At first glance, transfersomes appear to be remotely related to 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 biosurfactant) 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 topest part of the stratum corneum even they are applied non occlusively 5 (table-1). The reason for this is that mixed micelles are much less sensitive to the transpidermal 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 6,7.

ABSTRACT

Various new technologies have been developed for the transdermal delivery of some important drugs. Transdermal route will always remain a lucrative area for drug delivery. 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 ionic soluble bioactives. One of the very recent approaches is the use of ultra deformable carrier system (transfersomes®). Which is composed of phospholipid, surfactant, and water for enhanced transdermal delivery? The transfersomal system was much more efficient at delivering a low and high molecular weight drug to the skin in terms of quantity and depth. The system can be characterized by in vitro for vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm. Transfersome is an ultra deformable vesicle, elastic in nature which can squeeze itself through a pore which is many times smaller than its size owing to its elasticity. The uniqueness of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic drugs. These drugs find place in different places in the elastic vesicle before they get delivered beneath the skin.

Keywords: transfersomes, pharmacokinetic, Transdermal drug delivery, Occlusion, Elastic vesicles

INTRODUCTION

FDA approved the first transdermal patch products in 1981. These delivery systems provided the controlled systemic absorption of scopolamine for the prevention of motion sickness (Transderm-Scop, ALZA Corp.) and nitroglycerine for the prevention of angina pectoris associated with coronary artery disease (Transderm-Nitro). Over the last two decades, more than 35 transdermal products have been approved generating sales of $3.2 billion in 2002, which is predicted to rise to $4.5 billion in 2008. More recently, such dosage forms have been developed and/or modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and/or increase the permeability of the skin. These approaches include the use of penetration enhancers, supersaturated systems, prodrugs, liposomes and other vesicles1.

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).

The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is associated primarily with outermost stratum corneum layer of epidermis 2,3. The skin structure looks as if stratum corneum cells are embedded in a pool of intercellular lipid lamellae 4.

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.
Liposomes and niosomes are the vesicular carrier systems. Vesicular systems are gaining importance recently owing to their ability to act as a means of sustained release of drugs. These systems have several advantages: they can encapsulate both hydrophilic and lipophilic moieties, prolong half-lives of drugs by increasing duration in systemic circulation due to encapsulation, ability to target organs for drug delivery, biodegradability, and lack of toxicity.

Liposomes and niosomes are the vesicular carrier systems which have received a lot of attention over the last decade as carriers for transdermal drug delivery, in most cases transdermal drug penetration has not been achieved. Each vesicular carrier composition and the Transferosome transfer efficiency is low (less than 10%).

**VESICLES AS DRUG DELIVERY AGENTS**

Vesicular systems are gaining importance recently owing to their ability to act as a means of sustained release of drugs. These systems have several advantages: they can encapsulate both hydrophilic and lipophilic moieties, prolong half-lives of drugs by increasing duration in systemic circulation due to encapsulation, ability to target organs for drug delivery, biodegradability, and lack of toxicity. Liposomes and niosomes are the vesicular carrier systems which have received a lot of attention over the last decade as carriers for transdermal drug delivery, in most cases transdermal drug penetration has not been achieved. Each vesicular carrier composition and the Transferosome transfer efficiency is low (less than 10%).

**Transferosomes and Protransferomes**

Transferosomes and Protransferomes (Cevc et al, 1996) are more stable, high penetration due to high deformability, biocompatible and biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reach up to deeper skin layers. Transferosomes protect the encapsulated drug from metabolic degradation. They act as depot, releasing their content slowly and gradually.

**MECHANISMS OF TRANSDERMAL PERMEATION**

For a systemically-active drug to reach a target tissue, it has to possess some physico-chemical properties which facilitate the absorption of the drug through the skin and also the uptake of the drug by the capillary network in the dermal papillary layer. The rate of permeation dQ/dt across various layers of skin tissues can be expressed as:

\[ \frac{dQ}{dt} = P_s(C_d - C_r) \]

Where, \( C_d \) and \( C_r \) are respectively the concentrations of skin penetrate in the donor phase (stratum corneum) and the receptor phase (systemic circulation); and \( P_s \) is the overall permeability coefficient of the skin and is defined by

\[ P_s = \frac{K_s D_{ss}}{h_s} \]

Where, \( K_s \) = Partition coefficient of the penetrant, \( D_{ss} \) = Apparent diffusivity of penetrant, \( h_s \) = Thickness of skin.

Thus, permeability coefficient (Ps) may be a constant since \( K_s; D_{ss} \) and \( h_s \) terms in equation (2) are constant under the given set of conditions.

