INTRODUCTION

Since the last few years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs. The word “transfersome” and the underlying concept were introduced in 1991 by Gregor Cevc. The name ‘Transfero’ is derived from the latin word meaning to carry across and the Greek word ‘soma’ for a body. A transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultra deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. These vesicles serve as a depot for the sustained release of active compounds in the case of topical formulations, as well as rate-limiting membrane barrier for the modulation of systemic absorption in the case of transdermal formulations. Transfersomes consist of a phospholipids component along with a surfactant mixture. The ratio of individual surfactants and total amount of surfactants control the flexibility of the vesicle.

The uniqueness of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic to drugs. Transfersomes are applied in a non-occluded method to the skin and have been shown permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. Transfersomes can pass through even tiny pores (100nm) nearly as efficiently as water, which is 1500 times smaller.
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 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.

**Advantages**

1. High entrapment efficiency, for lipophilic drug it is near to 90%.
2. Can encapsulate both hydrophilic and lipophilic moieties.
3. Suitable as a carrier for low as well as high molecular weight drugs e.g., analgesic, corticosteroids, hormones, anticancer drugs, insulin, proteins, etc.
4. Can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.
5. Suitable for both systemic as well as topical delivery of drug.
6. Protect the encapsulated drug from metabolic degradation.
7. Biodegradability and lack of toxicity.

**Limitations**

1. Chemically unstable, highly susceptible to oxidative degradation.
2. Formulations are expensive.

**Mechanism of penetration of transfersomes**

After penetration through the outermost skin layers, transfersomes reach the deeper skin layer. From there, they are normally washed out into the blood circulation. If it is applied under suitable conditions, resulting in access to all body tissues.

![Figure 2: Penetration pathway of transfersomes](image)

The mechanism for penetration includes generation of “osmotic gradient” due to evaporation of water while applying the transfersomes on the skin surface. The transport of these elastic vesicles is thus independent of concentration. This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis. 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). 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.

**Additives and methods for preparation of transfersome**

Transfersomes composed of phospholipids like phosphatidyl choline which self assembles into lipid bilayer in aqueous environment and closes to forma vesicle. A bilayer softening component (such as a biocompatible surfactant or an amphiphile drug) is added to increase lipid bi layer flexibility and permeability. This second component is called as edge activator.

1. **Vortexing-sonication method**

In this 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.

2. **Suspension homogenization process**

In this process, transfersomes are prepared by mixing an ethanolic soybean phosphatidylycholine solution with an appropriate amount of edge-active molecule, e.g. sodium cholate. This prepared suspension is subsequently mixed with Triethanolamine-HCl buffer to yield a total lipid concentration. The resulting suspension is sonicated, frozen, and thawed for 2 to 3 times.

3. **Modified handshaking process**

In this process, the transfersomes are prepared by modified hand shaking, 'lipid film hydration technique'. Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking 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 vaporization of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature.

4. **Aqueous lipid suspension process**

In this process, Drug-to-lipid ratio in the vehicles is fixed between 1/4 and 1/9. Depending upon the particular formulation type, the composition is preferred. This would ensure the high flexibility of the vesicle membrane in comparison to the standard phosphatidylcholine vesicles in the fluid phase. Specifically, vesicles with the size ranging from 100-200 nm are prepared by using soyphosphatidylcholine with the standard deviation of size distribution (around 30%). This formulation could be prepared by suspending the lipids in an aqueous phase wherein the drug is dissolved.

5. **Centrifugation process**

In this process, phospholipids, surfactants and the drug are dissolved in alcohol. Then the solvent is removed by rotary evaporation under reduced pressure at 40°C. Final traces of solvent are removed under vacuum. Then the deposited lipid film is hydrated with the...
appropriate buffer by centrifuging at 60 rpm for 1 hour at room temperature. At room temperature, the resulting vesicles are swollen for 2 hours. The multilamellar lipid vesicles obtained which are further sonicated at room temperature\textsuperscript{16}.

**CHARACTERIZATION AND EVALUATION OF TRANSFERSOMES**

1. **Determination of vesicle diameter**
The vesicle size is one of the key issues during the manufacturing process of transfersomes. It gives important information about the control of the preparation technique and can be utilized for process optimisation. Very small vesicles (smaller than 40 nm) are prone to fusion processes due to the high curvature of their bilayer membrane. It can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method\textsuperscript{17}.

2. **Determination of Vesicle shape and type**
Transfersomes vesicles can be visualized by TEM, Phase contrast microscopy, etc\textsuperscript{18}.

