Review Article

TRANSFEROSOMES: Vesicular Carrier for both Hydrophilic and Lipophilic Drugs

Abstract:

The vesicular system has accessed significant importance towards a sustained drug delivery system in recent years. This article was designed to review the future of vesicular systems known as transferosomes. The vesicles are softer and highly deformable than liposomes.

The word Transferosomes is derived from Latin and Greek words called "Transferre" and "soma," respectively, where Transferre means "carry across" and Soma means "a body."

Transferosomes vesicles are carrying bodies for targeted transdermal drug delivery systems. It consists of phospholipid and an edge activator.

In transferosome, phospholipids vesicles act as transdermal drug carriers. It generates an osmotic gradient at the stratum corneum and helps the transferosomes penetrate through the stratum corneum by the transcellular route.

Transferosomes have a wide range of solubility, have a remarkable ability to penetrate through the skin, are compatible and biodegradable, and are highly expensive. But they are easily prone to chemical degradation and are highly expensive.

The transfersomes were formulated by handshaking, vortex, and freeze-thaw techniques.

Transferosomes have various Evaluation parameters such as Vesicle size distribution, the morphology of vesicle, Number of vesicles per cubic mm. Transferosomes have various applications like they can be applied in controlled release and sustained release formulation, transportation of lower and higher molecular weight drug molecules, target drug delivery system, and transdermal immunization.

Transferosomes have been ultra-deformable vesicles capable of subjecting a futuristic solution to all vesicular delivery and conventional delivery complications. It can carry any drug's molecular weight through the skin; due to its ultra-deformable characteristics feature, therefore, they are widely used to take protein and peptides. After doing all the evaluation studies, we can say that Transferosomes are the promising candidate for the vesicular system and can replace other novel vesicular systems. Thus it holds a bright future in novel drug delivery systems.

Keywords: *modified liposomes, vesicular delivery system, phospholipid, edge activator.*

1. INTRODUCTION [1-3]

In most cases, the conventional delivery system and some vesicular delivering systems like liposomes and niosomes cannot provide successful and productive treatment due to many reasons like first-pass metabolism, side effects, rejection of invasive therapy, and abysmal patient compliance. To overcome such problems, a novel approach was developed in the foam of transferosomes.

In 1991, transferosomes were first reported by Gregor Cevc as "self-adapting highly-deformable and flexible bilayer vesicles composed of phospholipids and surfactant." It is considered a modified version of liposomes. They differ from liposomes, as these Transferosomes are softer and highly deformable than liposomes.

The word Transferosomes is derived from Latin and Greek words called "Transferre" and "soma," respectively, where Transferre means "carry across" and Soma means "a body."

They act as artificial carriers and are designed in such a way so that they mimic at the cellular level, which makes them suitable for controlled and targeted drug delivery systems. They are complex vesicles with highly flexible and automated membranes (self-optimizing and self-adapting property of vesicular membrane), making them more deformable. The carrier aggregate consists of two main

components: liposomes, first phospholipids (an amphipathic molecule). When added into carrier water, it gets converted into a bilayer structure by self-assembly process and eventually foams a lipid vesicle. The second is a Bilayer softening agent (Surfactant), which helps to improve the permeability and flexibility of bilayer lipid vesicle. These vesicles can infiltrate through micro-porous barriers efficiently, even if the vesicles are more giant. They are self-optimized aggregates with ultra-flexible membranes, which can deliver the drug through the skin inter or intra- cellular membrane. Transferosomes are highly elastic and flexible, which helps them to overcome the skin penetration difficulty, which can be achieved by confining themselves within the intracellular sealing lipid layer of the stratum corneum.
The flexible nature of Transferosomes can prevent the risk of vesicle rupture in the skin. They can spontaneously infiltrate the stratum corneum via the transcellular route.

2. ADVANTAGES AND DISADVANTAGES OF TRANSFEROSOMES[4-5]

2.1 ADVANTAGES-

- *They can accommodate both hydrophilic and lipophilic drug substances due to hydrophilic and hydrophobic moieties.*
- *They can pass through narrow pore diameter (5-10 times lesser than their diameter) due to the deformable nature of Transferosomes.*
- *They act as a carrier for any molecular weight of a drug substance.*
- *They are biocompatible and biodegradable.*
- *The percentage of drug entrapment of transferosomes is around 90% in the case of hydrophilic drugs.*
- *They provide protective encapsulation of drugs from degradation.*
- *No extra additives are required.*
- *Useful for systemic and topical administration of drug substances.*
- *Chemically inert and non-toxic.*
- *They are the delivery of choice for sustained drug deliver*

2.2 DISADVANTAGES-

- *Chemically unstable and easily prone to oxidative degradation.*
- *Expensive*
- *The purity of phospholipids cannot be achieved in transferosomes.*

3. COMPOSITION OF TRANSFEROSOME[6-7]-

*Transferosomes consists of two main components. These are as follows, the structure of transferosomes is given in **figure 1**.*

- *Amphipathic molecule (Phospholipids), E.g., Phosphatidylcholine, to foam bilayer lipid vesicles.*
- *Edge activator (10-25% of Surfactant), which acts as a bilayer-softening agent and increases the flexibility and permeability of vesicles.*

- *Other components like solvents (4-10% ethanol) and an aqueous medium like water or phosphate buffer (pH-6.8-7).*

