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TRANSFEROSOMES- A NOVEL & UNIQUE VESICULAR CARRIERS IN THE DESIGN OF TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

Transdermal delivery systems have gained much interest in recent years owing to their advantages compared to conventional oral and parenteral delivery systems. They are non -invasive and self-administered delivery systems that can improve patient compliance and provide a controlled release of the therapeutic agents. The greatest challenge of transdermal delivery systems is the barrier function of the skin's outermost layer. Molecules with molecular weights greater than 500 Da and ionized compounds generally do not pass through the skin. Therefore, only a limited number of drugs are capable of being administered by this route. Encapsulating the drugs in transfersomes are one of the potential approaches to overcome this problem. They have a bilayered structure that facilitates the encapsulation of lipophilic and hydrophilic, as well as amphiphilic, drug with higher permeation efficiencies compared to conventional liposomes. Transfersomes are elastic in nature, which can deform and squeeze themselves as an intact vesicle through narrow pores that are significantly smaller than its size. This review aims to describe the concept of transfersomes, the mechanism of action, different methods of preparation and characterization and factors affecting the properties of transfersomes, along with their recent applications in the transdermal administration of drugs.

KEYWORDS: Transferosomes; deformable; transdermal delivery, transdermal patch.

INTRODUCTION

In recent years, research scenario goes toward the development of new type of drug delivery system with the objective of high therapeutic activity along with patient compliance. Many drug delivery systems are developed with improved therapeutic activity, but some complications arise with some delivery systems are not as such overcomes. Orally administered drugs experience a hostile environment in the gastrointestinal (GI) tract, where most drugs are degraded in variable pH conditions, or face solubility issues and most importantly first-pass metabolism. In case of parenteral preparation, disadvantages are a lack of drug reversal, hypersensitivity reaction, risk of infection, emboli and cost. Some drugs much bitter in taste, swallowing of such a bitter medication in oral delivery and pain associated due to needle in parenteral delivery make them less patient compliance1. From last few decades, considerable attention has been focused on the development of topical delivery of drugs because a number of advantages with this route. Skin in an average adult body covers a surface of approximately 2 m2 and total weight of 3 kg; it receives about one-third of the blood circulating among the body. Topical drug delivery means the application of drug to skin for localized effect and in transdermal drug delivery system (TDDS) skin is used as a potential route for the delivery of systemic action of drugs. TDDS is one of the systems with high patient compliance. Some potential advantages of transdermal route found over other conventional routes such as oral- and parenteral-like avoidance of first-pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter- and intra-patient variations and most importantly, it provides patients convenience. However, it also has some disadvantages such as possibility of local irritation effect, erythema, itching and low permeability in the stratum corneum. A major obstacle to dermal and transdermal drug delivery is the permeation characteristics of the stratum corneum, which limits drug transport, making this route of administration frequently insufficient for medical use4. Stratum corneum is the top layer of the epidermis consists of keratinized, flattened remnants of once actively dividing epidermal cells, impermeable to water and behaves as a tough flexible membrane. Many technologies and systems have been investigated to evade this barrier including electrophoresis, iontophoresis, chemical permeation enhancers, microemulsions, sonophoresis, as well as utilizing vesicular systems such as liposome, niosomes, ethosomes, and transfersomes, one of the most promising techniques is to formulate novel vesicular carriers for

delivery through the skin as it delivered drug at sustained or controlled manner5- 10. Among all these transfersomes appear promising. A new type of vesicular drug carrier system called transfersome. The term transfersomes and the underlying concept were introduced in 1991 by Gregor Cevc. In broadest sense, a transfersomes is a highly adaptable and stress-responsive, complex aggregate possessing an aqueous core surrounded by a complex of lipid bilayer. Transfersome is a term registered as a trademark by the German Company IDEA AG and used by it to refer to its proprietary drug delivery technology. The name means carrying body and is derived from the Latin word transferred meaning to carry across and the Greek word soma for a body. A transfersome carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potentially targeted, drug delivery. Most suitable form of transfersome is an ultra deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. In terms of delivering of drugs through transdermal route, To overcome all the above problems, a new type of vesicular carrier has been developed called transfersome which is capable of transdermal delivery of low as well as high molecular weight drugs.[1,2]

COMPARISON OF FEW LIPOIDAL PARTICULATE CARRIER AND THEIR APPLICATIONS

TYPE	COMPOSITION	FEATURE
Liposomes	Phospholipids: cholesterol: alcohol	The amphiphilic characteristic allows for the solubilization of both hydrophilic and lipophilic medicines, as well as the internalisation and amplification of bioactives.[8]
Transferosomes	Phospholipids: edge activators: alcohols: buffering agent: dye	Vesicles which are ultra deformable can deform and pass through constrictions that are 5 to 10 times smaller than the corresponding diameter without noticeable loss.[9]
Ethosomes	Phospholipids: ethanol	Its use as a combination of high concentrations of ethanol and phospholipids enhances the effect of deeper drug distribution and penetration in the skin.[10]
pharmacosomes	Phospholipids: dicholromethane	Colloidal dispersions of drug covalently bonded to lipids, which boosted entrapment efficiency; no drug loss due to leakage, and no drug incorporation difficulties.[11]

TRANSFERSOMES Vs OTHER CARRIER SYSTEM

Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. Transfersomes can deform and pass through narrow constriction without measurable loss. This high deformability gives better penetration of intact vesicles.

- Transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes. They differ vastly from commonly used liposomes in that they are much more flexible and adaptable.
- The extremely high flexibility of their membrane permits transfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersomes membrane.
- The high resulting aggregate deformability permits transfersomes to penetrate the skin spontaneously. This tendency is supported by the high transfersomes surface hydrophilicity that enforces the search for surrounding of high water activity.
- It is almost certain that the high penetration potential of the transfersomes is not primarily a consequence of stratum corneum fluidization by the surfactant because micellar suspension contains much more surfactant than transfersomes (PC/Sodium cholate 65/35 w/w %, respectively). Thus, if the penetration enhancement via the solubilization of the skin lipids was the reason for the superior penetration capability of transfersomes,
- To the topmost part of the stratum corneum even they are applied non- occlusively.
- The reason for this is that mixed micelles are much less sensitive to the trans-epidermal water activity gradient than transfersomes. Transfersomes differ in at least two basic features from the mixed micelles, first a transfersomes is normally by one to two orders of magnitude (in size) greater than standard lipid micelles. Secondly and more importantly, each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. Transfersomes thus carry water as well as fat-soluble agent in comparison to micelles that can only incorporate lipoidal substances.
- To differentiate the penetration ability of all these carrier systems10 proposed the distribution profiles of fluorescently labelled mixed lipid micelles, liposomes and transfersomes as measured by the Confocal Scanning Laser Microscopy (CSLM) 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.[2]

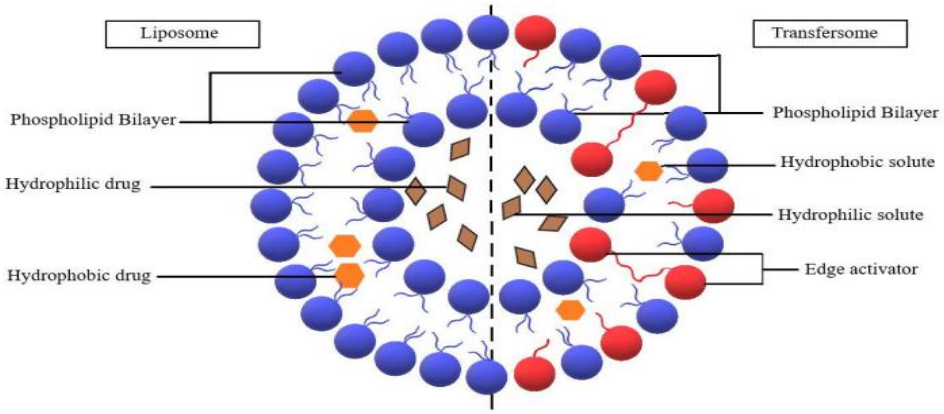
WHY ONLY TRANSFERSOMES FOR SKIN

Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self optimized and ultra flexible membrane properties, they are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency.

