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Transferosome: Novel Nanovesicular Drug Delivery System

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ABSTRACT

Transferosomes are one of the novel vesicular systems for transdermal delivery of the active substance. They are capable of transdermal delivery of drugs with a low and high molecular weight with the uniqueness of accommodating the hydrophilic, lipophilic, and amphiphilic nature of molecules. These are the second generation of flexible liposomes consisting of phospholipid and edge activator. Advantages of transferosomes are high entrapment efficacy, high deformability, use for both systemic as well as topical, biocompatible, biodegradable, protects the drug from degradation and easy to sale up as procedure of preparation is simple. The system can be characterized by vesicle shape and size, entrapment efficacy, degree of deformability, number of vesicles per cubic mm, drug content, permeability, turbidity measurement, surface charge-density, in-vitro drug release, in vitro skin permeation studies, physical stability, etc. Its application areas included delivery of NSAID, steroids, insulin, interferosomes, interleukin, transdermal immunization, peripheral drug targeting, carrier for other proteins and peptides, etc.

INTRODUCTION:

NDDS is the most suitable drug delivery system in which developing the therapeutic efficacy of preexisting as well as new drugs [Chandrakala et al., 2014. Hadgraft et al., 1989. Gros et al., 1980. Wokovich et al., 1989]. Novel vesicular systems allow the drug to control or sustain the release of conventional medicines [Tyle et al., 2003. El-Maghraby et al., 2009].

Oral drugs are degraded in variable pH conditions of the gastrointestinal tract (GIT). They also experience the first-pass metabolism. In the case of parenteral, disadvantages have lacked drug reversal, risk of infection, hypersensitivity reaction, emboli, and cost. Some oral drugs are bitter, the problem with swallowing and in case of parenteral pain due to needles makes these routes of administration less patient's compliance. Considering these problems, attention has been focused on a more advantageous topical route of administration. The transdermal drug delivery system (TDDS) is used as a potent route for the delivery of systemic action of drugs [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013. Madhumitha et al., 2020].

The vesicular system is getting importance due to its ability to act as a means of sustained release of the drug. A vesicular approach such as liposomes, niosomes, ethosomes, and transferosomes has the potential to overcome the skin barrier and has reported enhancing the permeability of the drug. Transferosomes are ultra deformable vesicles possessing an aqueous core surrounded by a complex lipid bilayer. [shabana et al., 2015. Chorasiya et al., 2019. Rai et al., 2017. Solanki et al., 2016. Zaafarany et al., 2010]. Vesicular systems reduce the cost of therapy by improving the bioavailability of drugs especially poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. Vesicular drug delivery systems reduce toxicity, reduces dose-related side effects, the high therapeutic efficacy of drugs for longer periods [Cevc et al., 1998. Modi et al., 2012]. The encapsulation of the drug in vesicular systems is predicted to prolong the drug in systemic circulation. [Cevc et al., 1998].

The term transferosomes and concept was introduced in 1991 by Gregor Cevc. Transferosomes is a term registered trademark by German company IDEA AG. The name means "carrying body" and is derived from the Latin word ' transferee means 'to carry across' and the Greek word 'soma' meaning 'a body'. Transferosomes are complex vesicles that have a highly flexible and self-regulating membrane which results from the vesicles more deformable [Prajapati et al., 2011. Rajan et al., 2011]. Transfersome is a special type of

liposome. They overcome the skin penetration problem by squeezing themselves along the intracellular sealing lipid of the stratum corneum. It penetrates the stratum corneum by either intracellular or transcellular route by a generation of osmotic gradient due to evaporation of water. Thus, transferosomes vesicles when applied on an open biological surface that is non-occluded skin tends to penetrate its barrier and migrate into the water-rich deeper strata [Piumitali et al., 2020. Fernandez-Garcia et al., 2020. Chouhan et al., 2017].

Flexible or elasticity of transferosomes membrane is achieved by mixing suitable edge activator (surface active component) in proper ratio. When applied on the skin, it exploits a hydrophilic pathway or pores between the cells, where it opens wide enough to permit the entire vesicle to pass through the stratum corneum along with the drug molecule. Transferosomes vesicles can cross microporous barrier efficiently, even if the porous are much smaller than vesicle size. Thus transferosomes carrier is an artificial vesicle designed to be like a cell vesicle and thus suitable for controlled and potentially targeted drug delivery [Venkatesh et al., 2014. Jain et al., 2003.Walve et al., 2011. Vijayalaxmi et al., 2015].

