REVIEW ARTICLE

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Niosomes as Drug Carriers

ALOK NAMDEO and N.K. JAIN*
Dept. of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M.P.) 470 003

The concept of carriers to deliver drugs to target organs and modify drug disposition has been widely discussed[1]. The majority of such reports have concerned the use of phospholipid vesicles or liposomes[2], which exhibit certain disadvantages, such as chemical instability, high cost and variable purity of lipids used, which militates against their adoption as drug delivery vehicle. Alternatives to phospholipids are thus of interest from the technical viewpoint and could also allow a wider study of the influence of chemical composition on the biological fate of vesicles[3].

MANY synthetic amphiphiles form vesicles[4], but as most of them are ionic and relatively toxic, they are generally unsuitable for use as drug carriers. Handjani-vila **et al**[5] first reported formation of vesicles on hydration of mixture of cholesterol and a single alkyl-chain non-ionic and non-toxic surfactant. Since then a number of non-ionic surfactants have been used to prepare vesicles viz. polyglycerol alkyl ethers[3,5,6], glucosyl dialkyl ethers[7], crown ethers[8], ester-linked surfactants[9], polyoxyethlene alkyl ethers[10,11], Brij[12,13] and a series of Spans and Tweens[12,13,14,15]. Resultant vesicles have been termed **Niosomes**.

Thus niosomes are 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 and exhibit a behaviour similar to liposomes **in-vivo**.

NIOSOMES : SALIENT FEATURES

(1) Niosomes entrap solute in manner analogous to liposomes.

(2) Niosomes are osmotically active and stable as well as they increase the stability of entrapped drug.

(3) Handling and storage of surfactants require no special conditions.

(4) Niosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.

(5) Niosomes exhibit flexibility in their structural characteristics (composition, fluidity, size) and can be designed according to the desired situation.

(6) Niosomes improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.

(7) They can be made to reach the site of action by oral, parenteral as well as topical routes.

(8) They allow their surface for attachment of hydrophilic group and can incorporate hydrophilic moieties in bilayer to bring about changes in the **in vivo** behaviour of niosomes.

*For Correspondence

(9) Niosomal dispersion in aqueous phase can be emulsified in non-aqueous phase to regulate delivery rate of drug and administer normal vesicles in external non-aqueous phase.

(10) Niosomal surfactants are biodegradable, biocompatible and non-immunogenic.

(11) Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

IN VIVO BEHAVIOUR OF NIOSOMES

In vivo niosomes have been found equiactive to liposomes in improving the therapeutic performance of drug[16] and their distribution in body follows the pattern of other colloidal drug delivery systems. Although tissues of extravasation; liver, lung, spleen and bone marrow are responsible for disposition of a major part of niosomes yet their level in liver is always significantly higher due to the natural vectoring process[6,16,17]. Variation in size also influences the pattern of niosome disposal from blood; large size niosomes may reside in lung due to alveolar retention and effect of alveolar phagocytic cells, while small sized vesicles, which can pass through fenestrations in liver sinusoidal epithelium, have better access to spleen[18,19].

It appears that, like liposomes, niosomes are also taken up intact by liver, and break down substantially to release the free drug which eventually re-enters the circulation and maintains the plasma drug level[17]. The effect of two doses of niosomal sodium stibogluconate given on successive days was additive, indicating that liver might act as depot of drugs[6].

Parthasarthi **et al**[13] found niosomes to be stable in plasma. However, non-ionic surfactants in higher concentration delipidize the low density lipoproteins[20]. Moser **et al**[21,22] found niosomes bearing hemoglobin to be physically stable with plasma protein component, Albumin and transferrin were identified and determined to absorb on vesicles without destabilizing them. Erythrocytes donate cholesterol to niosomes, particularly to cholesterol-free and cholesterol-poor niosomes, maintaining their integrity in body as well as keeping them less vulnerable to destabilisation[23].

CHARACTERIZATION OF NIOSOMES

i) Vesicle diameter

Niosomes, similar to liposomes assume spherical shape, its diameter can be determined using light microscope[13], photon correlation microscopy[17] and freeze-fracture electron microscopy[3].

ii) Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis[17], centrifugation[19] or gel chromatography[13]. The drug remaining entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100[3] and entrapment efficiency (EE) is expressed as (amount entrapped/Total amount added) X 100.

iii) In vitro release rate

Release of drug can be monitored by dialyzing niosomal suspension against buffer at definite temperature and determining the drug content of dialyzate[3].

