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SPHINGOSOME: A NOVEL VESICULAR DRUG DELIVERY SYSYTEM

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ABSTRACT

Sphingosomes are bilayered vesicles in which an aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of sphingolipids. It overcomes disadvantages of liposome, noisome and other vesicular drug delivery system. Sphingosomes are stable to acid hydrolysis and have better drug retention characteristics. They are mostly used for chemotherapy because various size and composition of sphingolipids. They are basically liposomal formulation based on sphingomyelin based cholesterol has several advantages when compared to other formulation. This review gives an idea about Sphingosomes as a promising vesicular drug delivery system to deliver therapeutic compound for all possible application.

KEYWORDS - Vesicular system, sphingolipids, Sphingosomes, sphingomyelin.

INTRODUCTION

In the past few decades, considerable attention has been focused on the development of novel drug delivery system (NDDS). The NDDS should ideally fulfill two prerequisites: Firstly, it should deliver the drug at rate directed by the needs of the body, over the period of treatment, secondly; it should channel the active entity to the site of action. [1, 2]Conventional dosage form does not fulfill this requirement. Now a days vesicle as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of valuein

immunology, membrane biology and diagnostic technique and most recently in genetic Engineering. [3, 4]

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphillic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphillic building blocks. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies (Bangham et al., 1965). For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of vesicular drug delivery systems. [1, 2, 3, 4]

Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduce of drug toxicity with no adverse effects.[5] Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and lipophilic drugs. Different novel approaches used for delivering the drug by vesicular system include liposomes, niosomes, sphingosomes, transferosomes and pharmacosomes. [5, 6]

SPHINGOSOMES

Liposome stability problems are of course much more severe so it is very important task to improve the liposomal stability. Liposomal phospholipid can undergo chemical degradation such as oxidation and hydrolysis either as a result of these changes or otherwise liposome maintained in aqueous suspension may aggregate, fuse, or leak their content. Hydrolysis of ester linkage will slow at pH value close to neutral. The hydrolysis may be avoided altogether by use of lipid which contains ether or amide linkage instead of ester linkage (such are found in sphingolipid) or phospholipid derivatives with the 2- ester linkage replaced by carbomoyloxy function. [3, 7]

Sphingosomes can be defined as colloidal, concentric bilayered vesicles where aqueous compartment is entirely enclosed by a bilayer membrane, mainly composed of natural or synthetic sphingolipids; that is, sphingosomes are liposomes that are composed of sphingolipids. Sphingosomes consist of sphingolipid(sphingomyelin) and cholesterol at acidic intraliposomal pH ratio of sphingomyelin and cholesterol varying in the range of 75/25mol%/mol% (55/45 mol%/mol% most preferably). Sphingosomes is more stable than the phospholipid liposome because of the following.[8, 9]

(i) Sphingolipid are built up by only amide and ether linkage. They are more resistant to hydrolysis than ester linkage of lecithin.

(ii) They also contain a smaller amount of double bonds then lecithin and thus less subjected to rancidity.

(iii) They also absorb a smaller amount oil then lecithin that in consequence change in geometry and diameter. [8, 9]

Advantages of Sphingosomes[8, 10, 11]

(i) Sphingosomes have better drug retention characteristics.

(ii) They can be administered by subcutaneous, intravenous, intra-arterial, intramuscular, oral, and transdermal routes of drug administration and so forth.

(iii) They provide selective passive targeting to tumor tissue.

Slow release of drug from extravasated sphingosomes increasedrug level within the tumor, extends drug exposure through multiple cell cycles and significantly enhance tumor cell killing. The immature neo vasculature within tumor is created during angiogenesis and has numerous imperfections, pores and discontinuities up to 800 nm in size, sphingosomes readily extravasate through these pores and accumulate within tumor and slowly release the encapsulated drugs. [10, 12]

(iv) Sphingosomes increase efficacy and therapeutic index of the encapsulated drug.

(v) Stability is increased via encapsulation.

(vi) Toxicity of the encapsulated drug is reduced.

(vii) Sphingosomes improve pharmacokinetics of the encapsulated drug simply by increasing the circulation time.