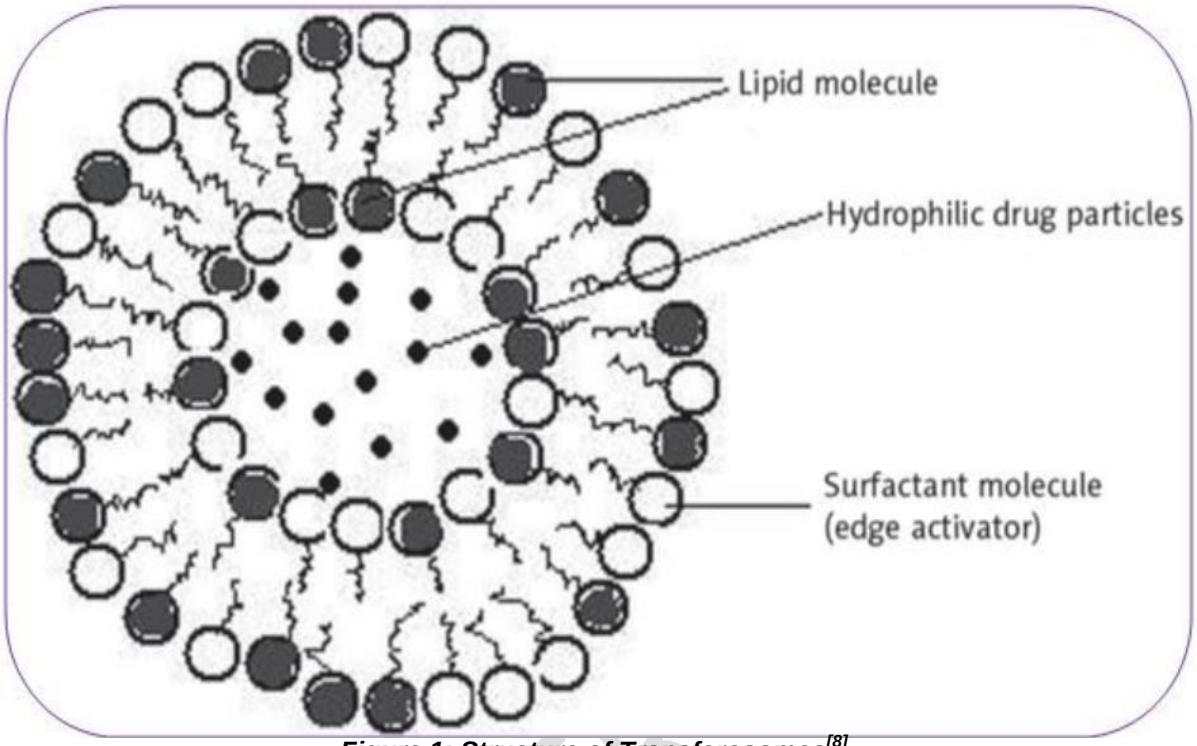

Figure 1: Structure of Transferosomes[8].

The composition required for the preparation of transferosomes is summarized in ***table 1****,*

TABLE 1- The formulation for the preparation of transferosomes

Ingredients	***Examples***	***Functions***
Phospholipids (Lecithin)	*Soya Phosphatidylcholine* *Egg Phosphatidylcholine*	*The main component of transferosome vesicle.*
Edge activator (Surfactant)	*Sodium deoxy Cholate* *Tween 80* *Span 80*	*The main component of transferosome vesicle.* *Provide flexibility*
Alcohol	*Ethanol* *Methanol*	*Solvent*
Dye	*Rhodamine-123* *Rhodamine-DHPE* *Nil red*	*For Confocal Scanning Laser Microscopy Study*
Buffering Agent	*Saline phosphate buffer (PH 6.5)* *7% v/v ethanol*	*hydrating medium*

4. MECHANISM OF ACTION[9-10]:

The Osmotic gradient exiting across different layers of skin is a crucial parameter for the improved skin penetration of Transferosomes.

There is less water content at the upper surface of the skin layer, and high-water content is present at the deeper skin layer. This difference in water content in different layers of the skin creates an osmotic gradient between different layers of skin.

Transferosome penetrates the deeper layer of the skin due to the development of osmotic gradient and also the edge activator or bilayer softening agents, which helps to deform the shape of the bilayer lipid layer (squeeze the bilayer lipid vesicle); due to this property, they can penetrate the layer of skin although been more significant in size.

