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Review Article

Nano-niosomes in drug, vaccine and gene delivery: a rapid overview

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Abstract

Niosomes, non-ionic surfactant vesicles (NSVs), are the hydrated lipids composed mainly of different classes of non-ionic surfactants, introduced in the seventies as a cosmetic vehicle. Nowadays, niosomes are used as important new drug delivery systems by many research groups and also they are effective immunoadjuvants which some commercial forms are available in the market. These vesicles recently used as gene transfer vectors too. This review article presents a brief explain about the achievements in the field of nano-science related to NSVs. Different polar head groups from a vast list of various surfactant with one, two or three lipophilic alkyl, perfluoroalkyl and steroidal chemical moieties may be utilized to form the proper vesicular structures for encapsulating both hydrophilic and hydrophobic compounds. The methods of niosome preparation, the vesicle stability related aspects and many examples about pharmaceutical applications of NSVs will be presented. The routes of administration of these amphiphilic assemblies are also discussed.

Keywords: Cholesterol, Drug delivery, Non-ionic surfactants, Nano-niosomes

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Introduction

The construction of the new phrase "nano-medicine" which is a term implying the application of nanotechnology for therapy and diagnosis (1), has made new branches in this field such as “pharmaceutical nanocarriers”. Several varieties of nanocarriers are available, such as nanoparticles, liposomes, solid lipid particles, micelles, surfactant vesicles, quantum dots and different nanodevices (2, 3). Liposome is a general phrase covering many classes of lipid vesicles. However, the term nano-liposome has recently been introduced to exclusively refer to nanoscale lipid vesicles (4). Higher ratio of surface area to volume of nanocarriers results in improved pharmacokinetics and biodistribution of therapeutic agents; therefore, they diminish toxicity by their preferential accumulation at the target site (5). On the other hand, nanocarriers at first improve therapeutic potential of drugs by facilitating intracellular delivery and prolonging their retention time either inside the cell (6, 7) or in blood circulation (8). The second available approach is to modify the composition of the systems, such as the incorporation of polyethylene glycol (PEG) to make stealth vesicle drug carriers or by reducing the size into nanoscale (9). Niosomes are vesicles composed mainly of hydrated non-ionic surfactants in addition to, in many cases, cholesterol (CHOL) or its derivatives. While most niosomes are in the nano or sub-micron (colloidal) size range, not many authors used the "nano-niosome" or "nanovesicle" term in their published articles which was due to introduction of new nanotechnology related phrases during the past few years. Niosomes are capable of encapsulating both hydrophilic and lipophilic substances where the former usually are either entrapped in vesicular aqueous core or adsorbed on the bilayer surfaces while the latter are encapsulated by their partitioning into the lipophilic domain of the bilayers. Cosmetic industry was the place for the first account of niosome production (10) after which a large number of niosome applications in drug delivery have been explored.

Non-ionic surfactants have more chemical stability against both oxidation and temperature in comparison to phospholipids, the main constituent of liposomes, thus requires less care in handling and storage (11, 12). Furthermore, greater versatility and lower cost make this type of vesicles more attractive in drug, gene and vaccine delivery (13). From the pharmaceutical manufacturing stand of view, the superiority of niosomes is the ease of their production in large scale without the use of pharmaceutically unacceptable solvents (14). Although the niosome has better chemical stability in storage but the physical instability during dispersion may be equivalent to that of the liposome. During dispersion, both liposomes and niosomes are at risk of aggregation, fusion, leakage of drugs, or hydrolysis of encapsulated drugs (15).

Chemical composition of niosomes

Surfactants

Following the application of some forms of energy such as mechanical or heating, the formation of niosomes is a self-assembly process due to high interfacial tension between aqueous medium and the lipophilic alkyl chain(s) resulted in the association of non-ionic surfactant monomers into vesicles (16). Concurrently, the hydrophilic head groups of amphiphilic molecules make water mediated interactions counter the previous formed force eventually results in bilayer formation.

