Indian Journal of Drugs, 2016, 4(2), 28-41 ISSN: 2348-1684

Review Article

PROTRANSFERSOME: ULTRAFLEXIBLE VESICULAR APPROACH FOR TRANSDERMAL DRUG DELIVERY SYSTEM

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Received: 09.02.2016
Accepted: 22.06.2016

Access this article online

Website:
www.drugresearch.in

Quick Response Code:

ABSTRACT

Transdermal drug delivery system appears to be a most promising delivery system due to their merits over conventional drug delivery system. Recently, various strategies have been used to augment the transdermal delivery of bioactive drugs. Mainly, they include iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancer, microneedles, vesicular system (liposomes, niosomes, elastic liposomes such as ethosomes & transfersomes) and provesicular system (proliposomes, proniosomes, protransfersomes). Among these strategies protransfersome appear promising. Protransfersome developed for transdermal delivery possessed superior skin penetration ability. In present study proultraflexible lipid vesicles "protransfersome" were proposed that will be converted into the ultraflexible vesicles, transfersomes in situ by absorbing water from the skin. The proposed system is more stable having higher entrapment efficiency, can be used as self-penetration enhancer, easy to scale up, better for dermal and transcutaneous delivery. This carrier comprises the advantage of unique elasto-mechanical characteristics of transfersomes that allow them to squeeze through the small pores across the skin, and hence affect into fast as well as enhanced drug permeation. Optimization can be done by selecting three process variables: effect of lecithin: surfactant ratio, effect of various solvents and effect of surfactants. The drug such as, Levonorgestrel, Cisplatin, Ketoprofen, Ketorolac, etc were formulated in Protransfersome Vesicular System. The System can be characterized for vesicle shape, size, entrapment efficiency, turbidity, and drug permeation across rat skin and were evaluated for their stability and bioavailability.

KEY WORDS: Protransfersome, Transfersome, optimization, ultraflexible vesicle, transcutaneous delivery, stability.

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.More recent,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 enhancer, supersaturated system, prodrug, liposomes and other vesicles[1]. There has been keen interest in the development of a novel drug delivery system. Novel drug delivery system aims to deliver the drug at a

rate directed by the needs of the body during the period of treatment, and channel the active entity to the site of action. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved. Consequently, a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. One of the possibilities for increasing the penetration of drugs through the skin is the use of vesicular systems such as liposomes, transfersome in order to increase biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs**.** Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to pediatric, geriatric and patients having dysphasia. Hence, transdermal dosage forms enjoy being the most patient compliant mode of drug delivery.[2]

More recent approach to drug delivery is to deliver the drug in to systemic circulation at a predetermined rate which is known as controlled release drug delivery system. Such systems helped to overcome the side effects associated with conventional system of medication, which require multidose therapy. In recent years, the development of transdermal dosage forms has been attracting increasing attention, owing to the several advantages that this administration route offers. Transdermal delivery system, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first pass metabolism, and a higher compliance**.** Transdermal therapeutic systems are defined as self-contained discrete dosage forms which when applied to the intact skin deliver the drug through the skin at a controlled rate to the systemic circulation. Thus it is anticipated that transdermal drug delivery system (TDDS) can be designed to input drugs at appropriate rates to maintain suitable plasma drug levels for therapeutic efficacy by using skin as the port of entry of drugs. Nevertheless, drug delivery via the skin is not an easy task because of the formidable barrier properties of the stratum corneum (SC). The majority of drugs do not appear to penetrate the skin at a sufficiently high rate to have therapeutic effectiveness[3].The most crucial reason for this is the inability of most of the drugs to pass the barrier nature of stratum corneum[4]. Skin structure is somewhat similar to the brick- mortar model. Corneocytes are like bricks surrounded by the mortar of the intercellular lipid lamellae[5]. Lipid lamellae, which is highly organized, plays important role in barrier properties of stratum corneum[6,7]. To overcome the problem of the stratum corneum barrier, various approaches can be adopted. First, application area can be enlarged; second, augmentation of the skin permeability and third, activation of concentration independent transport- driving forces. While the second approach belongs to penetration enhancers,the third approach is the domain of iontophoresis, jet- devices and more recently the drug carrier systems such as lipid vesicles (liposomes and proliposomes) and nonionic surfactant vesicles (niosomes and proniosomes). Though very novel, vesicles and especially the newer elastic, highly deformable vesicles are very promising in this regard for delivering wide variety of materials across the skin. 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.Vesicles have a unique structure which is capable of entrapping hydrophilic, lipophilic, amphiphilic and charged hydrophilic drugs. Vesicles are colloidal particles having a water filled core surrounded by a wall of lipids and surfactants (amphiphiles) arranges 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[8].