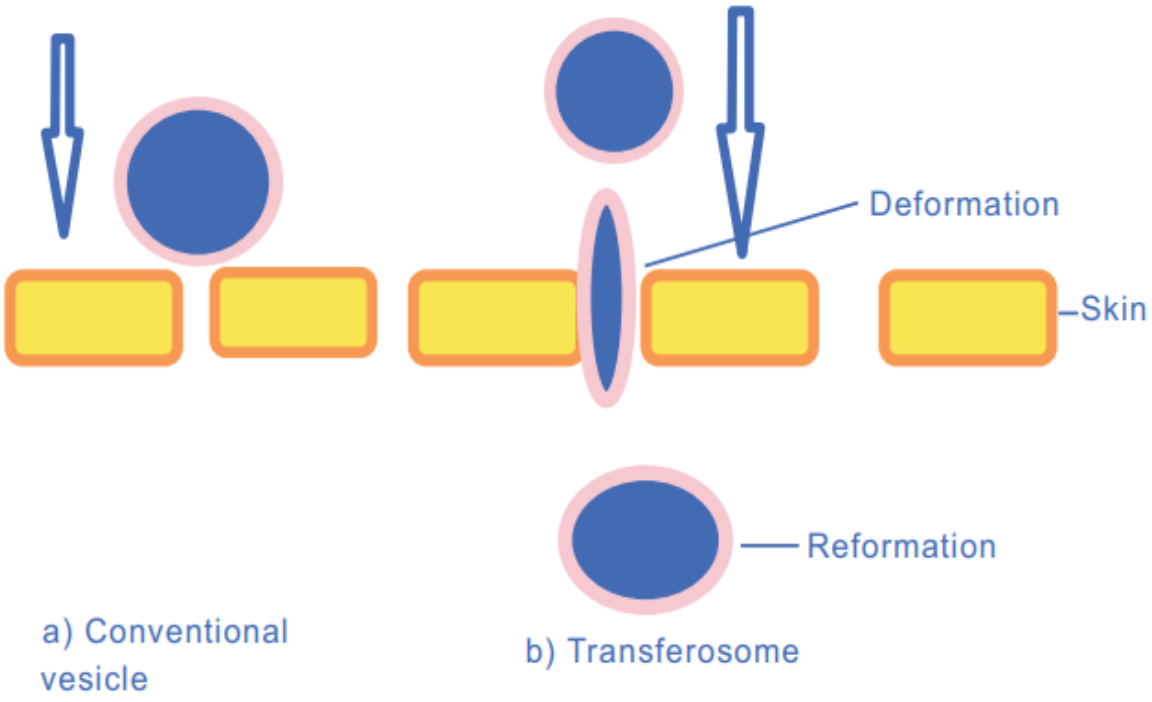

Figure 2: Diagrammatic representation of penetration of Transferosomes[11].

5. IDEAL CHARACTERISTICS OF TRANSFEROSOMES [12]:

- *It should be able to accommodate both hydrophilic and lipophilic drug substances.*
- *It should penetrate easily through the different layers of skin.*
- *They mimic as carriers for lower and higher molecular weight drug substances.*
- *They should be biocompatible and biodegradable.*
- *Chemically inert.*
- *Non-toxic and non-irritant.*
- *They should have protective action for drug substances.*

6. PREPARATION TECHNIQUES FOR TRANSFEROSOMES[13, 14]:

6.1. Hand Shaking Technique-*The technique is also known as the Rotary film evaporation technique, introduced by Bangham. This technique requires two main components, Phospholipid, and surfactant (edge activator), to prepare thin film vesicles (multi-laminar vesicle MLVs). The diagrammatic representation of this method is explained in* ***figure 3****.*

1. *Phospholipids and edge activators dissolved in an organic solvent (ethanol).*

2. *Evaporation of organic solvent occurs above the lipid transition temperature (50°C) using a Rotary evaporator, and the residual solvent is discarded by vacuum.*
3. *The thin film is hydrated using phosphate buffer pH- 6.5, containing a drug substance.*
4. *To prepare a small vesicle, these thin-film vesicles are sonicated at 4ºC for 30 minutes using a bath or probe sonicator.*
5. *The sonicated vesicle was homogenized by the extrusion process ten times.*

