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TRANSFERSOMES: A NOVEL TECHNIQUE FOR TRANSDERMAL DRUG DELIVERY

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ABSTRACT

With oral and parenteral drug delivery systems, poor patient compliance is a frequent problem observed in daily clinical practice. Transport of drug across the skin is best route of drug delivery, because the skin is largest human organ with total weight 3 kg and a surface of 1.5 -2.0 m^2. But the big hurdle in transdermal delivery of drug is the skin, the stratum corneum, the outermost envelop of the skin. Recently, various strategies have been used to augment to the transdermal delivery. Mainly, they include iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, micro needles, and vesicular system (liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes). Transfersomes possess an infrastructure consists of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. By using the concept of rational membrane design we have recently devised special composite bodies, they are called transfersomes. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. Transfersomes penetrate through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form due to its deformable nature. The system can be characterized by *in vitro* for vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm. 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 hormones, anticancer, insulin, gap junction protein, and albumin. The present review highlights the formulation aspects, characterization, and therapeutic applications of transfersomes.

KEYWORDS

Transfersomes, Phospholipids edge activator, Permeation enhancers and Ultra-deformable vesicles.

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INTRODUCTION

The term transfersomes and the underlying concept were introduced in 1991 by GregorCevc. In broadest sense, a transfersomes is a highly adaptable and stress-responsive, complex aggregatepossessing an aqueous core surrounded by a complex of lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-

regulating and self-optimizing. This enables the transfersomes to cross various transport barriers efficiently, and act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. Transfersomes is a term registered as a trademark by a German company IDEA AG, and used by it to refer its proprietary drug delivery technology. The name 'Transfero' is derived from the latin word meaning to carry across, and the Greek word 'soma' for a body. A transfersomes acts as a carrier or an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potentially targeted drug delivery. 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 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.[1]
To date many chemical and physical approaches have been applied to increase the efficacy of the material transfer across the intact skin, which includes the use of the penetration enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes and proliposomes) and nonionic surfactant vesicles (niosomes and proniosomes).
Vesicular systems show importance because of their ability to give sustained release action of drugs (Table No.1)[2]. These systems exhibit several advantages which include:

1. They can encapsulate both hydrophilic and lipophilic moieties.
2. Prolong half lives of drugs by increasing duration in systemic circulation due to encapsulation.
3. Ability to target organs for drug delivery.
4. Biodegradability and lack of toxicity.[3]

Transfersomes have a unique structure which is capable of entrapping hydrophilic, lipophilic, amphiphilicdrugs**.** Vesicles are colloidal particles having a water filled core surrounded by a wall of lipids and surfactants (amphiphiles) arranged in bilayer. If the proportion of water is increased, these amphiphiles can form one or more concentric bilayers. Hydrophilic drugs find a place in the internal aqueous environment while amphiphilic, lipophilic drugs get entrapped in the bilayered wall with electrostatic and/or hydrophobic forces. The flexible or deformable vesicles are called elastic vesicles or transfersomes.

Necessity of transfersomes for skin delivery

Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra-flexible membrane properties, they are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. The vesicular transfersomes are more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. This mechanism can be well understood from the figure 1. These are characteristic with transfersomes, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transfersomes membrane is governed by mixing suitable surface-active components in the proper ratios with phospholipids[4]. The resulting flexibility of transfersomes membrane minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under non-occlusive condition. Transferosomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties.[5] Figure 2. shows possible micro routes for drug penetration across human skin intracellular and transcellular. Bangham discovered liposomes in 1963, since then vesicular

Prolong half lives of drugs by increasing d

systems have attracted increasing attention[6]. But recently it has become evident that classic liposomes are of minor values in terms of penetration. Confocal microscopic studies have shown that intact liposomes are not able to penetrate into granular layer of epidermis but, they rather remain on the upper layer of stratum corneum. The modification of the vesicular compositions or surface properties can adjust the drug release rate and the deposition to the target site.[7]

Composition of transfersomes[8]

The transfersome is composed of two main aggregates namely,

1. Firstly, an amphipathic ingredient (phosphatidylcholine), in which the aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle.
2. Secondly, a bilayer softening component (such as a biocompatible surfactant oramphiphile drug) that increases lipid bilayer flexibility and permeability.

