ejpmr, 2015,2(3),218-233

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

TRANSFERSOMES: NOVEL APPROACH FOR TRANSDERMAL DELIVERY

Sakshi Sharma and Dr. Ujjwal Nautiyal*

Himachal Institute of Pharmacy, Rampur Ghat Road, Paonta Sahib, HP. 173025, India.

Article Received on 11/03/2015 **Article Revised on 02/04/2015** **Article Accepted on 23/04/2015**

***Correspondence for Author**

Dr. Ujjwal Nautiyal

Himachal Institute of Pharmacy, Rampur Ghat Road, Paonta Sahib, HP. 173025, India.

ABSTRACT

Transdermal drug delivery appears to be the most vital drug delivery system because of its merit over conventional systems. Various strategies can be used to augment the transdermal delivery which includes iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microneedles, and vesicular system (liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes).The transfersomal system was much more efficient among all these strategies. These can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. The system can be characterized by *in vitro* for vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm.The deformability characteristic of transfersomes give better permeation of drugs.

KEY WORDS: includes iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microneedles, and vesicular system.

INTRODUCTION

Transdermal Drug Delivery is one of the most efficient routes for the delivery of drug in to systemic circulation. The aim of successful formulation is to deliver the active substance at target organ with minimal discomfort and side effects. In this respect, transdermal route is preferred because of avoidance of hepatic first pass metabolism, typical peak trough plasma profile, ease of administration. However, the improvement in permeability of drug through the skin is always a difficult problem because of barrier function of human skin epithelia to exogenous substances. Therefore, the major challenge in topical administration is to increase the penetration of drug into the skin.[1]

Transdermal drug delivery system (TDDS) provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy. The principal of transdermal drug transport is to deliver drug across epidermis to achieve systemic effect over a prolonged period of time. The human skin is a readily accessible surface for drug delivery. Skin of an average adult body covers a surface of approximately 2 m^2 and receives about one-third of the blood circulating through the body. Over the past decades, developing controlled drug delivery has become increasingly important in the pharmaceutical industry. The human skin surface is known to contain an average, 10-70 hair follicles and 200 250 sweat ducts on every square centimeters of the skin area. It is one of the most readily accessible organs of the human body. There is considerable interest in the skin as a site of drug application both for local and systemic effect.[2]

In the past, the most commonly applied systems were topically applied creams and ointments for dermatological disorders and these show local action but occurrence of systemic side-effects with some of these formulations is indicative of absorption through the skin this concept lead to the birth of TDDS. Moreover, it over comes various side effects like painful delivery of the drugs and the first pass metabolism of the drug occurred by other means of drug delivery systems. TDDS has been a great field of interest in recent times. Many drugs which can be injected directly into the blood stream via skin have been formulated by TDDS.Transdermal drug delivery systems (TDDS) are defined as self contained, discrete dosage forms which, when applied to intact skin, deliver the drug(s), through the skin, at a controlled rate to systemic circulation. The transdermal route of administration is recognized as one of the potential route for the local and systemic delivery of drugs.[3]

Advantages

- Transdermal medication delivers a steady infusion of a drug over an extended period of time.
- An equivalent therapeutic effect can be elicited via transdermal drug input with a lower daily dose of the drug than is necessary, e.g. the drug is given orally.
- Self administration is possible with these systems.
- They are easily and rapidly identified in emergencies (e.g. unresponsive, unconscious or comatose patient) because of their physical presence, features and identifying markings.
- They can be used for drugs with narrow therapeutic window.
- Longer duration of action resulting in a reduction in dosing frequency.

- Increased convenience to administer drugs which would otherwise require frequent dosing.
- Improved bioavailability.
- More uniform plasma levels and maintain plasma concentration of potent drugs.
- Reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing interval.
- Flexibility of terminating the drug administration by simply removing patch from the skin.
- Improved patient compliance and comfort via non-invasive, painless and simple application.
- Avoid inter and intra patient variation and enhance therapeutic efficacy.

