Review Article

An updated review on transfersomes: a novel vesicular system for transdermal drug delivery

Abstract-
Transdermal route is an interesting option in this respect because a transdermalroute is convenient and safe, avoide first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological responses, avoiding the fluctuation in drug levels and inter and intra-patient variations.
However it has got its own limitations its inability to transport large molecules, inability to overcome the barrier properties of stratum corneum and many more.
Formulating the drug in a transfersome is one such approach to solve theseproblems. Transfersome, is an ultradeformable vesicle, elastic in nature which can squeeze itself through a pore which is many times smaller than its size owing to its elasticity.
Keywords- Transdermal route, transfersome, first pass metabolism.

Introduction

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 like the avoidance of first pass metabolism, predictable andextended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological responses, avoiding the fluctuation in drug levels and interand intra-patient variations [1].

The term Transfersome and the underlying concept were introduced in 1991 by Gregor Cevc. The name 'Transfero' is derived from the latin word meaning to carry across and the Greek word 'soma' for a body [2].

In the last few years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs. A transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesiclepossessing an aqueous core surrounded by the complex lipid bilayer (Wikipedia). Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. These vesicles serve as a depot for the sustained release of active compounds in the case of topical formulations, as well as rate-limiting membrane barrier for the modulation of systemic absorption in the case of transdermal formulations [3].

Transfersomal patch enhances the drug release potential of transdermal delivery systems and also increase the rate of skin permeation of the drug [4].

Advantages of transfersomes

1. Transfersomes can deform and pass through narrowconstriction (from 5 to 10 times less than their own diameter) without measurable loss.
2. They can encapsulate both hydrophilic and lipophilic moieties. They have high entrapment efficiency for lipophilic drug near to 90%.
3. They can be used for both systemic as well as topical delivery of drug.
4. They protect the encapsulated drug from metabolic degradation.
5. Transfersomes could act as efficient carriers for low as well as high molecular weight drugs e.g., analgesic, corticosteroids, hormones, anticancer drugs, insulin, proteins, etc.
6. The usage in transdermal delivery system arises due to their biocompatible and biodegradable nature, thus showing tremendous high entrapment efficiency. [5,6,7]

7. Biodegradability and lack of toxicity. [8]
8. High deformability of this system gives better penetration of intact vesicles.

Limitations of transfersomes

1. These are chemically unstable as they are highly susceptible to oxidative degradation [2].
2. Transfersomes formulations are expensive.
3. Purity of natural phospholipids is another criteriamilitating against adoption of transfersomes as drug delivery vehicles. [6,7,9]

Transfersomes v/s other carrier systems

Transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes. Transfersomes differ from commonly used liposomes in that they are much more flexible and adaptable as shown in Table 1. High flexibility of the transfersomes membrane is result of combination of atleast two lipophilic/amphiphilic components (phospholipidsplus bio surfactant) with sufficiently different packing characteristics into a single bilayer. This aggregate deformability permits transfersomes to penetrate the skin. 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. 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 each vesicular transfersomes contains a water filled core. In all these vesicles the highly deformable transfersomes transverse the stratum corneumand enter into the viable epidermis in significant quantity **[5,7,9]**.

Table 1: Comparision of different vesicles

S.N.	Method	Advantage	Disadvantage
1.	**Penetration Enhancers**	Increase penetration through skin and give both local and systemic effect	Skin irritation Immunogenicity, only for low molecular weight drugs
2.	**Physical methods e.g.iontophoresis**	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
3.	**Liposomes**	Phospholipid vesicle, biocompatible, biodegradable	Less skin penetration less stable
4.	**Proliposome**	Phospholipid vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of yesicles
5.	**Niosomes Proniosomes**	Non-ionic surfactants vesicles, greater stability, Will convert into noisome in situ, stable	Less skin penetration easy handling But will not reach up to deeper skin layer

6.	**Transfersomes and Protransfersomes**	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.	None, but for some limitations

Mechanism of penetration of transfersomes

Transfersomes are composed of phospholipids. A bilayer softening component called 'edge activator', i.e., biocompatible surfactants, are added to increase lipid flexibility and permeability [10].

The mechanism for penetration is the generation of "osmotic gradient" due to evaporation of water while applying the lipid suspension (Transfersomes) onthe skin surface. The transport of these elastic vesicles is thus independent of concentration. 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 which has 75% water content and nearly completely dry stratum corneum, near to the skin surface having 15% water content.The trans-epidermal hydration provides the driving force for the transport of the vesicles [11].

