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# Niosomes: An Exploration of A Novel Research Platform In Drug Delivery

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## ABSTRACT

The exploitation of the various approaches in the pharmaceutical field to develop a therapeutically efficient drug delivery system has lead to the exploration of niosomes as a suitable novel drug delivery system. Niosomes are vesicular systems made up of non-ionic surfactants with or without cholesterol and a charge inducing agent. Niosomes are similar to liposomes *in vivo.* Niosomes offer the advantages of having good stability, minimized toxicity, minimized side effects and are also economically feasible. In the present study, the various aspects on niosomal drug delivery system like the composition, methods of preparation, characterization, free drug removal, evaluation methods and applications in pharmaceutical field are explored. Niosomal formulations have been extensively studied and has showed enhanced therapeutic activity in the administration of drugs, hormones, markers and vaccines intended for systemic, topical, transdermal, ocular and targeted drug delivery. Considerable proven research in this efficient drug delivery system offers a pronounced scope for enhanced research with more drugs for targeted and site-specific drug delivery.

**Keywords:** Niosomes, non-ionic surfactant vesicles.

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INTRODUCTION

Since the advent of non-ionic surfactants, its application in pharmaceutical research on improving drug delivery has found an enormous growth. Handjani-Vila *et al* were the first to hydrate a mixture of cholesterol and a singly alkyl chain, non-ionic surfactant to result in the formation of vesicular system.[1] L'Oreal was the first to market and patent a niosomal formulation in 1975.

Niosomes or non-ionic surfactant vesicles or surfactant membrane vesicles are osmotically active and stable unilamellar or multilamellar vesicles wherein an aqueous solution is enclosed in highly ordered bilayer made up of non-ionic surfactant with or without cholesterol and dicetyl phosphate. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs.[2]

**Advantages of Niosomes[3,4,5]**

Niosomes have the following advantages over liposomes.

1) They act as potential carriers for delivery of drugs, antigens, hormones and other bioactive agents.
2) They have high chemical stability as they are less susceptible to oxidation.
3) They are relatively less toxic.
4) They have greater ease of production.
5) The production is economical.
6) They have wider formulation versatility.
7) Surfactants used for forming niosomes are biodegradable, non-immunogenic and biocompatible.
8) These vesicles improve the therapeutic efficacy of drugs by reducing the clearance rate, targeting to the specific site and by protecting the encapsulated drug.
9) Drug targeting reduces the dose which leads to subsequent decrease in side effects.
10) Encapsulation of the drug by niosomes is found to reduce the toxicity as demonstrated in the study on preparation of niosomes containing vincristine. It is also found to decrease the neurological toxicity, diarrhoea and alopecia following the intravenous administration of vincristine and increased vincristine anti-tumor activity in S-180 sarcoma and Erlich ascites mouse models.[5]

**COMPOSITION OF NIOSOMES**

Niosomes are made up of the following components:

**1) Non-ionic surfactants**

It is the main composition of niosomes which forms the bilayer called as lamella that is made up of hydrophobic and hydrophilic moiety. Various non-ionic surfactants have been investigated in the formulation of niosomes which includes crown ethers, perfluro alkyl group surfactants, sorbitan esters (Span 20,40,60,65,80 and 85), polysorbates (Tween 20,40,60 and 80), poly-24-oxyethylene cholesteryl ether (Solulan C24) and polyoxyethylene alkyl ethers (Brij 30,35,52,58,72,76,92 and 97).[6]

**2) Cholesterol**

Cholesterol, a steroid is included in the formulation of niosomes to stabilize them by providing rigidity due to alteration of steroidal rigid skeleton with surfactant molecules in the bilayer and thereby restricting the movement of carbons of hydrocarbon.

The bilayers of the vesicles are either in the liquid state or in the gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such as cholesterol. In the gel state, the alkyl chains are present in a well-ordered structure and in the liquid state; the structure of the bilayer is more disordered.