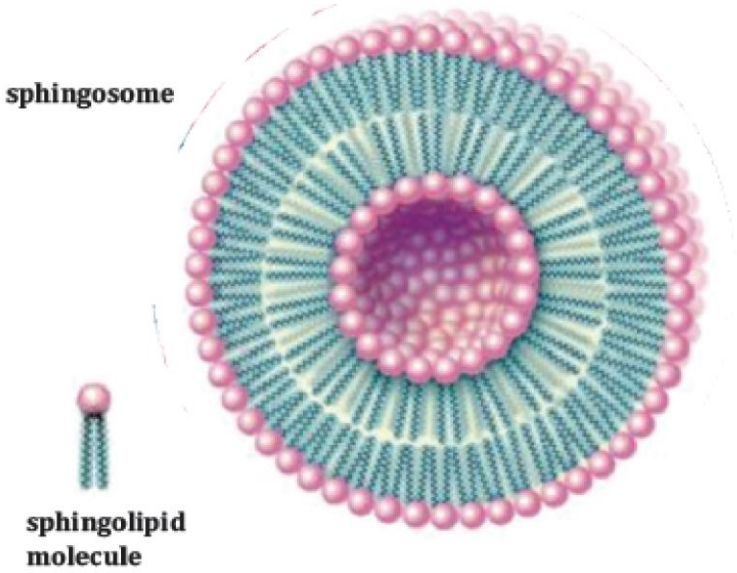
(viii) Design of sphingosomes is so flexible to allow coupling with site specific ligands to achieve active targeting.

(ix) Extended circulation time in vivo-Longer circulation time in plasma delivers more of the therapeutic agent to targeted site over a longer period of time. To stabilize lipid bilayer walls and retain active drug with in aqueous interior. This new sphingosomaltechnology increase rigidity of liposomal wall prolongs the circulating life of vesicle and significantly extends the duration of drug release. [10, 12]

Disadvantages of Sphingosomes-[8, 10]

(i) Since sphingolipids are expensive, sphingosomes are not economic.

(ii) Sphingosomes have poor entrapment efficiency.



"Fig 1"- Cross section view of sphingosomes

Classification of Sphingosomes [10, 11, 13]

Sphingosomes can be classified based on structural parameter like number of bilayer formed and diameter of their resultant vesicles. The sphingosomes are unilamellar or multilamellar and will typically have mean diameter of about 0.05 μ to 0.45 μ. More preferably diameter range is 0.05 to 0.2 μ.

1. Small unilamellar vesicles (SUV): It consists of single lipid bilayer and having diameter in size range 10nm-100nm.
2. Large unilamellar vesicles (LUV): It consists of single lipid bilayer. Having greater diameter than SUV. Having size range 100nm-1μm.
3. iii.Multilamellar vesicles (MLV): it consists of several bilayers of lipid and having size range 100nm-20μm.
4. Oligolamellar vesicles (OLV): bilayer is more than one but not as many as MLV's. Having size range 0.1-1μ.
5. Multivesicular vesicles (MVV): size range 100nm-20μm.
6. vi.Vesicles above 1 μm are known as Giant vesicles (GV).

Composition

Sphingosomes are the liposomal preparations which mainly differ in the lipid composition. They are having one or more membranes which comprise sphingolipids and cholesterol. The sphingolipid and cholesterol are typically present at a percentage molar ratio from 75:25 to 30:50 and most preferableratio is 55:45. Other lipids may also be present provided they

should not adversely affect the stability of the drug. Generally inclusion of other lipids will result in a decrease in sphingolipid/cholesterol ratio. [14, 15]

1)Sphingolipid

Sphingolipid have been known as cell component. Their name was given by J.L.W. Thudichum in 1884, because of their enigmatic nature. [11, 14]Sphingolipid contain a polar head attached to hydrophobic body. The sphingolipidbeing polar lipid is related to the composition and structure of human skin lipid, specifically in the epidermis layer. The sphingolipid obtained from natural source like mammals milk, preferably bovine milk, brain, egg yolk, erythrocytes from animal blood, preferably sheep. The sphingolipid may be synthetic or semi synthetic. The simplest sphingolipids are sphingosineand Ceramide which are scaffold and complex sphingolipid such as sphingomyelin(SM) and glycosphingolipid. Different types of sphingolipid can be used in sphingosomes and are described in table 1.

