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Review Article

Sphingosomes a novel appoach to vesicular drug delivery

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ABSTRACT

Vesicular systems have been realized as extremely useful carrier systems in various scientific domains. Sphingosomes is bilayered vesicles in which an aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of natural or synthetic sphingolipid. Sphingosomes solve the major drawback of vesicle system (liposomes, niosomes) like less stability, less in vivo circulation time, low tumor loading efficacy in case of cancer therapy. Sphingosomes are clinically used delivery system for chemotherapeutic agent, biological macromolecule and diagnostics. Due to flexibility in size and composition, different types of sphingosomes have been developed. The out come of this review is that sphingosomes represents a promising vesicular drug delivery system to delivers therapeutic compounds for a range of possible applications.

1. Introduction

In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). When the new drug or existing drug is given altering the formulation and administered through different route, this process is called as the novel drug delivery system. The NDDS should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a 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. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery [1].

Novel drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Novel drug delivery attempts to either sustain drug action at a predetermined rate, or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. It can also localize drug action by spatial placement of controlled release systems adjacent to, or in the diseased tissue or organ; or target drug action by using carriers or chemical derivatization to deliver drug to particular target cell type.

There are different types of pharmaceutical carriers are present. They are - particulate, polymeric, macromolecular, and cellular carrier. Particulate type carrier also known as a colloidal carrier system, includes lipid particles (low and high density lipoprotein-LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and vesicular like liposomes, sphingosome, niosomes pharmacosomes, virosomes [2-6] .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 [7]. Liposome vesicles will be understood to indicate structure having lipid containing membrane enclosing an aqueous interior. The structure can haveone or more lipid membrane unless otherwise indicated, although generally liposome will have only one

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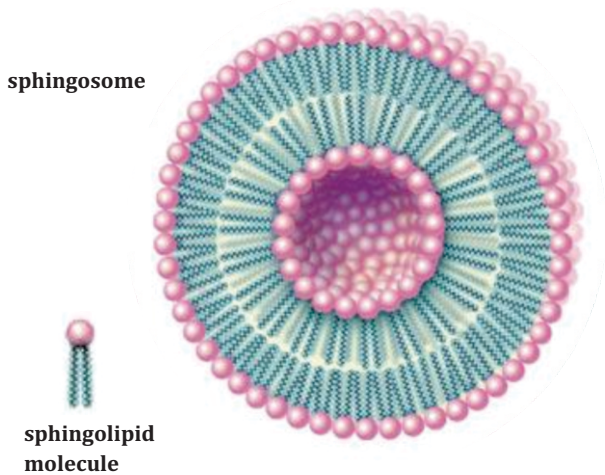
membrane. Such single layered referred to as unilamellar and multi layered liposome referred to as multi lamellar. The liposome that is preferred formed from lipids which when combined form relatively stable vesicles. Wide variety of lipids which can be used to generate the more stable liposome. Preferred lipid should be neutral or negatively charged phospholipid or sphingolipid and sterol such as cholesterol. The selection of lipid generally based on liposome size and stability of liposome in blood stream [8]. Liposomal drug delivery system is advantageous in the fulfillment of the aspects related to protection and control release of active moiety along with targeted drug delivery and cellular uptake via endocytosis [9-11]. Besides the merits of liposome also possess certain problems associated with degradation, hydrolysis [12], and oxidation [13], sedimentation, leaching of drug aggregation or fusion [14] during storage.

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 [15]. Thus sphingolipid are been nowadays used for the preparation of stable liposomes known as sphingosomes.

Sphingosome may be defined as "concentric, bilayered vesicle (figure I) in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid".

Figure I Cross section view of sphingosome



"In simple way we can say sphingosome is liposome which is composed of sphingolipid." [16]

Sphingosomes encapsulated technology was discovered at the University of British Columbia and subsequently developed by Inex Pharmaceutical Corp. In May 2006, Hana biosciences licensed 3 drug candidates utilizing this technology from Index [17]. Liposomal formulation based on sphingomyelin-based cholesterol has several advantages when compared to other formulation. The Sphingosomes are much more stable to acid hydrolysis, have better drug retention characteristics. Sphingosomes are administered in many ways these include parentral route of administration such as intravenous, intramuscular, subcutaneous, and intra-arterial. Generally it will be administered intravenous or some cases by inhalation. Often it will be administered into a large central vein, such as the superior vena cava and inferior vena cava to allow highly concentrated solution to be administered into large volume and flow vessels. Sphingosomes may be administered orally or transdermally [18,19].