The surfactants are characterized by the gel-liquid transition temperature, $T_c$. Cholesterol which is an amphiphilic molecule, orients itself by hydroxyl (-OH) group facing towards the aqueous phase and aliphatic chain towards the surfactant's hydrocarbon chain. In general the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other cholesterol decreases the chain orders of gel-state bilayers. At a high cholesterol concentration, the gel state gets transformed to a liquid-ordered phase.[7, 8]

**3) Charge inducing agents**

Charge inducing agents stabilizes the niosomes by increasing the surface charge density thereby preventing vesicle flocculation, aggregation and fusion. Stearyl amine and cetyl pyridiium chloride are used as positive charge inducers and dicetyl phosphate, dihexadecyl phosphate and lipoamine acid are used to provide negative charge to the vesicles. These act by increasing the interlamellar distance and thus increasing the entrapped volume of the vesicles.[9, 10]

**Preparation of Niosomes**

Non-ionic surfactant vesicles can be prepared using the following methods:

**1) Sonication method**

The lipophilic components are dissolved in an apolar solvent, such as a mixture of chloroform and methanol. The solvent evaporates overnight under vacuum, resulting in a surfactant film. The film is then hydrated with aqueous solution and the mixture is sonicated at $60^0$C for 3 min

using a probe sonicator. The method results in the formation of both large unilamellar vesicles (LUVs) and multilamellar vesicles (MLVs).[11, 12, 13]

**2) Ether injection method**

The oil soluble components are dissolved in diethyl ether and injected slowly through a needle into the aqueous phase maintained at $60^0$C. The method results in the formation of mainly multilamellar vesicles. The size of the vesicles can be made uniform by using suitable size needle but it has its limitations of limited solubility of materials in the ether and difficulty of removal of ether from the final formulation.[14, 15]

**3) Hand shaking method**

The apolar ingredients are dissolved in a mixture of chloroform and methanol. The solvent evaporates overnight under vacuum. The resulting surfactant film is hydrated with an aqueous solution and gently shaken at 50-$60^0$C to allow the formation of vesicles. If the drug is hydrophilic it can be added to the aqueous phase and if it is hydrophobic to the organic phase. The preparation method yields multilamellar vesicles.[16]

**4) Reversed phase evaporation**

The oil soluble components are dissolved in an apolar solvent such as chloroform. The aqueous phase is added and the mixture, sonicated to form an emulsion. The solvent evaporates overnight under vacuum. The resulting suspension is shaken to form vesicles. Mainly large multilamellar vesicles are formed.[15]

**5) Method as described by Handjani-Vila *et al***

The surfactants are added to the aqueous phase and the mixture is agitated to yield a homogenous lamellar phase. The suspensions are then homogenized by means of agitation or ultracentrifugation.[17]

**6) Micro fluidization method**

This is based on submerged jet principle wherein fluidized streams interact at ultrahigh velocities (up to 1700 ft/sec) in precisely defined micro channels within the interaction chamber to form niosomes. The method gives greater uniformity and smaller size vesicles.[18]

**7) Active Trapping techniques**

In this method the niosomes are prepared and then loaded with the drug maintaining the pH gradient or ion gradient to facilitate the uptake of drug by the niosomes. The resultant niosomes are found to have good entrapment, high drug lipid ratios and absence of leakage. The method is cost effective and suitable for labile drugs.[19]

**8) The bubble method**

This method is performed in the absence of organic solvents. All the components are dispersed in buffer in a round bottomed flask immersed in a water bath with controlled temperature. The flask is fitted with a homogenizer, thermometer and nitrogen supply, and is attached to a water cooled reflux. The dispersion is mixed with a shear homogenizer for 15 sec and then nitrogen is passed through it to form niosomes.[20]

**9) Trans-mebrane pH gradient (inside acidic) drug uptake process or remote loading method**

Multilamellar vesicles prepared at acidic pH by hand shaking method are then subjected to freeze-thaw cycle and later sonicated. Remote loading of drug is by adding aqueous solution of drug, pH adjusted to 7.0-7.2 and then the mixture is heated. Niosomes so formed shows greater entrapment efficiency and better retention of drug.[21, 22]

**Characterization of Niosomes[23, 24, 25, 26]**

The various methods used to characterize niosomes are summarized in table 1.

