Review Article

Transfersome: a vesicular drug delivery with enhanced permeation

Nurul Arfiyanti Yusuf[1,2], Marline Abdassah[1], Rachmat Mauludin[3], I Made Joni[4], Anis Yohana Chaerunisaa[1]*

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. [2]Department of Pharmaceutics, Sekolah Tinggi Ilmu Farmasi Makassar, Makassar, Indonesia. [3]Department of Pharmaceutics, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia. [4]Department of Physics, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, Indonesia.

Correspondence: Anis Yohana Chaerunisaa, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Jatinangor, Indonesia. anis.yohana.chaerunisaa@unpad.ac.id

ABSTRACT

Transdermal drug delivery offers numerous advantages in comparison to conventional delivery methods. However, the stratum corneum as the first layer has been the limiting barrier to some drugs so that no compilation permeable is applied to the skin. Permeation enhancers are now a solution for limitation in transdermal delivery systems, which are available as chemical or physically permeation enhancers. But sometimes these systems still have skin safety issues. Vesicle is a system that delivers hydrophilic and hydrophobic compounds and macromolecular drugs as well as targeted delivery to organs. Transfersome is an elastic vesicle with the capability of deforming its molecule while penetrating into the skin, which is effective in increasing the penetration of drugs with high and low molecular weights. Transfersomes are formed from phospholipids and surfactants known as edge activators. The flexibility of transfersome is affected by the ratio of surfactant and phospholipid. Transfersome has been prepared by various methods including high-pressure homogenization, reverse-phase evaporation, thin-film dispersion, sonication/vortex, and ethanol injection. Evaluation of transfersome included physical and chemical characterization, as well as permeation evaluation in both ex vivo and in vitro studies. In conclusion: Vesicular transfersome is a system that offers an increased effect of drug permeation into the skin with deforming capabilities due to the presence of edge activators. The surfactant and phospholipid ratio determines the ability of the system in enhancing drug permeation.

Keywords: Transdermal, Vesicular, Transfersome, Permeation

Introduction

The permeation of the drug into the systemic circulation across the skin is allowed through Transdermal delivery, thereby avoiding the first-pass effects of the liver during oral administration. The limitation of this administration is skin permeability to hydrophilic and lipophilic macromolecules and molecules [1]. Drug penetration in the skin can be disrupted by a barrier called the stratum corneum. The stratum corneum makes delivery not achieve the expected effectiveness.

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Yusuf NA, Abdassah M, Mauludin R, Joni IM, Chaerunisaa AY. Transfersome: a vesicular drug delivery with enhanced permeation. J Adv Pharm Educ Res. 2021;11(3):48-57. https://doi.org/10.51847/vrYnt7vHhp

Various methods are utilized for increasing the permeation of transdermal administration. In chemical methods, the use of permeation enhancers such as polyalcohol, pyrrolidone, fatty acids, terpenes, surfactants, etc. is applied . The physical method is also used through the skin by involving the disruption of the skin structure by iontophoresis, sonophoresis, or electroporation, ultrasound, microneedle. However, this physical method will affect skin safety for long-term use [1].

Vesicle is the most widely accepted formulation technique for transdermal drug delivery that increases hydrophilic and lipophilic permeation. In this system, macromolecular drugs act as drug carriers and provide controlled and sustainable drug delivery with safety for the skin due to biodegradable materials of macromolecules. Various types of vesicle delivery are being developed at this time such as liposome, ethosome, invasome, and ultra vesicle, namely transfersome, which can be deformed [2].

The new vesicular derivative "Transfersome" offers the ability to increase the permeation of some drugs. They are artificial vesicles designed to be involved in exocytosis. They are suitable for controlled or targeted drug delivery. It has been proposed as a new class of liposomes, which is also known as an elastic liposome or ultra-deformed liposome that can increase liposome penetration. Several studies have reported that transfersome is an effective carrier for non-invasive transdermal administration. Transfersome vesicles can penetrate through pores smaller than their diameter through the stratum corneum [3].

The most outer layer of the body that provides many functions like protection, homeostasis, and barrier to the external environment is the skin. The stratum corneum composed of 70-80% protein keratine and lipid, provides the principal barrier function of the skin [4]. The barrier of chemicals and biochemistry consists of macrophages, antimicrobial peptides, hydrolytic enzymes, acids, and lipids. Immunological barriers consist of cellular and humoral constituents of the immune system [5]. Stratum corneum is a barrier of the permeation rate from transdermal drug delivery, with inter-cell space composed of an exclusive lipid mixture [6]. The stratum corneum as the main barrier in transdermal administration has the obstructive property, making the drug delivery for medical use to be controlled.

Enhancer permeation

Physical enhancers: Iontophoresis is a non-invasive permeation technology that physically facilitates transdermal delivery of hydrophilic and ionic compounds across intact skin [7]. The ultrasound method interacts with the structural lipids present in the intercellular channels of the stratum corneum, which is similar to the postulated impacts of some chemical transdermal enhancers, which act through disrupting lipids. The summary of the method, including its advantages and disadvantages is shown in **Table 1**. The mechanisms of enhancers are different. Mostly, the common goal of the enhancers is disrupting the stratum corneum structure and creating "pores" that are large enough for the passage of molecules. The size of disruption generally is on the nanometer scale, to allow the delivery of small drugs. In some cases, macromolecules is also possible with the risk of causing some clinical damages.

