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Review Article

NIOSOMES – CHALLENGE IN PREPARATION FOR PHARMACEUTICAL SCIENTIST

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ABSTRACT

Target oriented drug delivery systems are the areas of the major interest in the modern pharmaceutical research. The selective drug delivery to the target tissues increases the therapeutic efficacy of the drug and reduces its undesirable effect to non target tissues.The concept of drug targeting or site specific drug delivery was introduced first time by Paul Elrich in 1909, when he reported 'magic bullet' to deliver a drug to the desired site of action without affecting the non target organs or tissues (Juliano, 1980) by associating the drug with a pharmacologically "inactive carrier" capable of conveying the drug selectively towards its target cells. Drug targeting is defined as the ability to direct a therapeutic agent specifically to the desired site of action with little or no interaction with non target tissues (Bremier, 1987*)*

The main goal of a site specific drug delivery system is not only to increase the selectivity and drug therapeutic index, but also to reduce the toxicity of the drug. (Widder *et al.*, 1982).

Keyword: Magic bullet, Niosomes, Inactive carrier, Drug therapeutic index.

INTRODUCTION

Target oriented drug delivery systems are the areas of the major interest in the modern pharmaceutical research. The selective drug delivery to the target tissues increases the therapeutic efficacy of the drug and reduces its undesirable effect to non target tissues. The concept of drug targeting or site specific drug delivery was introduced first time by Paul Elrich in 1909, when he reported 'magic bullet' to deliver a drug to the desired site of action without affecting the non target organs or tissues (Juliano, 1980) by associating the drug with a pharmacologically "inactive carrier" capable of conveying the drug selectively towards its target cells. Drug targeting is defined as the ability to direct a therapeutic agent specifically to the desired site of action with little or no interaction with non target tissues (Bremier, 1987*)*

The main goal of a site specific drug delivery system is not only to increase the selectivity and drug therapeutic index, but also to reduce the toxicity of the drug. (Widder *et al.*, 1982)

Vanlerbeghe *et al.* (1972) first reported the niosomes as a feature of cosmetic industry. In 1979, Handjanivila *et al.* reported that the hydration of a mixture of cholesterol and single alkyl chain, resulted in formation of non ionic surfactant vesicular systems (i.e. Niosomes).

These non ionic surfactant vesicles can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in the vesicular membrane made of lipid materials, which can be used to prolong the circulation of the entrapped drugs. Due to the presence of non ionic surfactant and the lipid, there is a better targeting of drug(s) to tumor, liver and brain. Thus, they are useful in targeting of the drug for treating cancers, parasitic, viral and other microbial diseases more effectively.

Merits of Novel Drug Delivery System

1. Reduction in the total amount of drug administered over the period of drug treatment. This contributes to the reduced incidence of systemic and local side effects.
2. Devoid of first pass metabolism and gastrointestinal tract degradation.
3. Improved patient compliance resulting from the reduction in the frequency of doses required to maintain the desired therapeutic response.
4. Targeting of the drug molecule towards the tissue (or) organ reduces the toxicity to the normal tissues.
5. Pulsatile and pH dependent systems release the drug whenever the body demands.
6. Biocompatibility.

Limitations of Novel Drug Delivery System

1. The drugs having biological half-life of 1 hr. or less are difficult to be formulated as sustained release formulations. The high rate of elimination of such drugs from the body needs an extremely large maintenance dose which provides 8-12 hrs of continuous therapy.
2. These products normally contain a large amount of drug. There is a possibility of unsafe over dosage, if the product is improperly made and the total drug contained therein is released at one time or over too short time interval.
3. If it is once administered, it may be difficult to stop the therapy for reasons of toxicity or any other reasons.
4. It may be unwise to include potent drugs in such systems.

