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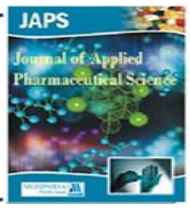

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NOVEL VESICULAR SYSTEM: AN OVERVIEW

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ABSTRACT

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. The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphillic building blocks are confronted with water. The vesicular system such as liposomes, niosomes, sphingosomes, ethosomes, transferosomes and pharmacosomes are used to improve the therapeutic index of both existing and new drug molecules by encapsulating an active medicament inside vesicular structure in one such system. It prolongs the existence of the drug in systemic circulation and finally reduces the toxicity. Such different systems are widely used in gene delivery, tumor targeting, oral formulations, in stability and permeability problems of drugs. 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 value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering.

Keywords: Vesicular system, controlled drug delivery, stability, applications.

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 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. 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 (Biju et al., 2006). Different types of pharmaceutical carriers such as particulate, polymeric, macromolecular and cellular are present. Particulate type carrier also known as 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, transferosomes, pharmacosomes, virosomes (Goldberg, 1983 and Poste et al., 1983).

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.

Vesicular drug delivery system has some of the advantages like:

- Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
- Improves the bioavailability especially in the case of poorly soluble drugs.
- Both hydrophilic and lipophilic drugs can be incorporated.
- Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

Liposomes are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecule. There are a number of components present in liposomes, with phospholipid and cholesterol being the main ingredients. Liposomes are often distinguished according to their number of lamellae and size. They are classified as small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs) and large multilamellar vesicles (MLVs) or multivesicular vesicles (MVVs).Large liposomes form spontaneously when phospholipids are dispersed in water above their phase transition temperature and the preparation of SUVs starts usually with MLVs, which then are transformed into small vesicles using an appropriate manufacturing technique, e.g. high-pressure homogenization.

The methods of preparation have been classified to the three basic modes of dispersions (Biju et al., 2006).

- Physical dispersion involving hand shaking and non-hand shaking methods.
- Solvent dispersion involving ethanol injection, ether injection, double emulsion vesicle method, reverse phase evaporation vesicle method, and stable plurilamellar vesicle method.
- Detergent solubilization.

The liposomes are characterized for their physical attributes i.e. size, shape, and size distribution, surface charge, percent capture, entrapped volume, lamellarity through freeze fracture microscopy and P-NMR, phase behavior, drug release, quantitative determination of phospholipids and cholesterol analysis. Liposome has been extensively investigated for their potential application in pharmaceutics; such as drug delivery, drug targeting; controlled release or increased solubility. They offer a substantial improvement in the therapeutic indices of the drug molecules entrapped in them. Due to their high degree of biocompatibility, liposomes have been used as delivery systems for an assortment of molecules. Applications of the liposomes are in the immunology, dermatology, vaccine adjuvant, eye disorders, brain targeting, infective disease and in tumour therapy. Liposomes as a potential delivery system for the oral administration of insulin have been extensively studied (Patel et al., 1982). It was observed by many scientists, that the liposomes had protective effects against proteolytic digestive enzymes like pepsin and pancreatin and they can increase the intestinal uptake of macromolecules and hence are capable of enhancing insulin uptake. Liposomes with a specifically modified design, i.e. long-circulating and especially actively targeting liposomes, stand a better chance in becoming truly tumor tropic carriers of photo sensitizers, and can hence be used successfully in photodynamic therapy.