Formation of niosomes requires an amphiphilic molecule composed of two main parts, a polar or hydrophilic head group and a non-polar or hydrophobic tail. This is obviously the ordinary structure of surfactant molecules, but in many cases the presence a wedge-shaped molecule such as CHOL is essential for turning the micellar structure of surfactant aggregates to bilayer arrangement (17). The lipophilic moiety of amphiphile molecule may contain one (18), two (19) or three (20-22) alkyl or perfluoroalkyl (23) groups or in some cases, a single steroidal group (24).

Alkyl ethers, alkyl esters, alkyl amides, fatty acids and amino acids are the main non-ionic surfactant classes used for niosome production. However, the most frequently used surfactants in niosomes formulations are sorbitan monoesters (Spans®, Fig. 1). The versatility of compounds capable of forming vesicle is due to the presence of different and various polar head groups attached to saturated or unsaturated alkyl chain(s) composed of 12 to 18 carbon atoms (C_{12}-C_{18}).

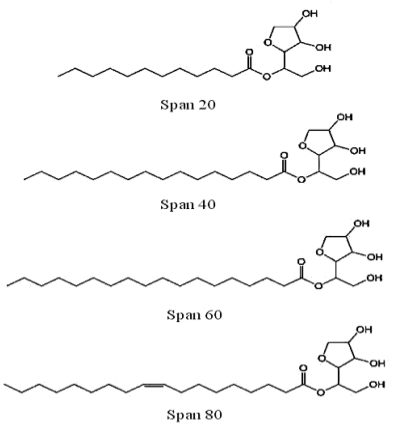

Figure 1. Chemical structure of most frequently used surfactants in niosomes formulations, sorbitan monoesters (Spans®).

Bilayer additives

Cholesterol

The most common additive found in niosomal systems is CHOL which is known to abolish the gel to liquid phase transition of liposomal and niosomal systems, resulting in less leakiness of the vesicles and improved niosomes stability (25). Surface pressure measurements on monolayers of nonionic surfactant/CHOL mixtures demonstrated a condensing effect of CHOL as evidenced by the decrease in the effective area per molecule as the CHOL content of the monolayer increased. This effect maybe attributed to the accommodation of CHOL in the molecular cavities formed by surfactant monomers assembled into vesicles and is responsible for the observed decreased permeability of CHOL-containing membranes compared to CHOL-free membranes (26). CHOL is used to complete the hydrophobic moiety of high HLB single alkyl chain non-ionic surfactants for vesicle formation (27). In general, it has been found that a molar ratio of 1:1 between CHOL and non-ionic surfactants is an optimal ratio for the formulation of physically stable niosomal vesicles (28). Some reports denote the formation of monohydrate or anhydrous CHOL crystals among the surfactant/CHOL bilayers (29). The minimum amount of CHOL required to form vesicles without evoking surfactant aggregates or other irregular structures depended on the type of surfactant and it's HLB (30).

Charged molecules

Charged molecules may be incorporated into vesicular formulation to enhance the electrostatic stability of vesicle, to increase the encapsulation or adsorption of charged molecules, to increase the transdermal iontophoretic transport of active materials (31) and to orient the vesicles for better specific interaction with target cells (32). Dicetyl phosphate (DCP) is the most used charged molecule introducing a negative charge in bilayers (33).

Polyethoxylated molecules

Solulan C24 (Fig. **6**), a polyethoxylated derivative of CHOL has also been used as surface modification material which contains a PEG moiety with molecular weight of approximately 1000 Da (34). It has a steric stabilizing effect on the non-ionic surfactant vesicles (35). Solulan C24 also increases the elasticity of some vesicle bilayers (36).

Yang et al. (37) used various molecular weights of PEG-cholesterols (Chol–PEG^m) for entrapping nimodipine in modified niosomes which showed greater accumulative release than that of plain niosomes over a period of 24 h. Incorporation of PEG in niosomal formulations also led to more physical stability of vesicles.