1.1 Definition of transdermal drug delivery system:

Transdermal therapeutic systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin at a controlled rate to the systemic circulation[9].

- **Importance of transdermal drug delivery system:**
  - Convenient and safe
  - Increased patient acceptability (Non-invasiveness)
  - Avoid GIT degradation and first pass effect
  - Avoid GIT disturbances due to drugs
  - Minimize side effects
  - Avoiding fluctuation

1.2 Vesicular approaches for transdermal drug delivery:

Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the ampiphilic nature, lipid vesicle may serve as non-toxic penetration enhancer for drugs. In addition, vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs[2].

1.2 Transfersomes (vesicular system):

The term Transfersome and the underlying concept were introduced in 1991 by Gregor Cevc.Since then, huge amount of research is going on worldwide on these elastic vesicles under different titles like flexible vesicles, ethosome, etc. In broadest sense, a Transfersome is a highly adaptable, stress-responsive and complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Transfersome is a term registered as a trademark by the German company IDEA AG, and used by it to refer to its proprietary drug delivery technology. The name means "carrying body", and is derived from the Latin word 'transferre', meaning 'to carry across', and the Greek word 'soma', for a 'body'. A Transfersome carrier is an artificial vesicle and resembles the natural cell vesicle. Thus it is suitable for targeted and controlled drug delivery[8].Transfersomes are vesicles, which are self-optimized aggregates with ultra-flexible membrane. These vesicular transfersomes are more elastic than the standard liposomes and thus well suited for the skin penetration[11].

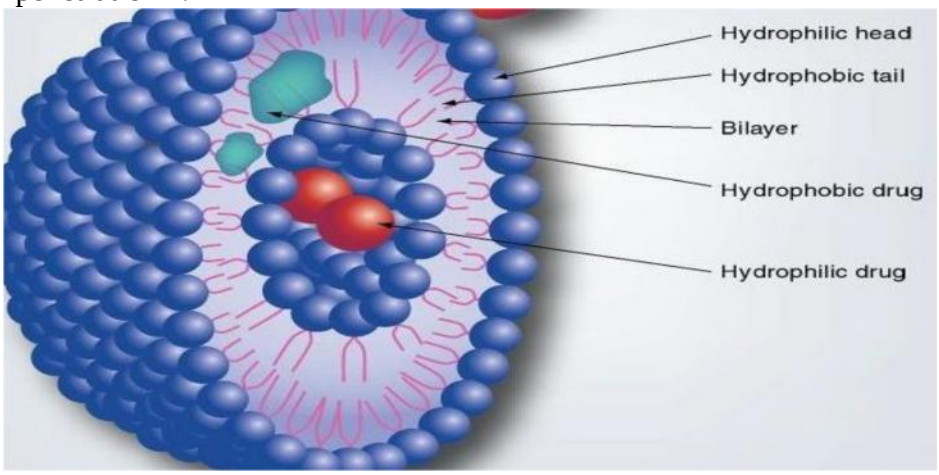

Figure 1: Structural of Transfersome[12]

1.3.1 Rationale for selecting the lipid vesicles (transfersomes) as a TDDS:

There are several instances where the most convenient drug intake methods, like oral route, were not feasible and alternative routes had to be sought. Although, intravenous administration of the medicament avoids many of these shortfalls (such as gastrointestinal and hepatic metabolism), its

invasive and apprehensive nature (particularly for chronic administration) has encouraged the search for alternative strategies.Transdermal Topical drug delivery offers several distinct advantages including relatively large and readily accessible surface area for absorption, ease of application and termination of therapy.