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.

Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour per cm2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients [12].

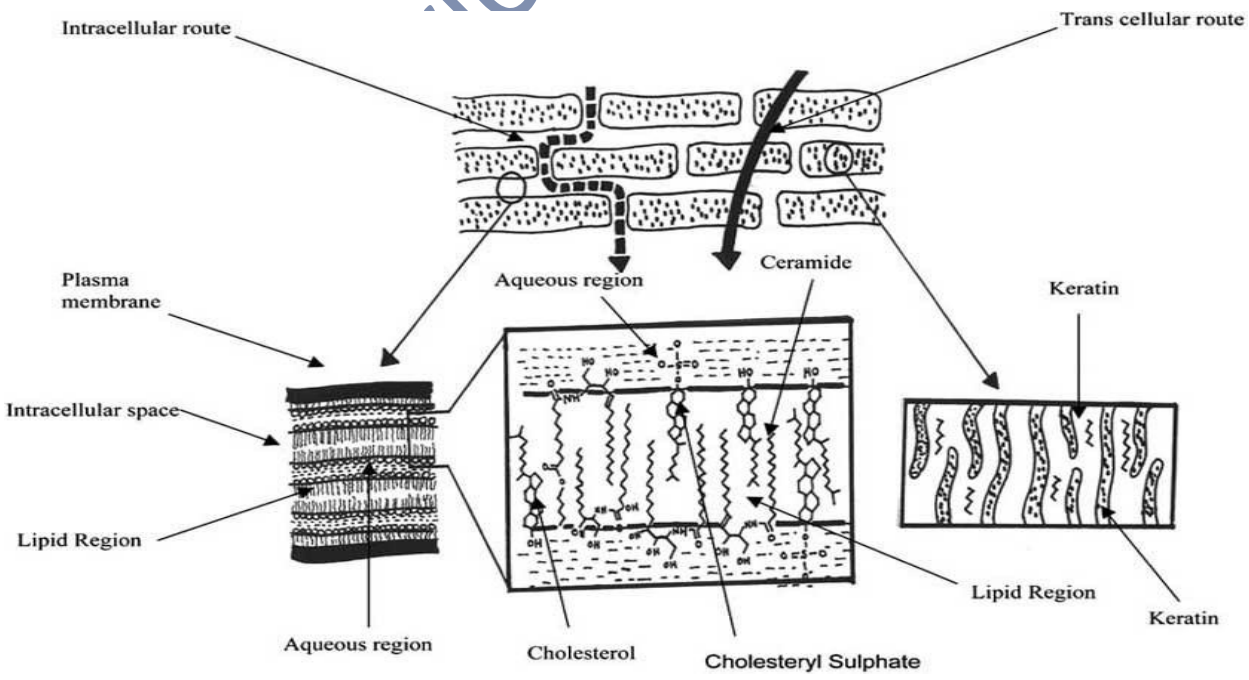

Fig 1: Diagrammatic Representation of The Stratum Corneum And The Intercellular And Transcellular Routes of Penetration.[13]

Propensity of penetration

The magnitude of the transport driving force, of course, also plays an important role:

Flow = Area x (Barrier) Permeability x (Trans-barrier) 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 [14].

Materials and methods for preparation of transfersome

Materials:

The most commonly used materials in the preparation of transfersomesare phospholipids, surfactants, alcohol, and buffering agents. Here, each material has its own importance.

Table 2: List of materials used in formulation of transfersomes [15-20]

Class	Example	Uses
Phospho-lipids	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; andsecondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane.

Preparation of lipid film

⇩

Hydration

⇩

Homogenization

⇩

Sonication

Figure 3: Flow diagram for preparation of Transferosomes

Methods:

Suspension homogenization process [2]

In this process, Transfersomes are prepared by mixing an ethanolic soybean phosphatidylcholine solution with an appropriate amount of edge-active molecule, e.g. sodium cholate. Thisprepared suspension is subsequently mixed with Triethanolamine-HCl buffer to yield a total lipid concentration. The resulting suspension is sonicated, frozen, and thawed for 2 to 3 times. Then brought to the desired size and measured by using photon correlation spectroscopy. The prepared vesicle suspension is ultimately sterilized by filtering through a 0.2 mm micro-porous filter. The final vesicle size is confirmed by the dynamic light scattering technique.

Modified handshaking process [21]

In this process, The transfersomes are prepared by modified hand shaking, 'lipid film hydration technique'. Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking abovelipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension was further hydrated up to 1 hour at 2-80°C.