**Table 1: Characterization of niosomes[23, 24, 25, 26]**

| S.No. | Characterization parameters | Method of analysis |
|---|---|---|
| **Physical characterization** | | |
| 1 | Vesicle shape and morphology | Transmission Electron Microscopy (TEM) or freeze fracture microscopy technique. |
| 2 | Mean vesicle size and Size distribution | Dynamic light scattering, zetasizer, photon correlation spectroscopy, laser light scattering, gel permeation and size exclusion chromatography. |
| 3 | Formation of bilayer | Formation of X-cross under light polarization microscopy. |
| 4 | Number of lamellae | NMR spectroscopy, small angle X-ray scattering and electron microscopy. |
| 5 | Membrane Rigidity | Mobility of fluorescence probe as function of temperature. |
| 6 | Membrane thickness | X-ray scattering analysis. |
| 7 | Viscosity | Ostwald's viscometer at room temperature. |
| 8 | Turbidity | UV-Visible diode array spectrophotometer. |
| 9 | Thermal analysis | Differential scanning calorimetry. |
| 10 | Osmotic shrinkage | Incubating in hypertonic salt solution and determining the reduction in vesicle size using optical microscopy. |
| 11 | Surface charge | Free-flow electrophoresis. |
| 12 | Phase behavior | Freeze-fracture electron microscopy, differential scanning calorimetry. |
| 13 | Electrical surface potential and surface pH | Zeta potential measurements and pH sensitive probes. |
| **Chemical characterization** | | |
| 1 | Cholesterol concentration | Cholesterol oxidase assay and High Performance Liquid Chromatography. |
| 2 | Cholesterol auto-oxidation | High Performance Liquid Chromatography and Thin Layer Chromatography. |

| | | |
|---|---|---|
| 3 | Osmolarity | Osmometer |
| **Biological characterization** | | |
| 1 | Sterility | Aerobic and anaerobic cultures |
| 2 | Pyrogenicity | Limulus Amoebocyte Lysate (LAL) test |
| 3 | Animal toxicity | Monitoring survival rates, histology and pathology. |

**Free drug removal**

The unentrapped drug can be removed by

a) Gel filtration using Sephadex G-50 column and elusion with phosphate buffered saline.[27, 28]

b) Centrifugation of the sample at 4000 rotations per minute and determining the amount of drug present in the surfactant after suitable dilutions.[29, 30]

c) Dialysis using a dialysis bag containing the niosomal suspension immersed in phosphate buffered saline and determining the amount of drug dialysed.[20]

**Entrapment efficiency**

Entrapment efficiency can be determined by disrupting the drug loaded vesicles after separation from unentrapped drug using Triton X-100[31] or n-propranolol[32] or 2.5% sodium lauryl sulphate[25] and determining the drug content after suitable dilutions.

***In vitro* release study**

The *in vitro* release study of drug from the niosomal suspension is determined by determining the amount of drug released through a dialyzing membrane placed in phosphate buffer saline under magnetic stirring.[33]

$$\text{Percentage of drug released} = \frac{\text{Amount of drug released}}{\text{Total amount of encapsulated drug}} \times 100$$

**4) Permeation study**

*In vitro* permeation study is performed using Franz diffusion cell through dehaired rat skin. In this 1 ml of the suspension is taken in the donor compartment and phosphate buffer in the receptor compartment which is stirred at 100 rpm and maintained at $37\pm0.5^{0}$C. Samples are withdrawn at periodic intervals and the amount of drug permeated is determined spectrophotometrically.[34]

**FACTORS AFFECTING THE CHARACTERIZATION OF NIOSOMES**

**1) Nature of the encapsulated drug**

Entrapment of drug in the niosomes resulted in increase in vesicle size due to the interaction of the solute with the surfactant bilayer.[35] Among a series of Spans and Tweens, the entrapment of water soluble drug (diclofenac sodium) is reported to be more with hydrophilic surfactants like

Tween 60.[36] Maximum entrapment is reported for slightly water soluble drugs (methotrexate) in lipophilic surfactant Span 60.[37]