- Transfersomes are amphiphilic in nature so able to accommodate both hydrophilic as well as lipophilic drugs.
- Transfersomes release the drug in a sustained manner for a prolonged period of time at a predetermined rate.
- Transfersomes can deform and pass through narrow constriction (from 5-10 times less than their own diameter) without measurable loss.
- Transfersomes can act as a carrier for low and high molecular weight drugs
- Transfersomes have high entrapment efficiency.
- Transfersomes are used for both, topical and systemic delivery of drugs
- They protect the encapsulated drug from metabolic degradation[13].

1.3.2 Advantages:

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[14].

1.3.3 Scope of transfersome:

Transfersome technology is best suited for noninvasive delivery of therapeutic molecules across open biological barriers. The Transfersome vesicles can transport across the skin, for example, molecules that are too big to diffuse through the barrier. Examples include systemic delivery of therapeutically meaningful amounts of macromolecules, such as insulin or interferon, across intact mammalian skin. Other applications include the transport of small molecule drugs which have certain physicochemical properties which would otherwise prevent them from diffusing across the barrier. Transfer some technology is the carrier's ability to target peripheral, subcutaneous tissue. This ability relies on minimisation of the carrier associated drug clearance through cutaneous blood vessels plexus, the non-fenestrated blood capillary walls in the skin together with the tight junctions between endothelial cells preclude vesicles getting directly into blood, thus maximizing local drug retention and propensity to reach the peripheral tissue targets[15].

1.3.4 Limitations of transfersomes:

- Chemically unstable
- Expensive
- Less purity of phospholipids
- Predisposition to oxidative degradation[2].

Table 1: Different Drugs Used and Results Obtained of Different Studies of Transfersomes For Transdermal Application[2]

Sr. No	System	Drug	Results	References
1	Transfersomes	Insulin	High entrapment efficiency, Improved transdermal flux.	Cevc et al., 2003
2	Transfersomes	Interferon-α	Efficient delivery means (because delivery by other routes is difficult).	Hofer et al., 2000

3	Transfersomes	Interleukin-2	Controlled release, reduce stability problem	Hofer et al., 2004
4	Transfersomes	Soluble proteins	Permits, non-invasive immunization.	Paul et al, 1998
5	Transfersomes	Hydrocortisne Dexamethasone	Increased biological potency, Prolonged effect, Reduced dosage	Cevc et al., 2004
6	Transfersomes	Triamcinolone acetonide	Both for local and systemic delivery.	Cevc et al., 2003
7	Transfersomes	Diclofenac Tetracaine Lidocaine	Non-invasive treatment of local pain on direct topical application	Cevc et al.,2001
8	Transfersomes	Oestradiol	Improved transdermal flux	Maghraby et al., 1999
9	Transfersomes	Tamoxifen	Improved transdermal flux	Gamal et al.,1999
10	Elastic liposome	Zidovudine	Sustained drug delivery	Jain et al., 2006
11	Transfersomes	Vaccine	Both for Local and Systemic delivery	Gupta et al., 2005

