

**TRANSFERSOMES: A SURROGATED CARRIER FOR TRANSDERMAL DRUG
DELIVERY SYSTEM**

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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 envelop of the skin that offers the principal hurdle for diffusion of hydrophilic ionizable bioactives. Recently, various strategies have been used to augment the transdermal delivery of bioactives. Mainly, they include iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microneedles, and vesicular system (liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes). Among these strategies transfersomes appear promising. 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.

Key Words: Transfersome, Undeformable Vesical, Skin Delivery, pharmacokinetic.

INTRODUCTION

The term Transfersome and the underlying concept were introduced in 1991 by Gregor Cevc. In broadest sense, a Transfersome is a highly adaptable and stress-responsive, complex 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 various transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents (Wikipedia).

Delivery via the transdermal route is an interesting option in this respect because a transdermal route is convenient and safe. This offers several potential advantages over conventional routes (Shaw et al., 1999) 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. To date many chemical and physical approaches have been applied to increase the efficacy of the material transfer across the intact skin, by use of the penetration enhancers, enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes and proliposomes) and nonionic surfactant vesicles (niosomes and proniosomes).

Transfersomes were developed in order to take the advantage of phospholipids vesicles as transdermal drug carrier. These self-optimized aggregates, with the ultra flexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. These vesicular transfersomes are several orders of magnitudes 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 as shown in fig 1. 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 (Cevc et al, 1991). 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 (Schatzlein et al, 1995). The following figure shows possible micro routes for drug penetration across human skin intracellular and transcellular (Panchagnula, 1997).

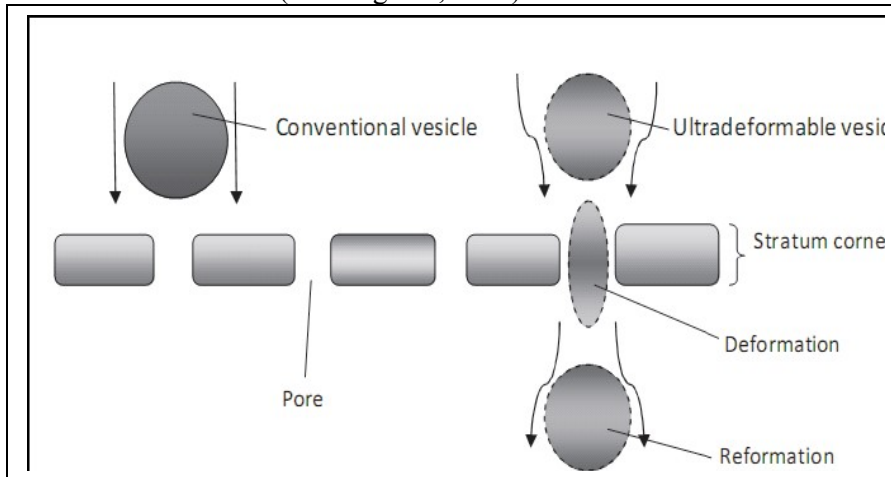


Fig 1: Schematic Diagram of the Two Microroutes Of Penetration.

The high and self-optimizing deformability of typical composite transfersomes membrane, which are adaptable to ambient stress allow the ultra deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into narrow pore. The transfersomes components that sustain strong membrane deformation preferentially accumulate, while the less adaptable molecules are diluted at sites of great stress. This dramatically lowers the energetic cost of membrane deformation and permits the resulting, highly flexible particles, first to enter and then to pass through the pores rapidly and efficiently. This behavior is not limited to one type of pore and has been observed in natural barriers such as in intact skin (Bain *et al*, Cevc *et al.*, 1996).

SALIENT FEATURES AND LIMITATIONS 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 as shown in fig 2. 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, 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 of pharmaceutically unacceptable additives (Jain, 2001).

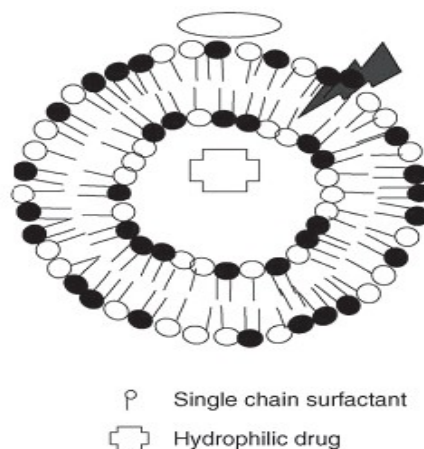


Fig 2: Undeformable Vesicle (Transferosome)

LIMITATIONS OF TRANSFERSOMES

Transfersomes are chemically unstable because of their predisposition to oxidative degradation. Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles. Transfersomes formulations are expensive (Jain, 2001).

COMPOSITION AND MECHANISM OF ACTION

The carrier aggregate is composed of at least one amphiphatic (such as phosphatidylcholine), which in aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle. By addition of at least one 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 optimized, 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. In its basic organization broadly similar to a liposome), the Transfersome thus differs from such more conventional vesicle primarily by its "softer", more deformable, and better adjustable artificial membrane.