FACTORS AFFECTING VESICLE SIZE, ENTRAPMENT EFFICIENCY AND RELEASE CHARACTERISTICS

i) Drug :

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayer thereby

increasing vesicle size[24]. In polyoxyethylene (PEG) coated vesicles, some drug is entrapped in long PEG chain thus reducing the tendency to increase the size[25].

Degree of entrapment is affected by the hydrophilic-lipophilic balance of a drug. For a series of Spans and Tweens, Raja Naresh **et al**[12] reported maximum entrapment of water-soluble drug, Diclofenac sodium in hydrophilic surfactant, Tween 60, and Chandraprakash **et al**[15] reported maximum entrapment of slightly water-soluble drug, Methotrexate in lipophilic surfactant, Span 60.

ii) Amount and type of surfactant :

The mean size of niosomes increases regularly with increase in the hydrophilic-lipophilic balance (HLB) from Span 85 (HLB 1.8) to Span 20 (HLB-8.6) because surface free energy decreases on increasing hydrophobicity of surfactant[26,27].

Yoshioka **et al**[27] reported linear correlation between concentration of lipid and entrapment efficiency. Phase transition temperature (Tc) of surfactant also effects entrapment efficiency i.e. Span 60 having higher Tc provides the highest entrapment[28].

iii) Cholesterol content and charge :

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency[27]. Presence of charge tends to increase the inter-lamellar distance between successive bilayers in multi-lamellar vesicle structure and leads to greater overall entrapped volume, vesicle size is slightly decreased as charge might increase the membrane curvature[28].

Presence of cholesterol in bilayer composition due to its membrane stabilizing activity[29], reduces permeability and improves retention of solute. Baillie **et al**[3] reported that incorporation of 50% cholesterol in surfactant composition reduces vesicle permeability to 5(6)- carboxyfluorescein (CF) by a factor of 10. Cholesterol has decreasing effect on gel-liquid transition temperature, at which rapid efflux of vesicle content occurs, it converts one well defined gel-liquid transition temperature of a pure surfactant to gel-liquid transition range[11].

iv) Method of preparation :

Methods of preparation of niosomes such as Hand Shaking, Ether Injection and Sonication, developed on the basis of liposome production technique have been reviewed by Khandare **et al**[30]. Hand shaking method form vesicles with greater diameter (0.35-13 μm)[15,18] compared to those prepared by Ether injection method (50-1000 nm)[30]. Sonication of MLV prepared by above methods, either with prove sonicator or bath type sonicator forms unilamellar vesicles with considerably reduced diameter. Increase in sonication time results in concomitant reduction in vesicle diameter. Hydrating the lipid above phase transition temperature of surfactant and vortexing during hydration help to reduce the size of vesicles prepared by Hand shaking method.

Stafford **et al**[24] extruded the niosomal dispersion prepared by hand shaking method through 0.1 μm Nuclepore polycarbonate membranes in series. After 8 such extrusions average diameter of vesicles was 135-340 μm. Small sized niosomes can be produced by Reverse Phase Evaporation [REV] method. In this, emulsion of aqueous phase in organic solvent containing lipid is prepared by sonication, followed by evaporation of organic solvent, resulting in formation of vesicles[13,31]. Microfluidization method[32] which gives greater uniformity and smaller size vesicles, is based on submerged jet principle wherein two fluidized streams interact at ultra high velocities (upto 1700 ft/sec) in precisely defined microchannels within the interaction chamber to form niosomes. Parthasarthy **et al**[13] prepared niosomes by Transmembrane pH gradient (inside acidic) drug uptake process (TmpH) or remote loading, in which multilamellar niosomes were prepared at acidic pH by

hand shaking method then subjected to freeze-thaw cycle and later sonicated. Remote loading of drug was done by adding aqueous solution of drug, pH was adjusted to 7.0-7.2 and then mixture heated. Niosomes so formed showed greater entrapment efficiency and better retention of drug.

Niosomes bearing 5(6)-carboxyfluorescein prepared by Ether injection method show entrapment efficiency significantly higher than those prepared by Hand shaking method or Sonication[3]. Compared to Hand shaking and Reverse phase evaporation methods, niosomes prepared by Remote loading method show greater entrapment efficiency and slower release of drug[13].

v) Osmotic effect :

Addition of hypertonic salt solution to suspension of niosomes brings about reduction in vesicle diameter with concomitant water efflux, which may be due to pumping out of vesicle content[3] whereas in hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicle, followed by faster release, which may be due to mechanical loosening of vesicles under osmotic stress[33].

