WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1694-1716. Research Article ISSN 2277– 7105

NOVEL MULTIPARTICULATE DRUG DELIVERY SYSTEM: A VERSATILE CONTROLLED RELEASE CARRIER FOR HYDROPHOBIC DRUGS

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Article Received on
05 May 2015,

Revised on 30 May 2015,
Accepted on 24 June 2015

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ABSTRACT

The development of safe and effective models for the delivery of any prophylactic or therapeutic agent remains an uphill task for pharmaceutical formulation developers. Drug molecules, nucleic acids, carbohydrates, proteins and a variety of other biological and chemical entities are used for attaining pharmacological benefits. However, the major challenge remains in the delivery of these agents to the specific site of action in a time-efficient manner. Among the many drug delivery systems developed, the nano scale technology of Lipospheres, cubosome, virosomes, ethosomes and transferosomes are the novel approaches. All these novel drug delivery systems are having their application with merits. And that why they all are widely used for newer approaches.

KEYWORDS: NDDS, Liposomes, Trasferosomes, Cubosome, Virosomes, Ethosomes, Controlled Release Drug delivery system.

TRASFEROSOMES

A Trasferosomes is a highly adaptable and stress-responsive. This enables the Trasferosomes to cross various transport barriers efficiently, and act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents.

INTRODUCTION

Transdermal drug delivery systems (TDDS) offer a number of potential advantages over conventional methods such as injectable and oral delivery. However, the major limitation of

TDDS is the permeability of the skin, it is permeable to small molecules, lipophilic drugs and highly impermeable to macromolecules and hydrophilic drugs. The main barrier and rate-limiting step for diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum (SC).

Recent approaches in modulating vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues. These approaches have resulted in the design of two novel vesicular carriers, ethosomes and ultra-flexible lipid-based elastic vesicles, transfersomes. Transfersomes are ultra-deformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. Transfersomes have recently been introduced, which are capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes are specially optimized, ultra-flexible lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact and then act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. Transfersomes 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 chlorate, sodium deoxychlorate, Span 80, and Tween 80, have been used as edge activators. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium, and large sized drugs.

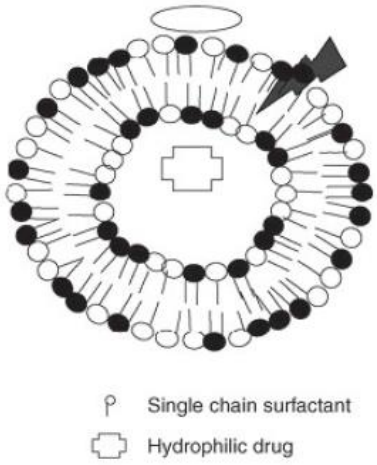


Figure1: Transferosomes

ADVANTAGES

Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.

1. They have high entrapment efficiency, in case of lipophilic drug near to 90%.
2. This high deformability gives better penetration of intact vesicles.
3. They can act as carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
4. Transfersomes possess infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
5. They act as depot, releasing their contents slowly and gradually.
6. They can be used for both systemic as well as topical delivery of drug.
7. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
8. They protect the encapsulated drug from metabolic degradation.
9. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.

DISADVANTAGES

1. Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
2. Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles.
3. Transfersomes formulations are expensive.

FORMULATION

Table 1: Different additives used in transferosomes

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline,eggphosphatidyl choline,dipalmitoyl phosphatidyl	Provides Flexibility
Surfactant	Sod.cholate,Sod.deoxycholate,Tween	Provides Flexibility
Alcohol	Ethanol, methanol	As a solvent
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium
Dye	Rhodamine-123 Rhoda mine-DHPE	For Confocal Scanning Laser study

METHOD OF PREPARATION

Rotary evaporation sonication method

- The lipid mixture (500mg) consisted of Phospholipase H 100, edge activator (surfactant) and drug (10 mg/ml) in different ratios was dissolved in an organic solvent mixture consisted of chloroform and methanol (2:1, v/v).
- Then placed in a clean, dry round bottom flask.
- The organic solvent was carefully evaporated by rotary evaporation under reduced pressure above the lipid transition temperature to form a lipid film on the wall of the flask.
- The final traces of the solvents were removed by subjecting the flask to vacuum overnight. The dried thin lipid film deposited on the wall of the flask was hydrated with a phosphate buffer solution (pH 6.4) by rotation for 1hr at room temperature at 60 rpm.
- The resulting vesicles were sonicated for 30 min in a bath to reduce the size of the vesicles & then stored at 4 C. Different edge activators in different molar ratios were used for the formulation of transfersomes.
- The liposomes (Phospholipid H 100: cholesterol, 7:3) that act as a control in the present study were prepared by the same method as described above.

EVALUATION PARAMETERS

% Entrapment Efficiency (EE %)

- The % Entrapment Efficiency of transfersomes was determined after separation of the non-entrapped drug.
- Entrapment efficiency in transfersomal formulations can be determined by Freeze thawing/centrifugation method.
- 1 ml samples of transfersomal dispersion were frozen for 24 hrs at −20 °C in eppendorff tubes.
- The frozen samples were then removed from the freezer and let to thaw at room temperature, then centrifuged at 14,000 rpm for 50 min at 4 °C.
- Transfersomal pellets were re-suspended in PBS (pH 6.4) and then centrifuged again.
- This washing procedure was repeated two times to ensure that the un-entrapped drug was no longer present.
- The supernatant was separated each time from transfersomal pellets and prepared for the assay of free drug.

