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Review

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Niosomes as vesicular carriers for delivery of proteins and biologicals

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Abstract

Over the past several years, treatment of infectious diseases and immunization has undergone a paradigm shift. Stemming from the nanobiotechnology research, not only a large number of disease-specific biologicals have been developed, but also enormous efforts have been made to effectively deliver these biologicals. Niosomes are vesicular systems prepared from self-assembly of hydrated non-ionic surfactants. Opinions of the usefulness of niosomes in delivery of proteins and biologicals range from unsubstantiated optimism to undeserved pessimism. This article reviews the current deepening and widening of interest of niosomes in many scientific disciplines, and their application in medicine particularly for the delivery of proteins (insulin, cyclosporine, bacitracin, trypsin), vaccines and antigens (bovine serum albumin, antigen tetanus toxoid, haemagglutinin). This article also presents an overview of techniques of noisome preparation, characterization of niosomes and their applications.

Keywords: Niosomes, Proteins, Biologicals, Vaccines, Oral delivery

Introduction

Recent advances in the fields of genetic research and biotechnology have resulted in endorsement of biologicals such as proteins and vaccines as a major class of therapeutic agents. Biologicals against debilitating diseases have proven remarkable in prevention of these diseases and have contributed significantly to an increase in life expectancy. Administration of these agents in their active state has been a formidable challenge to the biotechnological as well as pharmaceutical industries. Drug associated challenges such as poor bioavailability, suitable route of drug delivery, physical and chemical instability, and potentially serious side effects of these biologicals are some potential limitations on their successful formulation development. The nanobiotechnology i.e., combination of nanotechnology and biotechnology has proposed a new approach as a solution to their formulation problem in the form of niosomes [1, 2].

Protein delivery

DNA recombinant technology has made possible the large-scale production of these proteins, however, the ability to deliver these compounds systemically using convenient and effective delivery systems remains a challenge. Although the oral route is the most convenient and popular, protein delivery by this route is limited by low

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and erratic absorption, mainly due to degradation by proteolytic enzymes and poor permeability of the gastrointestinal mucosa. Many of these proteins are unable to accomplish their full range of therapeutic benefits when administered through the alternative routes due to presence of protease. There is a significant need for novel delivery systems that would retain the efficiency of these agents and deliver the drug at a controlled rate with prolonged biological activity [3]. When developing a novel delivery system, the biophysical, biochemical, and physiological characteristics of protein, as well as pharmacokinetics and pharmacodynamics are important to consider [4].

Vaccine delivery

Vaccination technology in controlling infectious diseases has made remarkable contributions to public health and quality of life, because it has been proven that vaccines have saved more lives than drugs [5]. Presently most vaccines for protection against both systemic and superficial pathogens are administered parentrally in order to avoid antigen degradation in the gut. Oral delivery of vaccines is attractive, since this offers a number of inherent advantages over the parenteral route, including increased patient compliance, ease and convenience of administration, reduced costs and minimal side effects. Most of the oral vaccines so far are based on either live attenuated organisms or employ synthetic peptides, proteins and polysaccarides. Due to their susceptibility to degradation during gastric and intestinal passage, these compounds require more sophisticated delivery systems. New vaccines based on recombinant proteins and DNA, are safer than traditional vaccines, but they are less immunogenic. Therefore, there is an urgent need for the development of potent and safe adjuvants and delivery systems that can be used with new generation of vaccines.