Table 1: Classification of sphingolipids based on source [10]

Sources	Name of sphingolipids
Egg, brain, milk	1.Sphingosine derivatives: D-erythrosphingosine, Sphingomyelin, Ceremides, brain sufatides 2.gangliosides: ovine braingangliosides, Porcine brain gangliosides
Soya bean	Glucosylceramides
Plant (yeast)	Phytosphingosine, D-ribo-Phytosphingosine-1-Phosphate, N-Acyl Phytosphingosine

Sphingolipids can also be classified as- [6, 7, 11]

1) Sphingoid bases

1. Sphing-4-enines (sphingosines)
2. Sphinganines
3. c.4-Hydroxysphinganines (phytosphingosines)
4. Hexadecasphinganine (Sphingoid base homologs and variants)
5. Sphingoid base 1-phosphates
6. Lysosphingomyelins and lysoglycosphingolipids
7. *N*-Methylated sphingoid bases

2) Ceramides

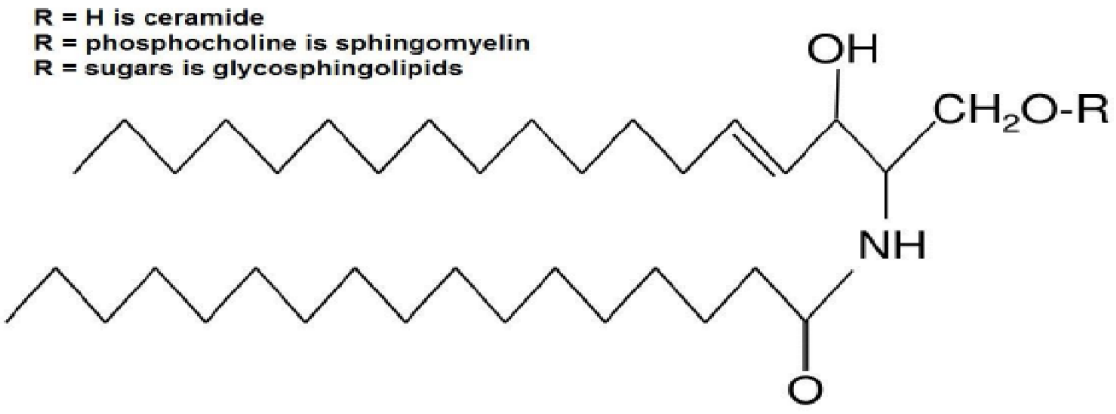
1. N-Acylsphingosines (ceramides)
2. j. N-Acylsphinganines (dihydroceramides)
3. k. N-Acyl-4-hydroxysphinganines (phytoceramides)

3) Neutral glycol sphingolipids

1. Simple Glc series (GlcCer, LacCer, etc.)
2. GalNAcβ1-3Galα1-4Galβ1-4Glc- (globo series)
3. GalNAcβ1-4Galβ1-4Glc- (ganglio series)
4. Galβ1-3GlcNAcβ1-3Galβ1-4Glc- (lacto series)
5. Galβ1-4GlcNAcβ1-3Galβ1-4Glc- (neolacto series)
6. GalNAcβ1-3Galα1-3Galβ1-4Glc- (isoglobo series)
7. GlcNAcβ1-2Manα1-3Manβ1-4Glc- (mollu series)
8. GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glc- (arthro series)

4) Acidic glycosphingolipids

1. Gangliosides
2. Sulfoglycosphingolipids (sulfatides)
3. Glucuronosphingolipids
4. Phosphoglycosphingolipids
5. Other Amphoteric glycosphingolipids



"Fig 2"- General structure of Sphingolipid

2) Cholesterol

Incorporation of sterol in sphingosomes bilayer can bring about major changes in the preparation of this membrane. Cholesterol does not by itself from bilayer structure, but can be

incorporated in to sphingolipid membranes in very high concentration up to 1:1 or even 2:1 molar ratio cholesterol to sphingolipid. Cholesterol incorporation increase the separation between the choline head group and eliminate the normal electrostatic and hydrogen bonding interaction.

The stability of sphingosomes can be increased by addition of stearylamine (SA) a positive charge inducing agent. Additional components may be added to the sphingosomes to target them to specific cell types. For example, the sphingosomes can be conjugated to monoclonal antibodies or binding fragments thereof that bind to epitopes present only on specific cell types, such as cancer-related antigens, providing a means for targeting the sphingosomes following systemic administration. Alternatively,ligands that bind surface receptors of the target cell types may also be bound to the liposomes. [14, 15]