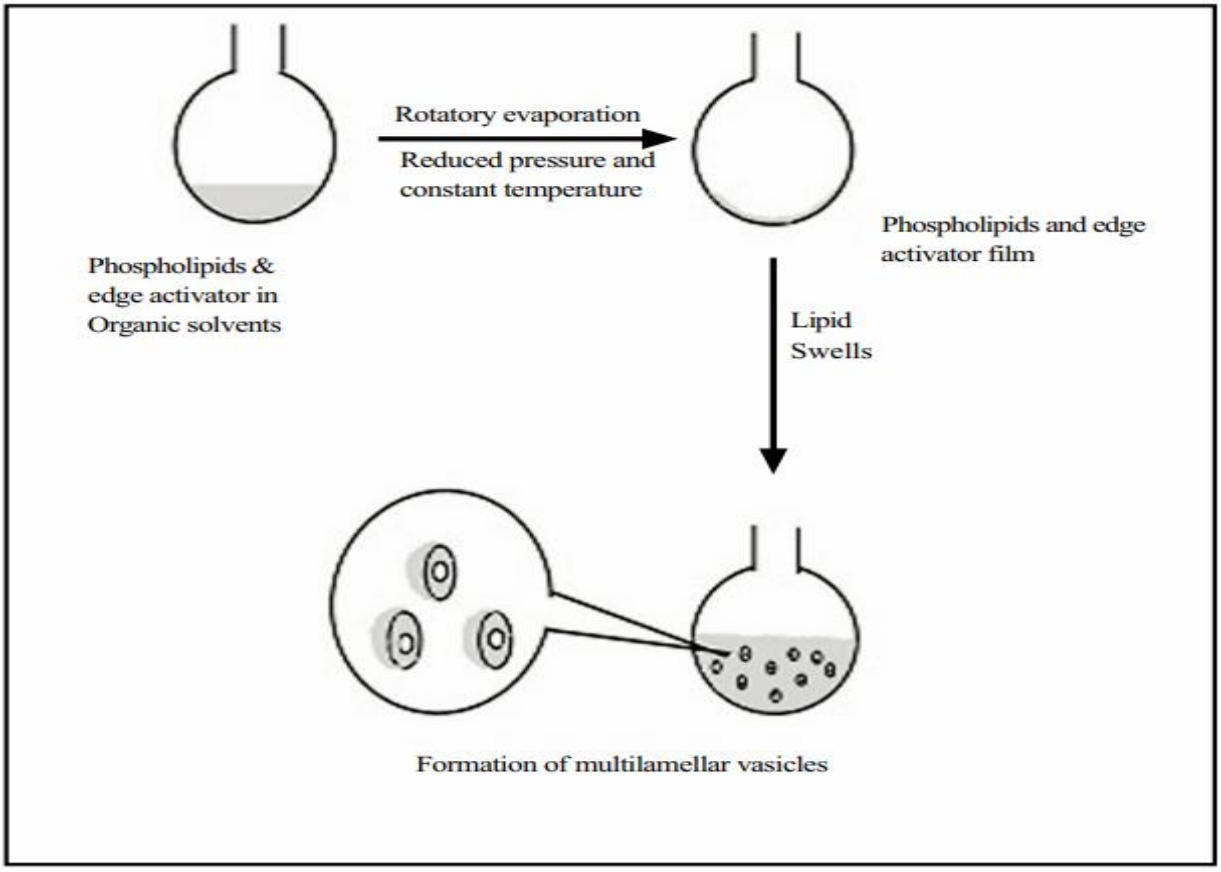

Figure 3: Handshaking technique [15]

6.2. Modified Handshaking method-

- *Required amount of Lecithin (phospholipid), surfactant (edge activator), and the drug were taken in the first beaker, and in the second beaker, the required amount of chloroform and ethanol was taken in 1:1 ratio respectively.*
- *Then the contents in the first beaker are dissolved into the second beaker.*
- *Now the entire mixture is kept for evaporation at a temperature above the transition temperature of lipid, to remove the organic solvent.*
- *The thin lipid film formed due to evaporation is kept overnight for the complete removal of organic solvent.*
- *The thin film is then hydrated with buffer followed by gentle shaking for 15 minutes.*
- *The suspension formed is further hydrated at 80°C for 1-2 hours. The method is summarized in the following flowchart given below.*

Lecithin + surfactant+ drug + Chloroform + ethanol (1:1}

↓ *Mixture is kept for evaporation*

Thin film formed was hydrated with buffer for 15 minutes.

↓

Suspension formed is further hydrated at 80^{O}C for 1-2 hour.

6.3. Reverse Phase Evaporation Technique: *The following steps for this method are as follow-*

1. *Phospholipids are dissolved in an organic solvent taken into the round bottom flask.*
2. *Edge activator (surfactant) is added into water under nitrogen purging.*
3. *Based on drug solubility, it can be added into water or an organic solvent.*
4. *Sonication is done until it forms a homogeneous mixture, and the mixture should remain homogenously for at least thirty minutes after sonication.*
5. *The organic solvent is then discarded under reduced pressure, due to which it gets converted to a viscous gel-like substance followed by vesicle formation.*
6. *Finally, the non-residual solvents and non-encapsulated material are discarded by the process of dialysis or centrifugation.*

6.4. Sonication Technique: *In this technique,*

1. *Phospholipid & surfactant (Edge activator) are blended vigorously by shaking and agitation to suspend the mixture into phosphate buffer containing the drug.*
2. *The suspension formed is then sonicated by using a bath sonicator or vortex.*
3. *Then, they are further squeezed out through membranes of different sizes to obtain desired size vesicles. The schematic diagram of this method is explained in* ***figure 4****.*