Vesicular systems: Niosomes

Among vesicular systems, opinions of the usefulness of niosomes in various biotechnological applications range from unsubstantiated optimism to undeserved pessimism. Niosomes offer an attractive mode of delivery for biological agents belonging to the class of proteins and vaccines, since they are able to overcome some inherent problems associated with these molecules. These problems include suitable route of delivery, physical as well as chemical instability, poor bioavailability, and potent side effects. Niosomes are organized non-ionic surfactant based vesicles formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures (Fig. 1). The assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat [6]. The result is an assembly in which the hydrophilic head groups enjoy maximum contact with the aqueous solvent and hydrophobic parts of the molecule are shielded from the same. Hydrophilic drugs can be encapsulated in the internal aqueous compartment while lipophilic and hydrophobic drugs can be associated with the bilayers of the vesicles. A large number of non-ionic surfactants with varying HLB have been used to prepare vesicles viz. polyglycerol alkylethers, glucosyl dialkyl ethers, crown ethers, polyoxyethylene ethers and esters, briz and a series of spans and tweens [7]. Cholesterol is one of the vital components and provides rigidity to the bilayer by making vesicles less leaky [8]. Both cholesterol content and HLB of the surfactant tends to affect the important vesicular properties, which should be optimized in the terms of better therapeutic efficacy and for the use of vesicles in pharmaceutical application. Thermodynamically stable vesicles are formed only in the presence of proper mixture of surfactants and charge inducing agents, which helps in electrostatic stabilization of vesicles. Such charge inducing agents include stearylamine, dicetyl phosphate and diacylglycerol. The low cost of ingredients, ease of large scale production, greater stability and resultant ease of storage of niosomes has lead to the exploitation of these systems as alternative to other micro- and nano- encapsulation technologies. Additionally, niosomes are

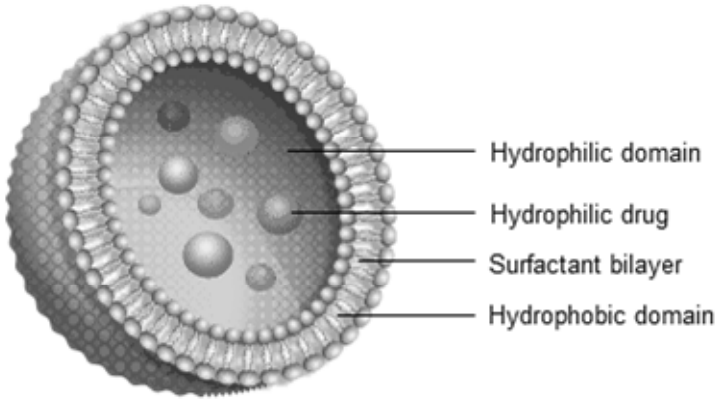

Fig. 1 Schematic representation of drug loaded niosome

biocompatible, non-immunogenic, biodegradable, non-toxic and capable of site specific delivery.

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Preparation of Niosomes

The most commonly used method for preparation of protein or vaccine loaded niosomes identified in the literature are summarized as follows.

Multilamellar vesicles (MLVs) - Film hydration is the simplest method and is widely used. In this method, the surfactants are dissolved in an organic solvent in a round-bottomed flask and a thin surfactant film is allowed to formed on the inside wall of the flask by removal of the organic solvent. An aqueous buffer containing drug is then added and the dry film is hydrated above the transition temperature (Tc) of the surfactant. Shaking (by hand or by vortex mixer) yields a dispersion of MLV. Dehydration-rehydration is another technique to produce MLVs with high entrapment efficiencies [9].

Large Unilamellar Vesicles (LUVs) - LUVs are prepared via reverse phase evaporation method wherein surfactants and cholesterol are dissolved in an organic solvent. The aqueous phase is added to the organic phase and the mixture is sonicated in order to form an emulsion, followed by slow removal of the organic phase. LUVs with relatively high entrapped aqueous volume are also produced by solvent injection technique where solutions of surfactant in solvents with high vapor pressure are injected into excess aqueous phase under reduced pressure.

Small Unilamellar Vesicles (SUVs) - MLV dispersions are converted into SUVs by application of external energy by either sonication using bath sonicator or a probe sonicator, or by high pressure homogenization using microfluidizer or by extrusion under high pressure using French pressure cell [9]. During application of energy the MLV structure is broken down and SUVs with a high radius of curvature are formed.