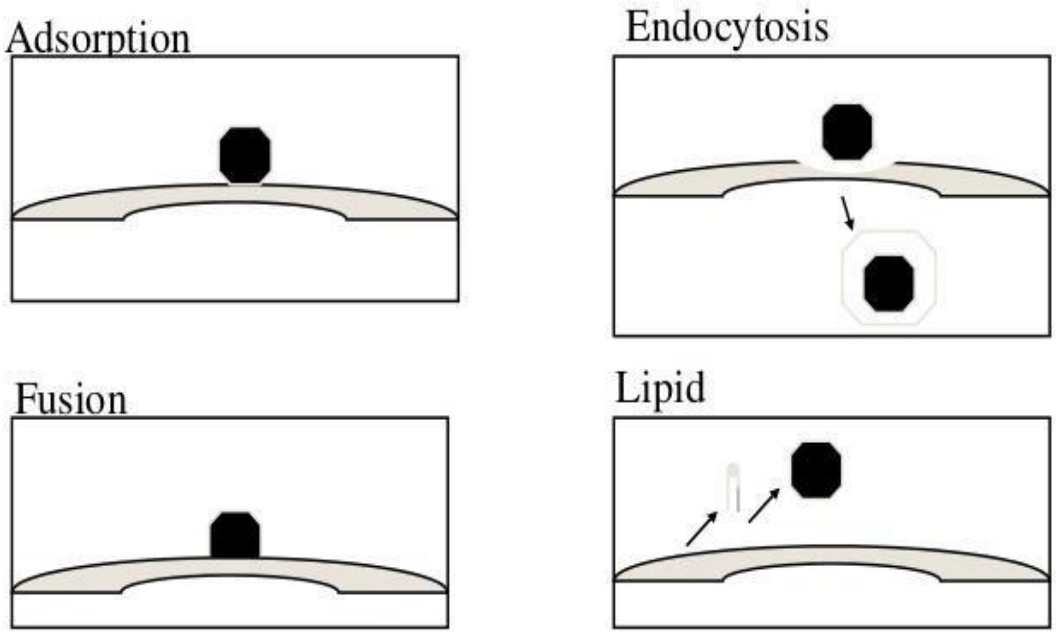
Table 2: Different additives employed in sphingosomes in transdermal route [16]

Additives	Uses	Examples
Lipid	Vesicles forming component	.Sphingolipid
Polyglycol	Skin penetration enhancer	Propylene glycol,transcutol
Cholestrol	Stabilizer	Cholestrol
Vehicle	As a gel former	Carbopol 934
Dye	For characterization study	6-Carboxy Fluroscence, Rhodamine-123 etc.

Transport Mechanism of Sphingosomes[8, 10,]

Small unilamellarsphingosomal vesicles interact with cell by following ways-

1. Stable adsorption:stable adsorption represents the association of intact vesicles with the cell surface. Such process mediated by non-specific electrostatic, hydrophobic or other forces. Or component presents at the vesicles or cell surface.
2. Endocytosis: endocytosis is the uptake of intact vesicles in to endocytotic vesicles and result, presumably in their delivery to the lysosomal apparatus.
3. Fusion: Fusion is the simple merging of vesicles bilayer with the plasma membrane bilayer, with components release of vesicle content in to the cytoplasmic space.
4. Lipid exchange: in this transfer of individual lipid molecular between vesicles and the cell surface without the cell association of aqueous of aqueous vesicle content.



"Fig 3'- Transport mechanism of Sphingosome [17]