- The drug content was determined spectrophotometrically using phosphate buffer saline (PBS) (pH 6.4) as a blank.
- The entrapment efficiency was defined as the percentage ratio of the entrapped drug concentration to the total drug concentration and calculated according to the following equation:

  % Entrapment Efficiency =

  (Total drug concentration – Free drug concentration/ Total drug concentration) X 100

Differential scanning calorimetry measurements (DSC)

- The DSC thermograms were recorded on a Schimadzu- DSC 50 differential scanning calorimetry.
- DSC was carried out for drug powder as well as for the plain transfersomes and drug loaded-transfersomes in the ratio of 90:10% (w/w).
- The analysis was performed on 40 µl or 1-mg samples sealed in standard aluminium pans. Thermograms were obtained at a temperature range from 0-300ºC and a scanning rate of 10 ◦C/min under nitrogen atmospher.
- Phosphate buffer (pH 7.4) was employed as reference.

In vitro drug release from transfersomes

- The in-vitro release of drug loaded transfersomes through an artificial cellophane membrane was determined by a simple dialysis method.
- The receptor medium was 100 ml PBS (pH 6.4) which was maintained at 37±0.2 ◦C and constantly stirred at 100 rpm in a thermostatically controlled water bath shaker.
- Transfersomes pellets equivalent to drug was placed in the donor compartment.
- Samples of specified volume were withdrawn from the receptor compartment at 0.5, 1, 2, 4, 6, 8 and 24 hrs.
- Intervals, and immediately replaced with an equal volume of fresh receptor solution.
- Triplicate experiments were conducted for each study and sink conditions were always maintained throughout the experiment.
- All samples were analyzed for NYSTATIN content spectrophotometrically against PBS (pH 6.4) as a blank.

Transmission electron microscopy (TEM)

- The surface appearance and shape of drug loaded transfersomes were analyzed by taking TEM photographs using transmission electron microscope.

- The transfersomes were dispersed in water and one drop of the diluted dispersion was placed on a carbon coated grid.
- The dispersion was left for 2 min, to allow its absorption in the carbon film, and the excess liquid was drawn off with filter paper. Subsequently, a drop of 2% ammonium molybdate was placed on the grid.
- The excess was removed with distilled water and the samples were examined by TEM.

***Ex-vivo* skin permeation studies**

Preparation of a rabbit skin

- Abdominal full-thickness skin was carefully removed from animals after sacrificing them.
- The hair was removed from the abdominal skin with the aid of an electric animal clipper and shaver.
- Care was taken not to damage the skin surface.
- The fat was removed with the aid of scissor and skin was washed and the excised full thickness rabbit skin samples were stored at 20ºC prior to use.
- The excised full thickness abdominal rabbit skin samples were equilibrated by soaking in buffer solution of pH 6.4 containing 0.02% sodium azide as preservative at 4±1ºC for about one hour before beginning of each experiment.

In Vitro permeation studies

- The transfersomal formulation which exhibites the highest % entrapment efficiency was chosen for *ex-vivo* permeation studies of drug through abdominal rabbit skin in comparison to liposomes, plain drug and commercial product (Nystatintatin ® cream, PHARAONIA).
- The in-vitro permeation of NYSTATIN from different formulations was determined by using a dissolution-dialysis apparatus.
- The shaved abdominal rabbit skin was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment and the dermal side facing downward to the receptor compartment with permeation area of 6.61 cm^2.
- The receptor compartment was then filled with 100 ml of PBS (pH 6.4) containing 0.02% sodium azide as preservative.
- The temperature of media was maintained at 37±0.5ºC. Amounts equivalent of drug of the tested transfersomal formulation were applied to the skin surface.

- The glass cylinder was attached to the dissolution apparatus by using parafilm and stirred at 100 rpm.
- 4 ml samples of the solution in the receptor compartment were removed for drug content determination at different time intervals up to 24 hrs. and replaced immediately with an equal volume of fresh receptor fluid every time.
- Samples were analyzed spectrophotometrically .The cumulative amount of drug permeated through abdominal rabbit skin per unit area (µg/cm2) was plotted against time (hrs.).
- The permeation parameters of NYSTATIN as steady-state transdermal fluxes (JSS), permeability coefficient (Kp) through the abdominal rabbit skin and enhancement ratio (ER) were calculated from the penetration data.
- The steady-state transdermal fluxes (JSS) of NYSTATIN through the abdominal rabbit skin were calculated from the slope of linear portion of the cumulative amount of drug permeated through unit area of the abdominal rabbit skin (ug/cm2) versus time plot.
- The permeability coefficient (kp) through the abdominal rabbit skin was calculated according to the following equations:

  Kp = JSS /Cd

  ER= Kp of test formulation / kp of plain drug

  Where Cd = the initial drug concentration in the donor compartment.