Disadvantages

- Many drugs especially drugs with hydrophilic structures permeate the skin too slowly to be of therapeutic benefit.
- The barrier function of the skin changes from one site to another on the same person, from person to person and also with age.
- Only small, lipophilic drugs can be delivered currently through the skin.
- Drug molecule must be potent because patch size limits amount that can be delivered.
- Not suitable for high drug doses.
- Adhesion may vary with patch type and environmental conditions.
- Skin irritation and hypersensitivity reactions may occur.
- Drugs that require high blood levels cannot be administered.
- Along with these limitations the high cost of the product is also a major drawback for the wide acceptance of this product. [4,5,6]

Anatomy and physiology of human skin

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface.

The skin of an average adult body covers a surface area of approximately 2 m^2 and receives about one third of the blood circulating through the body and serves as a permeability barrier against the transdermal absorption of various chemical and biological agent. It is one of the most readily available organs of the body with a thickness of only a few millimeters (2.97 ± 0.28 mm). The skin

- Separates the underlying blood circulation network from the outside environment.
- Serves as a barrier against physical, chemical and microbiological attacks.
- Acts as a thermostat in maintaining body temperature.
- Plays role in the regulation of blood pressure.
- Protects against the penetration of UV rays.[7,8]

As skin is major factor in determining the various drug delivery aspects like permeation and absorption of drug across the dermis. It is quite worthwhile to highlight some important characteristic of skin. The diffusion resistance of the skin is greatly dependent on its anatomy and ultra structure. Figure 1 shows the stratified organization of the skin. The composite structure of the skin is indicated by three distinct layers: the epidermis, dermis and subcutaneous fat layer **(Figure 1).**[9]

For the purpose of transdermal drug delivery, we can examine the structure and function of human skin categorized into following four main layers:

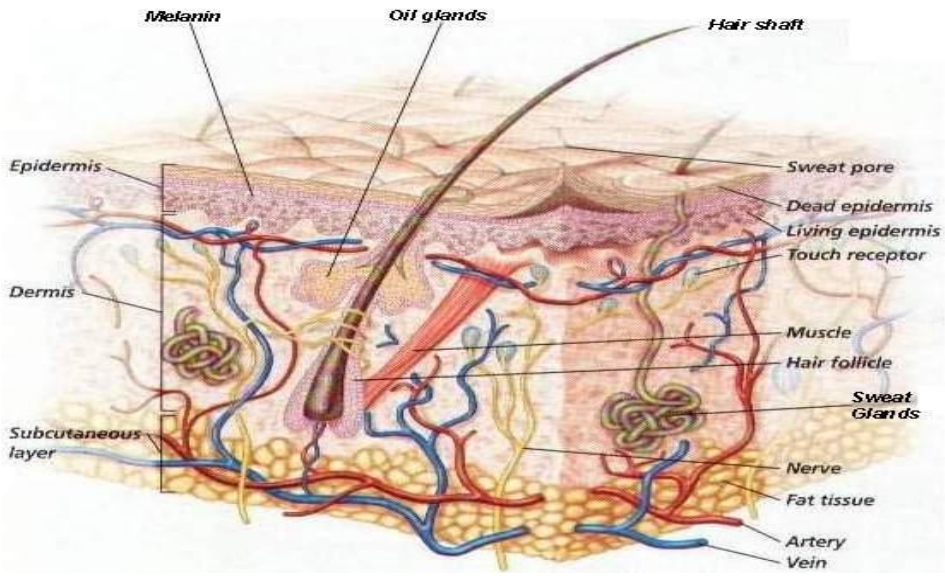

Figure 1: Standard organization of skin

- **Subcutaneous Fat Layer**

The subcutaneous fat layer or hypodermis, bridges between the overlying dermis and the underlying body constituents. In most areas of the body this layer is relatively thick, typically in the order of several millimetres. This layer of adipose tissue principally serves to insulate the body and to provide mechanical protection against physical shock. The subcutaneous fatty layer can also provide a readily available supply of high-energy molecules, whilst the principal blood vessels and nerves are carried to the skin in this layer.

