



TRANSFERSOMES: PREPARATION, CHARACTERIZATION & APPLICATION: A REVIEW

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ABSTRACT

A carrying structure for targeted transdermal medication administration is a Transfersomes. These particular liposomes are made of an edge activator and phosphatidylcholine. Creating the medication in a transfersome is one way to address these issues. A transfersome is an elastic, ultra deformable vesicle that can force itself through a pore several times smaller than its own size because of its elasticity. One kind of elastic or extremely flexible vesicle is called a transfersome. They are a popular new carrier for bioactives that are effectively delivered transdermally. Increasing the penetration of medications with low and high molecular weights is the main goal in creating these vesicles. When intimately processed, vesicles develop at a precise ratio of phospholipid and surfactant. The vesicle's flexibility is controlled by the ratios of each surfactant to the total amount of surfactants.

KEYWORDS; Transfersomes, Targeted Transdermal Medication Administration, Phosphatidylcholine.

INTRODUCTION

Transfersomes is a trademark registered by the German company IDEA AG, which refers to its proprietary drug delivery technology. The name means "carrying body" and is derived from the Latin word 'transfere', meaning 'to carry across' and the Greek word 'soma', meaning 'a body'. A *Transfersomes* carrier is an artificial vesicle designed to exhibit the characteristics of a cell vesicle or a cell engaged in exocytosis, exocytosis, and thus suitable for controlled and potentially, targeted drug delivery.^[1] A transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultra deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation.^[2] Transdermal delivery can improve both therapeutic efficacy and safety of drugs by more precisely, but it required spatial and temporal placement within the body to reduce both the size and number of doses necessary to achieve the objective of systemic medication through topical application to the intact skin surface.^[3] The system delivers the drug with high efficiency depending on the choice of administration or application. This system has several order magnitude of elasticity and flexibility over liposomal drug delivery which makes it favourable for efficient skin penetration and hence for the novel drug

delivery system. They overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. With the application of mechanical stress, they can enter through stratum corneum in self adapting manner because of their high vesicle deformability.^[4] A transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultra deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation.^[5] Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. Transfersomes can pass through even tiny pores (100nm) nearly as efficiently as water, which is 1500 times smaller.^[6] Due to the presence of first pass effect or medication interactions with other components of Gastro Intestinal tract (GIT) before absorption, an efficacious, successful therapy with no undesirable effects may not be possible in the majority of situations. Patient compliance is poor with these types of therapies. As a result, improved drug delivery methods have been studied in recent years in order to achieve the benefits of conventional treatment while avoiding the drawbacks. One promising method for avoiding pre-systemic metabolism and contact is the use of peptides. The presence of a skin barrier, on the

other hand, restricts or amplifies the relaxed penetration of various molecules when they are applied as creams, gels, or ointments. As a result, new carrier or vesicular based TDDS are needed to increase molecule penetrability through the skin barrier.^[7]

MECHANISM OF TRANSPORT

Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. Two mechanisms of action have been proposed.

- Transfersomes act as drug vectors, remaining intact after entering the skin.^[8]
- Transfersomes 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.^[9]

TRANSFEROSOMES

Transfersomes mainly composed of phospholipids like phosphatidyl choline which self assembles into lipid bilayer in aqueous environment and closes to form a vesicle. The main component in transfersome formulation is edge activator. It consists of single chain surfactant that causes destabilization of the lipid bilayer thereby increasing its fluidity and elasticity. Transfersomes are efficient in delivering the low molecular weight and as well as high molecular weight drugs through skin, consisting of hydrophobic and hydrophilic moieties together and has a result wide range of solubility. This high deformability gives better penetration of intact vesicle.^[10]

Transfersomes even called as ultra deformable vesicles for applying to skin holding a lipid bilayer with phospholipids and edge activator along with aqueous layer. Based on the lipophilicity the active substance is enclosed with in core or amongst the bilayer. In comparison to liposomes, transfersomes are having a

great capacity to touch whole deeper areas of skin once applied topically.^[11]

Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. Transfersomes can pass through even tiny pores (100nm) nearly as efficiently as water, which is 1500 times smaller. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.^[12]

The commonly used edge activator in this type of drug delivery system to increase flexibility are tween80, span80, sodium cholate, sodium deoxycholate etc. and the commonly employed phospholipids used for vesicle forming are soya phosphatidylcholine, Egg lecithin's and cholesterol etc. Examples of components of transfersomes are tabulated in Table 1.^[13]