Aqueous lipid suspension process [22]

In this process, Drug-to-lipid ratio in the vehicles is fixed between 1/4 and 1/9. Depending upon the particular formulation type, the composition is preferred. This would ensure the high flexibility of the vesicle membrane in comparison to the standard phosphatidylcholine vesicles in the fluid phase. Specifically, vesicles with the size ranging from 100-200 nm are prepared by using soyphosphatidylcholine with the standard deviation of size distribution (around 30%). This formulation could be prepared by suspending the lipids in an aqueous phasewherein the drug is dissolved. Then the usage of vigorous stirring or suspension homogenization (eg. sonication) techniques, the average vesicle size is reduced in the original suspension. Vesicles dimension is finally brought to the desired value by extruding the suspension through 100-200 nm pore filters and finally the optimal pH value of the suspension is adjusted.

Centrifugation process

In this process, Phospholipids, surfactants and the drug are dissolved in alcohol. Then the solvent is removed by rotary evaporation under reduced pressure at 40^{o}C. Final traces of solvent are removed under vacuum. Then the deposited lipid film is hydrated with theappropriate buffer by centrifuging at 60 rpm for 1 hour at room temperature. At room temperature, the resulting vesicles are swollen for 2 hours. The multi-lamellar lipid vesicles obtained which are further sonicated at room temperature.

Optimization of formulation containing transfersomes

There are various process variables which could affect the preparation and properties of the transfersomes. The preparation procedure was accordingly optimized andvalidated. The preparation of transfersomes involves various process variables such as:

1. Lecithin : surfactant ratio
2. Effect of various solvents (ethanol / isopropyl alcohol)
3. Effect of various surfactants (Span80, Tween80)
4. Hydration medium

Thus the optimization procedures are conducted by selecting entrapment efficiency of the drug **[22, 24, 27]**.

Characterization and evaluation of transfersomes

Vesicle morphology

Vesicle Diameter

Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filteredthrough 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 [23].

Vesicle Shape & Type

Transfersomes vesicles can be visualized by phase contrast microscopy, TEM, with an accelerating voltage of 100 kv. These vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope etc. The stabilityof vesicle can be determined by assessing the size and structure of vesicles over time. Mean size ismeasured by Dynamic light scattering (DLS) and structural changes are observed by TEM.

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 [5, 9].

Number of vesicle per cubic mm

This is an important parameter for optimizing the composition and other processvariables.

Non-sonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study. 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

$$N = A \times F \times 4000$$

Total number of transfersomes per cubic mm (N); Total number of transfersomes counted (A); Dilution Factor (F).

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 drugby use of minicolumn 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 (EE) = (Amount entrapped / Total amount added) × 100

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 programdepending upon the analytical method of the pharmacopoeial drug **[24]**.

Turbidity measurement

Turbidity of drug in aqueous solution can be measured using nephelometer **[5].**

Surface charge and charge density

Surface charge and Charge density of transfersomes can be determined using Zetasizer. Regarding the zeta potential measurements, all colloidal dispersions have a negative surface charge, containing Tween 80 which is a non ionic surfactant. The reason forthis result is that Tween 80 is a non-ionic surfactant while sodium cholate is anionic surfactant. It is speculated that the hydrocarbon tail of Tween 80 might be able to penetrate into the lipid bilayer. Thus, the incorporation of negative zeta potential increases the stability of the transfersomes.

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[34]. These 'Hydrotaxis' drives the vesicles from the relatively dry skin surface into water rich viable skin regions. However, the phenomena of occlusion prevent evaporation of water from the skin surface, thus affecting hydration force, eventually revealing that the occlusion imparts a disabling effect on vesicle permeation [25].

Confocal scanning laser microscopy (cslm) study

Conventional light microscopy and electron microscopy both face problemof 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 as –

1. Fluorescein- DHPE (1, 2- dihexadecanoyl- sn- glycero- 3- phosphoethanolamine- N- (5 - fluoresdenthiocarbamoyl), triethyl- ammonium salt)
2. Rhodamine- DHPE (1, 2- dihexadecanoyl- sn- glycero- 3-ogisogietgabikanube-NLissamineTmrhodamine- B- sulfonyl), triethanol- amine salt)
3. NBD- PE (1, 2- dihexadecanoyl- sn-glycero- 3- phosphoethanolamine- N- (7-nitro- Benz- 2-oxa- 1,3- diazol- 4- yl) triethanolamine salt)
4. Nile red.