- The vesicular transfersomes are more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. These are characteristic with transfersomes, because of the high vesicle deformability which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transfersomes membrane is governed by mixing suitable surface-active components in the proper ratios with phospholipids. The resulting flexibility of transfersome membrane minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under non-occlusive condition.
- Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties.[3,4]

SALIENT FEATURES OF TRANSFEROSOMES

Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility [Figure 1]. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability 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, and albumin. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes. They have high entrapment efficiency, in case of lipophilic drug near to 90%. They protect the enpsulated drug from metabolic degradation. They act as depot, releasing their contents slowlyand gradually. They can be used for both systemic as well as topical delivery of drug. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.[4,5]



Advantages of Transfersomes as Vesicle-based Transdermal Drug Delivery Systems

- Transfersomes carriers are composed of hydrophilic and hydrophobic moieties, which result in becoming a unique drug carrier system that can deliver therapeutic agents with wide range of solubility.
- Transfersomes are able to squeeze themselves through constrictions of the skin barrier that are very narrow, such as 5 to 10 times less than the vesicle diameter, owing to their ultra-deformability and elastic properties.
- High vesicle deformability facilitates the transport of drugs across the skin without any measurable loss in intact vesicles and can be used for both topical, as well as systemic, treatments.
- Transfersomes carriers are very versatile and efficient in accommodating a variety of agents nearly independent of their size, structure, molecular weight or polarity.
- They are made up of natural phospholipids and EAs, therefore promisingly biocompatible and biodegradable.
- Transfersomes can be used for the delivery of various active compounds, including proteins and peptides, insulin, corticosteroids, interferons, anesthetics, NSAIDs, anticancer drugs and herbal drugs.
- Transfersomes are an obvious choice for achieving a sustained drug release, as well as a predictable and extended duration of activity.
- They are capable of increasing the transdermal flux and improving the site specificity of bioactive agents.

- Avoiding the first-pass metabolism, which is a major drawback in oral drug administration, and result in optimized bioavailability of the drug.
- Minimize the undesirable side effects of the drug, as well as protect the drug from metabolic degradation; moreover, the utility of short half-life drugs.
- In most of the cases, a relatively high entrapment efficiency (EE) of nearly 90% of the lipophilic drug can be achieved by transfersomes. For transfersome formulations of diclofenac diethylamine (DDEA) and curcumin (CRM), the maximum entrapment efficiency achieved was over 90% for both DDEA and CRM transfersomes.
- They have the advantage of being made from pharmaceutically acceptable ingredients using standard methods but need to be designed and optimized on a case-by-case basis.
- Due to a short and simple production procedure, it is easy to scale-up.[5,6]

Table-1: Physicochemical, Biological properties of Drug:[4-6]

Physicochemical Properties of drug	Biological properties of drug
The drug should have a molecular weight less than 1000 Daltons	Drug should be very potent ,i.e. it should be effective in few mg/day
The drug should have affinity for both lipophilic and Hydrophilic	The drug should have short biological half-life.
Extreme partitioning characteristics are not conductive to successful drug delivery via the skin.	Tolerance to the drug must not develop under near zero order release profile of transdermal delivery.
Along with these properties the drug should be potent, having short half-life and be non-irritating.	The drug should not be irritant and non-allergic to human skin.
The drug should have low melting point.	The drug should be stable when contact with the skin
Dose is less than 50 mg per day, and ideally less than 10 mg per day.	They should not stimulate an immune reaction to the skin.

Limitations of transfersomes

- Transfersomes are considered as chemically unstable due to their tendency to oxidative degradation. The oxidation of transfersomes can be significantly decreased when the aqueous media is degassed and purged with inert gases, such as nitrogen and argon. Storage at a low temperature and protection from light will also reduce the chance of oxidation. Post-preparation processing, such as freeze-drying and spray-drying, can improve the storage stability of transfersomes.
- obstacle of utilizing transfersomes as a drug delivery system is the difficulty to achieve the purity of natural phospholipids. Therefore, synthetic phospholipids could be used as alternatives.
- The expensiveness of transfersomal formulations is associated with the raw materials used in lipid excipients, as well as the expensive equipment needed to increase Another manufacturing. Hence, the widely used lipid component is phosphatidylcholine, because it is relatively low in cost.[6,7]

Mechanism of Action of Transferosomes

According to current research, transfersomes are drug delivery systems that can pass through undamaged skin. The lipid's interaction with water causes the lipid to attract water molecules, causing hydration, and the lipid vesicles to migrate to the water-rich concentration part. This change in water content across the stratum and epidermis of the skin increases the trans dermal osmotic gradient, allowing transferosomes to penetrate the skin. As a result of its self optimizing deformability, once a transfersome reaches a pore, it can reversibly change its membrane role. Fig. 2 depicts the mechanism in visual form for easier comprehension. If transferosomes are applied to the skin under non-occlusive conditions, they can easily permeate the skin. To establish the transepidermal osmotic gradient across the skin, the skin must be non-occlusive.

- According to the literature, the Transferosomes' penetration mechanism is its moisture-seeking proclivity for deeper skin layers, also known as xerophobia (hydrotaxis). The moisture loss from the transferosomal formulation upon application to the skin causes this moisture seeking behaviour (non-occlusive state). The natural transdermal water activity variation across the skin layers creates a powerful force that activates the Transferosomes, causing the widening of intercellular connections and the formation of transcutaneous channels with a diameter of 20-30nm.
- Transferosomes to pass through the epidermal layers furthermore, the osmotic gradient created by body heat evaporating moisture from the skin's superficial layers is used as a driving force to facilitate the flexible passage of therapeutic agents from the site of application to the specific area for local or system therapies in effective therapeutic concentrations with minimal systemic toxicity.
- Transferosomes have a better penetration efficiency (through small skin pores) than traditional liposomes, but they have a similar bilayer structure that allows for the encapsulation of hydrophobic,

hydrophilic, and amphiphilic drugs. Transferosomes differ from liposomes in that their non-natural membranes are softer, more flexible, and ultra-deformable.