Skin retention study

- The ability of transfersomes to help retain the encapsulated drug within the skin (depot-effect) in comparison to liposomes, plain drug and commercial product was investigated by determining the amount of drug retained in the skin samples at the end of permeation studies.
- After performing the above mentioned *ex-vivo* permeation study for 24 hours, skin mounted on the diffusion cell was removed. The remaining formulation adhering to the skin was scraped with a spatula.
- The skin was cleaned with cotton piece dipped in saline solution and then gently dried by pressing between two tissue papers to remove any adhering formulation.
- Subsequently, the cleaned skin sample was mechanically shaked with 100 ml of PBS (pH 6.4) in water shaker bath at 37±0.5oC for 1 hr for complete extraction of the drug.
- The filtrate was removed and the drug content in the filtrate was determined spectrophotometrically at 306 nm using UV spectrophotometer.

APPLICATIONS

Table 2: Different additives used in transferosomes

S.No	Name of drug	Inference
1	Curcumin	Better permeation for anti-inflammatory activity
2	Indinavir sulfate	Improved influx for activity against acquired immune deficiency syndrome (AIDS)
3	Ketoprofen	Improved penetration for anti-inflammatory activity
4	Insulin	induce therapeutically significant hypoglycemia with good efficacy and reproducibility
5	Capsaicin	Increase skin penetration
6	Colchicine	Increase skin penetration
7	Vincristine	Increase entrapment efficiency and skin permeation
8	Interferon-α	Efficient delivery means (because delivery other route is difficult). Controlled release. Overcome stability problem.
9	Norgesterol	Improved transdermal flux
10	Tamoxifen	Improved transdermal flux
11	Methotrexate	Improved transdermal flux
12	Oestradiol	Improved transdermal flux
13	Tetracaine, Lignocaine	Suitable means for the noninvasive treatment of local pain on direct topical drug application.
14	Corticosteroids	Improved site specificity and overall drug safety.
15	Hydrocortisone	Biologically active at dose several times lower than currently used formulation.
16	Triamcinolone acetonide	Used for both local and systemic delivery.
17	Human serum albumin	Antibody titer is similar or even slightly higher than subcutaneous injection.
18	Stavudine	Improved the in vitro skin delivery of Stavudine for antiretroviral activity
19	Tetanus toxoid	For transdermal immunization

ETHOSOMES

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids, ethanol (in higher quantity) and water. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns (μ).

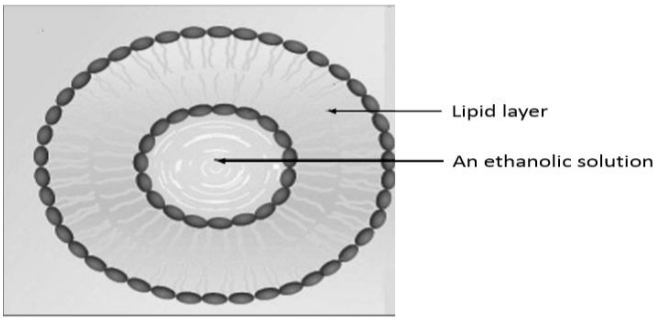


Figure 2: Ethosomes

INTRODUCTION

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Da can pass through it. To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Unlike classic liposomes, that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier.[4,5] Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes.

ADVANTAGES

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods

7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.