Table 1. Summary of Enhanced Permeation Transdermal Delivery

Methods	Advantages	Limitation
Chemical enhancer	Increases penetration through the skin; gives systemic and local effects [8]	Only for drugs with low molecular weight; skin irritation Immunogenicity [8]
Iontophoresis	Increasing the penetration of drug of intermediate size [7]	Low transfer efficiency; only for charged drugs [8]
Ultrasound	At low frequencies, increase skin permeability	does not induce the transport of high molecular weight proteins [9].
Microneedle	Permit the transfer of microparticles, supramolecular complexes, and macromolecules	The safety evaluation should be done due to minor skin abrasions that possibly occur in daily life
Vesicle Delivery	Improving the delivery of hydrophilic, poorly soluble, and macromolecular drugs; ability to target organ for drug delivery, biodegradable, lack toxicity [7]	Need special handling to maintain stability, the price is relatively expensive [7]

Vesicle delivery system

Vesicular delivery is one of the most widely used methods in the formulation of a delivery system through which vesicle skin can act as a drug carrier to deliver drug molecules that are trapped into the skin. These vesicles act as permeation enhancers following the permeation of intercellular lipid lamellae in the skin layer. Types of vesicle delivery system as well as its limitation and advantage are shown in **Table 2**.

Various literature shows the benefits of the vesicular system in increasing drug permeation. Conventional liposomes are limited to the upper surface with nominal penetration into the stratum corneum. Impaired lipid structure of the intercellular stratum corneum by phospholipids also increases drug permeation. Phospholipids are a group of lipids, which tend to form lipid bilayers due to their amphiphilic properties. Different flexible carrier systems such as ethosome, liposome, transfersome, niosome, flexisome, and aquasome were developed and successfully incorporated into the delivery system of skin for various therapeutic agents such as anticancers but some of the structural lipid carriers have been modified.

Table 2. Summary of Vesicle Delivery

Type of Vesicle Delivery	Composition Vesicle	Advantages	Limitation
Liposome	Phospholipid + Lipid	Phospholipid vesicle, biocompatible, biodegradable	Less skin permeation, less stable [10]
Niosome	Nonionic surfactant + Lipids	Non-ionic surfactants vesicles, more stability	Less skin permeation easy to handle
Ethosome	Phospholipid + Ethanol (up to 50%)	High permeation enhancer [11]	Skin irritation [10]
Invasome	Phospholipid + Ethanol + Terpene	High permeation enhancer than ethosome [12]	Less skin permeation than transfersome [12]
Transfersome	Phospolipid + Edge Activators	Suitable for hydrophilic and lipophilic drugs; proper for drugs with either low and high molecular weight; biodegradable; high skin penetration due to high deformability; biocompatible; and more stable	None [10]

Transfersome as vesicle delivery system

Transfersome is a type of vesicle with the ability to increase transdermal permeation in many drugs with low and high molecular weights. It can deform while penetrating into the skin and form a complex, which easily adapts to its environment and responsive to stress. Transfersomes form elastic vesicles, which can change shape when they pass through the cell membrane. The ultra deformable properties are obtained because the liquid core is surrounded by a complex bilayer lipid. The composition of transfersome as a bilayer makes them elastic vesicles. Therefore, they can pass through various transport barriers very efficiently and act as a drug delivery system for controlled release [3]. **Table 3** shows materials used in many formulations of transfersome.

Table 3. Materials in many types of Transfersome

No	Active Pharmaceutical Ingredient	Composition		Ref
		Phospholipid	Edge Activators	
1	Adapalene & Vitamin C	Lecithin soya	Tween 80, Sodium deoxycholate	[13]
2	Asenapine Maleate	Soy phosphatidylcholine (SPC)	Sodium deoxycholate	[1]
3	Baicalin	Soy Phosphatidylcholine(Lipoid S75)	Tween 80	[14]
4	Capsaicin	Phospholipon 90G	Tween 80	[15]
5	Cilnidipine	Phospholipon® 90G	Sodium cholate	[2]
6	Diclofenac Sodium	Soya Lecithin	Span 80	[16]
7	Diclofenac Sodium	Soya phosphatidylcholine (Emulmetik 930)	Tween 80	[8]
8	Diflunisal	L-alpha-Lecithin	Sodium Cholate	[10]
9	Dexamethasone	Soyaphosphatidylcholine	Sodium deoxycholate	[3]
10	DSPE-PEGPheo A (DPP)	Without lipid bilayer	Tween 80	[17]
11	Epigallocatechin-3-gallate (EGCG)	Soy Phosphatidylcholine	Sodium Cholate	[18]
12	Eprosartan mesylate	Phospholipid	Sodium Deoxycholate	[19]
13	Eprosartan mesylate	Phospholipid 90G	Sodium Deoxycholate	[20]
14	Phenylethyl resorcinol	L-a-phosphatidylcholine	Tween 20, 80, Span 20, 80, Sodium Deoxycholate	[12]
15	Genistein	Phosphatidylcholine (Lipoid S100)	Tween 80, Sodium Deoxycholate	[21]
16	Ginsenoside	Lipoid S75-3	Tween 80	[11]
17	Human Growth Hormone (hGH),	Lecithin soybean phospholipids	Sodium Deoxycholate, SLS, Brij 35	[22]
18	Ketoconazole	Phospholipon 90G & Lipoid S100	Tween 80	[23]
19	Ketoconazole	Lecithin	Tween 80	[24]
20	Lidocaine	Soybean phosphatidylcholine	Sodium Cholate, Span 80	[6]
21	Meloxicam	Phosphatidylcholine	Hexadecylpyridinium chloride(HPC), Sodium hexadecyl sulfates (SHS), sodium dodecyl sulfate (SDS), dodecylpyridinium chloride (DPC), Dicetylphosphate (DCP), stearylamine (SA)	[25]
22	Minoxidil & Caffeine	Soybean phosphatidylcholine	Tween 80 & 20	[26]
23	Ondansetron	Phosphatidylcholine	Sodium Taurocholate	[27]
24	Ostole	Soya Phosphatidylcholine	Tween 80	[28]
25	Ovalbumin	Soy Phosphatidylcholine	Sodium Cholate	[29]
26	Paromomycin Sulfate	Soya bean Phosphatidylcholine	Sodium Cholate	[30]
27	Monophosphoryl lipid A (MPL)	Egg Phosphatidylcholine	Tween 80	[31]
28	Piperin	Hydrogenated Phosphatidyl Choline	Span 80, Tween 80	[32]
29	Quercetin	Phosphatidylcholine	Tween 80	[33]
30	Raloxifen	Phospholipon 90G	Sodium deoxycholate	[34]
31	Raloxifen	Phospholipon 90G & 90H	Sodium cholate, Sodium deoxycholate	[35]
32	Raloxifen	Phospholipon 90G	Sodium cholate	[36]
33	Resveratrol	Soy phosphatidylcholine	Tween 80, Sodium cholate, Sodium deoxycholate	[37]
34	Resveratrol	Phosphatidylcholine	Tween 20, Plantacare® 1200 UP, and Tween 80	[38]
35	Risperidone	Soya Lecithin (L-a-phosphatidylcholine)	Sodium deoxycholate, Tween 80	[39]
36	Sertraline	Soya lecithin	Span 80	[40]
37	Sildenafil citrate	L-a-phosphatidylcholine	Span 80, Tween 80	[41]