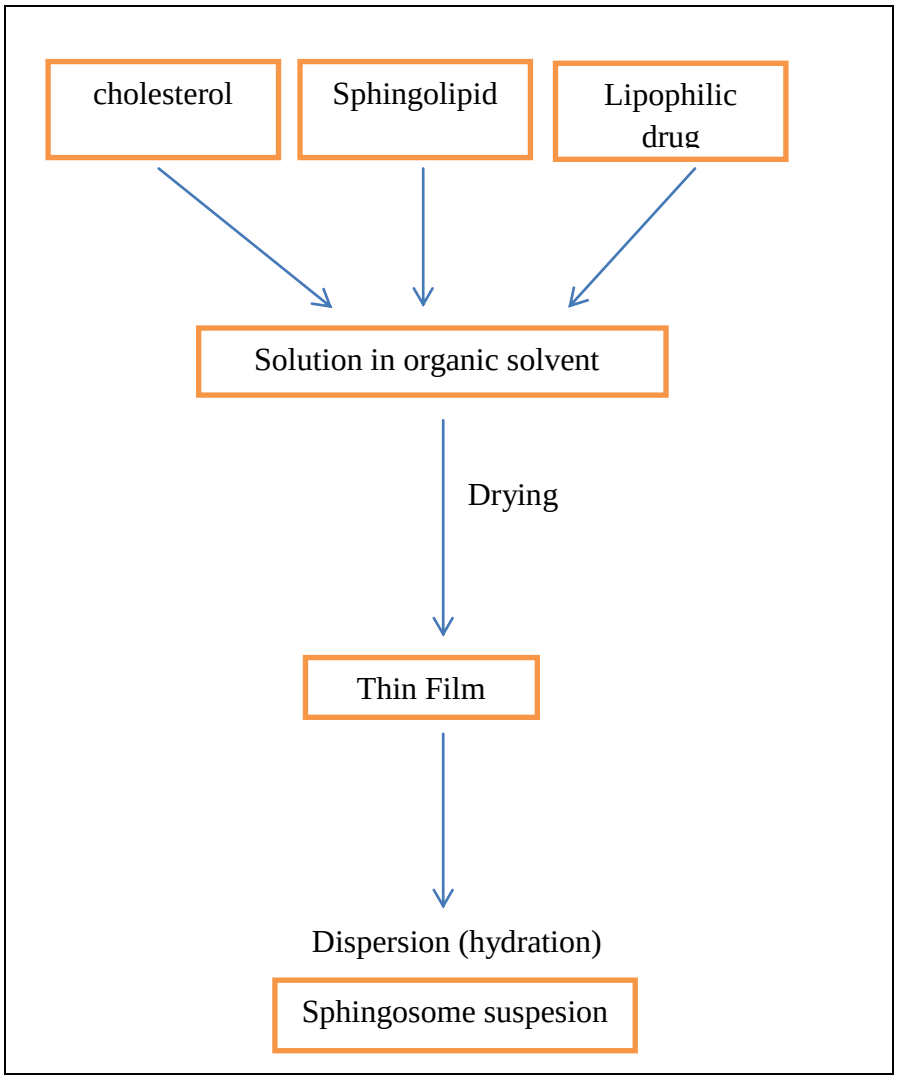
Method of Preparation of Sphingosome

Sphingosomes are lamellar vesicle systems whose preparation is similar to that of liposomes. The preparation of sphingosomes mainly involve loading of the drug into vesicles. For drug loading suitable conventional passive loading and active entrapment methods are being used.[14, 18, 19] The general methods for the preparation are listed below.

1. Lipid film Hydration Method [19,20]

Film method described by Bangham et.al 1965. In this method the mixture of appropriate amount of sphingolipid are casted as stack of film form this organic solution using flash rotary evaporator under reduced pressure (or by hand shaking) and then the casted film is dispersed in aqueous medium. Upon hydration the sphingolipid swell and peel off from the wall of round bottom flask and vasiculate forming multi lamellar sphingosomal vesicles (MLSV's). the mechanical energy required for swelling of sphingolipid in dispersion casted lipid film is imparted by manual agitation(hand shaking technique) or exposing the film to the stream of nitrogen for 15 minutes followed by swelling in aqueous medium without shaking (non shaking methods). The hand shaking method produce MLSV's, but the vesicles produced by non shaking method are large unilamellar sphingosomal vesicles. MLSV's

formed on hydration of sphingolipid could be further modified for their size and other characteristics. [10]



"Fig 4"- Steps in lipid film hydration method

2. Solvent Spherule Method. [21, 22]

The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which sphingolipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

3. Sonication Method. [23]

At high energy level the average size of sphingosome is further reduced. This was first achieved on exposure of MLSV's to ultrasonic irradiation and still remains the method most widely used for producing small vesicles. There are two method of sonication based on the

use of either probe or bath. Ultrasonic disintegrator bath sonicators are most widely used for preparation of small unilamellar vesicles.