1.4 Provesicular approach:

The vesicular approach i.e. Liposome in transdermal drug delivery system has been studied for many purposes but the unstable nature limits their use at clinical and industrial levels. In order to increase the stability of liposomes concept of proliposomes has been proposed. This approach has been extended to niosomes, which exhibit superior stability compared to traditional liposomes and overcome their limitations by proniosomal approach. But all approaches, because of poor skin permeability, breaking of vesicles, and leakage of drug, aggregation and fusion of vesicles are not much successful for effective transdermal drug delivery. To overcome problems of poor skin permeability recently introduced two new vesicular carrier system, transfersomes for non-invasive delivery of drugs into or across the skin. Transfersomes incorporated edge activators (surfactants) to influence the properties of vesicles and stratum corneum. Provesicular approach has been extended to the transfersome. Protransfersome developed for transdermal delivery which possess superior skin penetration ability and better stability. Each transfersomes consists of at least one inner aqueous compartment; this is surrounded by a lipid bilayer, but the problem in this carrier is self-stability. Transfersomes are also known as elastic liposomes, which pass through nanometer pores of the stratum corneum.So in present study liquid crystalline proultraflexible lipid vesicles (protransfersome) were proposed that will be converted into the ultraflexible lipid vesicles (transfersome) in situ by absorbing the water from the skin[16].

Table 2: Comparision between different Transdermal system[14]

Method	Advantage	Disadvantage
Penetration enhancers(WALTERS,1989)	Increase penetration through skin and give both local and systemic effect.	Skin irritation immunogenicity, only for low molecular weight drugs
Physical method e.g.Iontophoresis(Cevc et al,1995)	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
Liposomes (Hadgraft & Guy,1989)	Phospholipids vesicle,biocompatible, biodegradable	Less skin penetration less stable
Proliposome	Phospholipids vesicle,more stable than liposomes	Less penetration, cause aggregration and fusion of vesicles
Niosomes (Schreier & Bouwstra,1994) (Holland et al,1995) Proniosomes	Non-ionic suefactant vesicles, Greater stability, Will convert into noisome in situ, Stable	Less skin penetration easy handling But will not reach upto deeper skin layer
Transfersomes & Protransfersome	More stable, high penetration due to high deformability,biocompatibility and biodegradatable, 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

1.5 Protransfersome:

Protransfersome developed for transdermal delivery possessed superior skin penetration ability and better stability. Protransfersome are reported to have lamellar liquid crystalline structure which will be converted into the ultraflexible vesicles, transfersomes (also known as elastic liposomes), in situ by absorbing water[16].The main aim of study was to develop a transdermal delivery vehicle for sustained systemic delivery of drug using protransfersome system and to investigate the feasibility of using protransfersome as a transdermal drug delivery system. Encouraged with the potential of the concept of protransfersome for the enhancement of transdermal drug permeation, it was envisaged to solve the problem of transdermal delivery of BCS Class II combination using this novel carrier system.

1.5.1 Advantages:

- Protransfersome, may avoid many of the problems associated with aqueous dispersions of transfersome and minimize problems of physical stability (aggregation, fusion, leaking).
- The additional convenience of the transportation, distribution, storage, and dosing would make ‘Retransfers’ a promising industrial product.[11]