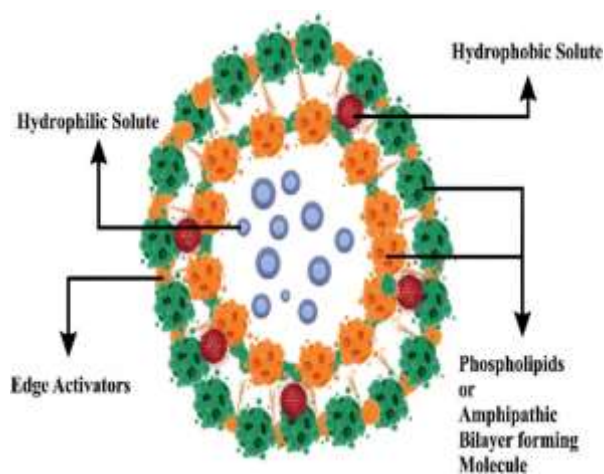


Fig. 1; Structure of transfersomes (copied from google.co.in).

Table 1: List of Transfersomes.

S. No.	Example	Class	Use
1.	Egg Phosphatidyl Choline Soya Phosphatidyl choline, , dipalmitoyl phosphatidyl choline	Phospholipids	Vesicles forming component
2.	Ethanol, methanol, isopropyl alcohol, chloroform	Solvents	As a solvent
3.	Sod. Cholate, Sod. Deoxycholate, Tween-80, Span 80, Tween 20	Surfactants	Vesicles forming component (Edge Activators)
4.	Saline phosphate buffer (pH 6.4), Phosphate buffer pH 7.4	Buffering agent	As a hydrating medium

TRANSFEROSOMES v/s OTHER CARRIER SYSTEMS^[14-15]

Transfersomes are generally differing from the mixed micelles. They are as following

1. In size, a transfersomes is greater than the standard lipid micelles.

2. Each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. As a result, transfersomes can carry water as well as fat-soluble agent in comparison to micelles that can only incorporate lipoidal substances.

- Transfersomes are different from commonly used liposomes as they are much more flexible and adaptable.
- Confocal Scanning Laser Microscopy (CSLM) can be used to differentiate the penetration ability of all these carrier systems in the intact murine skin. In all these vesicles the highly deformable transfersomes transverse the stratum corneum and enter into the viable epidermis in significant quantity.

METHOD OF PREPARATION OF TRANSFERSOMES

1) Rotary Film evaporation method

Modified hand shaking method is another name for this approach. In this approach, API, lecithin, and edge

activator are solubilised in a 1:1 mixture of chloroform and ethanol by manual shaking at a temperature higher than the lipid's transition temperature, and the resulting liquid is maintained for evaporation to remove the organic solvent. The thin lipid coating is left overnight to allow complete removal of the organic solvent. The film is then hydrated by rotating it at 60 RPM for 1 hour at room temperature with a pH 6.5 buffer. The leftover vesicles swell for 2 hours at room temperature. Small vesicles were made from leftover vesicles that had been sonicated at room temperature. Rotary Film Evaporation Method is given in Fig. 2.^[16]

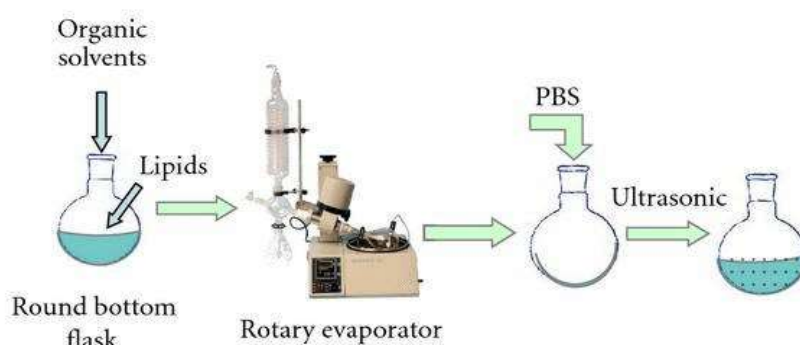


Fig. 2: Rotary Film evaporation method.

2) Thin film hydration technique

For the preparation of transfersomes thin film hydration technique is used, which comprised of mainly three steps.