- **Dermis**

The dermis has numerous structures embedded within it: blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicles and sebaceous glands) and sweat glands (eccrine and apocrine). Thus provides physiological support for the epidermis. The dermis (or corium) is typically 3–5 mm thick and is the major component of human skin. It is composed of a network of connective tissue, predominantly collagen fibrils providing support and elastic tissue providing flexibility, embedded in a mucopolysaccharide gel. In terms of transdermal drug delivery, this layer is often viewed as essentially gelled water and thus provides a minimal barrier to the delivery of most polar drugs, although the dermal barrier may be significant when delivering highly lipophilic molecules.

- **Epidermis**

The epidermis is approximately 100 □m thick in man and may be further classified into a number of layers. The stratum germinativum is the basal layer of the epidermis. Above the basal layer are the stratum spinosum, the stratum granulosum, the stratum lucidum and finally, the stratum corneum.

- **Stratum Corneum**

The stratum corneum or the horny layer is the rate limiting barrier that restricts the inward and outward movement of chemical substances consists of flattened keratin-filled cells (e.g., corneocytes). Upon reaching the stratum corneum, these cells are cornified and flatten. The corneocytes are then sloughed off the skin at a rate of about one cell layer per day, a process called desquamation. The man source of resistance to penetration and permeation through the skin is the stratum corneum. Stratum corneum is approximately 15-20 m thick over much of the human body and corneocytes are composed of cytoplasmic protein matrices comprising keratin embedded in the extracellular lipid. In the simplest sense, therefore, the skin may be

represented as a bilaminated membrane; and to reach the dermal vasculature (and rapid systemic distribution), a penetrating molecule must traverse both, the lipophillic environment of the stratum corneum and the aqueous environment of the underlying viable epidermis and upper dermis.[10,11,12,13,14,15]

Route of drug penetration through human skin

When a molecule reaches intact skin, it contacts cellular debris, microorganisms, sebum and other materials. The diffusant then has three potential entry routes to the viable tissue, through the hair follicles with their associated sebaceous glands, via the sweat ducts or across the continuous stratum corneum between these appendages.

Electron photo-microscopic examination shows that intracellular region in stratum corneum is filled with lipid reach amorphous material. During cornification the lipid composition shifts from polar to neutral constituents. In the dry stratum corneum intracellular diffusion volume may be as high as 5% and least 1% of the fully hydrated stratum corneum. This intra-cellular volume is at least an order magnitude larger than that (approximate 0-2%) estimated for the intra-appendageal pathway, thus, intracellular diffusion could be significant.

Both the structured lipid environment between the cells and the hydrated protein, within a corneocytes plays major role in skin permeability, cell membranes are probably of only minor consequences. These figures illustrate two potential routes for drug permeation **(Figure 2).**

- Intra cellular : between the cells and
- Trans cellular: across lipid rich region.

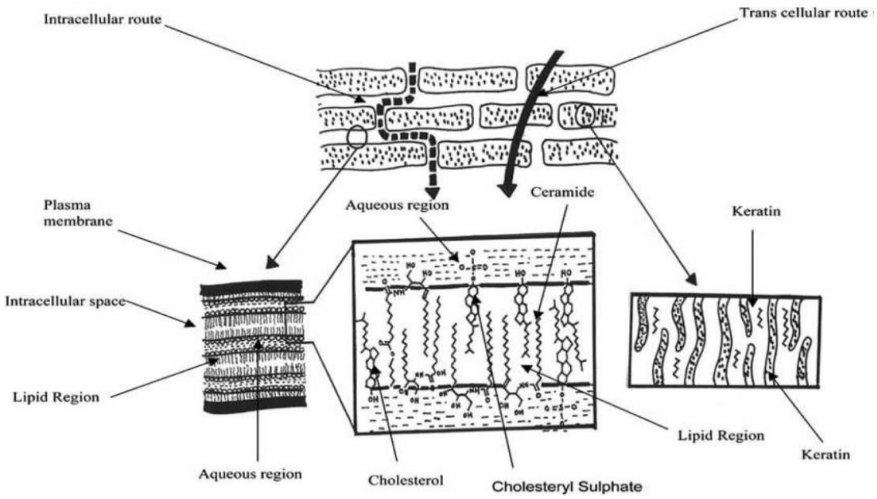

Figure 2: Intercellular and transcellular pathways for drugs and drug transport systems through the stratum corneum.[1]