NOVEL VESICULAR SYSTEM - MODIFICATIONS TO LIPOSOMES

Niosomes

Rigorous conditions required for handling liposomes under cryogenic atmosphere have prompted the use of non-ionic surfactant in vesicular drug delivery system, in place of phospholipids. Thus, the new vesicular delivery system consisting of unilamellar or multilamellar vesicles called niosomes, was introduced. Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomes can be changed or modified by the incorporation of other excipients like cholesterol, into the membrane and they can possess one or more lipid bilayers encapsulating an aqueous core. The bilayered vesicular structure is an assembly of hydrophobic tails of surfactant monomer, shielded away from the aqueous space located in the center and hydrophilic head group, in contact with the same. Niosome possess an infra structure consisting of hydrophobic and hydrophilic mostly together and so also accommodate the drug molecules with a wide range of solubility. Addition of cholesterol results in an ordered liquid phase formation which gives the rigidity to the bilayer, and results in less leaky niosomes. Dicetyl phosphate is known to increase the size of vesicles, provide charge to the vesicles, and thus shows increase entrapment efficiency. Other charge-inducers are stearylamine and diacylglycerol that also help in electrostatic stabilization of the vesicles. Niosomes have unique advantages over liposomes. Niosomes are quite stable structures, even in the emulsified form. Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency (Baille et al., 1985 and Yoshioka et al., 1994). They require no special conditions such as low temperature or inert atmosphere for protection or storage, and are chemically stable. Relatively low cost of materials makes it suitable for industrial manufacture. A diverse range of materials have been used to form niosomes such as sucrose ester surfactants and polyoxyethylene alkyl ether surfactants. Niosomes can entrap solutes in a manner analogous to liposomes. Niosomes are osmotically active and stable on their own, as well as increase the stability of the entrapped drugs (Rogerson et al., 1988 and Baille et al., 1984). Niosomes exhibits flexibility in their structural characteristics

(composition, fluidity and size) and can be designed according to the desired situation. Niosomes can improve the performance of the drug molecules by delayed clearance from the circulation, better availability to the particular site, just by protecting the drug from biological environment and by controlled delivery of drug at a particular site.

Method of preparation

Ether injection method

This method was reported in 1976 by Deamer and Bangham, in which a lipid solution in di-ethyl ether was slowly introduced into warm water typically the lipid mixture was injected into an aqueous solution of the material to be encapsulated (using syringe type infusion pump) at 55-65^{o}C and under reduced pressure. Vaporization of ether leads to the formation of single layered vesicles (SLVs) depending upon the conditions used, the diameter of vesicles varies.

Lipid film formation (Hand shaking method)

Surfactant/cholesterol mixture was dissolved in di-ethyl ether in a round bottom flask and ether was removed at room temperature under reduced pressure, in a rotary evaporator. The dried surfactant film was hydrated with aqueous phase at 50-60°C with gentle agitation; this method produces multilamellar vesicles (MLVs) with large diameter.

Sonication method

Aqueous phase was added to the surfactant/cholesterol mixture and the mixture was probe sonicated at 60°C for 3 minutes to produce niosomes.

Microfluidization

This is a recent technique to prepare small MLVS. A Microfluidizer is used to pump the fluid at a very high pressure (10,000psi) through a screen. Thereafter; it is forced along 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 niosomes.

Reverse phase evaporation

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 unilamellar niosomes. The vesicles formed are unilamellar and have a diameter of 0.5 µm.

Recently a great deal of interest is being shown in formulation of proniosomes. Proniosomes are dry formulations of surfactant coated carrier, which on rehydration and mild agitation give niosomes. Proniosomes have the advantage of circumventing the problems of physical stability such as aggregation, fusion and leaking, chemical stability such as hydrolysis, providing the convenience of transportation, distribution, storage and dosing. Proniosomes are usually prepared by dissolving spray coated surfactant in an organic solvent on to inert carriers such as sorbitol and maltodextrin.

Characterization

Niosomes are characterized for different attributes such as vesicle diameter using light microscope, photon correlation microscopy, freeze capture microscopy, entrapment efficiency, and *in vitro* release rate. Other aspects studied are drug stability, drug leakage in saline and plasma on storage, pharmacokinetic aspect, toxicity, etc.

Therapeutic applications of niosomes

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. Some of their therapeutic applications are discussed below.