Characterization of Niosomes

Niosomes are characterized chiefly for their size, morphology, charge, rigidity, homogeneity and drug loading capacity.

Vesicle size and morphology- Niosome size can range from around 20 nm to around 50 μm. Several techniques can be used to determine vesicle size and size distribution. Large niosomes, those with diameters over 1 μm, can be

adequately measured by light microscopy and the Coulter counter. Light microscopy offers the possibility of collecting information on particle shape, whereas the volume distribution of niosomes (>1 μm) in dispersions can be determined with Coulter counter. For vesicles in the submicron range, size can be assessed by electron microscopic analysis or by light scattering techniques. Electron microscopic analysis such as transmission electron microscopy or freeze-fracture techniques not only analyze niosome size, but also analyze number of bilayers. Further, scanning electron microscopy, atomic force microscopy and cryo transmission electron microscopy are also used to study the shape and surface characteristics of the niosomes.

Vesicle charge - The vesicle surface charge can play an important role in the behavior of niosomes *in vivo* and *in vivo*. In general, charged niosomes are more stable against aggregation and fusion than uncharged vesicles. In order to obtain an estimate of the surface potential, the zeta potential of individual niosomes can be measured by microelectrophoresis.
An alternative approach is the use of pH-sensitive fluorophores. More recently, dynamic light scattering have been used to measure the zeta potential of niosomes.

Bilayer Rigidity and Homogeneity- The biodistribution and biodegradation of niosomes are influenced by rigidity of the bilayer. Inhomogeneity can occur both within niosome structures themselves and between niosomes in dispersion and could be identified via. p-NMR, differential scanning calorimetry (DSC) and fourier transform-infra red spectroscopy (FT-IR) techniques. Recently, fluorescence resonance energy transfer (FRET) was used to obtain deeper insight about the shape, size and structure of the niosomes [10].
Niosomal drug loading and encapsulation efficiency- To determine drug loading and encapsulation efficiency, the niosomal aqueous suspension was ultracentrifuged, supernatant was removed and sediment was washed twice with distilled water in order to remove the adsorbed drug. The niosomal recovery was calculated as:

$$Niosomes\ recovery\ (\%) = \frac{Amount\ of\ Niosomes\ recovered \times 100}{Amount\ of\ polymer + drug + excipient}$$

The entrapment efficiency (EE) was then calculated using formula:

$$Entrapment\ efficiency\ (\%) = \frac{Amount\ of\ drug\ in\ Niosomes \times 100}{Amount\ of\ drug\ used}$$

The drug loading was calculated as:

$$Drug\ loading\ (\%) = \frac{Amount\ of\ drug\ in\ Niosomes \times 100}{Amount\ of\ Niosomes\ recovered}$$

Niosomal drug release - Recently, FRET was used to monitor release of encapsulated matters in niosomes by using separate niosomal suspensions incorporating donor and acceptor [10]. The simplest method to determine in vitro release kinetics of the loaded drug is by incubating a known quantity of drug loaded niosomes in a buffer of suitable pH at 37°C with continuous stirring, withdrawing samples periodically and analyzed the amount of drug by suitable analytical technique. Dialysis bags or dialysis membranes are commonly used to minimize interference.

Applications

Pulmonary delivery

Although inhaled glucocorticosteroid such as beclomethasone dipropionate (BDP) is the most effective therapy available for patients with asthma but the role of this drug in the management of chronic obstructive pulmonary disease (COPD) is less certain due to poor permeation through the hydrophilic mucus in order to reach the glucocorticoid receptor, located in the cytoplasm of bronchial epithelial cells. Pulmonary delivery of BDP through polysorbate 20 niosomes offers the advantages of sustained

delivery, an improved mucus permeation, targeted drug delivery and amplified therapeutic effect [11]. The clinical efficacy of therapeutic aerosols depends on the aerodynamic size distribution of the aerosol and the drug output from the nebulizer. The vesicular dispersions show the aerodynamic diameter comparable with the commercial product. In all conditions of nebulization, the niosomal dispersion seems to release a greater amount of BDP on filters (37%) with respect to BDP surfactant solution (25%) and BDP commercial product (35%). The results obtained by either TurboBoy nebulizer or Clenny nebulizer were similar. The rheology of the niosomal BDP formulation is also comparable to that of commercial product. In addition the non-ionic surfactant vesicles remarkably increase the permeation rate of BDP through the model mucosal barrier thus offering a better targeting of corticosteroids in the treatment of COPD.