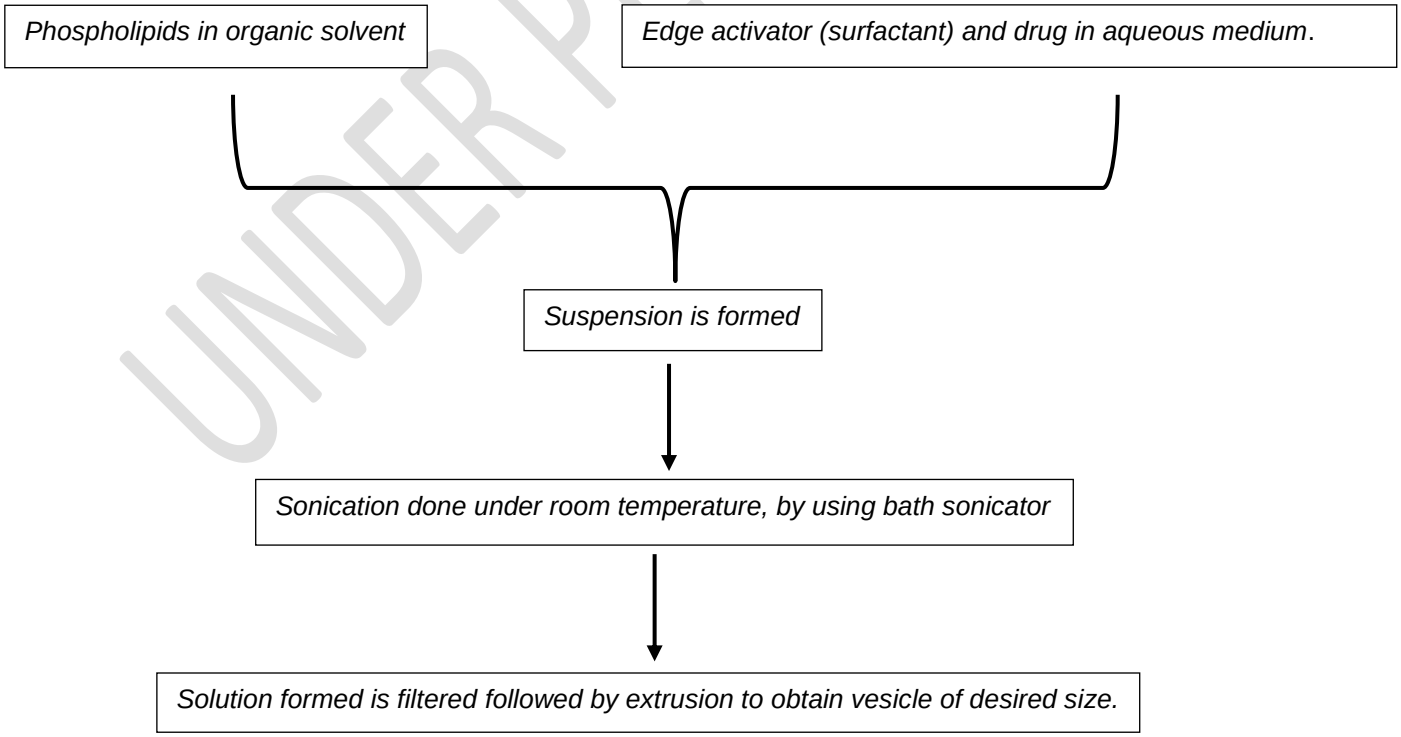

Figure 4: Sonication technique[16]

6.5. Ethanol Injection Technique: *Diagrammatic representation shown in* ***figure 5****.*

1. *Drug added into the aqueous medium is continuously stirred at a fixed temperature.*
2. *Ethanolic phospholipid and surfactant solution are injected into an aqueous medium drop by drop.*
3. *When the ethanolic mixtures contact the aqueous solution, the lipid molecules get precipitated and foam bilayer structure.*

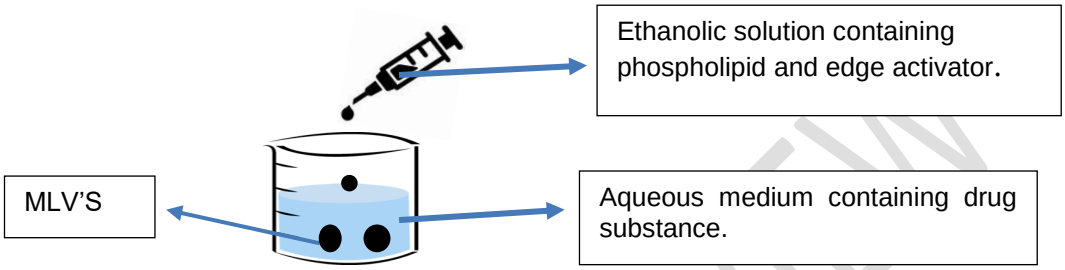

Figure 5: Ethanol Injection Method.

6.6. Freeze-Thaw Method: *this technique involves,*

- *Initially the MLV's suspension is subjected to a sudden change in temperature in an alternate manner, i.e, first at freezing temperature, liquid nitrogen (-30^{o}C) for 30 seconds followed by a high temperature in the water bath.*
- *This process is repeated 6-7 times, which results in the formation of small unilamellar vesicles.*

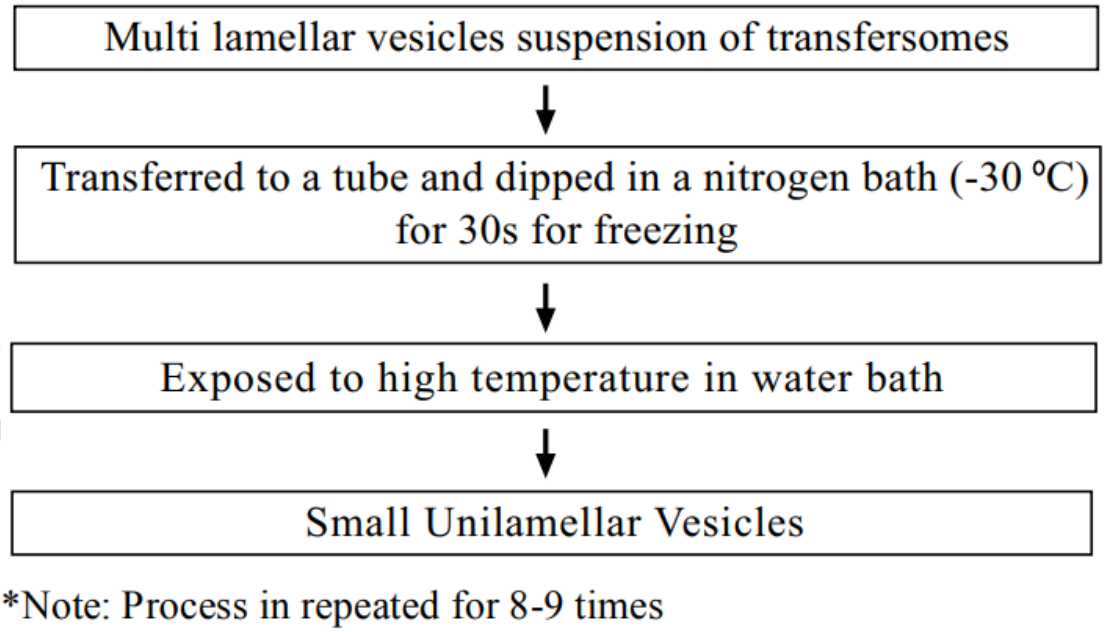

Figure 6: Freeze-Thaw technique [17]

7. Factors Affecting Transferosomes[18]:

7.1. Phospholipid: surfactant Ratio-

The ratio of phospholipid: edge activator should be optimized, as it affects the trapping efficiency, vesicle size, and permeability ability of Transferosomes.