1.1.General advantages of sphingosomes [20]

I. Provide selective passive targeting to tumor tissue.
ii. Increase efficacy and therapeutic index.
iii. Increase stability via encapsulation.
iv. Reduction in toxicity of the encapsulated agent.
v. Improve pharmacokinetic effect (increase circulation time).
vi. Flexibility to couple with site specific ligands to achieve active targeting.

1.2. Advantages over the phospholipid liposomes

i. It is more stable than the phospholipid liposome because [16]
a. Sphingolipid built up by only amide and ether linkage. They are more resistant to hydrolysis then ester linkage of lecithin.
b. They also contain less double bond then lecithin and thus less subject to rancidity.
c. They also absorb less oil then lecithin which in consequence change in geometry and diameter.

I.Extend the circulation time in vivo [17]

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 sphingosomal technology increase rigidity of liposomal wall prolongs the circulating life of vesicle and significantly extends the duration of drug release.

I.Have better tumor loading characteristics [17]

Slow release of drug from extravasated sphingosomes increase drug 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.

i. Have significantly better drug retention characteristics.
ii. Better acid stability [18,19].

1.3. Disadvantages

1. Higher cost of sphingolipid hinders the preparation and use of these vesicular systems.
2. Low entrapment efficacy.

CLASSIFICATION OF SPHINGOSOMES

Sphingosomes can be classified based on structural parameter like number of bilayer formed and diameter of their resultant vesicles [20,21]. 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.

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

COMPOSITION OF SPHINGOSOMES

Sphingosome are comprised of sphingolipid (sphingomyelin) and cholesterol and have an acidic intraliposomal pH ratio of sphingomylin and cholesterol varies in the range of 75/25 mol%/mol% (55/45 mol%/mol% most preferably). Liposomal formulation based on sphingomyelin and cholesterol has several advantages when compared to other formulation. The Sphingosomes are much more stable to acid hydrolysis, have better drug retention characteristics [18,19].

1.4. Sphingolipid

Sphingolipid have been known as cell component. Their name was given by J.L.W. Thudichum in 1884, because of their enigmatic nature [20]. Sphingolipid contain a polar head attached to hydrophobic body. The sphingolipid being 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 sphingosine and 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.

1.5. 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 [18].

Table 1: Classification of sphingolipid based on source

Natural Sphingolipids:	
Sources	**Name of sphingolipid**
Egg,brain,milk	**1.Sphingosine derivatives:** D-erythrosphingosine, Sphingomyelin, Ceremides, brain sufatides **2.gangliosides:** ovine braingangliosides, Porcine brain gangliosides
Soy-bean	Glucosylceramides
Plant (yeast)	Phytosphingosine, D-ribo-Phytosphingosine-1-Phosphate, N-Acyl Phytosphingosine
Natural Sphingolipids:	
Sphingosine derivatives:	D-erythro Sphingosine (synthetic) Sphingosine -1-Phosphate N,N-Dimethylsphingosine , Sphingomyelin , Glycosylated Sphingosine
OmegaLabeled Sphingosine:	Omega-Biotinyl Sphingosine , Omega-Biotinyl D-erythro-Sphingosine-1-Phosphate
Ceramide Derivatives:	Ceramides , D-erythro Ceramide-1Phosphate Glycosulated Ceramides , Fluorescent Ceramide
Sulfated Ceramide Derivatives	3-O-Sulfo-ß-D-C12-galactosylceramide (ammonium salt) 3,6-di-O-Sulfo-ß-D-C12-galactosylceramide (diammonium salt)
Sphinganine (Dihydrosphingosine)	Sphinganine-1-Phosphate ,Sphinganine (C20), D-erythro Sphinganine , N-Acyl-Sphinganine

2.Preparation Of Sphingosomes

Preparation of sphingosomes requires loading of drug in to vesicles. Loading can be either passive (streptokinase, urokinase) [6,22] or active.

Active loading is in many way preferably and a wide variety of therapeutic agents can be loaded in to sphingosomes with encapsulation efficacy approaching 100% by using transmembrane pH gradient [23,24]. In this method involve establishment of some form of gradient that draws lipophilic compounds in to the interior of vesicles where they can reside for as long as the gradient is maintained.

