TRANSFEROSOMES: A NOVEL CARRIER FOR ENHANCED DERMAL DELIVERY OF DRUG

Dr. Ranju Pal* and Dr. Manoj Pandey

Department of Surgical Oncology, BMHRC, Bhopal (M.P.), India.

ABSTRACT

The transdermal route of drug delivery has gained great interest of pharmaceutical research, as it circumvents number of problems associated with oral route of drug administration. The major barrier in transdermal delivery of drug is the skin intrinsic barrier, the stratum corneum, the outermost layer of the skin that offers the principal barrier for diffusion of drug. Recently, various strategies have been used to improve the transdermal delivery of drug. Mainly, they include iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microneedles, and vesicular system (liposomes, niosomes, elastic liposomes such as ethosomes and transferosomes). Among these strategies transfersomes appear promising candidate. Transfersomes are a form of elastic or deformable vesicle, which were first introduced in the early 1990s. Elasticity is generated by incorporation of an edge activator in the lipid bilayer structure. The original composition of these vesicles was soya phosphatidyl choline incorporating sodium cholate and a small concentration of ethanol. Transfersomes are applied in a non-occluded method to the skin and have been shown to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. They have been used as drug carriers for a range of small molecules, peptides, proteins and vaccines, both in vitro and in vivo. Transfersomes penetrate through the stratum corneum and the underlying viable skin into the blood circulation. This review highlights the various aspects of the transfersomes in the effective delivery of drug.

KEYWORDS: Transdermal delivery, Stratum corneum, Osmotic force, Transfersomes, Permeation enhancers.
INTRODUCTION
Molecules greater than 500 Da normally do not cross the skin. This prevents epicutaneous delivery of the high molecular weight therapeutics as well as non-invasive transcutaneous immunisation.\textsuperscript{[1]} Nowadays, the transdermal route has become one of the most successful and innovative focus for research in drug delivery, with around 40\% of the drug candidate being under clinical evaluation related to transdermal or dermal systems. The technology has a proven record of FDA approval since the first transdermal patch was approved in 1981. The market for transdermal products has been in a significant upward trend and this is likely to continue for the future. An increasing number of transdermal drug delivery products continue to deliver real therapeutic benefit to patients around the world.\textsuperscript{[2]}

There are several advantages offered by transdermal route, but only few drug candidates are administered via this route due to formidable barrier nature of stratum corneum.\textsuperscript{[3]} To overcome the constraint, a great number of vesicular approaches are under investigation and the major among them are elastic liposomes, ethosomes and transferosomes. Transfersome is a extremely adaptable and anxiety-responsive, complicated aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimising. This enables the Transfersome to cross a variety of transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. The carrier aggregate is composed of at least 1 amphiphat (such as phosphatidylcholine), which in aqueous solvents self-assembles into lipid bilayer that closes into a easy lipid vesicle. By addition of at least 1 bilayer softening component (such as a biocompatible surfactant or an amphiphile drug) lipid bilayer flexibility and permeability are greatly increased. The resulting, flexibility and permeability optimised, Transfersome vesicle can for that reason adapt its shape to ambient effortlessly and rapidly, by adjusting local concentration of each and every bilayer component to the local anxiety experienced by the bilayer. In its simple organization broadly similar to a liposome, the Transfersome thus differs from such more conventional vesicle primarily by its “softer”, far more deformable, and much better adjustable artificial membrane. One more advantageous consequence of strong bilayer deformability is the increased Transfersome affinity to bind and retain water. An ultradeformable and extremely hydrophilic vesicle usually seeks to steer clear of dehydration this may possibly involve a transport process related to but not identical with forward osmosis. For example, a Transfersome vesicle applied on an open biological
surface, such as non-occluded skin, tends to penetrate its barrier and migrate into the water-wealthy deeper strata to secure its adequate hydration. Barrier penetration entails reversible bilayer deformation, but need to not compromise unacceptably either the vesicle integrity or the barrier properties for the underlying hydration affinity and gradient to remain in place.

Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. There is provision for this, 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 achieved by mixing suitable surface-active components in the proper ratios.[4] 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 nonocclusive condition. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties.[5]

![Schematic Diagram of the Two Microroutes of Penetration.](image)

**Advantages of Transdermal Drug Delivery System (TDDS)**

The advantages of transdermal delivery over other delivery modalities are as follows:

- Avoidance of ‘first-pass’ metabolism of drugs.
- Peak plasma levels of drugs are reduced, leading to decreased side effects.[6]
- Reduction of fluctuations in plasma levels of drugs.
- Utilization of drug candidates with short half-life and low therapeutic index.[7]
- Easy termination of drug delivery in case of toxicity.
- Reduction of dosing frequency and enhancement of patient compliance.[8]
For transdermal drug delivery system to be effective, the drug must obviously be able to penetrate the skin barrier and reach the target site.\textsuperscript{[9]}

**Limitations**

Limitation for a drug candidate to be incorporated into a transdermal delivery system are:

• Higher molecular weight candidates (>500Da) fail to penetrate the stratum corneum.
• Drugs with very low or high partition coefficient fail to reach systemic circulation.
• High melting drugs, due to their low solubility both in water and fat. Such candidates cannot be delivered across the skin without effectively making suitable modifications in the conventional transdermal delivery systems.\textsuperscript{[10]}

**SCOPE OF TRANSFERSOMES**

Transfersome technology is best suited for non-invasive delivery of therapeutic molecules across open biological barriers. The Transfersome vesicles can transport across the skin, for example, molecules that are too big to diffuse through the barrier. Examples include systemic delivery of therapeutically meaningful amounts of macromolecules, such as insulin or interferon, across intact mammalian skin. Other applications include the transport of small molecule drugs which have certain physicochemical properties which would otherwise prevent them from diffusing across the barrier. Another attraction of the Transfersome technology is the carrier’s ability to target peripheral, subcutaneous tissue. This ability relies on minimisation of the carrier-associated drug clearance through cutaneous blood vessels plexus: the non-fenestrated blood capillary walls in the skin together with the tight junctions between endothelial cells preclude vesicles getting directly into blood, thus maximizing local drug retention and propensity to reach the peripheral tissue targets.\textsuperscript{[11]}

**Silent features of Transfersomes**

- 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 (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, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, 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 encapsulated drug from metabolic degradation.

They act as depot, releasing their contents slowly and 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.\textsuperscript{[12]}

**MATERIALS AND METHODS**

Materials commonly used for the preparation of transfersomes are summarized in Table.

**Table 1: Different additives used in formulation of transfersomes.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>Soya phosphatidyl choline</td>
<td>Vesicles forming Component\textsuperscript{[13]}</td>
</tr>
<tr>
<td></td>
<td>Dipalmitoyl phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distearoyl phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td>Surfactant</td>
<td>Sodium cholate</td>
<td>For providing flexibility\textsuperscript{[14]}</td>
</tr>
<tr>
<td></td>
<td>Sodium deoxycholate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tween-80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Span-80</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>Ethanol</td>
<td>As a solvent\textsuperscript{[15]}</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine-123</td>
<td>For CSLM study\textsuperscript{[16]}</td>
</tr>
<tr>
<td></td>
<td>Rhodamine-DHPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluorescein-DHPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nile-red</td>
<td></td>
</tr>
<tr>
<td>Buffering agent</td>
<td>Saline phosphate buffer (pH 6.4)</td>
<td>As a hydrating medium</td>
</tr>
</tbody>
</table>

All the methods of preparation of transfersomes are comprised of two steps. First, a thin film is prepared hydrated and then brought to the desired size by sonication; and secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane. The mixture of vesicles forming ingredients, that is phospholipids and surfactant were dissolved in volatile organic solvent (chloroform-methanol), organic solvent evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50\textdegree C for dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight. The deposited lipid films were hydrated with buffer (pH 6.5) by rotation at 60 rpm min\textsuperscript{−1} for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature. To prepare small vesicles, resulting LMVs were sonicated at room temperature or 50\textdegree C for 30 min. using a B-12 FTZ bath sonicator or probe.
sonicated at 40°C for 30 min (titanium micro tip, Heat Systems W 380). The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membrane.[17]

Fig 2: Undeformable Vesicle (Transferosome)

MECHANISM OF PENETRATION OF TRANSFERSOMES
Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. The reason for this high flux rate is naturally occurring "transdermal osmotic gradients" i.e. another much more prominent gradient is available across the skin.[18] This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content).[19] This gradient is very stable because ambient air is a perfect sink for the water molecule even when the transdermal water loss is unphysiologically high. All polar lipids attract some water this is due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration.[20,21] Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration.[22, 23] So when lipid suspension (transfersomes) is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and try to escape complete drying by moving along this gradient. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes composed of surfactant have more suitable rheologic and hydration properties.
than that responsible for their greater deformability less deformable vesicles including standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantages of the transepidermal osmotic gradient (water concentration gradient). Transfersome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer as shown in fig 3.