- Transferosomes are supramolecular units made up of a bilayer of amphipathic agent (phospholipid), and adding a bilayer unstiffening agent improves the elasticity and penetrability of the bilayer (edge activator). Alcohol is present in high or low concentrations in the formulations of several transferosomes as penetration enhancers and as solvating cosolvents. Vesicles are self-regulating and self-optimizing due to the shape of the lipid bilayer and the interdependency of local composition.
- Transferosomes can effectively and easily bypass many transport barriers.[7,8]

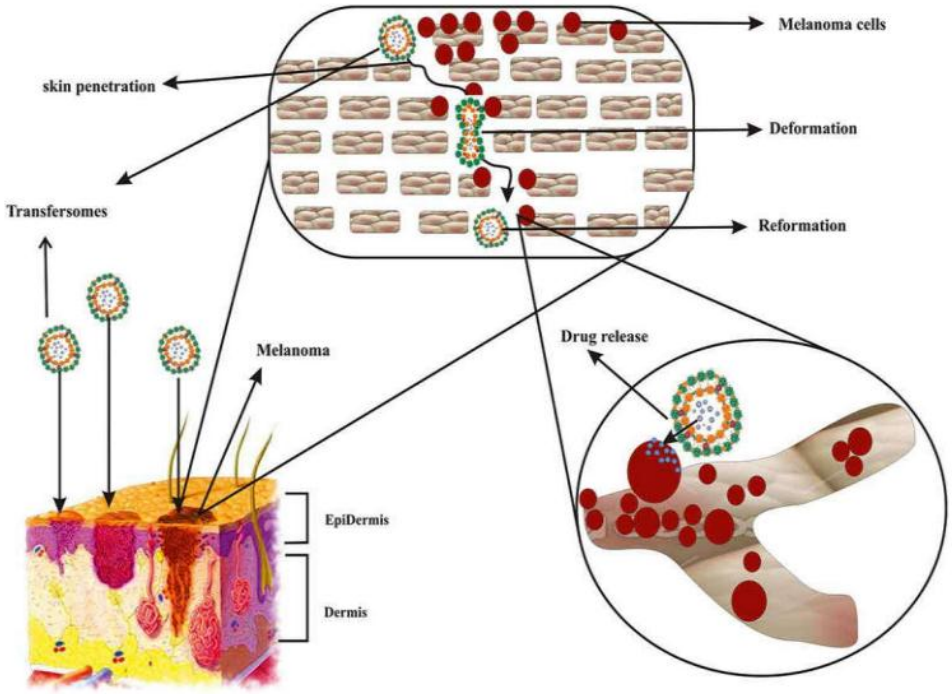


Fig. 2. Pictorial diagram of mechanism of action of transferosomes

COMPOSITION OF TRANSFERSOMES

The transfersome is composed of two main aggregates namely,

1. Firstly, an amphipathic ingredient (phosphatidylcholine), in which the aqueous solvents self-assemble into lipid bilayer that closes into a simple lipid vesicle. 2. Secondly, a bilayer softening component (such as a biocompatible surfactant or amphiphile drug) that increases lipid bilayer flexibility and permeability.
2. The resulting, flexibility and permeability optimized, transfersome vesicle can therefore adapt its shape easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer.
3. Therefore, the transfersome thus differs from such more conventional vesicle primarily by its "softer", more deformable, and better adjustable artificial membrane Table no 2.[8,9]

S.No	Class	Example	Use
1	Phospholipids	Soya phosphatidyl choline, egg phosphatidyl choline, dipalmitoylphosphatidyl choline	Vesicles forming component
2	Surfactants	Sod.cholate, Sod.deoxycholate, Tween-80, Span-80, Tween 20	Vesicles forming component
3	Solvents	Ethanol, methanol, isopropyl alcohol, chloroform	As a solvent,
4	Buffering agent	Saline phosphate buffer (pH 6.4), phosphate buffer pH 7.4	As a hydrating medium
5	Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	For CSLM study

Methods to Prepare Transferosomes

Rotary Film evaporation method

Modified hand shaking method is another name for this approach.

- In this approach, API, lecithin, and edge activator are solubilized 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.

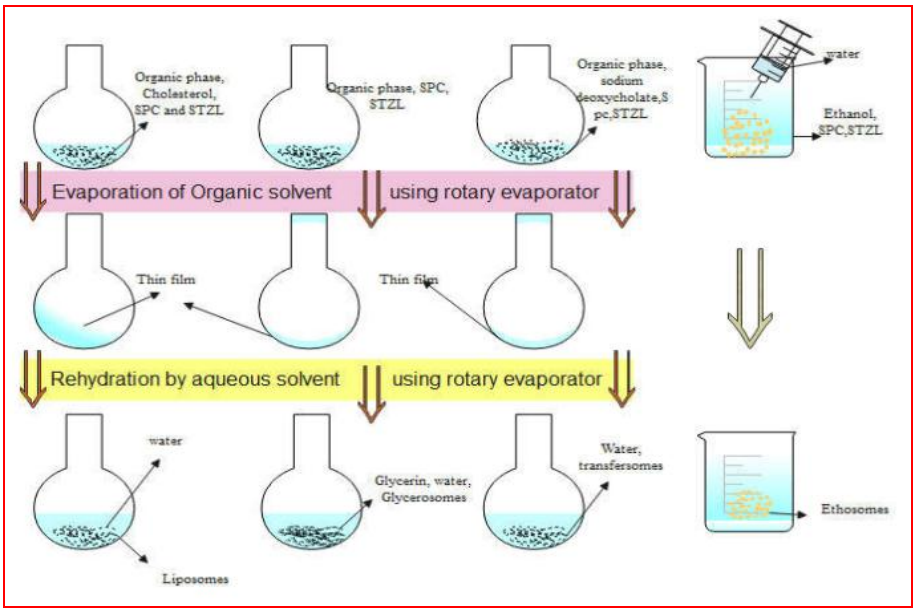


Fig no 3 Rotary film evaporation method.

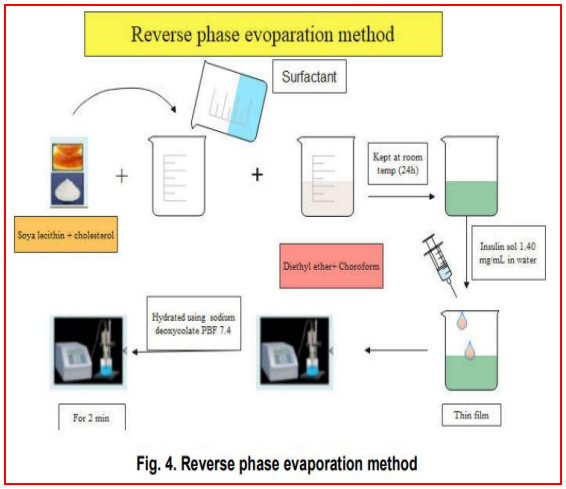


Fig. 4. Reverse phase evaporation method

Reverse phaseevaporation method

This method is carried out as follows: lipids and organic solvents were combined together in a round bottomed flask under nitrogen purging aqueous media containing edge activators.

- Depending on the drug's solubility, it's mixed with either a lipophilic or a lipophobic media. After sonication, the prepared material is left for 30 minutes until it appears to be a homogeneous combination.
- Organic phase is eliminated when pressure is kept to a minimum. The substance transforms into a viscous gel that creates vesicles. This method is depicted in Fig. 4.