Passive loading generally required addition of .drug to the buffer at the time of reconstitution step. This allowed drug to be entrapped within the vesicles interior when it will remain if it is not lipid soluble. Generally passive loading is applied for the sphingosome preparation. Various methods for passive loading utilized are as followed:

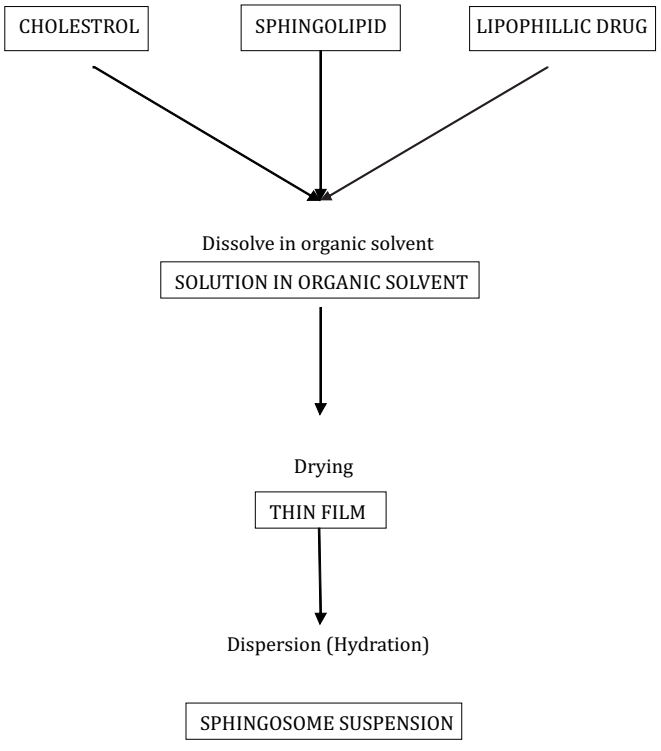
2.1.Classical method or Mechanical dispersion method

In mechanical dispersion method begin with a lipid solution in organic solvent and end up with lipid dispersion in water. The various components are typically combined by co-dissolving the lipid in an organic solvent and organic solvent is then removed by film deposition under vacuum. When all solvent removed the solid lipid mixture is hydrated using aqueous buffer. The lipid spontaneously swell and hydrate to form vesicle of sphingosomes. At this point methods incorporate some diverge processing parameter (sonication, freeze thawing and high pressure extrusion) in various ways to modify their properties [20].

2.2.Film method

Film method described by Bangham et.al 1965. In this method the mixture of appropriate amount of lipid 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 lipid 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 lipid 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 lipid could be further modified for their size and other characteristics [6]. The steps to sphingosome preparation could be understood by Figure II

Figure II Steps to sphingosome preparation



Extrusion technique is generally applied to reduce the size of sphingosomes. In this technique all the dispersion are extruded through polycarbonate membrane/ an asymmetric ceramic membrane, filter with core of 0.6μm (once) and 0.2μm (ten times). The dispersion subsequently freeze thaw ten times to increase the encapsulation efficiency of the sphingosomes. The non entrapped drug removed by ultracentrifugation for thirty minute at 55,000 rpm and 4ºC. The pellets subsequently redisperse in buffer.
Other method for size reduction of sphingosomes:

· Sonication: 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.

· French pressure cells: 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.

· Micro emulsification technique: Micro fluidizer pump is used to prepare small multilamellar vesicles. Micro fluidizer pumps the fluid at very high pressure 10,000 psi through 5μm orifices. After single pass size of vesicles is reduced to 0.1 and 0.2 μm in diameter [20,25,26].

TRANSPORT MECHANISM OF SPHINGOSOMES

2.3.Transport mechanism at cellular level

There are various ways by small unilamellar sphingosomal vesicles (SUSV's) interact with cell. These are as follows stable adsorption, endocytosis, fusion, lipid transfer [15]

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.

Endocytosis: endocytosis is the uptake of intact vesicles in to endocytotic vesicles and result, presumably in their delivery to the lysosomal apparatus.

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.

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.

3.Applications of sphingosomes

A wide variety of therapeutic compound may be delivered by the sphingosome. “Therapeutic compound” is meant to include example- nucleic acid, proteins, peptides, oncolytics, anti-infective, anxiolytics, psycho tropics, ionotrops, toxins such as gelonin and inhibitors of eukaryotic protein synthesis and the like.. Particularly preferred among the therapeutic compounds for entrapment in the sphingosomes are “lipophilic cations”. Among

these are therapeutic agents of the class of lipophilic molecules which are able to partition into the lipid bilayer phase of sphingosomes and which therefore are able to associate with the sphingosomes in a membrane form [18].

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.