Rationale for Site Specific Drug Delivery (Tomilinson, 1991)

1. To reach previously inaccessible domains e.g. intracellular site, bacteria, viruses parasites etc.
2. Exclusive drug delivery to the specific cells or diseased site in the body.
3. Reduction in the drug dose and side effects.
4. To control the rate and frequency of drug delivery at the pharmacological receptor.
5. To protect the drug and the body from one another until it reaches at the desired site of action.

Factors Affecting Vesicles Size, Entrapment Efficiency and Release Characteristics

a) Drug

The entrapment of the drug in niosomes results in an increase in 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 (Stafford *et al.*, 1988). In polyoxyethylene (PEG) coated vesicles, some drug are entrapped in long PEG chains thus reducing the tendency to increase the size (Chauhan, and Lawrence, 1989).

The degree of entrapment is affected by the hydrophilic-lipophilic balance of a drug. For a series of Spans and Tweens, Naresh *et al.*, 1994 reported the maximum entrapment of water-soluble drug (Diclofenac sodium) in hydrophilic surfactant, Tween 60. Chandraprakash *et al.*, 1993 reported maximum entrapment of

slightly water soluble drug - Methotrexate in lipophilic surfactant Span 60.

b) Amount and type of surfactant

The mean size of niosomes increases with increase in the hydrophilic-lipophilic balance (HLB) from Span 85 (HLB 1.8) to Span 20 (HLB 8.6) because of decrease in surface free energy and hence increasing hydrophobicity of surfactant (Yoshioka *et al*., 1992 and Yoshioka *et al*., 1994).

Yoshioka *et al*., 1992 reported linear correlation between concentration of lipid and entrapment efficiency. The phase transition temperature (Tc) of surfactant also effects entrapment efficiency i.e. Span 60 (having higher Tc) provides the highest entrapment (Yoshida *et al*., 1992).

c) Cholesterol content and charge

The inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency (Yoshioka *et al*., 1994). The presence of charge tends to increase the inter-lamellar distance between successive bilayers in multilamellar vesicles structure and leads to greater overall entrapped volume. The vesicle size is slightly decreased as the charge might increase the membrane curvature (Yoshida *et al*., 1992).

The presence of cholesterol in bilayer composition due to its membrane stabilizing activity, (Demel and Dekruyff, 1976) reduces permeability and improves retention of solute. Baillie *et al*. ,1985 reported that incorporation of 50% cholesterol in surfactant composition reduces vesicle permeability of 5(6) – carboxyfluorescein (CF) by a factor of 10. Cholesterol has a decreasing effect on gel-liquid transition temperature, at which rapid efflux of vesicle content occurs. It converts a well defined gel-liquid transition temperature of a pure surfactant to gel-liquid transition range (Hofland *et al*., 1992).

d) Osmotic effect

Addition of hypertonic salt solution to the suspension of niosomes brings about reduction in vesicle diameter with concomitant water efflux, probably due to pumping out of vesicle content (Baillie *et al*., 1985). In hypotonic salt solution, there is a initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicle, followed by faster release, due to mechanical loosening of vesicles under osmotic stress.

Stability of Niosomes

The factors affecting the stability of niosomes can be classified into the three categories-

- Physical stability
- Chemical stability
- Stability in biological fluids.

Physical stability

The niosomes can change their physical characteristics in several ways.

a. The particle size may change because of aggregate formation and fusion.
a. Occurrence of phase separation of bilayer components, upon storage.
b. Leakage of encapsulated material from niosomes.

The changes in particle upon storage of phosphatidyl choline containing niosomes over pharmaceutically relevant time intervals can be minimized by the selection of proper charge inducing agents. Mostly, negatively charged phospholipids (phosphatidyl glycerol) are used to stabilize the niosomes.

The phase separation may occur when the bilayer composition changes due to chemical degradation reactions or when the bilayer goes through temperature cycles (Lichtenberg and Bahrenholz, 1988). Sometimes, phase separation occurs *in vivo*, when bilayer components are selectively drawn from the bilayer plasma components (Scherphof, 1984). If this effect is undesired, the components that form more rigid bilayers are preferred. In other cases, one might wish to deliberately destabilize the niosomes *in vivo* so that a rapid release of the encapsulated drug is induced. An example of plasma destabilized niosomes is niosomes composed of phosphatidyl ethanolamine and oleic acid (New, 1990).

