COMPREHENSIVE REVIEW ON NOVEL PHOSPHOLIPID BASED NOVEL VESICULAR SYSTEM: TRANSFERSOMES

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Abstract

Transferosomes are one of the novel vesicular systems for transdermal delivery of active substance. They are capable of transdermal delivery of drug with low and high molecular weight with uniqueness of accommodating hydrophilic, lipophilic and ampiphilic nature of molecules. These are second generation of flexible liposomes consisting of phospholipid and edge activator. Advantages of transferosomes are high entrapment efficacy, high deform ability, use for both systemic as well as topical, biocompatible, biodegradable, protects drug from degradation and easy to sale up as procedure of preparation is simple. System can be characterized by vesicle shape and size, entrapment efficacy, degree of deformability, number of vesicles per cubic mm, drug content, permeability, turbidity measurement, surface charge-density, in-vitro drug release, in vitro skin permeation studies, physical stability etc. Its application areas included delivery of NSAID, steroids, insulin, interferosomes, interleukin, transdermal immunization, peripheral drug targeting, carrier for other proteins and peptides etc.

Keywords: Transferosomes, liposomes, Transdermal delivery, phospholipid and edge activator

Introduction:

NDDS is the most suitable drug delivery system in which developing the therapeutic efficacy of preexisting as well as new drugs [Chandrakala et al., 2014. Hadgraft et al., 1989. Gros et al., 1980. Wokovich et al., 1989]. Novel vesicular systems allow the drug to control or sustain the release of conventional medicines [Tyle et al., 2003. El-Maghraby et al., 2009].

Oral drugs are degraded in variable pH condition of the gastrointestinal tract (GIT). They also experience first pass metabolism. In case of parenteral, disadvantages are lacks drug reversal, risk of infection, hypersensitivity reaction, emboli and cost. Some oral drugs are bitter in taste, problem with swallowing and in case of parenteral pain due to needle makes these route of administration less patient's compliance. Considering these problems, attention has been focused on more advantageous topical route of administration. Transdermal drug delivery system (TDDS) is used as potent route for the delivery of systemic action of drug [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013.Madhumitha et al., 2020].

Vesicular system is getting importance due to their ability to act as a means of sustained release of drug. Vesicular approach such as liposomes, niosomes, ethosomes and transferosomes has the potential to overcome the skin barrier and have reported to enhance permeability of drug. Transferosomes are ultra deformable vesicle possessing

an aqueous core surrounded by complex lipid bilayer. [shabana et al., 2015. Chorasiya et al., 2019. Rai et al., 2017. Solanki et al., 2016. Zaafarany et al., 2010]. Vesicular systems reduces the cost of therapy by improving the bioavailability of drugs especially poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. Vesicular drug delivery systems reduces toxicity, reduces dose related side effects, high therapeutic efficacy of drugs for longer periods of time [Cevc et al., 1998. Modi et al., 2012]. The encapsulation of drug in vesicular systems is predicted to prolong the drug in systemic circulation. [Cevc et al., 1998].

The term transferosomes and concept was introduced in 1991 by Gregor Cevc. Transferosomes is a term registered trademark by German company IDEA AG. The name means “carrying body" and is derived from Latin word ' transfere' means 'to carry across' and Greek word 'soma' meaning 'a body'. Transferosomes are complex vesicles that have highly flexible and self regulating membrane which results the vesicles more deformable [Prajapati et al., 2011. Rajan et al., 2011]. Transfersome is special types of liposomes. They overcome the skin penetration problem with squeezing themselves along the intracellular sealing lipid of the stratum corneum. It penetrates the stratum corneum by either intracellular or trans cellular route by generation of osmotic gradient due to evaporation of water. Thus, transferosomes vesicles when applied on an open biological surface that is non-occluded skin, tends to penetrate its barrier and migrate into the water rich deeper strata [Piumitali et al., 2020. Fernandez-Garcia et al., 2020. Chouhan et al., 2017].