(a) At first, the vesicle forming ingredients phospholipids and surfactant were dissolved in volatile organic solvent. The organic solvent evaporated above the lipid transition temperature using rotary evaporator. Final traces of solvent were removed under vacuum for overnight. The deposited lipid films were hydrated with buffer by rotation at 60 RPM/min.^[17]

(b) The thin film is hydrated with buffer solution (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.^[18]

(c) To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min, using a bath sonicator or probe sonicator. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes (Fig:3).^[19]

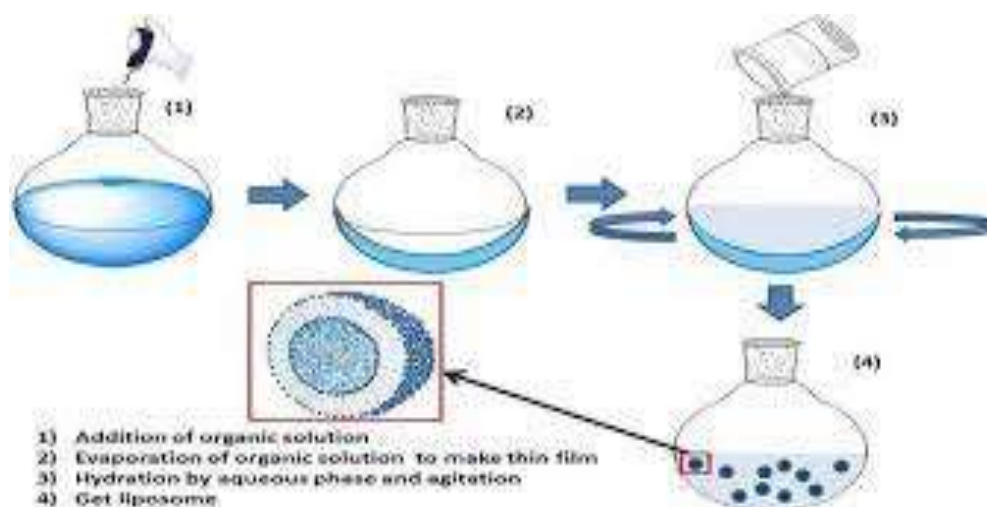


Fig. 3: Thin Film Hydration Technique.

3) Vortex/Sonication Method

The vortexing method involves mixing phospholipids, drug, and edge activator in a phosphate buffer saline (PBS) solution followed by vortexing of the mixture until a suspension milky white in colour is obtained. The

product is then subjected to sonication for a few minutes, followed by extrusion through a membrane filter made of polycarbonate with 100nm as the size of the pores.^[20] The process is illustrated in Figure 4.