Methods of niosome preparation

Generally there are two strategies for niosome or liposome preparation; the first set involves

dissolving the whole lipids in organic solvent(s) for molecular level mixing of the bilayer constituents, then removing the organic solvent and hydration of formed lipid thin films or surfaces by an aqueous medium. Film hydration (38), reverse phase evaporation (REV) (25, 39), ether injection (20, 26, 40), dehydration rehydration (DRV) (41), and solvent evaporation from double emulsion droplets (42) are the most common methods in which an organic solvent is exploited. The second strategy involves the direct mixing of lipids and hydration medium, usually in high elevated temperature, which has the advantage of not having the hazardous effects of residual of organic solvents on entrapped substance or biologically applied environments. The widely used and well-documented methods for vesicle production include heating and sonication of lipid (36), homogenization of lipids (43), lamellar liquid crystal transformation (44), heating (Mozafari method) (45), supercritical CO_2 (46), inert gas bubble (47), microfluidic hydrodynamic focusing (48) and the electroformation of vesicles which utilizes alternating electric fields to generate vesicles in aqueous solutions of the amphiphilic molecules (49).

Nano-niosomes in drug delivey

Nano-niosomes are currently used as versatile drug delivery systems with many pharmaceutical applications, including for oral, pulmonary, transdermal, parenteral, vaginal, nasal and ophthalmic route of administration.

Oral route

The in vivo distribution study of *Ginkgo biloba* extract nano-vesicles composed of Tween 80/Span 80/CHOL showed that the flavonoid glycoside content in heart, lung, kidney, brain, and blood of rats treated with niosomal carrier system was greater than those treated with the oral *Ginkgo biloba* extract tablet (50). Mean particle size of mentioned niosomes was in nano size range (141 nm) which resulted in both altered pharmacokinetic behavior and in vivo distribution of the plant extract. Di Marzio et al. (51) prepared polysorbate 20 nano-niosomes for oral delivery of unstable or poorly soluble drugs by film hydration associated with sonication in order to reduce the size down to sub-micron range. These vesicles were stable in different pH and in simulated gastrointestinal media with high mucoadhesion properties.

Parenteral route

Anticancer chemotherapy by using vesicular system have many benefits such as reduced organ toxicity (52), enhanced antineoplastic efficacy (53), prolonged circulation of vesicular carriers (54) and less mortality in patients (55). On the basis of these pharmaceutical and clinical facts, innovative niosomes made up of α,ω-hexadecyl-bis-(1-aza-18-crown-6) (bola) (Fig. 2), Span 80 and cholesterol (2:5:2 molar ratio) were prepared as suitable delivery systems for the administration of 5-fluorouracil (5-FU) (54).

Magnetic drug targeting to a specific organ or tissue is proposed on the assumption that magnetic fields are harmless to biological systems.

On the basis of this hypothesis, Tavano et al. (56) prepared Tween 60 and Pluronic L64 doxorubicin loaded magneto-niosomes with low toxicity and high targeting potential. Reducing the mean volume diameter and PEGylation of hydroxyl-camptothecin niosomes resulted in stealth effect and high antitumor activity of this chemotherapeutic agent (57).

Ribavirin niosomes were prepared by thin film hydration method using Span 60, CHOL, and DCP for liver targeting purpose (58). The results showed that the niosomal formulation significantly increased ribavirin liver concentration (6 fold) in comparison with ribavirin-free solution.

Mukherjee et al. (59) showed the superior stability and encapsulation efficiency of acyclovir in 200 nm niosomes in comparison to soya L-α-lecithin liposomes. They concluded that niosome could be a better choice for intravenous delivery of acyclovir.

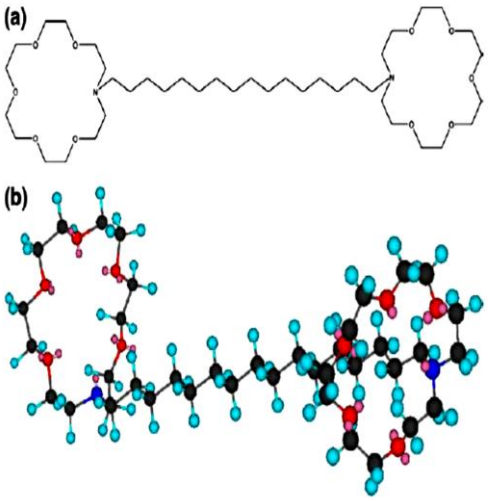

Figure 2. Chemical structure of bolaform surfactants: (a) Bola A-16, (b) Bola C-16 (54).