THEORIES AND APPROXIMATIONS IN MICELLE AND VESICLE FORMATIONS

Micelle formation Law of mass action

It was consider an aqueous solution of neutral amphiphilic molecules (i.e., non-ionic surfactants), each of which has a single alkyl chain as its hydrophobic tail. In general, amphiphiles can form aggregates of various sizes and shapes. We will assume each micelle is spherical and neglect the effects of fluctuations in micelle size and shape. Thus, we imagine that each surfactant molecule exists either as a monomer or as part of a spherical n-mer. We denote the number densities of the monomers and n-mers by $\rho_1$ and $\rho_n$, respectively, so that the total surfactant concentration is given by:

$$\rho = \rho_1 + n \rho_n$$

The concentrations of monomers and micelles are related by the law of mass action.

$$\rho_n a^3 = (\rho_1 a^3) \exp (-\beta G)$$

where: $\beta$ denotes inverse temperature (i.e., $\beta^{-1} = k_BT$), ‘$a$’ is a microscopic length that specifies the standard state convention, and is the driving force for assembly, namely, the free energy of the n-mer, $f_n$, relative to that of n monomers, $nf_1$. We take ‘$a$’ to be approximately the girth of a surfactant molecule.

$$\Delta G = f_n - nf_1$$

For large $n$, equation 3 implies the existence of a threshold concentration of surfactant molecules $\rho_{cmc}$, at which the density of aggregates becomes significant. Because this crossover is precipitous, its location is almost independent of the specific definition of the threshold as long as it is physically sensible. Specifically, to within corrections of order $n^{-1} \ln n$.

$$\ln \beta_{cmc} a^3 = \beta \Delta G \ln^*$$
The driving force per surfactant, $\Delta G/n$, is a function of $n$, and it is to be evaluated at the most probable aggregation number, $n^\ast$. This number is the value of $n$ that minimizes $\Delta G/n$.\cite{11}

**CHARACTERIZATION AND EVALUATION OF TRANSFERSOMES**

Transfersomes are characterized as follows using different methods.

**Vesicle size, size distribution and vesicle diameter**

Transfersomes can be visualized by transmission electron microscopy (TEM) and vesicle size and size distribution can be determined by dynamic light scattering (DLS) technique. 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 measurements.\cite{14}

**Vesicle shape and type**

Transfersomes vesicles can be visualized by using TEM, with an accelerating voltage of 100 kv. These vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope. Dynamic light scattering is also used for determining vesicle shape.\cite{11}

**Number of vesicle per cubic mm**

This is an essential parameter for optimizing the composition and other process variables. Transfersome formulations (without sonication) can be diluted five times with 0.9% of sodium chloride solution and studied with optical microscopy by using haemocytometer.\cite{11}

**Entrapment Efficiency**

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

$$\text{Entrapment efficiency} = \left( \frac{\text{amount entrapped}}{\text{total amount added}} \right) \times 100\%.$$\cite{12}

**Degree of Deformability or Permeability Measurement**

Degree of deformability is an important and unique parameter of transfersomal formulations because it differentiates transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum intact. 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. The degree of deformability was calculated by using the following formula, as reported by Berge vanden et al.\textsuperscript{[27]}

\[ D = J* \left( \frac{rv}{rp} \right)^2 \]

where,

- \( D \) = deformability of vesicle membrane
- \( J \) = amount of suspension, which was extruded during 5 min
- \( rv \) = size of vesicles (after passes)
- \( rp \) = pore size of the barrier

**Propensity of penetration**

The magnitude of the transport driving force plays an important role.

\[ \text{Flow} = \text{Area} \times (\text{Barrier \ Permeability}) \times (\text{Trans-barrier \ force}) \]

Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the same amount of lipids in a suspension.

**Confocal Scanning Laser Microscopy (CSLM) study**

A confocal microscope illuminates and detects the scattered or fluorescent light within the vesicles. A set of conjugated apertures, one for illumination and one for detection of light, function as spatial filters. In confocal microscopy, lateral and axial resolutions are enhanced when compared to standard light microscopy. The axial resolution is responsible for identifying the lodging position of the vesicles ever deep within the tissues. The main advantage of confocal microscope is its ability to optically section thick specimens. Real time video frame can be captured with a low light video camera which inturn can be connected to a video recorder. Video frames give a live demonstration of the pathway and nature of the transportation of vesicles. Fluorescent dyes for detection used are Calcien AM (for green fluorescent), Rhodamine – 123, Rhodamine – DHPE, fluoroscein – DHPE, nile red.