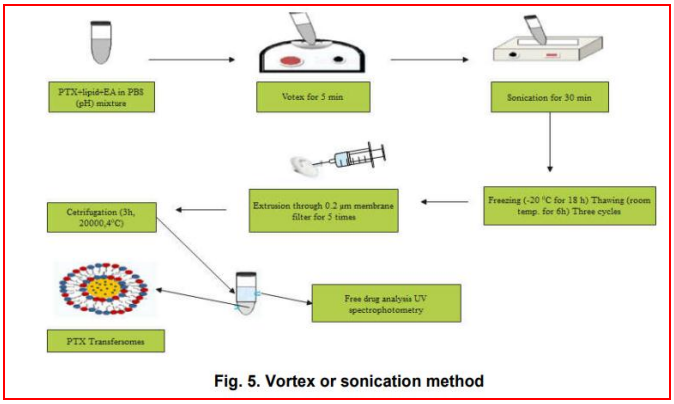


Fig. 5. Vortex or sonication method

Vortex or sonication method

- **Edge activators and phospholipids are assorted by continuous swirling in order to disperse in phosphate buffer in this procedure**.
- After forming a milky suspension, it is sonicated in a bath sonicator before being extruded through polycarbonate membranes. The process is shown in Fig. 5.

Ethanol Injection Method

- This method is more beneficial than others. The medication and water solution are warmed up at a consistent temperature with continuous agitation in this procedure. Phospholipids and edge activators are combined with an ethanolic solution in aqueous media and then reacted. Method is illustrated in Fig. 6

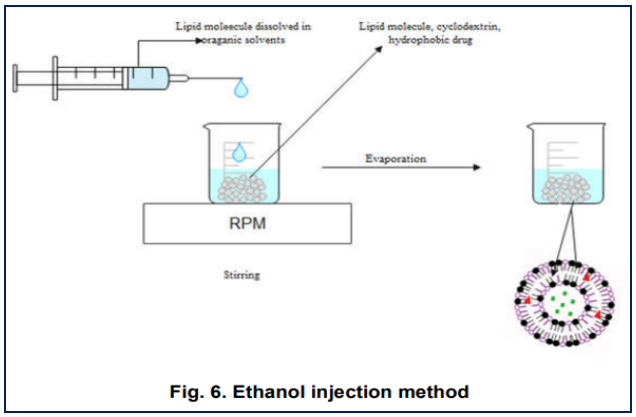


Fig. 6. Ethanol injection method

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 Fig. 5. This method is more beneficial than others. The medication and water solution are warmed up at a consistent temperature with continuous agitation in this procedure. Phospholipids and edge activators are combined with an ethanolic solution in aqueous media .Method is illustrated in 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.[9,10]

After the suspension has frozen, it is treated to a high temperature 8-9 rounds.

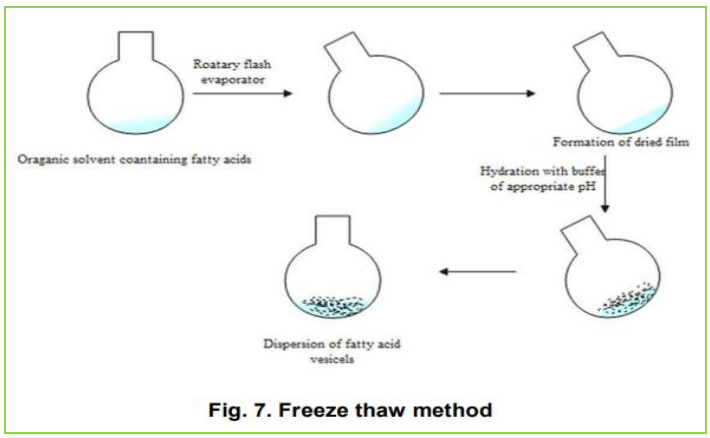


Fig. 7. Freeze thaw method

Factors Affecting Properties of Transfersomes

In the process of obtaining an optimized formulation of transfersomes, there are number of process variables that could affect the properties of the transfersomes. These variables basically involve the manufacturing of transfersomal formulations, which are identified as follows:

Effect of Phospholipids: Edge Activator Ratio

The phospholipid: Edge activator(lecithin:surfactant) should be an optimized ratio due to the fact that this greatly affects the entrapment efficiency, vesicle size and permeation ability. In general, it has been reported that the EE could be reduced due to the presence of a higher surfactant concentration. This may be due to the result of increased vesicles' membrane permeability because of the arrangement of surfactant molecules within the vesicular lipid bilayer structure, which could generate pores within the vesicular membrane and lead to an increased fluidity and prompt the leakage of the entrapped drug. A further increase in the edge activator content may lead to pore formation in the bilayer and a reduced permeation ability of the vesicles, whereas the incorporation of low concentrations of surfactants may result in growth of the vesicle size. In addition, the decrease in vesicles size at high phospholipid concentrations has been reported in various studies.

Effect of Various Solvents

Various solvents such as ethanol or methanol are used. Selection of the appropriate solvent depends on the solubility of all the formulation ingredients in the solvent and their compatibility with the solvent. Solvents used in the formulation can also exert their function as penetration enhancers that improve drug flux through the membrane For example, ethanol increases the permeation through different mechanisms, such as increasing the drug solubility in vesicles by acting as a solvent, moreover permeating into the stratum corneum and altering the solubility properties of the respective tissue and, consequently, improving the drug partitioning into the membrane.

Effect of Various Edge Activators

(Surfactants) Deformability, as well as the entrapment efficiency of transfersome vesicles, are affected by the type of edge activators used in their formulations. This could be due to the difference in the chemical structure of the EA. The three surfactants, including tween 80, span 80 and sodium deoxycholate, were used to prepare the transfersomes, and a reduction of the vesicle size was found when the higher surfactant concentration used. This might be due to the fact that the high surfactant concentrations (more than 15%) induce micelle formation rather than vesicle formation. for an example, the entrapment of a lipophilic drug would be enhanced with the use of a surfactant with a low HLB value. which leads to a higher volume of the hydrophobic bilayer domain that is available for the entrapment of hydrophobic drugs. The presence of surfactants can have an impact on the permeation property of transfersomes.

Effect of the Hydration Medium

The hydrating medium may consist of either water or saline phosphate buffer (pH 6.5–7). The pH level of the formulation should be suitable to achieve a balance between both the formulation properties and biological applications, as well as the route of administration. It is important to use the suitable pH of the hydration medium, which keeps the drug unionized to increase the entrapment and permeation of the drug.[10,11]

CHARACTERIZATION OF TRANSFERSOMES

The characterization of transfersomes is generally similar to liposomes, niosomes and micelles. Following characterization parameters have to be checked for transfersomes.

1. Vesicle size distribution and zeta potential Vesicle size, size distribution and zeta potential were determined by Dynamic Light Scattering Method (DLS) using a computerized inspection system by Malvern Zetasizer.[12]
2. Vesicle morphology 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.[12]
3. No. of vesicles per cubic mm This is an important parameter for optimizing the composition and other process variables. Nonsonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study.[7] 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) / Total number of squares counted.
4. Entrapment efficiency 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.[12]

The entrapment efficiency is expressed as: Entrapment efficiency = (Amount entrapped / Total amount added) ×100

5. Drug content The drug content can be determined using one of the instrumental analytical methods such as modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program depending upon the analytical method of the pharmacopoeial drug.[12]

6. Turbidity measurement Turbidity of drug in aqueous solution can be measured using nephelometer.[12]

7. Degree of deformability or permeability measurement In the case of transfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. 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.[12,16]

8. Penetration ability Penetration ability of Transfersomes can be evaluated using fluorescence microscopy.[17,16]

9. Occlusion effect Occlusion of skin is considered to be helpful for permeation of drug in case of traditional topical preparations. But the same proves to be detrimental for elastic vesicles. 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.[12]

10. Surface charge and charge density Surface charge and charge density of Transfersomes can be determined using zetasizer.[17]

11. *In-vitro* drug release In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from *In-vitro* studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32^0C and samples are taken at different times and the free drug is separated by mini column centrifugation. 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).[12,17]

12. In-vitro Skin permeation Studies Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50 cm2 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 0-40˚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 2.50cm2 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˚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.