Flexible or elasticity of transferosomes membrane is achieved by mixing suitable

edge activator (surface active component) in proper ratio. When applied on skin, it exploits hydrophilic pathway or pores between the cells, where it opens wide enough to permit entire vesicle to pass through stratum corneum along with drug molecule. Transferosomes vesicles can cross micro porous barrier efficiently, even if the porous are much smaller than vesicle size. Thus transferosomes carrier is an artificial vesicle designed be like a cell vesicle and thus suitable for controlled and potentially targeted drug delivery [Venkatesh et al., 2014. Jain et al., 2003.Walve et al., 2011. Vijayalaxmi et al., 2015].

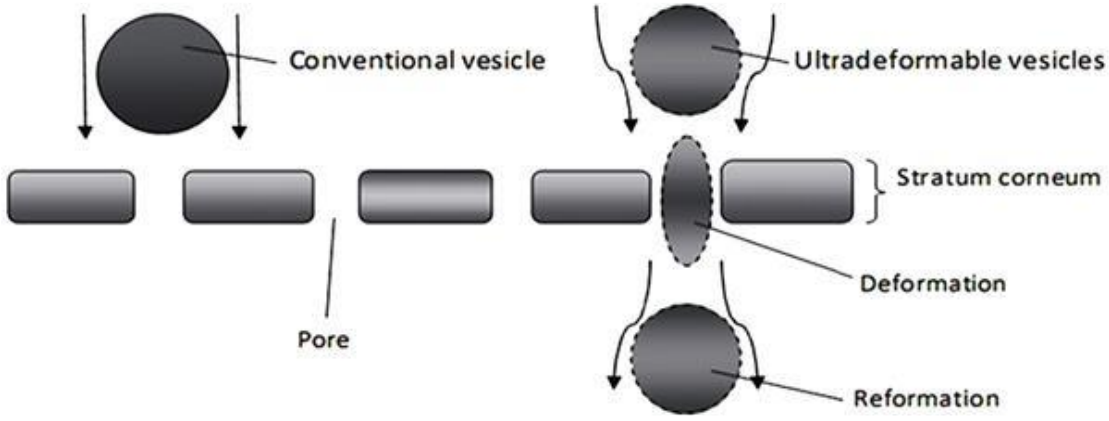

Figure 1 Mechanism of action of Transferosmes

Advantages: [Chourasiya et al., 2019. Reddy et al., 2015.]

- Delivers a study infusion of drug over extended period of time
- Can act as carrier for low as well as high molecular weight drugs
- Biocompatible and biodegradable as they are made up of natural phospholipid
- High deformability gives better penetration of intact vesicle
- High entrapment efficacy
- Release their content slowly and gradually and can be use for systemic and topical delivery of drug.

- More flexible and adaptable compared with liposomes
- Can accommodate hydrophilic, hydrophobic as well as ampiphilic drug molecule
- Protect the encapsulated drug from metabolic degradation
- Easy to scale up, as procedure is simple

Disadvantage: [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013.Madhumitha et al., 2020]

- Drugs which required high blood levels cannot be administered
- Barrier function of the skin changes from one site to another on same person, from person to person and also with age
- Drug must be potent because patch size limits amount that can be delivered
- Skin irritation or hypersensitivity reaction may occur
- Transferosomes are chemically unstable because of oxidative degradation make it predisposition
- Purity of natural phospholipids is another criteria
- Transferosomes formulations are expensive.

Mechanism of transport: [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013.Madhumitha et al., 2020]

At present, the mechanism of enhancing the delivery of active substance in and across the skin is not very well known. Proposed mechanisms are

- Transferosomes act as drug vector.
- Transferosomes acts as penetration enhancer, disrupting the highly organized

intracellular lipids.

Mechanism is complex and involved advanced principle of mechanism combined with material transport and hydration/ osmotic force.

Material for transferosomes: [Sharma et al., 2015. Jadupati et al., 2012. Subhash chandran et al., 2018.Kumar et al., 2015]

Transferosomes composed of phospholipid like phosphatidyl choline which self assembles into lipid bilayer in aqueous environment and closes to form vesicles. Biocompatible surfactant or an ampiphilic drug is added to increase bi layer flexibility and permeability. This second component is called as edge activator.

Edge activator consists of single chain surfactant that cause destabilization of lipid bilayer increasing its elasticity and fluidity.