MODIFIED NON-IONIC SURFACTANT VESICLES (NIOSOMES)

i) Discomes

These are disc shaped structures formed on mixing cholesteryl polyoxyethylene (Solulans) and vesicular dispersion. Unchegbu **et al**[34] observed them under the light microscope, existing under certain conditions of the phase diagram of non-ionic surfactant vesicles (NSVs) prepared from a hexadecyl diglycerol ether, cholesterol and dicetyl phosphate (DCP) (69:29:2) by Hand shaking and Sonication followed by incubation with various proportions of Solulan C24 at 74°C. Under these conditions, four different phases were distinct: a lamellar phase, a micellar phase, an uncharacterized coexistence phase and a novel phase called discome phase.

Dispersion in the Discome phase consist of large "Discomes" (30- 60 μm mean volume diameter). Lipids in the proportion (i.e. surfactant, cholesterol, Solulan C24, DCP, 49:19.5:29.5:2) produce discomes on sonication.

Discomes entrap water-soluble solute. Entrapment of 5(6)- carboxyfluorescein showed aqueous entrapment value of 3.603 ± 2.916% and release of 50% solute after 24 hour at room temperature. Large volume carrying capacity and minimal capacity allow them to be used in ophthalmology.

ii) Polymerized non-ionic surfactant vesicles :

Since vesicle systems are more or less thermodynamically unstable, proximity and regular orientation of surface active molecules at interface has been exploited to increase stability by controlled polymerization of vesicle forming non-ionic surfactant bearing a polymerisable residue[35,36]. Polymerisable surfactants used were:

(1) Dimethyl n-hexadecyl [{1- isocyanoethyl}carbonyloxy methyl] ammonium bromide.

(2) N,N (dihexadecanoyloxyethyl) maleyl amide.

(3) Dihexadecyl N,methyl N,maleyl ammonium bromide.

The vesicles prepared from these surfactants were polymerized by radiation or radical initiation. Polymerization restricts mobility of hydrocarbon core and hence improves the stability of niosomes. Size of the vesicles on polymerization remains unchanged while change in appearance depends upon location of polymerisable group.

Thus they combine advantages of polymers and membranes. They have stability and intriguing structural properties like polymers while retaining bene-

ficial fluidities and organizational abilities or membranes. In terms of drug delivery they might serve as unique polydisperse, time release carriers.

iii) Emulsified niosomal dispersion :

Yoshioka and Florence[37] formulated a range of vesicle-in-water-in- oil (V/W/O) emulsion from niosomes made from Spans (20,40,60,80) in the size range 600 nm - 3.4 μm, dispersed in water droplets of around 5-25 μm, themselves dispersed in an oil (octane, hexadecane, isopropyl myristate). This system showed release of CF slower than vesicle suspension and W/O emulsion. The release was affected by the HLB of surfactant, nature of oil and temperature of dialysis media. Thus by their appropriate choice, delivery rate of drug could be regulated.

This system allowed administration or application of vesicle in an external non-aqueous phase while maintaining normal vesicular structure in aqueous phase and can be of potential use in drug delivery or as vaccine vehicle. Albert **et al**[38] have patented a similar system for cosmetic application.

TOXICITY AND STABILITY

Non-ionic surfactants used in niosomes are non-toxic and no toxic effects have been reported so far in animal studies due to the use of niosomes as drug carrier.

Rogerson **et al**[19], in their experiment on 70 male NMRI mice, didn't report any fatalities that could be attributed to the preparation. The toxic side effects directly related to drug are also reduced.

Niosomes are stable structures, Jain[33] didn't observe any gross morphological changes on storage for three months. Yoshioka and Florence[37] found them stable even in emulsified form. Baillie **et al**[3] determined the stability in buffer and suggested that a substantial amount of entrapped solute would be retained under long term storage conditions.

NIOSOMALLY ENTRAPPED BIOACTIVE AGENTS

Various bioactive agents entrapped in niosomes are Sodium stibogluconate[6,9,18], Methotrexate[14,15,17], Vincristine[13], Doxorubicin[19], Diclofenac Sodium[12], Bovine Serum Albumin[39], 9-Desglycinamide 8- Arginine Vasopressin[28], Insulin[40], Estradiol[41], Antipyrine[42], Rifampicin[33], Hemoglobin[21,22].

CONCLUSION

Niosomes are more stable than liposomes and immense ability to alter pharmacokinetic profile of antileishmanial and anticancer drugs, NSAIDs antigens and peptides has made it to be an efficient delivery vehicle. Modifications of niosomes as discomes, sterically stabilized niosomes, polymerized vesicles and emulsified niosomal dispersion, although is a new field yet has great potential to emerge as controlled and sustained drug delivery vehicle with suitable properties of a vehicle. Thus to explore full utilization of this potential and promising drug delivery vehicle vigorous research inputs would be desirable.

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