**2) Amount and type of non-ionic surfactant**

The mean size of niosomes increases with increase in the hydrophilic-lipophilic balance (HLB value) from span 85 (HLB 1.8) to span 20 (HLB 8.6) due to decrease in surface free energy as a result of increase in hydrophobicity of surfactant.[38] A linear correlation is observed between concentration of lipid and entrapment efficiency.[39]

**3) Membrane additives**

Cholesterol when used in the molar ratio of 1:1 prevents the aggregation of niosomes by repulsive steric or electrostatic effects and leads to less leaking vesicles. Increase in cholesterol increases the hydrodynamic diameter and thus its entrapment efficiency.[8]

Presence of charge tends to increase the interlamellar distance between the successive bilayers of the multilamellar vesicle structure and leads to overall entrapped volume.[40] Dicetyl phosphate provides negative charge to the vesicles and is used to prevent the aggregation of hexadecyl diglycerol ether niosomes.

Stearic acid provides positive charge to the niosomes and thus used to prepare cationic niosomes.

**4) Method of preparation**

Niosomes prepared with Spans and cholesterol by lipid film hydration is found to give multilamellar vesicles whereas niosomes prepared by ether injection method is found to yield unilamellar vesicles or oligolamellar vesicles.

**5) Surfactant and lipid levels**

The surfactant/lipid ratio is generally 10-30 mM (1-2.5%w/w). Increasing the surfactant/lipid ratio increases encapsulation efficiency but results in a highly viscous system.[41]

**5) Hydration temperature**

The surfactants and lipids are characterized by the gel-liquid phase transition temperature ($T_c$). The hydration temperature should be above the $T_c$ of the system. Span 60 has a high phase transition temperature and low HLB value and thus forms stable vesicles without the formation of micelles.[40]

## PHARMACEUTICAL APPLICATIONS OF NIOSOMES

**1) Niosomes containing non-steroidal anti-inflammatory drugs**

Niosomal delivery of a number of anti-inflammatory drugs are studied and they show better extended release profile with reduced dose when compared with the plain drug leading to reduced side effects.

Stable, multilamellar, non-ionic surfactant vesicles (multilamellar vesicles) are prepared using interfacial polymerization by providing a polymer coat of poly (phthaloyl-L-lysine) for each niosome and thereby providing a rigid but diffusible multiple double barrier which controlled the release of diclofenac sodium. This is proved by *in vivo* studies in inflammed rat model compared with placebo multilamellar vesicles (MLVs).[42]

Diclofenac sodium niosomes are prepared by thin film hydration method and with *in vitro* drug release profile best fitted to Peppas equation. Niosomal vesicles acted as depot for diclofenac sodium exhibiting controlled release.[43]

Diclofenac sodium niosomes are prepared by lipid hydration method using three-level three-factor Box-Behnken experimental design to optimize the formulation. The optimized formulation prepared according to computer-determined levels provided an entrapment efficiency and controlled release profile, which approximated to the predicted values. The study proved the efficient applicability of experimental design methodology for characterization and optimization of formulation parameters affecting entrapment efficiency and drug release from diclofenac sodium niosomes.[44]

Aceclofenac niosomes are developed and optimized to improve its bioavailability that showed an extended release of the drug over a period of 72 h in all formulations. The best formulation containing Span 20 fitted to Peppas model.[45]

Ketoprofen niosomes are formulated and evaluated using *in vitro* everted rat intestine and *in vivo* anti-inflammatory activity in rats. It concluded that polysorbate 40 formulation as the best when compared with other formulations.[46]

Indomethacin loaded niosomes are formulated and evaluated. The results showed that the therapeutic effectiveness increased with niosomal encapsulation compared with free indomethacin in paw edema bearing rats.[47]

**2) Topical and transdermal delivery**

Flurbiprofen proniosomal transdermal carrier systems are formulated and evaluated using rabbit skin and cellophane membrane. The results concluded that diffusion through rabbit skin is slower than that through cellophane membrane due to slow diffusion properties. The proniosomal formulations controlled the diffusion rates to be faster compared with drug dispersed in HPMC gel and in distilled water respectively.[48]