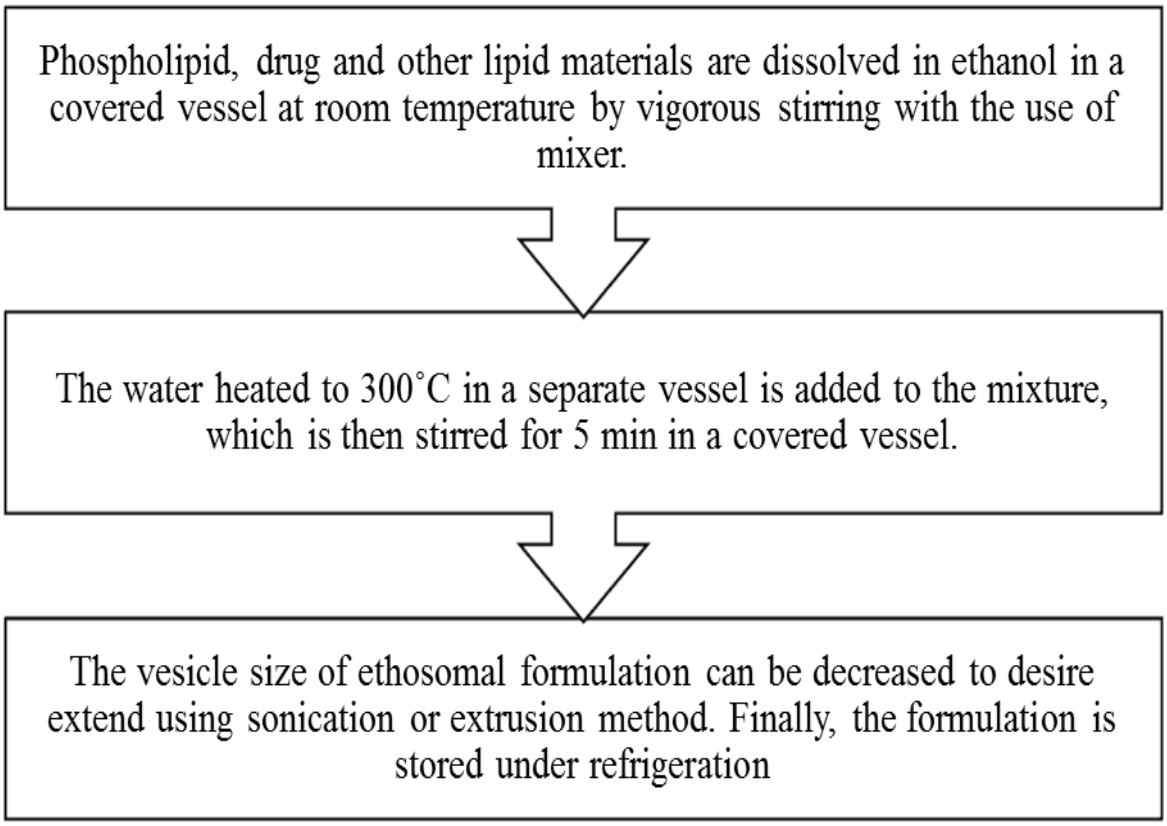
FORMULATION

Table 3: Different additives used in transferosomes

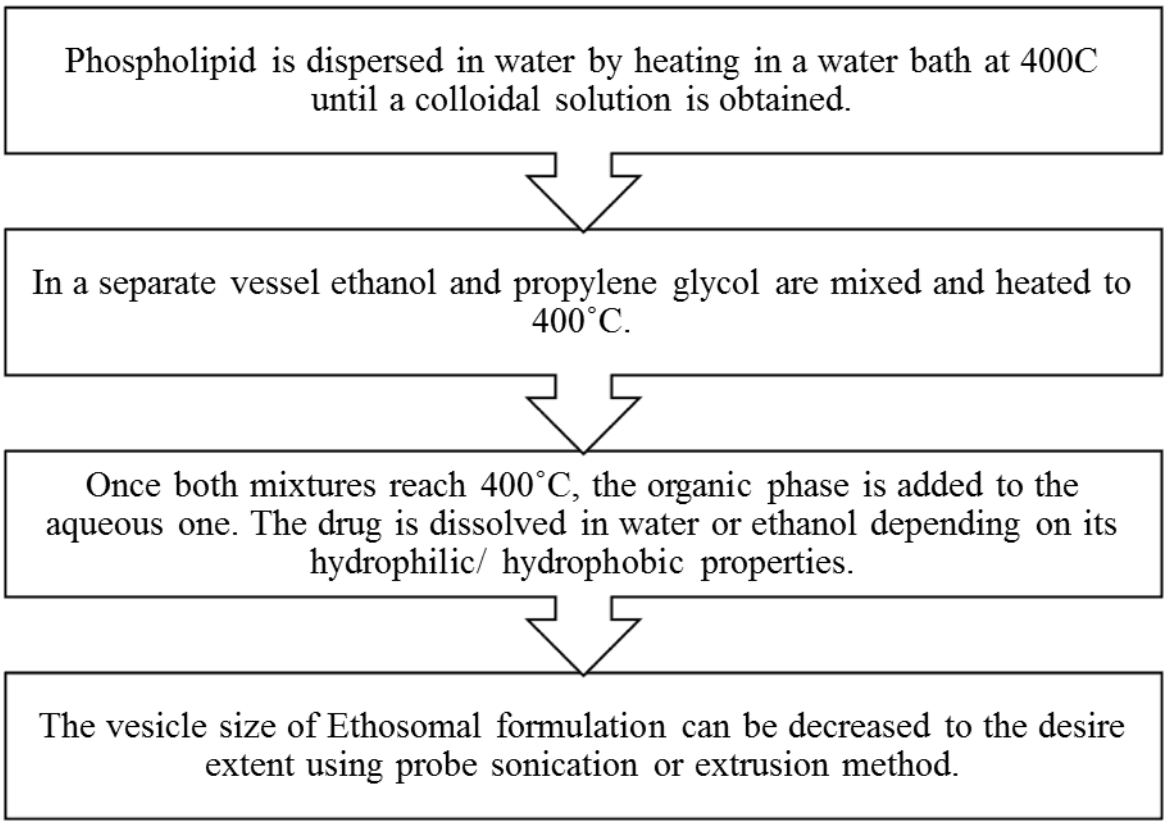
Class	Example	Uses
Phospholipids	Soya phosphatidyl choline,eggphosphatidyl choline,dipalmitoyl phosphatidyl choline	Provides Flexibility
Surfactant	Sod.cholate,Sod.deoxycholate,Tween	Provides Flexibility
Alcohol	Ethanol, methanol	As a solvent
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium
Dye	Rhodamine-123 Rhodamine-DHPE	For Confocal Scanning Laser study

METHODS OF PREPARATION ETHOSOMES

Cold Method



1. **Hot method**



EVALUATION PARAMETERS

1. **Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy**

- Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells.
- The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5).
- The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water).
- Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany).

2. **Vesicle-Skin Interaction Study by Fluorescence Microscopy**

- Fluorescence microscopy was carried according to the protocol used for TEM and SEM study.
- Paraffin blocks are used, were made, 5-µm thick sections were cut using microtome (Erma optical works, Tokyo, Japan) and examined under a fluorescence micro

Cytotoxicity Assay cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L Lglutamine at 37°C under a 5% CO_2 atmosphere.

- Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at540 nm.

3. Vesicle-Skin Interaction Study by TEM and SEM

- From animals ultra thin sections were cut (Ultracut, Vienna, Austria), collected and examined under transmission electron microscope.
- For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater.
- The sections were examined under scanning electron microscope.