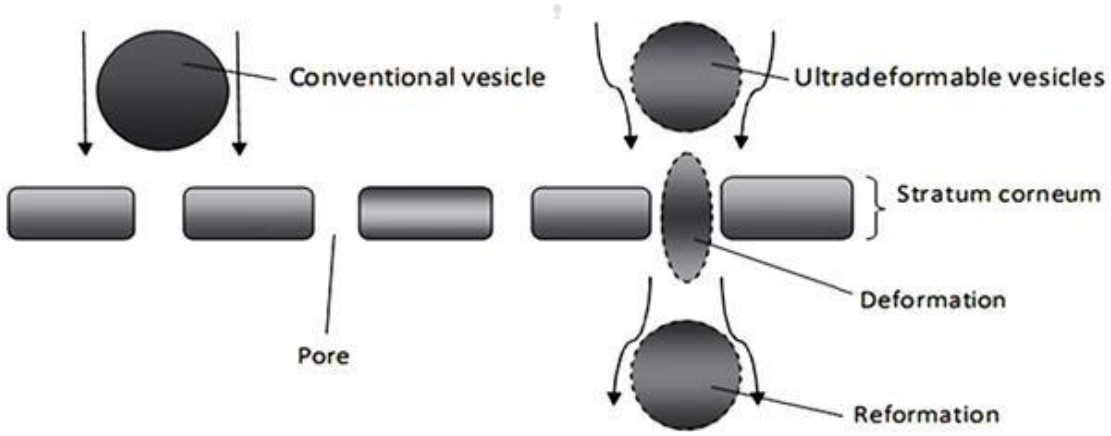

Figure No. 1: Mechanism of action of Transferosmes

Advantages: [Chourasiya et al., 2019. Reddy et al., 2015.]

- Delivers a study infusion of the drug over an extended period.
- Can act as a carrier for low as well as high molecular weight drugs.
- Biocompatible and biodegradable as they are made up of natural phospholipid.
- High deformability gives better penetration of intact vesicle
- High entrapment efficacy

- Release their content slowly and gradually and can be used for systemic and topical delivery of the drug.
- More flexible and adaptable compared with liposomes.
- Can accommodate hydrophilic, hydrophobic as well as amphiphilic drug molecule.
- Protect the encapsulated drug from metabolic degradation.
- Easy to scale up, as the procedure is simple.

Disadvantage: [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013. Madhumitha et al., 2020]

- Drugs that required high blood levels cannot be administered.
- The barrier function of the skin changes from one site to another on the same person, from person to person, and also with age.
- The drug must be potent because patch size limits the amount that can be delivered.
- Skin irritation or hypersensitivity reaction may occur.
- Transferosomes are chemically unstable because oxidative degradation make it predisposition.
- The purity of natural phospholipids is another criteria.
- Transferosomes formulations are expensive.

Mechanism of transport: [Chourasiya et al., 2019. Natsheh et al., 2020. Reddy et al., 2015. Eldhouse et al., 2016. Sachan et al., 2013. Madhumitha et al., 2020]

At present, the mechanism of enhancing the delivery of active substances in and across the skin is not very well known. Proposed mechanisms are

- Transferosomes act as drug vectors.
- Transferosomes acts as a penetration enhancer, disrupting the highly organized intracellular lipids.

The mechanism is complex and involved an advanced principle of mechanism combined with material transport and hydration/ osmotic force.

Material for transferosomes: [Sharma et al., 2015. Jadupati et al., 2012. Subhash chandran et al., 2018.Kumar et al., 2015]

Transferosomes composed of phospholipid like phosphatidylcholine which self assembles into lipid bilayer in an aqueous environment and closes to form vesicles. A biocompatible surfactant or an amphiphilic drug is added to increase bi-layer flexibility and permeability. This second component is called an edge activator.

Edge activator consists of single-chain surfactant that causes destabilization of lipid bilayer increasing its elasticity and fluidity.