Targeting of bioactive agents

To reticulo-endothelial system (RES): The cells of RES preferentially take up the vesicles. The uptake of niosomes by the cells is also by circulating serum factors known as opsonins, which mark them for clearance. Such localized drug accumulation has, however, been exploited in treatment of animal tumors known to metastasize to the liver and spleen and in parasitic infestation of liver (Malhotra and Jain, 1994).

To organs other than RES: It has been suggested that carrier system can be directed to specific sites in the body by use of antibodies, Immunoglobulins seem to bind quite readily to the lipid surface, thus offering a convenient means for targeting of drug carrier. Many cells possess the intrinsic ability to recognize and bind particular carbohydrate determinants and this can be exploited to direct carriers system to particular cells.

Neoplasia

Doxorubicin, the anthracyclic antibiotic with broad spectrum anti tumor activity, shows a dose dependant irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma. Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism.

Immunological application of niosomes

Niosomes have been used for studying the nature of the immune response provoked by antigens. Brewer and Alexander have reported niosomes as potent adjuvant in terms of immunological selectivity, low toxicity and stability (Brewer and Alexander, 1992).

Transdermal delivery of drugs by niosomes

Slow penetration of drug through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes. Jayaraman et al has studied the topical delivery of erythromycin from various formulations including niosomes on hairless mouse. From the studies, and confocal microscopy, it was seen that non-ionic vesicles could be formulated to target pilosebaceous glands (Jayaraman et al., 1996).

TRANSFEROSOMES

Liposomal as well as niosomal systems, are not suitable for transdermal delivery, because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation, and fusion of vesicles (Cevc et al., 1997). To overcome these problems, a new type of carrier system called "transferosome", has recently been introduced, which is capable of transdermal delivery of low as well as high molecular weight drugs (Schatzlein et al., 1995). Transferosomes are specially optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Each transferosome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of "edge activators" into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, span 80, and tween 80 have been used as edge activators. These novel carriers are applied in the form of semi-dilute suspension, without occlusion. Due to their deformability, transferosomes are good candidates for the non-invasive delivery of small, medium, and large sized drugs. Transferosomes are vesicles composed by phospholipids as the main ingredient (soya phosphatidylcholine, egg phosphatidylcholine, dipalmityl phosphatidylcholine, etc), 10-25% surfactants for providing flexibility (sodium cholate, tween 80, span-80), 3-10% alcohol as a solvent (ethanol, methanol) and hydrating medium consisting of saline phosphate buffer (pH 6.5-7).

Mechanism of penetration

Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of Stratum corneum. At present, the mechanism of enhancing the delivery of active substances in and across the skin is not very well known. Two mechanisms of action have been proposed (Jain and Jain, 2003 and Paul et al., 1998):

Transferosomes act as drug vectors, remaining intact after entering the skin. Transferosomes act as penetration enhancers, disrupting the highly organized intercellular lipids from Stratum corneum, and therefore facilitating the drug molecules penetration in and across the Stratum corneum.

The recent studies propose that the penetration and permeation of the vesicles across the skin are due to the combination of the two mechanisms. Depending on the nature of the active substance (lipophilic or hydrophilic) and the composition of the transferosomes, one of the two mechanisms prevails.

Method of preparation

Phospholipids, surfactants and the drug are dissolved in alcohol. The organic solvent is then removed by rotary evaporation under reduced pressure at 40ºC. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the appropriate buffer by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated at room temperature to get small vesicles.

Characterization

Visualization of transferosomes can be performed using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Particle size and size distribution can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). The drug entrapment efficiency of transferosomes can be measured by the ultracentrifugation technique. Vesicle stability can be determined by assessing the size and structure of the vesicles over time and drug content can be quantified by HPLC or other spectrophotometric methods. *In vitro* drug release can be measured using a diffusion cell or a dialysis method (Jain et al., and Touitou et al., 2001).

Therapeutic applications of transferosomes

They are used as a carrier for protein and peptides like insulin, bovine serum albumin, vaccines, etc.