Oral delivery

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) [12]. The kinetic of drug release is described by Baker and Lonsdale equation indicating diffusion based delivery mechanism. It thus appears that niosomes could be developed as sustained release oral dosage forms for delivery of peptides and proteins such as insulin. Yoshida et al investigated oral delivery of 9-desglycinamide-8-arginine vasopressin entrapped in niosomes in an in vitro intestinal loop model and reported that stability of peptide increased significantly. Manosroi *et al*., had demonstrated the enhanced entrapment of charged peptide drugs, bacitracin, insulin and bovine serum albumin in niosomes by modifying the vesicular charge compositions. Cationic, anionic and neutral niosomes were prepared from sorbitan monostearate (Span 60) or polyoxyethylene sorbitan monostearate (Tween 60), cholesterol (CHL), dimethyldioctadecylammonium bromide (DDAB) and/or dicetyl phosphate (DP). Anionic niosomes were oligolamellar membrane structure with the sizes of 40-60 nm whereas neutral niosomes and cationic niosomes showed the sizes of 0.1-1.3 μm and 100-150 nm, respectively. The highest entrapment efficiency of bacitracin, bovine serum albumin and insulin at 90.88, 72.94 and 87.15 was observed in anionic, neutral and cationic niosomes, respectively [13]. The results suggested the appropriate niosomal formulation to entrap the peptides with different charges and polarity for pharmaceutical application. In one of the first studies of its kind sucrose ester niosomes loaded with ovalbumin were found to cause a modest but significant increase in the level of specific antibodies after oral administration [14].

Vaginal delivery

Vaginal delivery of insulin niosomes reportedly prepared from Span 40 and Span 60 and nearly 250 nm in size were found to have enhanced hypoglycemic effects compared with subcutaneous administration of insulin solution to rats. Compared with subcutaneous administration of insulin solution, the relative pharmacological bioavailability and the relative bioavailability of insulin-Span 60 vesicles group were 8.43% and 9.61%, and insulin-Span 40 niosomes were 9.11% and 10.03% ($p > 0.05$). The results indicates insulin-Span 60, Span 40 niosomes had an enhancing effect on vaginal delivery of insulin [15]. Although the factors controlling the process for penetration of a portion of vaginally administrated niosomes into bloodstream from vaginal tract were not fully understood but the authors postulated that after encapsulation in niosomes, insulin became an active and efficiently therapeutic agent when administrated

vaginally and niosomes might be a good carrier for vaginal delivery of protein drugs.

Brain delivery

The vasoactive intestinal peptide (VIP) is a neuropeptide of the glucagon-secretin family with a widespread distribution in both the central and peripheral nervous system. It exerts diverse peripheral biological functions, such as anti-inflammatory and immunomodulatory effects, regulation of cell growth and differentiation and participation in the development of neural tissue [16]. VIP have interesting applications in the treatment of Alzheimer's disease. However, like most endogenous peptides, its potential therapeutic applications are limited by its failure to cross the blood–brain barrier (BBB) and by its rapid elimination after intravenous administration. The association of VIP with glucose-bearing niosomes allowed a significantly higher VIP brain uptake compared to control niosomes (up to 86%, 5 min after treatment). 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 [17]. Niosomes believed to curtail enzyme-induced inactivation of VIP, and the association of VIP with glucose-bearing niosomes also facilitates transport of VIP through BBB, leading to enhanced brain uptake. In conclusion, the study demonstrated that the administration of systemic glucose-bearing niosomal vesicles encapsulating VIP could deliver intact VIP to specific brain areas. Therefore, glucose-bearing vesicles represent a novel tool to deliver drugs across the BBB.