4. HPLC Assay

- The amount of drug permeated in the receptor compartment during *ex-vivo* skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water :acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1 mL/min by LC 10-AT vp pump (Shimadzu, Kyoto, Japan).
- A 20µl injection was eluted in C-18 column (4.6×150 mm, Luna, 54, Shimadzu) at room temperature.
- The column eluent was monitored at 271 nm using SPDM10A vp diode array UV detector.

5. Drug Uptake Studies

- The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µL medium was added.
- Cells were incubated with 100 µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

6. *Ex vivo* Skin Permeation Method

- The excised skin was placed on aluminium foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue.

- The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm^2 and 10 mL, respectively.
- The temperature was maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 mL of pH 6.5).
- Excised skin was mounted between the donor and the receptor compartment.
- Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin.
- Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1, 2, 4, 8, 12, 16, 20, and 24 hour time intervals and analyzed by high performance liquid chromatography 1(HPLC) assay.
- The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel.

7. Stability Study

- Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180.

APPLICATIONS

- Delivery of Anti-Viral Drugs
- Topical Delivery of DNA
- Transdermal Delivery of Hormones
- Delivery of anti-parkinsonism agent
- Transcellular Delivery
- Delivery of Anti-Arthritis Drug
- Delivery of Problematic drug molecules
- Delivery of Antibiotics

LIPOSHPERES

Liposphere formulation is an aqueous micro dispersion of solid water insoluble spherical micro particles of particle size between 0.01 and 100 μm in diameter. The lipospheres are made of solid hydrophobic triglycerides with a monolayer of phospholipids embedded on the surface of the particle.

INTRODUCTION

Liposphere formulation is appropriate for oral, parenteral and topical drug delivery system. The solid core containing a drug dissolved or dispersed in a solid fat matrix and used as carrier for hydrophobic drugs. Several techniques, such as solvent emulsification evaporation, hot and cold homogenization and high pressure homogenization have been used for the production of liposphere In addition, use of lipospheres for oral administration, it can protect the drug from hydrolysis, as well as improve drug bioavailability (Domb et al., 1996). Therefore, the present review articles focused on achievements of lipospheres formulation to deliver the drugs in the targeted sites.

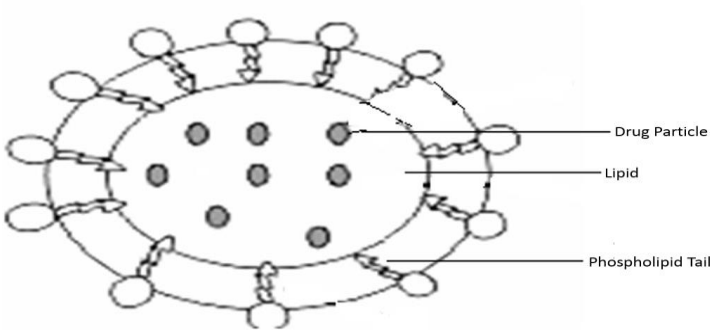


Figure 3: Lipospheres

ADVANTAGES

1. Solid lipid nanoparticles are nanosized lipid carriers in which lipidic core contain the drug in dissolved or dispersed state. These systems were designed to substitute polymeric carriers due to the inherent toxicity.
2. High dispersibility in an aqueous medium. Lipospheres are lipid based dispersion systems in which drug is dissolved or dispersed in lipidic core, the surface of which is embedded with emulsifier layer. Particle size of such lipid particles ranges from 0.2-100 micrometer (μm).
3. Extended release of entrapped drug after single injection.
4. Lipospheres exhibit enhanced physical stability due to avoidance of coalescence
5. Ease of preparation and scale up.
6. Low cost of ingredients.
7. High entrapment of hydrophobic drugs.
8. Controlled particle size.

9. Improving drug stability
10. Possibility for controlled drug release
11. Controlled particle size
12. High drug loading.
13. Reduced mobility of incorporated drug molecules responsible for reduction of drug leakage, circumvention of instabilities due to interaction between drug molecules and emulsifier film.
14. Static interface facilitates surface modification of carrier particles after solidification of the lipid matrix.
15. Lipospheres well comply with the needs of the drug development process, as for instance safety, stability, different application's fields (pharmaceutical, veterinary, cosmetic as well as food additives) and administration pathways (oral, mucosal and topical delivery), ease of modifying the release of APIs, taste masking ability, rapidity and availability of several processing techniques. Moreover, advances in solvent free process technologies have greatly improved the potential for successful lipid based formulations without surfactants included.

FORMULATION

The formulation of lipospheres approach utilizes naturally occurring biodegradable lipid constituents. The internal hydrophobic core of lipospheres is composed of lipids, especially triglycerides, while the surface activity of liposphere is provided by the surrounding phospholipid layer. The neutral fats, stabilizers are additionally used in the preparation of the hydrophobic core of the lipospheres.

Table 4: Different additives used in Lipospheres.