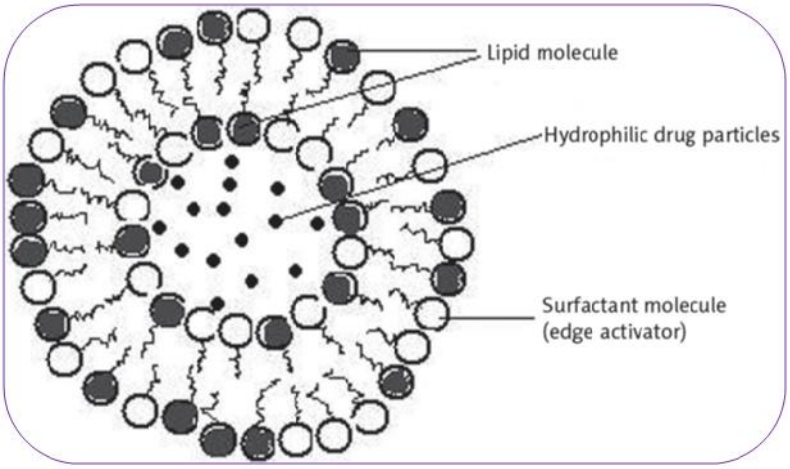

Figure 2: Structure of Protranfersome Vesicle

Table 3: Protransfersome of various Drugs and research outcome

Sr.No	Drug	Research outcome	References
1	Norgesterel	Ability as a self-penetration enhancer, easy to scale up and better for transdermal delivery as compared to proliposomes	S.Jain et al,2003
2	Levonorgesterel	Better skin permeation potential, better stability, higher entrapment efficiency	Subheet Jain et al,2005
3	Cisplatin	Better non-invasive delivery	Vandana Gupta et al,2010
4	Ketoprofen	Sustain and effective release rate.	Gaur Ajay et al,2013
5	Nifedifine	Better stability, greater intrapment efficiency	Murugesan Senthil Kumar,2014
6	Ketorolac	Simple production and facile scale up, higher entrapment efficiency	Tarkunde Sayali et al,2015

1.5.2 Mechanism of protransfersome:

For optimum drug delivery, a high degree of drug-vesicle association is essential so that appreciable quantity of drug could be carried by elastic vesicles across the stratum corneum.Subsequently, larger quantities of drugs will be released from the vesicles, thereby increasing the amount of free drug available for diffusion into the deeper skin layers. High entrapment efficiency of protransfersome gel is probably the reason for its better skin permeation.

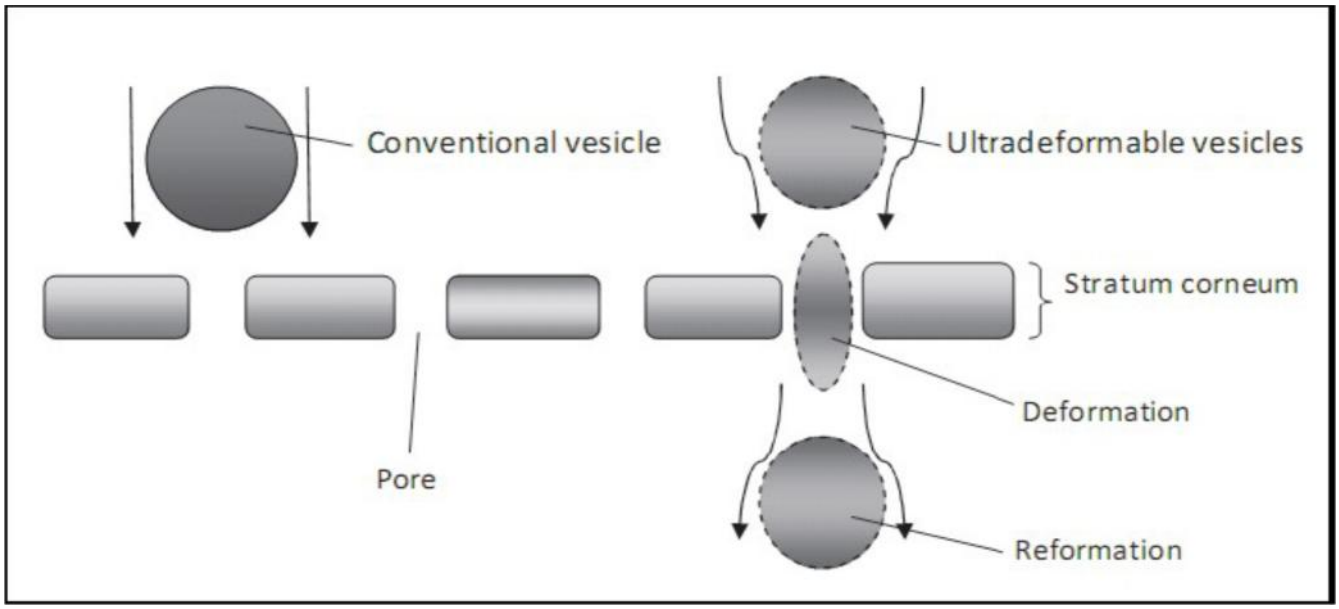

Figure 3: Comparative diagram of transport through conventional and ultra-deformable vesicle.