The resulting, flexibility and permeability optimized, transfersome vesicle can therefore adapt its shape easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer Figure 2. Therefore, the transfersome thus differs from such more conventional vesicle primarily by its "softer", more deformable, and better adjustable artificial membrane.

Composition of Transferosome

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.2.[9]

Preparation of transfersomes[10]

Thin film hydration method

In this method a thin film is prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in volatile organic solvent (Chloroform-Methanol). Organic solvent is then evaporated above the lipid transition temperature or 50°C using rotary evaporator. Final traces of solvent were removed under vacuum for overnight. A prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hour at the corresponding temperature. The resulting vesicles were swollen for 2 hours at room temperature. To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min. using a bath sonicator or probe sonicated at 40°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

Modified hand shaking, lipid film hydration technique [11]

Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid 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.

OPTIMIZATION OF FORMULATION CONTAINING TRANSFERSOMES[12]

There are various process variables which could affect the preparation and properties of the transfersomes. The preparation procedure was accordingly optimized and validated. The process variables depend upon the procedure involved for manufacturing of formulation. The preparation of transfersomes involves various process variables such as,

1. Lecithin
2. Surfactant ratio
3. Effect of various solvents
4. Effect of various surfactants
5. Hydration medium

Optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant.

Characterization of Transfersomes

The characterization of transfersomes is generally similar to liposomes, niosomes and micelles.[13]

Entrapment efficiency

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

The entrapment efficiency is expressed as:

$$EE = \frac{\text{Amount entrapped} \times 100}{\text{Total amount added}}$$

Drug content[14]

The drug content can be determined using one of the instrumental analytical methods such as modified high performance liquid chromatography method (HPLC).

Vesicle morphology[15]

Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements. Transfersomes vesicles can be visualized by TEM, phase contrast microscopy, etc. The stability of vesicle can be determined by assessing the size and structure of vesicles over time. Mean size is measured by DLS and structural changes are observed by TEM.

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.

No. of vesicles per cubic mm

This is an important parameter for optimizing the composition and other process variables. Non-sonicatedtransfersome 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

Confocal scanning laser microscopy study[16]

Both the conventional light microscopy and electron microscopy techniques faces 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:

1. To investigate the mechanism of penetration of transfersomes across the skin.
2. To determine histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways.
3. To compare and differentiate 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- 3ogisogietgabikanube-Lissamine Tmrhodamine-B- sulfonyl), triethanol- amine salt)
3. NBD- PE (1, 2- dihexadecanoyl- sn-glycero- 3-phosphoethanolamine- N- (7-nitro- Benz- 2- xa-1,3- diazol- 4- yl) triethanolamine salt)
4. 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 size distributions are noted after each pass by dynamic light scattering (DLS) measurements. The degree of deformability can be determined using the following formula,

Where,

$$D = J * \frac{rv}{rp}$$

J= the amount of the suspension extruded during 5min; rv = the size of the vesicle; rp = pore size of the barrier.

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.

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.

Physical stability

The initial percentage of the drug entrapped in the formulation was determined and were stored in sealed glass ampoules. The ampoules were placed at 4 ± 20°C (refrigeration), 25 ± 20°C (room temp), and 37 ± 20°C (body temp) for at least 3 months. Samples from each ampoule were analyzed after 30 days to determine drug leakage. Percentage drug lose was calculated by keeping the initial entrapment of drug as 100%.

***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 320°C and samples are taken at different times and the free drug is separated by mini column centrifugation. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

***In vitro* skin permeation studies**

Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50cm^2 was used for this study. In vitro drug study was performed by using goat skin in phosphate buffer solution (pH 7.4). Fresh abdominal skin of goat were collected from slaughterhouse and used in the permeation experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-40°C.

To perform skin permeation study, treated skin was mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50cm^2 and capacity of receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37± 0.50°C and stirred by a magnetic bar at 100RPM. At appropriate time intervals 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The

samples were analyzed by any instrumental analytical technique.

Skin deposition studies of optimized formulation

At the end of the permeation experiments (after 24hr), the skin surface was washed five times with ethanol: PBS pH 7.4 (1:1), then with water to remove excess drug from surface. The skin was then cut into small pieces. The tissue was further homogenized with ethanol: buffer solution pH 7.4 (1:1) and left for 6hr at room temperature. After shaking for 5 minutes and centrifuging for 5 minutes at 5000rpm, the drug content was analyzed after appropriate dilutions with appropriate phosphate buffer solution.