Transferosomes improve the site specificity, overall drug safety and lower the doses several times than the currently available formulations for the treatment of skin diseases. Because of their good penetration power and flexibility, transferosomes formulations are used for effective delivery of non-steroidal anti-inflammatory agents like ibuprofen and diclofenac. Transferosomes not only increase the penetration of diclofenac through intact skin, but also carry these agents directly into the depth of the soft tissues under the application site (Cevc, 1996 and Jain et al., 2005).

ETHOSOMES

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin (Akiladevi and Basak, 2010). Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water, which are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers. The size range of ethosomes may vary from tens of nanometers to microns (Touitou, 1996 and Patel, 2007).

Advantages of ethosomal drug delivery

In comparison to other transdermal & dermal delivery systems-

Enhanced permeation of drug through skin for transdermal drug delivery.

- Delivery of large molecules (peptides, protein molecules) is possible.
- Formulation contains non-toxic raw material.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in pharmaceutical, veterinary, cosmetic fields.
- Simple method for drug delivery in comparison to iontophoresis and phonophoresis and other complicated methods.

Mechanism of drug penetration

A possible mechanism for interaction between ethosomes and skin lipids has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect', whereby intercalation of the ethanol into intercellular lipids increases lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosome effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin.

Method of preparation

There are two methods which can be used for the formulation and preparation of ethosomes. Both of the methods are very simple and convenient and do not involve any sophisticated instrument or complicated process.

Hot method

In this method disperse phospholipid in water by heating in a water bath at 400^0C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat upto 400^0C. Add the organic phase into the aqueous phase. Dissolve the drug in water or ethanol depending on its solubility (Touitou, 1996 and Touitou, 1998). The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

Cold method

This is the most common and widely used method for the ethosomal preparation. Dissolve phospholipid, drug and other lipid materials in ethanol in a covered vessel at room temperature with vigorous stirring. Add propylene glycol or other polyol during stirring. Heat the mixture upto 300^0C in a water bath. Heat the water upto 300^0C in a separate vessel and add to the mixture and then stir it for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extent using sonication or extrusion method. Finally, the formulation should be properly stored under refrigeration.

Characterization

Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM), which determines skin penetration. Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta meter, which reflects the stability of vesicles. The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC). The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique. Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method, drug content is important in deciding the amount. The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer. The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Structure changes are observed by TEM. The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM) (Guo et al., 2000, Maghraby et al., 2000, Fry et al., 1978, Cevc et al., 1995, Vanden et al., 1997, Dayan et al., 1879-1885 and Toll et al.,2004).

Therapeutic applications of ethosomes

(Touitou et al., 2000, Touitou et al., 2001, Lodzki et al., 2003, Horwitz et al.,1999, Dkeidek and Touitou, 1999, Paolino et al., 2005, Dubey et al., 2007, Ehab et al., 2007). Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes mainly for the transdermal route of drug delivery. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier.

Table 1: Ethosomes as a carrier for drugs

Drug	Applications	Comments
Acyclovir	Treatment of Herpetic infection	Improved drug delivery
Zidovudine	Treatment of AIDS	Improved transdermal flux
Trihexypenidyl HCl	Treatment of Parkinsonian syndrome	Increased drug entrapment efficiency, reduced side effect & constant systemic levels
Erythromycin	Efficient healing of *S. aureus* -induced deep dermal infections	Improved drug penetration and systemic effect.
Insulin	Treatment of Diabetes	Improved therapeutic efficacy of drug

SPHINGOSOMES

Sphingosomes solve the major drawback of vesicle system (liposomes) 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 (Saraf et al., 2011).

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 (Allen et al., 1995, Kirpotin, 1998 and Mayer et al., 1998). Besides the merits liposome also possess certain problems associated with degradation, hydrolysis and oxidation, sedimentation, leaching of drug aggregation or fusion during storage. Liposome stability problems are of course much more severe therefore it is important 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. Thus, sphingolipid are been nowadays used for the preparation of stable liposomes known as sphingosomes.