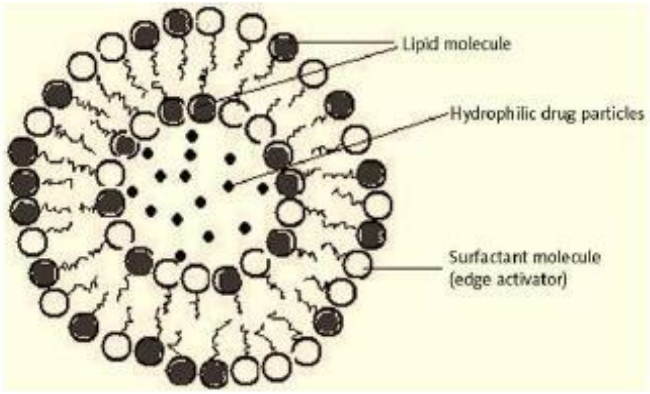

Figure No. 2: Structure of Transferosmes

Table No. 1: Composition of Transferosomes

Class	Example	Uses
Phospholipids	Soya phosphatidylcholine Dipalmitoyl phosphatidylcholine, disteroyl phosphatidylcholine	Vesicle forming agents
Surfactant	Sodium cholate, sodium deoxycholate, tween 80, span 80	Flexibility
Alcohol	Ethanol, methanol	solvent
Buffering agents	Saline phosphate buffer pH(6.4)	Hydrating medium
Dyes	Rhodamine-123, rhodamine DHPE, flourescein DHPE	CSLM study

A. **Thin film hydration technique** is employed for the preparation of transfersomes which are comprised of three steps: [Chourasiya et al., 2019. Reddy et al., 2015. Rajan et al., 2011]

1. A thin film is prepared from the mixture of phospholipids and surfactant by dissolving in volatile organic solvent (chloroform-methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 500C for dipalmitoylphosphatidylcholine) using a rotary evaporator. Final traces of solvent were removed under vacuum overnight.

2. A prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hrs. at room temperature.

3. To prepare small vesicles, the resulting vesicles were sonicated at room temperature or 50°C for 30 min. using a bath sonicator or probe sonicated at 4°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

B. Modified handshaking, lipid film hydration technique is also founded for the preparation of transfersomes which comprised following steps: : [Chourasiya et al., 2019. Reddy et al., 2015. Rajan et al., 2011]

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

2. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minutes at the corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8°C.

Characterization of Transferosomes: [Thakur et al., 2018. Sharma et al., 2012. Ali et al., 2020. Pena-Rodriguez et al., 2020]

1. Entrapment efficiency: [Gamal et al., 1999. Malakar et al., 2012]

The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by the first separation of the un-entrapped drug by use of the 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:

Entrapment efficiency = amount entrapped * 100 / total amount added

2. Drug content: [Fry et al., 1978]

The drug content can be determined using a modified high-performance liquid chromatography method (HPLC) method with help of a UV detector, column oven, auto sample, pump, and computerized analysis program depending upon the analytical method of the pharmacopoeial drug.

3. Vesicle morphology: [Subhash Chandran et al., 2018. Modi et al., 2012]

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 is 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 the 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.

4. Vesicle size distribution and zeta potential: [Subhash Chandran et al., 2018. Modi et al., 2012]

Vesicle size, size distribution, and zeta potential were determined by the Dynamic Light Scattering Method (DLS) using a computerized inspection system by Malvern Zetasizer.

5. No. of vesicles per cubic mm: [Gamal et al., 1999. Malakar et al., 2012]

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 the 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

6. Degree of deformability or permeability measurement: [Fry et al., 1978]

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.

7. Turbidity measurement: [Fry et al., 1978. Walb et al., 2009]

The turbidity of the drug in an aqueous solution can be measured using a nephelometer.

8. Surface charge and charge density: [Malakar et al., 2012]

The surface charge and charge density of transfersomes can be determined using a zeta sizer.

9. Penetration ability: [Malakar et al., 2012]

The penetration ability of Transfersomes can be evaluated using fluorescence microscopy.