Ophthalmic route

To minimize the problems associated with conventional eye drops, different ocular drug delivery devices have been investigated such as niosomes for brimonidine tartrate delivery in glaucoma management (60).

Pulmonary route

Drug delivery to lungs appears to be an attractive proposition on account of the large surface area of the alveolar region (61). Nanocarriers could be used for protection and more effective delivery of different therapeutics to respiratory tract. Proniosomes of the anti-asthma steroid beclometasone dipropionate were developed to generate niosomes that were suitable for aerosolization by eithr air-jet or vibrating-mesh nebulization methods (62). Proniosomes prepared by coating sucrose particles with Span 60/CHOL and nano-sized niosomes were produced by manual shaking of the resultant proniosomes in deionized water followed by sonication (median size 236 nm).

Nasal route

Priperm *et al.* (63) prepared melatonin encapsulated niosomes composed of Span 60/CHOL/sodium deoxycholate. Size reduction of melatonin niosomes was performed by extrusion through 100 nm polycarbonate membrane and intranasally administered nanovesicles could distribute melatonin to the liver, hypothalamus and testis of male rats.

Transdermal route

Preparation of vesicles could be a versatile technique to enhance topical penetration of applied drugs trough natural barrier layer, *stratum corneum*. The ability of a particle to diffuse through the *stratum corneum* (for particles of the same charge) depends mainly on its size and viscoelastic properties (64). Alvi et al. (65) reported that vesiculization of 5-FU not only improved the topical delivery, but also enhanced the cytotoxic effect of 5-FU in actinic keratosis and non-melanoma skin carcinoma. Mali et al. (66) used Span 60, Span 20, and Tween 20 with CHOL to prepare nano size vesicle of minoxidil. Niosome formulation prepared with 1:2 ratio of Span 60 and CHOL showed 17.21 ±3.2 % skin retention of minoxidil, which was six fold more than that of minoxidil gel as control. By preparation of nano-niosomes, both poor water solubility and low skin penetration of minoxidil were addressed.

A gel containing the novel Tween 61 elastic niosomes containing diclofenac diethyl-ammonium have not only showed physical and chemical stability for 3 months, but also exhibited high fluxes through rat skin and high *in vivo* anti-inflammatory activity in rat ear edema assay (67). Honeywell-Nguyen and Bouwstra (68) prepared 95 to 110 nm L-595 (sucrose laurate ester)/ PEG-8-L (octa-oxyethylene laurate ester) nano-niosomes as excellent transdermal carrier for pergolide.

In addition to nanovesicle encapsulation, some other methods were developed for enhancing transdermal transport of large molecules such as insulin. A combination techniques of charged nano-liposome encap-sulation of insulin and iontophoresis through rat skins with microneedle-induced micro-channels were resulted in 713.3 times higher transport of the protein than that of its passive diffusion (31).

Vaginal route

Two kinds of entrapped insulin vesicles with Span 40 and Span 60 were prepared by lipid phase evaporation and sonication methods with particle sizes of 242.5 nm and 259.7 nm, respectively (69). They concluded that vaginally administrated nano-niosomes might be a good carrier for protein drugs such as insulin.