Sphingosome may be defined as "concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid". 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. Sphingosomes may be administered orally or transdermally (Webb et al., 1996). 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 preferrably).

Advantages of sphingosomes (Vyas and Khar, 2002)

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

Longer circulation time in plasma delivers more of the therapeutic agent to targeted site over a longer period of time. This new sphingosomal technology increases rigidity of liposomal wall prolongs the circulating life of vesicle and significantly extends the duration of drug release. 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.

Method of preparation

Classical method or mechanical dispersion method

Mechanical dispersion method begins 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 diverse processing parameter (sonication, freeze thawing and high pressure extrusion) in various ways to modify their properties (Vyas and Khar, 2002).

Film method

Film method described by Bangham et al in 1965. In this method the mixture of appropriate amount of lipid are casted as stack of film from 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. 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 includes sonication, french pressure cells and micro emulsification technique.

Transport mechanism of sphingosomes

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 (Jain, 2001).

- Stable adsorption: Stable adsorption represents the association of intact vesicles with the cell surface. Such process is mediated by non-specific electrostatic, hydrophobic or other forces or component present 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 release of vesicle content in to the cytoplasmic space.
- Lipid transfer: Transfer of individual lipid molecular between vesicles and the cell surface without the cell association of aqueous vesicle content.

Therapeutic applications of 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.

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. 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.

Sphingosomes in cosmetic industry

Other therapeutic application of sphingosomes (Vyas and Khar, 2002)

I. Sphingosomes in antimicrobial, antifungal and antiviral (anti-HIV) therapy. E.g. ciprofloxacin, oflaxacin, vancomycin, amoxicillin, amphotericin B, iodoxuridine.

II. Sphingosomes may be used in gene delivery.
III. Sphingosomes may be used in enzyme immobilization.
IV. Sphingosomes may be used in immunology.

PHARMACOSOMES

Pharmacosomes bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicles. Pharmacosomes are the colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of the drug–lipid complex (Vaizoglu and Speiser., 1986). Because the system is formed by linking a drug (pharmakon) to a carrier (soma), they are called pharmacosomes. Pharmacosomes can pass through biomembranes efficiently and possess advantages over the use of other vesicular systems such as transferosome, liposomes, and niosomes. Any drug possessing a free carboxyl group or an active hydrogen atom (–OH, NH_2) can be esterified (with or without a spacer group) to the hydroxyl group of a lipid molecule, thus generating an amphiphilic prodrug, which will facilitate membrane, tissue, or cell wall transfer, in the organism. Any drug with a certain cut- off molecular weight may be formulated as pharmacosomes provided it has active functional groups to integrate with the vesicle-forming amphiphilic molecule.

An amphiphilic prodrug is converted to pharmacosomes upon dilution with water. The conjugate of drug with carrier produces a compound, which is amphiphilic in nature. The aqueous solution of these amphiphiles typically exhibits concentration dependent aggregation, which differs from that of most polar or ionic molecules. At low concentrations the amphiphile exists dispersed in the monomer state. As the monomer are added a critical micelle concentration is reached, leading to dramatic changes in the concentration dependence of many physical parameters, such as osmotic pressure, surface tension and electrical conductivity. The further increment in monomers may lead to variety of structures i.e. micelles of spherical or rod like or disc shaped or bilayered vesicles or cubic or hexagonal phases depending upon physiochemical interactions and thermodynamic variables of amphiphile. The prodrug conjoins hydrophilic and lipophilic properties thereby acquiring amphiphilic characteristics thus reduces interfacial tension, and, at higher concentrations, exhibit mesomorphic behavior. Because of a decrease in interfacial tension, the contact area increases, therefore increasing bioavailability. Pharmacosomes impart better biopharmaceutical properties to the drug, resulting in improved bioavailability. Pharmacosomes have been prepared for various non-steroidal anti-inflammatory drugs, proteins, cardiovascular and antineoplastic drugs. Developing the pharmacosomes of the drugs has been found to improve the absorption and minimize the gastrointestinal toxicity. The idea for the development of the vesicular pharmacosome is based on surface and bulk interactions of lipids with drug. The different membranes in body contain phosphatidylcholine, phosphatidyl-ethanolamine, ceramides and sphingomyeline. These resemble lipid prodrugs in physicochemical structure. Therefore, it can be expected that pharmacosomes can interact with biomembranes enabling a better transfer of active ingredient. This interaction can also change the phase transition temperature of biomembranes, thereby improving the membrane fluidity leading to enhanced permeation. Pharmacosomes are designed to avoid the usual problems associated with the liposomal entrapment of polar drug molecules like low drug incorporation, leakage and poor stability.