Transdermal delivery

Cyclosporin A glyceryl dilaurate:$C_{16}EO_{10}$: cholesterol niosomes increased the uptake of drug into deeper skin strata [18]. The niosomal vehicle also demonstrated deposition of alpha-interferon into pilosebaceous units of the hamster ear model [19]. The enhanced delivery of niosome encapsulated drugs through the stratum corneum has been observed [20, 21] and it therefore remains to elucidate the mechanism of this delivery. Small (100 nm) vesicular structures have been observed between the first and second layer of human corneocytes 48 h after incubation with dodecyl alcohol polyoxyethylene ether niosomes [22]. The same study reports the presence of niosomes in the deeper seemingly inaccessible areas of the skin and concludes that there was a reorganisation of the niosome membrane into individual monomers which on arriving at these deeper layers reformed into niosomes [22]. Based on the above studies, it does appear that transdermal delivery of protein and biologicals with niosomes appears promising and would require that the dose be applied in high concentration and within niosomes prepared from low phase transition surfactant mixtures. The mechanism of niosome penetration between bilayers appears to lie between destruction: reconstruction hypothesis [22] and the highly flexible hypothesis [23].

Vesicles with biological activity or with a targeting function in addition to carrier properties will have an added advantage. Aspasomes, vesicles prepared from ascorbyl palmitate, having antioxidant property also possess skin permeation enhancing property. Ascorbyl palmitate formed vesicles in combination with cholesterol and a negatively charged lipid (dicetyl phosphate) by film hydration method. The studies revealed that the antioxidant potency of ascorbyl moiety was better after converting ascorbyl palmitate into vesicles (Aspasomes). Further, azidothymidine (AZT) aspasomes showed significantly higher transdermal permeation compared to aspasomes-AZT aqueous dispersion and AZT-solution [24]. Thus, aspasomes may find applications as transdermal delivery of proteins particularly in disorders implicated with reactive oxygen species.

Systemic delivery

Polyethylene glycol (PEG) modified cationic niosomes were used to improve the stability and cellular delivery of oligonucleotides (OND). PEGylated cationic niosomes, composed of DC-Chol, PEG2000-DSPE and Span, offer some

advantages as gene carriers. Complexes of PEGylated cationic niosomes and OND showed a neutral zeta potential with particle size about 300 nm. PEG-modification not only increased the particle stability in serum but also enhanced the nuclease resistance of the loaded gene drug. Compared with cationic niosomes, complexation of PEGylated cationic niosomes with OND enhanced the cellular uptake of OND in serum [25]. The presence of PEG coated on the cationic niosomes not only contributes to the neutral zeta potential but also prevents serum proteins and nucleases from approaching the cargo. Thus, in terms of their stable physiochemical properties in storage and physiological environment, as well as low-cost and widely available materials, PEGylated cationic niosomes are promising drug delivery systems for improved OND potency in vivo.

In an another study, the preparation, characterization and activity of non-phospholipid vesicles based on polyglycerol alkyl ethers and on sorbitan monoalkyl ethers for the transport of aminoacid based molecules, such as trypsin, bovine basic pancreatic inhibitor and polylysine rich peptides derived from the herpes simplex virus type 1 (HSV-1) glycoprotein B were described [26]. Niosomes containing the indicated model drugs have shown to be more stable in term of size with respect to liposomes. In addition, niosomes, (i) are able to encapsulate the model drugs over 49%, (ii) are characterized by dimensions compatible with

applications on the mucous membrane, (iii) remain stable in size for at least 3 months and (iv) can release the model drug (after a slight lag time) in a controlled fashion as compared to that of the corresponding free solution.