Furthermore, higher skin permeation of PTG could be a result of better partitioning across the stratum corneum and to deeper layers of skin under the influence of transepidermal osmotic gradients. The osmotic gradient is developed because skin penetration barrier prevents water loss through the skin and maintains a water activity difference in viable parts of the epidermis (75% water content) and nearly completely dry stratum corneum near the surface (15% water content). PTG consists of polar lipids (PC) that have a tendency to attract water because of the energetically favorable interaction between the hydrophilic lipid residues and proximal water molecules. Hence, when PTG is applied on skin surface that is partly dehydrated by water loss due to evaporation, the lipid vesicles feel this osmotic gradient and try to escape complete drying by moving along this gradient resulting in faster partitioning of vesicles into the stratum corneum and other deeper layers of the skin[2].

MATERIALS AND METHODS

Materials commonly used for preparation of protransfersome are summarized in following table. Various procedure is available for the preparation of protransfersome. Protransfersomal gel (PTG) was prepared by using Phospholipids, Surfactant and Alcohol.

Table 4: Different additive and their role[17].

Class	Additives	Role
Phospholipids	Soya phosphatidyl choline Dipalmitoyl phosphatidyl Choline Distearoyl phoshadidyl choline	Vesicles forming component
Surfactant (edge activator)	Sod. Cholate Sod.deoxycholate Tween-80 Span-80	For providing flexibility
Alcohols	Ethanol Butanol Isopropanol	As a solvent
Buffering Agent	Saline phosphate buffer (pH 7.4)	As a hydrating medium

Preparation of protransfersomes:

1) 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. 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 7.4) 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[14].

2) 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 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[14].

3) Phase separation coacervation method: Accurately weighed or required amount of surfactant, carrier (lecithin), cholesterol and drug can be taken in a clean and dry wide mouthed glass vial (5 ml) and solvent should be added to it. All these ingredients have to be heated and after heating all the ingredients should be mixed with glass rod. To prevent the loss of solvent, the open end of the glass vial can be covered with a lid. It has to be warmed over water bath at 60-70^0 C for 5 minutes until the surfactant dissolved completely. The mixture should be allowed to cool down at room temperature till the dispersion gets converted to a gel.The apparatus for phase separation coacervation method[18].

3. OPTIMIZATION OF VARIABLES

The preparation of PT involves various formulation variables, which could affect the preparation and properties of the PT, but optimization was done by selecting three of them.

- Effect of Lecithin: Surfactant ratio
- Effect of various solvents
- Effect of various surfactants.
- Hydration medium.[2]

4. EVALUATION

4.1 Physical appearance:

The prepared protransferome was viewed by naked eye to characterize color and physical state Protransfersome was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics by spreading a thin layer of protransfersome on a slide and placing the cover slip on it[19].

4.2 Vesicle size and size distribution:

Determination of Zeta-Potential: The method involves the preparation of dispersion of protransfersomes in PBS (pH 7.4). Then this dispersion was filled in zeta cell and placed in the Zeta Sizer (Nano ZS, Malvern Instruments, UK) to determine the zeta-potential[20]**.**

4.3 Vesicle Shape and Surface Characteristics:

A) Optical microscopy:

Hydration of Protransfersome was performed with phosphate buffer saline (pH 7.4) with slight agitation to produce transfersome. A drop of transfersome suspension was placed on a slide and after placing cover slip observed under microscope. Photomicrographs were taken at 100X magnification.

B) Transmission electron microscope(TEM):

To further evaluate the surface characteristics of vesicle transmission electron microscopy (TEM) were performed.The transfersome suspension was negatively stained with a 1% aqueous solution of phosphotungustic acid (PTA). Transfersome suspension was dried on a microscopic carbon coated grid for staining. The excess solution was removed by blotting. After drying the specimen was viewed under the microscope.