38	Sildenafil citrate	L-a-phosphatidylcholine	Span 60 & 80	[42]
39	Sinomenine HCl	Egg phosphatidylcholine	Sodium deoxycholate	[43]
40	Tacrolimus	Lipoid E80	Sodium deoxycholate, Span 80, Tween 80	[44]
41	Timolol	Egg Lα phosphatidylcholine (EPC)	Tween 20	[45]
42	Timolol	L-a-phosphatidylcholine	Tween 80	[46]
43	Tocopherol	Lipoid S75	Tween 20,40,60,80	[47]
44	Valsartan	Phospholipon 90G	Sodium deoxycholate	[48]
45	Zolmitriptan	Soya Lecithin	Tween 80	[49]

Preparation of transfersome

Thin-film dispersion method

Thin-film dispersion method requires surfactants and phospholipids (as edge activators) to prepare a thin film. It is used to prepare multilamellar vesicles. A phospholipids and edge activators solution is made in a mixture of methanol and chloroform solvent. The solution is transferred into a round bottom flask with constant stirring and elevated temperature (higher than the lipids' glass transition temperature) at decreased pressure. Then, using aqueous media, a film of edge activator and lipids, formed on the walls flask is hydrated. The drug is dissolved in this media. During hydration, the lipids swell and form bilayer vesicles [48].

Reverse evaporation method

Lipids are dissolved in organic solvent in a round bottom flask. Aqueous media containing edge activators are added under nitrogen purging. Depending on the solubility, the drug can be added to the aqueous or lipid medium. The formed system is then sonicated until it becomes a homogeneous dispersion and is separated 0.5h after sonication. After that, the solvent is removed under reduced pressure and the system converts to a viscous gel followed by vesicle formation. The residual solvents and non-encapsulated materials are removed using the dialysis of centrifugation.

High-pressure homogenization method

Commonly, good-quality transfersomes are prepared by high-pressure homogenization method combining film dispersion method

Vortex/sonication method

Edge activators and phospholipids are mixed by vigorous shaking and agitation to be suspended in phosphate buffer. Then, the suspension is sonicated using a vortex or sonicator. This is further extruded through membranes of various sizes to obtain the vesicles of desired sizes

Ethanol injection method

The aqueous solution that contains the drug is heated with continuous stirring at a fixed temperature. The ethanolic solution of edge activators and phospholipids is poured dropwise into the aqueous solution. The lipid molecules precipitate and form bilayer structures as the solution comes in contact with aqueous media. This method has several advantages including simplicity, and reproducibility on scale-up [49].

The quality of transfersome after production can be conducted by the evaluation as follows:

Determination of the efficiency of entrapment

For estimating the amount of drug trapped in the vesicles, ultracentrifugation was used to separate the drug from the vesicular systems. Then, the supernatants are collected. Then, the quantity of unentrapped is determined [10]. The drug entrapment percentage (EE%) is calculated from 3 replicates as follows:

$$EE\% = \left[\frac{Total\ amount\ of\ drug\ added - Amount\ of\ untrapped\ drug}{Total\ a\ amount\ of\ drug\ added} \times 100\right] \quad (1)$$

Zeta potential, vesicle size, and size distribution measurements

The particle distribution and size, as well as zeta potential value of the vesicles, can be evaluated by dynamic light scattering (DLS) using a Zeta-Sizer. The sample is diluted with a proper medium at room temperature prior to measurements. All data are performed in triplicate [28].