13. Physical stability 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 ± 20C (refrigeration), 25 ± 20C (room temp), and 37 ± 20C (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%.[13,15]

14. Stability of Transfersomes The stability of transfersome vesicles can be determined by assessing the structure and the size of vesicles with respect to time. DLS and TEM can be used for the determination of the mean size and structural changes, respectively. The optimized transfersomal formulations can be stored in tightly sealed amber vials at different temperature conditions. According to ICH (International Conference on Harmonization) guidelines, under the stability testing of new drug substances and products, the general case for the storage condition is described as, for the long term, 25 ± 2 ◦C/60% relative humidity (RH) ± 5% RH or 30 ± 2◦C/65% RH ± 5% for 12 months and, for accelerated testing, 40 ± 2◦C/75% RH ± 5% for six months. Drug products intended for refrigeration should be subjected to long-term storage at a condition of 5 ± 3 ◦C for 12 months and accelerated study for 25 ± 2 ◦C/60% RH ± 5% RH for six months. A significant change for the drug product is defined as the failure to meet its specifications.

APPLICATION OF TRANSFERSOMES

1. Delivery of insulin: Transfersome is one of the successive ways to deliver such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient for patient. Encapsulation of insulin in transfersome (transfersulin) overcomes all problems arises with conventional insulin delivery. After application of transfersulin on the intact skin, therapeutic effect observed after 90-180 min, depending on the carrier composition.[16]

2. Delivery of corticosteroids: Problems arise with corticosteroids delivery is mask by incorporation it into transfersomes. Site specificity and overall drug of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose safety is achieved by transfersome encapsulation. Dose required for biological activity of corticosteroid is less by use of transfersomes technology.[16]

3. Delivery of proteins and peptides: Transfersomes have been widely used as a carrier for the transport of proteins and peptides also safely given by means of

transfersome technology. Proteins and peptide has problem is it is difficult to transfer into the body, are large biogenic molecules, GI tract degradation is problem arise when given orally. That's reasons why these peptides and proteins still given by means of injectables. A number of approaches have been developed to improve this condition. Transfersome is somewhat identical to that resulting from subcutaneous injection of protein suspension in terms of bioavailability. On repeated epicutaneous application, transfersome preparation of protein also induced a strong immune response. For example, the adjuvant immunogenic serum albumin in transfersomes, after several dermal challenges, is as active immunologically as is the corresponding injected proteo-transfersomes preparations.[17]

4. Delivery of interferon (INF): INF also delivered using transfersome as a carrier, for example, leukocyte-derived INF-α is a naturally occurring protein having antiviral, antiproliferative, and some immunomodulatory effects. Transfersomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Hafer et al. studied the formulation of transfersome containing interleukin-2 (IL-2) and INF-α for potential transdermal application. They reported delivery of IL-2 and INF-α promising by transfersomes insufficient concentration for immunotherapy.[18]

5. Delivery of anticancer drugs: Transfersome technology provides a new approach for cancer treatment, especially skin cancer. Result found to be favorable when methotrexate was tried for transdermal delivery using transfersome technology.[18]

6. Delivery of anesthetics: Application of transfersome containing anesthetics induces a topical anesthesia, under suitable conditions, within 10 min. Effect when we said in case of pain in sensitivity is nearly as strong (80%) as of a comparable.[19]

7. Delivery of non-steroidal anti-inflammatory drugs (NSAIDs) Problems arise with most of NSAIDs are a number of GI side effects. This can be overcome by transdermal delivery using transfersome. Further therapeutic products based on the transfersome technology, according to IDEA AG, are in clinical development.[18,19]

8. *Delivery of herbal drugs* Herbal drug also delivered by transfersome approach. XiaoYing et al. who shows the better topical absorption of transfersomes of capsaicin in comparison to pure capsaicin.[18]

Table No 4: Applications of Transferosomes.

S.NO	***Name of drug***	***Inference.***
1	Curcumin	Better permeation for anti-inflammatory activity
2	Indinavir sulfate	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).

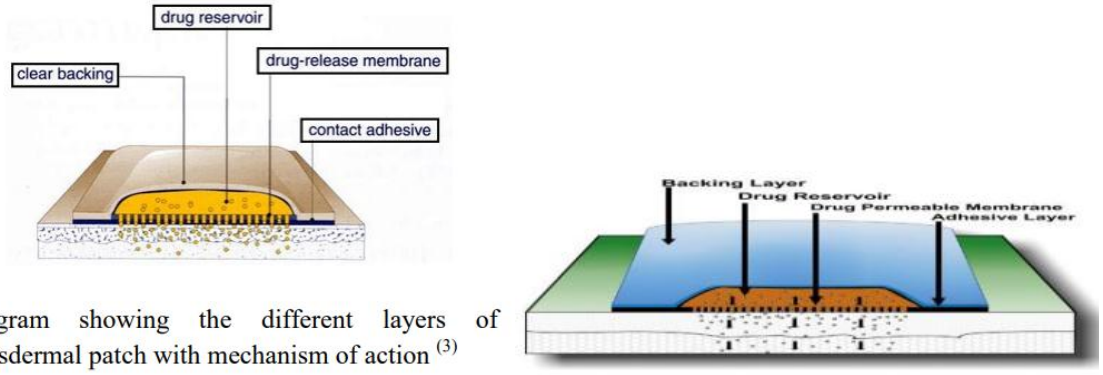


Diagram showing the different layers of transdermal patch with mechanism of action [3]

Fig no 8 Transdermal patch showing its different components.

A transdermal patch is defined as medicated adhesive patch which is placed above the skin to deliver a specific dose of medication through the skin with a predetermined rate of release to reach into the bloodstream. Today the most common transdermal

system present in the market mainly based on semipermeable membranes which were called as patches.

Mechanism of transdermal permeation: Transdermal permeation of a drug moiety involves the following steps: i. Sorption by stratum corneum ii. Permeation of drug through viable epidermis iii. Uptake of the drug moiety by the capillary network in the dermal papillary layer. iv. The drug must possess some physicochemical properties to reach target site via systemically through stratum corneum.[19]

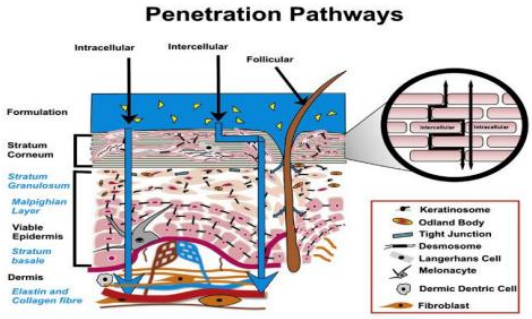


Fig no 9 Different routes of skin permeation.