Lipid	Stabilizers
Glyceryl monostearate Glyceryl monooleate Ethyl stearate Trilaurin Tristearin Tribehenin Tripalmitin Trimiristine Cetyl alcohol Cholesterol Stearic acid Hydrogenated vegetable oil	Gelatin 200 Bloom Pectin Carrageenan Carrageenan Carrageenan Polyvinyl alcohol Polyoxyethylene sorbitan trioleate Pluronic PE 8100 Lauryl sarcosine

Some biodegradable polymers are also used in the preparation of polymeric lipospheres to enhance the stability of lipospheres, which includes:

• Low molecular weight poly (lactic acid)

• Poly (caprolactone).

The phospholipids are used to form the surrounding layer of lipospheres includes.

• Phosphatidylethanolamine (PE).

• Soybean phosphatidylcholine (PCS)

• Dimyristoyl phosphatidylglycerol (DMPG)

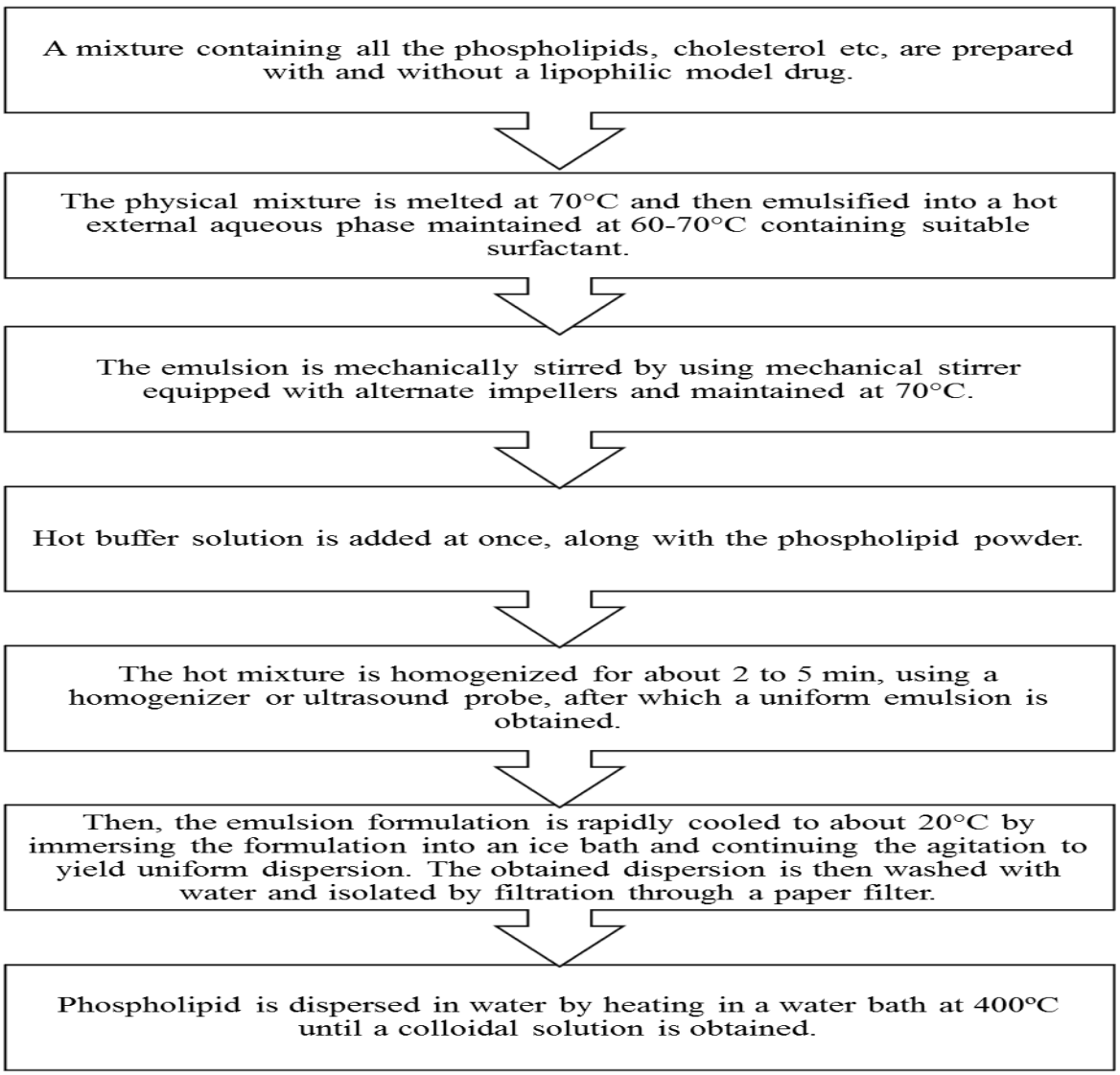
• Pure egg phosphatidylcholine (PCE)

• Food grade lecithin (96% acetone insoluble)

• Lipospheres for topical and veterinary applications.

METHODS PO PREPARATION

Melt dispersion technique



Solvent emulsification-diffusion technique

- In solvent emulsification-diffusion technique, the solvent used (e.g. benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, methyl acetate) must be partially miscible with water and this technique can be carried out either in aqueous phase or in oil.
- Initially, both the solvent and water were mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquid.
- When heating is required to solubilise the lipid, the saturation step was performed at that temperature.
- Then the lipid and drug were dissolved in water saturated solvent and this organic phase (internal phase) was emulsified with solvent saturated aqueous solution containing stabilizer (dispersed phase) using mechanical stirrer.
- After the formation of o/w emulsion, water (dilution medium) in typical ratio ranges from 1:5 to 1:10, were added to the system in order to allow solvent diffusion into the continuous phase, thus forming aggregation of the lipid in the nanoparticles.
- Here both the phase were maintained at same elevated temperature and the diffusion step was performed either at room temperature or at the temperature under which the lipid was dissolved.
- Throughout the process constant stirring was maintained. Finally, the diffused solvent was eliminated by vacuum distillation or lyophilization.