Morphology of transfersome

- Transmission electron microscopy (TEM)
  Morphological evaluation of vesicles can be performed by Transmission electron microscopy. The vesicle formulations have to be diluted at 1:100 (v/v) using water. Afterwards, surface shape and features are studied at proper magnification [50].
- SEM
  The morphological evaluation of the vesicles can also be conducted by scanning electron microscopy. A drop of vesicle sample is added on the glass. A gold sputter coater is applied to the samples [15].
- Freeze-Fractured microscopy
  This technique can be used to observe the morphology of the transfersomes. A drop of vesicle dispersion is placed on a copper block and is frozen quickly in nitrogen slash. The sample is then fractured in a freeze-fracture

apparatus continued by rotary-shadowed with platinum-carbon at 10°C. The shadowed surface is coated with carbon, and observed using a TEM [25].

- Optical Microscope
  The mean size of vesicle and morphology of the transfersomes is determined by using an optical microscope. The average size measured should be more than100 particles [25].

Number of vesicle per cubic

The vesicles are counted using optical microscopy and Neubauer chamber. Then, the number of vesicles in 1 ml of dispersions is calculated with the formula:

$$\text{Concentration} = \frac{Number\ of\ vesicle\ x\ 10000}{Number\ of\ square\ x\ dilution\ factor} \quad (2)$$

Thermal behavior

DSC is utilized to investigate thermal behavior. DSC is used to measure the phase transition temperature (T_m) of the drug and transfersome. The measurements are collected under a nitrogen atmosphere. The differential thermal curves for each sample is compared.

- DSC for Physicochemical Drug and Excipients In Transfersome
  DSC is used to identify and study the interaction and physicochemical compatibility of the drug with polymers, and diluents when used in transfersomes [1].
- DSC for studying the Transfersomal-treated and Untreated Skin
  The changes in the stratum corneum structure are assessed from the obtained thermograms [31].

$$\text{Hydration \%} = \frac{\text{Weight of hydrated stratum corneum} - \text{Weight of dry stratum corneum}}{\text{Weight of dry stratum corneum}} \quad (3)$$

FTIR (fourier transforms infrared)

FTIR spectra of the sample are used to detect functional groups, identify compounds, and analyze mixtures of analyzed samples without destroying the sample.

Turbidity measurement

The turbidity of transfersome formulation was determined using the buffer as blank. The transfersomes are diluted with water and sonicated for 5 minutes. The turbidity of the sample is then measured with a UV-vis spectrophotometer [40].

Drug loading

The drug loading percentage in transfersome is determined by high-speed centrifugation of the transfersomes. The supernatant is siphoned-off and free concentration of the drug is measured [6].

Determination of the drug content

The quantity of drug within the transfersomal dosage form is determined against ethanol as blank [40].

Interfacial behaviour studies of lipid monolayers

Investigations of the interfacial behavior of adjuvant and lipid monolayers are conducted using a Langmuir-Blodgett with an area (A) at ambient temperature [31].

Degree of deformability

The deformability measurement is a unique and important parameter of transfersomal formulations. It can differentiate transfersomes from other vesicular carriers such as liposomes. The deformability test is performed by the extrusion method. The particle size and distribution of vesicles are monitored by DLS measurement before and after filtration. The degree of deformability is determined using the formula [3]:

$$D = J(\frac{rx}{rp})^2 \quad (4)$$

Where,
D = vesicle membrane deformability
rp = pore size of the barriers
rx = vesicle size (after pass)
J = amount of suspension extruded within 5min

In vitro study of drug release

Drug release from transfersomes can be measured by a modified release drug study that measures drug release from a dialysis bag installed in the dissolution chamber [46].

Permeation study

- In vitro Permeation Study
  The in vitro drug release study can be done using Franz-diffusion cells. The membrane is put between the receptor and donor compartments and centrifuged at 100 rpm and 37 ± 0.5°C. At pre-determined time intervals, samples are collected and replaced with a fresh medium. The aliquot samples are filtered through a 0.45µm membrane [49]. In vivo permeation of active drug as transfersome delivery had been extensively studied and are shown in **Table 4**.
- Ex-Vivo Permeation Study
  Using Franz-type diffusion cells that fit with skin as a membrane, the in vitro skin permeation experiments are conducted. Firstly, the frozen skin is thawed at the room temperature. The reception solution is kept in a stirred circulating water bath. The skin in the Franz diffusion cells is equilibrated. Through plotting the mean cumulative permeated amounts/cm^2 of skin against time, the permeation profile for transfersome is obtained [49]. Ex vivo permeation of active drug as transfersome delivery has also been extensively studied **(Table 5)**.

Table 4. In Vitro Permeation Study of Transfersome

Active Pharmaceutical Ingredients	Instrument Permeation Study	Membrane	Instrument of Analysis	Year	Ref
Dexamethasone	A locally fabricated diffusion cell.	Cellophane membrane	HPLC	2003	[3]
Diclofenac Sodium	Franz diffusion cells	Cellulose membrane	HPLC	2017	[8]
Genistein	Franz diffusion cells	Cellulose membrane	HPLC	2019	[21]
Ketoconazole	Franz diffusion cells	Cellulose membrane	Spectrophotometer	2012	[24]
Minoxidil and Caffeine	Franz diffusion cells	Artificial membrane	Spectrophotometer	2018	[26]
Raloxifene Hydrochloride	Franz diffusion cells	Dialysis membrane	Spectrophotometer	2018	[35]
Resveratrol	Franz diffusion cells	Strat-M® Membrane(Merck, Darmstadt, Germany)	HPLC	2019	[38]
Sertraline	Franz diffusion cells	Cellulose membrane	HPLC	2012	[40]
Sildenafil	Franz diffusion cells	Synthetic nylon membrane	Spectrophotometer	2015	[41]
Timolol Maleate	Franz diffusion cells	Cellophane membrane	Spectrophotometer	2016	[46]
	Franz diffusion cells	Cellulose membrane	HPLC	2016	[45]
Tacrolimus	Franz diffusion cells	Nylon66 filters	HPLC	2013	[44]
Zolmitriptan	Franz diffusion cells	Dialysis membrane	Spectrophotometer	2018	[49]