In-vitro drug release

In vitro drug release study is performed for determining the permeation rate. For determining in vitro drug release, beaker method is used in which transfersomes suspension is incubated at 32°C using cellophane membrane and the samples are taken at different timesand then detected by various analytical techniques (UV, HPLC, HPTLC) and the free drug is separated by minicolumn centrifugation, then the amount of drug release is calculated **[26].**

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) **[5, 7]**.

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 goatwere 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 equalvolume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The samples were analyzed by any instrumental analytical technique **[27, 28]**.

***In vivo* fate of transfersomes & kinetics of transfersomes penetration**

After having penetrated through the outermost skin layers, transfersomes reach the deeper skin layer. From there, they are normally washed out into the blood circulation. If it is applied under suitable conditions, resulting in access to all body tissues. The kinetics of this action of an epicutaneous application depend upon the velocity of carrier penetration as well as on the speed of drug distribution **[29].**

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 thesuspension medium that must evaporate from the skin surface before the sufficiently strong trans-cutaneous chemical potential chemical potential or water activity gradient is established.

The lag phase is duration between the time of application and the time of drug appearance in the body. It is always quite long, complex and strongly sensitive to the type of drug and formulation administration. Mostly 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. 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 controlledto 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 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 measured. 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. The lidocaine-loaded transfersomes were analgesically active even when applied dermally. Maximum analgesic effect with the latter type of drug application was typically observed 15 minutes after the drug application. 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 **[30].**

1. Delivery of insulin:

By transferosomes 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 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 **[30,31].**

2. Delivery of corticosteroids:

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. Transferosomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for thetreatment of skin diseases **[32]**.

3. Delivery of proteins and peptides:

Transfersomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptides are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract and transdermal delivery suffers because of their large size. 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 bioavaibility obtained from transferosomes is somewhatsimilar to that resulting from subcutaneous injection of the same protein suspension. 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 **[33, 34]**.

.4. Delivery of Anticancer Drugs:

Anti cancer drugs like methotrexate were tried for transdermal delivery usingtransfersome technology. The results were favorable. This provided a new approach for treatment especially of skin cancer **[9, 35**].

5. Delivery of anesthetics:

Transfersome based formulations of local anesthetics- lidocaine and tetracaine showed permeation equivalent to subcutaneous injections, 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 **[9]**.

6. Delivery of Herbal Drugs

Transfersomes can penetrate stratum corneum and supply the nutrients locally to maintain its functions resulting maintenance of skin **[35, 36, 37].**

Table No.3: Applications of transfersomes

S. No	Name of drug	Inference
1	Curcumin	Better permeation for anti inflammatory activity
2	Indinavir sulfate	Improved influx for activity against acquired immune deficiency syndrome (AIDS)
3	Stavudine	Improved the in vitro skin delivery of Stavudine for antiretroviral activity
4	Norgesterol	Improved transdermal flux
5		

	Tetanus toxoid	For transdermal immunization
6	Hydrocortisone	Biologically active at dose several times lower than currently used formulation.
7	Interferon-α	Efficient delivery means (because delivery other route is difficult). Controlled release. Overcome stability problem.
8	Ketoprofen	Improved penetration for anti-inflammatory activity
9	Insulin	Induce therapeutically significant hypoglycemia with good efficacy and reproducibility
10	Capsaicin	Increase skin penetration
11	Vincristine	Increase entrapment efficiency and skin permeation
12	Methotrexate	Improved transdermal flux
13	Oestradiol	Improved transdermal flux
14	Colchicine	Increase skin penetration
15	Tetracaine, Lignocain	Suitable means for the noninvasive treatment of local pain on direct topical drug application.
16	Corticosteroids	Improved site specificity and overall drug safety.
17	Human serum albumin	Antibody titer is similar or even slightly higher than subcutaneous injection.

Discussion and 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 throughеven tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller.

Ultradeformable vesicles hold great prospective in delivery of huge range of drug substances which includes large molecules like peptides, hormones and antibiotics, drugs with poor

penetration due to unfavourable physicochemical characters, drugs for quicker and targeted action, etc. This carrier system does not depend upon the

concentration gradient and mainly works on the principle of hydrotaxis and elasto-mechanics. Transfersomes are highly deployed in the delivery of hormones, proteins, anticancer drugs, anesthetics and insulin transdermally.

All above discussed properties of this technology strongly advocate its good future in transdermal drug delivery. Drug release can also be controlled according to therequirement. Thus, this approach can overcome the problems which occur in conventional techniques.

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