Conventional formulations and novel approaches in transdermal delivery

The stratum corneum is the principal barrier to drug permeation across the skin. Consequently, there has been a concerted effort to investigate and develop novel strategies of maximizing the amount of permeant crossing this barrier. Innovative approaches focus on altering the drug vehicle interaction to enhance partitioning into the stratum corneum or modifying the structure of the stratum corneum to make it less resistance to drug diffusion.[2]

In recent years it has been shown that the skin is a useful route for drug delivery to the systemic circulation. Transdermal drug delivery system includes all topically administered drug formulations intended to deliver the active ingredients into the circulation. They provide controlled continuous delivery of drugs through the skin to the systemic circulation **(Figure 3).**[17]

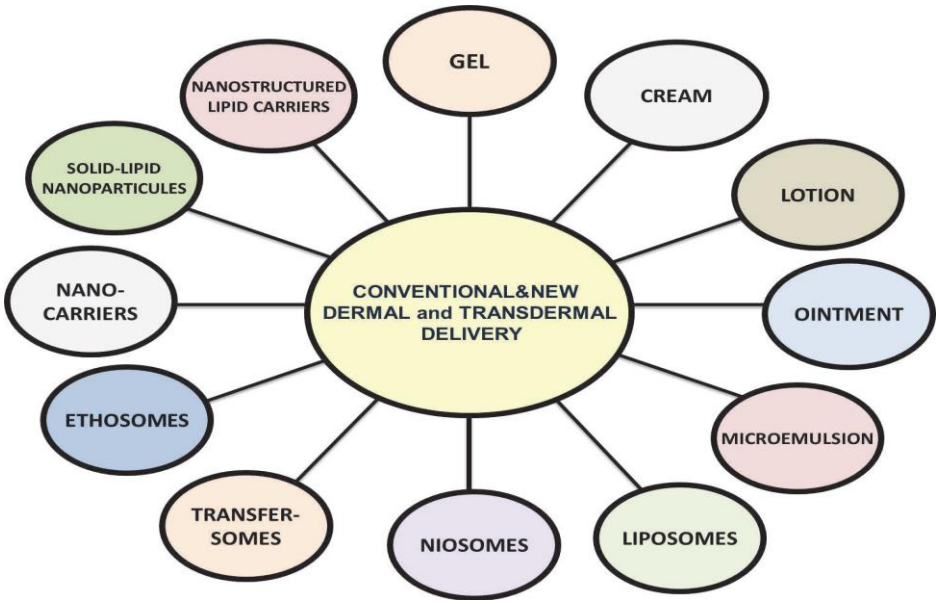

Figure 3: Schematic representation of the novel and conventional dermal drug delivery systems.

TRANSFERSOMES

In the last few years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs.[18]

By using the concept of rational membrane design we have recently devised special composite bodies, so-called Transfersomes which overcome the filtration problem.[19]The term transfersome and the underlying concept was introduced in 1991 by GregorCevc. Transfersomes are ultradeformable vesicles, elastic in nature, which can squeeze itself

through a pore which is many times smaller than its size owing to its elasticity. The name transfersome means “carrying body”, and it was derived from the Latin word 'transferre', meaning ‘to carry across’, and the Greek word ‘soma’, for a ‘body’.Transfersomes are artificial vesicles, and they are more deformable than standard Liposomes.Transfersome have been reported to enhance the transdermal delivery of drugs, when applied onto the skin non-occlusively.Transfersome usually composed of at least one inner aqueous compartment, which is surrounded by a lipid bilayer. It also possesses some specially tailored properties due to the incorporation of "edge activators “into the vesicular structure. Span 80, tween 80, sodiumcholate, sodium deoxycholate, are some surfactants that have been used as an edge activators. Transfersome are usually applied in the form of semi-dilute suspension.Because of their property of deformability, this vesicular system is a good candidate for the non-invasive delivery of small, medium, and large sized drugs.[20]

Characteristics of Transfersomes

- Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
- Transfersomes can deform and pass through narrow constriction (from 5 to10 times less than their own diameter) without measurable loss.
- This high deformability gives better penetration of intact vesicles.
- They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
- They have high entrapment efficiency, in case of lipophilic drug near to 90%.
- They protect the encapsulated drug from metabolic degradation.
- They act as depot, releasing their contents slowly and gradually.
- They can be used for both systemic as well as topical delivery of drug.
- Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.