C) Scanning electron microscope(Sem):

The shape, surface characteristics, and size of the transfersomes were observed by scanning electron microscopy (SEM). Weighed quantity of the protransfersome in a glass tube was diluted with 10 ml of phosphate buffer saline (pH 7.4). The lyophilized transfersomes were mounted on an aluminum stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd, Tokyo, Japan) equipped with a digital camera, at 20 kV accelerating voltage.[2]

4.4 Rate of hydration (spontaneity):

Spontaneity of transfersome formation is described as number of transfersome formed after hydration of protransfersomes for 20 minute. Approximately 20 mg of protransfersome was transferred to bottom of a small stoppered glass vial and spread uniformly around the wall of the glass vial with the help of the glass rod. Two ml phosphate buffer saline was added carefully along the walls of the vial and kept aside without agitation. After 20-minute suspension was withdrawn and diluted to 10 ml. one drop of diluted suspension was placed on Neubaur's chamber. The number of transfersomes eluted from protransfersome was counted at RBC subdivisions on Neubaur's chamber[21].

4.5 Drug entrapment efficiency:

To evaluate loading capacity of protransfersome system for drug, an accurately weighed quantity of protransfersome (100 mg) was dispersed into 10 ml phosphate buffer saline to produce transfersome suspension. The transfersome suspension was centrifuged at 18000 rpm in cooling centrifuge at 20 ^{0}C for 30 min to separate drug containing transfersome from unentrapped drug.Then the sediment of vesicle was resuspended in 1 ml 30% PEG-400 and 1 ml 0.1% Triton X 100 solution was added to it. Resulting solution was filtered and diluted to 100 ml with phosphate buffer saline and analyzed for drug content spectrophotometrically[11].

% EE = (Amount of drug entrapped / Total amount of drug added) ×100 ……. (Eq^n 1)

Drug content: The drug content can be determined using one of the instrumental analytical methods such as modified high performance liquid chromatography method (HPLC)[22,14].

4.7 Turbidity measurement:

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

4.8 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[8].

4.9 Degree of deformability or permeability measurement:

In the case of protransfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Protransfersomes 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 using the following formula: $E = J \times (rv/rp)2$ ……..(Eq^n 2)

Where, J= the amount of the suspension extruded during 5min, rv = the size of the vesicle, rp = pore size of the barrier[23].

4.10 *In-vitro* drug permeation study:

Permeation of protransfersome was studied using a Franz glass diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 1 cm^2 and 10 ml, respectively. The receptor compartment contained PBS (pH 7.4) and maintained at 370C ± 1°C by magnetic stirrer. Egg membrane was mounted between the donor and receptor compartment. PT (200mg) containing 1 mg of drug was applied to the surface of the egg membrane. Samples were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 48 hours and analyzed by UV-Visible Spectrophotometer. An equal volume of fresh PBS, pH 7.4 was replaced into the receptor compartment after each sampling[2].

4.11 *In-vi*vo evaluation:

In vivo studies were performed on female Sprague Dawley rats weighing 100 to 150 g. Four groups each comprising 9 animals were employed. The first group served as a control while the second, third and fourth groups received plain drug, optimized protransfersome, and proliposomal formulation, respectively. The quantity of drug in all the formulations was 500 µg. Rats were synchronized by injecting 1 mL of 0.1% copper acetate intraperitoneally. After 24 hours the formulation was applied to the dorsal surface of rats (1.0 cm^2). The treated areas of animals were protected by using nylon mesh, which was supported by plastic squares having small pores. Treated animals were kept in separate cages and housed in standard laboratory conditions. Food and water were provided ad libitum. Three rats from each group were killed by excessive chloroform inhalation after the first, fourth, and ninth day of application.All investigations were performed after approval by the animals ethical committee. The blood samples were collected from retro orbital plexus of the eye at defined time intervals. Each blood sample was centrifuged at 2000 rpm for 10 minutes and drug concentration after deproteinization with acetonitrile was determined by HPLC for plasma concentration determination[24].