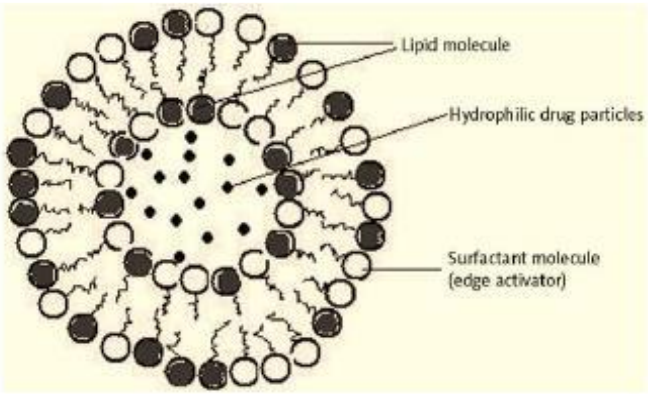

Figure 2 Structure of Transferosmes

Class	Example	Uses
Phosphpolipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline, disteroyl phosphatidyl choline	Vesicle forming agents
Surfectant	Sodium cholate, sodium deoxy cholate, tween 80, span 80	Flexibility
Alcohol	Ethanol, methanol	solvent
Buffering agents	Saline phosphate buffer pH(6.4)	Hydrating medium
Dyes	Rhodamine-123, rhodamine DHPE, flourescein DHPE	CSLM study

Table No.1 Composition of Transferosomes

A. **Thin film hydration technique** is employed for the preparation of transfersomes which comprised of three steps: [Chourasiya et al., 2019. Reddy et al., 2015. Rajan et al., 2011]

1. A thin film is prepared from the mixture of phospholipids and surfactant by dissolving in volatile organic solvent (chloroform-methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 500C for dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight.

2. A prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature.

3. To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min. using a bath sonicator or probe sonicated at 4°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

B. Modified hand shaking, lipid film hydration technique is also founded for the preparation of transfersomes which comprised following steps: : [Chourasiya et al., 2019. Reddy et al., 2015. Rajan et al., 2011]

1. Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent.

2. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minutes at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8°C.

Characterization of Transferosomes: [Thakur et al., 2018. Sharma et al., 2012. Ali net al., 2020. Pena-Rodriguez et al., 2020]

1. Entrapment efficiency: [Gamal et al., 1999. Malakar et al., 2012]

The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the un-entrapped

drug by use of mini-column centrifugation method. After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

Entrapment efficiency = amount entrapped * 100 / total amount added

2. Drug content: [Fry et al., 1978]

The drug content can be determined using modified high performance liquid chromatography method (HPLC) method with help of a UV detector, column oven, auto sample, pump, and computerized analysis program depending upon the analytical method of the pharmacopoeial drug.

3. Vesicle morphology: [Subhash chandran et al., 2018. Modi et al., 2012]

Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements. Transfersomes vesicles can be visualized by TEM, phase contrast microscopy, etc. The stability of vesicle can be determined by assessing the size and structure of vesicles over time. Mean size is measured by DLS and structural changes are observed by TEM.

4. Vesicle size distribution and zeta potential: [Subhash chandran et al., 2018. Modi et al., 2012]

Vesicle size, size distribution and zeta potential were determined by Dynamic Light Scattering Method (DLS) using a computerized inspection system by Malvern

Zetasizer.

5. No. of vesicles per cubic mm: [Gamal et al., 1999. Malakar et al., 2012]

This is an important parameter for optimizing the composition and other process variables. Non-sonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study. The Transfersomes in 80 small squares are counted and calculated using the following formula:

Total number of Transfersomes per cubic mm = Total number of Transfersomes counted × dilution factor × 4000

6. Degree of deformability or permeability measurement: [Fry et al., 1978]

Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension).Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements.

7. Turbidity measurement: [Fry et al., 1978. Walb et al., 2009]

Turbidity of drug in aqueous solution can be measured using nephelometer.

8. Surface charge and charge density: [Malakar et al., 2012]

Surface charge and charge density of transfersomes can be determined using zeta sizer.