Solvent evaporation technique

- This technique is an alternative to the melt dispersion technique and it is considered with the objective of possibly minimizing the exposure to high temperatures of thermolabile compounds, such as proteins and nucleic acids.
- This technique is based on the evaporation of organic solvent in which lipids are dissolved and allowing the formation of solid microparticles.
- In particular, the lipidic matrix is dissolved in an organic solvent such as ethyl acetate and maintaining the temperature at about 50-60°C and then emulsified with an external aqueous phase containing the surfactant.
- The resulting oil-in-water emulsion is stirred form 6 to 8 hr untill complete evaporation of the solvent.
- The dispersion are recovered by filtration through a filter paper, dried and stored.

Rotoevaporation method

- In this technique, lipid solution with drug is prepared in a round bottom flask containing 100 gram of glass beads (3 mm in diameter) mixed thoroughly till a clear solution is obtained.
- Then, the solvent is evaporated by using rotoevaporizer under reduced pressure at room temperature and a thin film is formed around the round bottom flask and the glass beads.
- Raise the temperature upto 40°C until complete evaporation of the organic solvent.
- Known amount of 0.9% saline is added to the vessel and the contents are mixed for 30min at room temperature and then the temperature is lowered to 10°C by placing in ice bath and mixing is continued for another 30min until lipospheres are formed.

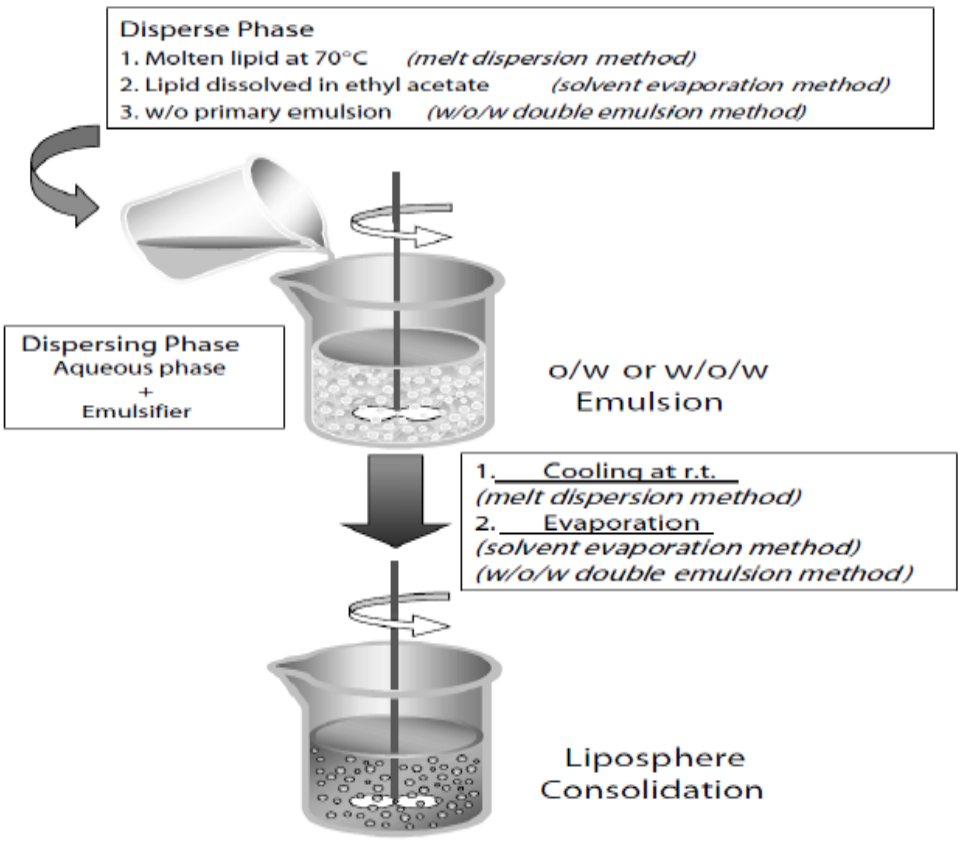


Figure 4: Schematic representation of the methods of production of Lipospheres by melt dispersion and solvent evaporation.

Multiple microemulsion method

- This method in which a solution of peptide is dispensed in stearic acid melt at 70ºC followed by dispersion of this primary emulsion into aqueous solution of egg lecithin, butyric acid and taurodeoxycholate sodium salt at 70ºC.

- Rapid cooling of multiple emulsion formed solid lipospheres with 90% entrapment of peptide.
- Sustained release is reported by multiple emulsification technique with inclusion of lipophilic counter ion to form lipophilic salt of peptide.
- Polymeric lipospheres have also been reported by double emulsification for encapsulation of antigen.