The permeability of bilayers is highly dependent on the physico-chemical properties of the bilayer, drug and the temperature (New, 1990). Three categories of drugs can be discerned (Berenholz and Crommelin, 1994).

- Highly hydrophilic, non-bilayer interacting drugs.
- Drugs with some lipophilicity.
- Strongly lipophilic drugs.

In category first, the presence of cholesterol in the bilayer of the egg phosphatidyl choline niosomes dramatically reduces the permeability (Crommelin and Van Bommel, 1984). For gel state bilayers, permeability is low with or without cholesterol. It is clear that if *in vivo* performance allows 'gel state' bilayers to be used, the shelf life of the niosomes in aqueous media with the proper pH might easily meet industrial demands. In the second category, the drug tends to be difficult to keep entrapped over periods of months as long as outside sink conditions prevail. In the third category, strongly lipophilic drugs have high affinity for the bilayer and therefore these drugs stay there over a long period of time, independently of the state of the bilayer.

As the final remark, the presence of hydrolysis or oxidation reduction products can affect bilayer properties. Although, lysophosphatidyl choline is known to be a lipid bilayer solubilizer, the solubilizing effect of lysophosphatidyl choline in degrading niosomes is neutralized by the simultaneous appearance of fatty acids in the bilayer (Grit and Crommelin, 1992).

Niosomes stored in freeze dried form: The niosomes stored in freeze dried form is preferred for proper *in vivo* performance of niosomes with long term stability. To maintain the particle size distribution after freeze drying-rehydration cycle, a cryoprotectant needs to be added. Different types of cryoprotectants and their possible mechanisms of action have been discussed by Crowe *et al*., 1987 and Ozer *et al*., 1988 for niosome stabilization. Usually, sugars are used as cryoprotectant, although other type of excipients also have been reported to exert cryoprotective effects (Crommelin and Van Bommel, 1984, Van Bommel, *et al*., 1984) A number of effects contribute to the cryoprotective action.

1) The formation of amorphous glass structures during the freeze drying process may avoid mechanical damage inflected by ice crystals. It is recommended to store these cakes below the glass transition temperature.

2) The sugars may interact with the polar head groups of the phospholipids and stabilize the membranes when the bilayer stabilizing water is removed by sublimation.

The proliposome concept: In proliposome formulations, liposomes are formed by hydrating lipids at the bed side. The dry lipids (coated as a film on the glass vessel wall in the form of a freeze dried cake) are hydrated by shaking with an aqueous medium just before injection (Dingle, 1978, Van Hoogevest and Fankhhauser, 1989, Payne, 1986, Payne *et al*., 1986).

Another way to improve stability of lipids is the covalently cross linking of the membrane by glutaraldehyde fixation or polymerization method (Leaver *et al*., 1983). The membrane stability is increased by imparting charge to the niosomes (Park *et al*., 1992).

Chemical stability

The stability of niosomes depends on the chemical stability of the lipid components and the bilayer components of niosomes, designed for carrying a drug or phospholipids. Usually, hydrolysis and peroxidation are the two degradation process which occurs with phospholipids (Welder *et al*., 1989). The analytical technique to monitor hydrolysis and oxidation reactions are reviewed (Grit *et al*., 1993, Kemps and Crommelin, 1988) as under:

Lipid hydrolysis: Grit *et al*., in 1989 and 1993 have described different variables that influence the hydrolysis reactions of phosphatidyl choline, the major phospholipids, in the most niosomal preparations and the charge inducing phospholipids phosphatidyl glycerol. Apart from pH, other experimental conditions like temperature, ionic strength, buffer species, and ultra sonication were reported to influence hydrolysis reactions. Many investigators choose the formation of lysophosphatidyl choline as a standard measure for the chemical stability to phospholipids. Since, the presence of lysophosphatidyl choline in lipid bilayer greatly enhances the permeability of niosomes, the most important method for minimizing this problem is the proper sourcing of the phospholipid to be used. They should be essentially free from any lyso-phosphatidyl choline to start with and free of any lipases.