Ketoprofen niosomes prepared with Span 60 showed slow and sustained release of the drug.[49]

Tretinoin loaded vesicles prepared by film hydration, extrution technique and sonication are evaluated for the influence of vesicle structure on the photostability of tretinoin compared with drug in methanol.[50]

In another study the influence of vesicle composition and preparation method on the vesicle structure (multilamellar, large unilamellar and small unilamellar vesicles), size distribution, entrapment efficiency and *in vitro* release of incorporated tretinoin are studied.[51]

Niosomal transdermal delivery of nimesulide in a 1% carbopol base is found to localize the drug to the skin for a prolonged period of time and enhance its anti-inflammatory activity compared with plain drug gel and marketed formulation.[52]

Dithranol loaded liposomes and niosomes are formulated and stabilized. Enhanced permeation is observed with dithranol in vesicles compared to cream base by *in vitro* permeation study using mouse skin.[53]

Proniosome gels of estradiol are formulated using sorbitans and polysorbates and the results concluded that two of the sorbitans showed increased estradiol permeation with rat skin.[54]

Ketoconazole niosomes are prepared by thin film hydration using different ratios of Tween 40 and 80 in a FAPG base ( stearyl alcohol -20%, stearic acid – 5% and propylene glycol – 75%) and concluded that niosomal formulation offers advantage over plain drug formulation.[55]

Ketoconazole niosomes prepared by ether injection technique are evaluated for *in vitro* (cup-plate method) and *in vivo* antifungal activity in rabbits compared to free ketoconazole. The results indicated that the niosomes have the potential to reduce the therapeutic dose of ketoconazole by improving its performance.[56]

Baclofen niosomal formulations are found to have improved bioavailability and muscle relaxant activity than the conventional topical vehicle.[57]

Gallidermin, an anionic drug showed increased stability by protecting the drug from oxidation environments when entrapped in niosomes. The drug loaded niosomes when incorporated in topical gel showed high accumulation of drug in the skin with no systemic side-effects.[58]

Enoxacin niosomal formulations are formulated and evaluated for physicochemical properties, stability and percutaneous absorption compared to liposomes. The results concluded that enoxacin niosomes can be used to modulate the delivery of drug without significant toxicity.[59]

Clotrimazole niosomes showed sustained and controlled release of the drug for local vaginal therapy. Evaluation of the vesicle gel system for antifungal activity and tolerability on tissue level in rat showed sustained and controlled release of the drug.[60]

Fluconazole niosomal formulations accumulated and formed localized drug depots for sustained release with enhanced cutaneous retention of the drug.[61]

The detrimental effects on repeated exposure of the marketed alcoholic gel of naftifine hydrochloride are overcome by formulating it as non-alcoholic niosomal gel formulations.[62]

Formulation of neutral vesicles of lidocaine with Tween 20 and cholesterol are better than liposomes and Tween 20 micelles.[63]

Niosomes formed from Brij and Spans are reported as a possible approach to improve the low skin penetration and bioavailability characteristics shown by conventional topical vehicle for minoxidil. The enhanced percentage of dose accumulated in the skin compared with commercial and control suggested that, niosomal topical minoxidil as a suitable alternative for hair loss treatment.[64]

Niosomes of rofecoxib are prepared by lipid film hydration technique, incorporated in a gel base and compared with plain drug gel. The lower flux value of niosomal gel as compared to plain drug gel across pig skin assured the prolonged drug release behaviour with sustained action.[65]

Niosomes of ciclopirox olamine (CPO), a broad spectrum antifungal drug are prepared by ethanol injection method to improve the poor skin penetration and residence that account for the long treatment regimes in cutaneous mycosis.[66]

**3) Antiplatelet activity**

Indomethacin niosomes showed sustained antiplatelet effect due to greater quantity of drug reaching the specific site of inhibition in the interior of the platelets and direct action on the cyclo-oxygenase system to prevent thromboxane formation.[67]