10. Occlusion effect: [Walb et al., 2009]

Occlusion of skin is considered to be helpful for the permeation of the drug in the case of traditional topical preparations. Hydrotaxis (movement in the direction) of water is the major driving force for the permeation of vesicles through the skin, from its relatively dry surface to water-rich deeper regions. Occlusion affects hydration forces as it prevents the evaporation of water from the skin.

11. Physical stability: [Walb et al., 2009]

The initial percentage of the drug entrapped in the formulation was determined and was stored in sealed glass ampoules. The ampoules were placed at 4 ± 2^0C (refrigeration), 25 ± 2^0C (room temp), and 37 ± 2^0C (body temp) for at least 3 months. Samples from each ampoule were analyzed after 30 days to determine drug leakage. Percent drug loss was calculated by keeping the initial entrapment of the drug as 100%.

12. *In-vitro* drug release: [Gamal et al., 1999. Subhash chandran et al., 2018]

In vitro drug release study is performed for determining the permeation rate. 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).

13. *In-vitro* Skin permeation Studies: [Sheo et al., 2010. Patel et al., 2009.]

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 goatskin in phosphate buffer solution (pH 7.4). The fresh Abdominal skin of the goat was collected from the 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. The skin was kept in an isopropyl alcohol solution and stored at 0-4^{0}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 the donor compartment exposed to the receptor compartment was 2.50cm^2 and the capacity of the receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37± 0.5^{0}C and stirred by a magnetic bar at 100RPM. Formulation (equivalent to 10mg drug) was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals, 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in the calculation of the release profile. The samples were analyzed by any instrumental analytical technique.

Application of transferosomes:

1. Delivery of

- large molecule/drugs: Interferon, Insulin [pandey et al., 2009]

- Anticancer drug[Hasibi et al., 2019. Rai et al., 2017]:Methotrexate [Sadarani et al., 2019], paclitaxel[Khan et al., 2019], 5-FU[Zhang et al., 2015], Toxifolin [Hasibi et al., 2019].

- Herbal drugs[Rose et al., 2016. Sarwa et al., 2016. Anwar et al., 2017. Zesioreani et al., 2017. Pavaloiy et al., 2020.]: Capsaicin

- NSAIDS : Acceclofenac [dudhipala et al., 2020]. Ketorolac [Prakash et al., 2019. Zhang et al., 2020].
- Anaesthetic[Omar et al., 2019. Bnyana et al., 2019]: Lidocaine[Omar et al., 2019]
- Anti-oxidant: resveratrol [Wu et al., 2019]
- Antidiabetic: Repaglinide [Vijayalaxmi et al., 2015]
- Antifungal: Flucanzole [tejaswini et al., 2016], Tacrolimus[Lei et al., 2013], Natamycin[Janga et al., 2019]
- Osteoporosis treatment: Roloxifen [Ashlesha et al., 2020. Mahmood et al., 2018]
- Sexual dysfunction: Sildenafil [Sayyad et al., 2017]
- Hypretension: Eprosartan[Ahad et al., 2017]
- Anti-leishmaniasis: Miltefosine [Dar et al., 2020]
- Antiviral: Lamivudine [Sudhakar et al., 2016], indinavir [Sheo et al., 2010]
- Ocular Delivery: aetazolamide[Eman et al., 2020]

2. **Carrier for**

- Interferon and interleukin ex. IL-2, INF-alpha [Natsheh et al., 2020]
- Protein and peptides ex. Human serum albumin [Zhenga et al., 2020. Celia et al., 2012. Kala et al., 2014]

3. **Transdermal immunization** ex. Hepatitis B vaccine[Gupta et al., 2010]

4. **Peripheral drug targeting** [Natsheh et al., 2020]

CONCLUSION:

The transdermal route of drug administration is not allowing the transport of high molecular weight drugs and therapeutic agents. Transferosomes are designed in such a way that they can squeeze themselves through skin pores irrespective of molecular weight. Transferosomes which are ultra deformable vesicles allows enhanced permeation of drug through the skin.

The composition of transferosomes vesicle is safe, advantageous with fewer demerits. These ensure reproducible and efficient transcutaneous carrier and drug target. Transferosomes accommodate drug molecules with a wide range of solubility. Transferosomes, thus differ from other vesicles by their softer, more deformable, better adjustable artificial membrane.