Results indicated clearly that vesicles remarkably partitioned deep into the stratum corneum and could reach the layers as deep as the stratum corneum viable epidermal junction within one hour. Vesicles were found housed within the intercellular regions as channel like structures. No other abnormalities were detected within the intercellular lipid lamellae. The
model drug was associated with vesicle material in the upper and central layers of stratum corneum. In the lower layers of stratum corneum, only vesicle material was there, not much of the drug. This clearly indicates that drug has been released from the vesicles before reaching the deeper layers. This indicates that the free drug molecules got released from the vesicles into viable skin layers.

Using confocal laser scanning microscopy (CLSM), it is possible with a high degree of precision to locate and study transport phenomena of fluorescent chemical substances in different materials. This technique is often used in evaluation of biological phenomena and transport studies through various biological membranes, e.g., the intestine and the skin. However, until now only a few studies using CLSM have focused on the application of technical pharmaceutical aspects. In this technique lipophilic fluorescent markers are incorporated into the transfersomes and the light emitted by these markers are used for the investigation of mechanism of penetration of transfersomes across the skin, for determining histological organization of the skin and for comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles. Penetration ability of transfersomes can be evaluated using fluorescence microscopy.[28]

**Number of Vesicles per Cubic mm**

This is the most important parameter for optimizing the composition and other process variables. Transfersomal formulation (without sonication) was diluted five times with 0.9% of NaCl solution, and the number of transfersomes per cubic mm was counted by optical microscopy by using a haemocytometer. The transfersomes in 80 small squares were counted and calculated by using the following formula.[27]

\[
\text{Total no. of Transferosomes per cubic mm} = \text{Total no. of Transferosomes counted X dilution factor X 4000/ Total no. of squares counted}
\]

**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°C and samples are taken at different times and the free drug is separated by mini column
centrifugation.\textsuperscript{[29]} 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).

\textbf{In Vivo Fate of Transfersomes and Kinetics of Transfersomes Penetration:} After having penetrated through the outermost skin layers, transfersomes reach the deeper skin layer, the dermis. From this latter skin region they are normally washed out, via the lymph, into the blood circulation and through the latter throughout the body, if applied under suitable conditions. Transfersomes can thus reach all such body tissues that are accessible to the subcutaneously injected liposomes. The kinetics of action of an epicutaneously-applied agent depends on the velocity of carrier penetration as well as on the speed of drug (re)distribution and the action after this passage. The most important single factors in this process are.

1. Carrier in-flow.
2. Carrier accumulation at the targets site.
3. Carrier elimination.

The onset of penetration-driving force depends on the volume of the suspension medium that must evaporate from the skin surface before the sufficiently strong trans-cutaneous chemical potential chemical potential or water activity gradient is established. Using less solvent is favorable in this respect. The rate of carrier passage across the skin is chiefly determined by the activation energy for the carrier deformation. The magnitude of the penetration driving force also plays a big role. This explains, for example, why the occlusion of an application site or the use of too strongly diluted suspension hampers the penetration process. Carrier elimination from the sub cutis is primarily affected by the lymphatic flow, general anesthesia or any other factor that affects this flow, consequently, is prone to modify the rate of transcutaneous carrier transport. While it has been estimated that approximately 10% of the cardiac blood flow pass through each gram of living skin tissue, no comparable quotation is available for the lymph. Further, drug distribution is also sensitive to the number of carrier used, as this may affect the rate of vehicle degradation and / or filtration in the lymph nodes. The lag between the time of application and the time of drug appearance in the body, therefore, is always quite long, complex and strongly sensitive to the type of drug and formulation administration.

In the best case, the skin penetration lag amounts to approximately 15 min. if rapidly exchanging agents such as local analgesics are detected right under the skin permeability barrier (Planas et al., 1992).\textsuperscript{[30]} Less rapidly exchanging molecules or molecules measured in
the blood compartment are typically detected with a lag time between 2 and 6 hr. depending on the details of drug formulation. Molecules that do not diffuse readily from the carriers or agents delivered with the suboptimal carriers normally fall in this category. The kinetics of vesicle penetration into and across the skin can be controlled to a large extent by fixing the physicochemical characteristics of the drug carrier suspension.