Vaccine and antigen delivery

A number of surfactants have documented immunostimulatory properties [27] and have been used as vaccine adjuvants. The adjuvanticity of niosomes prepared from 1-mono-palmitoyl glycerol, cholesterol, dicetyl phosphate-5:4:1 has been demonstrated in mice, on subcutaneous administration of ovalbumin or a synthetic peptide containing a known T-cell epitope [28] and bovine serum albumin [29]. The intraperitoneal administration of same niosome vesicle has also been shown to act as a vaccine adjuvant to severe combined immunodeficiency mice reconstituted with peripheral blood lymphocytes (PBL-SCID mice) [30].

A vesicle in water in oil (v:w:o) emulsion [31] prepared from Span 80 and cotton seed oil has been evaluated as an immunological adjuvant using the model antigen tetanus toxoid [32] . An increased secondary response (level of IgG1) was observed when the v:w:o formulation was administered by the intramuscular route in comparison with the vesicle formulation and the free antigen. Nonionic surfactant vesicles were also reported for nasal mucosal delivery of viral influenza vaccine antigen.

Bovine serum albumin (BSA) and haemagglutinin (HA) loaded niosomes have been formulated as multi-component organogels using the non-ionic surfactant sorbitan monostearate as gelator [33]. The complex gels were prepared by the addition of a hot aqueous niosome suspension to the organic solution of the gelator; a vesicle-in-water-in-oil (v/w/o) emulsion was produced which cools to an opaque, semi-solid, thermoreversible v/w/o gel [33]. Microscopy revealed that the microstructure consists of a tubular network of surfactant aggregates in the organic medium and the niosome suspension being dispersed in these surfactant tubules [33]. In vivo, a depot effect was observed following intramuscular administration of the gel containing the entrapped bovine serum albumin, cleared from the injection site over a period of days. The relatively short-lived nature of the depot was thought to arise due to interactions between the gel and the local interstitial fluid which results in gel disintegration in situ. Thus, the niosomes containing antigens are believed to be released from the organic gel. Immunogenicity studies showed that the v/w/o gel as well as one of the controls, the water-in-oil (w/o) gel, possess immunoadjuvant properties and enhance the primary and secondary antibody titres (of total IgG, IgG1, IgG2a and IgG2b) to haemagglutinin

antigen. As far as humoral immunity is concerned, the w/o gel showed stronger immunoadjuvant properties compared to the

Table:1 Proteins and biologicals formulated as niosomes.