Nano-niosomes as gene delivery vectors

Bilayer vesicles are biodegradable, less toxic, less immunogenic and activating lower levels of complement than the viral vectors; therefore utilizing of these kind of gene carriers are more convenient and safer than the viral vectors. Huang et al. (70) used cationic niosomes of sorbitan monoesters for delivery of antisense oligonucleotides (OND) in a COS-7 cell line among them Span 40 and 60 vesicles had more significant effect. However, positively charged particulates are prone to nonspecific interactions with plasma proteins, which resulted in destabilization, dissociation, and rapid clearance of gene/carrier complexes (71). For preparation of an effective non-phospholipid vesicular gene delivery vector, Huang et al. (72) hypothesized using PEGylated cationic niosomes. They used DSPE-mPEG 2000 for PEGylation of cationic niosomes and the resultant OND-vesicle complexes showed a neutral zeta potential with particle size about 300 nm. These complexes had less serum-protein binding affinity and particle aggregation in serum (72). On the other hand, the PEGylated niosomes showed a higher efficiency of OND cellular uptake in serum when compared with cationic niosomes. A new arising problem was reported by Manosroi et al. (73) which was the lower stability of luciferase plasmid (pLuc) encapsulated in Span 60 or Tween 61/dimethyl dioctadecyl ammonium bromide (DDAB)/CHOL in comparison to cationic liposomes. However DDAB/Tween 61/CHOL nanovesicles, made an effective cationic vector for pLuc delivery following the application of iontophoresis on the stratum corneum of rat skin (74). Later, this research team reported (75) successful transdermal absorption, gene expression and stability of tyrosinase plasmid (pMEL34)-loaded DDAB/Tween 61/CHOL nanovesicles as a promising topical delivery in vitiligo therapy. They also successfully expressed human tyrosinase plasmid (pAH7/Tyr) and increased melanin production in tyrosinase gene knocked out human melanoma (M5) cells and in tyrosine-producing mouse melanoma ($B_{16}F_{10}$) cells by loading the plasmid in elastic cationic niosomes (76).

Niosomes in vaccine delivery

Protein subunit vaccines

Development of new safe and effective vaccines is an important goal for many research groups in all over the world. Subunit proteins or DNA of various organisms are safer than live organism-based vaccines even they may show less efficacy. The use of adjuvanted systems have proven to enhance the immunogenicity of these subunit vaccines through protection (i.e. preventing degradation of the antigen *in vivo*) and enhanced targeting of these antigens to professional antigen-presenting cells (77). Brewer and Alexander (78) reported the first application on niosome antigen delivery for immunization of Balb/c mice against bovine serum albumin (BSA). They deduced that niosomes were potentially better stimulators of the Th1 lymphocyte subset than was Freund's complete adjuvant and by inference, potent stimulators of cellular immunity. Hassan et al (79) showed better immunogenicity with herpes simplex virus 1 antigen encapsulated in l-mono palmitoyl glycerol (MP)/-CHOL/DCP niosomes in mice. On the other hand, partial protection against homologous (type 2 herpes simplex virus HSV-2) challenge infection afforded to mice by HSV-2 antigen encapsulated niosomes (80) shows the importance of composition and method of niosomal adjuvant formulations. Yoshioka et al (81) formulated Span/CHOL/DCP niosomes containing tetanus toxoid (TT)

emulsified in an external oil phase to form a vesicle-in-water-in-oil (v/w/o) formulation. Initial studies of the system *in vivo* using cottonseed oil as the external oil phase, showed enhanced immunological activity over the free antigen or vesicles.

Encapsulation of BSA or haemagglutinin (HA) in v/w/o emulsion was also reported by Murdan (82). Immunogenicity studies showed that the v/w/o gel as well as the water-in-oil (w/o) gel as control, possess immunoadjuvant properties and enhance the primary and secondary antibody titres (of total IgG, IgG_1, IgG_{2a} and IgG_{2b}) to HA antigen. Chambers et al (83) reported a single subcutaneous dose of killed *Mycobacterium bovis* BCG in Brij® 52-based nano-niosomes (Novasome™) protected guinea pigs from lethal tuberculosis. Vangala et al. (84) incorporated three different protein antigens in positively charged niosomes made from MP/CHOL/ α,α´-trehalose 6,6´-dibehenate (TDB) or MP/CHOL/TDB/dimethyl-dioctadecylammonium (DDA). Antigens encapsulation led to increase in size of vesicles from submicron to larger (1-2.7 µm) ones which may be due to the high molecular weight of antigens, in addition to their high hydrophobic nature, causing the association of the proteins with the hydrophobic regions of the vesicle bilayers and possibly encouraging a degree of vesicle fusion or influencing the packing arrangements of the surfactants. Their results suggest that both DDA- and MP-based vesicular systems may be useful in enhancing the immunogenicity of the subunit vaccines, especially with the subunit antigen Ag85B-ESAT-6 against tuberculosis, for which a high cell-mediated Th1 immune response is essential (85). Vangala et al. (86) also reported DDA formulations incorporating TDB which showed markedly increased hepatitis B surface antigen specific splenocyte proliferation and elicited cytokine production concomitant with a strong T cell driven response, delineating formulations that may be useful for further evaluation of their clinical potential. Ferro and Stimson (87) used a gonadotrophin releasing hormone (GnRH) analogue, GnRH-glycs, linked to different carrier molecule and encapsulated in NSV formulations to immune-neutralisation of GnRH in male Sprague-Dawley rats. The results were encouraging to use NSVs as a non toxic immune adjuvant. Then, a modified GnRH peptide (CHWSYGLRPG-NH_2) was conjugated to TT and formulated with different adjuvants such as $C_{18}EO_2$/CHOL-/DCP niosomes (88). The best castration effect, depicted in production of IgG_{2b} antibody, was not as well by nano-niosomes as compared to sustained release poly(lactide-co-glycolide)/triacetin (PLGA) formulation.

