat liver and gallbladder disorders.	Hand Shaking Method	In-vivo	[92]
Zidovudine	Enhance zidovudine Entrapment and sustainability of release	Treat AIDS	Thin-filmHydration method	*In vitro*	[70]
Gentamicin sulphate	Prolongation of drug release	Antibiotic	Thin film hydration technique	In-vitro	[93]
Ampicillin	Increase antimicrobial activity	Antimicrobial	Film method	In-vitro	[53]
Beclomethasone dipropionate	Improve -inflammatory Activity	Treatment of inflammatory lung diseases	Thin layer evaporation	*In vitro*	[94]
Ammonium glycyrrhizinate	Improve the drug anti-inflammatory activity	Treatment of various inflammatory based diseases	Thin layer evaporation	*In vitro*/*in vivo*	[95]
Miconazole	Increase residence time of drug in the stratum corneum	Treatment of candida infections, fungal infections	Thin FilmHydration Technique	In-vitro	[96]
Nimesulide	Prolongation of drug release	Anti-inflammatory activity	-lipid film hydration method - ether injection technique	*In vitro*	[72]
Acetazolamide	Improve the low corneal penetration and bioavailability Promote absorption	Treatment of glaucoma	-reverse-phase evaporation -thin film hydration methods	*In vitro*/*in vivo*	[33]
Acyclovir	Prolonged activity Improve the oral Bioavailability	-treatment of Herps simplex virus -Varicelle-Zoster virus infections4	-hand shaking-ether injection process	In-vitro	[55]
Insulin	Sustained releaseIncrease absorption		Film hydration method	*In vitro*	[34]
Tyloxapol	Improve the drug bioavailability	Anti-tuberculosis	Sonication method	*In vitro*	[30]

chloride in the structure of Tween 61 provides the linkage of IgG antibody to vesicle surface. Conjugation of the monoclonal antibody to the specific cell receptors (CD44) was demonstrated using cultured fixed synovial lining cells expressing CD44 and showed the capability of immune-niosome binding to target antigens which might provide an effective method for targeted drug delivery [97].

Magnetic Niosomes: Niosomes show potential in combination of drug delivery and magnetic targeting in various applications particularly in cancer therapy [98]. The basic concept of using magnetic materials in cancer therapy is to direct drug-loaded magneto-niosomes to specific organ or tissue in the body by applying extracorporeal magnets [99]. Formulation of niosome in magnetically controlled drug targeting of doxorubicin is a good example to prove this ability of niosomal systems. Doxorubicin-loaded magneto-niosomal formulations were developed by encapsulating both anti-tumoral model drug and magnetic material (EMG 707 ferrofluid) into the niosome aqueous core. In addition, these formulations exhibited a controlled drug release without any additional toxicity due to incorporation of magnetic material into the niosomes [100].

Hyaluronic Acid Based Niosomes: Hyaluronic acid (HA) is a naturally occurring polymer present in the extracellular matrix and synovial fluids. Due to its biocompatibility and biodegradability nature, HA has been extensively studied in numerous methods of drug delivery [101]. HA can specifically bind to different cancer cells that over-express CD44, thus many scientists have focused on targeting ability of HA to tumor for anti-cancer therapeutics [102]. Kong *et al*. [98] used emulsion–evaporation method to prepare vitamin E loaded HA–niosome in order to combine transdermal and tumor targeting ability in one entity. HA–niosomes were efficient in transdermal permeation and showed enhancement in endocytosis to tumor cell compare to the chitosan nanoparticle which was selected as control to assess HA's enhancing endocytosis.

Gene Delivery: Although niosomes have been used in pharmaceutics since the 1980s, to date a few studies have focused on the application of niosomes for gene delivery. Since niosomes are biodegradable, biocompatible and nontoxic they have potential to be safely used in gene therapy [103]. Niosomes have been used as cutaneous gene delivery system especially for the treatment of a variety of skin diseases [104]. Huang *et al*. [105] reported