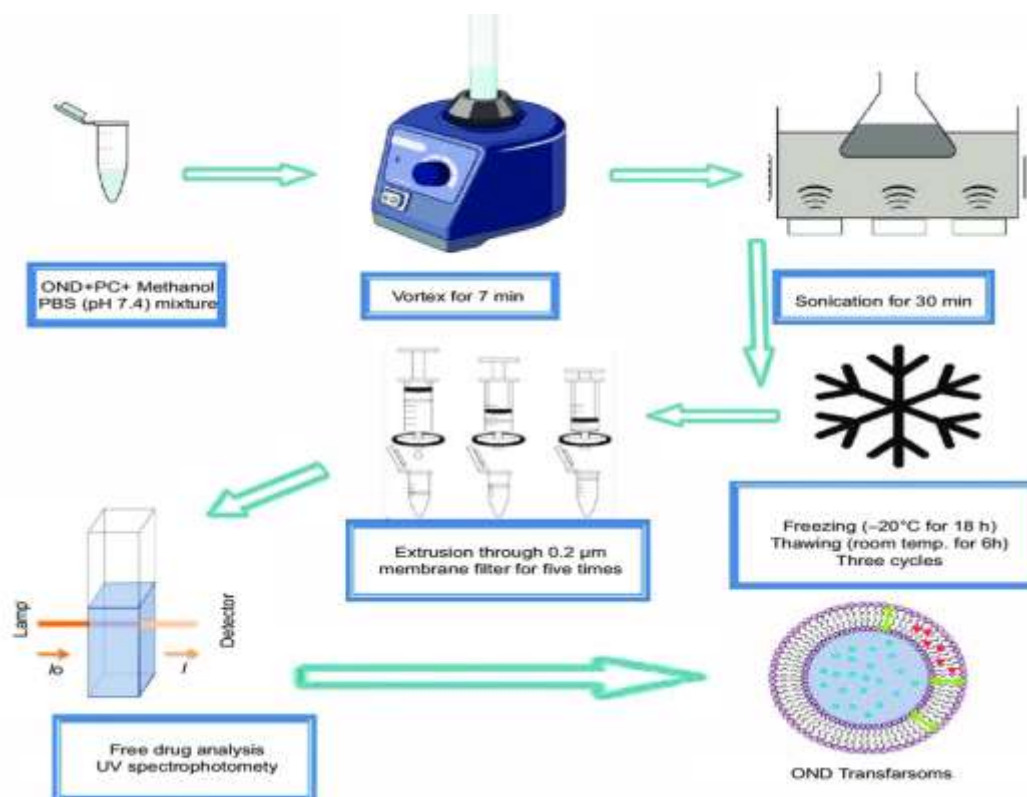


Figure 4: Vortex / Sonication Method.

4) Freeze thaw method

This process involves freezing the created multi lamellar vesicles suspension and then transferring it to a tube and dipping it in a nitrogen bath at -300 degrees Celsius for

30 seconds. After the suspension has frozen, it is treated to a high temperature 8-9 rounds.^[21-23] Diagrammatic view of this method is given Fig. 5.

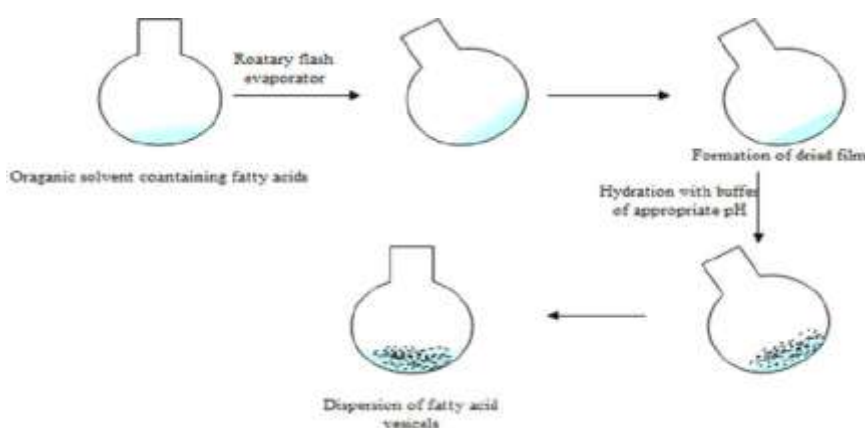


Fig. 5: Freeze thaw method.

5) Ethanol Injection Method

The technique is a popular technique used for the formulation of elastic liposomes. To begin the process, the drug is dissolved in an aqueous medium which is followed by heating the contents at a fixed temperature with constant stirring. Next, the solution of ethanol

containing edge activator as well as phospholipids is injected dropwise into the aqueous medium. When the ethanolic solution of phospholipids and EA is mixed with the aqueous solution, it results in the precipitation of the lipid molecules which in turn leads to the formation of bilayer structures.^[24-25]

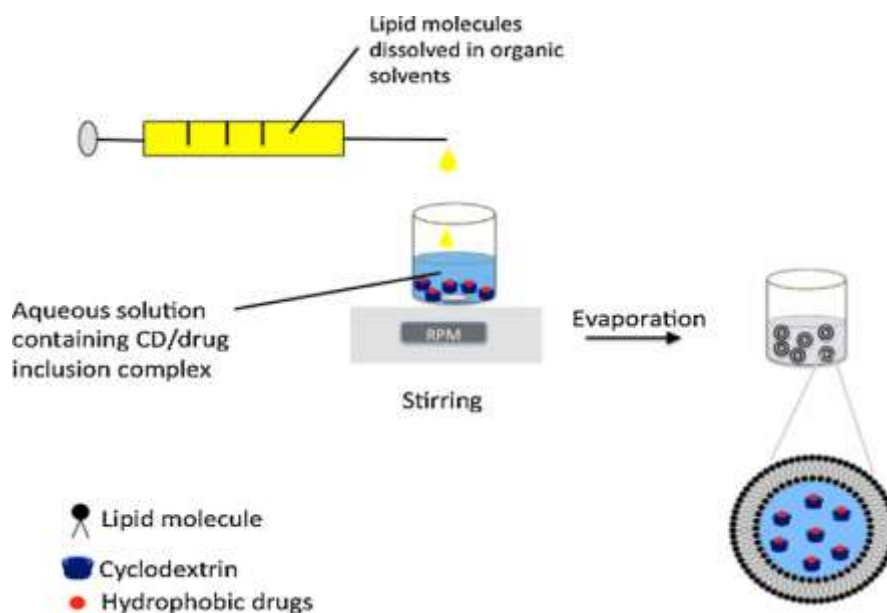


Figure 6: Ethanol Injection Method.