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

Once the transfersomes passes the outermost skin layers, they will go into blood circulation via the lymph and distributed throughout the body, if applied under suitable conditions. Transdermal transfersomes can supply the drug to 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 distribution and the action after this passage. The most important single factors in this process are:

I. Carrier in-flow.

II. Carrier accumulation at the targets site.

III. 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 favorable in this respect. The rate of carrier passage across the skin is chiefly determined by the activation energy for the carrier deformation. If penetration of transfersomes involves the occlusion of an application site or the use of too strongly diluted suspension then it will hamper 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.

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.[17]

Table No.1: Comparison of transfersomes with different vesicles

S.No	Method	Advantage	Disadvantage
1	Liposomes	Phospholipid vesicle, biocompatible, Biodegradable	Less skin penetration less stable
2	Proliposome	Phospholipid vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of vesicles
3	Physical methods e.g. iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
4	Niosomes	Non-ionic surfactants vesicles	Less skin penetration easy handling But will not reach up to deeper skin layer
5	Proniosomes	Greater stability, Will convert into noisome insitu, 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 upto deeper skin layers.	None, but for some limitations

Table No.2: Different additives used in formulation of transfersomes

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

Table No.3: Application 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	Ketoprofen	Improved penetration for anti-inflammatory activity
4	Insulin	induce therapeutically significant hypoglycemia with good efficacy and reproducibility
5	Capsaicin	Increase skin penetration
6	Colchicine	Increase skin penetration
7	Vincristine	Increase entrapment efficiency and skin permeation
8	Interferon-α	Efficient delivery means (because delivery other route is difficult). Controlled release. Overcome stability problem.
9	Norgesterol	Improved transdermal flux
10	Tamoxifen	Improved transdermal flux
11	Methotrexate	Improved transdermal flux
12	Oestradiol	Improved transdermal flux
13	Tetracaine, Lignocain	Suitable means for the noninvasive treatment of local pain on direct topical drug application.
14	Corticosteroids	Improved site specificity and overall drug safety.
15	Hydrocortisone	Biologically active at dose several times lower than currently used formulation.
16	Triamcinolone acetonide	Used for both local and systemic delivery.
17	Human serum albumin	Antibody titer is similar or even slightly higher than subcutaneous injection.
18	Stavudine	Improved the in vitro skin delivery of Stavudine for antiretroviral activity
19	Tetanus toxoid	For transdermal immunization