an effective delivery of antisense oligonucleotides (OND) via cationic niosomes of Spans in a COS-7 cell line with positive results on cellular uptake of OND. And further studies by incorporation of polyethylene glycol into OND/niosome complexes showed a higher efficiency of OND cellular uptake in serum which demonstrates positive results for gene delivery through niosomal formulations [103]. In another study conducted by Raghavachari and Fahl [106], nonionic liposomes provides an efficient delivery of beta-galactosidase or luciferase DNAs in rat skin cells. It is noted that generally DNA is a sequence of base-pairs of the four different nucleotide bases [107]. Niosomes also used for delivery of luciferase reporter and showed enhancement in transcutaneous permeation of a luciferase plasmid DNA through rat skin [108]. According to Vyas *et al*. [109], DNA encoding hepatitis B surface antigen (HBsAg) was encapsulated into niosomes of Span 85 and cholesterol. The immune stimulating activity of these niosomal formulations were studied in terms of the serum anti-HBsAg titre and also cytokine levels (IL-2 and IFN-c) were registered following the topical application of niosomes to Balb/c mice. The results revealed that niosomes can be used as DNA vaccine carriers for topical immunization which is simple, economical, stable, painless and potentially safe.

Anticancer Drug Delivery: Niosomes showed great potential in the targeted delivery of some anti-cancer drugs. Niosomes composed of a non-ionic surfactant, cholesterol and dicetyl phosphate encapsulating methotrexate (MTX) showed improvement in absorption of the drug from the gastrointestinal tract following oral ingestion; and a higher uptake of MTX into the liver following the intravenously administration of the niosomes as compared to methotrexate solution, administered either orally or intravenously [110]. Jain and Vyas [35] reported that high levels of MTX were found in the thoracic lymph following niosomal administration by this route as compared to administration through the intravenous route and the administration of the free drug via the peritoneal route. Doxorubicin niosomes composed of Span 60 showed improvement in the doxorubicin pharmacokinetics and tumoricidal activity after a single intravenous dose in the mouse adenocarcinoma as compare to the drug in solution. Improvement in anticancer activity or reduced toxicity of niosomal formulations of other anti-cancer agents such as vincristine [111] bleomycin [112] and paclitaxel [113] showed that niosomes can be used as efficient drug carriers for anticancer drugs.

Delivery of Peptide Drugs: Niosomal formulations used to deliver peptide drugs such as insulin [34] and OND [103]. Entrapment of insulin into niosomes protected it against proteolytic activity of á-chymotrypsin, trypsin and pepsin *in vitro*. The release of insulin was prolonged via niosomal formula prepared from Brij 92 and cholesterol and this formula retained 30% of insulin in niosomes after storage at refrigerated temperature for a period of 3 months [34]. Polyethylene glycol (PEG) modified cationic niosomes which were prepared by adding PEG2000-DSPE to the cationic niosomal dispersion showed a high efficacy in cellular uptake of OND in serum. The nuclease resistance of the encapsulated gene drug was enhanced using this niosomal carrier system due to the hydrophilic, sterically stabilized structure of PEG which prevents the near-approach of enzymes, thus protects OND from nuclease degradation [103].

Transdermal Delivery: The intercellular lipid barrier in the stratum corneum is dramatically looser and more permeable following treatment with vesicles and particles such as niosomes [20]. Encapsulation of 5-fluorouracil which is well-known for treatment of different forms of skin cancers into bola-niosomes showed an improvement of the cytotoxic effect by increasing the drug penetration of 8- and 4-folds with respect to a drug aqueous solution and to a mixture of empty bola-niosomes with a drug aqueous solution [65]. Niosomes also were used as a carrier for transdermal delivery of ketorolac (a potent nonsteroidal anti-inflammatory) and significantly improved drug permeation and reduced the lag time [48].

Conclusion and Outlook: Niosomes have been used in modern pharmaceutical industry due to their remarkable advantages over conventional vesicular delivery systems. Niosomes have the potential of being a new generation of delivery systems after the liposomes. The function of niosomes as targeted drug delivery systems has been studied and new classes of niosomes have been developed. A variety of drugs such as anti-cancer agents, peptide drugs, gene, etc. have been incorporated into niosomes in order to enhance their therapeutic performance and various preparation techniques have been developed to achieve this. It can be concluded that niosomes are very efficient delivery systems for incorporation/targeting of various therapeutically active compounds. Niosomes characteristics particularly entrapment efficiency may be affected by preparation techniques along with the nature of the incorporated drug, cholesterol content and the type of nonionic surfactant.

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