3.1.Sphingosomes in tumor therapy: Most of the medical applications that have reached the pre-clinical and clinical stages are in cancer. Ex. Vinorelbine (semi synthetic vinca alkaloid) sphingosomal product has reached in phase I clinical trials [17].

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.

Sphingosomal products such as Marqibo(TM) (sphingosomal vincristine) are loaded with active, cell cycle-specific anticancer agents that may benefit from increased targeting and long duration of drug exposure at the tumor site. Vincristine, vinorelbine and topotecan are approved cancer therapies which have been selected for sphingosomal formulation specifically for their ability to benefit from this novel encapsulation.

Vincristine (Oncovin(R); Eli Lilly and Company), a microtubule inhibitor, is approved for acute lymphoblastic leukemia (ALL) and is widely used as a single agent and in combination regimens for treatment for hematologic malignancies such as lymphomas and leukemias. Vinorelbine (Navelbine(R) GlaxoSmithKline), a microtubule inhibitor, is approved for use as a single agent or in combination with cisplatin for the first-line treatment of unresectable, advanced non-small cell lung cancer.

Topotecan (Hycamtin(R); GlaxoSmithKline), a topoisomerase I inhibitor, is approved for use in relapsed small-cell lung cancer and in relapsed ovarian cancer.

3.2.Sphingosomes as drug delivery vehicles

Sphingosomes generally refers to uni- or multilamellar lipid structures enclosing an aqueous interior, depending on the number of lipid membranes formed. Typically liposomes can be loaded with drugs, i.e. the drug is encapsulated in the interior of the vesicle, and/or drugs can be attached to the sphingosome or incorporated into the lipid bilayer. Such drug comprising liposomal formulations have been shown to have an increased efficacy in comparison to the free drug. For example, it has been shown that a liposomal formulation including the vinca alkaloid vincristine has a greater efficacy if compared to free vincristine and it shows less overall toxicity.

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 [27].

3.2.1.Sphingosomes 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 do epidermal lipid, they have characteristic that enhance their penetration.

3.2.2.Sphingosomes in ophthalmic drug delivery: A major problem in ocular therapeutics is the delivery of an optimal drug concentration at the site of action. The ocular drug bioavailability is often modified by the physical and chemical properties of a drug as well as by physical properties of the vehicle in which the drug is placed. Thus, the selection of vehicles has been limited and semisolid varieties, principally because of the anatomical construct of the conjunctival sac and the sensitivity of the cornea to foreign object. Amongst various vehicles and carrier, vesicles have gained considerable attention for ocular drug delivery [15].

Ex. In the treatment of acute and chronic herpetic keratitis, idoxuridine entrapped in sphingosomes is more effective than a comparable therapeutic regimen of unentraped drug.

3.2.3.Sphingosomes 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 [6,28].

3.2.4.Other therapeutic application of sphingosomes:

I. Sphingosomes in antimicrobial, antifungal and antiviral (anti-HIV) therapy [20,29].
ii. Ex. ciprofloxacin, oflaxacin, vancomycin, amoxicillin, amphotericin B, iodoxuridine.
iii. Sphingosomes may be used in gene delivery [20].
iv. Sphingosomes may be used in enzyme immobilization [20].
v. Sphingosomes may be used in immunology [20].

4.Future Aspects

The concepts of sphingosomes as drug or bioactive carrier still need further optimization. Researchers all world continue to put in their in improving vesicular system by making them steady in nature to prevent leaching of content, oxidation and their uptake by natural defence machanism. Genetic engineering aspect can be coupled to give newer dimension to the existing cellular drug carrier concept. Their potential pharmaceutical application include immobilization of enzyme, masking the taste of drug, enhancement of gastrointestinal absorption and as carrier for sustained release and transdermal drug delivery, treatment of drug overdosing. With the evolution of various newer techniques of preparation, stabilization, characterization of these system, they can serve as potential carrier for drug cosmetic and pharmaceutical agents.

5.Conclusions

Over the years, vesicular systems have been investigated as a major drug delivery system, due to their flexibility to be tailored for varied desirable purposes. Sphingosomes is bilayered vesicles in which an aqueous volume is entirely enclosed by membrane lipid bilayer mainly composed of natural or synthetic sphingolipid. Lipophilic cations are the preferred category which is to be encapsulated. Application of sphingosomes clinically used for delivery of chemotherapeutic compound, diagnostic purpose and in cosmetic industry. Sphingosomes is made up of lipid which is similar class of skin lipid so it is more compatible and safe to host cell 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 (GRAS status).

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