Advantages

Following are the advantages of transdermal patches,

1. Transdermal patches are easier to apply and remove hence they are user / patient friendly
2. They can be comfortably used in elderly, mentally retarded subjects and pediatric age groups without the risk of over dosage and adverse effects.
3. Dermal patches are relatively painless and completely noninvasive which makes them the first choice of selection for chronically ill patients.
4. Transdermal patch is the best route of drug administration for those drugs which gets broken down by gastric juice, poor absorption in gut and the drugs with short life span (e.g. hormones & peptides).[20]

Disadvantages

1. Skin being the outermost protective organ of the body with hydrophobic (water resistant) property. Hence the drugs with hydrophilic property cannot be delivered through transdermal patch and transdermal patches also fail in delivering the skin irritant drugs. This is one of the major disadvantage of the transdermal route of drug delivery system.

2. Dermal patch serves the best in delivering thedrug at the rate of < 5mg of molecule / day. Itslower down significantly in case of drug dosage delivery of about 10 – 25mg/ day and beyond 25 mg/ day the transdermal patches fail to deliver the drug. Hence this drug delivery system cannot be used in cases demanding high level of drug dosage maintenance in blood for longer periods.

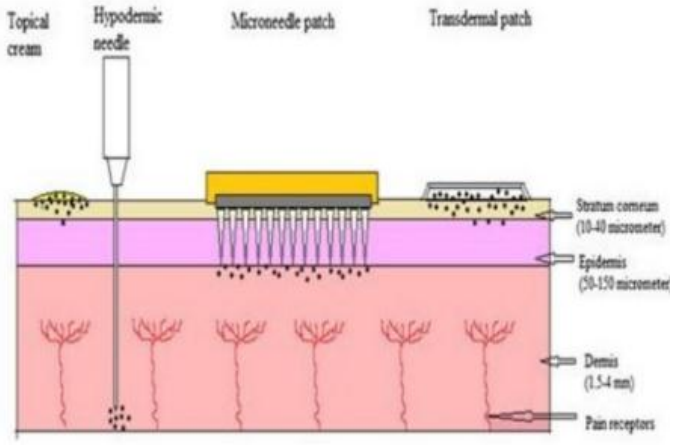


Fig no 10 The comparison of topical cream hypodermic needle, micro needle patch and transdermal patch.

Need For Transdermal Patches

Transdermal drug delivery is the delivery of medicament through the dermal route of administration by over coming to the first-pass metabolism, gastrointestinal complications, oral incompatibilities of various drugs.[2,6] This route of administration of drugs shows better plasma concentration with enhanced bioavailability compared to other formulations containing same medicament. The patches having more than one medicament are very useful for managing different disease states of the patient with minimal medication prescription, thereby achieving best outcomes with patient care-oriented therapy.[1,3,9] The major side effects such as Lacto-acidosis, drug toxicities can be reduced through using of transdermal patches.[5] This is patient-friendly as it is easy to use if any of the situations and even care takes can also administer to patients in case patient is having neurological and psychological diseases.[7,9,14,16,29] The transdermal system is a less painful route of administration, minimally invasive technique, ensuring better patient comfort for drug administration and increased to a great extent due to novel inventions and recent developments.

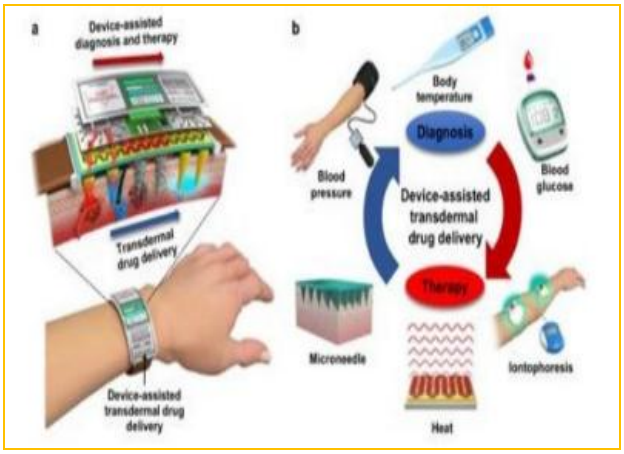


Fig no 11 schematic illustration of device transdermal drug delivery for patients. (b) the wearable device system monitoring of vital signs.

Advancement In Transdermal Drug Delivery System A break through in transdermal drug delivery system was seen in previous generations only focused on the areas for maximising the better utility of drug delivery system.[14,16] The recent growth in the area found to be personalized in medicine requires a new generation of the drug delivery.[14,18] Which aimed at controlled release and feed back induced transdermal drug release.[20] Novel material design and device fabrication bought advancement in variable devices for biomedical applications, giving a new opportunity in personalised health care.

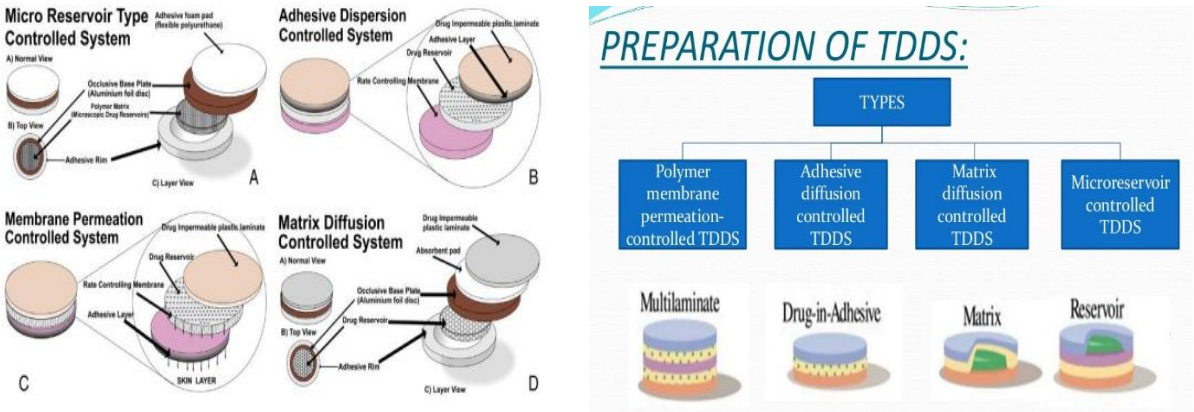


Fig no 12

(A): Showing the presence of microscopic spheres of drug reservoir,
(B) Development of adhesive dispersion controlled therapeutic system
(C)Diagrammatic representation of membrane permeation controlled system,
(D): Representation of matrix type transdermal system.

Several technologies have been successfully developed to provide a rate control over the release and the transdermal permeation of drugs.

These technologies are as follows:
Adhesive dispersion type system
- Membrane permeation controlled system
- Matrix diffusion controlled system:
- Microreservoir type controlled system:

Evaluation of Transdermal system
Interaction studies: The drug and polymer compatibility was characterized by means **of FTIR spectroscopy.**

Physical evaluation of transdermal system
Film thickness: The thickness of film is measured by using micro meter, electronic vernier callipers, with a, dial gauge, or screw gauge.

Percentage flatness: Film is cut in to strips, two from either end or one from the center. The length of these strips is measured to the nearest centimetre without applying any additional pressure.
% Constriction = $(\boldsymbol{Initial\ length - Final\ length})\ \boldsymbol{Initial\ length\ X\ 100}$

Folding endurance: folding endurance of patches can be determined by repeatedly folding a small strip of film (2 x 2 cm) at the same place till it breaks. The number of time film could be folded at the same place without breaking is the folding endurance value.