4. French Pressure Cell Method.[10, 24]

This is very useful method. In this extrusion of preformed sphingosome in French press under very high pressure. This technique yields rather uni or oligo lamellar sphingosomes. These sphingosomes are more stable as compared to sonicated vesicles

5. Solvent Injection Methods

The solvent injection methods involve the dissolution of the sphingolipid into an organic phase (ethanol or ether), followed by the injection of the sphingolipid solution into aqueous media, forming sphingosomes. [25]

a. Ether Infusion Method. [25, 26, 27]

The ether injection method differs from the ethanol injection method since the ether is immiscible with theaqueous phase, which is also heated so that the solvent is removed from the sphingosomal product. The method involves injection of ether-sphingolipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporizes upon contacting the aqueous phase, and the dispersed shingolipid forms primarily unilamellarsphingosome. An advantage of the ether injection method compared to the ethanol injection method is the removal of the solvent from the product, enabling the process to be run for extended periods forming a concentrated shingosomal product with high entrapment efficiencies

b. Ethanol Injection Method. [25, 28]

Themain relevance of the ethanol injection method resides in the observation that a narrow distribution of small sphingosomes (under 100 nm) can be obtained by simply injecting an ethanolicsphingolipid solution in water, in one step, without extrusion or sonication.

6. Detergent Removal Methods. [29, 30, 31, 32]

The detergents at their critical micelles concentrations have been used to solubilize sphingolipids. As the detergent is removed the micelles become progressively richer in sphingolipid and finally combine to form LUVs. The detergents can be removed by dialysis. The advantages of detergent dialysis method are excellent reproducibility and production of sphingosomes populations which are homogenous in size. The main drawback of the method

is the retention of traces of detergent(s) within the sphingosomes. Other techniques have been used for the removal of detergents: (a) by using Gel Chromatography involving a column of Sephadex G- 259 (b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-210 (c) by binding of octylglucoside (a detergent) to Amberlite XAD-2 beads.

7. Reverse Phase Evaporation Method. [6, 33]

The novel key in this method is the removal of solvent from an emulsion by evaporation. Water in oil emulsion is formed by bath sonication of a mixture of two phases, and then the emulsion is dried to a semi-solid gel in a rotary evaporator under reduced pressure. The next step is to bring about the collapse of certain portion of water droplets by vigorous mechanical shaking with a vortex mixture. In these circumstances, the lipid monolayer, which encloses the collapse vesicles, is contributed to adjacent intact vesicles to form the outer leaflet of the bilayer of large unilamellarniosomes. The vesicles formed are unilamellar and have a diameter of 0.5 μm.

8. Calcium-Induced Fusion Method.[34]

Calcium is added to SUV sphingosomes that induce fusion and cause formation of multilamellar vesicle. The addition of EDTA to the preparations results in the formation of LUV sphingosomes. The advantage of calcium induced fusion method is that macromolecules can be encapsulated , while their disadvantages is that LUV sphingosomes can only be obtained from acidic sphingolipids.

9. Microfluldization Method. [6, 36]

This is a recent technique to prepare small MLVS. A Micro fluidizer is used to pump the fluid at a very high pressure (10,000psi) through a screen. Thereafter; it is forcedalong defined micro channels, which direct two streams of fluid to collide together at right angles, thereby affecting a very efficient transfer of energy. The lipids can be introduced into the fluidizer. The fluid collected can be recycled through the pump until vesicles of spherical dimensions are obtained. This results in greater uniformity, small size and better reproducible sphingosomes.

10. Freeze-Thaw Method. [25, 37, 38, 39]

This new method was described for the preparation of sterile and pyrogen-free submicron narrow sized sphingosomes. It is based on the formation of a homogenous dispersion of sphingolipids in water-soluble carrier materials. Sphingosome-forming sphingolipids and

water-soluble carrier materials such as sucrose were dissolved in tert-butyl alcohol/water cosolvent systems in appropriate ratios to form a clear isotropic monophasesolution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at −40 °C for 8 h; primary drying at −40 °C for 48 h and secondary drying at 25 °C for 10 h. The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous sphingosome preparation.

Ideal Drug Characteristics for Sphingosomes

1. Low dose
2. Short biological half life
3. Higher dosing frequency
4. Less oral bioavailability
5. High lipophilicity

CHARACTERIZATION OF SPHINGOSOMES[1, 40, 41, 42, 43]

1)Vesicular characterization- Particle size, shape and zeta potential can be measure by using transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Dynamic light scattering (DLS) and Photon correlation spectroscopy (PCS)

2) Entraptment efficiency- It can be measured by ultracentrifugation technique.

3) Transition temperature- Transition temperature of vesicular sphingolipid system can be measured by differential scanning calorimetry.

4) Surface tension activity measurement- It can be measure by the ring method in a Du Nouy ring tensiometer.

5) Vesicle stability- It depends on size, structure of vesicles. Structure and shape changes observe using TEM.

6) Drug content- It can be done by using UV spectrophotometry and High performance liquid chromatography.