Table 5. In Vitro Permeation Study of Transfersome

Active Pharmaceutical Ingredients	Instrument Permeation Study	Membrane	Instrument Analysis	Year	Ref,
Adapalene	Franz diffusion cells	Goat skin	Spectrophotometer	2020	[13]
Asenapine maleate	Franz diffusion cells	Rats skin	HPLC	2016	[1]
Baicalin	Franz diffusion cells	Pig skin	HPLC	2018	[14]
Capsaicin	Franz diffusion cells	Rats skin	Confocal Laser Scanning Microscope	2015	[15]
Cilnidipine	Diffusion cells	Rats skin	Spectrophotometer	2019	[2]
Dexamethasone	Locally fabricated diffusion cell	Rats skin	HPLC	2003	[3]
Diclofenac Sodium	Franz diffusion cells	Rats skin	HPLC	2013	[16]
DPP {1,2-distearoyl-snglycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)2000] (DSPE-PEG(2000)-NH2)}	Franz diffusion cells	Mice skin	Confocal Laser Scanning Microscope	2016	[17]
Diflunisal	Franz diffusion cells	Rats skin	HPLC	2019	[10]
Epigallocatechin	Vertical diffusion cells	Rats skin	HPLC	2017	[18]
Eprosartan Mesylate	Franz diffusion cells	Rats skin	HPLC	2017	[19]
	Microneedle roller	Rats skin	HPLC	2017	[20]
Ginsenoside	Franz diffusion cells	Human cadaver skin	HPLC	2015	[11]
hGH (Human Growth Hormone)	Franz diffusion cells	Rats skin	ELISA	2019	[22]
Ketoconazole	Franz diffusion cells	Goat vaginal tissue	Spectrophotometer	2017	[23]
Lidocaine	Diffusion cell	Rats skin	Spectrophotometer	2019	[6]
Meloxicam	Side-by-side diffusion cell	Mice skin	HPLC	2013	[25]
Ondansetron	Vertical diffusion cells	Rats skin	HPLC	2018	[27]
Osthole	Side-by-side diffusion cell	Porcine skin	HPLC	2016	[28]
Ovalbumin	Franz diffusion cells	Porcine skin	Fluorescence spectroscopy	2017	[29]
Paromomycin	Franz diffusion cells	Mice skin	HPLC	2012	[30]
Phenylethyl Resorcinol	Franz diffusion cells	Pig skin	HPLC	2018	[12]
Peptide monophosphoryl lipid A	Franz diffusion cells	Pig skin	Fluorescence spectroscopy	2012	[31]
Piperin	Diffusion cell	Pig skin	Spectrophotometer	2012	[32]
Quercetin	Franz diffusion cells	Rats skin	Spectrophotometer	2020	[33]
Raloxifene Hydrochloride	Hanson diffusion cell	Rats skin	HPLC	2014	[34]
	Franz diffusion cells	Rats skin	Spectrophotometer	2018	[35]

	Franz diffusion cells	Rats skin	Spectrophotometer	2019	[36]
Resveratrol	Franz diffusion cells	Pig skin	HPLC	2013	[37]
Risperidone	Franz diffusion cells	Porcine skin	Spectrophotometer	2017	[39]
Sentraline	Franz diffusion cells	Rats skin	Spectrophotometer	2012	[40]
Sildenafil citrate	Franz diffusion cells	Rats skin	HPLC	2016	[42]
Sinomenine Hydrochloride	Franz diffusion cells	Rats skin	HPLC	2017	[43]
Tacrolimus	Franz diffusion cells	Rats skin	HPLC	2013	[44]
Tocopherol	Franz diffusion cells	Pig skin	HPLC	2018	[47]
Valsartan	Franz diffusion cells	Rats skin	HPLC	2012	[48]

1) The enhancement ratio (ER) is determined as follows [2]:

$$ER = \frac{Steady\ state\ flux\ of\ transfersome\ formula}{Steady\ state\ flux\ of\ control} \quad (5)$$

2) The cumulative amount of permeated drug is plotted as a function of time. The lag time (LT, h) and steady-state permeation rate (Jss) are calculated from the X-intercept and slope of the linear portion, respectively. The ER can be calculated as follows

$$\text{ER} = \frac{\text{Transdermal flux from vesicular formulation}}{\text{Transdermal flux from plain drug}} \quad (6)$$

3) The ER, permeability coefficient (Kp), and flux (Jmax) for the transport of drug from transfersomal compared to the marketed product) is calculated using this formula [13]:

$$\text{Jmax} = \frac{\text{Amount of drug permeate}}{\text{Time x Area of release membrane}} \quad (7)$$

$$Kp = \frac{Jmax\ drug\ concentration\ in\ the\ donor}{Initial} \quad (8)$$

$$ER = \frac{Jmax\ of\ test\ formulation}{Jmax\ of\ marketed\ product} \quad (9)$$

4) The cumulative amount of permeated drug per unit area can be plotted as a function of time. The flux can be calculated from the slope of the linear portion. The Kp of the drug across the membrane is calculated using relation derived from Fick's first diffusion law that is expressed as follows [16]:

$$K\,p = \frac{J}{C} \quad (10)$$

where C is the drug concentration and J is the flux in the donor compartment.