Limitations of transfersomes

- Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
- Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drugdelivery vehicles.
- Transfersomes formulations are expensive.[21]

Mechanism of penetration of transfersomes

The mechanism for penetration is the generation of "osmotic gradient" due to evaporation of water while applying the lipid suspension (Transfer-somes) on the skin surface. The transport of these elastic vesicles is thus independent of concentration. The trans-epidermal hydration provides the driving force for the transport of the vesicles. As the vesicles are elastic, they can squeeze through the pores in stratum corneum (though these pores are less than one-tenth of the diameter of vesicles).

- Interaction between hydrophilic lipid residues and proximal water makes the polar lipids to attract water molecules induce hydration, lipid vesicles move to the site of higher water concentration. The difference in water content across skin stratum and epidermis develops transdermal osmotic gradient that leads to penetration of transfersomes across skin.

- Transfersomes by enforcing its own route induce hydration that widen the hydrophobic pores of skin, through the widen pores there is gradual release of drug occurs that binds to targeted organ.

- Transfersomes act as penetration enhancers that disrupt the intercellular lipids from stratum which ultimately widens the pores of skin and facilitate the molecular interaction and penetration of system across skin **(Figure 4)**.[22]

Method of prepration of transfersomes

To prepare vesicles capable of different shape adaptability, the relative concentration of surfactants, which act as membrane- softening and - destabilizing agents (such as cholate or polysorbate), is varied. Two techniques have been generally used to prepare transfersomes, with slight modifications used by individual researchers.

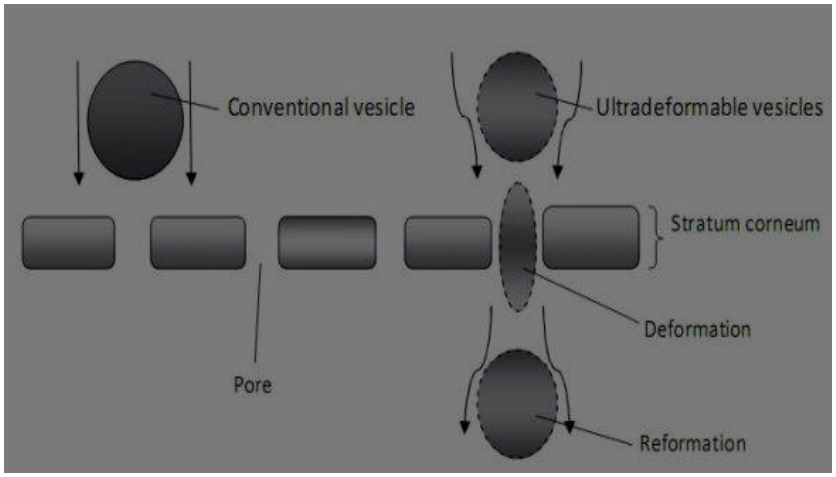

Figure 4: Two microroutes of penetration in biological membranes[23]

- **Vortexing-Sonication Method**

In the vortexing-sonication method, mixed lipids (i.e. phospha-tidylcholine, EA and the therapeutic agent) are blended in a phosphate buffer and vortexed to attain a milky suspension. The suspension is sonicated, followed by extrusion through poly-carbonate membranes 10. Cationic transfersomes have also been prepared by this method, which involves mixing cationic lipids, such as DOTMA, with PBS to obtain a concentration of 10 mg/ml followed by the addition of sodium deoxycholate (SDC). The blend is vortexed and sonicated, followed by extrusion through a polycarbonate (100-nm) filter 11.