The salient features of pharmacosomes

(Biju et al., 2006) Pharmacosomes provide an efficient method for delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects and also

reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs.

- Pharmacosomes are suitable for incorporating both hydrophilic and lipophilic drugs. The aqueous solution of these amphiphiles exhibits concentration dependent aggregation.
- Entrapment efficiency is not only high but also predetermined, because drug itself in conjugation with lipids forms vesicles and covalently linked together.
- Unlike liposomes, there is no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.
- Since the drug is covalently linked, loss due to leakage of drug, does not take place. However, loss may occur by hydrolysis.
- No problem of drug incorporation.
- Encaptured volume and drug-bilayer interactions do not influence entrapment efficiency, in case of pharmacosome. These factors on the other hand have great influence on entrapment efficiency in case of liposomes
- The lipid composition in liposomes decides its membrane fluidity, which in turn influences the rate of drug release, and physical stability of the system. However, in pharmacosomes, membrane fluidity depends upon the phase transition temperature of the drug lipid complex, but it does not affect release rate since the drug is covalently bound.
- The drug is released from pharmacosome by hydrolysis (including enzymatic).
- Phospholipid transfer/exchange is reduced, and Solublization by HDL is low.
- The physicochemical stability of the pharmacosome depends upon the physicochemical properties of the drug-lipid complex.
- Due to their amphiphilic behavior, such systems allow, after medication, a multiple transfer through the lipophilic membrane system or tissue, through cellular walls piggyback endocytosis and exocytosis.
- Following absorption, their degradation velocity into active drug molecule depends to a great extent on the size and functional groups of drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized in vivo pharmacokinetics.
- They can be given orally, topically, extra-or intravascularly.
- The volume of inclusion does not influence entrapment efficiency as in case of liposomes where volume of inclusion has great influence on entrapment efficiency.

Method of preparation

There are two methods which have been employed to prepare vesicles:

Hand-shaking method

In the hand-shaking method, the dried film of the drug–lipid complex (with or without egg lecithin) is deposited in a round-bottom flask and upon hydration with aqueous medium, readily gives a vesicular suspension.

Ether-injection method

In the ether-injection method, an organic solution of the drug–lipid complex is injected slowly into the hot aqueous medium, wherein the vesicles are readily formed. An alternative approach for producing pharmacosomes was recently reported in which a biodegradable micelle-forming drug conjunct was synthesized from the hydrophobic drug Adriamycin and a polymer composed of polyoxyethylene glycol and polyaspartic acid. This approach has the benefit that although it may be possible to dilute out the micelle, the drug will probably not precipitate because of the water solubility of the monomeric drug conjunct (Lawrence, 1994). Muller-Goymann and Hamann produced fenoprofen pharmacosomes using a modified technique that involved diluting lyotropic liquid crystals of amphiphilic drugs. Approaches have been done to attach drugs such as β-blockers to various glyceride-like groups and the resulting amphiphilic molecules have been spontaneously dispersed. They were labeled pharmacosomes because of their tendencies to form unilamellar vesicles. It was suggested that these molecules should enhance lymph transport. Zhang et al. optimized the preparation of 3', 5'-dioctanoyl-5-fluoro-2'-deoxyuridine pharmacosomes and found that the drug-phosphatidylcholine ratio, glycerol tristearate concentration and pluronic F-68 concentration, have an influence on the mean particle size, entrapment ratio, and drug loading (Zhang et al,. 2001).