5. APPLICATIONS

5.1 Controlled release and stability enhancement:

Protransfersome as drug delivery system have the potential for providing controlled release of the administered drug and increasing the stability of liable drugs.

5.2 Utilization of high molecular weight drugs:

Very large molecules incapable of diffusing into skin as such can be transported across the skin with the help of protransfersome.

5.3 Transport of drugs:

a) Percutaneous delivery of antitumor drug:

As a drug carrier, protransfersome was able to deliver antitumour drug whose percutaneos absorption is limited into the systemic blood circulation via the transdermal route and shows better transdermal delivery of drug for cancer therapy[17].Antitumour drugs like Cisplatin were tried for transdermal delivery using protransfersome technology. The results were favorable[19].

b) Transport of corticosteroids:

Protransfersome improves the site specificity and overall safety margin of corticosteroids which is difficult to maintain by other routes[25, 26].

c) Delivery of insulin:

By protransfersomes is the successful means of noninvasive 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 overcomes these entire problems. After application on the intact skin, the first sign of systemic hypoglycemia is observed after 90 to 180 min, depending on the specific carrier composition[27].

d) Delivery of protein and peptide:

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 bioavaibility obtained from protransfersomes is somewhat similar to that resulting from subcutaneous injection of the same protein suspension. The protransferosomal preparations of this protein also induced strong immune response after the repeated epicutaneous application[28, 29].

e) Delivery of interferon:

These system is 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. Protransfersomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs[30].

f) In anesthesia:

Application of anesthetics in the suspension of highly deformable vesicles 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 is last longer[31].

g) Delivery of NSAIDs:

NSAIDs are associated with number of GI side effects. These can be overcome by transdermal delivery using ultra-deformable vesicles. Studies have been carried out on Keterolac and Ketoprofen [2, 11, 32].

h) Delivery of herbal drug:

Protransfersomes can penetrate stratum corneum and supply the nutrients locally to maintain its functions resulting maintenance of skin.Protransfersome is useful for delivery of Herbal drug[33].

i) Transport of antihypertensive agent:

The problems associated with the oral bioavailability of antihypertensive drug could be overcome by incorporating it into a new transdermal proultraflexible drug carrier called as protransfersome[23].

5.4 Transdermal Immunization:

Another most important application of protransfersomes is transdermal immunization using protransfersomes loaded with soluble protein like integral membrane protein, human serum albumin, and gap junction protein. These approaches offer 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[31].

Table 5: Various applications of protransfersome

Sr.No	Drug	Category	References	Year
1	Norgesterel	Contraceptive Agent	S.Jain et al	2003
2	Levonorgesterel	Contraceptive Agent	Subheet Jain et al	2005
3	Cisplatin	Anti-tumour	Vandana Gupta et al	2010
4	Ketoprofen	NSAID	Gaur Ajay et al	2013
5	Nifedifine	Anti-hypertensive	Murugesan Senthil Kumar	2014
6	Ketorolac	NSAID	Tarkunde Sayali et al	2015

CONCLUSION

Vesicular systems have potential advantage in the field of pharmaceutical science. Nowadays the targeting of drug can be possible by vesicular approach. Protransfersome can be specially optimized vesicular system which converted into transfersome in situ by absorbing water from the skin. It is emerging vesicular system due to their abilities of site specificity, sustained release, systemic release possible, higher penetration power across skin, higher deformability, higher stability, encapsulation of higher molecular weight drug as comparsion of other vesicular system.2 The various drug such as NSAIDs, Anticancer, Antihypertensive, etc can be targeted to specific site by this approach. In future protransfersome reveals the potential advantages in the NDDS.

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