Kinetics of the transfersomes penetration through the intact skin is best studied in the direct biological assays in which vesicle associated drugs exert their action directly under the skin surface. Local analgesics are useful for this purpose, for determining the kinetics of penetration, various lidocaine loaded vesicles were left to dry out on the intact skin.

Corresponding subcutaneous injection is used as control. The animal's sensitivity to pain at the treated site after each application was then measured as a function of time. Dermally applied standard drug carrying liposomes or simple lidocaine solution have never caused any analgesic effect. It was necessary to inject such agent preparations to achieve significant pain suppression. In contrast to this, the lidocaine-loaded transfersomes were analgesic ally active even when applied dermally. Maximum analgesic effect with the latter type of drug application was typically observed 15 minutes after the drug application. A marked analgesic effect was still noticeable after very long time. The precise reach as well as kinetics of transfersomes penetration through the skin are affected by: drug carrier interaction, application condition or form, skin characteristics, applied dose.

**Tape stripping method**

Tape stripping technique is a widely accepted and it is used to examine the localization and distribution of substances within the stratum corneum. It is the simplest method for reducing the barrier imposed by the stratum corneum is to remove it. In this technique, an adhesive tape removes a layer of corneocytes. *In vivo*, removal of the stratum corneum by tape stripping is performed by the repeated application of adhesive tapes to the skin’s surface. This can be used to investigate stratum corneum cohesion *in vivo* by quantifying the amount of stratum corneum removed. Tape stripping method can be studied in combination with electron microscopy and FT-IR. There are different parameters that can affect the quantity of stratum corneum removed by a piece of tape, and these include tape stripping mode, skin hydration, cohesion between cells, the body site and inter-individual differences [31-34].
Stability Studies

Transfersomes stability was determined at 4°C and 37°C by TEM visualization and DLS size measurement at different time intervals (30, 45, and 60 days), following vesicles preparation.

APPLICATION OF TRANSFERSOMES

Transfersomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract. These are the reasons why these peptides and proteins still have to be introduced into the body through injections. Various approaches have been developed to improve these situations. The bioavailability obtained from transfersomes is somewhat similar to that resulting from subcutaneous injection of the same protein suspension. The transfersomal preparations of this protein also induced strong immune response after the repeated epicutaneous application, for example the adjuvant immunogenic bovine serum albumin in transfersomes, after several dermal challenges is as active immunologically as is the corresponding injected proteo-transfersomes preparations.

Delivery of insulin by transfersomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transfersomes (transfersulin) overcomes these entire problems. After transfersulin application on the intact skin, the first sign of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition.

Transfersomes have also been used as a carrier for interferons, for example leukocyctic derived interferone-α (INF-α) is a naturally occurring protein having antiviral, antiproliferive and some immunomodulatory effects. Transfersomes as drug delivery systems have the potential for providing controlled release of the administrated drug and increasing the stability of labile drugs. Hafer et al studied the formulation of interleukin-2 and interferone-α containing transfersomes for potential transdermal application. They reported delivery of IL-2 and INF-α trapped by transfersomes in sufficient concentration for immunotherapy.[35]

Another most important application of transfersomes is transdermal immunization using transfersomes loded with soluble protein like integral membrane protein, human serum albumin, gap junction protein. These approach offers at least two advantages, first they are
applicable without injection and second, they give rise to rather high titer and possibly, to relatively high IgA levels. Transferosomes have also used for the delivery of corticosteroids. Transferosomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose.\[13\]

Transferosomes beased cortiosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.

Application of anesthetics in the suspension of highly deformable vesicles, transferosomes, induces a topical anesthesia, under appropriate conditions, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transferosomal anesthetics last longer. Transferosomes has also been used for the topical analgesics, anaesthetics agents, NSAIDS and anti-cancer agents.

**CONCLUSION**

Transfersome technology is best suited for non-invasive delivery of therapeutic molecules across open biological barriers. The Transfersome vesicles can transport across the skin, for example, molecules that are too big to diffuse through the barrier. When tested in artificial systems transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller. The bio-distribution of radioactively labelled phospholipids applied in the form of transfersomes after 24 h is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. When used under different application conditions, transfersomes can also be positioned nearly exclusively and essentially quantitatively into the viable skin region.

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