- *At a higher concentration of surfactant, the trapping efficiency reduces and can also lead to the formation of pores within the bilayer vesicles.*
- *A lower concentration of surfactant can lead to an increase in vesicle size.*

7.2. Solvent effect:

The selection of different solvents relies on the solubility of ingredients (drug and excipients) in the formulations. The most commonly used solvents are ethanol and methanol.
Solvents also enhance drug permeability through the membrane, thus acting as permeation enhancers. E.g., Ethanol increases the permeability of drug substances by increasing drug solubility in vesicles or by altering the nature of the stratum corneum.

7.3. Effect of Edge Activators (Surfactant):

Edge activators strongly affect the Deformability and Entrapment Efficiency of Transferosomes. A high concentration of surfactant can lead to,

- *Micelle rather than vesicle formation if the concentration is more than 15%.*
- *Lead to increase the charge on vesicle surface, which reduces vesicle aggregation and increases stability.*
- *Reduce the entrapment efficiency of Transferosomes, as conc. Of surfactant increase, a more significant number of vesicles are formed, which lead to a higher volume of lipid bilayer part that can disrupt the vesicular membrane and cause leakage of the drug substance.*

7.4. Effect of Aqueous Medium:

The pH of the aqueous medium should be maintained so that the unionized drug can penetrate through the cell membrane because the lipid bilayer of Transferosomes vesicle act as lipid bilayer of the cell membrane and allows only unionized drug bound to lipid bilayer can penetrate through the cell membrane. The commonly used aqueous medium can be either water or phosphate buffer (pH-6.8-7.0).

8. Evaluation of Transferosomes[19]:

8.1. Vesicle Morphology (shape and size of vesicle)-

- *Vesicle diameter can be determined by the light scattering method.*
- *Shape of the vesicle can be visualized under the microscopy method (TEM AND SEM).*
- *The vesicle stability can be determined by assessing the size and shape of the vesicle.*
- *In the light scattering method, the sample solution is prepared by dissolving the sample into distilled water, then filtered through a membrane filter (0.2 mm pore size) and diluted with saline solution, then the size is measured.*

8.2. Number of vesicle/mm^3:

One of the significant parameters for optimizing the composition of Transferosomes.
Non-sonicated transferosomes are diluted 05 times with saline solution.
Hemocytometer can be used to determine the vesicles.
The transferosomes present in small squares are counted and calculated using the given formula,

Transferosomes per mm^3 =
*(Total no of transferosomes present * Dilution factor* 4000) / Total no of squares counted.*

8.3. Percentage Entrapment Efficiency:

Initially, all the un-entrapped drugs are separated from entrapped drugs using a column centrifugation method. After centrifugation, vesicles are disrupted using 50% n-propanol.

*Percentage Entrapment Efficiency = (Amount trapped / total amount added) * 100.*

8.4. Drug Content:

It can be measured using an analytical method like the HPLC method.

8.5. Turbidimetry measurement:

The turbidometry of the drug solution is measured by using Nephelometry.

8.6. Occlusion Effect:

The principle involved in the occlusion of transferosomes through the skin is constructive for drug penetration.
Hydro taxis (water movement) act as the main driving force for permeating vesicles through the skin.
Occlusion prevents the evaporation of water from the skin.

8.8. Drug Release studies:

- *It is performed to determine the permeation rate.*
- *It is defined as the time required to attain steady-state permeation.*
- *To determine the drug release, the suspension of transferosomes is incubated at 320ºC, the samples are taken at different times, and the free drug is separated by the column centrifugation method.*
- *The drug released is calculated as entrapped (100% tangled = 0% release).*

8.9. In-vitro Skin Permeation Studies: *Diffusion cell-like Franz diffusion cell was used for the study.*
It consists of a receiver compartment (capacity volume=50ml) and an effective diffusion area (2.50 $cm^{2)}$.
The study was conducted using goatskin in phosphate buffer (pH= 7.4).

- *abdominal skin of goat was collected from the source*
- *Hydrated in a saline solution (0.9% NaCl solution).*
- *Fatty layer was removed by rubbing with a cotton swab. Then the skin was kept in an isopropyl alcohol solution at 0-40ºC to preserve the skin.*
- *The skin was fixed on the receptor compartment end by keeping the stratum corneum facing the donor end.*
- *The receiver compartment containing 50ml of phosphate buffer, maintained under 37ºC.and stirred by a magnetic stirrer at 100RPM.*
- *Formulation was kept over the skin and covered the top of the diffusion cell.*
- *The study was performed.*

9. Application of Transferosomes:

9.1. Delivery of biological products (Insulin) [20, 21] :

Generally, insulin is administered through injection subcutaneously. However when Insulin is encapsulated in a transfersome carrier and then administered topically on the skin, the first symptoms of hyperglycemia are observed in 90 to 180 min of application which happens due to the composition of the carrier. Different studies have also been done on the preparation of transferosomes for other anti-diabetic drugs like metformin, repaglinide with improved skin permeation.
Malakar Jadupati et al.'s studies showed the delivery of insulin as transfersomal gel. The insulin permeation flux was disturbed due to several factors such as the ratio of lipid: surfactant, the texture of the skin, the lipophilicity of the drug, etc. The in-vitro permeation flux for the optimized gel was found to be 13.5 ± 0.21 µg/cm^2 /h and the experiment was carried out at porcine ear skin with a small margin of error value of 6.85 %.