- **Rotary Evaporation-Sonication Method**

The rotary evaporation–sonication method involves dissolution of phosphatidylcholine and EA in a blend of chloroform and methanol (2:1, v/v), followed by the removal of organic solvent using rotary evaporation under reduced pressure at 4008C. The film deposited is hydrated with a solution of the therapeutic agent in a suitable aqueous phase while rotating the flask for one hour at room temperature. The vesicles produced are left to swell for two hours at room temperature, followed by 30 min of sonication in a bath sonicator so as to decrease their volume. Extrusion of vesicles then occurs through a sandwich of 450- and 220-nm polycarbonate membranes, with the resulting vesicles being stored at 408°C.[24]

- **Modified hand shaking method**

First, Phospholipid, edge activator and the drug are taken in a clean, dry, round bottom flask, and then lipid mixture was dissolved in 3:1 v/v Chloroform: Methanol. The organic solvent was evaporated until complete dryness by hand shaking method. The deposited lipid film was hydrated with PBS (pH 7.4) by rotation in reverse direction for 1 hr. at room temperature and

the resulting vesicles were swollen for 2 hr. at room temperature to get large multi-lamellar vesicles (LMLV).

Table 1: Different materials used in prepration of transfersomes.[25,21]

Class	Example	Uses
Phospholipids	Egg phosphatidyl choline, soya phosphatidyl choline, dipalmitoylphosphatidyl choline	For formation of vesicles
Surfactant	Tween 80,Span 80,Sodium deoxycholate	Provides flexibility
Alcohol	Ethanol, Methanol	Used as solvent
Buffering agent	Phosphate buffer solution (pH 7.4)	As hydrating agent
Dye	Fluorescein-DHPE Nile red, Rhodamine-123,Rhodamine-DHPE	For CSLM study

Characterization of transfersomes

- **Entrapment Efficiency**

The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the unentrapped drug by use of mini- column centrifugation method (Wearner RR et al., 1988). After centrifugation, the vesicles were disrupted using 0.1% Triton X- 100 or 50% n-propanol. The entrapment efficiency is expressed as: Entrapment efficiency= (amount entrapped/ total amount added) ×100.

- **Vesicle Diameter**

Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements.

- **Vesicle Shape & Type**

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.

- **Number of Vesicle per cubic mm**

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

- **Confocal Scanning Laser Microscopy (CSLM) Study**

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

- **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.

- **Drug Content**

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

- **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.

- **In Vitro Drug Released**

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 320C 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).[26]

- **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 ± 20C (refrigeration), 25 ± 20C (room temp), and 37 ± 20C (body temp) for at least 3 months. Samples from each ampoule were analyzed after30 days to determine drug leakage. Percent drug lose was calculated by keeping the initial entrapment of drug as 100%.[27,28]

CONCLUSION

Transfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems.Transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller.

REFERENCES

1. Payal Khamora, Neelotpal Trivedi, Harish Chandrawanshi; Review on: Development of Novel Herbal System for Rheumatoid Arthritis; Panacea Journal of Pharmacy and Pharmaceutical Sciences, 2014; 2(3): 1-5.

2. Harunusman Patel, Dr. Upendra Patel, Bhavin Bhimani Daslaniya, Ghanshyam Patel; Transdermal drug delivery system as prominent Dosage forms for the highly; International Journal of Pharmaceutical Research and Bio-science, 2012; 1(3): 42- 65.
3. Jain A, Mishra A, Nayak S and Soni V; Transdermal delivery of antihypertensive agents: A tabular update. International Journal of Drug Delivery, 2011; 3: 1-13.
4. Sharma N, Agarwal G, Rana AC, Bhat Z and Kumar D: A Review: Transdermal drug delivery system: A tool for novel drug delivery system. International Journal of Drug Development and Research 2011; 3: 70-84.
5. Patel RP and Baria AH: Formulation and evaluation considerations of transdermal drug delivery system. International Journal of Pharmaceutical Research, 2011; 3: 1-9.
6. Vinod KR, Sarvani P, Banji D and Teja BB: Transdermal drug delivery system over coming challenges of popular drug delivery system. International Journal of Pharma World Research, 2010; 1: 1-14.
7. P.T. Pugliese. Physiology of the Skin, chapter 1, Allured Publishing Corporation, 2001: 1-2.
8. B. Alberts, D. Bray, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. Essential Cell Biology, 1998: 601.
9. Takanori Igarashi, Ko Nishino, and Shree K. Nayar The Appearance of Human Skin, the Skin Care Research Laboratory of Kao Corporation, Japan, 2006: 3-17.
10. W.F. Lever and G.Schaumburg-Lever. Histopathology of the skin. J.B.Lippincott Company, seventh edition edition, 1990.
11. D. Batisse, R. Bazin, T. Baldeweck, B. Querleux, and J.L. Leveque. Influence of age on the wrinkling capacities of skin. Skin Research and Technology, 2002; (8): 148–154.
12. Ting WW, Vest CD, Sontheimer RD Review of traditional and novel modalities that enhance the permeability of local therapeutics across the stratum conium. Int J Dermatol, 2004; 43(7): 538-47.
13. W.F. Lever and G.Schaumburg-Lever. Histopathology of the skin. J.B.Lippincott Company, seventh edition edition, 1990.
14. J.H. Chung. Photoaging in Asians. Photodermatol Photoimmunol. Photomed. 2003; (19): 109–121.
15. J.S. Koh, H. Kang, S.W. Choi, and H.O. Kim. Cigarette smoking associated with premature facial wrinkling: image analysis of facial skin replicas. International Journal of Dermatology, 2004; (41): 21–27.