Lipid peroxidation: Most of the phospholipid niosomal dispersions contain unsaturated acyl chains as a part of their molecular structures. These chains are vulnerable to oxidative degradation (lipid peroxidation) (Konings, 1984).

The peroxidation can occur during preparation, storage or actual use. Peroxidation of phospholipids produces the formation of cyclic peroxides and hydro peroxides. Peroxidation of the phospholipids may be minimized by a number of ways such as:

- Minimum use of unsaturated phospholipids.
- Use of nitrogen or argon to minimize exposure to oxygen.
- Use of light resistant container
- Removal of heavy metals (EDTA)
- Use of antioxidants such as □-tocopheorl or BHT.

It was reported that niosome of different lipid composition could be steam sterilized without substantial hydrolytic or oxidative degradation (Zuidam *et al*., 1993).

Stability in biological fluids

The inability of niosomes to retain entrapped substances when incubated in blood or plasma has been known for a decade. The instability of niosomes in plasma appears to be the result of transfer of bilayer lipids to albumin and high density lipoproteins (Theraesa *et al*., 1989). Both lecithin and cholesterol also exchanges with the membrane of red blood corpuscle. Niosomes are most susceptible to high density lipoprotein attack at their gel to liquid crystalline phase transition temperature. The susceptibility of niosomal phospholipids to lipoprotein and phospholipase attack is strongly dependent on niosome size and type. Generally, multilamellar vesicles are most stable whereas small lamellar vesicles are least stable.

The bile salts also destabilize the bilayer membrane structure, thereby, leading to release of the entrapped material.

In Vivo Behaviour of Niosomes

In vivo niosomes have been found equiactive to liposomes in improving the therapeutic performance of the drug (Hunter *et al*., 1988) and their distribution in body follows the pattern of their 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 higher due to the natural vectoring process (Hunter *et al*., 1988, Azmin *et al*., 1985). Variation in size also influences the pattern of niosomal disposal from the blood. The large size niosomes may reside in lung due to alveolar and effect of alveolar phagocytic cells, whereas, the small sized vesicles, can pass through fenestrations in liver sinusoidal epithelium and thus, have better access to spleen (Carter *et al*., 1989, Rogerson *et al*., 1988)

It appears that, like liposomes, niosomes are also taken up intact by the liver and substantial of the niosomes results in the release of the free drug which eventually re-enters the circulation and maintains the plasma drug level (Azmin *et al*., 1985). The effect of two doses of niosomal sodium stibogluconate given on successive days was additive, indicating that liver might act as a depot of drugs.

Niosomes Interactions with the Cells

Niosomes can interact with cells in many ways to cause niosome components associated with those cells (Gregoriadis, 1985, Roerdink *et al*., 1987). Five mechanisms are known for the niosome cell interactions as discussed below.

Intermembrane transfer: Intermembrane transfer to lipid components can take place upon close approach of two phospholipid bilayers without disruption of the niosomes of prejudicial of the membrane integrity. Interactions can take place between niosomes and lipoproteins and in certain circumstances, proceed to the extent of destroying the niosomes altogether.

Contact release: Contact release of aqueous contents of niosomes occurs where contact with the cells causes an increase in permeability of the niosome membrane. This leads to release of water soluble solutes in high concentration in the close vicinity of the cell membrane through which these solutes may pass under certain circumstances. This phenomenon can provide very effective means for introducing materials into specific cells without ingestion of the whole niosome.

Adsorption: Adsorption of niosomes to the cells surface occurs with little or no internalization of either aqueous or lipid components. It may take place either as a result of physical attractive forces or as a result of binding by specific receptors to ligands on the vesicle membrane.