Cevc et al., also reported that the permeation of drugs through the skin in transferomes carriers is 10 times greater than conventional vesicles. It was proved that the efficacy of transcutaneous delivery of insulin remains undisturbed by any previous therapy. The systemic normal glycemic value which lasts for a minimum of 16 hours was achieved in a non-invasive administration of insulin.

9.2. Delivery of steroids [22, 23] -

Delivery of corticosteroids can be achieved through transferosomes due to their site-specificity. A lesser dose is required for the delivery of steroids through transferosomes because at a lesser dose the transferosomes are biologically active.

Cevc et al., investigated two glucocorticosteroids i.e. dexamethasone and hydrocortisone, for biological properties. The biological indicator used was the minimum effective drug concentration required to reduce edema by 50 %. It was found that the minimum effective concentration for hydrocortisone in deformable carriers was minimized to 3-4 μg cm^{-2} from the drug concentration of 15 μg cm^{-2} as seen in cream and lotions. The potency was enhanced due to an increase in 20% drug potency. This type of delivery, suppress the edema caused by the drug by two folds.
The minimum effective concentration of dexamethasone was reduced by ten times compared to lotions and cream products. Also, the erosion property of drugs was lowered. Thus, the usage of transfersomes carrier delivery of corticosteroids has improved the therapeutic risk-benefit ratio and produced better targeting results.

9.3. Delivery of macromolecules (proteins and peptides) [24, 25] :

Proteins are macromolecules that are larger so it becomes difficult to administer them into the body through injection and the oral route is not an option, because they get degraded completely. The bioavailability of proteins delivery in transfersomes is similar to that of the bioavailability obtained from subcutaneous injection of proteins. The transfersome delivery also produces strong immune responses when applied epi-cutaneously. An example of this is serum albumin, despite having several dermal challenges it is active immunologically when delivered as a transfersome carrier into the skin.

9.4. Delivery of Interferon [26]:

Transfersomes can be a carrier to deliver biotechnological products such as Interferon-α, which are very difficult to deliver through other conventional methods, due to their short half-lives. A controlled release of interferon through transfersome can prevent stability issues.
A study done by Hafer et al., which showed delivery of IL-2 and INF- α with the help of transfersomes at an optimum concentration, was sufficient for immunotherapy.

9.5. Delivery of Anticancer agents [27, 28] :

Anticancer agents like methotrexate showed a futuristic application of transfersome technology, especially for treatment against skin cancers. Khan MA et al., prepared different formulations of 5-Fluorouracil transferosomes using different surfactants like tween 80 and span 80. Then these vesicles are evaluated for their particle size, shape, percentage entrapment efficiency of the vesicles, de-formability index, and in-vivo skin permeation studies. The optimized formulation was selected and incorporated into a gel made of a one percent solution of carbopol 940. Transfersomes containing 5-Fluorouracil showed the following results the optimum particle size was found to be 265.7 ± 2.05 nm with an entrapment efficiency of 68.7 ± 0.99 % and the highest deformability index of 28.8 ± 1.09. Maximum skin deposition was found to be (82.4%) and a transdermal flux of 22.56 μg/cm^2 /h. The transfersome gel showed even an improved skin penetration and deposition, compared to the marketed formulation. The transfersomal gel showed no symptoms of irritant nature to the skin. A conclusion was made based on the following studies that the transfersomal gel of 5-fluorouracil is a better treatment for skin cancer as it shows better absorption through the skin.

9.6. Delivery of Anesthetics [29]:

Transfersomes can produce anesthetic properties for less than 10 min when applied topically under certain conditions. The effect of transfersomal anesthetic has a longer effect than the subcutaneous bolus injection.
Planas, M. E. et al., conducted an experimental study of transdermal transferosome for common and local analgesics effects on the skin and permeability studies on rats and humans. A comparison between lidocaine liposomes and lidocaine tranferosomes was done. Subcutaneous solutions of 2%

lidocaine in liposomal or transfersomal solution type were prepared and when injected in rats, in case of liposomal type showed a strong analgesic effect initially for 6 - 8 min and the withdrawal time was approximately 35 sec. whereas in the case of analgesic transfersomes that were applied dermally, increased the reaction by greater than 70 s, which was 130% longer than in the control group that received a placebo or a standard aqueous lidocaine solution. Thus it was concluded that the transfersome type formulation was as effective as the SC injection containing the same dose of the drug and transfersomes stand out to be a very promising candidate for the treatment of local pain by topical application.

9.7. Delivery of NSAIDs [29]:

Transdermal transferosomes can be used to overcome gastrointestinal problems caused by NSAIDs. Some drugs like diclofenac and ketoprofen transferome formulation are already studied for their efficacy purpose, and ketoprofen transferosome formulation is already the approved by Swiss regulatory agency.

9.8. Delivery of Herbal Drugs [30, 31] :

Transfersomes vesicles of capsaicin were prepared by Xiao-Ying et al., whose studies indicated an enhanced absorption through the topical route when compared to pure capsaicin compound, it is due to the property of transfersome i.e. to deliver the nutrients locally by penetrating through the stratum corneum due to the presence of the surfactant in their formulation.

Transfersomes of curcumin are used for their anti-inflammatory property which also ensures better penetration of the drug into the skin. Indinavir sulfate transferomes also showed an improved penetration influx for the activity against the deadly acquired deficiency syndrome. Ketoprofen transfersomes just like curcumin, it has better penetration of the drug due to the presence of an edge activator which enhanced the anti-inflammatory action.