3. **Determination of 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\textsuperscript{18}.

4. **Determination of 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\textsuperscript{18}.

5. **Determination of entrapment efficiency**
The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency can be determined by separating the unentrapped drug. After centrifugation (to separate the unentrapped drug), the vesicle can be ruptured\textsuperscript{19}.

6. **Determination of drug content**
The drug content can be determined using one of the instrumental analytical methods such as modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program depending upon the analytical method of the pharmacopoeial drug\textsuperscript{19}.

7. **Turbidity measurement**
Turbidity of drug in aqueous solution can be measured using nephelometer\textsuperscript{21}.

8. **Surface charge and charge density**
Zetasizer is used to determine surface charge and charge density of transfersomes. Surface charge and Charge density of transfersomes can be determined using Zetasizer\textsuperscript{21}.

9. **Confocal scanning laser microscopy study\textsuperscript{22}**
In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose:

   a) Investigation of the mechanism of penetration of transfersomes across the skin.

   b) Determination of histological organization of the skin, shapes and architecture of the skin penetration pathways.

   c) Comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles.

10. **In-vitro drug release**
*In vitro* drug release study is performed for determining the permeation rate. For determining *in vitro* drug release, beaker method is used in which transfersomes suspension is incubated at 32°C using cellophane membrane and the samples are taken at different times and then detected by various analytical techniques (UV, HPLC, HPTLC) and the free drug is separated by minicolumn centrifugation, then the amount of drug release is calculated\textsuperscript{23}.

**APPLICATION OF TRANSFERSOMES**

1. **Delivery of insulin**
Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transfersomes (transfersulin) overcomes the problems of inconvenience, larger size (making it unsuitable for transdermal delivery using conventional method) along with showing 50% response as compared to subcutaneous injection\textsuperscript{24}.

2. **Delivery of corticosteroids**
Transfersomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutanously administered drug dose. Transfersomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases\textsuperscript{24}.

3. **Delivery of proteins and peptides**
Transfersomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptides are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract and transdermal delivery suffers because of their large size\textsuperscript{25}.

4. **Delivery of anticancer drugs**
Anti cancer drugs like methotrexate were tried for transdermal delivery using transfersome technology. The results were favorable. This provided a new approach for treatment especially of skin cancer\textsuperscript{25}.

5. **Delivery of anesthetics**
Transfersome based formulations of local anesthetics-lidocaine and tetracaine showed permeation equivalent to subcutaneous injections, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transfersomal anesthetics last longer.

6. **Delivery of herbal drugs**
Transfersomes can penetrate stratum corneum and supply the nutrients locally to maintain its functions resulting maintenance of skin\textsuperscript{25}.

**CONCLUSION**

Transdermal drug delivery system has several advantages but there is a major limitation, transportion
of the larger size molecule. That is why vesicular system like transfersomes are developed to overcome these limitations. This carrier system does not depend upon the concentration gradient and mainly works on the principle of hydrotaxis and elasto-mechanics. Transfersomes are highly deployed in the delivery of hormones, proteins, anticancer drugs, anesthetics and antibiotics, drugs with poor penetration due to unfavorable physicochemical characters. All above discussed properties of this technology conclude its good future in transdermal drug delivery.

REFERENCES

1. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Discovery Today: Technologies 2005; 2(1): 67-74. https://doi.org/10.1016/j.dttct.2005.05.003

2. Hoff HEJ, Bouwstra JA, Spies F, Gooris G, Nagelkerke JF. Interaction of liposomes and niosomes with human skin. J Pharm Sci 1994; 83, 1192-1196. https://doi.org/10.1016/0168-7162(94)90378-1

3. Fang YP, Tsai YH, Wu PC, Huang YB. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int J Pharm 2008; 336 (1-2): 44-52. https://doi.org/10.1016/j.ijpharm.2008.01.020

4. Prajapati ST, Patel CG, Patel CN. Transfersomes: A vesicular carrier system for transdermal drug delivery. Asian J Biochem Pharm Res 2011; 2 (1), 507-524. https://doi.org/10.3797/scipharm.1208-02

5. Kombath RV, Minumula SK, Sockalingam A, Subadhra S, Parre S, Reddy TR, David B. Critical issues related to transfersomes- novel Vesicular system. Acta Sci Pol Technol Aliment 2012; 11 (1), 67-82.