REFERENCES:

1. Ali, S., Gudipati, M., Nadendla, R. 2020. Current status and prospects of transfersomal drug delivery. *Indo American Journal of Pharmaceutical Sciences*, 07(01):1343-1350.
2. Ahad, A., Al-Saleh, A., Al-Mohizea, A., Al-Jenoobi, F., Raish, M., Yassin, A., Alam, A. 2017. Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of Eprosartan Mesylate. *Saudi Pharmaceutical Journal*, 25:1040–1046.
3. Anwar, E., Utami, T., Ramadon, D. 2017. Transfersomal gel containing green tea (Camellia sinensis L. Kuntze) leaves extract: increasing in vitro penetration. *Asian Journal of Pharmaceutical and Clinical Research*, 10(8):294-298.
4. Bnyana, R., Khana, I., Ehtezazia, T., Saleema, I., Gordona, S., O'Neillb, F., Roberts, M. 2019. Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic. *Journal of Pharmacy and Pharmacology*, 71 (10):1-12.
5. Chauhan, N., Kumar, K., Pant, N. 2017. An updated review on transfersomes: a novel vesicular system for transdermal drug delivery. *Universal Journal of Pharmaceutical Research*, 2(4):42-45.
6. Chaurasiya, P., Ganju, E., Upmanyu, N., Ray S., Jain, P. 2019. Transfersomes: a novel technique for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics*, 9(1):279-285.
7. Celia, C., Cilurzo, F., Trapasso, E., Cosco, D., Fresta, M., Paolino, D. 2011. Ethosomes® and transfersomes® containing linoleic acid: physicochemical and technological features of topical drug delivery carriers for the potential treatment of melasma disorders. *Biomedical Microdevices*, 14:119–130.
8. Cevc, G. 1998. Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance ant transport therapeutic amounts of insulin across the intact mammalian skin. *Biochemistry and Biophysics Acta*, 1368(2):01-15.
9. Chandran, S., Jaghatha, T., Wesley, J., Remya, S., Aparna, P. 2018. A review on transfersomes. *Indo American Journal of Pharmaceutical Sciences*, 05(04):2405-2411.
10. Chorasiya, L., Singh, S., Arora, K., Saxena, C. 2019. Transferosomes: A suitable delivery system for percutaneus administration. *Current Research in Pharmaceutical Sciences*, 09 (01):1-11.
11. Dar, M., McElroy, C., Khan, M., Satoskar, A., Khan, G. 2020. Development and evaluation of novel Miltefosine polyphenol co-loaded second-generation nanotransfersomes for the topical treatment of cutaneous leishmaniasis. *Expert Opinion on Drug Delivery*, 17(1):97-110.
12. De Rose, R., Cristiano, M., Celano, M., Maggisano, V., Vero, A., Lombardo, G., Di Francesco, M., Paolino, D., Russo, D., Cosco, D. 2016. PDE5 inhibitors-loaded nanovesicles: Physico-chemical properties and in vitro antiproliferative activity. *Nanomaterials*, 6:E92.
13. Dudhipala, N., Mohammed, R., Youssef, A., Banala, N. 2020. Effect of lipid and edge activator concentration on development of Aceclofenac-loaded transfersomes gel for transdermal application: in vitro and ex-vivo skin permeation. *Drug Development and Industrial Pharmacy*, 46(8):1-11.
14. El-Maghraby, GM., Williams, A. 2009. Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin. *Expert Opinion on Drug Delivery*, 6(2):149-63.
15. Eldhose, M., Mathew, F., Mathew, N. Transfersomes-A Review. *International Journal of Pharmacy and Pharmaceutical Research*, 6(4):436- 452.
16. Fernández-García, R., Lalatsa, A., Stats, L., Francisco Bolás-Fernández, M., Ballesterosa, P., Serrano, D. 2020. Transferosomes as nanocarriers for drugs across the skin: Quality by design from lab to industrial scale. *International Journal of Pharmaceutics*, 573:118817.