**4) Ocular delivery**

Chitosan or carbopol coated timolol maleate niosomes showed an extended release of the drug compared with marketed *in situ* gel forming solution of timolol.[68] Prolonged effect on intraocular pressure is observed with acetazolamide niosomes formulated with Span 60 and cholesterol.[69]

Cyclopentolate niosomes prepared by sonication are found to promote absorption of cyclopentolate by preferentially modifying the permeability characteristics of the conjunctival and scleral membranes.[70] Promising controlled release of the drug is observed from niosomal formulations of gentamicin for topical ophthalmic drug delivery.[71]

**5) Respiratory drug delivery**

Development of optimized formulation of all-trans retinoic acid is carried out for delivering the drug as inhaled aerosol formulation. The results concluded that, this will be an alternative approach for respiratory delivery of drug by aerolization.[72]

**6) Anti-tumor activity**

Niosomal formulations of the anti-cancer drug daunorubicin hydrochloride prepared by modified reverse evaporation process are studied to increase its therapeutic efficacy in Dalton's ascetic lymphoma.[73]

Methotrexate niosomes subjected for pharmacokinetic evaluation in tumour bearing mice showed that unilamellar vesicles prepared with Span 60 has maximum entrapment and a marked difference in pharmacokinetics compared with the unentrapped drug.[74]

5-Fluorouracil niosomes are prepared with Spans using different methods and the pharmacokinetic calculations concluded that niosomes can act as promising carriers of 5-fluorouracil.[75]

Intratumoral modified-release chemotherapy with fluorouracil/epinephrine injectable gel are prepared and evaluated to provide a non-surgical treatment alternative in selected patients with superficial squamous cell carcinoma.[76]

Vincristine niosomes prepared by different techniques using different surfactants are evaluated and concluded that the transmembrane pH gradient drug uptake process as the most satisfactory method yielding stable niosomes with Span 40-cholesterol (1:1).[77]

Negatively charged paclitaxel (PCT) niosomes are prepared using different surfactants (Tween 20, 60, Span 20, 40, 60, Brij 76, 78, 72) with dicetyl phosphate by film hydration method. High surface charges showed that niosomes can be suspended in water well and this is beneficial for their storage and administration. PCT released from niosomes by a diffusion controlled mechanism. The slow release observed from these formulations might be beneficial for reducing the toxic side effects of PCT. Reproducible niosomal formulations are produced in terms of size distribution, zeta potential and percentage drug loading values. The efficiency of niosomes to protect PCT against gastrointestinal enzymes (trypsin, chymotrypsin, and pepsin) for oral delivery is shown by the increased gastrointestinal stability of PCT prepared with Span 40 niosomes.[78]

**7) CNS targeting**

Improved efficacy against cerebral tumours has been observed with Temozolamide loaded niosomes which on pharmacokinetic evaluation proved its *in vivo* brain targeting in rats.[79]

Encapsulation of vasoactive intestinal peptide (VIP) within glucose-bearing niosomes mainly allowed a significantly higher VIP brain uptake compared with control niosomes (up to 86%, 5min after treatment). Distribution of intact VIP in the brain after injection of glucose-bearing niosomes showed a preferential location of radioactivity in the posterior and the anterior parts of

the brain. A homogeneous distribution of the vesicles is observed in the whole brain after the administration of control vesicles. Thus, the novel vesicular formulation of VIP delivers intact VIP to particular brain regions in mice and the glucose-bearing vesicles might be therefore a novel tool to deliver drugs across the blood-brain barrier (BBB).[80]

N-palmitoyl glucosamine bearing doxorubicin niosomal formulations are developed to target the drug to the brain. Stable, nano-sized vesicles with improved brain delivery are produced with preliminary *in vivo* studies.[81]

The low blood levels of folates are the prime cause for causing depression in Alzheimer's disease. Folic acid is a water soluble vitamin showing difficulty in crossing the blood brain barrier and thus is formulated as niosomal nasal drug delivery system to target the brain. The release of drug from niosomes follows anomalous diffusion with first order release kinetics. About 48.15% of drug is to be absorbed through nasal cavity at the end of 6 h in *ex-vivo* perfusion studies using rat model.[82]