7) Penetration study- It can be done by using confocal laser scanning microscopy (CLSM).

8) Permeation study- It can be done by incorporating sphingosome into gel. Diffusion studies of gel must be done using franz diffusion cell.

9) Sphingolipid-cholesterol interaction- It can be done by using differential scanning calorimetry and P31 NMR.

Application of Sphingosomes

Many drugs are delivered as sphingosomes.Sphingosomes may prove to be efficient carrier for targeting the drug to the site of action, because of being biodegradable, innocuous nature and being identical to biological membrane.

1) Sphingosome in tumor therapy

Sphingosomes increased drug concentration at the tumor site is associated with increased clinical activity. The link between drug exposure and anti-tumor efficacy is especially pronounced for cell cycle-specific agents such as vincristine, vinorelbine and topotecan, which kill tumor cells by interfering with mitosis at a precise step during the cancer cell cycle. Thus, this proprietary sphingosomal drug delivery platform encapsulates approved anticancer agents within the aqueous interior of small liposomes to potentially enhance the therapeutic index of these existing anticancer treatments. [10]

2) Sphingosome as a drug delivery vehicle

Sphingosome act as vehicle useful for the treatment of proliferative disease, immune disease, infectious disease, vascular disease, rheumatoid disease and inflammatory disease. The representative drugs include prostaglandins, amphoterecin B, methotrexate, cisplatin, vincristine, vinblastine, doxorubicin, camphothecin, ciprofloxacin, progesterone, testosterone, estradiol, beclometasone and esters vitamin-E, dexamethasone and other steroids. [44]

3) Sphingosome in cosmetic industry

Sphingosomes are also used in the cosmetic industry and drug delivered through transdermal route. The skin compatibility of topically applied sphingolipid is very high. Because of the membrane lipid of sphingosome belong to same class of chemical compound as epidermal lipid, they have characteristic that enhance their penetration. [10]

4) Sphingosome in ophthalmic delivery

Delivery of drug in accurate concentration in ophthalmic region is too difficult. Bioavailability of drug modified because of physicochemical properties of drug and

vehicle.Amongst various vehicles and carrier, vesicles have gainedconsiderable attention for ocular drug delivery. [7]

5) Sphingosome used for enzyme delivery

Many enzymes including streptokinase, urokinase esterase encapsulated in sphingosomes. Enzyme catalysis in sphingosomes has been used for variety of reaction such as synthesis of esters, peptides and sugar acetal transformation. [10]

Table 3: Therapeutic application of sphingosome

Drugs	Action	Application	Targeted disease
Vinorelbine [45]	Kill tumor cells by interfering with mitosis	In tumor therapy	Tumor site /Cancerous cell
Prostaglandins and other steroid [46]	Act as a Vehicle	As a drug delivery vehicles	For the treatment of prostaglandin disease,immunediseaseand infectious disease
Beclomethasone [7]	By enhancing the penetration of drugs	In Cosmetic Industry	Skin/Dermal therapy
Idoxuridine[7]	Drugs entrapped inside sphinosomes which posess optimum corneal and increase contact time	Occular drug delivery	Acute/Chronic herpatitics keatitis
streptokinase [47] Urokinase [48]	Proceed by sysnthesis of ester,peptide and sugar	Enzyme Delivery	Treatment of malnutrition

Marketed Products of Sphingosomes

Table 4: Marketed formulation of sphingosomes

Various marketed formulation	Drugs	company	Application
Margibo (TM), Oncovin (R)	Vincristine	Eli Lilly	Acute Lymphoblastic leukemia
Navelbine (R)	Vinorelbine	Glaskosmithkline	Lung cancer
Hycumtin (R)	Topotecan	Glaskosmithkline	Lung and ovarian cancer

CONCLUSION

Now a days vesicular drug delivery system is investigated as major drug delivery system. Extensive research is going on sphingosomes.Sphingosomes is bilayered vesicles in which an

aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of natural or synthetic sphingolipid.There is a great potential inutilizing these sphingosomes in ecology, biotechnology, medicine and pharmaceutical technology. Sphingosomes can be used in treatment of cancer, in cosmetics, ocular drug delivey, fungal infection etc. It has many advantages over liposomes.Sphingosomes is made up of lipid which is similar class of skin lipid so it is more compatible and safe to hostcell and there are no restrictions concerning their use neither in the EU nor for regulations of the US Food and Drug Administration; sphingosomes are generally accepted as safe.

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