17. Fry, D., White, J., Goldman, I. 1978. Rapid separation of low molecular solutes from liposomes without dilution. *Journal of Analytical Biochemistry*, 90:809-815.
18. Gros, L., Clark, W. 1980. The structure of skin. *The tissue of the body, 6th edition. London: ELBS and Oxford university press*, 296-313.
19. Gupta, N., Singh, P., Rawat, A., Dubey, P., Mahor, S., Vyas, S. 2010. Tetanus toxoid-loaded transfersomes for topical immunization. *Journal of Pharmacy and Pharmacology*, 57:295-301.
20. Hadgraft, J., Guy, R. 1989. Transdermal drug delivery. *Marcel Dekker, Inc., New York and Basel*, 35:296.
21. Hasibi, F., Nasirpour, A., Varshosaz, J., García-Manrique, P., Carmen Blanco-López, M., Gutiérrez, G., Matos, M. 2019. Formulation and Characterization of Taxifolin-Loaded Lipid Nanovesicles (Liposomes, Niosomes, and Transfersomes) for Beverage Fortification. *European Journal of Lipid Science and Technology*, 1900105:1-13.
22. Jadupati, M., Amites, G., Kumar, N. 2012. Transferosomes: an opportunistic carrier for transdermal drug delivery system. *International Journal of Pharmacy,* 3(3):35-38.
23. Janga, K., Tatke, A., Dudhipala, N., Balguri, S., Ibrahim, M., Maria, D., Jablonski, M., Majumdar, S. 2019. Gellan gum based sol-to-gel transforming system of natamycin transfersomes improves topical ocular delivery. *Journal of Pharmacology and Experimental Therapeutics*, 370(3):814-822.
24. Jain, S., Jain, P., Umamaheshwari, R., Jain, N. 2003. Transfersomes-A Novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. *Drug Development and Industrial Pharmacy,* 29:1013–1026.
25. Khan, I., Apostolou, M., Bnyan, R., Houacine, C., Elhissi, A., Yousaf, S. 2019. Paclitaxel-loaded Micro or Nano Transfersome Formulation into Novel Tablets for Pulmonary Drug Delivery via Nebulization. *International Journal of Pharmaceutics*, 19:1-28.
26. Kala, C., Firoz, S. 2014. A review on tranferosomes drug delivery systems. *Journal of Global Trends in Pharmaceutical Sciences*, 5(4):2118-2127.
27. Laxmi, V., Zafaruddin, M., Kuchana, V. 2015. Design and characterization of transferosomal gel of Repaglinide. *International Research Journal of Pharmacy*, 6(1):38-42.
28. Lei, W., Yu, C., Lin, H., Zhou, X. 2013. Development of Tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement in vivo. *Asian Journal of Pharmaceutical Sciences*, 8(6):336-345.
29. Mahmood, S., Chatterjee, B., Mandal, U. 2018. Nano Transfersomes vesicles of Raloxifene Hcl with Sorbitan-80: formulation and characterization. *Bioequivalence & Bioavailability International Journal*, 2(1);1-7.
30. Malakar, J., Sen S., Sen, K., Nayak, K. 2012. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. *Saudi Pharmaceutical Journal*, 20:355–363.
31. Mazyed, E., Abdelaziz, A. 2020. Fabrication of transgelosomes for enhancing the ocular delivery of Acetazolamide: statistical optimization, in vitro characterization and in vivo study. *Pharmaceutics*, 12:465.
32. Madhumitha, V., Sangeetha, S. 2020. Transfersomes: A Novel Vesicular Drug Delivery System for Enhanced Permeation through Skin. *Research Journal of Pharmacy and Technology*, 13(5):2020.
33. Modi, C., Bharadia, P. 2012. Transferosomes: New Dominants for Transdermal Drug Delivery. *American Journal of Pharmatechnology Research*, 2(3):71-91.
34. Natsheh, H., Touitou, E. 2020. Phospholipid vesicles for Dermal/Transdermal and nasal administration of active molecules: The effect of surfactants and alcohols on the fluidity of their lipid bilayers and Penetration Enhancement Properties. *Molecules*, 25:2959.
35. Omar, M., Hasan, O., Sisi, A. 2019. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. *International Journal of Nanomedicine*, 14:1551-1562.
36. Prajapati, S., Patel, C., Patel, C. 2011. Transfersomes: a vesicular carrier system for transdermal drug delivery. *Asian Journal of Biomedical and Pharmaceutical Science*, 2(1):507-524.
37. Piumitali, B., Neeraj, U., Jyotivardhan, J. 2020. Transfersomes- a nanoscience in transdermal drugs delivery and its clinical advancements. *International Journal of Nanoscience*, 19(04):1950033.
38. Pena-Rodríguez, E., Moreno, M., Blanco-Fernandez, B., González, J., Fernández-Campos, F. 2020. Epidermal delivery of retinyl palmitate loaded transfersomes: penetration and biodistribution studies. *Pharmaceutics*, 12:112.