Transfersomes tend to increase the skin penetration of certain phytoconstituents such as capsaicin and colchicine but also increase the percentage entrapment efficiency of certain phytoconstituents such as vincristine and vinblastine. Transfersomes also improved the transdermal flux of drugs like norgesterol, tamoxifen, methotrexate, and oestradiol. They provide an appropriate means of treatment of local pain by rationally using tetracaine and lignocaine on direct topical application.

Some of the important work done under the field of transferosomes is summarized in ***table 2****.*

Table 2- Example of some prepared transferosomes formulation

S no	Drug	Drug category	Study conducted	results	Reference
1	*18β-glycyrrhizic acid*	*Dermatitis*	*a) In vitro permeation study. b) In vivo anti-inflammation study.*	*a) 5 times greater than conventional vesicles. b) Shows anti-inflammatory activity.*	*[32]*
2	*5-Aminolevulinic acid*	*Photodynamic therapy*	*Skin retention study*	*b) improve drug retention*	*[33]*
3	*5-fluorouracil*	*Anti-Cancer*	*Skin permeation and deposition*	*a) Sustained drug release b) Enhanced flux rate.*	*[34]*
4	*Benzocaine*	*Local anesthetic*	*Entrapment efficiency*	*Increased drug Loading*	*[35]*
5	*Betamethasone*	*Steroid*	*Entrapment efficiency*	*Increased entrapment efficiency*	*[36]*
6	*Bleomycin*	*Skin cancer*	*a) In vitro keratinocytes cell line b) Stability study*	*a) Effective against human keratinocytes cell lines b) Stable at room temperature*	*[37]*
7	*Catechin*	*Antioxidant*	*a)Entrapment*	*a) greater*	*[38]*

			efficiency *b)Vesicle size* *c)Skin permeation* *d)Skin deposition*	*entrapment efficiency* *b) Small size* *c) Sustained drug released)* *Higher drug deposition*	
8	*Cetirizine*	*Anti-histaminic*	*In vivo study*	*More effective in atopic dermatitis.*	*[39]*
9	*Colchicine*	*Anti-Gout*	*a) In vitro skin permeation study* *b) Skin deposition study*	*a) 11 times more than that of the solution.* *b) 12.5 times better than compared to solution.*	*[40]*
10	*Curcumin*	*Anti-inflammatory herbal drug*	*In vitro permeation*	*Higher permeation*	*[41]*
11	*Cyclosporine A*	*Immunosuppressive*	*Drug delivery through mice skin*	*Successful delivery of drug through and into the mice skin as compared to the conventional vesicles*	*[42]*
12	*Dexamethasone*	*Corticosteroids*	*a) Drug release study* *b) In vivo study*	*a) Zero-order drug release.* *b) Better anti-edema activity.*	*[43]*
13	*Diclofenac sodium*	*NSAID*	*Skin permeation study*	*Ten times more concentration under the skin.*	*[44]*
14	*Griseofulvin*	*Anti-fungal*	*In vivo study*	*Curing of fungal disease was observed in animals within 10 days*	*[45]*
15	*Insulin*	*Hypoglycemic hormone*	*In vivo study*	*Longer hypoglycemia effect.*	*[46]*
16	*Interleukin 2*	*Cytokine*	*Skin permeation study*	*Carry IL-2 through the skin*	*[47]*
17	*Methotrexate (water-soluble)*	*Folic acid antagonist*	*In vitro skin permeation study*	*Greater drug permeation.*	*[48]*
18	*Metronidazole*	*Anti-amoebic*	*a) Entrapment efficiency* *b) In vitro permeation study*	*a) Lower entrapment efficiency* *b) Increased permeability*	*[49]*
19	*Oestradiol*	*Estrogens*	*Flux*	*Greater flux is achieved*	*[50,51]*
20	*Tetanus*	*Vaccine*	*a) Elasticity* *b) Immune response*	*a) Increased elasticity* *b) greater Immune response*	*[52]*

21	*Paclitaxel*	*Anti-neoplastic*	*Toxicity study*	*The maximum tolerated dose is 120 mg/kg*	*[53]*
22	*Stavudine*	*Antiviral*	*Skin permeation study*	*a) Lag time decreased b) 7.5 times greater transdermal flux*	*[54]*
23	*Lidocaine*	*Local anesthesia*	*Local anesthetic effect in human*	*Similar effect as obtained by subcutaneous injection*	*[55]*
24	*Heparin*	*Antithrombic*	*a) In vitro skin penetration b) In vivo localization*	*a) greater in vitro skin penetration b) greater in vivo localization.*	*[56]*
25	*Itraconazole*	*Antifungal*	*Skin retention study*	*Enhanced skin retention*	*[57]*

10. Conclusion:

After an extensive review of different literature reviews, it was found that Transferosomes have been ultra-deformable vesicles capable of subjecting a futuristic solution to all vesicular delivery and conventional delivery complications. It can carry any drug's molecular weight through the skin; therefore, they are widely used to take protein and peptides. They can easily penetrate through the stratum corneum of the skin, although having a larger size, by an excellent mechanism and due to the ultra-deformable and flexibility property of transferosomes. They are having numerous advantages over conventional delivery systems. After doing all the evaluation studies, we can say that Transferosomes is the promising candidate for the vesicular system and can replace other novel vesicular systems like liposomes, niosomes, and also conventional formulations. Thus it holds a bright future in novel drug delivery systems.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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