Drug	Administration route	Components	Preparation method	Application
Proteins and Peptides				
Beclomethasone	Pulmonary	Polysorbate 20, cholesterol	Film hydration	Sustained and targeted delivery, increased mucus penetration
Rh-Insulin	Oral	Polyoxyethylene alkyl ether, cholesterol, dicetylphosphate	Modified handshaking method	Protection against proteolysis, sustained release
Bacitracin	Oral	Span 60/Tween 61, cholesterol, dimethyldioctadecylammonium bromide/or dicetyl phosphate	Freeze dried empty liposome	Increased entrapment
Bovine serum albumin	Oral	Span 60/Tween 60, cholesterol, dimethyldioctadecylammonium bromide/ dicetyl phosphate	Freeze dried empty liposome	Increased entrapment
9- desglycinamide, 8-arginine vasopressin	Oral	-	Sonication	Stabilization of peptide
Insulin	Vaginal	Span 40 and Span 60	Phase evaporation with sonication	Enhanced hypoglycemic effects
Vasoactive intestinal peptide	Brain	*N*-Palmitoylglucosamine , Span 60, cholesterol, solulan C24	Handshaking followed by sonication	Significantly higher VIP brain uptake
Cyclosporine A	Transdermal	glyceryl dilaurate/ cholesterol/polyoxyethylene-10-stearyl ether	-	Target to pilosebaceous units
α-interferon	Transdermal	Glyceryl dilaurate/ cholesterol/ polyoxyethylene-10-stearyl ether	-	Target to pilosebaceous units
Azidothymidine	Transdermal	Aspasomes: ascorbyl palmitate, cholesterol, dicetyl phosphate	Film hydration method with sonication	Transdermal permeation
Trypsin	Systemic	Polyglycero-6-oleate, sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, cholesterol	Film hydration method	Stabilization of drug, controlled release
Bovine basic pancreatic inhibitor	Systemic	Polyglycero-6-oleate, sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, cholesterol	Film hydration method	Stabilization of drug, controlled release
Polylysine rich peptides	Systemic	Polyglycero-6-oleate, sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, cholesterol	Film hydration method	Stabilization of drug, controlled release
Oligonucleotide	Systemic	DC-Cholesterol, PEG 2000-DSPE and span	-	Enhanced cellular uptake
Vaccines and antigens				
Ovalbumin	Intragastric	Sucrose esters, cholesterol and dicetyl phosphate	Film hydration with sonication	Significant increase in antibody titres
Ovalbumin	subcutaneous	1-mono-palmitoyl glycerol, cholesterol, dicetyl phosphate	Dehydration-rehydration	vaccine adjuvant
T-cell epitope	intraperitoneal	1-mono-palmitoyl glycerol, cholesterol, dicetyl phosphate	Dehydration-rehydration	vaccine adjuvant
Antigen tetanus toxoid	intramuscular	Span 80 and cotton seed oil	Dehydration-rehydration	immunological adjuvant
Viral influenza vaccine antigen	Nasal mucosal	Surfactant, cholesterol, dicetyl phosphate	Dehydration-rehydration	-
Haemagglutinin	Intramuscular	Organogels, sorbitan monostearate	Vesicle-in-water-in-oil emulsion	Immunoadjuvant
DNA Vaccine	Oral	Span 60, cholesterol and stearylamine coated with a o-palmitoyl mannan	Reverse phase evaporation method	Protection from enzymatic degradation, enhanced affinity towards antigen presenting cells of Peyer's patches.

v/w/o gel, being effective at a lower antigen dose i.e 0.1 µg haemagglutinin antigen. Thus, niosomes may offer many advantages over other adjuvants in terms of immunological selectivity, low toxicity and stability [33].

Recently, mannosylated niosomes as oral DNA vaccine carriers for the induction of humoral, cellular and mucosal immunity were described. Niosomes composed of span 60, cholesterol and stearylamine were prepared by reverse phase evaporation method and were coated with o-palmitoyl mannan (OPM) in order to protect them from enzymatic degradation in the gastrointestinal tract and to enhance their affinity towards the antigen presenting cells of Peyer's patches [34]. Presence of OPM coating improves the stability of niosomes in GI tract. Although, the serum anti-HBsAg titer obtained after oral administration of OPM coated niosomal formulations was less as compared to that elicited by naked DNA and pure HBsAg administered intramuscularly, but the mice were seroprotective within 2 weeks and antibody level far above the clinically protective limit for humans was achieved. Further, intramuscular naked DNA and recombinant HBsAg did not elicited sIgA titer in mucosal secretions that was induced by oral administration of OPM coated niosomes. Similarly, OPM coated niosomes produced humoral (both systemic and mucosal) and cellular immune response upon oral administration. The study signifies the potential of OPM coated niosomes as DNA vaccine carrier and adjuvant for effective oral immunization [34].

Diagnostic imaging

Apart from the use of niosomes as various drug carriers one report in the literature details the evaluation of these systems as diagnostic agents. $C_{16}C_{12}G_7$ and $C_{16}G_3$ niosomes containing cholesterol and stearylamine encapsulating the radioopaque agent iopromide were found to concentrate in the kidneys on intravenous administration [35]. The presence of the positive charge on the niosome 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 $C_{16}C_{12}G_7$ [35]. Although the niosome formulation enhanced the opacity of this contrast agent, poor encapsulation efficiency agent was a problem with this system and clinically relevant enhancement of opacity was not achieved in this study.

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