Sonication method

- In this technique, the drug is mixed with lipid in a scintillation vial which is pre-coated with phospholipids.
- The vial is heated until the lipid melts, and then vortexed for 2min to ensure proper mixing of the ingredients.
- A 10 ml of hot buffer solution is added into the above mixture and sonicated for 10min with intermittent cooling until it reaches to the room temperature.

EVALUATION

A. Measurement of particle size and zeta potential

- Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size.
- PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by particle movement.
- This method covers a size range from a few nanometers to about 3 microns. PCS is a good tool to characterize nanoparticles.
- Electron Microscopy provides, in contrast to PCS and LD, direct information on the particle shape.
- The physical stability of optimized dispersion is generally more than 12 months. ZP measurements allow predictions about the storage stability of colloidal dispersion.

1. Photon Correlation Spectroscopy (PCS)

- It is an established method which is based on dynamic scattering of laser light due to Brownian motion of particles in solution/suspension.
- This method is suitable for the measurement of particles in the range of 3 nm to 3 mm.
- The PCS device consists of laser source, a sample cell (temperature controlled) and a detector.

- Photomultiplier is used as detector to detect the scattered light. The PCS diameter is based on the intensity of the light scattering from the particles.

2. Electron Microscopy

- Electron Microscopy methods such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to measure the overall shape and morphology of lipid nanoparticles.
- Permits the determination of particle size and distributions. SEM uses electrons transmitted from the surface of the sample while TEM uses electrons transmitted through the sample.

3. Atomic Force Microscopy (AFM)

- It is an advanced microscopic technique which is applied as a new tool to image the original unchanged shape and surface properties of the particles.
- AFM measures the force acting between surface of the sample and the tip of the probe, when the probe is kept in close proximity to the sample which results in a spatial resolution of up to 0.01 nm for imaging.

B. X-ray diffraction (powder X-ray diffraction) and differential scanning calorimetry (DSC)

- The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus permitting the degree of crystallinity to be assessed.
- Another method that is a little different from its implementation with bulk materials, DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies.

C. Determination of Incorporated Drug

- Amount of drug incorporated in lipospheres influences the release characteristics hence it is very important to measure the amount of incorporated drug.
- The amount of drug encapsulated per unit wt. of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium and this separation can be done by ultracentrifugation, centrifugation filtration or gel permeation chromatography.

- The drug can be assayed by standard analytical technique such as spectrophotometer, a spectroflurophotometry, HPLC or liquid scintillation counting.

D. Rheology

- Rheological measurements of formulations can perform by Brookfield Viscometer, using a suitable spindle number.
- The viscosity depends on the dispersed lipid content. As the lipid content increases, the flow becomes non-Newtonian from Newtonian.

E. Nuclear magnetic resonance (NMR)

- NMR can be used to determine both the size and the qualitative nature.
- The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle.

F. Entrapment efficiency

- Amount of drug loaded into lipospheres can be determined by first extracting the free drug (not encapsulated) by centrifugation into a suitable buffer solution.
- The encapsulated drug is then determined by dissolution-extraction of drug loaded Microparticles in a solvent which can dissolve the Microparticles completely

APPLICATIONS

1. The use of lipid nanocarriers provides a suitable way for the nasal delivery of antigenic molecules. In this sense, the design of optimized vaccine nanocarriers offers a promising way for nasal mucosal vaccination (Almeida and Souto, 2007).
2. Lipid nanoparticles have been extensively studied for the delivery of proteins and peptides.
3. For intravenous administration, the small particle size is a prerequisite as passage through the needle and possibility of embolism.
4. Lipospheres offer the opportunity of controlled drug release and the possibility to incorporate poorly soluble drugs (Attama et al., 2012).
5. The application of lipids as vehicle for the delivery of drugs has revolutionarized drug delivery with some old drugs with very serious side effects being safe for use. Some anti-inflammatory drugs for example, indomethacin, (SLM, SEFs) have shown that indomethacin lipid formulations could be used with minimal gastro-intestinal irritation.

6. Some anti-inflammatory drugs for example, indomethacin, (SLM, SEFs) have shown that indomethacin lipid formulations could be used with minimal gastro-intestinal irritation.
7. Lipospheres have been exploited for the delivery of anesthetics like lidocaine bupivacaine for the parenteral delivery of antibiotics like ofloxacin, norfloxacin, chloramphenicol palmitate and oxytetracycline and antifungal agents, such as Nystatintatin and amphotericin B ; for the parenteral delivery of vaccines and adjuvant.
8. Properties of lipospheres like film forming ability, occlusive properties; controlled release from solid lipid matrix resulting in prolonged release of drug and retarded systemic absorption of drugs; increasing the stability of drugs which are susceptible to extensive hepatic metabolism, make them attractive candidates for topical delivery .
9. Several categories of drugs like antibiotics, anti-inflammatory compounds, vasodilators, anticancer agents, proteins and peptides are being formulated as oral lipospheres.
10. These provide a novel and unique drug-delivery system they evade quick clearance by the immune system. They can target specific cells. Stealth lipospheres have been successfully tested in animal models with marker molecules and drugs. Antibody labelled stealth Lipobodies have shown increased delivery to the target tissue in accessible sites.
11. Agricultural application includes essential oil extraction from Artemisiaarboreseens L when incorporated in lipspheres, were able to reduce the rapid evaporation compared with emulsions and the systems have been used in agriculture as a suitable carrier of ecologically safe pesticides.

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