39. Pavaloiu, R., Shawkat, F., Bubueanu, C., Deaconu, M., Neagu, G., Shawkat, M., Anastasescu, M., Mihailescu, M., Matei, C., Nechifor, G., Berger, D. 2020. Polyphenolic extract from *Sambucus Ebulus L*. leaves free and loaded into lipid vesicles. *Nanomaterials*, 10:56;1-17.
40. Prakash, A., Pramod, K. 2019. The effect of Transferosomes on Skin Permeation of KetorolacTromethamine. *Advances in Pharmaceutical Research*, 1-13.
41. Patel, R., Singh, S., Singh, S., Sheth, N., Gendle, R. 2009. Development and characterization of Curcumim loaded transfersome for transdermal delivery. *Journal of Pharmarmaceutical Sciences & Research*, 1(4):71-80.
42. Pandit, A., Omase, S., Mute, V. 2020. A chitosan film containing quercetin-loaded transfersomes for treatment of secondary osteoporosis. *Drug Delivery and Translational Research*, 10(5):1495-1506.
43. Pandey, S., Goyani, M., Devmurari, V., Fakir, J. 2009. Transferosomes: A novel approach for transdermal drug delivery. *Der Pharmacia Letter*, 1(2):143-150.
44. Ravi, K., Singh, M., Bala, R., Seth, N., Rana, A. 2012. Transferosomes: A novel approach for transdermal drug delivery. *International Research Journal of Pharmacy*, 3(1): 20-24.
45. Reddy, Y., Sarvani, A., Ravishankar, V., Prakash, P., Ram Reddy, Y., Vijaya Bhaskar, N. 2015. Transferosomes-A novel vesicular carrier for transdermal drug delivery system. *Journal of Innovation in Pharmaceutical And Biological Sciences*, 2(2):193-208.
46. Rai, S., Pandey, V., Rai, G. 2017. Transfersomes as versatile and flexible nano-vesicular carriers in skin cancer therapy: the state of the art. *Nano Reviews & Experiments*,1-18.
47. Rajan, R., Jose, S., Mukund, V., Vasudevan, D. 2011. Transferosomes-A vesicular transdermal delivery system for enhanced drug permeation. *Journal of Advanced Pharmaceutical Technology & Research*, 2:138.
48. Sharma, S., Nautiyal, U. 2015. Transfersomes: novel approach for transdermal delivery. *European Journal of Pharmaceutical and Medical Research*, 2(3):218-233.
49. Sayyad, M., Zaky, A., Samy, A. 2017. Fabrication and characterization of Sildenafil citrate loaded transfersomes as a carrier for transdermal drug delivery. *Pharmacy & Pharmacology International Journal*, 5(2):37-46.
50. , Transfersomes And Ethosomes Bearing Lamivudine. *International Journal of Pharmaceutical Sciences and Research*, 7(10):4214-4221.
51. Sachan, R., Parashar, T., Singh, V., Singh, G., Tyagi, S., Patel, C., Gupta, A. 2013. Drug Carrier Transferosomes: A novel tool for transdermal drug delivery systems. *International Journal of Research and Development in Pharmacy and Life Sciences*, 2:309-316.
52. Shabana, A., Sailaja, K. 2015. Transfersomes-A novel approach in design of transdermal drug delivery system. *International Journal of Pharmacy and Chemical Research*,1(4):173-178.
53. Solanki, D., Kushwah, L., Motivale, M., Chouhan, V. 2016. Transferosomes-A Review. *World Journal of Pharmacy and Pharmaceutical Sciences*, 10(5): 435-449.
54. Sarwa, K., Mazumder, B., Rudrapal, M., Verma, V. 2015. Potential of Capsaicin-loaded transfersomes in arthritic rats. *Drug Delivery*, 22(5):638-46.
55. Sheo, D., Shweta, A., Ram, C., Ghanshyam, M., Girish, K., Sunil, K. 2010. Transfersomes- a novel vesicular carrier for enhanced transdermal delivery of stavudine: development, characterization and performance evaluation. *Journal of Scientific Speculations and Research*, 1(1): 30-36.
56. Sadarani, B., Majumdar, A., Paradkar, S., Mohanty, B., Chaudhari, P. 2019. Enhanced skin permeation of Methotrexate from penetration enhancer containing vesicles: In vitro optimization and in vivo evaluation. *Biomedicine & Pharmacotherapy*, 114:108770.
57. Sharma, A., Dubey, A., Gupta, P., Yadav, R., Saraogi, R. 2012. Transferosomes: Novel Drug Delivery System. *International Journal of Biological & Pharmaceutical Research*, 3(5):722-728.
58. Tejaswini, K., Swapna, S., Babu, A., Bakshi, V. 2016. Formulation and evaluation of fluconazole loaded transfersome gel. *International Journal of Science and Research Methodology*, 3(3):1-14.
59. Tyle, P. 2003. Drug delivery device, 3rd edition, Newyork and basel, Marcel Dekker, 13-18.
60. Trommer, H., Neubert R. 2006. Overcoming the stratum corneum, The modulation of skin penetration. *A review, Skin Pharmacology and Physiology*, 19(2): 06-21.
61. Thakur, N., Jain, P., Jain, V. 2018. Formulation development and evaluation of transferosomal gel. *Journal of Drug Delivery and Therapeutics*, 8(5):168-177.