Characterization

Similar to other vesicular systems, pharmacosomes are characterized for different attributes such as size and size distribution, nuclear magnetic resonance (NMR) spectroscopy, entrapment efficiency, *in vitro* release rate, stability studies, scanning electron microscopy, differential scanning calorimetry etc.

Therapeutic applications of pharmacosomes (Jain, 2003)

Bupranolol hydrochloride

The prodrug consisting of β-blocker bupranolol which is covalently linked to 1, 3-dipalmioyl-2-succinyl-glycerol. The resulting prodrug was amphipathic and dispersed readily in water above 30°C forming a smectic lamellar phase. The dispersion, similar to charged phospholipids showed continuous swelling with increasing water content and so in excess water region, the thermodynamically most stable structure was the unilamellar vesicles while oligomeric vesicles also formed. Enhanced effect on intraocular pressure in rabbits was observed after incorporation in pharmacosomes.

1-β-D-arabinofuranosylcytosine

MacCoss et al (1982) synthesized various amphipathic liponucleotide prodrugs, 1-β-D-arabinofuranosylcytosine 5'-diacylglycerols (containing either dimyristoyl, dipalmitoyl distearoyl free fatty acid side chains) and determined the aggregational and morphological characteristics of their sonicated dispersions in relation to change in temperature. The sonication at

low temperature gave turbid solutions containing large bilayer sheets. On rising the temperature, a transition temperature (t_g) was reached at which a stable three dimensional cross-linked network of small interlocking bilayer stacks was formed. Sonication at the temperature close to t_g produced small disc-shaped micellar structures. These micelles were shown to exist in another aggregational equilibrium consisting of stacking- destacking process. In contrast, the prodrug containing unsaturated fatty acid chain gave multilamellar liposomes. Improved biological activity after incorporation of drug into pharmacosomes was found in case of Taxol and Cytarabin.

Pindolol

Vaizoglu and Speiser, 1986 synthesized glycerol monostearate ester of weak base, pindolol via spacer (succinic acid) and isolated maleate salt as two isomers. This prodrug having structural similarity with lysolecithin reduced the interfacial tension between benzene/water and opalescent vesicular dispersion could be obtained by the film method and ether injection method. It was combined with tween-80 and MLVs could be prepared by the film method. Tween-80 was not found necessary for dispersion in preparing liposomes by the injection method. The freshly prepared pharmacosomes showed a particle size of 200nm and 240nm determined by transmission electron microscopy and photon correlation spectroscopy respectively, which upon sonication reduced to 90nm.The in-vivo fate of these vesicles, was studied in Beagle dogs by administering the vesicles and parent drug through oral and intravenous route and determining the plasma concentrations of unchanged pindolol. Plasma drug proile followed the one compartment open model,indicating rapid hydrolysis in body fluids. Three to five times higher concentrations of unchanged pindolol were observed following intravenous administration of the pharmacosomes rather than free pindolol. Urine data indicated lowering of renal clearance when pindolol was administered as pharmacosomes.

CONCLUSION

Vesicular systems have been realized as extensively useful carrier systems in various scientific domains. 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. In spite of certain drawbacks (fusion, aggregation), pharmacosomes still play an important role in the selective targeting, and the controlled delivery of various drugs. Further advantages of the vesicular system can be exploited by expanding this approach to additional drugs. Furthermore, the effect of covalent linkages and addition of spacer group on in vivo hydrolysis rate and subsequent pharmacokinetics is to be studied, in order to exploit more advantages of this system. Researches continue to put in their efforts in improving the vesicular system by making them steady in nature, in order to prevent leaching of contents, oxidation and their uptake by natural defense mechanisms. The system yet requires greater efforts towards investigating the non-bilayer phases and exploring the mechanism of action.

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