Fusion: Close approach of niosomes and cell membrane can lead to fusion of the two resulting in complete mixing of niosomal contents into the cytoplasm.

Endocytosis: The niosome is engulfed by the cell. The lysozyme present in the cytoplasm degrades or digests the membrane structure of niosomes thereby releasing the entrapped material into the medium.

Characterization of Niosomes

a) Vesicle diameter

Niosomes, similar to liposomes assume spherical shapes. Its diameter can be determined using light microscope (Parthasarathi et al., 1994), photon correlation microscopy (Azmin et al., 1985) and freeze-fracture electron microscopy (Baillie et al., 1985).

b) Entrapment efficiency

After preparing niosomal dispersion, the unentrapped drug is separated by dialysis (Azmin *et al*., 1985), centrifugation or gel chromatography (Baillie *et al*., 1985). The drug remaining entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 (Naresh *et al*., 1993) and entrapment efficiency (EE) is expressed in percent (amount entrapped/Total amount added) x 100.

The intercalation of cholesterol in the bilayers decreases the entrapment volume and thus entrapment efficiency. As the concentration of cholesterol increases, entrapment efficiency decreases (Yoshida *et al*., 1992).

c) Osmotic shrinkage

The osmotic shrinkage of vesicles can be determined by monitoring reduction in vesicle diameter, initiated by addition of hypertonic salt solution to niosomal suspension.

The niosomes prepared from pure surfactant are osmotically more sensitive in contrast to the vesicles containing cholesterol, confirming the membrane stabilizing activity of the lipid.

Niosomally Entrapped Bioactive Agents

A variety of drugs/active agents have been encapsulated in Niosomes. Table 2.1 summaries the various agents encapsulated in niosomes and the corresponding results:

Table 1: Agents encapsulated in Niosomes and Results Obtained

Drug	Result	References
Antipyrine	Niosomal drug extended the half life of the drug and small vesicles significantly altered the metabolic profiles of the drug	Al- Angari 1992
Bovine Serum Albumin	Niosomes elicit a stronger immune response and improved immunological selectivity. Show lower toxicity and more stability than conventional adjuvant.	Brewer and Alexander, 1992
DGAVP	Facilitated oral delivery of the peptide drug. Improved the stability of the peptide to gastric enzymes	Yoshida, *et al*., 1992
Diclofenac Sodium	Higher Anti-inflammatory activity of the niosomal drug when administered intraperitoneally and transdermally.	Naresh, *et al*., 1993
Doxorubicin	Niosomal drug had a prolonged half life, reduced cardiotoxicity and enhanced cytotoxic activity.	Rogerson, *et al*. 1988, Naresh *et al*.. 1993
Estradiol	*In vitro* transdermal delivery of niosomal drug resulted in higher diffusion rate of the drug through stratum corneum.	Hofland *et al*., 1992
Flurbiprofen & Piroxicam	Bio-availability improved both in case of oral and transdermal pre-aeration. Bioavailability improved in case of oral penetration only.	Reddy and Udupa 1993
Hemoglobin	Hemoglobin retained its oxygen carrying capacity when encapsulated in niosomes.	Moser *et al*. 1989, Moser *et al*. 1990
Humectants Sunscreens & Tanning Agents	Enhanced delivery of agents into the skin. Used as skin penetration enhancers in cosmetics.	Handjani Vila *et al*., 1979, Junginger *et al*., 1991

CONCLUSION

The success of liposomal system has stimulated the search for other vesicle forming amphiphiles. Non-ionic surfactant vesicles (niosomes) are among the first alternative materials studied for the drug delivery.

Niosomes are efficient carriers for controlled drug delivery, to entrap hydrophilic drugs in the larger interior aqueous layer , whereas, lipophilic drugs in the outer lipid bilayer. Since, the niosomes, are biodegradable and non toxic and hence, a good carrier for targeting of therapeutic agents at the site of interest with reduced systemic toxicity.

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