5) The permeation parameters including J, Kp, and ER of the drug in form of vesicles have been studied and calculated by El Salim *et al.* [10]. J (μg/cm².h) of the permeated drug from vesicles has been calculated from the slope of the plot of the cumulative quantity of drug permeated/cm² of the membrane at a steady-state against time. The steady-state Kp of the drug in the form of vesicles crossing membrane has been calculated as follows:

$$Kp = \frac{J}{C} \quad (11)$$

where J is the flux and C is the drug concentration in the donor compartment. The penetration enhancing effect of vesicles is calculated in terms of ER as follows:

$$ER = \frac{\text{Kp of drug in vesicular hydrogel}}{\text{Kp of drug in hydrogel}} \quad (12)$$

6) The cumulative amount (Q_t,μg/cm²) of drug that permeated through the membrane per unit area of skin is calculated as follows [12]:

$$Qt = \frac{CnV + \sum_{i-1}^{n-1} CiS}{A} \quad (13)$$

Where, Q_t is the cumulative amount of drug permeated (μg/cm²), A is effective diffusion surface area, S is the sampling aliquot volume, V is the volume of individual Franz diffusion cell (ml), C_i is the drug concentration determined at No.n sampling interval (μg/ml), and C_n is the drug concentration from n sampling interval (μg/ml). The Q_t amount is plotted as a function of time and the steady-state flux (J_{ss}) is calculated from the slope of the plot. The value of the Kp for the drug is calculated by the equation:

$$Kp = \frac{Jss}{C0} \quad (14)$$

Where C_0 is the initial concentration of drug in the donor compartment.

7) The formula for the amount of accumulated permeation is obtained using the following equation:

$$Q = \frac{V}{S} x\ (\sum Ci) \quad (15)$$

$$Q\% = \frac{Q}{Q0} x\ 100\% \quad (16)$$

V, C_i, and Q_0 are the volume of Franz diffusion cell, accepting liquid.

Drug retention

Evaluation of the drug retention in the skin is carried out by washing the skin with pH 7.4 PBS several times, cut into small pieces, and keeping in methanol for 24h to extract the drug deposited in the skin. The processed skin is then sonicated for 20minutes and centrifuged [16].

Penetration behaviour

Study on permeation behavior of the drug either in free form or it is as vesicles can be conducted by performing the following methods:

- CLSM
  Confocal laser scanning microscopy (CLSM) is used to assess the penetration behavior of the selected fluorescently-loaded vesicular formulations. CLSM study excised in membrane skin is treated with rhodamine-loaded optimized transfersomes formulation for 24h. After that, the excised rat skin is rinsed with DD water and subjected to glass slides. Skin morphology can be visualized by confocal microscope [3].
- Fluorescence microscopy
  Fluorescence microscopy can be carried out for confirming the penetration ability of vesicles containing the drug. Preparation of vesicles in the presence of 6-carboxyfluorescein and rhodamine provides the fluorescence labeling ability. The fluorescence marker-loaded formulation is topically applied to the skin. After 3hr of application, the skin is removed, cut into small pieces, fixed by conventional procedures, and evaluated under a fluorescence microscope. Skin treated with no formulation is used as the control [3].

Conclusion

Vesicular transfersome is a system that offers an increased effect of drug permeation into the skin with deforming capabilities supported by edge activators. The phospholipid and surfactant ratio determines the transfer permeation ability thus formulation of transfersome requires optimization of variables involved, including the process parameters and ratio of each of the components in the formulation.

Acknowledgments: The authors thank Directorate of Higher Education, Ministry of Education, Republic of Indonesia for funding the research project connected to this review as Grant for Disertation of Doctoral Student year 2020.

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Shreya AB, Managuli RS, Menon J, Kondapalli L, Hegde AR, Avadhani K, et al. Nano-transfersomal formulations for transdermal delivery of asenapine maleate: in vitro and in vivo performance evaluations. J Liposome Res. 2016;26(3):221-32.
2. Khatoon K, Rizwanullah M, Amin S, Mir SR, Akhter S. Cilnidipine loaded transfersomes for transdermal application: Formulation optimization, in-vitro and in-vivo study. J Drug Deliv Sci Technol. 2019;54:101303.
3. Jain S, Jain P, Umamaheshwari RB, Jain NK. Transfersomes - A Novel Vesicular Carrier for Enhanced Transdermal Delivery: Development, Characterization, and Performance Evaluation. Drug Dev Ind Pharm. 2003;29(9):1013-26.
4. Haque T, Talukder MMU. Chemical enhancer: A simplistic way to modulate barrier function of the stratum corneum. Adv Pharm Bull. 2018;8(2):169-79.
5. Baroni A, Buommino E, De Gregorio V, Ruocco E, Ruocco V, Wolf R. Structure and function of the epidermis related to barrier properties. Clin Dermatol. 2012;30(3):257-62. doi:10.1016/j.clindermatol.2011.08.007
6. Omar MM, Hasan OA, El Sisi AM. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: A promising approach for enhancement of skin permeation. Int J Nanomedicine. 2019;14:1551-62.
7. Hasan M, Khatun A, Fukuta T, Kogure K. Noninvasive transdermal delivery of liposomes by weak electric current. Adv Drug Deliv Rev. 2020;1-9. doi:10.1016/j.addr.2020.06.016
8. Malinovskaja-Gomez K, Espuelas S, Garrido MJ, Hirvonen J, Laaksonen T. Comparison of liposomal drug formulations for transdermal iontophoretic drug delivery. Eur J Pharm Sci. 2017;106:294-301.
9. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. Science. 1995;269(5225):850-3.
10. Abd El-Alim SH, Kassem AA, Basha M, Salama A. Comparative study of liposomes, ethosomes and transfersomes as carriers for enhancing the transdermal delivery of diflunisal: In vitro and in vivo evaluation. Int J Pharm. 2019;563:293-303.
11. Choi JH, Cho SH, Yun JJ, Yu YB, Cho CW. Ethosomes and transfersomes for topical delivery of ginsenoside Rh1 from red ginseng: Characterization and In Vitro evaluation. J Nanosci Nanotechnol. 2015;15(8):5660-2.
12. Amnuaikit T, Limsuwan T, Khongkow P, Boonme P. Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes. Asian J Pharm Sci. 2018;13(5):472-84. doi:10.1016/j.ajps.2018.02.004