**8) Hormone delivery**

Luteinising releasing hormone (LRH) niosomes are formed by varying the composition of the vesicle membrane consisting of hexadecyl diglycerol ether (C16G2), cholesterol, and poly-24-oxyethylene cholesteryl ether (Solulan C24) to give polyhedral, spherical and tubular niosomes. Polyhedral niosomes released more radiolabeled LHRH ([$^{125}$I] LHRH) than spherical/tubular niosomes in both muscle homogenate and plasma. In clearance experiments in the rat, following intramuscular injection, both polyhedral and spherical/tubular niosomes gradually released [$^{125}$I] LHRH into the blood, but some radioactivity remained at the injection site for 25 and 49 h, respectively. In contrast, [$^{125}$I] LHRH in phosphate buffered saline is completely cleared from the injection site at 2 h. The release of drug is sustained by both niosome formulations, but spherical/tubular niosomes possess more stable membranes than polyhedral niosomes due to the presence of cholesterol.[83]

**9) Vaccine delivery**

An adjuvant for tetanus toxoid is prepared as an aqueous dispersion of niosomes emulsified in an external oil phase forming the vesicle-in-water-in-oil (V/W/O) system using cottonseed oil as the external oil phase. Initial studies of the system *in vivo*, showed enhanced immunological activity over the free antigen or vesicles.[84]

Non-ionic surfactant vesicular carrier, i.e. niosomes, is evaluated for topical delivery of vaccines using hepatitis B surface protein as an antigen and cholera toxin B as an adjuvant. The study suggests that topical immunization with cholera toxin B as potential adjuvant for cutaneous

immune responses when co-administered with the HBsAg encapsulated niosomes and thus can be effective as topical delivery of vaccines.[85]

**10) Diagnostic markers**

Iopromide, a radiocontrast agent, is prepared and characterized in different gel and liquid crystalline formulations of liposomes and niosomes. Niosomes showed greater physical stability compared with liposomes. *Ex vivo* leakage of iopromide from vesicles is observed in human plasma and *in vitro* release fitted Higuchi model.[86]

Polymeric vesicles with encapsulated carboxyfluorescein are developed and evaluated using palmitoyl glycol chitosan and cholesterol (2:1) by weight, and then encapsulating within egg phosphatidylcholine, cholesterol (2:1) by weight, to yield liposomes or hexadecyl diglycerol ether to yield niosomes in vesicle system.[87]

**11) Anti-fertility activity**

An inclusion complex of plumbagin with hydroxypropyl beta-cyclodextrin entrapped in an aqueous layer of niosomes is prepared and evaluated. It concluded that an increase in stability and anti-fertility activity of the complex is observed, compared with control and niosomes with lipid layer entrapment.[88]

The anti-fertility activity of centchroman in niosomes is increased showing 83.3% protection against pregnancy with histopathological studies showing no side effects and toxic effects.[89]

**12) Anti-leishmanial therapy**

Liver and serum concentrations of antimony in mouse are determined after administration of sodium stibogluconate in the free, liposomal and niosomal form. High liver and low serum values are attained by the use of both vesicular formulations. Niosomal sodium stibogluconate is shown to be more active than free drug against experimental murine visceral leishmaniasis, an effect apparently dependent on maintaining high drug levels in the infected reticuloendothelial system.[90]

**13) As drug carriers**

**i) Anti-viral drugs**

Distribution of formulated zidovudine niosomes in lungs, kidney, heart, liver and spleen of mice are studied after i.v bolus injection. The formulation with Tween 80 is optimized with increased half-life, mean residence time and reduced leakage of drug at $4^0$C.[91]

Tenofovir niosomes are formulated using different compositions and evaluated. The results concluded that microfluidization can be used for further scale-up of the niosomes with very small mean vesicular sizes.[92]

Ribavirin niosomes enhanced up to 6 folds of liver targeting property, thereby proving to be effective at low doses compared with high doses of plain drug which have toxic side-effects.[93]

**ii) Anti-fungal drugs**

The oral bioavailability of griseofulvin is increased by sustained release of drug from niosomal formulation containing Span 60.[25]