62. Venkatesh, D., Kalyani, K., Tulasi, K., Priyanka, V., Ali, S., Kiran, H. 2014. Transferosomes: A novel technique for transdermal drug delivery. *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(4): 266-276.
63. Wokovich, A., Prodduturi, S., Doub, W., Hussain, A., Buhse, L. 1989. Transdermal drug delivery systems (TDDS) adhesion as a critical safety, efficacy and quality attribute. *European Journal of Pharmaceutics And Biopharmaceutics*, 64:1-8.
64. Walve, J., Bakliwal, S., Rane, B., Pawar, S. 2011. Transfersomes: A surrogated carrier for transdermal drug delivery system. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(1):201-214.
65. Walb, J., Bakliwalm, S., Rane, B., Pawar, S. 2009. Transferosomes A surrogated carrier for transdermal drug delivery. *Der Pharmacia Letter*, 1(2):143-150.
66. Wu, P., Li, Y., Kuo, Y., Tsai, S., Lin, C. 2019. Preparation and evaluation of novel transfersomes combined with the natural antioxidant Resveratrol. *Molecules*, 24:1-12.
67. Zhang, Z., Wang, X., Chen, X., Wo, Y., Zhang, Y., Biskup, E. 2015. 5-Fluorouracil-Loaded Transfersome as Theranostics in Dermal Tumor of Hypertrophic Scar Tissue. *Journal of Nanomaterials*, 10:1-9.
68. Zesiorani, N., Anwar, E. 2017. Transfersome gel formulation of an ethanol extracts of apples (malus domestica mill) containing antioxidants and in vitro penetration testing using Franz Diffusion cells. *International Journal of Applied Pharmaceutics*, 9(1):32-37.
69. Zhang, J., Froelich, A., Michniak-Kohn, B. 2020. Topical delivery of Meloxicam using liposome and microemulsion formulation approaches. *Pharmaceutics*, 12:282.
70. Zaafarany, E., Awad G., Holayel S., Mortada, N. 2010. Role of edge activators and surface charge in developing ultra deformable vesicles with enhanced skin delivery. *International Journal of Pharmacy*, 397: 164-72.
71. Zhenga, H., Xua, C., Feia, Y., Wanga, J., Yangb, M, Fangc, L., Weia, Y., Mua, C., Shenga, Y., Lia, F., Zhua, J., Taoa, C. 2020. Monoterpenes-containing PEGylated transfersomes for enhancing joint cavity drug delivery evidenced by CLSM and double-sited microdialysis. *Materials Science & Engineering C*, 113:110929.