Tensile strength: The tensile strength can be determined by using a modified pulley system. weight is gradually increased so as to increase the pulling force till the patch breaks. The force required to break the film is considered as tensile strength and it is calculated as kg/cm^2.
$\boldsymbol{Tensile\ Sterngth = Tensile\ Load / Cross\ section\ Area}$

Patch thickness: Patch thickness can be measured by using digital micrometer screw gauge at three different points and the mean value is calculated.

Elongation break test: The elongation break is to be determined by noting the length just before the break point. The elongation break can be determined by the formula:
Elongation break = $(\boldsymbol{Final\ length - Initial\ length})\ \boldsymbol{Initial\ length}$

Weight uniformity: weight uniformity is studied by randomly selected patches about 10 in number. A specified area of patch is to be cut in different parts of the patch and weighed in a digital balance.

Drug content: A film of required area (1 x1 cm / 2 x 2 cm etc.) is cut, put this small piece of film in to 100 ml buffer (pH 7.4 or 6.8 or as prescribed) and shaken continuously for 24 hours. Then the whole solution is ultrasonicated for 15 minute. After filtration, the drug is estimated spectrophotometric ally and the drug content is determined.

Percentage of moisture content: The films are weight individually and left in a dessicator containing anhydrous calcium chloride or activated silica at room temperature for 24 hours. Individually films are weighed repeatedly until they showed a constant weight
% moisture Content = $\boldsymbol{InitialWeight - FinalWeight\ FinalWeight \times 100}$

Percentage of moisture uptake: A weight film kept in a dessicator at room temperature for 24 hours is taken out and exposed to 84% relative humidity (a saturated solution of potassium chloride) in a dessicator until a constant weight for the film is obtained.
% $\boldsymbol{Moisture\ Uptake}$ = $(\boldsymbol{Final\ weight - Initial\ weight})\ \boldsymbol{Initial\ Weight \times 100}$

Water vapour transmission rate: Glass vials approx. 5 ml capacity of equal diameter were taken for transmission study. All vials washed thoroughly and dried in an oven completely. Weigh about 1 gm of anhydrous/ fused calcium chloride and kept in respective vials. Fix the films on the brim of vials and weigh individually then kept in closed dessicator containing saturated solution of potassium chloride to maintain humidity approx. 84%. The vials were weighed in 6, 12, 24, 36, 48, and 72 hours respectively.
$\boldsymbol{Transmission\ rate}$ = $(\boldsymbol{Final\ Weight - Initial\ Weight})\ \boldsymbol{Area \times Time \times 100}$

Content uniformity test: Select 10 patches but content is determined for individual patches. If 9 out of 10 showed content between 85-115% of the specified value and no one has shown 75-125% of the specified value, it means the test has been passed but if 3 patches shown the content between 75-125% then taken 20 additional patches and further test performed. If these 20 patches shown content between 85- 115%, then the patches passed the test.

Uniformity of dosage unit test : A patch of accurately weigh is cutted in to small pieces and transferred to volumetric flash containing specific volume of suitable solvent for dissolution of drug and then sonicated for a limited period of time for complete extraction of drug from pieces and then mark the volume with the same solvent. The solution obtained kept untouched for 1 hour to settle down then supernatant diluted as required. The dilute solution was filtered by membrane.

Polariscope examination: The instrument polariscope used to study the crystal structure of drug in a patch. A specifiec area of patch is cut and kept on the slide to observe that drug present in crystalline form or amorphous form.

Shear adhesion test: The cohesive strength of an adhesive polymer is determined by this test. The value of strength can be affected by the degree of cross linking, the molecular weight, the composition of polymer and the amount of tackifiers added. An adhesive coated patch is stacked on plate made of stainless steel and specified weight hung from the patch parallel to this plat. The time taken to pull off the patch from the plate determines the cohesive strength.

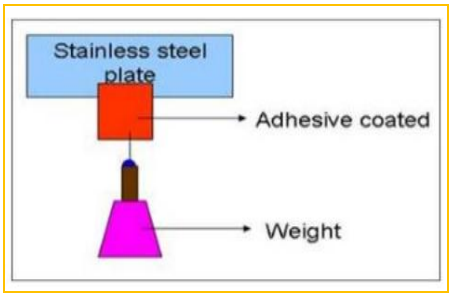


Fig no 13 Shear Adhesion test.

Peel adhesion *test:* The measure of patch strength between an adhesive and a substrate is defined as adhesion. The force required removing adhesive coating from the steel used as test substrate. The type and amount of polymer molecular weight and the composition of polymers determine the adhesive properties.

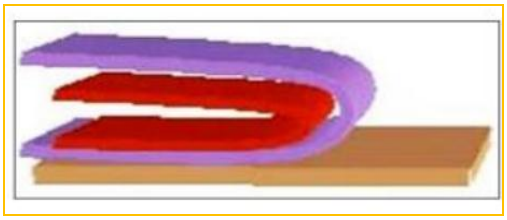


Fig no 14.

Tack properties: Tack is the ability of polymer to adhere to a substrate with little fingure pressure.

Tests for tack include

Thumb tack test: This is subjective test in which evaluation is done by pressing the thumb in to the adhesive. Experience is required for using the test.

Rolling ball tack test: **This test involves measurement of distance travelled by a stainless steel along the upward face of adhesive.** The diameter of ball is 7/16o inches and it released on inclined track More the distance travelled, less the tacky polymer.

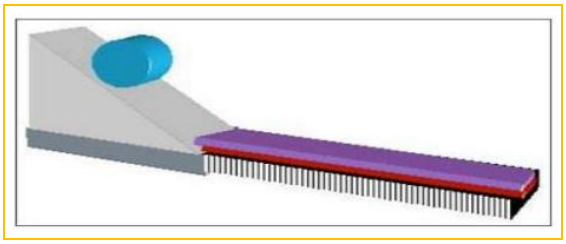


Fig no 15

Peel tack or quick stick test: The peel force is the force required to break the bond between the adhesive and the test substrate. The patch is pulled away from the substrate.

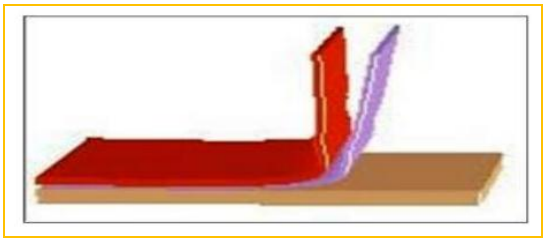


Fig no 16

***Probe tack test*:** In this, the tip of probe with defined surface roughness brought in to contact **with adhesive and when the bond is formed between the adhesive an probe, removal of probe** at a fixed rate away from the adhesive which break the bond. The force required to break the bond is recorded as tack and it is expressed in grams.

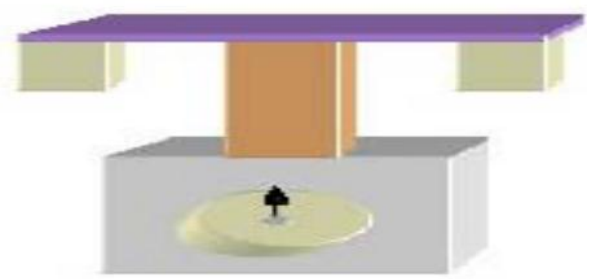


Fig no 17

Skin irritancy studies: The skin irritancy can be performed on healthy rabbits / mice albino / rats and potential of transdermal system can be evaluated by modified Draize test. The dorsal surface of given test animal is to be cleaned and remove the hair from the clean surface then applied rectified sprit. Applied the transdermal formulation over the clean surface for 24 hour. After this period, remove the formulation and observed the status of skin. The score are given from 0 to 4 depending the degree of erythema as follows: zero point given for no erythema, 1 point for slight erythema-(barely perceptible-light pink), 2 poinst for moderate erythema(dark pink), 3 points for moderate to severe erythema(dark pink) and 4 points for severe erythema (extreme redness).