Another beneficial consequence of strong bilayer deformability is the increased *Transfersome* affinity to bind and retain water. An ultradeformable and highly hydrophilic vesicle always seeks to avoid dehydration; this may 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-rich deeper strata to secure its adequate hydration. Barrier penetration involves reversible bilayer deformation, but must not compromise unacceptably either the vesicle integrity or the barrier properties for the underlying hydration affinity and gradient to remain in place.

Since it is too large to diffuse through the skin, the Transfersome needs to find and enforce its own route through the organ. The Transfersome vesicles usage in drug delivery consequently relies on the carrier's ability to widen and overcome the hydrophilic pores in the skin or some other (e.g. plant cuticle) barrier. The subsequent, gradual agent release from the drug carrier allows the drug molecules to diffuse and finally bind to their target. Drug transport to an intra-cellular action site may also involve the carrier's lipid bilayer fusion with the cell membrane, unless the vesicle is taken-up actively by the cell in the process called endocytosis (Wikipedia).

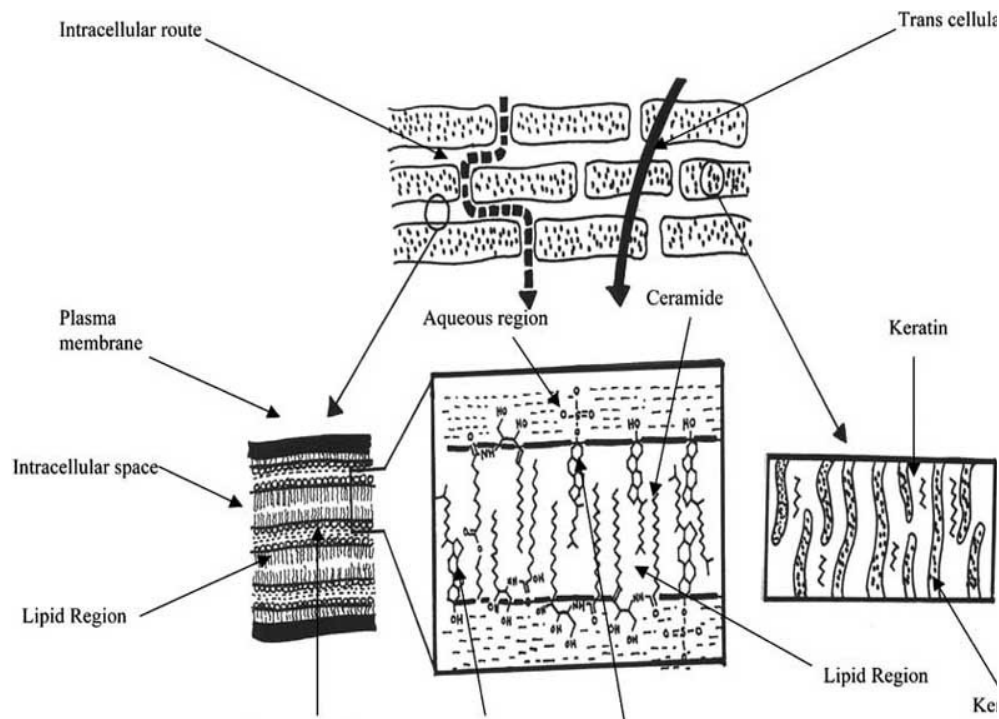


Fig 3: Diagrammatic Representation of The Stratum Corneum And The Intercellular And Transcellular Routes of Penetration (Heather., 2005).

PROPENSITY OF PENETRATION

The magnitude of the transport driving force, of course, also plays an important role: $\text{Flow} = \text{Area} \times (\text{Barrier}) \text{ Permeability} \times (\text{Trans-barrier}) \text{ force}$. Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the some amount of lipids in a suspension (jain., 2001).

MATERIALS AND METHODS

Materials which are widely used in the formulation of transfersomes are various phospholipids, surfactants, alcohol, dye, buffering agent etc different additives used in the formulation of transfersomes are summarized in table no. 1 (Wearner *et al.*, 1988, Rand *et al.*, 1988, Cevc *et al.*, 1997, Cevc *et al.* 1998., Gamal *et al.*, 1999)

Table no 1: Different Additives Used In Formulation of Transfersomes

CLASS	EXAMPLE	USES
Phospholipids	Soya phosphatidyl choline, egg phosphatidyl choline, dipalmitoyl phosphatidyl choline	Vesicles forming component
Surfactant	Sod. cholate, Sod. deoxycholate, Tween -80, Span-80	For providing flexibility
Alcohol	Ethanol, methanol	As a solvent
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium
Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	For CSLM study

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 °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⁻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 °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 (Fry *et al.*, 1978).