MECHANISM OF PENETRATION OF TRANSFERSOMES

After penetration through the outermost skin layers, transfersomes reach the deeper skin layer. From there, they are normally washed out into the blood circulation. If it is applied under suitable conditions, resulting in access to all body tissues.^[26]

The mechanism by which the active pharmaceutical ingredients are delivered by transfersomes is not very well known. It is however postulated that transfersomes disrupt the lipids present intercellularly within the stratum corneum and then permeate across the skin. As per reports in literature, besides the flexible as well as elastic nature of transfersomes which plays a pivotal role in their permeation, the hydration gradient among the topmost and deeper layers of the skin also has an important role in their enhanced permeation. It is reported that while undergoing squeezing through the tight junctions, they deform, which leads to their dehydration. Further, when these deformed transfersomes go deeper into the skin layers they get reformed by rehydration.^[27-29]

CHARACTERIZATIONS OF TRANSFERSOMES^[30-34]

The characterization of transfersomes is generally similar to liposomes, niosomes and micelles.

(a) Entrapment Efficiency

Using centrifugation method entrapment efficiency of un-entrapped drug was determined. The amount of drug entrapped (Total amt. of drug- unentrapped drug) in the vesicles was then determined by disrupting the vesicles using phosphate buffer, followed by filtration and amount of drug was quantified spectrophotometrically.^[30]

$$\text{Entrapment efficiency} = \left(\frac{\text{Amount entrapped}}{\text{Total amount added}} \right) \times 100$$

(b) Vesicle Shape and Type

Transfersomes vesicles can be visualized by Transmission Electron Microscopy, with an accelerating voltage of 100 kV. Transfersomes vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope.

(c) Number of Vesicle per Cubic mm

For optimizing the composition and other process variables in the formulation, it is an important parameter. Transfersomes formulations (without Sonication) can be diluted five times with 0.9% of sodium chloride solution and studied with optical microscopy by using haemocytometer.^[31]

(d) Penetration Ability

Fluorescence microscopy is used to evaluate the penetration ability of transfersomes.

(e) Surface Charge and Charge Density

Zetasizer is used to determine surface charge and charge density of transfersomes.^[32]

(f) Degree of deformability or permeability measurements

A permeability investigation will be used to characterise transfersomes. Pure water is utilised as a control for the deformability investigation, and the formed transfersomes are delivered through a series of pores with known sizes ranging from 50nm to 400nm. Following the entry of each size, DLS measurements can be taken.^[33]

(g) Drug content

The drug content can be calculated by using HPLC with UV detectors or Spectrophotometric methods.^[34]

The different parameters also used for the evaluation of transfersomes are

a) Zeta potential and Distribution of Vesicle size

For determination of zeta potential and distribution of vesicle size and diameter, dynamic light scattering (DLS) technique is used. Before determination, samples are diluted and then filtered through a 0.2 mm membrane filter.^[35]

b) Confocal scanning laser microscopy study

In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose: a. Investigation of the mechanism of penetration of transfersomes across the skin. b. Determination of histological organization of the skin, shapes and architecture of the skin penetration pathways. c. Comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles.^[36]

c) Degree of deformability or permeability measurement

Permeability study is a crucial and distinct parameter for characterizing transfersomes. To conduct this study, transfersomes preparation is passed through a sandwich of different micro-porous filters with pore diameters ranging from 50 nm to 400 nm. The size of particles as well as the distribution of sizes of transfersomes is measured after each pass using DLS.^[37]

d) In vitro drug release

To conduct the study, formulation is placed on a treated dialysis membrane mounted between the different compartments of the Franz diffusion cell (FDC). The receptor compartment is filled with a suitable release media. At regular time intervals, a sample is taken from the receptor and replaced with an equivalent amount of release media. The withdrawn sample is analyzed using a suitable analytical method to determine the percentage of drug release.^[38]