Confocal laser scanning microscopy(clsm): Depth of skin penetration of a patch can be assessed using CLSM. Transdermal formulation is applied non-occulsively for8 hours to the dorsal skin. The mice is sacrificed by heart puncture, dorsal skin is excised and washed with distilled water. The excised skin is then placed on aluminium foil and the dermal side of the skin is generally teased off any adhering fat and/ or subcutaneous tissue. These are then cut in to pieces of 1mm2 and tested for probe penetration. The full skin thickness is optically scanned at different increments through the z-axis of a CLS microscope.

Stability studies: The stability of active component is a major criterion in determining acceptance or rejection of transdermal system. The stability studies were performed as according to ICH guidelines as at different temperature and relative humidity 25-30^{o}C (60% relative humidity) and 45- 50^{o}C (75% relative humidity) over a period of 60 days. The sample were withdrawn at 0,3,6, and 9 weeks respectively and were analyazed for their physical appearance, drug content and in-vitro diffusion studies.

In-vitro release studies: The best available tool today which can at least quantitatively assure about the biological availability of a drug from its formulation is its in vitro dissolution test.

- Paddle over dics apparatus (USP apparatus)
- Cylindrical apparatus (USP apparatus)
- Reciprocating disc

In-vitro skin permeation and release kinetics studies: The design and development of transdermal patch is greatly influenced by in vitro studies. In-vitro studies greatly help in investigating the route of skin permeation and the rate of transfer through skin by which drug entered in to systemic circulation. These studies can easily performed and methodology used allowed flexibility in adapting the model in addressing different aspects involved in preliminary or feasibility studies in the development of transdermal patch.

Franz Diffusion Cell: The in-vitro skin permeation of transdermal patches can be studied using Franz diffusion cell(most commonly used) with an effective permeation area of 1.0cm^2 and receptor cell volume of 10 ml. The temperature is maintained at 32oC ± 1^{o}C. The receptor compartment is filled with 10 ml PBS and is constantly stirred in a magnetic stirrer at 100rpm. The skin is mounted on a receptor compartment with the stratum corneum side facing upward in to the donor compartment. Samples are withdrawn through the sampling port of the diffusion cell at predetermined time interval over 24 hours and are analysed.

IN-VIVO STUDIES: These studies are the true depiction of formulation performance. The variables which were not considered during in-vitro study taken in to account now**. In-vivo studies of transdermal system can be done by using following model ➢ Animal Models ➢ Human volunteers ➢ Biophysical Model.**[21]

Table1: Transdermal drugs approved by the US FDA:

year	Drug	Indication	Product Name
1979	Scopolamine	Motion sickness	Transderm-Scop
1981	Nitroglycerin	Angina pectoris	Transderm-Nitro
1984	Clonidine	Hypertension	Catapres-TTS
1986	Estradiol	Menopausal symptoms	Estraderm
1990	Fentanyl	Chronic pain	Duragesic
1991	Nicotine	Smoking cessation	Nicoderm, Habitrol, ProStep
1993	Testosterone	Testosterone deficiency	Testoderm
1995	Lidocaine/epinephrine (iontophoresis)	Local dermal analgesia	Iontocaine
1998	Estradiol/norethidrone	Menopausal symptoms	Combipatch

CONCLUSION

The use of the transdermal route has been well established in the past, and because of its inherent advantages, new methods for transdermal delivery are continuously being developed. The introduction of ultradeformable vesicles, transferosomes, will thus surely become an important step in relaunching the researches regarding the use of vesicles as transdermal drug delivery systems. In comparison to other transdermal delivery systems, the use of elastic vesicles has certain advantages: They allow enhanced permeation of drug through skin; their composition is safe and the components are approved for pharmaceutical and cosmetic use; they can increase the transdermal flux, prolonging the release and improving the site specificity of bioactive molecules; they can accommodate drug molecules with a wide range of solubility. Hence, enhanced delivery of bioactive molecules through the skin by means of an ultradeformable vesicular carrier opens new challenges and opportunities for the development of novel improved therapies. Thus, it could be concluded that the new ultra flexible drug carrier (transferosome) can overcome all the problems associated with the transdermal delivery as transferosomes itself are specially optimized vesicles having the capability of responding to an external stress by rapid and energetically inexpensive shape transformations.

FUTURE PERSPECTIVES: The high tolerability and efficiency of these vesicular systems open vast potential therapeutic uses. These nanocarriers might offer advanced local and systemic new therapies with agents that are unable to efficiently penetrate the stratum corneum via passive diffusion. The non steroidal anti-inflammatory drug (NSAID), ketoprofen, in a transferosome formulation gained marketing approval by the Swiss regulatory agency (SwissMedic). The product is expected to be marketed under the trademark Diractin. Further therapeutic products based on the transferosome technology, according to IDEA AG, are in clinical development.

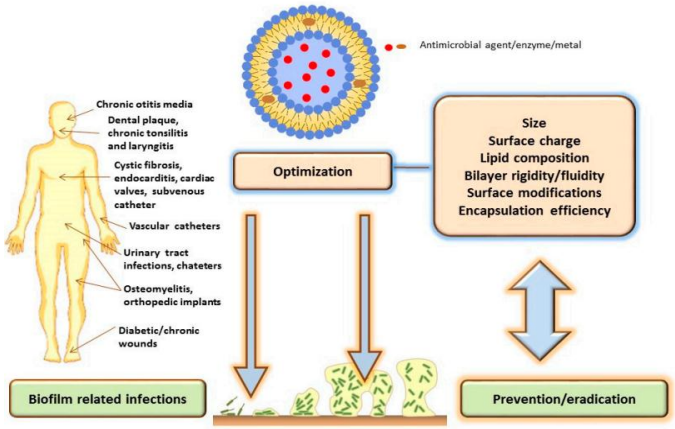


Fig no 17 Influence of transferosomal physicochemical properties on the biofilm delivery.

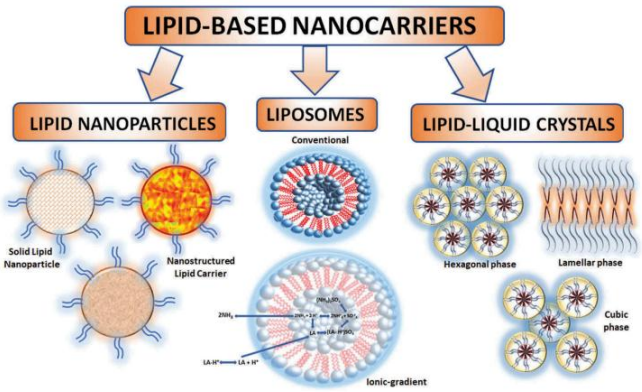


Fig no 18: Representation of lipid based nanocarriers described as DDs for local anesthetics.

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