CHARACTERIZATION OF TRANSFERSOMES

The characterization of transfersomes is generally similar to liposomes, niosomes and micelles (Nanda *et al.*, 2005, Jain *et al.*, 1998).

Entrapment Efficiency

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

Vesicle Diameter

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 (Gamal *et al.*, 1999).

Number of Vesicle per Cubic Mm

This is an important 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.

Confocal Scanning Laser Microscopy (CSLM) Study

Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose:

- For investigating the mechanism of penetration of transfersomes across the skin.
- For determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways for comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, Niosomes and micelles.

Different fluorescence markers used in CSLM study are

I. Fluorescein-DHPE (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(5-fluoresceinthiocarbonyl), triethylammonium salt).

II. Rhodamine-DHPE (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(10-Lissamine rhodamine B sulfonyl), triethanolamine salt).

III. NBD-PE (1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-Benz-2-oxa-1, 3-diazol-4-yl) triethanolamine salt).

IV. Nile red.

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 sizedistributions are noted after each pass by dynamic light scattering (DLS) measurements.

Turbidity Measurement

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

Surface Charge and Charge Density

Surface charge and charge density of transfersomes can be determined using zetasizer.

Penetration Ability

Penetration ability of transfersomes can be evaluated using fluorescence microscopy.

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 minicolumn centrifugation

(Fry *et al.*, 1978). 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).

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 or water activity gradient is established. Using less solvent is favourable 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 anaesthesia 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. 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 (Planas *et al.*, 1992).

TRANSFERSOMES Vs OTHER CARRIER SYSTEMS

At first glance, transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes. However in functional terms, transfersomes 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 and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus bio surfactant) with sufficiently different packing characteristics into a single bilayer. 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, one would expect an even better penetration performance of the micelles. In contrast to this postulate, the higher surfactant concentration in the mixed micelles does not improve the efficacy of material transport into the skin. On the contrary, mixed micelles stay confined to the topmost part of the stratum corneum even they are applied non occlusively (Chapman *et al.*, 1998).

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 (Gompper *et al.*, 1995, Wearner *et al.*, 1988). To differentiate the penetration ability of all these carrier systems (Rand *et al.*, 1989) 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. Chapman & Walsh (Cevc *et al.*, 1997) also showed that the former two types of aggregates are confined to the outer half of the horny layer, where the cellular packing and intercellular seals are already compromised by the desquamation process. Pure lipid vesicles or micelles seem to have access to the low-resistance pathway only and thus very seldom reach the lower stratum corneum or even get into the viable part of the skin in significant quantities.

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 transfersosomal 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 (Cevc et al, 1990). 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 leukocytic 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 administered 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 (Hafer et al,1999).

Another most important application of transfersomes is transdermal immunization using transfersomes loaded 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. Transfersomes have also used for the delivery of corticosteroids. Transfersomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose (Cevc et al, 1997). Transfersomes based cortiosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases (Cevc et al, 1997).

Application of anesthetics in the suspension of highly deformable vesicles, transfersomes, 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 transfersosomal anesthetics last longer. Transfersomes has also been used for the topical analgesics, anaesthetics agents, NSAIDS and anti-cancer agents.

Drug	Result
Norgesterol (Jain <i>et al.</i> , 1998)	Improved transdermal flux
Tamoxifen (Jain <i>et al.</i> , 1998)	Improved transdermal flux
Oestradiol (Maghraby <i>et al.</i> , 1998)	Improved transdermal flux
Topical analgesic and anesthetic agent (Tetracaine, lignocain) (Planas <i>et al.</i> , 1992)	Suitable means for the noninvasive treatment of local pain on direct topical drug application .
Corticosteroids Hydrocortosone Triamcinolone acetonide (Cevc <i>et al.</i> , 1997).	Improved site specificity and overall drug safety. Biologically active at dose several times lower than currently used formulation. Used for both local and systemic delivery.
Soluble proteins (Paul <i>et al.</i> , 1995) Gapjunction proteins Human serum albumin (Paul <i>et al.</i> , 1998) Integral membrane protein (Paul <i>et al.</i> , 1995)	Permits non-invasive immunization through normal skin. Antibody titer is similar or even slightly higher than subcutaneous injection.
Interferon- α Interleukin-2 (Hafer <i>et al.</i> , 1999)	Efficient delivery means (because delivery other route is difficult). Controlled release. Overcome stability problem.
Insulin (Cevc <i>et al.</i> , 1998)	High encapsulation efficiency. Transfer across the skin with an efficiency of >50%. Provide noninvasive means of therapeutic use

CONCLUSION

Transfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems. Transfersomes can pass through even tiny pores (100 nm) nearly as efficiently as water, which is 1500 times smaller. Drug laden transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm²h⁻¹). The systemic drug availability thus mediated is frequently higher than, or at least approaches 80-90%. The bio-distribution of radioactively labeled 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 positioned nearly exclusively and essentially quantitatively into the viable skin region.

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