e) In vitro skin permeation studies

To conduct the in vitro drug study, a Franz diffusion cell is utilized. For the permeation experiments, biological membranes such as goat skin or rat skin are used. To carry out the study, the skin is first treated to remove

hairs and adipose, after which it is horizontally mounted between the compartments of the Franz diffusion cell. The receptor compartment is filled with saline buffer (phosphate buffer pH 7.4), which is maintained under stirring at $37 \pm 0.5^\circ\text{C}$. Sample aliquots are withdrawn and used for determination of percentage drug permeated by suitable methods of analysis.^[39-40]

APPLICATION OF TRANSFERSOMES**a) Delivery of insulin**

Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transfersomes (transfersulin) overcomes the problems of inconvenience, larger size (making it unsuitable for transdermal delivery using conventional method) along with showing 50% response as compared to subcutaneous injection.^[41]

b) Delivery of corticosteroids

Transfersomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transfersomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.^[41]

c) Delivery of proteins and peptides

Transfersomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptides are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract and transdermal delivery suffers because of their large size.^[42]

d) Delivery of anticancer drugs

Anti cancer drugs like methotrexate were tried for transdermal delivery using transfersome technology. The results were favorable. This provided a new approach for treatment especially of skin cancer.^[42]

e) These are worthy transporter option to deliver the drug in to skin layers for treatment of dermal cancer.^[43]

f) These are applicable to deliver the herbal drugs, anticancer drugs and anaesthetics few applications are written in Table 2.^[44]

Table 2: Different kinds of drugs and their inference.

NAME OF DRUG	INFERENCE
Insulin	Persuade medicinally important hypoglycemia with good efficiency and reproducibility.
Capsaicin	Improved skin penetration.
Ketoprofen	Improved permeation for anti-inflammatory activity.
Norgesterol	Improved transdermal flux.
Curcumin	Improved penetration for anti-inflammatory activity.
Colchicine	Escalation in skin penetration.
Indinavir sulfate	Upgraded influx to fight against acquired immuno deficiency syndrome (AIDS).
Hydrocortisone	Biologically active at dose several times lower than currently used formulation.
Stavudine	Great in vitro skin delivery of Stavudine for antiretroviral action.

ADVANTAGES

- a) High entrapment efficiency, for lipophilic drug it is near to 90%.
- b) Can encapsulate both hydrophilic and lipophilic moieties.
- c) Suitable as a carrier for low as well as high molecular weight drugs e.g., analgesic, corticosteroids, hormones, anticancer drugs, insulin, proteins, etc.^[45]
- d) Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubilities. They can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without significant loss.^[46]
- e) High deformability of this system gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anaesthetic, corticosteroids, sex hormone, anticancer, insulin and albumin.
- f) They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.^[47]
- g) They exhibit high encapsulation of lipophilic drugs.
- h) Transfersomes enhance permeability of drugs due to their elastic and ultra-deformable properties.^[48]
- i) They can be used for both systemic as well as topical delivery of drugs.
- j) They have the potential to deliver both lipophilic and hydrophilic therapeutics.^[49]
- k) They have the potential to be easily scaled up due to their simple and short process of manufacturing.
- l) They have potential to provide sustained and predictable release as well as duration of action of the encapsulated drug.^[50]
- m) Transfersomes have the ability to enhance transdermal flux.^[51]

CONCLUSION

Compared to other vesicular systems, transfersomes offer a number of benefits, such as increased stability, deformability, and skin penetrating power. They can also be used to administer medications systemically. The transdermal route of medication delivery now has more options thanks to TFS. Through their ability to pass through the internal lipid of the stratum corneum, they can transport medicinal compounds with low and high molecular weights. A wide variety of pharmacological compounds, including big molecules like peptides, hormones, and antibiotics, as well as medications with low penetration owing to undesirable physicochemical characteristics, can be delivered via transfersomes with significant potential. The aforementioned characteristics of this technology indicate that transdermal drug administration will benefit greatly from it. It is evident that larger and/or more concentrated medicinal substances can be delivered through and into the skin by Transfersomes or elastic vesicles. There is insufficient evidence to support the movement of intact vesicles

outside of the stratum corneum, and the precise process of transport is yet unknown.

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