16. Morrow DIJ, McCarron PA, Woolfson AD and Donnelly RF: Innovative strategies for enhancing topical and transdermal drug delivery. The Open Drug Delivery Journal, 2007; 1: 36-59.
17. Latheeshjlal.L, P. Phanitejaswini, Y.Soujanya, U.Swapna,V.Sarika, G.Moulika, Transdermal Drug Delivery Systems: An Overview International Journal of PharmTech Research, 2011; 3(4): 2140-2148,
18. Kombath RavindranVinod, Minumula Suneel Kumar, Sockalingam Anbazhagan, Subadhra Sandhya, Parre Saikumar, Reddy Tera Rohit, David Banji; Critical issues related to transfersomes – novel vesicular system; Acta Sci. Pol., Technol. Aliment, 2012; 11(1): 67-82.
19. GregorCevc, Dieter Gebauer, Juliane Stieber, Andreas Schatzlein, Gabriele Blume; Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin; Elsevier Science B.V, 1998: 201-215.
20. Gyati Shilakari, Davinder Singh, Abhay Asthana; Novel vesicular carriers for topical drug delivery and their applications; Int. J. Pharm. Sci. Rev, 2013; 21(1): 77-86.
21. Sahil M. Gavali, Sharad S. Pacharane, Kisan R. Jadhav, Vilasrao J. Kadam; Clinical P transfersome: a new technique for transdermal drug delivery; International journal of research in pharmacy and chemistry, 2011; 1(3): 2231-2781.
22. Kumar Ravi ,Manvir Singh,Rajni Bala,Nimrata Seth, A C Rana; Transfersomes :A novel approach for drug delivery; International Research Journal of Pharmacy, 2012;3(1): 20-24.
23. D. Nagasamy Venkatesh, K. Kalyani, K. Tulasi, V. Swetha Priyanka, S. K. Abid Ali, H. C. Kiran; Transfersomes: a novel technique for transdermal drug delivery; International Journal of Research in Pharmaceutical and Nano Sciences 2014; 3(4): 266 - 276.
24. Deepika Singh; Ultradeformable system: a carrier for transdermal drug delivery; International Journal of Pharmaceutical Sciences and Research, 2013; 4(11): 4098-4014.
25. Walve J.R**,** Bakliwal S.R, Rane B.R, Pawar S.P; Transfersomes: a surrogated carrier for transdermal drug delivery system; International Journal of Applied Biology and Pharmaceutical Technology, 2011; 2(1): 204-213.
26. Akshat Sharma, Amit Dubey, Prashant Gupta, Reenu Yadav, Richa Saraogi; Transferosome: novel drug delivery system; International Journal of Biological & Pharmaceutical Research. 2012; 3(5): 722-728.

27. Sheo DM, Shweta A, Vijay KT, Ram CD, Aklavya S, Ghanshyam M; Enhanced Transdermal delivery of indinavir sulfate via transfersomes; Pharmacie Globale (IJCP), 2010; 1 (6): 1-7.
28. Panchagnula R; Transdermal delivery of drugs; Indian Journal Pharmacology, 1997; 29: 140-156.