13. Vasanth S, Dubey A, Ravi GS, Lewis SA, Ghate VM, El-Zahaby SA, et al. Development and Investigation of Vitamin C-Enriched Adapalene-Loaded Transfersome Gel: a Collegial Approach for the Treatment of Acne Vulgaris. AAPS PharmSciTech. 2020;21(2):1-7.
14. Manconi M, Manca ML, Caddeo C, Valenti D, Cencetti C, Diez-Sales O, et al. Nanodesign of new self-assembling core-shell gellan-transfersomes loading baicalin and in vivo evaluation of repair response in skin. Nanomedicine Nanotechnology, Biol Med. 2018;14(2):569-79.
15. Sarwa KK, Mazumder B, Rudrapal M, Verma VK. Potential of capsaicin-loaded transfersomes in arthritic rats. Drug Deliv. 2015;22(5):638-46.
16. Ghanbarzadeh S, Arami S. Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. Biomed Res Int. 2013;2013.
17. Park H, Lee J, Jeong S, Im BN, Kim MK, Yang SG, et al. Lipase-Sensitive Transfersomes Based on Photosensitizer/Polymerizable Lipid Conjugate for Selective Antimicrobial Photodynamic Therapy of Acne. Adv Healthc Mater. 2016;5(24):3139-47.
18. Avadhani KS, Manikkath J, Tiwari M, Chandrasekhar M, Godavarthi A, Vidya SM, et al. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. Drug Deliv. 2017;24(1):61-74.
19. Ahad A, Al-Saleh AA, Al-Mohizea AM, Al-Jenoobi FI, Raish M, Yassin AEB, et al. Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of eprosartan mesylate. Saudi Pharm J. 2017;25(7):1040-6. doi:10.1016/j.jsps.2017.01.006
20. Ahad A, Al-Saleh AA, Al-Mohizea AM, Al-Jenoobi FI, Raish M, Yassin AEB, et al. Pharmacodynamic study of eprosartan mesylate-loaded transfersomes Carbopol®gel under Dermaroller®on rats with methyl prednisolone acetate-induced hypertension. Biomed Pharmacother. 2017;89:177-84.
21. Langasco R, Fancello S, Rassu G, Cossu M, Cavalli R, Galleri G, et al. Increasing protective activity of genistein by loading into transfersomes: A new potential adjuvant in the oxidative stress-related neurodegenerative diseases? Phytomedicine. 2019;52:23-31.
22. Kateh Shamshiri M, Momtazi-Borojeni AA, Khodabandeh Shahraky M, Rahimi F. Lecithin soybean phospholipid nano-transfersomes as potential carriers for transdermal delivery of the human growth hormone. J Cell Biochem. 2019;120(6):9023-33.
23. Singh S, Verma D, Mirza MA, Das AK, Dudeja M, Anwer MK, et al. Development and optimization of ketoconazole loaded nano-transfersomal gel for vaginal delivery using Box-Behnken design: In vitro, ex vivo characterization and antimicrobial evaluation. J Drug Deliv Sci Technol. 2017;39:95-103.
24. Rajan R, Vasudevan DT. Effect of permeation enhancers on the penetration mechanism of transfersomal gel of ketoconazole. J Adv Pharm Technol Res. 2012;3(2):112-6.
25. Duangjit S, Opanasopit P, Rojarata T, Obata Y, Oniki Y, Takayama K, et al. Send Orders for Reprints to reprints@benthamscience.net The Open Conference Proceedings The Role of Deformable Liposome Characteristics on Skin Permeability of Meloxicam: Optimal Transfersome as Transdermal Delivery Carriers. Journal. 2013;4.
26. Ramezani V, Honarvar M, Seyedabadi M, Karimollah A, Ranjbar AM, Hashemi M. Formulation and optimization of transfersome containing minoxidil and caffeine. J Drug Deliv Sci Technol. 2018;44:129-35.
27. Habib BA, Sayed S, Elsayed GM. Enhanced transdermal delivery of ondansetron using nanovesicular systems: Fabrication, characterization, optimization and ex-vivo permeation study-Box-Cox transformation practical example. Eur J Pharm Sci. 2018;115:352-61.
28. Meng S, Zhang C, Shi W, Zhang X, Liu D, Wang P, et al. Preparation of osthole-loaded nano-vesicles for skin delivery: Characterization, in vitro skin permeation and preliminary in vivo pharmacokinetic studies. Eur J Pharm Sci. 2016;92:49-54. doi:10.1016/j.ejps.2016.04.033
29. Zhang Y, Ng W, Feng X, Cao F, Xu H. Lipid vesicular nanocarrier: Quick encapsulation efficiency determination and transcutaneous application. Int J Pharm. 2017;516(1-2):225-30. doi:10.1016/j.ijpharm.2016.11.011
30. Bavarsad N, Fazly Bazzaz BS, Khamesipour A, Jaafari MR. Colloidal, in vitro and in vivo anti-leishmanial properties of transfersomes containing paromomycin sulfate in susceptible BALB/c mice. Acta Trop. 2012;124(1):33-41.
31. Rattanapak T, Young K, Rades T, Hook S. Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: Characterisation and in vitro skin penetration. J Pharm Pharmacol. 2012;64(11):1560-9.
32. Vinod KR, Anbazhagan S, Kumar MS, Sandhya S, Banji D, Rani AP. Developing ultra deformable vesicular transportation of a bioactive alkaloid in pursuit of vitiligo therapy. Asian Pacific J Trop Dis. 2012;2(4):301-6. doi:10.1016/S2222-1808(12)60066-8
33. Pandit AP, Omase SB, Mute VM. A chitosan film containing quercetin-loaded transfersomes for treatment of secondary osteoporosis. Drug Deliv Transl Res. 2020:1-12.
34. Mahmood S, Taher M, Mandal UK uma. Experimental design and optimization of raloxifene hydrochloride loaded nanotransfersomes for transdermal application. Int J Nanomedicine. 2014;9:4331-46.
35. Joshi A, Kaur J, Kulkarni R, Chaudhari R. In-vitro and Ex-vivo evaluation of Raloxifene hydrochloride delivery using nano-transfersome based formulations. J Drug Deliv Sci Technol. 2018;45:151-8.
36. Waheed A, Aqil M, Ahad A, Imam SS, Moolakkadath T, Iqbal Z, et al. Improved bioavailability of raloxifene