A promising immunization effect was reported by Lezama-Davila (89) in C57BL/10 mice immunized with *L. m. mexicana* leishmanolysin (gp63).

For developing non parenteral niosomal vaccines, Rentel et al. (90) prepared sucrose ester niosomes for encapsulation of ovalbumine and administered the vesicular formulations through oral route in Balb/c mice. Significant increase in antibody titres was observed following oral vaccination with less hydrophilic vesicular formulation. Chattaraj and Das (91) entrapped hema-gglutinin antigens from three different influenza A strains in Span 4o or 60 niosomes for nasal mucosal delivery.

BSA-loaded niosomes composed of Span 60/Span 85/CHOL/stearylamine were coated with a modified polysaccharide O-palmitoyl mannan (OPM) for targeting them to Langerhan's cells, the major antigen presenting cells found in abundance beneath the stratum corneum (21). Measuring serum IgG titre and its subclasses (IgG_{2a}/IgG_1 ratio) elicited a significantly higher serum IgG titre upon topical application of mannosylated niosomes as compared with topically applied alum adsorbed BSA ($P < 0.05$). The mannosylated niosomes also were used orally for induction of the oral mucosal immunization against TT (92). Coating with OPM was carried out to protect antigen encapsulated vesicles from bile salts dissolution and enzymatic degradation in the

gastrointestinal tract and to enhance their affinity toward the antigen presenting cells of Peyer's patches. On the other hand, Gupta et al. (93) showed topically given TT containing transfersomes, after secondary immunization, could elicit immune response (anti-TT-IgG) that was equivalent to the one that produced following intramus-cularly alum-adsorbed TT-based immu-nization. The immunity response of Span 85/CHOL niosomes was weaker than trans-fersomes.

DNA vaccines

DNA entrapment in liposomes may be due to protection of genetic material in biological milieu, promoted greater homoral and cell-mediated immune responses against the encoded antigen in immunized mice (94). Parentral (95), topical (96) and oral (21) administration capability of NSV/DNA formulations made these systems as new non-toxic and effective vaccine delivery tools. Perrie et al. (95) reported the entrapment of nucleoprotein expressing plasmid of H3N2 influenza virus in NSVs and subcutaneous injection of the formulations resulted in better immunization of treated mice in comparison to naked DNA. Encapsulation of plasmid pRc/CMV-HBs(S) expressing sequence coding for the small proteins of the hepatitis B virus, HBsAg, in mannolysated niosomes signified the potential of these vesicles as DNA vaccine carrier and adjuvant for effective oral immunization against hepatitis B (21). Vyas et al. (96) formulated Span 85/CHOL niosomes encapsulating DNA encoding HBsAg and applied them topically in Balb/c mice. Elevation of serum anti-HBsAg titer and cyokines level (IL-2 and IFN-γ) indicated the efficacy of used topical vesicular vaccine delivery.

Recently, we reported the different positively charged micron-sized niosomal formulations containing sorbitan esters, CHOL and CTAB for the entrapment of autoclaved *Leishmania major* (ALM) (32). Inspite of large diameter of prepared vesicles, the results obtaind showed that the niosomes containing ALM have a moderate effect in the prevention of cutaneous leishmaniasis in BALB/c mice.

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