Nystatin niosomes demonstrated less nephrotoxicity and hepatotoxicity with higher level of drug in vital organs and pronounced efficacy in elimination of fungal burden in experimental animals compared with those treated with free nystatin.[94]

**iii) Anti-Tubercular drugs**

Niosomal formulations of rifampicin are developed with various Spans and cholesterol for sustained release of the drug. Formulation with Span 20 is found to have maximum drug release and that of Span 85 with minimum drug release.[95]

The cellular uptake by macrophage cells of isoniazid loaded niosomes is 61.80% which is sufficient to achieve effective treatment of tuberculosis. The formulations showed reduced dose, reduced toxicity, reduced dosing frequency and increased patient compliance.[96]

Isoniazid niosomal formulations showed sustained *in vitro* release of drug and lesser toxicity than the free drug *in vivo.*[97]

Niosomal encapsulated pyrazinamide showed maximum concentration of drug in the lungs, with reduced side effects, toxicity and drug resistance.[98]

The release pattern of anti-tubercular drugs like rifampicin and isoniazid showed fickian/diffusional release whereas pyrazinamide had non-fickian release mechanism from Triton-X 100 niosomes.[99]

**iv) Antibiotics**

Cefuroxime axetil bioavailability and stability in bile salts are improved by formulating it as niosomes.[100] Cefpodoxime proxetil niosomes showed controlled release of the drug with zero-order release kinetics, thus reduces the chances of dose dumping during usage.[101]

**v) Anti-Diabetic**

Gliclazide oral bioavailability is improved by formulation as niosomes with sustained release of the drug. The stability of the niosomes is attributed to the electrostatic repulsive forces which is indicated by high zeta potential.[102] Niosomal formulations of metformin are developed with the aim of sustaining the release of metformin so as to decrease its side effects and also reduce its dosing frequency.[103]

**vi) Haemoglobin**

Niosomal encapsulated haemoglobin is permeable to oxygen and hence can act as a carrier for haemoglobin in anaemic patients.[104]

**14) Gene therapy**

In this study neutral and negatively charged niosomes are used for DNA (PUC18 supercoiled plasmid) complexation. Different proportions of Span/Tween/Cholesterol with or without dicetylphosphate are utilized for niosome preparation by the film hydration method. The complexation percent of DNA-niosomes is as high as 80% in some formulations and the DNA is stable during complexation and extraction processes. The results concluded that negatively-charged niosomal systems can be used as a gene-delivery vector in the presence of $Ca^{2+}$.[105]

Polysorbate cationic niosomes (PCNs) comprising of nonionic surfactants (i.e., polysorbates) and cationic cholesterol are synthesized using film hydration method and developed as gene carriers. The binding capacity of PCNs toward oligodeoxynucleotides (ODN) is assessed by a gel retardation approach, which demonstrated that the ionic complexes are formed when 6 charge ratios reached to 4 or greater. Gene transfer study showed that the PCNs exhibited a high efficiency in mediating cellular uptake and transferred DNA expression. Based on these findings, PCNs may offer the potential to function as an effective gene delivery system.[106]

Disturbance in the synthesis of tyrosinase might be one of the major causes of vitiligo. The enhancement of tyrosinase gene expression and melanin production by loading the plasmid in elastic cationic niosomes are investigated in tyrosinase gene knocked out human melanoma (M5) cells and in tyrosine-producing mouse melanoma ($B_{16}F_{10}$) cells. The study demonstrated not only the enhancement of the expression of human tyrosinase gene by loading in elastic cationic niosomes, but also the potential application of this gene delivery system for the further development of vitiligo gene therapy.[107]

## CONCLUSION

Niosomal delivery has been widely explored for delivery of various drugs to enhance the bioavailability, extended release of the drug, increase the permeation of drugs through skin, to localize drug delivery for topical delivery, as targeted drug delivery to various organs and tumors and in gene therapy. Considerable research on this novel vesicular drug delivery proves it to be an efficient drug delivery and further explore it as a tool to deliver a wide range of drugs.

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