A constant rate of drug permeation achieved, if \( C_d > C_r \), then the equation (1) may be reduced to

\[ \frac{dQ}{dt} = P_s C_d \]

And the rate of skin permeation (dQ/dt) becomes a constant, if the Cd value remains fairly constant throughout the course of skin permeation. To maintain the Cd at a constant value, it is critical to make the drug to be released at a rate (Rr) which is always greater than the rate of skin uptake (Ra) i.e. \( R_r >> R_a \).
As expressed by equation (4), is thus reached -

\[
\frac{dQ}{dt}_m = C \varepsilon_P
\]

Apparently, the magnitude of \(\frac{dQ}{dt}_m\) is determined by the skin permeability coefficient (Ps) of the drug and its equilibrium solubility in the stratum corneum (Ces).

Fig. 2: The brick and mortar model.

Fig. 3: Comparative diagram of transport through conventional and ultra deformation vesicles.

**MATERIALS AND METHODS**

Materials commonly used for the preparation of transfersomes are summarized in Table-2.

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.

<table>
<thead>
<tr>
<th>CLASS</th>
<th>EXAMPLE</th>
<th>USES</th>
<th>REFERENCES</th>
</tr>
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<tbody>
<tr>
<td>Phospholipids</td>
<td>Soya phosphatidyl choline,</td>
<td>Vesicles forming</td>
<td>Cevc et al, 1997</td>
</tr>
<tr>
<td></td>
<td>Egg phosphatidyl choline,</td>
<td>component</td>
<td>Cevc, 1992b</td>
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<tr>
<td></td>
<td>Dipalmtoyl phosphatidyl choline</td>
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<td></td>
<td>Distearyl phosphatidyl choline</td>
<td></td>
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<tr>
<td>Surfactant</td>
<td>Sod. Cholate, Sod.deoxycholate</td>
<td>For providing flexibility</td>
<td>Schubert et al, 1986</td>
</tr>
<tr>
<td></td>
<td>tween-80, Span-80</td>
<td></td>
<td>Schubert et al,1988</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Ethanol</td>
<td>As a solvent</td>
<td>Cev et al, 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gamal et al,1999</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine-123</td>
<td>For CSLM study</td>
<td>Cev et al, 1995</td>
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<tr>
<td></td>
<td>Rhodamine-DHPE</td>
<td></td>
<td>Schatzlein &amp; Cevc, 1998</td>
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<tr>
<td></td>
<td>Fluorescein-DHPE</td>
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<tr>
<td></td>
<td>Nile-red</td>
<td></td>
<td></td>
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<tr>
<td>Buffering agent</td>
<td>Saline phosphate buffer (pH 6.4)</td>
<td>As a hydrating medium</td>
<td>Cev, 1993</td>
</tr>
</tbody>
</table>
CHARACTERIZATION OF TRANSFEROSOMES

The mechanical properties and transport ability of a vesicle can be studied by measuring stress or deformation-dependent vesicle bilayer elasticity and permeability changes. The pressure dependent area density of the Transferosomes suspension flux through a nano-porous filter can be determined for this purpose. For the proper Transferosomes vesicles, “Penetrability” increases non-linearly (usually sigmoidally) with the flux driving force (head pressure).

**Entrapment efficiency:** Entrapment efficiency can be determined by separating the unentrapped drug. After centrifugation (to separate the unentrapped drug), the vesicle can be ruptured. Then appropriate analytical technique can be used to determine the amount of entrapped drug.

**Turbidity measurement:** Turbidity of drug in aqueous suspension can be evaluated using nephelometer.

**Penetration ability:** Fluorescence microscopy is used to evaluate penetration ability of transferosomes.

**Surface charge and charge density:** Surface charge and charge density of transferosomes can be determined using zetasizer.

**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.

**Drug Content:** The drug content can be determined using a modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program.

**In Vivo and Kinetics of Transferosomes Penetration:** 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.

**APPLICATIONS**

Transferosomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Very large molecules incapable of diffusing into skin as such can be transported across the skin with the help of transfersomes. For example, insulin, interferon can be delivered through mammalian skin. 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 transferosomes (transfersulin) overcomes the problems of inconvenience, larger size (making it unsuitable for transdermal delivery using conventional method) along with showing 50% response as compared to subcutaneous injection. Transfersomes have also been used as a carrier for interferons like leukocytic derived interferon-α (INF-α).

Transferosomes have been widely used as a carrier for the transport of other 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 and transdermal delivery suffers because of their large size. Transferosomes help obtain somewhat similar bioavailability to subcutaneous injection. Human serum albumin or gap junction protein was found to be effective in producing the immune response when delivered by transdermal route encapsulated in transfersomes. Transport of certain drug molecules that have physicochemical which otherwise prevent them from diffusing across stratum corneum can be transported.
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 mm) 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 1000mg cm⁻² h⁻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 hr is essentially the same after an experimental period.

ACKNOWLEDGEMENT

The authors are thankful to the Bundelkhand University, Jhansi and Institute of pharmacy, Jhansi for providing necessary facilities to search the literature.

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Source of support: Nil, Conflict of interest: None Declared