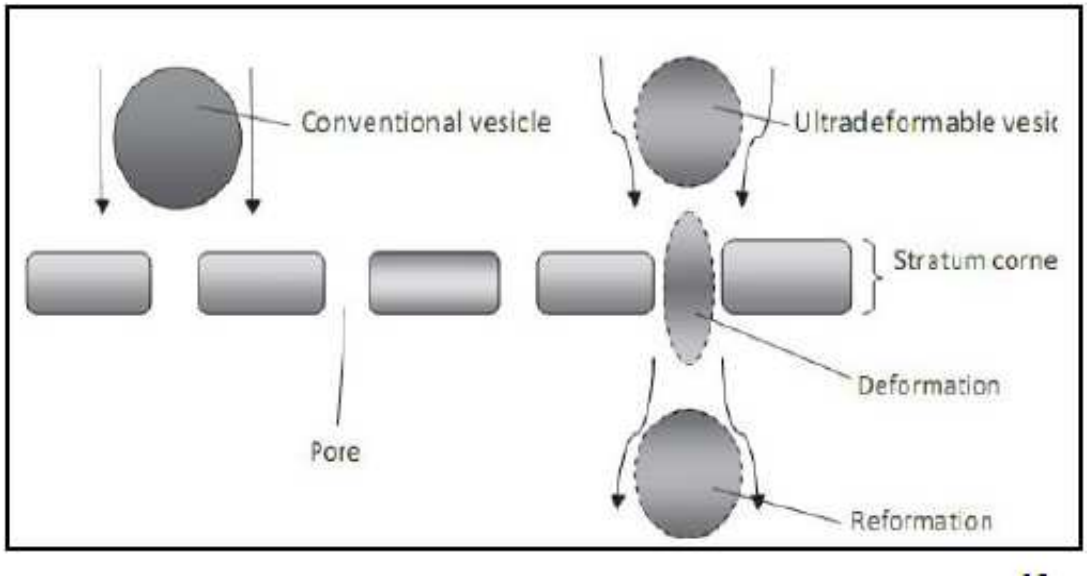

Figure No.1: Deformability of transfersomes in to skin pores

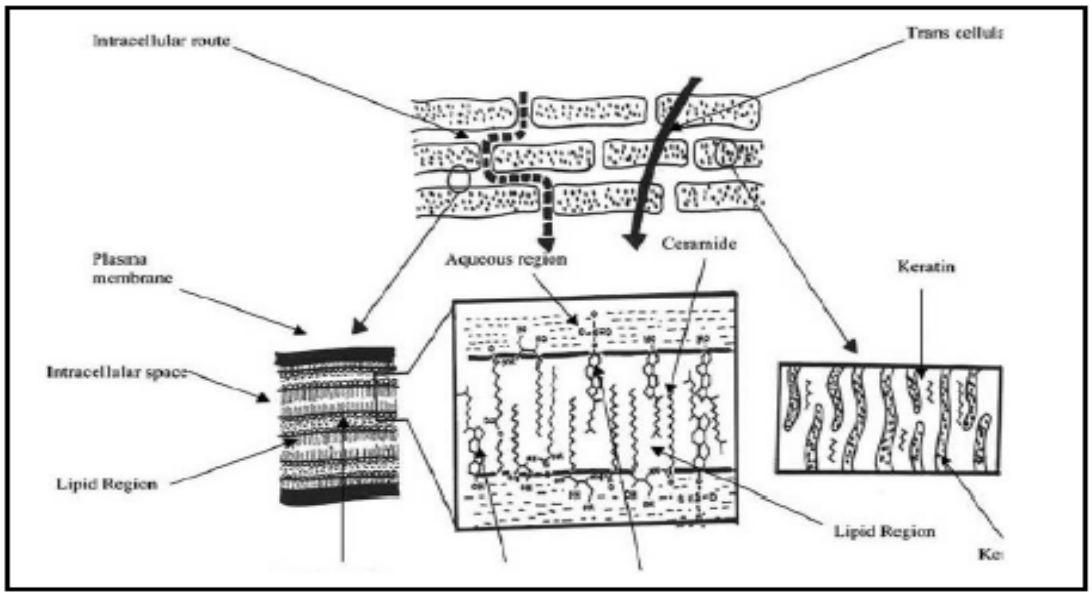

Figure No.2: Micro routes for drug penetration across human skin

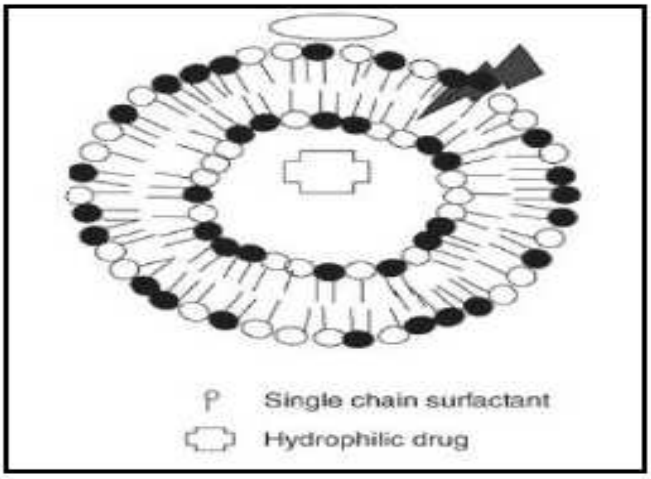

Figure No.3: Deformable transfersomes vesicle

CONCLUSION

Ultra-deformable vesicles can provide the novel solution for the transport related problems. They are free from the rigid nature of conventional vesicles and can transport even the large molecules. They work on number of mechanisms working together to provide an excellent carrier system for the drug transport. 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 loaded transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm2h-1). Ultra-deformable 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 unfavorable physicochemical characters, drugs for quicker and targeted action, etc. All above discussed properties of this technology strongly advocate its good future in transdermal drug delivery.

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