9. Penetration ability: [Malakar et al., 2012]

Penetration ability of Transfersomes can be evaluated using fluorescence microscopy

10. Occlusion effect: [Walb et al., 2009]

Occlusion of skin is considered to be helpful for permeation of drug in case of traditional topical preparations. Hydrotaxis (movement in the direction) of water is the major driving force for permeation of vesicles through the skin, from its relatively dry surface to water rich deeper regions. Occlusion affects hydration forces as it prevents evaporation of water from skin.

11. Physical stability: [Walb et al., 2009]

The initial percentage of the drug entrapped in the formulation was determined and were stored in sealed glass ampoules. The ampoules were placed at 4 ± 2^0C (refrigeration), 25 ± 2^0C (room temp), and 37 ± 2^0C (body temp) for at least 3 months. Samples from each ampoule were analyzed after 30 days to determine drug leakage. Percent drug lose was calculated by keeping the initial entrapment of drug as 100%.

12. In-vitro drug release: [Gamal et al., 1999. Subhash chandran et al., 2018]

In vitro drug release study is performed for determining the permeation rate. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

13. In-vitro Skin permeation Studies: [Sheo et al., 2010. Patel et al., 2009.]

Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50cm^2 was used for this study. In vitro drug study was performed by using goat skin in phosphate buffer solution (pH 7.4). Fresh Abdominal skin of goat were collected from slaughterhouse and used in the permeation

experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-4^{0}C.

To perform skin permeation study, treated skin was mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50cm^2 and capacity of receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37± 0.5^{0}C and stirred by a magnetic bar at 100RPM. Formulation (equivalent to 10mg drug) was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The samples were analyzed by any instrumental analytical technique.

Application of transferosomes:

1. **Delivery of**
    - large molecule / drugs: Interferon, Insulin [pandey et al., 2009]
    - Anticancer drug[Hasibi et al., 2019. Rai et al., 2017]:Methotrexate [Sadarani et al., 2019], paclitaxel[Khan et al., 2019], 5-FU[Zhang et al., 2015], Toxifolin [Hasibi et al., 2019].
    - Herbal drugs[Rose et al., 2016. Sarwa et al., 2016. Anwar et al., 2017.

Zesioreani et al., 2017. Pavaloiy et al., 2020.]: Capsaicin

- NSAIDS : Acceclofenac [dudhipala et al., 2020]. Ketorolac [Prakash et al., 2019. Zhang et al., 2020].
- Anaesthetic[Omar et al., 2019. Bnyana et al., 2019]: Lidocaine[Omar et al., 2019]
- Anti-oxidant: resveratrol [Wu et al., 2019]
- Antidiabetic: Repaglinide [Vijayalaxmi et al., 2015]
- Antifungal: Flucanzole [tejaswini et al., 2016], Tacrolimus[Lei et al., 2013], Natamycin[Janga et al., 2019]
- Osteoprosis treatment: Roloxifen [Ashlesha et al., 2020. Mahmood et al., 2018]
- Sexual dysfunction: Sildenafil [Sayyad et al., 2017]
- Hypretension: Eprosartan[Ahad et al., 2017]
- Anti-leishmaniasis: Miltefosine [Dar et al., 2020]
- Antiviral: Lamivudine [Sudhakar et al., 2016], indinavir [Sheo et al., 2010]
- Ocular Delivery: aetazolamide[Eman et al., 2020]

2. **Carrier for**
   - Interferon and interleukin ex. IL-2, INF-alpha [Natsheh et al., 2020]
   - Protein and peptides ex. Human serum albumin [Zhenga et al., 2020. Celia et al., 2012. Kala et al., 2014]
3. **Transdermal immunization** ex. Hepatitis B vaccine[Gupta et al., 2010]
4. **Peripheral drug targeting** [Natsheh et al., 2020]

Conclusion:

Transdermal route of drug administration is not allowing transport of high molecular weight drugs and therapeutic agents. Transferosomes are designed such a way that they can squeeze themselves through skin pores irrespective of molecular weight. Transferosomes which are ultra deformable vesicles, allow enhanced permeation of drug through skin. Composition of transferosomes vesicle is safe, advantageous with fewer demerits. These ensure reproducible and efficient transcutaneos carrier and drug target. Transferosomes accommodate drug molecules with wide range of solubility. Transferosomes, thus differ from other vesicles by its softer, more deformable, better adjustable artificial membrane.

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