6. Walve JR, Bakiwal SR, Rane BR, Pawar SP. Transfersomes: A surrogate carrier for transdermal drug delivery system. Int J App Biol Pharm Tech 2011; 2 (1): 201-214.

7. Modi CD, Bharadia PD. Transfersomes: New dominants for transdermal drug delivery. American J Pharmed Res 2012; 2 (3): 71-91.

8. Sheo DM, Shweta A, Ram CD, Ghanshyam M, Girish K, Sunil KP. Transfersomes- a novel vesicular carrier for enhanced transdermal delivery of stavudine: development, characterization and performance evaluation. J Scientific Spec Res 2010; 1 (30): 30-36. https://doi.org/10.3797/scipharm.1208-02

9. Patel R, Singh SK, Singh S, Sheth NR, Gendle R. Development and characterization of curcumin loaded transfersome for transdermal delivery. J Pharm Sci Res 2009; 1 (4): 71-80.

10. Shivanand P, Manish G, Viral D, Jaira F. Transfersomes: A novel approach for transdermal drug delivery. Scholars Research Library Der Pharmacia Lettre 2009; 1(2): 143-50.

11. Gregor C, Dieter G, Juliane S, Andreas S, Gabriele B. Ultra-flexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biophysica Acta 1998; 1368: 201-215. https://doi.org/10.1016/S0005-2736(97)00177-6

12. Maghraby EI, Williams GM, Barry BW. Skin delivery of oestradiol from lipid vesicles: importance of liposome structure. Int J Pharmaceutics 2000; 204: 1-5, 159-69. https://doi.org/10.1016/S0022-1694(00)00493-2

13. Trota M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. Int J Pharmaceutics 2004; 270: 119. https://doi.org/10.1016/j.ijpharm.2003.10.006

14. Woo HJ, Carraro C, Chandler D. Assembly of extended interfaces and micelles: Charge-frustrated models of amphiphilic mixtures. Faraday Discuss 1996; 104: 183-191. https://doi.org/10.1039/FD9960400183

15. Zhdanov RI, Podobed OV, Vlassov VV. Cationic lipid-DNA complexes-lipoplexes-for gene transfer and therapy. Bioelectrochemistry. 2002; 58: 53. https://doi.org/10.1016/S1879-0481(02)00132-9

16. Strittmatter P, Enoch HG. Formation and properties of 1000 Å-diameter, single-bilayer phospholipid vesicles. Methods Enzymol 1978; 52: 188-193.

17. Sullivan SM, Huang L. Enhanced delivery to target cells by heat-sensitive immunoliposomes. Proc Natl Acad Sci. 1986; 83: 6117. https://doi.org/10.1073/pnas.83.16.6117

18. Lum K, Chandler D, Weeks JD. Hydrophobicity at small and large length scales. J Phys Chem B 1999; 103: 4570-4577. https://doi.org/10.1021/jp984327n

19. Cevc G, Blume G, Schatzelein A. Transfersomes-mediated transdermal delivery improves the regio-specificity and biological activity of corticosteroids in vivo. J Control Release 1997; 45:211-226. https://doi.org/10.1016/S0168-3659(96)01566-0

20. Cevc G, Gebauer D. Hydration-driven transport of deformable lipid vesicles through fine pores and the skin barrier. Biophysical J 2016; 104 (18): 1010-1024. https://doi.org/10.1016/j.bpj.2016.05.032

21. Irfan M, Verma S, Ram A. Preparation and Characterization of Ibuprofen loaded transfersomes as a novel carrier for transdermal drug delivery. Asian J Pharm Clin Res 2012; 5(3): 162-165. https://doi.org/10.3797/scipharm.1208-02

22. Kumar K, Rai AK. Proniosomal formulation of Curcumin having Anti-inflammatory and Anti-arthritis activity in different experimental animal models. Die Pharmazie. 2012; 10:852-857. https://doi.org/10.1691/jph.2012.1164

23. Nanda A, Nanda S, Dhall M, Rao R. Transfersomes-a novel ultra-deformable vesicular carrier for transdermal drug delivery: Transdermal Delivery 2005; 5 (9). https://doi.org/10.4103/2231-0404.65524

24. Doaa A, Dalia R, Sally H and Mohamed N. Formulation and evaluation of fluconazole topical gel. Int J Pharm pharmaceutical sciences 2012; 4: 176-183.

25. Merin P. Eldhose, Flowerlet M, Neethusha J. Transfersomes - a review. Int J Pharm Pharm Sci 2016; 6(4): 436-452.