hydrochloride using limonene containing transdermal nano-sized vesicles. J Drug Deliv Sci Technol. 2019;52:468-76.
37. Scognamiglio I, De Stefano D, Campani V, Mayol L, Carnuccio R, Fabbrocini G, et al. Nanocarriers for topical administration of resveratrol: A comparative study. Int J Pharm. 2013;440(2):179-87.
38. Wu PS, Li YS, Kuo YC, Tsai SJJ, Lin CC. Preparation and evaluation of novel transfersomes combined with the natural antioxidant resveratrol. Molecules. 2019;24(3):1-12.
39. Das B, Sen SO, Maji R, Nayak AK, Sen KK. Transferosomal gel for transdermal delivery of risperidone: Formulation optimization and ex vivo permeation. J Drug Deliv Sci Technol. 2017;38:59-71.
40. Gupta A, Aggarwal G, Singla S, Arora R. Transfersomes: A novel vesicular carrier for enhanced transdermal delivery of sertraline: Development, characterization, and performance evaluation. Sci Pharm. 2012;80(4):1061-80.
41. Ahmed TA. Preparation of transfersomes encapsulating sildenafil aimed for transdermal drug delivery: Plackett-Burman design and characterization. J Liposome Res. 2015;25(1):1-10.
42. Badr-Eldin SM, Ahmed OAA. Optimized nano-transfersomal films for enhanced sildenafil citrate transdermal delivery: Ex vivo and in vivo evaluation. Drug Des Devel Ther. 2016;10:1323-33.
43. Wang J, Wei Y, Fei YR, Fang L, Zheng HS, Mu CF, et al. Preparation of mixed monoterpenes edge activated PEGylated transfersomes to improve the in vivo transdermal delivery efficiency of sinomenine hydrochloride. Int J Pharm. 2017;533(1):266-74.
44. Lei W, Yu C, Lin H, Zhou X. Development of tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement in vivo. Asian J Pharm Sci. 2013;8(6):336-45.
45. González-Rodríguez ML, Arroyo CM, Cózar-Bernal MJ, González-R PL, León JM, Calle M, et al. Deformability properties of timolol-loaded transfersomes based on the extrusion mechanism. Statistical optimization of the process. Drug Dev Ind Pharm. 2016;42(10):1683-94.
46. Morsi NM, Aboelwafa AA, Dawoud MHS. Improved bioavailability of timolol maleate via transdermal transfersomal gel: Statistical optimization, characterization, and pharmacokinetic assessment. J Adv Res. 2016;7(5):691-701. doi:10.1016/j.jare.2016.07.003
47. Caddeo C, Manca ML, Peris JE, Usach I, Diez-Sales O, Matos M, et al. Tocopherol-loaded transfersomes: In vitro antioxidant activity and efficacy in skin regeneration. Int J Pharm. 2018;551(1-2):34-41.
48. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M, Ali A. Formulation and optimization of nanotransfersomes using experimental design technique for accentuated transdermal delivery of valsartan. Nanomedicine Nanotechnology, Biol Med. 2012;8(2):237-49. doi:10.1016/j.nano.2011.06.004
49. Pitta SK, Dudhipala N, Narala A, Veerabrahma K. Development of zolmitriptan transfersomes by Box–Behnken design for nasal delivery: in vitro and in vivo evaluation. Drug Dev Ind Pharm. 2018;44(3):484-92. doi:10.1080/03639045.2017.1402918
50. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Evaluation of meloxicam-loaded cationic transfersomes as transdermal drug delivery carriers. AAPS Pharm Sci Tech. 2013;14(1):133-40.